

The effect of EDTA concentration on antimicrobial chitosan, EDTA, and polyvinyl alcohol films for inhibiting aerobic bacteria on stored tilapia fillets

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Abstract

This study proposes to create packaging films using a blend of polyvinyl alcohol (PVA), chitosan (CS), and ethylenediaminetetraacetic acid (EDTA) in order to inhibit bacterial growth on tilapia fillets during storage. The films were prepared in three formulations with varying weight ratios: PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4. The results showed that as the concentration of EDTA increased, the ΔE^* value, thickness, moisture content, and water vapor transmission rate of the films also increased. The morphology of the cross-sectional microstructure revealed a small spot in the PVA6/CS10/EDTA4 film, while the other formulations exhibited a homogeneous structure. FT-IR analysis indicated that the hydroxyl and carboxyl group peaks in the blended films (PVA, CS, and EDTA) were more prominent than in the individual components. Further, the tilapia fillets wrapped in the PVA6/CS10/EDTA4 film showed the greatest total color change but experienced less weight loss than the other films after storage at 4-7 °C for 7 days. The increased EDTA concentration in the wrapped film enhanced the inhibition of aerobic bacteria on the tilapia fillets. Therefore, the combination of PVA, CS, and EDTA in the film effectively inhibited bacterial growth and extended the shelf life of the tilapia fillets.

Keywords: Chitosan, Ethylenediaminetetraacetic acid (EDTA), Polyvinyl alcohol, Tilapia, Antimicrobial packaging

1. Introduction

In recent years, active packaging has emerged as a promising approach to enhancing food preservation. Among the various types, including UV-protective and antioxidant packaging, antimicrobial packaging has garnered the most attention due to its ability to prolong shelf life and to ensure food safety. The strategies for inhibiting microbial growth include reducing oxygen levels through scavengers or vacuum packaging [1], modifying package atmosphere [2], pre-treating meat with antimicrobial solutions [3], and incorporating antimicrobial agents directly into the packaging material [4].

Currently, extensive research is being conducted to develop antimicrobial packaging that is both user-friendly and highly effective in suppressing microbial growth. Various antimicrobial agents have been widely utilized, including chitosan, tannin, nisin, and ethylenediaminetetraacetic acid (EDTA), all of which exhibit antibacterial activity [5-7]. Naewkanya and Sowasod [5] demonstrated that tannic acid/polyvinyl alcohol (PVA) films inhibited bacterial growth on tilapia fillets for over seven days at $4 \pm 2^\circ\text{C}$, although higher concentrations caused discoloration [5]. In order to overcome such drawbacks, white or non-leaching agents are preferred.

Chitosan (CS), a polysaccharide derived from crustacean shells and fungi, is widely used in food, environmental, and medical industries due to its film-forming ability, biodegradability, and antimicrobial properties [8-10]. It is soluble in weak acids such as acetic or citric acid and is active against a broad range of microorganisms [11-13]. Its antimicrobial action involves disrupting microbial membranes and interfering with cellular functions [13]. Moreover, chitosan (poly-D-glucosamine) is exempt from the requirement of a tolerance for residues in or on all food commodities under the U.S. Environmental Protection Agency (EPA) regulation (40 CFR §180.1072), based on its demonstrated low toxicity and favorable safety profile [14]. This regulatory status further supports its suitability for food-related applications, particularly in food packaging systems.

EDTA is a metal-chelating agent used in foods to delay lipid oxidation and suppress off-odors, thereby extending product shelf life [15]. It is odorless, non-toxic, and water-soluble, with proven activity against Gram-negative and Gram-positive bacteria [7]. Its primary mechanism involves chelating divalent metal ions from bacterial membranes and weakening cell structures, leading to lysis [7]. However, due to its poor film-forming ability, EDTA must be blended with polymers such as PVA.

PVA is a biodegradable, flexible, and non-toxic synthetic polymer known for its excellent mechanical strength and film-forming capacity. These properties make it a suitable base for food-contact packaging materials [16, 17]. Recent studies have shown that

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blending PVA with CS and EDTA enhances antibacterial effectiveness. For instance, Hamza et al. [6] reported increased inhibition zone diameters for CS/EDTA and PVA/CS/EDTA composites compared to chitosan alone [6].

Tilapia (*Oreochromis niloticus*) is one of the most economically important freshwater fish in Thailand, with an annual production exceeding 250,000 tons in 2023, including 6,000 tons for export [18]. As the third most farmed fish worldwide [19], tilapia is in high demand. However, its high perishability limits storage life to less than five days at 4–10°C before bacterial counts exceed acceptable levels (6 log CFU/g) [5, 20]. Bacterial spoilage directly affects the appearance, texture, and odor of the fish, making microbial contamination a key concern for quality and safety.

