ANTIMICROBIAL AND PHYSICOCHEMICAL PROPERTIES OF METHYLCELLULOSE AND CHITOSAN FILMS CONTAINING A PRESERVATIVE

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ABSTRACT

The methylcellulose was mixed with chitosan as well as 4% of sodium benzoate or potassium sorbate to form a film. Investigations of the antimycotic activity of the film on Penicillium notatum and Rhodotorula rubra revealed that it possessed significant antifungal properties. At 25C, approximately 43-45% of the preservatives were released from the film to the glycerol-water mixture in the first 30 min. The maximum amount of preservative that could be released from the film at 25C was approximately 57-65%. At 4C, 38-39% of preservatives were released from the film within 30 min, and reached a maximum amount of 49% in approximately 6 h. The FT-IR spectrum showed that the ionic interaction between -COO⁻ of preservatives and -NH_3+ of chitosan existed in the film. However, the incorporation of preservatives did not affect the tensile strength and elongation property of the methylcellulose/chitosan film.

INTRODUCTION

The concept of "active packaging" has evolved many technologies for food preservation (Labuza and Breene 1989). The antimicrobial film is a type of active package such that the preservative released from the film deposits on the food surface and inhibits the microbial growth. A few reports concerning the antimicrobial film exist in the literature. The Farbwerke Hoechst Company of Germany (French patent No. 1557.949, 1967) coated sorbic acid on a polyethylene film (2-5 g/m^2) to extend shelf-life of packaged foods. Ghosh *et al.* (1977) developed a fungistatic wrapper made by coating grease paper with an aqueous dispersion of sorbic acid in 2% carboxylmethylcellulose, and observed

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that food could be preserved for a minimum of 10 days by simply wrapping it in the treated paper and further enclosing it in a polyethylene bag. The Dai Nippon Printing Company of Japan introduced an antimicrobial film (Ando *et al.* 1988). This film contains Zeolite, a metal containing aluminosilicate, which releases active oxygen to inhibit microbial activity in the packaged food. Many recent studies along the similar line, however, have focused on edible film. The edible film was coated on the food surface which prevented the diffusion of later applied preservative into the food, thus inhibiting the surface microorganisms (Vojdani and Torres 1989; Torres *et al.* 1985).

Direct incorporation of preservative into the film is an economical method for manufacturing antimicrobial packaging. However, the interaction between the preservatives and film forming material may affect the film casting, release of preservative and mechanical properties of the film. In this study, an antimicrobial film has been prepared by using the mixture of methylcellulose, chitosan and preservatives. Historically, cellulose and its derivative have yielded high quality films owning to the linear structure of the polymer backbone (Krumel and Lindsay 1976). However, a preliminary study revealed that methylcellulose film was readily dissolved in water. But, a mixture of methylcellulose and chitosan could yield a complex film with better water resistance. In addition, chitosan itself possesses antimicrobial property (Leuba and Stossel 1986; Hadwiger *et al.* 1986), and it may also provide the film with controlled release property (Miyazaki *et al.* 1981; Kawashima *et al.* 1985). However, the chitosan may also interact with the methylcellulose and preservatives which possess anionic groups (Mireles *et al.* 1992), thereby alters the physicochemical properties of the film and inhibit the release of preservatives. The main objectives of this study were to investigate the physicochemical and antimycotic properties of the methylcellulose/chitosan complex film incorporated with preservative in order to determine its efficacy as a potential "active packaging" material.

MATERIALS AND METHODS

Raw Materials and Reagents

Methylcellulose, potassium sorbate, sodium benzoate, and polyethylene glycol 400 were purchased from Sigma Co. (St. Louis, MO.). Other reagents included sorbic acid, benzoic acid (Merck Co. Darmstadt, F.R. Germany); acetic acid (ALPS Chem Co., Ltd., Taiwan); glycerol (Hayashi Pure Chemical Industries, Ltd. Osaka, Japan).

Chitosan Preparation

Crab shell was initially oven dried, ground and then passed through a 20

mesh screener. The particles were soaked overnight in 0.5N NaOH solution at room temperature to remove the adhered surface meat. After washing with water to neutrality, they were treated with 2N NaOH at 80C for 2 h to remove protein. The crab chitin thus obtained was allowed to react with 45% NaOH solution (NaOH soln/crab shell chitin V/W = 30/1) for 1 h at 100C to obtain 75% deacetylated chitosan (Chen 1987).

