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The Effect of Nisin and Clove Essential Oil on Shelf Life of Beef*

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Abstract: In this study, the effects of nisin and clove essential oil on shelf life of beef were investigated in order to evaluate their antimicrobial activities. It was determined that clove essential oil has a wide spectrum on Gram (+) and Gram (-) bacteria. *While L. monocytogenes* was the most resistant bacteria among the Gram (+) bacteria, *B. thermosphacta* was found to be the most sensitive bacteria. Out of Gram (-) bacteria, *Y. enterocolitica* was the most sensitive bacteria and *P. aeruginosa* was the most resistant one. The antimicrobial activities of clove essential oil and nisin on beef were tested both separately and in combination. Although clove essential oil was successful in vitro, its activity on prolonging shelf life of beef was found to be limited (4 days). Clove essential oil alone was more effective on the shelf life than that of combinations and nisin alone. The increase in concentrations indicates an antagonistic interaction between clove essential oil and nisin.

Key words: Antibacterial effect, Beef, Clove essential oil, Nisin, *Syzygium aromaticum.*

Sığır Eti Raf Ömrü Üzerine Karanfil Uçucu Yağı ve Nisinin Etkisi

Özet: Bu çalışmada, antimikrobiyal etkinliğini değerlendirmek üzere karanfil yağı ve nisinin kırmızı etin raf ömrüne etkisi araştırılmıştır. Karanfilin Gram (+) ve (-) bakteriler üzerinde geniş bir etki spektrumuna sahip olduğu belirlenmiştir. Gram (+) bakteriler arasında *L. monocytogenes* en dirençli bakteri iken, *B. thermosphacta* en hassas bakteri olmuştur. Gram (-) bakterilerden *Y. enterocolitica* en hassas, *P. aeruginosa* en dirençli bakteridir. Kırmızı et üzerinde karanfil yağı ve nisinin tek başlarına ve kombine edilerek antimikrobiyal etkinlikleri denenmiştir. İn vitro olarak başarılı olsa da, karanfilin kırmızı etin raf ömrünü uzatmadaki etkinliği sınırlı olmuştur (4 gün). Karanfil tek başına raf ömrü üzerine kombine kullanımlardan ve nisinden daha etkili olmuştur. Konsantrasyonların yükselmesi karanfil ile nisin arasında antagonistik bir etkileşime işaret etmektedir.

Anahtar kelimeler: Antibakteriyel etki, Kırmızı et, Karanfil uçucu yağı, Nisin, *Syzygium aromaticum.*

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INTRODUCTION

Foods should be preserved against the microbial spoilage throughout the storage periods. The rising demand of customers for chemical preservative-free natural products has directed researchers' interest to protective methods for reliable foods having better nutritional and organoleptic properties with high microbial quality (Goni et al., 2009). Concerns about the safety of synthetic additives have encouraged a more detailed study of plant resources. In addition to their aromatic effects, plants are known for their antioxidative, fungicidal and antibacterial effects on foods (Ova, 2006; Bilge Oral et al., 2010).

It has been reported that clove essential oil obtained by steam distillation of dried flower buds, contains as a main ingredient eugenol and other compounds like eugenol acetate, caryophyllene and α-humelene (Ayoola et al., 2008). In many in vitro studies, the antimicrobial effect of clove water infusion and that of essential oil have been studied with different methods. When the research data are analysed, it is understood that clove has an obvious bactericidal and bacteriostatic effect (Moreira et al., 2005; Ayoola et al., 2008; Gupta et al., 2008).

The other substance used in food industry as a natural antimicrobial is nisin. This peptide that belongs to antibiotics (Breukink and De Kruijff, 1999), shows its activity against most of the Gram-positive bacteria and also spore forms of Clostridium and Bacillus (Ova, 2006; Arauz et al., 2009). Further, the recognition of nisin as safe product, it is considered as the most important commercial bacteriocin (Coma, 2008). In addition to high solubility in low pH, it is resistant to heat and has high storage stability (Ova, 2006).

Although the antimicrobial activity of essential oils derived from herbs and spice has been proved by in vitro tests, many researches are required in order to investigate their activities on food. In this study, in order to prolong shelf life of beef, the combination of clove essential oil and nisin was applied on absorbent pads to investigate the microorganisms threatening food safety and causing economic loses and the potential impact of antimicrobial capacities of them especially on pathogenic bacteria causing food poisoning.

