Journal of Food Engineering 117 (2013) 247–254

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Development of protein-based bioplastics with antimicrobial activity by thermo-mechanical processing

Inmaculada Martínez [a,*], Pedro Partal [a], Moisés García-Morales [a], Antonio Guerrero [b], Críspulo Gallegos [a]

[a] Dpto. Ingeniería Química, Centro de Investigación en Tecnología de Productos y Procesos Químicos (Pro²TecS), Universidad de Huelva-Campus Excelencia CeiA3, Campus El Carmen, 21071 Huelva, Spain
[b] Dpto. Ingeniería Química, Facultad de Química, Universidad de Sevilla, 41012 Sevilla, Spain

ARTICLE INFO

Article history:
Received 22 October 2012
Received in revised form 1 February 2013
Accepted 17 February 2013
Available online 28 February 2013

Keywords:
Bioplastic
Biocide
Protein
Controlled-release
Rheology

ABSTRACT

This study focuses on the development of new bio-active protein-based bioplastics through a thermo-mechanical processing, which involves a first compounding step followed by compression-moulding of the resulting material into the desired shape. Two types of proteins, wheat gluten and egg-white albumen, and two different bioactive agents, formic acid and oregano essential oil, were selected. The effect of biocide addition on the material rheological response, its antimicrobial activity and biocide release behaviour have been assessed. Rheological tests demonstrated that formulation and processing may exert a notable effect on the material linear viscoelasticity. Kirby–Bauer tests carried out on four selected types of microorganisms revealed that oregano essential oil into a wheat gluten-based matrix may be suitable for applications where the active agent must be progressively delivered (for up to 7 days). Moreover, this biocide can inhibit microorganisms' growth even if the bioplastic is not in direct contact with the substrate.

1. Introduction

Replacement of synthetic polymers with new biodegradable materials is becoming an important challenge nowadays. The growing demand of petroleum along with the political circumstances in many of the most important producer countries have increased its price far away from those of previous decades (Shawkat and Huimin, 2004). Biopolymers, derived from agricultural sources (Irissin-Mangata et al., 2001; De Graaf, 2000), seem to be a promising alternative. Different vegetable (corn, wheat gluten, soy, etc.) and animal (milk, albumen, collagen, gelatin, etc.) proteins have been used to manufacture bioplastics (Jerez et al., 2007a,b; Pommet et al., 2003). Protein-based biomaterials may be an efficient way to produce biodegradable materials with a large range of functional properties. These applications include packaging, matrix for enzyme immobilization or controlled-release, etc. (Yu and Min, 2006; Suda et al., 2000).

A protein-based material could be defined as a stable three-dimensional macromolecular network stabilized and strengthened by hydrogen bonds, hydrophobic interactions and disulphide bonds (Pommet et al., 2003). However, as proteins themselves do not have sufficient plasticity to be handled, a plasticiser is required. Plasticisers are molecules with low molecular weight and volatility, which modify the three-dimensional structure of proteins by reducing the intermolecular forces and increasing the polymer chains mobility (Gennadios, 2002). Moreover, a plasticiser may reduce the bioplastic glass transition temperature (Pouplin et al., 1999; Irissin-Mangata et al., 2001; Matveev et al., 2000). Plasticizer incorporation into the protein matrix can be performed by following two different methods: (a) physico-chemical or "casting" method, using a chemical reactant to disrupt disulphide bonds (Gontard et al., 1993); and (b) thermoplastic processing, which consists of mixing proteins and plasticizer by a combination of heat and shear (Attenburrow et al., 1990; Hernandez-Izquierdo et al., 2008) and, depending on protein, of an additional stage involving further thermo-mechanical treatments (e.g. compression moulding) is required to achieve a suitable material (Song and Zheng, 2008; Min et al., 2008).

Studies on active packaging have been largely developed in the last recent years. Quintavalla and Vicini (2002) defined an active packaging material as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food". Principal active packaging systems involve oxygen scavenging, moisture absorption, carbon dioxide or ethanol generation and, finally, antimicrobials (Coma, 2008). Antimicrobial packaging reduces, inhibits, or retards the growth of pathogen microorganisms in packed foods (Vermeiren et al., 1999) through: (a) volatile and non-volatile antimicrobial agents incorporated into polymers;

* Corresponding author. Tel.: +34 959 21 99 97; fax: +34 959 21 93 85.
E-mail address: imgarcia@uhu.es (I. Martínez).

0260-8774/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jfoodeng.2013.02.014

and (b) coating or adsorbing antimicrobials onto polymer surfaces (Appendini and Hotchkiss, 2002). A variety of compounds, including organic acids, enzymes such as lysozyme, natural antimicrobials such as spices have been proposed for active food packaging (Tharanathan, 2003; Weng and Hotchkiss, 1992). However, the essential oils, which contain high concentrations of phenolic compounds such as carvacrol, eugenol and thymol, show the strongest antibacterial activity against foodborne pathogens (Burt, 2004; Lamber et al., 2001). Their antimicrobial properties have been demonstrated in numerous studies (Avila-Sosa et al., 2010; Emiroglu et al., 2010; Seydim and Sarikus, 2006). In addition, various organic acids and their salts (formic acid, sorbates, benzoates and propionates) are used to inhibit the microbial growths (Over et al., 2009; Ricke, 2003) and to increase the shelf-life of fresh dough together with cooling, because these products are packaged without thermal treatment.

