Hydroxypropyl Methylcellulose Films Incorporated with Antibacterial Cinnamaldehyde Microcapsules

Hui Wang, Hongyuan Sun, Jieyu He
Department of Food Science and Technology, Hainan Tropical Ocean University, Sanya 572022, China

ABSTRACT: Antibacterial hydroxypropyl methylcellulose (HPMC) films were prepared by incorporated with cinnamaldehyde microcapsules and their properties were studied. Cinnamaldehyde was encapsulated by chitosan and pectin through polyelectrolyte complexation. Encapsulation efficiency was 74.1% with 1:1 ratio of core and wall materials. Cinnamaldehyde release rate from microcapsule increases with the increase of temperature and humidity. The micrographs show more surface irregularity in HPMC films with the addition of cinnamaldehyde microcapsules. The tensile strength of HPMC films decreases with the increase of microcapsule content. The elongation at break of HPMC films is largest with 5% content of microcapsule. HPMC films with cinnamaldehyde microcapsules show good antibacterial activities and 10% addition is enough for control Staphylococcus aureus and E.coli. The results show possibility of antimicrobial food packaging using incorporation of cinnamaldehyde microcapsules into HPMC film.

KEYWORDS: Cinnamaldehyde, antibacterial, microcapsules, HPMC films

1 INTRODUCTION

Antimicrobial packaging material is one of the active packaging materials that can release antimicrobial compounds to the surface of food to extend the shelf life of products. Antimicrobial activity of film depends on the relationship between the release rate of antimicrobial and the growth rate of microbes. For consumption safety, extensive research has been focused on the incorporation of natural active compounds to packaging films (Muriel-Galet et al. 2012; Jiang et al. 2013). Spices and herb extracts such as vanillin, thymol and cinnamaldehyde are natural compounds that have been widely studied on their nature antimicrobial activities and found that they have potential for inhibition microorganism in foods. Moreover, these natural extracts are classed as generally recognized as safe (GRAS) food additives (Emiroglu et al. 2010; Lambert et al. 2001; Moreira et al. 2005). Unfortunately, most natural compounds are biologically instable because they are sensitive to the environment (water, oxygen, light) that destroy their antimicrobial activities. Currently, novel methods have been introduced in order to improve their stabilities and their bioavailability, by using encapsulation to prevent reactivity with the environment and minimal compounds loss during storage. Therefore encapsulation provides prolonged antimicrobial activity in the final product by graduately diffusion out of film (Leimann et al. 2009; Liolios et al. 2009; Dons et al. 2011; Chacon et al. 2006).

Microcapsule synthesized by polyelectrolyte complexation due to interaction between two oppositely charged polymers possesses series of distinct strengths, including oxidation retarding, heat-resistance and controlled-release. Based on these characteristics, the reaction has already been elucidated adequately and extensively, and applied in encapsulating flavor oil, nutritional ingredient and medicine (Singh et al. 2007; Maestrelli et al. 2012; Morris et al. 2010).

The overall objectives of the present research were to improve the antibacterial efficacy of edible film based on hydroxypropyl methylcellulose (HPMC) by incorporating encapsulated cinnamaldehyde. Cinnamaldehyde was encapsulated in polymers based on chitosan and pectin to improve their stabilities and their compatibility in HPMC film. The mechanical properties of edible HPMC films were also identified, and antibacterial efficacy was assessed against two food pathogenic bacteria.
2 EXPERIMENTAL

2.1 Microencapsulation of Cinnamaldehyde by polyelectrolyte complexation

Dissolve pectin in water under constant magnetic stirring at 45 °C to form 0.5% solution. Chitosan was dissolved in 1% (v/v) acetic acid solution to make up chitosan concentrations at 0.5%. Cinnamaldehyde and Tween 80 (2% of chitosan) were added to chitosan solution. The ratios of cinnamaldehyde and wall polymers were 1:1. The mixture was emulsified using a homogenizer at 10000 rpm for 5 min. The pH of the solution was adjusted to 5.

The pectin solution and emulsion were slowly dropped to water at same time under constant magnetic stirring for 30 min. The temperature was raised to 45 °C, glutaraldehyde (0.3 wt%) was then added to the solution under thorough and continuous mixing for 60 min. The temperature was decreased to 20 °C and the resulted material was stored at 7 °C overnight to promote decantation. Thereupon the encapsulated cinnamaldehyde microcapsules were filtered and dried at 50 °C.

