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The Effects of Acidified Sodium Chlorite and Acidified Sodium Chlorite Containing Sauce on *Listeria monocytogenes* in Chicken Chops

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SUMMARY This study was aimed to investigate the effects of acidified sodium chlorite (ASC) and ASC containing sauce on chicken chops contaminated with *Listeria monocytogenes.* Chicken chops were experimentally contaminated with *L. monocytogenes.* For the bacterial inhibition, the chops were treated with sauce, ASC (200 ppm and 1800 ppm) and ASC containing sauce. The groups are planned according to the proportions of decontaminants applied and the estimated shelf life of the product. Contaminated 8 groups chops (1 group contaminated control) were placed in plastic containers, and kept at 4 °C for 7 days. The samples were investigated in terms of *L. monocytogenes* at 0., 2., 3., 5. and 7. days. It was determined that there was statistically significant difference between groups in terms of number of *L. monocytogenes*. The highest decrease in number of *L. monocytogenes* was determined in group 6 on day 7 as 1.85 $\log_{10}$cfu/g. In this study, the highest antimicrobial effect on *L. monocytogenes* was found in chicken chops kept after holding it for 2 minutes in solution containing 1800 ppm ASC. In conclusion, ASC solution decreases the risk of *L. monocytogenes* in chicken chops.

Key Words: *Acidified Sodium Chlorite, Chicken Chop, Decontamination, Listeria monocytogenes, Sauce*

ÖZET

Asidifiye Sodyum Klorid ve Asidifiye Sodyum Klorid İçeren Sosun Broiler Pirzolalarında *Listeria monocytogenes* Üzerine Etkisi

Bu çalışmayla *Listeria monocytogenes* ile kontamine edilen broiler pirzolalarında asidifiye sodyum klorit (ASK) ve asidifiye sodyum klorit içeren sosun etkisinin incelenmesi amaçlanmıştır. Broiler pirzolaları *L. monocytogenes* ile deneysel olarak kontamine edilmiştir. Broiler pirzolaları bakteriyel azalma için ASK (1200 ppm ve 1800 ppm) ve ASK içeren sos ile muamele edilmiştir. Gruplar, uygulanan dekontaminantların oranlarına ve ürünün tahmini raf ömrüne göre planlanmıştır. Kontamine edilen 8 grup (1 grup kontaminasyon kontrol) plastik kaplara yerleştirilerek at 4 °C'de 7 gün depolanmıştır. Gruplar uygulanan dekontaminanatların oranlarına ve ürünün tahmini raf ömrüne göre planlanmıştır. Örnekler 0., 2., 3., 5. ve 7. günlerinde *L. monocytogenes* açısından incelenmiştir. *L. monocytogenes* sayısı açısından gruplar arasında farkın istatistiksel bakımdan önemli olduğu belirlenmiştir. *L. monocytogenes* açısından en büyük azalma 1.85 $\log_{10}$ kob/g ile depolamanın 7. gününde 6. grupta (1800 ppm ASK içeren solüsyon) belirlenmiştir. Bu çalışmada broiler pirzolalarında *L. monocytogenes* üzerine en büyük etkinin 1800 ppm ASK içeren solüsyonda 2 dk bekletilmesiyle sağlanmıştır. Sonuç olarak ASK broiler pirzolalarında *L. monocytogenes* riskini azaltmaktadır.

