

EFFICACY OF NANOSIL (HYDROGEN PEROXIDE-SILVER ION) AND PERACETIC ACID ON REDUCTION OF ESCHERICHIA COLI O157:H7 AND SALMONELLA ENTERITIDIS IN RAW VEGETABLES

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Abstract

This study aimed to evaluate the efficacy of peracetic acid and nanosil on reduction of E. coli O157: H7 and Salmonella enteritidis in raw mixed vegetables (radish, parsley basil, coriander, Allium Porum and peppermint). Raw vegetables were treated with 40, 60 and 80 mg/l peracetic acid and 120, 150 and 180 mg/l nanosil (a combination of hydrogen peroxide-silver ion) for 5 and 10 minutes. The reduction in the numbers of E. coli O157: H7 and Salmonella enteritidis inoculated in raw mixed vegetables were evaluated. The results showed no significant reduction in the number of E. coli O157: H7 and Salmonella enteritidis at different contact time ($p > 0.05$). In the optimized condition, Peracetic acid and nanosil reduced the number of E. coli O157:H7 to 1.56 and 2.04 log₁₀ CFU/g. The anti-microbial effect of nanosil and peracetic acid on Salmonella enteritidis was more noticeable. The reductions for peracetic acid were 1.46 and 1.65 log₁₀ CFU/g at the concentration of 80 mg/l and 2.02 and 2.24 log₁₀ CFU/g at the concentration of 180 mg/l for Nanosil. It is concluded that both peracetic acid and nanosil has a good bacteriostatic effect on the bacteria of vegetables. The antibacterial effect of Nanosil for reducing E.coli O157: H7 and Salmonella enteritidis is more than perascetic acid and it can be more effective on E.coli O157: H7.

Keywords: peacetic acid, nanosil, vegetable safety, antibacterial effect, disinfectant, food borne pathogen

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1. INTRODUCTION

Consumption of fresh products is increasingly growing due to their benefits to human health and development. Vegetables, as an important categories, provide essential ingredients such as vitamins, minerals and fiber required for better bodily function (Berbari et al., 2001, Beuchat and Ryu, 1997, López-Gálvez et al., 2009). However, given the possibility of contamination along production chain, mainly originated from soil, water and animals, these products are not completely safe for human consumption. (Berger et al., 2010, Beuchat, 2002, Gorny et al., 2006, Harris et al., 2003). Raw vegetables can carry microbial contamination which can reach approximately 10⁷ CFU/g. In the united states, bacterial contamination of vegetables was reported about 5 percent between 1973 and 2006, from which 60% caused by E.coli O157: H7 and 10

percent were associated with Salmonella spp (Bhagwat, 2006). The presence of various microorganisms in particular salmonella and E. coli O157: H7 in vegetables have been reported by earlier researchers (Gorski et al., 2011, Sant'Ana et al., 2011; Bohaychuk et al., 2009, Rúgeles et al., 2010). In Iran, the most important issue with raw vegetables is the contamination source from untreated or waste water used for irrigation. A report in 2006 showed that more than 20 million of the world's agricultural land were irrigated by untreated or waste water. According to Ministry of Health and official sources of Iran, more than 7000 hectares of agricultural land under cultivation of vegetables are irrigated with contaminated water (Hamilton et al., 2006, Omidvarnia et al., 2010). A wide variety of methods such as ozonation, use of hydrogen

peroxide and chlorine organic acids has been applied to disinfect fruits and vegetables (Alvarado-Casillas et al., 2007, Beuchat, 1998, Gil et al., 2009). Chlorine has been suggested at the concentrations of 50 to 100 ppm for washing fruits and vegetables, however, the probability of formation of carcinogenic compounds resulted from this substance has created serious concerns (Beuchat et al., 1998, Rodgers et al., 2004).

