

# Modifying the physical properties of dairy protein films for controlled release of antifungal agents



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## ABSTRACT

The effect of sodium chloride (NaCl) and pH of sorbitol-plasticized whey protein isolate (WPI) and sodium caseinate (NaCas) films on their mechanical and physical properties were examined. Moreover, antimicrobial films were prepared by incorporating different levels of potassium sorbate and natamycin in WPI films, plasticized with sorbitol, and the diffusion rates of the antimicrobial agents in a liquid medium were evaluated. The addition of NaCl resulted in a decline of Young modulus ( $E$ ) of the edible films and an increase of the elongation at break upon extension (%EB). For WPI films, reducing the pH of the casting solution down to 5.0 resulted in creation of WPI films with greater flexibility than those at pH 7.0, while the highest %EB values were observed at pH 6.0. The moisture uptake behavior increased with the addition of sodium chloride and the films made from casting the WPI-sorbitol solution containing 200 mM NaCl showed a greater moisture adsorption capacity at a given  $a_w$ . The addition of NaCl did not affect significantly the water vapor permeability with the exception of 300 mM NaCl. Whey protein films, made by casting a solution of pH 5.0 showed a significant increase in water vapor permeability (WVP), compared with films originated from solutions with pH 7.0 or 6.0. The addition of sodium chloride and the pH reduction of edible WPI films containing potassium sorbate resulted in reduction of the diffusion coefficient of the antimicrobial. In the case of films containing natamycin, adding sodium chloride did not affect the diffusion coefficient, whereas reducing the pH values of the film forming solution decreased this parameter. The diffusion rate of the two antifungal agents was inversely affected by the molecular shape/size of the component.

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## 1. Introduction

Edible biopolymer films have attracted an increasing amount of research interest and industrial attention as an alternative form of food packaging with reduced environmental and waste-disposal costs (Krochta & DeMulderJohnston, 1997). Edible films are used to prevent moisture permeation, limit gas transport (i.e.,  $O_2$ ,  $CO_2$ ), retard oil and fat migration, retain volatile flavored compounds, and improve mechanical handling of foods (Baldwin, Nisperoscarriedo, & Baker, 1995; Debeaufort, Quezada-Gallo, & Voilley, 1998; Kester & Fennema, 1986; Krochta & DeMulderJohnston, 1997; Miller & Krochta, 1997). Dairy proteins can be tailored to produce edible films which act as barriers to control transfer of moisture, oxygen and oxidizing or reducing agents, undesirable changes of pigments and appearance, and loss of volatile flavors and aromas (Torres, 1994). In particular, whey

protein films are transparent, insoluble in water, flexible and have a rather low permeability to gases (oxygen, carbon dioxide), aroma compounds and fat. However, these films do not provide protection from moisture loss due to their hydrophilic character. Lipids have been incorporated in whey protein films to improve moisture barrier properties by increasing hydrophobicity (McHugh, Aujard, & Krochta, 1994; McHugh & Krochta, 1994; Shellhammer & Krochta, 1997), but generally they cause a weakening of the film strength (Chen, 1995). Whey protein films are also known for their water insolubility which can be beneficial in the sustainability of the film matrix when applied on foods with relatively intermediate or high moisture.

Whey proteins form gels on heating which generate two types of network structures: fine-stranded and particulate (Langton & Hermansson, 1992; Stading & Hermansson, 1990). The isoelectric point of the whey proteins is around 5.2. At pH values between 4 and 6 the repulsive forces in the systems are relatively weak and thus white and opaque particulate gels are formed with network strand dimensions on the order of micrometers. The particulate gel network is composed of almost spherical aggregates linked

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together and forming the threads of the network. Langton and Hermansson (1992) have found that the fine-stranded gels formed at low pH ( $\text{pH} < 4$ ) are composed of short stiff strands, whereas the fine-stranded structures at high pH ( $\text{pH} > 6$ ) consist of longer, more flexible strands. Moreover, at pH 4–6 whey protein films were white and opaque, at  $\text{pH} < 4$  they were transparent and fragile, whereas at  $\text{pH} > 6$  the films were both transparent and flexible. Hence, a pH range from 5 to 7 is usually chosen to study how pH affects the mechanical and barrier properties of practically usable films.

Caseinates are generally accepted as non-ordered polymers, containing mostly random coil chain segments (Siew, Heilmann, Easteal, & Cooney, 1999). Sodium caseinate (NaCas) readily forms films, owing to its high water solubility, its random-coil structure, and the capacity to form chain aggregates via electrostatic, van der Waal's forces and hydrophobic interactions (McHugh & Krochta, 1994).

The incorporation of antimicrobial agents into edible films provides a novel means for enhancing the safety and shelf life of ready-to-eat foods. A wide variety of antimicrobials have been added to edible films and coatings to control microbiological growth and extend the shelf-life of the product (Gadang, Hettiarachchy, Johnson, & Owens, 2008; Lungu & Johnson, 2005; Martins, Cerqueira, Souza, Avides, & Vicente, 2010; Min, Harris, & Krochta, 2005; Mitrakas, Koutsoumanis, & Lazarides, 2008; Zinoviadou, Koutsoumanis, & Biliaderis, 2010). Antimicrobials used in the formulation of edible films and coatings must be classified as food-grade additives or compounds generally recognized as safe (GRAS) by the relevant regulations. Potassium sorbate and natamycin have a long history as GRAS food preservatives. Potassium sorbate is a widely used preservative in foods, particularly dairy products, meat, fish, bakery products, exhibiting inhibitory activity especially for fungi and yeasts. In food, it is effective in the concentration range 0.05–0.3 g/100 g (Vojdani & Torres, 1990). The World Health Organization has set the acceptable daily intake for sorbic acid at 25 ppm/Kg of body weight (Kabara, 1991). Therefore, a more effective use (antimicrobial activity vs. consumption intake levels) of the sorbates as antimicrobial agents is required.

In general, sorbates are highly soluble in aqueous media. The activity of sorbates is maximum at low pH values; nevertheless, they are equally effective at  $\text{pH} \sim 6.5$  (Buazzi & Marth, 1991; Sofos & Busta, 1993). Torres, Motoki, and Karel (1985) calculated the diffusion coefficient of sorbic acid in zein films and reported values 150–300 times lower than those measured in an intermediate moisture food model. The water activity ( $a_w$ ) of food has been found to have a significant impact on the diffusion of sorbic acid in foods with intermediate ( $a_w \approx 0.6$ – $0.7$ ) and/or high moisture content ( $a_w > 0.9$ ); the diffusion of sorbic acid increases with increasing  $a_w$  (Giannakopoulos & Guilbert, 1986). Redl, Gontard, and Guilbert (1996) studied the diffusion of sorbic acid in wheat gluten edible films with or without the presence of lipids. They found that the diffusion, following the Fick's second law, was the dominant mechanism of release of the sorbic acid from the films.

Natamycin is another widely used antimicrobial which protects the surface of dairy products against the development of mold (Reps, Drychowski, Tomasik, & Winiewska, 2002; Var, Erginkaya, Guven, & Kabak, 2006). According to the Codex Alimentarius, in the case of cheese the amount of natamycin which is allowed is 1 mg/dm<sup>2</sup>, and the antimicrobial should be completely absent in 5 mm depth. Natamycin is produced by strains of *Streptomyces natalensis* and in commercial formulations is typically mixed with 50% of lactose. The natamycin-enriched food products should be kept sealed in a cool place at a temperature below 15 °C, and should avoid direct exposure to sunlight (Reps et al., 2002; Var et al., 2006). Additionally, due to the low water solubility of natamycin, its

incorporation into a film would favor a good distribution into the cheese matrix (Reps et al., 2002).

The aim of this study was to investigate the mechanical properties, water sorption and water barrier phenomena, as well as the diffusion of potassium sorbate and natamycin in composite films made of blends with whey protein isolate and sodium caseinate. The influence of the three variables: concentration of sodium chloride, protein ratio and pH, were studied. Multivariate analyses were used to evaluate and interpret the results. Moreover, the influence of the mechanical properties of various edible films on the diffusion rate of the antifungal compound was evaluated in an attempt to control the release of the antimicrobials onto food matrices and thereby to affect the fungal growth.

