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Antibacterial effect of biodegradable active packaging on the growth of *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes* in fresh broccoli stored at 4 °C

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ABSTRACT

In this study, bioactive films were prepared using two types of film matrices based on i) methylcellulose (MC) and ii) a blend of polycaprolactone/alginate (PCL/ALG). Two antimicrobial formulations named as A [organic acids mixture + rosemary extract + Asian spice essential oil (EO)] and B [organic acids mixture + rosemary extract + Italian spice EO] were added in each type of films during casting. Broccoli florets were inoculated separately with *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium* to have a final microbial concentration of 5 logs CFU/g sample. Then, antimicrobial films were inserted into packaging containing inoculated broccoli and the package was then sealed under air in standard condition of storage for 12 days at 4 °C. It was found that films containing formulation A had better efficiency against *S. typhimurium*, with a significant reduction of bacterial concentration until total inhibition after 12 days of storage. In general, bioactive films showed a significant reduction and a good capacity to control the growth of *L. monocytogenes* and *E. coli* at short-term storage (4 days). Therefore, these results demonstrated the high antimicrobial potential of both types of films via the diffusion of antimicrobial volatiles on pathogenous bacteria in pre-cut vegetables.

1. Introduction

In recent years, the interest in active food packaging has been increasing significantly due to the fact that these systems are able to control the microbiological decay of perishable food products (Cha & Chinnan, 2004; Gemili, Yemenicioğlu, & Altınkaya, 2009). Many researches have been conducted to develop packaging strategies which are able to retain the active agent in the polymeric network and control its release (Gamage, Park, & Kim, 2009; Gemili et al., 2009; Imran, El-Fahmy, Revol-Junelles, & Desobry, 2010). It has been demonstrated that active packaging containing antimicrobial compounds can reduce, inhibit, or delay the growth of food-borne microorganisms (Appendini & Hotchkiss, 2002; Cha & Chinnan, 2004; Han, 2003). Antimicrobial agents can be incorporated into packaging systems through various methods i.e. (i) volatilized from an insert or a sachet placed in the package into the head space, (ii) sprayed or coated on food surfaces, (iii) sprayed, coated or chemically bound to the surface of the packaging film, (iv) formulated homogenously with the film polymers, or (v) designed to occupy pores or channels within the film (Appendini & Hotchkiss, 2002; Han, 2003).

To reduce the pollution problems caused by synthetic and non-biodegradable packaging films, edible or biodegradable packaging materials are encouraged to be developed, especially in case of antimicrobial packaging (Choi, Lee, & Park, 2006; Han, 2003; Salmieri & Lacroix, 2006). In this aspect, proteins such as soy protein isolates, corn zein, whey protein isolates (Choi et al., 2006; Le Tien et al., 2000), polysaccharides such as methylcellulose (MC), hydroxypropyl-cellulose (HPC) and carboxymethylcellulose (CMC), alginate (ALG), chitosan or synthetic biodegradable polyesters such as polycaprolactone (PCL) have been used as main materials for producing antimicrobial packaging films (Gazori et al., 2009; Gemili et al., 2009; Imran et al., 2010; Millette, Le Tien,

Abbreviations: MC, methylcellulose; HPC, hydroxypropylcellulose; CMC, carboxymethylcellulose; PCL, poly(ε-caprolactone); ALG, alginate; EO, essential oil; FWA, films without antimicrobial agents; MC-A, Methylcellulose film containing antimicrobial formulation A; MC-B, Methylcellulose film containing antimicrobial formulation B; PCL/ALG-A, Poly(ε-caprolactone)/alginate film containing antimicrobial formulation A; PCL/ALG-B, Poly(ε-caprolactone)/alginate film containing antimicrobial formulation B; PS, punctual strength; PD, puncture deformation; VO, vegetable oil.

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Smoragiewicz, & Lacroix, 2007; Nguyen, Gidley, & Dykes, 2008; Oussalah, Caillet, Salmieri, Saucier, & Lacroix, 2007; Ye, Needo, & Chen, 2008). For example, Millette et al. (2007) have applied nisin-containing alginate-based films on beef for inhibition of *Staphyloccocus aureus*. Results demonstrated that after 7 days of storage at 4 °C, a reduction of 0.91 and 1.86 log CFU/cm^2 was observed on sliced beef covered with films containing 500 or 1000 IU/mL of nisin, respectively. These results suggest that sterile, hydrophobic and biodegradable films including various amounts of nisin could be used efficiently to control the growth of pathogens or microorganisms responsible of spoilage at the surface of ground beef or other meat products (Millette et al., 2007).

In case of antimicrobial agents that are encapsulated into packaging materials, essential oils (EOs) and their derivatives are becoming more popular as naturally derived antimicrobial agents (Burt, 2004; Gamage et al., 2009; López, Sanchez, Batlle, & Nerin, 2007). Indeed, some spices or EOs have been successfully used as natural food preservatives in the food industry (Oussalah, Caillet, Saucier, & Lacroix, 2006; Oussalah, Calliet, Saucier et al., 2007; Oussalah, Calliet, Salmieri et al., 2007). However, the research regarding their incorporation into active films for preservation of vegetable and fruits is still limited due to lack of adequate process technology and stability concerns of bioactive agents (López et al., 2007).

