Journal of Applied Food Technology

Home page: https://ejournal2.undip.ac.id/index.php/jaft

Characterization of Layer-by-Layer Biodegradable Films Based on Hydroxypropyl Methylcellulose-Nanochitosan

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Abstract

This research investigates the physical and optical properties of single-layer and layer-by-layer biodegradable films composed of hydroxypropyl methylcellulose (HPMC) and nanochitosan. Initially, HPMC and nanochitosan were formulated as single layers at various concentrations, and subsequently, the selected formulas were utilized to produce a layer-by-layer film. The results indicate that the concentrations of 0.4% w/v HPMC and 0.5% w/v of nanochitosan were successfully assembled into a layer-by-layer biodegradable film. Assessment based on multiple parameters (thickness, moisture content, water vapor transmission rate, color, transparency, and biodegradability) reveals that the deposition of nanochitosan onto HPMC in a layer-by-layer configuration enhances most characteristics of single-layer HPMC films, with the exception of optical properties. Moreover, all samples were degraded within a seven-day observation period.

Article information:
Received: 15 November 2023
Accepted: 28 November 2023
Available online: 6 December 2023

Keywords:
biodegradable films
hydroxypropyl methylcellulose
layer-by-layer
nanochitosan

doi: 10.17728/jaft.20868

Introduction

Post-harvest handling refers to the process of preserving quality and extending shelf life. Post-harvest stages in a food supply chain need more attention to enhance products' worth and minimize food waste (Kuyu et al., 2019). Several efforts to develop post-harvest methods have been conducted, such as high-pressure processing (HPP) (Zarzecka et al., 2023), irradiation (Zhao et al., 2023), modified atmosphere packaging (MAP), and pulsed electric field (PEF) (Karki et al., 2023). However, simpler processes, such as edible coating, can be used to achieve an environmentally friendly approach.

Edible coatings are derived from natural substances and are easier to degrade (Jurić et al., 2023) yet provide adequate protection to prolong food substances. The edible coating creates a semi-permeable layer responsible for controlling the water vapor and oxygen transfer of food products (Maringgal et al., 2020). Several common materials are polysaccharides (starch, cellulose, pectin, and alginate), proteins (gelatine, soy protein, zein, and casein), and lipids (wax, fatty acids) (Yaashikaa et al., 2023). However, polysaccharides are the most abundant, cost-effective, and well-defined chemical structure, making them easier to access, predict, and modify.

Cellulose, a highly available polysaccharide, has not been fully utilized and possesses numerous derivatives. For instance, Hydroxypropyl methylcellulose (HPMC) is commonly used to produce edible coatings. HPMC could produce an edible coating with robust mechanical properties. However, hydrophilicity and hygroscopicity lead to low permeability properties; a previous study by Arnon-Rips & Poverenov (2018) and Jurić et al. (2023) introduced chitosan into HPMC to overcome it.

Chitosan is also popularly used to prepare edible coatings and has lower water contact than HPMC, resulting in lower hydrophilicity. Obtaining from the deacetylation of chitin, it also acts as an antimicrobial and anti-browning to food products (Pratama et al., 2019; Takeshita et al., 2021). HPMC and nanochitosan could form a strong hydrogen bond, enhancing HPMCs' permeability properties and, in most application cases, significantly lessening physical and quality losses (e.g., color, total antioxidant, and organic acids). In Jurić et al. (2023) study, HPMC and chitosan exhibited a glossier appearance. Modifying chitosan into nanoparticles, called nanochitosan, has improved its stability (Sam et al., 2022). However, different solvent and gelation behaviors make it challenging to accomplish these mixtures' compatibility and fine distribution.

Layer-by-layer (LbL) application method is proposed as an alternative. In its application, LbL does not mix those materials, which can alter their characteristics (Arnon-Rips & Poverenov, 2018); besides, it deposits material into a sandwich-like construction, allowing each material to work separately. LbL was previously studied by Yan et al. (2019), Hira et al. (2022), and Chen et al. (2023). Regrettably, the dipping method was used for all those studies. The dipping method remains the most common and widely used technique for applying edible coating solutions because of its simplicity and cost-effectiveness. However, Pham et al. (2023) stated that the dipping method is inefficient as it requires an excessive solution to immerse food products entirely. Also, the possibility of cross-contamination is higher as the same solution was used. On the other hand, the spraying method can be an alternative to address these issues (e.g., efficient application, minimal cross-contamination, and less wastage of the solution). Although it seems simple, many factors must be considered to produce edible coating solutions that can be effectively sprayed (Silva-Vera et al., 2018). The most crucial factor is viscosity. The edible coating solution's viscosity must be adjusted to a minimum possible to allow spraying while still effectively protecting food products. Additionally, a lower viscosity of the coating solution is preferable to expanding application techniques.

