# Quantitative investigation on the effects of chemical treatments in reducing *Listeria monocytogenes* populations on chicken breast meat

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## Abstract

The effectiveness of treatments for the reduction of *Listeria monocytogenes* surface-inoculated in chicken breast meat was investigated. The treatments consisted of dipping breasts in chemical solutions for 15 min: buffered phosphate (control), calcium hypochlorite, trichloroisocyanuric acid, sodium acetate, sodium lactate, L-lactic acid or trisodium phosphate. The rates of inactivation observed with the chlorine compounds were similar, and in the presence of 100 mg/l of active chlorine viability decreased by 4.41 log units, compared with less than 1.0 log unit decrease observed when cells were submitted to 45 mg/l of active chlorine. Sodium lactate at 2.5% was a more effective antilisterial agent, producing a reduction of 3.88 log units. The present study extends existing findings on the importance of the use of lactic acid, sodium acetate, sodium lactate, trisodium phosphate (chemically classified as GRAS) and chlorine compounds on the inactivation of *L. monocytogenes*.
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## 1. Introduction

Poultry meat constitutes a substantial portion of protein in present-day diets, hence the concern to market a safe, high quality product. The presence of pathogenic microorganisms, spoilage microorganisms, or both, in poultry is undesirable but unavoidable. Food-borne pathogens associated with poultry products include *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli*, *Yersinia enterocolitica*, and *Bacillus cereus* (Bremner & Johnston, 1996; Waldroup, 1996).

The highly publicized, fatal outbreaks of food-borne disease in North America caused by *L. monocytogenes* through consumption of post-processing contaminated meat products, related by Centers for Disease Control and Prevention, have increased consumer awareness and interest from public health authorities and the industry in improving sanitary conditions and controlling pathogens in meat production and processing (Samelis, Sofos, Kendall, & Smith, 2001). Strict and continuous adherence to good farming, manufacturing, and hygiene practices, as well as plant operation under an effective hazard analysis critical control points (HACCP) system, are the basis for controlling pathogen contamination in meat (Smulders & Greer, 1998; Sofos, 1993). This has renewed interest in meat decontamination technologies which are perceived as complementary to HACCP to improve the microbiological status of the

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carcasses at specific points in the process. Although enteric pathogens associated with animal and slaughter hygiene remain the main target organisms of decontamination, environmentally acquired pathogens, such as *L. monocytogenes*, may also be reduced at this early stage of meat processing (Samelis et al., 2001).

Among the technologies considered, the use of organic acids was identified as one the most practical options. Dilute solutions of organic acids (1–3%) are generally without effect on the desirable sensory properties of meat when used as carcass decontaminants. However, the use of organic acids is not applied widely. Within the Europe Union (EU), meat hygiene regulations do not allow any method of product decontamination other than washing with potable water. The major reason for this is the reluctance of legislators to grant permission for the adoption of this technology, as it is perceived to be a means of concealing or compensating for poor hygienic practices in the slaughterhouse (Smulders & Greer, 1998).

There have been limited reports of laboratory trials where organic acids have been applied directly to retail product in an effort to reduce indigenous microbial populations. Such studies have shown an increased storage life of veal tongues (Visser, Koolmees, & Bijker, 1988), minced beef (Niemand, Van Der Linde, & Holzapfel, 1983), and effective in the control of *L. monocytogenes* on the surface of frankfurters (Palumbo & Williams, 1994).

Despite regulatory approval it is evident that organic acid decontamination has not found acceptance or been widely applied in commercial practice, even in North America. Additional research is necessary to establish a set of treatment conditions that may permit a practicable reduction in bacterial contamination throughout the processing chain with a measurable effect on safety and storage life, without imposing any change in sensory properties.

This study was undertaken to investigate the efficacy of altering chlorine concentrations, sodium acetate, sodium lactate, ʟ-lactic acid or trisodium phosphate (TSP) in reducing populations of *Listeria monocytogenes* on laboratory-inoculated chicken breast meat.

## 2. Materials and methods

### 2.1. Preparation of bacterium inoculum

A strain of *L. monocytogenes* isolated previously from a chicken carcass in our laboratory (*L. monocytogenes* 1/2b) was used. *L. monocytogenes* strain 1/2b was subcultured at least twice by loop inoculation of 10-ml volumes of trypticase soy broth, which was incubated at 30 °C for 18 h to achieve viable cell populations of $10^{10}$ CFU/ml. An inoculum of *L. monocytogenes* was prepared by diluting 1.2 ml of the suspension with 1200 ml of sterile 0.1% (w/v) peptone water to yield $10^7$ CFU/ml.

