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RESEARCH ▪ ARAŞTIRMA

Could Decontamination with Salad Dressing effect on *S. aureus* Load?

Salata Sosu ile Dekontaminasyon S. aureus Yükü Üzerinde Etki Olabilir mi?

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ABSTRACT

Aim: This study was planned to determine the effects of mint and parsley extracts, vinegar, and chlorine on *S. aureus* load in lettuce leaves. It was also planned and carried out to evaluate the effectiveness of a salad dressing with appropriate organoleptic properties, developed by the researcher based on mint and parsley extracts.

Material and Method: The study was carried out on lettuce samples, which is a difficult vegetable to clean. *S. aureus* was inoculated on the lettuce before disinfection. 200 ppm/L of chlorine, vinegar, mint, and parsley extracts as 50 mL/L from each were used as treatments for 5 and 10 minutes. 50 mL/L of salad dressing, prepared using mint and parsley extracts, was used and the lettuce specimens were either dipped in and out of the salad dressing or kept in for 5 minutes. $p<0.05$ was accepted as statistically significant.

Results: According to the study results, the reduction levels in the *S. aureus* load caused by the disinfectants were found to be statistically significant ($p<0.05$). In comparison to the control group, the reductions in the *S. aureus* load by all applications were significant, and the best reduction was seen in 15 minutes of chlorine and vinegar applications ($p=0.01$). When the prepared salad dressing was analyzed, it was found that the reduction of *S. aureus* load by the salad dressing was statistically significant ($p<0.00$). The overall score of the salad dressing in terms of organoleptic properties was 3.9±0.96 out of 5 points.

Conclusion: The results of the study prove the disinfecting effect of natural extracts. It has also been shown that their use as salad dressing can effectively reduce microbial contamination during serving.

Keywords: Chlorine, disinfectant, mint, parsley, S. aureus

ÖZET

Amaç: Bu çalışma, özellikle servis aşamasındaki olası kontaminasyonlara karşı, nane ve maydanoz ekstraktları, sirke ve klorun marul yapraklarındaki S. aureus yükü üzerine etkilerini belirlemek amacıyla planlanmıştır. Aynı zamanda araştırmacı tarafından nane ve maydanoz ekstraktları temelli olarak geliştirilen, uygun organoleptik özelliklere sahip bir salata sosunun etkinliğini değerlendirmek amacıyla da planlanmış ve yürütülmüştür.

Gereç ve Yöntem: Çalışma, temizlenmesi zor bir sebze olan marul örnekleri üzerinde gerçekleştirilmiştir. Dezenfeksiyondan önce marul örnekleri üzerine S. aureus inokülasyonu gerçekleştirilmiştir. Dezenfektan olarak 200 ppm/L klor, sirke, nane ve maydanoz ekstraktlarının her birinden 5 ve 10 dakika süre ile 50 mL/L kullanılmıştır. Nane ve maydanoz özleri ile

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hazırlanan salata sosunun 50 mL/L miktarı kullanılmıştır. Marul örnekleri ya salata sosuna batırılıp çıkarılmış ya da 5 dakika bekletilmiştir. İstatistiksel anlamlılık düzeyi p<0.05 olarak kabul edilmiştir.

Bulgular: Çalışma sonuçlarına göre, dezenfektanların S. aureus yükünde neden oldukları azalma düzeyleri, kontrol grubuna göre, istatistiksel olarak anlamlı bulunmuştur (p<0.05). Kontrol grubu ile karşılaştırıldığında, tüm uygulamalarda S. aureus yükünde görülen azalma düzeyleri anlamlı bulunmuş ve en iyi azalma 15 dakikalık klor ve sirke uygulamalarında görülmüştür (p=0.01). Hazırlanan salata sosu üzerinde yapılan analizde, salata sosundan kaynaklanan S. aureus yüklerindeki azalma miktarları istatistiksel olarak önemli bulunmuştur (p<0.00). Salata sosu organoleptik değerlendirmede 5 üzerinden 3.90±0.96 puan almıştır.

