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Research Article

Antibacterial Activity and Physical Properties of Fish Gelatin-Chitosan Edible Films Supplemented with D-Limonene

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Fish gelatin-chitosan edible films with D-limonene were successfully prepared, which exhibited exceptional mechanical properties and antimicrobial activity. It has been demonstrated that water-soluble chitosan, fish gelatin, and D-limonene could be a candidate precursor to prepare low cost and high-performance edible food packaging material. The results showed that D-limonene in the films could effectively resist the penetration of light and water because of its hydrophobicity. Moreover, the elongation at break (EAB) increased with the addition of D-limonene, which indicated that D-limonene served as a strong plasticizer for the film. Microscopic characterization showed that D-limonene was uniformly distributed in the as-prepared film. And we found that the film exhibited strong antibacterial activity against *Escherichia coli* (*E. coli*). All the results indicate that the as-prepared film could be a promising food packaging.

1. Introduction

Over the past ten decades, synthetic polymer materials, such as polyethylene (PE) and oriented polypropylene (OPP), have become essential components in food packaging films. Because synthetic plastic films have high toughness and light weight, they have been extensively used as convenient materials in food packaging. However, synthetic plastic films are nonbiodegradable and contain many harmful components, thereby posing a severe global environment risk by generating a mass of refuse. More seriously, some molecules of synthetic polymer materials can infiltrate the food, resulting in a food safety problem. Therefore, exploration of edible packaging films has become a hot topic in recent years.

For the requirements for safety, low cost, and easy preparation with multifunctional properties, some natural biopolymer materials exhibit a unique structure that normal chemical synthesis could not achieve. Many researchers have already focused on natural materials such as polysaccharides [1, 2], proteins [3–5], lipids [6], or combinations of these materials [7]. Recently, many types of gelatin-chitosan edible films [8, 9] and the films incorporated with essential oils have been studied [10–12]. The water-soluble chitosan, fish gelatin, and D-limonene stimulated our interests to design the multifunctional films because of the following advantages. First, water-soluble chitosan with lower molecular weight has high solubility in acid-free aqueous media and it has been widely applied in pharmaceutics [13, 14] and food [15]. Second, fish gelatin and chitosan are useful compounds for the fabrication of edible films due to its film-forming properties at low temperature [16–18]. Third, D-limonene is an effective antibacterial agent [19, 20] and is hydrophobic in nature, so the incorporation of D-limonene into protein films could improve water resistance, impart flexibility, and prolong the quality guarantee period on food. Additionally, fish gelatin, water-soluble chitosan, and D-limonene are edible, inexpensive, and environmentally friendly materials with abundant production every year, because they are derived from natural byproducts, such as bones, cartilage, or tendons of fish, crab shells, and citrus plants, respectively.

In this work, we used fish gelatin and water-soluble chitosan as the matrix materials and D-limonene as an antibacterial agent to prepare the antibacterial edible films. Considering the strong smell of the D-limonene may affect

the taste or odor of the packaged food, a low concentration of D-limonene was used in films. Additionally, the multifunctional properties and antibacterial activity of the films were also evaluated. The results demonstrate that the as-prepared film exhibits attractive properties, thus suggesting that it may be a promising edible packaging film for food.

2. Materials and Methods

2.1. Materials. Commercial grade fish gelatin was obtained from Beijing Hangyang Health Technology Co., Ltd (China), water-soluble chitosan (90% degree of deacetylation) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China), and D-limonene (purity ≥ 95%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (China). Anhydrous glycerol (analytical grade) was purchased from Beijing Chemical Reagent Co. (China). Polyoxyethylene sorbitan monooleate (Tween-80) was obtained from Tianjin Guangfu Fine Chemical Research Institute (China). *Escherichia coli* (CMCC 44102) were acquired from Tianjin Industrial Microbial Test Center (China).

2.2. Preparation of Edible Films. Firstly, to prepare the film-forming solutions (FFS), gelatin powder was dissolved in distilled water and heated at 60°C for 30 min to obtain a solution concentration of 2% (w/w). Glycerol (0.5 g/g gelatin content) was added into FFS as a plasticizer. At the same time, 2% (w/w) water-soluble chitosan was dispersed into the mixture solution. Secondly, D-limonene that was mixed with Tween-80 at 20% (w/w, based on D-limonene) was added into FFS at a concentration of 0, 0.25, 0.50, 0.75, and 1.00% (w/w). Thirdly, the FFS was homogenized at 20,000 rpm for 3 min with a homogenizer (XHF-D, China). The dissolved air was removed using ultrasound (KQ3200B, China). Finally, an amount of 20 mL FFS was cast on rectangular plastic plate ($190 \times 65\,mm^2$) and dried at 25°C at 33% relative humidity (RH). Then, the dried films were peeled from the plate, stored up to equilibration in a desiccator at 33 ± 5% RH and 25°C, and then subjected to analyses.

