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The effect of high hydrostatic pressure, sodium nitrite and salt concentration on the growth of *Listeria monocytogenes* on RTE ham and turkey

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ABSTRACT

Growth of *Listeria monocytogenes* was evaluated for up to 182 days after inoculation on ready-to-eat (RTE) sliced ham and turkey breast formulated with sodium nitrite (0 or 200 ppm), sodium chloride (1.8% or 2.4%), and treated (no treatment or 600 MPa) with high hydrostatic pressure (HHP). HHP at 600 MPa for 3 min resulted in a 3.85–4.35 log CFU/g reduction in *L. monocytogenes*. With formulations at similar proximate analyses, one of the evaluation days (day 21) without HHP showed significantly greater growth of *L. monocytogenes* in ham than in turkey breast, but there were no significant differences on other evaluation days or with HHP. There were no differences in growth of *L. monocytogenes* due to sodium chloride level. Sodium nitrite provided a small, but significant inhibition of *L. monocytogenes* without HHP, but addition of sodium nitrite did not significantly affect growth of *L. monocytogenes* with use of HHP.

1. Introduction

Listeria monocytogenes is pervasive in many environments and has the unique ability among pathogens to grow at refrigerated temperatures, making it a pathogen of upmost concern for ready-to-eat (RTE) foods consumed without recooking (Farber & Peterkin, 1991; Kathariou, 2002). *L. monocytogenes* contamination for RTE meat items occurs mostly during post-lethality processes such as slicing, packaging, or other handling of product (Lin et al., 2006; Nesbakken, Kapperud, & Caugant, 1996; Vorst, Todd, & Ryser, 2006). An outbreak of listeriosis in 1998 that included approximately 108 illnesses and 18 deaths was traced to post-lethality contamination at a commercial processing facility (CDC, 1999; Graves et al., 2005).

Oven roasted turkey is a commercial product that has been reported to have greater likelihood of *L. monocytogenes* growth compared to other types of meats, such as bologna and salami (Lin et al., 2006). If turkey is contaminated with *L. monocytogenes*, it has the potential to grow to a high level in a short period of time (Lianou, Geornaras, Kendall, Scanga, & Sofos, 2007; Ojeniyi, Christensen, & Bisgaard, 2000). In the risk assessment published by Pradhan et al. (2009), *L. monocytogenes* had the greatest estimated growth rate and the shortest lag phase in turkey, compared to other products modeled. It is not clear why turkey meat has been reported to support more rapid growth than meat from other species though one possibility could be a difference in composition, particularly a greater moisture content.

Ham items have also been shown to support *L. monocytogenes* growth (Glass & Doyle, 1989; Lianou, Geornaras, Kendall, Belk, et al., 2007). However, in the risk assessment conducted by Pradhan et al. (2009), ham was found to have a reduced growth rate and an extended lag phase of *L. monocytogenes* compared to turkey or a generic deli meat product.

An expert panel made up of microbiologists, food scientists, and physicians from government, academia, and industry has recommended the use of post-packaging intervention treatments to reduce the risk of *L. monocytogenes* growing to high numbers in ready-to-eat products (Walls et al., 2005). High hydrostatic pressure (HHP) has been used for inactivation of pathogens in food items, with damage to the cell membrane being the primary mechanism of microbial destruction (Hugas, Garriga, & Monfort, 2002). HHP can also reduce the levels of vegetative bacteria without greatly affecting the flavor of foods (Cheftel & Culioli, 1997). High hydrostatic pressure treatment of meats after packaging has been shown to reduce the number of *L. monocytogenes* in hams (Aymerich, Jofre, Garriga, & Hugas, 2005; Jofre, Aymerich, Grebol, & Garriga, 2009; Marcos, Aymerich, Monfort, & Garriga, 2008; Pietrzak, Fonberg-Broczek, Mucka, & Windyga, 2007), cooked beef (Simpson & Gilmour, 1997), and cooked poultry items (Youart, Huang, Stewart, Kalinowski, & Legan, 2010). Researchers have also shown that sodium nitrite (Buchanan & Phillips, 1990; Buchanan, Stahl, & Whiting, 1989; Duffy, Vanderlinde, & Grau, 1994; Farber & Daley, 1994; Grau & Vanderlinde, 1992; McClure, Kelly, & Roberts, 1991; Schlyter, Glass, Loeffelholz, Degnan, & Luchansky, 1993; Vitas, Aguado, & Garcia-Jalon, 2004) and

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sodium chloride (Glass & Doyle, 1989; Legan, Seman, Milkowski, Hirschey, & Vandeven, 2004; McClure et al., 1991; Seman, Borger, Meyer, Hall, & Milkowski, 2002) can reduce the growth rate of *L. monocytogenes*.

