Whey Protein Film Composition Effects on Potassium Sorbate and Natamycin Diffusion

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ABSTRACT: To assess the ability of whey protein films to act as antimicrobial carriers, the effect of film composition on preservative diffusion was investigated. Preservative diffusion coefficients were measured at 24 °C in whey protein isolate (WPI) films with different WPI–glycerol plasticizer ratios (1:1 to 15:1), beeswax (BW) content, 0% to 40% w/w dry solids, and preservative addition of 0.3% (w/w) natamycin or 1.6% (w/w dry solids) potassium sorbate. Diffusion coefficients for potassium sorbate and natamycin were in the ranges 1.09×10^{-11} to 13.0×10^{-11} m²/s and 6.16×10^{-14} to 37.8×10^{-14} m²/s, respectively, and significantly decreased as the WPI-glycerol ratio increased. No significant difference in sorbate diffusion was seen with the addition of BW.

Keywords: whey, film, antimicrobial, diffusion, sorbate

Introduction

Whey protein films and coatings have been shown to be excellent aroma and oxygen barriers, as well as gloss enhancers (McHugh and Krochta 1994; Miller and Krochta 1997; Perez-Gago and Krochta 1999; Lee and others 2002). Recent studies have also shown whey protein films extend their usefulness as carriers of antimicrobials (Ozdemir and Floros 2001, 2003). Because whey protein coatings are water-based and easily formed, they have great potential in many commercial applications.

To assess the ability of a polymer film to act as an antimicrobial carrier, diffusion coefficients for the selected antimicrobials should be determined. To control the release of an antimicrobial, the film composition can be altered with the addition of a lipid or modification of the type and amount of plasticizer. Diffusion coefficients of potassium sorbate and sorbic acid have been measured in corn zein, wheat gluten, and whey protein films (Torres and others 1985; Redl and others 1996; Ozdemir and Floros 2001; Ozdemir and Floros 2003). In previous studies, the addition of various lipids lowered the sorbate diffusion coefficient. By changing the film composition, the film coating can be altered to release the antimicrobial at a desired rate that would be advantageous in a specific application.

Potassium sorbate and natamycin are currently used as antimycotic agents to prevent surface spoilage on Cheddar cheese. It has been hypothesized by industry that natamycin diffuses more slowly than sorbate in cheese because of the molecule's larger size and shape. A slower release and, therefore, a lower diffusion coefficient is desirable in this application to maintain a critical surface concentration of preservative.

This study dealt with the effects of glycerol and BW contents on the diffusion of potassium sorbate and natamycin in whey protein films. The objectives were to compare the diffusion coefficients of these 2 antimycotic agents and to explore the possibility of adjusting film formulation to achieve desired diffusion rates.

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Materials and Methods

Film formulation

Whey protein films were made with WPI (Bipro, Davisco Foods Intl., Le Sueur, Minn., U.S.A.), glycerol (Fisher Scientific Inc., Fair Lawn, N.J., U.S.A.), and potassium sorbate or natamycin (Sigma Chemical Co., St. Louis, Mo., U.S.A.). WPI films with several WPI–glycerol plasticizer ratios: (1:1, 3:1, 5:1, 10:1, 15:1) were made by mixing 10% (w/w) WPI solution with the appropriate amount of glycerol. The solutions were then degassed to remove air, heat-denatured in a 90 °C water bath for 30 min to produce water-insoluble films, and cooled immediately on ice. Once cooled, the preservative was added and the solution degassed. All films contained 0.3% (w/w) natamycin or 1.6% (w/w dry solids) potassium sorbate. These amounts were selected to minimize gelation of the film solution during mixing and film yellowing and discoloration, to measure a detectable level, and to be cost-effective. Previous experiments indicated that higher levels of sorbate (3%, 5%, 6%, and 7%) caused the film solution to gel and caused a visible film yellowing. The films were cast on high density polyethylene (HDPE) plates and dried overnight at about 24 °C. Films with 10:1 and 15:1 WPI-glycerol ratios were dried overnight at 40% relative humidity to prevent cracking. For each WPI-plasticizer ratio, 3 or 4 different films were separately made and tested.