2.3. Characterization of the Films

2.3.1. Structure and Texture Characterization. A morphological examination of the films was performed by scanning electron microscopy (SEM, HITACHI S-4700) at a voltage of 20.0 kV and with a digital camera. First, the as-prepared film was stored up to equilibration in a desiccator with silica gel for 1 month. Then, part of dry films was fractured in liquid nitrogen and fitted to aluminum stubs using electroconductive paste. In order to make the film conductive, the stub supporting samples were sputtered with gold and then the surface and cross-section morphologies of the film were observed.

2.3.2. Fourier-Transform Infrared Spectroscopy (FTIR). Spectra of the films were recorded between 400 and 4000 cm^{-1} using an attenuated total reflection (ATR) accessory with a diamond ATR crystal and 32 scans with resolution of 4 cm^{-1} as described by Wei et al. [21].

2.3.3. Thickness Measurement. The thickness of the film samples was determined using a thickness gauge (CH-1-ST, China). Five thickness measured values were randomly taken from each film and the average of all measurements was used in the calculations of mechanical properties and water resistance performance.

2.3.4. Mechanical Properties. The tensile strength (TS) and elongation at break (EAB) were determined with a slight modification using a Universal Testing Machine (UTM2502, China) as described by Iwata et al. [22]. The initial grip length and cross-head speed of ten film samples ($20 \times 120\,mm^2$) were set at 50 mm and 5 mm/min, respectively. The test was performed at 25 ± 0.5°C and 33 ± 5% RH.

2.3.5. Light Transmission and Film Opacity. According to Tongnuanchan et al. [23], the light transmission of composite film samples ($10 \times 50\,mm^2$) was measured at wavelengths between 200 and 800 nm with a UV-Vis spectrophotometer (TU-1810, China). The following equation was used to calculate the transparency of composite films:

$$\text{Transparency value} = \frac{(-\log T_{600})}{d}, \quad (1)$$

where T_{600} is the fractional value of transmittance at 600 nm and d is the film thickness (mm). The higher transparency value represents the lower transparency of the film.

2.3.6. Water Contact Angle (WCA) and Wettability. The wettability of the film was performed as described by Liu et al. [24] from water contact angle (WCA) measurements with the sessile drop technique using a goniometer (JC2000C1, China). A drop of approximately 5 μL of distilled water was dropped on the surface of films with a micrometer injector and contact angles were recorded immediately. The WCA values were assessed on the basis of time during 60 s. Three replicates were measured per film type.

2.3.7. Thermogravimetric Analysis (TGA). Under a nitrogen atmosphere, TGA measurements were assessed with a thermogravimetric analyzer (HCT-1, China) as described by Hosseini et al. [25]. Film samples of 2–10 mg were scanned from 25°C to 800°C. The heating rate was set at 10°C/min. The weight loss of samples was determined according to temperature.

2.3.8. Antibacterial Activity of Films. The antibacterial activity of fish gelatin-chitosan edible films supplemented with D-limonene was determined by the agar diffusion method as described by Ponce et al. [26]. Firstly, the as-prepared film was stored up to equilibration in a desiccator at 33 ± 5% RH and 25°C for 2 days. Secondly, bacterial strain was cultured in nutrient broth at 37°C overnight. At the same time, the film was cut into 12 mm circular disc. Finally, the as-prepared film was laid onto nutrient agar plate's surface, which have been seeded with 100 μL of inoculums containing approximately 1.0×10^8 CFU/mL of *E. coli*, whereafter the plates were incubated at 37°C. The zone of inhibition on medium was used for determining the antibacterial effects of

the films against typical bacteria including *E. coli*. The diameter of the inhibition zone surrounding the film discs was precisely measured using a Vernier caliper after 24 h incubation.

2.3.9. Moisture Content (MC) and Film Solubility (FS). Moisture content (MC) was measured by drying small film samples ($1 \times 40\ mm^2$) in dry oven for 24 h at 105°C according to Wu et al. [27]. The weights of film samples before and after the oven drying were recorded. Then, the films were put into a beaker with 30 mL of distilled water and kept for 24 h at 25°C. The film samples were then filtered using Whatman No. 1 filter paper. The papers plus insoluble film residues were desiccated for 24 h at 105°C. Moisture content (MC, %) and film solubility (FS, %) were determined by the following equations:

$$\begin{aligned} \text{MC}\,(\%) &= \left[\frac{(W_o - W_i)}{W_o}\right] \times 100\%, \\ \text{FS}\,(\%) &= \left[\frac{(W_i - W_j)}{W_i}\right] \times 100\%, \end{aligned} \tag{2}$$

where W_o is the initial weight of the film before drying, W_i is the weight of the film after drying, and W_j is undissolved desiccated film residue according to Li et al. [28]. The results are shown as an average of three replicates.

