The Effect of Some Natural Antimicrobial Substances on the Shelf Life of Beef

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ABSTRACT

This study compares the effect of natural and artificial antimicrobial substances (nisin, lysozyme, lactic acid, trisodium phosphate, cetylpyridinium chloride and acidified sodium chloride) with herbal extracts (*Origanum onites, Syzygium aromaticum, Rhus coriaria L.* and *Hibiscus sabdariffa L.*) on certain pathogenic microorganisms and investigates the feasibility of using trisodium phosphate, nisin, oregano and hibiscus to extend the shelf life of beef. The essential oils of oregano and clove demonstrated the strongest antimicrobial effect on *Listeria monocytogenes*, *Yersinia enterocolitica O3*, *Salmonella enteritidis* and *Staphylococcus aureus,* followed by water extracts of hibiscus and sumac. No statistical difference between the results of nisin group and physiological saline solution group with control group was found during beef shelf life trials (P>0.05). In addition, oregano and trisodium phosphate extended the shelf life by 3 days, and hibiscus by 8 days. It was concluded that hibiscus was a significant antimicrobial agent, but that it was not suitable for organoleptic reasons because using it directly on meat caused discoloration. The essential oil of oregano, on the other hand, could be used as an alternative to existing chemical decontaminants in order to extend shelf life and decontaminate beef.

Keywords: Beef, shelf life, *Hibiscus sabdariffa*, Oregano.

INTRODUCTION

Meat has a wide range of nutrients and is a food preferred by many humans around the world as a source of animal protein. However, meat is conducive to the reproduction of many microorganisms, and as a result it spoils very easily. It is also sensitive to spoiling chemical and enzymatic activity. The decomposition of the fat, protein and carbohydrates contained in meat result in odors, flavors and appearance that is unpleasant, making it unsuitable for human consumption. Therefore, meat spoilage must be controlled in order to maintain nutritional value, texture and taste and to extend the shelf life (1).

As supermarkets rapidly expand and develop, it is extremely important that meat be capable of being transported long distances without spoiling or losing characteristics like color, texture and nutritional value (2). In addition, consumers demand for natural, delicious and highly nutritious foods has encouraged many researchers to examine new preservative technologies, which has resulted in traditional approaches to preservation being replaced today by bio-preservatives and non-thermal techniques. Furthermore, the use of chemical preservatives is still drawing attention (3, 4, 5).

In this study, the *in vitro* effect was determined on selected pathogenic bacteria by essential oils (*Origanum onites, Syzygium aromaticum*) of some plants used as spices, water extracts (*Rhus coiaria L., Hibiscus sabdariffa L.*), the natural

antimicrobial agents such as lysozyme, nisin and lactic acid (LA) beside synthetic antimicrobial constituents: trisodium phosphate (TSP), cetylpyridinium (CPC) and acidified sodium chloride (ASC). Thereafter, the effect of oregano oil, hibiscus infusion, nisin and trisodium phosphate (TSP) upon extending potential of shelf-life of beefs in cold storage (4±1˚C) was investigated.

MATERIAL AND METHODS

Bacteria cultures

The following cultures were obtained from the Refik Saydam Culture Collection (RSKK) Center (Ankara, Turkey) for use in the study: *Listeria monocytogenes* (RSKK 475), *Yersinia enterocolitica O3* (RSKK 920), *Salmonella* Enteritidis (RSKK 538), *Staphylococcus aureus* (RSKK 25923).

Meat samples

The cattle beef (*musculus longissimus dorsi*) which was cut in the abattoir and offered for sell to a commercial enterprice (in Kars, Turkey) was brought to the laboratory under cold chain conditions. Thereafter aseptic conditions were adhered too. The beef was used by cutting to slices of approximately 1 cm thicknesses and 100 g in weight.

Table 1: Preparation of antimicrobial solutions

Solution	Preparation
Hibiscus extract	50 ml of tap water at 90-100˚C was added to 5g of hibiscus flower. This was kept enclosed at 20±1˚C for half an hour and then drained.
Sumac extract	50 ml of tap water at 90-100˚C was added to 5 g of sumac fruit. This was kept enclosed at 45±1˚C for half an hour. The individual pieces were crushed and then it was drained. The drained liquid was pasteurized for one minute at 85˚C.
Essential oil of clove	500 ml of distilled water was added to 50 g of the ground herb and then placed in a Clevenger device. The oils obtained after three hours of distillation were put into dark-colored enclosed bottles and kept in a refrigerator at 4˚C until they were used in the trials. In the trials, they were used at a concentration of 1.5% in distilled water with 0.1% Tween.
Essential oil of oregano	The oregano EO (*O. onites*) was provided by Türer Tarım Ltd.
Lysozyme	500 mg/l in distilled water
Nisin	1000 IU/ml in 0.02 N HCl
LA	2% in distilled water
TSP	12% in distilled water
CPC	0.5% in distilled water
ASC	12% in 0.9% citric acid

LA: lactic acid, TSP: trisodium phosphate, CPC: cetylpyridinium, ASC: Acidified sodium chloride.

Antimicrobial compounds

In previous studies, the essential oils of some spices have been shown to have a significant antimicrobial effect while for other studies spices water extracts were effective (6, 7). This study used the essential oils of the herbs oregano (*Origanum onites)* and clove (*Syzygium aromaticum*) and the water extracts of the herbs sumac (*Rhus coriaria L.)* and fruits and hibiscus (*Hibiscus sabdariffa L.*) flowers. Clove, sumac and hibicus plants were obtained from a spice market in Kars (Bağdat Ticaret Gıda Limited Şirketi Gimat Toptancılar Sitesi, Ankara, Turkey). The oregano EO (*O. onites*) was provided by Türer Tarım Ltd., Şti. (Türer Tarım ve Orman Ürünleri İthalat İhracat Sanayii ve Ticaret Limited Şirketi, Kavaklıdere Köyü, Bornova, İzmir, Turkey). The results of previous studies and the amounts which can be used legally were taken into consideration in determining the concentrations of the decontaminant solutions. Table 1 describes the methods by which the antimicrobial solutions were prepared.