Preparation of Solutions for Forming the Film

Solution A. Methylcellulose (4.5 g) and appropriate amount of preservative were mixed with 50 mL of hot water (ca 90C) and stirred until a uniform suspension was obtained. Further, 100 mL of ethanol and 0.5 mL of polyethylene glycol were added into the above suspension which was then heated to 80C and agitated for approximately 15 min to form solution A.

Solution B. Chitosan (1 g) and appropriate amount of preservative were dissolved in 100 mL of 1.5% acetic acid solution to form solution B.

Solution C. Solutions A and B were mixed in a ratio of 3:2 (v:v) to form solution C.

Following their preparation, the apparent viscosities (expressed in CPS) of these solutions were determined by the Brookfield synchroelectric viscometer (Model DVII, Brookfield Engineering Lab., Stoughton, MA) at 25C using LVF No. 4 spindle rotating at 30 rpm.

Preparation of Antimicrobial Films

The methylcellulose antimicrobial film was prepared by spreading solution A on a glass plate (20 × 20 cm) with a 2 mm spacer (Kamper and Fennema 1984). The film was dried on the plate at 80C for 20 min in an oven. After cooling, the film was peeled off from the plate, packed in a polyethylene bag and stored at room temperature for 3 to 4 days before use.

The antimicrobial chitosan film was prepared by spreading solution B on the glass plate with a 4 mm spacer. The film was first dried on the plate at 80C for 2 h and then allowed to set for 2 days at room temperature before peeling.

For the preparation of complex antimicrobial film, solution C was spread on the glass plate, dried on the plate at 80C for 1 h and then allowed to set for 2 days at room temperature before peeling.

Rates of Release of Preservatives from Methylcellulose/Chitosan Films

A water-glycerol solution prepared by mixing equal weight of distilled water and glycerol (W:W = 1:1) was used for preservative release rate study (Guilbert

et al. 1985; Giannkopoulos and Guilbert 1986). The water activity of the mixture was 0.80 as measured by a water activity analyzer (Novasina Thermoconstanter TH2/RTD-33/BSK, Defensor Co., Switzerland). The 50 mg of antimicrobial films were immersed in the water-glycerol solutions either at 4C or 25C. Sample solutions were taken periodically for analyzing the preservative content in the solutions. For determining the rates of release of preservatives from films to medium at 25C and various pH, 50 mg of films were immersed in the water-glycerol solution which was adjusted to pH 3 or 6 by NaOH or HCl solution, respectively. The potassium sorbate and sodium benzoate concentrations in the water-glycerol solution were determined spectrophotometrically at 257 nm and 224 nm, respectively (Rico-Pena and Torres 1991) (UV/VIS spectrophotometer, Spectronic 300 array, Milton Roy, Rochester, NY).

Determination of Antimicrobial Effect of Film

The agar diffusion test was used for determining the antimicrobial effect of films on microorganisms (Ellerman 1977; Halek and Gary 1989). The test organisms used were *Rhodotorula rubra* (CBS7014) and *Penicillium notatum* (ATCC, 11625). Both of the test organisms were supplied by the Food Industry Research and Development Institute (Shingchu, Taiwan). The *R. rubra* was first inoculated on a YM agar (Difco. Lab. Detroit, MI) slope, and incubated at 24C for 48 h. About 3 mL of sterile water was then transferred to the slope for suspending the growth and 0.1 mL of the suspension was spread evenly on a YM agar plate to form the assay plate. For *P. notatum*, the test organism was inoculated on a potato dextrose agar (PDA) slope. After incubating at 24C for 48 h, appropriate amount of sterile water, containing 0.01% Tween - 80 (Sigma Co., St. Louis, MO.) as surfactant, was added to suspend the growth. The 0.1 mL of suspension was then spread on PDA plate to obtain the assay plate.

The antimicrobial films were then cut into 1.5 in. diameter discs, placed on the assay plate and incubated at 24C, 90% RH for 72 h. The antimicrobial effect of the film was determined by observing the existence of clear zone at the contact area as well as around the discs.

The Fourier Transform Infrared (FT-IR) spectrometry was employed to analyze the functional groups of the film in order to determine the possible interactions between preservatives and chitosan. At 28 ± 1C, 55 ± 5% relative humidity, the transmittance of the chitosan and chitosan/preservative films between 500 and 4000 cm^{-1} was measured using the FT-IR spectrometer (Model No. FTS-40, Biorad Co., Boston).

Determination of Physical Properties of Antimicrobial Films

The thickness of the films was measured using a micrometer (Type SMD-540, Teclock Co., Japan). At least five measurements were taken for each film.

