

# Diffusion of Acetic and Propionic Acids from Chitosan-based Antimicrobial Packaging Films

B. OUATTARA, R.E. SIMARD, G. PIETTE, A. BÉGIN, AND R.A. HOLLEY

**ABSTRACT:** The diffusion of acetic or propionic acids from thin (44 to 54  $\mu\text{m}$ ) chitosan-based antimicrobial packaging films in which they were incorporated was measured after immersion of the films in water, and the effects of pH (5.7, 6.4, or 7.0) and temperature (4  $^{\circ}\text{C}$ , 10  $^{\circ}\text{C}$ , or 24  $^{\circ}\text{C}$ ) on diffusion were investigated. The kinetics of acetic- and propionic-acid release deviated from the Fickian model of diffusion. Diffusion was found to be unaffected by pH in the range of values tested, but a decrease in temperature from 24  $^{\circ}\text{C}$  to 4  $^{\circ}\text{C}$  resulted in a reduction of diffusion coefficients from  $2.59 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$  to  $1.19 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$  for acetic acid and from  $1.87 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$  to  $0.91 \times 10^{-12} \text{ m}^2 \cdot \text{s}^{-1}$  for propionic acid. The effect of temperature on diffusion was well ( $r^2 > 0.9785$ ) described by an Arrhenius-type model with activation energies of 27.19  $\text{J} \cdot \text{mole}^{-1}$  (acetic) and 24.27  $\text{J} \cdot \text{mole}^{-1}$  (propionic). Incorporation of lauric acid or essential oils (cinnamaldehyde or eugenol) into the chitosan film at the time of preparation produced a subsequent reduction in the diffusion of acetic or propionic acid, and maximum effects were obtained with lauric acid and cinnamaldehyde incorporated to final concentrations of 1.0% and 0.5% (w/w), respectively.

**Key Words:** diffusion, acetic, propionic, chitosan, packaging

## Introduction

OVER THE YEARS, A GREAT DEAL HAS BEEN LEARNED ABOUT microbial spoilage of meats and its control (Greer and Dilts 1992; Korkeala and Björkroth 1997; Renner and Labadie 1993). The bacterial species responsible for undesirable sensory changes such as sourness, slime, and gas production have been identified and found to belong to the genera *Acinetobacter*, *Brochothrix*, *Carnobacterium*, *Enterobacter*, *Lactobacillus*, *Leuconostoc*, *Moraxella*, *Pseudomonas*, and *Serratia* (Holley 1997; Korkeala and Björkroth 1997; Renner and Labadie 1993). Also, antimicrobial agents such as organic acids, bacteriocins, and spice extracts have been tested for their ability to control meat spoilage (Abu-groun and others 1993; Hotchkiss 1995; Miller and others 1993). In particular, substantial growth inhibition of meat spoilage bacteria was achieved by use of acetic, propionic, and lauric acids, as well as clove and cinnamon oils (Ouattara and others 1997a, 1997b).

Since microbial growth in solid and semisolid foods such as meat and meat products occurs 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 was found to have limited benefits because the active substances were neutralized on contact or diffused rapidly into the bulk of food, away from the surface (Siragusa and Dickson 1992; Torres and others 1985).

To overcome this problem, attempts are being made to develop active packages, in which antimicrobial agents are incorporated and slowly released at the food surface, where they remain at high concentrations for extended periods of time (Gennadios and others 1997; Hotchkiss 1995; Kester and Fennema 1986; Torres and others 1985). Although synthetic polymers can be used for this purpose, a recent review by Gennadios and others (1997) indicated a growing interest in edible coatings due to factors such as environmental concerns, need for new storage techniques, and opportunities for creating new markets for underutilized agricultural commodities with film-forming properties. Edible coatings prepared from polysaccharides, proteins, and lipids have already been proposed as carriers for various antimicrobial substances. For example, complete inhibition of *Listeria*

*monocytogenes* was obtained using nisin or pediocin fixed on a cellulose casing (Ming and others 1997), and organic acids immobilized in a calcium-alginate gel resulted in a 0.25 to 1.5 log unit reduction of *L. monocytogenes* on lean beef (Siragusa and Dickson 1992).

Chitosan, an aminopolysaccharide that has many applications in the fields of cosmetics, wound healing, dietetics, and wastewater treatment (Demarger-Andre and Domard 1994), is another edible polymer of interest for the preparation of antimicrobial coatings. Unlike chitin, from which it is prepared by deacylation, chitosan is water soluble. Chitosan films are easily prepared by evaporating dilute acid solutions of the polymer (Saitô and others 1987), and these films have been shown to be suitable for controlled release of drugs (Kaya and Picard 1996; Mi and others 1997; Pandya and Knorr 1991). In addition, chitosan has been shown to have antibacterial properties on its own (Darmadji and Izumimoto 1994). A study was therefore undertaken to investigate the feasibility of developing a chitosan-based antimicrobial packaging film, containing organic acids, for the preservation of meat products. This article reports on the extent and rate of diffusion of acetic and propionic acids from such chitosan films. Diffusion was measured in buffer to reproduce the aqueous environment normally encountered at the surface of vacuum-processed meats.

