Novel probiotic film with enhanced biological activity and probiotic viability in active packaging: application for fresh-cut apples and potatoes

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Abstract

A novel probiotic film based on gellan gum (GN), cranberry extract (CE), and *Lactococcus lactis* (LA) was developed in this work. The SEM and fluorescence image results revealed that GN/CE film containing LA was successfully fabricated. The addition of LA significantly improved the antibacterial activity of the film. The presence of CE strengthens the antioxidant activity and LA survivability in the film. The combination of LA (0~1.0%) and CE (0.5~1.0%) improved the mechanical property of the film through the formation of density structure. The colored writing patterns were successfully printed on the GN/LA/CE film. The best comprehensive properties were obtained with the film containing 2.0%LA and 0.5%CE. The GN/2.0%LA/0.5%CE film also showed the optimal preservation effect on fresh-cut potatoes and apples. The GN/2.0%LA/0.5%CE film with colored patterns was expected to be a novel probiotic film with improved biological property in active packaging.

Keywords: Cranberry extract; Green label; Gellan gum; *Lactococcus lactis*; Probiotic film;

Introduction

Nowadays, natural edible polymers packaging materials are a promising alternative to petroleum-based ones due to the former being environmentally friendly and biodegradable. Various natural edible polymers have been explored [1]. To broaden the application of edible film in food preservation, various natural agents have been intentionally added to the edible film for developing active packaging materials [2].

Recently, the incorporation of probiotics into the edible polymer to develop probiotic film has attracted more attention in the active packaging field. Because probiotics as gut-friendly live microorganisms that not only confer healthful effects on the host in adequate numbers but also reveal high antimicrobial activities through the production of bioactive substances or competition for nutrition [3]. Different probiotics with excellent antimicrobial activity have been used as effective antibacterial agents [4-7]. Among them, *Lactococcus lactis* (LA), a popular probiotic, has been listed as a generally safe (GRAS) food additive due to its excellent antibacterial activity and health benefits [8]. Some research revealed that the addition of LA could strengthen the antimicrobial property of the film, such as PVOH film [9], and starch/carboxymethyl cellulose film [10]. Hence, the LA could be considered to add into the edible film as a natural antimicrobial agent.

However, the antioxidant property of the probiotic film was not adequate, which could limit its application on easily oxidized food [7, 11, 12]. Settier-Ramírez et al. [9] also pointed out that the LA did not obviously affect the antioxidant property of the polyvinyl alcohol film because *L. lactis* produces limited antioxidants in the film. In addition, a big concern of the probiotic film is the survivability of probiotic embed in the film during storage period. Because the poor probiotic viability could significantly decrease its biological activity and health benefits [7]. Fortunately, various studies have demonstrated that the co-encapsulation of probiotic and bioactive compounds in a single matrix could increase the probiotic viability [13, 14]. Some bioactive compounds have been explored to improve the viability of probiotics, such as omega-3 oil, green tea extract, and resveratrol. Besides, the addition of bioactive compounds positively affected the antioxidant property of the film due to their scavenging ability on the free radicals [14, 15].

Cranberry extract (CE) was a natural bioactive compound-rich extract obtained from dried cranberry (*Vaccinium macrocarpon Ait.)*. CE contains a large amount of bioactive compounds, such as polyphenols, flavonoids, anthocyanins, and VC [16]. It has been widely used as a natural antioxidant in food due to it is safe and rich nutrition [17]. Previous studies also reported that the CE could improve the viability of LAB-related probiotics [18, 19]. Thus, the BE could be considered a bioactive compounds-rich substance to improve the probiotic viability and antioxidant activity of the film.

In addition, to broaden the application scope of the anthocyanin-rich film, our previous work has explored the possibility of printing different patterns on the nanocomposite film containing anthocyanin by using electrochemical writing [20]. These developed films with printed letters or patterns could be used as a promising alternative to petroleum-based labels to provide some essential product information to consumers. However, there are only few works regarding the development of printing patterns on anthocyanin/probiotic composite films.

Accordingly, the objective of this study was to develop a novel probiotic film with improved biological activity and probiotic viability. The gellan gum (GN), an important natural extracellular polysaccharide obtained from *Sphingomonas paucimobilis* fermentation, was used as the edible film-forming matrix in this work due to its low prices and good film-forming property. The effect of LA concentration and CE concentration on the structure, biological and mechanical properties of the film, and viability of *L. lactis* was explored. Furthermore, the colored writing patterns were printed on the probiotic film. Finally, the preservation effect of the probiotic film on easily oxidized and perishable food, including fresh-cut apples and potatoes, was also studied. This study would not only benefit the development of novel and effective packaging systems but also reduce white pollution.

2. Materials and methods

2.1. Materials

Dried cranberry (cultivar "Yageda") was obtained from Nanjing, China. Chemical agents, including gelatin (CP, water content ≤ 12%, pH=5.0~7.0), methanol (GR, purity >99.8%), Fluorescein Diacetate (FDA) (purity >99.99%), and Man, Rogosa, and Sharpe broth (MRS) (pH = 6.2 ± 0.2), and Tryptone Soy Agar (TSA) medium (pH = 7.3 ± 0.2) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Probiotic *Lactococcus lactis* (CICC 21028), and pathogenic microbe *Listeria monocytogenes* (ATCC 19114) were obtained from Shanghai yiyan bio-technology Co., Ltd. (Shanghai, China).

2.2. Preparation of cranberry extract (CE)

The extraction method of CE was measured following our previous method [21]. The dried cranberry was ground into powder and soaked in an alcohol (80%)-HCl (40 mM) mixture. The mixture was reacted in the dark for 1 h and centrifuged at 10000 rpm for 15 min. After that, the crude CE extraction was filtered four times with filter paper (Whatman No. 1) and then freeze-dried to achieve the CE powder. The obtained powder was stored at 4 ± 1 °C for further experiment.

2.3. Preparation of *L. lactis* culture

The *L. lactis* was cultured in MRS broth. The prepared *L. lactis* cultures were packed in Erlenmeyer flask and incubated at 30 °C for 24 h. The *L. lactis* culture was centrifuged at 6000 rpm for 15 min and then washed with sterile normal saline. Finally, the *L. lactis* suspension was centrifuged at 5000 rpm for 20 min to obtain the *L. lactis* pellet.

2.4. The fabrication of films

The pure GN solutions were prepared by dissolving 2 g of GN powder in 100 mL of distilled water at 98 °C, and expressed as GN/0%LA/0%CE. The GN solution enriched with LA was prepared by adding different amount of LA pellet (1.0 and 2.0 mg/10 mL) into GN solution at 30 °C, and expressed as GN/1.0%LA/0%CE and GN/2.0%LA/0%CE, respectively. The GN solution enriched with CE was prepared by adding different amount (0.5 and 1.0 mg/mL) of CE into GN solution at 50 °C, and expressed as GN/1.0%LA/0%CE and GN/2.0%LA/0%CE, respectively. The GN/LA/CE solution was prepared by adding different amount of CE (0.5 and 1.0 mg/mL) into GN solution at 50 °C. Then, the different amount of LA pellet (1.0 and 2.0 mg/ 10 mL) was added into the GN/CE solution when the solution was cooled to 30 °C, and expressed as GN/1.0%LA/0.5%CE, GN/1.0%LA1.0/0%CE, GN/2.0%LA/0.5%CE and GN/2.0%LA/1.0%CE, respectively. The film was prepared by casting the film-forming solution into a petri dish and drying in an oven at 30 °C for 24 h.

