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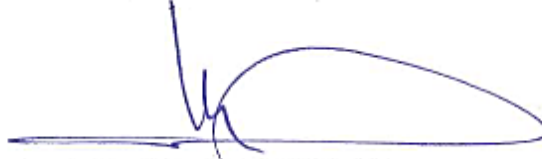
**THE EFFECTS OF SODIUM LACTATE, LACTIC
ACID AND ACETIC ACID, ALONE OR IN
COMBINATION, ON IMPROVING THE SHELF LIFE
OF CHICKEN DRUMSTICK AND THE SURVIVAL
OF *SALMONELLA* SPP. INOCULATED ON THE
CHICKEN DRUMSTICK**

MASTER'S THESIS

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APPROVAL PAGE



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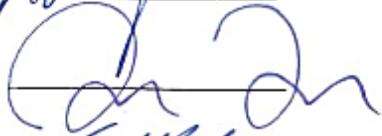
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DECLARATION OF ETHICS

I hereby declare that the works in this thesis have been conducted by myself; I had no ethically offensive behaviour in all stages of the thesis from planning, performing to the writing steps; I obtained all data and information by observing the academic and ethical principles; and I cited the relevant information, data or interpretations used in this thesis, except in the findings.

A handwritten signature in blue ink, appearing to read 'Goran Ali Habeeb', with a long horizontal stroke extending to the right.

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ELAZIĞ

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1. ABSTRACT

In this study, the effects of lactic acid, acetic acid and sodium lactate, alone and in combination, on the survival of *Salmonella* spp. and on the microbiological quality of chicken drumstick were investigated. The fresh chicken drumsticks were inoculated with *Salmonella* Typhimurium and *Salmonella* Enteritidis and divided into 8 groups as control (sterile tap water), 1% sodium lactate (SL), 1.5% lactic acid (LA), 1.5% acetic acid (AA), and their combinations. The drumstick samples were immersed into the treatment solutions for 5 min. Then the samples were stored at 4°C for 10 days and analyzed for aerobic psychrophilic colony (APC), *Pseudomonas* spp., lactic acid bacteria (LAB), *Salmonella* and pH level on days 0, 3, 5, 8, and 10.

The control, SL, LA and LA+ SL groups showed spoilage signs on day 5. On the same day, the numbers of APC, *Pseudomonas* and LAB in the AA, AA+ LA, AA+ SL and AA+ LA+ SL groups were lower ($<7 \log_{10} \text{CFU ml}^{-1}$) compared to the control and SL groups ($P<0.05$).

The LA and AA+ LA groups were significantly different from the control group in the *Salmonella* reductions of 1.2 and $0.9 \log_{10} \text{CFU ml}^{-1}$ on day 0 ($P<0.05$). The survival of *Salmonella* spp. in all groups was almost stable throughout the storage period, except for minor fluctuations.

As a result, shelf life of the chicken drumsticks that were immersed into solutions containing 1.5% AA (AA, AA+ SL, AA+ LA ve AA+ LA+ SL) for 5 min was at least 2 days longer compared to the control group. *Salmonella* spp. was relatively resistant to AA and LA probably due to the buffering capacity of the chicken skin and meat. It is concluded that decontamination of chicken

drumsticks with organic acid combinations will be useful in extending the shelf life of the product, and that increasing the acid concentration to be used while considering the organoleptic properties of the product can give better results.

Keywords: Chicken drumstick, Lactic acid, Acetic acid, Sodium lactate, Shelf life, *Salmonella* spp.



2. ÖZET

SODYUM LAKTAT, LAKTİK ASİT VE ASETİK ASİDİN TEK BAŞINA VEYA KOMBİNASYON HALİNDE KULLANILMALARININ TAVUK BUTLARININ RAF ÖMRÜ VE *SALMONELLA* SPP.'LERİNİN CANLILIĞI ÜZERİNE ETKİLERİ

Bu çalışmada laktik asit, asetik asit ve sodyum laktat'ın tek başlarına ve kombine halde kullanılmalarının tavuk butlarının mikrobiyolojik kalitesi ve *Salmonella* spp.'lerin canlılığı üzerindeki etkileri incelenmiştir. Taze tavuk butları *Salmonella* Typhimurium ve *Salmonella* Enteritidis inokulasyonu yapıldıktan sonra kontrol grubu (steril çeşme suyu), %1 Sodyum laktat (SL), %1,5 Laktik asit (LA), %1,5 Asetik asit (AA), ve bunların kombinasyonunu içeren 8 gruba ayrılmıştır. Her grup tavuk uygulama solusyonlarının içerisine 5'er dakikalığına daldırıldı. Sonra örnekler 4°C'de 10 gün muhafaza edildi ve muhafazanın 0, 3, 5, 8, ve 10. günleri aerob psikrofilik koloni (APK), *Pseudomonas*, laktik asit bakterileri, *Salmonella* spp., ve örneklerin pH değerleri yönünden analize alındı.

Muhafazanın 5. gününde kontrol, SL, LA ve LA+ SL gruplarının bozulma belirtileri gösterdikleri tespit edilmiştir. Aynı günde, AA, AA+ LA, AA+ SL ve AA+ LA+ SL gruplarının APK, *Pseudomonas* ve LAB sayılarının kontrol ve SL gruplarına göre daha düşük ($<7 \log_{10}$ kob ml⁻¹) oldukları ve bozulma belirtileri göstermedikleri görülmüştür ($P<0.05$).

Dekontaminasyon uygulamasının ilk gününde (0. gün), *Salmonella* üzerine sadece LA ve AA+ LA gruplarının 1,2 ve 0,9 $\log_{10}$ kob ml⁻¹ azalma miktarı ile kontrol grubuna göre farklılık gösterdikleri tespit edildi ($P<0.05$). Muhafaza süresi

boyunca tüm gruplardaki *Salmonella* spp. varlığı önemsiz dalgalanmalar haricinde neredeyse sabit kaldı.

Sonuç olarak, %1,5 AA içeren (AA, AA+ SL, AA+ LA ve AA+ LA+ SL) solusyonlarına 5'er dakika daldırılan tavuk butlarının raf ömrünün kontrol grubuna göre en az 2 gün uzun olduğu görüldü. Tavuk eti ve derisin kuvvetli tamponlama özelliğinden dolayı *Salmonella* spp., lerin nispeten AA ve LA'ya karşı direnç gösterdikleri tespit edildi. Organik asitlerin kombinasyonu ile yapılacak dekontaminasyon uygulamalarının tavuk butlarının raf ömrünün uzatılmasında faydalı olduğu ve ürünün organoleptik özellikleri de göz önüne alınarak kullanılacak asit konsantrasyonlarının artırılmasının daha iyi sonuçlar vereceği kanatine varıldı.

Anahtar Kelimeler: Tavuk butu, Laktik asit, Asetik asit, Sodyum laktat, Raf ömrü, *Salmonella* spp.

3. INTRODUCTION

3.1. General Information

Chicken meat is one of the important animal protein sources for human, and it is a very popular food commodity all over the world because of the low-cost production (1). It has many desirable nutritional properties such as low-fat content, high nutritional value, and a relatively high proportion of polyunsaturated fatty acids (2). It is a variety white meat, and it is separated from the red meat because of lower iron content (0.7 mg/100 g) in the case of comparison with mutton and beef (2 mg/100 g). The fat content and its proportion in cooked chicken change depending on whether cooked with skin on or off, in some parts and breeds and diet of birds. Breast meat contains less than 3 g fat/100 g while its proportion in dark meat (skin off) varies from 5 to 7 g/100 g. Only half of the fat is composed of the desirable monounsaturated fats, the less healthy saturated fats constitute only one-third. Poultry meat is regarded as a healthy meat because it does not contain the trans fats that cause coronary heart disease (3).

Production of broiler meat in Iraq has been relatively constant between 2010 and 2016 due to insufficient feed availability and weak biosecurity protocols. However, the production of broiler meat in Iraq is estimated to rise to 170 000 tons in 2017 with an increase of 5%. Iraqi broiler meat import is virtually unchanged by 663.000 tons in 2017. A slight increase in consumption of chicken meat at recent times is fulfilled by rising production. Traditional suppliers (Brazil and United States) continue to compete with the suppliers such as Turkey, Ukraine and Iran (4).

Table 1. Broiler meat productions of the some countries (1,000 Metric Tons) (4)

Total Production	2013	2014	2015	2016	2017
Brazil	12,308	12,692	13,146	12,910	13,250
European Union	10,050	10,450	10,890	11,533	11,700
China	13,350	13,000	13,400	12,300	11,600
India	3,450	3,725	3,900	4,200	4,400
Russia	3,010	3,260	3,600	3,730	3,870
Mexico	2,907	3,025	3,175	3,275	3,400
Argentina	2,060	2,050	2,080	2,055	2,086
Thailand	1,500	1,570	1,700	1,780	1,900
Turkey	1,758	1,894	1,909	1,900	1,950
Malaysia	1,458	1,584	1,633	1,671	1,690
Others	15,580	16,209	15,722	15,483	15,733
United States	16,976	17,306	17,971	18,261	18,596
Total	84,407	86,765	89,126	89,098	90,175

Table 2. Broiler meat consumptions of the some countries (1,000 Metric Tons)
(4)

Total Consumption	2013	2014	2015	2016	2017
China	13,174	12,830	13,267	12,344	11,650
Brazil	8,829	9,137	9,309	9,024	9,252
European Union	9,638	10,029	10,441	11,018	11,170
Russia	3,504	3,660	3,804	3,850	3,960
India	3,445	3,716	3,892	4,196	4,397
Mexico	3,582	3,738	3,960	4,061	4,144
Japan	2,209	2,228	2,321	2,386	2,425
Argentina	1,729	1,773	1,894	1,905	1,909
South Africa	1,556	1,572	1,640	1,665	1,695
Malaysia	1,494	1,624	1,677	1,731	1,750
Others	20,041	20,804	20,050	19,857	20,207
United States	13,691	14,043	15,094	15,331	15,576
Total	82,892	85,154	87,349	87,368	88,135

Spoilage of raw meat occurs in two ways during refrigeration: microbial growth and oxidative rancidity. Deterioration is further accelerated by some intrinsic factors including water activity and pH of fresh meat. In general, most of the fresh meat has a water activity value higher than 0.99 and the value of pH falls within the favorable pH range of spoilage bacteria of meat. As a result, extending the shelf life of perishable chicken products is a major concern to the poultry industry (5).

Poultry meat belongs to a class of highly perishable foods (6). If not properly handled and preserved, chicken meat and meat products support growth of spoilage and pathogenic bacteria. Spoilage of raw meat can occur during refrigerated storage. In this case, chicken meat loses its quality and it can cause a public health problem. It is a favorable host for bacterial growth. Raw chicken meat is a major source of spoilage bacteria, such as *Pseudomonas* spp., *Enterobacteriaceae* spp., lactic acid bacteria, and foodborne pathogens such as *Salmonellas* spp. (5).

3.2. Contamination Routes of Chicken Meat

Practically, the contamination of poultry meat by microorganisms begins in the slaughter line. Commonly, contamination of meat occurs during the several processing stages in poultry slaughterhouse, including scalding, defeathering (picking), evisceration, and chilling processes (7).

3.2.1. Scalding

Scalding is usually used to loosen the roots of the feathers. There are three methods including static hot water tanks which are composed of three tanks, counter-current hot water flow and aero- scalding. The last type is relatively new and expensive. Water of scalding tank results in microbial contamination due to each chicken introduces many millions of microorganisms in the water. This process may lead to a serious problem, resulting in excessive organic material in the scald water. Furthermore, high concentration of fecal material in scalding water is a problem because of excessive contact with the external surface of the chickens; this condition may result in increase of cross-contamination. Moreover,

bacteria presented in this dirty water may be transferred to the skin and into the open feather follicles during picking (7, 8).

