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Effects of Sumac (*Rhus coriaria* L), Oregano (*Oreganum vulgare* L.) and Lactic Acid on Microbiological Decontamination and Shelf-life of Raw Broiler Drumsticks

Leyla VATANSEVER* ✎ Murat GÜLMEZ* Nebahat ORAL* Abamüslüm GÜVEN* Salih OTLU**

* Kafkas University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kars – TURKEY
** Kafkas University, Faculty of Veterinary Medicine, Department of Microbiology, Kars – TURKEY

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Summary

In an attempt to improve the bacteriological quality and refrigerated shelf-life of broiler meat, 10 min surface wash treatments with sterile tap water, a steam distillate of oregano 10% (wt/v), water extract of sumac, and 2% (v/v) lactic acid were compared using a raw broiler drumstick model. The log10 cfu/g of the aerobic plate counts of psychrotrophs, mesophiles, *Enterobacteriaceae*, coliforms and faecal coliforms on the untreated control samples were determined to be 4.8, 3.6, 3.4, 2.8 and 1.6. The counts of same group of organisms, except coliforms and faecal coliforms, on sumac and lactic acid samples were 3.9, 2.8, 1 and 3.7, 2.6, 1, respectively. Number of coliforms and faecal coliforms was less than detectable level (<1 log cfu/g). There was no statistical difference between lactic acid and sumac ($p>0.05$) for all groups of bacteria counts. The shelf-life of the untreated, water treated and oregano treated samples was 7 days, whereas it was 14 days for sumac and lactic acid treated samples stored at 3±1ºC. With reference to color and odor, sumac appeared to be superior to lactic acid. However, more extensive studies on the subject should be done before recommend sumac as an alternative to lactic acid in antimicrobial interventions in poultry processing plants.

Key words: *Decontamination, Shelf-life, Broiler, Sumac, Lactic acid, Oregano*

Sumak (*Rhus coriaria* L), Kekik (*Oreganum vulgare* L.) ve Laktik Asit'in Broiler Butlarının Mikrobiyel Dekontaminasyonu ve Raf Ömrü Üzerine Etkisi

Özet

Broiler etinin bakteriyolojik kalitesinin ve soğuk muhafaza (3±1ºC) raf ömrünün uzatılması amacıyla, steril musluk suyu, kekiğin %10'luk buhar distilatı, sumağın su ekstraktı ve %2'lik laktik asit broiler butlarına 10 dakika yüzey yıkaması şeklinde uygulanarak bunların antimikrobiyel etkileri karşılaştırıldı. Kontrol örneklerinin psikrotrof ve mezofil bakteri sayısı ile *Enterobacteriaceae*, koliform ve fekal koliform mikroorganizma yükü sırasıyla 4.8, 3.6, 3.4, 2.8 ve 1.6 log kob/g olarak bulundu. Koliform ve fekal koliformlar hariç aynı bakteri gruplarının sumak ve laktik asit uygulanmış örneklerdeki düzeylerinin sırasıyla 3.9, 2.8, 1 ve 3.7, 2.6, 1 log kob/g olduğu, koliform ve fekal koliformların ise saptama sınırının (<1 log kob/g) altında kaldığı tespit edildi. Laktik asit ve sumak sonuçları arasında istatistiki olarak bir fark görülmedi ($P>0.05$). Raf ömrü; kontrol, su ve kekik gruplarında 7 gün iken sumak ve Laktik asit gruplarında yaklaşık 14 gün olarak tespit edildi. Örneklerin renk ve kokuları dikkate alındığında sumak grubunun Laktik asit grubundan daha iyi olduğu gözlendi. Bu sonuçlara rağmen L. asit yerine antimikrobiyel ajan olarak kanatlı üretiminde sumak kullanılmasını önermeden önce daha detaylı araştırmalar yapılmalıdır.

