pISSN 1229-1153 *J. Fd Hyg. Safety*
Vol. 28, No. 3, pp. 227~233 (2013)
http://dx.doi.org/10.13103/JFHS.2013.28.3.227

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Susceptibility of Foodborne Pathogens Isolated from Fresh-Cut Products and Organic Vegetable to Organic Acids and Sanitizers

Kyung Min Park, Minwoo Baek, Hyun Jung Kim, Byeong Sam Kim, and Minseon Koo*
Korea Food Research Institute, Gyeonggi 463-746, Korea
(Received January 10, 2013/Revised May 28, 2013/Accepted July 31, 2013)

ABSTRACT - In this study, we evaluated the ability of various disinfectants to suppress the growth of microorganisms in fresh-cut products and organic vegetable. The growth of more than 50% of *B. cereus* isolates were suppressed by 50% ethanol, 0.1% hydrogen peroxide, 0.4% sodium hypochlorite or 1% calcium oxide. *E. coli* generally showed high susceptibility to concentration of 10% ethanol, 0.4% sodium hypochlorite and 1% calcium oxide. Eighty percent or more of *S. aureus* isolates exhibited resistance to ethanol, hydrogen peroxide and sodium hypochlorite, but the isolates were susceptible to concentrations of 1% calcium oxide. All isolates evaluated in this study were sensitive to benzalkonium chloride (BAC) and growth in the presence of 2.0 μg/mL of BAC was completely inhibited. These pathogens showed widely different susceptibilities to different organic acids. Greater than 0.5% acetic acid and 2% and higher concentrations of malic acid and tartaric acid inhibited the growth of 60% of the isolates of *B. cereus*. Two percent acetic acid and tartaric acid inhibited 50% of the *S. aureus* isolates. Seventy percent of the *E. coli* isolates were resistant to malic acid and susceptible to 1% acetic acid and 10% tartaric acid. The antibacterial effects of the various sanitizers evaluated in this study were not only dependent on the type of disinfectant but also on the pathogen. Thus, it is important to select a sanitizer that is safe and effective at removing specific types of microorganisms.

Key words: Fresh cut produce, Organic vegetable, Antimicrobial agent, Foodborne pathogen

Introduction

Dietary consumption of vegetables and fruits has health benefit, and prevent or reduce the risk of some chronic disease. The benefits associated with fresh cut products have contributed to a substantial increase in the consumption of ready-to-eat vegetables. However, these changes in dietary habits, including the consumption of raw or minimally processed fruits and vegetables, have been accompanied by a number of foodborne illnesses attributed to fresh food[1]. This increase in consumption has thus been correlated with an increased frequency of outbreaks of illness associated with raw fruits and vegetables[2,3].

Escherichia coli, *Staphylococcus aureus*, *Salmonella* spp., and *Bacillus cereus* are major pathogens that found from raw vegetables in worldwide[4]. Thus, it is essential to use effective sanitizers against pathogenic microorganisms to minimize the risk of foodborne illness associated with fresh vegetables and fruits.

Ethanol, hydrogen peroxide (H_2O_2), and sodium hypochlorite (NaClO), which are sanitizers that can be use directly or indirectly on food, are the most widely used and studied sanitizers in Korea. However, chemical disinfectants can produce chemical residues on the surface of fruits and vegetables and was known potentially carcinogenic or mutagenic-products, such as haloacetic acid, that can be harmful to human health[5]. Therefore, there is a need for safer and more effective antimicrobial treatments in order to inactive microorganism of fruits and vegetables.

Recently, many natural organic acids have been studied as alternatives to chemical disinfectants. Organic produce must be cultivated with no agricultural chemicals agents, thus, organic acids are suitable for controlling microorganisms on organic produce. Because organic acids are "Generally Recognized As Safe" (GRAS) for treatment of food ingredients[6]. Various organic acids have been used to control microorganisms, and they are generally recognized as being effective and safe household sanitizers[7]. The antimicrobial activity of organic acids, including lactic, citric and acetic acids for searching alternative sanitizing method have evaluated against pathogens in much studies[7,12].

Washing is a critical step in fresh cut produce processing to inhibit the growth of microorganism and maintenance food quality. But prolonged and repeated exposed to a chemical disinfectant to remove microorganism may development

*Correspondence to: Minseon Koo, Korea Food Research Institute, Gyeonggi 463-746, Korea
Tel: 82-31-780-9161, Fax: 82-31-709-9876
E-mail: minsk@kfri.re.kr

resistance to the disinfectant itself against pathogens. Generally, a establishment of resistance to *L.monocytogenes* and *S. aureus* by frequent use of Benzalkonium chloride in food-processing is well known[11].