Determination of antimicrobial activity

Each of the bacteria cultures were inoculated in Brain Heart Infusion Broth (BHI, Oxoid CM 225, Basingstoke, UK) and incubated for 18 hours at 30˚C. Active cultures with bacterial density of at least $1x10^6$-$1x10^7$cfu/ml were used. In order to determine the antimicrobial effects of the herbal extracts on test microorganisms, 10 µl of 18-hour culture was added to each test tube containing 5 ml of antimicrobial agent while the control group contained 5 ml of physiological saline (PS). The test tubes were left at room temperature for 5 minutes, 1 hour and 24 hours to determine antimicrobial efficacy. At the end of this period, decimal dilutions of each test tube were prepared, and parallel inoculations were made into specific media to identify the microorganisms. Inoculation was performed using the spread method with LSA (Listeria Selective Agar Base, Oxoid CM 856, Basingstoke, UK) for *L. monocytogenes*, YSA (Yersinia Selective Agar Base, Oxoid CM 653, Basingstoke, UK) for *Y.enterocolitica O3*, BGA (Brilliant Green Agar, Oxoid CM 329B, Basingstoke, UK) for *S. enteritidis*, and BP (Baird Parker Agar Base, Oxoid CM 0275+ Egg Yolk Tellurite Emulsion Oxoid SR 0054, Basingstoke, UK for 24 hours at 37˚C) for *S. aureus*. LSA,

BGA and BP plates were incubated at 37˚C and YSA plates at 30˚C for 48 hours.

Shelf Life Trials

Essential oil of oregano and hibiscus infusion, as well as nisin and TSP were used in the shelf life trials. The essential oil of oregano was sprayed onto the meat while immersion was used for the infusion fluids. A total of six trial groups were prepared for the study. The control group consisted of meat samples that had not been processed in any way. One meat sample (1 cm thick weighing about 100 grams) was placed in each dish of polystyrene foam with an absorbing pad. It was then covered with polyethylene film and put in cold storage at 4˚C. Ten meat samples for the PS group were put in sterile bags that contained PS (200 ml) and after five minutes of this treatment, the meat samples were removed from the liquid and drained as much as possible. Then, they were packaged in the same way as the control group and put in cold storage at 4˚C. The same experimental arrangement set up for PS was prepared, but instead of PS a nisin group was formed using a nisin solution (1000 IU/ml), a TSP group using a TSP solution (12%) and a hibiscus group using a hibiscus infusion (10%). In the oregano group, application was made by spraying instead of immersion because essential oil of oregano was used to prevent the essential oils from having a negative effect on the smell and taste of the meat as they are more intense than the infusion solutions. Besides, methods of obtaining the essential oil are difficult and costly. After 5 ml of essential oil of oregano with a concentration of 1.5% was sprayed on absorbent pads in distilled water with 0.1% Tween 80, one meat sample was placed on each pad, and then they were packaged in the same way as the control group and put in cold storage (4±1˚C). Microbiological, chemical and organoleptic analysis was conducted on all of the groups on the following days of cold storage: 0, 3, 7, 8, 9, 10, 11, 12 and 17 days.

Table 2: Organoleptic evaluation form

Evaluation Criteria		Scoring: 1: Poor 9: excellent
The aroma arisen after addition of antimicrobial agent to the meat		
The effect of antimicrobial agent to the natural color of the meat		
Signs of spoiling or putrefaction in meat	Odor of the meat	
	Color of the meat	
	Stickiness of meat	
	Structural degeneration	
Effect of the antimicrobial agent on the general organoleptic characteristics of the meat		

Microbiological Analysis

Ten grams of the samples were weighed out under aseptic conditions and placed in sterile "Stomacher" bags for the microbiological analysis. Then, 90 ml of sterile physiological saline was added and homogenized for two minutes. After decimal dilutions of the samples had been prepared, they were inoculated on media specific to that group of microorganisms using the spread and pour plate techniques. Incubation was performed as follows: for total mesophilic aerobic bacteria, Plate Count Agar (PCA, Oxoid CM 325, Basingstoke, UK) for 48 hours at 30˚C; for total psychrotrophic aerobic bacteria, Plate Count Agar (PCA, Oxoid CM 325, Basingstoke, UK) for 10 days at 7˚C; for *Pseudomonas spp.,* Pseudomonas Agar Base (Oxoid CM 559,Basingstoke, UK) and C-F-C Supplement (Oxoid SR 103, Basingstoke, UK) for 48 hours at 30˚C; for lactic acid bacteria (LAB), de Man Rogosa Sharpe Agar (MRS, Oxoid CM 361, Basingstoke, UK) for 3-5 days at 30˚C; for *Enterobacteriaceae,* Violet Red Bile Glucose Agar (VRBG, Oxoid CM 485, Basingstoke, UK) for 48 hours at 35˚C; for coliform group bacteria, Violet Red Bile Lactose Agar (VRBL, Oxoid CM 107, Basingstoke, UK) for 24 hours at 37˚C; for Fecal Coliform Group Bacteria, Violet Red Bile Lactose Agar (VRBL, Oxoid CM 107, Basingstoke, UK) for 24-48 hours at 44.5˚C; for *S. aureus*, Baird Parker Agar (BP Oxoid CM 0275+ Egg Yolk Tellurite Emulsion Oxoid SR 0054, Basingstoke, UK) for 24 hours at 37˚C; for Enterococci, Slanetz and Bartley Agar (SB, CM 0377, Basingstoke, UK) for 24 hours at 37˚C; for *Brochotrix thermosphacta*, STAA Agar (Oxoid CM0881, Basingstoke, UK) for 24-48 hours at 25˚C (8, 9).

