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Behavior of *Listeria monocytogenes* at 7 °C in commercial turkey breast, with or without antimicrobials, after simulated contamination for manufacturing, retail and consumer settings

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Abstract

Uncured turkey breast, commercially available with or without a mixture of potassium lactate and sodium diacetate, was sliced, inoculated with a 10-strain composite of *Listeria monocytogenes*, vacuum-packaged, and stored at 4 °C, to simulate contamination after a lethal processing step at the plant. At 5, 15, 25 and 50 days of storage, packages were opened, slices were tested, and bags with remaining slices were reclosed with rubber bands; this simulated home use of plant-sliced and -packaged product. At the same above time intervals, portions of original product (stored at 4 °C in original processing bags) were sliced and inoculated as above, and packaged in delicatessen bags, simulating contamination during slicing/handling at retail or home. Both sets of bags were stored aerobically at 7 °C for 12 days to simulate home storage. *L. monocytogenes* populations were lower ($P<0.05$) during storage in turkey breast containing a combination of lactate and diacetate compared to product without antimicrobials under both contamination scenarios. Due to prolific growth of the pathogen under the plant-contamination scenario in product without lactate-diacetate during vacuum-packaged storage (4 °C), populations at 3 days of aerobic storage (7 °C) of such product ranged from 4.6 to 7.4 log cfu/cm^2. Under the retail/home-contamination scenario, mean growth rates (log cfu/cm^2/day) of the organism during aerobic storage ranged from 0.14 to 0.16, and from 0.25 to 0.51, in product with and without lactate-diacetate, respectively; growth rates in turkey breast without antimicrobials decreased ($P<0.05$) with age of the product. Overall, product without antimicrobials inoculated to simulate plant-contamination and product with lactate-diacetate inoculated to simulate retail/home-contamination were associated with the highest and lowest pathogen levels during aerobic storage at 7 °C, respectively. However, 5- and 15-day-old turkey breast without lactate-diacetate stored aerobically for 12 days resulted in similar pathogen levels (7.3–7.7 log cfu/cm^2), irrespective of contamination scenario.

Keywords: *Listeria monocytogenes*; Turkey breast; Antimicrobials

1. Introduction

Post-lethality treatment contamination of ready-to-eat (RTE) meat and poultry products with *Listeria monocytogenes* is a significant public health concern, as demonstrated by recent major listeriosis outbreaks (CDC, 1999, 2000, 2002) and risk assessment data (USDHHS-FDA-CFSAN/USDA-FSIS, 2003). Cross-contamination of RTE foods with the pathogen may occur both at the production and retail levels (Aguado et al., 2001; Van Coillie et al., 2004). Isolation of *L. monocytogenes* from a turkey processing plant, products, and cases of human listeriosis in Denmark demonstrated that end product contamination of turkey meat may pose high risk of infection for susceptible individuals (Ojeniyi et al., 2000). Molecular subtyping of food (prepared and handled in retail premises), environmental, and clinical isolates of *L. monocytogenes* in New York State, revealed that specific ribotypes of the organism, including ribotypes that have been linked to human disease, persisted over time in retail establishments (Sauders et al., 2004).

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Investigation of a large listeriosis outbreak in France, in 1992, linked to consumption of cooked pork tongue-in-jelly indicated that cross-contamination of other RTE products in delicatessen counters may have contributed to the magnitude of that particular outbreak (Jacquet et al., 1995). The potential of slicers and other utensils used in retail premises to serve as vehicles of cross-contamination of RTE meat and poultry products with *L. monocytogenes* also has been demonstrated (Humphrey and Worthington, 1990; Hudson and Mott, 1993; Lin et al., 2006; Vorst et al., 2006). Lin et al. (2006) reported that the transfer of the organism from an artificially contaminated slicer to deli meats was product-dependent, and more positive samples containing higher pathogen levels during storage at 4 °C were obtained for uncured oven-roasted turkey than for bologna containing potassium lactate and sodium diacetate or salami.

Numerous studies have been conducted in different countries to determine the prevalence and levels of *L. monocytogenes* in retail RTE products, and various incidence rates have been reported (Wilson, 1995; Uyttendaele et al., 1999; Aguado et al., 2001; Gombas et al., 2003; Van Coillie et al., 2004; Vitas et al., 2004; Angelidis and Koutsoumanis, 2006). Gombas et al. (2003) examined RTE foods collected from retail markets in the United States, and reported a *L. monocytogenes* prevalence of 0.89% in sliced luncheon meats. The observed trend for in-store-packaged luncheon meats, and deli and seafood salads to have higher incidence of the pathogen than manufacturer-packaged products, as reported by these investigators, may be regarded as indicative of the potential contribution of handling of RTE products at retail to their contamination with the organism. In addition to food-processing and retail establishments, the domestic environment and consumer food-handling practices also have been acknowledged as important risk factors for foodborne listeriosis (ILSI Research Foundation/Risk Science Institute, 2005; Yang et al., 2006). According to a recent consumer phase risk assessment, in which the data collected by Gombas et al. (2003) were used as initial contamination levels, approximately 0.3% of servings of deli meats were predicted to be contaminated with levels of *L. monocytogenes* higher than 10^4 cfu/serving at the time of consumption (Yang et al., 2006).

Risk assessment data indicate that control measures that prevent occurrence of high levels of *L. monocytogenes* at the point of consumption are expected to have the greatest impact on reducing the incidence of listeriosis (Nørrung, 2000; Chen et al., 2003). In order to prevent high levels of the pathogen in products that support its growth, information associated with its behavior in such products under common handling and storage conditions, preceding consumption, is required. Nevertheless, the majority of research studies have evaluated the behavior of *L. monocytogenes* or the efficacy of antilisterial hurdles during vacuum-packaged storage of RTE foods, and data on the fate of the pathogen under handling and storage conditions encountered at retail or in the home are limited.