MATERIALS and METHODS

Red Meat

In this study, *Musculus longissimus* dorsi obtained from cattle carcass stored for one day after slaughtering was used as material. The muscle sample was sliced into the portions as about 1 cm height and 100 g weight and they were treated with certain solutions to make test groups.

Reference Strains

Microorganisms used in the experiments on antibacterial effect were obtained from the isolates of a previous study (Sezer and Guven, 2009) and from Culture Collection of Refik Saydam (RSKK) Hygiene Centre. Each reference strain was inoculated into Brain Heart Infusion Broth (BHI, Oxoid CM 0225) and incubated at an appropriate temperature and atmosphere. During the experiments, the active broth cultures at 18^{th} h of their incubation were used. They were spread onto the selective agars for counting and were diluted to adjust them to 10^7 cfu/ml bacteria concentration.

Clove (*Syzygium aromaticum*) Essential Oil

Clove essential oil was obtained by steam distillation method in which clove was grinded and then 40 g of plant was mixed with 400 ml distilled water at Clevenger apparatus (Wisd Therm-Wise). The chemical composition of the oil was identified by GC-MS (Adams, 2004).

Nisin

Nisin (Maysa E 234 ™) used in experiments was dissolved at 0.002 N HCl according to desired

concentrations, sterilised with pore diameter of 0.22 µm micro filter and used as fresh without any delay.

The Minimum Inhibition Concentration (MIC) values of Clove Essential Oil and Nisin on Reference Strains

MIC values of clove oil and nisin against the bacterial cells were determined by the method of broth dilution (Nostro et al., 2001). For the clove oil groups, 1% of reference strain and clove oil at different concentrations were added to BHI broth containing 0.1% Tween 80. For Nisin groups, the stock solution of nisin at different concentrations and 1% of reference strain were added to BHI broth. Tubes were incubated at an appropriate temperature for each reference bacteria. At the ½., 2., 4. and 24. h of incubation, their inoculations were made in parallel in mediums specific for microorganism with the methods of spread and pour plate. The lowest essential oil or nisin concentration inhibiting the bacteria growth after 24 h incubation was identified as "Minimum Inhibition Concentration (MIC)". For that purpose, treatments were made for three times and the mean values were calculated.

The Effect of Clove Essential Oil and Nisin on Prolonging Shelf Life of Red Meat

Nine different groups as 1 control and 8 test ones were examined to determine the antimicrobial effects of clove oil and nisin. The pads in Group C (Control) had 5 ml of physiological saline (PS, 0.85 % NaCl). For the test groups, the absorbent pads were sprayed with 2 different concentrations of clove oil, 4 different combinations of clove oil and nisin and 2 different concentrations of nisin instead of PS (Table 1). Each group consisted of 9 trays for each days of experimental process.

Since the previous studies indicated that the concentrations of active agents to be applied to food should be higher than the ones identified as the MIC using the culture mediums (Burt, 2004; Stiles, 1996), the higher concentrations of clove oil or nisin than the MIC determined during the first part of the study were tried randomly and the most effective ones were used for food treatments.

Table 1. Test groups of the study
Tablo 1. Çalışılan test grupları

Groups	Content
Group C (Control)	0.85% physiological saline solution
Group 1	7% clove essential oil
Group 2	10% clove essential oil
Group 3	3.000 IU nisin
Group 4	6.000 IU nisin
Group 5	7% clove essential oil + 3.000 IU nisin
Group 6	7% clove essential oil + 6.000 IU nisin
Group 7	10% clove essential oil + 3.000 IU nisin
Group 8	10% clove essential oil + 6.000 IU nisin

The absorbent pads (MNM Hygiene Ped-90 x 135 mm) sprayed with different concentrations of clove oil and/or nisin and PS were placed onto the foam food packaging trays. The meat slices were laid on the top of pads. The stretch food packaging films were then placed over the package to seal the trays (Oral et al., 2009).

All groups were kept at 4 °C ± 1. On the day zero, 1, 3, 5, 7, 9, 11, 13 and 14 of the storage period, organoleptic, physical, microbiological and chemical analyses were carried out by taking samples of meat and tests were repeated for 3 times.