## Results and Discussion

### Film preparation and film characteristics

A precipitate formed when lauric acid was added to chitosan solutions prepared in diluted propionic acid, precluding the use of the lauric-propionic acid combination for film preparation. All other combinations of acetic or propionic acids with lauric acid, cinnamaldehyde, or eugenol led to homogeneous chitosan solutions that yielded uniform films. Films prepared with only acetic or propionic acid were  $44.4 \pm 3.9 \mu\text{m}$  and  $44.7 \pm 4.7 \mu\text{m}$  thick, respectively, while 10% to 34% thicker films were obtained after incorporation of lauric acid ( $53.7 \pm 5.1 \mu\text{m}$ ), cinnamaldehyde ( $53.9 \pm 5.0 \mu\text{m}$ ), or eugenol ( $51.8 \pm 1.7 \mu\text{m}$ ). Therefore, the thickness of chitosan-acetic (or propionic) acid films was purposely increased

in all experiments involving comparisons with films containing added lauric acid, cinnamaldehyde, or eugenol, in order to reduce the influence of thickness on diffusion characteristics. This was achieved by increasing the volume of film-forming solution deposited into the molds prior to drying.

All chitosan-based films absorbed large amounts of water upon immersion (100% to 250% of the initial film mass; Fig. 1); the extent of swelling being the lowest in films containing 1% lauric acid.

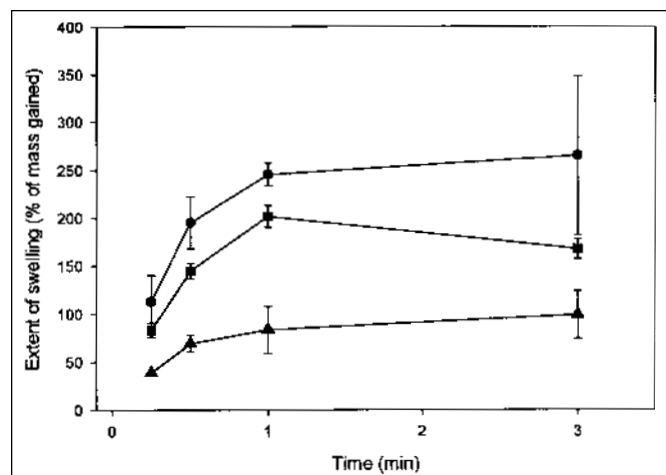
### Kinetics of organic-acid release from chitosan films

Diffusion of acetic acid from a plain chitosan film (containing no lauric acid, cinnamaldehyde, or eugenol) immersed into pH 6.4 sodium-phosphate buffer at 24 °C is represented in Fig. 2. The diffusion rate was maximum immediately after immersion and progressively decreased thereafter, until diffusion was complete (in about 200 min). All other diffusion curves were similar in shape, although diffusion rates varied with each set of experimental conditions (film composition, pH, and temperature). In all cases, linearity with respect to  $t^{1/2}$  of the initial portion of the curve ( $M_t/M_\infty < 2/3$ ) was weak ( $r^2$  as low as 0.6618). In contrast, a straight line always fitted the data well after a logit-log transformation ( $r^2 = 0.9649 \pm 0.0367$ ,  $a = 2.32 \pm 0.58$ , and  $b = -8.88 \pm 2.38$ ), indicative of a sigmoidal shape. In addition, the kinetics of acetic- or propionic-acid release from chitosan films was well described ( $r^2 = 0.9184 \pm 0.0400$ ) by Eq. 2, using  $D$  values calculated from Eq. 1. A better fit ( $r^2 = 0.9760 \pm 0.0157$ ) was obtained using Eq. 3 and rate constants  $k$ .

### Influence of pH and temperature on diffusion

Analysis of variance relative to the diffusion data (Table 1) indicated no effect ( $p > 0.05$ ) of pH on diffusion of acetic and propionic acids, as illustrated in Fig. 3. Consequently, fractional mass release data obtained under different pH conditions were pooled before evaluating the influence of temperature on diffusion.

Increasing the temperature from 4 to 24 °C resulted in a faster rate of diffusion for both acetic and propionic acids (Fig. 4). In particular, the time necessary to release half the amount of acetic acid initially contained in the chitosan film decreased from 77 s at 4 °C to 64 s at 10 °C and 42 s at 24 °C, while the corresponding times for propionic acid were 111 s (4 °C), 75 s (10 °C), and 52 s (24 °C), respectively. Also, the diffusion coefficient  $D$  of acetic acid, calculated with the half-time method (Eq. 1), and the corresponding rate constant  $k$  from Eq. 3 increased from  $1.19 \times 10^{-12}$  to  $2.59 \times 10^{-12}$   $m^2 \cdot s^{-1}$  and from  $9.2 \times 10^{-3}$  to  $19 \times 10^{-3}$   $s^{-1}$ , respectively, when temperature was increased from 4 to 24 °C (Ta-



**Fig. 1**—Swelling of chitosan/acetate-acid film containing 0% (●), 0.5% (■), or 1% (▲) lauric acid during immersion in buffer. Bars represent standard error around the mean ( $N \geq 3$ ).

