GIDA (2011) 36 (1): 9-15 *Research* / Araştırma

EFFICACY OF DIFFERENT DECONTAMINATION TREATMENTS ON MICROBIAL POPULATION OF LEAFY VEGETABLES

Ayhan Temiz[1*], Ufuk Bağcı[1], Sine Özmen Toğay[2]

[1]Hacettepe University, Faculty of Engineering, Food Engineering Department, Ankara, Turkey
[2]Çanakkale Onsekiz Mart University, Faculty of Engineering and Architecture, Food Engineering Department, Çanakkale, Turkey

Received / Geliş tarihi : 15.04.2010
Received in revised form / Düzeltilerek geliş tarihi: 23.06.2010
Accepted / Kabul tarihi: 30.06.2010

Abstract

In the present study, levels of microbial contamination on some leafy vegetables collected from local wholesale markets (Ankara, Turkey) were determined. The efficacies of different surface decontamination methods on these vegetables were also investigated. Average total mesophilic aerobic bacteria (MB) and total coliform (TC) counts were between 6.20 - 7.14 log cfu/g, and 3.39 - 4.91 log MPN/g, respectively. Of the sixteen samples analyzed, thirteen contained *Escherichia coli*. Dipping in tap water was not effective at reducing microbial populations on vegetables. Difference between chlorine (200 mg/L) and acetic acid (0.25 %, v/v) treatments in the reduction of MB and TC counts was found to be statistically insignificant. The highest reduction in both MB and TC counts was obtained by sequential treatment with acetic acid followed by chlorine. According to these results, acetic acid can be an alternative to chlorine for surface decontamination of leafy vegetables in food establishments and house level. Level of microbial decontamination can be increased by sequential dipping in different sanitizers.

Keywords: Leafy vegetable, natural microbial flora, surface decontamination, chlorine, acetic acid

DEĞİŞİK DEKONTAMİNASYON YÖNTEMLERİNİN YAPRAKLI SEBZELERDE MİKROBİYEL YÜK ÜZERİNE ETKİSİ

Özet

Bu çalışmada, Ankara'daki yerel pazarlardan toplanan bazı yapraklı sebzelerin mikrobiyel kontaminasyon düzeyleri belirlenmiştir. Çeşitli yüzey dekontaminasyon işlemlerinin bu sebzeler üzerindeki etkileri de ayrıca incelenmiştir. Ortalama toplam mezofilik aerobik bakteri (MB) ve toplam koliform (TC) sayıları sırasıyla 6.20 - 7.14 log kob/g ve 3.39 - 4.91 log EMS/g düzeyinde bulunmuştur. İncelenen 16 örneğin 13'ünde *Escherichia coli* tespit edilmiştir. Çeşme suyuna daldırma işlemi, sebzelerdeki mikrobiyel popülasyonun azaltılmasında etkili bulunmamıştır. MB ve TC sayılarındaki azalma açısından klor (200 mg/L) ve asetik asit (% 0.25, v/v) ile yıkama işlemleri arasında istatistiksel olarak önemli bir fark bulunmamıştır. MB ve TC sayılarındaki en fazla azalma, birbirini takip eden asetik asit ve klor uygulamasıyla elde edilmiştir. Bu sonuçlara göre de asetik asitin gıda işletmelerinde ve ev düzeyinde yapraklı sebzelerin yüzey dekontaminasyon uygulamalarında klora alternatif olabileceği ortaya çıkmıştır. Mikrobiyel dekontaminasyon düzeyi farklı sanitizerlerin ardışık uygulamaları ile arttırılabilecektir.

Anahtar Kelimeler: Yapraklı sebze, doğal mikrobiyel flora, yüzey dekontaminasyonu, klor, asetik asit.

* *Corresponding author* / Yazışmalardan sorumlu yazar ;
temiz@hacettepe.edu.tr (+90) 312 297 7100 (+90) 312 299 2123

INTRODUCTION

Consumption of leafy vegetables has increased in recent years because of their nutritional value and beneficial health effects (1, 2). The number of outbreaks of foodborne illnesses associated with consumption of fresh vegetables and fruits have increased due to the increase in demand for fresh produce (3, 4).

