ORIGINAL ARTICLE

Effects of lactic, malic and fumaric acids on *Salmonella* spp. counts and on chicken meat quality and sensory characteristics

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Abstract The aim of this work was to assess the effectiveness of dipping chicken breast in lactic, malic and fumaric acid 3% solutions for 15 s on *Salmonella* counts, as well as on chicken meat quality and sensory characteristics. All three treatments effectively reduced *Salmonella* counts. The values of *Salmonella* log reduction were 2.22, 1.55 and 1.30 log CFU/g for fumaric, malic and lactic treatments, respectively. Although fumaric acid was the most effective for reducing *Salmonell*a counts, chicken meat quality and sensory characteristics were significantly affected, even in cooked samples. Conversely, malic and lactic acids treatments caused minimal changes in chicken meat quality and sensory characteristics compared to control samples. This study shows effective alternatives to reduce *Salmonella* contamination on chicken breast fillets, although further studies should be considered to improve the effects on quality and sensory attributes.

Keywords Foodborne pathogen · Organic acids · Poultry meat · Color · Flavor · Texture

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Introduction

Salmonella is a zoonotic worldwide-distributed pathogenic bacteria. It is frequently found as a contaminant on chicken carcasses destined for human consumption and is considered one of the most important causes of food-borne disease globally (Adams and Moss 2008). Poultry meat is frequently associated with *Salmonella* outbreaks (Allerberger 2016; Antunes et al. 2016). Chickens often carry non-typhoidal *Salmonella* strains in their guts, which are plausible of contaminating meat surface during slaughter even when good processing practices are being followed (Padungtod and Kaneene 2005). Mckee (2011) has reported that only 3–4% broilers entering the slaughterhouses were positive for *Salmonella*, but 35% of the positive at the end of the plant process, clearly showing the spread of contamination at this stage.

Organic acids are widely used in the food industry as chemical sanitizers. It has been probed their antibacterial activity in several food matrices and they are generally recognized as safe (GRAS) for human consumption (Mani-López et al. 2012). Implementation of organic acids as antimicrobial agents in the food material depends on several characteristic properties of the acids such as chemical formula, physical form, pKa value, molecular weight, minimum inhibitory concentration, nature of the microorganism, buffering properties of the food, and acid-food exposure time (Moore et al. 2017; Dittoe et al. 2018; Coban 2019).

Lactic acid is one of the most widely accepted organic acids used for carcass decontamination by the food industry (Huffman 2002). Over the past ten years, many studies have reported lactic acid effectiveness against foodborne pathogens in meat (Ransom et al. 2003; Killinger et al. 2010). Malic acid has also been studied as a

sanitizer in foods. It has been reported that the combination of malic acid and acetic acid was able to significantly reduce *Salmonella* counts on chicken breasts (Olaimat et al. 2018). As to fumaric acid antimicrobial efficacy in foods, studies are scarce, however, its bactericide effect has been demonstrated in lettuce and apple cider (Comes and Beelman 2002; Kondo et al. 2006).

Few studies have been conducted in order to assess the effects of organic acids on chicken meat quality and sensory characteristics. Hecer and Guldas (2011) studied the effect of dipping treatment with lactic acid (0.5–1%) and fumaric acid (0.5–1%) in broiler wings and reported that the panelists did not perceive negative effects on the sensorial attributes of the treated samples. Zhu et al. (2016) demonstrated that the combined treatment of 0.5% of lactic acid and 1% of citric acid did not affect the physicochemical properties and sensory attributes of the quick-frozen chicken drumsticks during storage. To the best of our knowledge, no studies have been carried out to assess the effects of malic acid on meat quality and sensory characteristics of poultry products. The present study aimed to evaluate the effects of lactic, malic and fumaric acid not only on *Salmonella* spp. counts but also on chicken meat quality and sensory characteristics.

