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The occurrence of *Listeria monocytogenes* in retail ready-to-eat meat and poultry products related to the levels of acetate and lactate in the products

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ABSTRACT

Listeria monocytogenes is a psychrotrophic foodborne pathogen that has been isolated from ready-to-eat meat and poultry products (RTE meats). The purpose of this study was to quantify lactate and acetate levels in retail RTE meats that had been tested in a previous study for the presence of *L. monocytogenes* to correlate the occurrence of *L. monocytogenes* to the acid levels. Products were extracted after blending 50 g of each sample with de-ionized water, and the extracts were quantified for lactate and acetate using HPLC. In general, the concentrations of both acids in samples varied with product types and manufacturers ($p < 0.05$). The mean concentrations of lactate and acetate ranged from 10.71 to 23.03 mg/g (1.07–2.30%) and 0.66–1.56 mg/g (0.066–0.156%), respectively. The mean concentrations of lactate and acetate in *L. monocytogenes*-positive samples were 1.13–24.05 mg/g (0.11–2.4%) and 0–5.74 mg/g (0–0.574%), respectively. Results of this study indicate that RTE meats containing low levels of lactate were more likely to be positive for *L. monocytogenes* while samples with higher concentrations of lactate and acetate were less likely to be positive for the pathogen. Therefore, the addition of lactate and acetate as antimicrobials is helpful as part of an overall *Listeria* control program. However, a rigorous sanitation and an effective HACCP program are also essential for control of *Listeria*.

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1. Introduction

Listeria monocytogenes is a foodborne pathogen that causes listeriosis, a particularly severe illness for individuals with compromised immune systems, the elderly, pregnant women, and young children. In the U.S., the average annual incidence was 0.92 cases per 100,000 population for 2009–2011 (CDC, 2013) and approximated 1600 illnesses and 260 deaths were due to listeriosis (Scallan et al., 2011). The pathogen is psychrotrophic and can survive and grow in adverse conditions such as refrigeration temperature, low pH, and high salt concentrations (ICMSF, 1996). The contamination of *L. monocytogenes* in ready-to-eat (RTE) foods is a significant public health concern since these products are commonly consumed without prior cooking. Because it is a significant health concern, the U.S. regulatory agencies established a "zero tolerance" policy for *L. monocytogenes* in RTE foods (Gombas, Chen, Clavero, & Scott, 2003). Also, "*Listeria* rules" issued by the Food Safety and Inspection Services, U.S. Department of Agriculture, have encouraged the use of antimicrobial agents for controlling *L. monocytogenes* in RTE meat or poultry products (RTE meats).

Salts of lactic acid (lactate) and acetic acid (acetate) are widely used antimicrobials in meat products. There has been an increased interest in the anti-listerial activity of these two generally recognized as safe (GRAS) salts in processed meats, since their commercial application is simple and cost-effective. Lactate and acetate can be added as ingredients in meat products, applied on finished products by spraying or dipping (Samelis et al., 2001), and applied on packaging materials as antimicrobial packaging (Cagri, Ustunol, & Ryser, 2004; Ouattara, Simard, Piette, Begin, & Holley, 2000;

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Quintavalla & Vicini, 2002) for controlling the growth *L. monocytogenes*. The levels of lactate and acetate in processed meats permitted under USDA regulations are 4.8% and 0.25%, respectively (9 CFR Part 424; 21 CFR Part 184).

Studies examining the effects of lactate and acetate on *L. monocytogenes* were mainly to determine the effective levels and conditions of using both salts in various products (Abou-Zeid et al., 2007; Gonzalez-Fandos & Dominguez, 2006; Samelis et al., 2001). However, data on the actual levels and effectiveness of both additives in commercial products are limited and their applicability in the food industry has been questioned. The goal of this study was to determine the correlations between the concentrations of lactate and acetate and the occurrence of *L. monocytogenes* in retail RTE meats in the U.S.

2. Materials and methods

2.1. Sample selection

RTE meat samples used in this study were obtained from 8000 samples that were collected and refrigerated for a maximum of 24 h before sampling for *L. monocytogenes*, and then frozen at −70 °C until analysis for lactate and acetate levels. Approximately 75% of the samples were collected from major retail grocery chains (the top 50) and 25% of the samples came from smaller or regional grocery stores. All samples had been tested for *L. monocytogenes* by the USDA method (USDA, 2006). At the time of sample collection, information on each sample was collected to identify the type of meat or poultry, manufacturer (coded), curing, geographic location of sample collection, manufacturing information, and sell-by date. Samples were collected using a random number generator to identify point of purchase over an 18-month period. All products were collected and frozen at least 7 days before the sell-by date (Draughon, 2006; Oyarzabal et al., 2005).