To examine the effect of BW on potassium sorbate diffusion, WPI-BW films (20% and 40% BW dry solids basis) with 1:1 WPI-glycerol, containing 1.6% potassium sorbate were also made. Similar to making WPI films without BW, the WPI-glycerol solution was mixed, heat-denatured, and cooled. BW (Strahl and Pitsch, Bronson and Jabobs N.Z. Ltd., Auckland, New Zealand) was melted and then added to the WPI-glycerol solution, which had been heated to above the BW-melting temperature, 65 °C. The mixture was homogenized with a handheld high-shear probe mixer (Ultraturrax T25; IKA Labortechnik, Staufen, Germany) and cooled on ice. Potassium sorbate was added and the films were cast on Teflon plates. For both BW levels, 3 or 4 different films were separately made and tested.

Diffusion coefficient determination

Before a diffusion experiment, a film containing either potassi-

um sorbate or natamycin was conditioned overnight to 95% relative humidity, the water activity of Cheddar cheese. The film was then cut into a 5- × 5-cm square, and film thickness was measured at 4 different places using a micrometer (Mitutoyo, Kawasaki, Japan). The film was then placed between 2 pieces of a stainless-steel mesh screen to maintain a flat sheet geometry, and held in place with metal clips. Preservative diffusion in the film was measured by immersing the film in a continuously stirred 1000-mL volume of 20% glycerol solution (A_w = 0.94, the water activity of Cheddar cheese) at 24 °C. Potassium sorbate or natamycin concentration in the solution surrounding the film was determined at various time intervals by removing 0.7 mL and using UV spectroscopy at 250 or 317 nm, respectively. Approximately 23 samples for each potassium sorbate and natamycin experiment were taken. Potassium sorbate samples were taken at smaller intervals (15 to 120 s), while natamycin samples were taken at 5-min intervals based on initial experimental trials. Standard curves were used to determine concentrations.

Diffusion coefficients were determined from the data obtained using a relationship derived from the solution to Fick's s Law for a plane sheet (Crank 1975).

In the case of a film with initial uniform concentration equal to C_o and both surfaces at a constant concentration equal to C_1, the following solution for diffusion in a plane sheet can be used as shown in Crank (1975; section 4.3.2, p. 47–49). In this experiment, C_1 was assumed to be small enough to be equal to zero.

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\{-D(2n+1)^2\pi^2 t/l^2\} \quad (1)$$

where M_t = mass of diffusant released from the film at time t; M_∞ = mass of diffusant released from the film at infinite time; l = thickness of film.

For short times, when $M_t/M_\infty < 2/3$, the solution simplifies (Crank 1975) to:

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{l^2\pi}\right)^{\frac{1}{2}} \quad (2)$$

Equation 2 can be rearranged such that the slope of a plot M_t/M_∞ against $t^{1/2}$ (for $M_t/M_\infty < 2/3$) can be used to solve for D;

$$D = \left(\frac{slope * l}{4}\right)^2 \pi \quad (3)$$

Using these equations, diffusion coefficients were determined in 2 ways. A plot of M_t/M_∞ against time was generated from the experimental data. Fitting Eq. 1 to these data was done using the Nonlin function from MATLAB (The Mathworks, Natick, Mass., U.S.A.). Diffusion coefficients and confidence limits were determined. Additionally, a plot of M_t/M_∞ against $t^{1/2}$ was created from the experimental data. The slope of the data points for $M_t/M_\infty < 2/3$ was determined by fitting a straight line to the data using Microsoft Excel (Microsoft Corp., Redmond, Wash., U.S.A.). The slope was used to calculate the diffusion coefficient using Eq. 3. An r^2 value described the fit of the data. The diffusion coefficients from each method were compared.