2.2 Encapsulation efficiency and particle size

To determine the encapsulation efficiency, i.e., how much of cinnamaldehyde used in the formulation was admittedly encapsulated, the samples were dissolved in anhydrous ethanol in conical flask. The flask was sealed with a glass stopper, placed under ultrasound conditions at 30 °C for 15 min, then transferred to an oscillating water bath at 30 °C for 15 min. The solutions were used to determine cinnamaldehyde concentration using the UV spectrophotometric method at 305 nm wavelength. Encapsulation efficiency (EE) of the microcapsules was calculated as follows:

\[
EE(\%) = \frac{\text{Cinnamaldehyde in microcapsules}}{\text{Total cinnamaldehyde before encapsulation}} \times 100\%
\]

Particle size distributions of TPCHnano were measured using a laser particle sizer (Mastersizer 2000; Malvern, UK) based on a laser light-scattering technique. Each sample was measured in triplicate.

2.3 Evaporation of cinnamaldehyde from microcapsules in different humidity and temperature

0.5 g microcapsules were placed separately in two glass watch, stored in desiccator with different humidity (saturated MgNO₃ solution creating RH 50%, saturated K₂SO₄ solution creating RH 98%, saturated NaCl solution creating RH 75%) at 20 °C, and the lid was opened to change air every 12 hr for 5 min. The samples were taken to measure cinnamaldehyde contents every 24 hr.

0.5 g microcapsules were placed separately in two glass watch, stored in desiccator with RH 98%. The desiccator was placed in incubator at 20 °C, 30 °C, 40 °C, the lid was opened to change air every 12 hr for 5 min. The samples were taken to measure cinnamaldehyde contents every 24 hr.

2.4 Preparation of antibacterial HPMC film

1.5% Hydroxypropyl methylcellulose (HPMC) was dissolved into 100 mL of distilled water and rotary shaking was undertaken concurrently for 30 min, 10% of glycerol was added to the solution. Subsequently, the encapsulated cinnamaldehyde at 5%, 10%, 15%, 20% of HPMC was added and mixed for 5 min. Then the mixture was degassed under vacuum conditions and cast on plastic petri dish to dry at 50 °C. Film samples were stored in desiccators at 50% RH for further testing. All treatments were made in triplicate.

2.5 Determination of film microstructure

Morphology of films was examined by scanning electron microscopy (Shimadzu SS-550, Japan). The samples were gently and randomly broken and fixed upon an aluminium cylinder using a double-sided electric copper tape, and then the cylinder with films was coated with gold by sputter coater for 180 s at 4 mA to observe the micrographs of films.

2.6 Preparation of antibacterial HPMC film

1.5% Hydroxypropyl methylcellulose (HPMC) was dissolved into 100 mL of distilled water and rotary shaking was undertaken concurrently for 30 min, 10% of glycerol was added to the solution. Subsequently, the encapsulated cinnamaldehyde at 5%, 10%, 15%, 20% of HPMC was added and mixed for 5 min. Then the mixture was degassed under vacuum conditions and cast on plastic petri dish to dry at 50 °C. Film samples were stored in desiccators at 50% RH for further testing. All treatments were made in triplicate.

2.7 Tensile strength and elongation of film

Tensile strength was measured using a TAXT2 Stable Micro System texture analyzer (SMS, Surrey, UK) equipped with a 25 kg cell load using ASTM standard method D882-97: ASTM, 1997. Ten samples, 2.54 cm x 12 cm, were cut from each film. The initial grip separation and crosshead speed were set at 50 mm and 30 mm/min, respectively.

2.8 Antimicrobial activity

Testing of the antimicrobial activity of films was carried out using the plate-counting method. The films were cut into 50mm square rectangular pieces and sterilized in conical flask. The film without microcapsule was used as control. 1 ml aliquots of inoculums containing approximately 10⁵-10⁶ CFU/ml of Staphylococcus aureus or E.coli were dropped onto the films. The films were then incubated at 37 °C for 2 hr. Then 5 ml aliquots of sterile normal saline were added and shaken at 200
r/min for 15 min and the viable cell count was determined. The antibacterial rate was calculated from the following equation:

\[
\text{Antibacterial rate} = \left(\frac{A - B}{A}\right) \times 100\% ,
\]

where: A is average bacteria amount of the control film; B is average bacteria amount of the samples.

Experiments were done in triplicate.