Many chemical sanitizers, such as hydrogen peroxide[8], sodium hypochlorite[9], calcium oxide[10], and benzalkonium chloride[11], have already been investigated for efficacy against pathogens. However most previous studies has focused on the inhibitory effects of sanitizers on food deliberately inoculated with foodborne pathogens, while few studies have tested about effectiveness of disinfectants on microorganisms isolated from food itself.

Therefore, the present study performed to evaluate the susceptibility of foodborne pathogens, including *B. cereus, S. aureus,* and *E. coli* isolated from fresh product to various sanitizing agents. The objectives of this work were to compare the effectiveness of an organic acid against food pathogen isolate and recommend the appropriate use of sanitizers against the contaminating pathogen.

Materials and Methods

Source of foodborne pathogens

B. cereus (65 isolates), *S. aureus* (5 isolates), and *E. coli* (9 isolates) isolates in 96 fresh and organic vegetables were used[13]. All cultures maintained on tryptic soy agar (Merck, Darmstadt, Germany) slants at 4°C. Four standard cultures (*Escherichia coli* ATCC 10536, *Salmonella typhimurium*, ATCC 13311, *Bacillus cereus* ATCC 21772, and *Staphylococcus aureus* ATCC 33591) were used as control strains.

Antimicrobial agents

The chemical sanitizers, ethanol, sodium hypochlorite, calcium oxide and benzalkonium chloride, purchased from Sigma Chemical Co. (St. Louis, MO, USA). The organic acids included acetic acid, citric acid, fumaric acid, malic acid, lactic acid, tartaric acid, and pyruvic acid and purchased from Merck (Damstadt, Germany). All antimicrobial agents except calcium oxide diluted with sterile distilled water and filtered using a 0.2 μm membrane filter (Sartorius AG, Goettingen, Germany). Calcium oxide was diluted with dimethyl sulfoxide (Sigma).

Antimicrobial susceptibility test

Strain activation

Before the experiment, all strains in this study activated in tryptic soy broth (TSB, Merck, Darmstadt, Germany) and incubated for 24 h either 30°C *B. cereus* or 37°C *S. aureus* and *E. coli*. The activated culture reactivated in a fresh TSB. The final cell concentration of 10^6~10^7 CFU/mL used in this study determined using a turbidity of 0.5 McFarland (McF) in a Densichek (bioMérieux, Marcy I'Etoile, France).

Broth dilution method

Antimicrobial agents diluted in sterile water and added to the TSB at final concentrations of 10%, 30%, and 50% ethanol; 0.1%, 0.5%, and 1% hydrogen peroxide; 0.1%, 0.2%, and 0.4% sodium hypochlorite; and 1%, 3%, and 5% calcium oxide. Organic acids were tested at concentrations of 0.5%, 1%, 2%, 5%, and 10%. Antimicrobial susceptibility effect was determined by the broth dilution method as recommended by the CLSI[14]. A change in turbidity of the cultures was observed by measuring the absorbance using a Microplate reader (BioTek Instrument, Winoski, USA) at 600 nm. All experiments were performed in triplication for each isolate. And viability of bacteria was confirmed by plating on tryptic soy agar (TSA, Merck, Darmstadt, Germany).

The following calculation was used: inhibition rate (%) = 100 × (the number of strains with inhibited growth / the number of tested strains.)

Disk diffusion method

The antibacterial activity of benzalkonium chloride against isolates of *E. coli, S. aureus,* and *B. cereus* determined using the disc diffusion method by the CLSI. Inoculums of 10^5-10^6 CFU/mL were spread on the surface of Muller-Hinton agar (Hanil Komed, Gyeonggi-do, Korea). Sterile paper discs (6 mm diameter, Toyo Roshi Kaisha Ltd., Tokyo, Japan) were separately laid to the surface of the inoculated agar plates after being dropped the disinfectant solutions of different concentrations.

These plates were incubated for 24 h at either 37°C *S. aureus* and *E. coli* or 30°C *B. cereus*. After incubation, the diameter of the inhibition zones around the discs was measured a sensitivity of 0.05 mm.

Statistical analysis

The significance level $p < 0.05$ between the mean values was verified in the manner of an ANOVA test by a statistical analysis system (SAS Institute Inc., North Carolina, USA) program for statistical analysis of data.

Results

Effects of ethanol, hydrogen peroxide, sodium hypochlorite and calcium oxide

Table 1 show the inhibition efficacy of *B. cereus*, *E. coli* and *S. aureus* isolates by ethanol, hydrogen peroxide, sodium hypochlorite and calcium oxide.

