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Monitoring Hygienic Measures for Decreasing *Salmonella* Occurrence in Scalding Tank Water of a Turkey Slaughterhouse

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Abstract: The objective of this work was to test different treatments based on the temperature and acidification of scalding tank water throughout the day in a turkey slaughterhouse under industrial conditions in order to decrease the occurrence of *Salmonella*. After controlling the scalding tank water under usual conditions, the following measures were taken: (a) the temperature was increased to 60 °C and 70 °C for 15 min at the halfway point of the day; (b) the scalding water was acidified and six different initial pH levels were tested. Both measures which were tested (heating and acidification of scalding water) showed efficiency in reducing the occurrence of *Salmonella* during the scalding step. In order to prevent the disadvantages associated with the hardest measures in each case, we propose that scalding water be heated to 70 °C for 15 min without carcasses, which can be repeated if the disadvantages of the exposed costs and resources of processing are acceptable. Regarding acidification, a suitable measure would be an initial pH of 4.0 or any treatment that keeps the pH of the scalding water below 4.5, using acid that does not affect the final quality of the products and/or the elements involved.

Keywords: *Salmonella*; scalding; turkey slaughterhouse; acidification; mesophilic aerobic bacteria; *Escherichia coli*

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1. Introduction

Salmonella is one of the main microorganisms identified as a cause of food-borne outbreaks in the EU/EEA (87.9 cases/100.000 population). Animal-based food, especially poultry meat, is the main source of human salmonellosis [1]. Positive samples of *Salmonella* originate from different food products, mostly from meat and meat products, and notably from fresh meat from broilers and turkeys [2].

In the European Union (EU), and concretely in Spain, specific regulations have been developed for the control of food-borne zoonoses [3–5]. In turkey meat production, the focus has mainly been on the control of *Salmonella* spp. [6,7], with particular concern regarding *Salmonella* Enteritidis and *Salmonella* Typhimurium [2]. High prevalence rates can only be combatted through holistic management of the entire process. In slaughterhouses, all the processes must emphasize avoiding contamination of carcasses and reaching the maximum hygienic standards, and the *Salmonella* prevalence should be decreased in various slaughtering steps. European regulations (R 2073/2005) and different international recommendations have established that the absence of *Salmonella* in a 125 mL sample is required.

Scalding is performed to loosen the feathers prior to the defeathering step, and this process is conducted by immersing the slaughtered birds in warm water or through the use of a newly developed process that involves exposure to steam [8]. According to this author, turkeys are usually subjected to a process called soft scalding/semiscalding, which is performed at a water temperature of 50–53 °C for 1–3 min. The scalding and defeathering steps can support contamination. Ideally, a poultry scalder prepares carcasses for defeathering and reduces the bacterial and debris load on the skin of the carcass [9].

Controlling the temperature in a conventional water tank scalder is also a key aspect of keeping the bacterial load under control [8]. Indeed, scalding is considered both an important and troublesome step regarding contamination [10]. Irshad and Arun [11] report that careful equipment design is required for meat hygiene, since 1 g of soil material (e.g., dirt, fecal material) attached to the feathers can contain 10^8–10^9 microorganisms. It is important to minimize cross-contamination in the common bath used for scalding [12]. Physical variables to control this contamination are time and temperature, which influence washing and antimicrobial effects; the chemical variable is pH, which also influences the antimicrobial effect [11]. According to these authors, for quality pork and poultry meat production, methods of scalding and temperature selection need to be studied. In fact, places where cross-contamination occurs frequently in slaughterhouses include the scalding, defeathering, evisceration, and chilling steps [13]. Also, Russell [14] reports that time, temperature, pH, and the use of antimicrobial chemicals, among other factors involved in this step, are critical both in terms of maintaining product quality and minimizing the occurrence of enteric pathogens. Furthermore, the scalding water adheres to the feathers and to skin pores. For this reason, it is not strange to find different genera of microorganisms in scalding tank water [15]. In this sense, Buncic and Sofos [16] summarized reports demonstrating that in addition to water temperature, carcass contamination during scalding may be reduced by several measures, with the inclusion of approved chemicals to maintain pH at values which do not allow for *Salmonella* growth being among them [17–21].

