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Effect of Tea Polyphenols on Curdlan/Chitosan Blending Film Properties and Its Application to Chilled Meat Preservation

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Abstract: Incorporating phenolic acids into polysaccharide films improves their physical properties, in turn improving their potential commercial applicability as a preservation material for different foods. This study aimed to develop films from curdlan and tea polyphenols, and determine the effect of their contents on the water vapor permeability (WVP) and mechanical properties (tensile strength and elongation at break) of the films. Different ratios of tea polyphenols were incorporated into the curdlan-based films to improve their properties. The results obtained showed that the tensile strength and elongation at break of films were likely to be significantly decreased by adding tea polyphenols, especially at a content of 0.6%, which resulted in a 50% decrease. Meanwhile, the WVP and moisture content of the films was also decreased. However, a low WVP can prevent moisture loss from food. Other film properties, such as antioxidant efficiency, were also investigated. The results showed that the antioxidant potential of the film can be improved by tea polyphenols. The composite films were also applied to the preservation of chilled meat, which resulted in the shelf life being extended by about 3–5 days. Some properties, such as water resistance and DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity of the composite film, were improved.

Keywords: curdlan; tea polyphenols; films; meat

1. Introduction

Currently, the most commonly used packaging films on the market, are made of polyethylene (PE) and polyvinyl chloride [1]. As these films can cause major environmental damage, packaging films made from natural, nontoxic, and biodegradable materials have received increasing research interest [2].

Degradable packaging films are generally comprised of layers of biological macromolecules, such as polysaccharides, proteins, and fats [3]. Some biodegradable packaging films can be processed with packaged foods and used as special food ingredient carriers, preventing the migration of food components, and therefore, prolonging their shelf life [4]. Proteins, polysaccharides, and lipids are the three main matrices for the preparation of biodegradable packaging films with different features, but the properties of single component films are greatly restricted [5]. In recent years, many researchers have attempted to investigate compound materials for film preparation, achieving significant progress.

Curdlan is an extracellular polysaccharide produced by *Alcaligenes faecalis* through fermentation [6]. Curdlan readily disperses in water and can absorb more than 100 times its weight in moisture during heating [7]. When curdlan is heated to 60 °C, a heat-reversible low-strength gel is formed [7]. Curdlan

has excellent film forming properties, but the resulting film has poor mechanical performance, resulting in little research into this material [8].

Chitosan is the deacetylated product obtained from alkaline treatment of chitin [9]. Chitosan has many special features, including high viscosity, low toxicity, polyelectrolyte behavior, and film forming, antimicrobial, mucoadhesive, antibacterial, and metal chelating abilities [10–12]. Chitosan has been widely used in film packaging for fruits, vegetables, and eggs [13]. In an experiment preserving radish slices with chitosan film, Pushkala et al. reported that the weightlessness rate and respiratory rate of the treatment group were both lower than those of the control group [14]. Furthermore, the mechanical properties of chitosan films are influenced by chitosan properties. In general, a higher degree of deacetylation, molecular weight, and concentration of chitosan affords a film system with greater mechanical strength. However, the molecular weight has no obvious influence on the film's water vapor permeability (WVP) [15].

Tea polyphenols are the main active ingredients in tea, accounting for 15–30% of the dry weight of tea [16]. Tea polyphenols readily dissolve in water and ethanol, but are difficult to dissolve in organic solvents [17]. They also have many physiological activities, including free radical scavenging and antioxidative effects [18]. When applied in the food industry, the most valuable property of tea polyphenols is their antioxidant capacity [19]. Kumudavally et al. applied tea polyphenols to keeping mutton fresh, which resulted in a successfully extended shelf life [20]. Tang et al. found that tea polyphenols significantly inhibited the oxidation of fat in red meat and poultry [21].

Cold fresh meat is obtained by reducing the carcass's temperature to 0–4 °C shortly after slaughter [22]. Fresh meat is rich in nutrients and moisture, which provide a good environment for microorganism growth, in addition to increasing water loss and the oxidizing rate of meat products [23]. Low temperatures can accelerate the dry consumption of meat [24]. Biodegradable packaging films reduce the dry consumption rate and oxidation of meat products during storage, resulting in a prolonged shelf life [25]. Ouattar used organic acids or essential oils as bacteriostats in the chitosan coating solution [26]. A chitosan coating has been shown to inhibit bacteria in raw meat (*Lactobacillus* and *Enterobacteriaceae*). Kanatt et al. have also added peppermint extract to the chitosan coating solution as an antioxidant [27].

