



Effect of antimicrobial coatings on microbiological, sensorial and physico-chemical properties of pre-cut cauliflowers



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ABSTRACT

Six coatings containing different ratios of polysaccharides and antimicrobials were prepared and tested *in vitro* against *Listeria monocytogenes*. Best coating mixtures were then applied on vegetables and submitted to a sensorial analysis. Finally, their effect on the quality, the color and the consistency of vegetables during a one-week storage was determined. All selected coatings showed a total *in vitro* inhibition of bacteria at concentrations of 8–10 mL⁻¹. One formulation containing the antimicrobials induced vegetables to have similar characteristics (smell, taste and texture) as compared to the non-treated vegetables. Treatments with this coating generated minor changes concerning the respiration rate and no differences were visually observed on cauliflowers. Finally, *in situ* analyses showed a good antimicrobial effect and allowed a complete inhibition of *Listeria innocua* after seven days of storage at 4 °C.

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1. Introduction

Nowadays, ready-to-eat (RTE) products are increasingly in demand, mainly because of our lifestyle and their convenience. However, industrial processing can be a source of microbial contamination (Castro-Ibáñez et al., 2016; Zilelidou et al., 2015). During the last past years, food safety has become more important due to resulting illnesses, but also to related economic burdens. Indeed, it has been estimated that, in 2010, the total cost for *Listeria monocytogenes* illness was more than \$2 billion in the United States (Scharff, 2012). Since foodborne diseases can induce serious health threats, new methods controlling bacterial growth are being developed. Natural compounds such as essential oils (EOs) and organic acids have already proved their efficiency on a large scale of pathogenic bacteria such as *L. monocytogenes*, *Escherichia coli* O157:H7 or *Salmonella* Typhimurium (Boumail et al., 2013; Dussault et al., 2014). After internalization into cells, organic acids seem to dissociate into protons and anions (Ricke, 2003), leading to pH changes. As for EOs, their action on the membrane permeability results in a loss of metabolites and the denaturation of enzymes and proteins (Nazzaro et al., 2013). However, since the high volatility of those compounds may limit their use during time,

their immobilization in a polymer matrix can be used to increase their availability (Oussalah et al., 2004) but also to protect them from possible degradation due to humidity or oxygen (Ribeiro et al., 2015).

Methylcellulose (MC) is a water-soluble cellulose ether that is widely used for industry purposes. Indeed, its physico-chemical properties allow the formation of gels and coatings (Nasatto et al., 2015). It has been demonstrated that coatings based on MC can increase avocado shelf-life by reducing respiration rates and color changes (Maftoonazad and Ramaswamy, 2005). Starch (S), a polymer made of amylose and amylopectin, is also commonly used for the formation of edible coatings, since it increases firmness and enhances gel strength (Galus et al., 2012). Maltodextrins (MD) are produced after hydrolysis of S. Their properties depend on their dextrose equivalent (DE) value. Indeed, MD with a high DE will be more likely to produce an efficient encapsulation of bioactive compounds and will offer a better protection against oxidation (Jafari et al., 2008; Wang et al., 2015). MD has also been described as a polymer which possesses oxygen barrier and water retention properties amongst others (Chronakis, 1998).

The aim of this study was to evaluate the effects of the bioactive coating on the quality of fresh cauliflowers. Coatings containing different ratios of MC, MD and S were prepared and their antimicrobial properties were evaluated *in vitro*. Best coatings were then used to coat cauliflower florets which were submitted to sensorial evaluations in order to determine the level of

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appreciation of the smell, the texture and the taste. Physico-chemical parameters such as O₂/CO₂ content, colorimetry and consistency of coated cauliflowers were also measured. Finally, the antimicrobial effect of the coating was evaluated *in situ* on cauliflowers against *Listeria innocua*.

2. Materials and methods

2.1. Bacterial suspension

A mixture of five *L. monocytogenes* strains (Health Canada, Health Product and Food Branch, Ottawa, ON, Canada) were used (HPB 1043 1/2a, HPB 2371 1/2b, HPB 2558 1/2b, HPB 2569 1/2a, HPB 2812 1/2a), as well as a *L. innocua* ATCC 51742 strain. Bacteria were stored in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) containing glycerol (150 g L⁻¹) at -80 °C. Before utilization, bacteria were propagated twice in TSB for 24 h at 37 °C. *L. monocytogenes* strains were mixed together and this bacterial suspension was diluted to reach 10⁶ CFU (colony forming unit) mL⁻¹ for *in vitro* tests.

2.2. Antimicrobial formulation

The antimicrobial formulation was prepared using lactic acid (Sigma–Aldrich Ltd., Oakville, ON, Canada), citrus extract (Biosecure Lab Inc., Otterburn, QC, Canada), lemongrass EO (BSA Ingredients s. e.c./l.p., Montreal, QC, Canada) and Tween 80 (Sigma–Aldrich Ltd.) with the following ratio 100:10:1:2. This mixture was then homogenized at room temperature under sterile conditions, using a digital Ultra-Turrax T25 disperser (IKA Works Inc., Wilmington, NC, USA), at a speed of 1047 rad s⁻¹ for 60 s.