With the aim of finding novel biomaterials with antimicrobial activity, the present work studies the thermo-rheological response, biocide release behaviour and the inhibitory effects against four selected pathogens of protein-based bioplastics in which two types of active agents (formic acid and oregano essential oil) were incorporated. Selected proteins (wheat gluten or egg-white albumen) would also allow for taking advantage of the low solubility and high swelling in water of the resulting bioplastics in order to develop controlled-release matrix for bioactive agents. In this regard, previous works on bioplastic from these proteins have shown enhanced water resistance (protein solubility below 5%) and high water absorption capability (up to 300%) depending on material formulation and processing (Jerez et al., 2007b; Zárate-Ramírez et al., 2011).

2. Materials and methods

2.1. Materials

Two proteins from different sources were used in this research: (a) wheat gluten (WG), provided by RIBA S.A. (Spain), with 83 wt.% protein; and (b) egg white (EW) albumen, supplied by OVOSEC S.A. (Spain), with 73 wt.% protein. Glycerol (G), from Panreac Química, S.A. (Spain), was used as protein plasticiser. On the other hand, formic acid (FA) and oregano essential oil (OEO), from Panreac Química, S.A. (Spain) and Destilerías Muñoz Gálvez, S.A. (Spain), respectively, were used as antimicrobials. Both oregano essential oil and formic acid (in packaging) are listed as generally recognised as safe (GRAS) by the Food and Drug Administration. In addition, their uses as food additives and in packaging are regulated by EU (EC-No. 1333/2008 and EC-No. 1935/2004 regulations).

2.2. Samples preparation

Bioplastics compounding was performed in a Polylab torque-rheometer equipped with a Rheomix 3000p kneading tool (Thermo-Haake GmbH, Germany). This device allowed evolution of mixing temperature and torque to be monitored. Neither heating nor cooling was supplied to the kneading chamber (filled to 85% of its full capacity) during compounding. The procedure consisted in mixing protein (fine powder) with both biocide and plasticiser (liquids), by means of two rollers counter-rotating at 50 rpm. The mixing time applied, which depended on the type of protein, varied between 10 and 30 min. Subsequently, the resulting dough-like biomaterials were compression-moulded into rectangular specimens (50 mm length, 10 mm width and 3 mm thick) by applying, a gauge pressure of 100 bar, for 10 min, at two selected temperatures of 90 and 120 °C (Jerez et al., 2005a,b). Finally, specimens were allowed to cool down to room temperature inside the hot-plates press before removing from the mould. Afterwards, bioplastics were stored at 53% relative humidity (RH) before testing.

All the formulations studied present a 67 wt.% protein and 33% plasticizer + biocide blend, with the relative quantities of glycerol and biocide being adjusted so as to obtain a biocide content of 0, 5, 10 or 33 wt.% over the total weight of the bioplastic.

2.3. Testing procedures

Dynamic Mechanical Thermal Analysis (DMTA) tests were conducted on $50 \times 10 \times 3\ mm^3$ samples with a DMS 6100 (Seiko Instruments Inc., Japan) in double cantilever (bending) mode. In these tests, the storage modulus E' (elastic response) and loss modulus E'' (viscous response) of polymers are measured as a function of temperature (or frequency) as the polymer is deformed under an oscillatory load (stress). The complex modulus can be calculated as, $|E^*|^2 = |E'|^2 + |E''|^2$, measured in tension or flexure. Temperature sweep tests from 30 to 170 °C were carried out, within the linear viscoelasticity (LVE) region, at selected frequency and heating rate of 1 Hz and 2 °C/min, respectively.

Pure biocides volatility was studied by means of thermogravimetric analysis (TGA). Tests were carried out in a Q-50 analyser (TA Instruments, USA), on 5–10 mg samples which were heated from 30 to 250 °C at 10 °C/min, in N_2 atmosphere.

The Kirby–Bauer test (Boyle et al., 1973) was used in order to determine the antimicrobial activity of the two biocides proposed (formic acid and oregano essential oil). Round paper discs soaked in pure biocide or its corresponding 10 vol.% aqueous solution were set on a solidified agar culture medium inoculated with a solution of a selected microorganism. The four microorganisms studied, usually involved in food preservation and water pollution control processes, were: *Aspergillus niger* (fungus–mould) (wild strain), *Candida kefyr* (fungus–yeast) (wild strain), *Bacillus cereus* (bacteria gram-positive) CECT131 and *Escherichia coli* (bacteria gram-negative) CECT434. Antimicrobial test were carried out by disc diffusion method using 100 μl of bacterial suspension containing 2.0×10^8 CFU/ml in sterilized Petri dishes 90 mm in diameter. The paper discs (6 mm in diameter) were impregnated with 20 μl of each biocide dilution. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. The growth inhibition level exhibited by a selected microorganism for every different biocide tested can be quantitatively calculated by measuring the diameter of the inhibition zone, that is, the clear halo around the disc. Thus, K–B tests were carried at room temperature for 48 h, after which an average inhibition diameter was calculated for every combination of microorganism and biocide tested. The data were presented as mean ± standard deviation (SD) of three determinations. A probability value of $p < 0.05$ was considered significant.

A similar procedure was followed for the active bioplastics prepared, but replacing the paper discs by a square piece ($1 \times 1\ cm^2$) of that material. Alternatively, bioplastic antimicrobial activity was tested by placing the specimen under the lid of the Petry dish, so that the material is not in contact with the inoculated agar. In that case, inhibition would arise from the development of an antimicrobial atmosphere. Experiments were carried out in triplicate.

The transport rate, from the bioplastic samples into water, of the two active agents studied was evaluated by immersion of 1 g bioplastic in 100 mL water and taking samples of water at 24, 48, 72 h, and finally after 1 week at room temperature. Formic acid release was determined by acid/base titration and, as for OEO release, concentration in water of phenolic compounds (thymol and carvacrol) contained in this biocide was determined by means of a colorimetric assay involving the Folin–Ciocalteu reagent (FCR), and expressed in gallic acid equivalent (mg/L).