Effects of Chlorine Concentrations and Washing Conditions on the Reduction of Microbiological Contamination in Lettuce

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Received October 27, 2008; Accepted December 25, 2008

We established optimum washing and sanitizing conditions for fresh-cut lettuce using sodium hypochlorite to reduce microbial hazards. Reduction of microbial hazards, including total aerobic bacteria, *Escherichoa coli*, and *Staphylococcus aureus* at different sodium hypochlorite concentrations (0-500 ppm), immersion times (0-20 min), temperatures (0-30°C), and washing times (0-4 min) was evaluated. The optimum washing and sanitizing conditions using sodium hypochlorite for lettuce were determined as immersion at more than 50 ppm for 1 min at 20°C and washing twice for 30 sec after dipping. Application of these optimum conditions will improve safety and added value of lettuce as a fresh-cut-food without detrimental effects on sensory characteristics.

Key words: *fresh-cut-food, lettuce, sodium hypochlorite, washing optimum conditions*

Food consumption in Korea is changing toward convenience of use, better appearance, increased nutritional value, and better sanitation [Beuchat and Golden, 1985; Sakai S, 1995; Bae, 2001]. In Korea, like the USA and Europe, there is an increase in the amount and variety of vegetables that can meet the demands of consumers for freshness and convenience [Gardial *et al*., 1994; Marshall *et al*., 1995; Peter *et al*., 2004]. A reflection of these consumer demands is a new product category of 'fresh-cut-food', which is a raw farm product subjected to simple processes such as cleaning, peeling, cutting, and mincing or addition of food additives. Fresh-cut-foods include salads and fresh vegetables that can be consumed without preparation. Fresh-cut-foods are affected by changes in the plant respiration rate and enzyme activity due to tissue scarring that occurs during cutting and peeling. These types of foods are subject to contamination by pathogenic microorganisms during processing and distribution [Kumimoto, 1997]. In addition, since most fresh-cut-foods are used raw, like salads, bacterial safety is a concern [Kim *et al*., 2002]. In the USA, many cases of food-borne disease caused by fresh-cut-foods have been reported and *E. coli* O157:H7 was first confirmed in 1982 as a human host bacterium that causes food-borne illness in the USA [Riley *et al*., 1983]. Food-borne outbreaks of *Staphylococcus aureus* (*S. aureus*) occur in foods like lettuce and cucumber [Bergdoll, 1979]. Specific sources of food-borne illness due to fresh-cut-foods since 1990 are ranked by category from highest to lowest as salads, followed by fruits, germinating vegetables, cabbage, and carrots. By pathogen, from highest to lowest for cause of illness is *Salmonella*, *E. coli* O157:H7, *Shigella,* and *B.cereus*, and the frequency of occurrence is steadily increasing. A case of *Salmonella* food-borne occurrence in tomato was reported in 2008 [USFDA, 2008].

To prevent food-borne disease outbreaks, fresh-cut-foods should be protected from food-borne contamination

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Abbreviations: CFU, colony-forming unit; *E. coli*, *Escherichoa coli*; *S. aureus*, *Staphylococcus aureus*

doi:10.3839/jksabc.2009.048

during processing and storage [Manvell and Ackland, 1986]. Due to their characteristics, fresh-cut-foods should not be sterilized by heating, so chemical sterilization using halogen compounds, oxidizers, alcohols, and physical methods using radiation or UV irradiation are being used [Jung *et al*., 1996; No, 2003]. Among these, chemical methods are most widely used for convenience and cost effectiveness. In the USA, issues of safety regarding pathogenic microorganisms on fresh agricultural products are recognized, so most fresh agricultural products are processed with a no-rinse sterilizer. Compounds used with the approval of the U.S FDA as sterilizers for food surfaces include chlorine, chlorine dioxide, iodophors, quaternary ammonium compounds, and acid anions [Choi and Lee, 2008]. Among these, chlorines are most widely used for control of microorganisms in the food industry in Korea [Kim, 2001; Kim *et al*., 2002]. Sodium hypochlorite, an inorganic chlorine, is the most widely used chemical due to convenience. There is not much research relating to reduction of microorganisms in leafy vegetables [Takeuchi and Frank, 2000], so sterilizers are used indiscriminately without scientific verification of effective concentrations, optimal washing conditions, and sterilizing effects.