The presence of spoilage and pathogenic microorganisms on leafy vegetables has long been recognized (5). *Salmonella* spp. (6), Listeria monocytogenes (7), *Escherichia coli* O157:H7 (8), *Aeromonas hydrophila* (9), *Yersinia* spp. (10) and *Staphylococcus* aureus (1) have been isolated from vegetables which can become contaminated with these pathogens at any of several points from the field through to the time of consumption (11, 12, 13).

Effective and feasible sanitation methods are required to remove pathogens and also to prevent foodborne diseases associated with consumption of fresh vegetables and fruits (14). Although most processors and consumers assume that washing fresh vegetables and fruits will reduce the microbial load on their surface, studies have shown that water washing alone is not effective in reducing microbial population on the fresh vegetables (15, 16). Hypochlorite solution containing 50 – 200 mg/L free chlorine is the most widely used sanitizer in fresh produce processes. Chlorine creates about 1 to 2 log microbial reductions on fresh vegetables at commonly used concentrations (17, 18). Despite some safety concerns about the use of chlorine in food processes (e.g., formation of trihalomethanes), it has been accepted as a convenient and inexpensive sanitizer for use against many foodborne pathogens at the foodservice and household levels (19, 20). Acetic acid has been used as an alternative to chlorine for surface decontamination of vegetables. In household applications, vinegar can be used as an inexpensive acetic acid source (4, 5, 21).

The objectives of this study were to determine the level of microbial load on the surfaces of raw leafy vegetables (cos lettuce, arugula, parsley and lettuce) marketed in Ankara, Turkey and to evaluate the efficacy of different surface decontamination methods in the reduction of microbial load on the vegetables.

MATERIALS AND METHODS

Vegetable Samples

Cos lettuce (*Lactuca sativa* L. var. *longifolia*), arugula (*Eruca sativa*), parsley (*Petroselinum crispum*) and lettuce (*Lactuca sativa*) samples were purchased from the local wholesale markets in Ankara, Turkey and transported to the laboratory under refrigerated conditions at the day of the treatment. Sampling was performed four times for each vegetable and totally 16 vegetable samples were examined in the study. The outer damaged leaves and cores of lettuce and non-edible stems of arugula and parsley were aseptically removed and discarded. Then leaves of arugula, cos lettuce and lettuce were shredded into approximately 2-3 cm pieces of using a sterile stainless steel knife while parsley leaves used without cutting.

Preparation of Treatment Solutions

Tap water was sterilized for decontamination treatment. Chlorine solution (200 mg/L, pH 6.50) was prepared using commercial grade sodium hypochlorite (Ak-Kim, Istanbul, Turkey) and sterile deionized water. The free chlorine level was determined by the method given in the standard of Turkish Standards Institution (22). Acetic acid solution was prepared using glacial acetic acid (Merck, Darmstadt, Germany) and sterile deionized water at the concentration of 0.25 % (v/v, pH 3.48).

Decontamination Treatments of Vegetables

Decontamination treatments were performed by dipping 200 g of vegetable samples into two liters of the appropriate treatment solution by continuous stirring using a sterile stainless steel spatula. (I) Control group was not subjected to any surface decontamination method. The other groups were subjected to each decontamination solution for 10 min; (II) tap water, (III) 200 mg/L chlorine, (IV) 0.25 % (v/v) acetic acid and (V) sequential dipping using 0.25 % (v/v) acetic acid followed by 200 mg/L chlorine (sequential dipping treatment). Treated samples (III, IV and V) were subsequently rinsed with 2 liters of sterile deionized water in order to eliminate possible sensory quality defects caused by the use of acetic acid and chlorine as mentioned in the literature (2, 23, 24). At the end of treatments, samples were spin-dried in a sanitized home salad spinner for 1 min. All treatments were performed four times at room temperature (24±2 °C).