Microbiological Analysis

Ten g of meat samples were homogenised with 90 ml PS and their decimal dilutions in PS were prepared. Then, their inoculations were made in parallel in mediums specific for microorganism with the methods of spread and pour plate. The incubations were performed as follows: i) for faecal coliform group of bacteria, Violet Red Bile Agar for 24 h at 44.5 °C, aerobic, ii) for coliform group of bacteria, Violet Red Bile Agar for 24 h at 37 °C, aerobic, iii) for Enterobacteriaceae, Violet Red Bile Glucose Agar (Oxoid CM0485) for 24 h at 37 °C, aerobic, iv) for Pseudomonas spp. CFC Supplement added Pseudomonas Agar Base for 48 h at 30 °C,

aerobic, v) for total mesophilic aerobe bacteria (TMAB) Plate Count Agar (Oxoid CM0325) for 24 h at 30 °C, aerobic, vi) for total psychrophilic aerobic bacteria (TPAB) Plate Count Agar for 10 days at 7 °C, aerobic, vii) for S. aureus Egg yolk K-Tellurite added Baird Parker Agar Base for 24 h at 37 °C, aerobic, viii) for lactic acid bacteria (LAB) MRS Agar for 72 h at 30 °C, aerobic, ix) for Brochotrix spp. STAA Supplement added STAA Agar Base for 48 h at 22 °C, anaerobic and x) for sulphite-reducing bacteria SPS Agar (Merck 1.10235) for 24 h at 30 °C, anaerobic. After the incubation, the counting was done by evaluating the colonies found in mediums (Harrigan, 1998; Holzapfel, 1999).

Physico-chemical Analysis

pH Value

The pHs of meat samples, nisin, clove essential oil and their combinations prepared for the tests were measured with pH meter (Hanna H1221) and recorded.

Putrefaction Test

During the duration of cold storage, Eber test was applied on samples in order to determine the putrefaction. The principal in this test is the determination of ammoniac formed during the putrefaction. For that purpose, 2-3 ml of newly prepared Eber reagent was placed into a test tube. Pea-sized meat sample was inserted into a tube with the aid of a loop and it was kept therein for a while without any contact with reagent. The smoke from the piece of meat to the edge of test tube is originated from the presence of ammonium chloride and indicates the putrefaction of test sample. The intensity of smoke changes according to the level of putrefaction (Vural, 1992).

Organoleptic Analysis

This analysis was conducted according to Ruiz et al. (2001) with some modifications. Five assessors from Department of Food Hygiene and Technology Sensorial characteristics evaluated the samples. The same people were used for the evaluations throughout the study. Panellists were asked to evaluate the colour and odour intensities of samples, immediately after the package opening, half an hour later and after cooking.

Statistical Analysis

The data obtained from independent studies conducted in three times were analysed by one-way analysis of variance (ANOVA). TUKEY test was used for assessing differences between the groups. Statistical analyses were performed with Minitab 12 package.

RESULT

Clove Essential Oil Composition

The main component of the clove oil analysed by GC-MS was found to be 87.5% of eugenol, 8% of α-Humulene, (E, E) - 2.1% of α-farnesene, 1.4% of Δ-amorphene and 0.2% of caryophyllene oxide.

MIC Values of Clove Essential Oil and Nisin on Reference Strains

In this study, the antimicrobial effect of clove oil and/or nisin against the reference strains (except *P. aeruginosa*) was showed. The MIC values were presented in Table 2.

The Effect of Clove Oil and Nisin on Red Meat Shelf Life

In each day of experiment, the samples were analysed independently and it was found that the group (group 2) containing a high concentration of clove oil in TMAB count indicated more reduction. Other combination groups (groups 7 and 8) and test groups (3 and 4) containing two different concentration of nisin were ineffective with the results close to that of control group. No statistical difference was observed among the tests groups ($P > 0.05$). TMAB counts are given in Figure 1.

Table 2. MIC values of nisin and clove essential oil on microorganism
Tablo 2. Nisin ve Karanfil esansiyel yağının mikroorganizmalar üzerine MIC değerleri

Bacteria	Concentration of clove essential oil (%)	Concentration of nisin (IU)
*B.*thermosphacta	0.1	50.000
Y. enterocolitica O3	0.14	> 500.000
Y. enterocolitica O9	0.14	> 500.000
S. dysanteriae	0.2	> 500.000
S. Enteritidis	0.2	> 500.000
S. Typhimurium	0.2	> 500.000
B. subtilis	0.2	50.000
E. coli	0.26	> 500.000
Lc. Lactis	0.4	3.000
M. luteus	0.5	5.000
Leu. mesenteroides	0.5	3.000
Lb. casei	0.5	3.000
S. aureus	0.5	75.000
L. monocytogenes	0.7	7.500
P. aeruginosa	> 30 ineffective	> 500.000

Group 2 has a high reduction level on Enterobacteriaceae. Out of combination groups, group 5 had more reduction than those of other combination groups. Except for day zero of analysis, control group had the highest count of microorganisms out of all test groups on the other days of analyses (P>0.05). The counts of Enterobacteriaceae are given in Figure 2.

