International Journal of Food Microbiology 164 (2013) 54–59

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Predictive model for the reduction of heat resistance of *Listeria monocytogenes* in ground beef by the combined effect of sodium chloride and apple polyphenols

Vijay K. Juneja [a,*], Evrim Güneş Altuntaş [b], Kamuran Ayhan [b], Cheng-An Hwang [a], Shiowshuh Sheen [a], Mendel Friedman [c]

[a] U. S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA
[b] Ankara University, Engineering Faculty, Food Engineering Department, Ankara, Turkey
[c] U. S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center U. S. Department of Agriculture, Albany, CA 94710, USA

ARTICLE INFO

Article history:
Received 20 September 2012
Received in revised form 13 February 2013
Accepted 10 March 2013
Available online 21 March 2013

Keywords:
Listeria monocytogenes
Ground beef
Sodium chloride
Apple polyphenols
Heat resistance
Microbial food safety

ABSTRACT

We investigated the combined effect of three internal temperatures (57.5, 60, and 62.5 °C) and different concentrations (0 to 3.0 wt/wt.%) of sodium chloride (NaCl) and apple polyphenols (APP), individually and in combination, on the heat-resistance of a five-strain cocktail of *Listeria monocytogenes* in ground beef. A complete factorial design (3 × 4 × 4) was used to assess the effects and interactions of heating temperature, NaCl, and APP. All 48 combinations were tested twice, to yield 96 survival curves. Mathematical models were then used to quantitate the combined effect of these parameters on heat resistance of the pathogen. The theoretical analysis shows that compared with heat alone, the addition of NaCl enhanced and that of APP reduced the heat resistance of *L. monocytogenes* measured as D-values. By contrast, the protective effect of NaCl against thermal inactivation of the pathogen was reduced when both additives were present in combination, as evidenced by reduction of up to ~68% in D-values at 57.5 °C; 65% at 60 °C; and 25% at 62.5 °C. The observed high antimicrobial activity of the combination of APP and low salt levels (e.g., 2.5% APP and 0.5% salt) suggests that commercial and home processors of meat could reduce the salt concentration by adding APP to the ground meat. The influence of the combined effect allows a reduction of the temperature of heat treatments as well as the salt content of the meat. Meat processors can use the predictive model to design processing times and temperatures that can protect against adverse effects of contaminated meat products. Additional benefits include reduced energy use in cooking, and the addition of antioxidative apple polyphenols may provide beneficial health affects to consumers.

Published by Elsevier B.V.

1. Introduction

Listeria monocytogenes, a psychrotrophic and relatively thermotolerant pathogen, continues to be a significant threat to the safety of ready-to-eat (RTE) foods. The pathogen represents a major public health concern for consumers, the food industry, and regulatory agencies. The organism is widespread in the environment and has been isolated from a variety of raw and processed foods. The ability of *L. monocytogenes* to grow at refrigerated food storage conditions and to adapt to a variety of adverse environments, such as high salt and acidic foods, has made this organism difficult to control (Farber and Peterkin, 1991). The Centers for Disease Control and Prevention (CDC) has estimated that *L. monocytogenes* causes 1591 cases of foodborne listeriosis, 1455 hospitalizations, and 255 deaths annually in the United States (Scallan et al., 2011). The most susceptible individuals are expectant mothers, the very young with developing immune systems, and elderly and/or immunocompromised individuals (Painter and Slutsker, 2007). The unknown infectious dose in such individuals, combined with the high-frequency and magnitude of food recalls and outbreaks with high fatality rate, have been a challenging risk-management problem for the regulatory agencies and the food industry. This issue has prompted regulatory agencies in the US to implement regulations for meat processors that enforce a 'zero tolerance' policy for *L. monocytogenes* in cooked and RTE foods (Engel et al., 1990; U.S. Department of Agriculture — Food Safety and Inspection Service, 2003).

