International Journal of Food Microbiology 173 (2014) 62–71

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Functional properties and antifungal activity of films based on gliadins containing cinnamaldehyde and natamycin

Mari Pau Balaguer [a], Paula Fajardo [b], Hunter Gartner [c], Joaquin Gomez-Estaca [a], Rafael Gavara [a], Eva Almenar [c], Pilar Hernandez-Munoz [a,*]

[a] Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Grupo de Envases, Av. Agustín Escardino 7, 46980 Paterna, Valencia, Spain
[b] ANFACO-CECOPESCA, Campus Universitario 16, 36310 Vigo, Spain
[c] School of Packaging, Michigan State University, East Lansing, MI 48824-1223, USA

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 29 November 2013
Accepted 11 December 2013
Available online 19 December 2013

Keywords:
Bioplastics
Natural antimicrobials
Antifungal properties
Active packaging
Cheese

ABSTRACT

Gliadin films cross-linked with cinnamaldehyde (1.5, 3, and 5%) and incorporated with natamycin (0.5%) were prepared by casting, and their antifungal activity, water resistance, and barrier properties were characterized. Incorporation of natamycin gave rise to films with greater water uptake, weight loss and diameter gain, and higher water vapor and oxygen permeabilities. These results may be associated to a looser packing of the protein chains as a consequence of the presence of natamycin. The different cross-linking degree of the matrices influenced the natamycin migration to the agar test media, increasing from 13.3 to 23.7 (μg/g of film) as the percentage of cinnamaldehyde was reduced from 5% to 1.5%. Antifungal activity of films was assayed against common food spoilage fungi (*Penicillium* species, *Alternaria solani*, *Colletotrichum acutatum*). The greatest effectiveness was obtained for films containing natamycin and treated with 5% of cinnamaldehyde. The level of cinnamaldehyde reached in the head-space of the test assay showed a diminishing trend as a function of time, which was in agreement with fungal growth and cinnamaldehyde metabolization. Developed active films were used in the packaging of cheese slices showing promising results for their application in active packaging against food spoilage.

1. Introduction

The substitution of synthetic preservatives used in food products by natural antimicrobial compounds has been attracting much attention in the last decade (Burt, 2004; Del Nobile et al., 2012; Tajkarimi et al., 2010). Consumers are more aware of the importance of consuming healthy, great quality, and safety-assured food products, so food manufacturers need to find new ways of reducing food waste and sanitary risks associated with microbial contamination. In addition, extension of the shelf-life of food products is desired while complying with the legislation and without generating the marketing drawbacks associated with synthetic preservatives.

Natamycin (Fig. 1) is a fungicide of the polyene macrolide group produced by natural strains of *Streptomyces natalensis* or *Streptococcus lactis*. It is registered in EU food additive list with the number E-235. According to Regulation 1333/2008/EC on food additives, natamycin is only approved as a surface preservative for certain cheese and dried sausage products at a maximum level of 1 mg/dm^2 in the outer 5 mm of the surface, corresponding to 20 mg/kg. Its mechanism of action is binding to sterols (principally ergosterol) in the fungal cell membrane, and as bacteria lack sterols in their membrane they are insensitive to natamycin. Minimum inhibitory concentrations (MIC) of natamycin for molds range from 0.5 to 6 μg/mL, but some species require 10 to 25 μg/mL, and most yeasts are inhibited at concentrations from 1 to 5 μg/mL (Delves-Broughton et al., 2005).

The application of natamycin onto food surfaces by spraying or dipping is not always as effective as expected due to its inactivation by interaction with other components of the food matrix (Fucinos et al., 2012; Reps et al., 2002). Moreover, direct application of antimicrobials onto the food surface may result in a rapid migration to the food bulk, allowing surface fungal growth. The inclusion of natamycin in a polymer matrix for later sustained release to the surface of the food can ensure a minimal concentration of the antimicrobial capable of protecting the food against fungal growth. Moreover, incorporation of natamycin in a polymer matrix can reduce the loss of activity of the compound by interaction with food.