Further exploration of the spraying method for applying layer-by-layer edible coatings is still needed. The aim of this research is to investigate the physical and optical characteristics of biodegradable films that focuses on analyzing films constructed through single-layer and layer-by-layer methods utilizing a combination of HPMC (Hydroxypropyl Methylcellulose) and nanochitosan. An assessment will be undertaken on biodegradable films to expand examinations that cannot performed on solution form (e.g., physical, mechanical, and permeability characteristics) (Mileti et al., 2023). Therefore, the research will be carried out on single-layer and LbL biodegradable films based on HPMC and nanochitosan to understand their characteristics and potential for broadening LbL application.

Materials and Methods

Materials

The main materials were hydroxypropyl methylcellulose (HPMC) obtained from Prima Chemical & Packaging (Indonesia), and nanochitosan (Chitafood, Indonesia). Solvent materials namely distilled water and acetic acid purchased from Prima Chemical & Packaging (Indonesia). Supporting apparatus including hot plat stirrer (Sojilab HS-12), food dehydrator (Wirastar FD-10), viscometer (Viscometer ndj-8s), micrometer (Kris), color reader (Chnspec CS-10), oven (Memmert), and laboratory glassware.

Methods

Solution production

Solution productions were carried out on both hydroxypropyl methylcellulose (HPMC) and nanochitosan. HPMC solutions were made based on Jurić et al. (2023) with slight modifications on temperature and concentration. Solutions were made by dissolving 0.4%, 0.8%, and 1.2% (w/v) of hot distilled water (45 – 50℃), then stirred using a hot plate stirrer (Sojilab HS-12) for ±5 minutes, HPMC solution then cooled down until ±30℃.

Nanochitosan solutions were prepared following the method outlined by Jurić et al. (2023), with minor adjustments made to the concentration. Nanochitosan flakes were dissolved in 1% (v/v) acetic acids using 0.5%, 1.0%, and 1.5% (w/v), then stirred using a hot plate stirrer for ±7 – 10 minutes and cooled down until the solution turned clear (±15 – 20 minutes). All solutions were made and then measured using a viscometer (Viscometer ndj-8s) to determine their viscosity value.

Biodegradable Film production

Biodegradable film production was prepared on both hydroxypropyl methylcellulose (HPMC) and nanochitosan from the previous stage. Each of the HPMC and nanochitosan films were poured into a film mold with ±2-3 cm on thicknesses. The filled mold was then dried in a food dehydrator (Wirastar FD-10). The temperature was set at ±30℃ for 12 – 14 hours. Once the drying process was done, biodegradable film was successfully made. A conditioning stage at air-room temperature for ±1-3 hours was required until the films were easily peeled from mold. The biodegradable film peeled was then analyzed.

Layer-by-layer (LbL) Biodegradable Film Production

After evaluation from a single-layer biodegradable film, the chosen HPMC and nanochitosan concentration from the previous stage were applied to LbL biodegradable films. Single-layer of HPMC and nanochitosan, HPMC as the innermost layer (HPMC/nanochitosan), nanochitosan as the innermost layer (nanochitosan/HPMC), and single-layer composite (mixture ratio 1:1 of HPMC and nanochitosan) were made. The first layer must be thoroughly dried before the second layer is placed. The production of LbL was based on Yu et al. (2023)

Viscosity Analysis

Viscosity analysis was conducted in both HPMC and nanochitosan was made at every concentration.

Analysis was held based on the American Society for Testing and Material (ASTM) number D552-17 using a viscometer instrument (Viscometer ndj-8s). The solution was put into Beker glass, and then the spindle was submerged until the mark. Speed was set based on the initial trial, and press start until the values on the screen turned constant.

Thickness Analysis

Thickness analysis was conducted on every piece of film, both single-layer and layer-by-layer, using a micrometer instrument using ASTM-D1005-95. The examination was held on subtle surfaces, unbroken and unfolded. Analysis was made on ten different areas with an accuracy of 0.01 mm. The data is then given as the average of ten values measured at millimeter (mm) unit.

Moisture Content Analysis

Moisture contents were carried out using a gravimetric method based on the Official Method of Analysis of the Association of Official Analytical Chemists (AOAC) (2000). Biodegradable film sheets were cut into small pieces weighed in ranges between 0.3 – 0.5 grams (Weight A) and placed into porcelain cups that had previously been oven-dried overnight. Samples were put in a cup and then oven-dried for ±12 hours at 105℃ until constant weight (weight B) was reached (the difference was less than 0.002 grams). The calculation of moisture content was expressed on percentage (%) based on the equation:

$$Moisture\ Contents\ (\%) = \frac{Weight\ A - Weight\ B}{Weight\ \ A}\ x\ 100\%$$

Color Analysis

Color analysis was calculated following ASTM E1347-06 using Tristimulus Colorimetry (Chnspec CS-10). The instrument was calibrated using a black and white panel available from the device before use. Color analysis was carried out by putting the optic probe on a biodegradable film sheet. Results of color analysis were displayed on the screen using CIE L, a*, and b* values. Analysis was made on ten replications, and the data given were average of these replications.