Adequate heat treatment in food processing techniques remains the most effective intervention strategy to inactivate contaminating pathogens in RTE foods and is a critical control point during food processing. The published literature (Chhabra et al., 1999; Juneja and Eblen, 1999; Doyle et al., 2001; Mazzotta, 2001; Murphy et al., 2002; Juneja, 2003), provides evidence that the thermal death time values among various studies on *L. monocytogenes* differed considerably. Factors known to render the pathogens more sensitive or resistant to the lethal effect of heat include strain, age of culture, growth conditions, recovery media and food characteristics, such as water activity, pH, salt

* Corresponding author. Tel.: +1 215 233 6500; fax: +1 215 233 6697.
E-mail address: Vijay.Juneja@ars.usda.gov (V.K. Juneja).

0168-1605/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.03.008

levels, and presence of inhibitors (Doyle et al., 2001). The heat resistance of *L. monocytogenes* in foods increases with fat levels (MacDonald and Sutherland, 1993), pH (Juneja and Eblen, 1999), salt content (Juneja and Eblen, 1999), and absence of antimicrobials (Maisnier-Patin et al., 1995). The special properties of a specific food may help protect *L. monocytogenes* against heat lethality. The interactions of multiple food formulation factors on *L. monocytogenes* heat resistance have been quantified to develop predictive models that allow the estimation of the pathogen's survival for any changes in the combination of food formulation parameter values within the specific tested range. Such models are generally considered to be important tools for ensuring that thermal processes meet the prescribed log reductions of the contaminating pathogens and provide an increased understanding of how extrinsic and intrinsic factors affect their destruction (Chhabra et al., 1999; Maks et al., 2010; Camelia et al., 2011; Juneja et al., 2012).

Sodium chloride (NaCl or salt) is most commonly used in processed foods to preserve foods and to extend the shelf life. Sodium helps to bind ingredients, functions as a stabilizer, and improves taste and enhances the color of the food. Most processed meat products contain 2.75–3.25% NaCl, corresponding to 1.1–1.3% sodium (Maurer, 1983). Sufficient evidence exists to document the association of sodium electrolyte with hypertension or high blood pressure (Kerr and Nichaman, 1986; Appel et al., 2012). Current dietary guidelines for Americans recommend that adults should reduce daily sodium intake to less than 2.3 g per day (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). Because of public health concerns associated with high salt levels in the diet, consumers are increasingly demanding processed meats with reduced salt levels. Prophylactic measures to reduce sodium intake are necessary steps in the management of hypertension and the clinical symptoms associated with coronary heart disease and renal failure. The need to reduce the salt levels in meats could be satisfied by the use of other natural antimicrobials.

To place the results of the present study in proper perspective, we will also first briefly mention the following published studies on antimicrobial, antitoxin, antiviral, and anticancer cell effects of apple polyphenols. Apple polyphenols (a) inactivated pathogenic organisms in laboratory media (Fattouch et al., 2008; Wong et al., 2008; Du et al., 2011; Fratianni et al., 2011, 2012; Friedman et al., 2013); (b) inactivated pathogenic bacteria on leafy greens (Moore et al., 2011) and in grilled beef patties (Rounds et al., 2012); (c) inhibited the biological activities of bacterial Shiga 2 toxin (Quiñones et al., 2009; Rasooly et al., 2010); (d) protected mice against infection by the influenza virus (He et al., 2011); and (e) protected cells against carcinogenic and genotoxic damage (Gerhauser, 2008; Petermann et al., 2009).

In the present study, we evaluated this natural product for its ability to control *L. monocytogenes* in contaminated ground meat. The aim of this study was to develop a three-factor thermal death model for *L. monocytogenes* by quantitatively assessing and understanding the relative effects and interactions of NaCl and apple polyphenol (APP) in combination with heating temperature on the thermal inactivation of *L. monocytogenes* in 75% lean beef. The model presented will allow the food industry to incorporate acknowledged intrinsic barriers when formulating the product, and design reduced thermal processes based on the predictions that provide a margin of safety, thus achieving an adequate degree of protection against *L. monocytogenes*. To our knowledge, this is the first report on the ability of a natural antimicrobial (APP) to overcome sodium chloride-induced heat resistance of a foodborne pathogen in a meat matrix.

