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Combined effect of antimicrobial coatings, gamma radiation and negative air ionization with ozone on *Listeria innocua*, *Escherichia coli* and mesophilic bacteria on ready-to-eat cauliflower florets

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ABSTRACT

The objective of this study was to evaluate the effect of a bioactive edible coating combined with a low γ-radiation dose or negative air ionization (NAI) with ozone on the microbiological quality of ready-to-eat cauliflowers. Results showed that each treatment alone was effective on *Listeria innocua*, *Escherichia coli* and mesophilic bacteria. After 7 d, treatment with γ-radiation reduced *L. innocua* and *E. coli* of 1.8 and 3.6 log CFU g^{-1} respectively while NAI + ozone reduced *L. innocua* and *E. coli* of 2.0 and 2.8 log CFU g^{-1} respectively. Mesophilic bacteria were reduced of 1.8 log CFU g^{-1} after γ-radiation and 1.4 log CFU g^{-1} after NAI + ozone. This study demonstrated that the bioactive coating acts in synergy with γ-radiation, inducing no bacterial growth of *L. innocua* and *E. coli*, as well as a control of the growth of mesophilic bacteria during 7 d. The combination of bioactive coating and NAI + ozone induced an additive effect on *L. innocua*, *E. coli* and mesophilic bacteria, and suggests potential antioxidant properties of the coating.

1. Introduction

Elimination of pathogenic bacteria is a major concern for food industries. Indeed, Scallan et al. (2011) have estimated that 9.4 million illnesses were due to foodborne disease, causing 55,961 hospitalizations and more than 1,300 deaths. Among all the microorganisms, *Escherichia coli* and *Listeria monocytogenes* are frequently involved in microbial outbreaks. After harvest, fruits and vegetables can be considered as bacteria carriers which is why they undergo disinfection treatments (Ölmez and Kretzschmar, 2009). The use of chemical washing compounds such as peracetic acid can reduce the bacterial load but it is not enough to guarantee safety throughout the entire shelf life of food products (Alvaro et al., 2009; Dai et al., 2012; Siroli et al., 2015). Indeed, according to Erickson (2010), cross-contaminations are likely to occur while food is being processed but also after leaving processing facilities. Despite food safety measures, foodborne outbreaks still happen, leading to health threats and hospitalizations. Fruits and vegetables that are eaten raw can be an easy target, as confirmed by recent outbreaks which occurred on fresh vegetables for salads, cantaloupes or celery, respectively contaminated by *E. coli*, *Salmonella* and *Listeria* (Garner and Kathariou, 2016; Kozak et al., 2013).

Gamma-radiation is a cold process that can be used to assure food safety and is usually applied on packaged products. Irradiation provokes DNA double-strand breaks, which can lead to bacterial death (Hussain et al., 2014; Jeong et al., 2010). The maximum dose that can be applied on fresh vegetables should not exceed 1 kJ kg^{-1} (Komolprasert et al., December 2007/January 2008). However, some pathogens such as *L. monocytogenes* can still survive and need higher doses to be eliminated (Bari et al., 2006).

Ozone, an allotrope of oxygen, can be produced by using the corona method. Air is exposed to a high-voltage current, leading electrons to split after their excitation. Single atoms of oxygen will then combine with oxygen molecules, forming a new ozone molecule with a short half-life (Alencar et al., 2013; Kim et al., 1999; Shah et al., 2013). The antimicrobial action of ozone has been studied and several mechanisms have been listed (Kim et al., 1999). Indeed, it has been proposed that ozone induces changes in DNA of microorganisms, leading to their inactivation. Also, according to some authors, ozone would react on cell walls of bacteria by oxidizing major components (Beuchat, 1992). During the last past years, the use of ozone has been paired with negative air ionization. This new air-cleaning technology provides negative

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charges to particles, resulting in their accumulation on walls and floors (Holt et al., 1999). Challenger et al. (1996) showed that the use of negative ions also leads to the generation of hydrogen peroxide. Studies have been carried out on the survival of microorganisms on cantaloupe, showing that hydrogen peroxide treatments were effective against *L. monocytogenes* and total flora (Sapers et al., 2001; Ukuku and Fett, 2002).

Essential oils (EOs) are natural compounds known to possess strong antimicrobial properties due to their content in phenolic compounds and flavonoids (Oussalah et al., 2004). Burt (2004) has suggested that the hydrophobicity of EOs provokes changes in cell membrane permeability, resulting in a loss of ions and other cell contents. Lv et al. (2011) showed that a one hour-treatment with EOs such as oregano, bergamot or basil is also enough to damage cell membranes. Oussalah et al. (2006) studied the mechanism of action that occurred after treatment of bacteria with EOs. They showed that EOs are more likely to act on the cytoplasmic membrane, leading to physiological changes in bacteria. Indeed, losses of intracellular ATP and cell constituents were observed, confirming a disruption of bacteria membranes. Other effects such as denaturation of proteins and enzymes have been described to affect cell division mechanisms (Nazzaro et al., 2013). Organic acids can also be used as antimicrobial compounds since they are internalized in the cytoplasm before dissociating into protons and anions, affecting internal pH and cell components (Davidson and Taylor, 2007; Ricke, 2003). Recently, Boumail et al. (2016) have developed a bioactive coating which allowed a reduction of *L. monocytogenes* in vegetables.

