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Research article

INFLUENCE OF MARINATION ON *SALMONELLA* SPP. GROWTH IN BROILER BREAST FILLETS

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The aim of this study was to investigate the influence of marination, on the growth of *Salmonella* spp. in contaminated broiler breast fillets during storage. In the conducted study, broiler breast fillets were inoculated with a cocktail of different *Salmonella* strains and afterwards marinated in different solutions of table salt, sodium tripolyphosphate and/or sodium citrate. The total count of *Salmonella* spp. was examined on the 0, 3rd, 6th and 9th day of storage. Broiler breast fillets salted in 6% solution of table salt were used as the control. Proximate composition and salt content, pH value and a_w value, were determined as the meat quality parameters and parameters which can affect environmental conditions for bacterial growth, as well. Compared to initial contamination, *Salmonella* spp. count in marinated and salted fillets did not change significantly ($p < 0.001$) until the 3rd and 6th day of storage, respectively, but it increased significantly on the 9th day of storage. Marination of broiler breast fillets in different solutions of table salt, sodium tripolyphosphate and/or sodium citrate had a significant influence ($p < 0.05$; $p < 0.01$) on pH and a_w value, moisture, ash and salt content in marinated broiler meat, but there was no significant influence ($p > 0.05$) on protein and fat content in broiler meat. According to the results obtained it can be concluded that marination of broiler breast fillets in solutions containing table salt, sodium tripolyphosphate and/or citrate, in some way, can prolongate the lag phase of *Salmonella* spp. growth, where sodium citrate is more effective than sodium tripolyphosphate.

Key words: broiler meat, marinade, *Salmonella* spp., quality

INTRODUCTION

Contemporary lifestyle has led to increased demand for meat products, which beside the heat treatment does not require additional preparation in household conditions. Commercial, industrial marination of meat provides many benefits for both, producers and consumers. It can significantly decrease the meal preparing time, provide favorable sensory characteristics of the meat and furthermore, reduce the possibility of additional meat contamination in the household. On the other hand,

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responsibility of the manufacturers in terms of quality and safety of these products increases. Commercial marination of meat involves the addition of a water solution of table salt, phosphates and sometimes flavorings and other ingredients, by means of soaking, blending, tumbling or injecting [1]. Traditionally, meat has been marinated to improve flavor, tenderness and increase product shelf life [2]. Nowadays, it is known that marination affects the chemical, physical and microbiological quality of meat. What will be the effects of marination depends on the type of marinade, marination technique and quality of meat as a raw material as well. Industrial marination aims, above all, to improve the technological and sensory characteristics of meat. For this purpose, the most commonly used are alkaline marinades which consist of water, table salt and phosphates. Due to an increase in the pH value of meat, these marinades allow greater water uptake by the muscle, which eventually increases yield and juiciness of the meat. Moreover, marinades containing phosphates tend to reduce rancidity development and warmed-over flavor, increase shelf life, and improve the color of poultry meat [3]. Typical marinade solution for commercial broiler products is made of 90% water, 6% table salt (sodium chloride), and 4% sodium tripolyphosphate [1]. In the meat industry, citric acid and sodium citrate are widely used in the pH control, metal chelating, and preservation [4]. Sodium citrate has a positive effect on color stability [5] and juiciness of meat [6]. As a replacement for phosphates in “phosphate-free” meat products, sodium citrate increases the water holding capacity, which results in increased juiciness [2]. Several studies have confirmed that phosphates exhibit a bacteriostatic effect against Gram-positive bacteria (*Leuconostoc carnosum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus stearothermophilus*, *Bacillus brevis*, *Bacillus subtilis*, *Bacillus sphaericus*, *Bacillus spp.*, *Micrococcus luteus*, *Corynebacterium glutamicum*), but that bacteriostatic effect on Gram-negative bacteria (*Salmonella* Typhimurium, *Salmonella* Enteritidis, *Escherichia coli*) is not significant [7-14]. Organic acid salts like sodium acetate, lactate and citrate have antibacterial effects on different food pathogens, including *Staphylococcus aureus* and *Yersinia enterocolitica* [15], *Listeria monocytogenes*, *Escherichia coli* [15,16] and *Clostridium botulinum* [17]. In the recent years, more attention has been paid to investigations of different phosphates and other marinade ingredients on the microbiological status and meat quality.