In response to these challenges, this study aimed to investigate the effect of EDTA concentration on antimicrobial films composed of chitosan (CS), ethylenediaminetetraacetic acid (EDTA), and polyvinyl alcohol (PVA) for application in tilapia packaging. The films were characterized in terms of their physical and chemical properties—including moisture content, color, and water vapor permeability—and examined using scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). Their antimicrobial effectiveness was evaluated by assessing aerobic bacterial counts, weight loss, and visual changes in tilapia fillets during refrigerated storage at 4–7°C for seven days.

2. Materials and methods

2.1 Materials

Polyvinyl alcohol (PVA) with a degree of hydrolysis of 86–90% was purchased from Bangkok Chemical Co., Ltd. Shrimp chitosan (CS) powder was provided by Biolife ELAND, Thailand. Ethylenediaminetetraacetic acid (EDTA) with 99% purity was obtained from Bangkok Chemical Co., Ltd. Glycerol (99.5%) and acetic acid (CH₃COOH, 99.8%) were acquired from ANAPURE Co., Ltd., New Zealand. Aerobic bacteria count plates were purchased from 3M Petrifilm Co., Ltd.

2.2 Preparation of the film

The experimental conditions for the preparation of PVA, CS, and EDTA films are summarized in Table 1. A 6 wt% PVA solution was prepared by dissolving PVA in a 1% v/v acetic acid solution under magnetic stirring at 90°C for 30 minutes. Separately, a 10 wt% CS solution and an EDTA solution at appropriate concentrations were prepared in a 1% v/v acetic acid solution and stirred for 20 minutes at 35°C. For each film formulation, 0.3 g of CS (10 wt%), a designated amount of EDTA, and a calculated amount of PVA were combined to form a total of 3.0 g of solid content. These solids were dissolved in 50 mL of 1% (v/v) acetic acid. Acetic acid functioned as a crosslinking agent in all formulations. The PVA, CS, and EDTA solutions (50 mL) were combined and continuously stirred at 70°C until a visually homogeneous mixture was obtained. After 30 minutes, 2 mL of glycerol was added to the PVA/CS/EDTA solution and stirred for an additional 5 minutes at the same temperature to ensure complete dissolution and consistency of the final film-forming solution. Glycerol served as a plasticizer in order to enhance the flexibility of the resulting films.

In order to remove air bubbles, the mixtures were subjected to centrifugation (Model FC5706 Frontier, OHAUS, China) at 4,000 rpm for 1 minute. Subsequently, the PVA/CS/EDTA solutions were poured into Petri dishes (90 × 15 mm) and oven-dried at 35°C for 24 hours. Once dried, the films were carefully peeled off the Petri dishes, placed individually in zipper bags, and stored in a desiccator until further analysis and testing.

Table 1 Experimental conditions used to prepare the PVA, CS, and EDTA films

Sample	PVA (g)	CS (g)	EDTA (g)	PVA/CS/EDTA ^a
PVA6/CS10	2.7	0.3	0	90/10/0
PVA6/CS10/EDTA2	2.64	0.3	0.6	88/10/2
PVA6/CS10/EDTA4	2.37	0.3	0.12	86/10/4

^a Each formulation contained 3.00 g of total solid content (CS, EDTA, PVA) dissolved in 50 mL of 1% (v/v) acetic acid solution.

2.3 Characterization

2.3.1 Thickness and color measurement

The thickness of each film was measured using a micrometer (resolution 0.01 mm; model MC MW200-01, Mitutoyo, Japan). The thickness of each sample was measured at 3 different positions, and the average thickness was calculated.

The color of the films was measured using a colorimeter (Hunter Lab Digital Color Difference Meter, model YS 3020, 3nh, China) at room temperature [21]. The instrument was calibrated with a white standard ($L_0^* = 96.49$, $a_0^* = -0.23$, and $b_0^* = 0.3$) before measurements. The results were expressed as L^* (lightness), a^* (red/green), and b^* (yellow/blue) parameters. The total color difference (ΔE^*) was calculated using the differences between the color values of the samples and a white standard plate according to the following equation:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

For each film, three points of color value were taken at different positions, and the average values were determined.