The purpose of this study was to establish optimum washing and sanitizing conditions using a sodium hypochlorite solution treatment for fresh-cut lettuce to reduce microbial contamination.

Materials and Methods

Samples and inoculation. Lettuce was purchased from a local wholesale market in Ansung, Korea. Lettuce samples were inoculated with a suspension of *Escherichia coli* (*E. coli*) ATCC 10536 and *S. aureus* ATCC 6538 (0.1 mL in 100 g of lettuce) to a final concentration of 10^3-10^4 CFU/g, and dried on clean bench for sampling. Chlorine solutions were prepared using sodium hypochlorite (4% w/v, Yuhan Corporation, Seoul, Korea). These solutions were made just before the experiment and then used. To determine an optimum sodium hypochlorite concentration, lettuce (10 g) was immersed in a sterile beaker containing 200 mL of sodium hypochlorite (0-500 ppm) for 5 min following the School Foodservice Health Control Guidebook (for vegetables) and washed in 200 mL of tap water for 1 min. To determine an optimum immersion time in the sodium hypochlorite solution, lettuce (10 g) was immersed in a sterile beaker containing 200 mL of sodium hypochlorite (200 ppm) for 0-20 min and washed in 200 mL of tap water for 1 min. To determine optimum washing times, lettuce (10 g) was immersed in a sterile beaker containing 200 mL of sodium hypochlorite solution (200 ppm) for 5 min and washed in 200 mL of tap water from one to eight times for 30 sec. To determine an optimum sodium hypochlorite solution temperature (5, 10, 20, 30°C), lettuce (10 g) was immersed in a sterile beaker containing 200 mL of sodium hypochlorite solution (100, 200 ppm) for 1 min and washed in 200 mL of tap water for 1 min. Lettuce (10 g) was stomached in stomacher bags containing 90 mL of 0.1% peptone water. Additional dilutions were prepared in sterile 0.1% peptone water. Appropriate dilutions were pour-plated onto tryptic soy agar (TSA, Difco, Detroit, MI), violet red bile agar (VRBA, Difco), and mannitol salt agar (MSA, Difco) and were incubated at 37°C for 24±1 h. All experiments were replicated three times. Colonies were counted and the results were expressed as colony-forming unit (CFU)/g and converted to $\log_{10}$ CFU/g values.

Results and Discussion

Optimum concentrations of sodium hypochlorite solution as a disinfectant and sanitizer. Fig. 1 shows the reduction rate of microorganisms in lettuce at various concentrations (50-500 ppm) of a sodium hypochlorite solution. The initial contamination levels of total aerobic bacteria, *E. coli,* and *S. aureus* before treatment were 2.89, 3.75, and 3.89 log CFU/g, respectively. Treatment with 50 ppm sodium hypochlorite solution reduced these levels to 0.63, 1.53, and 1.63 log CFU/g, respectively. Total aerobic bacteria were not detected in lettuce after treatment with a 100 ppm sodium hypochlorite solution. *E. coli* and *S. aureus* were not detected after treatment with 250 and 300 ppm sodium hypochlorite solutions, respectively. Park *et al*. [2004] reported that treatment with a 200 ppm sodium hypochlorite solution for 5 min failed to reduce the level of *B. subtilis* by 90%, but treatment with a 200 ppm sodium hypochlorite solution for 1 min significantly reduced the level *of L. monocytogenes*.

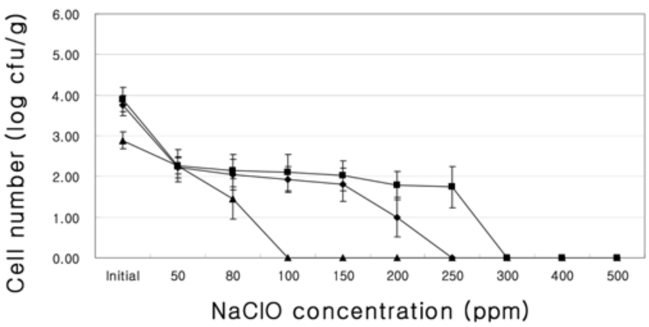

Fig. 1. Reduction rate of microorganisms in lettuce at different concentrations of a sodium hypochlorite solution. ◆, *E. coli*; ■, *S. aureus*; ▲, total aerobic bacteria.