Sonuç: Çalışmanın sonuçları, doğal ekstraktların dezenfektan etkilerini kanıtlar niteliktedir. Ayrıca salata sosu olarak kullanımlarının servis sırasında mikrobiyal kontaminasyonu azaltmada etkili olabileceği de gösterilmiştir.

Anahtar kelimeler: Klor, dezenfektan, nane, maydanoz, S. aureus

INTRODUCTION

Serving fresh fruits and vegetables whose various health benefits have been proven at organizations that provide catering services requires assessment and monitoring of possible risks especially in terms of food safety (1,2). To avoid contamination and reduce the risk of vegetables to be served without cooking, the phases of separation, washing, disinfection, final rinsing and serving are considered particularly critical control points (3,4).

Chlorine and chlorinated compounds not only show standard activity in usage at suitable doses and times, but they also have a broad bactericidal spectrum, and their inexpensiveness and mild scent are among the reasons why they are preferred. However, it was determined that chlorine does not degrade organic compounds and leaves residues on food (5-7). For this reason, in recent years, studies have been investigating usage of extraction fluids obtained from various spices and plants that contain natural antimicrobial compounds in their structures and natural products such as vinegar and lemon juice for disinfection (8-10).

The first scientific studies on the antimicrobial properties of vinegar may be traced back to the 19th century. Acetic acid, one of the main components of vinegar whose antimicrobial effects have been known since very early, shows a protective effect in foods especially against yeasts and bacteria (11).

The use of natural antimicrobial agents are one of the alternative methods in foods contaminated with microorganisms (12). It is known that thyme, clove, cinnamon, garlic, cilantro, rosemary, parsley, mint, lemon, sage and vanilla show a protective effect on foods by their single or combined use (8,10,13).

Serving constitutes a highly important, critical control point for foods that are offered for raw consumption, as in all foods, and as there is no other process where a possible risk of contamination can be eliminated at this point, it should be carefully focused on. Traditional disinfection methods such as chlorine cannot show an activity against contamination that may occur in the food after the stage of washing (14). Nevertheless, the fact that disinfectant products that can be obtained from natural sources may be added to raw foods as salad dressing may provide an important contribution in achieving continuation of food safety.

This study was planned and carried out to determine the activity of vinegar, mint and parsley extracts and a salad dressing created by the researcher from these extracts whose organoleptic characteristics were suitable against possible contaminations at the stage of serving.

MATERIAL AND METHOD

First Stage of the Study

In the study, lettuce (*Lactuca sativa* var. *longifolia* - Romaine variety) was selected as the food on which disinfection measures should be carried out.

Three different forms of vinegar available in the market that contained 2%, 4% and 6% acetic acid were utilized. According to a pilot study conducted by researchers before this study, as a result of the disinfection procedures performed with vinegar of different acetic acid content, no statistically significant difference was found between the reduction levels of total bacteria amounts ($p=0.36$) and it was decided to use the vinegar with 4% acetic acid, the most common on the market, for the disinfection of the obtained lettuce samples. The application amount of the vinegar was set at 50 mL/L.

The application dose of 200 ppm/L was chosen for chlorine, the efficacy of which was demonstrated in a previous study by the researcher (15). This application dose is also a frequently preferred amount in practice.

The mint and parsley extracts that were used for disinfection and prepared by the method of solvent extraction were commercially purchased. The amounts selected for their antimicrobial activities were 50 mL/L, and while selecting these amounts, previous studies that were carried on the topic were taken as a reference (16,17). It was decided to make the implementations in line with the information in the literature.

Using the indicated dosages of disinfectants and based on information found in previous studies (17,18), exposure times of 5 and 15 minutes were chosen for all disinfection methods used in the study.