Marination is especially used for poultry products [18]. Poultry meat, compared to other types of meat, in terms of nutritional value, has many advantages. However, raw poultry meat is a potential source of pathogenic bacteria. The most commonly isolated are *Salmonella* spp., *Campylobacter* spp., *Clostridium perfringens*, *Escherichia coli* O157 and *Listeria monocytogenes* [19]. Poultry meat is one of the most commonly identified sources of *Salmonella* outbreaks in humans worldwide. Cause of poisoning commonly referred to *Salmonella* Enteritidis, *S.* Typhimurium and *S.* Heidelberg [20,21]. In 2010, 2,636 confirmed cases of salmonellosis in humans were recorded in Serbia, from which 2,536 were serotyped. Studies conducted by Rašeta et al. [22] clearly indicate that *Salmonella* isolated from poultry carcasses, pose a risk to the health of people in Serbia. Moreover, the only legislation defined safety criteria for semi- processed

meat products, which is the marinated poultry meat, is the absence of *Salmonella* spp. (Commission Regulation No 2073/2005 on microbiological criteria for foodstuffs).

Therefore, the aim of this study was to investigate the influence of marination on the growth of *Salmonella* spp. in contaminated/inoculated broiler breast meat, but on chemical and physical quality parameters which can affect meat quality and environmental conditions for bacterial growth, as well.

MATERIALS AND METHODS

Deboned broiler breast meat (*m. pectoralis major*), skinless, was produced in a local poultry slaughterhouse. On the day of slaughter, broiler breast meat placed on ice was submitted to the laboratory, where it was filleted into pieces weighing approximately 0.1 kg and divided into four groups. For marinade, table salt with 99 to 99.5% sodium chloride (Solana dd Tuzla, Bosnia and Herzegovina), a “Carfosel Genius” commercial mixture of sodium tripolyphosphate (Prayon, Belgium) and tri-sodium citrate dihydrate ($C_6H_5Na_3O_7 \times 2H_2O$) (Merck, Germany) were used. Three different marinades and a solution of table salt (6% NaCl) as a control (I group) were prepared for the experiment. The first marinade consisted of water, 6% NaCl and 2% phosphate (II group), the second consisted of water, 6% NaCl and 2% citrate (III group) and the third one consisted of water, 6% NaCl, 1% phosphate and 1% citrate (IV group). For the preparation of the marinade, drinking water was used. Marinades have been prepared 24 hours before use, and afterwards refrigerated at 4 ± 1 °C.

For the experimental inoculation of the fillets, isolates of *Salmonella* Enteritidis (A1, A2, A3 and ATCC 13076) and *Salmonella* Typhimurium (ATCC 14028) were used. *Salmonella* Enteritidis A1 (Faculty of Veterinary Medicine, Belgrade, Serbia) was isolated from chicken liver, *S. Enteritidis* A2 (Institute of Meat Hygiene and Technology, Belgrade, Serbia) was isolated from mechanically deboned chicken meat, and *S. Enteritidis* A3 (Public Health Institute of Serbia “Dr Milan Jovanović Batut”, Belgrade, Serbia) was isolated from feces of a child affected by salmonellosis. A cocktail of these strains was prepared from overnight (18h) cultures (Brain Heart Infusion, Merck, Germany), and a final concentration of 5.42 log CFU/mL was obtained. Chicken breast fillets divided into four groups were inoculated with the *Salmonella* cocktail in the ratio of 1:10 (1 mL cocktail/10 g of meat). After inoculation, the meat was left for 30 min in order for bacterial cells to attach to the meat surface.

Contaminated broiler breast fillets were soaked in the marinades and solution of table salt, as a control, in the ratio of 1:2 (100g of meat/ 200 mL of marinade or table salt solution). Marination process lasted 4h at 4 ± 1 °C, with stirring at times. After 4 hours, the fillets were packed in sterile Stomacher bags and stored for 9 days under aerobic conditions at 4 ± 1 °C.