2.5. Characterization of composite films

2.5.1 Antibacterial and antioxidant properties of the films

The inhibition of films against harmful microorganisms growth was determined according to the previously reported method [22]. The *L. monocytogenes* was selected as the harmful microorganisms in this work due to its importance in foodborne illness in China. The *L. monocytogenes* was cultured in Tryptic Soy broth. The prepared *L. monocytogenes* cultures were packed in Erlenmeyer flask and incubated at 37 °C for 24 h. Then, 100 mL of this *L. monocytogenes* culture (~10^8 CFU/mL) was cultured onto TSA plates at 37 °C for 24 h. The antibacterial property of the film was evaluated by determining the diameter of the inhibition zone.

The DPPH scavenging ability of the film was measured to evaluate the antioxidant property in this work. Briefly, 30 mg of the film sample was mixed with 5 mL of 0.1 mM DPPH solution (dissolved in ethanol) and reacted for 45 min in the dark. The absorbance of the mixture was noted at 517 nm. DPPH scavenging activity of the film was obtained based on Eq. (1):

$$DPPH\ scavenging\ activity\ (\%) = [(A_0 - A_1)/A_0] \times 100 \qquad (1)$$

Where A_1 and A_0 signify the absorbance of the DPPH solution mixed with or without film extract solution.

2.5.2. Mechanical and optical properties

The absorbance of the films was measured at 600 nm wavelengths using a spectrophotometer. The opacity of film was evaluated based on the Eq. (2).

$$Opacity = A_{600}/x \quad (2)$$

Where A_{600} is the absorbance of the film at 600 nm, and x is film thickness.

The mechanical properties (Tensile strength (TS) and elongation percentage at break (EB)) of prepared films were determined according to the ASTM method (Standard D882-00, 2000) and calculated using Eq. (3) and (4):

$$TS = F/S \quad (3)$$

$$EB(\%) = \Delta l/l_0 \quad (4)$$

Where F is the maximum load; S is the initial cross-sectional area; Δl is film extension; l_0 is initial length.

2.5.3. Water vapor permeability (WVP)

The film's WVP was measured referred to the procedure of Yang, Zhai, Zhang, Shi, Huang, Li, Zou, Gong, Holmes, Povey and Xiao [21]. Firstly, the fabricated film sample was selected to cover the centrifuge tube (25 mL) containing a certain amount of distilled water. After that, the centrifuge tube was placed in the dryer. The weight of the centrifuge tube was weighted every two hours. The following formula obtained the WVP:

$$WVP = (\Delta m \times \Delta P)/(A \times \Delta t \times x) \quad (5)$$

Where Δm represents the weight change per unit time; Δp is the partial pressure difference between the two sides of the film; A is the transfer area; Δt is per unit time; x is the thickness of film.

2.5.4. The fluorescence and visual appearance

Films were stained with FDA (10 μg/mL) for 20 min at room temperature in the dark. The fluorescence of the films at 480 nm excitation wavelength was noted with a confocal laser microscope (Leica TCS SP5). The visual appearance of the film was obtained with a scanner.

2.5.5. Morphological properties

The surface of film morphology was characterized using scanning electron microscopy (JEOL, JSM-6360). All film samples were fixed on bronze stub using double-side adhesive and sputter-coated with gold in a vacuum evaporator.

2.6. Survivability of LA in the films

The bioactive film incorporated with LA was stored at 25 ± 1 °C and 75 % RH for 24 d, and the survivability of the LA was determined every four days. In brief, the probiotic films were aseptically transferred into the sterile saline solution and agitated for 45 min. After that, the LA solution of an appropriate concentration was spread on an MRS medium. The counts of LA were measured using platecount method. The relative survival rate of LA was obtained using Eq. (6) and (7):

$$\text{Survivability} = \log C_t - \log C_0 \quad (6)$$

$$\log C_t = \log C_0 - K \times t \quad (7)$$

Where C_0 is the number of initial viable *L. lactis* number, C_t is the number of viable *L. lactis* after a specific time of storage (CFU/g), t is the storage time (d), k is the inactivation rate (log CFU/d).

2.7. The procedure of printing patterns on the film

The written patterns on the film was conducted by electrochemical writing according to our previous method [20]. The GN hydrogels containing anthocyanin were selected in this work for electrochemical writing. The anode and cathode of the electrochemical analyzer (CHI660E, CH Instruments Co., Shanghai, China) were connected with platinum (Pt) needle (0.2 mm) and Pt plate, respectively. The Pt needle was contacted with the surface of the hydrogel that placed on the Pt plate and controlled by a DOBOT M1 robotic arm (Yuejiang Technology Co., Ltd., Shenzhen, China) with a move precision of 0.1 μm.

2.8. Biodegradable properties of films

The biodegradability of films in soil was measured according to the method reported by Lu, Li, Ma, Li, Jiang, Qin, Li, Li, Zhang and Wu [23]. Firstly, the square film samples (20 mm × 20 mm) were prepared and then placed in a natural environment in microorganisms-rich soil. The film figures were captured every 3 days to observe the visually decrease in their area.

2.9. Application for fresh-cut potatoes and apples

Fresh apples (cultivar "Hongfushi") and potatoes (cultivar "Huaen 1") with good appearance quality were obtained from local producers. The apples and potatoes were peeled and washed with distilled water. After that, the apple were cut into 15 mm × 30 mm pieces and the potatoes were cut into 20 mm × 20 mm pieces. All the pieces were washed with distilled water and then air-dried for 1 h. Then pieces samples were immersed in different film-forming solutions and air-dried again. The pieces only washed with distilled water served as the control. Finally, all fresh-cut samples were placed in a polyethylene tray and stored at 4 ± 1 °C and 75% RH for 6 days.

2.10. Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the significantly difference between the tested samples. All the presented results were the average of four measurements, and the mean values marked with different letters indicate significant differences (P <0.05).

3. Results and discussions

3.1. SEM morphology and florescence figures of the film

The SEM images of the surface of different films were presented in Fig. 1A. The surface of the pure GN film was smooth and flat. For the film only containing CE, the surface of GN/0.5%CE film presented a smooth and uniform morphology, indicating that CE has great compatibility with GN films. The incorporation of 1.0% CE make the surface of GN film became uneven. Similar rough structure was also observed in chitosan/jujube leaf extract [24] film and gelatin/corn starch/corn stigma extract film [15]. This was attributed to the fact that the increased CE concentration makes the insoluble particles not well dispersed in the GN matrix [25]. Notably, the addition of LA obviously changed the morphology of the surface of GN film, some LA cells were observed on the surface of the composite film. Li et al. [26] also observed that the edible film surface became rough with the addition of probiotics. Similar result was also observed in the GN-based film containing 2.0%LA. Notably, with the incorporation of CE, the surface of the GN/LA film became rough and dense, and it became rougher with the increasing concentration of CE. This result indicated the cross-linking between GN and LA was strengthened by the addition of CE. However, the surface of GN/2.0%LA/1.0%CE film became very rough due to the large accumulation of LA cells or excessive crosslinking between the different polymer chains. All GN/LA films presented a rough surface and cross-section morphology. Fortunately, no crack or phase separation was observed in all GN/LA/CE composite films. To check the presence and distribution of LA in the film, the fluorescence of the films was also measured (Fig. 1B). No fluorescence was observed in the figures of GN films without LA. The green fluorescence was evenly presented in GN/LA and GN/LA/CE films, indicating the active and good distribution of LA in the matrixes. For the film containing the same CE concentration, the number of green fluorescent dots in the 2.0% LA-loaded film was higher than that of 1.0%LA-loaded film. Notably, the distribution of green fluorescent in the GN/1.0%LA/1.0%CE and GN/2.0%LA/1.0%CE film became non-uniform, indicating the aggregation of the LA. This result was inconsistent with the SEM result. The high concentrations of CE (1.0%) may cause the aggregation of probiotics. This probiotic aggregation could be due to the increased roughness and unevenness of the film making probiotic cells accumulate in the depressions on the film's surface. Singh et al. [27] also found that *Lactobacillus rhamnosus* accumulate at the structural defects in cellulose-based films. These results indicated that the GN-based probiotic film containing LA/CE was developed successfully in this work. The incorporation of 1.0%CE/2.0%LA decreased the uniformity structure of the distribution of probiotics in the film.