## 2. Materials and methods

### 2.1. Materials

Powdered whey protein isolate (WPI) Bipro™ (92.08% w/w dry protein, fat 1.08% w/w, 4.08% w/w ash, 1.08% w/w lactose) was purchased from Davisco Foods (International, Le Sueur, MN, USA) and sodium caseinate (NaCas) was from Wako Chemicals (Japan). Sorbitol was obtained from Sigma Chemical (St. Louis, MO, USA). Inorganic salts (reagent grade), used to regulate the relative humidity environments, were obtained from Merck KGaA (Darmstadt, Germany). Potassium sorbate was obtained from Cheminova (Denmark) and natamycin from Danisco (Denmark).

### 2.2. Sample preparation

Whey protein isolate, sodium caseinate and their mixtures were dissolved in double distilled water under stirring. The total concentration of biopolymers in the aqueous solution was always kept constant at 5% (w/w). The protein solutions were placed in a water bath at 90 °C for 30 min to denature the proteins (WPI) and then immediately cooled in an ice water bath under continuous agitation. Sorbitol was used as a plasticizer at a constant concentration of 37.5% (sorbitol/(WPI + sorbitol)) on a dry solids basis, db. The presence of sorbitol was necessary to overcome the brittleness of WPI films. After heating, sodium chloride was added at different concentrations (0 mM, 50 mM, 100 mM, 200 mM, 300 mM, 500 mM), and also the pH of the solution was adjusted at 7.0, 6.0 and 5.0. In order to prepare antimicrobial films, appropriate amounts of the two antimicrobials (potassium sorbate and natamycin) were added into the film-forming solution under constant stirring for ~5 h to solubilize the antimicrobial compound. The solutions were placed at 4 °C for 24 h to remove air bubbles, and then portions of the solution (12.5 g) were cast on Petri dishes (diameter 8.5 cm) and were dried in an oven at 37 °C for ~24 h. The final concentrations of the antifungal agents were 1% and 0.1% w/w (in the film forming solution) for potassium sorbate and natamycin, respectively. An opaque bottle covered with aluminum foil was used for storing natamycin solution to eliminate the influence of light. All the samples were prepared with double distilled water.

### 2.3. Moisture sorption isotherms

Moisture sorption isotherms were determined according to Biliaderis, Lazaridou, and Arvanitoyannis (1999). Briefly, film samples (~300 mg) were placed in previously weighed dishes and dried in an air-circulated oven at 45 °C over silica gel until constant weight. The samples were kept in desiccators with different relative humidity levels (RH 11%, 33%, 43%, 53%, 64%, 75%, 84% and 94%) at 25 °C for 21 days in order to reach a constant weight (equilibrium moisture). The desired RH conditions were reached by using the

following saturated salt solutions: LiCl,  $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{Mg}(\text{NO}_3)_2 \times 6\text{H}_2\text{O}$ ,  $\text{NaNO}_2$ , NaCl, KCl and  $\text{KNO}_3$ , which give  $a_w$  values of 0.11, 0.33, 0.43, 0.53, 0.64, 0.75, 0.84 and 0.94, respectively (Rockland, 1960). The moisture content of the samples after storage was determined by drying at 110 °C for 2 h. The sorption data were fitted to the Brunauer–Emmett–Teller (BET) and the Guggenheim–Anderson–DeBoer (GAB) sorption isotherm models.

The BET model is described by the equation:

$$\frac{a_w}{(1 - a_w)m} = \frac{1}{m_m K} + \left[ \frac{K - 1}{m_m K} \right] a_w \quad (1)$$

where  $m$  is the equilibrium moisture content (g/100 g dry matter),  $m_m$  is the BET monolayer value and  $K$  is a constant; the constants  $m_m$  and  $K$  were calculated from the linear regression of the experimental data for  $a_w$  values up to 0.64.

The three-parameter GAB isotherm model is written as:

$$\frac{m}{m_m} = \frac{CKa_w}{(1 - Ka_w)[1 + (C - 1)Ka_w]} \quad (2)$$

where  $m$  is the equilibrium moisture content (g/100 g dry matter),  $m_m$  is the GAB monolayer value, and  $K$  and  $C$  are constants.

All measurements were performed in triplicate.

#### 2.4. Water vapor permeability

The water vapor permeability (WVP) of films was determined by the gravimetric method ASTM (E96-63T) at 25 °C, which is modified for the vapor pressure at film underside, according to McHugh, Avena-Bustillos and Krochta (1993). Initially, all films in the form of discs were equilibrated at 53% RH at 25 °C for 48 h. Afterward, they were used to seal the top of glass cups containing distilled water (RH 100%) and the cups were placed in an air-circulating oven with 53% RH by using a saturated solution of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  at 25 °C. The air gap inside the cup was ~1.2 cm and the film area exposed to moisture transmission was 13.8 cm<sup>2</sup>. The permeability of the films were determined as described by Kristo, Biliaderis, and Zampraka (2007). The water vapor permeability (WVP) was calculated as follows:

$$\text{WVP} = \text{Permeance} \times X$$

where  $X$  is the thickness of the film, as average thickness before and after the measurement of film permeance.

$$\text{Permeance} = \text{slope} / (A \times \Delta p)$$

where *slope* is given from weight loss vs. time,  $A$  is the exposed area of the film and  $\Delta p$  is the water vapor pressure differential across the film. The steady-state water vapor flow was achieved after ~8 h. The samples were weighed every hour. The slopes were calculated by linear regression and correlation coefficients for all data were >0.99. At least six replicates were tested for each film.

#### 2.5. Large deformation mechanical testing

Films were cut into dumbbell-like strips (height: 6 cm, width: 0.7 cm, narrow dimension 0.4 cm) and stored at different relative humidity environments (11%, 43% and 75%) for at least 10 days. The film thickness was measured with a hand-held micrometer at three different points and an average value was obtained. Mechanical measurements were performed with a Texture Analyzer, TA-XT2i (Stable Micro Systems, Godalming, Surrey, UK) in the tensile mode operated at ambient temperature and a crosshead speed of 60 mm/min. The Young's modulus ( $E$ ), the tensile strength ( $\sigma_{\text{max}}$ )

and the % elongation at break (% EB) were calculated from the load–deformation curves of the tensile testing (Lazaridou, Biliaderis, & Kontogiorgos, 2003). Average values of at least eight replicates are reported. The moisture content of the samples was determined by drying the film specimens at 110 °C for 2 h.

#### 2.6. Solubility

The solubility of edible films in water was determined as the percentage of film dry matter that solubilized after 24 h immersion in water at 25 °C (Gontard, Duchez, Cuq, & Guilbert, 1994). A piece of film (~0.1 g) was weighed and immersed in 40 ml of water upon stirring containing sodium azide (0.02% w/v) to avoid microbial growth. Subsequently, 4 ml of solution were taken and the absorbance value was determined with a spectrophotometer at 278 nm (Shimadzu UV-1800). The readings were recorded after the first 20 min and after 24 h.

#### 2.7. Estimation of diffusivity of the potassium sorbate and natamycin in protein films

Square film specimens (dimensions 2 cm × 2 cm, approximately 0.1 g) containing antimicrobials (natamycin and potassium sorbate) were cut using a sharp knife and four of them were placed in pre-weighed containers, and they were dried out at 70 °C for 24 h. Afterward, the four square specimens were immersed into a beaker containing 600 ml of water at 25 °C. Aliquots of the solution (4 ml) were removed from the beaker at various time intervals and potassium sorbate concentrations of the collected samples were measured at 255 nm spectrophotometrically.

In the case of natamycin, the four squared film specimens were immersed into an opaque beaker containing 300 ml distilled water at 25 °C, and 4 ml aliquots from the solution were taken at regular intervals for about 8 h to determine the concentration of diffused natamycin in solution. Natamycin has a characteristic UV spectrum with three absorption peaks at 291, 305 and 319 nm (Capitan-Valley, Checa-Moreno, & Navas, 2000). The wavelength of 319 nm was chosen for the measurements of the absorption of natamycin to avoid interference with the absorption from whey proteins.