2.3.2 Moisture content and water vapor transmission rate

The moisture content (MC) measurement method was adapted from the AOAC protocol [22]. Film specimens measuring 20 mm × 20 mm were prepared for the MC analysis. Each sample was pre-weighed (M_0) and subsequently dried in a hot air oven (Model SOV70B, KWF, China) at 105°C for 24 h until a constant weight (M_1) was achieved. All of the measurements were performed in duplicate. The moisture content of the films was determined using the following equation:

$$MC(\%) = \frac{M_0 - M_1}{M_0} \times 100 \quad (2)$$

The water vapor transmission rate (WVTR) of the films was determined using a method adapted from ASTM E96 standard method [23], with slight modifications. The film was sealed on top of 50 mL glass beakers (Duran) containing 30 mL of distilled water, with an estimated vertical distance of approximately 46 mm between the film and the water surface. The exposed film surface area was 0.00126 m². The prepared beakers were then placed in a hot air oven at 37 ± 1°C with a controlled relative humidity of 55 ± 1.5%. All of the measurements were conducted in duplicate. The covered beakers were weighed at hourly intervals over a period of 6 h. The WVTR was calculated using the following equation:

$$WVTR = \frac{\text{Mass lost}}{t \times \text{film area}} = \frac{\text{slope}}{\text{film area}} \quad (3)$$

where mass lost is the weight of water lost (g), t is the time of oven (d.), film area is the area of the exposed film surface (m²), and slope is the slope of the graph of the relationship between weight loss and time (g/day).

2.3.3 Infrared spectroscopy

The films were characterized using Fourier transform infrared spectroscopy (FTIR, Spectrum Gx, PerkinElmer Scientific, USA) in attenuated total reflection (ATR) mode. The spectra were recorded over the range of 4000 to 500 cm⁻¹ with a resolution of 4 cm⁻¹, based on a total of 16 scans.

2.4 Shelf-life study of the tilapia fillets after storage

2.4.1 Fish preparation and shelf-life study

Tilapia (*Oreochromis niloticus*) with an average weight of approximately 1 kg were purchased from Mae Kep Market in Rayong, Thailand. The fish were washed with tap water and then filleted. After filleting, the fish fillets were stored at 4-7°C for 15 hours. Subsequently, the fillets were washed again with running tap water, de-skinned, and cut into portions weighing approximately 5 g each. The fillet samples were obtained from the same fish, specifically from the upper part of the body, avoiding the gastrointestinal tract, gills, and blood-rich areas. The fillets were then divided into three test groups: (1) fish without packaging (control), (2) fish wrapped with PVA and CS film, and (3) fish wrapped with PVA, CS, and EDTA film. All of the samples were placed in Petri dishes and stored at 4-7°C for a period of 7 days. At 0, 3, 5, and 7 days, the samples were assessed for weight, color, and aerobic bacterial count. All of the experiments were conducted in duplicate.

2.4.2 Microbial analysis and enumeration

For the microbial analysis, a 5 g sample of a tilapia fillet was homogenized with 45 mL of 0.1% peptone water in a sterilized homogenizer (Otto model CP-390A) for 1 minute. The homogenate was then used for aerobic bacterial determination. A 1 mL aliquot of the homogenate was serially diluted (1:10), and 1 mL of each dilution was inoculated onto 3M Aerobic Petrifilm™ count plates. The plates were incubated at 35°C ± 1°C for 48 ± 3 h in an incubator (Dae Yang ETS Co., Ltd., Korea). Colony counts were expressed as log CFU/g.

3. Results and discussion

3.1 Physical appearance

Physical appearance is a critical property for packaging applications. Figure 1 shows the digital images of the PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 films, all of which exhibited a uniform light-yellow color, with good transparency and flexibility. The slight yellow coloration is attributed to the chitin content in chitosan [24]. The PVA6/CS10 and PVA6/CS10/EDTA2 films appeared smooth and clear, while the PVA6/CS10/EDTA4 film was also transparent but showed a slightly deeper yellow tone compared to the other formulations. The flexibility of all the films can be attributed to the addition of glycerol as a plasticizer [25].