As compared to controls, clove oil test groups indicated 1 logarithm reduction on the 7^{th} and 9^{th} days, 2.5 logarithms reduction on the 11^{th} and 13^{th} and 2 logarithms reduction on the 14^{th} day to the count of lactic acid bacteria of red meat in test groups. Group 8 in which nisin groups and both of two substances were used in high rates from the 9^{th} day had the lowest reduction level with the results close to that of controls. However, at least 1 logarithm variation was found between all experiment groups and control group in terms of their reduction levels especially in the last 3 days of analyses (P>0.05). The data for the count of lactic acid bacteria are shown in Figure 3.

Group 2 containing the highest concentration of clove oil on the count of *Pseudomonas spp.* indicated 0.5 logarithms variation on the day zero and 1 logarithm variation on the 1^{st} day as compared to that of controls. When the results for the last 3 days were analysed, the results of group 1 indicated 2-2.5 logarithms variation for reduction as compared to that of controls. Although groups 3 and 4 containing nisin indicated variations on the days of analyses, they could indicate/provide reduction of 1 logarithm as compared to that of controls (P>0.05). The counts of *Pseudomonas spp.* are given in Figure 4.

Although nisin groups indicated a successful reduction result for the first day of the study in which *Brochotrix spp.* was analysed, they could not give the same results for the following days, but the results were close to that of controls, like group 8. Though clove oil groups indicated variations based on the analysis days, they all reached to the highest reduction level. Thus, group 2 statistically reached a reasonable reduction level on the 13^{th} day as compared to those of group 4 and control group (P<0.05). Nonetheless, statistically there was no reasonable variation between the groups during the other days of analyses (P<0.05). The results for *Brochotrix spp.* are given in Figure 5.

pH value

The pH of clove oil used in this study was determined as 3.00 and that of nisin solution as 2.62. For pH, there was not any notable statistical difference between the control group and test groups during the storage period (P>0.05). The data for pH levels of test groups are given in Figure 6.

Putrefaction Test

No putrefaction was determined for the tests groups on the first 3 days of analyses where samples having putrefaction was labelled with (+) and those not having with (-) according to the results of Eber experiment. Control groups of 5 and 6 started to indicate signs of putrefaction from the 5^{th} day, while other samples indicated the same signs on the following days of analyses. The latest putrefaction

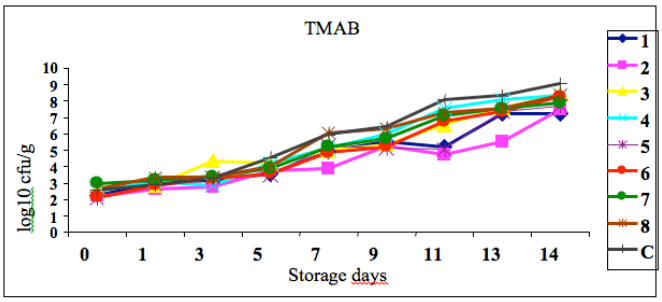

Figure 1. Total mesophilic aerobic bacteria (TMAB) counts in groups during storage (log10 cfu / g).

Şekil 1. Muhafaza esnasındaki toplam mezofilik aerobik bakteri (TMAB) sayıları (log10 cfu / g)**.**

1: 7% clove essential oil, **2:** 10% clove essential oil, **3:** 3.000 IU Nisin, **4:** 6.000 IU Nisin, **5:** 7% clove essential oil+ 3.000 IU Nisin, **6:** 7% clove essential oil+ 6.000 IU Nisin, **7:** 10% clove essential oil+ 3.000 IU Nisin, **8:** 10% clove essential oil + 6.000 IU Nisin, **C:** Control.