Fig. 1

3.2. The antibacterial and antioxidant properties

The antibacterial property of the films were presented in Fig. 2A. The pure GN has almost no inhibition effect on *L. monocytogenes*. The maximum antibacterial properties (14.9 ± 0.6 mm) were obtained with GN films containing 2.0%LA and 0.5%CE. For the film only incorporated with CE or LA, the antibacterial activity of the composite film significantly increased with the increased LA or CE concentration (P <0.05). This result indicated that the incorporation of LA or CE possessed a positive efficacy on the antibacterial property of the GN film. For the film both enriched with CE and LA, the combination of 1.0%LA or 2.0%LA with 0.5%CE significantly enhanced the antibacterial property of the film. Similar synergistic effect was also observed by Riešutė et al. [28], who pointed out that the combination of plant extract and lactic acid bacteria could improve the antibacterial property of the nutritional bar. The obviously increased antibacterial property in the CE/LA-loaded film was due to the presence of LA in GN matrix may produce nisin and bacteriocin to break the cell structure of pathogens [8]. In addition, the synergistic effect between the bioactive compounds in CE and LA could furtherly strengthen the inhibition effect [29]. However, when the CE concentration was up to 1.0%, the antibacterial property of the film containing CE and LA obviously decreased (P <0.05). This may be ascribed to that the excessive CE content changed the pH condition in the film so that inhibit the growth of probiotics and reduce the production of antibacterial substances, resulting in a decreased antibacterial property of the film [30]. Khalaphallah and Soliman [31] also reported that the positive effect of plant extract on probiotic viability depended on the concentration of plant extract. This result indicated that the incorporation of CE or LA favored the antibacterial property of the GN film, and the incorporation of 2.0% LA and 0.5% CE obtain the best antibacterial property.

The antioxidant property of the films were presented in Fig. 2B. The antioxidant property of GN/0%LA/0%CE film was 2.4 ± 0.2%, and it increased to 10.6 ± 0.7% by the incorporation of 1.0%LA. Notably, the incorporation of CE significantly affected the antioxidant property of the film (P <0.05). The satisfactory antioxidant property was obtained when the incorporation CE concentration was 1.0%. This improved antioxidant property of the film could be due to the presence of flavonoids, Vitamin C, and polyphenols in CE possessed excellent antioxidant capability [32]. It is interesting to observe that the antioxidant property of the film containing CE and LA seems to be only sensitive to CE concentration. For the composite film containing 0.5%CE or 1.0%CE, the addition of LA did not obviously affect the antioxidant property of the film (P >0.05). Contrarily, Li et al. [26] pointed out the incorporated probiotic could improve the antioxidant activity of the film because some LAB strains could produce some exopolysaccharides (EPS). However, in this work, the antioxidant property of the LA-loaded film hardly altered. Settier-Ramírez et al. [9] also observed the antioxidant property of the PVOH film did not change with the addition of LA because *L. lactis* produces limited antioxidants in the film.

Fig. 2

3.3. Optical property and visual appearance

The optical property is an essential parameter for food packaging material. Hence, the opacity and visual appearance of the films were studied in this work. The opacity of the GN/0%LA/0%CE film was 0.85 A/mm (Fig. 2C), and it significantly increased with the increasing incorporation of CE or LA (P <0.05). The result indicated that the incorporation of CE or LA could reduce the film's transmittance. For each GN-based film containing 1.0%LA or 2.0%LA, the incorporation of 0.5%CE did not affect the opacity of the composite film. Our previous studies also observed that the addition of low concentration red radish anthocyanin did not affect the transmittance of the gelatin/gellan gum based film [33]. The highest opacity value was observed in the GN/2.0%LA/1.0%CE composite film (3.0 A/mm). This was due to the presence of a large amount of colored group of LA in the film could block the light so that decrease the transmittance of the film.

The visual appearance of GN/0%LA/0%CE film presented bright white color (Fig. 2D). The incorporation of CE makes the pure GN film become light pink, and the color of the film became deepener and dark with the increasing CE concentration. The addition of LA increased the lightness of the GN film, and GN-based films only incorporated with LA became yellow. The color of probiotics film was mainly related to the density of probiotics [26]. The deepen color in GN/LA films was due to the presence of a higher LA concentration in the GN matrix block the passage of light. For each film, the addition of CE making the color of the film became light pink, and the color of the film further became pink with the increasing CE concentration. Similar finding was also observed in starch/polyvinyl alcohol/roselle extract film [34]. The opacity and color depth of the film increased with the increasing

CE or LA content. Even though the addition of 2.0% LA and 1.0%CE could enhance the biological activity of the film (Fig. 2A and 2B), however, it presented a negative effect on the opacity of the film.

3.4. TS and EB of the film

The mechanical property of the packaging material is a critical parameter to meet the external stress during the processing and transport process. As can be despite in Table 1, the TS of the pure GN film was 17.92 ± 0.8 MPa, and it obviously decreased with the incorporating LA (P <0.05). The lowest TS value (14.15 ± 1.2 MPa) was obtained with GN films containing 2.0% of LA. Similar result was also observed in the GN/LA/0.5%CE or GN/LA/1.0%CE films. Actually, the tensile strength of the composite films is related to the distribution and density of the incorporation polymer moleculars and their interactions [26]. The increased LA cells could aggregate in the matrix to decrease the compactness of the film (Fig. 1). Similar decreased TS was also found in the cassava starch/carboxymethylcellulose film incorporated with 2% lactic acid bacteria [26]. The TS value of the film significantly strengthened with the addition of CE, and it furtherly enhanced with the increasing concentration of CE. The highest TS (32.52 ± 1.2 MPa) was obtained with the film containing 1.0% LA and 1.0% CE. This satisfactory TS of the composite film was in accord with the fact that the presence of phenolic compounds in CE enhanced cross-linking and density in the polymer matrix (Fig. 1A) so that increase the TS of the film. The EB of the developed films was shown in Table 1. Generally, an enhanced TS results in a decrease in EB of the film. However, the EB of the composite film increased with the incorporation of 0.5%CE. This was because the addition of CE may form a layered structure so that increased the flexibility of the film. Zhai et al. [33] also observed that the increasing anthocyanin concentration increases the TS and EB of the gelatin/gellan Gum film. For the pure GN film or GN film containing CE, the incorporation of 1.0%LA did not affect the EB of the films. Similar finding was also studied by Settier-Ramírez et al. [9]. However, the incorporation of 2.0% LA significantly decreased the EB of the film. The lowest EB (9.15 ± 0.7%) was observed in the GN/2.0%LA/0%CE film. This decreased EB of the composite film was due to that the agglomeration of a large number of LA negatively affected the uniformity of the film (Fig. 1C) so that decrease the EB [10]. The satisfactory EB value was obtained when the film containing high-level value of CE concentration (0.5%-1.0%) and the low-level value of LA (0%-1.0%). Several works also indicated that the addition of high anthocyanin extract could improve the mechanical property of the film [10, 35, 36].This result revealed that the combination of CE (0.5~1.0%) and LA (0~1.0%) could enhance the mechanical property of the GN film.