This study was undertaken to evaluate the behavior of *L. monocytogenes* in uncured RTE turkey breast, commercially available with or without a mixture of potassium lactate and sodium diacetate, during aerobic storage at 7 °C under two contamination scenarios. The objective was to assess the levels of the organism to which consumers could be potentially exposed during home storage of contaminated product. Product was inoculated under conditions chosen to simulate contamination at production, or following storage and subsequent slicing/handling at the retail or home level. Product inoculated under each of the two contamination scenarios was stored aerobically at 7 °C for 12 days to simulate domestic storage conditions prior to consumption. Growth trends of the spoilage microflora of the product under the above conditions also were assessed.

2. Materials and methods

2.1. *L. monocytogenes* strains

A 10-strain composite was used in this study and included strains 558 (serotype 1/2, pork meat isolate), NA-1 (serotype 3b, pork sausage isolate), N-7150 (serotype 3a, meat isolate), N1-225 and N1-227 (serotype 4b, clinical and food isolates, respectively, associated with the same outbreak; CDC, 1999), R2-500 and R2-501 (serotype 4b, food and clinical isolates, respectively, associated with the same outbreak; CDC, 2001), and R2-763, R2-764 and R2-765 (serotype 4b, clinical, food and environmental isolates, respectively, associated with the same outbreak; CDC, 2002). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, New York). Selection of strains was based on their growth behavior in culture broth, as evaluated in a previous study conducted in our laboratory (Lianou et al., 2006), as well as on their origin. Strains with robust growth characteristics at 4 °C and outbreak-related isolates were primarily chosen, in compliance with recommendations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2005). All strains were available as refrigerated (4 °C) cultures on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, Maryland) supplemented with 0.6% yeast extract (YE; Acumedia, Baltimore, Maryland) slants, and working cultures were stored in this form and subcultured monthly. Strains were activated by transferring a single colony from PALCAM agar (Difco, Becton Dickinson) plates into 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson) supplemented with 0.6% YE, and incubating at 30 °C for 24 h. Activated cultures were subcultured (0.1 ml) into 10 ml of fresh TSBYE and incubated at 30 °C for 24 h.

2.2. Inoculum preparation

For inoculum preparation, 24-h cultures of each of the strains were centrifuged and washed separately as described in previous studies (Geornaras et al., 2005, 2006). The harvested cells were resuspended in 10 ml of turkey breast extract (each culture separately) and stored at 7 °C for approximately 3 days (60–72 h), to allow for acclimatization of the cells to a low-temperature food environment. The turkey breast extract was prepared from frozen (−20 °C) portions of product without potassium lactate–sodium diacetate. To prepare the extract, turkey breast was thawed (4 °C) overnight and then homogenized (Masticator, IUL Instruments, Barcelona, Spain) with distilled water for 2 min to yield a 10% (w/w) suspension of the product, which was subsequently passed twice through cheesecloth, autoclaved for 15 min and cooled to ambient temperature (25 °C) before use. The pH of product extract used for inoculum preparation was measured both before (6.39 ± 0.07) and after (6.40 ± 0.07) autoclaving using a digital pH meter with a glass electrode (Denver Instruments, Arvada, Colorado).

Following the acclimatization period, the cultures were combined and serially diluted in freshly prepared product extract to yield an inoculum level of 1–2 log cfu/cm^2 when 0.1 ml of inoculum was applied to each side of a piece of sliced turkey breast.

2.3. Product inoculation

Unfrozen turkey breast without or with potassium lactate (1.5%) and sodium diacetate (0.05%) was obtained from a commercial manufacturer and used within 5 days of production. The formulation of product without antimicrobials consisted of turkey breast, turkey broth, salt, modified food starch, sugar, carrageenan, sodium phosphate and flavor. The formulation of product containing antimicrobials consisted of turkey breast, water, potassium lactate, salt, sugar, carrageenan, sodium phosphate, sodium diacetate, and turkey flavor (turkey stock, modified food starch, salt, flavor). The fat and moisture contents of the product were determined according to the AOAC International official methods (960.39 and 950.46.B, respectively; AOAC, 1998). The fat content (%) of product with and without antimicrobials was 1.0 ± 0.3 and 0.9 ± 0.3, while the moisture content (%) was 76.2 ± 6.6 and 76.8 ± 0.6, respectively. Before inoculation, turkey breast was sliced, and slices (approximately 3 mm thick) were then cut into pieces (5 × 5 cm, 25 cm^2 per side). Pieces were inoculated under a biological safety cabinet as described by Geornaras et al. (2005).

Twelve pieces of inoculated product were stacked on top of each other and placed in vacuum bags (20 × 25 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch, Kansas City, Missouri), vacuum-packaged (Hollymatic Corp., Countryside, Illinois), and stored at 4 °C, to simulate post-lethality contamination at the processing plant. At 5, 15, 25 and 50 days of storage, packages were opened, pieces were tested (microbiological and chemical analyses), and bags with remaining pieces were reclosed by folding with rubber bands; this simulated purchase and domestic use of plant-sliced and -packaged product. Additionally, at the same above time intervals, portions of original unsliced product (stored at 4 °C in original processing vacuum packages) were sliced, cut, and inoculated as above, and 12 pieces of product stacked on top of each other were placed on delicatessen paper (20 × 27 cm, Glenvale Deli Wrap, Dixie Food Service, Georgia-Pacific, Atlanta, Georgia) and packaged in reclosable delicatessen bags (20 × 25 cm, Koch), simulating contamination during slicing/handling at retail or at home. Both sets of samples (i.e., vacuum bags that were opened and reclosed with rubber bands, and delicatessen bags) were left at ambient temperature (25 °C) for 90 min (Audits International, 1999; Kennedy et al., 2005) to simulate temperature abuse of the product (e.g., time lapse between purchase and home refrigeration, consumer handling, etc.). Bags were then stored at 7 °C for 12 days (Fig. 1). The latter storage conditions (i.e., aerobic storage at mildly abusive temperature) were applied to simulate home storage of pre-packaged, or sliced and packaged at retail, RTE turkey breast (NACMCF, 2005).