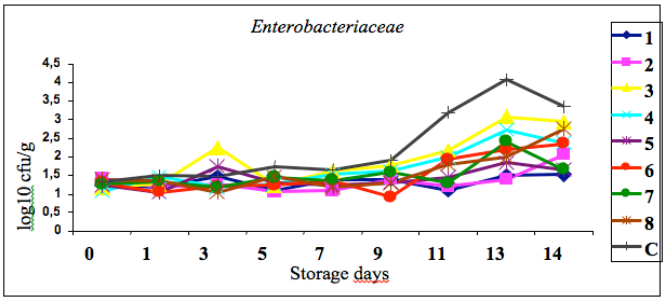

Figure 2. *Enterobacteriaceae* numbers in groups during the storage (log10 cfu / g).

Şekil 2. Muhafaza esnasında gruplardaki *Enterobacteriaceae* sayıları (log10 cfu / g).

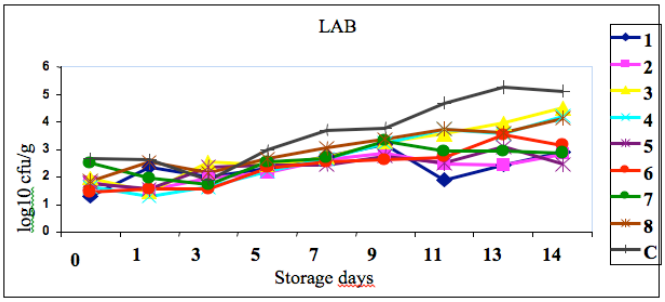

Figure 3. Lactic acid bacteria numbers in groups during the storage (log10 cfu / g).

Şekil 3. Muhafaza esnasında gruplardaki laktik asit sayıları (log10 cfu / g).

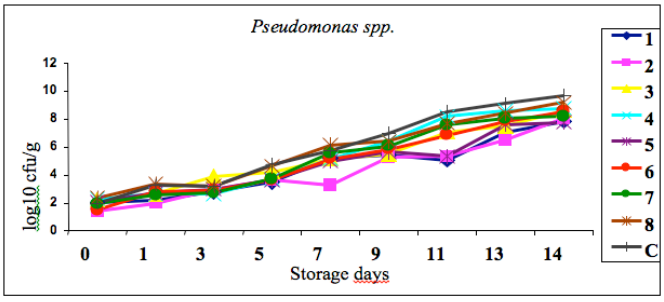

Figure 4. *Pseudomonas spp.* numbers in groups during the storage (log10 cfu / g).

Şekil 4. Muhafaza esnasında gruplardaki *Pseudomonas spp.* sayıları (log10 cfu / g).

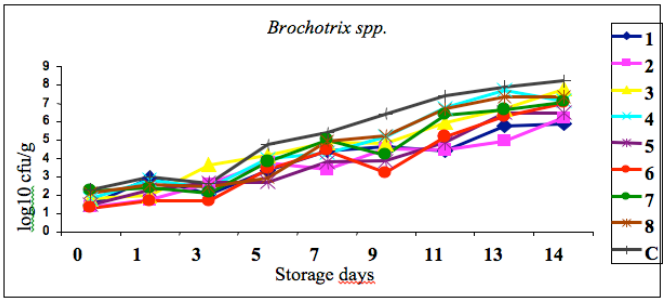

Figure 5. *Brochotrix spp*. numbers in groups during the storage (log10 cfu / g).

Şekil 5. Muhafaza esnasında gruplardaki *Brochotrix spp*. sayıları (log10 cfu / g).

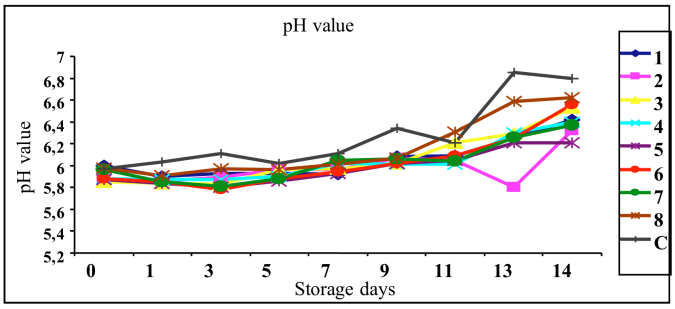

Figure 6. pH levels of test groups during storage.

Şekil 6. Muhafaza esnasında test gruplarının pH düzeyleri.

was observed in groups 1 and 5 with negative results on the 9th day of storage.

