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Antimicrobial effects of different combined non-thermal treatments against *Listeria monocytogenes* in broccoli florets

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ABSTRACT

The antilisterial effect of three non-thermal treatments in combination with a bioactive coating formulation on broccoli florets inoculated with *Listeria monocytogenes* was evaluated. The nanoemulsions of carvacrol, bergamot, lemon and mandarin essential oils (EO) were incorporated in native and modified chitosan coating formulations and the antilisterial effect in inoculated samples was evaluated. The modified chitosan based coating containing mandarin EO was the best treatment which caused a load reduction of 1.46 log CFU/g after 6 days of storage. The antilisterial effects of this coating formulation in combination with ozonated water, UV-C and γ-ray treatments on inoculated samples were evaluated during 13 days storage at 4 °C. The combined coating and ozonated water showed very high antilisterial effects at days 1 and 3; however, their antilisterial activity was reduced after day 5. The combined coating and UV-C did not show any additive effect against *L. monocytogenes* as compared to coating alone. The best antilisterial activity was obtained in the combined coating and γ-rays. This combined treatment caused an increase in relative radiation sensitivity of *L. monocytogenes* by 1.33-fold. Further, this combined treatment ensured microbial safety during storage with a reduction of *L. monocytogenes* by 2.5 log CFU/g after 13 days.

1. Introduction

According to the Center for Disease Control and Prevention (CDC), the annual incidence of serious foodborne illnesses is very high: CDC estimates that each year in the United States 48 million people get sick, 128,000 are hospitalized, and 3000 die due to foodborne diseases. Therefore, ensuring microbiological safety of food products, while maintaining their nutritional and organoleptic properties, is still a priority nowadays.

Vegetables can be easily contaminated: cutting or slicing operations increase tissue damage, causing the release of intracellular contents (González-Aguilar et al., 2009) that can support and increase the activity of pathogenic and saprophytic microorganisms. A frequent problem is contamination with *Listeria monocytogenes* (Beauchat, 1996), Gram-positive, rod-shaped bacteria which are widely distributed in the environment and are pathogenic to humans and animals (Hein et al., 2000). Heat treatments can ensure microbial inactivation, but frequently they unacceptably affect the nutritional and organoleptic properties of food. Consumers' demand for high-quality foods that are microbiologically safe and stable has awakened a growing interest in non-thermal preservation techniques (Espina et al., 2012). Particularly the combination of non-thermal methods with antimicrobial treatments can enhance the lethal effects of non-thermal processing, reduce the severity or the exposure time of non-thermal treatment needed to obtain a given level of microbial inactivation, preserve food physico-chemical properties without affecting the nutritional value (Raso and Barbosa-Cànovas, 2003).

In this context, the use of active edible coatings in combination with non-thermal treatments may represent a viable approach for achieving microbial stability and preserving quality. Due to their characteristics, in fact, edible coatings have been traditionally used to improve food appearance and maintain microbiology quality (Khwaldia et al., 2004). Chitosan is a polycationic polymer obtained by partial deacetylation of chitin, the most abundant natural carbohydrate after cellulose. Chitosan is non-toxic, biodegradable and biocompatible and is also easily modified by physical or chemical methods (Le Tien et al., 2003). Several studies reported the antimicrobial activity of chitosan and its derivatives in coating formulations (Kanatt et al., 2013; Bordenave et al., 2010). The mechanism of action of chitosan against bacteria has not been

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completely explained yet, but several hypotheses have been postulated: most likely, the antimicrobial activity can be attributed to a change in cell permeability due to interactions between the amine groups of chitosan and the electronegative charges on the cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (Papineau et al., 1991).

In addition to native chitosan, several derivatives have been studied for their antimicrobial activity, for example N-carboxybutyl chitosan (Muzzarelli et al., 1990), quaternary N-alkylchitosan (Jia, 2001). A new formulation was also elaborated, based on chitosan acylation with fatty acids derivatives, with the aim of enhancing the hydrophobic properties of the polymer (Han et al., 2008).

Essential oils have also gained interests as natural antimicrobial agents for food preservation against foodborne pathogens and spoilage bacteria (Caillet et al., 2006a,b). The antimicrobial activity of several essential oils and essential oil components has been successfully proved on different microorganism species (Di Pasqua et al., 2007; Gill and Holley, 2006). However, being essential oil constituents characterized by low solubility in water, their efficiency is promoted when encapsulated in appropriate delivery systems that can enhance their dispersibility in the aqueous part of foods, where microorganisms grow and proliferate (Weiss et al., 2009).

Recently, the use of nanoscale delivery systems for essential oils was shown to offer the potential of improving not only the physico-chemical stability of encapsulated bioactive compounds in foods, but also their bioactivity through the activation of passive mechanisms of cell absorption, owing to their subcellular size, therefore enabling the reduction of the dose of essential oils required to ensure antimicrobial activity in foods, minimizing the impact on aroma, flavor and taste (Donsì et al., 2011, 2012).