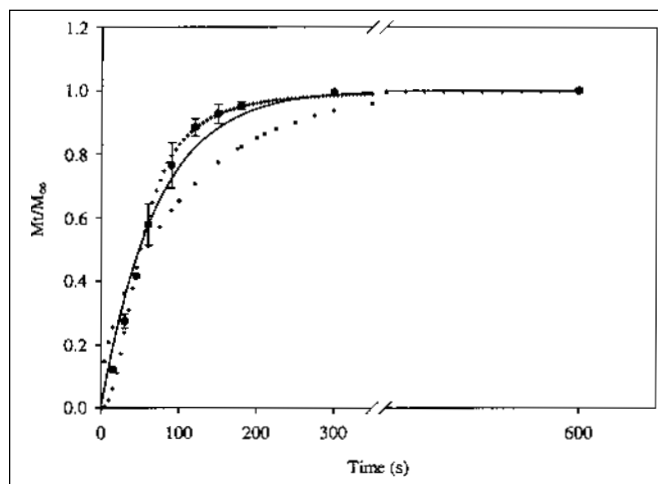
**Table 1**—Summarized results of variance analysis relative to the diffusion of acetic and propionic acids from chitosan films

	DF	P (F > F <sub>cal</sub> )	
		Acetic acid	Propionic acid
Temperature	2	0.0001	0.0001
pH	2	0.0930	0.0672
Time	9	0.0001	0.0001
Temperature*pH	4	0.2590	0.1560
Temperature*time	17	0.0001	0.0001
pH*time	18	0.2130	0.8840
Temperature*pH*time	33	0.9980	0.9890

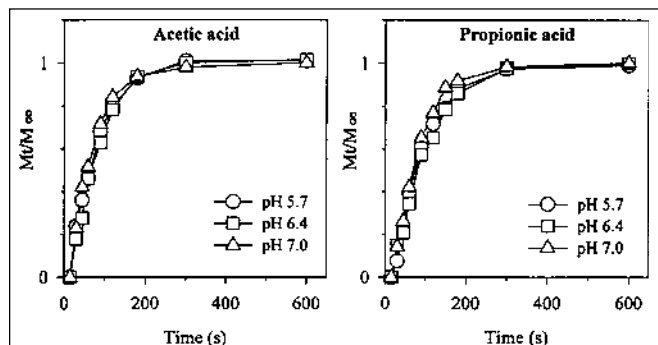
ble 2), and similar increases were observed with propionic acid. In addition, temperature dependence of the diffusion coefficients was well described by an Arrhenius plot (Fig. 5), with activation energies of 27.19 J.mole<sup>-1</sup> and 24.27 J.mole<sup>-1</sup> for acetic ( $r^2 = 0.9976$ ) and propionic acid ( $r^2 = 0.9785$ ), respectively.

### Effect of lauric acid, cinnamaldehyde, or eugenol on diffusion

Incorporating lauric acid into chitosan films at concentrations of 0.25%, 0.50%, and 0.75 % (w/w) had no effect on the diffusion of acetic acid from the films (Table 3). With the highest concentration of lauric acid (1%, w/w), a substantial reduction of  $D$  ( $1.84 \times 10^{-12}$   $m^2 \cdot s^{-1}$ ) and  $k$  ( $7.3 \times 10^{-3}$   $s^{-1}$ ) was observed, compared to the corresponding values measured in control films containing no lauric acid ( $D = 3.20 \times 10^{-12}$   $m^2 \cdot s^{-1}$ ,  $k = 1.7 \times 10^{-2}$   $s^{-1}$ ).



**Fig. 2**—Diffusion of acetic acid from a plain chitosan film (containing no lauric acid, cinnamaldehyde, or eugenol), at pH 6.4 and 24 °C. Bars represent standard error around the mean ( $N \geq 3$ ). Dotted and continuous lines represent predictions from Eq. 2 and 3, respectively, using diffusion coefficients calculated from Eq. 1. Cross-hair line represents the sigmoid that best fits experimental data.



**Fig. 3**—Effect of pH on the diffusion of acetic and propionic acids from plain chitosan films, measured at 10 °C

**Table 2—Influence of temperature on the diffusion of acetic and propionic acids from chitosan films**

Acid	Temperature (°C)	h <sup>a</sup> (10 <sup>-6</sup> m)	D <sup>b</sup> (10 <sup>-12</sup> m <sup>2</sup> .s <sup>-1</sup> )	k <sup>c</sup> (10 <sup>-3</sup> s <sup>-1</sup> )
Acetic	4	43.2	1.19 (1.16 to 1.22)	9.2 ± 0.4 <sub>A</sub>
	10	44.2	1.49 (1.37 to 1.68)	11.3 ± 0.6 <sub>B</sub>
	24	45.8	2.59 (2.30 to 2.73)	19.0 ± 0.8 <sub>C</sub>
Propionic	4	45.3	0.91 (0.85 to 0.95)	6.1 ± 0.3 <sub>A</sub>
	10	44.2	1.27 (1.22 to 1.35)	9.3 ± 0.6 <sub>B</sub>
	24	44.5	1.87 (1.77 to 1.89)	14.3 ± 0.8 <sub>C</sub>

a Film thickness  
 b Diffusion coefficient. Values in parentheses are lower and upper limits for D.  
 c Rate factor obtained by nonlinear regression. For each acid, k values with different letters (A, B, or C) are significantly different (p ≤ 0.05).