Anahtar Kelimeler: *Asidifiye Sodyum Klorid, Broiler Pirzolası, Dekontaminasyon, Listeria monocytogenes, Sos*

INTRODUCTION

The broiler carcass can be contaminated during slaughtering, scalding, defeathering, laying open, evisceration, cooling, splitting and packaging by personnel, water or devices-equipment (Arslan 2002). While central parts of meats obtained from healthy animals are sterile, outer parts may be contaminated with microorganisms depending on butchery hygiene (Mead 2004a). Because *Listeria monocytogenes* is one of the principle causative agents in foodborne diseases, it was chosen for the contamination of the material in our study. This bacteria is a gram-positive and facultative intracellular foodborne pathogen that causes listeriosis (Martín et al. 2014). The low microbiological limits are important for broiler meats kept in cold because these bacteria can reproduce at

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refrigerator temperature. For people whose bowel systems are sensitive, *L. monocytogenes* leads to encephalitis, septicemia and meningitis. Moreover, it leads to abort, congenital malformations and stillbirth in pregnant women (Pickering et al. 2012; Ahmed et al. 2015). It was emphasized that red and broiler meats in USA constitute the group with highest risk in terms of *L. monocytogenes*. Among samples obtained from sales points; *E. coli* O157:H7 was detected in 7% of turkeys and 12% of broilers while 24% of broiler carcasses and 12% of pre-cooked products were found to be positive in terms of *L. monocytogenes* (Mead 2004b).

Previous studies (Border et al. 1990; Mead 2004a), showed that more than 50% of processed broiler meats are positive in terms of *L. monocytogenes* while there are generally low numbers of *L. monocytogenes* uncooked broiler meats (<1 cfu/cm^2-skin). Presence of *L. monocytogenes* in foods and food processing locations was previously reported in Ireland (Leong et al. 2014). Yang et al. (2016) reported that presence of *L. monocytogenes* in fried meat was 2.47%.

For decontamination of broiler carcasses, biological and chemical materials and physical methods are used. Although many materials were tried for this purpose, only some of them have gained implementation fields in industry (Hwang and Beuchat 1995; Rodríguez-de-Ledesma et al. 1996; Mead 2004a;). Acidified sodium chlorite (ASC) is prepared by adding any acid into liquid sodium chloride solution. In America, Food and Drug Administration (FDA) approved the usage of ASC solution (500-1200 ppm) in final products for decontamination in red meats (USFDA 1998).

Natural food additives are generally more preferable in regard to food safety and this idea becomes more popular (Kim and Rhee 2013). Nowadays, many food matters are treated by sauces containing antimicrobial plants. For this reason, the aim of this study is to determine the effects of sauces containing plant material on the proliferation of *L. monocytogenes*.

This research was conducted in order to examine the effects of ASC and ASC containing sauce on L. monocytogenes in chicken chops which are experimentally contaminated.

MATERIALS and METHODS

In this research, 100 g-weight chicken chops were used. The samples were obtained from local markets. Chops were separated from skin and bones but not put into decontamination tank. Eight groups were clustered in research, one was control. The control group was formed from the contaminated chops without any decontamination processing. Research was repeated for 3 times and 40 pcs of chicken chops were used at each repeat. As decontamination material, sodium chloride (25% solution, Merck 8.14815) and citric acid (Merck 242.1000) were used. Solutions of ASC were prepared according to description of Özdemir et al. (2005). In our preliminary study showed that sauce groups containing 1200 ppm ASC had no sufficient antibacterial effects. For this reason, ASC was applied in the dose of 1800 ppm. Dose of ASC at 1800 ppm was used in some groups to evaluate the difference between the dose group of 1200 ppm which is the upper recommended limit for ASC in the USA and some other countries (USFDA 1998).

For sauce, the amounts and content of mixture is as follows; tomato paste (200 g), paprika paste (200 g), sunflower oil (180 ml), garlic (140 g), red pepper (15 g), thyme (15 g), black pepper (5 g), cumin (5 g), salt (45 g), natural lemon juice (195 ml).

By taking 2.64 ml of 1200 ppm ASC solution, it was placed in containers with 500 g chicken chops and 50 g sauce (10% of the weights of chops) mixture, and then mixed. In order to prepare the sauce containing 1800 ppm ASC 3.96 ml of solution which was covered to 250 ml was taken. The rest of process was same with those above. These solutions prepared were used in 10 minutes.

