Study on the Antimicrobial Activities of the Capsaicin Microcapsules

Fubao Xing,[1] Guoxiang Cheng,[2] Kejing Yi[2]

[1]*School of Sciences, Tianjin University, Tianjin 300072, China*
[2]*School of Materials Science and Engineering, Tianjin University, Tianjin 300072, China*

Received 31 March 3005; accepted 18 November 2005
DOI 10.1002/app.23766
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this study, capsaicin microcapsules were prepared by the complex coacervation of gelatin, acacia, and tannins. The antimicrobial activities of these microcapsules on the common microorganisms of food preservation, *Botrytis cinerea* and *Aspergillus niger*, were investigated. The factors affecting their antimicrobial effects, including the microcapsule concentrations, pH values, and release behavior were also examined. The results showed that the optimum pH for the antimicrobial effect was about 5.0, which might be related to the strongest protein-precipitating ability of tannins at this pH value. The inhibitory activity of the system originated from the synergistic actions of both capsaicin and tannins. The release behavior of the microcapsules had an important influence on the antimicrobial effect for the long shelf-life storage of foods. The present study indicated that the capsaicin microcapsules displayed potential antimicrobial applications in the food storage. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 1318–1321, 2006

Key words: *Botrytis cinerea*; *Aspergillus niger*; antimicrobial activities; microcapsules; capsaicin

INTRODUCTION

In recent years, there is an increasing consumer demand for avoiding foods prepared with preservatives of chemical substances. To meet this challenge, naturally derived compounds and plant extracts with functional antimicrobial properties are being investigated and exploited in controlling pathogens in food storage.[1–3]

Microencapsulation of plant extracts effective against microorganisms provides a promising method for food preservation and environmental protection. Microencapsulation technology has effective applications in many fields, including pharmacy,[4,5] agriculture, and environment.[6] The microencapsulation process involves the entrapment of the components of interest for a delivery system, protecting the efficiency of sensitive drug molecules from deteriorative reactions or adverse environmental conditions.[7] The microcapsule normally has a diameter of less than 1 mm, composed of outer wall materials and core substances, which can provide a mechanism for the controlled-delivery of its contents. Several microencapsulation methods are available for the preparation of microcapsules, but the physicochemical techniques such as complex coacervation and solvent evaporation have unique advantages in the microencapsulation of natural extracts because of their moderate conditions and easy adjustments properties.[7,8] The choice of a method depends mainly on the drug properties and the types of polymeric carriers. Natural polymers have unique advantages in microencapsulation because of their safety, biocompatibility and biodegradation,[8] and moderate processing conditions.[9,10] Using the electrostatic interactions of two oppositely charged polymer solutions, such as gelatin and acacia, microcapsules can be fabricated by complex coacervation.[9–11] Moreover, the interactions between the polymers are reversible, and can be adjusted to special requirements.[12]

Capsaicin (8-methyl-vanillyl-6-nonenamide) is the pungent, principal component of red pepper species (capsicum species). It is composed of three major functional moieties, namely, a vanilloid, an amide, and a hydrophobic side chain. Capsaicin and its synthetic derivatives have been extensively investigated in the fields of pharmacy,[13] neuroscience,[14] and antimicrobial drugs.[15] The plain and heated extracts were found to exhibit varying degrees of inhibition against microbes. Recently researchers have shown that capsaicin can inhibit the growth of some foodborne pathogenic bacteria.[16] In addition, capsaicin had a naturally occurring pungent odor that could prevent sea ani-

Correspondence to: G. Cheng (gxcheng@tju.edu.cn).
Contract grant sponsor: National Natural Science Foundation, China; contract grant number: 50373032.
Contract grant sponsor: Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions, Ministry of Education, China; contract grant number: 2002–123.

mals from attaching to marine structures, while being benign to the environment.[17] Using the method of microencapsulation, the release rates and the efficiency of these natural extracts of plants can be governed by the microcapsule particle size, the thickness, and the permeability of the wall. The practical way to adjust release rates over orders of magnitude is to vary the wall permeability through its crosslinking density and chemical composition.