3.5. Water barrier properties

The water barrier property of the film is essential for controlling the food quality during the storage and transport process. Hence, the WVP of the films were checked in this work and the results were shown in Table 1. For pure GN film, the incorporation of LA (1.0%~2.0%) or 0.5%CE did not significantly affect the WVP of the GN film. The WVP of the pure GN film obviously decreased to 4.27 ± 0.1 g·mm·m^{-2}·Kpa^{-1}·h^{-1} when the concentration of incorporated CE was 1.0% (P <0.05). This was because the increased density of the GN/1.0%CE/0%LA film's structure (Fig. 1A) could effectively reduce the passage of moisture. The WVP of the polymer film also related to the integrity of the matrix, hydrophilic, and the interactions between each component [37]. For each film containing 2% LA, the WVP of the film increased from 4.70 ± 0.1 to 4.81 ± 0.2 g·mm·m^{-2}·Kpa^{-1}·h^{-1} when the CE concentration increased from 0.0% to 1.0%. The high concentration of LA cells and CE could aggregate in GN so that forming rough and uneven structure (Fig. 1), resulting in an increased WVP of the film. Similar increased WVP was also observed in the gelatin/gellan gum/radish extract composite film [33].

Table. 1

3.6. Survivability of *L. lactis* in the film

3.6.1. *L. lactis* survivability during the drying process

Fig. 3A presented the survivability of *L. lactis* cells in the different films during the drying process. The viable cell numbers in all films decreased after drying at 30 °C for 24 h. This decreased viable cell numbers is in accord with the fact that the increased osmotic pressures during the drying process broke the cell structure of the microorganism [14]. Similar decreased viable probiotics numbers during the drying process was also studied in starch/carboxymethylcellulose edible film [26]. Generally, the suggested minimum doses in probiotic product was 10^6 CFU/g [38]. After the drying process, all developed films exhibited satisfactory viable cell numbers (> 6.0 log CFU/g). Regarding the GN/1.0%LA-based composite film containing different CE concentrations, the CE concentration did not affect the *L. lactis* viability in the GN film during the drying process. The similar finding was also studied in the GN/2.0%LA-based composite films. The concentration of *L. lactis* also did not affect the survivability of probiotics embedded in the film during the drying process, the viable cell numbers of all developed film containing LA decreased by ~1.0 Log CFU/g after drying process. The probiotic survivability in the film during the drying process is related to water evaporation rate, drying temperature, moisture content, and film-forming matrix types [39]. In brief, the LA concentration or CE concentration did not affect the probiotic survivability in the gellan gum film during the drying process in this work.

3.6.2. *L. lactis* survivability during the storage process

Probiotic viability during the storage process is an essential parameter for its health benefits and biological activity [38]. The survivability of the *L. lactis* in all films exhibited a decreasing trend thought the whole storage period (Fig. 3B). This reduction of viable cell numbers in all films was attributed to the increased osmotic pressures and decreased nutrients during the storage process [40]. In the initial 4 d, no significant difference was observed in the survivability of *L. lactis* in all films. After 24 d of storage, for each film containing the same concentration of LA, the addition of 0.5%CE significantly improved the survivability of the probiotics. This improved survivability of *L. lactis* was due to the demonstrated prebiotic ability of CE could promote the growth of LAB [41]. Carine Raddatz et al. [29] also reported that the presence of bioactive compounds, such as anthocyanin, phenolic, in the red onion residue extract could stimulate the growth of probiotics. Notably, for each film containing the same concentration of LA, the *L. lactis* survivability in the film decreased by the incorporation of 1.0% CE. Zhu et al. [42] also pointed out that the promotion effect of the anthocyanin on the probiotic viability first increased and then decreased with the increasing anthocyanin concentration. A high concentration of anthocyanin may change the pH condition in the film so that inhibit the probiotic growth. In addition, the addition of 1.0% CE causes the aggregation of probiotics in the film (Fig. 1B), which may intensify the space competition of probiotics. Notably, the addition of a certain amount (1%~2.0%) of LA did not affect the *L. lactis* viability in the film. The inactivation rate (K) of *L. lactis* in all prepared films was also presented in Fig. 3C. The inactivation of all probiotics film was conformed to the first-order kinetic formula. As we hypothesized, high concentrations of CE could reduce the viability of *L. lactis.* The GN/2.0%LA/1.0%CE film obtained the highest inactivation rate (0.2057) while that in GN/1.0%LA/0.5%CE film was 0.1819. This was because excessive concentration of quercetin in the CE may inhibit the growth of probiotics. C. Iyer and K. Kailasapathy [43] also observed that the quercetin decreased the growth of *Lactobacillus acidophilus*. In addition, the content and types of the compounds in the plant extract also affect its prebiotic activity [44]. This result revealed that the *L. lactis* survivability in the GN film depends on the CE concentration, and the incorporation of CE (0.5%) could promote the survivability of *L. lactis* in the GN-based film.

Fig. 3

3.7. Electrochemical writing on composite film

To provide essential manufacturing information to consumers, the colorful patterns were printed on the anthocyanin-rich probiotic films (Fig. 4A). The images of the hourglass with two colors (pink and yellow) were printed successfully on the GN based probiotic films containing 0.5% CE or 1.0% CE. This development of multicolor patterns on the anthocyanin-rich probiotic film was based on structure changes of anthocyanin molecular under different pH conditions [20]. The principle of the fabrication of the pink part of the hourglass pattern was due to that the structure of anhydrobase converted to flavylium ion under the low pH condition generated by positive electrolyte water reaction ($2H_2O - 4e^- = 4H^+ + O^2$) (Fig. 4B). The principle of the fabrication of the yellow part of the hourglass pattern was due to that the structure of anhydrobase converted to anhydrobase anion under the high pH condition generated by negative electrolyte water reaction ($4H_2O + 4e^- = 4OH^- + 2H_2$). This structure change of anhydrobase makes cranberry anthocyanin present different colors. Notably, the color of the hourglass patterns on the composite film containing 1.0% CE is darker than that of the film containing 0.5% CE. This was because a large number of anhydrobase anion or flavylium aggregates cause the deepening of the pattern color. The addition of different concentration of LA did not affect the electrochemical writing performance of the probiotic film. These colorful patterns could be used as a promising alternative to petroleum-based labels to provide some important product information to consumers, such as production date, shelf life. Furthermore, this patterned film could also broaden the application range of the probiotic film

Fig. 4

3.8. Degradability of films in soil

The photographs for checking the degradability of the composite film in soil was shown in Fig. 5. All the GN-based film could be degraded in soil within 24 days. As a hydrophilic material, the pure GN film was completely degraded within 15 days. This was because GN as a hydrophilic material could rapidly absorb water from the environment, so that accelerate the growth of degradation-related microorganisms [26]. The process of the film degradation in soil was slowed down with the incorporation of LA and CE, and the GN/2.0%CE/1.0%LA film has the longest degradation time in soil. Similar increased degradation time of the gelatin/corn stigma extract film was also studied by Boeira, Flores, Alves, Moura, Melo, Rolim, Nogueira-Librelotto and Rosa [15]. This due to the presence of antibacterial and antioxidant agents in the film could inhibit the microbial activity in the soil. In addition, the density, water content of the film, the microorganism types, and weather conditions also affected the biodegradability time of the film [26]. The increased density structure by the addition of CE and LA, and the increased moisture content and loss of anthocyanin due to the rain erosion at 9th and 18th days may also affect the degradability of the GN-based composite film. This result indicated GN-based film presented the satisfactory biodegradability in the natural environment, and the incorporation of LA and CE may prolong the degradation time of the film in soil.