The ratio  $M_t/M_\infty$  of the amount of antimicrobials released at time  $t$  ( $M_t$ ) and after infinite time ( $M_\infty$ ) was calculated and plotted as a function of time. Prior to these experiments, the thickness of each film ( $l$ ) was measured with a micrometer at six random locations on the film to the nearest 0.001 mm and the average of the six measurements was used in the diffusion coefficient calculations. The diffusion coefficients ( $D$ ) were calculated from the following solution of the second law of Fick (Crank, 1975) assuming that the antimicrobials were initially uniformly distributed in the thin films with negligible edge effects and the concentration in the solution (and thus at the film surface) was effectively zero:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} e^{-D(2n+1)^2 \pi^2 t / l^2} \quad (3)$$

In cases where  $M_t/M_\infty < 2/3$  the following equation (Crank, 1975) applies:

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

## 2.8. Statistical analysis

Statistical analysis was conducted by using the general linear model (GLM) of the SPSS software, Release 17.0. The Tukey's multiple comparisons test was used to determine any statistically significant differences between specific means at a 95% confidence interval.

## 3. Results and discussion

### 3.1. Moisture sorption isotherms

Water sorption isotherms (Fig. 1) were constructed for sorbitol-plasticized WPI films containing different concentrations of sodium chloride (0, 100 and 200 mM) and having different pH values (7.0, 6.0, 5.0) in the film forming solutions. The shape of the sorption isotherms of the studied systems was typical of many hydrophilic polymers (Biliaderis et al., 1999; Cho & Rhee, 2002; Cuq, Gontard, Aymard, & Guilbert, 1997; Kim & Ustunol, 2001; Kristo, Koutsoumanis, & Biliaderis, 2008). The moisture sorption isotherms for the films indicated that the equilibrium water content increased slowly with increasing  $a_w$  up to 0.53, above which there was a steep rise in moisture content. This behavior may originate from the ability of low molecular weight components to absorb water strongly at  $a_w > 0.53$ . At lower  $a_w$  the isotherms show a large flat section (Biliaderis et al., 1999; Kristo et al., 2008).

As shown in Fig. 1A, the water adsorption isotherms of whey protein films were affected by the concentration of sodium chloride. The equilibrium water content of WPI films containing sodium

chloride increased substantially with increasing the amount of sodium chloride. On the other hand, the moisture balance of the films at low and intermediate water activity was not considerably affected by the pH values of the film casting solutions (Fig. 1B).

The experimental sorption data were also fitted to GAB and BET equations (Equations (1) and (2)) and the calculated parameters of these models are given in Table 1.

The BET model is applicable only within the  $a_w$  range of 0–0.64 (Mathlouthi, 2001), while the three-parameter GAB is more popular because it provides an extension of the BET equation at  $a_w$  levels up to 0.94; i.e. including the properties of water adsorbed in multiple layers (Kapsalis, 1987). The monolayer moisture values derived from the BET model was slightly lower than those calculated by the GAB equation for the respective film matrix (Table 1). The highest values of monolayer moisture ( $m_m$ ) were observed for samples of whey proteins containing 200 mM sodium chloride. However, the C values decreased with the addition of sodium chloride in the whey protein films. This indicates that water is less strongly bound to the hydrophilic areas by adding sodium chloride. On the other hand, the  $m_m$  values and the C values increased with a decrease of pH from 7.0 to 5.0. The highest values of the parameter C suggests stronger binding between water molecules and the hydrophilic adsorption sites at pH 5.0. Lewicki (1997) concluded that when the K and C values of the GAB model fall within the intervals  $0.24 \leq K \leq 1$  and  $5.67 \leq C \leq \infty$ , the calculated monolayer values differ by no more than  $\pm 15.5\%$  from the true monolayer capacity. In the current study, the K values of the GAB model were similar for all the pH values examined and fell within the aforementioned ranges; however, the C values of films containing NaCl were much lower than 5.67.

### 3.2. Water vapor permeability (WVP)

The WVP values of the films along with their thickness are presented in Table 2. No significant difference in WVP was observed between films made from mixtures of various proportions of WPI and NaCas. Similar observations have been made by Longares, Monahan, O'Riordan and O'Sullivan (2005).

The observed differences in water vapor permeability between whey protein films without sodium chloride and those containing sodium chloride up to 200 mM were not statistically significant ( $P > 0.05$ ). However, the addition of 300 mM sodium chloride in the casting solution caused a significant reduction ( $P < 0.05$ ) of WVP. It is known that NaCl reduces the electrostatic repulsion between charged protein molecules and in this way it may facilitate a denser protein packing in the film polymeric matrix (Nayebzadeh, Chen, Dickinson, & Moschakis, 2006). Similarly, Park, Rhee, Bae, and Hettiarachchy (2001) reported that adding calcium chloride in soy protein films did not affect significantly the water vapor permeability coefficient, while the addition of calcium sulfate reduced the water vapor permeability. Also, Fang, Tung, Britt, Yada, and Dalglish (2002) reported that adding calcium chloride in

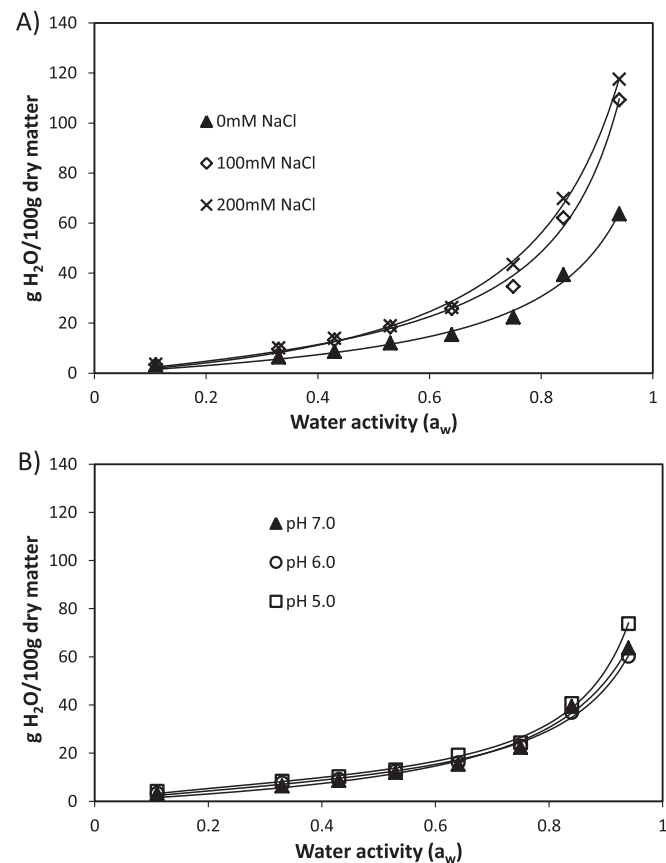


Fig. 1. Effect of sodium chloride A) and pH B) on the moisture sorption of sorbitol-plasticized WPI films. The solid lines represent the fit of the GAB model to the sorption data.

Table 1

Calculated parameters for water sorption data of WPI films containing sodium chloride and different pH values (25 °C) using the BET and GAB isotherm models.

Sample WPI = 5%	BET ( $a_w$ 0.11–0.64)			GAB ( $a_w$ 0.11–0.94)			
	$m_m$	K	$r^2$	$m_m$	K	C	$r^2$
WPI pH 7.0 + 0 mM	6.10	6.33	0.98	6.25	0.98	6.35	0.96
WPI pH 7.0 + 100 mM	11.05	2.95	0.99	11.69	0.96	2.90	0.99
WPI pH 7.0 + 200 mM	11.20	3.19	0.99	12.08	0.98	2.91	0.99
WPI pH 6.0 + 0 mM	6.35	7.88	0.99	6.97	0.95	6.62	0.98
WPI pH 5.0 + 0 mM	7.11	7.91	0.99	7.20	0.97	8.62	0.98

**Table 2**

Effect of various proportions of WPI and NaCas, NaCl and pH on the water vapor permeability (WVP) of sorbitol-plasticized films.