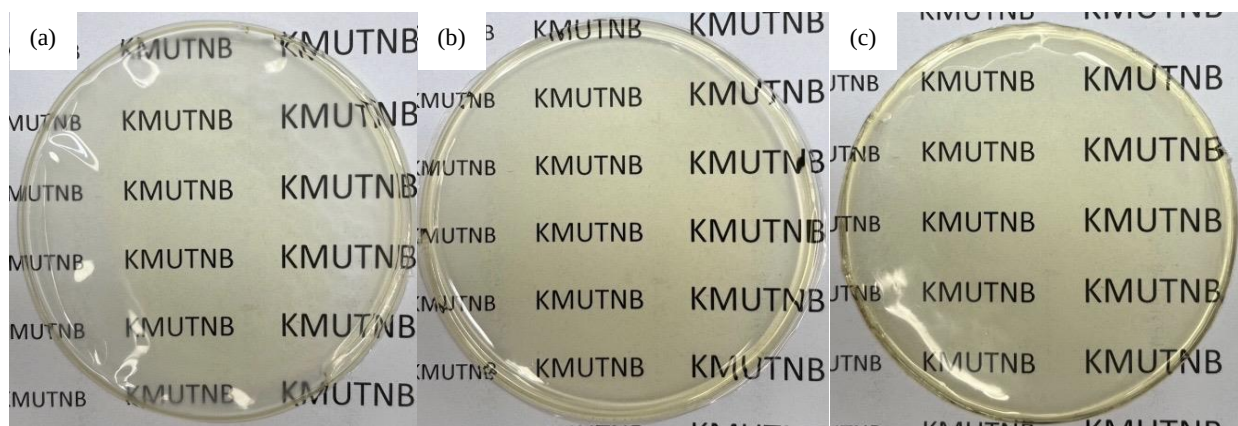


Figure 1 Digital images of the PVA6/CS10 (a), PVA6/CS10/EDTA2 (b), and PVA6/CS10/EDTA4 (c) film.

3.2 Thickness and color

The color of the film plays a crucial role in food packaging applications, as it can influence consumer purchasing decisions. The color values (L^* , a^* , and b^*), total color difference (ΔE^*), and the thickness of the PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 films are presented in Table 2. The objective evaluation of the film appearance is provided by the L^* , a^* , and b^* values, while the color change of the film relative to a standard white color is measured by ΔE^* . The results indicated slight variations in the L^* (brightness), a^* (red), and b^* (yellow) color values across the films. The ΔE^* values of the PVA6/CS10/EDTA4 films were higher than those of the PVA6/CS10/EDTA2 and PVA6/CS10 films. However, no statistically significant difference in the ΔE^* values was observed between the PVA6/CS10/EDTA4 and PVA6/CS10/EDTA2 films ($p > 0.05$). This suggests that the increase in the EDTA concentration in the films resulted in reduced brightness (L^*), accompanied by an increase in green (negative a^*) and yellow (positive b^*) color components. The increased yellowness can be attributed to the high molecular weight of EDTA and the pale-yellow color of the chitin naturally present in chitosan [24], resulting in a higher total color difference. Regarding the film thickness, the PVA6/CS10 film exhibited a lower thickness compared to the PVA6/CS10/EDTA2 and PVA6/CS10/EDTA4 films. No statistically significant differences in thickness were found between the PVA6/CS10/EDTA4 and PVA6/CS10/EDTA2 films ($p > 0.05$). The slight increase in film thickness with higher EDTA concentration can be attributed to the overall increase in material content. Similar trends were reported by Jones and Zivanovic [26], which reported that chitosan films containing EDTA had a higher average thickness compared to chitosan films without EDTA [27].

Table 2 Color parameters (L^* , a^* and b^*), total color difference (ΔE^*), and the thickness of the PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 film

Samples	L^*	a^*	b^*	ΔE	Thickness (mm)
PVA6/CS10	90.39±0.39 [*]	-2.32±0.07 ^a	9.37±0.48 ^a	11.12±0.62 ^a	0.586±0.07 [*]
PVA6/CS10/EDTA2	87.96±1.15 [*]	-2.99±0.72 ^a	17.67±6.58 ^a	15.94±5.83 ^b	0.654±0.05 [*]
PVA6/CS10/EDTA4	86.80±0.73 [*]	-3.42±0.03 ^b	22.56±1.42 ^b	16.85±2.44 ^b	0.667±0.10 [*]

^{*}No significant difference ($P > 0.05$) in the same column.

^{a, b} Different letters in the same column indicate significant difference ($P < 0.05$).

3.3 Moisture content and water vapor transmission rate

The hydrophilic characteristics of the PVA, CS, and EDTA films under high relative humidity conditions may lead to changes in their mechanical properties. Assessing water sensitivity is essential for evaluating the performance of food packaging materials. The MC and WVTR of the PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 films are presented in Table 3.

The MC quantifies the water resistance of films, especially under humid conditions. In this study, the MC values of all films were relatively high, as all components—CS, EDTA, PVA, and glycerol—possess hydrophilic properties [26, 27]. Additionally, the amine groups in CS are protonated from NH_2 to NH_3^+ in a dilute acidic film-forming solution, further increasing the hydrophilic nature of CS [28]. The MC values of the PVA6/CS10/EDTA2 and PVA6/CS10/EDTA4 films were higher than those of the PVA6/CS10 film, though the difference between the PVA6/CS10/EDTA4 and PVA6/CS10/EDTA2 films was not statistically significant ($p > 0.05$). The higher MC value in the films incorporating EDTA can be attributed to the presence of four carboxyl groups ($-\text{COOH}$) and two amino groups ($-\text{NH}_2$) in EDTA, which have a strong affinity for hydrogen and oxygen atoms in water molecules [29], thus increasing the MC value. The slight increase in the MC value of the PVA6/CS10/EDTA4 film compared to the PVA6/CS10/EDTA2 film was likely due to the higher EDTA content in the former.

