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Effects of combined treatments of irradiation and antimicrobial coatings on reduction of food pathogens in broccoli florets

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ABSTRACT

The effect of combined treatment of antimicrobial coatings and γ-radiation on reduction of food pathogens such as *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* Typhimurium was evaluated in broccoli florets. Broccoli florets were inoculated with pathogenic bacteria at 10^6 CFU/g. Inoculated florets were then coated with methylcellulose-based coating containing various mixtures of antimicrobial agents: organic acids (OAs) plus lactic acid bacteria metabolites (LABs), OA plus citrus extract (CE), OA plus CE plus spice mixture (SM), and OA plus rosemary extract (RE). Coated florets were irradiated with various doses (0–3.3 kGy), and microbial analyses were used to calculate the D_{10} value and radiosensitive relative. The coating containing OA plus CE was the most effective formulation for increasing the sensitization of *Escherichia coli* by 2.4 times as compared to the control without the antimicrobial coating. For *Salmonella* Typhimurium, coating containing OA plus LAB was the most effective formulation, increasing radiosensitivity by 2.4 times as well. All antimicrobial coatings had almost the same effect of increasing the sensitivity of *Listeria monocytogenes* (from 1.31 to 1.45 times) to γ-irradiation.

1. Introduction

Nowadays, consumer demands not only focus on microbiologically safe and stable food products but also require high quality products with enhanced attributes. Thus, food technologists and the food industry have combined their efforts in research to develop new technologies that can be used to maintain the safety as well as improve the safety and quality of food products (Artes et al., 2007).

Bacteriocins produced by lactic acid bacteria have been used as a promising approach to extending the shelf life of food. Some LAB bacteriocins exhibit inhibitory activity against *S.* Typhimurium and *E. coli* (Mahapatra et al., 2005; Millette et al., 2008). Many natural organic acids such as citric acid, propionic acid, and lactic acid have been used as natural antimicrobial agents for food preservation (Samelis et al., 2001). Moreover, many essential oils (EOs) have been confirmed their potentials as natural antimicrobial agents and many studies have used EOs in food products to inhibit the growth of food pathogens (Ouattara et al., 2001; Turgis et al., 2008, 2009).

Gamma-irradiation has been demonstrated as an excellent treatment method for reducing or eliminating foodborne pathogens such as *S.* Typhimurium and *E. coli* (Turgis et al., 2008). Food irradiation has been used to inhibit sprouting in vegetables, to control ripening in fruits, and to disinfect grains and spices and protect them from reinfection (Lado et al., 2002). It has been found that the combined treatments using antimicrobial compounds and γ-irradiation can increase the radiosensitivity of bacteria, resulting in lower radiation doses required for lethality (Ouattara et al., 2001).

In recent years, bioactive edible coatings have been developed to reduce the risk of contamination of processed products and to maintain the hygienic quality of these products (Nguyen et al., 2008). A coating matrix based on polysaccharides contains natural antimicrobial compounds that can interact with the food system, leading to an increase in shelf life. These bioactive coating formulations act as both selective barriers and vehicles for specific food additives such as antimicrobial agents, flavorings, and antioxidants (Han et al., 2004). Methylcellulose is cellulose derived biodegradable polymer commonly used as a film coating because of its low cost and ease of handling. Hydroxyl groups in cellulose are replaced by methoxyl groups after etherification, resulting in a significant decrease in hydrogen bonding that was responsible for the crystallinity of cellulose (Jingjid et al., 2006).

A variety of antimicrobial agents such as organic acids, bacteriocins, and EOs have been incorporated into edible coatings. The resulting bioactive coatings can protect the biological activity of the natural compounds, ensure adequate dispersion of the compounds on the surface of the food, and allow a controlled

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release of the compounds during storage (Lacroix and Ouattara, 2000). Research has been conducted on the use of essential oils (EOs) from spices combined with γ-irradiation to increase the radiosensitization of pathogenic bacteria such as *E. coli*, *S.* Typhi, and *L. monocytogenes* in carrots (Caillet et al., 2006a, 2006b). However, these studies did not include an evaluation of EOs in mixtures with other antimicrobial compounds. The objective of this study was to evaluate the sensitization of pathogenic bacteria on broccoli florets to γ-irradiation when treated by the combined antimicrobial coatings and various doses of γ-radiation. The antimicrobial coatings tested contained methylcellulose and various mixtures of antimicrobial agents such as bacteriocins, organic acids, terpenes, and polyphenolics compounds.

2. Materials and methods

2.1. Materials

2.1.1. Antimicrobial compounds

The spice mixture (SM) and the supernatant of lactic acid bacteria metabolites (LAB) were provided by BSA Food Ingredients s.e.c./l.p. (Montreal, Quebec, Canada), and the citrus extract (CE) was obtained from BioSecur Lab Inc. (Otterburn Park, Quebec, Canada). Rosemary extract (RE) (containing 40% rosmarinic acid, wt/wt) was obtained from P.L. Thomas & Co., Inc. (Morristown, NJ), the mixture of organic acids (OAs) was obtained from Kerry Ingredients and Flavors (Monterey, TN). All of these antimicrobials contain phenolic and/or terpenic compounds.