Second Stage of the Study

During the disinfection procedures, a 10-g lettuce specimen was used for each experiment. *S. aureus* strains obtained from Hatay Mustafa Kemal University Veterinary Faculty were inoculated on the lettuce. The *S. aureus* strains that were brought to the laboratory were firstly inoculated into Tryptic Soy Agar (TSA) and reanimated. Afterwards, they were taken into sterile spraying containers containing peptone solution by a loop and stirred. With the method of spraying, *S. aureus* was inoculated onto all lettuce specimens. The inoculated amount of *S. aureus* strains is 2.66×10^4 cfu/g (4.42 log cfu/g). After inoculation, lettuces, were treated with the specified disinfectants for the specified times. As a result, the effect of disinfectants on *S. aureus* was observed.

Third Stage of the Study

After the effect of each disinfectant on the bacterial load was evaluated separately, the production of salad dressing obtained from mint and parsley extracts, whose organoleptic properties were determined by sensory analysis, and which can be used in the service was started. After the salad dressing production was completed, the effect of this sauce on *S. aureus* load was evaluated. Due to its unique taste, smell and high acidity, vinegar was not used in dressing production. Chlorine was also excluded from the dressing for the purpose of obtaining a dosage that can be directly consumable.

Before the study, it was planned to include the amounts of mint and parsley extracts specified at the second stage in the dressing. During the trials, it was determined that the sharp smell and metallic taste of the parsley extract affected the edibility of the lettuce negatively. For this reason, the aim is to make it edible by adding cinnamon, sugar and pomegranate molasses to the salad dressing. Since no adverse effects of the mint extract were observed, the amount of mint extract was left at the level used in the disinfection procedures. During the finalization of the salad dressing, a tasting was performed by 5 nutritional experts who work at an academic level and have received training in sensory analysis. The following organoleptically preferred dressing contents emerged from the trials and tastings conducted:

Contents of the Produced Salad Dressing

- 4 mL parsley extract
- 50 mL mint extract
- 50 mL pomegranate molasses
- 4 g sugar
- 10 cinnamon bark strips

After the materials given above were mixed and left for 24 hours, the cinnamon strips were removed. The dressing was sieved before serving and served as chilled. The salad dressing, which was completed with the approval of 5 expert dietitians, was added to 10 g lettuce samples and tasted by a group of 16 experts for organoleptic evaluations. The panelists were asked to assess the organoleptic properties of the disinfected lettuce specimens based on the "Verbal Hedonic Scale Test" (5-point likert scale; 1: Not noticed, 2: Light, 3: Medium, 4: Strong, 5: Very strong) and a "Scoring Test" (19).

Afterwards, by dipping a 10-g lettuce specimen inoculated with *S. aureus*, into the dressing prepared with the contents specified above and removing immediately (waiting time of 0 minutes) and by leaving another specimen in the dressing for 5 minutes, two different specimens were subjected to microbiological analyses (*S. aureus*).

Statistical Analyses

The results of the study that was carried out are presented as the mean values of the data obtained out of two laboratory experiments. Logarithmic decrease was calculated in Microsoft Office Excel 2007.

The data were statistically analyzed by using the IBM SPSS Statistics 21 package software. In the analysis of the significance levels in the preliminary study, Kruskal-Wallis analysis of variance was used. Wilcoxon Test was applied in the statistical analysis of the reduction amounts observed in the lettuce specimens that were disinfected in the study. In the entire study, $p<0.05$ was accepted as statistically significant.

RESULTS

The reduction amounts caused in *S. aureus* load by the disinfectants in comparison to the control group are given in Figure 1 in units of log cfu/g.