Materials and methods

Inoculum preparation

Salmonella strains used in this work were identified as *Salmonella enterica* subsp. enterica serotype Typhimurium, Enteritidis, Thompson, Heidelberg and Schwarzengrund. Strains were originally isolated at different stages of the poultry chain and they were kindly provided by Dr. Pablo Chacana from Pathobiology Institute, INTA Castelar, Argentina. Cells in stationary phase of growth were prepared by individually subculturing one single colony from each serotype in 10 ml of Tryptic Soy Broth (TSB, Oxoid, UK) and incubating the preparation for 24 h at 37 °C. After incubation, 1 ml of each test tube was centrifuged at $3000 \times g$ for 5 min and pellets were twice washed with phosphate-buffered saline (PBS, pH 7.2, Oxoid). Finally, the pool of strains was prepared by mixing equal volumes of each serotype in PBS.

Sample preparation

Bags of skinless chicken breast were purchased from a local supermarket. After freezing they were irradiated with 10 kGy in order to eliminate the interference of local microbiota. This procedure was carried out in a semi-industrial irradiation facility (cobalt-60 source) at the Ezeiza

Atomic Center, National Commission of Atomic Energy of Argentina (CNEA), with an activity of 820 kCi. Irradiated chicken breasts were aseptically sliced with a sterile punch, to obtain samples of 25 cm^2 and 25 g. Samples were individually disposed on sterile petri dishes.

Organic acid solutions

For the 3 organic acids evaluated, the concentration used was of 3% w/v. The solutions of lactic acid (LA; Sigma-Aldrich, Canada), malic acid (MA; Biopack, Argentina) and fumaric acid (FA; Sigma-Aldrich, Canada) were prepared and handled according to manufacturer's recommendations. Regarding FA, it was kept at a temperature of 50 °C, to avoid its precipitation, over the whole procedure, from acid preparation to sample treatment. The other acids were utilized at room temperature.

Sample inoculation

For the inoculation procedure, 50 μl of *Salmonella* pool was applied onto the sample surface and evenly spread with a sterile Drigalsky spatula. Inoculated samples were allowed to dry for 40 min at room temperature in a biological safety cabinet (BSL-2) before treatment.

Treatments

Following aseptic procedures, samples were individually immersed for 15 s in 100 ml of the respective antimicrobial solution: AL 3%, AF, 3% and AM 3%. Controls were immersed for 15 s in 100 ml of sterile tap water, while another group of samples was left untreated. After treatment, samples were individually packed in sterile stomacher bags and kept at 4 °C overnight before microbiological analysis was performed.

Microbiological analysis

A total of 225 ml of 0.1% Peptone Water (PW, Biokar diagnostics, France) was added to each stomacher bag containing an individual sample. Immediately after, samples were passed through a Stomacher (easy Mix, AES, France) for 60 s and serially decimal dilutions were prepared. *Salmonella* counts were performed in Tryptic Soy agar (TSA, Biokar, France) as non-selective media and on XLD, as selective media. The values of log reduction were estimated by subtracting TSA counts of samples treated with the different organic acids from TSA counts of samples treated with tap water. Injured cells were expressed as percentages and calculated as the difference in microbial counts between TSA and XLD, divided by TSA counts and multiplied by 100.

Quality analysis

Cooking procedure

Samples were cooked at 80 °C using an electric convection oven (Oster, CKSTPA488, China) until reached 70 °C at the core. The internal temperature was monitored with T thermocouples and all samples were cooked at the same time.

Chromatic parameters analysis

The chromatic parameters of the raw and cooked chicken samples were determined using a colorimeter (model CR-400, Konica Minolta Sensing, Osaka, Japan) with a D 65 illuminant, 2° observer angle and calibrated using a standard white reflector tile. Five random points were measured on each piece. Results were expressed as lightness (L*), hue angle (h°), saturation index (C*), and color difference (ΔE) and were determined using the software of the colorimeter.

