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Active biodegradable sodium caseinate films manufactured by blown-film extrusion: Effect of thermo-mechanical processing parameters and formulation on lysozyme stability

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ABSTRACT

Sodium caseinate-based edible/biodegradable films containing lysozyme were prepared directly by extrusion processes to obtain antimicrobial films. Thermoplastic pellets containing 1% (w/w) lysozyme were blown by an industrial-like blown-film-extruder to obtain thermoplastic thin films. Lysozyme stability depended mainly on processing temperature and glycerol content. A correct choice of these parameters allowed obtaining pellets and films with 40.8 ± 0.5% and 26.4 ± 0.6% lysozyme residual activity, respectively. Whatever the storage temperature (−20 °C or room temperature), lysozyme activity of pellets remained constant for at least 5 weeks of storage. Antimicrobial activity against *Micrococcus luteus* was assayed in Tryptone Soya Broth (TSB): no bacterial growth was observed for 25 h at 37 °C in the presence of 0.05 g.L^{-1} pellets, while a 2.4 log increase of bacterial population was observed in the presence of control pellets without lysozyme. Traditional properties of films such as their water sorption and mechanical properties were also investigated and proved that addition of lysozyme at a 1% concentration did not affect these properties as well as their ultimate aerobic biodegradability. The application potential of such edible antimicrobial films is discussed in relation with these properties.

1. Introduction

The globalization of food markets and increasing concerns on food stability and control lead researchers and industrial companies to develop active and intelligent packaging materials. Biodegradable films as carriers of active compounds are promising applications of active food packaging (Han and Gennadios, 2005). Antimicrobial biodegradable films provide not only the opportunity to extend shelf-life of foods by preventing contamination or growth of unwanted microorganisms, but also provide the possibility to reduce environmental impact of conventional packaging materials (Chen, 1995; Krochta and De Mulder-Johnston, 1997; Hernandez-Izquierdo, 2008). Such films can be obtained mainly by two processes. The "wet process" is based on the preparation of a film-forming solution by homogeneous dispersion or solubilization of biopolymer and successive evaporation of the solvent on a substrate (Gontard and Guilbert, 1996). The "dry process" involves thermomechanical processes such as extrusion and compression molding. These methods are getting documented more and more in the literature for the elaboration of hydrocolloids-based biomaterials (Belyamani et al., 2014; Guerrero et al., 2012; Hanani et al., 2012; Hernandez-Izquierdo and Krochta, 2009; Jerez et al., 2005, 2007a, 2007b; Martinez et al., 2013; Nam et al., 2007; Pommet et al., 2005; Ture et al., 2012; Zhang et al., 2001). Krochta (2002) suggested that an efficient production by conventional extrusion equipment would certainly encourage the commercialization of biodegradable or edible films. One bottleneck to the elaboration of biodegradable films incorporating antimicrobial agents such as enzymes by thermo-mechanical processes such as extrusion is due to their limited thermal stability. Del Nobile et al. (2009) published

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one of the rare studies challenging the fabrication of active films using recyclable or biodegradable polymers such as high density polyethylene (HDPE), polylactide (PLA) and polycaprolactone (PCL) by dry extrusion processes. Nam et al. (2007), on the other hand, successfully showed the possibility to obtain antimicrobial edible films formulated with pea starch and lysozyme by twin-screw extrusion process. These authors assayed the residual enzymatic activity of lysozyme after twin-screw extrusion. They observed that lysozyme activity is significantly affected by temperature and shear stress induced by thermomechanical processes.

Hen egg white lysozyme (E1105) is a widely used enzyme authorized for food preservation in European Union under 2008/1333/EC Regulation on food additives. It has also been accepted as an antimicrobial agent in casings and in ready-to-eat products by Food and Drug Administration (2000) in the U.S.A. lysozyme exhibits a strong antimicrobial activity against most Gram-positive and some Gram-negative bacteria. It damages peptidoglycans in bacterial cell wall by catalyzing hydrolysis of β-1–3 glycosidic linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine. Lysozyme was used for the first time by Appendini and Hotchkiss (1997) in antimicrobial films based on polyvinyl alcohol (PVOH), nylon, and cellulose triacetate. It has also been incorporated in chitosan films (Duan et al., 2007; Duan et al., 2008; Park et al., 2004; Yuceer and Caner, 2014), starch- (Fabra et al., 2014) and zein-based films (Arcan and Yemenicioglu, 2013; Gucbilmez et al., 2007; Mecitoglu et al., 2006; Unalan et al., 2011). These studies focused either on the incorporation of lysozyme in a film-forming solution to obtain stand-alone active films by solution-casting or on the functionalization of thermoplastic films by post-processing coating treatments with aqueous lysozyme solutions.