Results and Discussion

Potassium sorbate diffusion

The release of potassium sorbate from a selected whey protein film formulation (WPI-Gly = 3:1) can be seen in Figure 1. As shown, almost all of the potassium sorbate was released from the film in 500 s. Potassium sorbate diffusion coefficients are summarized in Table 1. The 2 diffusion coefficient determination methods produced some differences in diffusion coefficients, which had an effect on the significant differences among film formulations. Using a Fisher least significant difference (LSD) test, significant differences could be seen among film formulations using both diffusion coefficient determination methods (Table 1). Figure 2 shows the fit of Eq. 1 to the same sorbate experimental data shown in Figure 1 (WPI-Gly = 3:1). R^2 values ranged from 0.900 to 0.996. Fitting Eq. 1 to all the data obtained from a given film formulation is likely to give more accurate diffusion coefficients. As seen in Figure 2, only 4 to 5 data points, taken before $M_t/M_\infty \leq 2/3$, could be used for the slope-diffusion method using Eq. 2.

As the WPI-glycerol ratio increased, the films became less flexible.

Table 1—The effect of whey protein isolate (WPI)-glycerol ratio on potassium sorbate diffusion coefficient[a]

WPI-glycerol (w/w)	D × 10^{-11} (m^2/s)[b]	r^2	D × 10^{-11} (m^2/s)[c]
1:1	9.73a	0.9945 0.9932 0.9918 0.9062	9.24a
3:1	6.67ab	0.9000 0.9871 0.9587 0.9996	6.47a
5:1	2.27bc	0.9522 0.9943 0.9062	2.23b
10:1	1.09c	0.9418 0.9534 0.9944	1.16b

[a]Different letters indicate significant differences for ($P < 0.05$) using least significant difference (LSD) test.
[b]Diffusion coefficient from Eq. 3, average of 3 or 4 trials.
[c]Diffusion coefficient from curve fit of Eq. 1, average of 3 or 4 trials.

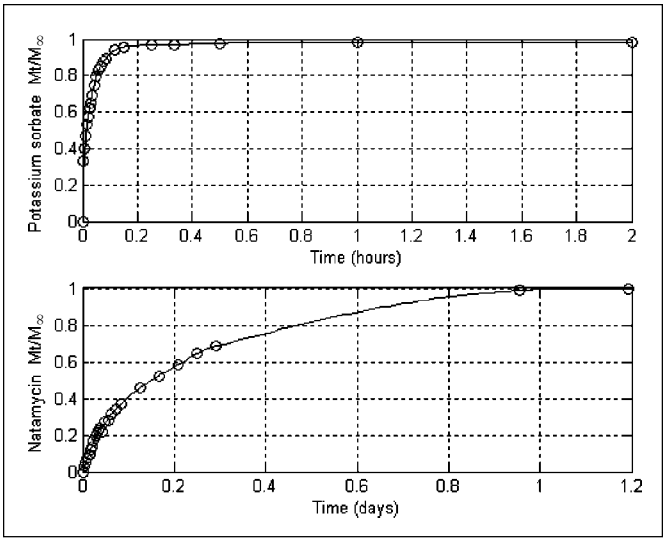

Figure 1—The release of potassium sorbate and natamycin from a whey protein film. M_t = mass of preservative released from film at time t; M_∞ = mass of preservative released from film at infinite time.

Table 2—Measured diffusion coefficients of antimicrobials in edible films

Antimicrobial	Polymer-plasticizer	Receiving solution	D × 10^{-12} (m^2/s)	T (°C)	Reference
Acetic acid	Chitosan	0.2 *M* sodium phosphate	2.59	24	Ouattara and others 2000
Propionic acid	Chitosan	0.2 *M* sodium phosphate	1.87	24	Ouattara and others 2000
Potassium sorbate	WPI-sorbitol	Water-glycerol A_w = 0.8	53.8 to 97.6	25	Ozdemir and Floros 2001
Potassium sorbate	WPI-sorbitol-BW	Water-glycerol A_w = 0.8	53.8 to 97.6	25	Ozdemir and Floros 2003
Potassium sorbate	WPI-glycerol (1:1 to 10:1)	Water-glycerol A_w = 0.94	11.6 to 92.4	24	Franssen 2002
Sorbic acid	Wheat gluten–glycerol (5:1)	Distilled water	7.6	20	Redl and others 1996
Sorbic acid	Wheat gluten–BW-glycerol (5:1)	Distilled water	5.6	20	Redl and others 1996
Sorbic acid	Wheat gluten–AM-glycerol (5:1)	Distilled water	3.2	20	Redl and others 1996
Sorbic acid	AM	Distilled water	0.27	20	Redl and others 1996
Sorbic acid	BW	Distilled water	0.00024	20	Redl and others 1996
Sorbic acid	Corn zein–glycerol (4:1)	50% Water-glycerol	0.3 to 0.7	24	Torres and others 1985
Natamycin	WPI-glycerol (1:1 to 15:1)	Water-glycerol Aw = 0.94	0.063 to 0.378	24	Franssen 2002
Nisin	Wheat gluten–glycerol; corn zein–glycerol	Distilled water	0.65 to 110	5 to 45	Teerakarn and others 2002