Fig. 5

3.9. Application of the film on fresh-cut potatoes and apples

To evaluate the preservation effect of developed films on susceptible to bacterial infection and oxidized food, the fresh-cut apple and potatoes was selected in this work. The appearance quality of the fresh-cut potatoes and apples were shown in Fig. 6A and 6B. At the beginning of storage, all fresh-cut fruits are full and without damage, and the surface of all the fresh-cut apples and potatoes are bright color and healthy. After storage for 6 d, the obviously surface browning and severe shrinkage due to water loss was observed in the untreated fresh-cut potatoes and apples. The pure GN treatment presented a little effect on improving the quality of the fresh-cut samples. Notably, the visible browning degree in the GN/CE/LA treated fresh-cut potatoes and apples was obviously lower over that of untreated samples, and the GN/2.0%LA/0.5%CE treated samples exhibited the optimal effect. This valid inhibition effect could be in accord with the fact that the presence of CE and LA in the GN/LA/CE films with excellent antioxidant and antibacterial properties (Fig. 2A and 2B) could effectively inhibit the growth of pathogen and scavenge free radical (Fig. 6C), so that decrease the browning degree and maintain good appearance quality of the fresh-cut apples or potatoes. In addition, the improved barrier properties also positively control the water vapor exchange so that slow down the process of decay. This result proved that the GN-based film incorporated with 0.5%CE and 2.0%LA expected to be an effective active packaging material for fresh-cut apples and potatoes.

Fig. 6

4. Conclusion

A novel probiotic film with improved biological and probiotic viability based on GN, LA, and CE was fabricated in this work. The SEM and fluorescence image results revealed that the GN-based film containing LA/CE was prepared successfully. The incorporation of 1.0%CE and 2.0%LA positively affect the antioxidant and antibacterial properties of the film while it had a negative effect on the film's optical property and biodegradability. The addition of 0.5%CE significantly improved the LA survivability in the GN film during the storage period. Furthermore, the probiotic film with colorful patterns was successfully developed in this work, and the color depth of the patterns was sensitive to the CE concentration. The best comprehensive properties were obtained with the film containing 2.0%LA and 0.5%CE. The GN/2.0%LA/0.5%CE film also obtained the best preservation effect on fresh-cut apples and potatoes. Hence, GN/2.0%LA/0.5%CE probiotic film with green labels is expected to be a novel active packaging material for food preservation.

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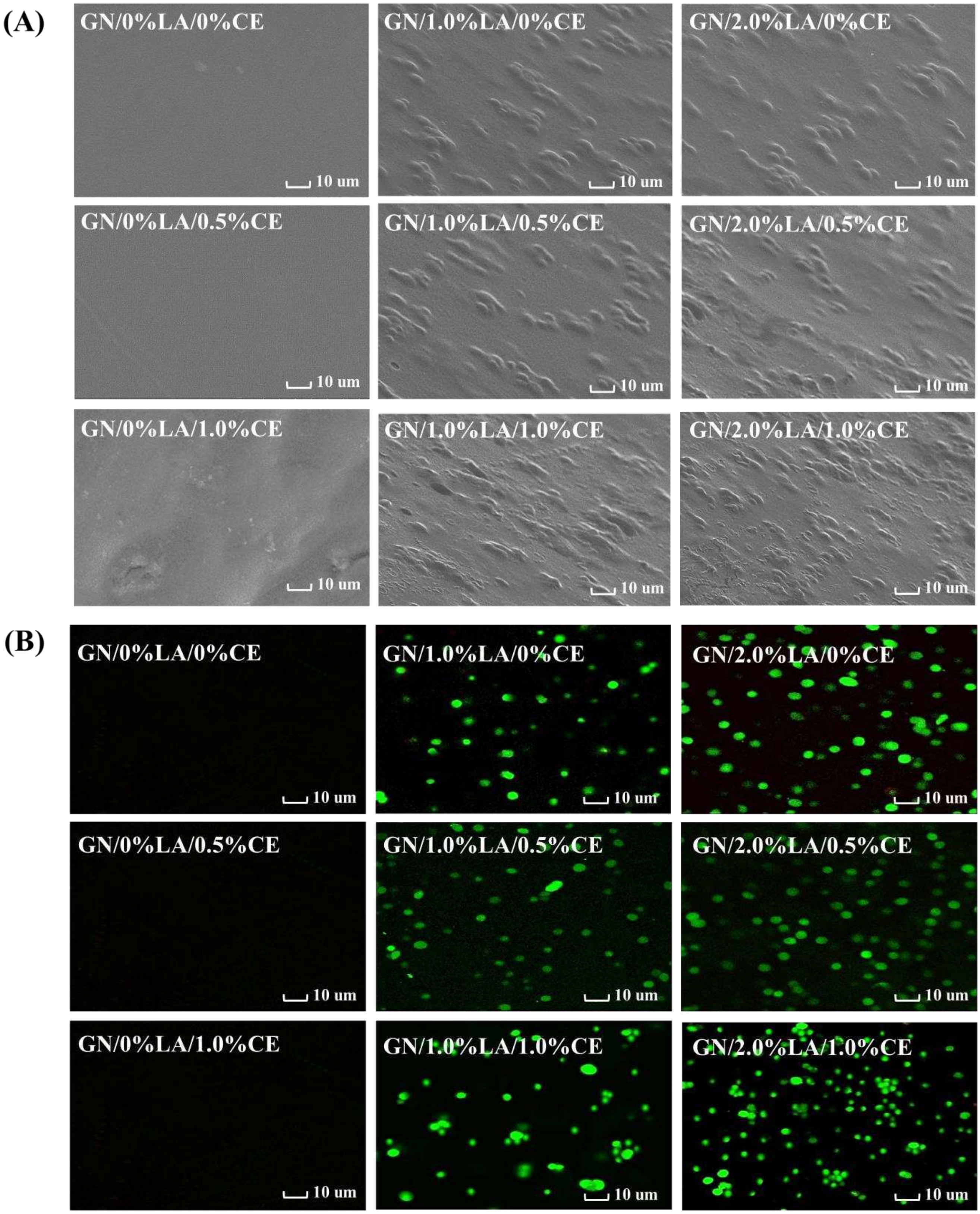
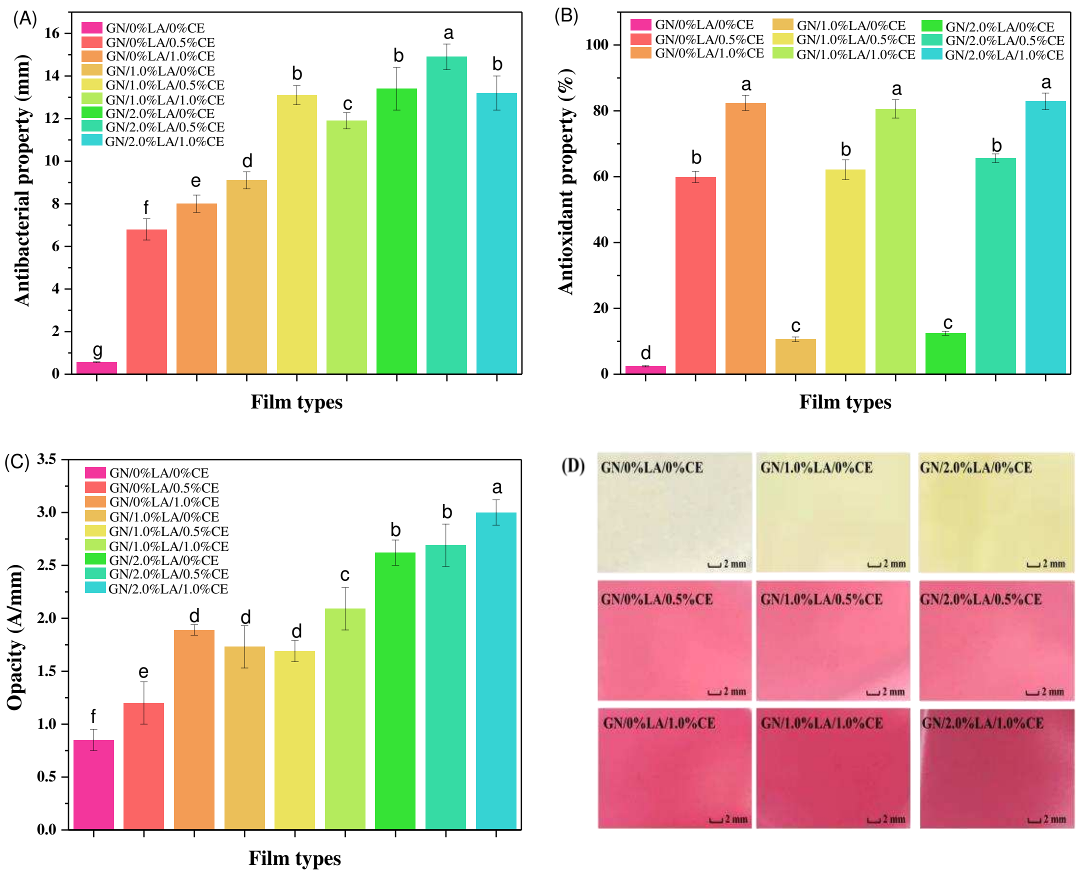
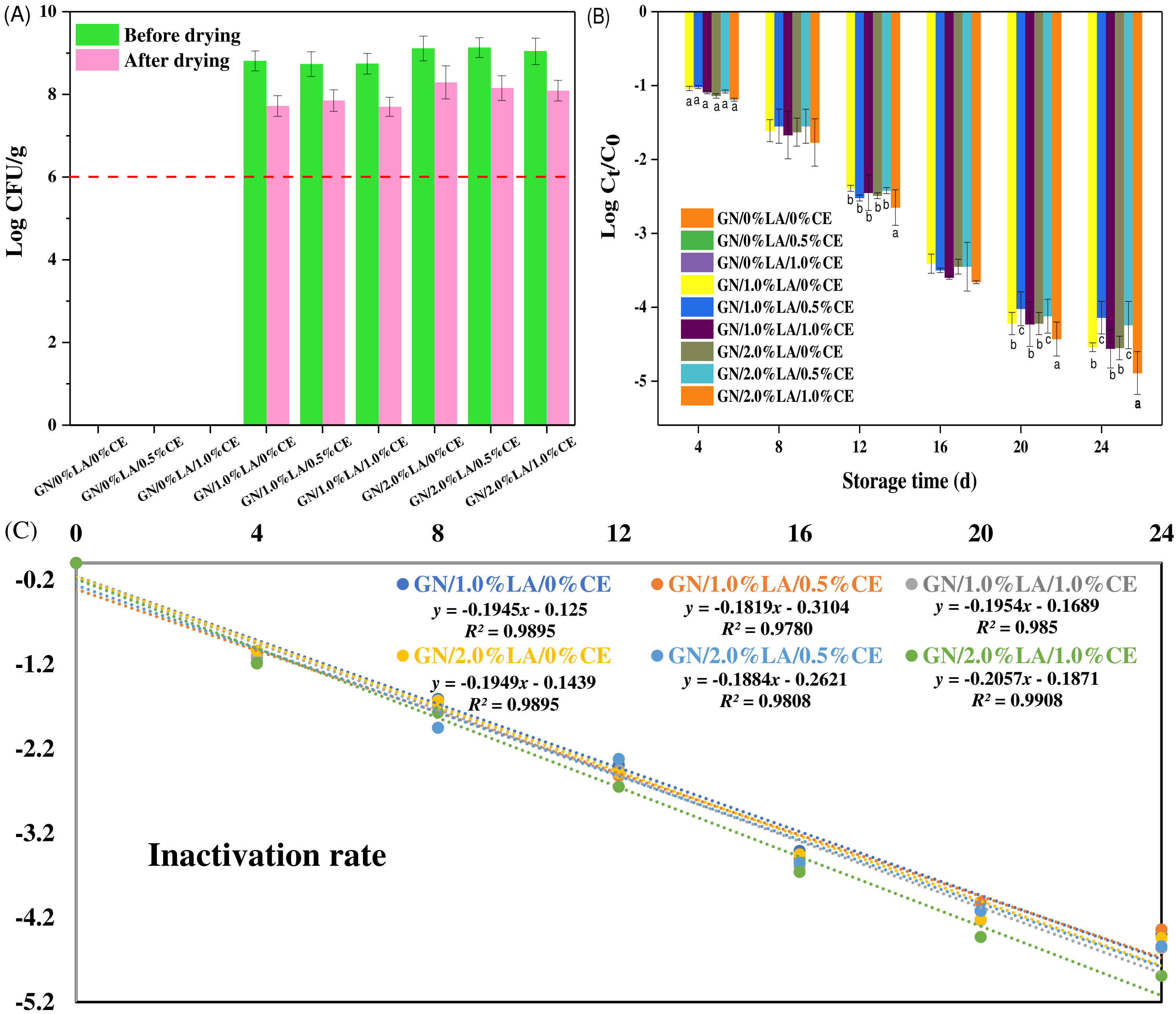