3 RESULTS AND DISCUSSION

3.1 Morphology and particle size

Figure 1 shows the small spherical objects with wall materials around cinnamaldehyde oil, which demonstrates that the encapsulation of cinnamaldehyde with chitosan and pectin as wall material through polyelectrolyte complexation was successfully accomplished. The microcapsules exhibits round formats. The assorted sizes of microcapsules vary from 1 to 100 µm. The encapsulation efficiency was 75%.

Figure 1. Micrographs of cinnamaldehyde microcapsules with wall materials of chitosan and pectin; The core/wall material ratio is 1:1.

3.2 Release rate measurement of cinnamaldehyde from microcapsule

Figure 2 shows the release behavior of cinnamaldehyde from microcapsule at 30 ºC at different humidity. The cinnamaldehyde release rate falls down continuously with the heating time, but their release rate is different. The release rate is highest at 98% RH, and reaches 64% after 96 hr, and is only 41% at 50% RH. The reason is that the wall polymers of microcapsule are hydrophilic, they have larger pore space at high humidity, which makes easy for cinnamaldehyde to release from microcapsules.

Figure 3 shows the release behavior of cinnamaldehyde from microcapsule at 75% humidity at different temperature. The cinnamaldehyde release rate increases with the increase of temperature, but there is no much difference.

3.3 Film microstructure
Figure 4. Scanning electron micrograph of (a) HPMC film, (b) HPMC film containing 5% microcapsule, (c) HPMC film containing 10% microcapsule, (d) HPMC film containing 20% microcapsule.

Figure 4 illustrates the scanning electron micrograph of HPMC films containing various contents of encapsulated cinnamaldehye. It is observed that the reference film (without encapsulated cinnamaldehye) is free of air bubbles, smooth and has a homogeneous surface (Figure 4a). The micrographs show increased surface irregularity with the addition of encapsulated cinnamaldehye. The cinnamaldehye microcapsule is more intense on the surface as the content of microcapsule increases, resulting in a more noticeably irregular surface.

3.4 Tensile strength of films

The tensile strength and elongation at break of HPMC films are depicted in Figure 5. The results show that the addition of cinnamaldehye microcapsules influences the mechanic properties of the film. The tensile strength of HPMC films decreases with the increase of microcapsule content. No significant decrease occurs in the tensile strength of film when microcapsule content is lower than 10%. The microcapsule content above 15% caused greater reduction of tensile strength. The film without microcapsules has maximum tensile strength (16.5MPa). The tensile strength of the film decreases to 4.3MPa when the microcapsule content is 20%.

The elongation at break of HPMC films increases from 19.3 to 24.4% as the content of microcapsule increases from 0 to 5%. The addition microcapsule above 15% results in a marked decrease of elongation.

3.5 Antimicrobial activity of HPMC films with cinnamaldehye microcapsules

Table 1 is antibacterial rates of films for *Staphylococcus aureus* and *E.coli*. The film without microcapsules shows no inhibition effect of *Staphylococcus aureus* and *E.coli*. The addition of cinnamaldehye microcapsules has the significant antibacterial effect. The film with 5% microcapsules has 83.4% antibacterial rate for *Staphylococcus aureus* and 71.5% for *E.coli*. The antibacterial rate increases with the increase of microcapsules. No bacteria grow with 10% microcapsule contents. The results show that 10% addition is enough for control bacteria.

<table>
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<tr>
<th>Cinnamaldehye microcapsule contents in film</th>
<th>Antibacterial rate for S. aureus</th>
<th>Antibacterial rate for E.coli</th>
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<tbody>
<tr>
<td>0%</td>
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<tr>
<td>5%</td>
<td>83.4%</td>
<td>71.5%</td>
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<td>20%</td>
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CONCLUSIONS

Cinnamaldehye was encapsulated by chitosan and pectin through polyelectrolyte complexation. The cinnamaldehye release rate from microcapsule increases with the increase of temperature and humidity. The release rate reaches 64% at 98% humidity at 30 °C after 96 h. The tensile strength of HPMC films incorporated with cinnamaldehye microcapsules decreases with the increase of microcapsule content. 10% addition of cinnamaldehye microcapsules in film is enough for control *Staphylococcus aureus* and *E.coli*. The results show possibility of antimicrobial food packaging using incorporation of cinnamaldehye microcapsules into HPMC film.

ACKNOWLEDGEMENTS

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REFERENCES