Proteins are very interesting biopolymers for biodegradable or edible films thanks to their good gas (Bourlieu et al., 2009; Cuq et al., 1998; Pochat-Bohatier et al., 2006) and aroma (Debeaufort and Voilley, 2002) barrier properties under dry conditions. Sodium caseinate (NaC) is one of the most studied proteins in edible films due to its excellent film-forming properties and high nutritional value. Numerous antimicrobial agents including essential oils (Atares et al., 2010), organic acids (Arrieta et al., 2013, 2014), bacteriocins (Cao-Hoang et al., 2010a, 2010b) and lysozyme (Mendes de Souza et al., 2010) have been incorporated into NaC films to obtain biodegradable edible packaging films with enhanced protective functions. However, all these antimicrobial NaC films were prepared by a solvent-casting method, while most of commercial plastic films are prepared by extrusion-blowing. Interestingly, Belyamani et al. (2014) recently reported for the first time the possibility to obtain NaC-based edible thin films (~30 μm) by blown-film extrusion. In a recent study, Jbilou et al. (2014) reported that increasing glycerol and reducing moisture content in corn flour-based formulations exerted a protective effect against lysozyme inactivation during single-screw extrusion. This would open the possibility to prepare corn flour-based antimicrobial materials retaining more than 60% of the initial activity of lysozyme by thermomechanical processes such as extrusion. Therefore, the goal of the present study was to prepare NaC-based biodegradable edible antimicrobial films incorporating lysozyme by blown-film extrusion. Preparation of NaC-based films is a two-step process: first, casein-based thermoplastic pellets incorporating lysozyme were prepared by twin-screw extrusion, then these lysozyme-containing pellets were introduced in a blown-film extruder. The influence of glycerol content in the formulation and of twin-screw extrusion temperature on the residual activity of lysozyme in pellets and films subsequently prepared was studied in order to define formulation and processing parameters allowing to prevent lysozyme inactivation. Thereafter, the antimicrobial activity against a lysozyme-sensitive bacterial strain, *Micrococcus luteus*, of the most active films was assayed. The lysozyme activity of films was assayed for 25 weeks storage at either room temperature or −20 °C to check their stability. Traditional properties of films such as water sorption and mechanical properties were also measured. Finally, the aerobic biodegradability of the most active films was measured to estimate whether the kinetics of biodegradation were affected by the presence of active lysozyme. To our knowledge, the present manuscript is the first one reporting the preparation of lysozyme-containing active NaC films by extrusion-blowing. Therefore, the technical possibility to optimize formulation and process parameters to prepare active films incorporating lysozyme based on other film-forming proteins by extrusion-blowing should also be investigated.

2. Materials and methods

2.1. Materials

Sodium caseinate native powder was from EPI Ingredients (Ancenis, France) and hen egg white lysozyme and glycerol were from Sigma–Aldrich (St Louis, Mo., USA).

2.2. Preparation of sodium caseinate pellets and films with or without (control) lysozyme

Sodium caseinate control pellets and films without lysozyme were prepared as previously described by Belyamani et al. (2014). A co-rotating twin-screw extruder (Clextral BC 21, Firminy, France) was used for the elaboration of pellets. The machine consists of nine zones heated independently and cooled with recycled water. Each screw measures 900 mm of length and 25 mm of diameter. The sodium caseinate powder was introduced without any treatment into the first zone of the extruder using a K-Tron gravimetric feeder (Coperion K-Tron, Niederlenz, Switzerland). Plasticizers were added into the second zone using a piston pump (PP9, PCM pumps, Salon-de-Provence, France). The temperature along the screw ranged between 40 °C and the maximum temperature corresponding to each formulation (Table 1).

In order to prepare pellets containing lysozyme, a preliminary mixture of sodium caseinate powder and lysozyme to the desired concentration (1% w/w) was prepared. The powder mixture was simply shaked for 5 min in a closed plastic container to ensure the homogenization. This sodium caseinate-lysozyme mixture was directly introduced in twin-screw extruder.

Glycerol and water were used as plasticizer agents; the glycerol rate was evaluated according to following equation:

$$\%\ \text{glycerol} = \frac{\text{weight}_{\text{glycerol}}}{\text{w eight}_{\text{glycerol}} + \text{weight}_{\text{sodium caseinate}}} \times 100 \qquad \text{(Eq. 1)}$$

Pellets, with or without lysozyme were then used to prepare antimicrobial or control blown films with a blown-film extruder (Diani, Cassano, Italy). Pellets were conditioned till equilibrium in an environmental chamber at 80% relative humidity (RH) at 30 °C before extrusion-blowing. Residence times in twin-screw extruder and blown-film-extruder were 2.5 and 7 min, respectively.

Table 1 lists the composition of all samples studied with maximal processing temperatures and nomenclatures used.

2.3. Determination of pellet and film compositions following extrusion and blowing

2.3.1. Moisture isotherms of sodium caseinate pellets and films

The water vapor adsorption isotherms at 30 °C were determined for NaC control pellets (extruded at 65, 85, or 100 °C) and the corresponding blown films (blown at 80 °C). Lysozyme-containing pellets extruded at 65 °C and corresponding films were