Properties and Antimicrobial Activities of Starch-Sodium Alginate Composite Films Incorporated with Sodium Dehydroacetate or Rosemary Extract

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ABSTRACT: Antimicrobial films were prepared with oxidized and acetylated corn starch–sodium alginate by incorporating sodium dehydroacetate or rosemary extract. Films incorporated with sodium dehydroacetate $\geq$ 0.1% or rosemary extract $\geq$ 0.3% had an anti-*Escherichia coli* effect. *Aspergillus niger* could be effectively inhibited by the incorporation of sodium dehydroacetate $\geq$ 0.3%. Rosemary extract showed no inhibitory effect on *Aspergillus niger*. Sodium dehydroacetate and rosemary extract reduced the tensile strength and elongation at break, and increased the water vapor permeability of the films. Sodium dehydroacetate made the films more greenish–yellow with the increase of sodium dehydroacetate concentration. The color of the films became darker and more reddish–yellow as rosemary extract was increased. Fourier transform infrared (FTIR) spectra analysis revealed that sodium dehydroacetate and rosemary extract reduced starch crystallinity. The surface of the films became rougher as a result of an addition of sodium dehydroacetate and rosemary extract. These findings had potential applications in prolonging food shelf life based on different needs.

KEYWORDS: antimicrobial films; starch; FTIR; sodium dehydroacetate; rosemary extract

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INTRODUCTION

Severe environmental pollution caused by plastic food packaging has pushed considerable researches towards edible and biodegradable films made from natural polymers. Films carrying food additives, such as antioxidants, antimicrobial agents, would be the tendency of functional food packaging in the future.[1] The development of active packaging systems with desirable physicochemical properties could serve as carriers and provide a controlled release of antimicrobials over an extended period of time.[2,3] Among natural polymers, starch has been considered as one of the most promising candidates for future materials because of its low price, abundance, and thermoplastic behavior.[4] Unfortunately, starch exhibits several disadvantages such as a strong hydrophilic character (water sensitivity) and poor mechanical properties compared to conventional synthetic polymers.[5] An effective approach to cut back on these shortcomings was to use modified starch. Antimicrobial and physical properties of films with various antimicrobials have been investigated.[6–9] However, there have been very few reports on antimicrobial oxidized and acetylated corn starch-based films.

Sodium dehydroacetate is recognized by Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a safe food preservative and has a relatively broad spectrum of antibacterial activity against food-borne pathogens and spoilage organisms. It has been widely used in Europe and America for many years. Rosemary (R. officinalis L.) from the Lamiaceae family, is well known for its antioxidative properties and is used to flavor food and beverages, as well as for several pharmaceutical purposes.[10] Rosemary has an antimicrobial as well as an antioxidative activity.[11] It could be potentially used as alternative food additives to prevent food spoilage and the contamination with Listeria monocytogenes.[12] Rosemary extract with 30% of carnosic acid had the best antimicrobial efficiency of all tested rosemary extracts against different bacteria and yeasts.[13]

This study was to improve the antimicrobial efficiency of biodegradable films based on oxidized and acetylated corn starch by incorporating sodium dehydroacetate and rosemary extract. The objectives of this study were to investigate the antimicrobial activities of oxidized and acetylated corn starch–sodium alginate composite films on *E. coli* and *A. niger* which were incorporated with sodium dehydroacetate and rosemary extract. The mechanical and physical properties were also described.

EXPERIMENTAL

Materials

Oxidized and acetylated corn starch, provided by Zhucheng Xingmao Corn Development (Weifang, China) was used to prepare starch films. Glycerol (Gly) was purchased from Chemical Reagent (Tianjin, China) and used as a plasticizer. Sodium alginate was obtained from Yantai Feng Yuan Seaweed (Yantai, China). Sodium dehydroacetate was purchased from Qingdao Dawei Biological Engineering (Qingdao, China). Rosemary extract was purchased from Hainan Super biotech (Hainan, China). *E. coli* ACCC 10141 was purchased from the Agricultural Culture Collection of China. *A. niger* Asp-1 was provided by College of Food Science and Engineering, Shandong Agricultural University.

Film Preparation

Totally, 0.7 g of sodium alginate and 6.3 g of oxidized and acetylated corn starch were dispersed in 70.55 g of H_2O, moderately stirred for 20 min at room temperature. After an addition of 2.45 g of glycerol, the suspension was heated in a water bath at 80°C for 40 min by mixing continuously with an electric stirrer (Jintan Zhongda Instrument Plant, Jiangsu, China) at 300 rpm. After cooling, sodium dehydroacetate was added to reach a final concentration of 0, 0.1, 0.3, 0.5, and 0.7% (w/w) or rosemary extract of 0, 0.3, 0.6, 0.9, and 1.2% (w/w). Before antimicrobial agents were added into film solution, sodium dehydroacetate dissolved in 20 g of H_2O and the rosemary extract dissolved in 20 g of H_2O with an overnight agitation. The obtained solutions were degassed in a vacuum desiccator. Film forming solution was poured on teflon-coated glass plates (24 cm × 13 cm) and dried at 50°C in an oven for at least 3 h. The films were carefully peeled from one side to another with tweezers. Then, the films were stored in polyethylene bags for further use.