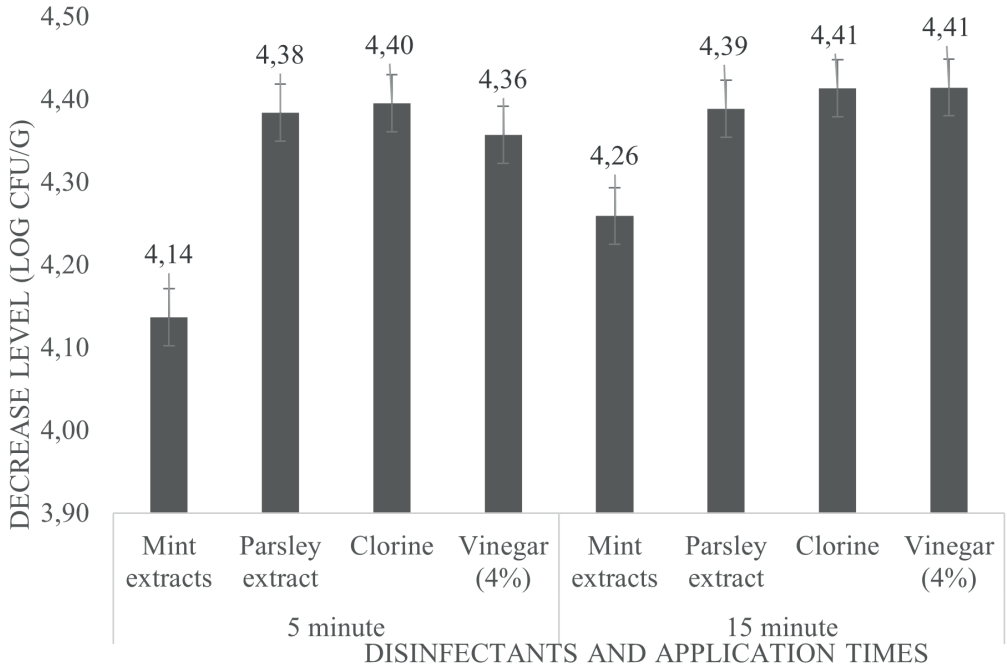

Figure 1. Mean reduction amounts of the lettuce specimens subjected to disinfection in comparison to the control group in terms of *S. aureus* loads (log cfu/g)

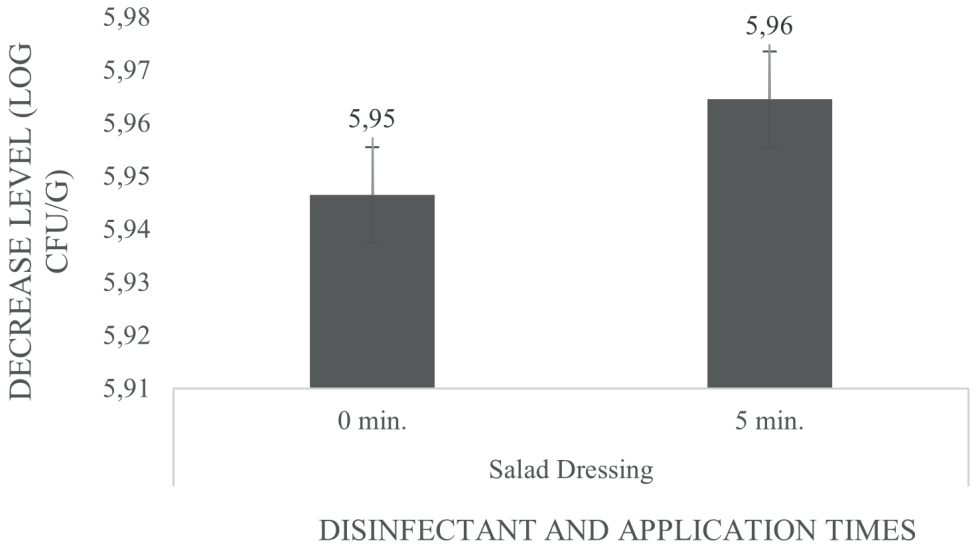

Figure 2. Mean reduction amounts of the lettuce specimens subjected to momentary disinfection in the salad dressing in comparison to the control group in terms of *S. aureus* loads (log cfu/g)

