

## Physical Properties and Antimicrobial Activity of Chilled Meat Pads Containing Sodium Carboxymethyl Cellulose

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**ABSTRACT:** An improved chilled meat pad consisting of nonwoven fabric containing essential oils or active powders, coated by a sodium carboxymethylcellulose (CMCNa) layer was prepared successfully. In the formation of CMCNa film, effects of four acids and pH were examined to obtain optimal results in terms of water, saline, and simulative blood sorption. Fourier Transform Infrared spectroscopy, scanning electron microscopy, and differential scanning calorimetry were used to evaluate the CMCNa film. Also, antimicrobial activities were examined in relation to water loss, aerobic bacterial count, and pH. When the pH value was 4.6 adjusted by citric acid, CMCNa film had the largest absorbent capacity. In the first 6 days, pads with essential oils were superior to pads with powders but inhibiting effect decreased with essential oil releasing. The pad with clove powder gained the best inhibiting effect and could extend the shelf life of chilled meat to 10 days. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 000: 000–000, 2012

**KEYWORDS:** CMC film; crosslinking; swelling; water sorption; essential oil; essential powder

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### INTRODUCTION

Over the years, a great deal has been learned about microbial spoilage of meats and its control.<sup>1–3</sup> Various antimicrobial agents, such as organic acids and essential oils, have been shown to control spoilage.<sup>4,5</sup> When fresh meats are packaged in retail plastic trays, dripping of product juices occurs making such packages unattractive to consumers. Edible coatings could hold in juices, prevent dripping, and enhance product presentation.<sup>6</sup> In addition, edible coatings prepared from polysaccharides, proteins, and lipids have already been proposed as carriers for various antimicrobial substances.<sup>7,8</sup>

As microbial contamination of meat and meat products occur primarily at the surface, attempts have been made to delay spoilage by use of antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods have limited benefits because the active substances are neutralized on contact or diffuse rapidly from the surface into the food mass.<sup>9,10</sup> The use of packaging films containing antimicrobial agents could prove more efficient, by slowing the migration of the agents away from the surface, thus helping maintain high concentrations where they are needed.<sup>11</sup> For example, Ouattara et al.<sup>12,13</sup> prepared antimicrobial films by inclusion of various organic acids and essential oils into a chitosan matrix and investigated the ability of these

films to inhibit the growth of indigenous or inoculated bacteria onto the surfaces of vacuum-packed cured meat products.

CMC is anionic, cold, and hot water soluble cellulose ether, usually available in its sodium salt form.<sup>14</sup> CMC has excellent film forming properties, whose films are tough, flexible, totally transparent, and highly sensible to water presence but resistant to fats and oils.<sup>15,16</sup> Crosslinking treatments can be used to decrease the water solubility of cellulose ethers.<sup>17</sup> CMC of a degree of substitution 0.7 is usually used in such applications.<sup>18</sup> Now coating formulations for fresh fruits and vegetables containing CMC and sucrose fatty acid esters have been commercialized.<sup>19</sup> Moreira et al.<sup>20</sup> analyzed the effect of a CMC coating during drying process on the quality of butternut squash slices and observed a slight improvement in weight loss and ascorbic acid retention in comparison with control samples without any coating. Wambura et al.<sup>21</sup> analyzed the effects of a CMC-based edible coating containing rosemary and tea extracts on the reduction of lipid oxidative rancidity. Reduction in oxidation of 66.1% and 10.4% was observed for samples roasted and coated with CMC films formulated with extracts of rosemary and tea, respectively, when compared with uncoated sample.

However, inclusion of CMC in edible meat coating compositions has not been extensively studied. Funk applied a coating

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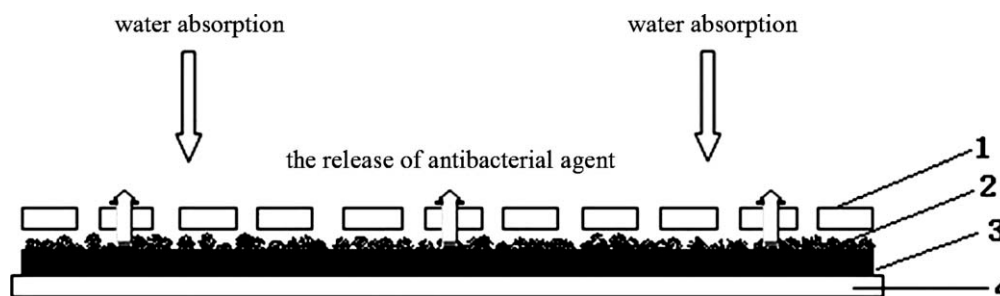


Figure 1. Microstructure of an antimicrobial pad with superabsorbent.

of an aqueous solution of the hydrogen form of CMC on breaded chicken parts before skillet-frying and on chicken parts before roasting. In both cases, no significant differences were detected between coated and uncoated samples in terms of cooking losses, moisture and fat contents, and sensory scores.<sup>22</sup> A study was therefore undertaken to investigate the feasibility of a chilled meat pad consisting of organic acids, essential oils or powders, and sodium carboxymethylcellulose (CMCNa) film for the preservation of meat products.