Before experimental contamination, the presence of *Salmonella* spp. in broiler breast meat was analyzed according to the EN ISO 6579: 2002/A1:2007 method. In

contaminated meat, count of *Salmonella* spp. was performed on the 0, 3rd, 6th and 9th day of storage, according to the method recommended by Pathania et al. [23]. On the testing day, from each group of fillets (I, II, III, IV), 20 g of meat was sampled, in triplicate. To each sample, 180 mL of Buffered Peptone Water (BPW), (Merck, Germany) was added and homogenized 30 sec in a Stomacher. The count of *Salmonella* spp. in contaminated fillets was determined by applying 0.1 mL of appropriate sample dilution on the surface of Xylose Lysine Tergitol-4 agar (XLT4), (Merck, Germany). Inoculated plates were incubated for 24h at 37°C. After incubation, the characteristic black colonies of *Salmonella* spp. were counted. The results of microbiological examinations are expressed as log CFU/g of meat.

Chemical analyzes were performed on the day of the experiment setup (0 day). The proximate composition of the chicken fillets was determined using standard ISO methods. Protein content ($N \times 6.25$) was quantified by the Kjeldahl procedure on Kjeltac 8400 Analyzer Unit (Foss, Sweden), according to ISO 937:1978 method. Moisture content was determined by drying at 103 ± 2 °C to constant mass (ISO 1442:1998). Free fat content was determined by extraction with petroleum ether in a Soxhlet apparatus (ISO 1444:1996). Ash content was determined by combustion of the sample at 550°C to constant weight (ISO 936:1998), while salt content was determined by silver nitrate titration, according to ISO 1841-1: 1996 method.

The water activity (a_w) values of chicken fillets were determined on the 0 and 9th day of storage. The a_w value was calculated from water-phase salt (WPS) with the equation used by Giménez and Dalgaard [24]:

$$a_w = 1 - 0,0052471 \times WPS - 0,00012206 \times WPS^2$$

Water-phase salt (WPS) was calculated from the total salt and moisture content of the meat, by using the equation:

$$WPS (\%) = \% \text{ salt} / \% \text{ salt} + \% \text{ moisture} \times 100$$

The pH values of chicken fillets were determined on the 0, 3rd, 6th and 9th day. Determination of the pH value in marinated fillets was performed directly, with “Testo 150” pH- meter (Testo, Germany), according to ISO 2917:1999 method.

Statistical analysis of the results was elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

RESULTS

The counts of *Salmonella* spp. in broiler breast fillets during 9 days of storage are presented in Table 1. Before experimental contamination, broiler breast fillets were free of *Salmonella* spp. On the 0 day of storage, there were no significant differences

($p > 0.05$) between *Salmonella* spp. counts in marinated (II, III, IV group) and salted breast fillets (I group), but on the 3rd and 6th day of storage significant differences ($p < 0.01$; $p < 0.05$) between groups were found. Until the 6th day of storage, *Salmonella* spp. count was the lowest in control group I and the highest in group IV. From 6th to 9th day of storage, *Salmonella* spp. count in all tested groups reached count of more than 5 log CFU/g. Contrary to previous results, on the 9th day of storage the lowest count was in group II and highest in group III, where the *Salmonella* spp. count was significantly higher ($p < 0.05$) from the *Salmonella* spp. count in group IV.

Table 1. The changes of *Salmonella* spp. count (log CFU/g) in broiler breast fillets during storage

Group	Storage days			
	0	3 rd	6 th	9 th
	$\bar{X} \pm Sd$			
I	3.37±0.55	2.92±0.30 ^{aA}	3.07±0.45 ^{AB}	5.91±0.32
II	3.65±0.39	3.29±0.34 ^B	4.32±0.34 ^{AC}	5.62±0.48
III	3.64±0.35	3.57±0.54 ^a	3.34±0.27 ^{CD}	6.18±0.55 ^a
IV	3.50±0.36	3.64±0.17 ^{AB}	4.40±0.48 ^{BD}	5.44±0.29 ^a

Legend: same letters A-D $p < 0.01$; a $p < 0.05$; \bar{X} – Mean value; Sd – Standard deviation

Comparing the storage days, a statistically significant difference in the *Salmonella* spp. count within each group was tested (Table 2). Within group I, significant difference in *Salmonella* spp. count was not established until the 6th day of storage. However, difference in *Salmonella* spp. count between 9th day and the other two comparing days was significant ($p < 0.001$). In marinated breast fillets (II, III and IV group), there was no significant difference ($p > 0.05$) in *Salmonella* spp. count between the 0 and 3rd day of storage ($p > 0.05$). On the other comparing days, *Salmonella* spp. count in marinated fillets (II, III and IV group) were changed significantly, at different significance levels of $p < 0.001$ or $p < 0.01$.