	Thickness ( $\mu\text{m}$ )	WVPC ( $\text{g}^*\text{s}^{-1}\text{m}^{-1}\text{Pa}^{-1}$ ) $\times 10^{10}$
<i>WPI:NaCas</i>		
5:0	131	20.2 $\pm$ 3.5 <sup>b</sup>
4:1	135	18.8 $\pm$ 2.6 <sup>b</sup>
1:4	130	17.3 $\pm$ 1.6 <sup>b</sup>
<i>NaCl (WPI = 5%)</i>		
0	131	20.2 $\pm$ 3.5 <sup>b</sup>
50	139	23.1 $\pm$ 1.4 <sup>b</sup>
100	143	22.8 $\pm$ 1.1 <sup>b</sup>
200	184	23.4 $\pm$ 2.6 <sup>b</sup>
300	189	11.4 $\pm$ 2.7 <sup>a</sup>
<i>pH (WPI = 5%)</i>		
5:0 pH 7.0	131	20.2 $\pm$ 3.5 <sup>b</sup>
5:0 pH 6.0	133	15.6 $\pm$ 1.1 <sup>a</sup>
5:0 pH 5.0	163	61.2 $\pm$ 8.5 <sup>c</sup>

\* Different letters within the same column indicate significant differences ( $P < 0.05$ ).

when protein films had no statistically significant effect on the permeability coefficient up to 10 mM calcium chloride used.

The condition of the RH gradient applied in this study was 100%/53% (interior/exterior of the cell). The average value of WVP at pH 6.0 is lower than pH 7.0 ( $P < 0.05$ ). Moreover, whey protein films at pH 5.0 showed a significant increase in WVP. Perez-Gago and Krochta (1999) reported that pH does not affect significantly the rate of water vapor permeation in whey protein films, except in the area around the isoelectric point ( $\text{pI} \sim 5.2$ ). In addition, the high values in WVP observed at approximately the isoelectric point of whey protein may be due to incomplete removal of air bubbles due to the increased viscosity of the WPI solution.

### 3.3. Tensile properties

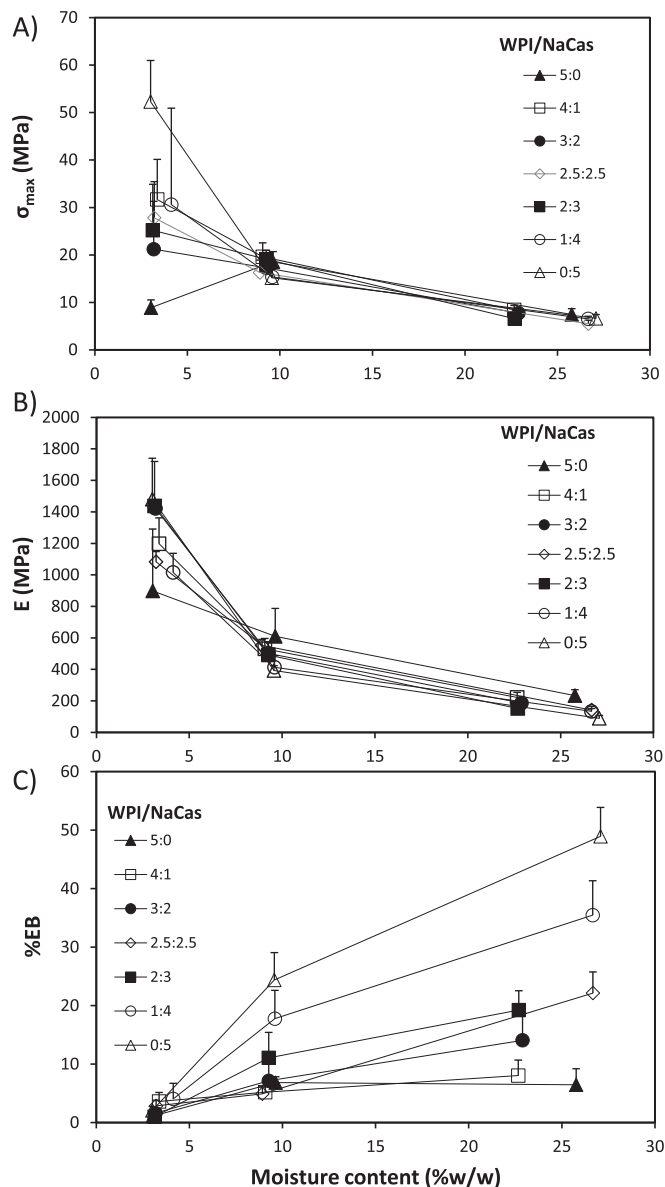
#### 3.3.1. Effect of WPI:NaCas mixture

The profiles of large deformation mechanical properties under tensile mode of sorbitol-plasticized WPI films, as affected by film moisture content and polymer composition in the blends of WPI and NaCas, are illustrated in Fig. 2. The effect of water content of WPI:NaCas blend films is depicted at three different RH levels (11%, 43% and 75%) at 25 °C.

At low water content, films showed very high values of tensile modulus ( $E$ ) (about 0.8–1.5 GPa), maximum tensile strength ( $\sigma_{\text{max}}$ ) and low values of % elongation at break (%EB) at room temperature. These features usually characterize materials at the glassy state. Similar behavior has been reported in previous studies on complex systems of food biopolymers with low water content (Biliaderis et al., 1999; Lazaridou & Biliaderis, 2002; Lazaridou et al., 2003; Longares, Monahan, O'Riordan, & O'Sullivan, 2004; Marzec & Lewicki, 2006). Sodium caseinate samples (WPI:NaCas 0:5) at low moisture showed low values of  $E$  and  $\sigma_{\text{max}}$ , but extremely high %EB values (almost 50%) when the moisture content of the films was  $\sim 28\%$  w/w (Fig. 2). The tensile mechanical properties of blend films with moisture content  $> 8\%$  seems to be controlled mainly by the polymer which is at higher proportion. Therefore, films containing higher levels of sodium caseinate (whey protein/sodium caseinate 1:4 and 2:3) showed properties resembling those of sodium caseinate films, i.e., a gradual decline in  $E$  and  $\sigma_{\text{max}}$  and an increase in %EB. Overall, sodium caseinate seems to be responsible for the flexibility of the composite protein films.

#### 3.3.2. Effect of NaCl

The maximum tensile strength increased with an increase in moisture content up to 10% for the WPI films and for the films



**Fig. 2.** Influence of water content of whey protein isolate (WPI)/sodium caseinate (NaCas) blend (WPI:NaCas) films with various weight polymer ratios on (A) tensile strength,  $\sigma_{\text{max}}$ , (B) tensile modulus,  $E$ , and (C) % elongation at break, %EB, as determined from large deformation mechanical testing of the sorbitol-plasticized WPI films.

containing NaCl as can be seen in Fig. 3A. On further hydration, water appeared to act as a typical plasticizer by reducing the measured  $\sigma_{\text{max}}$ . On the other hand, the Young modulus showed a progressive decline with increasing moisture content. Such behavior is common upon the addition of small molecules that act as plasticizers in a biopolymer matrix. Similar observations have been made by Park et al. (2001), with the addition of calcium chloride in soy protein films, which revealed a reduction of  $E$  and increase of %EB with increasing concentration of calcium chloride.

Increasing the salt concentration up to 100 mM in the casting solution increases the %EB. Moreover, at high moisture content, high %EB values were observed for all the samples containing NaCl. The NaCl reduces the electrostatic repulsion between molecules and in this way it causes a denser microstructure of the film matrix (Nayebzadeh et al., 2006), resulting in a more ductile mechanical response. The  $\sigma_{\text{max}}$  of the films was not significantly affected.