Physicochemical Analysis

The Eber's reagent was used to qualitatively determined spoilage of the samples (10, 11). Two to three ml of prepared Eber's reagent was added to test tubes. A meat sample about the size of a chickpea (1x1cm) was inserted into the test tube using forceps and kept there for few seconds without coming into contact with the reagent. The emis-

Table 3 : Antibacterial efficacy of the trial groups against *Y. enterocolitica*

Period	Bacteria counts (log cfu/ml) (mean ± SD) in the trial groups										
	Control	TSP	CPC	LA	ASC	Nisin	Lysozyme	Oregano	Clove	Sumac	Hibiscus
5th minute	6.36±0.01[a]	<1[b]	<1[b]	<1[b]	3.60±0.09[c]	<1[b]	6.32±0.13[a]	<1[b]	<1[b]	4.12±0.07[d]	<1[b]
1st h	6.58±0.01[a]	<1[b]	<1[b]	<1[b]	2.55±0.07[c]	<1[b]	4.58±0.23[d]	<1[b]	<1[b]	<1[b]	<1[b]
24th h	6.87±0.01[a]	<1[b]	<1[b]	<1[b]	<1[b]	<1[b]	3.51±0.16[c]	<1[b]	<1[b]	<1[b]	<1[b]

Table 4: Antibacterial efficacy of the trial groups against *S. aureus*

Period	Bacteria counts (log cfu/ml) (mean ± SD) in the trial groups										
	Control	TSP	CPC	LA	ASC	Nisin	Lysozyme	Oregano	Clove	Sumac	Hibiscus
5th minute	6.87±0.01[a]	4.26±0.08[b]	<1[b]	<1[b]	6.60±0.28[d]	3.47±0.29[e]	6.73±0.40[ad]	<1[b]	<1[b]	3.76±0.13[f]	6.69±0.04[ad]
1st h	6.91±0.01[a]	<1[b]	<1[b]	<1[b]	4.79±0.12[c]	<1[b]	6.50±0.04[a]	<1[b]	<1[b]	3.47±0.12[d]	4.73±0.11[c]
24th h	8.12±0.04[a]	<1[b]	<1[b]	<1[b]	1.82±0.27[c]	<1[b]	4.69±0.32[d]	<1[b]	<1[b]	<1[b]	<1[b]

Table 5: Antibacterial efficacy of the trial groups against *L. monocytogenes*

Period	Bacteria counts (log cfu/ml) (mean ± SD) in the trial groups										
	Control	TSP	CPC	LA	ASC	Nisin	Lysozyme	Oregano	Clove	Sumac	Hibiscus
5th minute	7.02±0.02[a]	0.94±0.87[b]	<1[b]	<1[b]	6.75±0.17[a]	3.44±0.09[d]	6.90±0.08[a]	<1[b]	<1[b]	<1[b]	6.70±0.36[a]
1st h	7.11±0.01[a]	<1[b]	<1[b]	<1[b]	5.45±0.24[c]	<1[b]	6.53±0.06[d]	<1[b]	<1[b]	<1[b]	5.17±0.13[e]
24th h	6.14±0.01[a]	<1[b]	<1[b]	<1[b]	2.34±0.24[c]	<1[b]	<1[b]	<1[b]	<1[b]	<1[b]	<1[b]

Table 6: Antibacterial efficacy of the trial groups against *S. typhimurium*.

Period	Bacteria counts (log cfu/ml) (mean ± SD) in the trial groups										
	Control	TSP	CPC	LA	ASC	Nisin	Lysozyme	Oregano	Clove	Sumac	Hibiscus
5th minute	6.61±0.01[a]	<1[b]	<1[b]	<1[b]	6.58±0.11[a]	<1[b]	6.60±0.02[a]	<1[b]	<1[b]	4.83±0.08[c]	<1[b]
1st h	6.53±0.04[a]	<1[b]	<1[b]	<1[b]	6.56±0.23[a]	<1[b]	6.55±0.03[a]	<1[b]	<1[b]	<1[b]	<1[b]
24th h	6.71±0.02[a]	<1[b]	<1[b]	<1[b]	2.87±0.06[c]	<1[b]	4.40±0.22[d]	<1[b]	<1[b]	<1[b]	<1[b]

[abcd:] Those with a different superscript from the average on the same line were statistically different ($p<0.05$)
LA: Lactic acid; TSP: trisodium phosphate; CPC: cetylpyridinium; ASC: Acidified sodium chlorite

sion of smoke was accepted as a positive reaction (12). The pH values of meat homogenates from each group were measured using a pH meter (Thermo-Orion 3 Star, Thermoscıentıfic-Singapore).

Organoleptic Analysis

Organoleptic analyses were performed by four female individuals of 30 to 35 years of age who were working in department of food hygiene and technology. The criteria used as the basis of the organoleptic assessment and the rating system are provided in Table 2. A nine-point hedonic scale (1: poor; 9: excellent) was used in the evaluation (13).