Texture profile analysis

The texture profile was evaluated using the Warner–Bratzler test. Measurements were carried out in a Texture Analyzer Stable MicroSystems (model TA-XT2i, Surrey, U.K.) at 25 °C. The assay parameters used were: test speed 1 mm/s, a cutting distance of 30 mm, a trigger force of 5 g, and a load cell of 50 kg. Cooked samples were cut into sticks of 1.5 cm in diameter and 1.5 cm in thickness, parallel to the muscle fiber orientation. Firmness (maximum cutting force, g) and work of shear (area under the force–deformation curve, g.s.) were determined using the Texture Expert software.

Sensory analysis

The visual color evaluation was carried out with raw samples and by a test of difference against a control using two blind controls (Muñoz et al. 1992). Twenty consumers randomly selected evaluated the color following verbal scale with 7 points: "much clearer than R (control samples)" (− 3), "quite clearer than R" (− 2), "slightly clearer than R" (− 1), "no difference of color concerning R" (0); "Slightly darker than R" (1); "Quite darker than R" (2) and "much darker than R" (3). The test was developed in a cabinet with standardized light (Verivide, CAC 120, UK) and repeated twice with an intermediate time of 15 min. The flavor test was carried out by a triangular similarity test with a panel of semi-trained evaluators (32, without repetition). Each evaluator received three series of cooked chicken samples and identified which was

the different sample. Data analysis was carried out by the comparison of the correct responses (identify of the different samples) with the table data based in the binomial distribution (minimum number of responses required to declare significance at the defined α level) (Lawless and Heymann 2010).

Experimental design and statistical analysis

Experiments were conducted independently three times, with three samples per test in each replicate. The effects of organics acids on *Salmonella* counts and quality parameters (assays 2.7.1 and 2.7.2) were analyzed using a one-way ANOVA analysis (Adhikari et al. 2020; Mohan and Purohit 2020). Treatments that showed a $P < 0.05$ indicated significant effects. Based on the data of the Levene test, the mean values were compared through a comparison test (Tukey or Thamane) to determine significant differences ($P < 0.05$). Regarding, the visual color evaluation data were analyzed using a two-factor ANOVA (samples—evaluator) followed by a Dunnet test to determine significant differences ($P < 0.05$) between samples (Rogers 2017). The software used for the analyses was SPSS version 21 (SPSS Inc., Chicago, Ill, USA).

Results

Microbiological analysis

The mean *Salmonella* counts in TSA and XLD, the log reductions and the percentages of injured cells after treatments are shown in Table 1. No significant differences ($P > 0.05$) were observed between samples treated with tap water and untreated samples nor in TSA neither in XLD. *Salmonella* counts of samples treated with FA were significantly different ($P < 0.05$) from samples treated with tap water and from samples treated with MA and LA in both, TSA and XLD. The log reduction achieved for these samples was 2.22 log CFU/g and the percentage of injured cells was 17%. *Salmonella* counts of samples treated with MA were significantly different ($P < 0.05$) from samples treated with tap water but similar to samples treated with LA in both, TSA and XLD. The log reduction achieved for these samples was 1.55 log CFU/g and the percentage of injured cells was of 11%. Finally, the log reduction achieved for samples treated with LA was 1.30 log CFU/g and the percentage of injured cells was 11% ($P < 0.05$).