### 2.2. Sample inoculation and treatment application

A total of 84 samples of frozen chicken breast meat were purchased from supermarkets located in Salvador (Brazil). Samples were transported in an insulated cold box filled with ice from their place of collection to the laboratory for experimental treatments and defrosted under refrigeration at 4 °C for 24 h and after that at room temperature (23 °C) for 30 min before being used.

Disinfectant levels added to the breast meat were as follows: calcium hypochlorite or trichloroisocyanuric acid, 45, 60, 70 and 100 mg/l of active chlorine; sodium acetate, 0.2% and 0.4%; sodium lactate, 1.8% and 2.5%, ʟ-lactic acid, 4% (55 °C at source); trisodium phosphate (TSP), 12%. The concentrations of organic acids and salts and TSP used in this study were that considered generally recognized as safe for use on foods and chlorine concentrations were that used in previous studies with *L. monocytogenes* Scott A (Alves, Loura, Almeida, & Almeida, 2001).

Iodometric method (APHA, 1992) was used for total available chlorine immediately before the application of the all chlorinating solutions.

Disinfectant pH values were measured by directly inserting the pH electrode (model HI 98107, HANNA) into the solutions and adjusted (calcium hypochlorite solutions) with glacial acetic acid.

For each treatment, one package containing three breasts was used. Using sterile forceps, two breasts were immersed separately for 10 min in suspensions of $10^7$ CFU/ml of *L. monocytogenes* 1/2b at room temperature (23 °C). After inoculation, one breast was dipped in sterile buffer phosphate (control) and the other in disinfectant solution for 15 min at room temperature (treatment). The third breast was used to carry out an investigation for the presence of *L. monocytogenes* occurring naturally on chicken breast meat. The breast meat was then drained at 4 °C for 2 h. Immediately after draining was completed, samples were tested for quantitative determination of *L. monocytogenes* using the three-tube most probable number (MPN) technique (Peeler, Hought, & Rainosek, 1992). The MPN procedure was chosen because it is the only effective way to obtain quantitative data when the level of *L. monocytogenes* in the sample is lower than 25–50 CFU/g (Buchanan, 1990).

### 2.3. Most probable number of Listeria monocytogenes

A 25 g sample of each chicken breast obtained in laminar flow hood was placed in an aluminum cup containing 225 ml of *Listeria* enrichment broth (LEB) with 0.5%

sodium thiosulphate to neutralize residual chlorine when necessary, and then homogenized using a homogenizer for 2 min. Decimal dilutions from homogenate were prepared in 0.1% (w/v) peptone water ($10^{-2}$ and $10^{-3}$). Triplicate 10-ml LEB homogenates were transferred to sterile empty tubes. The same homogenate was used to prepare $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions in LEB broth in triplicate. After being incubated at 30 °C for 24 h, an aliquot of 0.1 ml from each tube was transferred to other tubes containing 10 ml of Fraser broth, which were then incubated at 35 °C for 48 h. To confirm the most probable number/ g (MPN/g) of *L. monocytogenes*, the broth cultures that changed colour from straw to black were streaked on lithium chloride–phenylethanol–moxalactam medium containing ferric citrate ammoniac (0.05%) and esculin (0.1%) supplemented with antibiotics (LPM) and incubated for 24 h at 35 °C. At least five typical dark esculin-positive-colonies from each plate were submitted to the catalase test, Gram stain, morphology, motility at 25 °C on sulphite–indol-motility medium (SIM) and β-hemolysis on 5% horse blood agar plates to confirm their identity as *L. monocytogenes* (Donnelly, Brackett, Doores, Lee, & Lovett, 1992). A table was used to calculate the MPN of *L. monocytogenes* (AOAC, 1996). All microbiological media were obtained from Difco Laboratories, Detroit, MI.

### 2.4. Data analysis

Two replicate experiments were conducted, with three samples evaluated per replicate, and all bacterial MPN/g obtained were transformed to logarithms before computing means and standard deviations.

## 3. Results and discussion

*Listeria* species were not isolated from original samples of frozen chicken breast meat investigated in the present study.

### 3.1. Inactivation of L. monocytogenes by chlorinating solutions

The data in Table 1 shows the effects of calcium hypochlorite and trichloroisocyanuric acid treatments on the surface of chicken breast meat.

Addition of either calcium hypochlorite or trichloroisocyanuric acid at 60, 70 and 100 mg/l reduced the viability of cells of *L. monocytogenes*. The rates of inactivation observed with the two compounds were similar, and in the presence of 100 mg/l of active chlorine viability decreased by 4.41 log units for both compounds, compared with less than 1.0 log unit decrease in viability that was observed when cells were submitted to 45 mg/l of active chlorine.