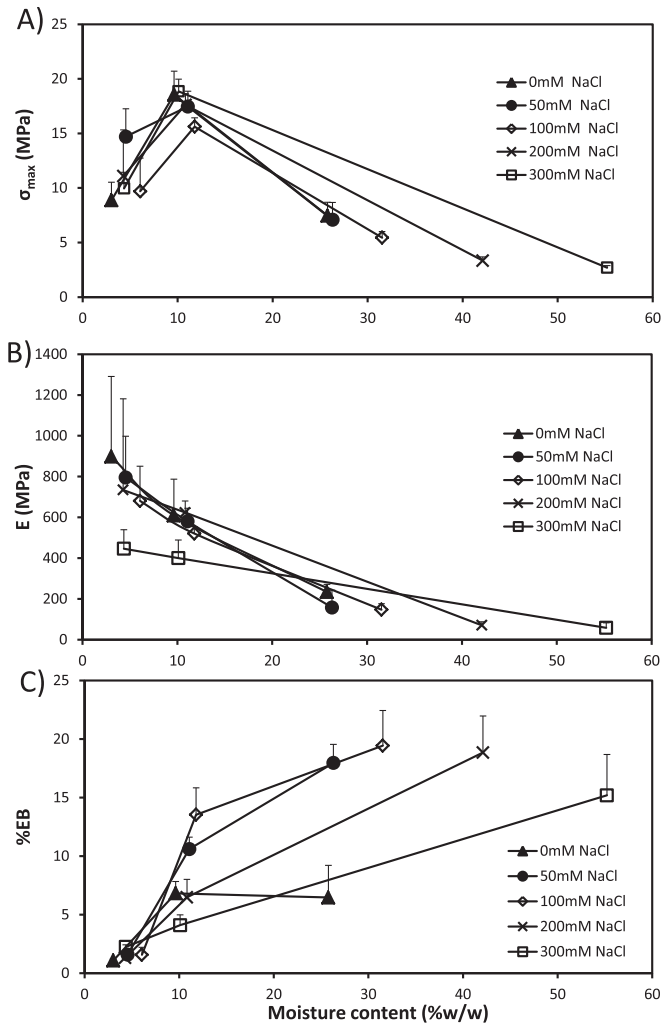


Fig. 3. Effect of sodium chloride concentration and water content on (A) tensile strength,  $\sigma_{max}$ , (B) tensile modulus,  $E$ , and (C) % elongation at break, %EB, as determined from large deformation mechanical testing of the sorbitol-plasticized WPI films.

Moreover, films which were stored at 75% relative humidity absorbed more moisture with increasing the concentration of sodium chloride.

On the other hand, the addition of sodium chloride in sodium caseinate films led to a decrease in  $E$  and  $\sigma_{max}$  (Fig. 4) at low moisture contents. Thus, the addition of sodium chloride appears to produce less rigid caseinate films especially at low moisture contents. That is, with the addition of 100 mM NaCl in the film casting solution a drastic reduction of  $E$  (from 1500 MPa to 540 MPa) and  $\sigma_{max}$  (from 52 MPa to 16 MPa) was observed at ~4% water content, while the addition of 200 mM NaCl resulted in even lower values of  $E$  and  $\sigma_{max}$  for films exposed to similar levels of relative humidity. Sodium caseinate particles are much bigger than whey protein particles and this may have affected the microstructure (homogeneity) and thus the respective mechanical response. When the sodium caseinate films were stored at 75% relative humidity, the films adsorbed more water which may have a plasticizing effect. The % elongation at break of films with 100 mM NaCl was higher than the values of all other samples. Similar results were reported by Fang et al. (2002) who found an increase in %EB up to 10 mM  $Ca^{2+}$  concentration, in whey protein films.

Microscopic observations of the protein films revealed that an inhomogeneous microstructure appeared as NaCl was added

(results not shown). Moreover, the films became opaque macroscopically, implying the formation of aggregates at the micro-level. The non-homogeneous microstructure and the forces between the protein molecules may have a significant impact on the final overall mechanical properties of the films. Further research is needed in this area to fully understand the effects of electrolyte addition in casted biopolymer films on their microstructure and mechanical properties.

### 3.3.3. Effect of pH

The pH decrease from 7.0 to 5.0 caused a pronounced reduction of  $E$ , from 898 MPa to 332 MPa, at ~3% water content (Fig. 5A). However, whey protein films with pH 6.0 had only slightly lower  $E$  values than those of films with pH 7.0 at a similar hydration level. With increasing water content of the samples, the differences ( $E$ ,  $\sigma_{max}$ ) between films became smaller. On the other hand, the %EB increased by changing the pH, with the highest impact being at pH

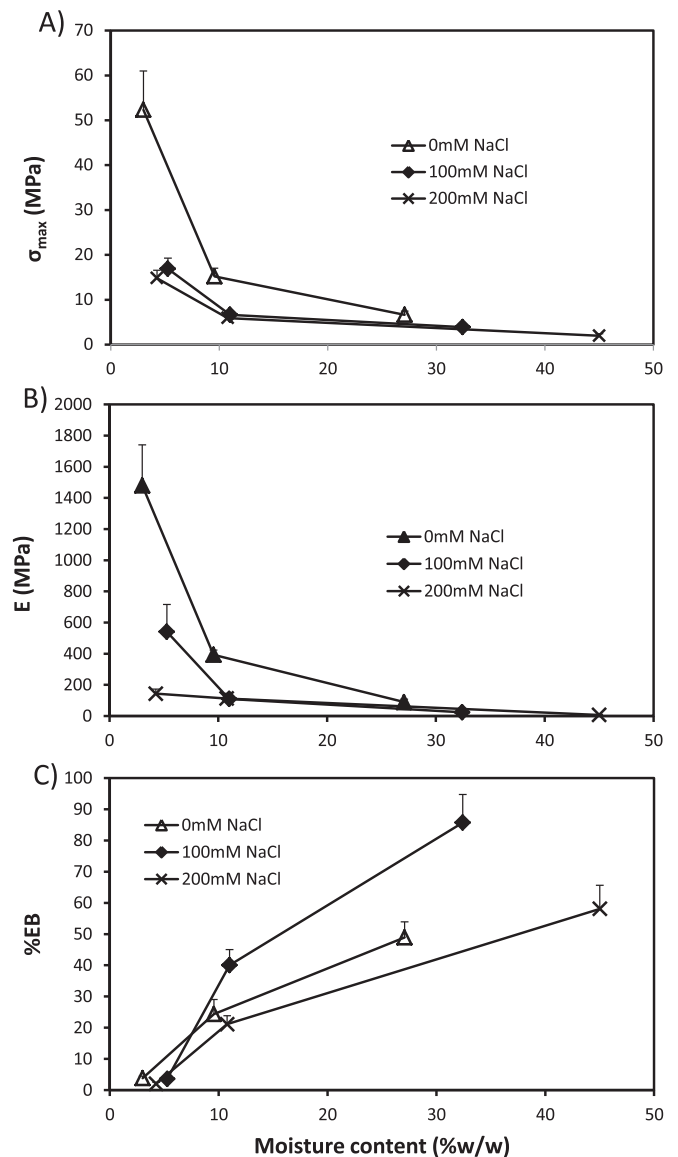


Fig. 4. Effect of sodium chloride concentration and water content on (A) tensile strength,  $\sigma_{max}$ , (B) tensile modulus,  $E$ , and (C) % elongation at break, %EB, as determined from large deformation mechanical testing of the sorbitol-plasticized NaCas films.

