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Decontamination of Pig Carcasses with Organic Acids †

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Abstract: This study aims to evaluate the efficiency of two organic acids as decontaminants in pig carcasses. A mix of *Salmonella* Typhimurium and *Salmonella* Derby bacterial suspensions were inoculated in rind samples of about 25 cm^2 at two concentrations, 1.42 × 10^5 CFU/cm^2 (Suspension A) and 4.92 × 10^6 CFU/cm^2 (Suspension B). Samples were decontaminated by spraying with one of two organic acids at each of two concentrations: 2% or 5% of lactic acid or citric acid. Five different times were analyzed, 30 min, 6, 12, 24 and 48 h. For each condition, three samples were used, totaling 120 samples, together with the 30 corresponding control samples. In control samples, an increase of 1.36 log CFU/cm^2 (Suspension A) and 1.43 log CFU/cm^2 (Suspension B) was obtained after 48 h. With the application of lactic acid (2% and 5%) and citric acid (2% and 5%), lower counts were obtained over time. According to the obtained counts, lactic acid (5%) presented better results. There was an increase of 0.51 log CFU/cm^2 (Suspension A) and 0.23 log CFU/cm^2 (Suspension B), over 48 h of storage. The results showed a bacteriostatic effect for lactic and citric acid for Suspension A and B.

Keywords: contamination; carcass surface; organic acid; pork

1. Introduction

All over the world thousands of people continue to die after consuming contaminated food. Contamination can occur from pathogenic bacteria, viruses or parasites [1]. Pathogens frequently associated with foodborne diseases are *Campylobacter*, *Salmonella* spp., *E. coli*, *Yersinia* and *Listeria monocytogenes* [2,3].

Salmonella is an enteric Gram-negative bacterium, facultative anaerobic, and does not form spores. It grows between 8 and 45 °C, at pH between 4 and 9, and with an a_W range between 0.98 and 0.99. This microorganism is resistant to drying, and can survive for a long time in dust [4].

The occurrence of microorganisms on carcasses' surface can result from soiling through intestinal contents, contact with contaminated utensils, bad practices by handlers or even the poor quality of air and water [5]. *Salmonella* finds favorable multiplication conditions on fresh meat [4].

Salmonellosis, is the second most common gastrointestinal infection. In 2018, 91.857 cases were confirmed [2].Several food-borne outbreaks were associated with pork meat and its derivatives [6]. In 2018, according to the frequency of outbreaks published by EFSA, 4.9% of 709 outbreaks were associated with pork meat [2].

Different strategies have been developed, either to prevent microorganism contamination or to eliminate it. Several studies have been developed to test the effectiveness of decontamination treatment of carcasses surfaces [1]. Physical, chemical and biological treatments have been tested, individually and together. The spraying of carcasses with chemical substances, such as organic acids, has been the subject of extensive studies. Organic acids are naturally part of the constitution of some foods, and in others they are added as preservatives [7].

The aim of this work was to evaluate the effectiveness of two decontaminating substances on the surface of pig carcasses, previously inoculated with a mix of *Salmonella Typhimurium* ATCC 14028 and *Salmonella* Derby. In order to achieve this objective, the work was divided into four phases: preparation of two standardized concentrations for *Salmonella* mix; inoculation of the rind samples; spraying pre-inoculated surfaces with lactic acid (2% and 5%) and citric acid (2% and 5%); and finally, evaluation of the effectiveness of decontaminants over 48 h.

2. Material and Methods

2.1. Inoculum Preparation

To obtain the mix for *Salmonella* spp., two strains, *Salmonella* Typhimurium ATCC 14028 (collection strain) and *Salmonella* Derby (isolated from a slaughterhouse), were used. The cultures were preserved at −20 °C in Brain Heart Infusion supplemented with 25% (*v*/*v*) of glycerol. Bacterial strains were subcultured individually in Brain Heart Infusion and incubated at 37 °C for 24 h, followed by subculture to Xylose Lysine Deoxycholate agar and Hektoen Enteric agar followed by incubation at 37 °C for 24 h. Isolated colonies were subcultured in BHI and incubated at 37 °C for 24 h.