Anahtar sözcükler: *Dekontaminasyon, Raf ömrü, Broiler, Sumak, Laktik asit, Kekik*

✎ İletişim (Correspondence)
☎ +90 474 242 68 07/1123
✉ lvatansever@hotmail.com

INTRODUCTION

Contamination of poultry meat with food borne pathogens remains an important public health issue, because it can lead to illness if there are malpractices in handling, cooking or post-cooking storage of the product [1]. It has been reported that, surface decontamination with chlorine, alkaline or organic acids improves the hygiene of the poultry carcass and prolongs its refrigerated shelf-life [2,3]. However, it is important that, any potential treatment applied for this purpose should not alter the sensory characteristics of the meat. Although lactic acid has become the organic acid most commonly used in commercial practice to improve the bacterial safety and refrigerated shelf-life of carcasses [4-6], undesirable changes may occur in chicken carcasses treated in this way [7]. In spite of modern improvements in slaughter hygiene and food production techniques, there is still a need for new methods of reducing or eliminating food borne pathogens [7,8].

The antibacterial activities of spices and essential oils have been known for a long time, however the relatively recent enhancement of interest in *"green"* consumerism has lead to a renewal of scientific interest in these substances and their antibacterial properties and potential applications in food reviewed and investigated intensively by scientists [8-10]. Sumac (*Rhus coriaria* L.) and oregano (*Oreganum vulgare* L.), two of these spices are known to contain naturally occurring compounds with antimicrobial properties. The bacteriostatic/bactericidal effect of various extracts of sumac and oregano on foodborne bacteria including pathogens has been demonstrated in many *in vitro* studies [10-14]. However a few studies have addressed the effect of these compounds on pathogens associated with muscle foods [15]. Thus, the present work was undertaken to compare the surface decontamination and shelf-life activities of sumac and oregano with those of the most commonly used organic decontaminant, lactic acid, in a broiler drumstick model.

MATERIAL and METHODS

Preparation of meat surface decontaminants

Water Extract of Sumac: Fifty g of ripened (reddish brown) native sumac fruits bought from a local retailer in Kars, Turkey, and they were placed in a sterile plastic bag with 500 ml of sterile distilled water and left at 45ºC for 12 h. The bag was then squeezed by hand to crush its contents. The crushed contents were then filtered through into cheesecloth to an Erlenmeyer flask.

Steam Distillate of Oregano: A 50-g sample of oregano, bought from a local retailer in Kars, Turkey was ground in an omnimixer. The hydrosol of ground plant was obtained after 1 h in a steam-distillation apparatus with 500 ml of distilled water (1:10, wt/vol). Then, the oil was removed. Hydrosol was kept in sterile bottles under refrigerated conditions until use.

Lactic acid: A 2.0% lactic acid in sterile distilled water, (v/v), was prepared from a 98% concentration (Sigma, L6402).

Water: Steril tap water was used.

All decontaminant fluids were prepared freshly on the day before use and sterilized by filtering using a 0.22 µ syringe filter, then stored at 3±1ºC.

Sample collection and allocation to treatments

The procedure described below was replicated three times in consecutive weeks. In each replication, a box of 20 kg drumsticks was bought from a slaughterhouse in Erzurum, Turkey, and transferred under cold storage to the lab within 3 h. Then, 60 drumsticks from the box were selected and grouped randomly into five separate sterile bags of 12 drumsticks.

Surface decontamination and shelf-life study

One group of 12 drumsticks was not decontaminated and was used as control. The other 4 groups were decontaminated separately by addition of 200 ml of water, oregano, sumac or lactic acid. The amount of decontamination fluid was ca. 10 ml for 100 g of drumstick. Each bag was slowly shaken and/or rotated by hand for 10 min. The each drumstick was then allowed to drain briefly into the bag, before being transferred separately to a previously weighed sterile stomacher bag. Two of the 12 drumsticks in each group were randomly selected immediately after

10 min surface decontamination (day 0), weighed and used for microbiological and sensory analysis. All the separately bagged drumsticks were stored at 3±1ºC until analysis time. Two of the drumsticks were removed from each group on days 4, 7, 10 and 14th of cold storage time then weighed and used for microbiological and sensory analysis.