The mechanical strength of the antimicrobial films (25 × 100 mm, thickness 50 ± 5 μm) was measured by using Instron (Series IX, automated materials testing system I.II, Instron Co., Chicago). The tensile strength as well as rate of elongation of the films were both tested at 50 mm/min of crosshead speed (Test Method A, ASTM, 1990). The measurements were performed on two sheets of film which were prepared separately. At least six samples were taken from each sheet of film for the measurements. The Duncan multiple range test was used for analyzing the data statistically (Duncan 1955).

RESULTS AND DISCUSSION

Compatibility and Interactions among Preservatives, Methylcellulose and Chitosan

Methylcellulose is known to be compatible with many water-soluble polymers, such as alginate, starch and polyvinyl alcohol (Grover 1986). Although chitosan is soluble only in acidic water solution, it also exhibited good compatibility with methylcellulose. In the present experiment, pH of the chitosan/methylcellulose mixture was 4.3, while the amino group of chitosan (pKa 6.3-6.7) was positively charged and the carboxyl group of preservative was negatively charged (Rinaudo and Domard 1989). Thus, ionic interactions between preservative and chitosan are likely to exist, which are expected to reduce the intramolecular electrostatic repulsion in the chitosan molecules and facilitated formation of intramolecular hydrogen bonding. Consequently, the chitosan molecules might have a more compact structure, which resulted in lowering the viscosity of the solution due to the reduced friction between chitosan molecules (Kienzle-Sterzer *et al.* 1985; Rinaduo and Domard 1989). In addition, Grover (1986) has reported that the viscosity of methylcellulose solution would reduce as a result of increase in the concentration of electrolytes in the solution. Therefore, addition of preservative in the chitosan/methylcellulose mixture resulting in lowering the viscosity (Table 1) was not unexpected.

Figure 1 depicts the infrared spectra of chitosan film with as well as without preservatives. The absorption peaks around 2800 cm^{-1} (peak A), 1640 cm^{-1} (peak B), and 1560 cm^{-1} (peak C) corresponded to the NH_3^+ stretching, asymmetric bending, and symmetric bending of chitosan, respectively. Although all three films showed absorption at 1380 cm^{-1} the chitosan films containing preservative had a relatively larger absorption peak at this wavenumber (peak D). Since the absorption at 1380 cm^{-1} corresponds to a symmetric carboxyl ion group ($-coo^-$) of preservative (Silverstein *et al.* 1991), the spectra suggested that the preservatives in the film were in the form of carboxylate anion. In addition, there was no absorption at 1710cm^{-1} (the C=0 stretch of the -COOH group) in

TABLE 1.
EFFECT OF PRESERVATIVES ON THE VISCOSITY OF METHYLCELLULOSE AND CHITOSAN SOLUTIONS.

Kinds of solution	Viscosity* (25 C) (cps)x10^3
Chitosan	1.7^{a}
Methylcellulose	14.5^{bc}
Chitosan/methylcellulose	16.4^{b}
Chitosan/methylcellulose+sodium benzoate (4%)	14.2^{bc}
Chitosan/methylcellulose+potassium sorbate (4%)	13.2^{c}

abc Means superscripted with different letters are significantly different at 5% level.
* The Brookfield No. 4 spindle rotating at 30 rpm was used for the viscosity measurement.