## MATERIALS AND METHODS

### Preparation of CMCNa Film with an Ester Crosslinking Method

The films were obtained by the casting technique: Preparation of CMCNa film was done by dispersing CMCNa powder with average M.W. 250,000 (Ruqen Co, USA) in water, stirring at a high speed until completely soluble to a concentration of 2.5%. Then, add edible acetic acid, citric acid, lactic acid, and malic acid to adjust CMC sols of different pH values.<sup>23</sup> Then 150 g of sol was dispensed on the surface of Petri dishes (9 cm of diameter) and dried in a controlled temperature chamber at 70°C. After the constitution of the film, it was separated from the dish to obtain stand-alone films. All the samples were conditioned inside desiccators.

### Determination of CMCNa Film Sorption

About 0.1 g CMCNa films were dipped into distilled water at 25°C for 24 h to ensure the complete swelling of CMCNa films, and then filtered with sieve cloth 100 meshes to remove absorbed water before measurement. Water sorption  $Q_{\text{water}}$  was calculated with the following equation:  $Q_{\text{water}} = (M_a - M_b)/M_b$ , where  $M_a$  was the equilibrium weight of films, and  $M_b$  was the weight of dried films.

In the case of main conditions unchanged, we used normal saline and simulative blood instead of distilled water to the determination of saline sorption and simulative blood sorption. The composition of simulative blood were deionized water 88.14%, glycerol 10.00%, NaCl 1.00%,  $\text{Na}_2\text{CO}_3$  0.40%, and CMC 0.46%. Saline sorption  $Q_{\text{salt}} = (M_c - M_d)/M_d$ , where  $M_c$  was the equilibrium weight of films in normal saline, and  $M_d$  was the weight of dried films. Simulative blood sorption  $Q_{\text{blood}} = (M_e - M_f)/M_f$ , where  $M_e$  was the equilibrium weight of films in simulative blood, and  $M_f$  was the weight of dried films.

### Physical Characterization of CMC Films

**Fourier Transform Infrared Spectroscopy.** Samples were prepared by cutting the film into pieces and grinding the pieces with potassium bromide and laminating. The IR spectra were recorded

at room temperature with a Nicolet Nexus 470 Fourier Transform Infrared (FTIR) spectrometer at a resolution of  $4 \text{ cm}^{-1}$  in the range  $400\text{--}4000 \text{ cm}^{-1}$ .

**Scanning Electron Microscopy.** Film samples were ground into powder, fixed onto metallic sample holders, and coated with gold in  $0.1\tau$  vacuum degree. The cross-section morphologies were observed with magnifications of  $500\times$  on a Hitachi X-650 scanning electron microscopy (SEM).

**Differential Scanning Calorimetry.** The thermal properties of the film samples with the weight about 6 mg were performed by a DSC-204-F1 (NETZSCH, Germany) under a nitrogen atmosphere with a flow capacity of  $20 \text{ mL min}^{-1}$  from 50°C to 400°C at a heating rate of  $10^\circ\text{C min}^{-1}$ .

### Preparation of Antimicrobial Pad with Super Absorbent

Four antimicrobial pads with superabsorbent were prepared by adding clove powder, cinnamon powder, clove oil, and cinnamon oil, respectively. The release paper, absorbent films, essential oil or active powders, and a collagen-bonded nonwoven fabric (Shanghai Pengwei Packing Material Co., China) were put in order as Figure 1 to prepare a preservative pad with length and width  $15 \text{ cm} \times 15 \text{ cm}$ . A total of 1.8 g essential powders were spread evenly over the film; 18 mg cinnamon oil and 189 mg clove oil dissolved in ethanol (1: 10 v/v) were dropped slowly on the nonwoven fabric, respectively. Finally, nonwoven fabric was fixed to the release paper. Cinnamon oil and clove oil extraction rates were 1%–2% and 14%–21% under normal circumstances.

### Preparation of Meat Samples

The meat was purchased at a local market. In sterile operating conditions, the slices of 100 g meat ( $3 \times 3 \text{ cm}^2$ ) were cut and placed onto antimicrobial pads (three pieces in each pad). The liquid in excess was eliminated. Then, the pads were wrapped with polyvinyl chloride film and stored at 4°C for the subsequent antimicrobial experiments.<sup>24</sup> The pieces without antimicrobial agents were used as control systems.

### Determination of Antimicrobial Activities

Antimicrobial activities of pads were evaluated by means of the analysis of the development of microbial flora, pH, and water loss. All the assays were performed in triplicate.