2.3.10. Water Vapor Permeability. According to Clarke et al. [29], water vapor permeability (WVP) was measured gravimetrically using the wet bottle method with some minor adjustments. 8 g anhydrous calcium chloride ($CaCl_2$), as a desiccant, was placed in each test cup to maintain a 0% RH. Then, the bottleneck was sealed with the test film (5 cm^2 film area). Then, the bottle was placed at 25°C and 33% RH in desiccators. The weight of the bottle was measured over 24 h and recorded at 2 h intervals using an analytical balance. All tests were carried out in triplicate. The WVP of the edible films was calculated using the following equations:

$$\text{WVP}\left(\text{gKPa}^{-1}\text{m}^{-1}\text{s}^{-1}\right) = \frac{(\Delta m \times d)}{(A \times t \times \Delta p)}, \tag{3}$$

where Δm is the weight gain of the cup (g), d is the average thickness (m) of film, A is the exposed film (m^2) area, t is the time (s), and Δp is the partial water vapor pressure variance across the two sides of the film.

2.3.11. Statistical Analysis. All experiments were performed in triplicate. The data were subjected to one-way analysis of variance (ANOVA) and statistical analysis was carried out by means of the SPSS 19.0 package (IBM, New York). The least significant difference test was applied for all statistical analyses. The data was expressed as the mean ± SD (standard deviation).

3. Results and Discussion

3.1. Structure and Texture Characterization. SEM images of surface and freeze-fractured cross-sections of edible films based on fish gelatin and chitosan supplemented with D-limonene at a concentration of 1.00% (w/w) are illustrated in Figure 1. Regardless of D-limonene, it can be observed that all the fish gelatin-chitosan films had a compact, glabrous, and uniform surface without porous and granular structure, indicating that a well-organized structure was formed. Additionally, the cross-section of the control film has an even and dense structure without flaws or pores. The results suggested that the chitosan and fish gelatin were dispersed evenly in the film-forming solution. Compared with the control film, the cross-section of fish gelatin-chitosan edible films supplemented with D-limonene showed a denser structure. Moreover, the existence of pores or cracks might be attributed to the volatility of few of essential oil during the drying process. A similar effect was observed by Tongnuanchan [23]. In addition, it can be observed from the digital images (Figure 1) of the films that the control films and fish gelatin-chitosan edible films supplemented with D-limonene both have excellent flexibility. Thus, film structure could be effectively associated with the properties of film, especially film solubility and water vapor permeability of as-prepared films.

3.2. Fourier-Transform Infrared Spectroscopy (FTIR). The FTIR spectra of the D-limonene and fish gelatin-chitosan edible films containing D-limonene at selected concentrations are presented in Figure 2. The spectrum of the control film (Figure 2(b)) showed major peaks at approximately 3296.07 cm^{-1} (amide-A, N-H stretching), 2921.01 cm^{-1} (amide-B, C-H stretching), 1659.85 cm^{-1} (amide-I, C=O stretching), 1556.56 cm^{-1} (amide-II, N-H bending), and 1241.64 cm^{-1} (amide-III, C-N and N-H stretching). As shown in Figure 2, these fish gelatin-chitosan edible films supplemented with D-limonene also have characteristic bands in the spectra region, which are similar to those of the control fish gelatin-chitosan films. These spectra were in agreement with Hosseini et al. [30]. As observed in Figure 2, some of the peaks are shifted to higher or lower wavenumbers with the supplement of D-limonene. For instance, the peaks of amide-A shifted to higher wavenumbers more likely suggested the decline of hydrophilic group in the edible films due to the possible formation of hydrogen bonds between fish gelatin and chitosan. The shift of the amide-I band to a lower wavenumber in D-limonene-supplemented films, compared to the control film, is attributed to the presence of disordered molecular structure. Moreover, one band (at 1039.24 cm^{-1}) can be observed that is possibly associated with a skeletal stretching vibration of C=C bonds. These peaks become sharper with increasing D-limonene content, presumably due to an even distribution into the interior of both protein and polysaccharide domains or due to the interactions of fish gelatin or chitosan with D-limonene. Thus, it is possible that the incorporation of D-limonene slightly transformed the molecular structure and intermolecular interactions in the film.