The effects of incorporating cinnamaldehyde or eugenol into chitosan films on the diffusion of acetic or propionic acid from the films are summarized in Table 4. Cinnamaldehyde (0.50%, w/w) produced the maximum effect with D values of 2.02 x 10<sup>-12</sup> m<sup>2</sup>.s<sup>-1</sup> for acetic acid and 1.74 x 10<sup>-12</sup> m<sup>2</sup>.s<sup>-1</sup> for propionic acid, compared to 3.63 x 10<sup>-12</sup> m<sup>2</sup>.s<sup>-1</sup> and 2.75 x 10<sup>-12</sup> m<sup>2</sup>.s<sup>-1</sup> in control films (containing only acetic or propionic acid), respectively. Incorporation of eugenol (0.50%, w/w) reduced the D value of acetic acid to 2.30 x 10<sup>-12</sup> m<sup>2</sup>.s<sup>-1</sup>, but no effect was observed on the diffusion of propionic acid.

**Discussion**

Theoretically, the release of acetic and propionic acids from chitosan films immersed in water could be described by the swelling-controlled model for drug release previously reported by Malley and others (1987) and Armand and others (1987). According to this model, water first enters the chitosan matrix and

**Table 3—Influence of lauric acid on the diffusion of acetic acid from chitosan film<sup>a</sup>**

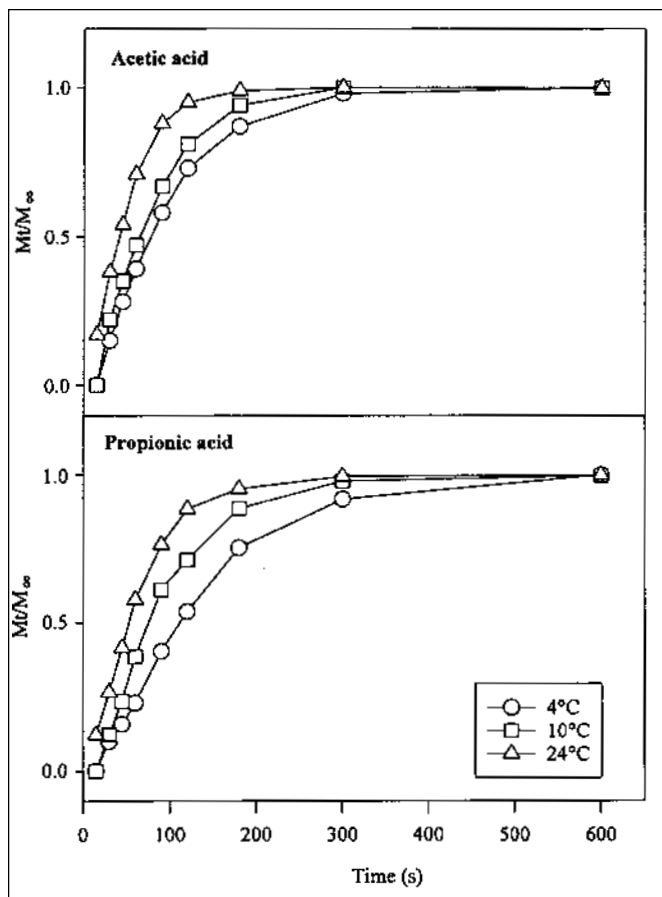
Concentration of lauric acid in films (% w/w)	h <sup>b</sup> (10 <sup>-6</sup> m)	D <sup>c</sup> (10 <sup>-12</sup> m <sup>2</sup> .s <sup>-1</sup> )	k <sup>d</sup> (10 <sup>-3</sup> s <sup>-1</sup> )
0.00 (Control)	56.0	3.20 (2.84 to 3.51)	17.0 ± 1.6 <sub>A</sub>
0.25	49.0	2.73 (1.92 to 2.98)	15.6 ± 0.6 <sub>A</sub>
0.50	49.8	2.29 (1.73 to 3.10)	13.7 ± 0.9 <sub>AB</sub>
0.75	59.7	2.56 (2.54 to 2.58)	14.8 ± 1.3 <sub>A</sub>
1.00	56.2	1.84 (1.66 to 1.90)	7.3 ± 0.8 <sub>B</sub>

a The measurements were done at pH 6.4 and 24 °C.  
 b Film thickness  
 c Diffusion coefficient. Values in parentheses are lower and upper limits for D.  
 d Rate factor obtained by nonlinear regression. For each acid, k-values with different letters (A, or B) are significantly different (p ≤ 0.05).

**Table 4—Effects of cinnamaldehyde and eugenol on the diffusion of acetic and propionic acids from chitosan films<sup>a</sup>**

	Concentration of cinnamaldehyde or eugenol in films (% w/w)	h <sup>b</sup> (10 <sup>-6</sup> m)	D <sup>c</sup> (10 <sup>-12</sup> m <sup>2</sup> .s <sup>-1</sup> )	k <sup>d</sup> (10 <sup>-3</sup> s <sup>-1</sup> )
Acetic acid	0.00 (Control)	59.0	3.63 (3.14 to 4.25)	16.6 ± 1.2 <sub>A</sub>
	Cinnamaldehyde 0.25	59.0	2.99 (2.52 to 3.38)	12.7 ± 1.2 <sub>B</sub>
	0.50	57.0	2.02 (1.93 to 2.16)	9.2 ± 0.8 <sub>C</sub>
	Eugenol 0.25	51.5	2.77 (2.55 to 2.99)	15.8 ± 1.2 <sub>AB</sub>
	0.50	54.0	2.30 (2.23 to 2.46)	13.3 ± 0.5 <sub>B</sub>
Propionic acid	0.00 (Control)	51.3	2.75 (2.51 to 2.82)	18.2 ± 1.6 <sub>A</sub>
	Cinnamaldehyde 0.25	52.0	2.70 (2.46 to 3.05)	14.4 ± 0.9 <sub>A</sub>
	0.50	47.7	1.74 (1.41 to 2.16)	12.3 ± 1.4 <sub>B</sub>
	Eugenol 0.25	50.0	2.50 (2.45 to 2.57)	16.2 ± 1.7 <sub>A</sub>
	0.50	51.8	2.58 (1.98 to 3.10)	13.8 ± 0.9 <sub>A</sub>