Antimicrobial Activities

Culture Preparation. Four loops of *E. coli* from beef extract and peptone agar slant were taken and inoculated into 50 mL of sterile beef extract and peptone broth in a 200-mL flask. Four loops of *A. niger* from potato dextrose agar slant were taken and inoculated into 50 mL of sterile potato and dextrose broth in a 200-mL flask containing some sterile glass beads. The flask containing *E. coli* was then incubated in an HYG-IIa Incubator (Shanghai, China) at a shaking speed of 150 rpm at 37°C for 24 h and the flask containing *A. niger* at a shaking speed of 150 rpm at 28°C for 48 h.

Antimicrobial Test. The inhibitory zone test on solid medium was used to determine the antimicrobial effects of films on *E. coli* and *A. niger*. Biodegradable film was cut into discs (diameter = 6.0 mm) with a punch and a disc was placed carefully into each petri dish containing solid medium. These petri dishes had been previously seeded with 0.2 mL of tested bacteria. The concentrations of the *E. coli* and *A. niger* seeding culture were10^8–10^9 CFU/mL. The petri dishes with *E. coli* were incubated at 37°C for 24 h and those with *A. niger* at 28°C for 48 h. The diameter of the "zone of inhibition" around the film discs was measured in triplicate. The "zone of inhibition" was reported as the whole area subtracted from the film disc area. Each sample was conducted three times.

Film Thickness

The thickness of films was measured with a digital micrometer caliper (0–25 mm, 0.001 mm, Guanglu Digital Measurement and Control, Guilin, China) at five random locations on the film. Mean thickness values for each sample were calculated and used to measure tensile strength (TS) and water vapor permeability (WVP).

Mechanical Properties

Mechanical properties of the films were determined by tension tests, with a TA-XT2i texture analyzer (Stable Micro System, UK), according to ASTM D882-02[14] with some modifications. All of the tested film sheets, equilibrated at 23 ± 2°C and 53% relative humidity [$Mg(NO_3)_2$ saturated solution] for at least 48 h prior to test, were cut into strips (15 mm × 80 mm) with a sharp knife. The initial distance between the grips was 50 mm. The test speed was 1 mm/s. All measurements were replicated six times. The tensile strength (TS, MPa) and elongation at break (e_b, %) were calculated with the following equations[15]:

$$TS = L_P/a \times 10^{-6}\ \text{MPa} \tag{1}$$

In this equation, L_p was the peak load (N), and a was the cross-sectional area of samples (m^2).

$$e_b = \Delta L/l \times 100\% \tag{2}$$

In this equation, $\triangle L$ was the increase in length at breaking point (mm), and l was the original length (mm).

Water Vapor Permeability

Water vapor permeability (WWP) of the films was measured according to GB1037 with a PERME™ W3/030 Automatic Water vapor permeability Tester (Languang, Jinan, China). Film specimens were conditioned for 48 h in a desiccator at 23 ± 2°C and 53% relative humidity [$Mg(NO_3)_2$ saturated solution] before analysis. The testing area of each film was 33.00 cm^2. The test temperature and relative humidity were 38°C and 90%, respectively. The weighing interval was 120 min. Water vapor permeability of each sample was averaged from three separate tests.

Color

A CHROMA METER CR-400 (Konica Minolta Sensing) was used to determine the color changes of the films. Measurements were performed by placing the films on the standard white plate five times at different locations on each specimen. The L^* a^* b^*color system was used, where L^* was 0 for black and 100 for white, a^* values indicated red (+) to green (−), and b^* values indicated yellow (+) to blue (−).The colorimeter was calibrated with a standard white plate (L^* = 87.7, a^* = +0.3158, b^* = +0.3225). Total color difference ($\triangle E^*$) was calculated with the following equations:

$$\Delta L* = L*_{\text{sample}} - L*_{\text{standard}} \tag{3}$$

Table I. Antimicrobial Activity of Starch–Sodium Alginate Composite Films Incorporated with Sodium Dehydroacetate or Rosemary Extract

Antimicrobial agents	Concentration (%)	Inhibitory zone (mm^2)	
		Escherichia coli	*Aspergillus niger*
Sodium dehydroacetate	0.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
	0.10	5.07 ± 1.07^{d}	0.00 ± 0.00^{c}
	0.30	16.78 ± 8.10^{c}	542.24 ± 69.6^{b}
	0.50	32.85 ± 4.71^{b}	$789.78 \pm 83.8^{a,b}$
	0.70	46.21 ± 0.92^{a}	986.79 ± 49.90^{a}
Rosemary extract	0.00	0.00 ± 0.00^{e}	0.00 ± 0.00^{a}
	0.30	10.75 ± 3.08^{d}	0.00 ± 0.00^{a}
	0.60	47.12 ± 0.56^{c}	0.00 ± 0.00^{a}
	0.90	56.31 ± 7.94^{b}	0.00 ± 0.00^{a}
	1.20	74.77 ± 5.50^{a}	0.00 ± 0.00^{a}