Microbiological analyses

Each drumstick was mechanically deboned in its bag by using sterile surgical scissors and the bones were discarded. The deboned meat was trimmed and then weighed. The volume of sterile buffered peptone water BPW required to make a 10^{-1} dilution was added to bag and stomached for 2 min. Then serial decimal dilutions were prepared in BPW. Plate count agar (PCA, Oxoid CM 463) was spread plated for psychrotrophs, and the plates were incubated at 7ºC for 10 days. The same medium was also spread plated for aerobic mesophilic counts (AMC), and the plates were incubated at 30ºC for 3 days, aerobically. Violet red bile glucose agar (VRBG, Oxoid CM 485) was pour plated for *Enterobacteriaceae*, and the plates were incubated at 30ºC for 72 h, aerobically. Violet red bile lactose agar (VRBL, Oxoid CM 107) was pour plated for presumptive coliforms, and an overlay procedure applied to the plates using 15 ml of melted VRBL at 45ºC. The plates were incubated at 35ºC for 24 h, and then the typical colonies were enumerated. The VRBL was also pour plated for presumptive faecal coliforms and the plates were incubated at 44.5ºC for 1 day. Colonies that were round, red to pink, 0.5-2 mm in diameter, and surrounded by a red to pink halo on VRBL and VRBG were counted. The mean cfu/g and log cfu/g of the three replications were then calculated.

Analysis of pH and acidity of the decontamination fluids

The pH of treatment solutions was measured by pH meter (HANNA Instruments, Italy). The acidity of treatment fluids was determined by titration technique using 0.1 N NaOH.

Sensory evaluation of the samples

Sensory evaluation of the drumsticks was performed by a six member panel composed of staff from the laboratory. The same individuals took part in each evaluation, and had no information about which product was being tested. Special attention was given to the color and the odor of the product during opening of the pack and the samples were rated as acceptable or unacceptable.

Statistical analyses

Data obtained from three replicates of the study were subjected to one-way analysis of variance (ANOVA). Differences among the mean values of various treatments were determined by the Tukey's post hoc test. The significance defined at $P<0.05$. Statistical analysis were made using SPSS 9.05 program (SPSS. Inc., Chicago, III).

RESULTS

In an attempt to improve the bacteriological quality and refrigerated shelf-life of broiler meat, the effect of treatments with water, oregano, 10% sumac and 2% lactic acid was investigated using a broiler drumstick model in comparison with untreated samples. Immediately after a 10 min surface wash treatment (day 0), and at 4, 7, 10 and 14th day of cold storage, psychrotrophs, AMC, *Enterobacteriaceae*, coliforms and presumptive faecal coliforms of samples were counted.

Results of microbiological analyses

The effect of treatments on logarithmic counts of psychrotrophs, mesophilic aerobes, *Enterobactericeae*, coliforms and faecal coliforms is presented in Figure 1a, b, c, d, e.

The initial (day 0) psychrotrophs (log cfu/g) of broiler drumsticks were 4.8, 4.7, 4.8, 3.9 and 3.7 in untreated, water, oregano, sumac and lactic acid treated samples, respectively *(Fig 1a)*. By day 14 of storage, the number of psychrotrophs of lactic acid and sumac treated samples were still ca. 2 log cfu/g lower than that of other treatments.

AMC of broiler drumsticks at day 0 was 3.6, 3.7, 3.8, 2.8 and 2.6 (log cfu/g) for untreated, water, oregano, sumac and lactic acid treated samples, respectively *(Fig 1b)*.

The initial *Enterobacteriaceae* of broiler drumsticks ranged from 1 (log cfu/g) in lactic acid

and sumac treated samples to 3.4 (log cfu/g) in untreated samples *(Fig 1c)*.

The initial numbers of coliforms and presumptive faecal coliforms in broiler drumstick was lower than the detection limit (<1 log cfu/g) in sumac and lactic acid treated samples while they were 2.8, 2.6, 2.6 and 1.6, 1.5, 1.3 (log cfu/g) in untreated, water and oregano treated samples, respectively *(Fig 1d, e)*.