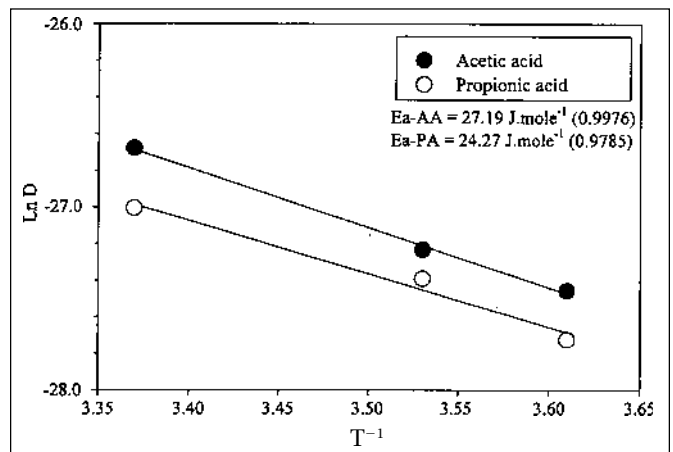
a The measurements were done at pH 6.4 and 24 °C.  
 b Film thickness  
 c Diffusion coefficient. Values in parentheses are lower and upper limits for D.  
 d Rate factor obtained by nonlinear regression. Statistical analyses were done separately for acetic and propionic acids, and k values with different letters (A, B, or C) are significantly different (p ≤ 0.05).



**Fig. 4—Effect of temperature on the diffusion of acetic and propionic acids from plain chitosan films (all pH pooled.)**

dissolves the organic acids, thus allowing their subsequent release from the polymer. The diffusion of acetic and propionic acids is therefore expected to increase with increasing penetration of water into the chitosan film, to finally reach a plateau when the matrix is saturated with water (Armand and others 1987); this was essentially confirmed by the experimental results obtained in the present study.

In reality, the situation is more complex. Many interactions occur during diffusion from polymers to liquids. In particular, liquid uptake generally causes polymers to swell (Peppas and Brannon-Peppas 1994; Armand and others 1987). Also, Lim and Tung



**Fig. 5—Arrhenius plots and activation energies of acetic (Ea-AA) and propionic (Ea-PA) acids incorporated in chitosan films. Values in parentheses are the coefficients of regression (r<sup>2</sup>).**

(1997) reported a time-dependant relaxation process resulting from the swelling stress that occurred during the diffusion of liquid into polymers. As a result, migration rates change continuously, and diffusion is difficult to analyze mathematically (Gnanasekharan and Floros 1997).

In this study, the initial portions of the diffusion curves were not found to be linear with the square root of diffusion time, contrary to the predictions of the general Fick's law of diffusion. This indicates that the release of acetic and propionic acids from chitosan films is not entirely determined by diffusion (Peppas 1985). Additional evidence of the non-Fickian nature of the phenomenon was provided by the sigmoidal shape of the diffusion curves, as already mentioned by Lim and Tung (1997). Also, the fractional mass release, plotted as a function of time, was better represented by an exponential rise to a maximum (Eq. 3) than by the classical solution (Eq. 2) to Fick's law, proposed by Crank (1975). These results differ from those of Redl and others (1996) who reported a typical Fickian behaviour for the diffusion of sorbic acid from wheat gluten and lipid-based films, with correlation coefficients greater than 0.99. The discrepancy is probably related to differences in swelling properties of wheat gluten (5%) and chitosan films (more than 100% in the present study), since Piron and others (1997) reported a change in diffusion pattern from Fickian to non-Fickian behavior, as chitosan became fully hydrated. It is also worth noting that Vojdani and Torres (1989) observed Fickian behavior when potassium sorbate diffused through fully swollen chitosan films. Therefore, the non-Fickian behavior observed in the present study is most likely due to simultaneity of swelling (due to water uptake) and outward diffusion of the acetic or propionic acid.

Demarger-Andre and Domard (1994) reported that in chitosan/carboxylic acid solutions or films, interactions were purely electrostatic, without any complexation processes. These interactions are facilitated when both chitosan and organic acids are protonated, that is, when pH values are lower than the pK of chitosan, which is 6.3 (Mi and others 1997), and higher than the pK of acetic and propionic acids (4.8 and 4.9, respectively). Based on that hypothesis, the release of acetic and propionic acids from chitosan films should be increased when pH increases from 5.7 to 7.0. This was not observed in the present study, suggesting that the diffusion process was not completely controlled by the electrostatic interactions.

