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Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli

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ABSTRACT

Antimicrobial properties of chitosan (CH) coatings and CH enriched with bioactive compounds (BC) and essential oils (EO) were determined by *in vitro* and *in vivo* assays on minimally processed broccoli. The efficiency of CH plus BC/EO in improving the safety of broccoli was tested against the native microflora. Also, its effects on the survival of *Escherichia coli* and *Listeria monocytogenes* inoculated in broccoli were evaluated.

In vitro assays performed in tea tree, rosemary, pollen and propolis demonstrated significant inhibitory effects on *E. coli* and *L. monocytogenes* counts while pomegranate and resveratrol presented reduced activity. *In vivo* application of these BC on broccoli exerted a bacteriostatic effect on mesophilic and psychrotrophic populations except for rosemary. The application of CH alone or enriched with BC/EO resulted in a significant reduction in mesophilic and psychrotrophic counts. Between days 5 and 7, significant reductions (2.5 log) were observed in samples treated with CH + BC. The enrichment with BC improved the antimicrobial action of CH. The application of these coatings did not introduce deleterious effects on the sensory attributes of broccoli.

CH coatings enriched with BC/EO were a good alternative for controlling not only the microorganisms present in broccoli, but also the survival of *E. coli* and *L. monocytogenes*.

1. Introduction

In recent years, the demand for broccoli for fresh or ready to eat salad consumption has increased greatly (Vallejo, García-Viguera, & Tomás-Barberán, 2003). The main problem that makes fresh cut broccoli a highly perishable product is the ease of microbial growth (Rivera-Lopez, Vasquez-Ortiz, Ayala-Zavala, Sotelo-Mundo, & Gonzalez-Aguilar, 2005). Cutting or slicing operations greatly increase tissue damage and cause the released of intracellular contents (González-Aguilar et al., 2009). The release of cellular substrates supports and increases the activity of pathogenic and saprophytic microorganisms. This is why the development of new technologies to reduce broccoli deterioration and safety problems is much needed.

There is a new tendency in food technology preservation that consists of developing materials with film-forming capacity and antimicrobial properties which help improve food safety and shelf life. Edible coatings, formed with Generally Recognized As Safe materials, offer several advantages over synthetic materials, such as being biodegradable and environmentally friendly (Tharanathan, 2003). Moreover, some edible coatings have the potential to improve food appearance and delay or inhibit the growth of pathogenic and spoilage microorganisms (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Quintavalla & Vicini, 2002).

The incorporation of antimicrobial agents in coatings is emerging as a promising technology, as it establishes contact with food and inhibits the growth of microorganisms present on the surface (Santiago-Silva et al., 2009).

In this context, chitosan coatings result adequate for their application in food preservation (Dutta et al., 2009). The chitosan coating creates a semipermeable barrier that controls gas exchange and reduces water loss, thereby maintaining tissue firmness and reducing microbial decay of harvested vegetables for extended periods (Devlieghere, Vermeulen, & Debevere, 2004; Dong, Cheng, Tan, Zheng, & Jiang, 2004; Thommohaway, Kanlayanarat, Uthairatanakij, & Jitareerat, 2007).

Various natural compounds could be used to improve the antimicrobial activity of chitosan coatings. The essential oils and

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bioactive compounds are an attractive option of natural preservatives. The information available on their biological activity in edible coatings is still scarce.

There are a few scientific works describing the effects of chitosan edible coatings enriched with biopreservatives on minimally processed broccoli to control microbial spoilage and to ensure the vegetable's safety. The present study had the objective to develop and evaluate the antimicrobial effect of chitosan edible coatings enriched with bioactive compounds (BC) and essential oils (EO). Native microflora evolution (mesophilic and psychrotrophic) of fresh cut broccoli was followed during refrigerated storage. Also, the effects of chitosan coatings combined with BC or EO on the survival and growth of *Escherichia coli* and *Listeria monocytogenes* inoculated in broccoli were evaluated. Moreover, as the sensory quality is the property with greater impact on purchase decision it is essential to evaluate how the coating treatment impacts the sensory quality of the product.

2. Materials and methods

2.1. Plant material

Broccoli heads (*Brassica oleracea* L. var. *Italica*) were directly obtained from a local producer in Mar del Plata, Argentina. Heads were immediately transported to the laboratory within 1 h of harvesting, in refrigerated containers with polyfreezer (refrigerated gel for maintaining cold chain, Thermics Argentina SA). Before the application of the chitosan coating, heads were separated into florets and stems and rinsed with chlorinated water (100 μL/L) for 3 min, then washed by immersion in tap water for 1 min and drained.

2.2. Essential oils and bioactive compounds

The essential oils used in this work were purchased from Nelson and Russell (London, England), which supplies food grade oils. The essential oils used for *in vitro* test were: tea tree (*Melaleuca alternifolia*), rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), lemon (*Citrus limonum*), oreganum (*Origanum vulgare*), calendula (*Calendula officinalis*) and aloe vera (*Aloe ferox*). The bioactive compounds used in this study were: bee pollen (Crinway S.A., Argentina), ethanolic extract of propolis (Jurisich, Argentina), pomegranate dried extract (*Punica granatum* L.) and resveratrol (3, 4′, 5-Trihydroxy-*trans*-stilbene). Pomegranate and resveratrol (Sigma) were initially dissolved in 1 mL of DMSO (Biopack, Argentina).