Nanosil is a disinfectant solution and composed of hydrogen peroxide and silver ions that is produced by kimia faam, an Iranian pharmaceutical company. The presence of silver ions in addition to the catalytic role kills bacteria. Oxygen released from hydrogen peroxide destroys the protective membranes of bacteria and penetrate into the cells. This product is completely safe and according to the manufacturer, do not have any harmful effect on environment or human health (Kimia faam. Co/www.kimiafaam.com). Based on our literature review, there is no information on the antimicrobial effects of nonosil on food-borne pathogens. Considering that the main compounds are hydrogen peroxide and small amount of silver ion (30 ppb), therefore, the concentration tested in this study are sourced from earlier studies conducted on antimicrobial effects of hydrogen peroxide. Another disinfectant is peracetic acid. It is a commercial product which is made of a mixture of hydrogen peroxide and acetic acid (Kitis, 2004). It is broken down to oxygen and acetic acid and finally converted to carbonic anhydride. The use of this substances is permitted in many countries for treating and disinfecting fruits and vegetables (Rovira et al., 2012). in the USA, its application in the concentration of 80 ppm is allowed (FDA, 2001). a number of studies evaluated the effects of peracetic acid on reducing *Listeria monocytogenes*, *Salmonella* spp. and *E. coli* O157: H7 in vegetables including lettuce (Dai et al., 2012), carrots (Ruiz-Cruz et al., 2007), spinach (Neal et al., 2012) and cabbage (Lee et al., 2014) and reported that its capability to reduce microbial load in the range of 1 to 4 log₁₀ CFU/g. This study aimed to reduce

microbial load of raw vegetables which are commonly consumed in Iran by using peracetic acid and hydrogen peroxide-silver ion (Nanosil).

2. MATERIAL AND METHODS

2.1. Bacterial strains, growth condition and preparation

Bacterial strains including *Escherichia coli* O157:H7 (ATCC 35150) and *Salmonella enteritidis* (ATCC 13076) were obtained from Department of Microbiology, Tehran University of Medical Sciences. All strains were cultured individually in tryptone soy agar (TSA) at 37 ° C for 24 h. Three days before each experiment, each of bacterial cultures were cultured individually in tryptone soy broth (TSB) at 37 ° C for 24 h. Then 9 ml of each culture was transferred into sterile tube. The cells of each strain were collected by centrifuging at 1600×g at 20° C for 20 min. After centrifuging, pellets of each microorganism was dissolved in 5 ml of buffered peptone water. Finally, all pellets were dissolved in 1 liter of water to form microbial suspension approximately at the concentrations of 8-9 log₁₀cfu/g.

2.2. Sample Collection and inoculation of vegetables

Equal mixtures of fresh raw vegetables including radish, parsley basil, coriander, *Allium Porum*, and peppermint were purchased from center of fruits and vegetables of Tehran city. All the samples were placed into sterile bags and transported to the laboratory in the shortest time. Dirt and mud of vegetables were cleaned and cut into equal pieces and they washed and finally placed under the hood for 1hours. The dipping method was used for microbial inoculation in order to achieve more surface contact between vegetables and inoculation solution. 200 grams of mixed vegetables suspended in inoculation solution containing concentrations of 10⁹-10⁸ log₁₀cfu/g for 2 minutes. Lastly, the inoculated samples were put at room temperature for 1 hour to be dried.

2.3. Preparation of disinfectant solutions

Commercial solution of peracetic acid (purity of 38.5 to 40%) were purchased from Merck co (Germany). In the present study, peracetic acid was used at the different concentrations of 40, 60 and 80 ppm for 5 and 10 minutes. The final concentration of peracetic acid in the working solution was determined by titration reduction-oxidation with ceric sulfate. Commercial nanosil solution was obtained from the pharmaceutical company of Kimia faam with purity of 50%, and the concentrations of 120, 150 and 180 mg/l for 5 and 10 minutes was used as offered by manufacturer.

2.4. Disinfectant treatment

200 grams of the inoculated vegetables were treated with disinfectant solution at known concentrations. After treatment for specified time, the samples were washed using deionized water to remove residual disinfectant.

2.5. Microbial Analysis

Nine milliliter of 0.1% peptone water was added to the samples (10 grams) inside a sterile bag and agitated for 2 minutes. After that, 1 ml of the sample was added into the mixture in sterile condition to prepare serial dilutions. Salmonella enteritidis and E. Coli O157:H7 were cultured in XLD agar and sorbitol macconkey agar respectively incubated at 37 ° C for 24 hours. Negative controls (Inoculated vegetables without disinfectant treatment) was applied in parallel with the tested samples for all experiments.