Transparency Analysis

Transparency analysis was done using spectrophotometry UV-Vis based on ASTM D1746-15. Samples were first shaped into cuvette-size ± 4 x 1 cm, then read the absorbance at 600 nm wavelength (A600). The calculation of transparency was expressed in %T using the calculation:

$$\%T\ = 10^{(2-A600)}$$

Water Vapor Transmission Rate (WVTR) Analysis

WVTR analysis was held using ASTM-E96-00 methods. Analysis was carried out using an oven-dried porcelain cup filled with oven-dried silica gel and closed tightly by the biodegradable film sheet. Cups are then arranged at an atmosphere-modified desiccator to maintain the humidity stability. Every hour until 10 data reached, cups were weighed using an analytical balance. WVTR values were calculated using the equation:

$$WVTR = \frac{Weight\ difference\ (gram)}{Surface\ area\ (m^2)\ x\ time\ (hour)}$$

Biodegradability Analysis

Biodegradability was based on Beghetto et al. (2020) since there is no standard method for soil burial tests. Samples were cut into 2 x 2 cm pieces and then buried under 4 cm of soil for seven days. The observation was conducted once every 24 hours.

Table 1. Characteristics evaluation of solutions and single-layer biodegradable films of HPMC and Nanochitosan

Treatment	Viscosity (mPa.s)	Thickness (mm)	Moisture content (%)	WVTR ($g/h.m^2$)	Color		
					L	a*	b*
Hydroxypropyl methylcellulose (HPMC)							
HPMC 0.4%	36.8 ± 3.2^a	0.05 ± 0.01	10.92 ± 1.71^a	2.65 ± 0.20	89.98±0.47^b	-0.58±0.21^a	5.99±2.38^c
HPMC 0.8%	670.7 ± 19.1^b	0.05 ± 0.01	12.49 ± 1.61^{ab}	2.42 ± 0.40	89.71±0.23^c	-0.70±0.12^a	5.74±0.37^b
HPMC 1.2%	2243 ± 24.2^c	0.06 ± 0.01	16.13 ± 4.32^b	2.38 ± 0.14	92.81±1.55^a	1.70±0.63^b	1.55±0.62^a
Nanochitosan							
Nanochitosan 0.5%	17.4 ± 0.5^a	0.04 ± 0.01^a	20.09 ± 0.20^b	0.73 ± 0.17	88.95±0.55	-0.35±0.13^a	7.76±1.33
Nanochitosan 1.0%	49.8 ± 1.6^b	0.06 ± 0.01^b	19.56 ± 0.85^{ab}	0.63 ± 0.09	90.05±3.49	1.92±1.38^b	6.96±1.55
Nanochitosan 1.5%	77.7 ± 1.6^c	0.07 ± 0.02^b	18.91 ± 0.67^a	0.62 ± 0.17	89.79±4.03	1.94±1.01^b	7.36±2.07

*HPMC = hydroxypropyl methylcellulose; WVTR = water vapor transmission rate
*Results showed as mean ± standard deviation (n = 6)
*Different superscript letters in the same column indicate the significant differences ($p < 0.05$).