The meat surface tissue sections of  $3 \text{ cm}^2$  were swabbed with sterile peptone water and the microbial load was resuspended in 4 mL of peptone water. The serial dilutions from the meat surface tissue were prepared in peptone water. Total viable aerobic bacterial counts were determined by the pour plate method, using plate count agar (PCA, Merk, Darmstadt, Germany). The inoculated

**Table I.** Effects of Edible Acids and pH Values for CMCNa Sol on Water Absorbency, Normal Saline Absorbency, and Simulative Blood Absorbency of CMCNa Film

	Acid	pH					
		5.4	5.1	4.8	4.6	4.4	4.2
$Q_{\text{salt}}$	Acetic acid	72.9 ± 0.05	107.5 ± 0.55	104.9 ± 0.39	136.4 ± 0.24	83.9 ± 0.37	95.1 ± 0.15
	Citric acid	161.58 ± 0.12	137.33 ± 0.34	116.08 ± 0.09	122.67 ± 0.39	37 ± 0.23	4.83 ± 0.22
	Lactic acid	116.5 ± 0.05	113.5 ± 0.76	112.5 ± 0.84	27.4 ± 0.12	35 ± 0.10	10.7 ± 0.35
	Malic acid	118.7 ± 0.14	104.1 ± 0.38	61.6 ± 0.91	27 ± 0.18	18 ± 0.32	4 ± 0.33
$Q_{\text{blood}}$	Acetic acid	80.7 ± 0.32	73.5 ± 0.33	50.8 ± 0.49	59.3 ± 0.20	50.3 ± 0.34	72.1 ± 0.23
	Citric acid	66.42 ± 0.45	71.58 ± 0.89	76 ± 0.59	115.25 ± 0.42	77.83 ± 0.61	70.17 ± 0.64
	Lactic acid	69.5 ± 0.29	61.4 ± 0.03	49.6 ± 0.38	73.4 ± 0.91	58.8 ± 0.93	32.9 ± 0.28
	Malic acid	66.9 ± 0.71	91.2 ± 0.73	62.8 ± 0.19	68.8 ± 0.39	75.8 ± 0.71	49.1 ± 0.29
$Q_{\text{water}}$	Acetic acid	-	-	-	0	0	0
	Citric acid	-	-	-	144.08 ± 0.33	63.67 ± 0.37	8.92 ± 0.86
	Lactic acid	-	-	-	209.8 ± 0.74	183 ± 0.98	152.6 ± 0.57
	Malic acid	-	-	-	68.8 ± 0.29	75.8 ± 0.22	49.1 ± 0.39

Data, followed by their standard deviations, are means of three experiments. Treatment means were separated using the Student's *t* test ( $P > 0.05$ ).

plates were incubated at 37°C for 2 days for total viable counts. All counts were expressed as log<sub>10</sub> cfu g<sup>-1</sup>.<sup>25</sup> The evolution of pH and water loss in the meat tissue was determined throughout storage to evaluate global quality of meat samples. The pH was measured with a 6 mm diameter penetration glass electrode connected to a pH meter Solution Analyzer 5800-05 (Cole-Parmer Instrument Co., Vernon Hills, IL). Water loss was evaluated gravimetrically.

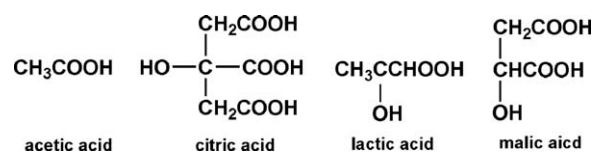
## RESULTS AND DISCUSSION

### Effects of Edible Acids on CMCNa Films Absorbent Capacity

When CMCNa was dissolved in water, its associative structure turned to swell and chains turned to stretch. Through a thorough stretch, displacement, and transfer, polymer chains formed an ordered and oriented structure to increase tightness and crystallinity of films. In the heating process, polymer chains might make a crosslinking reaction to form a three-dimensional network structure between —COOH and —OH. In the experiment when the pH value of CMCNa sol was 5.7, the absorbent capacities of normal saline and simulative blood were 118.33 and 64.83, respectively, but films were completely dissolved in distilled water. From Table 1, we saw that films of different pH values treated with acetic acid swelled and dissolved in distilled water, but films of pH 4.6–4.2 treated with three other kinds of acids just swelled, maintaining a modal integrity in water. Bégin and Van Calsteren<sup>26</sup> reported that films made from acetic acids were hard and brittle, whereas those from lactic and citric acids were soft and could be stretched.<sup>8</sup> When CMCNa sol was obtained at pH 4.6 with lactic acid, water absorbency of the film reached up to 209.8.

