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Sodium lactate, sodium diacetate and pediocin: Effects and interactions on the thermal inactivation of *Listeria monocytogenes* on bologna☆

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ABSTRACT

The effects and interactions of temperature (56.3–60 °C), sodium lactate (SL; 0–4.8%), sodium diacetate (SD; 0–0.25%) and pediocin (0–10,000 AU) on *Listeria monocytogenes* on bologna were studied and a predictive inactivation model was developed. Bologna was manufactured with different SL/SD concentrations in the formulation, dipped in pediocin solution and treated at different temperatures using combinations of parameters determined by central composite design. D-values were calculated and analyzed using second order response regression. Predicted D-values were also calculated. The observed D-values for *L. monocytogenes* on bologna ranged from 2.10 to 35.59 min. Temperature alone decreased predicted D-values from 99.02 min at 56.3 °C to 44.71 min at 60.0 °C. Adding SL decreased D-values (85.43–22.71 min) further; however, heat and SD combined was the most effective for reducing *L. monocytogenes* on bologna. An SD level of 0.25% at 58.2 °C had the overall lowest predicted D-value (15.95 min). Combination treatments increased or decreased D-values, depending on the temperature. Pediocin (2500 and 5000 AU) and heat decreased D-values, but exhibited a protective effect at higher concentrations (≥7500 AU). The results showed that interactions between additives in formulations can vary at different temperatures/concentrations, thereby affecting thermal inactivation of foodborne pathogens in meat products. Hence, food processors should modify food formulations carefully, and verify with adequate testing so that product safety is not compromised.

1. Introduction

Post-process contamination of food products by *Listeria monocytogenes* is a concern to ready-to-eat (RTE) meat processors because there is a risk of serious illness to consumers. Between 1998 and 2000, and in 2002, multi-state listeriosis outbreaks involving contaminated RTE meat and poultry products altogether caused 125 illnesses, 17 deaths and 8 miscarriages or stillbirths (CDC, 2002, 2000, 1999).

L. monocytogenes is able to colonize processing plants, survive under unfavorable conditions and has been isolated from various areas in food processing facilities (Samelis and Metaxopoulos, 1999). Areas in food processing environments providing niches for *L. monocytogenes* include hollow rollers on conveyors, spaces between close fitting parts, worn or cracked seals, valves and switches, and saturated insulation; the source is often limited to a very specific growth site that can lead to contact surface contamination during production, typically a specific production line (Tompkin, 2002). Floor drains can also be a niche for listeriae and also a point of contamination in a food processing plant and possibly in food products (Zhao et al., 2006). Sanitation procedures are not always effective at removing *L. monocytogenes* because the pathogen can form biofilms (Eckner, 1990). For these reasons, there is a risk for post-process contamination of RTE food products within the processing plant. Because *L. monocytogenes* can grow at refrigeration temperatures, and RTE meats are often consumed with little or no reheating, illness can result from consuming the contaminated product.

Methods to reduce or inhibit *L. monocytogenes* growth on post-processed RTE meat products have been studied during recent years. Using antimicrobials in RTE meat formulations has been shown to inhibit *L. monocytogenes* (Lianou et al., 2007; Barmpalia et al., 2005; Glass et al., 2002). Currently, the U.S. Department of Agriculture (USDA, 2000) permits adding sodium or potassium lactate up to levels of 4.8% and sodium diacetate up to 0.25% to meat and poultry product formulations to inhibit microbial growth. Other

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antimicrobials such as bacteriocins and organic acids applied as a spray or dip treatment may also reduce or inhibit *L. monocytogenes* growth on RTE meats (Uhart et al., 2004; Glass et al., 2002; Samelis et al., 2001). Hurdle technology combines multiple techniques or interventions at a lower intensity helping to preserve taste and nutritional quality while targeting harmful microorganisms. Many studies on the combined effect of heat and antimicrobials on *L. monocytogenes* have been published (Mangalassary et al., 2007; Chen et al., 2004; Juneja, 2003; Samelis et al., 2001; McMahon et al., 1999). However, studies on effects of combinations of lactate and diacetate with bacteriocins on *L. monocytogenes* are limited.

The objective of this study was to understand the effects and interactions of heating temperature (56.3–60 °C), sodium lactate (0–4.8%), sodium diacetate (0–0.25%) and pediocin (0–10,000 AU) on *L. monocytogenes* inoculated on bologna and develop a predictive inactivation model.

2. Methods

2.1. Bacterial strain and culture preparation

L. monocytogenes LM 101M (serotype 4b, beef and pork sausage isolate) was used for this study. Of the four strains previously studied in our laboratory, *L. monocytogenes* LM 101M was found to be the most resistant to the antimicrobials tested (Uhart et al., 2004) and hence, selected for further investigation. Stock cultures were maintained in cryovials (Pro-Lab Diagnostics, Austin, TX) at −80 °C and activated by transferring 100 μl into tryptic soy broth containing 0.6% yeast extract (TSBYE; Difco, Sparks, MD) and incubating at 37 °C for 24 h. Strains were subcultured twice in TSBYE. For experimental use, *L. monocytogenes* LM 101M was grown overnight in 25 ml TSBYE at 37 °C with shaking.