The suspensions of each strain were obtained by centrifugation at 10,000× *g* for 10 min at 4 °C in a Sigma 3k18 centrifuge. The supernatant was decanted and the pellet suspended in sterile 0.9% isotonic saline. This entire process was repeated three times. Standardized concentrations were determined by optical density (O.D.) for 1.5 absorbance at 600 nm. After that, the strains were mixed with the same volume and adjusted for 0.1 and 1.5 followed by successive decimal dilutions (1:10) in 0.9% isotonic saline and confirmation of viable cells of the microorganism in Xylose Lysine Deoxycholate agar. The plates were incubated at 37 °C for 24 h. Standardized concentrations were 1.42×10^5 μL/cm^2 and 4.92×10^6 μL/cm^2 for 0.1 and 1.5 respectively.

2.2. Sampling

The samples used in this work were collected from a slaughterhouse. For their preparation, a laminar flow chamber inserted in a refrigerated room (10 °C) was used to prepare pieces of rind with 25 cm^2. Then, they were placed in sterile petri dishes and duly identified.

2.3. Inoculation and Decontamination Procedure

Inside the laminar flow chamber, the samples were inoculated using automatic pipettors and sterile tips. They were inoculated with 20 μL of bacterial suspension in 5 different points of the rind sample, making up 100 μL of inoculum per sample. The inoculum was spread over the surface (25 cm^2) and the samples were stored at 7 °C for 20 min before decontamination. The decontamination procedure consisted of spraying the samples with the prepared decontaminant. About 0.6 mL was sprayed. In this work, lactic acid and citric acid were used as decontaminants, each in concentrations of 2 and 5%. The analysis was performed at 20 min, 6, 12, 24 and 48 h.

2.4. Enumeration of Salmonella spp.

For each storage time, enumeration of S. Typhimurium was carried out by dilution of each samples in 40 mL of sterile 0.9% isotonic saline, followed by maceration in a stomacher for 90 s at room temperature. Sample dilutions (1:10) were spread on Xylose Lysine Deoxycholate agar. The plates were incubated at 37 °C, for 24 h.

3. Results and Discussion

Influence of Organic Acids on Salmonella spp. *Counts over Time*

Table 1 shows *Salmonella* spp. counts (mean and standard deviation), for samples with inoculated concentration of approximately 1.42 ×10^5 µL/cm^2 (Suspension A) and application of organic acids during storage.

Table 1. Counts of Salmonella spp. (log UFC/cm^2, mean and standard deviation) over 48 h (Suspension A) with organic acids application log CFU/25 cm^2.

Suspension A	Control	Lactic Acid 2%	Lactic Acid 5%	Citric Aacid 2%	Citric Acid 5%	Effect
30 min	4.06 ± 0.05 ^{a}	4.23 ± 0.25	4.12 ± 0.13	4.28 ± 0.61	4.20 ± 0.42	n.s.
6 h	4.14 ± 0.06 ^{a}	3.98 ± 0.33	4.21 ± 0.37	4.25 ± 0.70	4.22 ± 0.38	n.s.
12 h	4.84 ± 0.62 ^{ab}	4.44 ± 0.22	4.33 ± 0.23	4.48 ± 0.21	4.56 ± 0.35	n.s.
24 h	5.39 ± 0.20 ^{bA}	4.27 ± 0.28 ^{B}	4.13 ± 0.17 ^{B}	4.36 ± 0.20 ^{B}	4.24 ± 0.17 ^{B}	***
48 h	5.42 ± 0.03 ^{b}	4.88 ± 0.52	4.63 ± 0.78	5.13 ± 0.23	4.86 ± 0.64	n.s.
Effect	***	n.s.	n.s.	n.s.	n.s.	

n.s.—non-significant; for storage time effect (columns), means with different letters (small letters) differ significantly: * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$. For acid effect (lines), means with different letters (capital letters) differ significantly: * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$.

In control samples, it was verified a highly significant increase in *Salmonella* counts over time, increasing 1.36 log CFU/cm^2. The greatest increase was observed between 6 and 12 h, of 0.7 log CFU/cm^2.