3.3. Thickness. Thickness of edible films based on fish gelatin and chitosan supplemented with D-limonene is shown in Table 1. The incorporation of D-limonene led to a slight increase in thickness of the edible films. According to Table 1, an 11.4% increase in the thickness value (40.2–44.8 μm) is

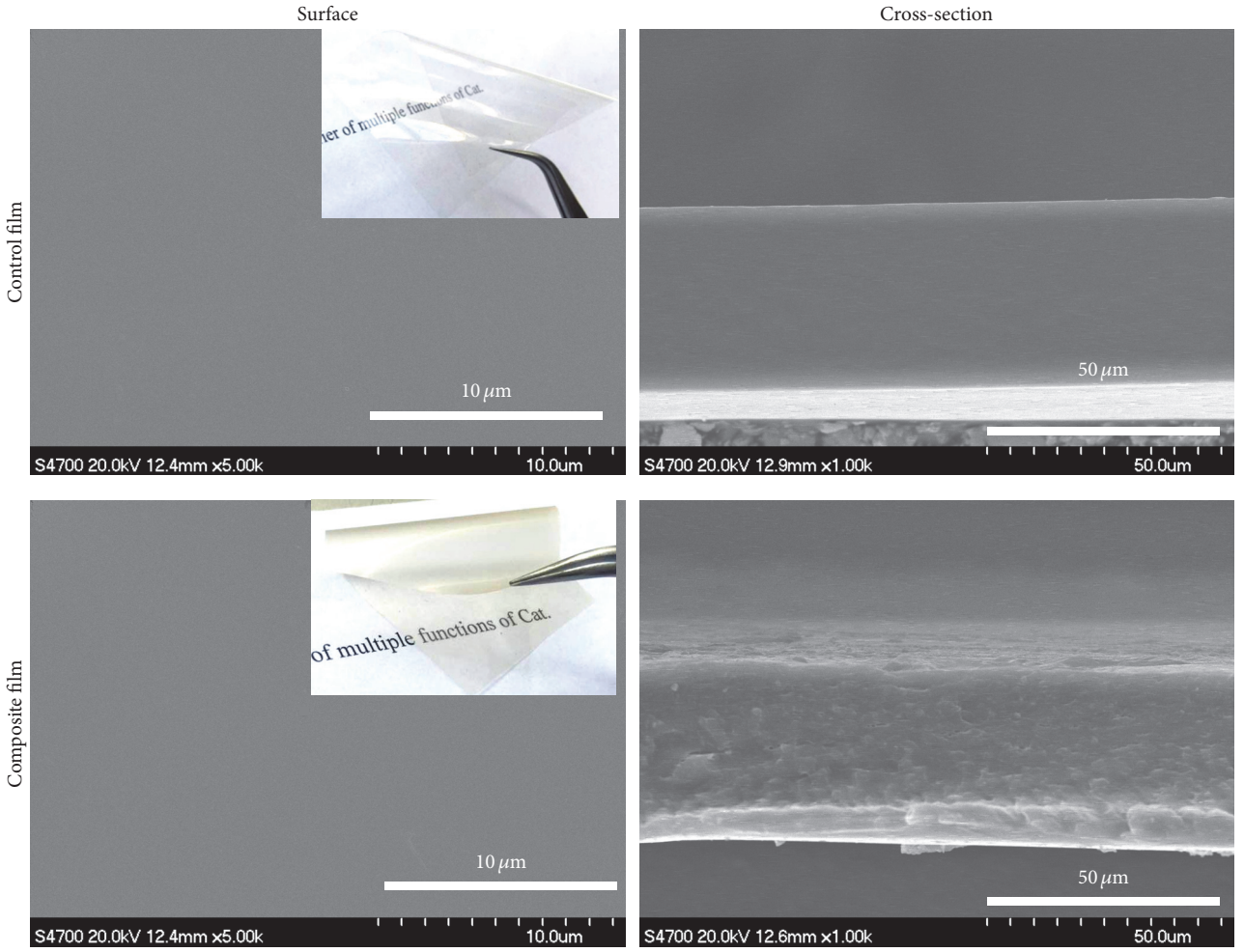


Figure 1: SEM micrographs of the surface and cross-sections of a fish gelatin-chitosan edible film with 1.00% (w/w) D-limonene added and of the control film.

Table 1: Thickness, tensile strength (TS), and elongation at break (EAB) of fish gelatin-chitosan edible films supplemented with different concentrations of D-limonene and of the control film.