RSKK 472 *L. monocytogenes* 1/2b, RSKK 474 *L. monocytogenes* 3a, RSKK 475 *L. monocytogenes* 4b, RSKK 476 *L. monocytogenes* 4c and RSKK 02028 *L. monocytogenes* strains obtained from Refik Saydam Public Health Center were used in research. Target contamination level on chop surfaces was 10^{6}-10^{7} cfu/g, the contaminated solution containing 10^{7}–10^{8} cfu/ml *L. monocytogenes* (mixture of 5 strains) were applied on all of chop surfaces with sterile brush. After inoculation, the chops were held for 4 min to provide adhesion of the pathogen to the surface. Then, in order to determine the level of contamination, samples were taken and microbiological inoculation was conducted. The groups without ASC implementation were placed in polyethylene containers after being kept in ASC solution for 2 minutes and then containers were closed. All of the groups were kept at 4 °C for 7 days. Samples were investigated in terms of *L. monocytogenes* at 0, 2, 3, 5, and 7 days.

The groups were formed as the following; 1) control (contaminated with 5 strains, without ASC or sauce application), 2) the sauce without any ASC was applied, 3) kept for 2 minutes in solution containing 1200 ppm ASC, 4) kept for 2 minutes in solution containing 1200 ppm ASC and then sauce was applied, 5) the sauce with 1200 ppm ASC was applied, 6) kept for 2 minutes in solution containing 1800 ppmASC, 7) kept for 2 minutes in solution containing 1800 ppm ASC and then sauce was applied, 8) the sauce with 1800 ppm ASC was applied.

Analysis of chops was performed according to the method of USDA-FSIS (2002). The pH values of the samples were measured in order to investigate the microbial load with respect to the changes in pH values. pH values of samples (at 25±1 ºC) were determined (at 0, 2, 3, 5 and 7 days) through pH-meter (pH2001, JP Selecta, Spain). Statistical Analysis was conducted by using Statistical Analysis System (SAS) package software (Version 8, 1999, SAS Institute Inc., Cary, NC, USA). Data was subjected to variance analysis (ANOVA). Averages were parsed according to the method of Fisher's Least Significant Difference-LSD. Statistical significance was determined as $P<0.05$.

RESULTS

The effects of ASC and the sauce against *L. monocytogenes* on chicken chops were given in Table 1. In general, the differences between groups were found to be significant ($P<0.05$). In groups 6 and 7, significant difference was determined between day 0 and other days ($P<0.05$). In other 6 groups, no difference was determined among the storage days ($P>0.05$). The highest decrease was found in group 6 with value of 1.85 $\log_{10}$cfu/g on day 7 whereas, the lowest decreased was found in group 4 with value of 0.10 $\log_{10}$cfu/g on day 7 (Table 1). The effect of ASC might be decreased by sauce. The pH values determined during storage in groups contaminated with *L. monocytogenes* are given in Table 2.

Table 1. The Effects of acidified sodium chlorite and sauce on *Listeria monocytogenes* in chicken chops during storage at 4 °C (log_{10}cfu/g, n=120)