Ten percent ethanol inhibited the growth of 43% of *B. cereus* and 66% of *E. coli* isolates, and 30% ethanol had

Table 1. Bactericidal effects of ethanol, hydrogen peroxide, sodium hypochlorite and calcium oxide against pathogens isolated from fresh-cut produce and organic vegetables by the broth dilution method

Treatment	Concentration (%)	B. cereus (n = 65)	E. coli (n = 9)	S. aureus (n = 5)
ethanol	10	43.1 ± 1.54[1)f2)]	66.7 ± 0.00[e]	20 ± 0.00[g]
	30	47.7 ± 1.54[e]	77.8 ± 0.00[b]	20 ± 0.00[g]
	50	56.2 ± 2.31[d]	83.3 ± 5.56[a]	20 ± 0.00[g]
hydrogen peroxide	0.1	59.2 ± 0.77[1)b2)]	44.4 ± 0.00[d]	20 ± 0.00[e]
	0.5	61.5 ± 0.00[b]	50.0 ± 5.58[c]	20 ± 0.00[e]
	1	66.2 ± 1.54[a]	50.0 ± 5.58[c]	20 ± 0.00[e]
sodium hypochlorite	0.1 (100 ppm)	2.3 ± 0.77[1)e2)]	0.0 ± 0.00[f]	0.0 ± 0.00[f]
	0.2 (200 ppm)	29.2 ± 1.54[c]	0.0 ± 0.00[f]	20.0 ± 0.00[d]
	0.4 (400 ppm)	75.4 ± 0.00[b]	100.0 ± 0.00[a]	20.0 ± 0.00[d]
calcium oxide	1	97.7 ± 0.77[1)ab2)]	94.4 ± 5.56[ab]	80.0 ± 0.00[c]
	3	99.2 ± 0.77[ab]	100 ± 0.00[a]	90.0 ± 0.00[b]
	5	100 ± 0.00[a]	100 ± 0.00[a]	90.0 ± 0.00[b]

[1)]Percentage of positive samples based on inhibiting the growth of pathogens isolated from tested sample.
[2)]Mean values with different superscripts are significantly different at $p < 0.05$.

inhibitory efficacy against 47% of *B. cereus* and 77% of *E. coli* isolates. The 50% solution of ethanol was the most effective disinfectant for the *E. coli* isolates. The inhibition rate of *S. aureus* isolates was not change by the ethanol concentration.

The inhibition effects of microorganisms with hydrogen peroxide (H_2O_2) were between 44% and 66%. 0.1% H_2O_2 inhibited approximately 66% of *B. cereus* isolates. Meanwhile, 0.1%, 0.5% and 1% H_2O_2 significantly inhibited *E. coli* by up to 50% and inhibited *S. aureus* by less than 20%.

At concentrations of 400 ppm sodium hypochlorite (NaOCl) presented strong antimicrobial activity against *E. coli* and *B. cereus*. NaOCl inhibited *B. cereus* by 75% and suppressed the growth of *E. coli* by 100%, but it did not inhibit *S. aureus* strains by more than 20%.

Calcium oxide showed better disinfecting efficiency than ethanol, hydrogen peroxide and sodium hypochlorite. A solution of 1% calcium oxide eliminated up to 80% of the tested pathogens. Three percent calcium oxide inhibited *B. cereus*, *E. coli* and *S. aureus* by 99%, 100% and 90%, respectively. Five percent calcium oxide caused 100% growth inhibition of the *B. cereus* isolates.

Effects of benzalkonium chloride

The bactericidal of benzalkonium chloride evaluated by the disc diffusion method, and the results are shown in Table 2. The susceptibility of benzalkonium chloride found to be microorganism-dependent in this study.

Additionally, based on the diameters of the inhibition zones for the three microorganisms, benzalkonium chloride exhibit the best antimicrobial activity on *B. cereus*, followed by *E. coli* and *S. aureus*. At a concentration of 0.1 μg/mL, 98.8% of *B. cereus* isolates and 88.9% of *E. coli* isolates were sensitive to BAC. Meanwhile, all *S. aureus* isolates were inhibited by 0.1 μg/mL of BAC.

