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Characterization of *Citrus* pectin edible films containing transglutaminase-modified phaseolin

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ABSTRACT

The growing social and economic consequences of pollution derived from plastics are focusing attention on the need to produce novel bioprocesses for enhancing food shelf-life. As a consequence, in recent years the use of edible films for food packaging is generating a huge scientific interest. In this work we report the production of an edible hydrocolloid film made by using *Citrus* pectin and the protein phaseolin crosslinked by microbial transglutaminase, an enzyme able to covalently modify proteins by formation of isopeptide bonds between glutamine and lysine residues. The films were characterized and their morphology was evaluated by both atomic force microscopy and scanning electron microscopy. Mechanical properties and barrier properties to CO_2, O_2 and water vapor have demonstrated that these films possess technological features comparable to those possessed by commercial plastics. It is worth noting that these characteristics are maintained even following storage of the films at 4 °C or −20 °C, suggesting that our bioplastics can be tailored to protect food at low temperature. Moreover, gastric and duodenal digestion studies conducted under the same conditions found in the human digestion system have demonstrated that transglutaminase-containing films are regularly digested encouraging an application of the proposed materials as food coatings.

1. Introduction

As the pollution levels derived from commercial plastics rise rapidly in the EU, the food industry is heavily pushing the development of new innovative products which allow consumers to better sustain their health and well-being. Consumers are becoming increasingly aware of the relationship between pollution and health, emphasizing a need for the production of novel biodegradable plastics with the double aim of enhancing the shelf-life of many food-based products and of reducing the amount of plastic packaging that causes environmental concerns. The challenge is to target nanosciences toward the development of natural structures for food protection.

For this reason edible films based on biopolymers are gaining a great interest in the scientific community because of their biodegradable nature and for their potential use in food industry (Cagri, Ustunol, & Ryser, 2004). In recent years, in fact, several research projects have been carried out to develop edible films based on natural products coming from both agriculture and food industry wastes (Giosafatto, Mariniello, & Ring, 2007; Mariniello, Giosafatto, Di Pierro, Sorrentino, & Porta, 2007; Mariniello, Giosafatto, Moschetti, et al., 2007).

Packaging systems exert the function of food protection by influencing the transport with which low molecular weight substances, responsible for the deterioration of the product, permeate through the packaging. These systems promote an extension of the shelf-life of the product, improving its quality and organoleptic characteristics. Edible films have long been known to exert a protective effect for fruit and vegetables delaying dehydration, reducing respiration, improving the structural quality, helping to preserve the volatile compounds and reduce microbial spoilage (Campos, Gerschenson, & Flores, 2011). Another interesting feature of these materials is their ability to reduce bacterial and fungal infections of semi-processed meat products as well as of ready-to-eat fish (Campos et al., 2011). From a structure point of view, the edible films can be classified as (1) hydrocolloid, (2) lipid and (3) composite (Donhowe & Fennema, 1994). Hydrocolloid films are composed of proteins, cellulose derivatives, pectin (Pec) and other polysaccharides; lipid films consist of waxes, acylglycerols and fatty acids, while the composite films generally contain both lipid and

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hydrocolloid components. Among the polysaccharides, cellulose, starch, alginate, chitosan, Pec and their derivatives are commonly used for the synthesis of these types of films (Donhowe & Fennema, 1994).

In this work we have developed hydrocolloid edible films made of *Citrus* Pec and the protein phaseolin (Ph) modified or not by the enzyme microbial transglutaminase (TG). The use of transglutaminases (EC 2.3.2.13) has been proposed for their ability to catalyze intra and/or intermolecular isopeptide bonds between the γ-carboxamide group of glutamine (acyl donor) and ε-amino group of lysine residues (acyl acceptor) (Cozzolino et al., 2003; Di Pierro, Sorrentino, Mariniello, Giosafatto, & Porta, 2011; Porta, Mariniello, Di Pierro, Sorrentino, & Giosafatto, 2011; Valdivia et al., 2006). In fact, in our previous investigations we have demonstrated that Ph is able to act as an effective substrate for TG (Mariniello, Giosafatto, Di Pierro, et al., 2007; Mariniello, Giosafatto, Di Pierro, Sorrentino, & Porta, 2010; Mariniello, Giosafatto, Moschetti, et al., 2007). The Pec/Ph films were characterized according to their mechanical and barrier properties to water vapor, CO_2 and O_2. The films were analyzed also by Atomic Force Microscopy (AFM), a powerful tool used to evaluate film surface topography (a qualitative parameter) and roughness (a quantitative parameter) (Ghanbarzadeha, Oromiehib, & Razmi-Radb, 2008). Nanoscale measurements by AFM allow to study the influence of different factors on film hardness, elasticity and permeability, extremely useful for designing high-performance edible food packaging. Film morphology characterization was also performed by Scanning Electron Microscopy (SEM). In addition, digestibility studies, carried out under physiological conditions were performed in order to propose these innovative edible films as new candidates for protecting different kinds of food addressed to the human consumption.