3.2.2. Mechanical Defeathering (picking)

The defeathering process has important microbiological implications. The scouring or flailing of carcasses with a large number of flexible rubber 'fingers' causes widespread diffusion of bacteria. Experiments with a readily identifiable 'marker' organism have displayed that at least 200 carcasses can be cross-contaminated during the plucking process from a single inoculated bird (7).

3.2.3. Evisceration

Evisceration is carried out manually in the smaller processing plants, while larger ones use automatic equipment involving several various machines, each concerned with a specific operation (7). During the evisceration process, intestines of chicken may be torn or broken up, and fecal material spreads to the inside or outside of the carcass, to other carcasses, and to processing equipment.

3.2.4. Chilling

In high capacity processing plants, chicken carcasses with a body temperature that is not less than 30 °C reach the chilling stage and need to be refrigerated quickly and efficiently to inhibit the growth of any foodborne pathogens present and delay the growth of psychrotrophic spoilage bacteria. Since there are large numbers of chickens in the cooling system at any given time, there are opportunities for cross-contamination (7).

Furthermore, each of the scalding and defeathering processes always is able to remove the epidermis of chicken skin, thus during the evisceration process and chilling operations, the new surfaces can be colonized by other bacteria.

Finally, during the refrigerated storage, psychrotrophic bacteria are capable of surviving and multiplying and may cause spoilage of raw chicken meat. Therefore, psychrotrophic microorganisms play a significant role in the spoilage of raw poultry meat (9).

3.3. Spoilage of Chicken Meat and Meat Parts

Cross-contamination occurs in the final product during chicken meat processing. Although products that are exposed to excessive microbial contamination are not desirable in point of public health and storage quality, the presence of spoilage and pathogenic microorganisms on poultry carcasses throughout the slaughter processes is inevitable (10). There is increasing concern about the safety of chicken meat because of its underlying association with pathogens. Commonly, microbial loads on fresh chicken meat should be reduced to low levels to allow extending its shelf life. Fresh chicken meat that contained low levels bacteria is profitable for consumers, retailers and processors. The prevalence and numbers of spoilage microorganisms on poultry carcasses can be controlled by an integrated control strategy including preventive systems such as the Hazard Analysis and Critical Control Point (HACCP) principles throughout the production chain (11). Microbial spoilage of chilled products is regarded as a major issue that causes important financial losses for the chicken industry. When raw chicken meat is stored under aerobic refrigerated conditions, the microorganisms that are predominate in spoilage are *Pseudomonas* spp., these groups of microorganisms are considered as the major spoilage bacteria of poultry meat (10). However, pathogens such as *Salmonella*, which is commonly associated with poultry meat and meat products, may result in problems of

mortality and morbidity worldwide. *Salmonella* infections may result in undesirable impact such as illness, medical costs, productivity lost, disability and deaths (12).

3.4. *Salmonella* spp. in Chicken Meat

Salmonella belongs to a genus from family *Enterobacteriaceae*, and *Enterobacteriaceae* is recognized as aerobic and facultative anaerobic, non-spore-forming, Gram-negative and motile pathogenic bacteria. It can multiply and grow at environmental temperatures ranging from 6 to 45°C with a pH range of 4 to 9, and grow well at water activities (a_w) more than 0.94 (13).

Salmonella is one of the most common pathogens transmitted by poultry meat, and causing problems of mortality and morbidity worldwide. Chickens in poultry house that are symptom-free carriers of *Salmonella* are not identified during routine hygiene and veterinary inspections of slaughter stock and meat (14). *Salmonella* spp. are generally distributed in nature and they survive well in a variety of foods. Poultry, eggs and dairy products are the commonest vehicles of human salmonellosis. Salmonellosis is still regarded as a significant public health problem (15).

After slaughtering, the carcasses contaminated with *Salmonella* spp. dramatically increase. Contamination of chicken carcasses with *Salmonella* reaches to its highest before cooling process. Therefore, it can be likely that chicken meat reaching consumers can be contaminated with *Salmonella*. Chai et al. (14) reported that chicken is likely to be the main sources of *Salmonella* Enteritidis. Despite numerous attempts to avoid or reduce contamination by *Salmonella* on the slaughter line and during treatment through processing, the

search for techniques to control or eliminate *Salmonella* spp. from the poultry carcasses is still ongoing.

Chicken is one of the animal meats that is most widely consumed in the world, and because of the various operations in chicken slaughter and processing as mentioned above, it is also known as an important reservoir of *Salmonella* spp. (16). Hence, the poultry industry is considered as the major source of foodborne salmonellosis (17-20). Guran et al. (17) found that *Salmonella* prevalence in the skin of chicken drumstick, chicken breast and thighs were 41%, 44.7% and 40.9% in their study, respectively, while Mazengia et al. (19) noted 12% *Salmonella* prevalence for chicken drumstick. Usually, several factors that result in the perishable of raw chicken meat and meat products including high pH (5.5–6.5), enriched nutrient composition, and water activity (0.98– 0.99) support multiplication of pathogenic bacteria (21). *Salmonella* spp., such as *Salmonella* Enteritidis, is commonly isolated from chicken meat (Table 3).

Table 3. Prevalence of *Salmonella* Enteritidis isolated from nine stages of the broiler chicken supply chain (16).

Source of isolates	Stages of isolates	Total no. of samples	Number of <i>Salmonella</i> – positive isolates %	Number of isolates
Breeder farms	Breeder	150	2	-----
Broiler farms	5-day-old broiler	100	8	<i>S. Enteritidis</i> (8)
	20-day-old broiler	100	13	<i>S. Enteritidis</i> (12)
	Adult broiler	290	33	<i>S. Enteritidis</i> (14)
Abattoir	Pre-cleaning	100	13	<i>S. Enteritidis</i> (8)
	Post-cleaning	103	15	<i>S. Enteritidis</i> (12)
	After chilling	99	26	<i>S. Enteritidis</i> (25)
	Segmented chicken	80	33	<i>S. Enteritidis</i> (18)
Retail markets	Retail chicken	126	29	<i>S. Enteritidis</i> (19)
Total		1148	172	<i>S. Enteritidis</i> (116)

3.5. Chemical Decontamination of Chicken Meat with Organic Acids

Always, some microorganisms may cause a large economic loss because of product loss or illnesses in human. Hence, keeping the safety and quality of the chicken meat and extending the shelf life of chicken products are very important issues for the poultry industry. The decontamination of poultry carcasses can be helpful in reducing human foodborne infections and increasing the shelf life of the product. However, the hygiene processes to prevent contamination should never

be neglected. Thus, poultry meat products have to be safe, have a low spoilage rate, and have the right composition packaging, color, taste and appearance (22).

Decontamination of poultry carcasses or poultry meat parts seems to be the only possibility for the production of pathogen free products. As mentioned above, however, the decontamination strategy should not be a method of first option to eliminate bacteria during or after processing, and hygiene procedures should not be neglected. Decontamination treatments are able to be roughly divided into three types: chemical, physical and combinations of the two (23).

In this regard, many preservation methods and chemical additives have been used to eliminate the pathogens and extend the shelf life of chicken meat. On the other hand, there is an increasing concern about the use of chemical preservatives to eliminate or inhibit spoilage and pathogenic microorganisms in foodstuff. Hence, more and more people prefer natural and minimally processed food products (21). Therefore, it becomes essential to find suitable organic decontaminants that are able to inhibit the bacterial growth to keep the poultry meat safe.

Most organic acids are capable of freely moving throughout bacterial cells due to their small molecular size or mass and simple structure, and have bacteriostatic or bactericidal properties depending on the physicochemical characteristics of the external environment and the physiological status of the target microorganism. The antimicrobial action of weak organic acids, especially lactic acid and acetic acid, has been widely studied and suggested to depend on (i) the pH lowering effect solely; (ii) the extent of dissociation of the acid; and (iii) a specific effect related to the acid molecule (24).

The relative importance of each one of the above contributions had been always a point of argument and frequent debate among researchers. Although the exact antibacterial mechanisms have not been completely explained, all information about bacterial inactivation by applying of weak organic acids has been attributed traditionally to the capacity of the lipophilic, un-dissociated acid molecules so as to penetrate the membrane of bacteria. Different acids effect bacterial growth differently because of their degree of dissociation, and only un-dissociated acid molecules penetrating into the bacterial cell show antimicrobial properties. Un-dissociated acid molecules penetrating into the bacterial cell release a proton (H^+) which reduces the internal pH value of the bacterial cell, thus slowing down metabolic activity (25).

Undissociated organic acids possess high antimicrobial activity, much stronger than those exerted by their dissociated forms, and the extent of disintegration (i.e., the concentration of undissociated acid) and, thus, the antimicrobial effectiveness of an organic acid in solution is determined by its pK_a and the pH of the external medium. Lower pH levels favor an uncharged, an undissociated state of the compound with the latter being able to passively cross the cell membrane and entering the cell (23-26).

3.5.1. Sodium Lactate

Sodium lactate (SL) is the sodium salt of lactic acid, and primarily has bacteriostatic effect (27). Organic acid salts are accepted as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), and they are allowed to be directly added to various foods to control the microbial growth and extend the shelf life of the products (28).

Sodium lactate can be used as a preservative agent. The use of aqueous solution of SL (2.5%) has been found efficient against the growth of various spoilage microorganisms. SL was reported to delay lipid oxidation and extend the shelf life of marinated meat during refrigerated storage (28). SL is usually added to meat and poultry products, and it is advisable to use it as a flavor enhancer in cooked meat and poultry products. SL, which is also used as a pH-control agent, reduces the water activity and inhibits the bacterial growth particularly in *Lactobacilli* (29).

As a result of a study, application of sodium lactate into meatball had a little effect in the reduction of microorganism (30). It is reported that SL shows primarily bacteriostatic effect similar to other salts derived from organic acids (23, 31). Deumier and Collignan (32) reported that there were no significant differences in the numbers of lactic acid bacteria, *Salmonella* spp. and *Listeria monocytogenes* of the chicken fermented sausage treated with 1.8% SL when compared to the control. In another study (33), the growth of background microflora in beef bologna with 2.5% SL was delayed during storage at 5°C.

3.5.2. Lactic Acid

Lactic acid (LA) is a naturally occurring compound in muscles, and it is one of the organic acids. LA is approved as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), and is allowed to directly add to various foods to inhibit the microbial growth and to extend the shelf life of products (34, 35). In addition, it has been shown that LA has some antibacterial effects on the main spoilage microorganisms, and it is effective in reducing microbial counts on poultry meat (36). Various researchers have shown that LA

that is used as a food acidulant can inhibit the growth of some food borne pathogens or can accelerate their inactivation (36-38). Some researchers reported that the rate of inactivation of food borne pathogens depends not only on the environmental pH, but also on the type and concentration of the acid used (37). Lindsay (37) reported that LA has been applied as a preservation method by increasing the acidity of foods. Therefore, LA is most commonly used in foods along with their basic physical and chemical properties, including their dissociation constant pKa value of pH 3.86. In general, the antimicrobial activity of organic acids increases as the environmental pH approaches the pKa (39).