Table 2. Antimicrobial effects of benzalkonium chloride (0.1 ug/mL) against pathogens isolated from fresh-cut produce and organic vegetables by the disc[1)] diffusion method

Zone diameter (mm)	No. of positive isolates (%)		
	B. cereus (n = 65)	*E. coli* (n = 9)	*S. aureus* (n = 5)
> 7	1 (1.5)[2)]	1 (11.1)	0
7-9	14 (21.5)	5 (55.6)	0
10-12	29 (44.6)	3 (33.3)	2 (40.0)
13-15	13 (20.0)	0	1 (20.0)
16-18	4 (6.2)	0	2 (40.0)
19-21	4 (6.2)	0	0

[1)]Disc diameter = 6 mm
[2)]Number and percent of positive samples

Effects of acidic disinfectants

In this study, acetic acid, malic acid and tartaric acid were selected in a preliminary bactericidal test using standard cultures of *S. aureus* ATCC 33591, *B. cereus* ATCC 21772 and *E. coli* ATCC 10536.

They exhibited better antibacterial activity than fumaric acid, lactic acid, pyruvic acid and citric acid against *S. aureus*, *B. cereus* and *E. coli*. (data not shown). The bactericidal effects of the selected organic acids on pathogen are shown in Table 3. The bactericidal effects against isolates increased with the concentration of the organic acids. The susceptibility of *E. coli and B. cereus* to acetic acid showed better than *S. aureus*. In particular, 2% acetic acid suppressed the growth of all *E. coli* and 92% of *B. cereus* isolates, and each pathogen showed strong susceptibility relative to *S. aureus*

Table 3. Bactericidal effects of different concentrations of organic acid against pathogen isolates in fresh-cut produce and organic vegetables

Organic acids	Conc. (%)	Inhibition rate (%)		
		B. cereus (n = 65)	*E. coli* (n = 9)	*S. aureus* (n = 5)
Acetic acid	0.5	65.4 ± 0.77[1)d2)]	10.0 ± 10.00[g]	0.0 ± 0.00[h]
	1	84.6 ± 3.08[bc]	80.0 ± 0.00[c]	33.3 ± 11.11[f]
	2	92.3 ± 1.54[ab]	100.0 ± 0.00[a]	55.6 ± 0.00[e]
Malic acid	2	68.5 ± 0.77[b]	0.0 ± 0.00[e]	55.6 ± 0.00[c]
	5	77.7 ± 0.77[ab]	30.0 ± 10.00[d]	55.6 ± 0.00[c]
	10	79.2 ± 2.31[a]	30.0 ± 10.00[d]	72.2 ± 5.56[ab]
Tartaric acid	2	63.8 ± 3.85[cd]	40.0 ± 0.00[e]	55.6 ± 0.00[d]
	5	79.2 ± 2.31[b]	40.0 ± 0.00[e]	61.1 ± 5.56[d]
	10	87.7 ± 0.00[a]	70.0 ± 10.00[c]	61.1 ± 5.56[d]

[1)] Mean values plus and minus the standard deviation in inhibition rate (%)
[2)] Mean values with different superscripts are significantly different at $p < 0.05$

(P < 0.05). In the present experiment, 0.5%, 1%, and 2% acetic acid showed to produce up to 50% inhibition of *S. aureus*.

Malic acid had a weak inhibitory effect on *E. coli* relative to *S. aureus* and *B. cereus*. Solutions of only 2% malic acid suppressed the growth of up to 50% of *B. cereus* and *S. aureus* isolates. Two percent malic acid did not inhibit the growth of *E. coli*, but 5% and 10% malic acid slightly inhibited 30% of *E. coli* isolates.

The addition of tartaric acid inhibited the growth of microorganisms. Sixty-three percent of *B. cereus*, 40% of *E. coli* and 55% of *S. aureus* isolates were affected by the antimicrobial activity of 2% tartaric acid. Increasing the tartaric acid concentration led to a reduction in the number of *B. cereus* and *E. coli* isolates. However, no increase in inhibition rate was observed in *S. aureus* strains for concentrations greater than 5% tartaric acid.

Discussion

These experiments were performed to compare the susceptibility of various disinfectants on pathogen isolates from fresh-cut products and organic vegetable. All of the sanitizers were capable of inhibiting *B. cereus*, *S. aureus* and *E. coli*, but the bactericidal activity was different.

E. coli isolates presented strong inhibition effect in 10% ethanol relative to the other pathogens. Furthermore, the antibacterial effect of ethanol on *E. coli* and *B. cereus* significantly increased with increasing concentration, and 80% or more of *E. coli* isolates showed inhibition of growth in ethanol of 50%. On the other hand, the bactericidal effect of ethanol against *S. aureus* was the lowest. These findings are similar to results from a study by Kim *et al*.[10)] reported that a range of 5% to 20% ethanol did not suppress the growth of *S. aureus*. Ethanol showed a relatively poor antimicrobial effect against *S. aureus* compared with *E. coli* and *B. cereus*. Additionally, 60% to 70% ethanol killed gram-negative bacteria such as *E. coli* by inhibiting the cross-linkage of peptidoglycan and inducing cellular lysis. On the other hand, ethanol had weak activity against gram-positive bacteria[15)]. Generally, foodborne pathogens were more effectively inhibited when exposed to 50% than 20% to 40% ethanol solution[16)]. However, this study found that 50% ethanol inhibited poorly *B. cereus* and *S. aureus.*