The WVTR values ranged from 3670.48 to 4712.38 g/day·m². The PVA6/CS10/EDTA4 film exhibited the highest WVTR, followed by the PVA6/CS10/EDTA2 and PVA6/CS10 films. The increase in WVTR after adding EDTA can be attributed to its hydrophilic nature, which enhances the film's ability to attract water molecules [30], thereby facilitating water vapor transmission.

Table 3 Moisture content (MC) and water vapor transmission rate (WVTR) of the PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 film

Samples	MC (%)	WVTR (g/day m ²)
PVA6/CS10	43.94±0.70 ^a	3670.48±35.02 ^a
PVA6/CS10/EDTA2	52.60±2.35 ^b	4338.10±84.85 ^b
PVA6/CS10/EDTA4	53.88±0.10 ^b	4712.38±45.79 ^c

^{a, b, c} Different letters in the same column indicate significant differences ($p < 0.05$).

3.4 Morphological property

The morphology of the PVA, CS, and EDTA films at various ratios was examined using scanning electron microscopy (SEM). The cross-sectional microstructures of the PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 films at two different magnifications are presented in Figure 2. The results indicated that the PVA6/CS10 and PVA6/CS10/EDTA2 films exhibited smooth, homogeneous, and uniform surfaces without any visible pores. This can be attributed to the strong molecular interactions between the functional groups of PVA, CS, and EDTA, which promote homogeneity and prevent pore formation [30]. In contrast, the PVA6/CS10/EDTA4 film, while free of pores, displayed small spots on the surface. This phenomenon suggests that some EDTA content may not have dissolved completely during the film-forming process. This incomplete dissolution could be due to the limited crosslinking ability of acetic acid, which contains a small number of carboxyl groups ($-\text{COOH}$) and is therefore less capable of facilitating crosslinking between the functional groups in the polymer chains, such as amino groups ($-\text{NH}_2$) or hydroxyl groups ($-\text{OH}$) [31].