With the application of organic acids, counts were lower over time compared with control samples. In all samples, it was verified an increase in counts over time with a smaller increase in samples with organic acids addition. When lactic acid 2% was added, the greatest increase, of 0.61 log CFU/cm^2, was observed between 24 and 48 h. For lactic acid 5%, the same was verified, with an increase of 0.50 log CFU/cm^2 between 24 and 48 h. Despite lactic acid 5% presented lower counts compared to lactic acid 2%, no significant differences were observed between them. Comparing with the control, highly significant differences were observed at 24 h.

Similarly, for citric acid 2% counts, the highest increase (0.77 log CFU/cm^2) was between 24 and 48 h, the same occurring in the 5% concentration, with an increase of 0.62 log CFU/cm^2. When compared to control samples, significant differences occur at 24h. Between acid concentrations, no significant differences were observed.

After 48 h, in the inoculated samples, when compared with control samples, it was verified a greater reduction with the use of lactic acid at 5%, and a smaller reduction when using citric acid at 2%.

Figure 1 shows the graph that represents the evolution of *Salmonella* spp. during storage.

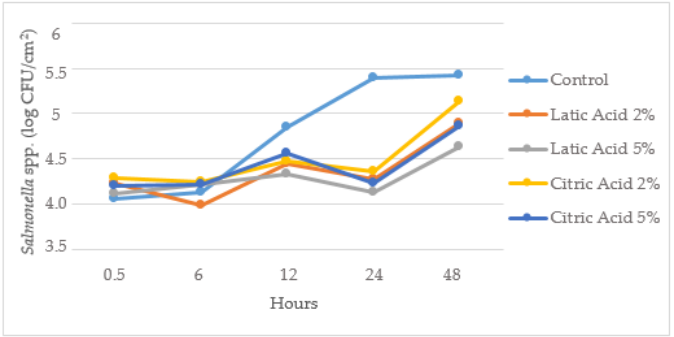

Figure 1. Evolution of Salmonella spp. (log CFU/cm^2) over storage time, after decontamination with Suspension A.

Analyzing the Figure 1, a bacteriostatic effect is suggested for both lactic acid and citric acid in the first 24 h of application. As can be observed, between 24 and 48 h there was an exponential growth. This bacteriostatic effect could be explained by the ability of acids to penetrate the cell membrane, dissociate into the more alkaline interior and acidify the cell's cytoplasm [8].

Table 2 shows *Salmonella* spp. counts (mean and standard deviation), for samples with inoculated concentration of approximately 4.92×10^6 µL/cm² (Suspension B) and application of organic acids during storage.

Table 2. Counts of Salmonella spp. (log CFU/cm², mean and standard deviation) over 48 h with organic acids application log CFU/cm².

Suspension B	Control	Lactic Acid 2%	Lactic Acid 5%	Citric Acid 2%	Citric Acid 5%	Effect
30 min	5.48 ± 0.07 ^{a}	5.14 ± 0.24 ^{a}	5.12 ± 0.16	5.29 ± 0.20 ^{a}	5.56 ± 0.94	n.s.
6 h	5.67 ± 0.14 ^{a}	5.14 ± 0.75 ^{a}	5.10 ± 0.62	6.01 ± 0.17 ^{ab}	5.78 ± 0.11	n.s.
12 h	6.51 ± 0.08 ^{bA}	5.56 ± 0.31 ^{abAB}	5.17 ± 0.42 ^{B}	5.90 ± 0.28 ^{abAB}	5.37 ± 0.53 ^{B}	**
24 h	6.57 ± 0.20 ^{bcA}	5.56 ± 0.25 ^{abBC}	5.07 ± 0.16 ^{C}	5.83 ± 0.22 ^{abB}	5.47 ± 0.07 ^{BC}	***
48 h	6.91 ± 0.10 ^{cA}	6.64 ± 0.27 ^{bABC}	5.35 ± 0.32	6.52 ± 0.53 ^{bABC}	5.98 ± 0.19 ^{B}	***
Effect	***	**	n.s.	**	n.s.	

n.s.—non-significant; for storage time effect (columns), means with different letters (small letters) differ significantly: * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$. For acid effect, (lines), means with different letters (capital letters) differ significantly: * $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$.

