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Antifungal properties of gliadin films incorporating cinnamaldehyde and application in active food packaging of bread and cheese spread foodstuffs

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ABSTRACT

Gliadin films incorporating 1.5, 3 and 5% cinnamaldehyde (g/100 g protein) were tested against food-spoilage fungi *Penicillium expansum* and *Aspergillus niger* in vitro, and were employed in an active food packaging system for sliced bread and cheese spread. Gliadin films incorporating cinnamaldehyde were highly effective against fungal growth. *P. expansum* and *A. niger* were completely inhibited after storage in vitro for 10 days in the presence of films incorporating 3% cinnamaldehyde. Indeed 1.5% cinnamaldehyde was sufficient in the case of *P. expansum*. The amount of cinnamaldehyde retained in films after storage for 45 days at 20 °C and 0% RH was also sufficient in most cases to prevent fungal growth in vitro. Active food packaging with gliadin films incorporating 5% cinnamaldehyde increased the shelf-life of both sliced bread and cheese spread. Mold growth was observed on sliced bread after 27 days of storage at 23 °C with active packaging, whereas in the control bread packaged without the active film fungal growth appeared around the fourth day. In the cheese spread, no fungi were observed after 26 days of storage at 4 °C when the product was packaged with the active film. However, growth of fungi was observed in control packaged cheese after 16 days of storage. This work demonstrates a noteworthy potential of these novel bioplastics incorporating natural antimicrobial compounds as innovative solutions to be used in active food packaging to extend shelf-life of food products.

1. Introduction

Nowadays, food industry challenges are focused on diverse requirements. On the one hand, the fulfillment of consumers' demands aimed at increasing food quality from the sensory and nutritional points of view without raising its cost. On the other hand, the reduction of food waste and the improvement of food safety by controlling the growth of food-borne and food-spoilage microorganisms while reducing the use of synthetic preservatives that are associated with health risks and microbial resistance (Shannon, 2000). Fungi are able to grow on diverse foods inducing the development of off-flavors, acidifying, fermenting, discoloring, disintegrating, rotting and rendering nutritious commodities unpalatable or unsafe due to the formation of pathogenic or allergenic toxins (Filtenborg et al., 1996; Pitt and Hocking, 2009).

One novel approach to achieve these needs is based on the use of natural antimicrobial compounds combined with the design of new carrier materials or devices. These active systems should incorporate the antimicrobial agent, trigger its release once necessary, control its rate of release thereby exerting either lethal or inhibitory effects against food pathogens or spoilage microorganisms present in foodstuffs.

Although herbs and spices have been used for centuries in food preservation, a renewed scientific interest has occurred for the last twenty years (Kalemba and Kunicka, 2003). It has been demonstrated that some of their constituents possess greater antimicrobial activities than the essential oils themselves (Burt, 2004; Friedman et al., 2002; Lopez et al., 2007b), hence the use of individual compounds derived from essential oils could reduce the amount of material required for antimicrobial activity. Moreover, numerous individual components of essentials oils, either extracted from plant material or synthetically manufactured, are also categorized as flavoring agents by the European Commission, and as generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA).

Cinnamaldehyde is an aromatic α,β-unsatured aldehyde, and the major component in essential oils from some cinnamon species (Singh et al., 2007; Wang et al., 2009). It is registered in the EU flavoring list (FL no. 05.014[1]), and the FDA has classified it as a synthetic GRAS flavoring substance for its intended use (reg no. 182.60[2]).

Cinnamaldehyde has been shown to exert antimicrobial activity against a wide range of microorganism including bacteria, yeasts, and

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[1] http://ec.europa.eu/food/food/chemicalsafety/flavouring/database.
[2] http://www.ecfr.gov/cgi-bin/text-idx?sid=58029aa65152691d14eab45977de0fb1&c=ecfr&tpl=/ecfrbrowse/Title21/21tab_02.tpl.

molds (Burt, 2004; Gutierrez et al., 2009; Lopez et al., 2007b; Pauli and Knobloch, 1987; Xing et al., 2010), and to be more effective than other cinnamon essential oil constituents (Khan and Ahmad, 2011; Matan et al., 2011; Utama et al., 2002), especially in vapor phase (Lopez et al., 2007b). Thus, the main advantage of cinnamaldehyde is that direct contact is not required for antimicrobial activity. Its microbicidal activity may be ascribed to the high electrophilic properties of the carbonyl group adjacent to the double bound that make this compound particularly reactive with nucleophiles, such as protein sulfhydryl and amino groups of the microorganism (Neri et al., 2006). Recently, Manso et al. (2013) hypothesized that cinnamon essential oil induces incomplete formation of the conidia of *Aspergillus flavus*, resulting in inhibited growth, and alternatively, it is also possible that the essential oil causes damage to the conidia after the conidiophore has been formed, resulting in an unstructured vesicle head. These observations are consistent with those observed by other authors when fungi were exposed to essential oils. Abnormalities in the cell wall structure, disrupted and aggregated hyphae, loss of cytoplasm, strong decrease in the number of conidia, and conidiophores with anomalous development have been reported (Helal et al., 2006; Lopez-Malo et al., 2002; Tolouee et al., 2010).