2.6. Statistical Analysis

The viable cell colonies were counted as log colony forming units (CFU) per gram of samples and subjected to R statistical software for further analysis using the nonparametric test of Kruskal-Wallis.

3. RESULTS AND DISCUSSION

The effect of different concentrations of peracetic acid and nanosil on the reduction of E. Coli O157: H7 is shown in Table 1.

Peracetic acid at the concentrations of 40, 60 and 80 ppm for 5 min caused 0.87, 0.94 and 1.14 log₁₀CFU/g reduction in E. coli O157: H7, respectively.

The treatment of vegetables with the same concentrations for 10 min resulted in more slight reductions of 0.97, 0.96 and 1.56 log₁₀ CFU/g in E. coli O157: H7, respectively. It is clearly evident that the application of peracetic acid at higher concentrations would be significantly more effective for disinfection. But the reduction of E. coli O157: H7 in none of the concentrations wasn't significant (p>0.05).

An earlier study showed that 40 ppm peracetic acid at 10 minutes reduced 0.3 log₁₀CFU/g of Escherichia coli O157: H7 in lettuce (Oh et al., 2005). Neal et al.(2012) also reported that treatment spinach with 80 ppm peracetic acid caused 1.1 log₁₀CFU/g reduction in Escherichia coli O157:H7 (Neal et al., 2012). In another study, peracetic acid was used at different concentrations of 30, 40 and 50 ppm which reduced Escherichia coli O157:H7 to 2.13, 2.33 and 2.47 log₁₀CFU/g in lettuce respectively Keeratipibul et al., (2011). Observed that reduction of bacteria by nanosil in concentrations of 120 to 180 ppm more than peracetic acid. So that at concentration of 120 ppm and 5 minute 1.09 log₁₀CFU/g decreased. At a concentration of 180 ppm and 10 minutes reduction of E. coli. O157: H7 reached to 2.04 log₁₀CFU/g.

Huang and Chen (2011) treated baby spinach with hydrogen peroxide (3%) for 5 min and reported 1.5 log₁₀CFU/g reduction in E. coli. O157: H7 (Huang and Chen, 2011). It seems that the increase in temperature has an effect on increasing yield of hydrogen peroxide.

It is reported that 2% of hydrogen peroxide solution at a temperature of 50 ° C and 90 seconds decreased 4 log₁₀CFU/g of E.coli.O157:H7 and salmonella on fresh-cut melons (Ukuku, 2004).

The effect of different concentrations of peracetic acid and nanosil on the reduction of salmonella enteritidis is shown in Table 2.

Table1. The effect of different concentrations of peracetic acid and nanosil on the reduction of E. Coli O157: H7

Reduction of E. coli O157: H7 (log CFU / g) (mean ± SD)						
Nanosil			Peracetic acid			
180	150	120	80	60	40	Concentration (mg/l) Time(min)
1.84±0.05	1.33±0.04	1.09±0.03	1.14±0.09	0.94±0.02	0.87±0.04	5
2.04±0.03	1.45±0.07	1.17±0.03	1.56±0.03	0.96±0.08	0.97±0.01	10

Table 2.The effect of different concentrations of peracetic acid and nanosil on the reduction of salmonella enteritidis

Reduction of salmonella enteritidis (log CFU / g) (mean ± SD)						
nanosil			Peracetic acid			
180	150	120	80	60	40	Concentration (mg/l) Time(min)
2.02±0.07	1.52±0.04	1.01±0.04	1.46±0.03	1.04±0.03	1.03±0.06	5
2.24±0.07	1.53±0.03	1.12±0.03	1.65±0.03	1.19±0.02	1.05±0.02	10

The effect of peracetic acid on the reduction of Salmonella enteritidis was more significant than of Escherichia coli. peracetic acid at the concentration of 40 ppm with contact times of 5 and 10 min resulted in 1.03 and 1.05 log₁₀CFU/g reduction in Salmonella enteritidis. In one study reported that at concentration of 40 ppm at 5 and 10 minute decreased 0.9 and 0.8 log₁₀CFU/g of salmonella in lettuce (Ge et al., 2013). Peracetic acid at the concentration of 80 ppm showed to be more effective on the reduction of salmonella enteritidis than E. coli O157:H7. Neal et al (2012) reported that peracetic acid at the concentration of 80 ppm reduced salmonella in spinach to 0.8 log₁₀CFU/g (Neal et al., 2012).