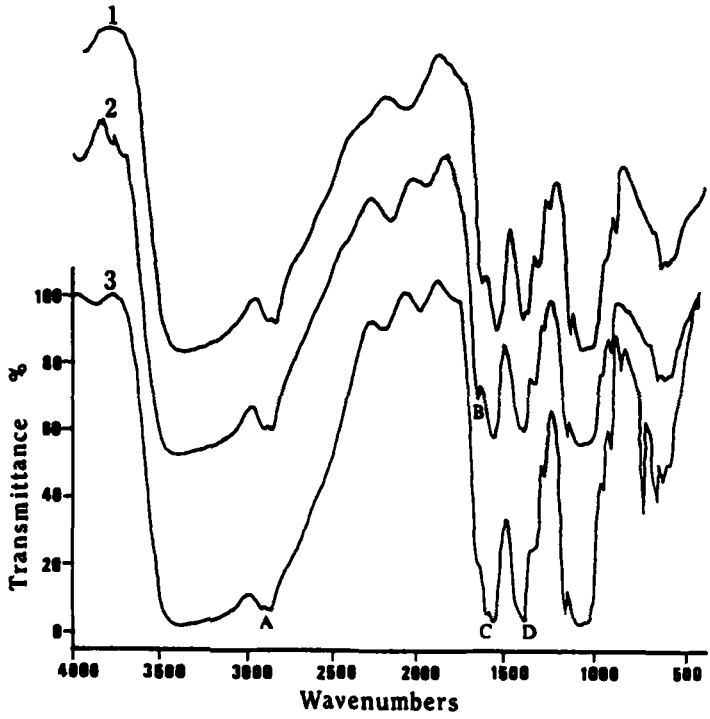

FIG. 1. INFRARED SPECTRA OF CHITOSAN FILMS INCORPORATED WITH SODIUM BENZOATE OR POTASSIUM SORBATE

1. Chitosan film; 2. chitosan/potassium sorbate film; 3. chitosan/sodium benzoate film. A. NH stretch (2800 CM); B. asymmetric NH bend (1640 CM); C. symmetric NH bend (1560 CM); D. symmetric carboxy stretch (1380 CM).

any of the spectrum in Fig. 1, which also implied that most of the carboxyl groups in the films existed as carboxylate anion (Samuels 1981). Based on the results of FTIR, it appeared that the possible electrostatic interaction between the amino group of chitosan and the carboxyl group of preservative could exist.

Antimicrobial Properties of the Films

The antimicrobial properties of the films were evaluated by noting whether there was inhibition of bacterial growth at the film/medium interface, along with the existence of clear inhibitory zone around the film after incubation. The results of these investigations revealed that the chitosan film itself could inhibit the microbial growth at the film/medium interface (Table 2). The antimicrobial property of chitosan is likely to be due to the interaction between the NH_3^+ of chitosan and cell membrane, which results in altering the permeability of the

TABLE 2.
THE ANTIMICROBIAL ACTIVITIES OF THE FILMS MADE OF METHYLCELLULOSE, CHITOSAN AND PRESERVATIVES.

Types of edible film	Inhibition of inhibitory zone	Contact area
CF	+	—
MF		—
CF+2% benzoate	+	—
CF+2% sorbate	+	—
CF+5% benzoate	+	+
CF+5% sorbate	+	+
MF+2% benzoate	+	+
MF+2% sorbate	+	+
CF/MF	+/—	—
CF/MF+4% benzoate	+	+
CF/MF+4% sorbate	+	+

CF : chitosan film
MF : methylcellulose film
CF/MF : chitosan and methylcellulose complex film
+ : represents an inhibitory effect
— : represent no inhibitory effect
+/— : represents a little inhibitory effect

membrane (Leuba and Stossel 1986). The methylcellulose films containing 2% preservatives yielded clear zone at the film/medium interface as well as the area around the disc. However, the chitosan film containing 2% of preservative did not result in a clear inhibitory zone around the film disc. This observation could be attributed to the interaction between chitosan and preservatives and consequently prohibited the release of latter. The clear inhibitory zone was observed only until the concentration of preservatives in the chitosan film reached 5%; presumably the binding sites for preservative in chitosan were saturated at this concentration. The complex film comprising of methylcellulose and chitosan, containing 4% preservatives, could release the antimicrobial agents and form clear inhibitory zone during incubation.

Rate of Release of Preservatives from Film

The main aim for the development of antimicrobial film is to inhibit growth of microorganisms on the surface of food. One of its major potential application lies in the storage of semimoist foods which contain 20-50% moisture. The rates of release of preservatives were hence measured in the water-glycerol solution at water activity of 0.8 (Guilbert *et al.* 1985; Giannakopoulos and Guilbert 1986; Rico-Pena and Torres 1991).

Regardless of the temperature difference, the initial rates of release of preservatives from the methylcellulose/chitosan complex film were observed to be high. It was noted that, within the first 30 min, approximately 40% of the preservative in the film was released (Fig. 2 and 3). High initial rate of release of preservative could inhibit the microbial growth at the early stage of storage; however, it also resulted in a high preservative concentration at the food surface and hence increased its diffusion rate from the surface into the foodstuff due to the high concentration gradient. Such high diffusion rate might in turn reduce the antimicrobial effect of the film for long term storage. The chitosan in the film was supposed to provide the film with controlled release property (Miyazaki *et al.* 1981; Kawashima *et al.* 1985). The present result was, however, unsatisfactory. More detailed study along this line is deemed necessary.

At 4C, the maximum amount of the preservative released from the film was approximately 50% and increased to 60% at 25C. High temperature increases the solubility of the preservative, and probably weakens the ionic interaction between preservative and chitosan, thereby increasing the amount of preservative being released. The rates of release of preservatives at pH 3 and pH 6 were not significantly different ($P < 0.05$). However, both sodium benzoate and potassium sorbate were mostly in the form of undissociated acid at low pH, whereas they were present in the form of salt at high pH. Since only the acid form of preservative possesses antimicrobial effect, more preservatives may be incorporated into the antimicrobial film when the film is to be used for low acid foods.