Microbiological Analysis

After decontamination treatments 25 g of each sample was immediately weighted in a sterile stomacher bag and 225 ml of buffered peptone water (PW, Merck, Germany) was added into the bag and homogenized using Seward 400 laboratory stomacher (West Sussex, UK) at medium speed for 1 min. Serial dilutions were made by using PW. The efficacies of the surface decontamination methods were examined by determination of the numbers of total mesophilic aerobic bacteria (MB), total coliforms (TC) and *Escherichia coli*. Total mesophilic aerobic bacteria were enumerated in plate count agar (PCA, Merck, Germany) following the pour plate method by incubation at 37 °C for 48 h (25, 26).

The numbers of TC and *E. coli* were determined by using three tube most probable numbers (MPN) method. Appropriate dilutions were inoculated into Fluorocult® Lauryl Sulfate Tryptose (LST-MUG, Merck, Germany) broth with Durham tube and incubated at 37 °C for 24 to 48 h. One loopful culture from the tubes with gas formation at 24 and 48 h was transferred to brilliant green bile broth (BGB, Merck, Germany) and incubated at 37 °C for 24 to 48 h. The BGB tubes with gas and turbidity formation were used for the enumeration of coliform by using the MPN table. For the determination of *E. coli* numbers, gas and turbidity positive LST-MUG broth tubes at 24-48 h were examined for blue fluorescence at a wavelength of 366 nm using a hand held UV lamp (CAMAG, Muttenz, Switzerland). As a confirmation, indole test was performed by using Kovac's reagent. The gas, fluorescence and indole positive LST-MUG broth tubes were used for the determination of *E. coli* number by using the MPN table (27-29).

Statistical Analysis

All experiments were replicated four times. Data were subjected to Duncan's test using SPSS version 11.5 (SPSS Inc., Chicago, USA) to determine if significant (P<0.05) differences existed between decontamination treatments.

RESULTS AND DISCUSSION

The effects of different surface disinfection methods treatments on cos lettuce, arugula, parsley and lettuce samples are presented in Table 1 and 2. In this study, the levels of initial MB (6.20 - 7.14 log cfu/g) of the cos lettuce, arugula, parsley and lettuce samples were found fairly high. These results are similar with the study of various researchers. For example, Abadias et al. (30) reported the mean mesophilic aerobic bacteria counts of iceberg, lettuce hearts and romaine samples as 4.6, 4.4 and 6.0 log cfu/g, respectively. Similarly, Allende et al. (18) found high number of total mesophilic aerobic bacteria (6-8 log cfu/g) in salad leaves (lettuce and escarole).

Table 1. Effects of disinfection treatments on total mesophilic aerobic bacteria counts (log cfu/g)* on cos lettuce, arugula, parsley and lettuce samples

	Control	Tap water	Chlorine (200 ppm)	Acetic acid (0.25%, v/v)	Acetic acid (0.25%, v/v) + Chlorine (200 ppm)
Cos lettuce	6.60±0.12^{A**}	5.98±0.24^{AB}	5.12±0.62^{BC}	5.58±0.69^{BC}	4.65±0.91^{C}
Arugula	6.20±0.08^{A}	5.45±0.35^{B}	4.77±0.33^{C}	4.70±0.17^{C}	3.93±0.33^{D}
Parsley	7.14±1.02^{A}	6.31±0.91^{A}	5.64±0.86^{A}	5.99±1.06^{A}	5.30±1.12^{A}
Lettuce	6.40±0.34^{A}	5.72±0.48^{AB}	5.22±0.44^{BC}	5.08±0.53^{BC}	4.93±0.52^{C}

*Values are mean ± SD population recovered (log cfu/g) (n=4)
**Different capital letters in the same raw significantly different (P<0.05)

Table 2. Effects of disinfection treatments on total coliform counts (log MPN/g)* on cos lettuce, arugula, parsley and lettuce samples