Table 1 Salmonella counts in TSA and XLD in chicken breast of untreated samples and samples treated with tap water, fumaric, malic and lactic acid

Treatment	TSA (log CFU/g)	XLD (log CFU/g)	Log reductions (log CFU/g)	Injured cells (%)
Untreated	8.09 ± 0.38 a	7.90 ± 0.43 a	N/A	N/A
Tap water	8.09 ± 0.45 a	7.70 ± 0.21 b	0	5
Fumaric acid 3%	5.87 ± 0.63 c	4.86 ± 0.72 d	2.22	17
Malic acid 3%	6.54 ± 0.20 bc	5.83 ± 0.19 c	1.55	11
Lactic acid 3%	6.79 ± 0.16 b	6.03 ± 0.17 c	1.30	11

Results are expressed as mean (SD); n = 9 per treatment

a, b, c Interventions with no common letter differed significantly ($P < 0.05$; one-way ANOVA)

Quality analysis

Chromatic parameters and the texture profile analysis

Chromatic parameters and the texture profile are shown in Figs. 1 and 2, respectively. Overall, FA, MA, and LA caused a significant increase in L*, h, and C* values ($P < 0.05$) in raw samples. Only samples treated with MA presented a similar h than samples treated with tap water. The color difference (ΔE) found between samples treated with tap water and samples treated with FA was 11.9, with MA was 4.47 and with LA was 3.35. As to cooked samples, small differences ($P \leq 0.05$) in L* values were found among samples treated with tap water, FA, and LA. Only cooked samples treated with MA (< L* values) were significantly different ($P < 0.05$) from samples treated with tap water. As to C* and h, the FA treatment caused the highest effect on these parameters, while significant differences ($P > 0.05$) were not found between samples treated with LA and tap water. The color difference (ΔE) found between control samples treated with tap water and samples treated with FA was 2.5, with MA was 3.65 and with LA was 1.03.

Regarding the texture profile, FA and LA treatments caused a slight increase in firmness and work of shear, while a decrease was observed in samples treated with MA. However, significant differences ($P > 0.05$) were not found with the samples treated with tap water.

Sensory analysis

As to the visual color evaluation, panelists perceived significant differences ($P < 0.05$) among samples treated with FA, MA, and samples treated with tap water. As to samples treated with LA no differences were perceived with samples treated with tap water ($P < 0.05$). Regarding flavor, the panelists indicated that samples treated with FA were significant different ($P < 0.05$) from samples treated with

water, while samples treated with MA and LA were perceived as similar to samples treated with tap water.

Discussion

In the present study, Salmonella log reduction after dipping chicken breast in LA 3% solution for 15 s was 1.30 log CFU/g. Similar results were reported by Ilhak et al. (2018) who evaluated the reduction of Salmonella Typhimurium after dipping skinless chicken breast in LA 4% solution for 1 min and informed a reduction of 1.4 log CFU/g. This finding was very interesting as Ilhak et al. (2018) evaluated a longer exposure time (1 min vs 15 s) and a higher LA concentration (4 vs 3%) than those evaluated in the present study. The fact of achieving similar reductions with shorter exposure time and lower LA concentration represents an advantage since, under these conditions; it is less likely that the decontamination treatment adversely affects the general appearance and nutritional content of the poultry products. Likewise, shorter decontamination treatment times are more feasible with regard to integration of decontamination as an in-line operation during processing (Riedel et al. 2009). The application method also appears to have a significant impact on the decontamination efficacy of organic acids solutions. Ramirez-Hernandez et al. (2018) evaluated the effectiveness of a 15 s LA 2.84% treatment applied by spray to reduce Salmonella in chicken breasts and reported that no significant reduction was observed. In this case, LA concentrations were similar to our study and the exposure time was the same as ours (15 s). The main difference, however, was that Ramirez-Hernandez et al. (2018) used a spray method while we used a dip one. Based on these findings, dipping treatment appears to be more effective than spraying. This has been previously reported by Kim and Slavik (1995) and Wolf et al. (2012).