Lactic acid was commonly used to inhibit the multiply of numerous types of meat spoilage bacteria, including Gram-negative species of the families *Pseudomonaceae* and *Enterobacteriaceae*. Furthermore, it improved the shelf life by reducing microbial loads on chicken meat and red meat (38, 40-42). Smaoui et al. (40) investigated the effects of sodium lactate and lactic acid on microbiological characteristics of marinated chicken thighs and reported that the aerobic plate counts were reduced by about 1 log and 2 log CFU⁻¹g, respectively with 0.2 and 1% LA, compared to the control samples, and the use of 1% LA or 3% SL increased the shelf life by two days. In a review on antimicrobial activity of decontamination treatments for poultry carcasses (42), it was reported that by 0.25-2% LA treatment, aerobic bacteria and coliforms were reduced on poultry carcasses by 0.8–2.3 and 0.2–3.0 orders of magnitude, respectively. Increasing the lactic acid concentration in the immersion solution from 2% to 8% increased the *Salmonella* Typhimurium reductions by 0.8–2.2 orders of magnitude,

respectively. European Union legislation has allowed the use of lactic acid and their salts as an additive in chicken meat products (43).

3.5.3. Acetic Acid

Acetic acid (AA) is one of the organic acids naturally occurring during the spoilage of fruit and certain other foods by the bacterium *Acetobacter*. It is commonly known as vinegar and shows antimicrobial properties. Therefore, it is most commonly used in food preservation technology (44). The pKa value of AA is pH 4.76. The proportion of undissociated acidity of AA is 98.5% when pH reached to 3.0 (45). According to Adam's report (46), AA (pKa 4.76) is a weaker acid than LA (pKa 3.86) because of interactive effects of different pKa. LA decreases the pH, in consequence of that increasing the proportion of AA in the undissociated state and increasing its antimicrobial effect.

AA has been used alone and in combination with other preservation methods for the decontamination of fresh chicken meat and commonly in the poultry industry (42, 47, 48). In a review on antimicrobial activity of decontamination treatments for poultry carcasses (42), it was reported that AA treatment yielded reductions between 0.2 and 2.0, 0.6 and 2.3, and 0.8 and 2.0 orders of magnitude for aerobic bacteria, Enterobacteriaceae and *Salmonella* spp., respectively. Furthermore, increasing the AA concentration in the immersion solution from 0.3% to 0.6% increased the Enterobacteriaceae reduction by 1.4 log CFU ml⁻¹ (42).

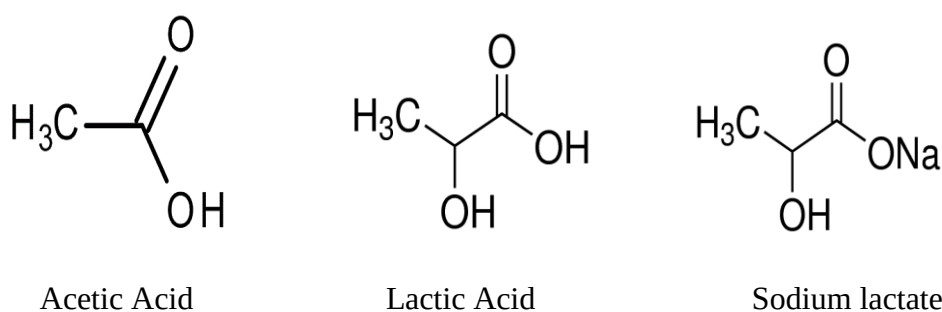


Figure 1. Chemical structure of acetic acid, lactic acid and sodium lactate commonly used in meat and poultry products (35).

3.5.4. Combined Use of Organic Acids

One possible method for reducing spoilage and pathogenic bacteria on raw poultry meat is the use of weak acids that are known as organic acids and their salts, which are normally considered safe. Hence, in many developed countries allow the use of tartaric acids, ascorbic, acetic, citric and lactic, and their salts. These acids are a natural component of many fermented food products (21, 23, 24, 35). It has been reported that applications of organic acids like acetic acid, lactic acid, propionic acid, citric acid, and benzoic acid for decontaminating meat surfaces are used without causing any health problems (36-38).

There is much information on the effectiveness of organic acids on reducing *Salmonella* on poultry meats, however, there is less information on the effect of mixed organic acid solutions on the survival of *Salmonella* on poultry carcasses or their parts during storage. Some researchers have studied to determine the effect of various mixtures of organic acids on the spoilage and pathogenic microorganisms on the surface of poultry carcasses. They have noted that the combination of organic acids may improve the microbiological quality of poultry meat (26, 48-51).

Smaoui et al. (40) evaluated the microbiological effect of different sodium lactate and lactic acid combinations on the marinated chicken thigh, and they demonstrated that the addition of 0.9% sodium lactate and 0.09% lactic acid to marinated chicken can delay the spoilage of the product. Burfoot and Mulvey (52) sprayed 4% lactic acid that was buffered to pH 3.7 using sodium lactate on commercial chicken and turkey carcasses, and they showed that the application of a buffered lactic acid can be a suitable method for reducing microbial counts on poultry carcasses. Mikolajczyk (26) investigated the effect of various mixtures of organic acid solutions (acetic, ascorbic, citric, lactic and tartaric acid) on the survival of *Salmonella* on turkey carcasses. The author found that immersion the turkey carcass into a solution composed of equal parts of 1% tartaric acid, 1% lactic acid, and 1% acetic acid for 15 min gave a promising option for reducing the risk of the presence of *Salmonella*. Crist et al. (53) conducted a research investigating the effect of 2.5% sodium lactate and acetic acid mixture on the shelf life of fresh Italian pork sausage, and they reported that sodium lactate/acetic acid mixture allowed approximately 4 extra days of shelf life when compared to control group.

Acetic and lactic acids can be characterized by bactericidal and bacteriostatic properties, while the salts (sodium lactate) are primarily bacteriostatic. The un-dissociated state of the acid molecule is primarily responsible for the antimicrobial activity (24, 38, 44). The antimicrobial efficacy of sodium lactate, lactic acid and acetic acid has been intensely studied by many researchers (26, 51, 52). However, there is not much research investigating the

combined effect of organic acids or acid salts on the shelf life of meat or meat products, especially on chicken meat or meat parts.

The specific goals of this study are (i) to evaluate the antimicrobial efficacy of sodium lactate, lactic acid and acetic acid, alone and in combination, on aerobic psychrophilic bacteria, *Pseudomonas* spp., lactic acid bacteria and *Salmonella* spp. on chicken drumstick, and (ii) to improve the microbial quality and extend the shelf life of chicken drumstick by applying the combination of sodium lactate, lactic acid and acetic acid.



4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Chicken Drumsticks

For each of the three trials, 44 fresh chicken drumsticks with skin (each one has a weigh 100 - 150 gram, and the drumsticks had no abnormalities such as presence of feathers, trauma and fracture) that were produced one or two days ago were purchased from a local supermarket on the day of the experiments. The samples were transported to the laboratory in 20 minutes, and stored in the refrigerator at 4°C until starting the experiments. Throughout the experiments, a total of 132 chicken drumsticks were used.

4.1.2. Sodium Lactate

Sodium lactate (CAS number 72-17-3) solution (60% w/w) was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

4.1.3. Lactic Acid

L(+) - Lactic acid (CAS number: 79-33-4) solution (88-92%) was purchased from Sigma (Sigma-Aldrich, Seelze, Germany).

4.1.4. Acetic Acid

Acetic acid (CAS number: 64-19-7) (100%) was purchased from Sigma (Sigma-Aldrich, Seelze, Germany).

4.2. Methods

4.2.1. Preparation of *Salmonella* spp. Inoculum

In the present study, one *Salmonella* Enteritidis (RSKK 92 (Turkish Public Health - Turkey) and two *Salmonella* Typhimurium (NCTC 12416 and NCTC 74) strains were used. Each of *Salmonella* strains was grown in tryptic soy broth of 10

ml at 37°C for 18 h. Cultures were then centrifuged at $4,192 \times g$ for 10 minutes at 5°C, and the formed pellets were washed with 0.1% sterile peptone water before re-centrifuging to remove organic residues. After second centrifugation, the supernatant was removed and the pellets of each strain were re-suspended in an aliquot of 0.1% sterile peptone water. These suspensions were combined in a single tube and completed to 10 ml with 0.1% sterile peptone water. This mixture of *Salmonella* inoculum was used immediately.

4.2.2. Inoculation of *Salmonella* spp. on the Drumstick Samples

Prior to the inoculation procedure, two randomly selected drumstick samples were taken, and tested for the existence of indigenous *Salmonella* spp., for the inoculation, the *Salmonella* suspension of 0.25 ml was spread on the each drumstick sample using a sterile L-shaped spreader. After inoculation, the drumsticks were held at room temperature for 10 min for bacterial attachment, and two samples were taken and analyzed for the enumeration of *Salmonella* spp., the numbers of aerobic psychrophilic bacteria, *Pseudomonas* spp. and lactic acid bacteria.

4.2.3. Decontamination Treatments

The chicken samples in each batch were divided into eight groups, each containing five drumsticks with skin. After inoculation procedure, each group of drumstick samples was dipped into the sterile glass beaker containing 500 ml (for each one) of one of the following sterile decontamination solutions (v/v) at ambient temperature for 5 min. Decontamination solutions (treatment groups) and their pH levels were as follows:

- 1- Control (sterile tap water),

- 2- 1.5% Lactic acid (pH 2.3)
- 3- 1.5% Acetic acid (pH 2.75)
- 4- 1% Sodium lactate (pH 6.96)
- 5- 1.5% Lactic acid + 1% Sodium lactate (pH 3.33)
- 6- 1.5% Acetic acid + 1% Sodium lactate (pH 3.72)
- 7- 1.5% Lactic acid + 1.5 Acetic acid (pH 2.20)
- 8- 1.5% Lactic acid + 1.5% Acetic acid + 1% Sodium lactate (pH 3.28)

After the treatments, the chicken drumsticks were left to drain at room temperature for 10 minutes. After draining, each drumstick was individually placed in separate styrofoam plates and wrapped with cling film, and stored at 4°C for 10 days. A total of 132 drumstick samples were used during the study.

4.2.4. Microbiological Analysis

Microbiological analyses were carried out on days 0 (after the dipping treatment), 3, 5, 8 and 10. One drumstick sample from each group was analyzed on each sampling day. Briefly, 100 ml of 0.1% sterile peptone water (PW) was added into a sterile stomacher bag containing the chicken drumstick sample, and the stomacher bag was shaken by manually massaging for 1 min. Then, a 1 ml of the fluid part was taken from the stomacher bag and serially diluted in sterile tubes, each one containing 9 ml of 0.1% PW. The serial dilutions were used for the following microbial analysis. Microbiological analyzes were conducted by using surface plating method in double plates.

4.2.4.1. Enumeration of *Salmonella* spp.

Xylose Lysine Deoxycholate (XLD) agar (HiMedia, Mumbai India) was used for the enumeration of *Salmonella* spp.. Plates were incubated in aerobic conditions at 35°C for 24–36 hours, and characteristic colonies were counted (54).

4.2.4.2. Enumeration of Aerobic Psychrophilic Colonies

Plate count agar (PCA) LAB-M, Merck (E. Merck, Darmstadt, Germany) was used for the enumeration of aerobic psychrophilic colonies. Plates were incubated at 6.5°C for 10 days, and colonies were counted (55).

4.2.4.3. Enumeration of *Pseudomonas* spp.

Pseudomonas Selective Agar (Merck, Darmstadt, Germany) that was supplemented with *Pseudomonas* CFC Selective Supplement (Merck, Darmstadt, Germany) and added 5 ml glycerin was used for the enumeration of *Pseudomonas* spp. Plates were incubated at 25°C for 2 days. After incubation period was completed, the colonies were counted. Then, three colonies from each plate were randomly selected and subjected to oxidase test (Bactident Oxidase, Merck, Darmstadt, Germany). According to the results of the oxidase test (*Pseudomonas* spp. is oxidase positive), the numbers of *Pseudomonas* spp. were calculated (55).