Storage Days	GROUPS							
	1st group	2nd group	3rd group	4th group	5th group	6th group	7th group	8th group
0.	6.83 ± 0.18^{Ax}	6.54± 0.15^{ABx}	6.00± 0.18^{ABx}	5.76± 0.23^{Bx}	6.39± 0.15^{ABx}	6.02±0.79^{ABx}	5.59± 0.41^{Bx}	6.46±0.15^{ABx}
2.	7.08± 0.34^{Ax}	6.77± 0.33^{ABx}	5.86± 0.03^{Bx}	5.37± 0.14^{BCx}	6.83± 0.10^{Ax}	4.17± 0.15^{Cy}	4.93± 0.11^{Cy}	6.61± 0.45^{ABx}
3.	7.11± 0.51^{Ax}	6.64± 0.34^{ABx}	5.25± 0.08^{BCx}	5.83± 0.06^{Bx}	6.85± 0.37^{Ax}	4.39± 0.05^{Cy}	4.32± 0.04^{Cy}	6.61± 0.49^{ABx}
5.	7.47± 0.56^{Ax}	6.87± 0.77^{Ax}	5.41± 0.16^{BCx}	5.80± 0.10^{Bx}	6.84± 0.49^{Ax}	4.29± 0.17^{Cy}	4.81± 0.13^{Cy}	6.99± 0.65^{Ax}
7.	6.94± 0.09^{Ax}	6.56± 0.18^{ABx}	5.41± 0.09^{Bx}	5.66± 0.15^{Bx}	6.42± 0.09^{ABx}	4.17± 0.04^{Cy}	4.86±0.06^{BCy}	7.03± 0.49^{Ax}

ABC; means in the samel ine with different superscripts are statistically different ($P < 0.05$).

xy ; means in the same column with different superscripts are statistically different ($P < 0.05$).

1st group: Control Group (No ASC or sauce application), 2nd group: Sauce without any ASC content was applied, 3rd group: Kept for 2 minutes in solution containing 1200 ppm ASC, 4th group: Kept for 2 minutes in solution containing 1200 ppm ASC and then sauce was applied, 5th group:Sauce with 1200 ppm ASC content was applied, 6th group: kept for 2 minutes in solution containing 1800 ppmASC content, 7th group: kept for 2 minutes in solution containing 1800 ppm ASC and then sauce was applied, 8th group: Sauce with 1800 ppm ASC content was applied.

Table 2. pH values of the groups during storage at 4 °C (n = 120).

Storage days	GROUPS							
	1st group	2nd group	3rd group	4th group	5th group	6th group	7th group	8th group
0.	6.53 ± 0.06	5.73 ± 0.07	5.13 ± 0.06	5.17 ± 0.07	5.25 ± 0.07	5.07 ± 0.06	5.14 ± 0.04	5.21 ± 0.03
2.	6.74 ± 0.06	5.84 ± 0.06	5.19 ± 0.08	5.25 ± 0.07	5.31 ± 0.08	5.15 ± 0.05	5.26 ± 0.04	5.33 ± 0.07
3.	6.83 ± 0.06	5.93 ± 0.08	5.25 ± 0.07	5.34 ± 0.05	5.41 ± 0.04	5.23 ± 0.08	5.32 ± 0.03	5.37 ± 0.03
5.	6.92 ± 0.04	6.06 ± 0.06	5.35 ± 0.05	5.43 ± 0.03	5.53 ± 0.06	5.25 ± 0.06	5.34 ± 0.06	5.41 ± 0.07
7.	6.97 ± 0.03	6.15 ± 0.08	5.46 ± 0.06	5.55 ± 0.07	5.64 ± 0.07	5.36 ± 0.04	5.45 ± 0.05	5.54 ± 0.05

Sauce pH; 3.24, ASC pH; 2.38

DISCUSSION

This research was conducted in order to examine the effects of ASC solution and sauce containing ASC on *L. monocytogenes* in keeping chicken chops contaminated with that pathogen at 4 °C. Del Rio et al. (2007), stated that an important residual effect was observed in number of *L. monocytogenes* and *Salmonella* when keeping drumsticks, which were hold in solution containing 1200 ppm ASC for 15 minutes, in storage for 0, 1, 3, and 5 days. In this study, it was determined that ASC solution in groups 6 and 7 provides antibacterial effect on *L. monocytogenes*.