[a–e]Different lowercase letters in the same column for the same antimicrobial agent indicated significant differences ($P < 0.05$). Data shown in mean ± standard deviation ($n = 3$).

$$\Delta a* = a*_{\text{sample}} - a*_{\text{standard}} \tag{4}$$

$$\Delta b* = b*_{\text{sample}} - b*_{\text{standard}} \tag{5}$$

$$\Delta E^* = [(\Delta L*)^2 + (\Delta a*) + (\Delta b*)^2]^{0.5} \tag{6}$$

Fourier Transform Infrared Spectra Analysis
The Fourier transform infrared (FTIR) spectra of the films were measured in a Thermo Fisher Scientific (USA) Nexus 670 spectrometer attached to a Smart iTR diamond ATR accessory in the wavelength range of 600–4000 cm^{-1}.The resolution was 4 cm^{-1}.

Scanning Electron Microscopy
The morphology of film surface was examined by a QUANTA FEG 250 scanning electron microscope (SEM) (FEI, USA) operated at 5.0 kV. Film samples were mounted on a bronze stub and sputter-coated with a layer of gold prior to taking image.

Statistical Analysis
SPSS 17.0 was used for the statistical analyses. Data were subjected to analysis of variance (ANOVA), and comparison of means was carried out by Duncan's multiple-range test ($P < 0.05$).

RESULTS AND DISCUSSIONS

Antimicrobial Assay
The antimicrobial activities of starch–sodium alginate composite films incorporated with sodium dehydroacetate or rosemary extract against *E. coli* and *A. niger* were shown in Table I and Figure 1. The control composite film did not show any inhibitory effect on *E. coli* nor on *A. niger*. In this study, the composite films incorporated with sodium dehydroacetate or rosemary extract had significantly antimicrobial effect on *E. coli* ($P < 0.05$). The inhibition zone increased with the increased concentration of sodium dehydroacetate or rosemary extract. These results showed that *E. coli* was sensitive to sodium dehydroacetate and rosemary extract. The release of sodium dehydroacetate or rosemary extract covering the composite film sheets could inhibit the microorganisms. When the concentration of sodium dehydroacetate was 0.1%, no inhibitory effect on *A. niger* was shown. However, significant inhibition on *A. niger* was observed ($P < 0.05$) when the concentration was increased to 0.3%. The inhibitory zone of *A. niger* increased with the increase of sodium dehydroacetate concentration. This may be caused by the release of sodium dehydroacetate. The higher concentration of sodium dehydroacetate, the more release. When the adding concentration was 0.1%, the release concentration was less than minimal inhibitory concentration. Therefore, no inhibitory zone was observed. When sodium dehydroacetate concentration was no less than 0.3%, the inhibitory zone on *A. niger* of the films was much larger than on *E. coli*. An explanation for this may be that sodium dehydroacetate had much better inhibitory effect on *A. niger* than on *E. coli*. The minimal inhibitory concentration of sodium dehydroacetate to inhibit *A. niger* was lower than *E. coli*.[16] The antimicrobial mechanism of sodium dehydroacetate was the disturbance of microbial cell and the ion entering into the cell, resulting in restrained microbial breathing.[17] The composite films incorporated with rosemary extract had no inhibitory effect on *A. niger*. Table I showed that the inhibitory effects of composite films incorporated with rosemary extract on *E. coli* and *A. niger* differed greatly. The difference was related to the sensitivity of *E. coli* and *A. niger* to rosemary extract. The differences in sensitivity may be caused by different cell structure or function. The phenolics were believed to be the major group of compounds responsible for the antimicrobial activities of most plant extracts.[18] The mechanism of the action for the antimicrobial activities of phenolic compounds present in herbaceous and woody plants had not been fully defined. However, previous studies had investigated the mechanism. The activities had been attributed to the inhibition of extracellular enzymes, the deprivation of the substrates required for growth, and the inhibition of oxidative phosphorylation or iron deprivation.[19]

Mechanical Properties
The tensile strength (TS) and elongation at break (e_b) of the starch–alginate composite films incorporated with sodium dehydroacetate and rosemary extract were summarized in