2.4. Microbiological analyses

Samples were analyzed on days 0, 5, 15, 25 and 50 of refrigerated (4 °C) vacuum-packaged storage, and on days 0, 3, 6, 9 and 12 of aerobic storage (7 °C) following 5, 15, 25 and 50 days of vacuum-packaged storage of the product (either sliced and inoculated, or unsliced; Fig. 1). Two consecutive pieces from each bag were transferred aseptically to a sterile 24 oz bag (Whirl-Pak®, Nasco, Modesto, California) containing 50 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone), and shaken vertically 30 times (Samelis et al., 2001; Barmpalia et al., 2004). Appropriate serial dilutions in 0.1% buffered peptone water (Difco, Becton Dickinson) were surface plated on the following media: TSAYE for the enumeration of total mesophilic and total psychrotrophic microbial populations; PALCAM agar for the enumeration of *L. monocytogenes*; and Rose Bengal Chloramphenicol agar (RBC; Difco, Becton Dickinson) for the enumeration of yeasts and molds. Moreover, presumptive lactic acid bacteria (LAB) were enumerated by pour-plating in de Man Rogosa Sharpe agar (MRS; Biotrace International Inc., Bothell, Washington) as described in previous studies (Barmpalia et al., 2004, 2005). Colonies were counted after incubation at 25 °C for 72 h (TSAYE-total mesophiles, MRS) or 7 days (RBC), at 30 °C for 48 h (PALCAM), or at 7 °C for 14 days (TSAYE-total psychrotrophs).

2.5. pH and water activity measurements

Each sample, after being plated, was homogenized (Masticator, IUL Instruments) for 2 min, and the pH of the homogenate was measured using a digital pH meter

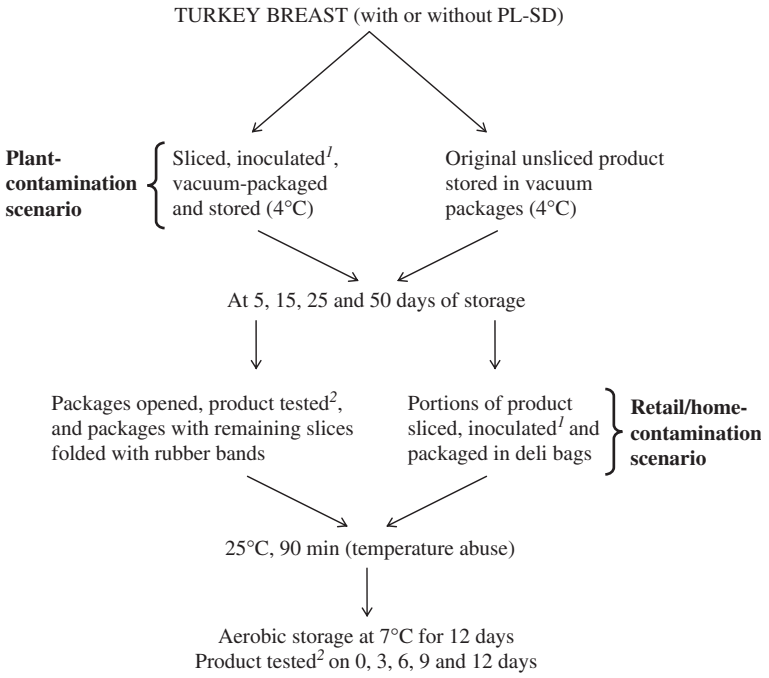

Fig. 1. Inoculation of turkey breast with *Listeria monocytogenes* under conditions simulating contamination at plant or retail/home, and storage under simulated home storage conditions. PL: potassium lactate; SD: sodium diacetate; [1]10-strain composite of *L. monocytogenes* (1–2 log cfu/cm^2); [2]microbiological and chemical analyses.

with a glass electrode (Denver Instruments). Furthermore, at 0, 5, 15, 25 and 50 days of vacuum-packaged storage (4 °C), a turkey breast piece was transferred to a Whirl-Pak® bag containing distilled water (1:10), homogenized for 2 min, followed by pH measurement as described above. Water activity (a_w) of turkey breast was measured during vacuum-packaged storage using an AquaLab (model series 3, Decagon Devices Inc., Pullman, Washington) water activity meter (Georgnaras et al., 2005, 2006).

2.6. Data analysis

The experiment was conducted twice with different product batches and, for each replication, three individual samples were analyzed at each sampling time ($n = 6$). Microbiological data were converted to log cfu/cm^2 and, for samples inoculated to simulate retail/home-contamination, growth rates during aerobic storage (7 °C) were determined using the linear regression function of Microsoft Excel. Data were analyzed using the mixed model procedure of SAS® (SAS, 2002). Means and standard deviations were calculated and least-squares means were separated using the pairwise *t*-test at $\alpha = 0.05$.