	Control	Tap water	Chlorine (200 ppm)	Acetic acid (0.25%, v/v)	Acetic acid (0.25%, v/v) + Chlorine (200 ppm)
Cos lettuce	4.91±0.67^{A**}	4.20±0.30^{AB}	3.47±0.51^{BC}	3.96±0.56^{B}	2.84±0.77^{C}
Arugula	3.39±0.77^{A}	3.10±0.80^{AB}	1.77±0.71^{C}	1.97±0.53^{BC}	1.39±0.23^{C}
Parsley	4.73±0.48^{A}	3.75±0.19^{B}	2.66±0.71^{C}	3.67±0.29^{B}	2.49±0.56^{C}
Lettuce	3.74±1.14^{A}	2.99±1.17^{A}	2.29±1.43^{A}	2.69±1.02^{A}	2.37±1.39^{A}

*Values are mean ± SD population recovered (log cfu/g) (n=4)
**Different capital letters in the same raw significantly different (P<0.05).

The presence of high level of TC is undesirable although these are part of the natural microflora on vegetables (31). High microbial loads of TC were found on the samples examined. Mean TC counts of control samples of romaine lettuce, arugula, parsley and lettuce were 4.91, 3.39, 4.73 and 3.74 log MPN/g, respectively. In contrast, Cruz et al. (32) and Soriano et al. (1) reported lower coliform counts (ranged from <0.47 to >3.38 log MPN/g and 0.65 to 1.55 log MPN/g, respectively) on lettuce samples.

The incidence of *E. coli* was fairly high on the samples. Among the sixteen vegetable samples analyzed, thirteen tested positive for *E. coli*. The population of *E. coli* was between 0.60 and 4.32 log MPN/g in parsley and arugula samples respectively (data not shown). The presence of *E. coli* is commonly used as an indicator of fecal contamination in foods and its existence indicates the likely presence of pathogens such as *Salmonella*, *Shigella* and *E. coli* O157:H7 (17, 30, 32). Thus, determination of *E. coli* is important in the food safety point of view. Meldrum et al. (33) reported that 3.9 % of the salad vegetables (cabbage, cucumber, lettuce, onion, tomato) analyzed contained >2 log cfu/g *E. coli*. On the contrary, McMahon and Wilson (9) couldn't isolate *E. coli* from the organic vegetables (carrot, mushroom, tomatoes, cherry tomatoes, pepper, courgette, asparagus, red onion, onion, spring onion, potato, alfalfa sprouts, water cress, lettuce, cucumber, broccoli, cabbage, celery, cauliflower and turnip) examined. These high MB and TC counts and high incidence of *E. coli* on leafy vegetables could possibly be originated from natural flora of soil, contaminated irrigation water, manure and animals (12).

Washing vegetables with water is the most used decontamination method in house level, although its decontamination efficacy is very low. In this study, the maximum reduction with the tap water in MB and TC counts were 0.83 log cfu/g and 0.98 log MPN/g (in parsley), respectively. However, there was no significant difference in the counts of MB (except arugula) and TC (except parsley) after the tap water treatment compared to control. Similarly, Allende et al. (18) found that water washing reduced the initial population of total mesophilic aerobic bacteria about 1 log in fresh cut lettuce and escarole. Various studies had also shown that decontamination efficacy of water on vegetables was not more than 1 log (5, 20).

The decontamination efficacy of chlorine was found to be significant (P<0.05) at reducing MB and TC counts. MB and TC counts were reduced by 1.18-1.49 log cfu/g and 1.44-2.07 log MPN/g, respectively. The results obtained in our study for chlorine are also consistent with those reported by other researchers. Allende et al. (18) reported about 2 log reduction in MB and TC counts following 100 mg/L free chlorine (pH 6.5) treatment for 1 min on lettuce samples. Delaquis et al. (17) reported about 1 log and 2 log reductions in MB counts following 100 ppm free chlorine (pH 8.7) treatments of lettuce samples for 1 min at 4 °C and at 50 °C, respectively. Washing lettuce samples with chlorinated water (200 ppm free chlorine, for 15 min) reduced the MB and TC counts by 2.63 and 1.91 log, respectively (5). Vandekinderen et al. (2) reported maximum 0.5 - 2 log reduction in TC counts following 20-200 mg/L free chlorine (pH 6.0) treatment for 1-5 min on carrot samples.