For suspension B, in the control samples, it was observed an increase in *Salmonella* spp. counts of 1.43 log CFU/cm² over the storage period, with the largest increase between 6 and 12 h, of 0.84 log CFU/cm². Statistically, in the control samples there was a highly significant increase during storage.

In lactic acid 2%, over time, the increase in counts was highly significant, particularly between 24 and 48 h of 1.08 log CFU/cm². For lactic acid 5%, the increase was not significant. Both concentrations, when compared to the control, show significant differences at 12, 24 and 48 h.

In citric acid 2%, the increase of *Salmonella* spp. counts was very significant, more relevant between 24 and 48 h, with an increase of 0.69 log CFU/cm². At the highest concentration of the acid under analysis, there are no significant differences in the increase in counts over time. However, in a similar way to what happened with the lowest concentration, there is a more pronounced increase between 24 and 48 h of 0.51 log CFU/cm². There were no significant differences between 2% and 5%, and when comparing samples decontaminated with either concentration with the control samples, lower counts were observed

With the application of organic acids, counts reduction of *Salmonella* spp. were observed over time, and at both acid concentrations when compared to the control. After 48 h of storage, it was found a greater reduction with the application of lactic acid at 5%, and a smaller reduction when using citric acid at 2%.

Figure 2 shows the evolution of *Salmonella* spp. counts during the storage period after decontamination with different acids and concentrations.

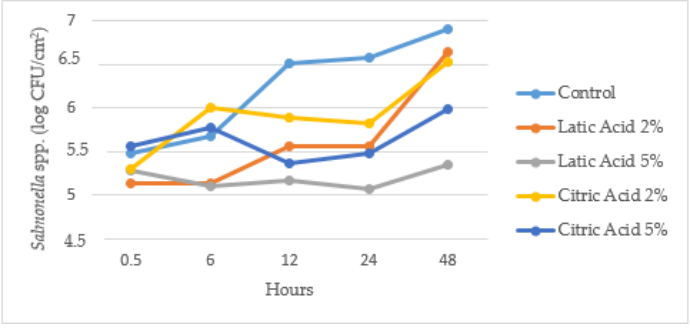

Figure 2. Salmonella spp. (log CFU/cm²) over storage time, after decontamination with Suspension B.

During the first 24 h of storage, reduction in counts were observed when compared to the control, except for citric acid 5%. Thus, it was possible to conclude that both lactic acid and citric acid have a bacteriostatic effect. Similarly, to the results obtained in the inoculum concentration 1.42×10^5 μL/cm^2, between 24 and 48 h of application, there seems to be an exponential development. As explained in the previous inoculum concentration, this effect is due to the fact that the acid acidifies the cell's cytoplasm.

In another study, the effectiveness of lactic, acetic and citric acid in concentrations of 1%, 2% and 3% was studied in beef inoculated with *Salmonella* spp., *Listeria* spp. and *E. coli*. For *Salmonella* spp., and for a 3% concentration, after 30 min of inoculated, there were reductions of 2.34 and 2.35 log CFU/g were obtained for lactic and citric acid, respectively [9].

In another study ground meat samples were inoculated with a mix of *Salmonella* spp. to test the effectiveness of organic acids (lactic acid and peroxyacid) and ultraviolet light. The treatments were applied before grinding, and for lactic acid 5%, reductions of 3.13 log CFU/g were obtained, which were not considered significant [10].

In a study that used chicken meat as a study sample, *Salmonella* Typhimurium ATCC 14028 was inoculated (in two concentrations, 10^2 and 10^6), and later decontaminated with lactic, acetic and citric acid in a concentration of 1% in both. For lactic and citric acid, reductions of 66% and 51% were observed, respectively for the lowest inoculation concentration. For the highest inoculation, reductions of 88% and 72% were observed [11].

4. Conclusions

Considering the obtained results, it was possible to observe that lactic acid at 5% achieved better results regarding *Salmonella* spp. counts. On the other hand, citric acid at 2% was the one where smaller reductions found.

It is possible to conclude that both acids, in both concentrations, have a bacteriostatic effect on *Salmonella* spp.

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