D-limonene in FFS (%, w/w)	Thickness (μm)	TS (MPa)	EAB (%)
None	40.2 ± 2.2^{a}	35.55 ± 1.18^{a}	2.44 ± 2.37^{a}
0.25	40.7 ± 0.4^{b}	30.98 ± 0.95^{b}	3.30 ± 4.78^{a}
0.50	41.1 ± 1.7^{c}	27.52 ± 2.34^{c}	4.04 ± 2.00^{b}
0.75	42.3 ± 2.1^{d}	23.98 ± 2.89^{bc}	5.21 ± 3.54^{c}
1.00	44.8 ± 4.8^{bc}	21.48 ± 1.37^{b}	5.61 ± 2.87^{c}

Values are given as the mean ± SD ($n = 3$).
Different small letters indicated significant differences among means in the same column ($P < 0.05$).

achieved by adding 1.00% (w/w) of D-limonene to the edible films. The result suggested that thickness of edible films was influenced by the addition of D-limonene. In general, thickness of edible films is related to the volatility of essential oils. However, essential oils, like D-limonene, are volatile and unsolvable in water. The oil droplets were dispersed in the film network, thereby resulting in lowering the interaction between fish gelatin and chitosan in the edible films and destroying the compactness of films. Additionally, a 24% higher mass is achieved by adding 1.00% (w/w) of D-limonene to the edible films in theory, but thickness of the as-prepared film has an 11.4% increase on the same concentration of D-limonene. As a consequence, the higher density of structure in edible films than control film was formed as indicated by the increased edible film thickness. The thickness differences between the control and films containing D-limonene were in accordance with the edible films microstructure observed by SEM.

Table 2: Light transmission (%) and transparency of fish gelatin-chitosan edible films containing different concentrations of D-limonene and of the control film.

D-limonene in FFS (%, w/w)	Wavelength (nm)							Transparency values
	280	350	400	500	600	700	800	
None	54.07	80.17	83.57	85.7	86.40	86.70	86.07	1.45 ± 0.038^{a}
0.25	38.97	70.94	75.90	80.3	82.50	83.93	85.03	1.63 ± 0.025^{b}
0.50	23.17	51.20	57.87	63.9	67.70	70.40	72.57	3.41 ± 0.013^{c}
0.75	19.03	59.07	42.80	48.13	52.80	56.97	60.90	4.87 ± 0.047^{b}
1.00	17.07	35.27	38.40	41.97	44.83	47.47	50.00	6.60 ± 0.029^{a}

Values are given as the mean ± SD ($n = 3$).
Different small letters indicated significant differences among means in the same column ($P < 0.05$).

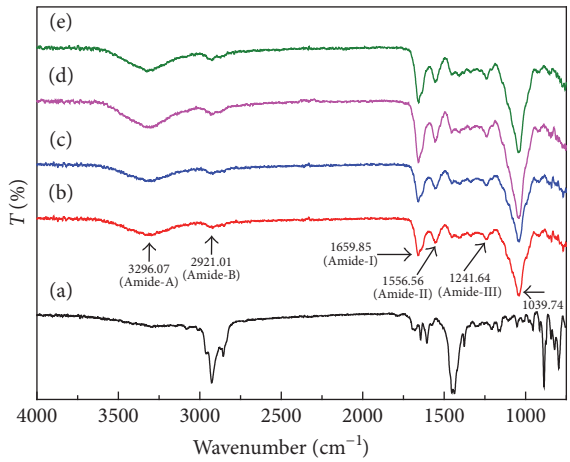


Figure 2: FTIR spectra of D-limonene (a) and fish gelatin-chitosan edible films supplemented with (b) 0, (c) 0.25, (d) 0.50, and (e) 1.00% (w/w) D-limonene.

3.4. Mechanical Properties. Tensile strength (TS) and elongation at break (EAB) of fish gelatin-chitosan edible films containing D-limonene at selected concentrations are shown in Table 1. The addition of D-limonene results in lower TS and higher EAB of the edible films. As shown in Table 1, when 1.00% (w/w) D-limonene was added to the film matrix, TS of the composite film shows a marked reduction (up to 40%). This is attributed to a hindrance effect on hydrogen bond cross-linking caused by recombination of the polysaccharide layer and the gelatin-based layer. The molecular network in the films was destroyed, which explains the drop in TS of the film.

As shown in Table 1, EAB increases with increasing concentrations of D-limonene, ranging from 2.44% (control) to 5.61% for films with 1.00% (w/w) D-limonene. The results indicated that D-limonene functioned as plasticizer in resulting edible films. D-limonene, as an oil phase, could penetrate into the film matrix, thus increasing the volume of spaces between macromolecules chains and providing the greater mobility. A similar result was observed by Arfat et al. [31] when they supplemented basil leaf essential oil into films.