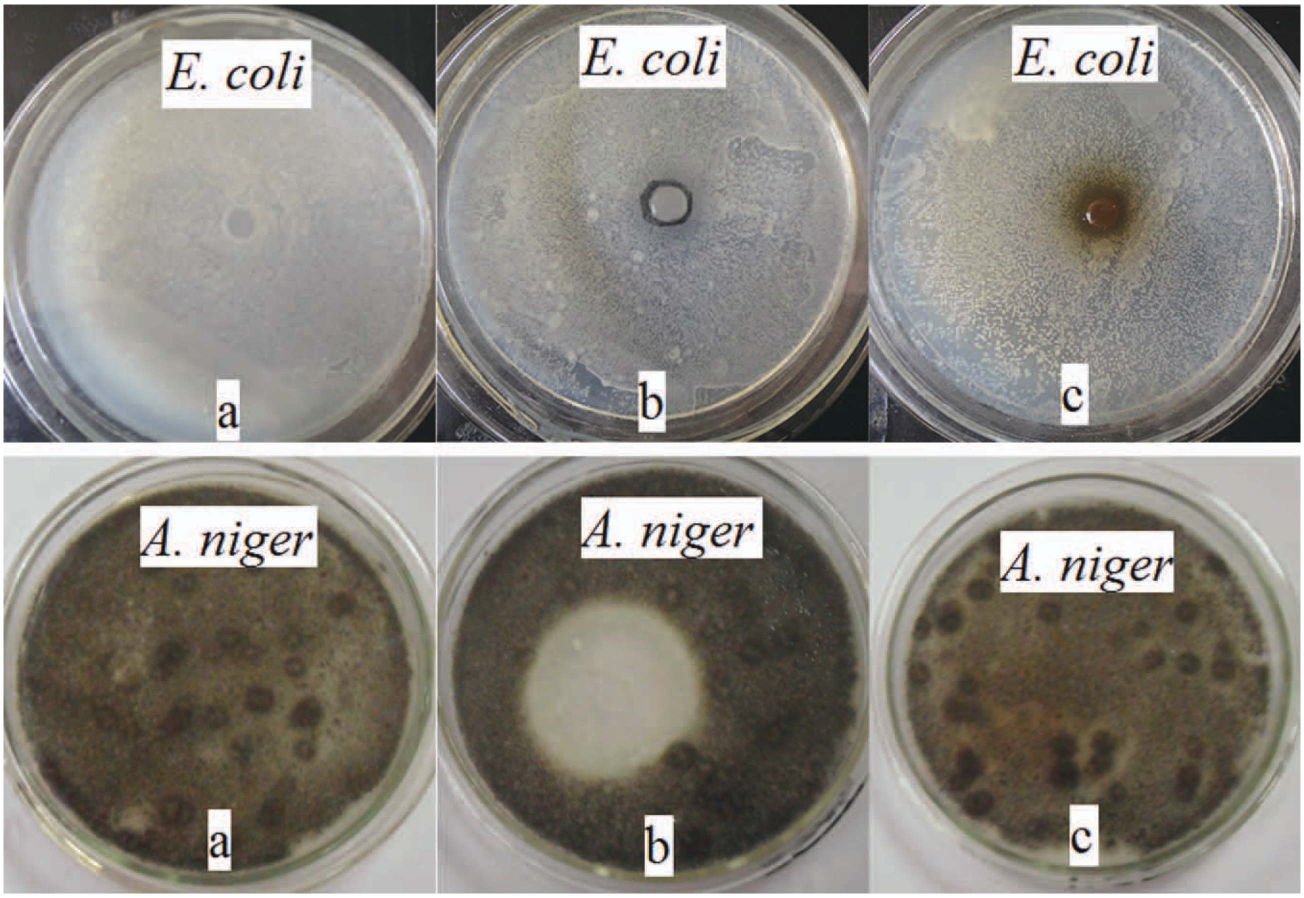

Figure 1. Representative pictures of inhibitory zones of starch-sodium alginate composite films without or with antimicrobial agent. (a, film without antimicrobial agent; b, films containing 0.7% sodium dehydroacetate; c, films containing 1.2% rosemary extract). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2. It can be observed that TS and e_b values of control films were higher than those of the films with sodium dehydroacetate or rosemary extract. TS and e_b of the composite films incorporated with sodium dehydroacetate exhibited similar trend with the addition of rosemary extract. Incorporation of sodium dehydroacetate or rosemary extract had negative effects on the mechanical properties of the composite films. A similar result that potassium sorbate could decrease the TS of tapioca-starch edible films was also reported.[20] Sodium dehydroacetate could easily be fit into starch chains and inhibit the bonding between molecules of polymers. A research found a similar result that all the tensile properties of the edible HPMC incorporated with kiam wood extract decreased significantly ($P < 0.05$).[21] This could be attributed to the incomplete dispersal of the kiam wood extract in the polymer matrix, which is caused by the incompatibility of kiam wood extract and HPMC biopolymer.