This work deals with two of the factors with notable influence on bacterial growth: temperature and pH. The objective of our work is to test different treatments based on these factors in scalding tank water throughout the day in a pilot line of a turkey slaughterhouse under real conditions in order to decrease the occurrence of *Salmonella*.

2. Materials and Methods

2.1. Experimental Design and Sampling

Before testing the different treatments of scalding tank water, the status of *Salmonella* occurrence during a labor week (six days) in a pilot line of a turkey slaughterhouse was analyzed under real conditions. Sampling was carried out throughout each labor day to evaluate the evolution of this parameter according to the scalding time. Samples were taken every two hours, considering t_i as the moment at which the first carcass completed the scalding process. The scalding tank's water temperature was 52 ± 1 °C, as is usual in this kind of slaughtering process [9,22]. The temperature was continuously monitored using four probes at different points in the scalding tank (20,000 L). The process was continuously controlled, avoiding declines in temperature using air blowers and heating coils. The turkey carcasses weighed between 12 and 16 kg. The immersion time for the carcasses in the scalding tank water was 3 min, and bled carcasses progressed upstream to the scalding water. In this way, organic matter adhered to feathers was swollen in the scalding tank. According to European legislation, slaughter is organized according to the prevalence of *Salmonella* in farms. Turkeys from *Salmonella*-positive farms are slaughtered last.

Later, the process was repeated with two heating treatments (60 °C and 70 °C for 15 min) applied to scalding water (with no carcasses) at the halfway point of the labor day (Figure 1). Finally, acidification of the scalding tank water was conducted (for testing this last measure, samples were taken every 2 h throughout the day of slaughter).

During the process, the scalding tank was filled every day with clean water and continuously renewed at a rate of 1–1.5 L/turkey. For sampling, sterile bottles (125 mL) were used. Samples were taken from the middle of the scalding tank in order to guarantee homogeneity in the water conditions for the parameters to be studied. All the previous slaughter steps were carried out according to the current European legislation in an accredited turkey slaughterhouse.

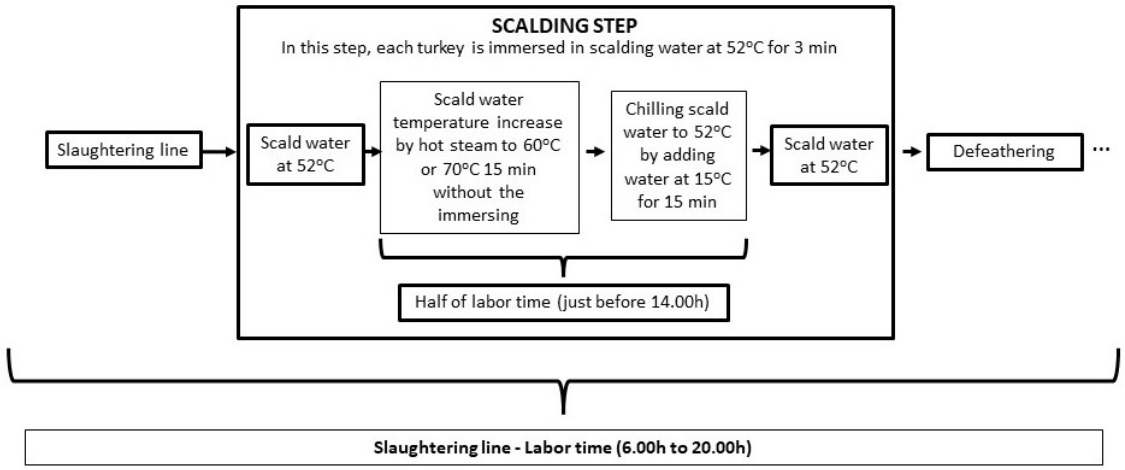

Figure 1. Flow chart of the process of heating the scalding water to test its influence on *Salmonella* occurrence.

2.2. Heating of Scalding Water

After the first step, described in the previous section, the experience was repeated two times. At the halfway point of the labor day (just before the third sampling point), the water temperature was increased to 60 °C and 70 °C, respectively, for 15 min to evaluate the occurrence of *Salmonella*. The heating was carried out without carcasses in the scalding water by applying hot steam. After this treatment, the temperature was returned to the usual scalding parameter (52 °C) by adding clean water at 15 °C (usually, 15 min is needed to return to these conditions).