As a sanitizing agent, organic acids are used as an alternative to chlorine in decontamination of fresh vegetables. Acetic acid (vinegar) is the most widely used acidulant in salads to give zest or tang flavor (34). Reductions in the counts of MB and TC on vegetables treated with 0.25% acetic acid were significant ($P<0.05$). The average reductions were about 1.25 log cfu/g and 1.12 log MPN/g in MB and TC counts, respectively. As seen from Table 1 and 2, the best decontamination results with the use of acetic acid were achieved in arugula (1.50 log cfu/g and 1.42 log MPN/g). Akbas and Ölmez (24) reported that dipping of lettuce in 0.5 % acetic acid for 2 min significantly ($P<0.05$) decreased (1.3 log cfu/g) the number of *E. coli*, and the change in the acetic acid concentration (from 0.5 to 1.0 %) had no significant effect on reduction level. Chang and Fang (21) compared the effectiveness of acetic acid to reduce the *E. coli* O157:H7 for 5 min at 25 °C in lettuce samples. They reported that 5% acetic acid reduced the *E. coli* count (~3 log) significantly. On the other hand, acetic acid concentrations of 0.05% and 0.5% had no significant effects. Nascimento et al. (5) reported 3.37 and 3.91 log reductions in MB counts following 2% and 4% acetic acid treatments of lettuce samples for 15 min. The reductions in TC counts were found as >2.25 log with 2% and 4% acetic acid treatments.

Overall statistical analysis indicated that sequential dipping in acetic acid and chlorine solution was the most effective method in reducing the MB and TC counts. Initial MB counts reduced by 1.96, 2.27, 1.84 and 1.48 log cfu/g on cos lettuce, arugula, parsley and lettuce samples, respectively; where in the case of TC counts the reduction levels were determined as 2.07, 2.0, 2.24 and 1.45 log MPN/g. Statistically, no significant differences were observed between sequential dipping treatment and individual applications of chlorine and acetic acid on MB counts except from arugula sample. However, better reduction levels were observed numerically by sequential dipping treatments than the others (Table 1). Similarly, application of sequential dipping and individual use of chlorine showed no significant differences in the reduction of TC counts. However, in the view of the reduction rate, the sequential dipping treatment gave relatively higher reduction in TC counts than chlorine treatment except lettuce samples as seen in Table 2. In agreement with our results, Singh et al. (35) showed that microbial quality could be improved more when the washing regime was repeated with different sanitizer.

In parsley samples, no significant decreases were determined in the level of MB although there were up to 1.84 log cfu/g reductions after decontamination treatments. Similarly, the reduction in TC counts were found insignificant in lettuce samples, although there were up to 1.45 log MPN/g reduction compared to control after decontamination treatments.

Since the initial *E. coli* levels were very low (Table 3) and standard deviations of replicate experiments were also very high in all tested samples, the effect of decontamination treatments on *E. coli* could not be determined.

Table 3. Mean *E. coli* counts (log MPN/g)* in control samples

	Control
Cos lettuce	2.12±1.19
Arugula	1.17±0.71
Parsley	1.99±2.18
Lettuce	1.79±0.94

*Values are mean ± SD population recovered (log cfu/g) (n=4)

In conclusion, the high counts of MB and TC were determined on the leafy vegetables. Also high incidence of *E. coli* was found on the samples. It is very difficult to remove or kill the microorganisms after the produce is contaminated. Therefore Good Agricultural Practices must be applied by farmers in the field level in order to prevent this microbial contamination.