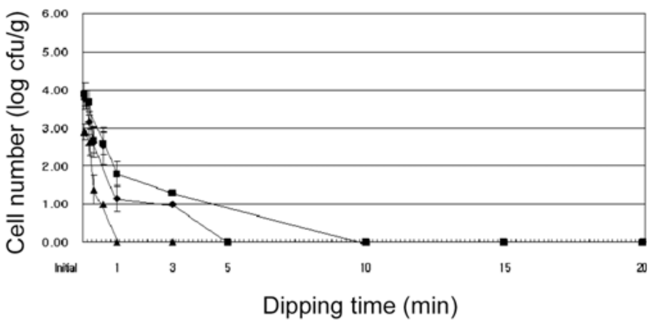

Fig. 2. Reduction rate of microorganisms in lettuce at different immersion times in a sodium hypochlorite solution (200 ppm). ◆, *E. coli*; ■, *S. aureus*; ▲, total aerobic bacteria.

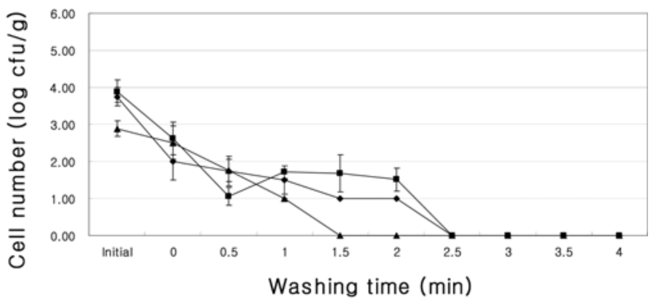

Fig. 3. Reduction rate of microorganisms in lettuce at different washing times (min) with tap water after treatment with a sodium hypochlorite solution (200 ppm). ◆, *E. coli*; ■, *S. aureus*; ▲, total aerobic bacteria.

Jeong *et al.* [2006] 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 sodium hypochlorite solution, and to 1 CFU/mL after treatment with a 100 ppm solution. The level of *S. aureus* in lettuce was reduced from 51 CFU/mL to 31 CFU/mL after treatment with a 20 ppm sodium hypochlorite solution while treatments with 50 and 100 ppm solutions reduced the levels to 39 and 6 CFU/mL, respectively. Kim [2005] found that initial contamination levels of total aerobic bacteria and coliforms in lettuce of 6.13 log CFU/g and 3.85 log CFU/g, respectively, were reduced to 3.24 -4.42 log CFU/g after immersion in chlorine solutions in a commercial facility. Grag N *et al.* [1990] reported that total aerobic bacteria in lettuce was reduced from 6.04 log CFU/g to 3.47 log CFU/g after immersion in a 300 ppm chlorine solution. However, lettuce treated with highly concentrated solutions of sodium hypochlorite can suffer tissue damage, changes in taste, and a chlorine odor. Theses problems resulted in a 200 ppm sodium hypochlorite concentration to be set as the legal maximum permissible concentration in Korea.