[a]AM = acetylated monoglycerides; BW = beeswax; WPI = whey protein isolate.

Additionally, a significant decrease in sorbate diffusion was seen (Table 1). By decreasing the relative amount of plasticizer, the potassium sorbate diffusion coefficient was lowered by a factor of 8.93, almost an order of magnitude. This decrease is hypothesized to be related to the increased film stiffness. As the plasticizer amount decreased, the free volume available for sorbate diffusion decreased, reducing both the film flexibility and the sorbate diffusion coefficient. Diffusion coefficients for sorbate and sorbic acid in other polymers are compared with our results in Table 2. The glycerol-plasticized WPI films from our study had sorbate diffusion coefficients similar to those of WPI films plasticized with sorbitol (Ozdemir and Floros 2001, 2003). Compared with sorbate diffusion in WPI films, sorbic acid diffusion in wheat gluten and corn zein films was found to be slower (Torres and others 1985; Redl and others 1996). Inherent properties of the polymer film, different film conditioning, and different experimental conditions, including temperature and diffusion receiving solution, all likely affect diffusion.

Diffusion coefficients for WPI-BW films are summarized in Table 3. With the addition of BW at 20% and 40% levels, no significant difference in sorbate diffusion was seen. These data are in contrast to effect of BW addition to wheat gluten films, where decreased sorbic acid diffusion was seen (Table 2). In pure BW films, as reported by Redl and others (1996), sorbic acid diffusion was lower by a factor of 10^4 than in WPI films or WPI-BW films. However, the sorbic acid diffusion coefficient in wheat gluten–BW films was not as low as in pure BW. Addition of other lipids such as acetylated monoglycerides showed greater decreases in sorbic acid diffusion in wheat gluten films. Other types of lipid addition to WPI films have the potential to affect the sorbate diffusion coefficient and could be investigated. The data also suggest that the addition of 40% BW was not at a critical volume at the given particle size to affect the potassium sorbate diffusion. Further studies at different particle sizes and higher amounts of BW could be investigated.

Table 3—The effect of beeswax addition on potassium sorbate diffusion coefficient[a]

WPI-glycerol (w/w)	%BW	D × 10^{-11} (m^2/s)[b]	r^2	D × 10^{-11} (m^2/s)[c]
1:1	0	9.74a	0.9945 0.9932 0.9918 0.9062	9.26a
1:1	20	11.4a	0.9786 0.9709	11.2a
1:1	40	12.3a	0.9289 0.9918 0.9852	13.0a

[a]Different letters indicate significant differences for ($P < 0.05$) using least significant difference (LSD) test.
[b]Diffusion coefficient from Eq. 2, average of 2 to 4 trials.
[c]Diffusion coefficient from curve fit of Eq. 1, average of 2 to 4 trials.