Similar findings were reported by Lin, Huang, Cornell, Lin, and Wei, 1996, who observed that *L. monocytogenes* washed off the fish cubes at high-level inoculation was not completely eliminated by the three chlorinating solutions used (aqueous chlorine, chlorine dioxide obtained in situ and commercial chlorine dioxide), even at 400 ppm.

Tsai, Schade, and Molineux, 1992 showed that poultry chlorinating chiller water at 40 mg/l of chlorine reduced *L. monocytogenes* in poultry by only 37–50% in 3–5 min. At least 100 mg/l of chlorine was needed in order to achieve a 99% (two log units) reduction. Such results may be attributed to variation in chlorine demand, which represents the capability of chilled water or the treatment solutions (used in our study) to consume chlorine within a designated time. Chlorine demand is affected by factors such as the presence of organic compounds and interactions between such compounds and chlorine. The competition for available chlorine between bacterial constituents and chicken components affects bactericidal activity (Tsai et al., 1992).

The findings of the present study concurred with those of previous studies where modest microbial reductions on chicken breasts after exposure to similar chlorine concentrations were observed (Mead, Hudson, &

Table 1
Effect of chlorine solutions on viability of *L. monocytogenes*, serotype 1/2b, on chicken breast meat[a]

| Treatment | Concentration (mg/l) | Mean log MPN/g[b] | Log cycles reduction |
|---|---|---|---|
| Control | – | 6.59 ± 0.28 | – |
| Calcium hypochlorite | 45 | 6.42 ± 0.23 | 0.17 |
| (pH = 5.0) | 60 | 3.81 ± 0.12 | 2.78 |
| | 70 | 3.32 ± 0.12 | 3.27 |
| | 100 | 2.18 ± 0.11 | 4.41 |
| Trichloroisocyanuric acid | 45 | 6.52 ± 0.38 | 0.07 |
| (pH = 3.5) | 60 | 4.04 ± 0.12 | 2.55 |
| | 70 | 3.11 ± 0.55 | 3.48 |
| | 100 | 2.18 ± 0.11 | 4.41 |

± Standard deviation.
[a] Time of exposure = 15 min.
[b] Mean log of most Probable Number/g (6 observations).

Hinton, 1993; Wabeck, Schurall, Evancho, Heck, & Rogers, 1968).

### 3.2. Inactivation of L. monocytogenes by organic acids and salts

Organic acid rinses have been proposed as effective, inexpensive carcass interventions (Castillo, Lucia, Goodson, Savell, & Acuff, 1998; Dickson & Anderson, 1992; Dorsa, Cutter, & Siragusa, 1997), with lactic or acetic acid being the most commonly used compound in beef carcass decontamination. Their ability to reduce pathogens or other organisms of faecal origin on beef surfaces has been extensively studied (Castillo et al., 1998; Castillo et al., 2001; Castillo, Lucia, Mercado, & Acuff, 2001).

Salts of organic acids (primarily lactates) are approved for use as food ingredients and have been utilized traditionally to enhance the quality of cooked or cured meat products. Thus, they have been employed as emulsifiers, colour and flavour enhancers, humectants, and to control pH (Houtsma, de Wit, & Rombouts, 1993). The limit of acceptability of sodium lactate would appear to be 4% since panelists noted a mild throat irritation at acid levels of 4% (Papadopoulos, Miller, Ringer, & Cross, 1991).

The results found in this study revealed that sodium lactate, sodium acetate and L-lactic acid caused a decrease in initial population of L. monocytogenes inoculated on chicken breast meat (Table 2).

### 3.2.1. Effects of L-lactic acid

Immediately after treatment with 4% lactic acid at 55 °C, 2.38 log fewer Listeria could be recovered from chicken breast meat in comparison to untreated equivalent (Table 2). Results obtained by Greer and Dilts (1995) when they studied the effects of 3% lactic acid on the growth of L. monocytogenes 4b Scott A no. 3, demonstrated that immediately following the treatment, one log fewer L. monocytogenes could be recovered from

pork lean tissue than from the water treated controls. The same authors using an initial bacterial load ($10^4$–$10^5$/cm$^2$), selected an acid treatment at 3%, 55 °C for optimal effectiveness. It is generally accepted that the bactericidal effects of lactic acid are due to the ability of undissociated acid to penetrate the bacterial cell membrane. More of the acid would be undissociated at a lower rather than a neutral pH.