Statistical Analysis

Antimicrobial efficacy trials were repeated at five different times, shelf life trials were repeated three times and their averages were calculated and transformed to $\log_{10}$ base. The data was analyzed using one-way analysis of variance (ANOVA). Test of homogeneity of variances was carried out. The LSD (Least Significant Difference) test was used to determine difference between the groups. Statistical analyses were conducted with the SPSS (SPSS 16.0, Inc., Chicago, IL, USA) package program. P value< 0.05 was considered to be statistically significant.

RESULTS

Antimicrobial efficacy trials

The *in vitro* antimicrobial efficacy of natural and artificial antimicrobial agents against four tested pathogens was determined at different intervals. The *in vitro* antibacterial efficacy of the antimicrobial agents against the test bacteria are provided in Tables 3, 4, 5 and 6. The TSP, CPC, LA and nisin

solutions tested at the end of the five-minute waiting period applied during the trials completely inhibited strains of *Y. enterocolitica* and *S. typhimurium.* While CPC and LA solutions completely inhibited strains of *S. aureus* and *L. monocytogenes,* TSP and nisin achieved a significant reduction in *S. aureus* and *L. monocytogenes* counts, demonstrating a significant difference over the control group ($p<0.05$). Lysozyme, however, was not effective against any of the tested strains ($p>0.05$). ASC, on the other hand, reduced the counts of *Y. enterocolitica* and *S. aureus* strains ($p<0.05$), but had no significant antimicrobial effect against *L. monocytogenes* and *S. typhimurium* ($p>0.05$).

When the antimicrobial efficacy of herbal extracts was evaluated, the essential oils of oregano and clove had a significant antimicrobial effect on all of the bacteria tested at the end of the 5-minute waiting period ($p<0.05$). While the sumac infusion completely inhibited the *L. monocytogenes* bacteria, a significant reduction was also observed with the other test bacteria ($p<0.05$). The hibiscus infusion had a significant antimicrobial effect against *Y. enterocolitica* and *S. typhimurium* bacteria ($p<0.05$). However it was not effective against *S. aureus* and *L. monocytogenes* in the first 5 minutes ($p>0.05$), but was effective at the end of 1 and 24 hours periods ($p<0.05$).

Beef shelf life trials

The results of microbiological analysis conducted on different days for the six groups, which included the control group and the PS group, are provided in Figures 1-8.

On the first day (day 0) total mesophilic bacteria count in the control group nisin and TSP samples was determined to be 3.63 log cfu/g, whereas the hibiscus and oregano groups were found to be under the detectable limit (<1 log cfu/g) ($P<0.01$). On day seventeen of cold storage, the total mesophilic bacteria count was lowest in the hibiscus group 4.82 log cfu/g), while the control group, nisin, TSP and oregano groups were 11.42, 11.19, 8.31, and 8.40 log cfu/g, respectively.

On the initial day (day 0) total psychrotrophic bacteria count in the control group, nisin and TPS was 2.75 log cfu/g, TSP, whereas in the hibiscus and oregano groups it was found to be below the detectable limit (<1 log cfu/g) ($P<0.01$). On day 17 of cold storage, the total psychrotrophic bacteria count of the meats treated with hibiscus was found to be 4.50 log cfu/g. On the first day (day 0) *Pseudomonas spp.* Total counts in the control group was 2.50 log cfu/g, and the other groups were found to be under the detectable limit (<1 log cfu/g) ($P<0.01$). In samples from the hibiscus group on day 17, the *Pseudomonas spp.* total count was 4.43 log cfu/g while samples from the control, nisin, TSP and oregano groups were 11.15, 10.95, 8.10 and 8.20 log cfu/g respectively.

On the first day (day 0) lactic acid bacteria (LAB) count was found to be below the detectable limit (<1 log cfu/g) in all groups including the control group. In samples from the control and nisin group on day 17, the LAB total count was 9.53 and 9.41 log cfu/g while samples from the

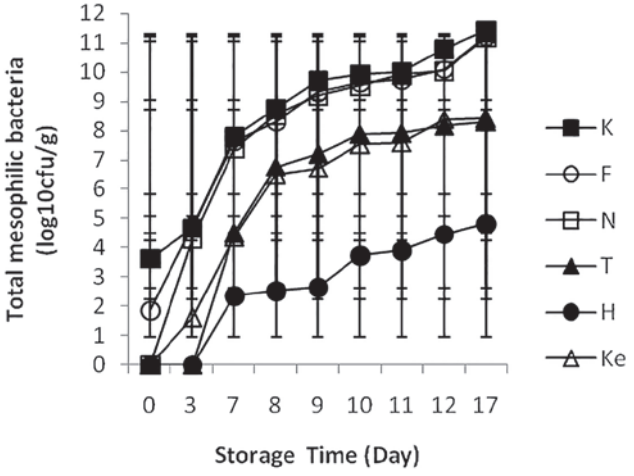

Figure 1: Total mesophilic bacteria count (log 10 cfu/g) during cold storage (4±1°C).

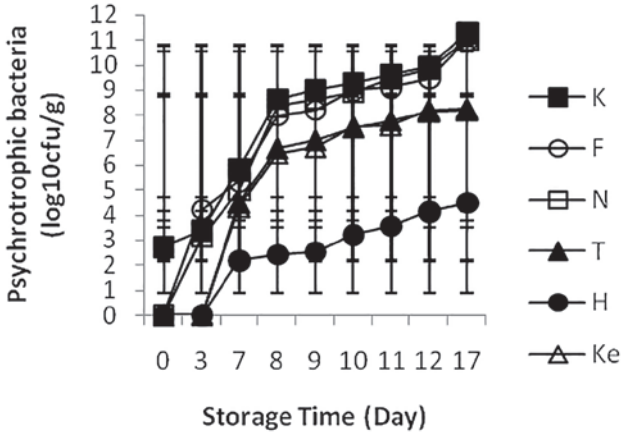

Figure 2: Psychrotrophic bacteria count (log 10 cfu/g) during cold storage (4±1°C).