3. Results and discussion

3.1. L. monocytogenes populations under the plant-contamination scenario

In turkey breast without lactate-diacetate, *L. monocytogenes* populations (log cfu/cm^2) increased from 1.6 on day-0 to 7.3 on day-25 of storage in vacuum packages (4 °C), and approximately the same levels were observed on day-50 (Fig. 2). Extensive growth of *L. monocytogenes* in processed poultry products formulated without antimicrobial compounds and stored at refrigeration or abusive temperatures has been demonstrated in numerous investigations (Glass and Doyle, 1989; Wederquist et al., 1994; Beumer et al., 1996; Islam et al., 2002; Zhu et al., 2005). In addition to the favorable pH and a_w conditions for microbial growth in poultry products, the profuse growth of the organism in turkey breast in the present study may be attributed to the fact that this particular product was uncured. Inhibition of growth of *L. monocytogenes* exerted by curing ingredients such as sodium nitrite, as well as lower prevalence of the organism in cured than in uncured meat products have been documented (Grau and Vanderlinde, 1992; Duffy et al., 1994; Farber and Daley, 1994; Vitas et al., 2004).

In turkey breast with lactate-diacetate, *L. monocytogenes* populations were lower ($P < 0.05$) compared to those on product without antimicrobials at each sampling period during vacuum-packaged storage (4 °C), and increased from 1.7 log cfu/cm^2 on day-0 to 5.3 log cfu/cm^2 on day-50 of storage (Fig. 2). The antilisterial efficacy of potassium or sodium salts of lactic acid and sodium acetate or diacetate used as formulation ingredients in RTE meat products, either individually or in combination, has been demonstrated by numerous studies (Bedie et al., 2001; Mbandi and Shelef, 2002; Samelis et al., 2002; Seman et al., 2002; Stekelenburg, 2003; Barmpalia et al., 2004, 2005; Georgnaras et al., 2006), and by relatively fewer investigations in

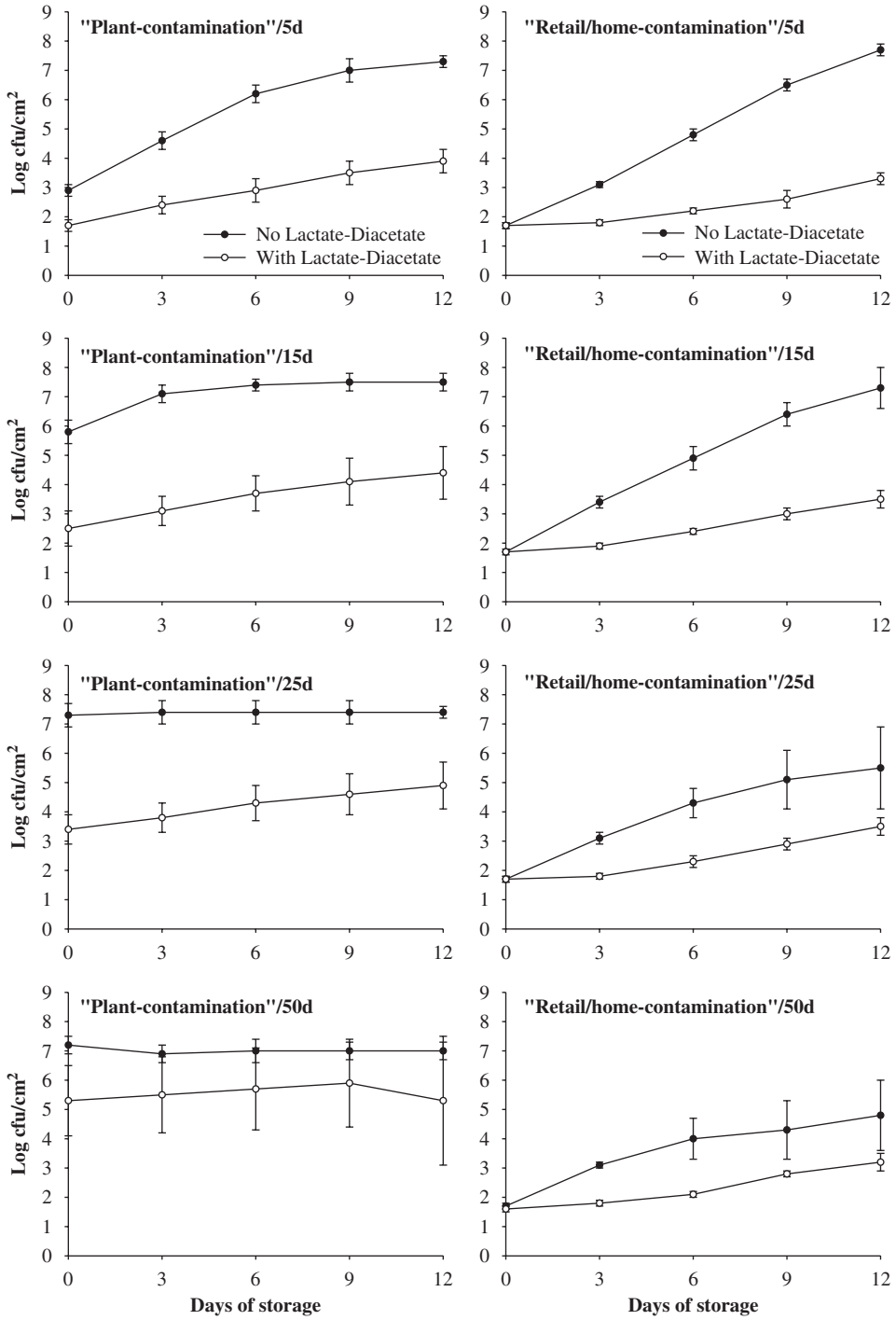

Fig. 2. Mean populations (log cfu/cm^2 ± standard deviation, $n = 6$) of *Listeria monocytogenes* (PALCAM agar) on turkey breast slices with or without potassium lactate–sodium diacetate stored aerobically at 7 °C for 12 days, (i) at 5, 15, 25 and 50 days of vacuum-packaged storage at 4 °C following inoculation (1–2 log cfu/cm^2) with the pathogen (''Plant-contamination''), or (ii) sliced and inoculated (1–2 log cfu/cm^2) with the pathogen after 5, 15, 25 and 50 days of vacuum-packaged storage of unsliced product at 4 °C (''Retail/home-contamination'').

processed poultry products (Schlyter et al., 1993a, b; Wederquist et al., 1994; Zhu et al., 2005).