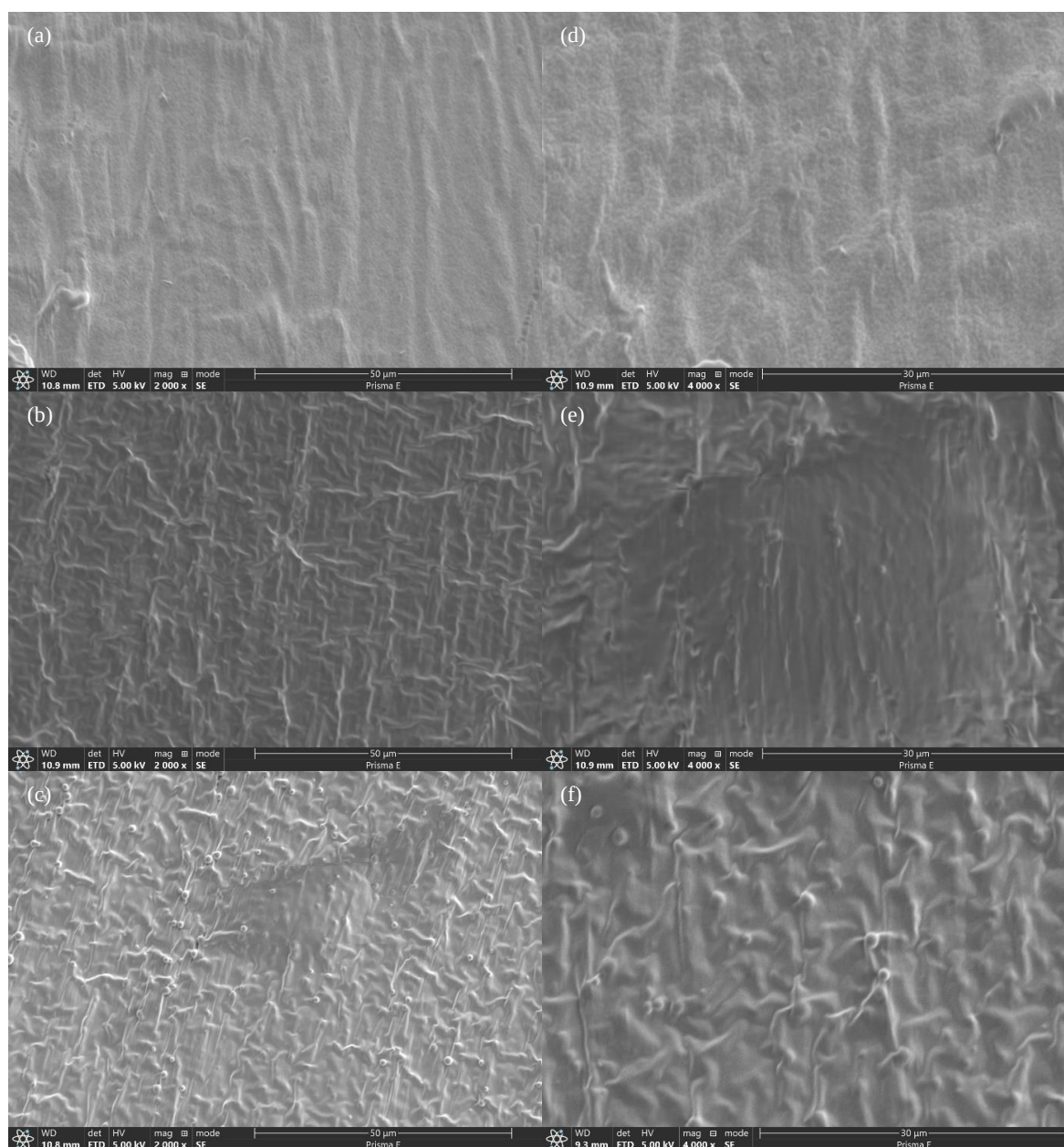


Figure 2 SEM micrographs of the cross-section film at 2000x (a) PVA6/CS10, (b) PVA6/CS10/EDTA2, (c) PVA6/CS10/EDTA4, and 4000x (d) PVA6/CS10, (e) PVA6/CS10/EDTA2, and (f) PVA6/CS10/EDTA4

3.5 Chemical structure characterization

The structural and spectroscopic changes of the films, analyzed using Fourier-transform infrared (FTIR) spectroscopy, are shown in Figure 3. The FTIR spectra of the CS, EDTA, and blended films (PVA6/CS10 and EDTA at 2–4% w/w) are presented for comparison. The FTIR spectra of CS exhibited a broad -OH stretching absorption band between 3600 and 3000 cm^{-1} , the amino group (-NH₂) stretching between 3360 and 3290 cm^{-1} , C-H stretching between 2990 and 2850 cm^{-1} , and an absorption peak at 1583 cm^{-1} representing -C=O stretching. Additionally, the absorption peak at 1000 cm^{-1} corresponded to the C-O-C glycosidic ring. For EDTA, the FTIR spectra revealed a broad -OH stretching absorption band between 3600 and 3300 cm^{-1} . The spectra also displayed an absorption peak at 1612 cm^{-1} , attributed to -C=O stretching, 1476 cm^{-1} for CH₂ bending, 1394 cm^{-1} for -OH bending, and an absorption peak between 1250 cm^{-1} and 1020 cm^{-1} corresponding to -C-N stretching. The FTIR spectrum of the PVA6/CS10 film closely resembled that of the CS/EDTA/PVA blend films across various ratios. All of the blended films exhibited a broad -OH stretching band at approximately 3300 cm^{-1} , indicating strong intramolecular and intermolecular -H bonding [32]. Notably, compared to the peaks of CS and EDTA powders without a cross-linking agent, a shift in the peak at 2938 cm^{-1} was observed, which corresponds to the asymmetric stretching of CH₂. This shift is attributed to the incorporation of -O-CH₂-O- groups within the crosslinked structure [33]. The absorption peak at 1036 cm^{-1} , observed in the blend films, shifted from the CS powder, signifying C-O-C stretching of the ether groups. Moreover, the absorption peak at 1640 cm^{-1} , representing -C=O stretching of the amide groups, was associated with water solubility, and this peak correlates with the moisture content. In the PVA6/CS10/EDTA2 film, this peak was more pronounced than in the PVA6/CS10/EDTA4 film, suggesting that the cross-linking agent was more effective in connecting CS, EDTA, and PVA. However, the addition of 4% EDTA to the CS/PVA blend led to a decreased absorption peak for -C=O stretching at 1640 cm^{-1} , indicating that insufficient crosslinking had occurred in order to properly bond the included materials [29].