All *L. monocytogenes*-positive samples and a cross section of *Listeria*-negative samples were selected. A total of 1883 samples were tested for lactate and acetate. Samples represented different categories of processed meats and poultry products including uncured and cured poultry products and pork and beef products that were sliced at retail deli or pre-packaged in USDA- or state-inspected plants. Some samples were categorized as mixed products since they were prepared from a mixture of beef, pork and/or poultry. Samples were obtained from four states (California, Georgia, Minnesota, and Tennessee) participating in FoodNet and PulseNet and representing geographic diversity in the U.S.

2.2. Sample extraction

Lactate and acetate contents in the RTE meat samples were analyzed according to the procedures of Nassos, Schade, King, and Stafford (1984) and Friedrich (2002). The analytical procedure consisted of sample extraction, clean-up, and separation of acids using high performance liquid chromatography (HPLC). Samples (50 g) were added with 450 ml of de-ionized water and homogenized in a blender at high speed for 2 min. The homogenized samples were filtered with Whatman No. 113 filter paper under vacuum. An aliquot (filtrate) of 50 ml of each sample was mixed with 100 ml of 0.5 N perchloric acid in a 200 ml flask and allowed to stand for 5 min at room temperature to precipitate protein. The sample was filtered again with Whatman No. 4 filter paper under vacuum to remove the protein. The extracts (about 20 ml) were stored in vials at 4 °C until HPLC analysis. A final filtration through 0.45 μm Millipore membrane filter was performed prior to injection into the HPLC system.

Extraction and recovery of lactate and acetate were validated. Percentage recovery was determined by adding known concentrations of standards to RTE meat samples, and the samples analyzed using the method described above. Duplicate non-spiked samples were analyzed to quantify the background concentrations of lactate and acetate. Recovery percentage was calculated by the formula: (amount of analyte recovered)/(amount of analyte added + background analyte amount)*100%. The average recovery percentage of lactate and acetate was 91.81 ± 5.50% and 92.64 ± 6.80%, respectively.

2.3. HPLC analysis

Lactate and acetate were analyzed by a Dionex HPLC system (Dionex Corp, Sunnyvale, CA) equipped with a GP50 gradient pump, an AS50 Auto-sampler, and a PDA-100 UV detector. The acids were separated on an ion-exclusion column (Aminex HPX-87H) with guard column containing a cartridge of the same ion exclusion resin (Bio-Rad Laboratories, Hercules, CA). The mobile phase was 0.005 M H_2SO_4 with a flow rate of 0.6 ml/min. A 20 μL sample was injected into the HPLC and the data were collected with PeakNet software (Dionex Corp, Sunnyvale, CA) on a personal computer interfaced with the HPLC system.

Standard solutions of AA (Acros Organics, Morristown, NJ) and LA (Sigma, St. Louis, MO) were analyzed under the same conditions to establish standard curves. The identity and concentrations of AA and LA in the samples were confirmed by the retention time and calculated based on the regression analysis of the standard curves (Fig. 1).

2.4. Statistical analysis

The correlations between the amounts of lactate and acetate and the presence of *L. monocytogenes* in products was tested for Spearman correlation and analyzed by Dummy regression analysis (Statistical Analysis System, SAS Institute Inc., Cary, NC). Dummy regression analysis allowed class variables, which included products (such as beef, pork, poultry, and mixed) to be used in the regression analysis. Differences were considered significant when the associated p value was less than 0.05. A completely randomized design (Statistical Analysis System) was also used to compare means of lactate, acetate, and lactate + acetate in RTE meats among manufacturers, products types, and curing.

3. Results and discussion

3.1. Levels of lactate and acetate in RTE meats

All data presented refers to manufacturers by letters A-Z to preserve anonymity. For manufacturers with <10 samples, all data for samples were grouped and designated as “ZZ” (Table 1). If a sample was positive, the manufacturer was designated as a “capital letter” along with a “p” for positive and a number indicating the sequential order in which the sample was taken over the 18-month collecting period (Table 2).

Levels of lactate and acetate in approximately 1200 *L. monocytogenes*-negative RTE meat samples collected are shown in Table 1. The concentrations of lactate and acetate in RTE meats by manufacturer (A-ZZ) are shown along with the total lactate + acetate for each manufacturer's samples. The concentrations varied widely even within a same manufacturer's products. For example, lactate levels in samples from Manufacturers A and B ranged from 1.29 to 59.53 mg/g and 5.93–50.46 mg/g, respectively (Table 1). Acetate levels for products from a single manufacturer ranged from 0 to 9.59 mg/g. Since some products may not have lactate/acetate added in formulation or during processing, the