The values of TS and e_b decreased with the increase of sodium dehydroacetate or rosemary extract concentration. Except the e_b value of the films incorporated with rosemary extract at a concentration of 1.2%, the decreasing trend was significant ($P < 0.05$). The values of TS and e_b for the composite films incorporated with sodium dehydroacetate decreased from 3.02 to 0.66 MPa and from 52.92 to 33.62%, respectively when the sodium dehydroacetate increased from 0.1 to 0.7%. This maybe attributed to the interaction between sodium dehydroacetate and the polymers, resulting in a change of the starch network in the films. This also could be proved by FTIR in the later study. The values of TS and e_b for the composite films incorporated with rosemary extract were decreased from 2.34 to 1.33 MPa and from 52.90 to 43.47%, respectively when the rosemary extract increased from 0.3 to 1.2%. This was possibly due to the presence of rosemary extract as an additive. This might lead to the development of a heterogeneous film structure, resulting in the decrease in TS and e_b of the films. In addition, rosemary extract might weaken the strong intermolecular interaction between the polymers in composite films. The density of the intermolecular interaction decreased in the materials while the increase in the free volume between polymer chains resulted in the deterioration in mechanical properties.[22]

Water Vapor Permeability

The main function of biodegradable or edible films was often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product. Therefore, water vapor permeability should be as low as possible.[21]

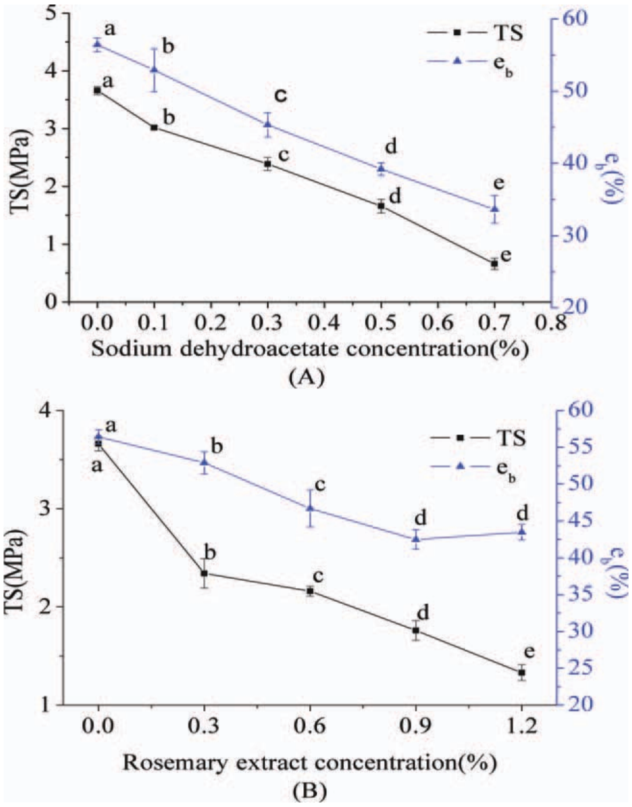

Figure 2. Tensile strength and elongation at break of starch–sodium alginate composite films as a function of sodium dehydroacetate (A) and rosemary extract (B) concentration (error bars were standard error of the mean of six measurements from separate films). Different lowercase letters in the same curve indicated significantly different groups determined by Duncan's test ($P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The effects of sodium dehydroacetate or rosemary extract on the water vapor permeability properties were shown in Figure 3. In this study, the water vapor permeability properties were affected by the incorporation of sodium dehydroacetate and rosemary extract. The water vapor permeability of the control films was lower than that of the composite films containing sodium dehydroacetate and rosemary extract. From Figure 3(A) we could observe the values of WVP markedly elevated with the increase of sodium dehydroacetate concentration ($P < 0.05$). When sodium dehydroacetate concentration increased from 0.1 to 0.7%, the WVP of composite films increased considerably from 2.41×10^{-12} to 2.92×10^{-12} g cm cm^{-2} s^{-1} Pa^{-1}.This behavior could be attributed to structural modifications of the starch network. The network became less dense, therefore, it is more permeable.[23,24]

In Figure 3(B), the WVP of composite films increased from 2.31×10^{-12} to 2.68×10^{-12} g cm cm^{-2} s^{-1} Pa^{-1} when the rosemary extract increased from 0.3 to 1.2%. The primary contents of rosemary extract were hydrophilic; therefore, adding rosemary extract introduced hydrophilic groups into the films. The increase of WVP values with the increase of rosemary extract concentration could be attributed to the introduction of hydrophilic groups when rosemary extract was added into films. Introducing hydrophillic additives, prone to adsorption and desorption of water molecules, was known to enhance the water vapor permeability of hydrocolloid-based films.[22,25]

Color

Color of the film may influence the consumer acceptability of a product.[26] The values of L^*, a^*, b^*, and $\triangle E^*$ were recorded in Table II. There were no obvious differences ($P < 0.05$) in lightness (expressed as the L^* value) among the composite films containing different concentration of sodium dehydroacetate. The values of a^* of the films decreased with the increase of sodium dehydroacetate concentration. The values of a^* of the composite films without and with 0.1% sodium dehydroacetate were not significantly different from a statistical view point ($P > 0.05$). However, the decrease of a^* of the films was significant ($P < 0.05$) when the concentration of sodium dehydroacetate increased from 0.3 to 0.7%. The value of b^* and $\triangle E^*$ increased with the increase of the sodium dehydroacetate concentration. These results indicated that the sodium dehydroacetate kept the composite films light greenish yellow.