The antimicrobial effects of nanosil on salmonella enteritidis was clearly higher than E. coli.O157:H7. The highest reduction observed at the concentrations of 150 and 180 ppm for salmonella enteritidis which was declined to 2.04 log₁₀CFU/g. Ukuku showed the treatment of fresh-cut melons with hydrogen peroxide (2.5 - 5%) caused 3

log₁₀CFU/g reduction in Salmonella spp (Ukuku, 2004).

In the present study, the differences between the applied concentrations for each sanitizer were not statistically significant. However, there were significant differences between the used concentrations (40, 60 and 80 ppm) of peracetic and the concentrations (150 and 180) applied for (p<0.05). Also, significant differences were observed between peracetic acid at the concentrations of 40 and 60 ppm and nonosil at the concentrations of 120 and 180 ppm (p<0.05) (Fig1 and 2). Results also showed no significant difference in the reduction of Escherichia coli (p = 0.213) Salmonella Enteritidis (p = 0.375) between contact times of 5 and 10 min for all the concentrations applied in this study. So it appears that time has not any effect on the reduction of bacteria. Figures 1 and 2 compare the effect of different concentrations of nanosil (120,150 and 180 ppm) and peracetic acid (40, 60, and 80 ppm) on the reduction of E. coli O157: H7 and Salmonella Enteritidis with the contact times of 5 and 10 minutes.

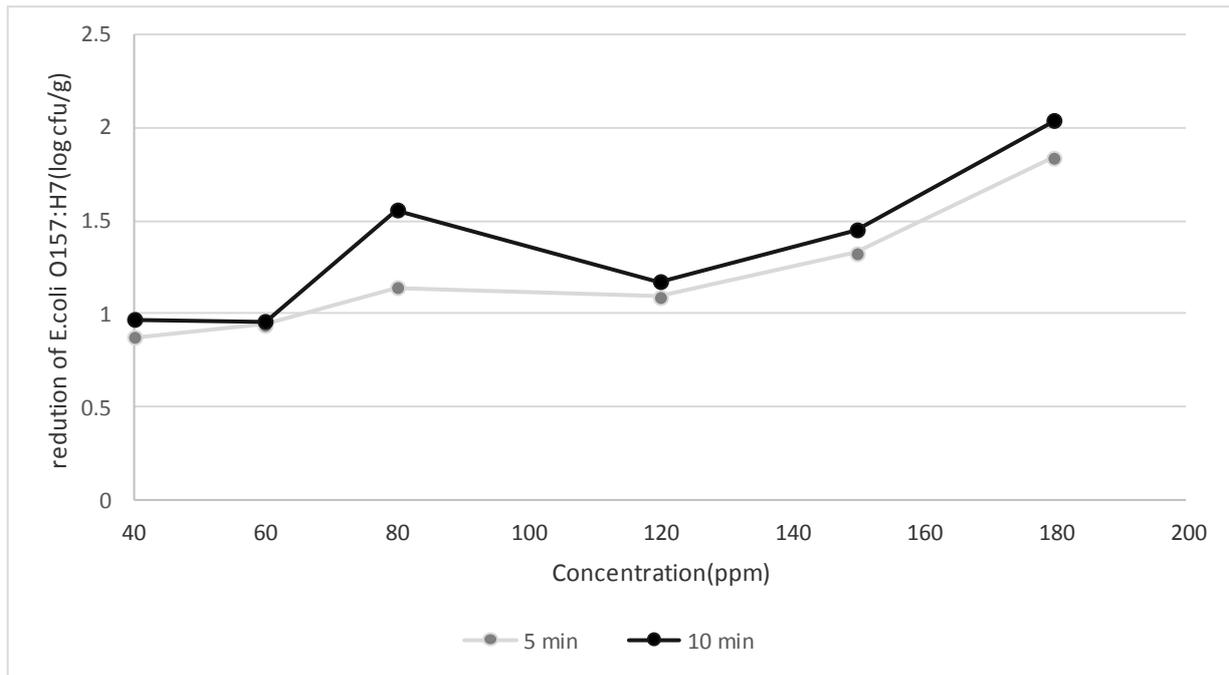


Fig1. The comparison of the used concentrations of nanosil (120,150 and180 ppm) and peracetic acid (40, 60 and 80 ppm) on the reduction of E. coli O157:H7 with contact time of 5 and 10 minutes.