3.5. Light Transmission and Film Transparency. Generally speaking, it is significant that an edible film could protect food from the effects of light, particularly UV radiation. Table 2 shows the transmission of UV and visible light at wavelength range of 200–800 nm of fish gelatin-chitosan films supplemented with D-limonene. It is noteworthy that the light transmission of edible films supplemented with D-limonene is much lower in both the UV radiation range and visible range compared with those of the control film. This finding indicates that edible films supplemented with D-limonene are capable of delaying the lipid oxidation in food systems that is caused by UV light. The result (Table 2) also shows that edible films supplemented with D-limonene have better light barrier properties for visible light. Moreover, D-limonene is able to hinder light transmission through films. Ahmad et al. [32] have already proved that light scattering at the interface of oil droplets inserted in the polymer matrix is the most likely reason for the reduced light transmission.

The edible films supplemented with D-limonene have higher opacity than the control film, which is related to the decrease in light transmission when the film is supplemented with D-limonene. The D-limonene in the film matrix remains in the oil droplets, which can scatter light and lower the transparency. When the concentration of D-limonene in the edible film increases, the intensity of light scattering is greater due to the extent of dispersion of the oil droplets, and transparency is decreased, which improves the light barrier properties. Additionally, transparency of edible film supplemented with D-limonene might be also related to the degree of drying of the as-prepared film. Figure 1 shows the transparency of fish gelatin-chitosan edible films supplemented with D-limonene being similar to the control film, but the transparency of edible films is lower than the control film as shown in Figure 5. This phenomenon might be ascribed to the volatilization of water or some D-limonene in edible films during the preservation.

3.6. Wetting Properties. The hydrophilic nature of fish gelatin-chitosan films presents the main barrier to this type of film being an ideal packaging material. The value of the WCA is a measurement of the water sensitivity of the surface. Generally, if the WCA is lower than 65°, the surface can be described as hydrophilic; if the WCA is higher than 65°, the surface can be characterized as hydrophobic. According to Figure 3, adding D-limonene to the film causes a significant increase in the WCA, from 46.5° for the control film to 68.5° for a film with 0.25% (w/w) D-limonene. It is apparent from Figures 3(b), 3(c), 3(d), and 3(e) that the WCA slightly

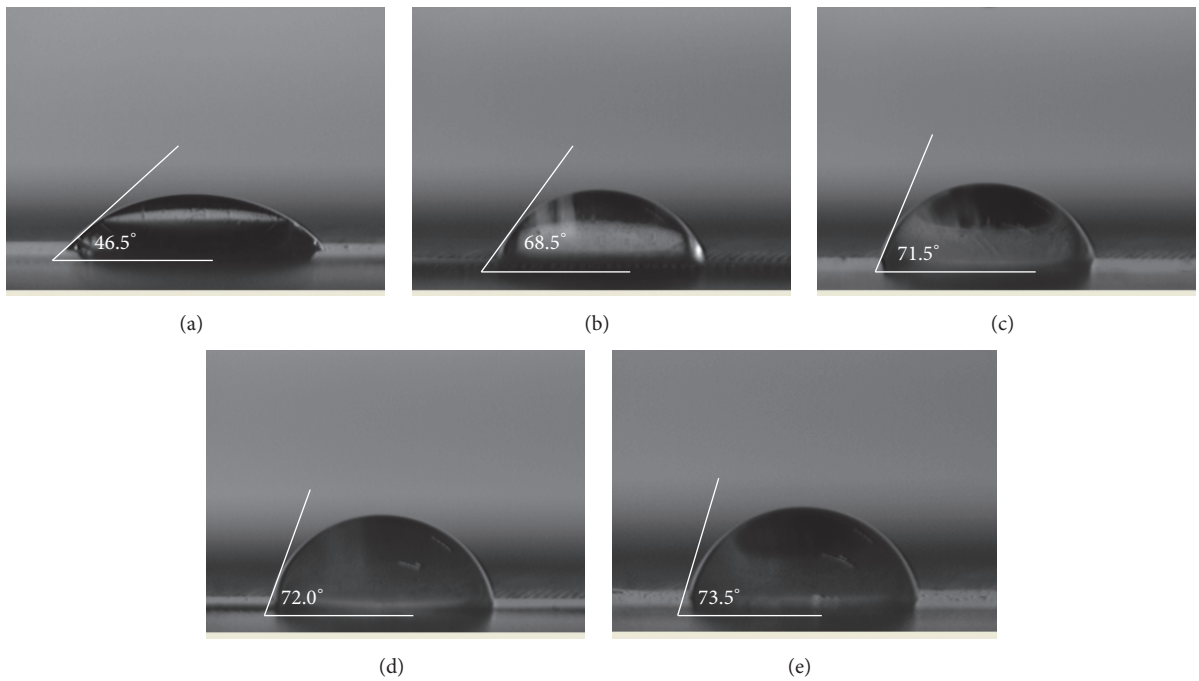


Figure 3: The water contact angle (WCA) of (a) the control film and of the fish gelatin-chitosan edible films supplemented with (b) 0.25, (c) 0.50, (d) 0.75, and (e) 1.00% (w/w) D-limonene.