On each analysis day, the populations of all five bacterial groups of sumac and lactic acid treated samples remained at significantly lower levels than those of the untreated, water and oregano treated samples ($p<0.05$) *(Fig 1a, b, c, d, e)*. The most significant antimicrobial activity of sumac and lactic acid was displayed against presumptive faecal coliforms when compared with the other bacterial groups during cold storage. The presumptive faecal coliform levels of the sumac and lactic acid treated samples were still below detection limit (<1 log cfu/g) at the 14th day of cold storage whereas the results of untreated, water and

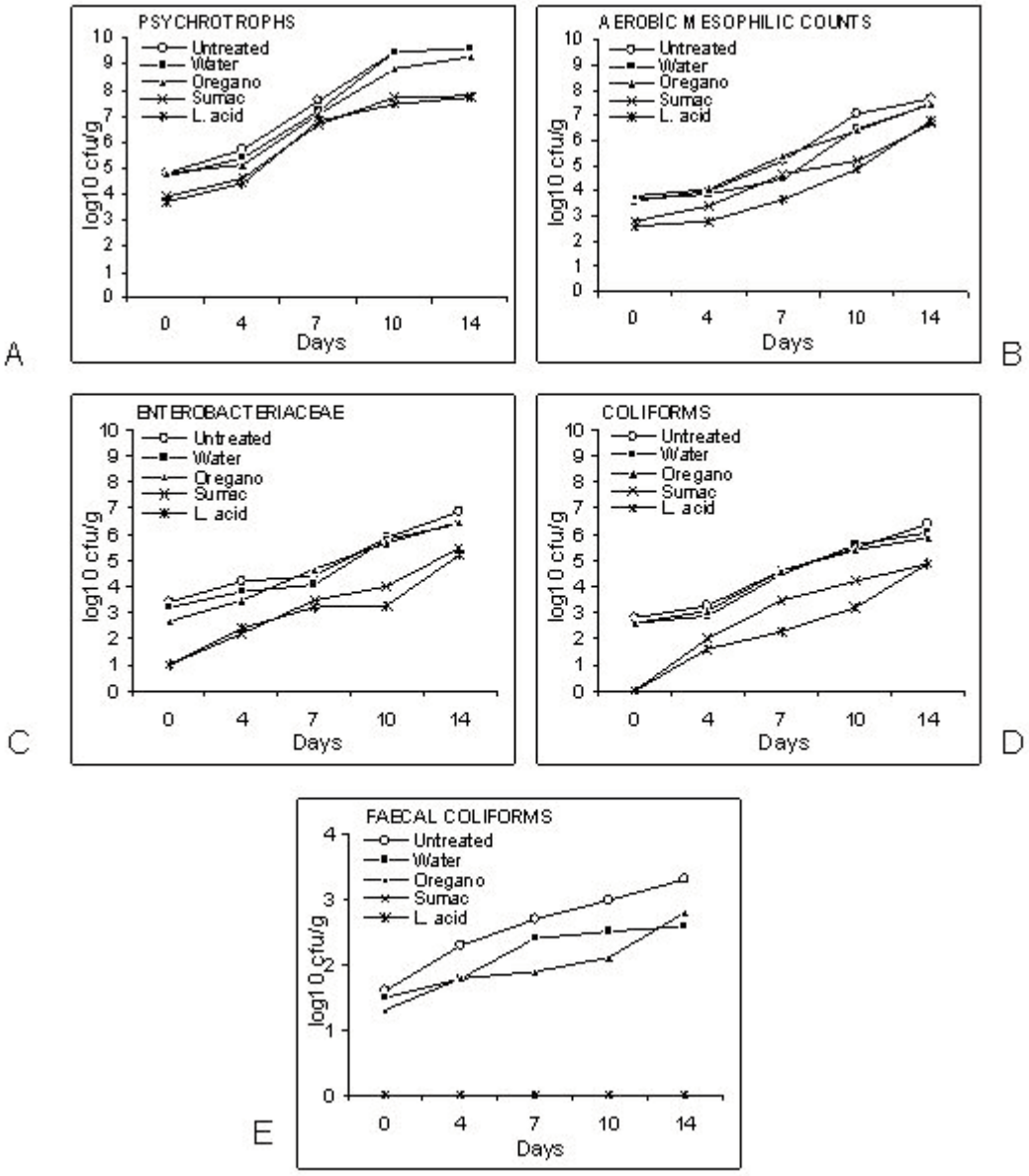

Fig 1. The separate colony counts of bacterial populations during cold storage (3±1°C). Water (sterile tap water), oregano (steam distillate of oregano, *Oreganum vulgare* L), sumac (water extract of sumac fruits, *Rhus coriaria* L, 10%, wt/vol) and lactic acid (2%, vol/vol)