As expected, the fate of *L. monocytogenes* during aerobic storage following the plant-contamination scenario was affected by its behavior during the preceding vacuum-packaged storage. Pathogen populations attained at 5, 15, 25 and 50 days of vacuum-packaged storage of the product at 4 °C constituted the day-0 counts of aerobic storage at 7 °C (Fig. 2). Due to the prolific growth of the organism in turkey breast without lactate-diacetate during storage in vacuum packages, pathogen populations attained within 3 days of subsequent aerobic storage ranged from 4.6 to 7.4 log cfu/cm^2; in product packages that were opened at 25 and 50 days of vacuum storage, pathogen levels of

approximately 7.0 log cfu/cm^2 were already present on day-0 of aerobic storage (Fig. 2). Since the infectious dose for *L. monocytogenes* is strongly associated with parameters such as the food matrix, strain virulence and host susceptibility (McLauchlin et al., 2004), relatively low doses cannot be excluded from causing infection to high-risk population groups (Farber and Peterkin, 1991). With *L. monocytogenes* growth to a level observed in this study, post-processing plant-contamination of uncured turkey breast without antimicrobials may result in potentially hazardous pathogen levels within 0–3 days of subsequent home storage of opened packages. Although shelf life is usually a quality measurement, the above observations indicate that establishing safety-oriented "sell-by" or "use-by" dates by processors of uncured poultry products should be also considered when no antilisterial interventions are in place. Public health concerns associated with extended shelf life of certain foods, as well as the potential value of safety-based date labeling for the control of *L. monocytogenes* in refrigerated RTE products, have been acknowledged (NACMCF, 2005).

In turkey breast containing lactate-diacetate, pathogen populations at 12 days of aerobic storage ranged from 3.9 to 5.3 log cfu/cm^2 with increasing age of the product (length of previous refrigerated vacuum-packaged storage). On the one hand, in 5- and 15-day-old product, pathogen levels of approximately 3 log cfu/cm^2 were reached within 6 and 3 days of aerobic storage, respectively, while populations of approximately 4 log cfu/cm^2 were observed within 12 and 9 days, respectively. On the other hand, slower growth of the pathogen was observed in 25-day-old product, while in 50-day-old product, although initial pathogen levels were 5.3 log cfu/cm^2, no further growth ($P \geqslant 0.05$) was observed during the 12-day storage period (Fig. 2). The latter observation could be attributed to the high levels of spoilage microflora encountered during vacuum-packaged, and consequently, aerobic storage of turkey breast with lactate-diacetate, as demonstrated by the comparative evaluation of the counts obtained on PALCAM agar and TSAYE (Figs. 2 and 3). Although lower ($P < 0.05$) *L. monocytogenes* populations were observed in turkey breast containing lactate-diacetate compared to product without antimicrobials, the observations made here indicate that these antimicrobial compounds, depending on concentrations applied (USDA-FSIS, 2000), may not be sufficient to completely inhibit growth of the organism. Therefore, consumer exposure to potentially hazardous levels of the pathogen may not be avoided; such levels under this contamination scenario could be reached either due to prolonged shelf life of the product, or due to extended home storage of opened product packages by the consumers (Fig. 2).

3.2. *L. monocytogenes* populations under the retail/home-contamination scenario

Pathogen populations (log cfu/cm^2) increased from 1.6–1.7 on day-0 to 3.2–3.5 and 4.8–7.7 on day-12 of aerobic storage (7 °C) in product with and without lactate-diacetate, respectively (Fig. 2). In turkey breast without lactate-diacetate, significant ($P < 0.05$) increases in pathogen levels were observed within 3 days of aerobic storage, resulting in populations of 3.1–3.4 log cfu/cm^2. Lactate-diacetate inhibited ($P < 0.05$) growth of the organism, and populations of approximately 3 log cfu/cm^2 were reached only at 9 or 12 days of aerobic storage. Mean growth rates (log cfu/cm^2/day) of *L. monocytogenes* during aerobic storage of turkey breast of different age (length of vacuum-packaged storage of original product before slicing and inoculation) are shown in Fig. 4, and ranged from 0.14 to 0.16, and, from 0.25 to 0.51, in product with and without lactate-diacetate, respectively. Growth rates of the pathogen in turkey breast without antimicrobials decreased with product age: the organism grew slower ($P < 0.05$) in product that was sliced and inoculated after 25 and 50 days than in product sliced and inoculated after 5 and 15 days of vacuum-packaged storage (Fig. 4). This observation could be attributed to growth of spoilage microorganisms observed during storage (4 °C) in vacuum packages of original unsliced turkey breast without lactate-diacetate; total microbial counts on day-0 of aerobic storage of product sliced and inoculated with the pathogen at different points of its vacuum storage appeared to increase (Fig. 3).

Although, the growth potential of *L. monocytogenes* has been evaluated in various products and at different storage temperatures, there is limited information relative to the behavior of the organism under conditions similar to those encountered during handling and storage of foods at retail or in the home (Burnett et al., 2005; Yang et al., 2006). Burnett et al. (2005) reported that a 14-day aerobic storage period of turkey breast at 5, 7 or 10 °C (conditions chosen to simulate additional handling at retail) supported prolific growth of the pathogen. Growth rates of the organism (log cfu/g/day) were 0.45, 0.83 and 1.53 during storage at 5, 7 and 10 °C, respectively, and were higher than those observed for ham and cold-smoked salmon, particularly at 7 and 10 °C. As indicated by the data presented here, contamination of uncured turkey breast with *L. monocytogenes* during slicing/handling at retail or home could pose a health risk for susceptible individuals during home storage of the product. Nevertheless, this risk can be reduced considerably when antimicrobials are incorporated in the formulation of the product and when proper food-handling practices (i.e., short-term retail/home storage) are followed.