Optimum immersion time. Fig. 2 shows the reduction rate of microorganisms in lettuce immersed for 20 min in a 200 ppm sodium hypochlorite solution. Levels of total aerobic bacteria, *E. coli,* and *S. aureus* in lettuce washed with tap water were reduced 0.28, 0.61, and 0.22 log CFU/g, respectively. However, the levels of microorganisms after washing with tap water subsequent to immersion in a 200 ppm sodium hypochlorite solution for 10 sec were reduced 1.5, 1.14, and 1.22 log CFU/g, respectively. Total aerobic bacteria, *E. coli,* and *S. aureus* were not detected when lettuce was immersed in a 200 ppm sodium hypochlorite solution for 1, 5, and 5 min. Cho *et al.* [2004] observed that treatment with a 100 ppm chlorine solution for 2 min reduced the initial the levels of total aerobic bacteria and *E. coli* in cabbage by 2 log CFU/g. Kim *et al.* [2004] reported that treatment with a 100 ppm chlorine solution for 3-5 min reduced levels of total aerobic bacteria and coliforms from 6.88 log CFU/g and 5.71 log CFU/g to 5.51 log CFU/g and 3.28 log CFU/g, respectively. The Korean Food Code specifies that vegetables in a lunch box product should be immersed in a 100 ppm sodium hypochlorite solution for 10 min based on the idea that initial vegetable microorganism levels of 3-4 log CFU/g will be sufficiently reduced by immersion for 1-5 min in a chlorine solution. Fig. 3 shows the reduction rate for microorganisms in lettuce with different washing times using tap water after treatment with a sodium hypochlorite solution. Total aerobic bacteria, *E. coli,* and *S. aureus* in lettuce treated with a 200 ppm sodium hypochlorite solution without rinsing were reduced 0.38, 1.74, and 1.27 log CFU/g, respectively. Washing for 30 sec after treatment with a 200 ppm sodium hypochlorite solution reduced the initial levels 1.12, 2.00, and 2.83 log CFU/g, respectively. Total aerobic bacteria, *E. coli,* and *S. aureus* were not detected when lettuce was immersed in a sodium hypochlorite for 1.5, 2.5, and 2.5 min, respectively. Kim and Kim [2005] reported that the initial contamination level of total aerobic bacteria ($1{\times}10^7$ CFU/g) was proportionally reduced with an increase in the washing time. Immersion in a chlorine solution is an important process for reducing the number of microorganisms in salad. However, textural and sensory characteristics of vegetables were degraded with washing for more than 2 min.

Optimum temperature. Fig. 4 shows the reduction rate for microorganisms in lettuce at various temperatures (5, 10, 20, and 30°C) of a sodium hypochlorite solution. Temperature affected the reduction levels of total aerobic bacteria, *E. coli,* and *S. aureus* in lettuce after treatments with 100 and 200 ppm sodium hypochlorite solutions. According to Zhuang *et al.* [1995], when warm tomatoes (26 to 40°C) were immersed at 20-22°C, the reduction

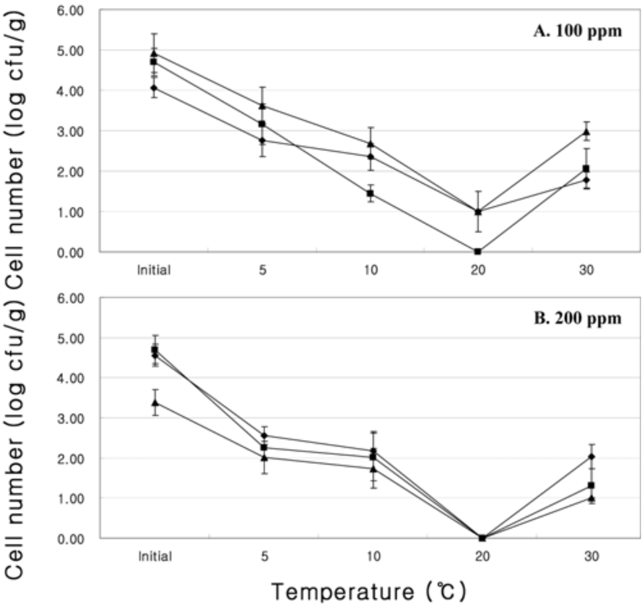

Fig. 4. Reduction rate of microorganisms in lettuce at different temperatures of a sodium hypochlorite solution. ◆, *E. coli*; ■, *S. aureus*; ▲, total aerobic bacteria.

level was decreased due to the generation of negative pressure when a vegetable is stored at a temperature lower than the temperature of the vegetable. The temperature of wash-water should be higher than the temperature of the vegetables it in order to minimize uptake of microorganisms into the vegetable tissue. We found that the levels of total aerobic bacteria, *E. coli,* and *S. aureus* in lettuce were influenced by the concentration of a sodium hypochlorite solution, the immersion time, the temperature, and the washing time. Use of optimum sanitation conditions will improve the safety and the added value of fresh-cut lettuce without affecting the sensory characteristics.

Acknowledgment. This work was supported by a grant from the Korea Food and Drug Administration in 2008 (08062sicpuman012) and the Gyeonggi Regional Research Center (20080577).

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