2.3. Acidification of Scalding Water

Seven experiments at different initial pH levels were carried out to evaluate the influence on the evolution of microbial indicators (*Escherichia coli* and mesophilic aerobic bacteria (MAB)) and *Salmonella* throughout the day of slaughter, taking into account that the deposits of organic matter along the process progressively increased the pH of the water.

To adjust the initial pH of the scalding water, a glycolic acid solution (Adic509H, Adiquimica, Barcelona, Spain) was used. The acidification was carried out by pumping different volumes of acid according to the initial pH value.

2.4. Determination of Salmonella, Mesophilic Aerobic Bacteria (MAB), and E. coli

To determine *Salmonella*, the Vidas^{TM}Up *Salmonella* protocol (BioMèrieux, Madrid, Spain) was used in accordance with ISO 16140, according to Bird et al. [23]. Decimal dilutions of 25 mL of the samples were made with buffered peptone water. Then, 1 mL of *Salmonella* supplement previously reconstituted with 14 mL of 70% ethanol was added and vortexed. Subsequently, the mixture was incubated for 18–24 h at 41.5 ± 1 °C. After incubation, 0.5 mL of the enrichment broth was transferred to the sample well of the cartridge, heated in the VIDAS® Heat & Go heating block for 5 ± 1 min, and allowed to cool for 10 min. Finally, the VIDAS® test was performed, which lasted 48 min, and determined the presence or absence of *Salmonella* as positive or negative, respectively.

To determine the occurrence and counts of MAB and *E. coli*, Tempo EC and Tempo AC Tests (BioMérieux, Madrid, Spain) were used according to Crowley et al. [24] and Crowley et al. [25], respectively. The Tempo system protocol used a vial of culture medium and a card with a transfer tube specific to this test. The culture medium was inoculated with the sample, and the inoculated medium was transferred into a card containing 48 wells of 3 different volumes: 2.25, 22.5, and 225 µL. The card was hermetically sealed and incubated for 40–48 h. The microorganisms which were present were detected with a fluorescent signal. The Tempo Reader detected the signal and calculated the number of microorganisms

present in the sample, in accordance with calculations based on the most probable number (MPN) method [15,22].

2.5. Sensory Tests

Throughout the experiment (three times), routine sensory tests were carried out on carcasses (or cut pieces) after processing, washing, and 24 h of airing at 0–4 °C (cutting temperature). These tests were performed on samples subjected to the most severe treatment conditions. Twenty samples were taken: ten whole carcasses (the outer surfaces and the inner cavities after evisceration were studied) and ten to be cut (breasts and thighs, the main cutting pieces, were studied). Whole carcasses were kept with skin, and cut pieces without skin. For seven days (the usual shelf life of this kind of product), parameters were monitored by an expert panel. For this purpose, 100 g of each sample were taken, without skin, always from the same area and with the same thickness.

The parameters which were assessed were: general overview (normal, freshness loss, silt surface, or other), meat entirety (normal, PSE, DFD, or other), color (normal, pale, blackened, occurrence of stains, bone blackening, greenish areas, or other), odor (normal, mold, acid, rotten, or other), and taste (normal, acid, hardened meat, juicy, or other).

2.6. Statistical Analyses

Statistical analysis was carried out using the SPSS 15.0 Software package (IBM Company, Armonk, NY, USA). The results were compared according to the different parameters studied. For this purpose, a non-parametric chi-square (X^2) test with a level of significance of 95% ($p < 0.05$) was performed. In the case of dichotomic parameters (occurrence or absence of *Salmonella*), a Q Cochran test was carried out.

3. Results

The results regarding the presence of *Salmonella* in scalding water over the course of the experiment are shown in Figure 2.

Once *Salmonella* occurred, it remained in the scalding water until the end of the experiment. This is generally the case under normal conditions. Directly after applying the treatments by increasing the temperature of the scalding water, the results showed an absence of *Salmonella* in all cases (t_i + 8 h). However, when the treatment used a temperature of 60 °C, two hours later, *Salmonella* reappeared on five of the six days of the experiment (83.3%). After treatment at 70 °C, *Salmonella* remained absent for longer, even until the end of the day in 33.3% of the cases. The influence of the treatment showed statistical differences ($p < 0.05$) in terms of the impact of the decrease in the occurrence of *Salmonella*. The effects of decreasing the pH of scalding water on the occurrence of *Salmonella* and microbial indicators are shown in Table 1 and Figures 3 and 4.