Salmonella log reduction after dipping chicken breast in a MA 3% solution for 15 s was 1.55 log CFU/g. Tamblyn and Conner (1997) evaluated the effectiveness of a 15 s
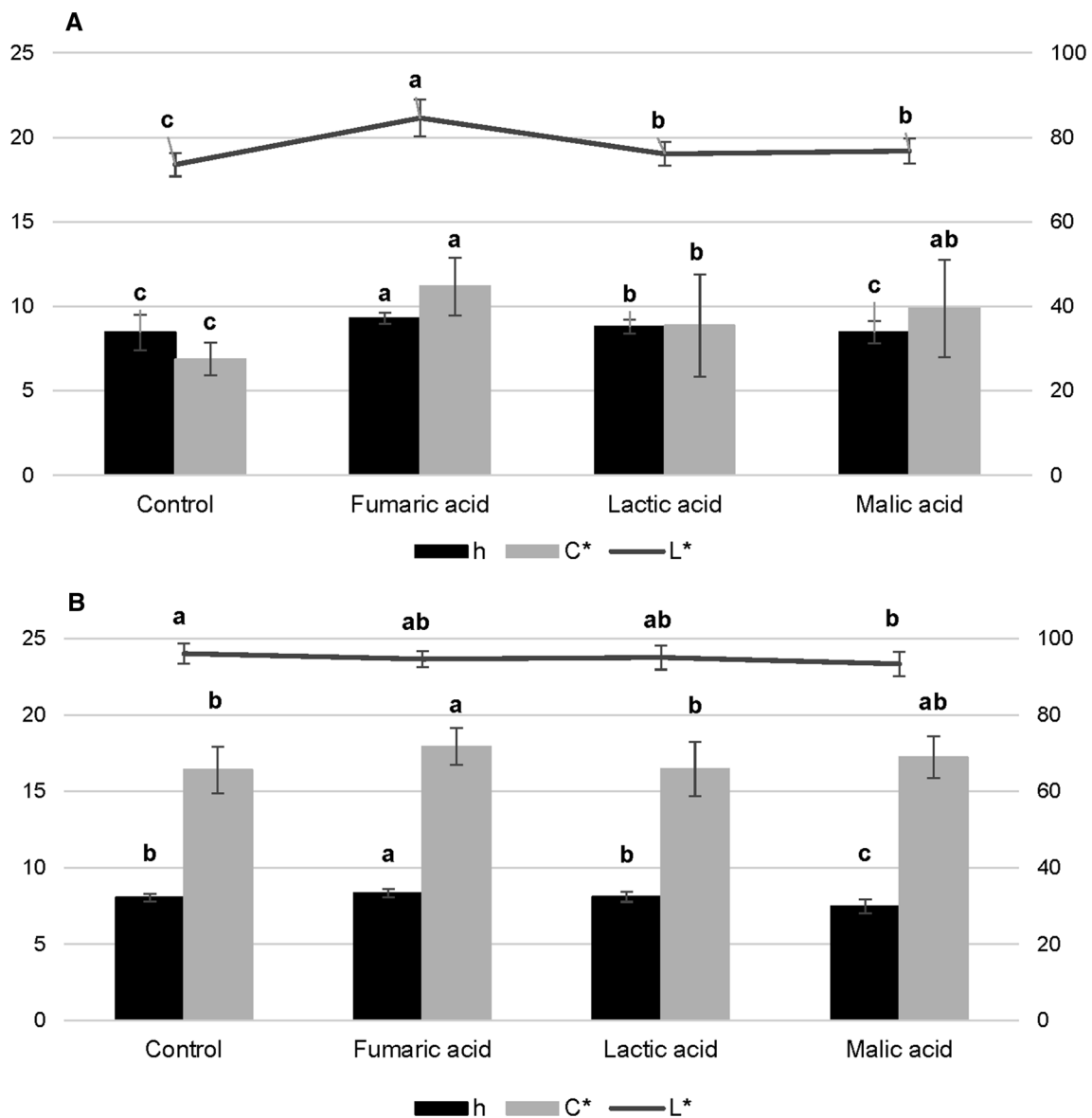
Fig. 1 Effect of organic acid treatments on chromatic parameters of chicken meat: **A** raw samples and **B** cooked samples. (a–c) Interventions with no common letter differed significantly ($P < 0.05$) using one-way ANOVA