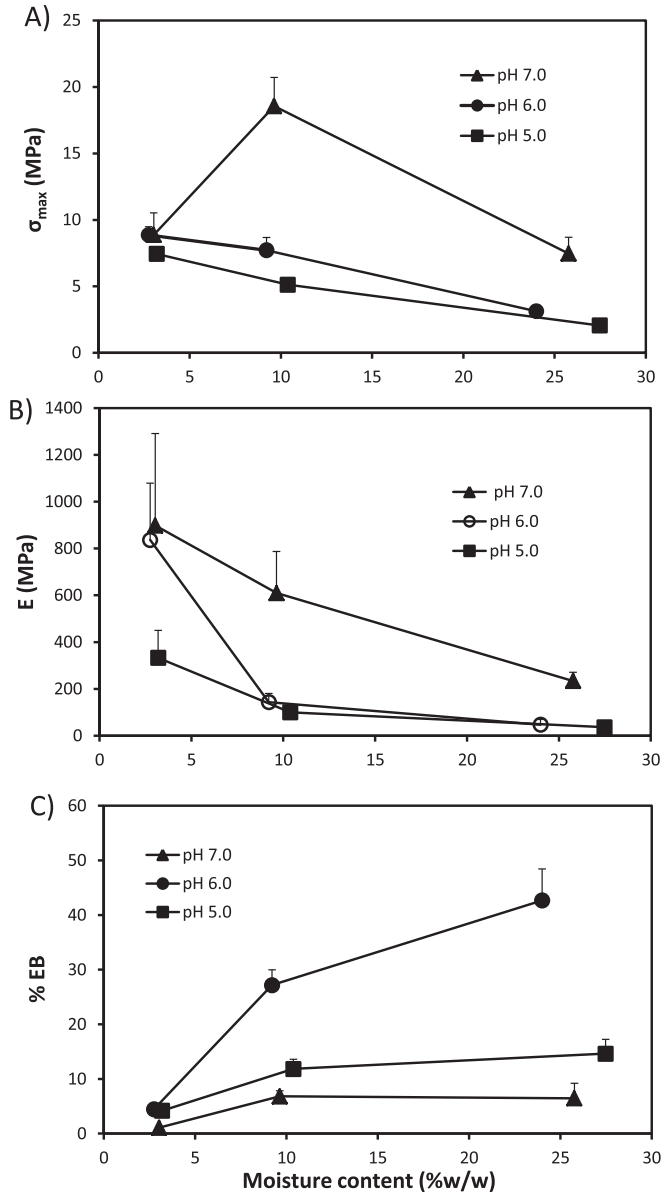


Fig. 5. Influence of pH and water content on (A) tensile strength,  $\sigma_{max}$ , (B) tensile modulus, E, and (C) % elongation at break, % EB, as determined from large deformation mechanical testing of the sorbitol-plasticized WPI films.

6.0. At low humidity environments, there were no significant differences among films obtained by casting WPI solution of different pH values. Reducing the pH to 5.0 resulted in the formation of films with slightly greater flexibility than those with pH 7.0, but not from those with pH 6.0. This could be explained by the fact that at reduced pH, the protein is less negatively charged. This leads to weaker electrostatic repulsions between the protein molecules and it results in translucent/opaque films with very inhomogeneous microstructure. It is believed that the generated non-homogeneous structure may be responsible for the modified mechanical properties.

3.4. Diffusion of antimicrobials

The effect of sodium chloride and pH on the diffusion of potassium sorbate and natamycin from the whey protein films into a water medium was also examined upon continuous stirring. The

edible films were kept intact during the shaking in the liquid medium and attempts were made not to stack to the walls of the glass vessel for a more uniform diffusion of the antifungal agents.

3.4.1. Potassium sorbate diffusion

The kinetics of diffusion-release of potassium sorbate from whey protein films made from solutions containing different concentrations of NaCl (0 mM, 100 mM and 200 mM) and having different pH values (7.0, 6.0 and 5.0) are depicted in Fig. 6, whereas estimates of the diffusion coefficient are summarized in Table 3.

The diffusion coefficient of potassium sorbate, which was measured at ambient temperature at 25 °C and at pH 7.0, was  $\sim 7.23 \times 10^{-7} \text{ cm}^2/\text{s}$ . This value was 10-fold greater than that reported by Redl et al. (1996) for the diffusion of sorbic acid incorporated in edible wheat gluten films ( $7.6 \times 10^{-8} \text{ cm}^2/\text{s}$  at 20 °C). Compared to synthetic polymers, such as LDPE, the diffusion coefficient of potassium sorbate in whey protein films was also one order of magnitude higher. The diffusivity of potassium sorbate in LDPE films was determined by Han and Floros (1998) to be  $1.83 \times 10^{-8} \text{ cm}^2/\text{s}$  at 25 °C.

As shown in Fig. 6, the release of potassium sorbate from whey protein films was initially fast, then decreased and finally reached a plateau. In general, the total release of potassium sorbate from the polymer proceeded within a few minutes after immersing the film in the liquid medium. This behavior was most likely due to the high water solubility of potassium sorbate and the hydrophilicity of the

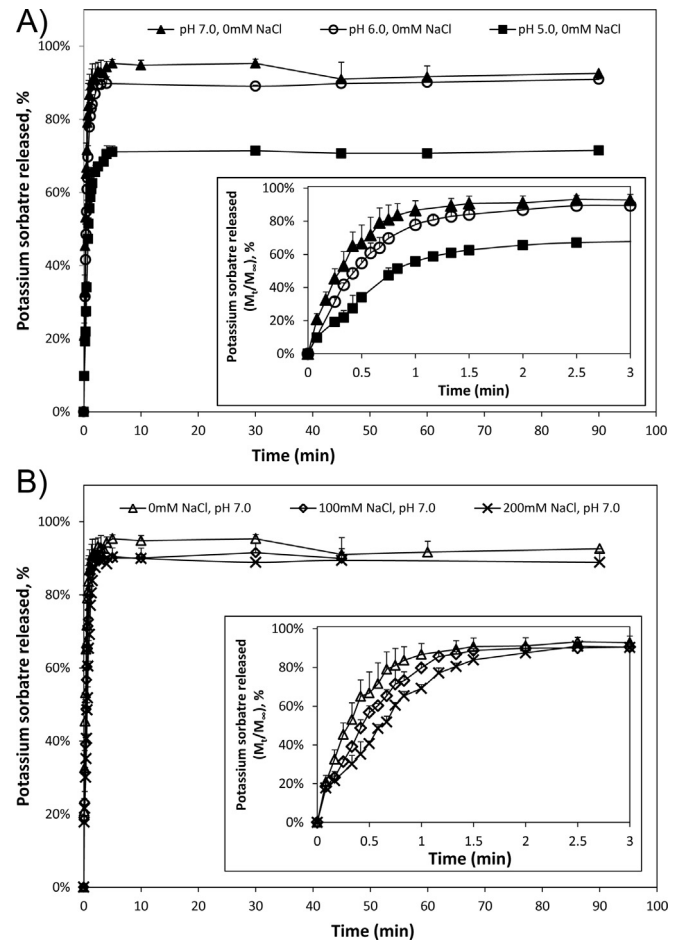


Fig. 6. Diffusion kinetics of potassium sorbate in whey protein films A) at different pH values of the film casting solution and B) by addition of NaCl. The insets show the same data at earlier stages of the experiments.

**Table 3**  
Impact of sodium chloride and pH on the diffusion coefficient of potassium sorbate and natamycin at 25 °C.

Antifungal substance	Diffusion coefficients (cm <sup>2</sup> /s) in WPI films (5%) at 25 °C				
	0 mM NaCl, pH 7.0	100 mM NaCl, pH 7.0	200 mM NaCl, pH 7.0	0 mM NaCl, pH 6.0	0 mM NaCl, pH 5.0
Potassium sorbate ( $\times 10^{-7}$ )	7.23 $\pm$ 1.56 <sup>b</sup>	4.49 $\pm$ 0.33 <sup>ab</sup>	4.44 $\pm$ 0.72 <sup>ab</sup>	4.48 $\pm$ 0.95 <sup>ab</sup>	3.11 $\pm$ 0.28 <sup>a</sup>
Natamycin ( $\times 10^{-9}$ )	8.48 $\pm$ 0.83 <sup>b</sup>	9.13 $\pm$ 2.58 <sup>b</sup>	9.85 $\pm$ 1.93 <sup>b</sup>	3.07 $\pm$ 0.28 <sup>a</sup>	2.21 $\pm$ 0.62 <sup>a</sup>

\* Different letters within the same column indicate significant differences ( $P < 0.05$ ).

polymer matrix; the latter facilitates solvent absorption when the film surface is in contact with the water. Such a diffusion behavior may result in a reduced effectiveness of the film during storage for long periods (Ouattara, Simard, Piette, Begin, & Holley, 2000).

The release of potassium sorbate was also affected by the addition of sodium chloride (Fig. 6B). This may be related to the electrostatic screening of the protein molecules by the salts which can induce structural changes in the polymeric film lattice, making the migration of antimicrobials incorporated in the film a more complex phenomenon (Ouattara et al., 2000).