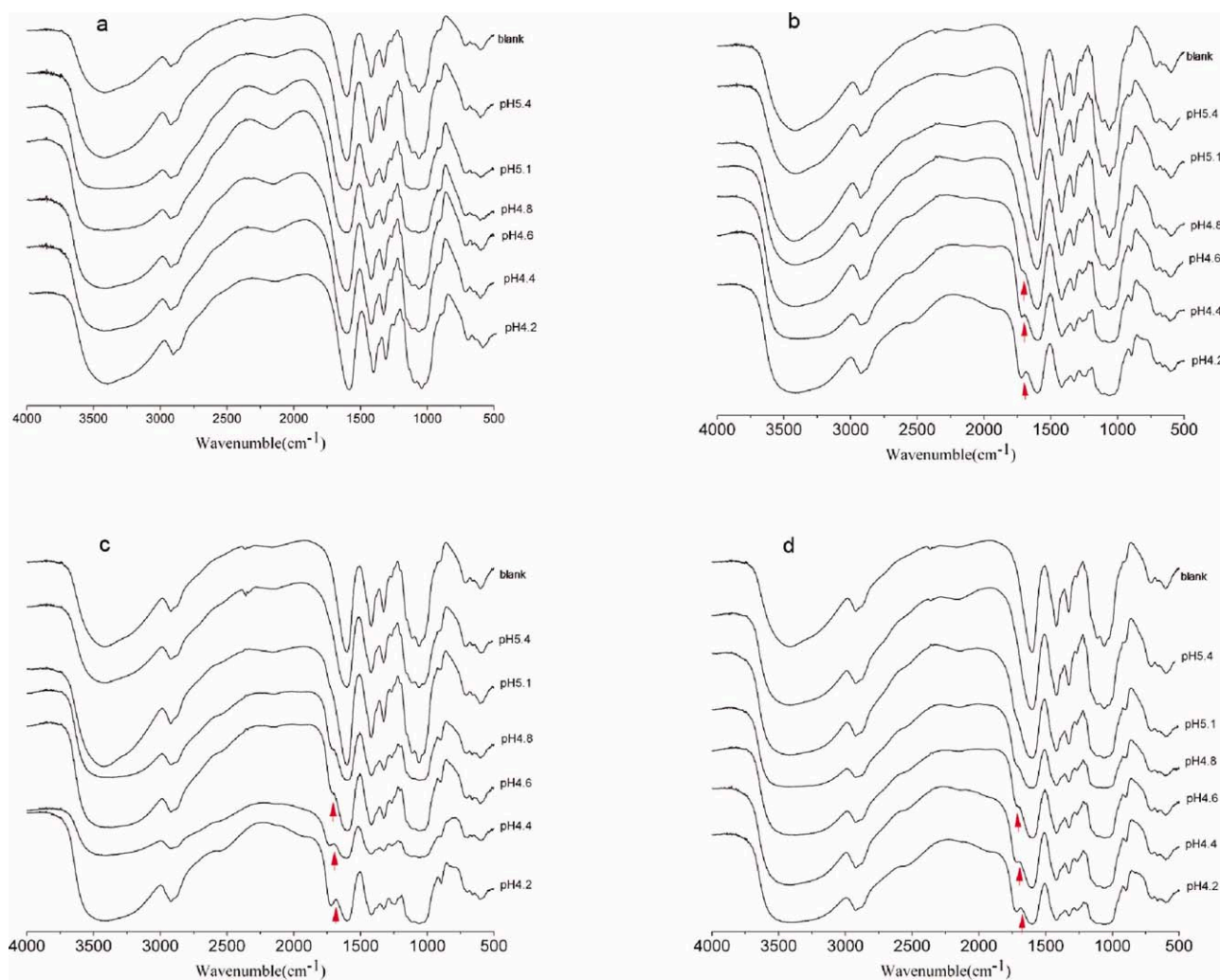
So, a conclusion could be led to that the conformation of films in distilled water was closely related to pH values of CMCNa sol and types of edible acids. The ionized form occurred more likely as  $pK_a$  of carboxylic groups in polysaccharides was about 3.2–3.4. Below  $pK_a$  values, carboxylic acid groups were in the form of COOH. As pH of the solution increased above  $pK_a$  value, COOH became ionized COO<sup>-</sup>, and the resulting electrostatic repulsion caused films to swell.<sup>27,28</sup> It might indicate that these films exhib-

ited pH-responsive character below 7.<sup>29</sup> In acid medium, gel structure was devoid of charge, which made it extremely compact, as demonstrated for the CMC polysaccharide in the free form,<sup>27</sup> because of the presence of several hydrogen bonds. Hydrogen bonds in CMC hydrogels affected pH-dependent water sorption. Barbucci et al.<sup>30</sup> observed that an increase occurred in water sorption, together with pH increasing for two CMC hydrogels with 25% and 50% crosslinking. Except acetic acid, the other three acids were  $\alpha$ -hydroxy acids. In the film-forming process, highly active hydroxyl groups on the  $\alpha$ -carbon atom were also involved in a crosslinking reaction, which made a more compact three-dimensional network structure.



In the experiment to determine saline sorption, films of pH 5.4–4.6 with citric acid were the best, for absorbent values of four samples were more than 100 (Table 1), while three acetic acid samples of pH 5.1–4.6, three lactic acid samples of pH 5.4–4.8, and two malic acid samples of pH 5.4–5.1 had similar results. When pH was adjusted to 5.4 with citric acid, films of saline sorption reached up to 161.58. Compared with saline sorption, simulative blood sorption was relatively low. Films acidulated by citric acid had the best result. When pH was adjusted to 4.6 with citric acid, film samples of simulative blood sorption reached up to 115.25. Citric acid had been reported to be more inhibitive and effective depends on its pH and concentration.<sup>31</sup>

CMC network imbibed a significant amount of solution also as a result of osmotic pressure developed inside the ionized gel to correspond with Donnan equilibrium theory.<sup>32</sup> CMC film was very sensitive to the presence of even a small amount of salt. It



**Figure 2.** FTIR spectra of CMCNa films after (a) acetate acid, (b) citric acid, (c) lactic acid, and (d) malic acid treatment. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

could be explained by the fact that CMC was a polyelectrolyte, so its hydrogel maintained this feature. The degree of swelling decreased continuously with the increasing ionic strength of the solution.<sup>33</sup> As the ionic strength of the medium increased, the amount of the sodium salt of the carboxylate groups increased. This state of affairs caused the hydrogels to shrink resulting in the release of water.<sup>29</sup> Chang<sup>34</sup> reported that the swelling ratios decreased quickly in physical saline water and in synthetic urine. The charge screening effect caused by cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$ ) in physical saline water and synthetic urine could induce a clear decline of anion–anion electrostatic repulsions, leading to a decrease of the osmotic pressure between hydrogel network and the external solution.<sup>35</sup>

According to all characteristics of sorption, we could get the best film by regulating the pH of CMCNa sol to 4.6 with citric acid. Because water, sorption, saline sorption and simulative sorption were all beyond 115, which met requirements of super absorbent films.