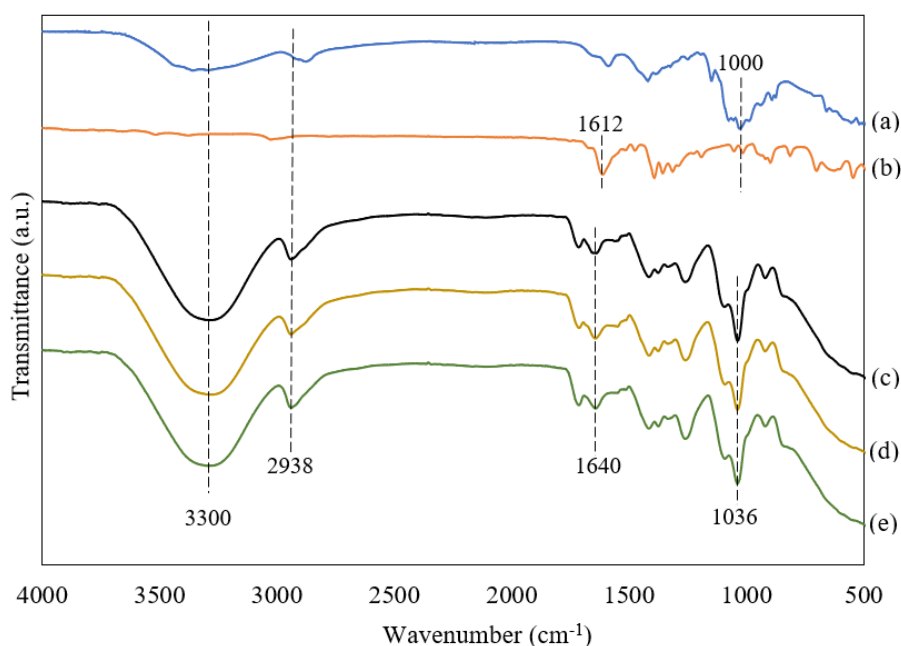


Figure 3 FTIR spectra of (a) CS powder, (b) EDTA powder, (c) PVA6/CS10, (d) PVA6/CS10/EDTA2, and (e) PVA6/CS10/EDTA4 blend

3.6 The testing of film on tilapia fillets after storage

3.6.1 The color and weight of tilapia fillets after storage

The color of fish fillets plays a critical role in assessing the visual appeal of the food, influencing consumer purchasing decisions. Therefore, color measurement is essential. Table 4 presents the color values (L^* , a^* , and b^*), total color difference (ΔE^*), and weight of the tilapia fillets after 7 d of storage at 4-7°C, comparing non-wrapped (control) fillets and those wrapped with PVA, CS, and EDTA films at various ratios. The L^* , a^* , and b^* values provide an objective evaluation of the fillets' appearance, while ΔE^* measures the color change of the fillets from the initial day to day 7. On the first day, the tilapia fillets had L^* , a^* , and b^* values of 45.51 ± 0.65 , -1.653 ± 0.27 , and 5.765 ± 1.27 , respectively, exhibiting a pinkish-white color. As storage time progressed, the ΔE^* values of all the tilapia fillets increased, which can be attributed to the release of pigments from the fish tissues or oxidation, leading to a darker surface [34]. The fillets wrapped with the PVA6/CS10/EDTA4 film exhibited a higher total color difference than those wrapped with PVA6/CS10, PVA6/CS10/EDTA2, or the non-wrapped (control) fillets. Despite the increased color change, the tilapia fillets wrapped with PVA6/CS10/EDTA4 film experienced less weight loss compared to other conditions after 7 d of storage at 4-7°C. This can be attributed to the film's thickness, which likely contributed to a slight reduction in weight loss during storage.

Table 4 Color parameters (L^* , a^* , and b^*), total color difference (ΔE^*), and the weight of the tilapia fillets after being stored at 4-7 °C for 7 d in non-wrapped (control) and wrapped with film

Samples	L^*	a^*	b^*	ΔE^*	Weight at day 7**
control	42.17 ± 2.02^a	-1.39 ± 0.23^a	7.68 ± 3.05^a	5.51 ± 0.20^a	2.34 ± 0.01^a
PVA6/CS10	36.54 ± 1.71^b	-1.29 ± 0.25^b	0.98 ± 0.35^b	10.62 ± 1.27^b	2.36 ± 0.01^a
PVA6/CS10/EDTA2	35.75 ± 1.04^c	-1.58 ± 0.14^c	3.46 ± 0.47^c	10.72 ± 1.32^b	2.38 ± 0.01^a
PVA6/CS10/EDTA4	32.75 ± 0.57^d	-1.80 ± 0.27^d	0.07 ± 0.00^d	14.32 ± 0.57^c	2.45 ± 0.04^b

** tilapia fillets at the initial day weigh at 5 g.

^{a, b, c, d} Different letters in the same column indicate significant differences ($p < 0.05$).

