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Physical and thermo-mechanical properties of whey protein isolate films containing antimicrobials, and their effect against spoilage flora of fresh beef

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ABSTRACT

The effectiveness of antimicrobial films against beef's spoilage flora during storage at 5 °C and the impact of the antimicrobial agents on the mechanical and physical properties of the films were examined. Antimicrobial films were prepared by incorporating different levels of sodium lactate (NaL) and ε-polylysine (ε-PL) into sorbitol-plasticized whey protein isolate (WPI) films. The moisture uptake behavior and the water vapor permeability (WVP) were affected only by the addition of NaL at all concentrations used since an increased water uptake and permeability were observed with the addition of NaL into the protein matrix. An increase of the glass transition temperature (5–15 °C) of the sorbitol region, as determined by Dynamic Mechanical Thermal Analysis (DMTA), was caused by the addition of ε-PL into the WPI specimens. Instead, incorporation of NaL into the protein matrix did not alter its thermo-mechanical behavior. The addition of NaL at concentrations of 1.0% and 1.5% w/w in the film-forming solution resulted in a decline of maximum tensile strength (σ_{max}) and Young modulus (E). A decrease of E and σ_{max}, accompanied with an increase in elongation at break (%EB), was also observed with increasing ε-PL concentration, at moisture contents higher that 10% (w/w). The antimicrobial activity of the composite WPI films was tested on fresh beef cut portions. The maximum specific growth rate (μ_{max}) of total flora (total viable count, TVC) was significantly reduced with the use of antimicrobial films made from 0.75% w/w ε-PL in film-forming solutions ($p < 0.05$), while the growth of Lactic Acid Bacteria was completely inhibited. Significant inhibition of growth of the total flora and pseudomonads was also observed with the use of films made with protein solutions containing 2.0% w/w NaL. These results pointed to the effectiveness of the antimicrobial whey protein films to extend the shelf life of fresh beef.

1. Introduction

The increased interest in "ready to eat" and easy to consume products enhances the obligation for greater control on food quality and safety. Outbreaks of foodborne diseases brought the necessity for alternative methods in controlling microbial growth in food products (Appendini & Hotchkiss, 2002). A new trend in food preservation consists of the use of active packaging in order to enlarge the safety margin and reassure high quality products. Antimicrobial packaging materials can effectively control the microbial contamination of solid or semi-solid food products by inhibiting the growth of spoilage or pathogenic microorganisms on the surface of the food. Incorporation of antimicrobial compounds into films results in decreased diffusion rates from the packaging material into the product, thus assisting the maintenance of high concentrations of the active ingredient where it is required (Kristo, Koutsoumanis, & Biliaderis, 2008). Antimicrobial packaging and its applications in the food industry has been thoroughly reviewed recently (Cagri, Ustunol, & Ryser, 2004; Cha & Chinnan, 2004; Coma, 2008; Gennadios, Hanna, & Kurth, 1997; Ozdemir & Floros, 2008; Quintavalla & Vicini, 2002).

Lactate salts such as sodium lactate (NaL) are widely used as flavor enhancers in meat and poultry products, contributing to increased cooking yields and water holding capacity (Aymerich, Jofre, Garriga, & Hugas, 2005; Lungu & Johnson, 2005; Shelef, 1994). Various salts of lactic or other organic acids have also demonstrated antimicrobial activity in laboratory media or food products (Barmpalia et al., 2005; Koutsoumanis et al., 2004; Mbandi & Shelef, 2001, 2002; Samelis et al., 2001). Surprisingly, more pronounced antibacterial effects of lactates in meat than in broth have been reported (Shelef, 1994). NaL has been characterized Generally Recognised as Safe (GRAS) and is usually added at a level of 2–3% based on the finished product weight (Kristo et al., 2008).

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E-polylysine (ε-PL) is a cationic homopolymer of 25–35 L-lysine units interlinked by a peptide bond between the carboxyl and ε-amino groups of L-lysine residues (Hiraki, Hatakeyama, Morita, & Izumi, 1998; Yoshida & Nagasawa, 2003). This compound is heat stable even under acidic conditions and exhibits a wide antimicrobial activity against Gram(+) and Gram(−) bacteria, yeasts and moulds (Hiraki, 2000; Shih, Shen, & Van, 2006; Shima, Matsuoka, Iwamoto, & Sakai, 1984; Yoshida & Nagasawa, 2003). Additionally, it has been suggested that ε-PL is able to suppress dietary fat adsorption from the small intestine by inhibiting pancreatic lipase activity (Kido et al., 2003). The safety of ε-PL as a food additive has been demonstrated by experiments in rats and for this reason it has been used in Japan for the preservation of fish sushi, cooked vegetables, noodles and other products (Hiraki et al., 2003). In 2004, this compound was recognised as safe (GRAS) by the U.S. Food and Drug Administration for use as an antimicrobial agent in cooked or sushi rice at levels up to 50 mg/kg of rice (USFDA, 2004).