Broccoli is a highly perishable product and yellowing is a major limitation to its shelf life and quality. It is considered as one of the highly nutritional vegetables and it contains important levels of vitamins, antioxidants and anti-carcinogenic compounds (Lemoine, Civello, Chaves, & Martínez, 2009). Several methods have been used to extend the postharvest life and maintain the quality of fresh product including modified atmosphere packaging (Bastrash, Makhlouf, Castaigne, & Willemot, 1993), food irradiation (Grolichova, Dvorak, & Musilova, 2004), hot air treatment (Lemoine et al., 2009) or high pressure processing (Marcos, Aymerich, Dolors Guardia, & Garriga, 2007). Therefore, research on preservation and/or increase of the shelf life of broccoli by antimicrobial packaging is a challenge, especially in case of natural extracts encapsulated in hydrocolloid-based matrices.

Therefore, the objective of this study was to develop biodegradable antimicrobial films containing two antimicrobial formulations and to evaluate their potentials in controlling/inhibiting the growth of foodborne pathogens in fresh-cut vegetable. Broccoli was used as a food model for this application of diffusion film treatment. Different natural extracts were incorporated into MC- and PCL/ALG-based films and the antimicrobial activity of these films were evaluated against food borne bacteria (*Escherichia coli* O157:H7, *S. typhimurium* and *Listeria monocytogenes*) during the storage of broccoli at 4 °C.

2. Materials and methods

2.1. Materials

2.1.1. Broccoli florets

Broccoli was purchased from a local supermarket (IGA, Laval, Quebec, Canada). Broccoli heads were cut into florets which were then packaged in 0.5-mil metallized polyester-2-mil ethylene vinyl acetate copolymer bags (205 × 355 mm; thickness: 62 µm; oxygen transmission rate at 23 °C: 0.7 cc/m^2/24 h; water vapour transmission rate at 38.7 °C/90% RH: 1.2 g/m^2/24 h; Winpak Division Ltd, Montreal, Quebec, Canada). The packaged florets were stored at 4 °C overnight until irradiation treatment. The packaged florets were sterilized by gamma-irradiation at 10 kGy (dose rate of 16.74 kGy/h) 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. The sterilized broccoli florets were then stored at 4 °C.

2.1.2. Antimicrobial extracts

The antimicrobial formulations were composed of organic acids mixture containing mostly acetic acid (8.7 g/100 mL), rosemary extract and Asian EO (to prepare formulation A) vs Italian EO (to prepare formulation B) respectively. The organic acids mixture was obtained from Kerry Ingredients and Flavours (Monterey, Tennessee, USA), rosemary extract (containing 40 g/100 g rosmarinic acid) was obtained from P.L. Thomas & Co., Inc. (Morristown, New Jersey, USA). The Asian and Italian EOs were provided by BSA Food Ingredients s.e.c./l.p. (Montreal, Quebec, Canada). The Asian essential oil mixture was mainly composed of nutmeg, lemongrass and citral and the Italian essential oil mixture contained mainly oregano, pimento berry and lemongrass. The EO samples were stored in the dry and dark place at room temperature during the experiments. The shelf-life of EOs is 1 year under storage conditions.

2.1.3. Materials for film preparation

MC, ALG (from brown algae, low viscosity; 39 g/100 g guluronic acid) and PCL (average molecular weight of 1250 g/mol; melting point approximately 40 °C) were purchased from Sigma–Aldrich Canada Ltd (Oakville, ON, Canada) and were used as polymeric bulk for film preparation. Fig. 1 shows the chemical structure of MC (a), ALG (b) and PCL (c). Tween®80 and glycerol were purchased from Laboratoire Mat (Montreal, Quebec, Canada). Vegetable oil (VO; sunflower oil) was purchased from a local grocery.

2.2. Preparation of antimicrobial biopolymer films

2.2.1. Preparation of antimicrobial formulations

A preliminary experiment was conducted to evaluate the antimicrobial effects of different formulations against pathogenic bacteria in broccoli samples. The antimicrobial formulations that were able to reduce 90% population (one log CFU) on broccoli were selected (data not shown). A sensory analysis was also carried out to determine the maximal concentration of active compounds in formulations that did not affect the organoleptic properties of the broccoli (data not shown). The most formulations (named as A and B) were selected from the preliminary experiment and the sensory analysis. Antimicrobial formulations were prepared in order to obtain a concentration of 60 g/L organic acids mixture, 13.5 g/L rosemary extract, and 6 g/L Asian EO (formulation A) or 6 g/L Italian EO (formulation B) in the final film-forming suspension prior to casting. The mixtures were homogenized at room temperature using an Ultra-Turrax disperser (T25 model; IKA® Works Inc., Wilmington, NC, USA) at 12,000 rpm for 1 min immediately followed by 24,000 rpm for 1 min.

2.2.2. PCL/ALG-based antimicrobial films

An aqueous solution containing 30 g/L ALG and 20 g/L glycerol was prepared at room temperature under stirring. The antimicrobial formulation (A or B) was incorporated into the ALG/Glycerol suspension, under stirring at room temperature. Then, 10 g/L PCL was added under molten state to the mixture under vigorous stirring for 5 min at 45 °C. The polymer suspension was homogenized using an IKA® T25 digital Ultra-Turrax disperser (IKA® Works Inc.) at 24,000 rpm for 1 min, by maintaining the temperature at 45 °C. Films were then cast by applying 20 mL of the film-forming suspension onto Petri dishes (50 × 9 mm; VWR International, Ville Mont-Royal, QC, Canada) and allowed to dry for 24 h, at 20 °C and 35% relative humidity (RH) before peeling.