and a 2 min dip treatments in 4% MA solution but on chicken skin instead of chicken breast. Cited authors reported no reduction in *Salmonella* counts after the 15 s treatment and a 1.46 log CFU/g reduction after the 2 min treatment. Conversely, Mohan and Pohlman (2016) reported a reduction of 2.23 log CFU/g of coliform populations after dipping beef trimmings in a 3% MA solution for 15 s. As the antimicrobial activity of organic acids depends on the pH and the buffering capacity of the food matrix, the discrepancies between results may be attributed to the type of treated tissue (Lianou et al. 2012). Olaimat et al. (2018) evaluated a dip treatment in a 0.5% MA solution for 5 min to decontaminate chicken breasts and reported a reduction lower than 0.5 log CFU/g in *Salmonella* population after

1 day of storage and a reduction of 2.7 log CFU/g after 10 days of storage. The lower reductions reported by Olaimat et al. (2018) at day 1 may be due to the lower concentration in MA solution (3 vs 0.5%) that could not be compensated by the higher exposure time (5 min vs 15 s). However, after 10 days of storage, the reductions were higher than those reported in the present study. These results suggest that an increase in *Salmonella*´s reduction may be expected after several days of storage. This is due to the fact that organic acids not only exhibit an immediate bactericidal effect but also a residual effect (Lianou et al. 2012). Further studies should be done to demonstrate this hypothesis.

Fig. 2 Effect of organic acid treatments on the texture profile of chicken meat
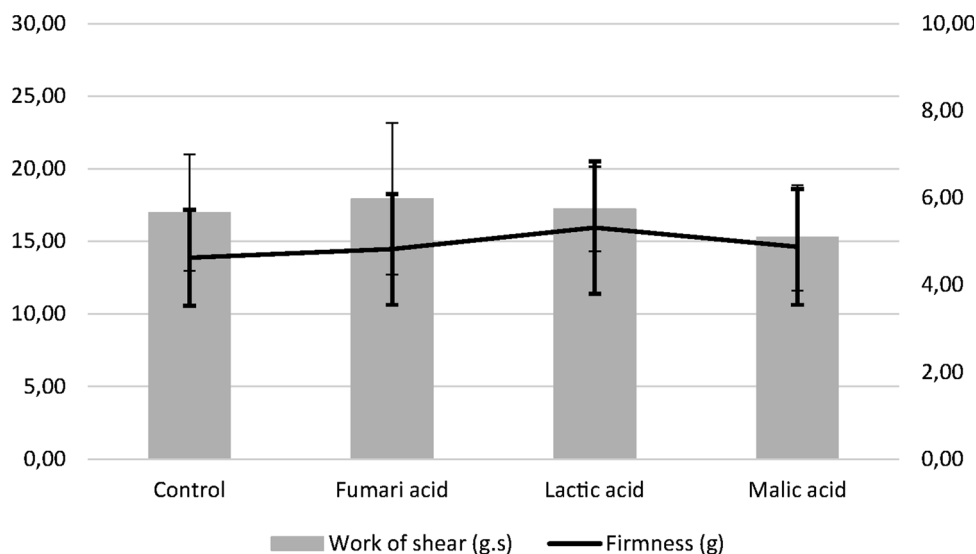
Salmonella log reduction after dipping chicken breast in a FA 3% solution for 15 s was 2.22 log CFU/g. To the best of our knowledge, no previous studies have been conducted to assess the effectiveness of FA in reducing *Salmonella* population in poultry products. However, some studies demonstrated its effectiveness as an antimicrobial treatment. Tango et al. (2015) reported that a concentration of 0.25% of FA was capable of reducing, to an undetectable level, an in vitro *Salmonella* Typhimurium culture of 8–9 log CFU/ml. Other studies demonstrated its effectiveness against *Salmonella* Typhimurium on fresh cut lettuce and against *Escherichia coli* O157:H7 in apple cider (Comes and Beelman 2002; Kondo et al. 2006). Based on these results, FA appears as an interesting alternative to conventional interventions frequently used for poultry product decontamination.