Organoleptic Analysis

For the groups of clove oil and of its combination with nisin, the surface of meat samples contacted with dry pad indicated whitening colour from the first day onwards. However, the change in colour did not make a notable difference as compared to the concentration of clove oil and it was steady for the following days. The odour of putrefaction started earlier in test groups containing nisin and high concentration of nisin and clove than that of other groups and it continued increasingly until the end of storage period. The clove is known as a spice having a strong odour and aroma. When the pockets were opened for the first time, the odour of clove oil, though being not strong but perceivable, was sensed. Panellists reported that the odour concerned was not at a disturbing level. There was not an obvious difference in terms of the level of odour in groups within which different concentrations of clove were used. After 1 h of storage, when the pockets were opened, there was no reasonable (noticable) difference between all the test groups. In general, it was reported that the odour of cloves in the fat of meat samples was clearer than that of the clove odour of meat.

For the experiments of boiling and frying samples of test groups (1, 5 and 6) containing 7% of clove oil, it was reported that when pan lid was opened, the odour of clove was more significant than that of raw meat samples. When tasted, the odour and aroma of clove became clearer on the pharynx. The same situation was observed to be much stronger for the samples of groups (2, 7 and 8) containing 10% of clove oil.

DISCUSSION

The aim of this study was to prevent the interaction of antimicrobial agents with meat's chemical structure and chemical and organoleptic changes that likely to be seen on meat and to provide the continuity of activity from the sprayed pad during the storage period. For this reason, the substances investigated were not applied on meat directly but on the pads by spraying. In antimicrobial effect tests, it was proved that while the activities of essential oil were found higher in in vitro studies, their activities applied on food were lower (Brul and Coote, 1997). There are many factors that restrict the effect of antimicrobial substance in organic systems and it is very difficult to control these factors concurrently (Davidson and Parish, 1989). The antimicrobial activity of plant origin depends on many factors such as the extraction method of essential oils, the amount of inoculums, growth phase, culture media used, pH, packing procedure and internal characteristics of food (Brandi et al., 2006). Studies on the antimicrobial effects of plants in vitro against the pathogens have been noteworthy. It was observed that the clove performed 0.1% MIC against *E. coli* O157:H7, 0.1% MIC against *S. Typhimurium*, 0.05% MIC against *S. aureus* and 0.2% MIC against *L. monocytogenes. While L. monocytogenes* was observed to be the most resistant bacteria, *S. aureus* was determined

to be the most sensitive one (Oussalah et al., 2007). The activities of ten essential oils, *S. aureus, S. Epidermidis, B. subtilis, B. cereus, Bacillus spp., L. monocytogenes, M. luteus, E. coli, P. aeruginosa* and *Klebsiella spp.* were observed and Gram (-) bacteria were found to be resistant against many of the essential oils except for cinnamon and clove. The MIC value of clove essential oil was observed between 2.5% and 5%. It was considered that the most sensitive bacterium for the clove is *B. cereus* (with 24 mm of inhibition zone), while the most resistant bacterium is *P. aeruginosa* (with 0.0 mm zone of inhibition) (Gupta et al., 2008). Among the microorganisms tested according to the MIC values, *Y. enterocolitica*, followed by *B. cereus* and *S. aureus* was identified as the most sensitive microorganism. In parallel with the studies in question, *P. aeruginosa* was found to be the most resistant bacteria against the clove oil according to the results obtained from the test bacteria.

L. monocytogenes was identified as the most resistant bacteria with the highest MIC value of 0.7% among the Gram (+) bacteria. Lactic acid bacteria were influenced moderately with 0.4 - 0.5% MIC levels. Bacterium *B. thermosphacta* was found to be the most sensitive one among the Gram (+) bacteria. In this study, it performed a high resistance more than many of the Gram (-) bacteria with the MIC level of 0.5%. *S. aureus* with the MIC level of 0.5% was found to be more resistant than the *B. cereus* (0.2%). The present results of clove evaluated *in vitro* showed that the clove was effective on Gram (-) and Gram (+) bacteria except for *P. aeruginosa* exhibiting a wide range of antibacterial activity with successful results of low concentration of oil ratios. Nisin combined with clove did not indicate any activity on the Gram (-) bacteria. Nisin though being effective on these strains required using an inhibitory concentration of minimum 500,000 IU, a very high rate that is not economical. Although nisin indicated a large reduction at the first half hour of storage at levels determined as MIC, the total inhibition was observed at the 6^{th} h. It was proved that the reduction level observed at the 6^{th} h decreased to the 24^{th} h at concentrations below the MIC levels at which nisin lost its effect. Besides, nisin was effective on the Gram (+) bacteria tested with the MIC levels; 5,000 IU for *M. luteus,* 7.500 IU for *L. monocytogenes,* 50,000 IU for *B. subtilis,* 75,000 IU for *S. aureus* and 50.000 IU for *B. thermosphacta.* Nisin was not a successful preservative alone observed herein where red meat was packed with nisin-sprayed pads.