In this study, the microcapsules of capsaicin were prepared by the complex coacervation of gelatin, acacia, and tannins. The antimicrobial activities on common microorganisms of food preservation were investigated in the paper. The factors affecting their antimicrobial effects, including pH values, concentrations, and release behavior, were also examined. The present study indicated that the capsaicin microcapsules displayed potential antimicrobial applications in the food storage.

MATERIALS AND METHODS

Materials used in the microcapsule preparation

Gelatin A was purchased from Tianjin Third Chemical Factory (Tianjin, China). Acacia was obtained from Shanghai Chemical Company (Shanghai, China). Capsaicin was supplied by Nanjing Tianshu Biological Company (Nanjing, China). All other agents were of analytical grade. All aqueous solutions were prepared using distilled water.

Preparation of capsaicin microcapsules by complex coacervation of gelatin, acacia, and tannins

Preparation and characterization of the microcapsules were described elsewhere.[10] The procedure can be summarized as follows: 0.8 g capsaicin was dispersed in 40 mL of 5% (w/v) acacia solution with 15 mL of 1% (mol/mol) HEC solution as a surfactant and sonicated for 30 min (power of the ultrasonic instrument is 50 W, model KQ-50). Then the resulting emulsion was poured into 40 mL of 5% (w/v) gelatin solution, stirred at a rate of 350 rpm at 50 °C. The coacervation procedure was maintained for 1 h to obtain small aggregates, after adding 5% (v/v) aqueous solution of glacial acetic acid to adjust pH to 4.2. Afterwards, the temperature was lowered to 5–10 °C and the pH was adjusted to 8–9 by adding 5% (mol/mol) aqueous solution of sodium hydroxide. The small aggregates were then crosslinked using 5% (w/v) glutaraldehyde solution. After several washings with deionized water, the microcapsules were treated with 10% (w/v) aqueous solution of tannins for about 10 h at 30 °C. Finally, the microcapsules were washed several times by deionized water and dried in vessels at 30 °C.

The microcapsules had a mean diameter of 23.99 μm, drug loading content of 19.84%, and encapsulation efficiency of 88.21%.

Microorganism strains

The strains of microorganisms used in this study were *Botrytis cinerea* and *Aspergillus niger*. The fungal strains were isolated, stored, and maintained by normal subculture techniques at Tianjin Academy of Sciences (China). *B. cinerea* and *A. niger* were isolated from diseased strawberries and rice respectively. Inoculated plates were held at 23 °C for 4 days. The cultures were transferred to potato dextrose agar (PDA) slants and maintained at 4 °C until use. The fungi were identified according to its physiological and morphological characteristics.

Fungi culture methods

Fungi inocula were prepared by growing cells in PDA at 28 °C, with vigorous agitation in a rotary shaker at 250 rpm until a thick mycelium was formed (usually 48 h). The mycelium was introduced into 0.1% bacteriological peptone solution, using an inoculating loop. The resulting suspension was shaken at 250 rpm in the shaker.

Antimicrobial tests

The antimicrobial activity of the capsaicin microcapsules was carried out by disc diffusion method.[3,18] The capsaicin microcapsules were diluted at required concentrations in sterile water. Sterile filter paper (diameter 6 mm) was impregnated with 100 μL of the microcapsules at different concentrations, and placed in the center of the agar plate, on which the test microorganisms were uniformly inoculated. For control, discs were impregnated with sterile water. After approximately 30 min standing, the plates were inverted and incubated at 28 °C for 48 h. The diameter of the clear zone shown on plates was measured using calipers and expressed in millimetres as its antimicrobial

TABLE I
Different Capsaicin Contents of the Microcapsules on the Antimicrobial Activities (Inhibition Zone Diameter, mm)

	Capsaicin contents of the microcapsules (μg mL^{-1})				
Strains	150	310	590	890	1170
B. cinerea	–[a]	5.2 ± 1.0	8.5 ± 0.6	10.6 ± 1.5	11.0 ± 1.2
A. niger	–	5.4 ± 1.2	9.2 ± 0.8	11.3 ± 2.0	12.1 ± 1.7
Control	–	–		–	–

Values are expressed as mean ± standard error.
[a]No antimicrobial activity.