Table 1. Evolution of *Salmonella* determination after applying acidification (with different initial pH values) to scalding tank water.

	t_i	t_i + 2 h	t_i + 4 h	t_i + 6 h	t_i + 8 h	t_i + 10 h	t_i + 12 h	t_i + 14 h	t_i + 16 h
pH	5.5	5.65	5.75	5.86	6.05	6.15	6.35	6.38	6.45
Salmonella	-	-	-	+	+	+	+	+	+
pH	5.0	5.06	5.23	5.42	5.55	5.61	5.87	5.96	6.16
Salmonella	-	+	-	+	+	+	+	+	+
pH	4.5	4.65	4.78	4.96	5.02	5.25	5.39	5.56	5.75
Salmonella	-	-	-	-	+	+	+	-	+
pH	4.0	4.12	4.37	4.51	4.75	4.86	4.91	5.05	5.26
Salmonella	-	-	-	-	-	+	+	+	+
pH	3.5	3.58	3.76	3.89	4.15	4.27	4.36	4.46	4.65

Table 1. *Cont.*

	t_i	t_i + 2 h	t_i + 4 h	t_i + 6 h	t_i + 8 h	t_i + 10 h	t_i + 12 h	t_i + 14 h	t_i + 16 h
Salmonella	-	-	-	-	-	-	-	+	+
pH	3.00	3.19	3.25	3.43	3.66	3.89	3.96	4.15	4.42
Salmonella	-	-	-	-	-	-	-	-	-

-: Absence of *Salmonella*; +: Occurrence of *Salmonella*.

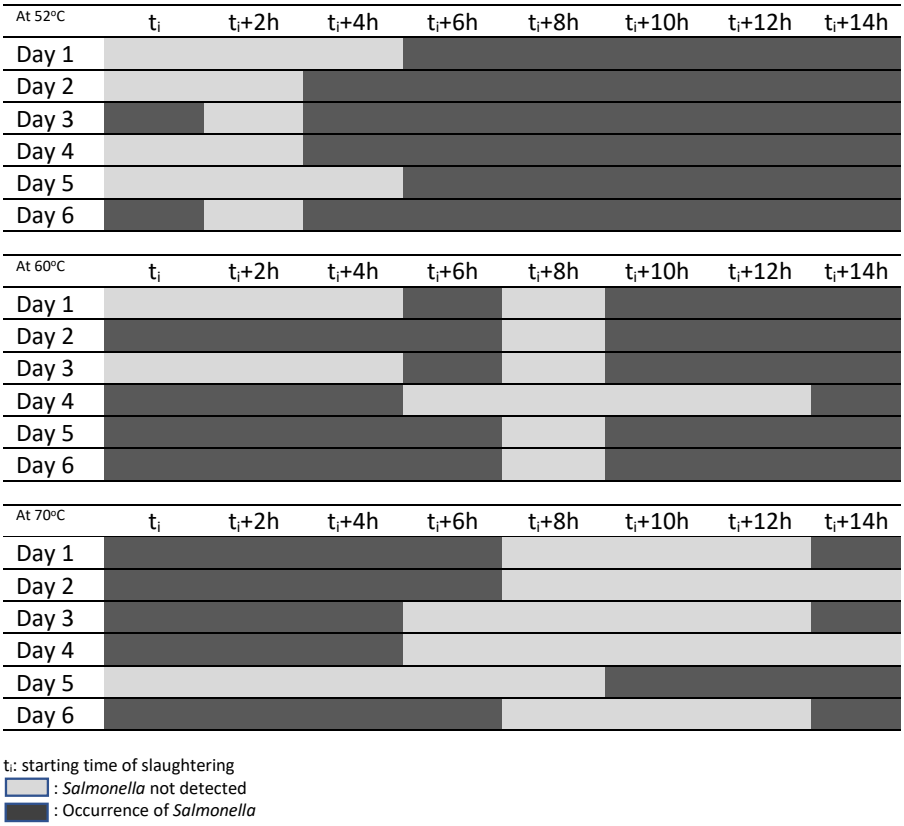

Figure 2. Occurrence of *Salmonella* in scalding tank water throughout 2 h intervals during a slaughter day.