Biopolymers based on renewable resources present a noteworthy potential to retain and release active compounds (Cha and Chinnan, 2004). These biopolymers can be employed in the development of antimicrobial active packaging films which can extend the shelf-life of packaged foods and at the same time contribute to the use of environmentally-friendly materials. In this regard, some biopolymers have been proposed as carriers of natamycin, *e.g.* cellulose (de Oliveira et al., 2007; Pires et al., 2008), wheat gluten and methyl cellulose (Ture et al., 2008), chitosan (Ce et al., 2012; Fajardo et al., 2010), blends of alginate and chitosan (da Silva et al., 2012), whey protein (Pintado et al., 2010; Ramos et al., 2012), and alginate and pectin (Krause Bierhalz et al., 2012).

* Corresponding author. Tel.: +34 96 3900022; fax: +34 96 3636301.
E-mail address: phernan@iata.csic.es (P. Hernandez-Munoz).

0168-1605/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.12.013

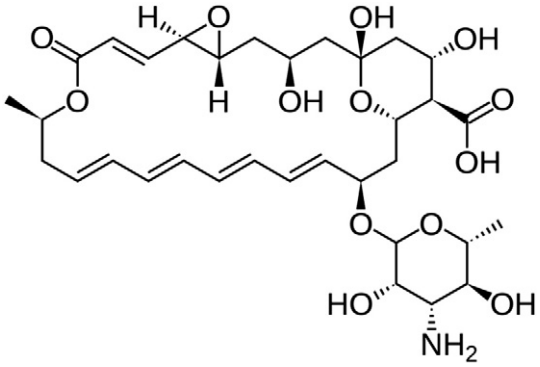

Fig. 1. Chemical structure of natamycin.

The cross-linking degree of the polymer employed as carrier of natamycin could be used as a mechanism to modulate its release into the medium. Films based on gliadins chemically modified by treatment with cinnamaldehyde possess different functional properties depending on the cross-linking degree achieved (Balaguer et al., 2011a, 2011b, 2013b). Moreover, it has been shown that a part of the cinnamaldehyde incorporated in the gliadin matrix does not cross-link the protein and remains entrapped in the matrix until being released under certain environmental conditions.

The aim of this work has thus been (i) to incorporate natamycin into gliadin films treated with cinnamaldehyde, (ii) to study how natamycin affects some functional properties of the films, (iii) to estimate the release of cinnamaldehyde and natamycin from the films, and (iv) to evaluate the antifungal effectiveness of the developed films *in vitro* and in different kinds of cheese.

2. Materials and methods

2.1. Reagents and microbial strains

Crude wheat gluten (≥80% protein), glycerol, ethanol, hydrochloric acid, and cinnamaldehyde, all laboratory grade, were supplied by Sigma (Madrid, Spain). Pimaricin supplied by VGP, S.L. (Barcelona, Spain) as the source of the active agent natamycin, consisted of 50% natamycin and 50% lactose. In this work, unless otherwise indicated, percentage of natamycin will be expressed as levels of pure natamycin (without lactose).

Potato Dextrose Agar (PDA) was purchased from Sigma (Sigma-Aldrich Corp., Saint Louis, MO, USA).

Fungal strains of *Colletotrichum acutatum* and *Penicillium* species were originally isolated from diseased blueberry fruit, and decayed bread, respectively. *Alternaria solani* was provided by the Department of Plant Pathology of Michigan State University (East Lansing, MI, USA).

2.2. Film formation and characterization

2.2.1. Gliadin-rich fraction extraction from wheat gluten

The gliadin-rich fraction was extracted from wheat gluten according to Hernandez-Munoz et al. (2003). Briefly, 100 g of wheat gluten was dispersed in 400 mL of 70% (v/v) ethanol/water mixture, stirred overnight at room temperature, and centrifuged at 5000 rpm for 20 min at 20 °C. The supernatant containing the gliadin-rich fraction was collected and used as the film-forming solution.