2. Experimental procedures

2.1. Materials

Phaseolus vulgaris L. beans were purchased from a local supermarket. Chemicals for electrophoresis were from Bio-Rad (Segrate, Milano, Italy). Microbial TG (Activa WM), derived from the culture of *Streptoverticillium* sp., was supplied by Ajinomoto Co. (Japan). *Citrus* Pec, trypsin from porcine pancreas (product T0303, activity 17,000 U/mg protein), chymotrypsin from bovine pancreas (product C7762, activity 58 U/mg protein), pepsin from porcine gastric mucosa (product P6887, activity 4220 U/mg protein), soybean Bowmann-Birk trypsin-chymotrypsin inhibitor, bile salts, and all other reagents were purchased from Sigma Chemical Company (Pool, Dorset, UK). Chemicals were of analytical grade, unless specified.

2.2. Methods

2.2.1. Ph purification

Ph was isolated from *P. vulgaris* beans by using the ascorbate-NaCl procedure described by Sun and Hall (1975) and modified by Mariniello, Giosafatto, Di Pierro, et al. (2007). To achieve maximum extraction of phaseolin, the extraction steps were repeated three times, and to maximize precipitation of phaseolin, the samples were kept in the dark at 4 °C for 30 min and then centrifuged for 20 min. The purified protein was dissolved into distilled water at a concentration of 7 mg mL^{-1}.

2.2.2. TG preparation

The enzyme solution was prepared by dissolving the commercial preparation (containing 1% of TG and 99% of maltodextrins) in distilled water at a concentration of 180 mg mL^{-1} and the mixture was centrifuged at 10,000 × *g* for 2 min to remove precipitates. The specific activity of the enzyme was 92 U/g. Estimation of enzymatic activity was carried out by a colorimetric hydroxamate assay according to Pasternack et al. (1998).

2.2.3. Citrus Pec preparation

3.2 g of *Citrus* Pec were dissolved in 200 mL of distilled water. The solution was stirred until the Pec was completely solubilized. Then the pH of the solution was adjusted to 5 by using HCl 3 N.

2.2.4. Film forming procedure

Two different kinds of films were prepared: Pectin and Phaseolin based-films (Pec/Ph films) and Pectin and Phaseolin-based films made in the presence of TG (Pec/Fas/TG films). Films were cast by pouring the solution into 5 cm diameter polystyrene Petri dishes. For Pec/Ph films, 12.5 mL of Pec solution (2%, w/w) were mixed with 2.6 mL of Ph solution and spread into the plates. Pec/Ph/TG films were obtained by adding 0.35 U of the enzyme to the final solution of Pec and Ph. All the samples were prepared in the presence of 6% glycerol (with respect to protein content). In fact, preliminary experiments, aimed to study the effect of different amounts (6%, 12% and 24%) of plasticizer, demonstrated no differences on film features at higher glycerol concentrations. The solutions were allowed to dry at 37 °C for 18 h under air circulation. Dried films were peeled intact from the casting surface and conditioned at 50% RH and at 25 °C for 48 h before being tested.

2.2.5. Protein determination

Protein determination was carried out by the Bio-Rad Protein Assay (Bio-Rad), using bovine serum albumin as standard (Bradford, 1976).

2.2.6. Film characterization

Thickness: Film thickness was measured using an electronic digital micrometer with a sensitivity of 2 μm (Metrocontrol, Srl, model HO62). Film strips were placed between the jaws of the micrometer and the gap was reduced until the minimum friction was measured. Mean thickness (mm) was determined from the average of measurements at five locations.

Film water vapor permeability: Film water vapor permeability (WVP) was evaluated by a gravimetric test according to ASTM E96 (1993) by means of a Fisher/Payne permeability cup (Carlo Erba, Italy) as described by Di Pierro, Mariniello, Giosafatto, Masi, & Porta (2005). Silica gel (3 g) was introduced into each cup, and a film sample disk with a diameter of about 6 cm was placed on top of the cup and sealed by means of a ring kept in place by three tight clamps. The film area exposed to vapor transmission was 10 cm^2. The assembled cups were weighted and then placed in a desiccator containing a saturated KCl solution that provided a constant water activity of 0.8434 at 25 °C. The desiccator was stored in a Heareus thermostated incubator at 25.0 ± 0.1 °C. Cups were weighed at scheduled times and the water vapor transmission rate through the film was estimated by the linear portion of the diagram obtained by plotting the weight increment of the cup as a function of time. It was assumed that steady-state was reached once the regression analyses made by using the last four data points resulted in $r^2 \geq 0.998$. The WVP was calculated from the equation

$$\mathrm{WVP} = \frac{X}{A\Delta p}\frac{dm}{dt}$$

where dm/dt is the slope of the cup weight *versus* time once steady state was reached, X is the film thickness, A is the film exposed area, and Δp is the water vapor pressure across the film. By assuming that the vapor pressure inside the cup, due to the presence of silica gel, can be taken as equal to zero, Δp becomes equal to the vapor pressure inside the desiccator given by the product of the water activity and water saturation pressure (P0) at 25 °C (P0) 3.167 kPa).