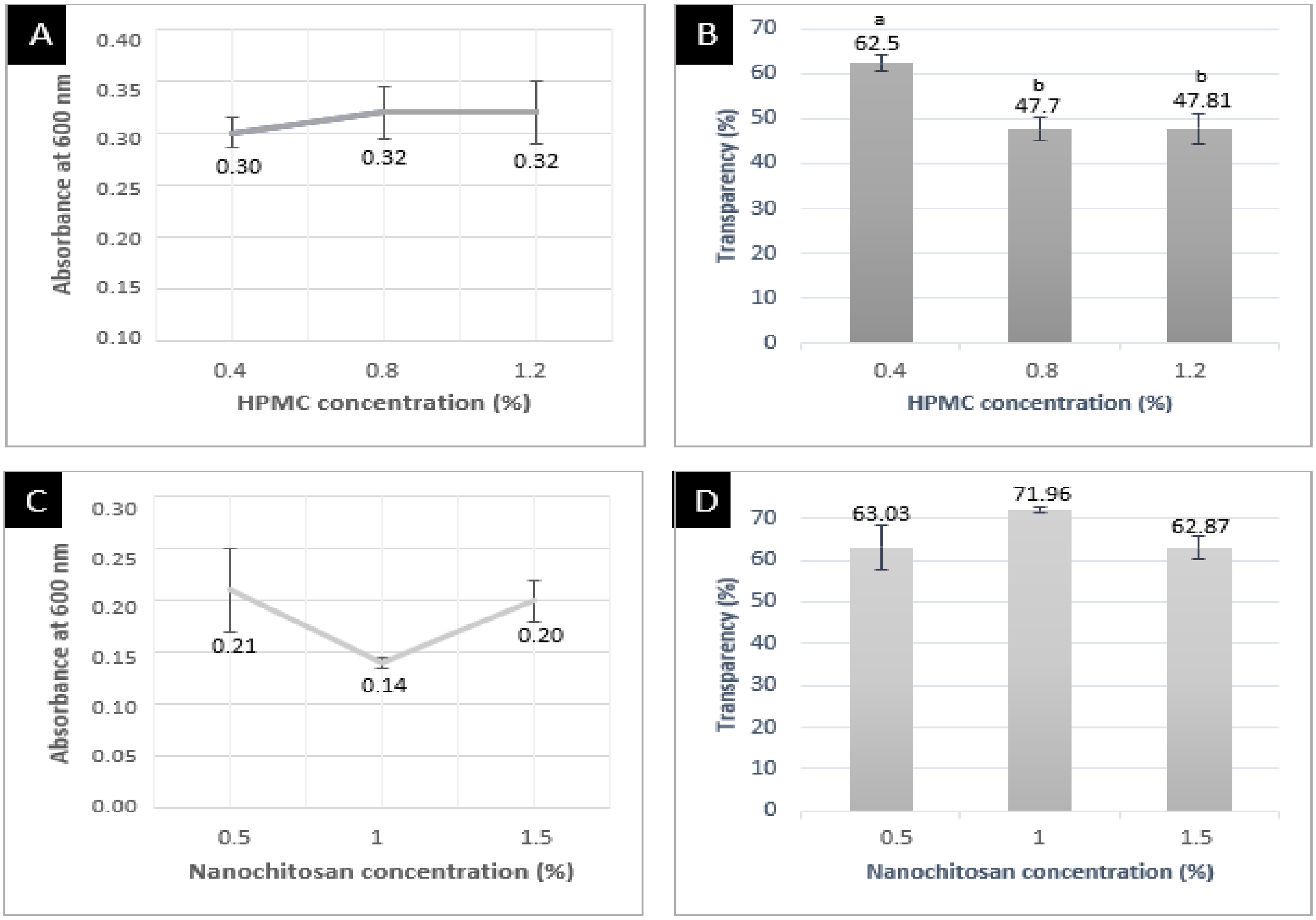

Figure 1. Optical analysis of single-layer biodegradable film including (A) absorbance at 600 nm wavelength and (B) transparency of hydroxypropyl methylcellulose (HPMC) single-layer biodegradable film, (C) absorbance at 600 nm wavelength and (B) transparency of nanochitosan single-layer biodegradable film

Biodegradable Film Characteristics

The solution was then made into biodegradable film through the drying process. In this process, precipitation occurs, transforming suspensions into a thin and unbroken sheet. The evaluation of the biodegradable film, including thickness, moisture content, water vapor transmission rate (WVTR), color (L, a*, and b* values) (see Table 1), transparency (see Figures 1 and 2), and biodegradability (see Figure 3).

Results and Discussion

Solutions Characteristics

Characteristics of the solution were determined using viscosity measurements, expressing the consistency of the liquid. Viscosity values (see Table 1) obtained from this study range from 36.8 mPa.S to 2243 mPa.S and 17.4 mPa.S to 77.7 mPa.S for hydroxypropyl methylcellulose (HPMC) and nanochitosan respectively. The addition of concentration significantly increased the viscosity of both HPMC and nanochitosan ($p < 0.05$). A higher viscosity value indicates a thicker liquid, which is reasonable since adding material concentration results in more total dissolved solids in suspensions (Sancakli et al., 2021). At the lowest concentration of HPMC, the viscosity was 36.8 mPa.S, surpassing nanochitosan, which registered only 17.4 mPa.S. The gelatinization behavior of a material might influence it. HPMC is known as the gelling agent, having groups of hydroxyls that establish a robust viscous suspension (Wang et al., 2021). On the other hand, nanochitosan only forms a gel through amino groups (Takeshita et al., 2021). The addition of concentration in HPMC resulted in more significant values of viscosity, which were 670.7 mPa.S and 2243 mPa.S from 0.8% and 1.2% of HPMC (w/v) respectively, owing to the addition of hydrophilic groups on the solutions. At the same time, the addition of concentration in nanochitosan also resulted in higher viscosity from 49.8 mPa.S to 77.7 mPa.S at 1% and 1.5% of nanochitosan (w/v), respectively. It is worth mentioning that the various viscosity values cannot be generalized as the best or the worst but rather depend on methods of further applications.

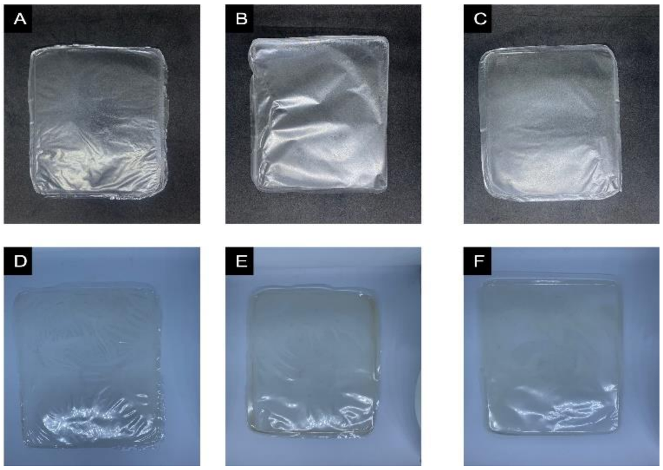

Figure 2. Single-layer biodegradable film of different concentrations of (A) 0.4% (w/v), (B) 0.8% (w/v), and (C) 1.2% (w/v) of hydroxypropyl methylcellulose (HPMC) and (C) 0.5% (w/v), (D) 1.0% (w/v) and (E) 1.5% (w/v) of nanochitosan.