The "date marking" requirements of the 2005 Food Code, proposed as a means of controlling the temperature-time combination for retail cold holding of potentially hazardous RTE foods after opening of the package, allow for a maximum of 7 days of storage at 5 °C or 4 days at 7 °C. These cold holding provisions were developed so as not to allow 1 log of *L. monocytogenes* growth (USDHHS-PHS-FDA-CFSAN, 2005). Under the conditions of this study and under the retail/home-contamination scenario of

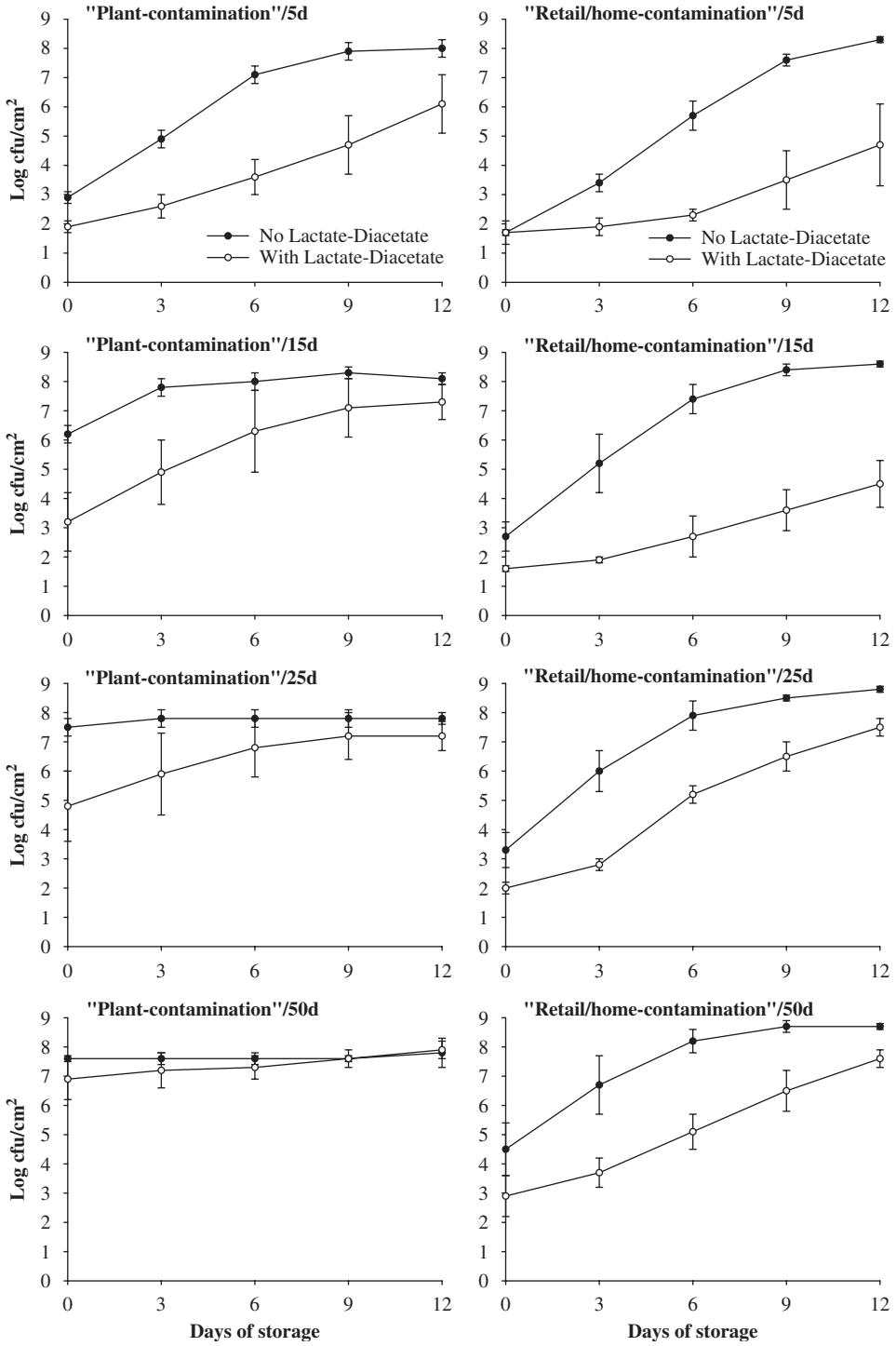

Fig. 3. Mean total psychrotrophic microbial populations ($\log$ cfu/cm$^2 \pm$ standard deviation, $n = 6$) (TSAYE) on turkey breast slices with or without potassium lactate–sodium diacetate stored aerobically at 7 °C for 12 days, (i) at 5, 15, 25 and 50 days of vacuum-packaged storage at 4 °C following inoculation (1–2 $\log$ cfu/cm^2) with *Listeria monocytogenes* (''Plant-contamination''), or (ii) sliced and inoculated (1–2 $\log$ cfu/cm^2) with *Listeria monocytogenes* after 5, 15, 25 and 50 days of vacuum-packaged storage of unsliced product at 4 °C (''Retail/home-contamination'').

turkey breast without lactate-diacetate, observed increases of the pathogen within 3 days of aerobic storage at 7 °C were >1 $\log$ cfu/cm^2. Hence, re-evaluation of the above requirements may be needed with respect to refrigerated retail storage of uncured poultry products without antimicrobials. According to the NACMCF, it might be necessary for safety-based date labeling to be applied at various points in the food chain; labeling at the consumer and food-handler level of the format ''use within *x* days'' of opening or purchase, appears to be very promising in controlling *L. monocytogenes* when coupled with good temperature control (NACMCF, 2005). Such a concept