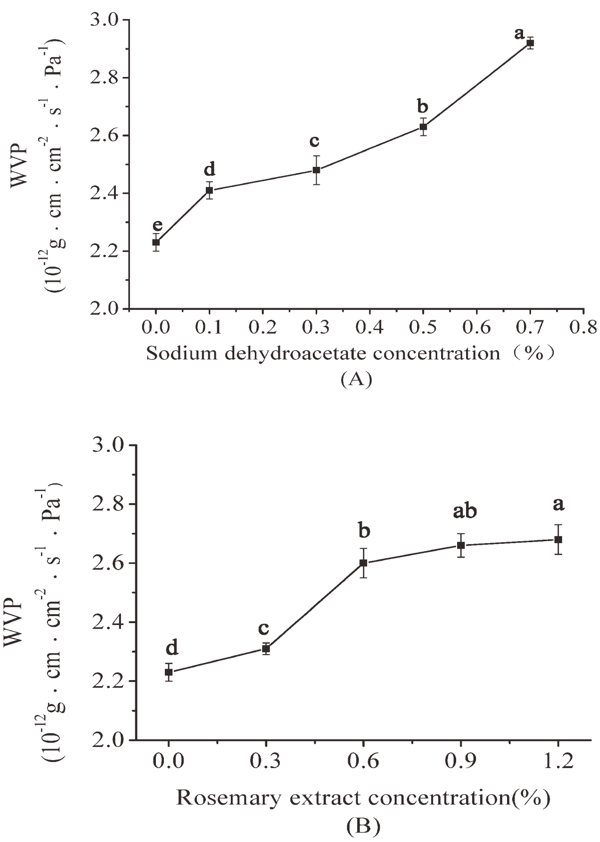

Figure 3. Water vapor permeability of starch–sodium alginate composite films as a function of sodium dehydroacetate (A) and rosemary extract (B) concentration (error bars were standard error of the mean of three measurements from separate films). Different lowercase letters in the same curve indicated significantly different groups determined by Duncan's test ($P < 0.05$).

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Table II. Color of Starch–Sodium Alginate Composite Films as a Function of Sodium Dehydroacetate or Rosemary Extract Concentration

Antimicrobial agents	Concentration (%)	L^*	a^*	b^*	ΔE^*
Sodium dehydroacetate	0.00	95.45 ± 0.34^a	-0.22 ± 0.013^a	3.08 ± 0.24^d	8.25 ± 0.24^c
	0.10	95.44 ± 0.29^a	-0.33 ± 0.022^a	3.23 ± 0.077^d	8.28 ± 0.27^b
	0.30	95.44 ± 0.23^a	-0.69 ± 0.19^b	4.02 ± 0.54^c	8.65 ± 0.27^b
	0.50	95.39 ± 0.11^a	-1.31 ± 0.089^c	5.17 ± 0.20^b	9.23 ± 0.058^a
	0.70	95.25 ± 0.025^a	-1.61 ± 0.24^d	5.80 ± 0.59^a	9.53 ± 0.41^a
Rosemary extract	0.00	95.45 ± 0.34^a	-0.22 ± 0.013^c	3.08 ± 0.24^d	8.25 ± 0.235^d
	0.30	88.17 ± 2.25^b	1.07 ± 0.10^b	23.37 ± 4.32^c	23.36 ± 4.25^c
	0.60	81.34 ± 0.63^c	1.52 ± 0.20^b	37.53 ± 0.76^b	37.8 ± 0.85^b
	0.90	79.48 ± 1.97^c	2.32 ± 0.90^a	40.01 ± 2.27^b	40.57 ± 2.62^b
	1.20	73.57 ± 1.99^d	2.83 ± 0.91^a	46.93 ± 1.96^a	48.76 ± 2.47^a

[a–e]Different lowercase letters in the same column for the same antimicrobial agent indicated significant differences ($P < 0.05$). Data shown in mean ± standard deviation ($n = 5$).

The addition of rosemary extract influenced the color of the films (Table II). The composite films without rosemary extract looked clear and transparent. The darkness of films incorporated with rosemary extract were significantly ($P < 0.05$) increased as indicated by the lower L^* values compared with control film. The results demonstrated that a^* and b^* values increased as the content of rosemary extract increased (Table II). The rosemary extract changed from light brown to a reddish-yellow. The greater difference in $a^*$and b^*values of the films with rosemary extract was possibly due to the phenolics compounds that contributed to the reddish and yellowish colors.