2.1.2. Polymeric matrix

Methylcellulose (MC) was purchased from Sigma–Aldrich Ltd. (Oakville, Ontario, Canada). Tween 80 and glycerol were purchased from Laboratoire Mat (Beauport, Quebec, Canada), and vegetable oil (100% pure sunflower oil) was provided by Sobeys Inc. (Mississauga, Ontario, Canada).

2.1.3. Vegetables

Broccoli heads were purchased from a local supermarket (IGA, Laval, Quebec, Canada). Broccoli heads were cut into florets, and florets were then packaged in Winpak bags (Winpak Division Ltd., Montreal, Quebec, Canada). The packaged florets were sterilized by irradiation at the Canadian Irradiation Center using a UC-15 A (SS canister) underwater calibrator (Nordion Inc., Kanata, Ontario, Canada) equipped with a ^{60}Co source. A radiation dose of 10 kGy was applied to sterilize the broccoli florets. The packages were then stored at 4 °C.

2.2. Bacteria and inoculation process

2.2.1. Pathogenic bacteria preparation

L. monocytogenes HPB 2812 serovar 1/2a was isolated from homemade salami by the Health Products and Foods Branch of Health Canada (Ottawa, Ontario, Canada). *E. coli* O157:H7 (EDL933) and *S.* Typhimurium (SL 1344) were obtained from Prof. Charles Dozois (Institut National de la Recherche Scientifique, Institut Armand-Frappier, Laval, Quebec, Canada). All bacterial cultures were maintained at 80 °C in TSB containing glycerol (10%, v/v). Before each experiment, stock cultures of pathogenic bacteria were propagated through two consecutive 24 h growth cycles in tryptic soya broth (TSB) at 35 °C and washed twice in saline solution (0.85%, w/v) to obtain working cultures containing approximately 10^9 CFU/ml.

2.2.2. Inoculation process

An inoculation bath was prepared separately for each pathogen (*L. monocytogenes*, *S.* Typhimurium, and *E. coli*) by adding these microorganisms to 3 l of sterile 0.85% NaCl (w/v) solution to reach a population of approximately 10^6 CFU/g on broccoli florets. The packaged broccoli florets were opened under sterile conditions, and each floret was dipped into the inoculation bath and stirred gently for 5 min. After inoculation, the florets were dried on aluminum foil for 30 min before treatment with the coating containing the bioactive agents.

2.3. Preparation of bioactive coatings and irradiation treatment

2.3.1. Preparation of bioactive coatings

MC was poured into a beaker containing warm water (60 °C) to obtain a final concentration of 2% (w/v) and then subjected to constant stirring for MC pregelatinization. The suspension was placed in an ice bath and stirred vigorously to ensure complete solubilization. One percent vegetable oil, 0.5% glycerol, and 0.025% Tween 80 were added at room temperature and stirred vigorously for 5 min. The bioactive agents were then incorporated into the coating formulation and stored at 4 °C until application. In this study, the florets were separated into five groups: control (uncoated), coating without antimicrobials (CWA), OA plus LAB (OA+LAB), OA plus CE (OA+CE), OA plus CE plus SM (OA+CE+SM), and OA plus RE (OA+RE).

2.3.2. Antimicrobial coating of broccoli

The broccoli florets (which were inoculated with different pathogenic bacteria) were dipped in baths containing various antimicrobial coatings, drained and dried on aluminum for 90 min. A 25 g sample of florets was packaged in 0.5-mil metallized polyester-2-mil ethylene vinyl acetate copolymer bags (205 mm × 355 mm; Winpak), and all samples were left overnight at 4 °C until irradiation.

2.3.3. Irradiation treatment

Each sample was irradiated at room temperature with the UC-15 A underwater calibrator at 16.74 kGy/h. Radiation doses were 0–3.3 kGy. Samples were analyzed immediately after irradiation. Microbial analysis of samples was conducted to verify the radiosensitivity of the bacteria.

2.4. Microbiological analysis

Each floret sample was weighed (15 g) and homogenized for 2 min at 2300 rpm in 45 ml of sterile peptone water (0.1%, w/v) with a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From this homogenate, serial dilutions were prepared, plated onto tryptic soy agar (TSA) (Difco, BD), incubated for 24 h at 37 °C, and processed for bacterial enumeration. The minimum detection level was 10 CFU/g.

Relative radiation sensitivity was determined using the following equation: relative radiation sensitivity $\sim$(D_{10} of control sample)/(D_{10} of sample treated with antimicrobial compounds). D_{10} is defined here as the radiation dose required to reduce the population of viable bacteria inoculated onto the florets by 90% (1 log CFU). For the D_{10} calculation, the kinetics of bacterial destruction with or without antimicrobial compounds was evaluated by linear regression. Bacterial counts (log CFU per gram) were plotted against radiation doses, and the reciprocal of the slope of the trend line was extracted from the plot.

2.5. Statistical analysis

All experiments were done with two replicates. For each radiation dose, three samples per treatment were evaluated.