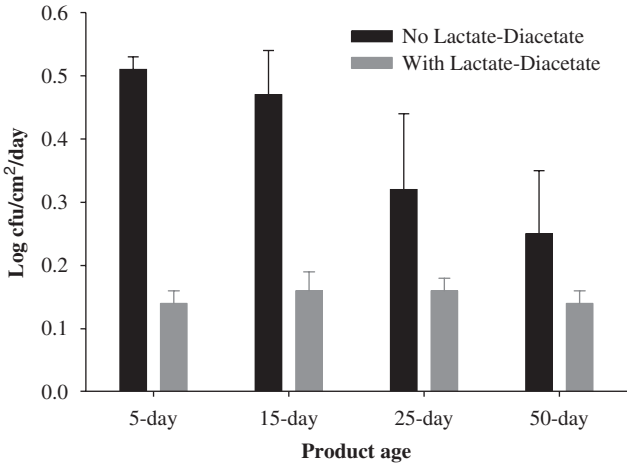

Fig. 4. Mean growth rates (log cfu/cm^2/day ± standard deviation, $n = 6$) of *Listeria monocytogenes* (PALCAM agar) on the surface of turkey breast with or without potassium lactate–sodium diacetate stored aerobically at 7 °C for 12 days, after being sliced and inoculated (1–2 log cfu/cm^2) with the pathogen at 5, 15, 25 and 50 days of vacuum-packaged storage of unsliced product at 4 °C.

can be particularly important in the case of RTE foods such as the one used in the present study, which, due to repeated use by the consumers followed by refrigerated storage, are highly susceptible to recontamination and growth of the pathogen.

Overall, product without antimicrobials inoculated to simulate plant-contamination and product containing lactate-diacetate inoculated to simulate retail/home-contamination were associated with the highest and lowest levels of *L. monocytogenes* during aerobic storage at 7 °C, respectively (Fig. 2). Due to pathogen growth observed both in product without and with (although at slower rate) lactate-diacetate during vacuum-packaged storage (4 °C), the plant-contamination scenario was generally associated with higher pathogen levels during aerobic storage (7 °C) than the retail/home-contamination scenario. Nevertheless, aerobic storage of 5- and 15-day-old product without antimicrobials for 12 days resulted in similar levels of the organism (7.3–7.7 log cfu/cm^2), irrespective of contamination scenario (Fig. 2). Data on storage times of RTE foods at retail or in the home, to be used in assessing the risk associated with growth of *L. monocytogenes* before consumption, are limited. According to the findings of a consumer-based study in Sweden, the majority of the participants reported that they stored opened packages of ham for 3 days to 1 week, but storage times as long as 2 weeks or based on the "best-before" date on the product label or on personal judgment also were reported (Marklinder et al., 2004). Based on the results of the present study, such practices may contribute to exposure of susceptible individuals to levels of *L. monocytogenes* able to cause infection. According to recent risk assessment findings, domestic food-handling practices can increase the mean mortality (death/serving) from consumption of deli meats for the intermediate-age US population (total population excluding elderly and pregnancy-associated groups) by as much as 10^6 times, and inadequate refrigeration temperature followed by storage time were determined to be the practices contributing the most to increased risk of listeriosis (Yang et al., 2006). However, the effect of storage temperature and time on mean mortality was different in different types of deli meats, with opened packages of deli meats demonstrating higher risk than unopened vacuum packages or fresh-sliced products.

Although the present study was only a simulation of contamination at the processing or retail/home level, the collected data indicate that home storage of plant-contaminated product may result in consumer exposure to similar or higher levels of the organism compared to product contaminated subsequently in the food chain, depending on application and efficacy of antilisterial interventions, product age and length of storage. A survey, undertaken to determine the prevalence and concentrations of *L. monocytogenes* in RTE foods collected from retail markets in Maryland and northern California, demonstrated a trend for manufacturer-packaged products to have higher levels of the organism than in-store-packaged products (Gombas et al., 2003). According to Yang et al. (2006), contamination level of *L. monocytogenes* in deli meats at retail represented the input parameter with the highest correlation with mortality in a consumer phase risk assessment approach. However, when assessing the relative risk of listeriosis associated with RTE meat or poultry products that are either purchased pre-sliced and pre-packaged or sliced and packaged at retail, prevalence of the pathogen, consumption data and consumer preferences also need to be considered. Gombas et al. (2003) reported that the incidence of *L. monocytogenes* tended to be higher in in-store-packaged luncheon meats (2.7%) than in manufacturer-packaged products (0.4%), supporting findings of additional investigations indicating the high likelihood of retail premises to serve as a source of cross-contamination of RTE products with the organism (Uyttendaele et al., 1999; Aguado et al., 2001; Van Coillie et al., 2004).

3.3. Spoilage microflora

The high similarity in total mesophilic (data not shown) and total psychrotrophic (Fig. 3) microbial counts obtained on TSAYE during storage in vacuum packages and under aerobic conditions, demonstrated that the spoilage microflora of the product was composed almost exclusively by psychrotrophs. Comparative evaluation of the counts obtained on PALCAM agar (Fig. 2), TSAYE (Fig. 3) and MRS agar (data not shown) illustrated growth of spoilage microflora, and primarily presumptive LAB, after 25 and 50 days of vacuum-packaged storage (4 °C) of product with and without lactate-diacetate, respectively. LAB bacteria, and primarily members of the genera *Lactobacillus* and *Leuconostoc*, constitute the major bacterial group associated with spoilage of refrigerated

vacuum-packaged processed meats (Borch et al., 1996; Samelis et al., 2000; Barmpalia et al., 2005). Although effective antilisterial treatments have been shown to provide inhibition against this bacterial group during refrigerated vacuum-packaged storage of RTE meats (Barmpalia et al., 2005), lactate-diacetate did not inhibit growth of presumptive LAB under the conditions of this study.