(A)
GN/0%LA/0%CE
GN/1.0%LA/0%CE
GN/2.0%LA/0%CE
GN/0%LA/0.5%CE
GN/1.0%LA/0.5%CE
GN/2.0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/1.0%CE
10 um
(B)
GN/0%LA/0%CE
GN/1.0%LA/0%CE
GN/2.0%LA/0%CE
GN/0%LA/0.5%CE
GN/1.0%LA/0.5%CE
GN/2.0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/1.0%CE
10 um

Figure 2



(A)
Antibacterial property (mm)
Film types
GN/0%LA/0%CE
GN/0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/0%CE
GN/1.0%LA/0.5%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/0%CE
GN/2.0%LA/0.5%CE
GN/2.0%LA/1.0%CE
(B)
Antioxidant property (%)
Film types
(C)
Opacity (A/mm)
Film types
(D)
GN/0%LA/0%CE
GN/1.0%LA/0%CE
GN/2.0%LA/0%CE
GN/0%LA/0.5%CE
GN/1.0%LA/0.5%CE
GN/2.0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/1.0%CE
2 mm

Figure 3



(A)
Before drying
After drying
Log CFU/g
GN/0%LA/0%CE
GN/0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/0%CE
GN/1.0%LA/0.5%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/0%CE
GN/2.0%LA/0.5%CE
GN/2.0%LA/1.0%CE
(B)
Log C_t/C_0
Storage time (d)
GN/0%LA/0%CE
GN/0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/0%CE
GN/1.0%LA/0.5%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/0%CE
GN/2.0%LA/0.5%CE
GN/2.0%LA/1.0%CE
(C)
GN/1.0%LA/0%CE
y = -0.1945x - 0.125
R^2 = 0.9895
GN/1.0%LA/0.5%CE
y = -0.1819x - 0.3104
R^2 = 0.9780
GN/1.0%LA/1.0%CE
y = -0.1954x - 0.1689
R^2 = 0.985
GN/2.0%LA/0%CE
y = -0.1949x - 0.1439
R^2 = 0.9895
GN/2.0%LA/0.5%CE
y = -0.1884x - 0.2621
R^2 = 0.9808
GN/2.0%LA/1.0%CE
y = -0.2057x - 0.1871
R^2 = 0.9908
Inactivation rate