Among the most effective phenolic acids, tea polyphenols and gallic acid have been shown to possess good potential antimicrobial activity for film applications, which can be attributed to the presence of a phenol ring. The antimicrobial activity of tea polyphenols and gallic acid-incorporated biodegradable films has been evaluated, but data on the application of antimicrobial biodegradable films incorporated with phenolic acids to real food systems are limited. Therefore, this study aimed to produce composite polysaccharide–phenolic acid films (curdlan, chitosan, and tea polyphenols) with improved performance, including improved tensile strength, elongation at break, water vapor transmission rate, antibacterial activity, and oxidation resistance. The effect of tea polyphenols on the films was determined by applying them to the preservation of chilled meat.

2. Materials and Methods

2.1. Materials

Curdlan ($(C_6H_{10}O_5)_n$, 70–80 kDa) [28] was purchased from Kirin Holdings(Tokyo, Japan). Medium-viscosity chitosan (deacetylation degree, ≥85%; molecular weight, 400 kDa) was purchased from Shanghai Ruiyong Biological Technology Co., Ltd (Shanghai, China). Tea polyphenols (purity, ≥98%) were purchased from Shanghai Source Leaf Biotechnology Co., Ltd (Shanghai, China). Sodium hydroxide, glycerol, acetic acid, and hydrochloric acid, all of analytical-grade purity, were purchased from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). All other chemicals were of reagent grade.

2.2. Film Preparation

Curdlan (1.5 wt %) was dissolved in 0.25 mol/L NaOH solution, and chitosan (1.5 wt %) was dissolved in 1 wt % acetic acid solution. Certain amounts of the curdlan(80 mL) and chitosan solutions (20 mL) were mixed. Then, glycerol (30% of base material content) and different quantities of tea polyphenols (g/g chitosan) were added, with the final solution adjusted to pH 4–5 using acetic acid and sodium hydroxide. After stirring and degassing by ultrasonication, the film-forming liquid was poured onto polytetrafluoroethylene plates (18 cm × 18 cm) and allowed to dry at 25 °C and 50% relative humidity (RH) for 24 h, in a constant temperature and humidity box. To control the films' thickness, the volume of each film-forming solution was fixed at 100 mL. After formation, the films were peeled off the plates and conditioned at a controlled temperature and RH for 24 h in a constant-temperature humidity chamber prior to testing. The film's thickness was measured using a vernier caliper, and it' thickness is about 0.02–0.05 mm.

2.3. Film Properties

2.3.1. Mechanical Properties

The tensile strength (TS) and elongation at break (EB), which reflect the mechanical properties of films, were determined using a texture analyzer (TA.XT Plus, Stable Micro System, London, UK) equipped with a tension grip (A/TG) according to the method by Park et al. [29]. The film samples were cut into strips with scissors (80 mm × 20 mm). Before testing, the thickness of each film sample was measured using a micrometer (Shanghai, China) with a precision of 0.001 mm. The thicknesses reported are averages of six random measurements. The film specimens were mounted on the self-tightening roller grips of the testing machine and stretched at a rate of 0.8 mm/s until breaking. The analysis was performed at ambient temperature and a relative humidity of 50% ± 5%. This test was performed on at least eight replicates of each film formulation. TS and EB were calculated according to Equations (1) and (2), respectively:

$$\mathrm{TS} = F_{max}/(L \times W), \tag{1}$$

$$\mathrm{EB} = 100\% \times (L_{max} - L_0)/L_0, \tag{2}$$

where $F_{\max}$ is the maximum tension sustained when the film breaks, L is the film thickness, W is the film width, L_0 is the initial grip separation distance, and $L_{\max}$ is the grip separation distance when the film breaks.

2.3.2. Water Vapor Permeability (WVP) and Moisture Content (MC)

Water vapor permeability (WVP) was measured using the method by Peng Yong, with some modifications, and employed the simulation cup method [30]. Anhydrous calcium chloride powder (20 g, particle size of less than 2 mm) was added into the test cup, with a distance between the calcium chloride powder and cup rim of less than 5 mm. The composite film was used to seal the test cup mouth. The thickness of each film was measured at six randomly chosen points using a micrometer and the average was taken. The cups were placed in a chamber at 25 °C and 75% humidity (RH), and the internal and external sides of the film were kept at a confirmed water vapor pressure difference. The cups were removed and weighed every 12 h until the mass change was less than 0.001 g. This measurement was repeated three times for each group. WVP was calculated using Equation (3):

$$\mathrm{WVP} = \mathrm{mL}/\mathrm{At}\Delta\mathrm{P}, \tag{3}$$

where m is the mass of moisture that permeated through the film (g), A is the permeation area (m^2), L is the film thickness (mm), t is the permeation time (s), and ΔP is the pressure difference between the two sides of the film (Pa).