The results obtained in this study showed that the levels of microbial contamination of the leafy vegetables surfaces were fairly high and tap water had limited or no effect on reducing these microorganisms. The use of acetic acid or vinegar could be preferred instead of chlorine for surface decontamination in home applications and foodservice establishments. Compared to the single dipping treatments, sequential dipping method showed the best reduction rate on microbial load of leafy vegetables.

REFERENCES

1. Soriano JM, Rico H, Moltó JC, Mañes J. 2000. Assessment of the microbiological quality of lettuce served in University restaurants. *Int J Food Microbiol*, 58, 123-8.

2. Vandekinderen I, Van Camp J, Devlieghere F, Veramme K, Denon Q, Ragaert P, De Meulenaer B. 2008. Effect of decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (Daucus carota L.). *J Agr Food Chem*, 56, 5723-5731.

3. Behrsing J, Winkler S, Franz P, Premier R. 2000. Efficacy of chlorine for inactivation of Escherichia coli on vegetables. *Postharvest Biol Tec*, 19, 187–192.

4. Sengun IY, Karapinar M. 2004. Effectiveness of lemon juice, vinegar and their mixture in the elimination of Salmonella typhimurium on carrots (Daucus carota L.). *Int J Food Microbiol*, 96, 301-305.

5. Nascimento MS, Silva N, Catanozi M, Silva KC. 2003. Effects of different disinfection treatments on the natural microbiota of lettuce. *J Food Protect*, 66, 1697–1700.

6. Viswanathan P, Kaur R. 2001. Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. *Int J Hyg Envir Heal*, 23, 205–213.

7. Cordano AM, Jacquet C. 2009. Listeria monocytogenes isolated from vegetable salads sold at supermarkets in Santiago Chile: Prevalence and strain characterization. *Int J Food Microbiol,* 12, 176–179.

8. Abong'o BO, Momba MNB, Mwambakana JN. 2008. Prevalence and antimicrobial susceptibility of Escherichia coli O157:H7 in vegetables sold in the Amathole district, Eastern Cape Province of South Africa. *J Food Protect*, 71, 816–819.

9. McMahon MAS, Wilson IG. 2001. The occurence of enteric pathogens and Aeromonas species in organic vegetables. *Int J Food Microbiol*, 70, 155–162.

10. Rimhanen-Finne R, Niskanen T, Hallanvuo S, Makary P, Haukka K, Pajunen S, Siitonen A, Ristolainen R, Poyry H, Ollgren J, Kuusi M. 2006. Yersinia psudotuberculosis causing a large outbreak associated with carrots in Finland. *Epidemiol Infect*, 137, 342–347.

11. Szabo EA, Scurrah KJ, Burrows JM. 2000. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce Lett Appl Microbiol, 30, 456–460.

12. Beuchat LR. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect*, 4, 413–423.

13. Harris L, Farber J, Beuchat L, Parish M, Suslow T, Garrett E, Butsa F. 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comp Rev Food Sci* F,. 2, 78–141.

14. Karapinar M, Sengun IY. 2007. Antimicrobial effect of koruk (unripe grape – Vitis vinifera) juice against Salmonella Typhimurium on salad vegetables. *Food Control*, 18, 702–706.

15. Beuchat LR, Nail BV, Adler BB, Clavero MRS. 1998. Efficacy of spray application of chlorine in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J Food Protect*, 61, 1305–1311.

16. Sapers GM. 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technol Biotech*, 39(4), 305–311.

17. Delaquis PJ, Fukumoto LR, Toivonen PMA, Cliff MA. 2004. Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biol Tec*, 31(1), 81–91.

18. Allende A, Selma MV, López-Gálvez F, Villaescusa R, Gil MI. 2008. Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce, *Postharvest Biol Tec*, 49, 155-163.

19. Parish M, Beuchat L, Suslow T, Haris L, Garrett E, Farber J, Butsa F. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comp Rev Food Sci* F, 2, 161-173.