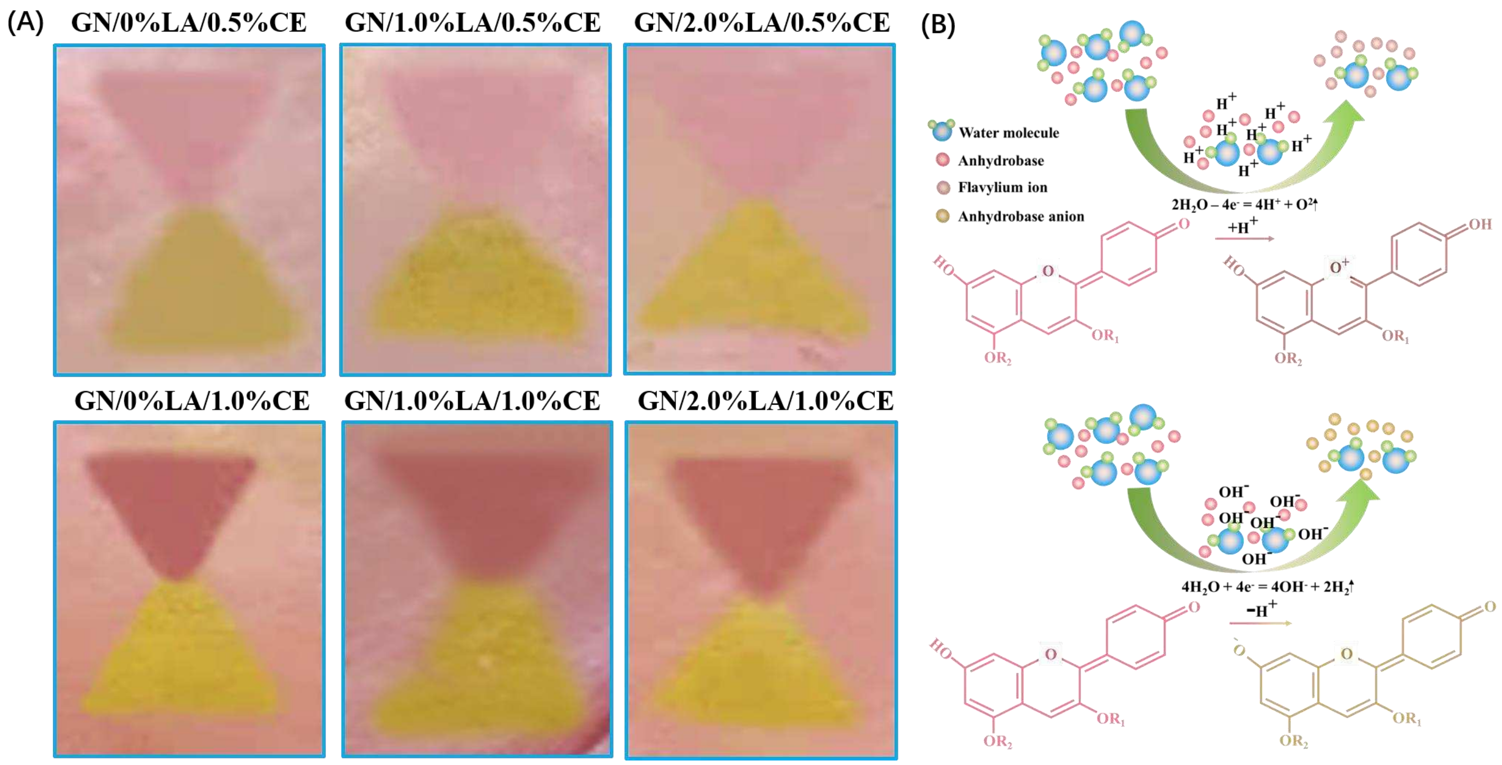
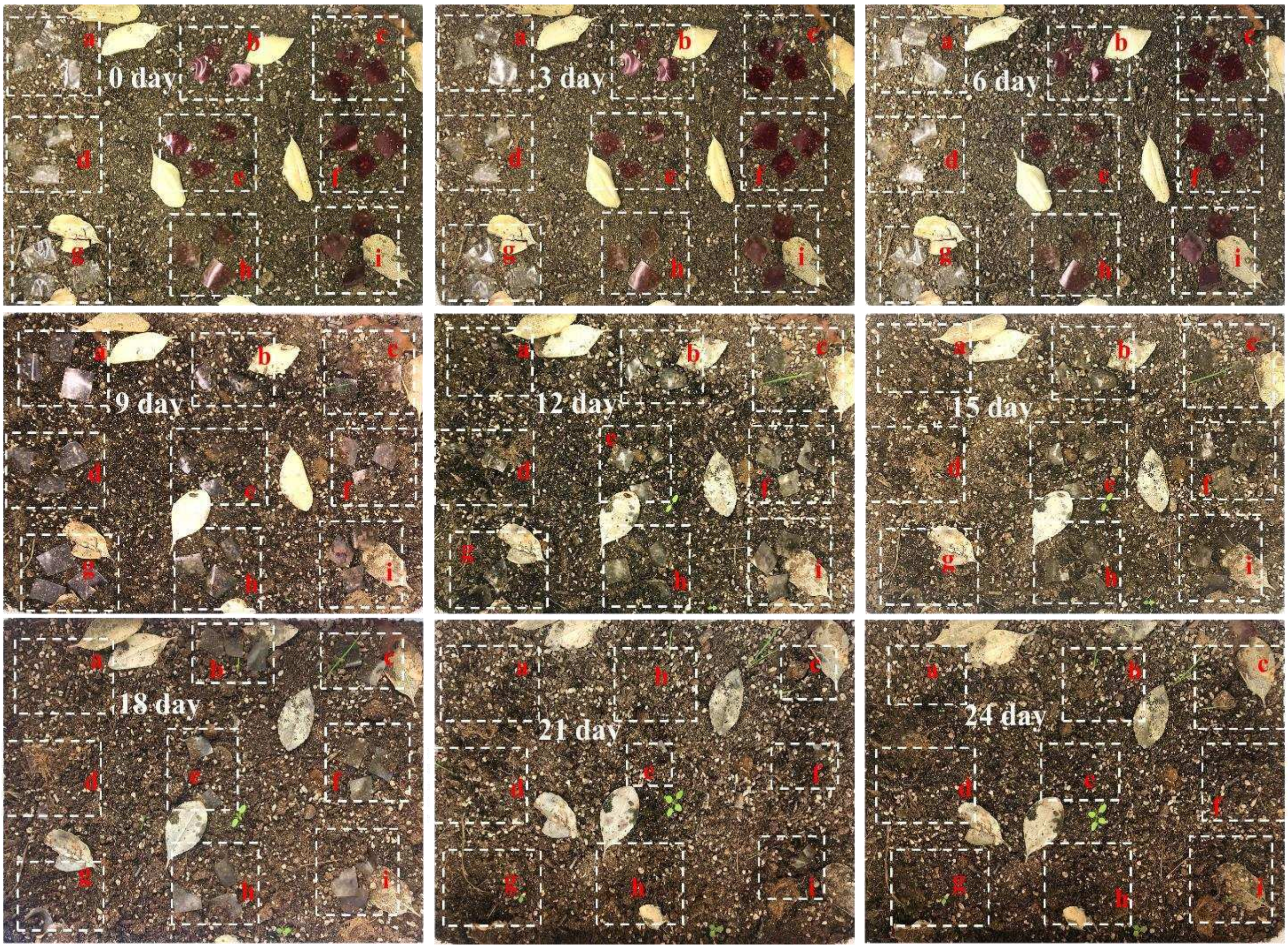

(A)
GN/0%LA/0.5%CE
GN/1.0%LA/0.5%CE
GN/2.0%LA/0.5%CE
GN/0%LA/1.0%CE
GN/1.0%LA/1.0%CE
GN/2.0%LA/1.0%CE
(B)
Water molecule
Anhydrobase
Flavylium ion
Anhydrobase anion
2H2O – 4e- = 4H+ + O2↑
+H+
-H+
4H2O + 4e- = 4OH- + 2H2↑

