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ORIGINAL ARTICLE

Antimicrobial activity of chitosan coatings and films against *Listeria monocytogenes* on black radish

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Abstract The antibacterial activity of chitosan coatings prepared with acetic or lactic acid, as well as of composite chitosan–gelatin films prepared with essential oils, was evaluated in fresh shredded black radish samples inoculated with *Listeria monocytogenes* ATCC 19115 and *L. monocytogenes* ATCC 19112 during seven days of storage at 4 °C. The chitosan coating prepared with acetic acid showed the most effective antibacterial activity. All tested formulations of chitosan films exhibited strong antimicrobial activity on the growth of *L. monocytogenes* on black radish, although a higher inhibition of pathogens was achieved at higher concentrations of chitosan. The antimicrobial effect of chitosan films was even more pronounced with the addition of essential oils. Chitosan–gelatin films with thyme essential oils showed the most effective antimicrobial activity. A reduction of 2.4 $\log_{10}$ CFU/g for *L. monocytogenes* ATCC 19115 and 2.1 $\log_{10}$ CFU/g for *L. monocytogenes* ATCC 19112 was achieved in the presence of 1% chitosan film containing 0.2% of thyme essential oil after 24 h of storage.

PALABRAS CLAVE
Actividad antimicrobiana;
Rábano negro;
Coberturas y películas de quitosano;
Aceites esenciales;
Listeria monocytogenes

Actividad antimicrobiana de coberturas y películas de quitosano contra *Listeria monocytogenes* en rábano negro

Resumen Se evaluó la actividad antimicrobiana de coberturas del quitosano y de películas compuestas de quitosano-gelatina en muestras frescas de rábano negro cortado inoculadas con las cepas de *Listeria monocytogenes* ATCC 19115 y ATCC 19112, almacenadas durante 7 días a 4 °C. Las primeras fueron preparadas con ácido acético o ácido láctico, las segundas con aceites esenciales. Las coberturas de quitosano preparadas con ácido acético mostraron la actividad antimicrobiana más eficaz. Todas las formulaciones de películas de quitosano exploradas mostraron una fuerte actividad antimicrobiana sobre el crecimiento de *L. monocytogenes*, aunque la mayor inhibición de

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estos patógenos se logró con las mayores concentraciones de quitosano. La actividad antimicrobiana de las películas de quitosano fue mayor con la adición de aceite esencial. Las películas de quitosano-gelatina con aceite esencial del tomillo fueron las que mostraron la actividad antimicrobiana más eficiente. A las 24 h de almacenamiento, la película con 1% de quitosano y 0,2% de aceite esencial de tomillo produjo una reducción de 2,4 log10 UFC/g en *L. monocytogenes* ATCC 19115, y de 2,1 log10 UFC/g en *L. monocytogenes* ATCC 19112.

Introduction

Nowadays, an increasing number of consumers realize the importance of fresh vegetables in their daily diet. Vegetables represent a significant source of vitamins, minerals and dietary fibers[24]. Moreover, it may be useful in the prevention of various diseases, especially when consumed fresh[14,34]. For these reasons, the increased consumption of vegetables has led to the development of the special sector that deals with the treatment of minimally processed vegetables, which have been trimmed, peeled, cut, and packaged for distribution and consumption. Since minimally processed vegetables normally do not contain any preservatives and have not been subjected to any heat or chemical treatments, they must be kept at low temperature or refrigerated storage. They can be found in the market in the form of sliced cabbage, carrots, lettuce or mixtures thereof.

During the growing process, when the outer surfaces of vegetables come in contact with soil, irrigation water, environment in the packing plants, surfaces of transportation trailers or hands of packing workers, they may be contaminated with pathogenic microorganisms[1,6,11]. Bacteria may be transferred from the external vegetable surfaces to the edible portions during cutting and dividing, thus contaminating fresh-cut products[28,32]. In addition, minimally processed products may be re-contaminated through cross-contamination and improper handling during their distribution[31]. *Listeria monocytogenes* is one of the microorganisms that represent a risk to public health. This bacterium possesses the capacity to develop at the low temperatures[13] mainly used for storing minimally processed products and may multiply in vegetables in large quantities causing listeriosis, a severe disease in humans.

Listeriosis is a serious infection caused by eating food contaminated with the *L. monocytogenes* bacterium, particularly ready-to-eat foods, including dairy, meat and poultry, and some fruit and vegetable products. The disease primarily affects pregnant women, newborns, and adults with weakened immune systems. Although *L. monocytogenes* rarely causes foodborne disease outbreaks, it represents a major food safety concern due to the high mortality rate associated with listeriosis[2]. One of the largest listeriosis outbreaks ever occurred in several states of the USA in 2011, and was caused by the consumption of fresh cantaloupe[7]. This outbreak caused 146 illnesses in 28 states, including 30 deaths and 1 miscarriage.

Traditional food preservation techniques based on thermal treatments can provide microbiological stability of food; however, they are not appropriate for fresh-cut, minimally processed vegetables. To allow the microbiological stability of minimally processed vegetables in the market today, researchers are looking for non-thermal treatments that do not affect the physicochemical properties and nutritional value of these products.

A convenient agent that can provide microbiological stability of minimally processed vegetables extending shelf life and improving the quality of these products may be chitosan, a natural biopolymer which possesses unique biodegradability and bioactivity properties[17]. It is obtained by partial deacetylation of chitin, the second most widespread polysaccharide on Earth after cellulose. Several studies have reported the antimicrobial and antioxidant characteristics, as well as non-toxicity of chitosan[3,16,18,36]. The specific antimicrobial activity of chitosan in coating formulations has been previously investigated, providing evidence that it can be attributed to its molecular weight and its deacetylation degree[9]. The activity of chitosan can be related to a change in cell permeability; the interactions between the amino groups of chitosan and the electronegative charges on the cell surface lead to the leakage of intracellular electrolytes and protein constituents[23].

The incorporation of different antimicrobial constituents, such as essential oils or organic acid, into the polymer matrices can notably change and/or improve antimicrobial and some physicochemical properties, such as mechanical properties, color or water vapor barriers, as has been described for composite films[25,26] and for coatings in fruit applications[4]. Chitosan can be used in the form of edible coatings or films having a variety of advantages over synthetic materials, such as biodegradability, edibility, biocompatibility, also the quality of being environmentally friendly[21]. Several authors have studied the antimicrobial activity of chitosan in different food products[8,29,33,35]. However, there is no data referring to the antimicrobial activity of chitosan on black radish. Radish (*Raphanussativus* L.) belongs to the family Cruciferae and is grown for its edible root. In traditional kitchen, black radish is often used in winter salads. Grated fresh root, spiced with vinegar and oil, is a local specialty. This vegetable can be used as a stand-alone salad or in combination with other fruits and vegetables such as carrots, cabbage, apples, etc. Black radish mixed with honey is one of the most commonly used alternative folk medicines, suitable to treat coughs and bronchitis. Therefore, in a large number of handbooks and instructions in the field of phytotherapy, homeopathy and self-treatment, it is a very powerful hepatoprotective and well-known healing medicine in the treatment of liver disorders and bile ducts[15].