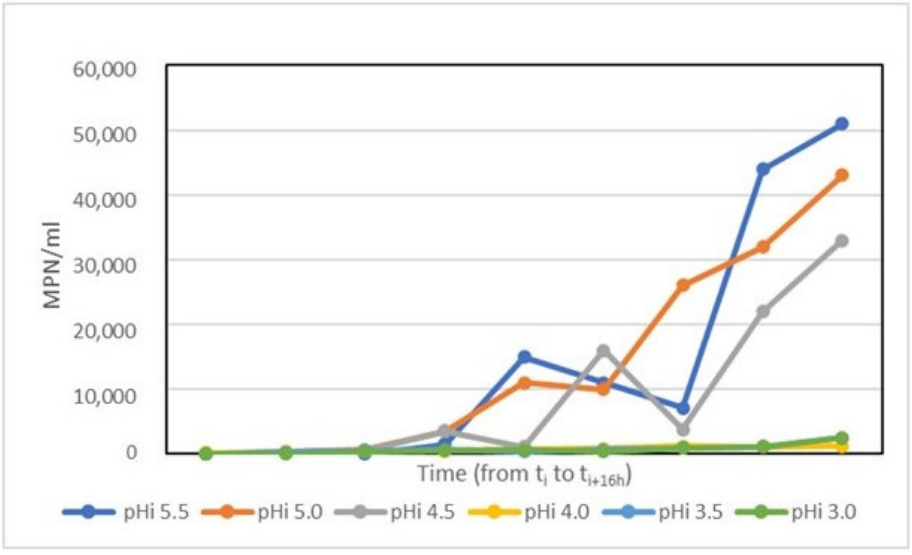

Figure 3. Evolution of *E. coli* occurrence in scald water during scalding step from different pH initial.

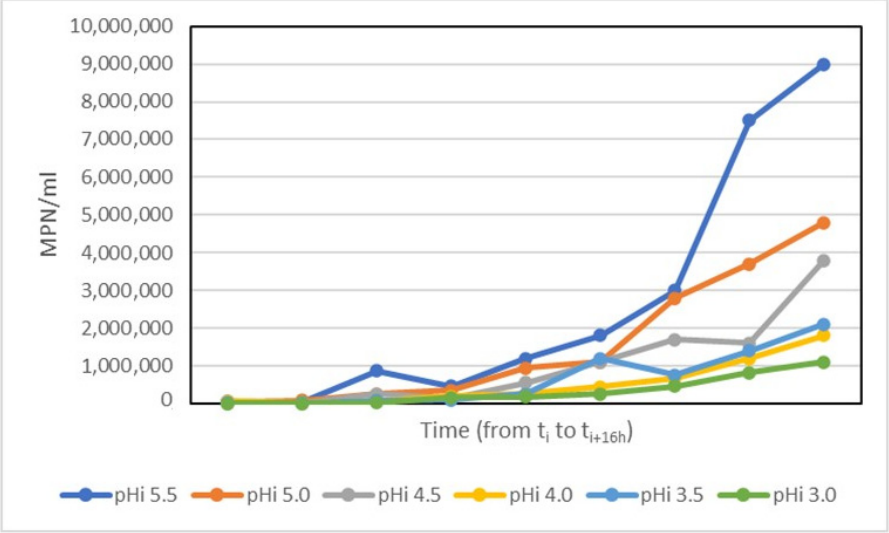

Figure 4. Evolution of mesophilic aerobic bacteria in scald water during scalding step from different pH initial.

As expected, the pH increased throughout the scalding process due to the accumulation of organic matter. The microbial indicators showed consistency with the expected results (Figures 3 and 4). The evolution of *E. coli* showed a clear difference between cases where the initial pH was either above or below 4.5. With initial pH values of 3.0, 3.5, and 4.0, *E. coli* evolution remained at a minimum slope ($p < 0.05$). Also, in the case of mesophilic aerobic bacteria (MAB), the gradient of the curves between the initial pH series was evident. The occurrence/absence of *Salmonella*, which was the main parameter, confirmed the effectiveness of this measure. In this respect, the occurrence/absence of *Salmonella* is clearly influenced by acidification.