There have been many studies on the plants and species and on their synergistic or antagonistic effects of various combinations of their active ingredients (Burt, 2004; Goni et al., 2009; Zhang et al., 2009). Herein, the combination of clove oil and nisin has been studied for the first time. The antimicrobial effect determined in low concentration groups of nisin and clove was not observed in high concentration test groups of nisin and clove oil. Although it was known that nisin and clove had a synergistic effect on different substances within which they were combined, their combination did not yield a high antimicrobial effect herein. Furthermore, nisin and clove reduced the effect of each others at the test groups where they were used in high concentrations with the results close to those of test groups. However, the mechanism to be interpreted as an antagonistic effect for this conclusion is yet unknown. The reasons that nisin is reported to be more active at lower pH (Sezer and Guven, 2009) and that the activity of clove rises at the environments with higher pH (Devi et al., 2010) were considered as one of the probable factors which cause reduction in the antimicrobial activity of these two substances' combinations.

The use of essential oils at effective concentrations may raise concerns about the changes in the organoleptic characteristics of food. It is necessary to know the lowest concentrations having efficient antimicrobial effects, the levels of

safety and toxicity for a practical addition of essential oils without affecting sensory quality (Oussalah et al., 2006). When the pockets were opened for the first time during the storage period, the odour of clove oil, though being not strong but perceivable, was sensed. There was not an obvious difference in terms of the odour at the groups for which different concentrations of clove were used. It was reported that the odour of cloves that was not at a disturbing level was a good one. Nevertheless, at the beginning test groups containing the clove oil received a lower score of acceptability as compared to those of control and nisin groups. Instead of chemical additives of antibacterial effective herbs and spices in the food industry, many studies have been required to use them technologically. The fact that there are still many unexplained interactions to be studied and combinations emphasize ongoing studies and more extensive others to be conducted on the subject in future (Rios and Recio, 2005).

Overall, it was determined that the strong antimicrobial effect of a broad-spectrum of clove occurred suddenly *in vitro*. In terms of the antimicrobial activity, it was observed that the clove oil was more successful than nisin and clove oil + nisin combination and that it had an effect on microflora of red meat. Nisin alone decreased the microbial count of the samples at about 1 logarithm rate at the beginning; however this difference between test and control groups disappeared at the end of storage period. Generally, in test groups where nisin and clove oil was combined; the group (group 5) where low amounts were used was more successful than the other combinations but it provided lesser reduction than the samples where only the clove oil was used. Antibacterial activity of the groups (group 7 and 8) where the highest amounts were used was low and they were ineffective in general with the results close to control group. Considering the undesired findings of present trials in which high concentrations of clove oil and nisin were combined together, it was concluded that there might be an antagonistic interaction between these agents. However, when their lower concentrations were combined they worked well. Therefore, the antimicrobial effect of the combination did not improve by increasing their levels used. Hence, in future studies, the reasons of that inferior results could be investigated to elucidate the underlying mechanisms that may exist.

Although the clove yielded successful results *in vitro*, its activity on prolonging the shelf life of beef was found to be limited. Though many substances or essential oil were studied in combination with the clove, no study on its combination with nisin has been found yet. But, the results concerning the reduced activity by raising concentrations in the combined groups was considered interesting. The clove considered yielding more effective results when it was applied on the surface, performed an immediate effective antimicrobial activity with quite effective results in the first half hour of *in vitro* MIC test period. The clove oil is a potential natural antimicrobial with its immediate effect in food industry. Therefore, the clove and its different concentrations should be studied further with various methods so that unknown facts on the subject could become clearer.

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REFERENCES

Adams RP., 2004. Identification of essential oil components by Gas Chromatography / Quadrupole Mass Spectroscopy. Allured, Carol Stream, IL, USA.

Arauz LJ., Jozala AF., Mazzola PG., Pena TCV., 2009. Nisin biotechnological production and application: A review. Trends Food Sci. Tech., 20, 146-154.