4.2.4.4. Enumeration of Lactic Acid Bacteria

De Man Rogosa Sharpe (MRS) Agar, LAB-M, (E. Merck, Darmstadt, Germany) was used for the enumeration of lactic acid bacteria. Colonies of lactic acid bacteria were counted after the plates were incubated at 28°C for 2 days (55).

4.2.5. Determination of pH

After the microbiological analyses were completed, the pH values of the rinsing solution of the samples were measured by using a pH meter (Selecta pH 2001, J.P. Selecta, s.a, Barcelona, Spain).

4.2.6. Statistical Analysis

Analysis of the microbiological data and pH values were carried out using SPSS 22 software (IBM, SPSS Statistics, Version 22). The numbers of each group of bacteria were converted to logarithmic values (log CFU/ml rinsate) before calculating means and performing statistical analyses. The data were subjected to analysis of variance (ANOVA) appropriate to replicate × treatment groups × sampling times to determine fixed effects and interactions between variables. Bonferroni test was used for multiple comparisons between the groups. Statistical significant level was expressed as $P \leq 0.05$.

5. RESULTS

5.1. Changes in the Numbers of Microorganisms on the Chicken Drumstick Samples

All analyses were composed of three independent trials. Mean numbers of aerobic psychrophilic colonies, *pseudomonas* spp., lactic acid bacteria and *Salmonella* spp. of the chicken drumstick samples decreased after immersion into treatment solutions (alone or in combination) for 5 minutes. Mean counts of aerobic psychrophilic colony, *Pseudomonas*, lactic acid bacteria and *Salmonella* of the chicken drumstick samples treated with various chemicals were shown in Figure 2 and Table 4, Figure 3 and Table 5, Figure 4 and Table 6, Figure 5 and Table 7, respectively. No indigenous *Salmonella* spp. was found in the chicken drumstick samples used in the study. The pH results of the samples were shown in Figure 6 and Table 9.

5.1.1. Counts of Aerobic Psychrophilic Colonies (APC)

The mean number of total aerobic psychrophilic colony on the control sample was $5.4 \log_{10} \text{ CFU ml}^{-1}$. After the decontamination treatments (on day 0), the count of APC of the samples decreased between 0.5 and $1.2 \log_{10} \text{ CFU ml}^{-1}$ depending on the decontamination solutions, except for the sample treated with 1%SL (Figure 2 and Table 4). After on day 0, the count of APC in all of the groups continuously increased during the storage time at 4°C , and significant differences were observed between the storage days ($P < 0.05$). On day 3, APC numbers of the control and 1% SL groups were $6.9 \log_{10} \text{ CFU ml}^{-1}$, and APC numbers of 1.5% AA, 1.5% AA+ 1% SL, 1.5% AA+ 1.5% LA and 1.5% AA+

1.5% LA+ 1% SL groups were significantly lower compared to the control and 1% SL groups on day 3 ($P<0.05$).

It was detected that the counts of APC of the control, 1% SL, 1.5% LA and 1.5% LA+1% SL groups was above $7 \log_{10}$ CFU ml⁻¹ on day 5. APC counts of the samples treated with 1.5% AA + 1.5% LA and 1.5% AA + 1.5% LA + 1% SL were significantly lower when compared to the control group on day 5 ($P<0.05$).

On day 8, the control, 1% SL and 1.5% LA+1% SL groups were not analyzed because those samples clearly showed spoilage indications (bad smell and slime layer on the samples). Although the 1.5% LA, 1.5% AA and 1.5% AA+ 1% SL groups have slight bad smell (no slime layer), all samples were submitted to the microbiological analyze. Analyze results of those samples showed that APC numbers of the 1.5% LA, 1.5% AA and 1.5% AA+ 1% SL reached to 8.2, 7.9 and $8.3 \log_{10}$ CFU ml⁻¹, respectively. APC numbers of the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL were $7.8 \log_{10}$ CFU ml⁻¹. No significant differences were observed between the groups on day 8 ($P>0.05$).

On day 10, the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL groups showed the spoilage indications and their APC numbers were 8.8 and $8.9 \log_{10}$ CFU ml⁻¹, respectively (Figure 2 and Table 4).

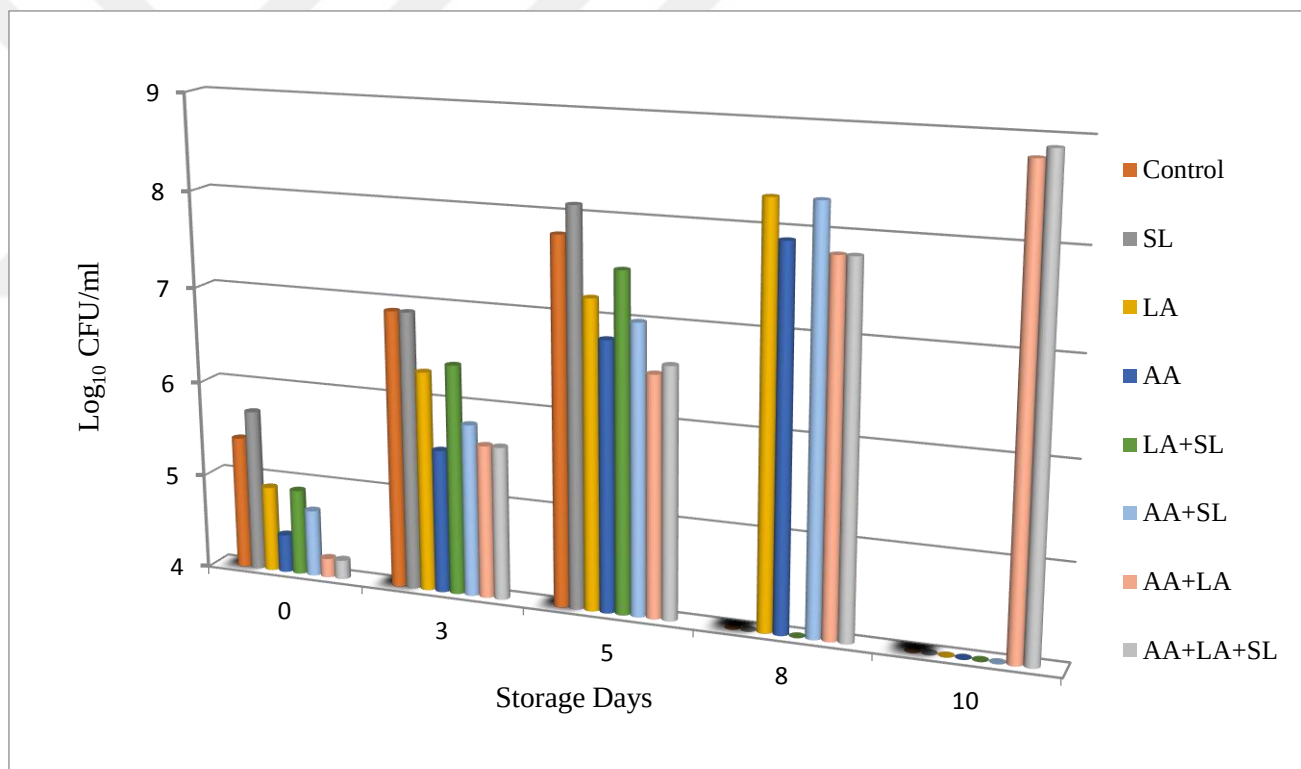


Figure 2. The mean numbers of aerobic psychrophilic colonies of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C ($\log_{10}$ CFU ml^{-1} rinsate)

Table 4. The mean numbers of aerobic psychrophilic colonies of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C ($\log_{10}$ CFU ml⁻¹ rinsate $\pm$ SD)

Treatment Groups	Storage days				
	0	3	5	8	10
Control	5.4 ^{BCv} $\pm$ 0.24	6.9 ^{Cw} $\pm$ 0.26	7.8 ^{CDx} $\pm$ 0.21	NA	NA
1% SL	5.7 ^{Cv} $\pm$ 0.16	6.9 ^{Cw} $\pm$ 0.22	8.1 ^{Dx} $\pm$ 0.19	NA	NA
1.5% LA	4.9 ^{ABv} $\pm$ 0.28	6.3 ^{BCw} $\pm$ 0.29	7.2 ^{ABCx} $\pm$ 0.08	8.2 ^{Ay} $\pm$ 0.26	NA
1.5% AA	4.4 ^{Av} $\pm$ 0.25	5.5 ^{Aw} $\pm$ 0.49	6.8 ^{ABx} $\pm$ 0.09	7.9 ^{Ay} $\pm$ 0.29	NA
1.5% LA + 1% SL	4.9 ^{ABv} $\pm$ 0.14	6.4 ^{BCw} $\pm$ 0.21	7.5 ^{BCDx} $\pm$ 0.24	NA	NA
1.5% AA + 1% SL	4.7 ^{ABv} $\pm$ 0.14	5.8 ^{ABw} $\pm$ 0.17	7.0 ^{ABCx} $\pm$ 0.09	8.3 ^{Ay} $\pm$ 0.37	NA
1.5% AA + 1.5% LA	4.2 ^{Av} $\pm$ 0.29	5.6 ^{ABw} $\pm$ 0.26	6.5 ^{Ax} $\pm$ 0.12	7.8 ^{Ay} $\pm$ 0.14	8.8 ^{Az} $\pm$ 0.14
1.5% AA + 1.5% LA + 1%SL	4.2 ^{Av} $\pm$ 0.36	5.6 ^{ABw} $\pm$ 0.17	6.6 ^{Ax} $\pm$ 0.17	7.8 ^{Ay} $\pm$ 0.17	8.9 ^{Az} $\pm$ 0.12

^{ABCD}: Values with different superscripts within the same column are significantly different (P<0.05)

^{vwxyz}: Values with different superscripts within the same row are significantly different (P<0.05)

NA: Not Analyzed

SL: Sodium lactate

LA: Lactic acid

AA: Acetic acid

5.1.2. Counts of *Pseudomonas* spp.

The mean number of *Pseudomonas* spp. on the control group was $5.4 \log_{10}$ CFU ml⁻¹. After the decontamination treatments (on day 0), the count of *Pseudomonas* spp. of the samples reduced between 0.7 and $1.5 \log_{10}$ CFU ml⁻¹ depending on the decontamination solutions, except for the sample treated with 1% SL (Figure 3 and Table 5).

After on day 0, the count of *Pseudomonas* colony in the all samples continuously increased during the storage time at 4°C, and significant differences were observed between the storage days ($P < 0.05$). On day 3, *Pseudomonas* numbers of the control and 1% SL groups were 6.8 and $6.9 \log_{10}$ CFU ml⁻¹, respectively, and *Pseudomonas* numbers of 1.5% LA, 1.5% AA, 1.5% LA+1% SL, 1.5% AA+ 1% SL, 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL groups were significantly lower when compared to the control and 1% SL groups on day 3 ($P < 0.05$).

It was detected that the counts of *Pseudomonas* colony of the control, 1% SL and 1.5% LA+1% SL groups was above $7 \log_{10}$ CFU ml⁻¹ on day 5. *Pseudomonas* counts of the samples treated with 1.5% AA, 1.5% AA+1% SL, 1.5% AA + 1.5% LA and 1.5% AA + 1.5% LA + 1% SL were significantly lower when compared to the control and 1% SL groups on day 5 ($P < 0.05$).