The purpose of this study was to determine the extent to which species, salt concentration, sodium nitrite concentration, and HHP influence the growth of *L. monocytogenes* in RTE sliced meats.

2. Materials and methods

2.1. Product manufacture

Fresh trimmed ham muscles from the inside (*semimembranosus*) and knuckle (*rectus femoris*, *vastus intermedius*, *vastus lateralis*, and *vastus medialis*), as well as fresh trimmed turkey breast (*pectoralis major* and *pectoralis minor*) were utilized for the respective products. All raw materials were used within 2–4 days after harvest and were ground to 0.3175 cm immediately before use. After grinding, meats for both turkey and ham were mixed for ca. 1 min in a Blentech Auto Chef Silver Ribbon blender (Blentech Corp., Rohnert Park, CA) to assure homogeneity and randomly assigned to 1 of 4 treatments. Formulations consisted of two salt concentrations (1.8% and 2.4% of formulation) and either 0 or 200 ppm sodium nitrite (with 500 ppm sodium erythorbate) based on meat weight for both turkey and ham products. The remaining part of the formulations for each of the salt and nitrite treatments consisted of 1% dextrose (ADM Corn Processing, Decatur, IL), 0.4% sodium tripolyphosphate (Nutrifos O-88 — ICL Performance Products LP, St. Louis, MO), and water/ice (to target total moisture of 77% in final product). Formulations were calculated as close as possible to equalize moisture, fat, and protein levels in both species, based on values from initial screening of raw materials, to allow assessment based on the variables of species type, salt level, and presence/absence of nitrite, independent of proximate composition.

Dry ingredients were dissolved in 85% of the water using a Lightnin mixer (Model S1UO3A, Lightnin, Rochester, NY) and additional water/ice was added to achieve a temperature of 28 °F in the pickle solution. The pickle solution was then added along with the meat materials to the Blentech mixer and blended under vacuum for 20 min at 30 rpm. After blending, the mixed batter was held for 18 h at 2 °C and stuffed into 8.38 cm diameter, non-permeable casings (Viscofan, Danville, IL). All items for each replication were cooked together via steam heat for 45 min at 54 °C, 45 min at 63 °C, 45 min at 71 °C, and ca. 1 h at 80 °C to an internal temperature of 74 °C. After reaching final temperature, cooked logs were showered with ca. 21 °C water for 30 min and then chilled in a 1 °C cooler to reach an internal temperature of <4 °C within 6 h of cooking.

Within 1 week after chilling, the casings were removed and each product treatment was sliced into 11 gram slices. Slices were stacked and bulk packaged into ~2 pound nitrogen gas flushed packages. Because several studies have shown that *L. monocytogenes* growth may be affected by high levels of lactic acid bacteria (Buchanan & Klawitter, 1992; Farber & Daley, 1994; Foegeding, Thomas, Pilkington, & Klaenhammer, 1992), all samples were treated with high pressure at 600 MPa for 10 min to greatly decrease the number of vegetative organisms potentially acquired during slicing/packaging. Preliminary experiments confirmed the reduction of most vegetative microorganisms by HHP treatment prior to inoculation of the samples.