The film was heated to constant weight in an oven at 100 °C, and the dry matter content was measured. MC was calculated as the percentage weight loss of the total mass during drying. Three films samples were tested for each water content value.

2.3.3. Antioxidant Activity

The antioxidant efficiency of the composite film was evaluated using a DPPH free radical scavenging assay based on a reported method with some modifications [31]. Briefly, the composite film was cut into 20 mm × 20 mm pieces and placed in a beaker, followed by distilled water (100 mL), and the mixture was stirred using a magnetic stirrer. A sample (1 mL) was transferred to a test tube, and distilled water (1 mL) was added as the blank control, followed by an ethanol solution of the stable DPPH radical (4 mL, 150 μ;mol/L). The mixture was then incubated in the dark for 1 h. The absorbance of the resulting solution (A_{sample}) was measured at 516 nm. All measurements were performed in triplicate. The free radical scavenging activity was defined as the decrease in DPPH radical absorbance, and calculated by the following Equation [32]:

$$\text{DPPH free radical scavenging activity} = \left(1 - A_{sample}\right) \times 100\% / A_{control}, \quad (4)$$

where A_{control} is the absorbance of initial DPPH concentration and A_{sample} is the absorbance of the DPPH concentration remaining in the sample.

2.3.4. Scanning Electron Microscopy (SEM) Analysis

The film morphology was analyzed by SEM [28]. The composite film was cut into 6 mm × 1 mm pieces after drying to a constant weight at 100 ± 2 °C. The film was broken in liquid nitrogen to form natural fracture layers, and attached to a metal plate using double-sided adhesive. Gold plating was formed by ion sputtering. The surface and cross-sectional film morphologies were characterized at an accelerating voltage of 7 × 1.0 kV.

2.4. Application to Chilled Meat Preservation

2.4.1. Packing Chilled Meat with the Blending Film

Cold fresh meat (black pork tenderloin) was divided into equal portions (80 g each) and packed using the blending film. Five sample groups (Table 1) were wrapped with different composite films and stored in the refrigerator at 4 °C for 20 days. The samples were analyzed every two days until the end of storage. The indicators measured included the total bacterial count, pH, color, total volatile base nitrogen (TVB-N), and thiobarbituric acid (TBARS). Each sample group was prepared in triplicate and measured in parallel.

Table 1. The grouping form.

Group	Content of Tea Polyphenols(w/w)
control	blank control
L.0	0%
L.1	0.6%
L.2	1.8%
L.3	3%

Note: the blank control was cold fresh meat without composite film.

2.4.2. Antibacterial Activity Analysis

The total number of colonies was the number of colonies formed in samples of 1 mL (or 1 g) under certain conditions (such as medium composition, temperature, and time, pH, oxygen demand) after treatment [33]. The antibacterial activities of the films against bacteria (such as *Staphylococcus*

aureus, *Escherichia coli*, *Pseudomonas*, *Acinetobacter*, *Brochothrix thermosphacta*, *Salmonella bacillus subtilis*, and *Listeria monocytogenes*) were determined using the flat colony counting method with some modifications [34]. These strains were used because they are the most common bacteria in meat products, can cause meat products to spoil, and can even affect human health [35]. One indicator of chilled meat deterioration is a bacterial count exceeding 10^6 /g.

Samples (25 g) were placed in a sterile homogeneous cup with phosphate buffer (225 mL) or physiological saline and homogenized for 1–2 min at 8000–10,000 rpm, or put in a sterile homogeneous bag with diluent (225 mL) and beaten for 1–2 min with a slap homogenizer. This process was continued until the sample homogenization reached 1:100. The homogenized samples were cultured with agar medium and the total number of colonies was counted. All experiments were performed in triplicate.

2.4.3. Determination of the total volatile basic nitrogen (TVB-N)

The meats' freshness, as represented by the total volatile basic nitrogen (TVB-N) level, was determined using semi micro nitrogen determination method in GB/T 5009.44-2003 [36]. This method is based on the principle that protein is decomposed under the action of enzymes and bacteria to produce alkaline nitrogen-containing substances, such as primary amines and secondary amines. These substances are volatile and can be distilled out in alkaline solution, with the content calculated by standard acid titration. The TVB-N content was calculated using Equation (5):

$$X = [14C(V_1 - V_2)/M] \times 100\,, \quad (5)$$

where X is the TVB-N content in the sample (mg/100 g), V_1 is the volume of hydrochloric acid consumed in the sample titration (mL), V_2 is the volume of hydrochloric acid consumed by the blank reagent (mL), C is the concentration of the hydrochloric acid solution used for titration (mol/L), M is the sample weight (g), and 100 is the unit conversion factor.