The percentages of injured *Salmonella* cells after treatment were 17% for FA and 11% for MA and LA. Regarding LA, similar results were reported by Dickson and Siragusa (1994) who informed 15% of injured *Salmonella* cells after washing beef tissue with 1% LA solution. The presence of injured cells in a food can pose major public health concerns, since many bacterial pathogens can become more resistant to cooking and other commonly used microbial reduction strategies as a result of sublethal injury (Wesche et al. 2009).

LA was the most effective treatment at preserving chicken meat quality and sensory characteristics, followed by MA and FA treatments. Regarding the chromatic parameters of raw samples, all 3 organic acids evaluated caused significant differences compared to the samples treated with tap water. Samples treated with FA were the most affected, presenting a pinker color with greater intensity and clarity than the samples treated with tap water. These results were, in general, consistent with those

observed in the sensory analysis. The panelists perceived color differences among samples treated with tap water and samples treated with FA and MA while no differences were perceived between samples treated with tap water and samples treated with LA. Several authors had reported that after the use of organic acids, the pH value of the samples decreases (Kim and Slavik 1995; Lim and Mustapha 2004; Harris et al. 2012). That could explain the changes in the chromatic parameters of raw samples observed in our experiment. The decrease in pH value causes protein denaturation, giving a paler appearance to treated meat. Given the fact that we did not include measurements of pH values as part of our methodology, we can only speculate about the possible existence of a causal relationship between those two observed results. After cooking, these color differences decreased to the point that LA treated samples were similar to samples treated with tap water. Regarding flavor, the panelists indicated that samples treated with FA were significantly different from samples treated with water, while samples treated with MA and LA were perceived as similar to samples treated with tap water.

Little information is available regarding the effects of organic acids treatments on quality parameters of chicken breasts treated under conditions similar to the ones defined in the present study. Therefore, comparison between assays should be interpreted cautiously. As to the use of LA, our results were in agreement with those reported by González-Fandos and Dominguez (2006) and, Hecer and Guldas (2011). González-Fandos and Dominguez (2006) reported that the sensory properties (odor, color, texture, and overall appearance) of poultry legs were not affected by the LA treatments at 0.22 M ($\sim$ 2% w/v) and 0.11 M ($\sim$ 1% w/v) for 5 min. Hecer and Guldas (2011) reported that treatments using concentrations of 0.5–1% for 10 min, did not affect sensory properties of chicken wings. Hecer and

Guldas ([2011]) also evaluated the effects of FA treatment and reported that, after dipping broiler wings in a 0.5–1% FA solution, panelists did not detect undesirable effects on the sensory attributes. Regarding MA, González-Fandos and Naiara ([2015]) reported that after MA treatments (1% and 2% for 5 min) on chicken legs, non-undesirable effects on quality characteristics (color and odor) were detected. Likewise, Skřivanová et al. ([2011]) reported that the sensory analysis revealed no changes in the appearance or odor of the raw chicken skin treated with MA (0.5% for 60 s). These results differed from our results as in our case, panelists perceived color differences among samples treated with tap water and samples treated with FA and MA. This discrepancy may be due to the lower concentration of FA and MA solution used by González-Fandos and Dominguez ([2006]), Hecer and Guldas ([2011]) and Skřivanová et al. ([2011]) compared to the concentration used in the present study (0.5–1–2% vs 3%).

Conclusion

Dipping skinless chicken breast in a 3% lactic, malic or fumaric acid solutions for 15 s significantly reduced *Salmonella* counts. Lactic and malic acid treatments caused minimal changes while fumaric acid treatment caused significantly changes in chicken meat quality and sensory characteristics.

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