2.3. Preparation of coatings

Stock solutions of MD (dextrose equivalent 16.5–19.5), MC (powder form, viscosity of 1.5 Pa s for a 10 g L⁻¹ solution at 20 °C—Sigma–Aldrich, Oakville, ON, Canada) and modified S (National Starch & Chemical Company, Bridgewater, NJ, USA) were prepared at a concentration of 20 g L⁻¹ distilled water. Suspensions were autoclaved at 80 °C for 15 min for pre-gelatinization and sterilization, then cooled in an ice bath to ensure complete solubilization. The high viscosity of MC was used to provide a coating matrix, allowing the coating to adhere to vegetables but also limiting the dripping. Glycerol was also used as a plasticizer at a set concentration of 7.5 g L⁻¹. Coatings were homogenized at a speed of 1047 rad s⁻¹ for 60 s with a digital Ultra-Turrax T25 disperser.

2.4. In vitro test—minimum inhibitory concentration (MIC)

The effect of relative concentrations of MD and S with/without MC (2.5 g L⁻¹) was evaluated on the MIC by insuring a total polymer

content of 10 g L⁻¹ and the different composition are presented in Table 1. The antimicrobial formulation was added to reach final concentrations of 0; 0.8; 8; 10; 12; 14; 16 and 34 mL L⁻¹ in the coating. The *in vitro* screening of the MIC was evaluated using 96-wells (flat-bottom wells) microtiter plates. One plate was used for the control coating while the others were used for measuring the effect of the coating on *L. monocytogenes* at 10⁶ CFU mL⁻¹. The control plate was prepared as follows according to Dussault et al. (2014). The first column was filled with 250 μL of TSB and column 2–9 were filled with 125 μL of TSB and 125 μL of coating containing the antimicrobial formulation (from 0 to 34 mL L⁻¹ respectively). A quantity of 15 μL of peptone water was then added to each well of the 9 columns. The other plates were prepared with a similar design but peptone water was replaced with 15 μL of the bacterial suspension. Plates were then incubated at 37 °C for 24 h. Bacterial growth was detected by absorbance at 595 nm using a microtiter plate reader (ELISA reader, CLX800–Biotek Instruments) and compared to the negative turbidity control. The lowest concentration that inhibited the growth of the bacteria was determined as the minimal inhibitory concentration (MIC).

2.5. Sensorial evaluation

The sensorial evaluation was performed with 65 panelists who were asked to evaluate the odor, the texture and the taste of 8 samples. Cauliflowers (*Brassica oleracea* L. var. *botrytis* L.) were purchased from a local supermarket, cut into ready-to-eat size florets and dipped for 30 s in the selected coatings. Each side of the cauliflowers was allowed to dry on foil for 10 min. Cauliflowers were deposited in cups, randomly identified with a 3-digit number and refrigerated overnight before organoleptic evaluation. Panelists were asked to eat unsalted crackers and drink water between samples. The answers were based on a 9 point hedonic scale, from 9 (“like extremely”) to 1 (“dislike extremely”). Samples with scores lower than 5 were considered as not appreciated.

2.6. Physico-chemical properties of coated cauliflowers

The oxygen/carbon dioxide amount released as well as the color, the resistance of penetration and the consistency of cauliflowers were measured during storage in plastic desiccators (VWR, Mississauga, ON, Canada) at 4 °C.

2.6.1. Respiration rates

A headspace oxygen and carbon dioxide analyzer (Illinois Instruments Inc., IL, USA) was used to measure the gas content surrounding vegetables during storage. The headspace analyzer was calibrated with concentrations of 209 mL L⁻¹ of oxygen and 0 mL L⁻¹ of carbon dioxide and a filter was used to prevent bringing humidity in the analyzer. Packaged air was taken during 30 s and the O₂ and CO₂ percentages were displayed. Respiration rates were calculated according to modified equations used by Bhande et al. (2008):

$$R_{O_2} = \frac{(G_{O_2})_t - (G_{O_2})_{t+1}}{\Delta t}$$

$$R_{CO_2} = \frac{(G_{CO_2})_{t+1} - (G_{CO_2})_t}{\Delta t}$$

where R_{O_2} is the consumption of O₂ (mL L⁻¹ h⁻¹), R_{CO_2} is the production of CO₂ (mL L⁻¹ h⁻¹), G_{O_2} and G_{CO_2} are the O₂ and CO₂ gas concentration (mL L⁻¹) respectively and Δt is the time storage difference between samples (h).

Table 1

Ratio (g/L) of MD, S and MC in studied bioactive coating formulations^a and their *in vitro* antimicrobial effect against *L. monocytogenes*.

| Coating ^a | MD | S | MC | MIC (mL L ⁻¹) ^b |
|----------------------|-----|-----|-----|--|
| A | 0 | 10 | 0 | 10 |
| B | 0 | 7.5 | 2.5 | 10 |
| C | 2.5 | 5.0 | 2.5 | 8 |
| D | 5.0 | 2.5 | 2.5 | 8 |
| E | 7.5 | 0 | 2.5 | 8 |
| F | 10 | 0 | 0 | 8 |

^a Coating formulations were also composed of water, glycerol (7.5 g L⁻¹) and the antimicrobial formulation (concentration between 0 and 34 mL L⁻¹).

^b MIC: minimal inhibitory concentration.

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