2.4.4. Evaluation of Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation was measured using a TBARS assay according to the method described by Qianying with slight modifications [37]. First, a standard curve of malondialdehyde (MDA) was constructed using titration end point (TEP) solutions of different concentrations. The standard curve was constructed using the MDA (malondialdehyde) content in the test tube as the abscissa and the absorbance value measured at 532 nm as the ordinate.

The TBARS values of cold fresh meat samples were measured as follows. Samples (5 g) were added to 15 mL of solution A (prepared from trichloroacetic acid (75 g), EDTA (1 g), and propyl gallate (1 g) made up to a constant volume of 1 L with double-distilled H_2O) and rendered homogeneous by filtration. A 5 mL aliquot of the filtrate was transferred to a plugged test tube, followed by solution B (5 mL), mixed evenly, and reacted for 40 min in a water bath at 100 °C. The absorbance was then measured. The MDA content was calculated according to the MDA standard curve, while the TBARS value was expressed as mg MDA/kg.

2.4.5. Color

A CR-400 colorimeter Tokyo, Japan) was used to determine the meat surface color properties. The instrument was corrected using the whiteboard after self-inspection (Y = 94.0, x = 0.3131, y = 0.3193), adopting a D65 light source, 8 mm measurement diameter range, and 2° visual angle to determine the surface color of cold fresh meat (lightness, L*; red degree, a*; and yellow degree, b*). Three samples from each chilled meat group were measured. For each sample, five points were randomly selected and the average result was obtained.

2.4.6. pH

The pH was determined according to Chinese Standard GB/T 9695.5-2008. Boneless loin (10 g) was homogenized (PT-MR2100, Kinematica, Luzern, Switzerland) in distilled water (90 mL). The pH values were then measured on each sampling day using a digital pH meter (Orion 3-Star Plus, Thermo Scientific, Waltham, MA, USA). All readings were performed in triplicate.

2.5. Statistical Analysis

All measured data were obtained using at least three groups in parallel. Data were analyzed by single factor analysis of variance and Duncan's multiple comparison test ($p < 0.05$) using SPSS 16.0 analysis software (version 20, IBM), and the results are presented as mean ± standard deviation of triplicate measurements.

3. Results and Discussion

3.1. Film Properties

3.1.1. Mechanical Properties

As shown in Table 2, the tensile strength (TS) of the composite film decreased significantly after adding tea polyphenols, especially when the tea polyphenol content was 0.6%, resulting in a 50% decrease. This phenomenon might be attributed to the film polymer network being destroyed by tea polyphenols. We speculated that, when tea polyphenols were not added, some of the curdlan was cross-linked with chitosan to form a certain network structure. However, after adding tea polyphenols, the cross-linked structure of curdlan and chitosan was destroyed, leading to weakened mechanical properties. However, with increasing tea polyphenol content in the film structure, tea polyphenols, chitosan, and curdlan also became partially cross-linked, resulting in the film having increased mechanical strength. Adding tea polyphenols significantly also reduced the elongation at break (EB), indicating that tea polyphenols influenced the intermolecular structure and reduced the molecular chain mobility.

Table 2. TS, EB, WVP, and MC of curdlan/chitosan blended film.

Content of Tea Polyphenols	TS	EB	WVP × 10^{-11} ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	MC %
0%	40.02 ± 0.16^{a}	20.03 ± 0.19^{a}	12.0 ± 1.0^{a}	30.29 ± 1.64^{a}
0.6%	20.24 ± 0.35^{c}	20.76 ± 0.24^{a}	5.3 ± 0.2^{c}	14.0 ± 0.7^{c}
1.8%	25.73 ± 1.05^{b}	14.9 ± 0.7^{b}	5.6 ± 0.3^{b}	14.3 ± 0.8^{b}
3.0%	23.45 ± 0.63^{c}	6.0 ± 0.6^{c}	4.4 ± 0.3^{c}	14.1 ± 0.6^{c}

Note: letters a–c indicate significant differences ($p < 0.05$), with different letters in the same column indicating significant differences, and the same letters indicating no significant differences.

