Postharvest Biology and Technology 49 (2008) 294–300

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: *In vitro* and *in vivo* studies

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ARTICLE INFO

Article history:
Received 2 July 2007
Accepted 20 February 2008

Keywords:
Film-forming solutions
Edible coatings
Oleoresins
Native microflora
Listeria monocytogenes
Peroxidase and polyphenoloxidase
Enzymatic browning

ABSTRACT

Consumers demand less use of chemicals on minimally processed fruits and vegetables so more attention has been paid to the search for naturally occurring substances able to act as alternative antimicrobials and antioxidants.

The susceptibility of the native microflora of butternut squash and *Listeria monocytogenes* was analyzed by *in vitro* assays using (a) film-forming solutions (chitosan, carboxymethyl cellulose and casein), (b) oleoresins (olive, rosemary, onion, capsicum, cranberry, garlic, oreganum and oreganum + carvacrol 5%) and (c) film-forming solutions enriched with oleoresins. Film-forming solutions did not show significant antimicrobial properties. The oleoresins with meaningful antimicrobial activity against both squash native microflora and *L. monocytogenes* were olive and rosemary. In general, film-solutions containing 1% of different oleoresins showed limited antimicrobial effects against these indicator microorganisms. *In vitro* antioxidant properties were measured on different crude vegetable extracts. The enzyme source proved to affect peroxidase (POD) and polyphenoloxidase (PPO) susceptibility to the film-forming solutions. Most oleoresins significantly affected POD activity, regardless of the enzyme source. When the film-forming solutions were enriched with oleoresins, the latter lost, or retained their potential to reduce POD and PPO activities.

In vivo experiments were focused on the treatments offering potential antibacterial and antioxidant benefits. The use of chitosan coatings enriched with rosemary and olive oleoresins applied to butternut squash did not produce a significant antimicrobial effect, however antioxidant effects were observed during the first day, exerting POD inhibition for up to 5 d of storage. Both oleoresins and chitosan enriched with them exerted significant antioxidant activities over PPO throughout 5 d of storage. The use of chitosan enriched with rosemary and olive did not introduce deleterious effects on the sensorial acceptability of squash.

Chitosan enriched with rosemary and olive improved the antioxidant protection of the minimally processed squash offering a great advantage in the prevention of browning reactions which typically result in quality loss in fruits and vegetables.

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1. Introduction

The use of edible films and coatings in food protection and preservation has recently increased since they offer several advantages over synthetic materials, such as being biodegradable and environmentally friendly (Tharanathan, 2003).

Currently, studies dealing with edible films with antimicrobial properties are on the increase. These films could prolong the shelf life and safety of foods by preventing growth of pathogenic and spoilage microorganisms as a result of their lag-phase extension and/or their growth rate reduction (Quintavalla and Vicini, 2002). Moreover, antimicrobials imbedded in films can be gradually released on the food surface, therefore, requiring smaller amounts to achieve the target shelf life (Min and Krochta, 2005).

The ability of edible films to retard moisture, oxygen, aromas and solute transport may be improved by including additives such as antioxidants, antimicrobials, colorants, flavours, fortifying nutrients and spices in film formulation (Pranoto et al., 2005). Given the fact that consumers demand less use of chemicals on minimally processed fruits and vegetables, more attention has been

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doi:10.1016/j.postharvbio.2008.02.013

paid to the search for naturally occurring substances able to act as alternative antimicrobials and antioxidants. The addition of natural antioxidants derived from vegetable extracts as a way of increasing the shelf life of food products has become increasingly popular. It has also improved the stability of lipids and lipid-containing foods, thereby preventing sensorial and nutritional quality loss (Hemeda and Klein, 1990; Ozcan, 2003; Ponce et al., 2004; Sebranek, 2004). Along these lines, Botsoglou et al. (2002) indicated that the essential oils included in edible films could reduce water vapor permeability.

Spice oleoresins (natural plant extracts) constitute the true essence of spices in their most concentrated form, containing volatile as well as non-volatile components. Besides, they are the preferred and most convenient substitutes for raw spices in the food processing industry.

The isolated non-volatile components consist of several chemical compound groups, such as carotenoids, steroids, alkaloids, anthocyanins, glycosides, etc. This fraction can be essential for taste, colour, mouthfeel, texture and antioxidant properties of foods. Oleoresins differ from essential spice oils as they count on all the flavouring ingredients of a particular spice. They are free from bacteria and may be standardized to a desired degree of flavour strength.

When edible films are enriched with essential oils, the drying temperatures usually employed to form the edible coating are high enough to volatilize a high percentage of the aromatic components. The advantage of substituting essential oils for their corresponding food grade oleoresins could lie in the introduction of other non-volatile components, positively affecting food quality. Experiments carried out on the antimicrobial and antioxidant properties of spices and herbs and their compounds have been well documented. This issue shows interest even at present; however, the information available on their biological activity in edible films is still scarce.