On day 8, the control, 1% SL and 1.5% LA+1% SL groups were not analyzed because those samples clearly showed spoilage indications (bad smell and slime layer on the samples). Although the 1.5% LA, 1.5% AA and 1.5% AA+ 1% SL groups have slight off odor (no slime layer), all samples were submitted to the microbiological analyze. Analyze results of those samples showed that

Pseudomonas colony numbers of the 1.5% LA, 1.5% AA and 1.5% AA+ 1% SL reached to 7.5, 7.5 and 7.6 log₁₀ CFU ml⁻¹, respectively. *Pseudomonas* colony numbers of the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL were 7.3 and 7.2 log₁₀ CFU ml⁻¹, respectively. No significant differences were observed between the groups on day 8 (P>0.05).

On day 10, the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL groups showed the spoilage indications and their *Pseudomonas* colony numbers were 8.6 and 8.8 log₁₀ CFU ml⁻¹, respectively (Figure 3 and Table 5).

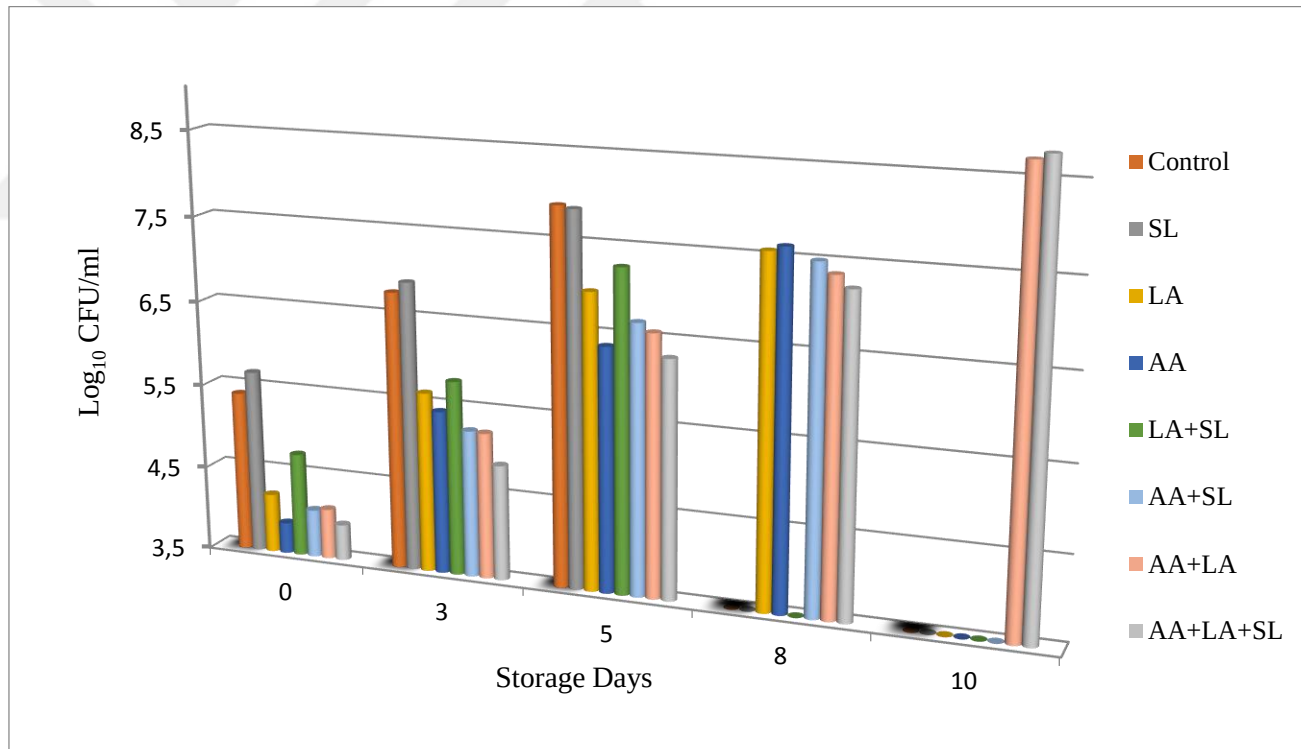


Figure 3. The mean numbers of *Pseudomonas* spp. of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C ($\log_{10}$ CFU ml^{-1} rinsate)

Table 5. The mean numbers of *Pseudomonas* spp. of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (log₁₀ CFU ml⁻¹ rinsate ± SD)

Treatment Groups	Storage days				
	0	3	5	8	10
Control	5.4 ^{BCw} ± 0.41	6.8 ^{Cx} ± 0.42	7.9 ^{By} ± 0.39	NA	NA
1% SL	5.7 ^{Cw} ± 0.47	6.9 ^{Cx} ± 0.45	7.9 ^{By} ± 0.19	NA	NA
1.5% LA	4.2 ^{Aw} ± 0.22	5.6 ^{ABx} ± 0.29	7.0 ^{ABy} ± 0.21	7.5 ^{Ay} ± 0.09	NA
1.5% AA	3.9 ^{Aw} ± 0.09	5.4 ^{ABx} ± 0.39	6.4 ^{Ay} ± 0.26	7.6 ^{Az} ± 0.19	NA
1.5% LA + 1% SL	4.7 ^{ABw} ± 0.17	5.8 ^{Bx} ± 0.29	7.2 ^{ABy} ± 0.29	NA	NA
1.5% AA + 1% SL	4.0 ^{Aw} ± 0.33	5.2 ^{ABx} ± 0.17	6.7 ^{Ay} ± 0.17	7.5 ^{Az} ± 0.21	NA
1.5% AA + 1.5% LA	4.1 ^{Av} ± 0.43	5.2 ^{ABw} ± 0.21	6.6 ^{Ax} ± 0.17	7.3 ^{Ax} ± 0.26	8.6 ^{Ay} ± 0.08
1.5% AA + 1.5% LA + 1%SL	3.9 ^{Av} ± 0.17	4.8 ^{Aw} ± 0.12	6.2 ^{Ax} ± 0.21	7.2 ^{Ay} ± 0.4	8.8 ^{Az} ± 0.09

^{AB}: Values with different superscripts within the same column are significantly different (P<0.05)

^{vwxyz}: Values with different superscripts within the same row are significantly different (P<0.05)

NA: Not Analyzed

SL: Sodium lactate

LA: Lactic acid

AA: Acetic acid

5.1.3. Counts of Lactic Acid Bacteria (LAB)

The mean number of lactic acid bacteria on the control sample was 5.1 $\log_{10}$ CFU ml^{-1} . After the decontamination treatments (on day 0), the count of LAB of the samples reduced between 0.1 and 0.4 $\log_{10}$ CFU ml^{-1} depending on the decontamination solutions, except for the samples treated with 1% SL, 1.5% AA+ 1% SL and 1.5% LA + 1% SL (Figure 4 and Table 6).

After on day 0, the count of LAB in all the samples continuously increased during the storage time at 4°C, and significant differences were observed between the storage days ($P < 0.05$). On day 3, LAB numbers of the control and 1% SL groups were 6.8 and 7.0 $\log_{10}$ CFU ml^{-1} , respectively, and LAB numbers of 1.5% AA, 1.5% AA+ 1% SL, 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL were significantly lower when compared to the control and 1% SL groups on day 3 ($P < 0.05$).

It was detected that the counts of LAB colony of the control, 1% SL, 1.5% LA and 1.5% LA+1% SL groups was above 7 $\log_{10}$ CFU ml^{-1} on day 5. LAB counts of the samples treated with 1.5% AA, 1.5% AA+1% SL, 1.5% AA + 1.5% LA and 1.5% AA + 1.5% LA + 1% SL were significantly lower when compared to the control group on day 5 ($P < 0.05$).

On day 8, the control, 1% SL and 1.5% LA+1% SL groups were not analyzed because those samples clearly showed spoilage indications (bad smell and slime layer on the samples). Although the 1.5% LA, 1.5% AA and 1.5% AA+ 1% SL groups have slight bad smell (no slime layer), all samples were submitted to the microbiological analyze. Analyze results of those samples showed that LAB numbers of the 1.5% LA, 1.5% AA and 1.5% AA+ 1% SL reached to 7.9, 7.7 and

7.4 log₁₀ CFU ml⁻¹, respectively. LAB numbers of the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL were 7.1 and 7.0 log₁₀ CFU ml⁻¹. Significant difference was observed between the 1.5% LA group and the 1.5% AA+ 1.5% LA+ 1% SL group on day 8 (P<0.05).

On day 10, the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL groups showed the spoilage signs and their LAB numbers were 7.6 and 7.5 log₁₀ CFU ml⁻¹, respectively (Figure 4 and Table 6).



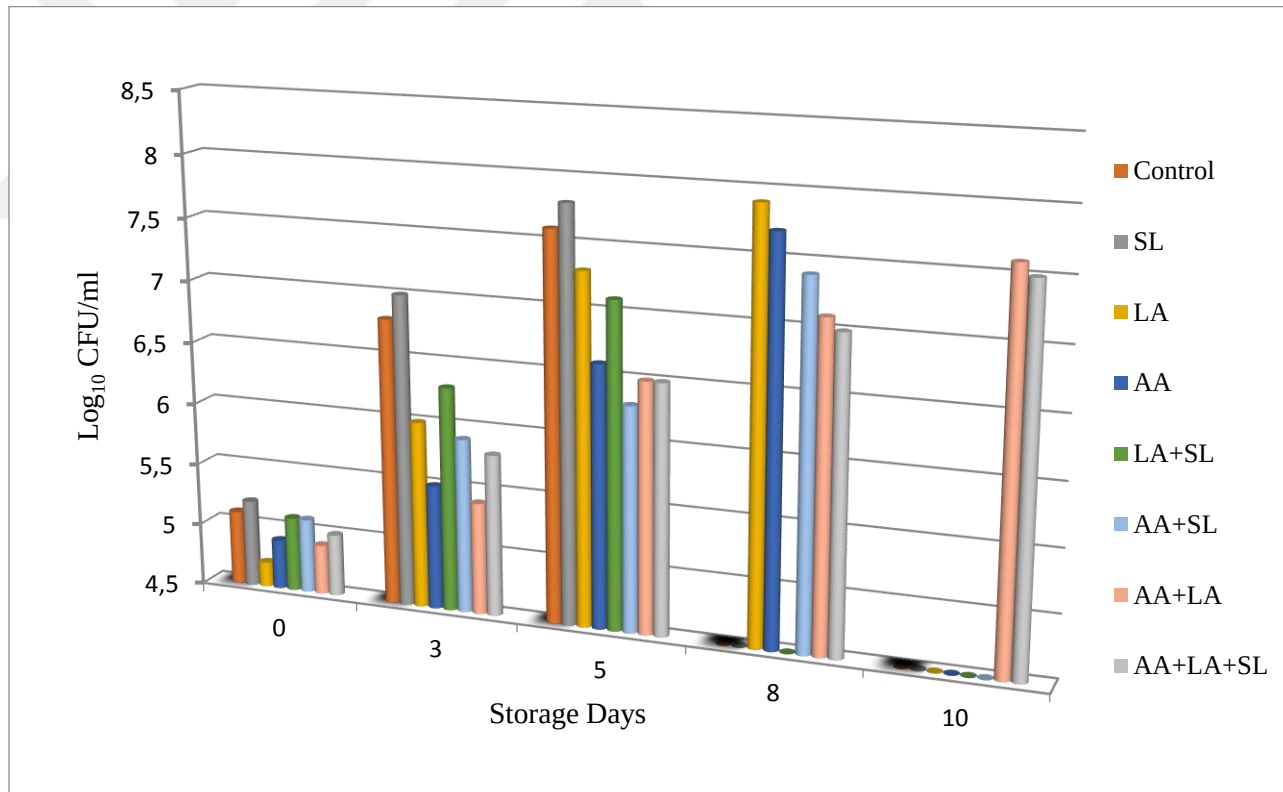


Figure 4. The mean numbers of lactic acid bacteria of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (log₁₀ CFU ml⁻¹ rinsate)