Changes in populations of presumptive LAB during aerobic storage (7 °C) were variable (data not shown), even within samples of the same product type and contamination scenario and at the same time interval, suggesting that factors such as frequency, homogeneity, initial levels and types of contamination may affect the natural microflora of the product. Spoilage of aerobically stored product inoculated to simulate plant-contamination was mainly due to presumptive LAB. Growth of these presumptive LAB during vacuum-packaged storage of turkey breast containing lactate-diacetate, and the resulting high levels during aerobic storage may explain the reduced or no significant growth of *L. monocytogenes* during aerobic storage of 25- or 50-day-old product, respectively (Fig. 2). It has been shown that changing the storage environment from microaerophilic or anaerobic to aerobic resulted in faster growth of LAB, as well as in replacement of some members of this bacterial group by others (Samelis et al., 2000). Under the retail/home-contamination scenario, significant increases in counts of presumptive LAB were observed after 9 or 12 and 6-12 days of aerobic storage in product with and without lactate-diacetate, respectively.

Colonies growing on RBC agar were almost exclusively yeasts. Initial levels of yeasts were 0.2 and -0.2 log cfu/cm^2 in turkey breast with and without lactate-diacetate, respectively, and overall, no significant ($P \geqslant 0.05$) increases were observed during vacuum-packaged storage of product inoculated to simulate plant-contamination. Under the same contamination scenario and during aerobic storage, significant growth of yeasts, when observed, was encountered after 6 or 9 days. Growth of yeasts during aerobic storage was also observed under the retail/home-contamination scenario, with significant increases encountered after 6–12 and 6 or 9 days of storage in product with and without lactate-diacetate, respectively. The potentially important contribution of yeasts to spoilage of processed poultry products during refrigerated aerobic storage, as reported by others (Ismail et al., 2000), can be also demonstrated in the present study, even in the presence of antimicrobials. Specifically, in turkey breast with lactate-diacetate that was sliced and inoculated with *L. monocytogenes* after 50 days of storage in vacuum packages, populations of yeasts (log cfu/cm^2) increased from 0.2 on day-0 to 5.6 on day-12 of aerobic storage at 7 °C.

3.4. Chemical and physical properties

Differences between pH values of samples suspended in MRD and distilled water were small (0.01–0.12 units) and pH changes during storage appeared to follow similar trends (data not shown). Sodium diacetate has been known to act as an acidifier when added to the formulation of RTE meats, while salts of lactic acid do not affect the product pH and have been used as pH control agents (Shelef, 1994; Bedie et al., 2001). The initial pH of turkey breast with and without lactate-diacetate was not considerably different in this study, with the pH values of samples suspended in MRD being 6.18 ± 0.02 and 6.26 ± 0.10, respectively. The pH of unsliced turkey breast (both with and without lactate-diacetate), as well as of sliced (inoculated) product containing lactate-diacetate did not change noticeably during storage in vacuum packages (4 °C). However, the pH of sliced product without antimicrobials decreased to 5.76 ± 0.12 after 50 days of vacuum-packaged storage, reflecting microbial growth, most likely that of *L. monocytogenes* and presumptive LAB. Although growth of presumptive LAB was also observed during storage in vacuum packages of product with lactate-diacetate, the more pronounced decrease of pH in product without antimicrobials might have been either due to the prolific growth of *L. monocytogenes*, or due to the predominance of different LAB species in the two products. Consequently, the pH values of samples without antimicrobials inoculated to simulate plant-contamination were generally lower ($P < 0.05$) during subsequent aerobic storage (7 °C) compared to samples with lactate-diacetate or to samples, both with and without antimicrobials, inoculated to simulate retail/home-contamination. In product with lactate-diacetate inoculated to simulate plant-contamination and in product without antimicrobials inoculated to simulate retail/home-contamination, pH decreases ($P < 0.05$), when observed, were encountered after 9 or 12 and 6 days of aerobic storage, respectively. The pH of product containing lactate-diacetate and inoculated to reflect retail/home-contamination did not change significantly during aerobic storage.

The initial a_w of turkey breast was 0.969 ± 0.002 and 0.974 ± 0.002 for samples with and without lactate-diacetate, respectively. The presence of potassium or sodium salts of lactic acid in the formulation of RTE meats has been shown to reduce their a_w (Bedie et al., 2001; Stekelenburg, 2003; Barmpalia et al., 2005). However, in the present study, the a_w of turkey breast with lactate-diacetate was only slightly lower than that of product without antimicrobials. The a_w values of turkey breast, either sliced or unsliced, did not change significantly during refrigerated vacuum-packaged storage and were similar ($P \geqslant 0.05$) in product with and without lactate-diacetate (data not shown).

4. Conclusions

The results demonstrated that the behavior of *L. monocytogenes* in uncured turkey breast during simulated home storage (aerobic storage at 7 °C) depended on contamination scenario and presence of antimicrobials in

the formulation of the product. Plant-contamination (contamination soon after processing) of product without antimicrobials and retail/home-contamination of product containing potassium lactate–sodium diacetate were determined to be the worst and best case scenario, respectively, relative to pathogen levels encountered during subsequent aerobic storage of the product at 7 °C. *L. monocytogenes* populations were lower in product containing potassium lactate–sodium diacetate compared to product without antimicrobials, under both contamination scenarios. Moreover, the results of this study indicated that avoidance of extended shelf life and home storage may reduce considerably the risk of consumer exposure to high levels of the pathogen. The information provided here should be useful in risk assessments and in establishing safety-oriented date labeling for refrigerated RTE meat and poultry products.

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