### Structure Characterization

**FTIR Analysis.** FTIR was of importance in the study of the molecular structure. From Figure 2, we could see that acid-

treated CMC films had the same chemical bonds with unmodified ones basically. The absorption band at  $3437.97\text{ cm}^{-1}$  was assigned to the stretching of  $-\text{OH}$ ;  $2923.87\text{ cm}^{-1}$  were the stretching of  $-\text{CH}_3$  and  $-\text{CH}_2$ ;  $1603.80\text{ cm}^{-1}$  and  $1420.79\text{ cm}^{-1}$  belonged to asymmetric and symmetric stretching of  $-\text{C}=\text{O}$ , respectively;  $1327.01\text{ cm}^{-1}$  was the bending of  $\text{C}-\text{O}$ ;  $1059.65\text{ cm}^{-1}$  was the stretching of  $\text{C}-\text{O}-\text{C}$ ;  $601.74\text{ cm}^{-1}$  was the bending of  $-\text{OH}$ .

From Figure 2(a), we saw that no new peaks occurred in the spectrum of films treated by acetic acid, which indicated that acetic acid had almost no contribution to a crosslinking reaction and verified a reason why films completely dissolved in water. However, in Figure 2(b–d), peak intensity or position changed obviously. After citric acid, lactic acid, and malic acid treatment, peaks of  $-\text{OH}$  at  $3437.97\text{ cm}^{-1}$  broadened due to the large number of hydrogen bonds involving  $-\text{OH}$  and  $-\text{C}=\text{O}$  groups with the reduction of pH values. In addition, a new absorption peak emerged at  $1720\text{ cm}^{-1}$  (around  $1603.80\text{ cm}^{-1}$ ) with pH values lower than 4.8. Tomihata and Ikada reported that the most prominent difference in the spectrum

between the noncrosslinked and the crosslinked hyaluronic acid film was noticeable at a wavenumber of  $1700\text{ cm}^{-1}$ , which was assigned to the carbonyl group most likely of ester bond. Intermolecular formation of ester bonds between the hydroxyl and carboxyl groups belonging to different polysaccharide molecules led to crosslinking.<sup>36</sup> This phenomenon proved again that the variety of edible acids effected on absorbent capacity of CMC films and was consistent with results that films of pH 4.6–4.2 treated with three  $\alpha$ -hydroxy acids just swelled, maintaining a modal integrity in water (Table 1). The reason could be that highly active hydroxyl groups on the  $\alpha$ -carbon atom were also involved in a crosslinking reaction.

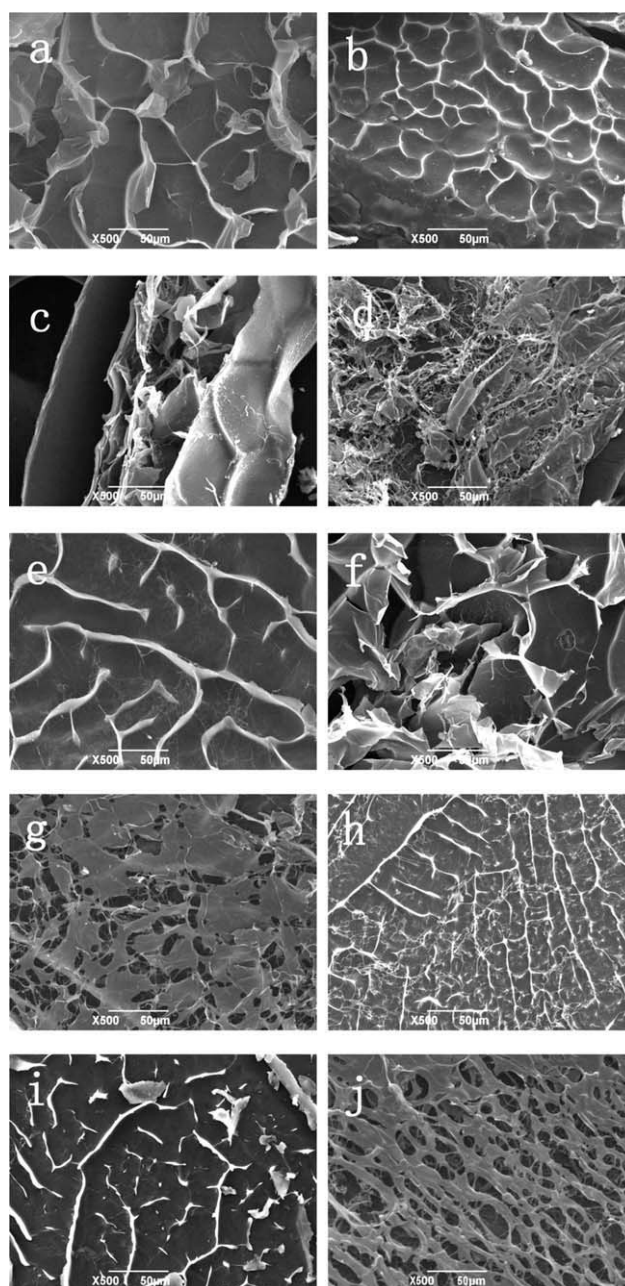
**SEM Analysis.** Figure 3 showed the SEM images of the cross-section of the CMC film samples. The cross-sections of the samples exhibited macropores architecture. a: was the blank without acid treatment; b–d: belonged to films of pH 4.6, 4.4, and 4.2 treated by citric acid, respectively; e–g: were films of pH 4.6, 4.4, and 4.2 treated by lactic acid; h–j: were films of pH 4.6, 4.4, and 4.2 treated by malic acid.