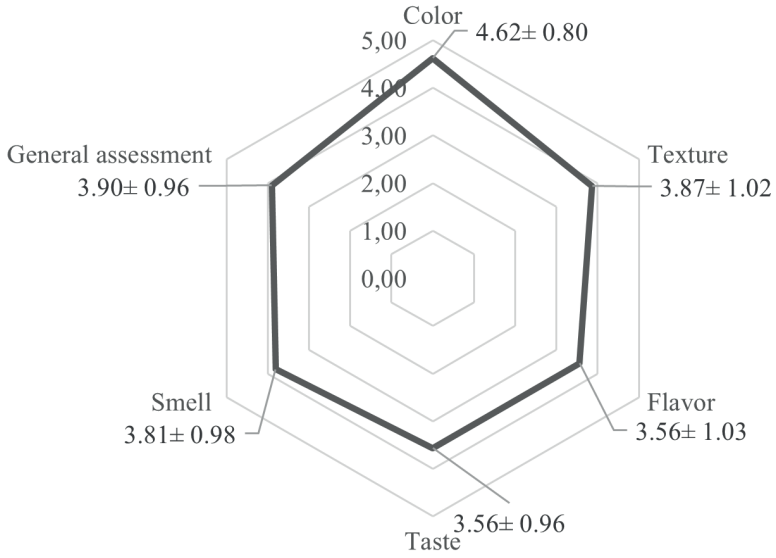

Figure 3. Mean and standard deviation values of the sensory properties of the salad dressing

The reduction levels in *S. aureus* load caused by the disinfectants were found to be statistically significant ($p<0.05$). In comparison to the control group, the reductions in the *S. aureus* load by all applications were significant, and the best reduction was seen in 15 minutes of chlorine and vinegar applications ($p=0.01$).

The reduction levels in *S. aureus* loads (Figure 2) caused by the prepared salad dressing were statistically significant ($p<0.05$). In comparison to the control group in terms of the reduction amounts in *S. aureus* loads as a result of 0 and 5 minutes of salad dressing application, the reduction levels in all applications were significant, and it was observed that the application of 5 minutes of treatment was more effective in reducing the amount of *S. aureus* ($p=0.01$).

The mean score and standard deviation values (over 5 points) regarding the sensory analysis of the prepared salad dressing are shown in Figure 3. The general assessment of the salad dressing was as 3.90±0.96 over 5 points.

Table 1. Panelist assessment of the salad dressing based on the verbal hedonic scale test (n=16)

Description	n	%
Not noticed	1	6.3
Light	4	25.0
Medium	5	31.2
Strong	6	37.5

In the hedonic scale test, it was asked to select the word that best described the taste, smell and texture of the salad dressing, and the word was selected as 'light' by 25.0% of the individuals and 'strong' by 37.5% (Table 1).

DISCUSSION

Chlorine and chlorinated compounds are at the top of the most frequently utilized disinfectants in foods that are served raw, as they are economical and relatively easily accessible (20). There are many studies in which a decrease is detected with the use of chlorine in bacterial load (21,22). In this study, after application of chlorine for 5 minutes, the reduction level in *S. aureus* was found to be statistically significant ($p<0.05$). The inhibitory or antimicrobial activity of chlorine may vary depending on the amount of the hypochlorous acid within the water contacting the microorganisms. Hypochlorous acid is the free chlorine form that has the highest bactericidal activity against pathogen microorganisms found prevalently in fresh fruits and vegetables (23).

However, it is known that chlorine leaves residues as a result of unsuitable usage applications (24,25). Due to its proven activity and prevalent usage, chlorine was selected as one of the disinfectants in this study, but in addition to this, due to the concerns of residues, organic acid, and plant extracts were also used as alternative natural disinfectants.

Among organic acids that are naturally found in the structure of fruits and vegetables, acetic acid in vinegar is among the most frequently used organic acids as a disinfectant (26). In studies, it has also been determined that vinegar or its active ingredient, acetic acid have effects on many bacteria such as *E. coli* and *Salmonella* (2,27). In this study, usage of vinegar containing 4% acetic acid was one of the best disinfectant treatments that reduced the levels of *S. aureus* loads. Acetic acid, one of the main components of vinegar whose antimicrobial effects have been known since very early, shows a protective effect in foods especially against yeasts and bacteria. Additionally, the phenolic compounds found in vinegar also have qualities that support antimicrobial activity (11).