Table 2. The statistical significance of differences in *Salmonella* spp. count within groups, comparing storage days

Comparing days	Group			
	I	II	III	IV
	P			
0-3	ns	ns	ns	ns
0-6	ns	**	***	**
0-9	***	***	***	***
3-6	ns	***	ns	***
3-9	***	***	***	***
6-9	***	***	***	**

Legend: *** $p < 0.001$; ** $p < 0.01$; ns – not significant

Data for proximate composition (proteins, fat, moisture and ash content) and salt content in salted and marinated breast fillets are presented in Table 3. The obtained results indicated that marination has no significant influence ($p>0.05$) on protein and fat content in marinated fillets, but has significant influence on moisture, ash and salt content. Moisture content in marinated breast fillets was significantly ($p<0.01$; $p<0.05$) lower compared to moisture content in salted breast fillets. Ash content was significantly ($p<0.01$) higher in samples of group III and group IV, compared to ash content in control group I and samples of group II, as well. Furthermore, salt content in samples of group III and IV was significantly ($p<0.01$; $p<0.05$) higher than salt content in samples of group I and group II.

Table 3. The proximate composition and salt content in broiler breast fillets on the 0 day of storage

Group	Proteins (%)	Fat (%)	Moisture (%)	Ash (%)	Salt (%)
	$\bar{X} \pm Sd$				
I	16.33±0.51	1.92±0.20	78.10±0.13 ^{Ab}	3.65±0.07 ^{Aa}	2.60±0.05 ^{Aa}
II	16.00±0.33	1.92±0.20	77.58±0.11 ^b	3.62±0.09 ^{BC}	2.54±0.09 ^{BC}
III	16.15±0.34	1.92±0.20	77.53±0.26 ^a	4.40±0.09 ^{ABD}	3.35±0.09 ^{ABD}
IV	16.62±1.05	2.00±0.31	77.54±0.94 ^A	3.84±0.12 ^{aCD}	2.79±0.18 ^{aCD}

Legend: same letters A-D $p<0.01$; a-b $p<0.05$; \bar{X} – Mean value; Sd – Standard deviation

Table 4 shows the pH values of broiler breast fillets during 9 days of storage. As it was expected, pH values of marinated samples were significantly higher ($p<0.01$) compared to pH values of control samples. Similar data were obtained on the 3rd, 6th and 9th day of storage, when statistically significant differences ($p<0.01$) between pH values of marinated and salted breast fillets were established. From the beginning to the end of the study, the pH values were gradually increasing in all four groups of samples.

Table 4. pH values of broiler breast fillets during storage

Group	Day of storage			
	0	3 rd	6 th	9 th
$\bar{X} \pm Sd$				
I	5.94±0.02 ^{ABC}	6.15±0.02 ^{AB}	6.06±0.02 ^{ABC}	6.38±0.01 ^{AB}
II	6.09±0.01 ^{ADE}	6.20±0.02 ^C	6.21±0.03 ^{AD}	6.45±0.03 ^{CD}
III	6.04±0.01 ^{BDF}	5.98±0.07 ^{ACD}	6.13±0.05 ^{BDE}	6.28±0.05 ^{ACE}
IV	6.25±0.03 ^{CEF}	6.25±0.01 ^{BD}	6.20±0.02 ^{CE}	6.58±0.08 ^{BDE}

Legend: A-F statistical significance of $p\leq 0.01$; \bar{X} – Mean value; Sd – Standard deviation

Data for water activity (a_w) in salted and marinated breast fillets are shown in Table 5. On the 0 day, the lowest a_w value was established in the samples of group III. This

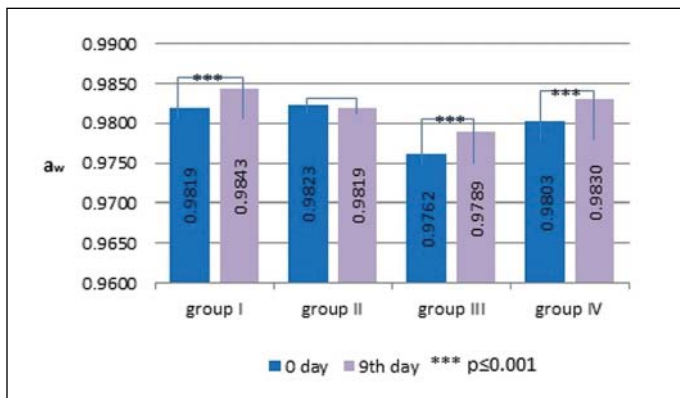
value was significantly lower ($p < 0.01$) from a_w values in group II and group IV and control group I, as well. The same significance of differences was observed on the 9th day of storage.