Hydrogen peroxide is an effective and well-known disinfectant that has bactericidal efficacy due to its strong oxidizing powder[17)]. However, hydrogen peroxide can leave a residue on the surface of fruits and vegetables and is potentially harmful to humans[18,19)]. In this study, 1% H_2O_2 suppressed the growth of approximately 66% of *B. cereus*, 50% of *E. coli* and 20% of *S. aureus* isolates. Therefore, hydrogen peroxide is a better inhibitor of *B. cereus* and is less effective at inhibiting *S. aureus* relative to *B. cereus* and *E. coli*.

Sodium hypochlorite (NaOCl) is widely used for reducing the bacterial contamination of fruits, vegetables and fresh produce[20)]. However, with chlorine treatment, organic agents in the washing water rapidly inactive chlorine[21,22)]. Therefore, pre-cleaning prior to chlorine treatment is required to chlorine compounds to be effective. In this study, 400 ppm NaOCl had the highest antibacterial effect against *E. coli* and *B. cereus,* however, showed a weak inhibitory effect of only 20% against *S. aureus* isolates.

Previous study demonstrated that low concentrations of NaOCl had bactericidal effects on the growth of *S. aureus*, *B. cereus* and *E. coli*[9,23)]. Lee *et al*.[23)] reported that the level of *S. aureus* in lettuce was reduced from 51 CFU/mL to 31 CFU/mL after treatment with a 20-ppm NaOCl solution, and treatment with 100-ppm solutions reduced the level to 6 CFU/mL. Jeong *et al*[9)] reported that initial *E. coli* contamination levels of 39 CFU/mL in lettuce were reduced to

29 CFU/mL after treatment with a 50-ppm NaOCl solution and to 1 CFU/mL after treatment with a 100-ppm solution. The current study reported that 100-ppm to 200-ppm NaOCl concentrations did not show the inhibition effect against *E. coli* and *B. cereus* and *S. aureus* isolates found to be resistant to even relatively high concentrations of NaOCl. This discrepancy might be due to differences in the experimental conditions or the properties of the test bacteria. Additionally, microorganisms isolated from commercially available food may be adapted to disinfectants after repeated exposure in the food-processing environment.

Scallop shells considered to be waste products that cause environmental problems. However, by using heat treatment, calcium carbonate, the main component of the scallop shell, is converted to calcium oxide (CaO), which has antibacterial properties[24]. In various studies, the presence of high concentrations of calcium in solution interfered with enzyme activity and thus inhibited the growth of microorganisms[25]. Treatment with 1% calcium oxide killed more than 80% of three pathogen isolates in this study. In particular, CaO was effective for the inhibition of *S. aureus* compared to other sanitizers.

Similar results were obtained by Kim *et al*[10], who observed that CaO showed better disinfecting efficiency against *B. cereus* than chlorine and hydrogen peroxide. CaO is not commonly used as disinfectant for washing the fresh cut vegetable and fruit in food processing industry yet. Thus CaO might show the best antimicrobial activity against most of *B. cereus*, *E. coli* and *S. aureus* isolates in this study.

BAC, an active quaternary ammonium compound (QAC), is widely used as a disinfectant in the medical and food-processing environment to eliminate and prevent the spread of unwanted microorganisms. However, the frequent use and misuse of QACs, such as the use of high concentrations of the disinfectant or insufficient rinsing and exposure times, has raised concerns regarding whether QAC-resistant bacteria from food and the food environment may emerge[26].

QAC resistance of Staphylococci in the food industry and in humans is well known. Ase *et al.*[11] and Heir *et al.*[27] reported that 10% to 13% of Staphylococci isolated from areas of the food industry and *Listeria monocytogenes* isolated from fish product showed resistance (MIC ≥ 3.0 μg/mL) to BAC. The minimum inhibitory concentration (MIC) guideline for QACs-resistant bacteria is over 3.0 μg/ml, and the MIC guideline for QACs-sensitive bacteria is under 2.0 μg/mL[26]. All isolates used in this study were sensitive to BAC, and a concentration of 2.0 μg/mL was completely inhibitory. The failure to detect BAC-resistant pathogens was thought to be due to different source of the samples. Pathogen isolates in this study were isolated from fresh vegetables that had been packed by minimal processing after harvesting. Therefore, the bacteria had little chance to contact BAC, a disinfectant used in the food-processing environment, thus all of the strains could be sensitive to BAC.