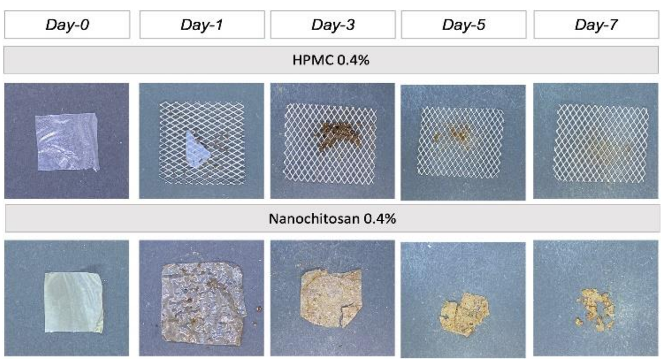

Figure 3. Representation of biodegradability on hydroxypropyl methylcellulose (HPMC) 0.4% (w/v) and nanochitosan 0.5% (w/v) single layer during seven days of observation

Thickness is a fundamental evaluation to determine the terms 'biodegradable films'; Warkoyo et al. (2022) stated that biodegradable films should be less than 0.25 mm in thickness. The thickness of the films was evaluated using a micrometer and expressed in mm. Thickness measurements were performed on ten points to avoid biases from unsymmetrical surfaces. The data is presented in Table 1. All treatments demonstrated thickness results of less than 0.25 mm. HPMC thickness ranged from 0.05 mm for 0.4% and 0.8% concentration of HPMC (w/v) and increased to 0.06 mm with the addition of concentration (1.2% HPMC (w/v)). Statistical analysis shows no significant difference at each concentration ($p > 0.05$). Differing from HPMC, nanochitosan films' statistical calculation showed the difference at each concentration ($p < 0.05$). Thickness in nanochitosan films ranged from 0.04 mm to 0.07 mm, following the addition of concentration in HPMC and nanochitosan films. Similar trends were identified in prior studies conducted by Yu et al. (2023). Higher concentrations led to increased dry matter and reduced water content, resulting in greater evaporation during the drying process, consequently enhancing the thickness of the films (Ghadermazi et al., 2019). Furthermore, thickness also defines the feasibility and opacity of biodegradable film products (Lim et al., 2021).

The biodegradable film's moisture content (MC) expresses the total percentage of water contained in the products. HPMC films' moisture content ranges from 10.92% to 16,13%, while the nanochitosan is 20,09% to 18,91%, considered to have low moisture content (16.48 – 23.96%) based on Warkoyo et al. (2022)**.** The statistical evaluation showed that different HPMC and nanochitosan concentrations significantly influence the moisture content value ($p < 0.05$). Adding HPMC concentration resulted in higher moisture content, whereas the nanochitosan lowered the moisture content. The trend difference between HPMC and nanochitosan film is probably related to the existence of hydrophilic groups in HPMC accounted by hydroxyl groups (Chiaregato et al., 2023). Hydrophilic groups exhibit affinity to water, increasing HPMCs' moisture content with the addition of the concentration. The water contact angle could be investigated to indicate a hydrophilic degree of biodegradable films (Ngo et al., 2020).

The lowest moisture content of nanochitosan (18.91%) was higher than HPMC's highest (16.13%), probably caused by the different solvents used in the production. Acetic acid, a solvent of nanochitosan, requires a higher boiling point temperature than water, which is used as the HPMC solvent (Ahammed et al., 2023). The higher boiling point of acetic acid causes less water to evaporate during drying. Consequently, higher moisture content occurred on the product.

The moisture content of films suggested product durability; therefore, it needs to be maintained low. In general, the lower moisture content of biodegradable film could protect food longer due to the lack of nutrition for microbial growth (Bradford et al., 2020). Water is recognized as the leading cause of food deterioration (Yadav et al., 2022). Although moisture content can contribute to food deterioration, the crucial parameter for maintaining the quality of food products in the biodegradable film is the water vapor transmission rate (WVTR) (Roshandel-hesari et al., 2022). WVTR exhibits the rate at which water vapor will permeate through a material over a specific period (Sultan et al., 2021). WVTR results of this study are presented in Table 1. The WVTR of 0.4% HPMC (w/v) was 2.65 g/h.m2 and reduced to 2.42 g/h.m2 and 2.38 g/h.m2 in the higher concentration (0.8% and 1.2% of HPMC (w/v)) respectively. On the other hand, WVTR values of nanochitosan range from 0.73 g/h.m2 to 0.62 g/h.m2. The addition of concentration in HPMC and nanochitosan did not significantly influence the WVTR values ($p > 0.05$). However, it is still necessary to understand the results. The graphs showed higher HPMC and nanochitosan concentrations lead to lower WVTR values. The addition of concentration seems responsible for lowering the WVTR value of biodegradable films. The decreased value might be prompted by the greater amount of HPMC and nanochitosan in the products. HPMC is a hygroscopic material that absorbs environmental moisture (Zhou et al., 2021). Meanwhile, compared to HPMC, chitosan showed lower hygroscopicity in a prior study (Yang et al., 2020).