20. Keskinen LA, Burke A, Annous BA. 2009. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157:H7 from lettuce leaves. *Int J Food Microbiol*, 132 (2–3), 134–140.

21. Chang JM, Fang TJ. 2007. Survival of Escherichia coli O157:H7 and Salmonella entrica serovar Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against E. coli O157:H7. *Food Microbiol*, 24, 745–751.

22. Anon 2002. Turkish Standards Institution (TSE) 2002. Water quality - Determination of free chlorine and total chlorine - Part 3: Iodometric titration method. Turkish Standards Institution Publications No. TS 6230 EN ISO 7393-3, Ankara, Turkey.

23. Bett LK. 2002. Evaluating sensory quality of fresh cut fruits and vegetables. In: Fresh Cut Fruits and Vegetables: Science, Technology and Market, L. Olusola (Ed.), CRC Press LLC, New York, pp. 436.

24. Akbas MY, Olmez H. 2007. Inactivation of Escherichia coli and Listeria monocytogenes on iceberg lettuce by dip treatments with organic acids. *Lett Appl Microbiol*, 44, 619–624.

25. Harrigan W.F. 1998. Laboratory Methods in Food Microbiology. 3rd Edition, Academic Press, San Diego, USA, 532 p.

26. Temiz, A. 2000. Genel Mikrobiyoloji Uygulama Teknikleri. 3. Baskı, Hatiboğlu Yayınevi, Ankara, Türkiye, 291 p.

27. FDA. 2002. BAM: Enumeration of Escherichia coli and the Coliform Bacteria. http://www.fda.gov/food/scienceresearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm064948.htm (Accessed 01 March 2004).

28. Feng PCS, Hartman PA. 1982. Fluorogenic assays for immediate confirmation of Escherichia coli. *Appl Environ Microb*, 43, 1320-1329.

29. Anonymous, 2005. Merck Gıda Mikrobiyolojisi Uygulamaları. Ed: A.K. Halkman. Başak Matbaacılık Ltd. Şti., Ankara, Türkiye, 358 p.

30. Abadias M, Usall J, Anguera M, Solsona C, Viñas I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int J Food Microbiol*, 123, 121–129.

31. Doğan-Halkman HB, Çakır İ, Keven F, Worobo RW, Halkman AK. 2003. Relationship among fecalcoliforms and Escherichia coli in various foods. *Eur Food Res Technol*, 216, 331–334.

32. Cruz AG, Cenci SA, Maia MCA. 2008. Microbiological hazards involved in fresh-cut lettuce processing. *J Sci Food Agr*, 88, 1455–1463.

33. Meldrum RJ, Little CI, Sagoo S, Mithani V, McLauchlin J, Pinna E. 2009. Assesment of the microbiological safety of salad vegetables and sauces from kebab takeaway restaurants in the United Kingdom. *Food Microbiol*, 26, 573–577.

34. Sengun IY, Karapinar M. 2005. Effectiveness of household natural sanitizers in the elimination of Salmonella typhimurium on rocket (Eruca sativa Miller) and spring onion (Allium cepa L.). *Int J Food Microbiol*, 98, 319-315.

35. Singh N, Singh RK, Bhunia AK, Stroshine RL. 2002. Efficacy of Chlorine dioxide, Ozone, and Thyme essential oil or a sequential washing in killing Escherichia coli O157:H7 on lettuce and baby carrots. *LWT-Food Sci Technol*, 35, 720–729.

Author Instructions

GIDA (2009) 34 (1): 59-63

www.gidadernegi.org / English / The Journal of FOOD /Author Instructions

Manuscript Submission and Copyright Release Form

GIDA (2009) 34 (1): 67

www.gidadernegi.org / English / The Journal of FOOD /Manuscript Submission and Copyright Release Form

Final Check List

GIDA (2009) 34 (1): 68

www.gidadernegi.org / English / The Journal of FOOD /Final Check List

can be reached from those addresses. Authors must read carefully the author instructions and prepare the manuscript accordingly.