From Figure 3, we saw that films without acidification were with loose structures, large pores, and thin walls, which were completely dissolved after swelling. However, after acid treatment, their surface morphology underwent an obvious change, whose wall was thickening, and whose pore was reducing, due to strength of hydrogen bonds and crosslinking degree increasing between CMC chains. It was also confirmed by infrared spectra. When pH was adjusted to 4.6 by three  $\alpha$ -hydroxy acids, wall thickness and cross-linking degree of films reached a maximum, with a most regular network structure.

In addition, a clear layered structure was appearing in Figure 3(e,h). The reason could be that the electrostatic repulsions caused by the ionic character of the carboxylate anions  $\text{COO}^-$  in CMC had enlarged the space in the networks of hydrogels. The numerous water molecules could easily diffuse into hydrogels to form pores, leading to the higher swelling ratio.<sup>34</sup> After films swelling, the expansion of the layered structure might increase distances between layers, so water could be well absorbed and stored in the structure, which just explained the phenomenon when CMC hydrogels was obtained at pH 4.6 with lactic acid, water absorbency of the film reached up to a maximum (209.8).

However, with the pH value decreasing, crosslinking degree between intermolecular reduced, resulting in the decline of water absorbency. Because excessive acid would make degradation of CMC and reduction of molecular weight, and inhibit a crosslinking reaction. Krochta and Nisperos-Carriedo<sup>37</sup> reported the barrier and mechanical properties of cellulose-based films were dependent on the molecular weight of cellulose, higher the molecular weight, better is the properties. So, we speculated that CMC films treated with each acid had a different optimum pH.

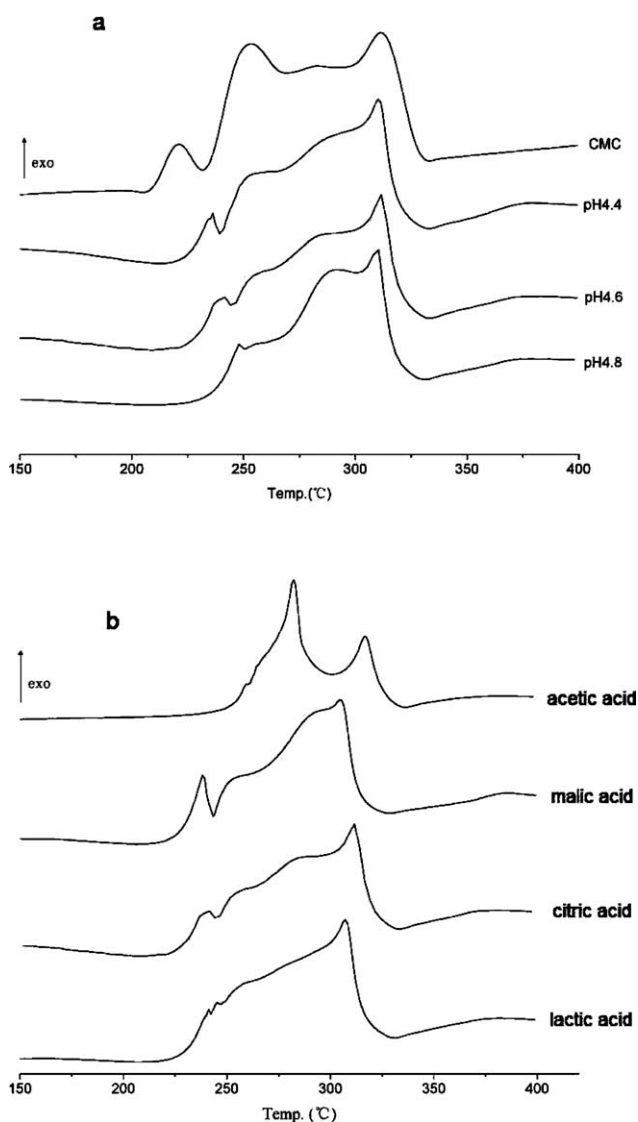
**Differential Scanning Calorimetry Analysis.** Figure 4(a) was the differential scanning calorimetry (DSC) of CMCNa films treated by citric acid at pH4.8, 4.6, and 4.4. The range of CMCNa decomposition temperature was  $229.0^\circ\text{C}$ – $311.4^\circ\text{C}$ . The exothermic peak at about  $221^\circ\text{C}$  was created by the breakage of