Moreover, there was a significant difference in potassium sorbate release among the three pH values of the film casting solutions investigated (Fig. 6A). At pH 6.0 and 5.0, the diffusion rate of potassium sorbate decreased as well as the residual quantity of potassium sorbate trapped in the film matrix was higher. This seems more pronounced in films where the pH was adjusted to 5.0 that is close to the isoelectric point of whey proteins where a more compact polymeric matrix is expected.

### 3.4.2. Natamycin diffusion

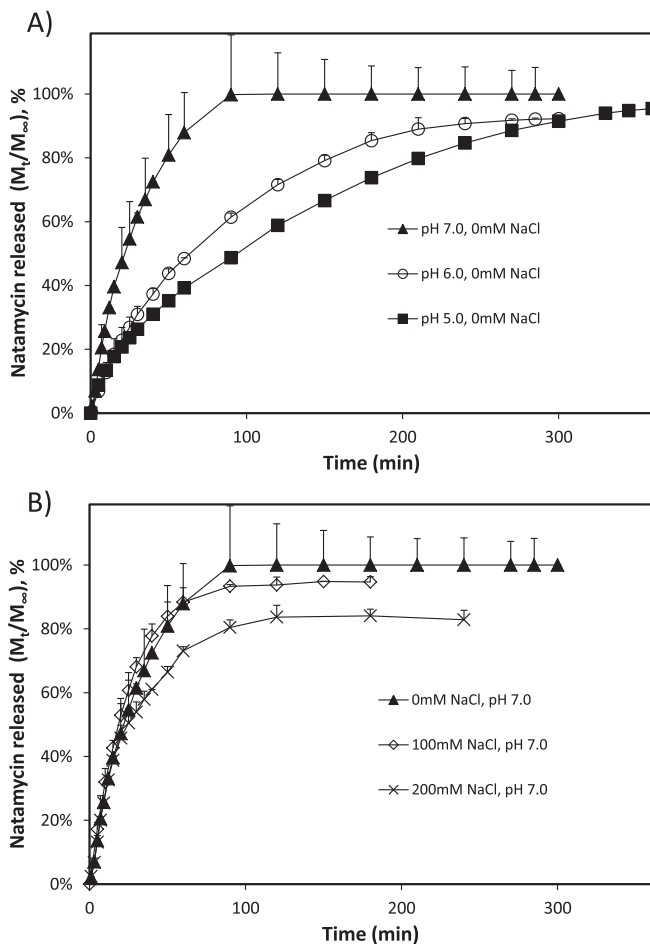
The kinetics of natamycin release from WPI films at different NaCl concentration and pH of the film casting solution are shown in Fig. 7. The release of natamycin was significantly slower than the release of potassium sorbate; the respective diffusion coefficients (~two orders of magnitude lower than those for sorbate) are summarized in Table 3.

The slowest release of natamycin was also observed at pH 5.0, which is close to the isoelectric point of whey proteins. The addition of sodium chloride did not appear to considerably affect the diffusion rate. Because of its larger molecular size, the diffusion of natamycin may be unaffected by small changes in free volume as a result of changes in ionic strength. Overall, the diffusion coefficient of natamycin was several orders of magnitude smaller than that of potassium sorbate. This may be a reflection of the bulkier size and shape of natamycin compared with the smaller, more linear potassium sorbate. Moreover, natamycin has very low solubility in water and therefore its diffusion is restricted. Another behavior that was also evident is the retention of the natamycin in the film matrix. This phenomenon was more pronounced when sodium chloride was added at a concentration of 200 mM, while in other film preparations occurred to a lesser extent.

The effectiveness of antimicrobial films is related not only to the release of the antimicrobial from the film, but also to the diffusion of the antimicrobial compound inside a particular food. For instance, Ouattara et al. (2000) showed that the retention of acetic acid in the chitosan films and its effectiveness is also related to the surface features of the product where the film is applied, i.e. the Bologna sausages (which contain less water than ham and pastrami) show a better retention of acetic acid during storage.

## 4. Conclusions

The present study indicated that the protein ratio, the addition of sodium chloride, and the reduction of pH alter the mechanical and physical properties of dairy protein films which can serve as effective carriers of antimicrobial agents. The results of this study clearly demonstrated that addition of sodium chloride into the whey protein isolate film matrix did not affect the WVP of the films, while it induced a plasticizing effect, as evidenced by a reduction of the  $E$  and an increase of elongation at break upon extension. On the other hand, reduction of pH in the film forming solution to 5.0, assisted a higher moisture uptake and resulted in higher water vapor permeability. The moisture uptake behavior increased with the addition of sodium chloride and films from WPI solutions containing 200 mM NaCl showed a higher water adsorption capacity. The diffusion coefficient of potassium sorbate in WPI films was reduced with the addition of sodium chloride and pH reduction in the film casting solution, while the diffusion coefficient of natamycin decreased only by the change of pH. In addition, the retention of the antimicrobials in the film matrix was increased by increasing the salt concentration or reducing the pH. Generally, the release rate of natamycin was significantly lower than that of potassium sorbate. These results clearly point to the potential of modifying the structural and mechanical properties of edible films in order to modulate the retention and release of the antimicrobials



**Fig. 7.** Diffusion kinetics of natamycin in whey protein films A) at different pH values of the film casting solution and B) by addition of NaCl.

onto food matrices and thereby to affect the fungal growth on the product.