3.1.2. Water Vapor Permeability and Moisture Content

Changes in the WVP and MC of the composite film containing tea polyphenols are shown in Table 1. The WVP and MC of the film were reduced by adding tea polyphenols, with decreases of more than 50% observed. A low WVP can prevent moisture loss from food. Meanwhile, trace tea polyphenols produced obvious effects. When the tea polyphenol content was increased from 0% to 0.6%, the WVP and MC of the film decreased significantly. Moisture is necessary for microorganism growth. The low moisture content in the film was not conducive to the growth and reproduction of microorganisms, resulting in an extended food shelf life. However, further increasing the tea polyphenol content produced no significant changes in the moisture content.

3.1.3. Antioxidant Properties

The DPPH radical scavenging capacity is a standard method for evaluating the antioxidant capacity of substances. Generally, strong DPPH radical scavenging activities imply a high resistance to oxidation. The DPPH free radical scavenging ability of the conventional composite film is shown in Figure 1. Tea polyphenols are well known to be potent antioxidants that can scavenge free radicals and reactive oxygen species (ROS). Therefore, the antioxidant ability of films can be improved by adding tea polyphenols. This change might also be attributed to an enhanced hydrogen and electron-donating ability after conjugation with tea polyphenols, as reported in previous studies [38]. In this experiment, tea polyphenols were added into the curdlan/chitosan composite film. The release rate of tea polyphenols was controlled by changing the film-forming conditions, which could improve the tea polyphenol stability. The experiment showed that the antioxidant capacity of the composite film without tea polyphenols was very poor and almost impossible to detect, similar to the results of Peng Yong [39]. As shown in Figure 1, the antioxidant capacity of the composite film with tea polyphenols added was significantly improved. Similar results have been observed by Yen and Chen et al., who reported that the DPPH free radical scavenging ability could reach 87.72%, basically without effect [40]. Therefore, adding antioxidant phenolics to chitosan was suggested to be a useful approach to producing novel antioxidants.

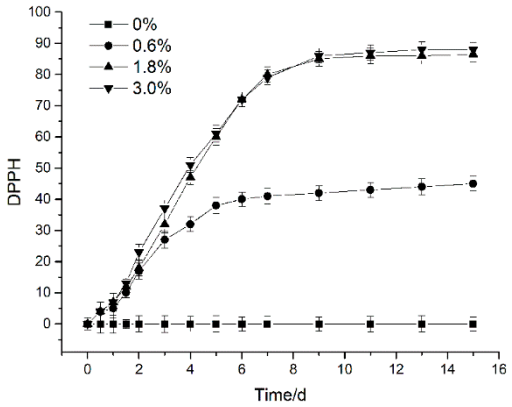


Figure 1. DPPH scavenging ability of film containing tea polyphenols.

3.1.4. Scanning Electron Microscopy (SEM) Analysis

The film morphology is closely related to its physical properties. Scanning electron microscopy (SEM) images of the curdlan/chitosan composite film are shown in Figure 2, where Figure 2a–d show composite films with 0%, 0.6%, 1.8%, and 3.0% tea polyphenol contents, respectively. Observation of the composite film's cross-section showed a crack in the composite film without tea polyphenols added, indicating that the molecular structure of the composite film was not compact, and that adding tea polyphenols promote the film's integrity. This might be due to tea polyphenols binding to curdlan or chitosan molecules, which would cause the film's surface to become smooth. Compared with Figure 2b–d, Figure 2b shows a denser structure and a smoother surface. The possible reason is that with the increase of the content of tea polyphenols, some tea polyphenols do not combine with curdlan or chitosan, leading to many free tea polyphenols macromolecules, so the surface of the film becomes rough.