When the initial pH was 5.5, *Salmonella* occurred after 6 h. When the initial pH was 5.0, *Salmonella* was regularly detected after 6 h. When the initial pH was 4.5, it was detected after 8 h. If the initial pH was 4.0, *Salmonella* was detected after 10 h. When the initial pH was 3.5, occurrence was observed after 14 h, and when the initial pH was 3.0, the result was the absence of *Salmonella* throughout the process. The results of the acidification of the scalding water show a statistically significant influence ($p < 0.05$). Sensory tests showed that all of the parameters which were studied remained "Normal", with no defaults reported, as shown in Table 2.

Table 2. Results of sensory tests.

	1st Day Shelf Life				Half Shelf Life				End of Shelf Life				
Sample	**Ov**	**Ent**	**Col**	**Od**	**Ov**	**Ent**	**Col**	**Od**	**Ov**	**Ent**	**Col**	**Od**	**T**
Carcass 1	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 2	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 3	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 4	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 5	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 6	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 7	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 8	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 9	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcass 10	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 1	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 2	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 3	N	N	N	N	N	N	N	N	N	N	N	N	N

Table 2. *Cont.*

	1st Day Shelf Life				Half Shelf Life				End of Shelf Life				
Sample	**Ov**	**Ent**	**Col**	**Od**	**Ov**	**Ent**	**Col**	**Od**	**Ov**	**Ent**	**Col**	**Od**	**T**
Breast 4	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 5	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 6	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 7	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 8	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 9	N	N	N	N	N	N	N	N	N	N	N	N	N
Breast 10	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 1	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 2	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 3	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 4	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 5	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 6	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 7	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 8	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 9	N	N	N	N	N	N	N	N	N	N	N	N	N
Thigh 10	N	N	N	N	N	N	N	N	N	N	N	N	N

Ov: General overview—N: Normal. Ent: Meat entirety—N: Normal. Col: Colour—N: Normal. Od: Odour—N: Normal. T: Taste—N: Normal.

4. Discussion

Turkeys can carry a large number of microorganisms from their skin and feathers into scalding water, and there is also some degree of involuntary defecation that adds a significant amount of fecal bacteria to the water [26]. Because of this, there may be an initial build-up reflected in microbial counts, but depending on water use and temperature, the microbial content should be controlled and the number of organisms present should be relatively constant [26].

According to Buhr et al. [8], scalding at an excessively high temperature should be avoided to prevent partial cooking of the breast muscle surface, which leads to white streaks and toughening of the meat. Hotter or prolonged scalding may be better for plucking and *Salmonella* control, but excessive scalding would increase the toughness of turkey breast meat or cause weight loss [8,27]. According to Schilling et al. [22], in general, hard scalding can cause discoloration in the thick skin of young birds. However, Mead [26] argues that turkeys and ducks require the use of higher temperatures ("hard" scalding) to detach the more difficult feathers. Other authors have reported other possibilities, such as three sequenced scalding tanks that reduce temperature sequentially, to solve these problems [28], but these have not shown consistent results in terms of *Salmonella* control and require more economic resources. In our work, the temperature increase was not properly applied to the carcasses, but to the scalding water in the middle of the process. After the scalding water returned to its usual temperature (52 °C), the scalding process continued. A clear influence on the *Salmonella* counts after heating was observed (Figure 2).

For other microorganisms, laboratory tests were conducted on poultry carcasses artificially inoculated with *Escherichia coli* K12 and *Campylobacter jejuni* AR6. Using a pilot system developed with batch immersion, an overall reduction of 1.31 log CFU/cm^2 for *E. coli* K12 counts was observed after 20 s at 80 °C treatment; and a 1.66 log CFU/cm^2 reduction for *C. jejuni* AR6 was achieved by a 30 s treatment at 75 °C [29].

According to Mead [26], the survival of bacteria in scalding water is also affected by the pH, which is usually around 6.0, due to the dissociation of ammonium urate present in feces. This author considers this pH value to be close to the optimum for the heat resistance of *Salmonella*, and reports alkalinization data under laboratory conditions, as well as mentioning a reduction in total viable counts and coliforms in a processing plant [30]. However, the drawbacks are related to the handling of carcasses with the products used

for alkalinization. On the other hand, no results have been reported with the application of acidification under industrial conditions. In this context, and taking into account the obtained results, the acidification of scalding tank water seems to be a potential option.