The rate of diffusion of acetic and propionic acids from chitosan films increased with increasing temperatures, in the 4 to 24 °C range (this study). Similarly, increased rates of diffusion for potassium sorbate through various polysaccharide films, including chitosan, methylcellulose, and hydroxypropyl methylcellulose were observed as temperatures were increased from 5 °C to 40 °C (Vojdani and Torres 1989, 1990). Also, in the same temperature range (5 to 40 °C), Giannakopoulos and Guilbert (1986) reported an increase in the apparent diffusion coefficients of sorbic acid incorporated in gel cubes from  $3.57 \times 10^{-11}$  to  $1.50 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$ . The dependency of diffusion on tempera-

ture is generally explained by temperature effects on the solubility of diffusing molecules in films, on the nature of adhesive forces at interfaces, and on molecular mobility (Vojdani and Torres 1990; Myint and others 1996). The fact that diffusion can be described by an Arrhenius equation (this study) suggests that the effect of temperature is thermodynamic in nature, essentially controlled by the ratio of energy provided to activation energy (Daniels and Alberty 1972), and that no morphological modification of the chitosan film is involved (Redl and others 1996).

Since the release of hydrosoluble components from polymer films in which they are incorporated is dependent on the simultaneous entry of water (Vasquez and others 1997), inclusion of hydrophobic compounds into hydrophilic chitosan films was expected to reduce diffusion by slowing down film hydration. Indeed, diffusion of acetic acid was decreased in chitosan films containing 1.0% lauric acid or 0.5% cinnamaldehyde or eugenol, in line with the results of previous reports on the diffusion characteristics of lipid-polysaccharide films. For example, Redl and others (1996) found that the addition of beeswax or acetylated monoglyceride to wheat-gluten films resulted in a 20% to 50% reduction in diffusion coefficients for sorbic acid. Also, the addition of various fatty acids has been found to reduce potassium-sorbate permeability of methylcellulose or hydroxypropyl methylcellulose films (Vojdani and Torres 1990) and water-vapor permeability of chitosan films (Wong and others 1992). Reduction of diffusion by hydrophobic substances is thought to be due to impairment of water uptake (Vasquez and others 1997) and to modifications to the chitosan structure leading to an increase in network tortuosity (Callegarin and others 1997; Redl and others 1996), which may affect other geometric features, such as pore constrictions or blind porosity, thereby limiting molecular transport through the network (Papadokostaki and others 1997).

## Conclusions

THE ULTIMATE PURPOSE OF THIS STUDY WAS TO EVALUATE IF THE rate of diffusion of acetic and propionic acids from a thin chitosan film would be sufficiently slow to envision using the film on processed meats for controlled release of the acids. In this regard, no firm conclusion can be drawn from the results obtained. The acids were always completely released from the chitosan matrix in a short time (5 to 10 min) after immersion in buffer, but, because the release mechanism appears to be controlled by entry of water into the matrix, diffusion of the acids onto meat surfaces, where water content is limited, is expected to be slower. The efficacy of chitosan-based antimicrobial films, containing acetic or propionic acids, in delaying spoilage during storage of processed meats will therefore have to be evaluated in real situations, in separate experiments. Best results are expected at refrigeration temperatures and with chitosan films containing cinnamaldehyde or lauric acids, since diffusion of acetic or propionic acids in buffer (this study) was found to proceed at a slower rate under these conditions.

## Materials and Methods

### Chitosan films

Chitosan films containing organic acids were prepared by dissolving practical grade (85% deacylated) chitosan from crab shells (Sigma Chemical, St. Louis, Mo., U.S.A.) in aqueous solutions (1%, w/v) of acetic or propionic acids (Fisher Scientific, Nepean, Ontario, Canada) to a final concentration of 2% (w/v), which typically required overnight stirring. Alternatively, lauric acid (> 99% pure; Sigma Chemical), eugenol, or trans-cinnamaldehyde (> 99% pure; both from Aldrich Chemical, Milwau-

kee, Wis., U.S.A.) were added to the chitosan-acid solutions to final concentrations (w/v) of 0.25, 0.50, 0.75, or 1% (lauric acid) and of 0.25 or 0.50% (cinnamaldehyde or eugenol). All solutions were subsequently filtered through a coarse glass filter, and 100 mL of each solution were poured into a 20 cm × 20 cm × 0.5 cm Plexiglas mold at room temperature (24 °C ± 1 °C), except for solutions containing lauric acid, which were heated to 70 °C before casting. Molds and their contents were then placed in an 80 °C oven (BT-23 Isotemp, Fisher Scientific) until all water was evaporated (constant weight), which typically took 4 to 5 h. During that time, about 50% of the acid ini-

tial contents were also lost through evaporation, so that final acid concentrations in the chitosan films were about 1.2 mg.cm<sup>-2</sup>. Finally, the dried films were cooled, and their thickness was determined with a hand-held micrometer (Model ID-110 ME; Mitutoyo, MFG, Japan).

### Diffusion experiments

Diffusion experiments were conducted in 500-mL glass beakers containing 200 mL of 0.2 M sodium-phosphate buffer at 3 different pH values (5.7, 6.4, or 7.0) and maintained at temperatures of 4 °C, 10 °C, or at room temperature (24 °C ± 1 °C). Square pieces (3 × 3 cm) of the films under study were inserted between 2 square polyethylene grids (3.5 × 3.5 cm) for support, and these grids were immersed in the buffer, which was kept agitated to obtain uniform dispersion of acetic or propionic acid diffusing from the chitosan film. Samples of the buffer solution were taken periodically, and the concentrations of acetic or propionic acid were determined by high-performance liquid chromatography (Waters HPLC system composed of a 771 plus autosampler, an U6K injector, and a 600E pump; Waters Corporation, Milford, Mass., U.S.A.). Peak separation was achieved through an Ion Guard precolumn and an Ion 300 polymeric column, both from Interaction Chromatograph (San Jose, Calif., U.S.A.), using a 0.005 N sulfuric-acid solution as the mobile phase, at a flow rate of 0.5 mL.min<sup>-1</sup>. Detection was done at 210 nm, on a 991 Photodiode Array UV Detector (Waters Corporation).