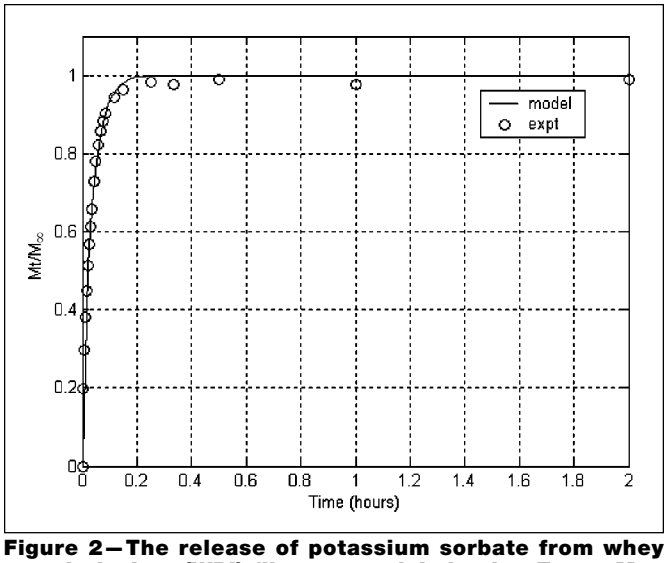

Figure 2—The release of potassium sorbate from whey protein isolate (WPI) films as modeled using Eq. 1. M_t = mass of preservative diffused at time t; M_∞ = mass of preservative diffused at infinite time.

Natamycin diffusion

Release of natamycin is shown in Figure 1 for one film formulation (WPI-Gly = 3:1). The release of natamycin was significantly slower than the release of potassium sorbate. Natamycin diffusion coefficients are summarized in Table 4. As with potassium sorbate diffusion coefficients, a decrease in natamycin diffusion coefficient was found with an increase in the WPI-glycerol ratio. By adjusting the plasticizer amount, the natamycin diffusion coefficient was lowered by as factor of 12. However, only the initial change in the WPI-glycerol ratio was significantly different. Further increase of the ratio resulted in no further significant decrease in diffusion. The larger shape and size of natamycin may be unaffected by further smaller changes in free volume. Natamycin diffusion coefficients

were several orders of magnitude smaller than those for potassium sorbate. This is hypothesized to result from the large, bulky size and shape of natamycin compared with the smaller, more linear potassium sorbate.

In summary, the results of this study indicate that whey protein films have some potential to be antimicrobial carriers. They have the ability to carry and release potassium sorbate and natamycin, with the release rates affected by plasticizer content. Natamycin was found to have a significantly smaller diffusion coefficient than potassium sorbate, most likely because of its larger size and bulky shape. These dramatically different diffusion coefficients would influence the effectiveness of these preservatives in WPI films by affecting the amount of time a preservative would remain at the film surface to be active against microorganisms.

Adjustment of film formulation was shown to modify diffusion characteristics. Increasing the WPI-glycerol ratio significantly decreased the diffusion coefficients of potassium sorbate and natamycin, producing slower preservative release. Thus, there is the potential for customizing diffusion characteristics by changing plasticizer amount and possibly type. At the levels studied, the addition of BW showed no significant change in potassium sorbate diffusion. However, higher BW amounts as well as other lipids should be investigated for their effects.

Table 4—The effect of the WPI-glycerol ratio on natamycin diffusion coefficient[a]

WPI-glycerol (w/w)	D × 10^{-14} (m^2/s)[b]	r^2	D × 10^{-14} (m^2/s)[c]
1:1	37.6a	0.9771 0.9620 0.9229 0.9604	37.8a
3:1	8.50b	0.9788 0.9932 0.9549	8.68b
5:1	6.16b	0.9945 0.9727 0.9491	6.34b
10:1	6.19b	0.9335 0.9587 0.9716	6.39b
15:1	6.26b	0.9538 0.9771 0.9549	6.28b

[a]Different letters indicate significant differences for ($P < 0.05$) using least significant difference (LSD) test.
[b]Diffusion coefficient from Eq. 2, average of 3 or 4 trials.
[c]Diffusion coefficient from curve-fit of Eq. 1, average of 3 or 4 trials.

Conclusions

By custom formulating films and coatings, controlled release of antimicrobials can be optimized for specific food applications. The ability to design a film formulation with desirable controlled release characteristics is critical to effective engineering of food-coating applications. Further research of film formulation effect on antimicrobial diffusion will give a greater understanding of the potential of edible films and coatings as antimicrobial carriers.

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