K: control group; H: hibiscus group; N: nisin group; T: trisodium phosphate group; Ke: oregano group; F: physiological saline group

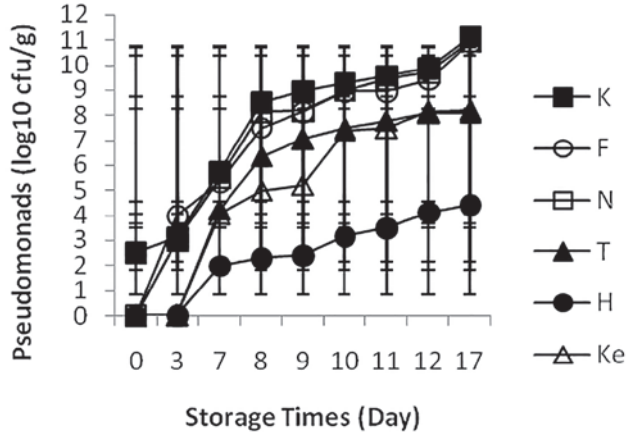

Figure 3: *Pseudomonads* bacteria count (log 10 cfu/g) during cold storage (4±1˚C).

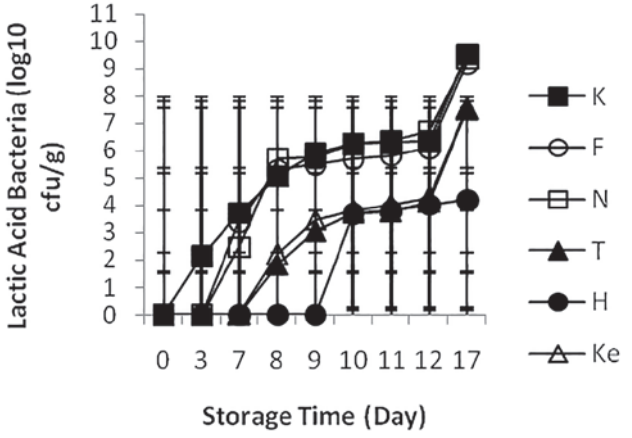

Figure 4: Lactic acid bacteria count (log 10 cfu/g) during cold storage (4±1˚C).

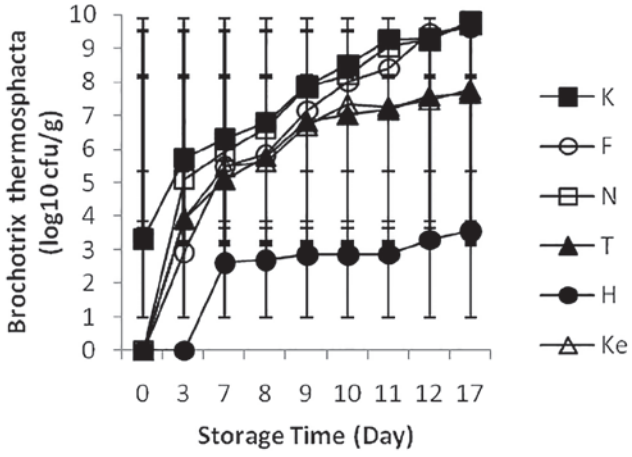

Figure 5: *Brochotrix thermosphacta* bacteria count (log 10 cfu/g) during cold storage (4±1˚C).

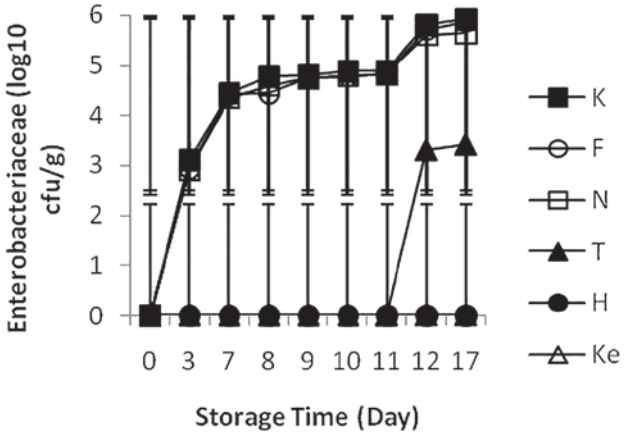

Figure 6: *Enterobacteriaceae* bacteria count (log 10 cfu/g) during cold storage (4±1˚C).

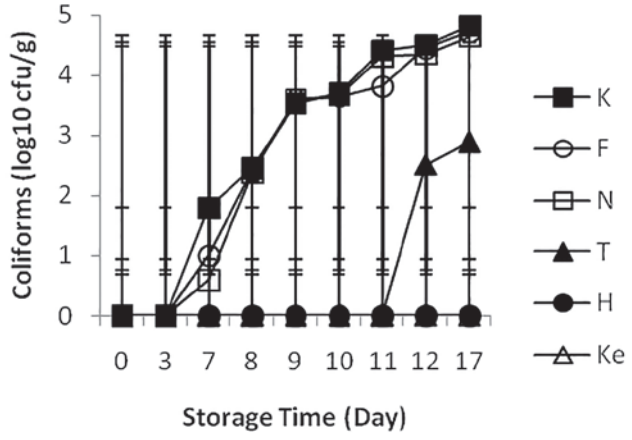

Figure 7: Coliform bacteria count (log 10 cfu/g) during cold storage (4±1˚C).