Şekil 1. Bakteri gruplarının soğuk muhafaza sırasındaki (3±1°C) ayrı koloni sayımları. Su (Steril çeşme suyu), kekik (oreganonun buhar distilatı, *Oreganum vulgare* L), sumak (sumağın su ekstraktı, *Rhus coriaria* L, 10%, wt/vol) ve laktik asit (2%, vol/vol)

oregano treated samples were 2.7, 2.4 and 1.9 at the 7th day, and 3.3, 2.6 and 2.8 at the 14th day, respectively *(Fig 1e)*.

Results of pH and acidity

The pH of the oregano, sumac and lactic acid was determined as 3.4, 2.7 and 2.2, and the acidity (Lactic acid %) were determined as 0.45, 2.25 and 1.53, respectively.

Results of sensory analyses

The untreated, water and oregano treated samples had an unacceptable off odor at the 7th day of cold storage, whereas the sumac and lactic acid treated samples had not, even at 10 days. The shelf-life of the sumac and lactic acid treated samples was 3 to 5 days longer than that of the samples in the other three groups. All the panelists found that the sumac treated samples had preserved more natural and acceptable color and odor than the lactic acid treated samples. The lactic acid treated samples were found to have a marked acidulous odor and pale color compared to the control samples.

DISCUSSION

The shelf-life of chicken meat depends on the level of its microbial decontamination. Therefore enhancing the storage quality of chicken carcasses by reducing spoilage-causing microorganisms is a very important objective of food technologists and microbiologists [16,17]. Lactic acid is one of the most widely studied of the organic acids currently used in the meat industry [18]. Surface treatment of chilled poultry with lactic acid is known to reduce initial bacterial counts and to causes a delay in the start of the logarithmic phase of their growth [3]. In this study, although lactic acid appeared to achieve the most effective decontamination of the drumsticks, there was no statistical difference between lactic acid and sumac ($p>0.05$). Sumac was as effective as lactic acid in prolonging the refrigerated shelf life of raw poultry. However, the pale color and acidulous odor which developed in the lactic acid treated drumsticks were not present in the sumac treated samples. Such undesirable effects of lactic acid on meat surfaces at decontamination doses have been demonstrated by many other researchers [19-21]. The color of the sumac treated drumsticks was determined to be superior to that of the other treatment groups. This advantage of sumac may be important for poultry processors and for consumers.

Although counts of Enterobacteriaceae alone appeared to represent the effectiveness of such a decontamination technique in terms of the safety and shelf-life of poultry meat, more detailed studies need to be conducted on this subject. Although sumac appeared to be as effective as lactic acid against hygiene indicator bacteria, including Enterobacteriaceae and faecal coliforms in this study, additional studies should be conducted on the decontamination of primary foodborne pathogenic bacteria using sumac at different stages of processing.

Scientists reviewed and investigated the antimicrobial properties of the essential oils of spices, including oregano [8,9]. As documented in the review, oregano is one of the leading source of antimicrobial essential oil [8]. Thus, we compared two of the best known natural and safe antimicrobial sources, oregano and sumac, with lactic acid, the most accepted commercial natural antimicrobial. Some kinds of extract of oregano, such as hydrodistillate, have been reported to have effective antimicrobial properties [22,23]. The oregano has also been found to be a good antimicrobial substance in *in vitro* studies [24,25]. However, in this study oregano did not prove to be an effective decontaminant of the drumsticks. This may be due to differences in the materials or the method used for decontamination. There were no differences between the effects of water and oregano on the decontamination and shelf-life of the samples. Burt [8] also indicated that essential oils which were well in *in vitro* studies could be less effective in foods.

The use of certain spice hydrosols as antimicrobial agents may be exploitable to prevent the bacterial deterioration of stored foods, as long as the taste impact is acceptable in the targeted foods [25]. Sumac may be an alternative decontaminant to use by poultry processors, since it seems to be superior to lactic acid in terms of the color and odor developed on the surface of the poultry meat.

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