This study found that BAC was more effective against *E. coli* than against *S. aureus* and *B. cereus*. The outer membrane surface of gram positive strains, such as *S. aureus*, reduces the penetration of sanitizers, thus, the difference in the outer membrane structure between gram positive and negative bacterial is likely the cause of reducing sensitivity by BAC[28].

Organic food was cultivated without using of agricultural chemicals and chemical sanitizers, and organic acids may use as an alternative safe sanitizer for controlling foodborne pathogens on this type of produce. This study demonstrated the antimicrobial effects of organic acids as an alternative to chemical sanitizers against pathogens isolated from organic vegetables. The bactericidal effects against pathogen isolates increased with the concentration of the organic acids. However, the susceptibility to organic acids was different depending on the type of pathogen. The susceptibility of *S. aureus* to acetic acid, malic acid and tartaric acid were better than that to chemical sanitizer such as ethanol, hydrogen peroxide and sodium hypochlorite. In particular, 2% acetic acid led to inhibition of most of *E. coli* and *B. cereus* strains, which is a relatively high level of antimicrobial activity relative to *S. aureus* ($P < 0.05$). Similar results were found by Minor *et al*[29]. They demonstrated that acetic acid had greater inhibitory activity on the growth of microorganism than did lactic acid, citric acid, and phosphoric acid. *B. cereus* and *S. aureus* isolates showed higher susceptibility to malic acid and tartaric acid than did *E. coli*.

According to Jo *et al*,[30] tartaric acid completely inhibited the growth of pathogenic *E. coli*. This finding differed from the results of the present study. In this study, *B. cereus* was more inhibited than *E. coli* and *S. aureus* at a concentration of 10% tartaric acid. Taken together, the findings support the previous observations regarding the efficacy of organic acids in controlling the growth of food related pathogens[31,32]. Hsiao *et al.* [33] reported that *B. cereus* cultures were inhibited by concentrations of tartaric acid and citric acid greater than 10 g/L. In particular, they demonstrated that *B. cereus* appeared to be relatively sensitive to many organic acids, including acetic acid, citric acid, tartaric acid, and malic acid. Acidic disinfectants killed the pathogens by breaking the bonds nucleic acids and by precipitating proteins. The antimicrobial activity of organic acids, meanwhile, is attributed to a reduction of pH by the ionization of undissociated acid molecules. A low external pH can disrupt the substrate transport system by altering cell membrane permeability[34,35].

The detection rate for *B. cereus* (67.7%) in this study was higher than for *E. coli* (9.4%) and *S. aureus* (5.2%). The common sanitizers for inactivation *B. cereus* isolates in the food industry are sodium hypochlorite[36,37] and hydrogen per-

oxide[37] due to sporicidal properties. The applied commercial amount is between 50 and 200 ppm of free chlorine of NaOCl[38] and between 1-3.5% of hydrogen peroxide[37].

In this study, acetic acid (0.5%), malic acid (2%) and tartaric acid (2%) showed similar inhibition effect against *B. cereus* with 1% H_2O_2 and were more effective than 200 ppm NaOCl. It is suggested that washing by organic acid could be as effective as hydrogen peroxide and sodium hypochlorite.

Conclusion

When populations of microorganism are exposed to a high concentration of an antimicrobial agent, such as ethanol, hydrogen peroxide, sodium hypochlorite and organic acid, living cells are eliminated than at low concentrations of an agent. The antibacterial effects of sanitizers are thus not only dependent on the type of disinfectants and pathogens but also on their concentrations. Some bacteria are naturally unaffected by antimicrobial products and become resistant to antibiotics as a result of spontaneous changes in their DNA or gene transfer from by an unknown mechanism. It is thus important to select a disinfectant that is effective at removing specific types of microorganisms by suppressing the development and persistence of resistance.

Bases on the findings of this study, CaO and organic acids may be useful in reducing the presence of pathogens isolated from fresh cut product and organic vegetables. However, we suggest that using an organic acid to wash vegetable is a suitable method for the elimination of microorganisms. Organic acids can serve as an alternative to commercial disinfectants for the quality assurance of organic produce because these chemicals are inexpensive, safe, and offer high inhibitory effectiveness.

However, foodstuff can still create disinfectant-resistant microorganisms. Therefore, additional experiments are needed to investigate the development of antimicrobial resistance among pathogens in fresh produce to supply clear guidelines for the safe and effective use of disinfectants.