### 3.2.2. Effects of sodium acetate and sodium lactate

Addition of either sodium acetate or sodium lactate reduced the viability of cells of L. monocytogenes. Sodium lactate at 1.8 revealed a reduction of Listeria of 2.38 log units whereas the same salt at 2.5% was a more effective antilisterial agent, producing a reduction of 3.88 log units (Table 2).

The use of 4.8% sodium lactate as a flavouring agent and as a means of inhibiting certain pathogens in cooked meat and poultry products has been approved by Food Safety Inspection Service, US Department of Agriculture (Mbandi & Shelef, 2001). However, the agency has stated that no evidence was available for the efficacy of these high concentrations of salt to inhibit pathogens. Mbandi and Shelef (2001) demonstrated that the combination of sodium lactate and sodium diacetate, at concentrations lower than the maximum approved for use and well within those considered acceptable organoleptically, was bactericidal to Salmonella enteritidis and bacteriostatic to L. monocytogenes.

According to Stekelenburg and Kant-Muermans (2001), L. monocytogenes was best inhibited by the addition of sodium lactate at 2.5–3.3% but also the addition of 0.2% sodium diacetate. The authors demonstrate that the addition of sodium lactate to a cooked ham product, in amounts between 2.5% and 3.3%, showed a shelf life extending effect, without clear sensory disadvantages. Unlike our results, the authors also found that 0.2% sodium acetate was a more effective antilisterial agent than 1.8% and 2.5% sodium lactate ($P < 0.05$).

Table 2
Effect of organic acids, salts and trisodium phosphate on viability of L. monocytogenes, serotype 1/2b, on chicken breast meat[a]

| Treatment | Concentration (%) | Mean log MPN/g[b] | Log cycles reduction |
|---|---|---|---|
| Control | – | 6.42 ± 0.11 | – |
| Sodium acetate | 0.2 | 4.04 ± 0.14 | 2.38 |
| (pH = 3.1) | 0.4 | 3.82 ± 0.51 | 2.60 |
| Sodium lactate | 1.8 | 4.04 ± 0.14 | 2.38 |
| (pH = 2.4) | 2.5 | 2.54 ± 0.25 | 3.88 |
| L-lactic acid (55 °C) | 4.0 | 4.04 ± 0.28 | 2.38 |
| (pH = 2.6) | | | |
| Trisodium phosphate | 12.0 | 4.04 ± 0.12 | 2.38 |
| (pH = 12.8) | | | |

± Standard deviation.
[a] Time of exposure = 15 min.
[b] Mean log of most probable number/g (6 observations).

## 3.3. Inactivation of L. monocytogenes by trisodium phosphate

A process using a food grade orthophosphate (trisodium phosphate, TSP) to reduce viable *Salmonella* in chicken meat has been approved by the US Department of Agriculture (Giese, 1993). TSP is generally recognized as safe and, under the conditions proposed for treatment (immersion of processed poultry in 8–12% (w/v) solutions of TSP for 15 s), significant reductions have been achieved in *Salmonella*, *E. coli*, *Campylobacter* spp., *Pseudomonas*, spoilage microbes, and *S. aureus* on chicken carcasses without affecting the sensorial quality of the product (Somers, Schoeni, & Wong, 1994). It is known that *L. monocytogenes* is among those most resistant to TSP. According to Dickson (1988), Dickson, Cutter, and Siragusa (1994), Hwang and Beuchat (1995) and Somers et al. (1994), this resistance is due to *L. monocytogenes* high tolerance to alkaline pH values.

In this study trisodium phosphate (TSP) 12% was effective against *L. monocytogenes*. Mean MPN expressed as log MPN exhibited reduction of 2.38 (Table 2). This is in agreement with previous study that have reported differences between *L. monocytogenes* counts in chicken skin samples immersed in water and those treated with TSP ranged from 2.10 (8% TSP-treated samples) and 3.63 (12% TSP treated samples) log cycles (Capita, Alonso-Calleja, García-Fernández, & Moreno, 2001).

The present work extends existing findings on the importance of the use of lactic acid, sodium acetate, sodium lactate and trisodium phosphate (chemically classified as GRAS) on the inactivation of *L. monocytogenes*. Organic acid rinses or immersion of chilled cuts of chicken meat before processing appear to provide an additional measure of safety by reducing levels of pathogens, if present, in poultry processing after the cooler. Lactic acid may be a more appropriate choice for food treatments as it lacks acute and chronic toxicity and which has led to its widespread use as a food preservative and decontaminating agent.

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