**Figure 3.** a: SEM photographs of CMCNa films without acid treatments (b–d) SEM photographs of pH 4.6, 4.4, and 4.2 for CMCNa films with citric acid treatment. (e–g) SEM photographs of pH 4.6, 4.4, and 4.2 for CMCNa films with lactic acid treatment. (h–j) SEM photographs of pH 4.6, 4.4, and 4.2 for CMCNa films with malic acid treatment.

ether bond and intermolecular hydrogen bond, while around  $311^\circ\text{C}$  was from the breakage of glycosidic bond and oxidation of  $\text{H}_2\text{O}$ ,  $\text{CO}_2$ , and  $\text{CO}$ .<sup>38,39</sup> Comparing the three pictures in Figure 4(a), we found as the pH reduced, an exothermic peak of ether about  $250^\circ\text{C}$  moved to a lower temperature area. It would lead to the breakage of ester bonds and a decline in crosslinking, which conformed to the observation of FTIR and SEM.

Figure 4(b) was the DSC of CMC films treated with acetic acid, citric acid, lactic acid, and malic acid when pH got 4.6. It was



**Figure 4.** a: DSC thermograms of CMCNa films after citric acid treatment. b: DSC thermograms of CMCNa films after different acid treatments.

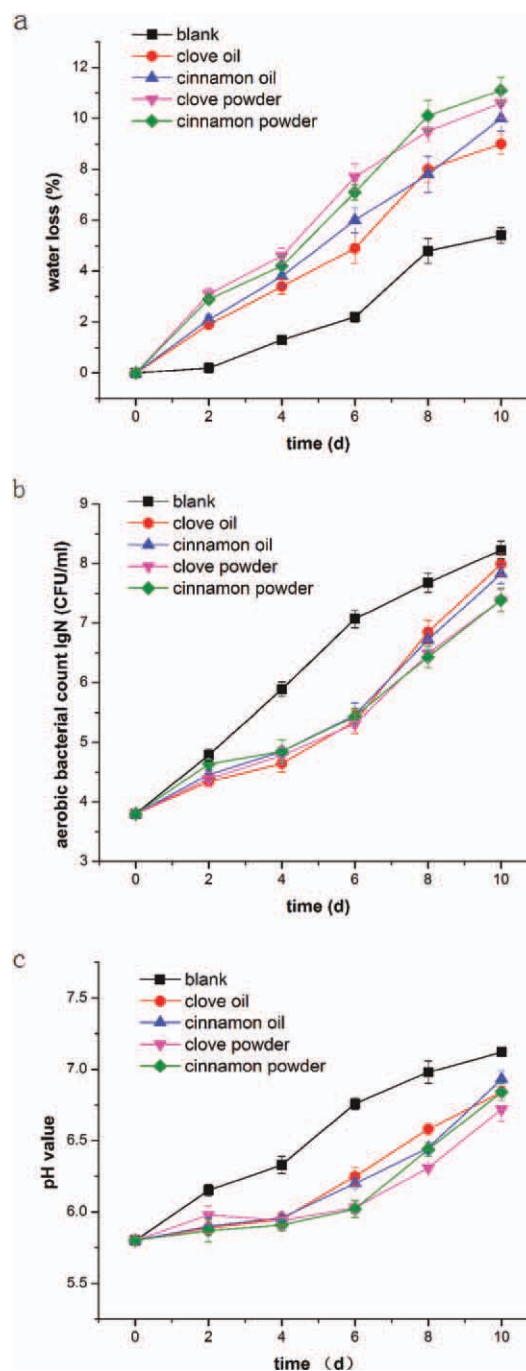
clear that initial peak intensities of films which acidized by malic acid, citric acid, and lactic acid decreased obviously, even that acidized by lactic acid peaks disappeared indeed, which explained that when CMCNa sol was pH 4.6 with lactic acid, water absorbency improved to a maximum (209.8) again. The phenomenon also meant a crosslinking reaction of CMC film was also influenced by the variety of acids. The film acidulated by malic acid would be decomposed at 238.9°C and 305.3°C. The film acidulated by citric acid had an exothermic peak at 311.5°C. The breaking temperature of glycosidic bond which acidulated by lactic acid was 307.7°C.

#### Antimicrobial Effects of Pads with Super Absorbent

**Water Loss.** Water loss measured the ability of films to bind water and fat after protein denaturation and aggregation.<sup>40</sup> The film mainly absorbed free water rather than combination water in the musculature. We could see from Figure 5(a) that with the extension

of storage time, water loss showed an upward trend overall. In the early storage, water loss between each group was relatively similar. In the late time, water loss showed a larger increase and finally tended to be stable. Throughout the whole period, water loss of powder pads was more than oil pads, while the blank was with a minimum.