Table 6. The mean numbers of Lactic acid bacteria of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C ($\log_{10}$ CFU ml⁻¹ rinsate $\pm$ SD)

Treatment Groups	Storage days				
	0	3	5	8	10
Control	5.1 ^{Ax} $\pm$ 0.14	6.8 ^{CDy} $\pm$ 0.29	7.6 ^{Cz} $\pm$ 0.19	NA	NA
1% SL	5.2 ^{Ax} $\pm$ 0.17	7.0 ^{Dy} $\pm$ 0.22	7.8 ^{Cz} $\pm$ 0.31	NA	NA
1.5% LA	4.7 ^{Ax} $\pm$ 0.17	6.0 ^{ABCy} $\pm$ 0.09	7.3 ^{BCz} $\pm$ 0.29	7.9 ^{Bz} $\pm$ 0.12	NA
1.5% AA	4.9 ^{Ax} $\pm$ 0.12	5.5 ^{Ax} $\pm$ 0.42	6.6 ^{ABy} $\pm$ 0.25	7.7 ^{ABz} $\pm$ 0.39	NA
1.5% LA + 1% SL	5.1 ^{Ax} $\pm$ 0.08	6.3 ^{BCDy} $\pm$ 0.39	7.1 ^{ABCz} $\pm$ 0.37	NA	NA
1.5% AA + 1% SL	5.1 ^{Ax} $\pm$ 0.12	5.9 ^{ABxy} $\pm$ 0.43	6.3 ^{Ay} $\pm$ 0.09	7.4 ^{ABz} $\pm$ 0.37	NA
1.5% AA + 1.5% LA	4.9 ^{Ax} $\pm$ 0.29	5.4 ^{Ax} $\pm$ 0.12	6.5 ^{ABy} $\pm$ 0.08	7.1 ^{AByz} $\pm$ 0.14	7.6 ^{Az} $\pm$ 0.24
1.5% AA + 1.5% LA + 1%SL	5.0 ^{Aw} $\pm$ 0.09	5.8 ^{ABwx} $\pm$ 0.42	6.5 ^{ABxy} $\pm$ 0.41	7.0 ^{Ayz} $\pm$ 0.48	7.5 ^{Az} $\pm$ 0.14

^{ABCD}: Values with different superscripts within the same column are significantly different (P<0,05)

^{wxyz}: Values with different superscripts within the same row are significantly different (P<0,05)

NA: Not Analyzed SL: Sodium lactate LA: Lactic acid AA: Acetic acid

5.1.4. Counts of *Salmonella* spp.

The average inoculation level of *Salmonella* colonies on the control sample was $5.4 \log_{10}$ CFU ml⁻¹. After the decontamination treatments (on day 0), the counts of *Salmonella* colonies of the samples reduced between 0.2 and 1.2 $\log_{10}$ CFU ml⁻¹ depending on the decontamination solutions, except for the sample treated with 1%AA+ 1%SL (Figure 5 and Table 7). After day 0, the numbers of *Salmonella* in the groups were nearly stable during the storage time, and no significant differences were observed between the storage days ($P>0.05$). On day 3, the group with the lowest *Salmonella* count was the group of 1.5% AA+ 1.5% LA by the number of $4.6 \log_{10}$ CFU ml⁻¹, however, no significant differences were observed between the control group and other treatment groups ($P>0.05$).

On day 5 of refrigerated storage, the combination of 1.5% AA + 1.5% LA had the best antimicrobial efficacy on the *Salmonella* spp. compared to control by reduction of $0.8 \log_{10}$ CFU ml⁻¹ ($P<0.05$). The numbers of *Salmonella* in the samples analyzed on day 8 were between 4.4 and $5.1 \log_{10}$ CFU ml⁻¹ and no significant differences were observed between the groups ($P>0.05$).

On day 10, *Salmonella* colony numbers of the 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL were 4.6 and $4.7 \log_{10}$ CFU ml⁻¹, respectively (Figure 5 and Table 7).

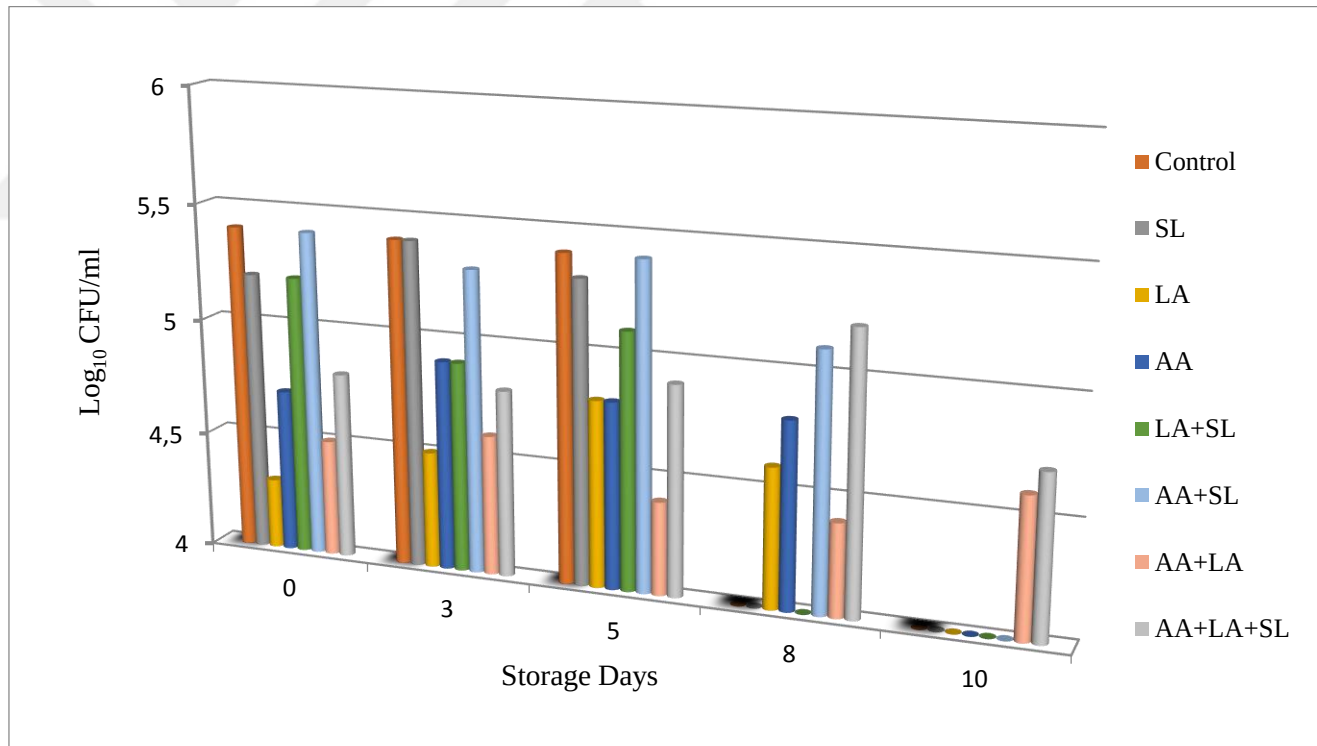


Figure 5. The mean numbers of *Salmonella* spp. of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C ($\log_{10}$ CFU ml⁻¹ rinsate)

Table 7. The mean numbers of *Salmonella* spp. of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (log₁₀ CFU ml⁻¹ rinsate ± SD)

Treatment Groups	Storage days				
	0	3	5	8	10
Control	5.4 ^{Cx} ± 0,09	5.3 ^{ABx} ± 0,24	5.3 ^{Bx} ± 0,12	NA	NA
1% SL	5.2 ^{BCx} ± 0,08	5.4 ^{Bx} ± 0,25	5.3 ^{Bx} ± 0,16	NA	NA
1.5% LA	4.2 ^{Ax} ± 0,21	4.8 ^{ABx} ± 0,22	4.8 ^{ABx} ± 0,28	4.6 ^{ABx} ± 0,15	NA
1.5% AA	4.7 ^{ABCx} ± 0,22	4.9 ^{ABx} ± 0,22	4.7 ^{ABx} ± 0,26	4.8 ^{ABx} ± 0,12	NA
1.5% LA + 1% SL	4.9 ^{ABCx} ± 0,24	4.9 ^{ABx} ± 0,14	5.1 ^{ABx} ± 0,09	NA	NA
1.5% AA + 1% SL	5.4 ^{Cx} ± 0,08	5.3 ^{ABx} ± 0,24	5.4 ^{Bx} ± 0,08	5.1 ^{Bx} ± 0,17	NA
1.5% AA + 1.5% LA	4.5 ^{ABx} ± 0,14	4.6 ^{Ax} ± 0,12	4.5 ^{Ax} ± 0,25	4.4 ^{Ax} ± 0,28	4.6 ^{Ax} ± 0,08
1.5% AA + 1.5% LA + 1%SL	4.8 ^{ABCx} ± 0,36	4.8 ^{ABx} ± 0,05	4.9 ^{ABx} ± 0,17	4.8 ^{ABx} ± 0,14	4.7 ^{Ax} ± 0,05

^{ABC}: Values with different superscripts within the same column are significantly different (P<0.05)

^x: Values with the same superscript within the same row are not significantly different (P>0.05)

NA: Not Analyzed

SL: Sodium lactate

LA: Lactic acid

AA: Acetic acid

5.2. pH Values of the Rinsing Solutions of the Chicken Drumstick Samples

The changes in the pH level of the drumstick samples treated with various decontamination solutions were shown in Figure 6 and Table 8. The mean pH level of the rinsing solution of the control samples was 6.78 on day 0. In the treated samples except for the 1% SL group, pH levels decreased between 1.76 and 1.02 units depending on the treatment solutions, and significant differences were observed between the control and the other groups ($P<0.05$). The lowest pH levels were observed in the samples treated with 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL by the pH levels of 5.02 and 5.03, respectively.

On day 3, except for 1% SL group, the pH levels of the groups treated with the decontamination solutions increased and approached to the control and 1% SL groups. No significant difference was observed between the control and the treatment groups in point of pH level on day 3 ($P>0.05$).

On day 5, pH levels of the rinsing solutions of the control, 1%SL, 1.5% LA and 1.5% LA+ 1% SL groups were above 7.0, and the groups with the lowest pH level were 1.5% LA+ 1.5% AA and 1.5% AA+ 1.5% LA+ 1% SL by the pH levels of 6.72 and 6.83, respectively. The pH levels of these groups were still below 7.0 by the pH levels of 6.85 and 6.79 on day 8, respectively. No significant difference was observed between the groups on day 8 ($P>0.05$).

The pH levels of the rinsing solutions of the 1.5% LA+ 1.5% AA and 1.5% AA+ 1.5% LA+ 1% SL groups were 7.17 and 7.18 on day 10, respectively.