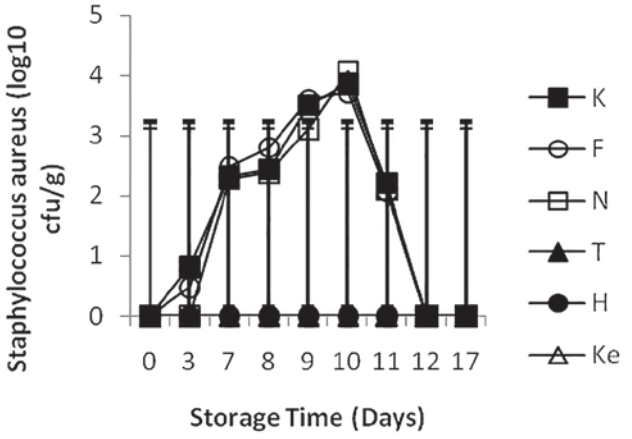

Figure 8: *Staphylococcus aureus* bacteria count (log 10 cfu/g) during cold storage (4±1˚C).

K: control group; H: hibiscus group; N: nisin group; T: trisodium phosphate group; Ke: oregano group; F: physiological saline group

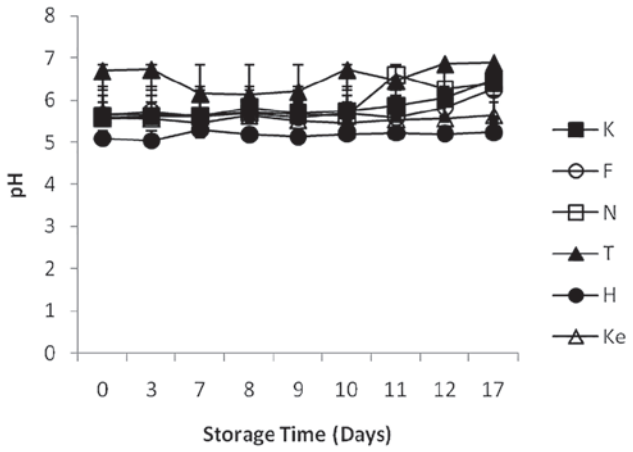

Figure 9: pH values during cold storage (4±1°C).
K: control group; H: hibiscus group; N: nisin group; T: trisodium phosphate group; Ke: oregano group; F: physiological saline group

TSP, the hibiscus and oregano groups were found to have total counts of 7.51, 4.20 and 7.55 log cfu/g respectively (P>0.05). On the first day (day 0) *B. thermosphacta* the total count in the control group was 3.32 log cfu/g, and in the other groups it was below the detectable limit (<1 log cfu/g). On the initial day (day 0) *Enterobacteriaceae* total count was found to be below the detectable limit (<1 log cfu/g) in all groups. In samples from the control and nisin group, the *Enterobacteriaceae* total count was 5.92 and 5.64 log cfu/g respectively on day 17. In samples from the TSP group, the total count was 3.41 log cfu/g, but it was below the detectable limit (<1 log cfu/g) in the hibiscus and oregano groups, resulting in a significant difference compared to the other groups (P<0.01). On the first day (day 0) coliform counts were under the detectable limit (<1 log cfu/g) in all groups just as was the case with *Enterobacteriaceae*. On the initial day (day 0), day 12 and day 17 *S. aureus* counts were under the detectable limit (<1 log cfu/g) in all groups. No microorganisms from the fecal coliform or enterococci groups could be isolated in any of the control or trial groups.

Physicochemical Changes

According to the results of the Eber's test conducted on the groups, the meat samples in the control, PS and nisin groups were spoiled on day 7, the meat samples from the TSP and oregano groups on day 11. The test results conducted on day 17 for the hibiscus group were negative. The pH values of the samples treated with TSP and hibiscus differed significantly from that of the control group (p<0.01). The pH values of the samples were recorded for the duration of cold storage and are presented in Figure 9.

Organoleptic Analysis

When the effect of the antimicrobial substances used in the groups was examined, TSP, hibiscus and nisin did not affect the odor of the meat at all, while in the oregano group, the specific aroma of oregano was intense, especially during the first days of storage. In subsequent days, oregano oil was almost non-detectable because of its volatile nature and was judged to have no effect that would bother the consumer. An examination of the effect that the antimicrobial substances used in the groups had on the color of the meat showed that TSP, nisin and oregano did not affect the color of the meat, but in the hibiscus groups, the pale reddish color of hibiscus was pronounced at the beginning but towards the end of the storage period, the meat samples developed a dark red or purple color. On the eighth day of storage, spoilage and putrefaction had commenced in the control, PS and nisin groups. There was discoloration and the meat samples which developed a sticky surface. Samples from the nisin group were spoiling on day 8 and on day 12 in the TSP and oregano groups while the hibiscus group showed no evidence of spoilage even on day 17.

DISCUSSION

This study examined the effectiveness of some natural and artificial antimicrobial substances (nisin, lysozyme, lactic acid, trisodium phosphate, cetylpyridinium chloride and acidified sodium chloride) as well as some herbal extracts (*Origanum onites, Syzygium aromaticum, Rhus coriaria L.* and *Hibiscus sabdariffa L.*), consumed as spices or teas, as antibacterial agents against food-based pathogenic microorganisms. The feasibility of using hibiscus infusion, nisin, trisodium phosphate and essential oil of oregano, which all had a significant antimicrobial effect, to extend the shelf life of beef were deemed promising and the study was continued in this direction. The antimicrobial effects of essential oils from herbs have been examined in several *in vitro* studies (3, 7, 14). From this research, it was clear that very different results could be obtained. These differences may be attributed to the type of essential oil, its composition and concentration, the type and number of microorganisms it affects, the composition of the substrate and storage conditions (15).