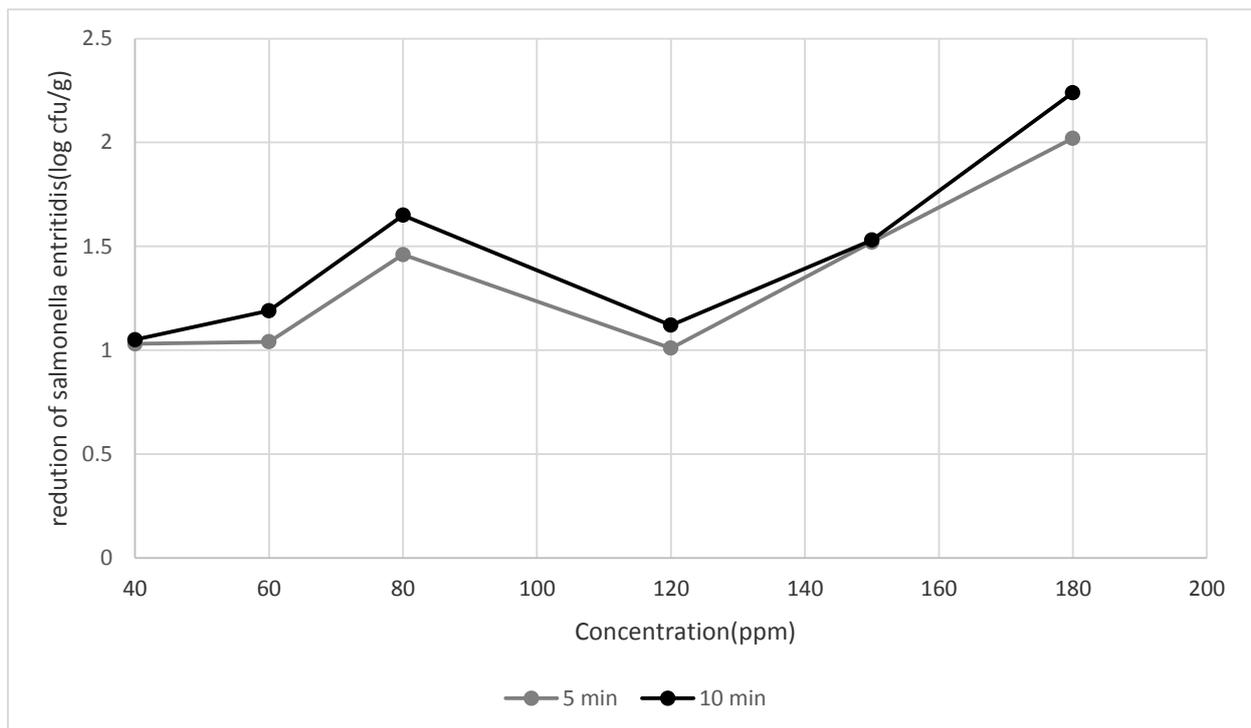


Fig1. The comparison of the used concentrations of nanosil (120,150 and 180 ppm) and peracetic acid (40, 60 and 80 ppm) on the reduction of Salmonella Enteritidis with contact time of 5 and 10 minutes.

4. CONCLUSIONS

Our results showed that nanosil solution has a greater impact on the reduction of E coli O157:

H7, however, peracetic acid, even at the highest concentration, did not show a significant antimicrobial effect. In addition, considering no significant differences in the

reduction of bacteria between contact times at low concentrations, the greatest reductions in microflora would be achieved when higher concentrations of sanitizers are used in combination with greater contact time.

5. ACKNOWLEDGEMENT

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