2. Materials and methods

2.1. Additives

Commercial apple polyphenol powder containing ~80% phenolic compounds was obtained from Apple Poly LLC (Morrill, NE, USA). Salt was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Bacterial strains and preparation of inocula

L. monocytogenes strains Scott A (clinical isolate), H7762 (hot dog outbreak isolate), MF27137 (steer/heifer isolate), MF38521 (ground chicken), and MF46869 (pork sausage isolate) were maintained at −70 °C in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) containing 10% (v/v) glycerol (Sigma-Aldrich Co., St. Louis, MO, USA). To propagate the cultures, vials were thawed at room temperature. An aliquot (1.0 ml) of the resuspended thawed culture was added to 10 ml of sterile brain heart infusion broth (BHI; Difco) in 50 ml tubes. The cultures were incubated for 24 h at 37 °C. Activated cultures (0.1 ml) were transferred again into BHI (10 ml) and incubated for 24 h at 37 °C. These cultures were maintained in BHI at 4 °C for two weeks when a new series of cultures were activated individually from the frozen stock.

To prepare inocula for conducting thermal inactivation studies, each culture (0.1 ml) was transferred into BHI (50 ml) in 250 ml flasks, and incubated for 18 h at 37 °C. Late stationary phase cells were harvested by refrigerated centrifugation at 5000 ×*g* for 15 min and washed twice in 0.1% (w/v) peptone water (PW). The inoculum (about 8–9 $\log_{10}$ CFU/ml) re-suspended in PW (2 ml) was enumerated by spiral plating (Autoplate 4000 Spiral Plater, Spiral Biotech, Gaithersburg, MD, USA) of appropriate dilutions in PW (0.1%), in duplicate, onto tryptic soy agar (TSA, Difco) plates to verify the initial population. Plates were incubated for 24 h at 37 °C before counting colonies. Equivalent populations of each isolate were combined in a sterile conical vial to obtain a five-strain mixture of *L. monocytogenes* (ca. 8 $\log_{10}$ CFU/ml) prior to inoculation of meat.

2.3. Preparation and inoculation of meat

Raw 75% lean ground beef, used as the heating menstruum, was obtained from a commercial source. The meat was separated into portions for different treatments and mixed thoroughly with NaCl (0.0 to 3.0%; w/w) and/or APP (0.0 to 3.0%; w/w) using KitchenAid Mixer, Model K5SSDWH, (KitchenAid Co., St. Joseph, MI, USA). The meat (50 g/bag) was placed into PrimeSource 8 × 12 vacuum pouches (BUNZL-Koch Supplies, Kansas City, MO, USA) and vacuum-sealed. Thereafter, five of these bags were placed in barrier pouches (Bell Fibre Products, Columbus, GA, USA), vacuum-sealed, frozen and irradiated (25 kGy) to eliminate background microflora. Irradiation was performed using a self-contained ^{137}Cs Irradiator (Lockheed Georgia Co., Marietta, GA, USA) at the Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA. Random samples were analyzed for sterility by spiral plating.

The cocktail of five strains of *L. monocytogenes* (0.1 ml) was added to completely thawed, irradiated ground meat (50 g) so that the final concentration of cells was approximately 7 $\log_{10}$ cfu/g. The inoculated meat was pummeled with a Seward laboratory stomacher 400 (UK) for 2 min to ensure homogeneous distribution of the organisms in the meat sample, as confirmed in preliminary studies. Ground meat samples (3 g), in duplicate, were then weighed aseptically into 20 × 25 cm sterile filter stomacher bags (BagPage^{+}, Interscience Laboratories Inc., Rockland, MA, USA). Negative controls consisted of meat samples bags inoculated with only 0.1 ml of 0.1% PW without *L. monocytogenes* cells. Thereafter, the sample bags were compressed into a thin layer by pressing them against a flat surface, thereby achieving approximately 1–2 mm thickness, excluding most of the air and preventing air pockets, and then heat sealed.

2.4. Experimental design

A complete factorial design (3 × 4 × 4) was employed to determine the effects and interactions of heating temperatures (57.5, 60, 62.5 °C), NaCl concentration (0.0, 1.0, 2.0, 3.0%), and APP concentration (0.0, 1.0, 2.0, 3.0%). Heating studies for each of the 48 variable combinations, selected at random, were performed in duplicate and repeated. A total of 96 survivor curves were obtained from the experimental data. Subsequently, the data collected were used to develop a predictive model that describes