요 약

신선편의 샐러드와 유기농 채소에 오염되어 있는 식중독균인 *B. cereus, S. aureus* 그리고 *E. coli*를 대상으로 살균소독제와 유기산에 대한 감수성을 평가하여 신선편의 식품의 미생물학적 안전성의 확보를 위한 올바른 세척제와 사용량을 제시하고자 하였다.

*S. aureus*는 2% acetic acid, malic acid, tartaric acid와 1% CaO에 대하여 고농도의 염소계, 과산화수소계 보다 높은 살균 효과를 보였고 *E. coli*의 경우에는 1% acetic acid 와 CaO처리에 의해 80%의 분리주가 감소하여 50% 에탄올과 400 ppm 차아염소산나트륨과 대등한 감소율을 보였다. *B. cereus* 분리주에 대한 1% CaO, 0.5% acetic acid, 2% malic acid와 tartaric acid 처리는 200 ppm의 차아염소산나트륨보다 높은 감소율을 나타내었고, 1%의 과산화수소와 대등한 감소율을 나타내었다. 본 연구에 따라 천연 물질 유래 살균 소독제인 CaO와 유기산은 기존의 화학적 살균제를 대체할 수 있는 천연 살균 소독제로 활용될 수 있을 것이고 특히 환경 친화적이고 안전한 유기산은 고품질의 신선편의 채소를 안전성으로 공급하는데 효과적인 것으로 판단되었다.

Acknowledgments

This study was supported by the Korea Food Research Institute, Republic of Korea.

References

1. Niemira BA, Fan X, Sokorai KJB.:Irradiation and modified atmosphere packaging of endive influences survival and regrowth of *Listeria monocytogenes* and product sensory qualities. *Int. J. Radiat. Phys. Chem.*, **72**, 41- 48 (2005).
2. McMahon, M. A. S. Wilson, I. G.:The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Int. J. Food Microbiol.*, **70**, 155-162 (2001).
3. Bhagwat, A. A.: Rapid detection of *Salmonella* from vegetable rinse-water using real-time PCR. *Food Microbiol.,* **21**, 73-78 (2004).
4. Korean Food and Drug Administration. http://e-stat.kfda.go.kr. Seoul, Korea. (2010).
5. Lazarova V, Janex M, Fiksdal L, Oberg C, Barcina I, Pommepuy M.: Advanced wastewater disinfection technologies: short and long term efficiency. *Water Sci. Technol.,* **38**, 109-117(1998).
6. Park SH, Choi MR, Park JW, Park KH, Chung MS, RYU SR, Kang DH.: Use of Organic Acids to Inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on Organic Fresh Apples and Lettuce. *J. Food Sci.*, **76**, 6 (2011).
7. Akbas MY, Ölmez H.: Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. *Lett. Appl. Microbiol.*, **44**, 619-624 (2007).
8. Park HK, Kim SB.: Microbial reduction of fresh vegetables by treatment of sanitizing regents. *Korean J. Food & Nutr.,* **17**, 436-441 (2004).
9. Jeong JW, Kim TK, Park JW, Lee KG.: Antimicrobial activity of sterilizer for the exclusive of vegetable against *Escherichia coli* and *Staphylococcus aureus*. *Food Engin. Progr.,* **10**, 221-225 (2006).
10. Kim IJ, Kim YS, Ha SD.: Bacteriocidal effect of CaO (Scallop-shell powder) on natural microflora and pathogenic bacteria in lettuce. *J. Fd. Hyg. Safety.*, **21**, 60-64 (2006).