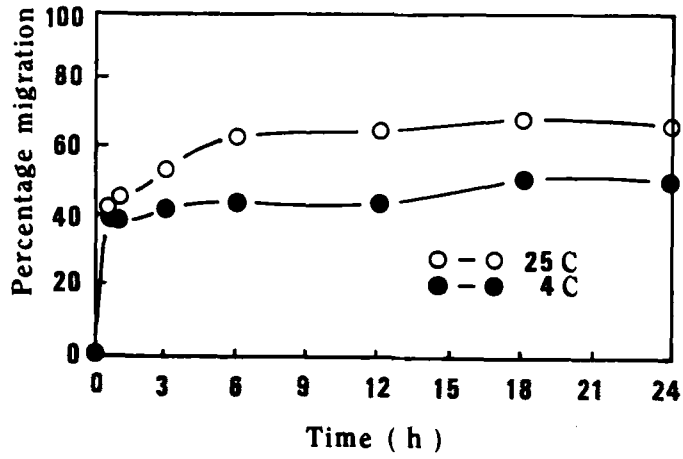

FIG. 2. EFFECT OF TEMPERATURE ON THE MIGRATION RATE OF SODIUM BENZOATE FROM THE METHYLCELLULOSE/CHITOSAN FILM INTO A GLYCEROL-WATER MIXTURE MODEL SYSTEM AT WATER ACTIVITY 0.8

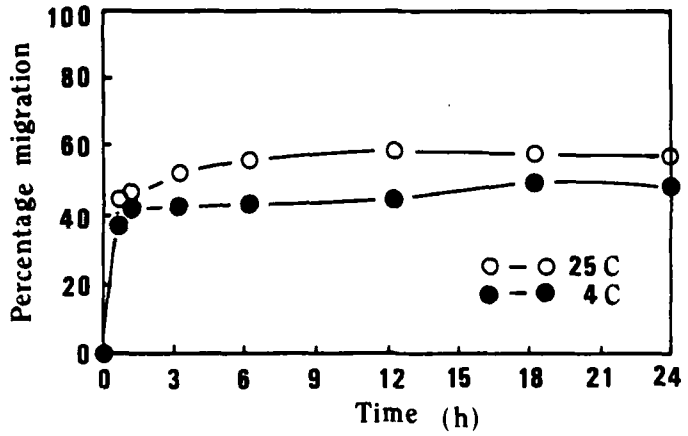

FIG. 3. EFFECT OF TEMPERATURE ON THE MIGRATION RATE OF POTASSIUM SORBATE FROM A METHYLCELLULOSE/CHITOSAN FILM INTO A GLYCEROL-WATER MODEL SYSTEM AT WATER ACTIVITY 0.8

Table 3 lists the tensile strength and elongation rate of the methylcellulose/chitosan antimicrobial films. It was found that the mechanical properties of the films were not changed significantly ($P>0.05$) because of the incorporation of preservatives. In general, the antimicrobial film showed relatively good tensile strength, but poor elongation property, as compared to the mechanical properties of commonly used plastic films.

TABLE 3.
EFFECT OF PRESERVATIVES ON THE MECHANICAL PROPERTIES OF THE FILMS MADE OF METHYLCELLULOSE, CHITOSAN, AND PRESERVATIVES

Type of film	Tensile strength	Elongation (kg/mm^2) (%)
CF/MF	2.8±0.2	19.6±6.1
CF/MF+Benzoate(4%)	3.8±0.6	28.5±6.8
CF/MF+Sorbate(4%)	3.0±0.1	22.5±4.6

CF/MF: chitosan/methylcellulose composite film
* Means within the same column are not significantly different ($P>0.05$)
** Data were obtained by testing on the 50±5 um (ca. 2 mil) film

CONCLUSION

In the present study, it has been observed that the packaging film prepared from methylcellulose, chitosan, and preservative possesses antimicrobial property. Although the rate of release of preservative from the film was too high to maintain a proper preservative concentration at food surface for a long period of time, the film also possesses antimicrobial activity when contacted with food, thereby extending the shelf-life of packaged foods.

It should also be pointed out that the release of preservative from the film depends on the migration of water or water vapor from food to the film in order to dissolve the preservative and facilitate its diffusion to the food surface. The film may hence be suitable only for those food items which have relatively high moisture content.

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