2.2.2. Chemical modification of gliadins

In preliminary studies, the use of cinnamaldehyde as a protein cross-linker was found to be strongly dependent on the pH (Balaguer et al., 2011a,b, 2013a). Initially, the pH of the gliadin film forming solution was brought to 2.0 with HCl as the most suitable for polymerization. Then, chemical modification of gliadins was conducted by adding different concentrations of cinnamaldehyde to the film-forming solution, namely 1.5% (G1.5C_pH2), 3% (G3C_pH2) and 5% (G5C_pH2) (g cinnamaldehyde/100 g protein). Glycerol was added as plasticizer at 25% (g glycerol/100 g protein). For films incorporating natamycin (NT-GXC_pH2), 0.5% of natamycin was added to the film-forming solution (g natamycin/100 g protein). The mixture with all the reagents was stirred at room temperature for 30 min.

2.2.3. Film production and conditioning

In order to have 0.01 g of protein/cm^2, measured volumes of the film-forming solution were poured onto a horizontal flat Pyrex tray to allow water and ethanol to evaporate. The films were dried at 37 °C for 24 h. The dried films were peeled off the casting surface. The film thickness was measured using a micrometer (Mitutoyo, Kanagawa, Japan) with a sensitivity of ±2 μm. The mean thickness was calculated from measurements taken at ten different locations on each film sample. The films were stored 1 month at 30% RH and 23 °C before testing. The surface density of the resulting films was 0.0195 ± 0.0065 g/cm^2.

2.2.4. Water uptake, weight loss and diameter gain

Film specimens were cut into circles with a diameter of 2.5 cm (initial diameter, $Ø^i$), and dried over P_2O_5 in a desiccator until they reached constant weight. At this point the moisture content in the film samples was assumed to be close to zero. Samples were accurately weighed (initial dry weight, W_d^i) and immersed in test tubes containing 10 mL of distilled water. The tubes were shaken in an orbital shaker at 180 rpm at 23 °C for 24 h. The film specimens were then removed from the solutions, blotted with absorbent paper to remove any remaining water from the surface, and weighed (final wet weight, W_w^f). The diameter (final diameter, $Ø^f$) was measured using a Vernier caliper. The film samples were replaced in the desiccator with P_2O_5 until they reached a constant weight (final dry weight, W_d^f). The percentage of water uptake, weight loss and diameter gain of the films was calculated as:

$$\text{Water uptake } (\%) = \frac{W_w^f - W_d^f}{W_d^f} \cdot 100 \quad (1)$$

$$\text{Weight loss } (\%) = \frac{W_d^i - W_d^f}{W_d^i} \cdot 100 \quad (2)$$

$$\Delta Ø(\%) = \frac{Ø^f - Ø^i}{Ø^i} \cdot 100. \quad (3)$$

2.2.5. Barrier properties

2.2.5.1. Water permeability. Water vapor transmission rate [kg/(m^2·s)] through the films was measured using a Mocon PERMATRAN-W® Model 3/33 (Lippke, Neuwied, Germany). It operates according to ASTM F1249 (ASTM, 2006) and uses an infrared sensor. The film samples were double-masked with aluminum foil, leaving a circular uncovered effective film area of 5 cm^2. Testing was performed at 23 °C with a relative humidity gradient of 50% to 0% (in dry nitrogen) across the film. At least four samples of each type of film were measured. The water vapor permeability coefficient in (kg·m)/(m^2·s·Pa) can be calculated from the transmission rate values measured as follows:

$$P = \frac{Q \cdot l}{A \cdot t \cdot \Delta p} \quad (4)$$

where Q is the amount of permeant (kg) passing through a film of thickness l (m) and area A (m^2), t is the time (s), and Δp is the partial pressure differential across the film (Pa). Δp is calculated from the water vapor partial pressure at 23 °C and a RH gradient of 50–0%.