In this study, 1.5% concentration of oregano and 1.5% concentration of clove oil had a significant antibacterial effect against all of the tested bacteria. Hammer *et al*. (14), reported that only clove oil in concentrations > 2% was effective against *S. typhimurium* and against *S. aureus* bacteria at concentrations < 2%. Baydar *et al*. (7), reported that even 0.5-1% concentrations of oregano oil were quite effective against pathogens. This study found that the hibiscus water extract inhibited all of the test bacteria at the end of the 24-hour period. Wong *et al*. (16), reported that hibiscus extract had a broad spectrum effect against Gram positive and Gram negative bacteria, and that it was important for future research as a natural antimicrobial agent. It has been claimed that TSP is more effective against Gram negative pathogens like *Salmonella*, *Campylobacter* and *E. coli* than it is against Gram positive bacteria such as *L. monocytogenes* (17). In this *in vitro* study, it exhibited a significant effect against Gram negative bacteria (*Y. enterocolitica* and *S. typhimurium*), inhibiting them completely at the end of a five-minute period. A study that examined the antimicrobial effect of nisin in beef showed that it provided an initial reduction but that its effect weakened over time (18, 19). On its own, nisin failed to provide effective preservation, but it is said to be more effective when combined with other compounds. Govaris *et al.* (20), reported that nisin at a rate of 500 and 1000 IU/g had no effect against *S. enteritidis* in minced sheep meat, but that it was effective when combined with 0.9% oregano oil. In this study, nisin reduced the initial microorganism count but was not effective on days 3, 5, 7, 9, 11, 12 and 17 of storage.

Pohlman *et al*. (21) reported that a 10% concentration of TSP reduced the total bacteria count in beef by 0.61 log. Özdemir (22) reported that TSP achieved a reduction of approximately 1 log in the total bacterial count in the skin of chicken breasts on day 0 compared to the control group and that the *Pseudomonas spp.* and *enterobacteriaceae* count was generally below the detectable limit (< 2.0 $\log_{10}$ cfu/g) for the duration of the storage period compared to the control group. In this study, however, the initial total mesophilic and psychrotrophic count in the control and PS groups was 3.63 and 1.85 log cfu/g respectively, but it was below the detectable limit (< 1 cfu/g) in the group treated with TSP. There are differences between the results obtained from other research, and the results of this study, and it was considered that the reason for this difference may be due to the initial microbial load and application methods. After all, it has been reported that the effect of TSP varies depending on the concentration of the solution, temperature, length and manner of treatment (23, 24).

In this study, the initial total mesophilic bacteria count in the samples treated with 1.5% oregano oil was beneath the detectable limit (< 1 cfu/g), but in the samples from the control and PS groups, this number was 3.63 and 1.85 log cfu/g respectively. A number of studies have been conducted on the ability of essential oil of oregano to extend the shelf life of food, and most of them have obtained successful results in terms of its microbial effect (9, 25, 26, 27).

This study found that water extract of hibiscus had the most significant effect on the shelf life of beef due to its significant antimicrobial characteristics. Even on day 17 of the storage period, total mesophilic bacteria, total psychrotrophic, *Pseudomonas spp.,* LAB and *B. thermosphacta* counts were 4.82, 4.50, 4.43, 4.20 and 3.57 log cfu/g respectively and the *Enterobacteriacea,* coliform and *S. aureus* counts were below the detectable level (< 1 cfu/g).

As a result of the organoleptic analysis, it was determined that the smell of oregano was intense in the first few days but in subsequent days decreased so that it would not be objectionable. Even though hibiscus had a significant antimicrobial effect, the organoleptic characteristics of the meat samples in the hibiscus group were found unacceptable due to their color. The water extract of hibiscus (*H. sabdariffa L.*) is consumed around the world either as a hot tea or a cold drink. It is also used as a food coloring in the production of Roselle jelly (28). Hibiscus extract is said to be very effective against Gram positive and Gram negative bacteria (16, 29). In this study, the hibiscus water extract exhibited a significant inhibitory effect both *in vitro* and in shelf life trials. Shelf life was determined to be 8 days in the control group and 17 days for samples from the hibiscus group.

In the light of the results obtained, it was concluded that essential oil of oregano extended shelf life significantly. The water extract hibiscus was found to be promising as a natural antimicrobial agent that could be an alternative to synthetic food additives that extend shelf life and consequently improve the microbial quality of meat if the negative discoloration it causes in meat can be eliminated. The possibility that the color problem that hibiscus causes in fresh meat might not be noticeable in meat products such as salami, sausages and wieners and there is a need for detailed research of this issue.