### Fractional mass release and diffusion coefficients of acetic or propionic acid

The fractional mass release is the ratio  $M_t/M_\infty$  of the mass  $M_t$  of acid released in the buffer at time  $t$  to the maximum amount of acid that can be released, that is, the mass  $M_\infty$  of acid released after an infinite time period. Whether or not  $M_t/M_\infty$  was directly proportional to  $t^{1/2}$  was first evaluated, as linearity would indicate compliance with the general law of diffusion (Crank 1975; Peppas 1985). The diffusion coefficients  $D$  (m<sup>2</sup>.s<sup>-1</sup>) of acetic and propionic acids were later calculated using the half-time method equation (Lim and Tung 1997),

$$D = 0.049h^2 / t_{0.5} \quad (1)$$

where  $h$  is the film thickness (m), and  $t_{0.5}$  is the time (s) at which  $M_t = 0.5 M_\infty$ .

Theoretical values of fractional mass release as a function of time  $t$  were calculated by 2 methods. In the 1<sup>st</sup>, the diffusion coefficients  $D$ , obtained with Eq. 1, were substituted in Crank's equation (Crank 1975)

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

In the 2<sup>nd</sup>, an exponential rise to a maximum was used (Lim and Tung 1997),

$$M_t / M_\infty = 1 - \exp(-kt) \quad (3)$$

where  $t$  is the diffusion time,  $M_t$  and  $M_\infty$  are the amounts (mg.cm<sup>-2</sup>) of organic acids released from films at time  $t$  and at equilibrium, respectively, and  $k$  is the rate constant (s<sup>-1</sup>).

In order to evaluate the temperature dependence of diffusion, an Arrhenius activation energy equation was used,

$$D = D_0 \exp(-E_a / RT)$$

in which  $D_0$  is a constant (m<sup>2</sup>.s<sup>-1</sup>),  $E_a$  is the activation energy (J.mole<sup>-1</sup>),  $R$  is the universal gas constant (8.314 J.mole<sup>-1</sup>.K<sup>-1</sup>), and  $T$  is the absolute temperature (°K).

### Data analysis

The initial portions of the diffusion curves ( $M_t/M_\infty < 2/3$ ) were tested for linearity with respect to  $t^{1/2}$  using the SAS-GLM (general linear) procedure (SAS Institute, Cary, N.C., U.S.A.). The overall kinetic data were analysed by the NLIN (nonlinear) procedure to determine the rate constant of the kinetic equation ( $Y = 1 - \exp(-kX)$ ). Rate constant ( $k$ ) values at different temperature or concentration of hydrophobic compounds were tested for significant differences using the Wald statistic (Agresti 1996). Diffusion curves were also tested for sigmoidal shape by evaluating linearity after a logit-log transformation,  $\ln[(M_t/M_\infty)/(1 - (M_t/M_\infty))] = a + b \ln(t)$ . Finally, the GLM procedure of SAS- was used to evaluate the significance of the main effects of temperature, pH, time, and their interactions.