Fourier Transform Infrared Spectra Analysis

FTIR spectroscopy was used to examine an interaction between starch and sodium dehydroacetae or rosemary extract. FTIR spectra of composite films incorporated with different levels of sodium dehydroacetae or rosemary extract were shown in Figure 4. The broad absorption band at 3304–3355 cm^{-1} and the peaks at 2928–2932 cm^{-1} were assigned to the stretching of —OH groups and C—H stretching in starch. The band 1600–1652 cm^{-1} was due to the C=O bond stretching (amide I).The peaks were attributable to C—H bending in starch in the ranges of 1410–1422cm^{-1}. The peaks observed at 1078–1081 and 1023–1030 cm^{-1} were characteristics of C—O—H and C—O—C stretchings. In Figure 4, the changes of the absorption band at 3304–3355 cm^{-1} were obvious between the films with and without antimicrobial agents. The peak was sharper with the increase of antimicrobial agents concentration. The broad band caused by —OH groups appeared at 3304–3355 cm^{-1}, which may be attributed to the complex vibrational stretches associated with free, inter- and intramolecular bound hydroxyl groups that made up the gross structure of starch.[27] Therefore, the change of the peak indicated an increase of free —OH groups. Some researchers contributed it to the dissociation of starch molecules which was consistent with the decrease of starch crystallinity in films.[28] They concluded that the addition of antimicrobial agents disturbed the crystalline of starch and the crystallinity of starch was the vital factor to determine the mechanical and permeable properties of starch films. The similar results of mechanical and permeable properties of starch films in our study could also be explained by the conclusion. In Figure 4(A), the double peaks appeared at 1600–1652 cm^{-1} when the addition of sodium dehydroacetate was no less than 0.3%, indicating that vibrational coupling appeared between the C=O group of starch and sodium dehydroacetate. The peak at 1410–1422

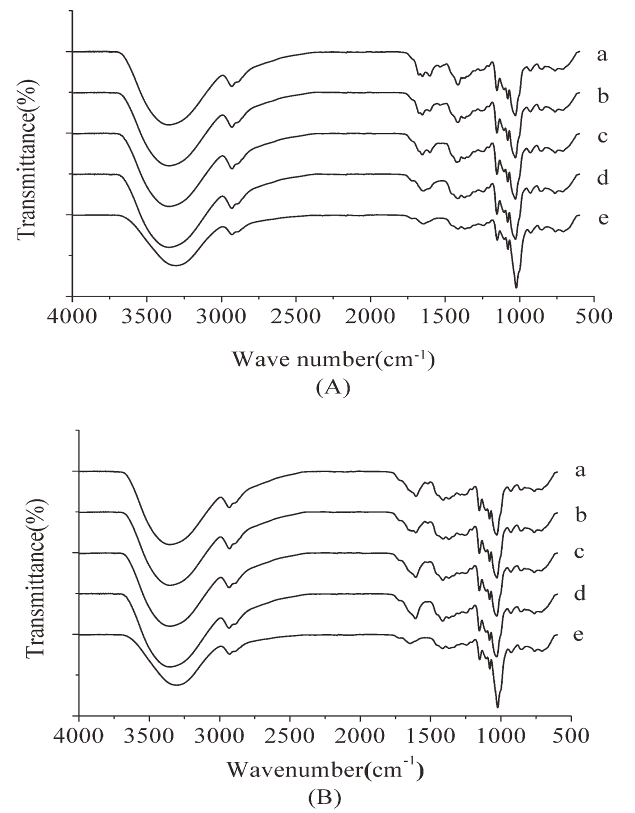

Figure 4. FTIR spectra of starch–sodium alginate composite films incorporated with different concentration of sodium dehydroacetate (A) and rosemary extract (B). (A): a- 0.7% sodium dehydroacetate; b- 0.5% sodium dehydroacetate; c- 0.3% sodium dehydroacetate; d- 0.1% sodium dehydroacetate and e- 0.0% sodium dehydroacetate. (B): a- 1.2% rosemary extract; b- 0.9% rosemary extract; c- 0.6% rosemary extract; d- 0.3% rosemary extract and e- 0.0% rosemary extract.