## References

- Baldwin, E. A., Nisperoscarriedo, M. O., & Baker, R. A. (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Critical Reviews in Food Science and Nutrition*, 35(6), 509–524.
- Biliaderis, C. G., Lazaridou, A., & Arvanitoyannis, I. (1999). Glass transition and physical properties of polyol-plasticized pullulan-starch blends at low moisture. *Carbohydrate Polymers*, 40(1), 29–47.
- Buazzi, M. M., & Marth, E. H. (1991). Mechanisms in the inhibition of *Listeria monocytogenes* by potassium sorbate. *Food Microbiology*, 8(3), 249–256.
- Capitan-Valley, L. F., Checa-Moreno, R., & Navas, N. (2000). Rapid ultraviolet spectrophotometric and liquid chromatographic methods for the determination of natamycin in lactoserum matrix. *Journal of AOAC International*, 83(4), 802–808.
- Chen, H. (1995). Functional properties and applications of edible films made of milk proteins. *Journal of Dairy Science*, 78(11), 2563–2583.
- Cho, S. Y., & Rhee, C. (2002). Sorption characteristics of soy protein films and their relation to mechanical properties. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 35(2), 151–157.
- Crank, J. (1975). *The mathematics of diffusion* (2nd ed.). New York: Oxford University Press Inc.
- Cuq, B., Gontard, N., Aymard, C., & Guilbert, S. (1997). Relative humidity and temperature effects on mechanical and water vapor barrier properties of myofibrillar protein-based films. *Polymer Gels and Networks*, 5(1), 1–15.
- Debeaufort, F., Quezada-Gallo, J. A., & Voilley, A. (1998). Edible films and coatings: tomorrow's packagings: a review. *Critical Reviews in Food Science and Nutrition*, 38(4), 299–313.
- Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science*, 67(1), 188–193.
- Gadang, V. P., Hettiarachchy, N. S., Johnson, M. G., & Owens, C. (2008). Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a Turkey frankfurter system. *Journal of Food Science*, 73(8), M389–M394.
- Giannakopoulos, A., & Guilbert, S. (1986). Sorbic acid diffusivity in relation to the composition of high and intermediate moisture model gels and foods. *Journal of Food Technology*, 21(4), 477–485.
- Gontard, N., Duchez, C., Cuq, J. L., & Guilbert, S. (1994). Edible composite films of wheat gluten and lipids – water-vapor permeability and other physical-properties. *International Journal of Food Science and Technology*, 29(1), 39–50.
- Han, J. H., & Floros, J. D. (1998). Simulating diffusion model and determining diffusivity of potassium sorbate through plastics to develop antimicrobial packaging films. *Journal of Food Processing and Preservation*, 22(2), 107–122.
- Kabara, J. J., & Eklund, T. (1991). Organic acids and esters. In G. G. W. & N. J. Russell (Eds.), *Food preservatives* (pp. 23–58). Glasgow, Scotland: Blackie and Son Ltd.
- Kapsalis, J. G. (1987). Influences of hysteresis and temperature on moisture sorption isotherms. In L. B. Rockland, & L. R. Beuchat (Eds.), *Water activity: theory and applications to food* (pp. 173–215). New York: Marcel Dekker.
- Kester, J. J., & Fennema, O. R. (1986). Edible films and coatings – a review. *Food Technology*, 40(12), 47–59.
- Kim, S. J., & Ustunol, Z. (2001). Solubility and moisture sorption isotherms of whey-protein-based edible films as influenced by lipid and plasticizer incorporation. *Journal of Agricultural and Food Chemistry*, 49(9), 4388–4391.
- Kristo, E., Biliaderis, C. G., & Zampraka, A. (2007). Water vapour barrier and tensile properties of composite caseinate-pullulan films: biopolymer composition effects and impact of beeswax lamination. *Food Chemistry*, 101(2), 753–764.
- Kristo, E., Koutsoumanis, K. P., & Biliaderis, C. G. (2008). Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on *Listeria monocytogenes*. *Food Hydrocolloids*, 22(3), 373–386.
- Krochta, J. M., & DeMulderJohnston, C. (1997). Edible and biodegradable polymer films: challenges and opportunities. *Food Technology*, 51(2), 61–74.
- Langton, M., & Hermansson, A. M. (1992). Fine-stranded and particulate gels of beta-lactoglobulin and whey-protein at varying pH. *Food Hydrocolloids*, 5(6), 523–539.
- Lazaridou, A., & Biliaderis, C. G. (2002). Thermophysical properties of chitosan, chitosan–starch and chitosan–pullulan films near the glass transition. *Carbohydrate Polymers*, 48(2), 179–190.
- Lazaridou, A., Biliaderis, C. G., & Kontogiorgos, V. (2003). Molecular weight effects on solution rheology of pullulan and mechanical properties of its films. *Carbohydrate Polymers*, 52(2), 151–166.
- Lewicki, P. P. (1997). The applicability of the GAB model to food water sorption isotherms. *International Journal of Food Science and Technology*, 32(6), 553–557.
- Longares, A., Monahan, F. J., O'Riordan, E. D., & O'Sullivan, M. (2004). Physical properties and sensory evaluation of WPI films of varying thickness. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 37(5), 545–550.
- Longares, A., Monahan, F. J., O'Riordan, E. D., & O'Sullivan, M. (2005). Physical properties of edible films made from mixtures of sodium caseinate and WPI. *International Dairy Journal*, 15(12), 1255–1260.
- Lungu, B., & Johnson, M. G. (2005). Fate of *Listeria monocytogenes* inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate, and sodium lactate at 4 degrees C. *Journal of Food Protection*, 68(4), 855–859.
- Martins, J. T., Cerqueira, M. A., Souza, B. W. S., Avides, M. D., & Vicente, A. A. (2010). Shelf life extension of ricotta cheese using coatings of galactomannans from nonconventional sources incorporating nisin against *Listeria monocytogenes*. *Journal of Agricultural and Food Chemistry*, 58(3), 1884–1891.
- Marzec, A., & Lewicki, P. P. (2006). Antiplasticization of cereal-based products by water. Part I. Extruded flat bread. *Journal of Food Engineering*, 73(1), 1–8.
- Mathlouthi, M. (2001). Water content, water activity, water structure and the stability of foodstuffs. *Food Control*, 12(7), 409–417.
- McHugh, T. H., Aujard, J. F., & Krochta, J. M. (1994). Plasticized whey-protein edible films – water-vapor permeability properties. *Journal of Food Science*, 59(2), 416–423.
- McHugh, T. H., Avenabustillos, R., & Krochta, J. M. (1993). Hydrophilic edible films – modified procedure for water-vapor permeability and explanation of thickness effects. *Journal of Food Science*, 58(4), 899–903.
- McHugh, T. H., & Krochta, J. M. (1994). Water-vapor permeability properties of edible whey protein–lipid emulsion films. *Journal of the American Oil Chemists Society*, 71(3), 307–312.
- Miller, K. S., & Krochta, J. M. (1997). Oxygen and aroma barrier properties of edible films: a review. *Trends in Food Science & Technology*, 8(7), 228–237.
- Min, S., Harris, L. J., & Krochta, J. M. (2005). *Listeria monocytogenes* inhibition by whey protein films and coatings incorporating the lactoperoxidase system. *Journal of Food Science*, 70(7), M317–M324.
- Mitrakas, G. E., Koutsoumanis, K. P., & Lazarides, H. N. (2008). Impact of edible coating with or without anti-microbial agent on microbial growth during osmotic dehydration and refrigerated storage of a model plant material. *Innovative Food Science & Emerging Technologies*, 9(4), 550–555.
- Nayebzadeh, K., Chen, J. S., Dickinson, E., & Moschakis, T. (2006). Surface structure smoothing effect of polysaccharide on a heat-set protein particle gel. *Langmuir*, 22(21), 8873–8880.
- Ouattara, B., Simard, R. E., Piette, G., Begin, A., & Holley, R. A. (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *International Journal of Food Microbiology*, 62(1–2), 139–148.
- Park, S. K., Rhee, C. O., Bae, D. H., & Hettiarachchy, N. S. (2001). Mechanical properties and water-vapor permeability of soy-protein films affected by calcium salts and glucono-delta-lactone. *Journal of Agricultural and Food Chemistry*, 49(5), 2308–2312.
- Perez-Gago, M. B., & Krochta, J. M. (1999). Water vapor permeability of whey protein emulsion films as affected by pH. *Journal of Food Science*, 64(4), 695–698.
- Redl, A., Gontard, N., & Guilbert, S. (1996). Determination of sorbic acid diffusivity in edible wheat gluten and lipid based films. *Journal of Food Science*, 61(1), 116–120.
- Reps, A., Drychowicki, L. J., Tomasik, J., & Winiewska, K. (2002). Natamycin in ripening cheeses. *Pakistan Journal of Nutrition*, 1, 243–247.
- Rockland, L. B. (1960). Saturated salt solutions for static control of relative humidity between 5-degrees-C and 40-degrees-C. *Analytical Chemistry*, 32(10), 1375–1376.
- Shellhammer, T. H., & Krochta, J. M. (1997). Whey protein emulsion film performance as affected by lipid type and amount. *Journal of Food Science*, 62(2), 390–394.
- Siew, D. C. W., Heilmann, C., Easteal, A. J., & Cooney, R. P. (1999). Solution and film properties of sodium caseinate/glycerol and sodium caseinate/polyethylene glycol edible coating systems. *Journal of Agricultural and Food Chemistry*, 47(8), 3432–3440.
- Sofos, J. N., & Busta, F. F. (1993). Sorbic acid and sorbates. In A. L. Branen, & P. M. Davidson (Eds.), *Antimicrobials in foods* (pp. 49–94). New York: Marcel Dekker.
- Stading, M., & Hermansson, A. M. (1990). Viscoelastic behaviour of beta-lactoglobulin gel structures. *Food Hydrocolloids*, 4(2), 121–135.
- Torres, J. A. (1994). *Edible films and coatings from proteins*. In N. S. Hettiarachchy, & G. R. Ziegler (Eds.), *Protein functionality in food systems* (Vol. 9). New York: Marcel Dekker.
- Torres, J. A., Motoki, M., & Karel, M. (1985). Microbial stabilization of intermediate moisture food surfaces. I. Control of surface preservative concentration. *Journal of Food Processing and Preservation*, 9(2), 75–92.
- Var, I., Erginkaya, Z., Guven, M., & Kabak, B. (2006). Effects of antifungal agent and packaging material on microflora of Kashar cheese during storage period. *Food Control*, 17(2), 132–136.
- Vojdani, F., & Torres, J. A. (1990). Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings – effect of fatty-acids. *Journal of Food Science*, 55(3), 841–846.
- Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2010). Physical and thermo-mechanical properties of whey protein isolate films containing antimicrobials, and their effect against spoilage flora of fresh beef. *Food Hydrocolloids*, 24(1), 49–59.