2.2. Bologna and antimicrobials

The bologna used in this study was manufactured by Viskase Inc. (Darien, IL) using the following ingredients: boneless beef chuck (36.2%), regular pork trim (34.95%), water (16.72–24.84%), spice (2.3%), salt (1.4%), prague powder (0.17%), sodium lactate (SL; 0–4.8%) (Wilke International, Lenexa, KS) and sodium diacetate (SD; 0–0.25%) (Niacet Corp., Niagara Falls, NY). The bologna was sliced and packaged after manufacturing. Ten batches were manufactured with different SL and SD concentrations according to the central composite design (CCD) matrix (Table 1). ALTA 2341™ (Quest International, Hoffman Estates, IL), a fermentation product having pediocin activity was used as a dip and was not part of the bologna formulation.

2.3. Bologna sample preparation

The bologna slices were removed from the freezer (−20 °C) and placed in the refrigerator (4 °C) to thaw overnight. Two hours before inoculation, the bologna was removed from the refrigerator and kept at room temperature.

The pediocin solution was prepared by mixing the appropriate amount of ALTA 2341 in sterile deionized water. Each bologna slice (~25 g) was dipped in the pediocin solution for 5 min, and placed on aluminum foil to dry for 20 min at room temperature. Afterwards, each side of the bologna slice was inoculated with 500 μl of the *L. monocytogenes* overnight culture (10^9 CFU/ml), spread evenly using a cell spreader and dried for 5 min.

Each bologna slice was placed into a Food Saver® bag (Tilia, San Francisco, CA) and vacuum sealed using a Food Saver® sealer. An uninoculated bologna slice was also vacuum sealed in a bag on which a small amount of silicone sealant (Silicone II; GE Sealants and Adhesives, Huntsville, AL) had been placed and allowed to dry. This slice was used for monitoring the temperature by inserting a T-type thermocouple (Omega Engineering, Inc., Stanford, CT) through the silicone area onto the uninoculated bologna surface.

2.4. Experimental design

A CCD was chosen to determine the effects and interactions of the five levels of antimicrobials and temperature. Four factors and five factor levels were used: SL: 0, 1.2, 2.4, 3.6 and 4.8%; SD: 0, 0.0625, 0.125, 0.1875 and 0.25%; ALTA 2341: 0, 2500, 5000, 7500

Table 1
Central composite design with observed and predicted D-values and upper limit of predicted D-values for *L. monocytogenes* on bologna.

Combination	Sodium lactate (SL)	Sodium diacetate (SDA)	Pediocin	Temperature	Observed D-values	Predicted D-value	Upper limit of predicted D-value
1	0.0	0.1250	5000	58.2	4.17	10.44	15.81
2	1.2	0.0625	2500	57.2	33.53	29.34	34.75
3	1.2	0.0625	2500	59.1	11.61	8.57	13.90
4	1.2	0.0625	7500	57.2	35.59	30.31	35.72
5	1.2	0.0625	7500	59.1	12.16	9.09	14.42
6	1.2	0.1875	2500	57.2	8.30	8.74	14.15
7	1.2	0.1875	2500	59.1	2.70	1.54	6.88
8	1.2	0.1875	7500	57.2	9.49	9.27	14.68
9	1.2	0.1875	7500	59.1	2.63	1.62	6.96
10	2.4	0.0000	5000	58.2	7.70	17.03	22.40
11	2.4	0.1250	0	58.2	9.09	10.78	16.16
12	2.4	0.1250	5000	56.3	26.97	30.50	35.78
13	2.4	0.1250	5000	58.2	7.07	6.80	13.83
14	2.4	0.1250	5000	60.0	2.10	3.84	9.16
15	2.4	0.1250	10000	58.2	6.63	9.95	15.32
16	2.4	0.2500	5000	58.2	4.73	4.41	5.79
17	3.6	0.0625	2500	57.2	25.44	23.46	28.87
18	3.6	0.0625	2500	59.1	5.53	3.73	9.07
19	3.6	0.0625	7500	57.2	23.27	22.57	27.98
20	3.6	0.0625	7500	59.1	5.98	2.39	7.73
21	3.6	0.1875	2500	57.2	12.78	13.59	19.00
22	3.6	0.1875	2500	59.1	4.90	7.44	12.77
23	3.6	0.1875	7500	57.2	12.52	12.26	17.67
24	3.6	0.1875	7500	59.1	3.17	5.66	10.99
25	4.8	0.1250	5000	58.2	9.91	8.65	14.02