In our work, and considering that the results do not possess repeatability because of the experimental design (carried out under industrial conditions) and the different microbial loads of origin, Table 1 shows that the decrease in pH was paralleled by a decrease in the detection of *Salmonella* throughout the scalding process, also affecting the hygiene indicators (MAB and *E. coli*) (Figures 3 and 4). The accepted pH limit for *Salmonella* (pH = 4.5) [31] is consistent with our results, as only in one case was *Salmonella* detected below this value (4.46, after 14 h (see Table 1)). This is consistent with the opinion of Buncic and Sofos [16], in which alkaline (9.0) or acidic (3–4) pH levels reduce *Salmonella*'s heat resistance, while organic matter and associated uric acid derived from poultry feces in the tank reduce pH values, maintain them near neutrality, and favor the growth of *Salmonella*.

The pH at which *Salmonella* is detected is different in each case and depends on the initial pH. This initial pH seems to be more influential in preventing the occurrence of *Salmonella*, and the lower the initial pH, the longer the time before the occurrence of *Salmonella*. According to Irshad and Acun [11], the amounts of dry matter and microorganisms in scalding water increase over time. Furthermore, the adding of a high number of poultry carcasses into scalding tanks will result in the contamination of the water within a short period of time [32]. Once the accumulation of organic matter and microbial contamination is sufficiently high, this build-up can lead to an increased occurrence of *Salmonella*, lessening the effect of pH. In addition, *Salmonella* sometimes uses mechanisms to survive unfavorable pH conditions, and can reach pH homeostasis [33] or the ATR (acid tolerance response), which protects *Salmonella* spp. at low pH levels (pH 3 to 4). This mechanism is activated when environmental pH values are between 6.0 and 5.5 and when pH homeostasis fails [34]. Due to these different characteristics, the effective control of *Salmonella* has become a complex process [35]. All of these factors may influence the fact that, although *Salmonella* is progressively detected later when decreasing initial pH (except in the case of pH_i = 4.5), it is also detected at lower pH values than in the case of the immediately higher initial pH.

Okrend et al. [36] reported the addition of 1% acetic acid to the scalding water, lowering the pH to 3.38. The authors reported that the odor was quite pungent, but the effect on the death rate of the two studied salmonellae was marked. This sensory effect was not described with a reduction in the acetic acid concentration to 0.1% (pH 4.38), but resulted in less marked, but still impressive, microbial results. Also, Sakhare et al. [37] assessed the efficacy of microbial decontamination treatments (with acetic and lactic acids) at each step during the processing of broiler chickens, minimizing the chances of cross-contamination from scalding water. The appearance of the carcasses was not affected. In our work, no defaults are reported in the sensory analyses. The carcasses' contact times were not long, and the carcasses remained unplucked. Afterwards, carcasses were washed several times to be aired later. Also, we took into account the fact that turkey products are consumed without skin.

According to our results, and as far as the acidification treatment of the scalding water is concerned, the best result was achieved with a starting point of pH = 3 (preventing a progressive increase due to organic matter), or even slightly higher (4.0) if a progressive dose of scalding water was applied (controlling the pH level by means of a probe line). It is not advisable to apply acidification below pH = 3 to avoid damage to equipment and installations and to prevent potential sensory modifications. Although, in our experience, sensory defaults were not found, it would be advisable to perform a deeper sensory analysis of carcasses under the selected conditions before their final implementation. In this sense, it is remarkable that under pH 4.5, *Salmonella* was not detected in 98.1% of measurements.

5. Conclusions

Both measures tested herein showed efficiency in reducing the occurrence of *Salmonella* during the scalding step. In order to prevent the disadvantages associated with the hardest measures in each case, we proposed that scalding water be heated to 70 °C, which could be

repeated if the disadvantages of exposed costs and resources of processing are acceptable. Regarding acidification, a suitable measure would be an initial pH of 4.0 or any treatment that keeps the pH of the scalding water below 4.5, using acid that does not affect the final quality of the products and/or the elements involved. Further studies should be carried out to combine these measures with other possibilities, such a system of continuous removal of organic matter, as well as sensory analysis of selected conditions.

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