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Inhibition of *Listeria monocytogenes* on cooked cured chicken breasts by acidified coating containing allyl isothiocyanate or deodorized Oriental mustard extract

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ABSTRACT

Ready-to-eat meats are considered foods at high risk to cause life-threatening *Listeria monocytogenes* infections. This study screened 5 *L. monocytogenes* strains for their ability to hydrolyze sinigrin (a glucosinolate in Oriental mustard), which formed allyl isothiocyanate (AITC) and reduced *L. monocytogenes* viability on inoculated vacuum-packed, cooked, cured roast chicken slices at 4 °C. Tests involved incorporation of 25–50 μl/g AITC directly or 100–250 mg/g Oriental mustard extract in 0.5% (w/v) κ-carrageenan/2% (w/v) chitosan-based coatings prepared using 1.5% malic or acetic acid. *L. monocytogenes* strains hydrolyzed 33.6%–48.4% pure sinigrin in MH broth by 21 d at 25 °C. Acidified κ-carrageenan/chitosan coatings containing 25–50 μl/g AITC or 100–250 mg/g mustard reduced the viability of *L. monocytogenes* and aerobic bacteria on cooked, cured roast chicken slices by 4.1 to >7.0 $\log_{10}$ CFU/g compared to uncoated chicken stored at 4 °C for 70 d. Coatings containing malic acid were significantly more antimicrobial than those with acetic acid. During storage for 70 d, acidified κ-carrageenan/chitosan coatings containing 25–50 μl/g AITC or 250 mg/g mustard extract reduced lactic acid bacteria (LAB) numbers 3.8 to 5.4 $\log_{10}$ CFU/g on chicken slices compared to uncoated samples. Acidified κ-carrageenan/chitosan-based coatings containing either AITC or Oriental mustard extract at the concentrations tested had the ability to control *L. monocytogenes* viability and delay growth of potential spoilage bacteria on refrigerated, vacuum-packed cured roast chicken.

1. Introduction

Listeria monocytogenes is a Gram-positive, non-spore-forming, motile, facultatively anaerobic, rod-shaped bacterium. The organism is commonly found in many environments including soil, water, manure, dust, silage as well as animals and it can grow over a wide range of temperatures (−0.4–45 °C), pH values (4.3–9.6), salt concentrations (≤10%), and water activities > 0.93 (McClure et al., 1997; Pellicer et al., 2011). These characteristics enable the pathogen to survive and multiply in the food processing environment and in/on fresh and processed foods and make *L. monocytogenes* a significant problem for the food industry. The heat treatment used for the manufacture of ready-to-eat (RTE) meat and poultry products is sufficient to eliminate *L. monocytogenes*; however, cooked products can be re-contaminated with the organism during subsequent processing steps (FSIS, 2014). Todd and Notermans (2010) reported that the prevalence of *L. monocytogenes* in RTE meat products in the US and Canada ranged from 0.4 to 71% and 0.0–21%, respectively.

L. monocytogenes is an opportunistic pathogen that can cause severe infection in humans. In healthy people listeriosis is characterized by flu-like symptoms; however, serious complications like meningitis, septicemia, spontaneous abortion or infection of the newborn may also occur in susceptible individuals (FSIS, 2014). Foodborne listeriosis outbreaks are rare, but the mortality rate can be high (20–30%), and may reach 75% in pregnant women, neonates, and immunocompromised adults (Jalali and Abedi, 2008). In the US, listeriosis accounts for about 1600 illnesses annually (Scallan et al., 2011). In 2008, a significant listeriosis outbreak occurred in 7 provinces in Canada which was linked to delicatessen meat and caused 57 illnesses with 23 deaths (Farber et al., 2011).

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Some plant-derived natural antimicrobials have been successfully used as food preservatives (Holley and Patel, 2005). Mustard, including the black cultivar (*Brassica nigra* L.), Oriental (*B. juncea* L.), and white or yellow types (*Sinapis alba* or *B. hirta*), belong to the family *Brassicaceae* which also includes cabbage, broccoli, horseradish, wasabi and cauliflower. These plants contain secondary nitrogen and sulfur-containing metabolites called glucosinolates which can protect the plant from parasitism or infection upon their hydrolysis. The glucosinolate sinigrin, present in Oriental mustard, is hydrolyzed by endogenous plant myrosinase upon physical injury in the presence of moisture to yield allyl isothiocyanate (AITC) which is lethal toward major foodborne pathogens such as *Escherichia coli* O157:H7, *L. monocytogenes*, *Salmonella* spp. and *Campylobacter jejuni* (Luciano and Holley, 2009; Olaimat and Holley, 2013; Olaimat et al., 2014a). In deodorized mustard which is devoid of myrosinase, sinigrin is converted into AITC by the action of bacterial myrosinase-like enzyme(s) present in *E. coli* O157:H7, other VTEC *E. coli*, *Salmonella*, *L. monocytogenes* and *C. jejuni* (Herzallah et al., 2011; Luciano and Holley, 2011; Olaimat and Holley, 2014; Olaimat et al., 2014a). Therefore, it is probable that hydrolysis of sinigrin in Oriental mustard by *L. monocytogenes* could be used to reduce the viability of this same pathogen on RTE poultry meats.