Table 5. Water activity (a_w) values in broiler breast fillets during storage

Groups	Day of storage	
	0	9 th
	$\bar{X} \pm Sd$	
S	0.982 ^{AB} ± 0.001	0.984 ^{ABa} ± 0.001
SF	0.982 ^{CD} ± 0.001	0.982 ^{AC} ± 0.001
SC	0.976 ^{ACE} ± 0.001	0.979 ^{BCD} ± 0.001
SFC	0.980 ^{BDE} ± 0.001	0.983 ^{dD} ± 0.001

Legend: ^{A-E} statistical significance of $p \leq 0.01$; ^a statistical significance of $p \leq 0.05$; $\bar{X} \pm$ – Mean value; Sd – Standard deviation

Changes of a_w values during storage, within the control group I and experimental groups (II, III and IV) are shown in Graph 1.



Graph 1. Statistical significance of differences in a_w values change during storage

Comparing the a_w values on the 0 and 9th day of storage, significant differences ($p < 0.001$) were established within group III, group IV and the control group I, where a_w values were increasing during storage.

DISCUSSION

Salmonella species are highly resistant microorganisms, able to survive longer periods in foods where its multiplication for some reason is not possible [25]. The optimum pH values for the growth of most *Salmonella* species ranged from 6.5-7.5, but they can grow in the range of 4.5-9.0. *Salmonella* species also survive in the food sample with is

$a_w \geq 0.94$ [26]. Results for the proximate composition and salt content have shown that marination of broiler breast fillets in solutions of table salt, sodium tripolyphosphate and/or sodium citrate contributed to the change in moisture, ash and salt content in chicken fillets but had no influence on the protein and fat content compared to control (6% table salt solution). Marination of broiler breast fillets in a solution of 6% table salt and 2% sodium citrate has significant influence on the salt content in the meat, with a resulting significant decrease of a_w values in group III samples. However, determined a_w values in all tested groups were well above the limit value of 0.94 and were not a limiting factor for the growth of *Salmonella* spp. in marinated broiler meat.

Investigations on the pH values in salted and marinated fillets have shown that the pH values of marinated fillets were significantly higher than the pH value of salted fillets. Carroll et al. [27] examined the influence of marination on the pH value of turkey fillets. According to their data, there was no statistically significant difference between the pH value of fillets marinated in a solution of 1.5% NaCl and 0.75% sodium citrate, and control samples marinated in a solution of 1.5% NaCl and 0.45% tripolyphosphate. Ergezer and Gokce [28] achieved the most effective increase in pH value of turkey breast meat with a combination of 2% NaCl and 2% sodium tripolyphosphate. Our study has shown that the pH values in group II samples were significantly higher compared to pH values in the fillets from group III. During 9 days of storage, the most significant increase in pH value was achieved by a combination of 6% NaCl, 1% sodium tripolyphosphate and 1% citrate, and these values were significantly higher compared to pH values in the control and other two groups. These data are in accordance with the research of Killefer et al. [29], who found that the ultimate pH values of pork treated with saline solution, phosphate and citrate were higher compared to the control samples treated with phosphates only.

Perko- Mäkela et al. [30] have reported that a single factor such as the pH value can reduce survival of bacteria such as *Campylobacter jejuni* in marinades but that the effect of pH is limited on marinated chicken products, which is confirmed by our survey. We found that marination has no significant influence on the reduction of *Salmonella* population in marinated broiler breast fillets, but results indicates that the growth of *Salmonella* spp. in marinated broiler meat could be delayed. Shelef and Seiter [31] have reported the suppression of microbial growth by polyphosphates and suggested that the antimicrobial activity of these compounds can be attributed to their ability to chelate cations essential for microbial growth. However, the pH value increased gradually during storage which was probably the reason for an increase in the number of *Salmonella* in all groups tested. Long et al. [32] found that the bacteriostatic effect of phosphates is not significant, and that is displayed when using acidic phosphates or a combination of phosphates with other additives, such as nisin, EDTA, sodium chloride, nitrate, eritorbat and others, as confirmed by our survey. Lee et al. [15] after testing the antimicrobial activity of citrate and acetate *in vitro*, concluded that citrate has a weak antimicrobial activity against gram-negative bacteria, and that the combination of citrate and acetate does not increase this activity. Similar results were obtained in

