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Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef

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ABSTRACT

Antimicrobial films were prepared by incorporating different levels of oregano oil (0.5%, 1.0%, and 1.5% w/w in the film forming solution) into sorbitol-plasticized whey protein isolate (WPI) films. The moisture uptake behavior and the water vapor permeability (WVP) were not affected by the addition of oregano oil at any of the concentrations used. A reduction of the glass transition temperature (~10–20 °C), as determined by dynamic mechanical thermal analysis (DMTA), was caused by addition of oil into the protein matrix. A decrease of Young modulus (E) and maximum tensile strength (σ_{max}) accompanied with an increase in elongation at break (%EB) was observed with increasing oil concentration up to a level of 1.0% (w/w). Wrapping of beef cuts with the antimicrobial films resulted in smaller changes in total color difference (ΔE) and saturation difference (Δ_{chroma}) during refrigeration (5 °C, 12 days). The maximum specific growth rate (μ_{max}) of total flora (total viable count, TVC) and pseudomonads were significantly reduced ($P < 0.05$) by a factor of two with the use of antimicrobial films (1.5% w/w oil in the film forming solution), while the growth of lactic acid bacteria was completely inhibited. These results pointed to the effectiveness of oregano oil containing whey protein films to increase the shelf life of fresh beef.

1. Introduction

Whey proteins are a by-product of the cheese-making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Nowadays, great efforts are being made to find new uses for whey proteins, e.g. production of edible films (Anker, Stading, & Hermansson, 1998). Edible or biodegradable films constitute a convenient means to prolong the shelf life of foods and increase their quality without contributing to environmental pollution. Apart from acting as selective barriers for moisture, gas and solute migration, these films may operate as carriers of many functional ingredients. Such ingredients may include antioxidants, antimicrobial agents, flavors, spices and colorants which improve the functionality of the packaging materials by adding novel or extra functions (Salmieri & Lacroix, 2006). Antimicrobial packaging and its applications in the food industry has been thoroughly reviewed (Cagri, Ustunol, & Ryser, 2004; Cha & Chinnan, 2004; Coma, 2008; Gennadios, Hanna, & Kurth, 1997; Ozdemir & Floros, 2004; Quintavalla & Vicini, 2002). 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 they are required (Kristo, Koutsoumanis, & Biliaderis, 2008).

Essential oils (EOs) extracted from plants or spices are rich sources of biologically active compounds such as terpenoids and phenolic acids. It has been long recognised that some of the EOs have antimicrobial properties (Burt, 2004; Nychas, 1995; Shelef, 1983). Within a great variety of EOs, oregano oil that contains large amounts of carvacrol is considered to be one of the most active plant extracts against pathogens (López, Sánchez, Batlle, & Nerín, 2005, 2007). The hydroxyl group present in the structure of phenolic compounds confers antimicrobial activity and its relative position is very crucial for the effectiveness of these natural components; this can explain the superior antimicrobial activity of carvacrol compared to other plant phenolics. Although most of the EOs are classified as Generally Recognized as Safe (GRAS) their use as food preservatives is often limited due to flavouring considerations since effective antimicrobial doses may exceed organoleptically acceptable levels. However, incorporation of oregano oil in edible films seems rather appealing since, due to the decreased diffusion rate of the active compounds smaller amounts will be required to accomplish the desired antimicrobial effect.

In the present study, fresh beef cuts were wrapped in WPI films containing oregano oil at three different levels. The effectiveness of the films against the beef's spoilage flora during storage at 5 °C was investigated. Additionally, the impact of the oregano oil on the mechanical and physical properties of the films was examined since the functional behavior of the films is a combination of both their antimicrobial and physico-chemical properties.

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2. Materials and methods

2.1. Film preparation

Bipro, a whey protein isolate (WPI) (Davisco Foods International), was dissolved in distilled water under continuous stirring to obtain film forming solutions of either 8% (w/w) for preparing thick specimens for dynamic mechanical thermal analysis (DMTA) or 5% (w/w) concentration for the rest of the measurements. 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 to assist the establishment of a cross-linked polymeric network structure. This process is necessary to obtain a flexible film that retains its structural integrity in 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 (St. Louis, MO, USA) was added as a plasticizer at the 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. Oregano oil (*Origanum vulgare* sp. *Hirtum*, Ecopharm, Greece) at 0.5%, 1.0% and 1.5% (w/w) ratios was added to the film forming solutions. Equivalent amounts were also added for preparation of the DMTA samples in order to obtain the same oil concentration on a dry basis. The solutions were homogenized at room temperature for 2 min at 13,000 rpm and 2 min at 19,000 rpm using an Ultra-Turrax (T-25 basic, IKA, Werbe). The solutions were then 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. Film thickness was determined using a manual micrometer at five random positions on the film to obtain an average value.

2.1.1. 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 (Durango et al., 2006) or the 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 α_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.

Measurements were performed at least in triplicate.

2.1.2. 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 (15.20 cm^2), previously equilibrated at 53% RH for 48 h, were sealed into cups containing distilled water and the cups were placed in an air-circulated oven at 25 °C equilibrated at 53% RH using a saturated solution of $MgCl_2 \times 6H_2O$ (Merck KgaA, Darmstadt, Germany). Film permeability was essentially determined according to 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.

2.1.3. Dynamic mechanical thermal analysis

Thick WPI specimens ($0.5 \times 0.6 \times 0.15$ cm^3) prepared for DMTA analysis were conditioned at various RH's (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 measurement at 110 °C for 2 h. The DMTA 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 periodical displacement of 16 μm). The T_g of the samples was determined as the peak in tan δ at 3 Hz.

2.1.4. Large deformation mechanical testing

Films were cut in dumbbell form strips and stored at appropriate RH's (11%, 23%, 43%, 53% and 75%) for at least 10 days to obtain films with different moisture contents. Film thickness was measured 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 modulus (E), tensile strength (σ_{max}) and % elongation at break (%EB) were calculated from the load–deformation curves of tensile testing (Lazaridou, Biliaderis, & Kontogiorgos, 2003). The data represent averages of measurements of at least eight samples. The moisture content of the samples, after storage, was determined by drying at 110 °C for 2 h.

2.2. Meat sample preparation and storage

Freshly cut beef was purchased from a local retail store. The meat was divided in small pieces ($2.1 \times 2.5 \times 1$ cm) and these were wrapped in cross-shaped antimicrobial films that covered the entire meat surface. Samples that were not covered with the films served as controls. The meat samples were placed into a sterile plastic dish covered with plastic film and stored in high precision (±0.2 °C) low-temperature incubators (model MIR 153; Sanyo Electric Co., Ora-Gun, Gumma, Japan) at 5 °C; all samples were evaluated periodically for color and microbiological quality (0, 2, 4, 6, 8, 10 and 12 days).

2.2.1. Colorimetric measurements

The changes in color of the beef pieces wrapped in the antimicrobial films were evaluated by measuring the L^*, a^* and b^* parameters using a portable colorimeter (Chroma Meter, model CR-400; Minolta, Osaka, Japan). The measured color parameters were used to calculate changes in total color (ΔE) and saturation difference (Δ_{chroma}) (Boakye & Mittal, 1996), according to the following equations:

$$\Delta E = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2}$$

$$\Delta_{chroma} = (a_0^{2*} + b_0^{2*})^{1/2} - (a^{2*} + b^{2*})^{1/2}$$

The colorimeter was calibrated using a white standard plate. For each treatment four samples were measured and on each beef piece four readings were made.