Since incorporation of AITC or mustard powder as ingredients may affect the physicochemical and organoleptic properties of processed poultry meat, an antimicrobial film or coating as a delivery vehicle appeared to be a more productive approach. Chitosan has good film-forming and antimicrobial properties, while κ-carrageenan has gelling, thickening, emulsifying and stabilizing characteristics. The mixture of these two compounds showed good coating properties, acted as a gas barrier and delayed release of incorporated antimicrobials (Pinheiro et al., 2012). In earlier work, κ-carrageenan/chitosan-based coatings containing AITC or Oriental mustard extract were effective against *C. jejuni* and *Salmonella* on uncooked chicken breasts (Olaimat and Holley, 2015; Olaimat et al., 2014a). It was thought that these antimicrobial coatings might be beneficial for the prevention of foodborne illnesses caused by *L. monocytogenes* in RTE meat products. Malic acid was considered for inclusion in antimicrobial formulations because previous work in broth showed that it enhanced the antimicrobial activity of mustard extract (Olaimat and Holley, 2014). Therefore, the objectives of the current study were i) to determine the minimum inhibitory (MIC) and bactericidal (MBC) concentrations of AITC against 5 *L. monocytogenes* strains at 4 °C, ii) to screen the 5 *L. monocytogenes* strains for their ability to hydrolyze pure sinigrin via bacterial myrosinase, iii) to use edible κ-carrageenan/chitosan-based coatings (prepared with malic or acetic acid) containing AITC or Oriental mustard extract to reduce *L. monocytogenes* viability on cooked, cured sliced roast chicken.

2. Materials and methods

2.1. Chemicals

AITC (94%), chitosan (molecular weight: 100,000–300,000 Da; deacetylation degree: 75–85%), and HPLC grade tetrabutylammonium hydrogen sulphate (TBA), were from Acros Organics (Morris Plains, NJ, USA). Sinigrin from horseradish, glycerol (>99%) and κ-carrageenan from *Eucheuma cottonii* were from Sigma–Aldrich Co. (St. Louis, MO, USA). Acetonitrile HPLC grade, malic acid, acetic acid and hydrochloric acid (HCl) were from Fisher Scientific Co. (Fair Lawn, NJ, USA).

2.2. Bacterial strains and culture preparation

Five *L. monocytogenes* strains (2-138, 2-243, GLM-1, GLM-3, GLM-5) from the culture collection of the Food Science Department, University of Manitoba were stored in Brain Heart Infusion (BHI) broth (Oxoid Ltd., Basingstoke, England) containing 25% (v/v) glycerol at −80 °C. One loopful from each strain was streaked on *Listeria* Selective Agar Base with *Listeria* Selective Supplement (LSA, Oxoid) and incubated at 37° C for 48 h. A single colony of each *L. monocytogenes* strain was transferred to BHI broth and incubated overnight at 37 °C. Then, 0.1% (v/v) of this culture was transferred to fresh BHI broth and incubated as before to get stationary phase cells. Freshly prepared single cultures of *L. monocytogenes* were used in a sinigrin hydrolysis test. A mixture of *L. monocytogenes* cultures was prepared by combining 8 ml of each of the 5 freshly cultured strains in a sterile container and used for inoculation of cooked, cured chicken in experiments to evaluate the antimicrobial activity of coatings.

2.3. MIC and MBC of AITC against L. monocytogenes strains at 4 °C

The MIC and MBC of AITC against *L. monocytogenes* strains were determined in 9 ml screw-capped tubes containing 7.92 ml Mueller-Hinton (MH) broth (pH 7.2) (Oxoid) with 1.25–640 ppm AITC and then 0.08 ml of each strain of *L. monocytogenes* (8 $\log_{10}$ CFU/ml) was added to yield 6 $\log_{10}$ CFU/ml in tubes which were mixed and incubated with shaking at 200 rpm (Junior Orbit Shaker; Lab-Line Instruments Inc., Melrose Park, IL, USA) for 10 d at 4 °C. The lowest concentration at which no visible growth occurred was identified as the MIC, while the MBC was the lowest concentration which reduced the initial numbers 3 $\log_{10}$ CFU/ml.

2.4. Screening of L. monocytogenes strains for sinigrin degradation

Mueller Hinton (MH, Oxoid) broth containing approximately 1000 ppm sinigrin was prepared by mixing 10 ml of a filter-sterilized (0.22 μm, Millipore, Cork, Ireland) sinigrin stock solution (50,000 ppm) with 490 ml sterile MH broth (pH 7.2). Then, 9.9 ml of MH broth with sinigrin was transferred to sterile 12 ml screw-capped tubes containing 0.1 ml of 8 $\log_{10}$ CFU/ml of individual stationary phase cultures of *L. monocytogenes* strains. Tubes containing sinigrin and cultures were incubated with shaking at 200 rpm (Junior Orbit Shaker; Lab-Line Instruments Inc., Melrose Park, IL, USA) for 21 d at 25 °C. A negative control without *L. monocytogenes* was prepared by mixing 0.1 ml sterile distilled water with 9.9 ml MH broth containing sinigrin. Samples of 1 ml were taken and filter-sterilized in sterile HPLC vials at 0, 1, 2, 3, 7, 14 and 21 d to measure the remaining sinigrin using a reversed phase-liquid chromatograph (RP-HPLC, model 2695, Waters Corporation, Milford, MA, USA) based on the method described by Lara-Lledó et al. (2012). Briefly, TBA, acetonitrile and double-distilled water were used as HPLC solvents which were sterilized using 0.45 mm Millipore nylon filters (Fisher Scientific, Nepean, ON, Canada) and degassed for 30 min in a Bransonic bath (Branson, 5510, Grass Valley, CA, USA). The sample injection volume was 10 μl. Sinigrin was quantitatively determined using a calibration curve (100–1500 ppm) established with a sinigrin standard under the same analytical conditions.

2.5. Preparation of antimicrobial coatings

An aqueous mustard extract was prepared as described by Olaimat et al. (2014a). κ-Carrageenan/chitosan coatings were prepared by adding 0.5% (w/v) κ-carrageenan to sterile distilled water with stirring for 1 h at 60 °C. After cooling to room