This research aims to determine: (1) the antimicrobial activity of chitosan, casein and carboxymethyl cellulose films, alone as well as enriched with oleoresins, on the native microflora of butternut squash and on *Listeria monocytogenes*; (2) the antioxidant properties of these coatings; and (3) their antimicrobial and antioxidant effectiveness when applied to butternut slices (studies *in vivo*).

2. Material and methods

2.1. Film reagents

Sodium caseinate (9×10^{-3} kg kg^{-1}) and carboxymethyl cellulose were obtained from Merck (Darmstadt, Germany); and food grade glycerol from Mallinckrodt (Paris, KY, USA). Medium molecular weight chitosan was supplied by Aldrich Chemical Co. (Milwaukee, WI, USA).

2.2. Preparation of film-forming solutions

A 5% sodium caseinate aqueous solution was prepared by gradually adding sodium caseinate to distilled water and stirring continuously for 3 h at refrigerated temperature. Glycerol was included to reach a glycerol/protein ratio of 0.25 (Schou et al., 2005).

Chitosan solution was prepared by dissolving 20 g of chitosan in 1 kg of 1% acetic acid and 1% glycerol solution. To achieve complete chitosan dispersion, the solution was stirred overnight at room temperature and centrifuged to remove impurities. It was then sterilized at 121 °C for 15 min (Park et al., 2004).

The carboxymethyl cellulose coating was prepared by solubilizing carboxymethyl cellulose powder (0.75%) in a water–ethyl alcohol mixture (31/11) at 75 °C under stirring for 15 min. Ethyl alcohol was used to reduce drying time and produce a transparent and shiny carboxymethyl cellulose coating. Then glycerol was added (1.9%) and the solution was stirred for another 10 min under the same conditions and cooled (Maftoonazad and Ramaswamy, 2005).

2.3. Oleoresins

The oleoresins used in this work were provided by Pionherb, Buenos Aires, Argentina. Food grade oleoresins were obtained by alcohol steam distillation starting from fresh vegetables. The oleoresins used were rosemary (*Rosmarinus officinalis*), oreganum (*Origanum vulgare*), olive (*Olea europea*), capsicum (*Capsicum frutescens*), garlic (*Allium sativum*), onion (*Allium cepa* L.) and cranberry (*Vaccinium oxycoccus*).

2.4. Agar diffusion method

The sensitivity of the native microflora of butternut squash (*Cucurbita moschata* Duch) and *L. monocytogenes* to film components, oleoresins and film components enriched with oleoresins was determined by the agar diffusion method. An inhibition zone assay was conducted by inoculating Brain Heart Infusion (BHI) (Britania, Buenos Aires, Argentina) agar with an overnight culture of the indicator microorganisms. Seventy μL of the different solutions were poured into agar wells (5–6 mm diameter) following the methodology described by Coma et al. (2002). Table 1 describes the experimental conditions assayed.

Two types of control were utilized: distilled water control and acidic water control (pH 5.0). The latter aimed at determining the possible role of acid pH values on the inhibition of indicator microorganisms. The dishes were incubated at 37 °C for 1–2 d and the inhibition zones were measured. The sensitivity to the different antimicrobial solutions was classified by the diameter of the inhibition halos as: not sensitive, diameters less than 8 mm; sensitive, diameters 9–14 mm; very sensitive, diameters 15–19 mm; and extremely sensitive, diameters larger than 20 mm (Ponce et al., 2003). Each assay was performed in duplicate in two separate experimental runs.

Native microflora of butternut squash was prepared with 10 g of raw material macerated in 90 mL phosphate buffer solution (0.1 mol L^{-1}) with a Stomacher 400 Circulator Homogenizer (pH 7.2) in agreement with Ponce et al. (2003); and then incubated for 3 h at 37 °C. *L. monocytogenes* indicator microorganism was supplied by CERELA (Centro de Referencia de Lactobacilos, Tucumán, Argentina). This culture was kept refrigerated on tryptic soy agar (TSA) supplemented with 0.5% of yeast extract during the experiment. Before *L. monocytogenes* was used, the pathogen was cultured in BHI for 1 d at 37 °C. Immediately before each experiment, approximately 0.1 mL of culture was transferred to 90 mL of BHI at two consecutive 1 d intervals.

Table 1
Experimental conditions for the agar diffusion method

Antimicrobial solutions assayed	Indicator microorganisms
Casein solution Chitosan solution Carboxymethyl cellulose solutions Oleoresins at 1% concentration: olive, rosemary, onion, capsicum, cranberry, garlic, oreganum, oreganum + carvacrol 5% Combinations of film-forming solutions with oleoresins	Native microflora of butternut squash, *Listeria monocytogenes*

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