Treatments are usually combined to induce synergies and increase antimicrobial effects. However, very little research has studied the effect of gaseous ozone and EOs on bacteria. Whangchai et al. (2006) studied the effect of ozone combined with citric or oxalic acids. They concluded that gaseous ozone should be followed by another antimicrobial treatment to prevent later contaminations. Thus, the use of negative air ionization with ozone (NAI + ozone) during the whole storage represents an innovative technique to control bacterial growth. The aim of this study was to evaluate and compare the antimicrobial effects of γ-radiation or NAI + ozone in combination with a bioactive coating on ready-to-eat cauliflowers during storage.

2. Materials and methods

2.1. Bacterial suspension

Under sterile conditions, 25 g of cauliflower were immersed in 75 mL of peptone water (1 g L^{-1}) and then, homogenized for 1 min using a Stomacher Lab-Blender 400 (Laboratory Equipment, London, UK). A quantity of 1 mL of this resulting mixture was incubated in 9 mL of Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, MI, USA) at 37 °C for 24 h. Those bacteria extracted from cauliflower were used to prepare a stock solution of mesophilic bacteria. *E. coli* ATCC 8739, *Listeria* innocua ATCC 51742 and mesophilic bacteria were stored at −80 °C in TSB in presence of glycerol (150 g L^{-1}). Before each experiment, bacteria were propagated through 2 consecutive cycles of 24 h in TSB at 37 °C. The cultivated cultures were centrifuged at 5,000 *g* for 15 min and the collected pellets were washed twice in peptone water (1 g L^{-1}) to obtain working cultures containing approximately 10^9 CFU (colony forming unit) mL^{-1}.

2.2. Preparation of vegetables

Cauliflowers were purchased from a local supermarket, cut into florets (20–25 g) and packaged in 12.7 μm metalized polyester-50.8 μm ethylene vinyl acetate copolymer bags (Winpak Division Ltd., Montreal, QC, Canada). Sterilization was done at the Canadian Irradiation Center by γ-radiation, using a UC-15 A (SS canister) underwater calibrator (Nordion Inc., Kanata, ON, Canada) equipped with a ^{60}Co source. A radiation dose of 10 kJ kg^{-1} was delivered at a dose rate of 16.95 kJ kg^{-1} h^{-1}. Vegetables were then stored at 4 °C.

2.3. Bioactive coating

The mixture of antimicrobial compounds was prepared according to Boumail et al. (2016). The bioactive coating was prepared as described by Boumail et al. (2016) and contained 2.5 g L^{-1} of methylcellulose (MC), 7.5 g L^{-1} of maltodextrin (MD), 7.5 g L^{-1} of glycerol (Sigma-Aldrich Ltd.) and 34 g L^{-1} of the antimicrobial compounds.

2.4. Bacterial radiosensitization

The D_{10} is defined as the radiation dose required reducing 90% population (reduction of 1 log CFU g^{-1}) of viable *E. coli* and *L. innocua* on cauliflowers. Control and coated cauliflowers were inoculated with *E. coli* or *L. innocua* in order to reach approximately 10^6 log CFU g^{-1} on vegetables. Samples were stored at 4 °C for 15 h, allowing the bioactive coating to act on bacteria. Irradiation treatment were then performed with doses from 0 to 1 kJ kg^{-1} for *E. coli* and from 0 to 2.4 kJ kg^{-1} for *L. innocua*. For microbiological analysis, cauliflowers samples (25 g) were immersed in 50 g of peptone water (1 g L^{-1}) and homogenized using a stomacher. Serial 10-fold dilutions were made and 1 mL of each dilution was spread on petri dishes before medium was poured. MacConckey culture media supplemented with sorbitol was used for *E. coli* and Palcam supplemented with ceftazidime (20 mg L^{-1}), acriflavin (5 mg L^{-1}) and polymixin B (10 mg L^{-1}) was used for *L. innocua*. Petri dishes were incubated at 37 °C for 48 h before bacterial enumeration. Detection level was calculated as 0.48 log CFU g^{-1} (3 CFU g^{-1}). Bacterial counts were plotted against radiation doses and the D_{10} value was calculated according to Eq. (1).

$$D_{10} = \frac{1}{a} \quad (1)$$

where *a* is the slope of the trendline extracted from the plot.

2.5. Negative air ionization (NAI) with ozone

The ionizer/ozonator was set to produce minimal amount of ozone (volume of 428 mg m^{-3}) and negative ions (ranging from −0.2 to −0.4 mV) during the whole experiment. The residual ozone content was measured and recorded using a Portable Ozone Analyzer Series 500 (Aeroqual Limited, Auckland, New Zealand) that can detect up to 1,070 mg m^{-3}. Data were collected using Aeroqual S500 v6.0 software (Aeroqual Limited). The negative ions content was measured and recorded with an Air Ion Counter (AlphaLab Inc., Salt Lake City, UT, USA) and data were collected using AlphaApp 1.0.19 (AlphaLab Inc.).

2.6. Antimicrobial effect of combined treatments during storage

Cauliflowers were treated with the bioactive coating and then inoculated by adding 500 μL of a diluted bacterial suspension (10^6 log CFU mL^{-1}) on 20 g of cauliflowers. Final concentrations on cauliflower were 3.2 log CFU g^{-1} for *L. innocua* and 3.6 log CFU g^{-1} for *E. coli* and for mesophilic bacteria. After inoculation, samples were divided into 2 groups. One group was stored at 4 °C for 15 h and then irradiated with a dose of 0.25 kJ kg^{-1} before storage during 7 d. The other group was stored at 4 °C under ionized/ozonated air during 7 d. Microbiological analysis were performed as described in Section 2.4 at days 0, 1, 2, 4 and 7. Tryptic Soy Agar