Films, coatings, nanoparticles or devices containing cinnamaldehyde have been produced using a wide variety of raw materials, including cellulose (Sanla-Ead et al., 2012), chitosan (Brasil et al., 2012; Ouattara et al., 2000), pectin (Brasil et al., 2012; Ravishankar et al., 2012; Wang et al., 2010), starch (Ben Arfa et al., 2007; Kechichian et al., 2010), soy protein (Ben Arfa et al., 2007; Gamage et al., 2009), poly(lactic-co-glycolic acid) (Gomes et al., 2011; Zodrow et al., 2012), PP, PE/EVOH (Lopez et al., 2007a), and wax paraffin (Rodriguez-Lafuente et al., 2010; Rodriguez et al., 2008). However, as far as we know there are no studies reported in the literature regarding the use of wheat protein-based materials as carriers of cinnamaldehyde for the development of antimicrobial films.

Most of the active packaging systems containing cinnamaldehyde or cinnamon oil aimed at food applications have shown promising results when tested in vivo. Mild et al. (2011) incorporated cinnamaldehyde in apple-based edible films used to wrap chicken breast and observed great reductions of *Campylobacter jejuni* population without impairment of the sensorial properties of the wrapped product (Du et al., 2012). Reductions in *Escherichia coli* O157:H7 and *Salmonella enterica* inoculated in chicken breast, and *Listeria monocytogenes* inoculated in ham were also achieved with these apple-based films containing cinnamaldehyde (Ravishankar et al., 2009). Bologna, regular cooked ham, or pastrami were packaged with chitosan-based films containing cinnamaldehyde; as a result of the application of these films a delay or complete inhibition of the growth of Enterobacteriaceae and *Serratia liquefaciens* was obtained (Ouattara et al., 2000). A paper coating based on cinnamon essential oil was highly effective against *Alternaria alternata* inoculated in cherry tomatoes and there were no sensorial changes detected (Rodriguez-Lafuente et al., 2010). Shelf-life of fresh-cut papaya was prolonged by coating with a layer-by-layer edible assembly made of chitosan and pectin incorporating a microencapsulated beta-cyclodextrin/cinnamaldehyde complex (Brasil et al., 2012). Microbial counts of radish, broccoli, and alfalfa sprouts stored in oPP/PE film packages coated with soy protein isolate containing cinnamaldehyde were significantly reduced (Gamage et al., 2009). Cinnamon essential oil incorporated into solid wax paraffin and used as a paper coating showed a strong inhibitory effect on the growth of *Rhizopus stolonifer* inoculated in bread (Rodriguez et al., 2008). However, the antimicrobial effect of cinnamon powders added to cassava-starch films for packaging of bread slices could not be determined since the physico-chemical properties of these biodegradable films were affected by the high relative humidity of bread (Kechichian et al., 2010). No scientific references were found related to the use of cinnamaldehyde in the active packaging of cheese.

The present study is aimed to evaluate the in vitro effectiveness against food-contaminating fungi (*Penicillium expansum* and *Aspergillus niger*) of bioplastics films made from wheat gliadins incorporating cinnamaldehyde, and to provide evidence of their applicability in the design of active food packaging systems for sliced bread and cheese spread foodstuffs.

2. Materials and methods

2.1. Reagents and microbial strains

Crude wheat gluten (≥80% protein), cinnamaldehyde, glycerol, ethanol, and, hydrochloric acid, all laboratory grade, were supplied by Sigma (Madrid, Spain).

Malt Extract Agar (MEA) was purchased from Scharlau (Scharlab S.L., Barcelona, Spain).

P. expansum CECT 2275 and *A. niger* CECT 20156 were used for testing the antimicrobial activity of gliadin films incorporating cinnamaldehyde.

2.2. Gliadin-rich fraction extraction from wheat gluten

The gliadin-rich fraction was extracted from the wheat gluten according to the method described by Hernandez-Munoz and Hernandez (2001). 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. The amount of protein extracted was around 12–14% (g protein/100 g film-forming solution).

2.3. Chemical modification of gliadins

Several cinnamaldehyde concentrations were added 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 to the film-forming solutions at 25% (g glycerol/100 g protein). The pH of the mixture containing protein, glycerol, and cinnamaldehyde was adjusted to 2.0 with HCl, the most suitable for producing the cross-linking reaction of gliadins by means of cinnamaldehyde (Balaguer et al., 2013, 2011a, 2011b), and the mixture was stirred for 30 min to produce a complete homogenization.

2.4. Film formation

The film-forming solution was poured onto a horizontal flat Pyrex tray or onto the lid of the plastic Petri dish to allow water and ethanol to evaporate. The weight of film-forming solution used to form the film was calculated in order to obtain a density of 0.01 g protein/cm^2. The films were dried at 37 °C for 24 h, and further conditioned at 50% RH for 20 h to facilitate peeling off the casting surface. They were stored at 0% RH and 20 °C until use.

The film thickness was measured using a micrometer (Mitutoyo, Kanagawa, Japan) with a sensitivity of ±2 μm. The mean thickness was 100 ± 12 μm, calculated from measurements taken at ten different locations on each film sample. The surface density of the films was 0.015 ± 0.002 g/cm^2.

2.5. Culture preparation

P. expansum and *A. niger* were grown on MEA in plastic Petri dishes (9 cm diameter) for 7 days at 30 °C. Conidia were then collected by flooding the surface of the plates with sterile peptone water and gently scraping the mycelial surface with a spatula. Ten mL of this suspension was transferred to sterile plastic tubes, which were shaken to obtain a homogenous suspension of conidia. The conidial suspensions were adjusted to 1×10^6 spores/mL. The Neubauer improved method (Bright-