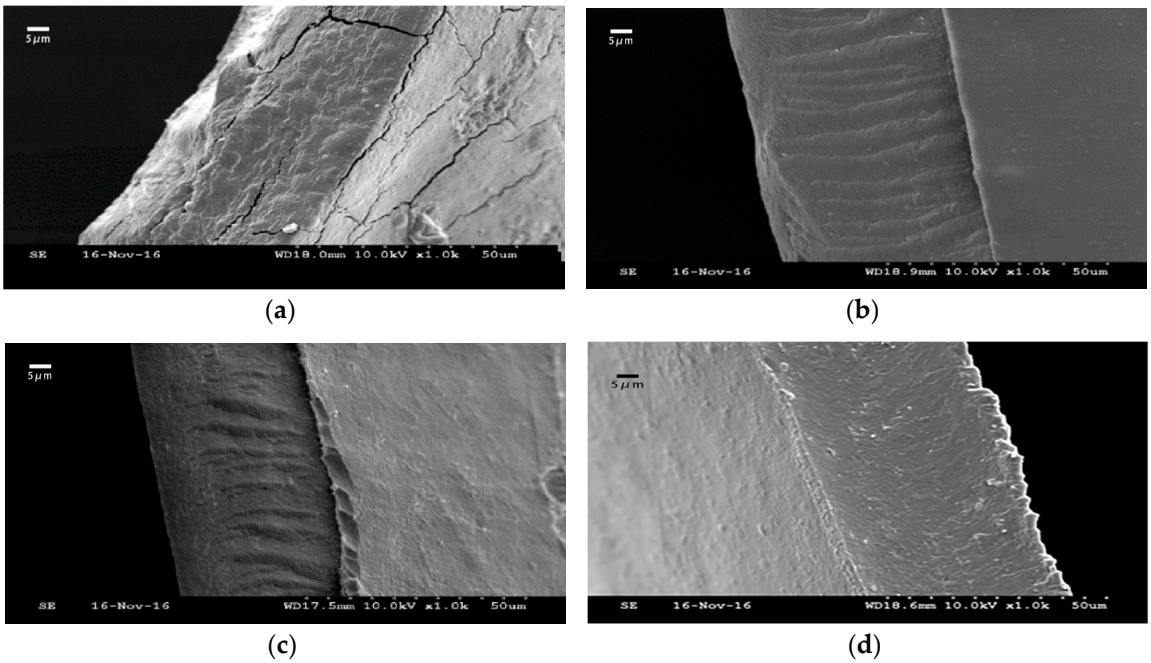


Figure 2. SEM images of composite films with tea polyphenol contents of (**a**) 0%; (**b**) 0.6%; (**c**) 1.8%, and (**d**) 3.0%.

3.2. Application to Chilled Meat Preservation

3.2.1. Microbial Assay

Common bacteria in pork can be divided into spoilage bacteria (*Pseudomonas*, *Acinetobacter*, *Brochothrix thermosphacta*) and pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella*) [41]. The antibacterial properties of films against these bacteria were studied. As shown in Table 3, the total colony number of each treatment group increased with time when cold fresh meat was stored at 4 °C. Compared with the control group and the L.0 group, the total colony number of treatment groups L.1, L.2, and L.3 increased slowly, with no significant difference among the three groups throughout the storage period ($p > 0.05$). In the early stages of storage, the total colony growth rates of the L.0 group and control group were similar. However, on day 7, the L.0 group growth rate had decreased significantly, while that on day 9 had increased again. The tea polyphenols showed an obvious bacteriostatic effect on the storage of cold fresh meat, but the inhibitory effect of low-concentration chitosan was not obvious in the early stages of storage. When the storage time reached 7 d, the effect of chitosan began to play a role, such that when the storage time was further prolonged, the bacteriostatic effect decreased again. After a storage time of 3 d, the logarithm of the total colony number in the control and L.0 groups had exceeded 4.0, becoming grade-two fresh meat. The logarithm of the total colony number had reached six, which proved that the meat was spoiled. The control and L.0 groups had become spoilt meat by days 7 and 9, respectively. The total colony number of the other three treatment groups was not more than 10^6 CFU/g at the end of the storage time, but the logarithm of the total colony number in the L.1 group was 5.25, which was close to meat spoilage.

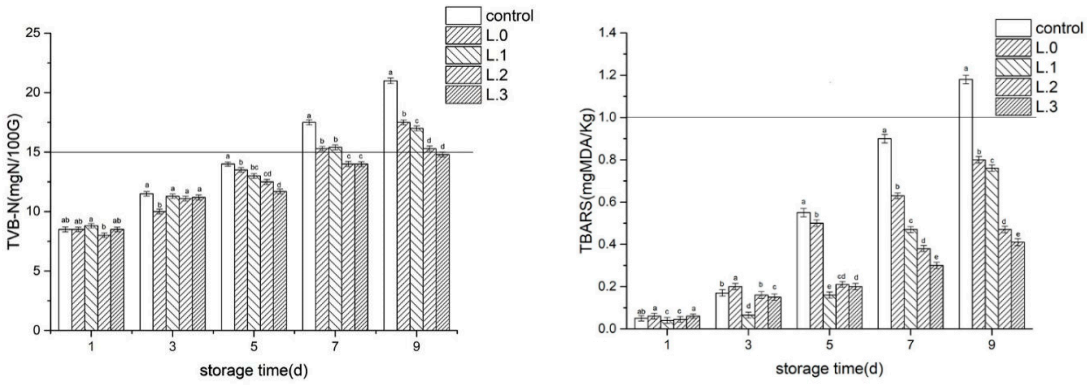
Table 3. Parameter fresh pork during storage at 4 °C for 9 d.