Regarding the optical characteristics, color and transparency measurements were conducted. The results of the color evaluation in this study are presented in Table 1, while the transparency is in Figure 1. The color evaluation was expressed in L, a*, and b* values. The L parameters, measuring the lightness of the films, varied from 89.71 to 92.81 in HPMC and 88.95 to 90.05, indicating the high brightness of the films. Statistical reports show there was a significant difference ($p < 0.05$) in HPMC films and no significant difference ($p > 0.05$) in nanochitosan films. Higher L values were obtained from higher HPMC concentration and lower nanochitosan concentration. The a* value parameter expresses greenness-redness values. HPMC films a* value range from -0.58 to 1.70, indicating the greener color, while nanochitosan ranges from -0.35 to 1.94, indicating the redder color. On the other hand, the b* value parameter represents blueness-yellowness. In this study, HPMC b* values varied from 1.55 to 5.99 in HPMC, bluer than nanochitosan results, which ranged from 6.96 to 7.76. Nanochitosan contained astaxanthins, pigments from crustaceans corresponding in red-yellowish color. The increase of a* and b* values might be associated with the presence of color pigments in the material (Warkoyo et al., 2022).

Transparency measurements were carried out using 600 nm wavelength in spectrophotometry. %T was measured to identify transparency, implying the clarity of the film by the transmittance of light (Zhao et al., 2022). Higher transparency value was needed to produce clear and see-through films linking to the physical food product looks. The transparency value of HPMC films ranges from 47.70% to 62.50%, while nanochitosan ranges from 62.87% to 71.96%. Generally, the transparency of HPMC films decreased with increasing concentration (see Figure 1A-B). On the contrary, results from nanochitosan films showed a higher value of %T along with the concentration addition (see Figure 1C-D). Figure 2A-C disclosed a biodegradable film picture to confirm the optical results from the naked eye, HPMC can produce transparent films on 0.4% concentration of HPMC (w/v); however, blurry films were obtained with the addition of concentration, probably due to inhomogeneous solutions. It can be found that homogenous energy to dissolve 0.4% HPMC (w/v) and 1.2% HPMC (w/v) cannot remain the same. Additionally, increasing the solids content can increase the refractive index, thereby reducing transparency (Chen et al., 2022). Meanwhile, nanochitosan films display transparent films with no significant difference ($p > 0.05$) at various concentrations (see Figure 2D-F), indicating a homogenous solution at all concentrations. Further investigations, such as surface morphologies, are needed to verify.

Biodegradability is also an essential parameter to biodegradable films' environmental-friendly claims. Soil burial tests were conducted for seven days to evaluate the resilience of films in time and the environmental degradation of films (Apriliyani et al., 2020). However, due to residual soil in the film samples, weighing examination could not accurately occur. Thus, observation is only held in physical appearance. The study was previously successfully held by Beghetto et al. (2020). As presented in Figure 3, HPMC films only required approximately three days to decompose entirely, while nanochitosan films could not fully decompose after seven days. The hydrogen bond between HPMC and water requires less energy to break down than the ionic interactions between nanochitosan and acetic acid (Qiao et al., 2021).

Table 2. Characteristic evaluation of layer-by-layer biodegradable films

Treatment	**Thickness (mm)**	**Moisture Content (%)**	**WVTR (g/h.m^2)**	**Color**		
				L	**a***	**b***
HPMC 0.4%	0.04 ± 0.00	11.44 ± 1.14^{a}	5.91 ± 0.50^{c}	92.56 ± 0.45^{e}	1.13 ± 0.33	2.19 ± 0.29^{a}
Nanochitosan 0.5%	0.04 ± 0.00	20.81 ± 0.56^{c}	4.15 ± 0.51^{a}	86.04 ± 0.69^{a}	-0.20 ± 0.14	6.28 ± 1.19^{c}
Nanochitosan 0.5% / HPMC 0.4%	0.06 ± 0.01	17.87 ± 0.42^{b}	4.31 ± 0.99^{ab}	88.93 ± 0.87^{d}	0.72 ± 0.36	6.40 ± 1.27^{b}
HPMC 0.4% / Nanochitosan 0.5%	0.05 ± 0.00	16.96 ± 1.04^{b}	5.17 ± 0.62^{bc}	90.20 ± 0.54^{b}	0.65 ± 0.34	4.45 ± 0.83^{c}
HPMC 0.4% + Nanochitosan 0.5%	0.04 ± 0.00	16.74 ± 0.42^{b}	6.09 ± 0.36^{c}	89.86 ± 2.05^{bc}	0.52 ± 0.61	4.00 ± 0.78^{b}

*HPMC = hydroxypropyl methylcellulose; WVTR = water vapor transmission rate
*Results showed as mean±standard deviation (n = 4)
*Different superscript letters in the same column indicate the significant differences ($p < 0.05$).