However, the lack of a dosage amount to be used also leads to doubts about whether its use ensures effective disinfection, especially in catering establishments about vinegar (28). But, the results of this study showed that a 15-minute application of 50 ml/L vinegar containing 4% acetic acid effectively reduced microorganisms that are common indicators of hygiene, as well as *S. aureus*, which is an indicator of contamination caused by personnel.

The usage areas of extracts obtained from plants show much diversity. These areas include protective effects against chemicals, regulation of kidney, and bowel functions and antioxidant properties (29,30). In addition to these, another usage area of plant extracts is their antimicrobial activity (31). Many plants may be used for these purposes. Parsley and mint are just two of these plants that are commonly used in food.

Among the parsley extracts applied in concentrations of 0.3%, 0.6% and 1.2%, it was found that especially the concentrations of 0.6% and 1.2% showed a significant disinfecting effect against *Staphylococcus aureus*, *Salmonella enterica, Pseudomonas aeruginosa* and *Escherichia coli* ($p<0.05$) (32). It has been confirmed in other studies to be effective on many microorganisms, and a similar mint extract has also been detected (33, 34). Even, in one study, it was stated that increasing the application time of peppermint extract increased its effectiveness (35). In this study, it was found that the parsley and mint extracts led to a significant decrease in *S. aureus* in different application procedures. The antimicrobial effect mechanism of

essential oils obtained from plants is mainly caused by the hydrophobic structures that allow them to penetrate the cell membrane and mitochondria of the bacterium. Phenolic compounds are the main substances that have this effect in the essential oils of plants (13). Despite the limitations of not determining the phenolic content of the plant extracts in the study, the results of the study show that the extracts of parsley and mint have a good disinfectant effect.

Consumption of fruits and vegetables in food service establishments requires assessment and monitoring of potential risks, particularly with regard to food safety (3). In Brazil, the microbial contents of products ready for consumption that are served raw such as lettuce, spinach, cabbage, and parsley were examined, and the psychotropic aerobic bacterium number of 96.7% of the foods and coliform bacterium number of 81.5% were found to be >5 log cfu/g. Additionally, it was determined that 53.1% of the specimens contained *E. coli*, 3.7% contained *Listeria* spp., and 1.2% contained *Salmonella* spp. (36).

In this study, it was thought that usage of the plant extracts that were used as disinfectants in combination with a salad dressing would protect salads from microorganisms at the stage of serving which is a critical checkpoint. Some studies have stated that combined usage of plant extracts is more effective than their single usage, and their effects may be increased by up to 1.29 times when they are used this way (37,38). However, in comparison to the microorganism reduction amounts observed in the single usage of the plant extracts as disinfectants, it was observed that the reduction level caused by the salad dressing was lower. This may be due to the fact that the amount of parsley extract used in the preparation of the salad dressing was limited due to its very pungent taste and odor. The extract obtained from plants contains natural components. For this reason, no rinsing is required after disinfection, as is the case with chlorine. However, some plant extracts may have a sharp smell or taste that is not desirable to the consumer. In comparison to antimicrobial activity studies that may be more frequently encountered, sensory analysis studies carried out with plant extracts are highly rare (39). In this study, the overall impression of the salad dressing applied to the lettuce leaves for consumption was rated at 3.9 points out of 5 points. When making the salad dressing, it was not a problem to use the amount of mint extract that was also used as a disinfectant. However, the amount of the parsley extract was highly reduced due to its sharp taste and smell, and different materials were also added to mask the existing sharpness.

Consequently, to avoid the concerning effects of chlorine that is prevalently used as a disinfectant, it may be a good alternative to use extracts that are obtained from plants. Such that, a salad dressing that is prepared by using these extracts may help the continuation of the protective effect even at the stage of serving. Nevertheless, to be able to establish a standard regarding the disinfectant activity of plant extracts, more studies are needed. This also applies to studies on salad dressings to achieve organoleptic properties that may be desirable to consumers and to induce a disinfectant effect at the same time.

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Conflict of interest ▪ Çıkar çatışması: *The authors declare that they have no conflict of interest. ▪ Yazarlar çıkar çatışması olmadığını beyan ederler.*

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