Evaluation Project	Processing Group	Storage Time/d				
		1	3	5	7	9
logarithm of the total colony number	Control	4.02^{a}	4.85^{a}	5.68^{a}	6.25^{a}	7.15^{a}
	L.0	3.51^{a}	4.25^{e}	4.84^{c}	5.25^{d}	6.24^{a}
	L.1	3.25^{b}	3.43^{e}	3.75^{c}	4.25^{a}	5.25^{a}
	L.2	3.31^{c}	3.42^{e}	3.81^{d}	4.16^{b}	4.85^{a}
	L.3	3.42^{c}	3.55^{d}	3.62^{a}	4.08^{e}	4.67^{d}
pH	Control	6.15^{a}	6.13^{a}	6.20^{a}	6.29^{a}	6.65^{a}
	L.0	6.05^{c}	5.93^{b}	6.11^{e}	6.20^{c}	6.37^{b}
	L.1	5.90^{b}	5.78^{c}	5.92^{d}	6.03^{d}	6.15^{e}
	L.2	6.08^{c}	6.12^{e}	6.08^{d}	6.13^{d}	6.14^{a}
	L.3	6.08^{c}	6.06^{b}	6.08^{a}	6.03^{e}	6.05^{d}
lightness (L*)	Control	35.41^{a}	34.13^{a}	33.54^{a}	33.48^{a}	33.45^{a}
	L.0	35.30^{d}	34.52^{d}	54.51^{e}	34.02^{a}	33.71^{b}
	L.1	34.75^{c}	34.08^{a}	34.00^{b}	33.71^{c}	33.45^{e}
	L.2	34.32^{b}	33.75^{c}	33.79^{e}	33.25^{c}	32.75^{e}
	L.3	34.23^{a}	33.95^{b}	33.69^{b}	33.68^{e}	33.31^{c}
red degree (a*)	Control	10.63^{a}	10.25^{a}	9.01^{a}	11.05^{a}	13.52^{a}
	L.0	10.81^{e}	11.53^{c}	9.92^{c}	12.90^{b}	14.07^{a}
	L.1	11.52^{c}	12.02^{d}	10.74^{a}	12.21^{c}	12.15^{c}
	L.2	11.34^{d}	12.19^{d}	11.28^{c}	10.57^{a}	12.18^{c}
	L.3	9.66^{c}	10.09^{d}	10.13^{e}	10.88^{b}	11.49^{c}
yellow degree (b*)	Control	6.15^{a}	6.65^{a}	7.72^{a}	8.19^{a}	7.22^{a}
	L.0	5.75^{a}	6.15^{c}	6.65^{b}	7.49^{d}	6.92^{e}
	L.1	6.28^{e}	6.43^{c}	7.02^{b}	7.22^{d}	6.65^{e}
	L.2	6.14^{d}	6.38^{e}	6.75^{d}	7.23^{a}	6.65^{c}
	L.3	5.88^{d}	6.25^{a}	6.65^{c}	6.67^{c}	6.65^{d}

Note: letters a–e indicate significant differences ($p < 0.05$), with different letters in the same column indicating significant differences, and the same letters indicating no significant differences.

3.2.2. TVB-N

TVB-N is an index for the freshness of regenerated meat. TVB-N determination in chilled meat from different treatment groups is shown in Figure 3a. According to Chinese Standard GB-9959.2-2008 [42], the meat products were corrupted when the TVB-N value of a sample reached 15 mg/100 g. The TVB-N values of the five groups increased with time ($p < 0.05$). However, compared with the control group, the other four groups showed smaller increases in the TVB-N values. Under the storage conditions of 4 °C, the TVB-N values after 1 d and 3 d showed no significant differences. From day 5, the TVB-N value for the control group was significantly higher than those of the other four groups using the composite film ($p < 0.05$), and the gap increased with time. From day 9, the TVB-N value of the L.0 group was observed to be higher than those of the three groups coated with tea polyphenols. This result was also in agreement with the TBARS results discussed in the next section. Notably, after a storage time of 9 d, the TVB-N value of group L.3 still did not exceed 15 mg/100 g.



(**a**) (**b**)

Figure 3. Effects of different treatments of chilled fresh pork on TVB-N (**a**) and TBARS (**b**) values during storage at 4 °C for 9 d period.