Layer-by-Layer Biodegradable Film Characteristics

Based on the examination of the single-layer biodegradable film, 0.4% HPMC (w/v) and 0.5% nanochitosan (w/v) were chosen based on viscosity, WVTR, and L value to produce layer-by-layer (LbL) biodegradable films. Single-layer composite films (a mixture of HPMC and nanochitosan using a 1:1 ratio) were included as the comparison. The evaluation assessment of LbL films, including thickness, moisture content, WVTR, color (L, a*, b* values) (see Table 2), transparency, and biodegradability (see Figure 4). Thickness measurements on LbL films remain the same at the initial stages. The data presented in Table 2 averages ten points of four repetitions. Similar results were found in LbL films compared to the previous stage. Single-layer HPMC was the thinnest, followed by single-layer nanochitosan, composite, HPMC/nanochitosan, and nanochitosan/HPMC, which were the thickest. Increases in thickness by adding nanoparticles were also found in previous studies by Chen et al. (2022), Perera et al. (2022), and Khater et al. (2023). It is reasonable since nanochitosan has smaller particles, resulting in diffusion to HPMC films when deposited as the outermost layer, generating a thinner layer than HPMC as the outermost. Strong interaction between these two materials could also influence less evaporation during drying, resulting in a thicker layer than a single layer.

The moisture content of LbL films is presented in Table 2. Single-layer nanochitosan was 20.81%, higher than single-layer HPMC, which was 11.44%, similar to the initial investigation. Ranging from 16.74% to 17.87%, the merger of two materials registered no significant difference ($p > 0.05$). The lowest moisture content of the combination was obtained from the composite of HPMC and nanochitosan with a 1:1 ratio. Adding HPMC lowers the moisture content of single-layer nanochitosan due to the strong intermolecular hydrogen bonds (Yu et al., 2023). Further investigation, such as Fourier-Transform Infrared Spectroscopy (FT-IR) and X-ray Diffraction (XRD), is needed for confirmation.

The water vapor transmission rate (WVTR) of LbL films was similar to the initial stage. WVTR values were ranged between 4.15 g/h.m2 and 6.09 g/h.m2. The merger of a combination of these two materials registered a slight yet significant ($p < 0.05$) difference. As the outermost and innermost layer in LbL films, nanochitosan addition successfully lowers the WVTR value of single-layer HPMC. It is reasonable since nanochitosan could reduce HPMC's water contact due to solid hydrogen bond reactions. Additionally, incorporating nanochitosan into HPMC would form a zigzag path, suppressing vapor transmission (Yu et al., 2023).