11. Ase B, Sundheim G, Langsrud S, Rorvik L.: Occurrence and a possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. *Int. J. Food Microbio.,* **62**, 57-63 (2000).
12. Zhang S, Farber JM.: The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiol.*, **13**, 311-21 (1996).
13. MS, Jeong A, Kim HJ, Lee N, Oh S, Kim Y, Chun HS, Koo M.: Microbiological quality of fresh-cut produce and organic vegetables. Kor. J. Food Sci. Technol., **43**, 91-97 (2001).
14. CLSI. Performance standards for Antimicrobial Disk Susceptibility Tests. 10th ed. CLSI document M02-A10. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA (2009).
15. Knight, D. and Cooke, M. 2002. The Biocide Business: Regulations, Safety and Applications. Wiley-VCH, Germany.
16. Ingram LO.: Mechanism of lysis of *Escherchia coli* by ethanol and other chaotpic agents. *J. Bacteriol.*, **146**, 331-336 (1981).
17. Juven, B.J., M.D. Pierson.: Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation. *J. Food Prot*., **59**(11), 1233-1241 (1996).
18. Korean Food and Drug Administration. 2010. Korea Food Additives Code. Seoul, Korea.
19. Park HK, Kim SB. 2004. Microbial reduction of fresh vegetables by treatment of sanitizing regents. *Korean J Food & Nutr* 17: 436-441.
20. Beuchat LR, Nail BV, Adler BB, Clavero MRS.: Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. *J. Food Prot.*, **61**, 1305-1311 (1998).
21. Eifert JD, Sanglay GC.: Chemistry of chlorine sanitizers in food processing. *Dairy Food Environ. Sanit*., **22**, 534-538 (2002).
22. Bloomfield S.: Chlorine and iodine formulations. In Handbook of disinfectants and antiseptics. Joesph A. ed. Marcel Dekker. Inc. New York. 135-158 (1996).
23. Lee YS, Jo SH, Cho SD, Kim GH, Kim YM, Lee DH, Park SH, Bae DH, Chung MS, Bahk GJ, Ha SD.: Effects of Chlorine Concentrations and Washing Conditions on the Reduction of Microbiological Contamination in Lettuce. *J. Korean Soc. Appl. Biol. Chem*., **52**(3), 270-274 (2009).
24. Sawai J, Miyoshi H, Kojima H.: Sporicidal kinetic of *Bacillus subtilis* spore by heated scallop shell powder. *J. Food Prot*., **66**, 1482-1485 (2003).
25. Droby S, Wisniewski ME, Cohen L, Weiss B, Touitou D, Eilam Y and Chalutz E.: Influence of $CaCl_2$ on *Penicillium digitatum*, Grapefruit Peel Tissue, and Biocontrol Activity of *Pichia guilliermondii. Phytopathol*., **87**(3), 310 -315 (1997).
26. Langsrud, S., Sidhu, M.S., Heir, E., Holck, A.L.: Bacterial disinfectant resistance: a challenge for the food industry. *Int. Biodeterioration & Biodegradation*., **51**, 283-290 (2003).
27. Heir E, Sundheim G, Holock AL.: The qacG gene on plasmid pST94 confers resistance to quaternary ammonium compounds in staphylococci from the food industry. *J. Applied of Bacteriol*., **86**, 378-388 (1999).
28. Russell AD.: Bacterial resistance to disinfectants: present knowledge and future problems. *J. Hosp. Infect*., **43**, 57-68 (1998).
29. Minor TE, Marth EH.: Growth of *staphylococcus aureus* in acidified pasteurized milk. *J. Milk Food Technol.*, **33**, 516-520 (1970).
30. Jo, S.C., A.R. Rim, H.J. Park, H.G. Yuk, S.C. Lee.: Combined treatment with silver ions and organic acid enhances growth-inhibition of *Escherichia coli* O157: H7. *J. Food Control*., **18**, 1235-1240 (2006).
31. Souza, E.L., Barros, J.C., da Conceição, M.L., Gomes Neto, N.J., Costa, A.C.V.: Effect of Organic Acids on *Escherichia coli* O157:H7 and *Staphylococcus aureus* Contaminated Meat. *The Open Microbiol. J*., **3**, 121-127 (2009).
32. de Souza, E. L., de Barros, J. C., da Conceição, M. L., Gomes Neto, N. J., Da Costa, A. C. V.: Combined applications of Origanum vulgare L. essential oil and acetic acid for controlling the growth of *Staphylococcus aureus* in foods. *Braz. J. Microbiol.*, **40**, 387-393 (2009).
33. Hsiao, C. P., Siebert, K. J.: Modeling the inhibitory effects of organic acids on bacteria. *Int. J. Food Microbiol*., **47**, 189-201 (1999).
34. Conner DE, Scott VN, Bernard DT.: Growth, inhibition and survival of *Listeria monocytogenes* as affected by acidic conditions. *J. Food Prot.*, **53**, 652-655 (1990).
35. Ahn YS, Shin DH.: Antimicrobial effects of organic acids and ethanol on several foodborne microorganisms. *Korean J. Food Sci Technol*., **31**, 1315-1323 (1999).
36. Russell AD.: Bacterial spores and chemical sporicidal agents. *Clin Microbiol Rev.*, **3**(2), 99-119 (1990).
37. Hoxey, E. V. and Thomas, N. (1999), Gaseous sterilization, in Russell, A. D., Hugo, W. B.and Ayliffe, G. A. J. Principles and Practice of Disinfection, Preservation and Sterilization, Blackwell Science Ltd, Oxford.
38. Francis G.A., Thomas C, O., Beirne D.: The microbiological safety of minimally processed vegetables, *Int J Food Sci Technol*.; **34**, 1-22 (1999).