During our research, the antimicrobial effect on number of *L. monocytogenes* increased (0.10 - 1.85 log_{10}cfu/g) in parallel with storage duration with usage of solutions containing 1200 ppm and 1800 ppm ASC. The highest reduction was found to be between control group and groups immersed into ASC solutions on day 7 in group 6 with 2.77 log_{10}cfu/g reduction. Similarly, 2.86 log_{10}cfu/g of reduction was found between in group 8 to which sauce containing 1800 ppm ASC and in group 6 immersed into ASC solution the end of storage duration. These results showed similarities with those reported by Del Rio et al. (2007), Lim and Mustapha (2004), and Özdemir et al. (2005).

No study about the combinations of sauces and ASC solutions were found during conducted literature researches. In addition, sauces prepared by soy has been used. Moon and Rhee (2016), reported that thymol and carvacrol added soy sauce has considerably decreased the number of *L. monocytogenes*. Burt (2004) reported that Gram (+) bacteriae are more sensitive to carvacrol and tymole**.** In the present sauce, the sauced groups thyme has no effects on *L. monocytogenes.* In group 4 where sauce was applied after sinking in solution containing 1200 ppm ASC, 1.28 log_{10}cfu/g decrease in number of *L. monocytogenes* in proportion to control group was found at the end of day 7. Similarly, in group 5 where sauce containing 1200 ppm ASC was applied, 0.52 log_{10}cfu/g decrease in number of *L. monocytogenes* in proportion to control group was found at the end of day 7. However, in group 7 where sauce was applied after sinking in solution containing 1800 ppm ASC, 2.08 log_{10}cfu/g decrease in number of *L. monocytogenes* in proportion to control group was found at the end of day 7. Differently, in group 8 the sauce containing 1800 ppm ASC, the number of *L. monocytogenes* on day 7 has increased at a level of 0.57 log_{10}cfu/g compared to day 0. The lower antibacterial effects of implementation of sauce containing ASC can be attributed to that the materials used in sauce mixture acted as barrier by cohering on surfaces of samples, or that they conjoined with materials in sauce combination, or that they reacted with materials in sauce combination. The highest antimicrobial effect among groups was determined in samples kept at 4 °C after sinking in solution containing 1800 ppm ASC.

In groups where only sauce was applied, the obtained results were similar with those in control group. The used sauce composition had no antimicrobial effect on *L. monocytogenes*. We believe that its reason is that the concentrations used in sauce composition were not enough in order to create antimicrobial effect. Zarai et al. (2013), reported that black pepper (due to containing of

piperine and piperic acid) is efficient on Gram (-) bacteria more than Gram (+) bacteria. Ting and Deibel (1992), reported that black pepper, garlic and red pepper have no antimicrobial effect on reproduction of *L. monocytogenes* up to 3% concentration. The carvacrol and thymol contained by thyme has the effect of elimination of *L. monocytogenes* and other pathogens when added in soy sauce (Moon and Rhee 2016). Garlic has thiosulphinate compounds which have antimicrobial effects (Rhoades et al. 2013). In the present study, the effects of tyhyme, garlic and black pepper could not be determined on *L. monocytogenes* in regard to dose and the amount.

The increases in pH value depending on storage duration in this study can be attributed to increase in microbial reproduction. Koutsoumanis et al. (2004) reported that the proliferation of *L. monocytogenes* needs pH 5.1 at 15 °C. As there is inclination to increase in pH during storage, no negative effects on *L. monocytogenes* were determined. In the present study, the groups (6 and 7) which was directly added 1800 ppm ASC had the lowest pH levels. The samples that kept in low temperature inhibited *L. monocytogenes* by increasing ASC concentration.

CONCLUSION

According to results obtained in this study, it was determined that the implementations showed antimicrobial effect on *L. monocytogenes* in some groups. This effect is ranged as immersing in ASC solution, sauced up after immersing in ASC solution and implementation of sauce containing ASC. During this study, the solution containing 1800 ppm ASC (immersion method) created the highest effect on *L. monocytogenes*. It was revealed that the effective methods can be used for eliminating the bacterial risks arising from broiler meats. It is thought that better effects can be obtained by changing the rates of spices.

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