increased from 68.5° to 73.5° with an increase of D-limonene concentration, which results from the hydrophobic nature of D-limonene. This coincides with the results reported by Hosseini et al. [33].

3.7. Thermogravimetric Analysis (TGA). TGA thermograms of fish gelatin-chitosan edible films supplemented with different concentrations of D-limonene are illustrated in Figure 4. The films underwent two mass-loss steps. The first one, in the temperature range of 50–110°C, corresponds to the loss of absorbed water and other volatile compounds in the films. The second one, in the temperature range 236–315°C, is attributed to the chemical degradation of fish gelatin, chitosan, glycerol, and loss of D-limonene from the films. The degradation temperature (T_d) of the control film was 315°C, and T_d for films with 0.25, 0.50, 0.75, and 1.00% (w/w) D-limonene were 313, 314, 316, and 317°C, respectively. D-limonene only had a slight effect on T_d. Therefore, the thermal stability of fish gelatin-chitosan edible films was not influenced by adding D-limonene.

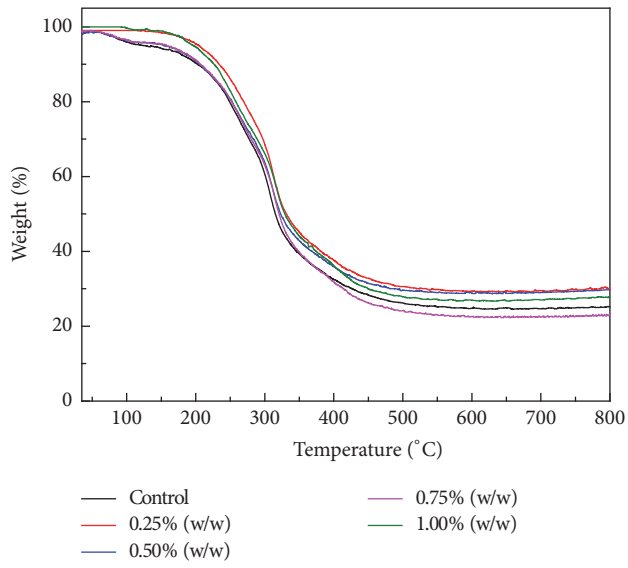
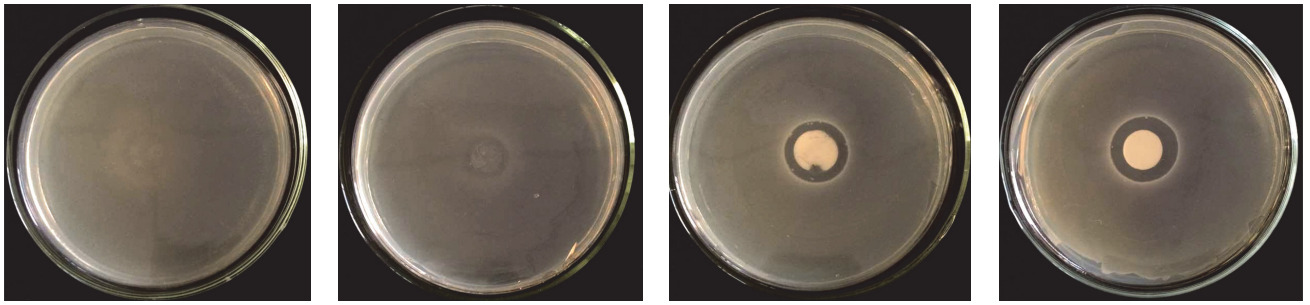


Figure 4: TGA thermograms of control (a) and fish gelatin-chitosan edible films supplemented with (b) 0.25, (c) 0.50, (d) 0.75, and (e) 1.00% (w/w) D-limonene.