REFERENCES

1. Dave, D., and Abdel, E. G.: Meat spoilage mechanisms and preservation techniques: A critical review.Am. J. Agri. & Biol. Sci. 6: 486-510, 2011.
2. Nychas, G.J.E., Skandamis, P.N., Tassou, C.C. and Koutsoumanis, K.P.: Meat spoilage during distribution. Meat Sci. 78: 77-89, 2008.
3. Burt, S.: Essential oils: their antibacterial properties and potential applications in foods-A review. Int. J. Food Microbiol. 94: 223-253, 2004.
4. Devlieghere, F., Vermeiren, L. and Debevere, J.: New preservationtechnologies: possibilities and limitations. Int. Dairy J.14: 273-285, 2004.
5. Zhou, G. H., Xu, X. L. and Liu, Y.: Preservation technologies for fresh meat-A review. Meat Sci. 86:119-128, 2010.
6. Aksoy, A., Güven, A. and Gülmez, M.: Decontamination and shelf-life potential of some herbal ınfusions and hydrodistillates on chicken meat. Kafkas Üniv. Vet. Fak. Derg. 17: 137-143, 2011.
7. Baydar, H., Sağdıç, O., Özkan, G. and Karadoğan, T.: Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey. Food Cont. 15: 169-172, 2004.
8. Harrigan, W. F.: Laboratory methods in food microbiology, (4th ed.). Academic press. California, USA, 1998.
9. Chouliara, E., Karatapanis, A., Savvaidis, I. N. and Kontominas, M. G.: Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat stored at 4 °C. Food Microbiol. 24: 607-617, 2007.
10. Tanikawa, E., Akiba, M. and Motohiro, T.: Studies on the manufacture of canned crab. III. Discussion of methods for determining freshness of meat of Erimacrus isenbeckii. Bull. Fac. Fisheries, Hokkaido Univ. 4:14-22, 1953.
11. Çolak, H., Uğurluay, G., Nazlı, B. and Bingöl, E. B.: The effect of humıdıty absorbıng fılters used as packıng materıal on the shelf lıfe of turkey meat. İstanbul Üniv. Vet. Fak. Derg. 37: 107-116, 2011.
12. Vural, N.: Besin analizleri, Ankara Üniv. Ecz. Fak. Yayın no: 69 Ankara, Türkiye, 1992.
13. Patsias, A., Chouliara, I., Badeka, A., Savvaidis, I. N. and Kontominas, M. G.: Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes. Food Microbiol. 23: 423-429, 2006.
14. Hammer, K. A., Carson, C.F. and Riley, T.V.: Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985-90, 1999.
15. Skandamis, PN. and Nychas, G.-J.E.: Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pH and oregano essential oil concentrations. Appl. Environ. Microbiol. 66: 1646-1653, 2000.
16. Wong, S.K., Lim, Y.Y. and Chan, E.W.C.: Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected hibiscus species. Ethno. Leaflets. 14: 781-96, 2010.
17. Keener, K.M., Bashor, M. P., Curtis, P. A., Sheldon, B. W. and Kathariou, S.: Comprehensive review of *campylobacter* and poultry processing. Compr. Rev. Food Sci. Food Saf. 3: 105-116, 2004.
18. Cutter, C.N. and Siragusa, G. R.: Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. 11: 481–489, 1994.
19. Cutter, C.N. and Siragusa, G. R.: Growth of *Brochothrix thermosphacta*in ground beef following treatments with nisin incalcium alginate gels. Food Microbiol. 14: 425–430, 1997.
20. Govaris, A., Solomakos, N.A., Pexara A. and Chatzopoulou, P.S.: The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella* Enteritidis in minced sheep meat during refrigerated storage. Int. J. Food Microbiol. 137: 175-180, 2010.
21. Pohlman, F.W., Stivarius, M.R., McElyea, K.S. and Waldroup, A.L.: Reduction of *E. coli, Salmonella* Typhimurium, coliforms, aerobic bacteria, and improvement of ground beef color using trisodium phosphate or cetylpyridinium chloride before grinding. Meat Sci. 60: 349-356, 2002.
22. Özdemir, H.: Effects of trisodium phosphate on aerobic mesophilic bacteria, psychrotrophic bacteria, *Pseudomonas* spp. and Enterobacteriaceae on chicken breast skin. Ankara Üniv. Vet. Fak. Derg. 53: 61-63, 2006.
23. Bolder, N.M.: Decontamination of meat and poultry carcasses. Trends Food Sci. Technol. 8: 221-227, 1997.
24. Capita, R., Alonso-Calleja, C., Sierra, M., Moreno, B. and Garcia-Fernandez, M.D.C.: Effect of trisodium phosphate solutions washing on the sensory evaluation of poultry meat. Meat Sci. 55: 471-474, 2000.
25. Oral, N., Vatansever, L., Sezer, Ç., Aydın, B., Güven, A., Gülmez, M., Başer, K.H.C. and Kürkçüoğlu, M.: Effect of absorbent pads containing oregano essential oil on the shelf life extension of overwrap packed chicken drumsticks stored at four degrees celsius. Poult. Sci., 85: 1466-1471, 2009.
26. Mahmoud, B.S.M., Yamazaki, K., Miyashita, K., II-Shin, S., Dong-Suk, C. and Suzuki, T.: Bacterial microflora of carp (*Cyprinuscarpio*) and its shelf-life extension by essential oil compounds. Food Microbiol. 21: 657–666, 2004.
27. Tsigarida, E., Skandamis, P. and Nychas, G.-J.E.: Behaviourof *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essentialoil at 5°C. J. Appl. Microbiol. 89: 901–909, 2000.
28. Nman, N.M. and Onyeke, N.G.: Chemical composition of two varieties of Sorrel (*Hibiscus sabdarifa L.*), calyces and the drink made from them. Plant Foods Hum. Nutr. 58: 1-7, 2003.
29. Mamun, A., Khatun, M.H., Nesa, M.L., Islam, M.R. and Munira, M.S.: *In vitro* evaluation of the antibacterial, cytotoxic and ınsecticidal activities of *Hibiscus sabdariffa* fruits. Libyan Agric. Res. Cen. J. Intl. 2:144-149, 2011.