Figure 5

Click here to access/download;Figure;Fig. 5.docx



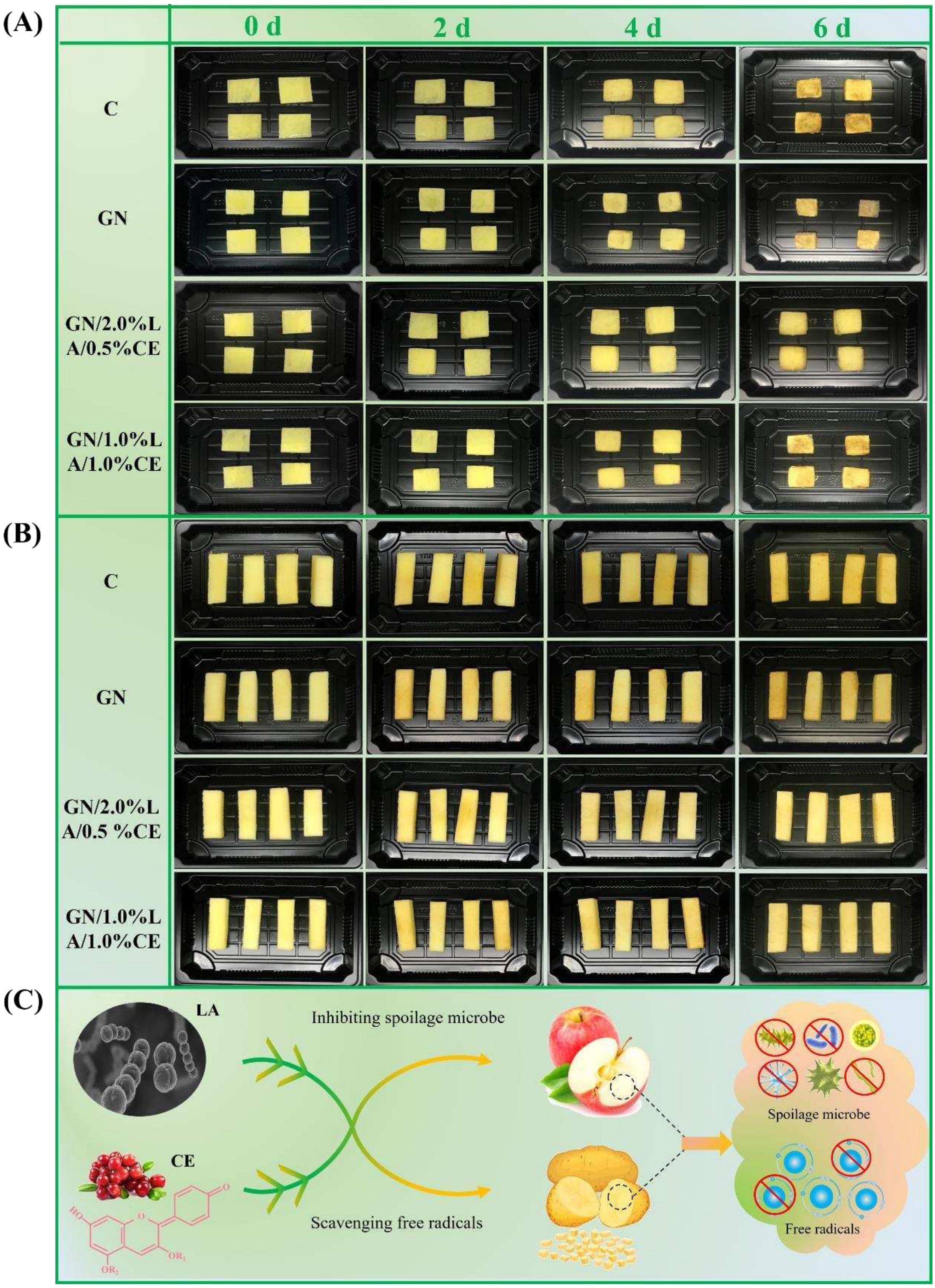

(A)
0 d
2 d
4 d
6 d
C
GN
GN/2.0%LA/0.5%CE
GN/1.0%LA/1.0%CE
(B)
C
GN
GN/2.0%LA/0.5 %CE
GN/1.0%LA/1.0%CE
(C)
LA
Inhibiting spoilage microbe
CE
Scavenging free radicals
Spoilage microbe
Free radicals

Fig. 1. The SEM morphology (A) and florescence images (B) of the different films.

Fig. 2. The antibacterial (A), antioxidant (B), optical (C) and appearance (D) properties of the different films.

Fig. 3. The survivability of *L. lactis* in the film during drying process (A) and storage process (B), and the first-order kinetic formula of inactivation (C).

Fig. 4. The colorful patterns (A) and electrochemical writing principle (B) on the different films

Fig. 5. The biodegradability of the films in soil in natural for 24 days. (a: GN/0%LA/0%CE films, b: GN/0%LA/0.5%CE films, c: GN/0%LA/1.0%CE films, d: GN/1.0%LA/0%CE films, e: GN/1.0%LA/0.5%CE films, f: GN/1.0%LA/1.0%CE films, g: GN/2.0%LA/0%CE films, h: GN/2.0%LA/0.5%CE films, i: GN/2.0%LA/1.0%CE films)

Fig. 6. The preservation effect of different films on fresh-cut potatoes (A) and apples (B) during the storage period.

Table 1. The tensile strength (TS) and elongation percentage at break (EB) and water vapor permeability (WVP) of the films.

Film samples	TS (MPa)	EB (%)	WVP ($g\cdot mm\cdot m^{-2}\cdot Kpa^{-1}\cdot h^{-1}$)
GN/0%LA/0%CE	17.92 ± 0.8^{e}	12.32 ± 1.3^{c}	4.71 ± 0.1^{a}
GN/0%LA/0.5%CE	22.62 ± 1.2^{d}	19.0 ± 0.8^{a}	4.66 ± 0.3^{a}
GN/0%LA/1.0%CE	31.70 ± 1.4^{a}	19.23 ± 1.0^{a}	4.27 ± 0.1^{b}
GN/1.0%LA/0%CE	17.12 ± 2.0^{e}	12.47 ± 1.0^{c}	4.63 ± 0.3^{a}
GN/1.0%LA/0.5%CE	22.60 ± 1.3^{d}	18.50 ± 0.6^{a}	4.57 ± 0.1^{a}
GN/1.0%LA/1.0%CE	32.52 ± 1.2^{a}	17.9 ± 2.1^{a}	4.30 ± 0.2^{b}
GN/2.0%LA/0%CE	14.15 ± 1.2^{f}	9.15 ± 0.7^{d}	4.70 ± 0.1^{a}
GN/2.0%LA/0.5%CE	25.50 ± 0.8^{c}	15.44 ± 1.2^{b}	4.65 ± 0.2^{a}
GN/2.0%LA/1.0%CE	28.17 ± 1.7^{b}	15.10 ± 1.0^{b}	4.81 ± 0.2^{a}

Values are expressed as mean ± standard deviation (n = 4). Different small letters indicate significant differences.

Credit author statement

Zou Xiaobo: Conceptualization, Methodology. Yang Zhikun: Data curation, Writing-Original draft preparation. Zhai Xiaodong: Reviewing and Editing. Shi Jiyong: Conceptualization, Methodology. Li Zhihua: Conceptualization. Methodology. Huang Xiaowei: Reviewing and Editing. Wang Tao: Reviewing and Editing. Li Chuang: Conceptualization, Methodology. Megan Povey: Conceptualization, Methodology. Yunyun Gong: Conceptualization, Methodology. Melvin Holmes: Conceptualization, Methodology.

Conflict of Interest

Xiaobo Zou declares that he has no conflict of interest. Zhikun Yang declares that he has no conflict of interest. Jiyong Shi declares that he has no conflict of interest. Xiaodong Zhai declares that he has no conflict of interest. Xiaowei Huang declares that she has no conflict of interest. Zhihua Li declares that he has no conflict of interest. Tao Wang declares that he has no conflict of interest. Li Chuang declares that she has no conflict of interest. Yunyun Gong declares that she has no conflict of interest. Melvin Holmes declares that he has no conflict of interest. Megan Povey declares that she has no conflict of interest.