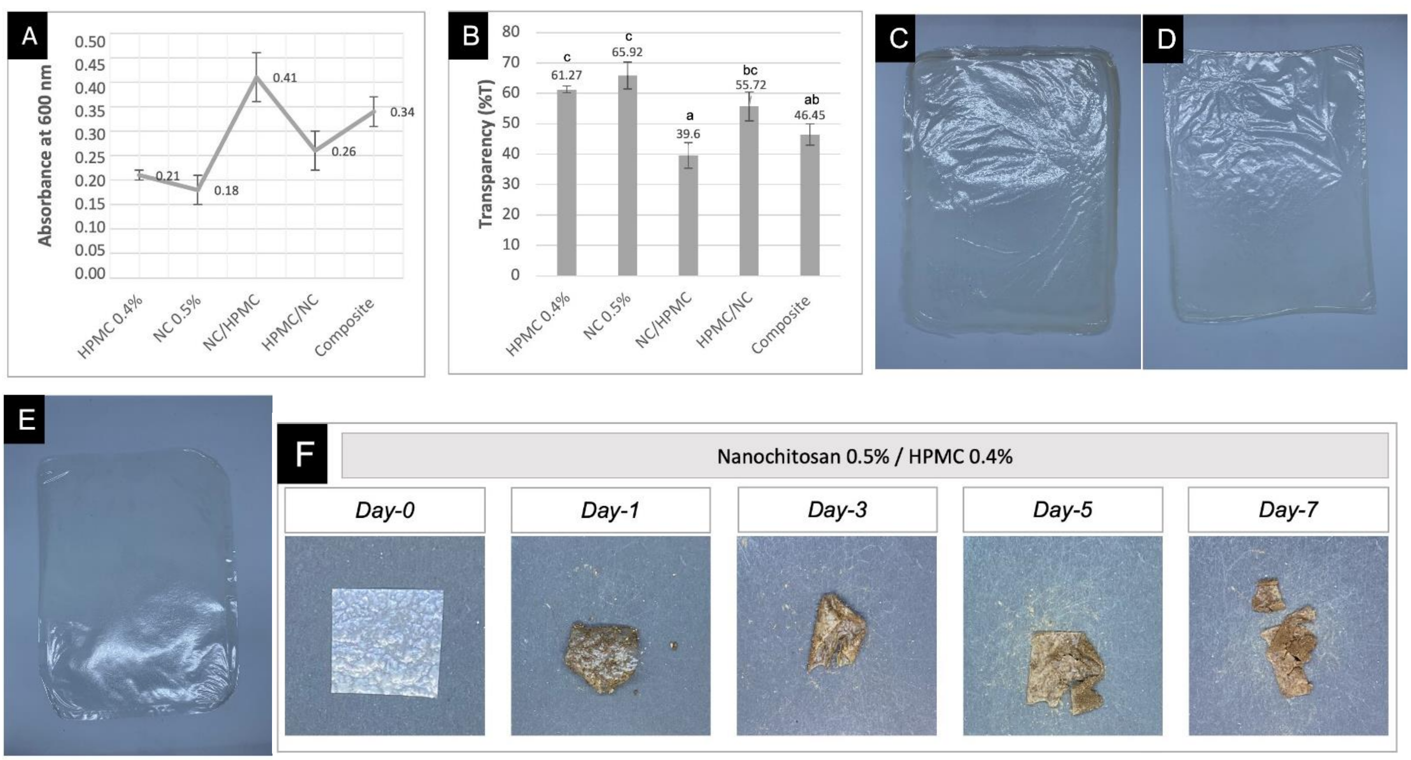

Figure 4. The physical appearance of layer-by-layer biodegradable film (A) nanochitosan (NC) / hydroxypropyl methylcellulose (HPMC); (B) HPMC/NC; and (C) composites. Optical measurements of absorbance at 600 nm wavelength (D), transparency (E), and biodegradability (F) of layer-by-layer biodegradable film.

In terms of optical characteristics, color and transparency were also carried out on LbL films. The color results resembled those of the initial examination, with the L values of HPMC at 92.56, higher than nanochitosan, which measured only 86.04. It is mainly due to the presence of astaxanthins, red-yellowish pigments in crustaceans as the raw material of nanochitosan (Yarnpakdee et al., 2022), leading to a darker color of films. On the other hand, the combination of these materials on LbL and composites ranged between 92.56 to 86.04. The highest results were obtained from HPMC/nanochitosan, followed by composite and nanochitosan/HPMC, which were the darkest. Generally, the addition of nanochitosan to HPMC as both the outermost and innermost layer, however, lowers its value (see Figure 4A-C). The* value of LbL film was positive except for single-layer nanochitosan, which showed the redness of its films ($p < 0.05$). The astaxanthin pigments were still responsible for its case. Furthermore, astaxanthins also generated a higher value of b*, resulting in yellower films ($p < 0.05$). HPMC were transparent polymers; however, adding other materials could alter their properties (Ghadermazi et al., 2019). The merger of nanochitosan to HPMC as the innermost, outermost, and mixture resulted in darker, redder, and yellower films. Its color results could impact food appearance during further application on food products (Puscaselu et al., 2020).

Additionally, the transparency measurements were carried out using 600 nm wavelength to define the clarity of LbL films. The results were delivered in Figure 4A-B as graphs. A higher percentage of T demanded the definition of its purities. A similar finding was found in LbL films compared to the initial stage. Single-layer nanochitosan (65.91%) films were the highest, showing clear and see-through films compared to others. Followed by HPMC (61.27%), and the combination was lower than its single-layered. Ranging between 55.72% and 39.60%, LbL of HPMC/nanochitosan is the highest of the other combinations, followed by composite and nanochitosan/HPMC. Despite adding nanochitosan could influence to lowered transparency of HPMC, indeed, LbL were homogenously assembled as depicted in Figure 4C-E.

Regarding biodegradability, similar findings compared to the initial stages also occurred (see Figure 4F). The soil burial test ran for seven days, and single-layer HPMC was easier to degrade than other samples. On the contrary, nanochitosan was the hardest among them all. The combination of HPMC and nanochitosan resulted in between; after seven days, the LbL and composites did not break down like single-layer HPMC completely but had more resilience than nanochitosan. Its discovery had practical implications for expanding the solution application. For example, the usage of edible coating, which requires immediate dilution after washing, or food wrapped, which requires resilience characteristics to protect stronger.

Conclusion

The optimal concentrations of hydroxypropyl methylcellulose (HPMC) and nanochitosan in a single layer were identified as 0.4% and 0.5%, respectively. Employing these specific concentrations, Layer-by-layer (LbL) films were successfully produced. These LbL films demonstrated enhancements in thickness, water vapor transmission (WVTR), and biodegradability. However, in contrast to their single-layer characteristic, the color parameter of the LbL films exhibited a reduction. Additionally, this investigation highlights the potential utilization of LbL films in food items, particularly within the agricultural commodities.

Acknowledgments

The authors thank the Directorate General of Higher Education, Research, and Technology, Ministry of Research, Technology and Higher Education Indonesia for the funding project of “Penelitian dan Pengabdian Kepada Masyarakat” year 2023.

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