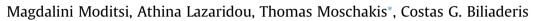
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Modifying the physical properties of dairy protein films for controlled release of antifungal agents



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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

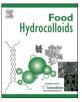
Edible biopolymer films have attracted an increasing amount of research interest and industrial attention as an alternative form of food packaging with reduced environmental and waste-disposal costs (Krochta & DeMulderJohnston, 1997). Edible films are used to prevent moisture permeation, limit gas transport (i.e., O₂, CO₂), retard oil and fat migration, retain volatile flavored compounds, improve mechanical handling of foods (Baldwin, and Nisperoscarriedo, & Baker, 1995; Debeaufort, Quezada-Gallo, & Voilley, 1998; Kester & Fennema, 1986; Krochta 8 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 (pH < 4) are composed of short stiff strands, whereas the fine-stranded structures at high pH (pH > 6) consist of longer, more flexible strands. Moreover, at pH 4–6 whey protein films were white and opaque, at pH < 4 they were transparent and fragile, whereas at 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 pH ~ 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/dm², 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 $^{\circ}$ 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

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