In contrast to the large amount of information on the use of various antimicrobial films for controlling meat pathogens, little is known about their effect on the spoilage microflora of these products. In the present study fresh beef cuts were wrapped into WPI films containing NaL or ε-PL at two different levels. The effectiveness of these films against beef's spoilage flora during storage at 5 °C was studied. Additionally, the impact of the antimicrobial agents on the mechanical and physical properties of the films was studied since the overall performance of the films depends strongly on their physicochemical properties.

2. Materials and methods

2.1. Film preparation

Bipro, a whey protein isolate (Davisco Foods International, Le Sueur, MN, USA), was dissolved in distilled water under continuous stirring to obtain film-forming solutions of 5% (w/w). The protein solutions were placed in a water bath at 90 °C for 30 min while being stirred continuously; heating the protein is essential for the formation of intermolecular disulfide bonds. This process is necessary to obtain a flexible film via covalent and non-covalent cross-linking that retains its integrity at high moisture environments (Le Tien et al., 2000; Vachon et al., 2000). Solutions were then rapidly cooled in an ice water bath to avoid further denaturation, and sorbitol (Sigma, St. Louis, MO, USA) was added as a plasticizer at a constant concentration of 37.5% (sorbitol/(WPI + sorbitol)). Such a concentration of sorbitol was necessary to overcome the brittleness of WPI films, which otherwise are very difficult to handle without breaking. Appropriate amounts of the antimicrobials were added in the film-forming solution resulting in a final concentration of 1.0%, 1.5% and 2.0% (w/w) for NaL (50% solution, Merck KGaA, Germany) and 0.25%, 0.50% and 0.75% (w/w) for ε-PL (Chisso Corp., Tokyo, Japan). The solutions were kept overnight at 4 °C to remove air bubbles. Portions of 12.5 g solution were cast on Petri dishes (φ 8.5 cm) and allowed to dry in an oven at 35 °C for ~24 h. In order to prepare thick specimens for the dynamic mechanical thermal analysis (DMTA), Petri dishes (φ 13.0 cm) were completely filled with the protein solution and allowed to dry in an oven at 35 °C for ~72 h. Film thickness was determined using a manual micrometer at 5 random positions on the film to obtain an average value.

2.2. Moisture sorption isotherms

Moisture sorption isotherms were determined for all films according to Biliaderis, Lazaridou, and Arvanitoyannis (1999). Film samples (~300 mg) were placed in previously weighed aluminum dishes and dried at 45 °C in an air-circulated oven over silica gel (Sigma–Aldrich GmbH, Germany) until constant weight. The samples were subsequently kept in desiccators over saturated salt solutions of known relative humidity (RH) at 25 °C for 21 days, a time sufficient to reach constant weight and hence practical equilibrium. The moisture content of samples, after storage, was determined by drying at 110 °C for 2 h. The obtained data were fitted to the Brunauer–Emmett–Teller (BET) or Guggenheim–Anderson–DeBoer (GAB) sorption isotherm models.

The BET model is given by the equation:

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

where 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:

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

where m_m is the GAB monolayer value, and K and C are constants.

All sorption measurements were performed at least in triplicate.

2.3. Water vapor permeability

Water vapor permeability (WVP) measurements of films were conducted at 25 °C using the ASTM (E96-63T) procedure modified for the vapor pressure at film underside according to McHugh, Avena-Bustillos, and Krochta (1993). Film discs, previously equilibrated at 53% RH for 48 h, were sealed in cups containing distilled water and the cups were placed in an air-circulated oven at 25 °C that was equilibrated at 53% RH using a saturated solution of $MgCl_2 \times 6H_2O$ (Merck KgaA, Darmstadt, Germany). Film permeabilities were determined as described by Kristo, Biliaderis, and Zampraka (2007). The steady-state water vapor flow was reached within 1 h for all films. Slopes were calculated by linear regression and correlation coefficients for all reported data were >0.99. At least five replicates of each film type were tested for WVP.

2.4. Dynamic mechanical thermal analysis

Thick WPI specimens ($0.5 \times 0.6 \times 0.15$ cm^3) prepared for DMTA analysis were previously conditioned at various RH environments (33, 43, 53 and 75%) over saturated salt solutions for at least one month. The moisture content of each film was evaluated by drying the sample after the DMTA measurement at 110 °C for 2 h. The thermo-mechanical measurements were performed with a Mark III analyzer (Polymer Labs. Loughborough, UK) operated in the single cantilever bending mode (heating rate 2°C min^{-1} and a strain level equal to a maximum displacement of 16 μm). The DMTA thermal scans were performed at five frequencies, i.e. 1, 3, 5, 10 and 20 Hz, and the T_g values were taken as the peaks in tan δ of the protein polymeric matrix.

2.5. Large deformation mechanical testing

Films were cut in dumbell form strips and stored at appropriate RH environments (11%, 23%, 43%, 53% and 75%) for at least 10 days to obtain specimens with different moisture contents. Film thickness was evaluated at three different points with a hand-held micrometer and an average value was obtained. Samples were analyzed with a TA-XT2i instrument (Stable Micro systems, Godalming, Surrey, UK) in the tensile mode operated at ambient temperature and a crosshead speed of 60 mm min^{-1}. Young's