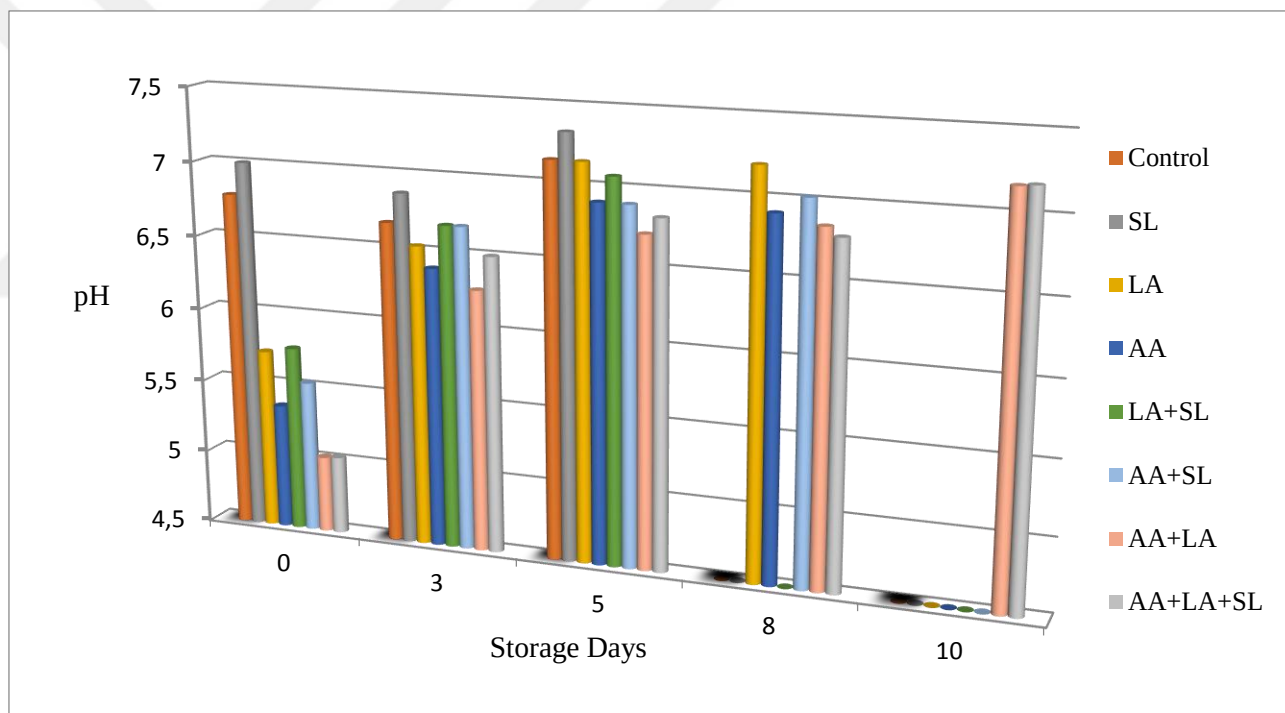


Figure 6. The mean pH values of the rinsing solutions of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C.

Table 8. The mean pH values of the rinsing solutions of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (pH $\pm$ SD)

Treatment Groups	Storage days				
	0	3	5	8	10
Control	6.78 ^{Cxy} $\pm$ 0.09	6.67 ^{ABx} $\pm$ 0.09	7.15 ^{ABy} $\pm$ 0.15	NA	NA
1% SL	7.0 ^{Cxy} $\pm$ 0.08	6.87 ^{Bx} $\pm$ 0.17	7.33 ^{By} $\pm$ 0.17	NA	NA
1.5% LA	5.72 ^{Bx} $\pm$ 0.16	6.53 ^{ABy} $\pm$ 0.21	7.15 ^{ABz} $\pm$ 0.15	7.20 ^{Az} $\pm$ 0.04	NA
1.5% AA	5.35 ^{ABx} $\pm$ 0.19	6.39 ^{ABy} $\pm$ 0.23	6.90 ^{ABz} $\pm$ 0.20	6.91 ^{Az} $\pm$ 0.19	NA
1.5% LA + 1% SL	5.76 ^{Bx} $\pm$ 0.28	6.68 ^{ABy} $\pm$ 0.10	7.07 ^{ABy} $\pm$ 0.13	NA	NA
1.5% AA + 1% SL	5.53 ^{ABx} $\pm$ 0.25	6.68 ^{ABy} $\pm$ 0.20	6.90 ^{ABy} $\pm$ 0.08	7.02 ^{Ay} $\pm$ 0.10	NA
1.5% AA + 1.5% LA	5.02 ^{Ax} $\pm$ 0.12	6.27 ^{Ay} $\pm$ 0.20	6.72 ^{Ayz} $\pm$ 0.35	6.85 ^{Az} $\pm$ 0.11	7.17 ^{Az} $\pm$ 0.19
1.5% AA + 1.5% LA + 1%SL	5.03 ^{Ax} $\pm$ 0.21	6.53 ^{ABy} $\pm$ 0.12	6.83 ^{AByz} $\pm$ 0.12	6.79 ^{Ayz} $\pm$ 0.10	7.18 ^{Az} $\pm$ 0.14

^{ABC}: Values with different superscripts within the same column are significantly different (P<0.05)

^{xyz}: Values with different superscripts within the same row are significantly different (P<0.05)

NA: Not Analyzed SL: Sodium lactate LA: Lactic acid AA: Acetic acid

6. DISCUSSION

Chicken meat and meat products are among the foods with high nutritional value, and its production is comparatively more inexpensive and widely available than red meat (56). However, poultry meat can be highly contaminated with spoilage or pathogenic bacteria because of its production that cause cross-contamination during different operations in the slaughterhouse. Therefore, elimination of foodborne pathogens and extension of shelf life by reducing spoilage microorganisms on chicken meat are the prime goal of the poultry meat industry. For this goal, many chemical agents have been studied on chicken carcasses and carcass parts (21, 42, 49, 57). However, consumers have demanded foods that organic antimicrobial agents are used for the food preservation.

The results shown in Figure 2 and Table 5 revealed that total aerobic psychrophilic colonies (APC) increased during the storage time, because of their ability of growth in the refrigerated temperature. On day 0, the reduction in the number of APC was observed in all groups treated with the chemical solutions, except for the group treated with 1% SL. The statistical reduction in APC was observed in the group treated with 1.5% acetic acid (AA) compared to the control group. The results showed that AA is more effective than lactic acid (LA) on the APC. This may be because of their pK_a levels. In general, the antimicrobial activity of organic acids increases when the environmental pH is close to the pK_a level of the organic acid (45). Proportion of total acid undissociated at different pH values has been published by International Commission on Microbiological Specifications for Foods (ICMSF). As it is shown in Table 9, as the environmental pH level increases the proportion of un-dissociated acid decreases.

Table 9. Proportion of total acid un-dissociated at different pH values (expressed as percentages) (45).

<u>Organic acids</u>	pH values				
	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
Acetic acid	98.5	84.5	34.9	5.1	0.54
Lactic acid	86.6	39.2	6.05	0.64	0.06

The pKa value of AA and LA are pH 4.76 and 3.86, respectively. The pH of the samples treated with AA and LA are 5.35 and 5.72 on day 0, respectively (Table 8). The pH level of the samples treated with AA was more close to the pKa value of AA. Hence, the high efficacy of AA on the APC can be attributed to the pH of the samples and pK_a value of AA. It was also reported that acetic acid is more effective than lactic acid against bacteria at similar pH values because acetic acid is less dissociated at the same pH level than lactic acid (25). Although AA showed numerically more reduction in the APC count compared to LA, there was no significant difference between the AA and LA in point of the bacteriostatic effect on the APC (P<0.05).

In the present study, the combination of AA with LA and the combination of AA, LA and SL showed significant reduction in count of APC by 1.2 and 1.2 log₁₀ CFU ml⁻¹ respectively, compared to the control group (P<0.05). However, no synergistic effects were observed when compared to AA or LA alone. Sodium lactate (SL) did not show any bacteriostatic effect on the APC. Milillo and Ricke (58) demonstrated that a minimum inhibitory concentration of SL for bacterial decontamination of chicken drumstick was higher than 1.25%.

The reason for no effect of SL on the APC can be attributed to the low concentration (1%) of SL used in our study. On day 3, APC numbers of the groups treated with 1.5% AA, 1.5% AA+ 1% SL, 1.5% AA + 1.5% LA and 1.5% AA + 1.5% LA + 1% SL were significantly lower than the control and 1% SL groups ($P<0.05$). However, LA no significantly reduced APC when compared to control and SL groups. The pH levels of the samples treated with the decontamination solutions exceeded the pH 6 (Table 8) because of the buffering capacity of the chicken skin and meat. In those pH levels (>6.0), the undissociated acid proportion of AA and LA is very low, and because of that reason, increase in the number of APC may be expected.

On day 5, the control and 1% SL groups had a slightly bad odor but not 1.5% LA, 1.5% LA+ 1% SL and 1.5% AA+ 1% SL groups. The results showed that the numbers of APC of the control and 1% SL groups was close to $8 \log_{10}$ CFU ml⁻¹, and the number of APC of the 1.5% LA, 1.5% LA+ 1% SL and 1.5% AA+ 1% SL groups were between 7 and $7.5 \log_{10}$ CFU ml⁻¹ depending on the groups. Even though numerically lower APC count was observed in the 1.5% AA + 1.5% LA and 1.5% AA + 1.5% LA + 1%SL groups, they were not significantly different when compared to the 1.5% AA and 1.5% LA groups ($P>0.05$).

The control sample and the samples treated with 1% SL and 1.5% LA+ 1%SL had a bad odor and slime layer on day 8. Because the sensorial defect of these samples, they were not analyzed. Although the other groups had a slightly bad odor they did not have slime layer, and they were analyzed. Results showed that all groups had more than $7.8 \log_{10}$ CFU ml⁻¹ APC count and there was no significant difference between the groups ($P>0.05$). On day 10, the samples

treated with 1.5% AA+ 1.5% LA and 1.5% AA+ 1.5% LA+ 1% SL showed sensorial deterioration and their APC counts were close to $9 \log_{10}$ CFU ml⁻¹.

Hecer and Sözen (59) exposed poultry breast meats to 0.5% lactic acid, 0.3% acetic acid and 2% sodium lactate for 10 min and then stored the samples at 4°C for four days. They reported that the most effective bacterial decrease for APC was observed in the samples treated with 0.5% lactic acid by $0.72 \log_{10}$ CFU g⁻¹ on day 1. They also found that the control samples spoiled on day 4 while the numbers of APC of the samples treated with 0.5% LA, 0.3% AA and 2% SL were 5.8, 5.8 and 5.6 $\log_{10}$ CFU g⁻¹, respectively. İlhak et al. (54) sprayed chicken drumstick with 2% LA and 2% SL for 30 sec and then stored the samples at 4°C for four days. They reported that the numbers of APC of the drumstick samples treated with 2% LA and 2% SL were 7.3 and 7.4 $\log_{10}$ CFU ml⁻¹ on day 4, respectively. The results of both studies are more or less similar to the findings of the present study. The reason for the small differences between the studies might be because that Hecer and Sözen (59) used chicken breast meat and application time of 10 min, and İlhak et al. (54) used spray method for 30 sec.

Chicken meat is considered as a good environment for aerobic microbiota and a consortium of bacteria including *Pseudomonas* spp.. It has been reported that when chicken meat undergo spoilage at low temperatures, the primary spoilage bacteria is also *Pseudomonas* spp. (10, 60-62). Fresh poultry meat stored at aerobic condition and refrigeration temperatures is susceptible to the growth of bacteria such as *Pseudomonas* spp..