TABLE II
Ambient pH Values on the Antimicrobial Activities (Inhibition Zone Diameter, mm)

	pH value				
Strains	4.0	5.0	6.0	7.0	8.0
B. cinerea	7.6 ± 0.9	8.5 ± 0.6	7.1 ± 1.0	5.3 ± 1.1	–[a]
A. niger	8.0 ± 0.7	9.2 ± 0.8	7.1 ± 1.0	–	–
Control	–	–	–	–	–

The capsaicin content of the microcapsules in the test was 590 μg mL^{-1}. Values are expressed as mean ± standard error.

[a]No antimicrobial activity.

activity. Each test was performed in triplicate on at least two separate occasions.

For tests of pH effect, the culture media was adjusted to pH 4, 5, 6, 7, 8 by the addition of 1*N* citric acid or 1*N* sodium hydroxide.

For tests of release behavior, the given dose of capsaicin microcapsules was released at a specific time to investigate the antimicrobial effects using the aforementioned method.

RESULTS AND DISCUSSION

The capsaicin content of microcapsules on the antimicrobial effect

The effect of different capsaicin contents on antimicrobial activity is shown in Table I. In dose–response study, *B. cinerea* and *A. niger* were less inhibited by capsaicin at concentrations lower than 590 μg mL^{-1}. The sensitivity of *B. cinerea* and *A. niger* to the capsaicin microcapsules was similar. With the increase in the capsaicin concentration in the microcapsules, the inhibition zone diameter of the system also increased to a certain extent, but displaying a relative slowly increasing trend in antimicrobial effect. When the concentration of capsaicin reached 1170 μg mL^{-1}, the antimicrobial effect became stable. As observed in the inhibition test on PDA, the activity effect of the microcapsules on the microorganisms was dependent on the capsaicin concentrations. In addition, as tannins are well known to possess general antimicrobial properties, the use of tannins could improve the antimicrobial effects in the system. So, the inhibitory activity of the system originated from the synergistic actions of both capsaicin and tannins.

Ambient pH on the antimicrobial effect

Acid pH was an important parameter influencing the ability of pathogens to cause disease. When other experiment conditions such as temperature, concentration were constant, the antimicrobial effects of capsaicin microcapsules were tested adjusting pH values to determine optimal parameters. Acidity affected the growth conditions of the producing fungi, so had effects on the inhibitory activity. The results were shown in Table II.

The optimum pH for the antimicrobial effect was about 5.0, which might be due to the strongest protein-precipitating ability of tannins at this pH value through the combinative interactions of hydrogen bonding and hydrophobic effects.[19–21] Tannins were also found to be toxic to filamentous fungi, yeasts, and bacteria. The mode of antimicrobial action might be related to its ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins.[22]

The antimicrobial activity of the capsaicin microcapsules with time

One of the distinct advantages of the microencapsulation lies in the facts that the release rate can be delayed and the efficiency of these plant extracts can be improved over a relatively long duration. The practical way to adjust release rates over orders of magnitude is to vary the permeability of polymer matrix through its crosslinking density and chemical composition. The antimicrobial activity of microcapsules with time was also investigated in the paper. The results were shown in Table III.

The release behavior of the capsaicin microcapsules led to the increasing content of capsaicin in the system, so the antimicrobial activity also demonstrated smooth improvement, but the influential degrees showed some difference in a specific strain or between

TABLE III
Capsaicin Contents and Antimicrobial Activity of Microcapsules with Time (Inhibition Zone Diameter, mm)

	Time (h)				Capsaicin content (μg mL^{-1})		
Strain	0 (590)	12 (740)	24 (850)	36 (950)	48 (1160)	60 (1280)	72 (1390)
B. cinerea	8.5 ± 0.6	9.4 ± 0.9	10.4 ± 1.0	10.8 ± 1.2	11.0 ± 0.8	11.9 ± 1.2	12.4 ± 1.6
A. niger	9.2 ± 0.8	9.9 ± 0.7	10.6 ± 1.1	11.6 ± 0.9	11.9 ± 0.9	12.1 ± 1.0	12.5 ± 1.3
Control	–	–	–	–	–	–	–

The original capsaicin content of the microcapsules in the test was 590 μg mL^{-1}. Values are expressed as mean ± standard error.