In parallel, physical non-thermal treatments have known an increasing development, with the aim of ensuring microbial safety without undesirable sensorial and nutritional changes, such as color degradation, softening of tissues, and vitamin losses on foods. Recently, new processing technologies have been developed, based on ozone treatments, UV-C irradiation and gamma ray irradiation, which are capable of prolonging product shelf life without the unfavorable effects of severe heating (Alexandre et al., 2011).

Owing to its powerful oxidizing effect, ozone is one of the most potent disinfectant agents (Güzel-Seydim et al., 2004). Ozone is appropriate for several applications in food industry, such as food surface hygiene and preservation, equipment and food plant sanitation and reuse of waste water. In several studies ozone has been found to be effective against a wide spectrum of microorganisms, including viruses, gram-negative and gram-positive bacteria, spores and fungi (Manousaridis et al., 2005).

Several studies highlighted that γ-irradiation is an excellent process for reducing or eliminating foodborne pathogens (Caillet et al., 2006b; Turgis et al., 2009) by breaking DNA chemical bonds, or altering the membrane permeability and other cellular functions (Lopez-Gonzales et al., 1999). It has also been shown that the combined use of antimicrobial compound incorporated or not in edible coating and γ-irradiation can increase the radiosensitivity of bacteria, resulting in lower radiation doses required for lethality (Caillet et al., 2006a,b; Ouattara et al., 2001; Vu et al., 2012).

Another postharvest physical treatment developed in recent years that can be used to ensure fruits and vegetables safety is the UV-C irradiation (Alexandre et al., 2012). Its germicidal activity against several pathogens, due to the formation of pyrimidine dimers in DNA strands, crosslinks of aromatic amino acids leading to membrane depolarization and abnormal ionic flow, has been reported in several studies (Sommer et al., 1996; Wright et al., 2000). It has also been demonstrated that UV-C illumination can delay postharvest fruit senescence and especially control decay in different fruit and vegetable species (Erkan et al., 2001). For example, UV-C exposition decreases the activity of enzymes involved in tomato cell wall degradation and delays the fruit softening (Barka et al., 2000).

The aim of this study is the evaluation of antimicrobial effect of combined non-thermal treatments against *L. monocytogenes* on broccoli florets. In particular, the combined non-thermal treatments will be based on the use on an antimicrobial edible coating, based on native and modified chitosan incorporating nanoemulsions of different essential oil, in combination with a physical non-thermal treatment, such as ozone treatment, UV-C exposition and γ-ray irradiation. This approach is innovative both in the development of novel antimicrobial edible coatings, and in the study of the possible synergistic effect of its use in combination with a physical non-thermal treatment.

2. Materials and methods

2.1. Bacterial preparation

The five *L. monocytogenes* strains (Health Canada, Health Products and Food Branch, Ottawa, Canada) used in this study were isolated from foodborne outbreak (Table 1).

Before each experiment, stock cultures of 5 strains of *L. monocytogenes* were propagated separately through two consecutive 24 h growth cycles in tryptic soy broth (TSB, Difco) at 37 °C. Subsequently, 2 ml from each culture broth were combined together. The combined broth of mixed strains was centrifuged at 4000 g for 20 min at 4 °C and washed twice in sterile saline solution (0.85% w/v) to eliminate residual components of media culture. The obtained biomass was then suspended in sterile saline water, to obtain a working culture containing approximately 10^9 CFU/ml.

2.2. Characterization of the essential oils

The analysis of the essential oils was carried out in a GC–MS Finnigan-Focus (Thermo-Fisher Scientific, UK), as previously described (Donsì et al., 2012). Briefly, an RTX-5 SIL MS capillary column (30 m long, 0.25 mm i.d. and 0.25 μm film thickness) was used with a cross-linked stationary phase of polyethylene glycol (Restek). The chromatographic conditions were as follows: He as the carrier gas; the injector in split-mode with a split flow of 20 ml/min and a temperature of 250 °C; the temperature of the ion source was 250 °C; the temperature of the transfer line was 260 °C. The compounds were separated using a temperature program with an initial oven temperature of 80 °C for 10 min and a temperature gradient of 25 °C/min to a final temperature of 250 °C, which was maintained for 10 min. 3 μl of the sample was injected, using the split technique. The ionization was produced by electronic impact at 70 eV. The eluted compounds were identified using the retention times and by comparing their mass spectra with a spectral library of known standard compounds. The identification was carried out in full scan mode between 40 and 400 amu.

Table 1
Source and serotypes of *L. monocytogenes* strains used in the study.

Strain	Serotype	Source
HPB2558	½b	Beef hot dogs
HPB2812	½a	Homemade salami
HPB1043	½a	Turkey frank factory isolate
HPB2569	½a	Cooked cured sliced turkey
HPB2371	½b	Raw turkey