3.6.2 Total aerobic bacteria count of tilapia fillets after storage

On the initial day, the number of aerobic bacteria counted on the tilapia fillets was 3.699 log CFU/g, which was well below the upper limit for fresh fish consumption proposed by the ICMSF (1992), set at 6 log CFU/g [28]. The number of aerobic bacteria (log CFU/g) in the tilapia fillets stored at 4-7°C for 7 d, both unwrapped (control) and wrapped in PVA and CS film (PVA6/CS10) or in CS, EDTA, and PVA film (PVA6/CS10/EDTA2 and PVA6/CS10/EDTA4), is shown in Table 5. The results indicated that the aerobic bacteria count in the control samples increased with storage time, surpassing the ICMSF's upper limit by day 7. For the tilapia fillets wrapped with PVA and CS film or PVA, CS, and EDTA films, the number of aerobic bacteria increased from day 1 to day 5, but then decreased from day 5 to day 7, remaining below the 6 log CFU/g threshold. This suggests that the antibacterial compounds in the films may have been protected from inactivation by the enzymes in the food [35]. Additionally, the tilapia fillets wrapped in PVA6/CS10/EDTA4 film exhibited greater inhibition of aerobic bacteria than those wrapped in PVA6/CS10/EDTA2 and PVA6/CS10 films after 7 d of storage at 4-7°C. This increased inhibition can be attributed to the high antimicrobial activity of CS against both Gram-positive and Gram-negative bacteria [10-12]. Furthermore, the incorporation of EDTA enhanced the inhibition of Gram-negative bacteria, as EDTA acts as a metal chelator, sequestering divalent cations that are essential for maintaining the stability of the outer membrane of Gram-negative bacteria [7].

Hamza et al. (2019) investigated the antibacterial activity of nanofibers composed of PVA, CS, and EDTA, and their study showed that the combination of PVA, CS, and EDTA nanofibers exhibited a significantly larger inhibition zone against *Staphylococcus aureus* on agar plates compared to nanofibers composed of only CS and PVA, highlighting the antibacterial efficacy of this combination [36]. Similarly, Belfiore et al. (2007) demonstrated that the metal ion chelating agent EDTA is capable of binding to Mg^{2+} and Ca^{2+} ions found in the cell wall of Gram-positive bacteria or the outer membrane of Gram-negative bacteria. This interaction disrupts the cell's stable structure, allowing CS to enter the cells more easily. Therefore, the combination of PVA, CS, and EDTA film contributed to inhibiting the bacterial growth in the tilapia fillets [37].

Table 5 Changes in the aerobic bacteria counted (log CFU/g) in the tilapia fillets stored at 4-7 °C for 7 d in non-wrapped (control) and wrapped with film

Samples	Storage time (day)			
	0	3	5	7
control		4.602±0.015	5.806±0.010	7.021±0.029
PVA6/CS10	3.699±0.012	4.681±0.026	5.748±0.022	4.892±0.004
PVA6/CS10/EDTA2		3.847±0.014	5.556±0.017	3.975±0.003
PVA6/CS10/EDTA4		3.782±0.005	4.406±0.036	3.712±0.006

4. Conclusion

In the present study, packaging films were developed based on PVA, CS, and EDTA in order to inhibit bacterial growth on tilapia fillets during storage. The film formation results showed that all of the films had a uniform light-yellow color with good transparency and flexibility. The ΔE^* value of the PVA6/CS10/EDTA4 film was higher than that of the PVA6/CS10/EDTA2 and PVA6/CS10 films. Additionally, the thickness of the PVA6/CS10 film was lower compared to both the PVA6/CS10/EDTA2 and PVA6/CS10/EDTA4 films. The moisture content and water vapor transmission rate of the PVA6/CS10/EDTA4 film were also the highest among all formulations. The morphological analysis of the cross-sectional microstructures showed that while the PVA6/CS10/EDTA4 film had a small spot, the other films exhibited a smooth, homogeneous, and uniform surface. The FT-IR analysis revealed that the hydroxyl and carboxyl group peaks in the blended films (PVA, CS, and EDTA) were more pronounced than in the individual components, indicating enhanced water solubility. In the shelf-life test, the tilapia fillets wrapped in PVA6/CS10/EDTA4 film showed the greatest total color difference change but experienced the least weight loss compared to the other films after being stored at 4-7°C for 7 days. On the initial day, the aerobic bacterial count of the tilapia fillets was below the upper limit (6 log CFU/g) according to the ICMSF standard. However, after 7 days of storage, the aerobic bacteria count in the control samples exceeded the ICMSF standard, while the tilapia fillets wrapped with PVA6/CS10, PVA6/CS10/EDTA2, and PVA6/CS10/EDTA4 films maintained bacterial counts below the ICMSF threshold. Among these, the PVA6/CS10/EDTA4 film was the most effective in inhibiting the growth of the aerobic bacteria in the tilapia fillets. Thus, the combination of PVA, CS, and EDTA films effectively inhibited bacterial growth and extended the shelf life of the tilapia fillets. For future work, the impact of these films on sensory attributes, including taste, texture, and odor, should be evaluated in order to ensure consumer acceptance.

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6. References

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