[a]No antimicrobial activity.

the tested microorganisms. In addition, the release rates and the efficiency of the microcapsules can be further governed by the preparation conditions to obtain optimal parameters.

CONCLUSIONS

In this study, the antimicrobial activities of the capsaicin microcapsules on common microorganisms of food preservation, *B. cinerea* and *A. niger*, were investigated. The factors affecting the antimicrobial effects, including the microcapsule concentrations, pH values, and release behavior, were also examined in the paper. The results showed that the optimum pH for the antimicrobial effect was about 5.0, which might be due to the strongest protein-precipitating ability of tannins at these pH values. The release behavior of the microcapsules had an important influence on the antimicrobial effect for the short-term storage of foods. The present study indicated that the capsaicin microcapsules displayed potential antimicrobial applications in the food storage.

References

1. Corine, D. D.; Michel, D.; Joëlle, Q. L. J Ethnopharmacol 2000, 71, 307.
2. Dykes, G. A.; Amarowicz, R.; Pegg, R. B. Food Microbiol 2003, 20, 211.
3. Hsieh, P. C.; Mau, J. L.; Huang, S. H. Food Microbiol 2001, 18, 35.
4. Soppimath, K. S.; Aminabhavi, T. M.; Kulkarni, A. K.; Rudzinski, W. E. J Control Release 2001, 7, 1.
5. Perumal, D. Int J Pharm 2001, 218, 1.
6. Perrin, B. Pestic Outlook 2000, 4, 68.
7. Mayya, K. S.; Bhattacharyya, A.; Argillier, J. F. Polym Int 2003, 52, 644.
8. Genta, I.; Perugini, P.; Conti, B.; Pavanetto, F. Int J Pharm 1997, 152, 237.
9. Stott, P. W.; Williams, A. C.; Barry, B. W. J Control Release 1996, 41, 215–227.
10. Xing, F. B.; Cheng, G. X.; Yang, B. X.; Ma, L. R. J Appl Polym Sci 2004, 91, 2669.
11. Schmitt, C. A.; Sanchez, C. A.; Thomas, F. B.; Hardy, J. A. Food Hydrocolloids 1999, 13, 483.
12. Li, R. H. Adv Drug Delivery Rev 1998, 33, 87.
13. Degim, I. T.; Uslu, A.; Hadgraft, J.; Atay, T.; Akay, C.; Cevheroglu, S. Int J Pharm 1999, 179, 21.
14. Terashima, S. I.; Ogawa, K. Brain Res 2002, 958, 468.
15. Cichewicz, R. H.; Patrick, A. T. J Ethnopharmacol 1996, 52, 61.
16. Dorantes, L.; Colmenero, R.; Hernandez, H.; Mota, L.; Jaramillo, M. E. Int J Food Microbiol 2000, 57, 125.
17. Jorge, M. T.; Abraham, G. C.; Enrique, R. C. J Ethnopharmacol 1999, 64, 241.
18. Hayes, A. J.; Markovic, B. Food Chem Toxicity 2002, 40, 535.
19. Kawamoto, H.; Mizutani, K.; Nakatsubo, F. Phytochemistry 1997, 46, 473.
20. Naczk, M.; Amarowicz, R.; Zadernowski, R.; Shahidi, F. Food Chem 2001, 73, 467.
21. Osborne, N. J. T.; McNeill, D. M. J Sci Food Agric 2001, 81, 113.
22. Cowan, M. M. Clin Microbiol Rev 1999, 12, 564.