3.2.3. TBARS

TBARS is an important index for measure the degree of lipid oxidation in meat products [43]. TBARS is among the most important factors relating to declining meat quality, such as the formation of undesirable rancid flavor and poisoning. During storage, free radicals from lipid oxidation were created by oxygen attack at double bonds in fatty acids. TBARS values above 1 mg MAD/kg pork indicate that the meat is corrupted according to Chinese Standard GB-9959.2-2008 [42]. As shown in Figure 3b, TBARS values of each sample group increased with storage time. Compared with groups using the composite film packaging, the TBARS values of the control group increased faster ($p < 0.05$). At the beginning of the experiment, the TBARS value of each treatment group showed little difference. When the storage time was 5 d, the TBARS values of the control and L.0 group had increased significantly. In contrast, the L.1 group showed an increasing TBARS value from day 7, while those of groups L.2 and L.3 remained stable. In addition to the control group, the TBARS values of the four treatment groups wrapped with composite films did not exceed 1 mg MDA/kg during the storage period of 9 d.

3.2.4. pH

The composite film was applied to chilled meat preservation. The pH value can indirectly reflect meat quality. During meat storage, the formation of volatile gases (such as trimethylamine and ammonia) produced by either microbial or endogenous enzymes can lead to an increase in pH [44]. At a storage temperature of 4 °C, the pH value of each sample initially decreased and then increased, which might be due to the activity of acid microorganisms in the cold fresh meat during the early stages of storage. With prolonged storage time, the pH value of each treatment group increased continuously. According to Chinese standard GB-T 9959.2-2008 [42], chilled meat is spoiled at pH 6.4. The pH value of the control, L.0, and L.1 groups increased relatively quickly, while the pH values of groups L.2 and L.3 changed only slightly. Therefore, the compound film with added tea polyphenols can stabilize the pH of chilled fresh meat. According to the national standard [42], the metamorphic meat pH value was over 6.4. During the storage period of 9 d, the pH value of samples treated with the composite film containing tea polyphenols was below 6.4 (Table 3).

3.2.5. Color

The desirability of food for consumer purchase is largely dependent on its color, because it is the most direct parameter reflecting meat freshness. As shown in Table 3, the L* value of chilled meat decreased with storage time. The L* values of the control and L.0 groups were slightly higher than those of the other treatment groups at the beginning of storage, which might be due to the release of

tea polyphenols onto the cold fresh meat surface. No significant differences in the L* values were observed at other storage times. The L* values of the groups treated with composite films containing tea polyphenols were more stable and showed little change, indicating that adding tea polyphenols into the composite film had little effect on the L* value of cold fresh meat throughout storage, in agreement with Guoqing et al. [45].

The a* value indicates the degree of redness, which is mainly influenced by the color and oxidation degree of myoglobin in the cold fresh meat [46]. Some studies have shown that a* is an important index for measuring the degree of oxidation in meat products [47]. As shown in Table 3, the changing trend for the a* value was the same in each treatment group, showing a decrease midway through storage, followed by an increase. The a* values of the control and L.0 groups showed significantly larger changes than those of the L.1, L.2, and L.3 groups on day 9 ($p < 0.05$). In contrast, the a* values of the three treatment groups using composite films with added tea polyphenols were more stable, indicating that color protection and antioxidant abilities of these films were excellent.

During cold fresh meat storage, the b* value, which indicates the degree of yellowness, initially increased and then decreased with time, reaching the maximum value at a storage time of 7. The control group showed a significantly higher b* value compared with the other treatment groups (Table 3). High b* values indicated that the oxidation degree of cold fresh meat and the bacterial content had increased. The b* value of the composite film treatment groups increased slowly, indicating that the composite film slowed the oxidation of cold fresh meat and inhibited microbial reproduction. At a storage time of 9 d, the b* values of each treatment group began to decline, which might be due to the partial degradation of chilled meat oxidation products produced in the initial stages of storage.

4. Conclusions

Using a 4:1 (w/w) curdlan/chitosan composite film as carrier with added tea polyphenols afforded an active antioxidant edible composite film. Performance analysis showed that the obtained curdlan/chitosan composite film has a high tensile strength, which is important for practical applications, and WVP and WC% values that indicate water resistance. The film can reduce gas exchange and water loss inside and outside of the packaging. Adding tea polyphenols significantly improved the DPPH free radical scavenging activity, which reached 87.72%, while the water vapor transmission coefficient of the composite film decreased.

During cold fresh meat storage, microorganism growth was inhibited by the composite film of polysaccharide and chitosan as the substrate, but the composite films with added tea polyphenols showed a more obvious effect. The microbial contents of the three treatment groups containing tea polyphenols during storage were within the fresh evaluation standards for meat products.

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