3.8. Antibacterial Activity. The antibacterial activity of fish gelatin-chitosan edible films containing D-limonene at different concentrations against *E. coli* is presented in Figure 5 and Table 3. As shown in Figure 5, the control film showed no inhibition zone. Meanwhile, *E. coli* grew slightly on the surface of fish gelatin-chitosan films, demonstrating that the fish gelatin-chitosan films have no antibacterial activity against *E. coli*. It might be attributed to the molecular structure of water-soluble chitosan. The molecules might have no ability to adhere to the surface of bacteria. Similar results were found by Qin et al. [34]. However, as the concentration of D-limonene increased in the edible films, the zone of inhibition increased significantly (Figures 5(d)). The fish gelatin-chitosan edible film containing the highest concentration of D-limonene (i.e., 1.00%, w/w) effectively inhibited the growth of *E. coli*, producing halos about 22 mm (Table 3). These results are in concordance with Hosseini et al. [33]. The investigation indicated that the ability of D-limonene to



(a) (b) (c) (d)

Figure 5: Photographs of the antibacterial activity of the control film (a) and of the fish gelatin-chitosan edible films supplemented with (b) 0.25, (c) 0.50, and (d) 1.00% (w/w) D-limonene.

Table 3: Antibacterial activity of fish gelatin-chitosan edible films supplemented with different concentrations of D-limonene and of the control film.

D-limonene in FFS (%, w/w)	None	0.25	0.50	0.75	1.00
Inhibition zone for *E. coli* (mm)	ND	ND	14.7 ± 1.1^{a}	16.3 ± 0.6^{b}	22.0 ± 1.2^{c}

Values are given as the mean ± SD ($n = 3$). ND represents not detected.
Different small letters indicated significant differences among means in the same column ($P < 0.05$).

Table 4: Moisture content (MC), film solubility (FS), and water vapor permeability (WVP) of fish gelatin-chitosan edible films supplemented with D-limonene and of the control film.

D-limonene in FFS (%, w/w)	MC (%)	FS (%)	WVP $\times 10^{-7}$ ($gKPa^{-1}\,m^{-1}\,s^{-1}$)
None	5.49 ± 0.65^{b}	71.12 ± 044^{a}	3.997 ± 0.034^{a}
1.00	4.26 ± 0.82^{c}	54.07 ± 0.63^{b}	2.567 ± 0.048^{b}

Values are given as the mean ± SD ($n = 3$).
Different small letters indicated significant differences among means in the same column ($P < 0.05$).

inhibit microorganisms depended on the content of the D-limonene in films. The mechanism of antibacterial activity of D-limonene is presumably attributed to its ability to penetrate through the cell wall of microorganism and to affect the viability of the cells. Antimicrobial action of D-limonene is suggested to occur through its interaction with specific cell components. However, it is required that the exact antimicrobial mechanism of D-limonene needs to be further investigated. In summary, these results proved that the fish gelatin-chitosan edible films supplemented with 16.13% (w/w) D-limonene (in total dry matter) had the potential to inhibit target microorganisms.

3.9. Moisture Content (MC) and Film Solubility (FS). MC and FS of fish gelatin-chitosan edible films supplemented with D-limonene are revealed in Table 4. Both MC and FS of the edible films containing D-limonene were lower than the control film. FS of the control film (without D-limonene) was 71.12 ± 0.44%, which obviously reduced (up to 54.07 ± 0.63%) when D-limonene was added into fish gelatin-chitosan film. The high MC and FS of control films are ascribed to the hydrophilicity of fish gelatin and water-soluble chitosan. This characteristic could benefit to develop instant noodle packaging. However, the lower MC and FS of the as-prepared film supplemented with D-limonene might result from the hydrophobicity of D-limonene and the stronger molecular network structure in the as-prepared films. It would contribute to extending quality guarantee period of films.

3.10. Water Vapor Permeability (WVP). WVP revealing water vapor barrier properties of the edible films as influenced by D-limonene incorporation are shown in Table 4. WVP of the edible films decreased with increasing amount of D-limonene, compared with the control films. The results demonstrated that WVP values of the edible films depend on the hydrophilic/hydrophobic ratio of the film component. D-limonene, as a hydrophobic oil phase, uniformly distributes to the edible film, which could hinder the water to transfer through the edible films. Thereby the lower WVP of edible films supplemented with D-limonene was obtained.

4. Conclusions

Fish gelatin-chitosan edible films supplemented with D-limonene were developed. Compared with control film (without D-limonene), the developed films showed excellent antibacterial activity. Moreover, incorporation of D-limonene into the fish gelatin-chitosan film effectively improved their ductility, water vapor barrier, and light barrier properties. The

research results showed that D-limonene could be uniformly distributed in the films. When the concentration of D-limonene in dry films reached 16.13% (w/w), the edible films exhibited significant antimicrobial effect against *E. coli*. Given the high FS of the film, the as-prepared film can be mainly applied as instant noodle packaging or interlayer of food packaging.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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