Ayoola GA., Lawore FM., Adelowotan T., Aibinu IE.,

Adenipekun E., Coker HAB., Odugbemi TO., 2008. Chemical analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). Afr. J. Microbiol. Res., 2, 162-166.

Bilge Oral N., Vatansever L., Duman Aydın B., Sezer C., Guven A., Gulmez M., Baser KHC., Kurkcuoglu M., 2010. Effect of oregano essential oil on biofilms formed by Staphylococcus and Escherichia coli. Kafkas Univ. Vet. Fak. Derg., 16 (Suppl-A): 23-29.

Brandi G., Amagliani G., Schiavano GF., De Santi M., Sisti M., 2006. Activity of Brassica oleracea leaf juice on food borne pathogenic bacteria. J. Food Protect., 69, 2274-2279.

Breukink E., De Kruijff B., 1999. The antibiotic nisin, a special case or not?, Biochim. Biophys. Acta., 1462, 223-234.

Brul S., Coote P., 1999. Preservative agents in foods mode of action and microbial resistance mechanisms. Int. J. Food Microbiol., 50, 1-17.

Burt S., 2004. Essential oils: Their antibacterial properties and potential applications in foods - a review. Int. J. Food Microbiol., 94, 223-253.

Coma V., 2008. Bioactive packaging technologies for extended shelf life of meat-based products. Meat Sci., 78, 90-103.

Davidson PM., Parish ME., 1989. Methods for testing the efficacy of food antimicrobials. Food Technol., 1, 148-155.

Devi KP., Nisha SA., Sakthivel R., Pandian SK., 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J. Ethnopharmacol., 130, 107-115.

Goni P., Lopez P., Sanchez C., Gomez-Lus R., Becerril R., Nerin C., 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem., 116, 982-989.

Gupta C., Garg AP., Uniyal RC., Kumari A., 2008. Antimicrobial activity of some herbal oils against common food-borne pathogens. Afr. J. Microbiol. Res., 2, 258-261.

Harrigan WF., 1998. Laboratory methods in food microbiology. 4th ed. Academic press. California, USA.

Holzapfel WH., 1999. Culture media for non-sporulating Gram-positive food spoilage bacteria. In "Culture media for food microbiology, progress in industrial microbiology" Ed., JEL Corry, GDW Curtis, RM Baird, 34, 89-94, Amsterdam.

Moreira MR, Ponce AG., Del Valle CE., Roura SI., 2005. Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT-Food Sci. Technol., 38, 565-570.

Nostro A., Bisignano G., Cannatelli MA., Crisafi G., Germano MP., Alonzo V., 2001. Effects of Helichrysum italicum extract on growth and enzymatic activity of Staphylococcus aureus. Int. J. Antimicrob. Ag., 17, 517-520.

Oral N., Vatansever L., Sezer Ç., Aydın B., Güven A., Gülmez M., Başer KHC., Kürkcüoğlu M., 2009. Effect of absorbent pads containing oregano essential oil on the shelf life extension of overwrap packed chicken drumsticks stored at four degrees celsius. Poultry Sci., 85, 1466-1471.

Oussalah M., Caillet S., Saucier L., Lacroix M., 2006. Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. Meat Sci., 73, 236-244.

Oussalah M., Caillet S., Saucier L., Lacroix M., 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food Control, 18, 414-420.

Ova G. 2006. Koruyucular. In "Gıda Katkı Maddeleri", Ed., T. Altuğ Meta Basım Matbaacılık, İzmir, 105-134.

Rios JL., Recio MC., 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol., 100, 80-84.

Ruiz JA., Guerreo L., Arnau J., Guardia MD., Esteve-Garcia E., 2001. Descriptive sensory analysis of meat from broilers fed diets containing vitamin E or β- carotene as antioxidants and different supplemental fats. Poultry Sci., 80, 976-982.

Sezer C., Güven A., 2009. Investigation of bacteriocin production capability of lactic acid bacteria isolated from foods. Kafkas Univ. Vet. Fak. Derg., 15, 45–50.

Stiles ME., 1996. Biopreservation by lactic acid bacteria. Review. Anton. Leeuw., 70, 331–345.

Vural N., 1992. Besin Analizleri. Ankara Üniversitesi, Eczacılık Fakültesi, Yayın no: 69, Ankara.

Zhang H., Kong B., Xiong YL., Sun X. 2009. Antimicrobial activities of spice extract against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4 ^{0}C. Meat Sci., 81, 686-692.