### References

- Abugroun HA, Cousin NA, Judge MD. 1993. Extended shelf life of unrefrigerated prerigor cooked meat. *Meat Sci* 33:207-229.
- Aresti A, editor. 1996. An introduction to categorical data analysis. New York: Wiley. 312 p.
- Armand JY, Magbard F, Bouzou J, Rollet J, Taverdet JL, Vergnaud JM. 1987. Modelling of drug release in gastric liquid from spheric galenic forms with eudragit matrix. *Int J Pharm* 40:33-41.
- Callegarin F, Quezada Gallo J-A, Debeaufort F, Voilley A. 1997. Lipids and biopackaging. *J Am Oil Chem Soc* 74:1183-1192.
- Crank J, editor. 1975. The mathematics of diffusion. 2<sup>nd</sup> ed. Oxford, England: Oxford University Press. 414 p.
- Daniels F, Alberty RA. 1972. Chemical kinetics. In: Alberty RA, editor. Physical chemistry. New York: John Wiley & Sons. p 325-380.
- Darmadji P, Izumimoto M. 1994. Effect of chitosan in meat preservation. *Meat Sci* 38:243-254.
- Demarger-Andre S, Domard A. 1994. Chitosan carboxylic acid salts in solution and in the solid state. *Carbohydr Polym* 23:211-219.
- Gennadios A, Hanna MA, Kurth LB. 1997. Application of edible coatings on meats, poultry and seafoods: a review. *Lebensm-Wiss Tech* 30:337-350.
- Giannakopoulos A, Guilbert S. 1986. Determination of sorbic acid diffusivity in model food gels. *J Food Tech* 21:339-353.
- Gnanasekharan V, Floros JD. 1997. Migration and sorption phenomena in packaged foods. *Crit Rev Food Sci Nutri* 37:519-559.
- Greer GG, Dilts BD. 1992. Factors affecting the susceptibility of meatborne pathogens and spoilage bacteria to organic acids. *Food Res Int* 25:355-364.
- Holley RA. 1997. Asymmetric distribution and growth of bacteria in sliced vacuum-packaged ham and bologna. *J Food Prot* 60:510-519.
- Hotchkiss JH. 1995. Safety consideration in active packaging. In: Rooney ML, editor. Active food packaging. Bishopbriggs, Glasgow: Blackie Academic & Professional. p 238-253.
- Kaya VM, Picard G. 1996. Stability of chitosan gel as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater. *Biore-source Tech* 56:147-155.
- Kester JJ, Fennema OR. 1986. Edible films and coatings: a review. *Food Tech* 40(12):47-59.
- Korkeala HJ, Björkroth KJ. 1997. Microbial spoilage and contamination of vacuum-packaged cooked sausages. *J Food Prot* 60:724-731.
- Lim L-T, Tung MA. 1997. Vapor pressure of allyl isothiocyanate and its transport in PVDC/PVC copolymer packaging film. *J Food Sci* 62:1061-1066.
- Malley I, Bardon J, Rollet M, Taverdet JL, Vergnaud JM. 1987. Modelling of controlled drug-release in case of carbopol-sodium salicylate matrix in gastric liquid. *Drug Dev Ind Pharm* 13:67-79.
- Mi F-L, Her N-L, Kuan C-Y, Wong T-B, Shyu S-S. 1997. Chitosan tablets for controlled release of theophylline: effect of polymer-drug wet or dry blending and anionic-cationic interpolymer complex. *J Appl Polym Sci* 66:2495-2505.
- Miller AJ, Call JE, Whiting RC. 1993. Comparison of organic acid salts for *Clostridium botulinum* control in an uncured turkey product. *J Food Prot* 56:958-962.
- Ming X, Weber GH, Ayres JW, Sandine WE. 1997. Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. *J Food Sci* 62:413-415.
- Myint S, Daud WRW, Mohamad AA, Kadhum AAH. 1996. Temperature-dependent diffusion coefficient of soluble substances during ethanol extraction of clove. *J Am Oil Chem Soc* 73:603-610.
- Quattara B, Simard RE, Holley RA, Piette GJ-P, Bégin A. 1997a. Inhibitory effect of organic acids upon meat spoilage bacteria. *J Food Prot* 60:246-253.
- Quattara B, Simard RE, Holley RA, Piette GJ-P, Bégin A. 1997b. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int J Food Microbio* 37:155-162.
- Pandya Y, Knorr D. 1991. Diffusion characteristic and properties of chitosan coacervates. *Process BioChem* 26:75-81.
- Papadokostaki KG, Amarantos SG, Petropoulos JH. 1997. Kinetics of release of particulate solutes incorporated in cellulosic polymer matrices as a function of solute solubility and polymer swellability. I. Sparingly soluble solutes. *J Appl Polym Sci* 67:277-287.
- Peppas NA. 1985. Analysis of Fickian and non Fickian drug release from polymers. *Pharm Act Helv* 60:110-111.
- Peppas NA, Brannon-Peppas L. 1994. Water diffusion and sorption in amorphous macromolecular systems and foods. *J Food Eng* 22:189-210.

- Piron E, Accominotti M, Domard A. 1997. Interaction between chitosan and uranyl ions. Role of physical and physicochemical parameters on the kinetics of sorption. *Langmuir* 13:1653-1658.
- Redl A, Gontard N, Guilbert S. 1996. Determination of sorbic acid diffusivity in edible wheat gluten and lipid films. *J Food Sci* 61:116-120.
- Renner M, Labadie J. 1993. Fresh meat packaging and meat quality. Review paper. *Proc Int Congr Meat Sci and Tech* 39:361-387.
- Saitô H, Tabeta R, Ogawa K. 1987. High-resolution solid-state  $^{13}\text{C}$  NMR study of chitosan and its salts with acids: conformational characterization of polymorphs and helical structures as viewed from the conformation-dependent  $^{13}\text{C}$  chemical shifts. *Macromolecules* 20:2424-2430.
- Siragusa GA, Dickson JS. 1992. Inhibition of *Listeria monocytogenes* on beef tissue by application of organic acids immobilized in a calcium alginate gel. *J Food Sci* 57:293-296.
- Torres JA, Motoki M, Karel M. 1985. Microbial stabilization of intermediate moisture food surfaces. I. Control of surface preservative concentration. *J Food Proc Pres* 9:75-92.
- Vasquez B, San Roma J, Peniche C, Cohen ME. 1997. Polymeric hydrophilic hydrogels with flexible hydrophobic chains. Control of the hydration and interactions with water molecules. *Macromolecules* 30:8440-8446.
- Vojdani F, Torres JA. 1989. Potassium sorbate permeability of polysaccharide films: chitosan, methylcellulose and hydroxypropyl methylcellulose. *J Food Proc Eng* 12:33-48.
- Vojdani F, Torres JA. 1990. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings: effect of fatty acids. *J Food Sci* 55:841-846.
- Wong DWS, Gastineau FA, Gregorski KS, Tillin SJ, Pavlath AE. 1992. Chitosan-lipid films: Microstructure and surface energy. *J Agri Food Chem* 40:540-544.
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