2.3. Preparation of coating-forming solutions

Medium molecular weight Chitosan (deacetylation degree (DD) = 98%) was supplied by ACOFAR (Argentina), and food grade glycerol from Mallinckrodt (Paris, KY, USA). Chitosan solutions (2 g/100 mL) (Xu, Kim, Hanna, & Nag, 2005) were prepared by dispersing chitosan powder in acetic acid solution (1 mL/100 mL) with magnetic stirring at 23 °C. To achieve complete chitosan dispersion, the solution was stirred overnight at room temperature and centrifuged to remove impurities. Then, it was sterilized at 121 °C for 15 min (Park, Daeschel, & Zhao, 2004). Glycerol was added as plasticizer to obtain flexible coatings that could be folded and manipulated without breakage. Glycerol content was added to achieve a glycerol/chitosan (Gly/CH) weight ratio of 0.28.

2.4. Culture maintenance and inoculum preparation

E. coli O157:H7, ATCC 43895 (American Type Culture Collection), provided by CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos, La Plata, Argentina) and *L. monocytogenes* provided by CERELA (Centro de Referencia de Lactobacilos, Tucumán, Argentina) were used. A stock culture was maintained on tryptic soy broth (Britania, Buenos Aires, Argentina) at 4 °C. Before it was used, the O157:H7 and *L. monocytogenes* were cultured in Brain–Heart Infusion (BHI, Britania, Buenos Aires, Argentina) for 24 h at 37 °C. 0.1 mL of culture was transferred to 9.9 mL of BHI at two consecutive 24 h intervals immediately before each experiment.

2.5. In vitro assay

2.5.1. Preparation of broccoli native microflora

Native microflora from broccoli was prepared from 10 g of raw material macerated in 90 mL of phosphate buffer solution (0.1 mol/L), using a Stomacher 400 Circulator Homogenizer (pH 7.2) and incubated overnight at 37 °C, in agreement with the procedure reported by Moreira, Ponce, del Valle, and Roura (2007).

2.5.2. Determination of sensitivity

The sensitivity of the broccoli native microflora to different EO and BC was determined by the agar diffusion method. Sterile paper discs (Whatman N° 40; 6.0 mm in diameter, Britania) were soaked with pure tea tree and rosemary EO, pure pollen and propolis extract and diluted (60 mg/mL) pomegranate and resveratrol. DMSO was included as a negative control for pomegranate and resveratrol. Then, the paper discs were placed on the surface of the inoculated BHI agar plates. The dishes were incubated at 37 °C for 24–48 h and the zones of inhibition were measured. The sensitivity to the different biopreservatives was classified by the diameter of the inhibition halos as: not sensitive, for diameter less than 8 mm; sensitive, for diameter 9–14 mm; very sensitive, for diameter 15–19 mm and extremely sensitive, for diameter larger than 20 mm (Moreira, Ponce, Del Valle, & Roura, 2005; Ponce, Fritz, Del Valle, & Roura, 2003). Each assay was performed in duplicate on 3 separate experimental runs.

2.5.3. Tube-assay method

Test tubes with 5 mL of BHI broth were inoculated with 1 mL of inoculum obtained from the native microflora of broccoli (approximately 10^4–10^5 CFU/mL). Then, 4 mL of CH coating-forming solutions and acetic acid solvent (2 mL/100 mL) were added. At 0 h and after 24 h incubation at 37 °C the optical density of the broths at 610 nm was measured with the UV–Visible spectrophotometer (Shimadzu Corporation UV 1601 PC UV–Visible, Kyoto, Japan) (Moreira, Roura, & Ponce, 2011). Each assay was performed in duplicate on 3 separate experimental runs.

2.5.4. Microdilution agar plate method

Aliquots of 10 mL of Luria–Bertani broth (LB, triptone 1 g/100 mL, yeast extract 0.5 g/100 mL and NaCl 1 g/100 mL) were agitated vigorously with the BC or EO to achieve different final biopreservative concentrations (0.5–8.0 μL/mL for tea tree and rosemary; 60–180 μg/mL for pomegranate and resveratrol; and 1.0–40.0 μL/mL for pollen and propolis). DMSO (3 μL/mL) was included as a negative control for pomegranate and resveratrol, taking into account the maximum concentration used to dissolve these BC. Then, 100 μL of an overnight culture of *E. coli* and *L. monocytogenes* were added. Inoculated solutions were mixed followed by incubation at 37 °C during 32 h. The viable *E. coli* and *L. monocytogenes* counts were monitored as follows: 0.1 mL sample of each treatment were spread on the surface plating on LB agar. The plates were incubated at 37 °C for 24–48 h and the numbers of colonies were determined. Microbial counts were expressed as log CFU/mL. Each assay was performed in duplicate on 3 separate experimental runs.