the study of Sallam *et al.* [33] who, after testing the antimicrobial and antioxidant activity of sodium acetate, lactate and citrate, concluded that sodium acetate is more effective compared to sodium lactate and sodium citrate.

In our trial, *Salmonella* spp. count in marinated and salted fillets did not differ significantly until the 3rd and 6th day of storage, respectively. However, to the end of study, *Salmonella* spp. counts increased significantly and values above 5 log CFU/g were established in all tested groups. During 9 days of storage, the most significant increase in pH value was achieved by a combination of 6% NaCl, 1% sodium tripolyphosphate and 1% citrate, and these values were higher compared to pH values in the control and other two groups. It seems that these relatively high pH values contributed to an increase of *Salmonella* spp. in samples of group IV.

From the results obtained, it can be concluded that sodium tripolyphosphate and sodium citrate have an influence on moisture and salt content in marinated breast fillets and consequently affect the change in a_w value of meat. Solution of table salt and sodium citrate reduces the a_w value in marinated fillets, but not at a level which could entirely inhibit the growth of *Salmonella* spp. The most effective increase was in pH value, and consequently an increased juiciness of the breast fillets was achieved by marination in a solution of table salt, sodium tripolyphosphate and sodium citrate. While improving quality, this combination of salts stimulates intensive *Salmonella* spp. growth, probably because of the lower citrate concentration in this marinade. The marinade which consisted of table salt and sodium tripolyphosphate did not show an inhibitory effect on *Salmonella* spp. growth. Lower *Salmonella* spp. count in salted fillets is probably a consequence of table salt ability to cause cellular plasmolysis. These results emphasize the importance of adhering to strict hygienic and quality control standards throughout the processing of marinated broiler meat.

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UTICAJ MARINIRANJA NA RAST *SALMONELLA* SPP. U MESU GRUDI BROJLERA

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Cilj ovog rada bio je da se ispita uticaj mariniranja na rast *Salmonella* spp. u kontaminiranom mesu grudi brojlera tokom skladištenja. U sprovedenom istraživanju, fileti grudi brojlera su inokulisani mešanom kulturom različitih *Salmonella* serotipova, a zatim su marinirani u različitim rastvorima kuhinjske soli, natrijum tripolifosfata i/ili natrijum citrata. Ukupan broj *Salmonella* spp. određivan je nultog, 3., 6. i 9. dana skladištenja. Fileti grudi brojlera potapani u 6% rastvor kuhinjske soli korišćeni su kao kontrola. Osnovni hemijski sastav i sadržaj soli, pH i a_w vrednost mesa, određivani su kao parametri kvaliteta mesa, ali i kao parametri koji mogu da utiču na rast bakterija u mesu. U odnosu na početnu kontaminaciju, broj *Salmonella* spp. u mariniranim i soljenim filetima nije se značajno menjao ($p < 0,001$) do 3., odnosno 6. dana skladištenja, ali se značajno povećao do 9. dana skladištenja. Mariniranje fileta grudi brojlera u različitim rastvorima kuhinjske soli, natrijum tripolifosfat i/ili natrijum citrata imalo

je značajan uticaj ($p < 0,05$; $p < 0,01$) na promenu pH vrednosti mesa, sadržaj vlage i pepela i sadržaj soli u mariniranom mesu, ali nije utvrđen značajan uticaj ($p > 0,05$) na promenu sadržaja proteina i masti u mesu brojlera. Na osnovu dobijenih rezultata može se zaključiti da mariniranje mesa grudi brojlera u rastvorima koji sadrže kuhinjsku so, natrijum tripolifosfat i/ili citrat, na neki način, može da produži lag fazu rasta *Salmonella* vrsta, gde je natrijum citrat efikasniji od natrijum tripolifosfata.