One possible explanation for the observed effect could be that the lipid-soluble polyphenol provided a hygroscopic effect,



**Figure 5.** Changes in water loss (a), aerobic bacterial count (b), and pH (c) during storage. Values represent mean  $\pm$  standard deviation of mean ( $n = 3$ ). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

thereby reducing moisture loss from the inter- and intra-muscular fibers. The use of lipid-soluble antibacterial agents could also maintain membrane integrity of muscle fibers and reduce moisture loss.<sup>41</sup> Hayes et al.<sup>42</sup> found that the addition of lutein, sesamol, ellagic acid, and olive leaf extract to pork patties had no significant effect on cook loss, pH, or water holding capacity.

**Aerobic Bacterial Count.** It could be appreciated that meat with antimicrobial agents developed a lower population of native flora during storage in comparison with the blank. In the first 6 days, aerobic bacterial count in the blank took on a rapid increase, while other groups presented a relatively slow growth. Groups of oil and powders had a similar inhibition, and clove oil had a slightly better effect. On the 4th day and 6th day, values of the blank were close to 6 and 7.07, respectively. At this time, meat corruption began to appear. After 6 days, values of powder groups were lower than that of oil groups. When it came to the 8th day, the blank, clove oil, cinnamon oil, clove powder, and cinnamon powder on the value of aerobic bacterial count were 7.68, 6.85, 6.72, 6.49, and 6.43, respectively.

The essential oils from clove and cinnamon showed the strongest antibacterial activity against all food borne pathogens and spoilage bacteria tested.<sup>43,44</sup> Clove oil contained a high eugenol (70%–90%) content, which is an antimicrobial compound having wide spectra of antimicrobial effects against enterobacteria.<sup>45</sup> Cinnamon oils contained high concentrations of trans-cinnamaldehyde, a well-known antimicrobial compound and also contained linalool, eugenol, and other phenolic compounds. Previous studies had also identified trans-cinnamaldehyde as the major antibacterial constituent of cinnamon oil.<sup>46</sup> The electro-negativity of trans-cinnamaldehyde interfered in biological processes involving electron transfer and reacts with nitrogen-containing components, such as proteins and nucleic acids, and therefore, inhibited the growth of the microorganisms.<sup>47</sup> Ojagh et al.<sup>48</sup> found a chitosan coating enriched with cinnamon oil could maintain trout fillet shelf life till the end of the storage period (Day 16) without any significant loss of texture, odor, color, or overall acceptability and without significant microbial growth, while control samples had a shelf life of only 12 days. Cinnamon essential oil used in gelatin coatings could maintain the quality of refrigerated rainbow trout fillets over a period of 20 days.<sup>49</sup> A total of 0.10% clove oil v/v could be against bacterial strains inoculated experimentally in irradiated minced meat and against natural microbiota found in minced meat samples.<sup>50</sup> The application of 1% clove oil (v/w) to frankfurter surfaces or the inclusion of cloves or clove oil in the frankfurters, coupled with low temperature storage could reduce the potential of *Listeria monocytogenes* contamination and growth without significantly changing flavor.<sup>51</sup>

**pH.** pH could act as indicators of meat freshness as it started with low value at the early stage of storage which meant the nutritional state was still good and then increased when meat had been stored for certain period of time.<sup>52</sup> During the later postmortem changes, pH increased due to the accumulation of alkaline derived from microbial action and the increase of pH indicated that the muscle began to putresce. From Figure 5(c),

pH values of each group increased slowly at first and then gradually sped up. At the 4th day, the pH value of the blank was 6.33. After 6 days, the value was up to 6.76, at this time, the meat had been corrupt. This phenomenon occurred in groups of oil and powders when it came to 10th day. Besides, a trend was observed that pH value of powder groups were less than oil groups, which was consistent with the phenomenon of aerobic bacterial count.

Cinnamon had better effects on inhibiting bacterial growth and maintaining values of pH of northern snakehead fish than untreated ones.<sup>53</sup> Naveena et al.<sup>54</sup> reported that buffalo meat steaks dipped in 2% v/v lactic acid + 0.1% v/v clove or 2% v/v lactic acid + 0.1% v/v clove + 0.5% w/v vitamin C had significantly ( $P < 0.05$ ) lower pH than those dipped in distilled water.

## CONCLUSION

After  $\alpha$ -hydroxy acid treatment, such as citric acid, lactic acid, and malic acid, films could improve absorbency to a large extent. In the experiment, we got films of super absorbent by regulating pH of CMCNa sol to 4.6 with citric acid, for absorptions of water, saline, and simulative blood were all beyond 115. FTIR, SEM, and DSC analysis showed that acetic acid neither affected the structure of CMC film nor facilitated ester crosslinking reaction. When CMCNa sol was obtained at pH 4.6 with lactic acid ( $\alpha$ -hydroxy acid), crosslinking degree of films reached a maximum. Excessive acid would make degradation of molecular weights, inhibiting a crosslinking reaction. As for water loss, aerobic bacterial count and the pH value, powder groups had a better inhibitory effect than other groups, which could extend the shelf life of chilled meat to 10 days without other fresh-keeping technologies that related to the slow release of effective antimicrobial ingredients.

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