2.2. Microbiological procedures

Each *L. monocytogenes* culture was grown overnight (18–24 h) in tryptic soy broth (TSB) at 35 °C and tested for purity on modified oxford agar (MOX) (VWR, Batavia, IL). After <1 week storage at 2 °C, individual 11 gram ham or turkey slices were removed from bulk packages and repackaged into 13×29 cm packages (oxygen transmission rate = 3.5 cc/100 sq. in./day; Ultravac Solutions, Kansas City, MO) for inoculation. *L. monocytogenes* strains used for the study were ATCC 7644, NCTC 10890, ATCC 19112, ATCC 19114, and ATCC 19115 (Microbiologics, St. Cloud, MN). Equal amounts of each strain were mixed into a common cocktail used for the inoculation. Based on prior testing, a count of 10^9 CFU/ml of *L. monocytogenes* in the overnight cultures was used for calculation of further dilutions. The following dilutions were made using sterile peptone water to achieve three targeted levels of inoculum. First, a 1:10 dilution of the cocktail (10^9 CFU/ml) in 90 ml of sterile peptone water (10^8 CFU/ml) was inoculated via pipette at 0.01 ml (10 μl) on each 11-gram slice to achieve a final count of approximately 10^5 CFU/g (5 log inoculation). Second, a 1:100 dilution of the 10^8 CFU/ml dilution (10^6 CFU/ml) in 90 ml sterile peptone water was inoculated via pipette at 0.01 ml (10 μl) on each 11-gram slice to achieve a final count of approximately 10^3 CFU/g (3 log inoculation). Finally, a 1:100 dilution of the 10^6 CFU/ml dilution (10^4 CFU/ml) in 90 ml sterile peptone water was inoculated via pipette at 0.01 ml (10 μl) on each 11-gram slice to achieve a final count of approximately 10^1 CFU/g (1 log inoculation). After inoculation, all samples were vacuum-sealed on a Multivac packaging machine (model A300; Multivac, Kansas City, MO.). The 1 log inoculation was used to represent what might be a level of contamination actually occurring in a processing facility and the potential impacts of a low number of organisms on lag phase and growth of the organism on the samples. The 3 log inoculation was included to assess a potentially worst-case level of contamination, and the 5 log inoculation was included to evaluate the relative impact of the experimental treatments.

The inoculated samples were randomly assigned to one of two groups consisting of 1) non-HHP samples and 2) HHP (180 s) treated samples. The experiment was replicated three times with microbial analyses completed in triplicate for each replication on each testing date. The samples of all treatments were stored at 4.4 °C throughout the duration of the experiment. The evaluations of the non- HHP samples were conducted on days 0, 5, 7, 14, 19, 21 and 28 after inoculation because growth was relatively rapid in these samples, while the HHP-treated samples were evaluated at days 0, 28, 56, 91, 119, 154, and 182 after inoculation and HHP treatment to confirm that very limited growth occurred beyond what might be considered a typical shelf life of 90 to 120 days.

For the non-HHP treated group, the samples were inoculated and the day 0 microbial measurements conducted for each treatment at ca. 2 h post-inoculation. All dilutions were made by adding 99 ml of Butterfield's phosphate buffer to each 11-gram sample, stomaching, making further dilutions as needed, and then plating on MOX agar via direct plating. All counts were run in triplicate and reported as CFU/g. For the HHP-treated samples on day 0, the products were pressure treated (600 MPa for 180 s) and microbial counts conducted in triplicate at ca. 2 h after pressure treatment (ca. 3–4 h after inoculation), using the same dilutions and plating procedures as the non-HHP samples. The MOX plates for all treatments were incubated for 48 h at 35 °C then examined for the presence or absence of growth. All counts were completed using direct plating methods. All test sample dilutions (1:10) were kept in snap-cap cups and refrigerated at 4.4 °C for later enrichment of the sample, if needed. An uninoculated, negative sample was also prepared for all treatments for each of the testing days to verify testing methods.

If no colonies were detected on MOX agar (<10 CFU/g), then enrichment of the pathogen was completed from the stored samples using USDA methods (USDA, 2009). Briefly, 25 ml of dilution sample was added to 225 ml of UVM broth and incubated for 24 h at 30 °C, then 0.1 ml of UVM broth/sample was transferred into 10 ml of Fraser broth with 0.1 ml ferric ammonium citrate and incubated for 48 h at 35 °C. Tubes were evaluated for the presence (positive) or absence (negative) of a darkening color. The positive enrichment was considered as 1 log CFU/g and the negative enrichment was considered as −0.39 log CFU/g (25 ml of total 110 ml sample was enriched {22%}, and 22% of original 11 g slice is 2.42 g, therefore less than