The results shown in Figure 3 and Table 5 revealed that the reductions in the numbers of *Pseudomonas* spp. in the treatment groups were similar to the

APC counts of the groups. The results showed that AA was more effective than LA on *Pseudomonas*. As mentioned above, this may occur because of pK_a values of the organic acids used. The high efficacy of AA can be attributed to the pH level of the samples that is close to the pK_a level of AA. Although AA showed numerically more reduction in *Pseudomonas* spp. count compared to LA during the storage, there was no significant difference between the AA and LA in point of the bacteriostatic effect on *Pseudomonas* spp. ($P>0.05$). In addition, combining the AA, LA and SL did not show further bacteriostatic effect on *Pseudomonas* on the chicken drumstick when compared to use AA and LA alone. The control and 1% SL groups had a significantly higher number of *Pseudomonas* than those the other treatment groups ($P<0.05$).

As mentioned above, the control and 1% SL groups had a slightly bad odor but not 1.5% LA and 1.5% LA+ 1% SL groups on day 5. Jay et al. (62) reported that bad odor in chicken meat can be detected when the surface bacterial number (especially *Pseudomonas* spp.) is between 7.2-8.0 $\log_{10}$ CFU cm^{-2} , and then slime layer on the surface can be detected when the surface bacterial number is about 8.0 $\log_{10}$ CFU cm^{-2} (62, 63). On day 5, the numbers of *Pseudomonas* of the control and SL groups were 7.9 $\log_{10}$ CFU ml^{-1} while the numbers of *Pseudomonas* of the 1.5% LA and 1.5% LA+ 1% SL were 7.0 and 7.2 $\log_{10}$ CFU ml^{-1} . The high numbers of the *Pseudomonas* observed in the control and 1% SL groups explain the reason of the bad odor in these groups. The samples treated with 1.5% AA, 1.5% AA + 1% SL, 1.5% AA + 1.5% LA and 1.5% AA + 1.5% LA + 1%SL showed significantly lower number of *Pseudomonas* when compared to the control and SL groups ($P<0.05$). Throughout the storage of 5 days, the

results showed that *Pseudomonas* numbers of the groups containing AA were numerically lower than those the other groups containing no AA. It can be said that AA is more effective than LA on *Pseudomonas* on the chicken drumstick. The numbers of *Pseudomonas* of the samples analyzed on day 8 were above 7 log₁₀ CFU ml⁻¹, and as mentioned above those groups were showing slight spoilage indicators. On day 10, the numbers of *Pseudomonas* of the samples treated with the combinations of AA, LA and SL were above 8.5 log₁₀ CFU ml⁻¹.

The microbial shelf life of chicken meat is defined by aerobic bacteria such as *Pseudomonas* spp., and the product is spoilt when the counts of these bacteria reach to 7-8 log₁₀ CFU/g (62). Certainly, initial bacterial load of the product has a great effect on the shelf life of the product. In the present study, the initial bacterial load of the control group was 5.4 log₁₀ CFU ml⁻¹, which was pretty high. The findings of the present study showed that the numbers of APC and *Pseudomonas* of the control group were 6.9 and 6.8 log₁₀ CFU ml⁻¹ on day 3, respectively. It can be said that the control group spoiled before day 5.

Bostan et al. (64) kept poultry breast meats in 0.6% lactic acid, 1% acetic acid and 1% acetic acid + 0.3% lactic acid for 10 min and then stored the samples at 4°C for ten days. They reported that most effective bacterial decrease for *Pseudomonas* spp. was observed in the samples treated with 1% acetic acid + 0.3% lactic acid by 0.79 log₁₀ CFU carcass⁻¹ on day 1. They also found that the control samples spoiled on day 2 while the numbers of *Pseudomonas* spp. of the samples treated with 0.6% lactic acid, 1% acetic acid and 1% acetic acid + 0.3% lactic acid were 6.5, 6.9 and 6.6 log₁₀ CFU carcass⁻¹, respectively. Bolton et al. (65) kept chicken skin sample with 1-5% lactic acid for 15 sec and then stored the

samples at 4°C for 3 days. They reported that the numbers of *Pseudomonas* spp. of the drumstick samples treated with 1% lactic acid and 5% lactic acid were 7.8 and 6.7 log₁₀ CFU cm⁻² on day 3, respectively. The results of both studies are more or less similar to the findings in the present study. The reason for the small differences between the studies might be because that Bostan et al. (64) used chicken breast meat and application time of 10 min, and Bolton et al. (65) used immersion method for 15 sec.

Like other bacteria, the growth of lactic acid bacteria (LAB) depends upon various factors including water activity of the meat, pH, oxygen and CO₂ level, and storage temperature etc. Although they are not the primary spoilage bacteria such as *Pseudomonas* spp., LAB may exist in much quantity on spoiled chicken meats and other poultry products stored at refrigeration temperatures (66).

In the present study, none of the treatments showed bactericidal effect on LAB on day 0 (Figure 4 and Table 6, and therefore, no significant differences were observed between the groups ($P>0.05$). It can be said that LAB is more resistant to LA and AA compared to *Pseudomonas*. On day 3 and 5, LAB numbers of the control and 1% SL groups rapidly increased compared to the other groups. Consequently, LAB numbers of the groups containing AA, alone and in combination were significantly lower when compared to the control and 1% SL groups ($P<0.05$). AA treated chicken drumsticks had consistently low number of LAB than those of the groups treated with LA. According to this result, it can be asserted that AA shows bacteriostatic effect on LAB on chicken meat, and it is more effective than LA.

Bolton et al., (65) treated chicken skin sample with 1-5% lactic acid and 1-5% citric acid for 15 sec and then stored the samples at 4°C for 3 days. They reported that no significant differences were observed between the groups in point of the number of LAB immediately after application, and they found that citric acid was more effective than LA throughout the experiment.

The microbial decontamination with organic chemicals has a great potential for safety of chicken meat and reducing foodborne pathogen risks related to chicken meat consumption (67). The most important factor affecting the microbiota of meat is temperature, the refrigerated temperature for chicken drumstick would be the first hurdle that *Salmonella* have to overcome. *Salmonella* spp. can not grow well in the environmental temperature lower than 5°C. The temperature range for growth of *Salmonella* on raw ground chicken with natural microflora is between 10–40°C (68). In the present study, the chicken drumstick samples were stored at 4°C. Because the storage temperature was below than 5°C, it was not expected *Salmonella* growth on the samples during the storage period. As seen in the results, the number of *Salmonella* on control samples did not change during the storage (Figure 5 and Table 7). Treatment with 1.5% LA and 1.5% AA+ 1.5% LA decreased *Salmonella* numbers by 1.2 and 0.9 log₁₀ CFU ml⁻¹ on the chicken drumstick samples when compared with the control sample on day 0, respectively (P<0.05). No significant differences were observed in the treatment groups when compared to the control group on day 3 (P>0.05). The number of *Salmonella* in the 1.5% AA+ 1.5% LA group was 4.5 log₁₀ CFU ml⁻¹ and showed significant difference when compared to control group by reduction of 0.8 log₁₀ CFU ml⁻¹.

When the results were analyzed, it can be said that none of the decontamination solutions used in the present study decreased the *Salmonella* number satisfactorily. Nagel et al. (69) and Lee et al. (70) reported that it was not easy to remove *Salmonella* spp. from the folded areas and follicles on chicken skin. İlhak et al. (54) did not observe a significant reduction in the number of *Salmonella* on chicken drumstick sprayed with 2% LA for 30 sec.

Mani-López et al., (35) reported that the sensitivity of bacteria to the antimicrobial effect of acetic acid, lactic acid or sodium lactate increases with a decrease in pH level of food. It has been noted that *Salmonella* can multiply and grow at a pH range of 4 to 9, and they have ability for adaptation to acidic environment (71). In the present study, the pH of the samples treated with organic acids was above 5 immediately after the treatments. After day 0, pH of the samples reached above 6.0. In these pH levels, it is unlikely that *Salmonella* will be affected. As seen in Table 8, the numbers of *Salmonella* in the groups treated with the organic acids were stable throughout the storage days.

As mentioned before, it is not easy to remove *Salmonella* spp. from the folded areas and follicles on chicken skin. Tan et al., (72) noted that chicken skin and chicken meat shows buffering effects which may impact the survival of *Salmonella* on chicken meat when treated with acids. Thanks to the physical structure of chicken skin and the high buffering capacity of the chicken meat, *Salmonella* may be protected from the effect of organic acids. The reason of the less reductions observed in the number of *Salmonella* in the treated groups can be explained in this way.

Mikolajczyk (26) investigated the effect of various mixtures of equal parts of organic acid solutions (acetic, ascorbic, citric, lactic and tartaric acid) on the survival of *Salmonella* on turkey breast meat. The author found that immersion the breast meat into a solution composed of equal parts of 1% acetic acid, 1% lactic acid, and 1% tartaric acid for 15 min resulted in 2 log MPN/ml reduction in the number of *Salmonella* after storage at 4 °C for 6 days. Lecompte et al., (73) treated chicken skin with 5% lactic acid for 1 min and found that the number of *Salmonella* Enteritidis on the chicken skin decreased 2.38 log₁₀ CFU ml⁻¹ after 7 day storage. Chaine et al., (74) treated chicken skin with 5% lactic acid for 1 min and found that the number of *Salmonella* Enteritidis on the chicken skin decreased 1.4 log₁₀ CFU cm² immediately after the treatment. Anang et al., (75) dipped chicken breast meat into 1.5% LA for 10 and 20 min, and reported that the reduction levels of *Salmonella* Enteritidis were 0.81 log₁₀ CFU g⁻¹ for 10 min and 0.98 log₁₀ CFU g⁻¹ for 20 min dipping. As understood from these studies and the other similar studies, the reducing effect of organic acids on *Salmonella* spp present on chicken meat can change from insignificant level to about 2.5 log₁₀ CFU depending on the concentration of acids (1-5%), duration of treatment (1-20 min) and application (spraying or dipping) methods. In our study, the reduction levels of *Salmonella* spp. in the groups treated with organic acids, alone and in combined form, were changed between 0 and 1.2 log₁₀ CFU ml⁻¹, which were similar to the findings of other researchers.

As a result, decontamination of chicken drumstick with %1 SL displayed no advantage for extending the shelf life of the samples and reducing *Salmonella* spp., and combining 1% SL with 1.5% LA and 1.5% AA did not show synergistic

or additive antibacterial effect on the spoilage bacteria and *Salmonella* spp. when compared to LA and AA used alone. The shelf life of the drumstick samples treated with 1.5% AA, 1.5% AA+ 1.5% LA, 1.5% AA+ 1.5% LA+ 1% SL were at least 5 days at 4°C. However, combining AA with LA and LA+SL did not provide a significant benefit for extending the shelf life of the samples and reducing *Salmonella* spp. when compared to AA used alone.

The shelf life of chicken drumsticks treated with the decontamination solutions containing AA (alone or in combination) extended at least 2 days when compared to the control group. *Salmonella* spp. was relatively resistant to AA and LA at a concentration of 1.5% probably due to the buffering capacity of chicken skin and meat. Buffering capacity of the product should not be neglected when organic acids are used as decontamination agent. The antimicrobial activity of organic acids increases as the environmental pH decreases. Increasing the acid concentration would probably give better results in point of extension of shelf life of the product and elimination of *Salmonella* spp. However, the use of organic acid at high concentrations can negatively affect the organoleptic properties of the product.

In conclusion, the shelf life of chicken drumstick will be longer if the initial bacterial load of the product is low and if the hygiene rules are followed during delivery and storage of the product. In addition to these practices, decontamination of chicken drumstick with the organic acids can provide an extra contribution to the prolongation of the shelf life and the protection of the public health. In the present study, the effects of AA, LA and SL, alone and in combination, on natural flora and the shelf life of chicken drumstick and on the

survival of *Salmonella* on chicken drumstick were demonstrated. The provided information from this study will contribute to the literature knowledge.



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