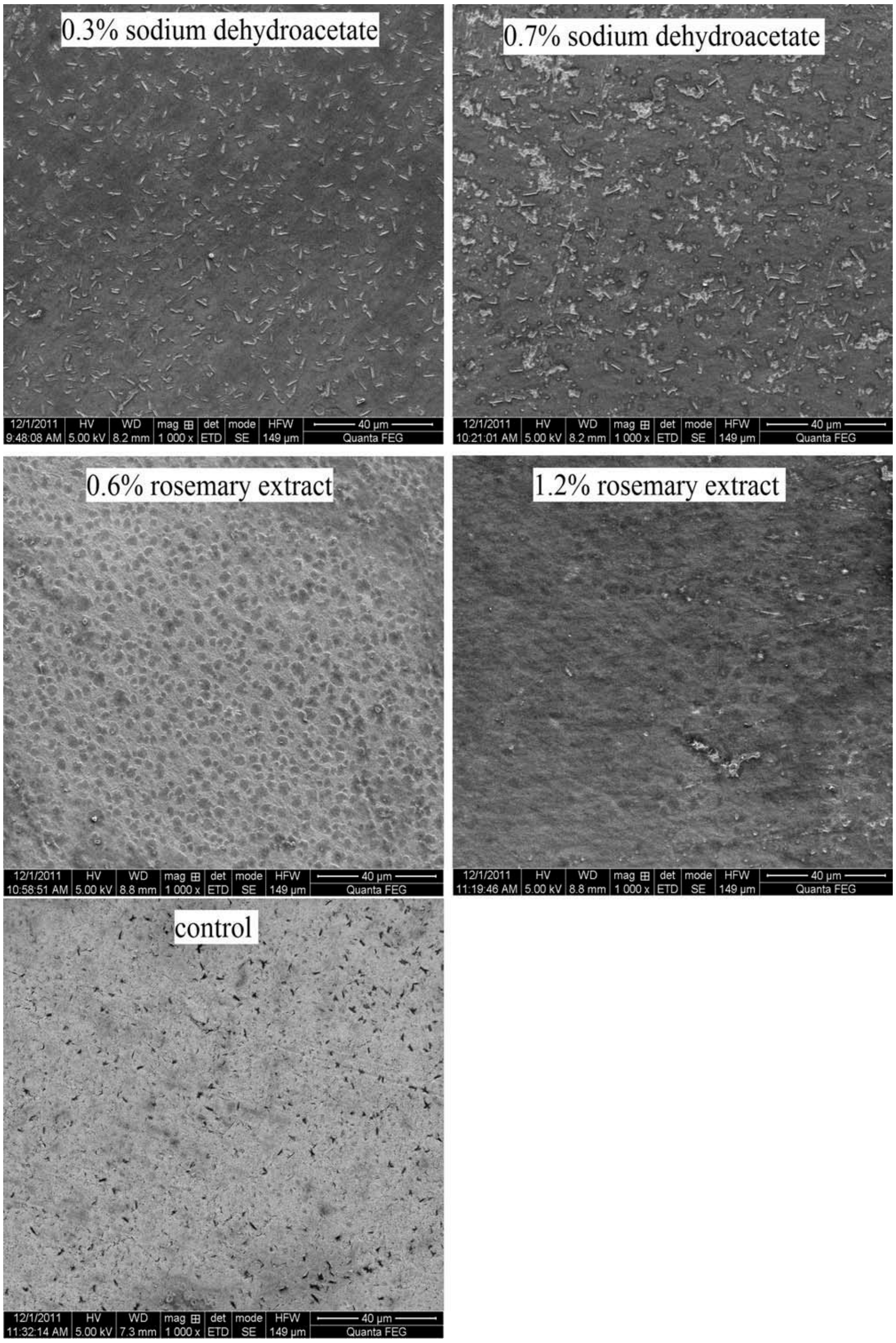

Figure 5. Scanning electron micrograph of starch–sodium alginate composite films incorporated with different concentration of sodium dehydroacetate or rosemary extract.

cm^{-1} shifted to higher wave numbers with the increase of sodium dehydroacetate concentration and to lower wave numbers with the increase of rosemary extract concentration. The shifting of C—O—H and C—O—C bands of the films containing antimicrobial agent to higher wave numbers indicated that the interactions between the various functional groups were weaker than those in the control films. Weaker interactions of the functional groups were also reflected on the tensile strength data.

Morphology of the Films

The morphology of the antimicrobial starch–sodium alginate composite films incorporated with sodium dehydroacetate or rosemary extract were investigated by a scanning electron microscopy (SEM). Figure 5 showed the surface of antimicrobial starch–sodium alginate composite films without and with different concentration sodium dehydroacetate or rosemary extract. The addition of antimicrobial agents made the films rougher. The film surface became rougher with the increase of antimicrobial agents concentration. The distribution of antimicrobial agents in the antimicrobial films as affected by the sodium dehydroacetate or rosemary extract content were compared. The film containing 0.3% sodium dehydroacetate was homogeneous; however, when then concentration increased to 0.7% some sodium dehydroacetate formed small aggregates. This also could be observed in the films containing 1.2% rosemary extract.

CONCLUSIONS

Antimicrobial films have been successfully prepared with oxidized and acetylated corn starch–sodium alginate incorporated with different levels of sodium dehydroacetate or rosemary extract. Films incorporated with sodium dehydroacetate had an anti-*Escherichia coli* effect. The inhibitory zone increased significantly ($P < 0.05$) with the increase of sodium dehydroacetate concentration. *Aspergillus niger* could be effectively suppressed when the concentration of sodium dehydroacetate was not less than 0.3%. Incorporation of sodium dehydroactate deteriorated the mechanical property of the films and elevated water vapor permeability. Sodium dehydroacetate made the films light yellowish green. Films incorporated with rosemary extract had great anti-*Escherichia coli* effect but no inhibitory effect on *Aspergillus niger*. Incorporation of rosemary extract lowered the mechanical property of the films and enhanced water vapor permeability. The color of the films became darker and more yellow-reddish as rosemary extract was increased. Fourier Transform Infrared (FTIR) spectra analysis indicated that sodium dehydroacetate and rosemary extract reduced starch crystallinity, resulting in the deterioration of the mechanical and barrier properties. Sodium dehydroacetate and rosemary extract incorporated in starch–sodium alginate composite films provided the films with a rougher surface than pure starch–sodium alginate composite films did. Our results indicated that antimicrobial films incorporated with sodium dehydroacetate was a good option to prolong the shelf life of food for its antimicrobial property although the mechanical and barrier properties need to be improved.

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