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Combined effects of ultrasound and surfactants to reduce *Bacillus cereus* spores on lettuce and carrots

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ABSTRACT

This study was performed to compare the effectiveness of ultrasound treatment singly and in combination with surfactants as an alternative method to conventional sanitizers containing chlorine for reducing numbers of *Bacillus cereus* spores on fresh produce. A cocktail of three strains of *B. cereus* (10876, ATCC 13061, and W-1) spores was inoculated onto iceberg lettuce and then treated with ultrasound for 0, 5, 10, 20 and 60 min. Five minutes was found to be an adequate ultrasound (40 kHz, 30 W/L) treatment time which also caused no damage to lettuce leaf surfaces as observed through a field-emission scanning electron microscope (FE-SEM).

Iceberg lettuce and carrots were inoculated with a cocktail of three strains of *B. cereus* spores and treated with combinations of ultrasound and various concentrations (0.03 to 0.3%) of surfactant (Tween 20, 40, 60, 80 and Span 20, 80, 85) solutions for 5 min. The efficacy of the combination of ultrasound and surfactant increased depending on the hydrophile–lipophile balance (HLB). The most effective treatment for reducing levels of *B. cereus* spores was the combination of ultrasound and 0.1% Tween 20, yielding reductions of 2.49 and 2.22 log CFU/g on lettuce and carrots, respectively, without causing deterioration of quality. These reductions were 1 log greater than those obtained by immersion in 200 ppm chlorine for 5 min.

Further research for elimination of *B. cereus* spores involving study of spore adhesion and removal mechanisms from food surfaces is needed, as well as devising an industrial-scale ultrasound system for the food industry.

1. Introduction

Bacillus cereus, a Gram-positive, spore-forming, motile, aerobic rod-shaped bacterium, is an important foodborne pathogen causing diarrheal and emetic types of food poisoning. This organism has been often isolated from foods and implicated in many foodborne outbreaks involving raw and pasteurized milk and dairy products (Ahmed et al., 1983), skim milk powder (Kim and Goepfert, 1971), fresh vegetables and ready-to-eat vegetable-based foods (Harmon and Kautter, 1991; Kaneko et al., 1999; King et al., 1991; Roberts et al., 1982).

Research studies involving ultrasound processing for the food industry have been increasing since cavitation is a fundamental principle for destroying and removing microorganisms from fresh food surfaces (García et al., 1989; Ordoñez et al., 1987). Ultrasound treatment can reduce levels of bacterial spores by inactivating their enzymes, though spores are generally known to be more resistant than vegetative cells. Regarding this effect, critical processing factors of ultrasound treatment are variable and involve multiple factors such as characteristics of the ultrasonic waves, treatment time, the type of pathogens and food. However, the effects are not exerted enough for inactivation of bacterial spore when using ultrasound alone (Butz and Tauscher, 2002). Recently, it was demonstrated that a combination treatment of ultrasound and organic acid could significantly reduce levels of *E. coli* in juice (Salleh-Mack and Roberts, 2007). Mostly, the bath type ultrasound in the range of 20–100 kHz has been utilized by industry because ultrasound at these frequencies can generate a more powerful cavitation phenomenon. However, ultrasound treatment has limitations for application by industry because cavitation phenomenon may be detrimental to prejudicial effects on food quality (Scouten and Beuchat, 2002; Seymour et al., 2002).

Chemical sanitizers are commonly used to control foodborne pathogens on produce. For example, chlorine and hydrogen peroxide are easy to handle, very inexpensive, and are soluble in water and stable over a long storage time (Russell, 2001). However, *B. cereus* spores are reportedly less permeable to antimicrobial agents than are the corresponding vegetative cells because spore DNA is able to protect

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from these agents of DNA damage (Setlow and Setlow, 1993; Setlow, 1995). For a similar reason, a major characteristic of *B. cereus* spores is their high heat resistance (Dufrenne et al., 1994; Pendurkar and Kulkarni, 1989). Hydrophobic properties of *B. cereus* spores may potentially reduce sanitizer contact and penetration. Therefore, these bacteria are important in the produce industry since they can survive most chemical and heat processing.

Surfactants consisting of amphipathic molecules can reduce interfacial tension (Gross and Logan, 1995). Based on the same theory, surfactants can cause a diminution of hydrophobic interactions which are involved in bacterial attachment (Paul and Jeffrey, 1985a,b). Hydrophobic interactions also play a major role in spore adhesion of *Bacillus*-spp. (Flint et al., 2001; Parkar et al., 2001). For instance, Amato (1993) proved that the combination of surfactant and sanitizer has more efficacy against spores of *Penicillium expansum* than using sanitizer alone. Other earlier studies demonstrated that $NaCl/NaHCO_3$ solution removed *E. coli* O157:H7 from cut lettuce leaves (Janes et al., 1999) and surfactant/bicarbonate solution detached *E. coli* O157:H7 from lettuce leaves (Hassan and Frank, 2003). Based on these studies, we hypothesized that if the attachment process is influenced by hydrophobic interactions, the hydrophobicity of the surfactants will be associated with their effectiveness in bacterial removal.

Therefore, we initiated this study to investigate the efficacy of surfactants that act as hydrophobic interaction disruptors in combination with ultrasound for reducing levels of *B. cereus* spores on fresh produce surfaces of lettuce, carrots, potatoes, cucumbers and apples.

2. Materials and methods

2.1. Bacterial strains and spore production

Three bacterial strains of *B. cereus* (ATCC 10876, ATCC 13061, and W-1), were obtained from the bacterial culture collection of Seoul National University (Seoul, South Korea) for this study and used for all experiments. Spores of each strain were produced by culturing on brain-heart infusion (BHI) agar (Difco) for 3 weeks at 20 °C until at least 80% of the cells had sporulated, as determined by microscopic examination. Spores were harvested by depositing 1–2 mL of sterile water onto the surface of BHI culture plates and gently rubbing with a sterile swab. Pooled suspensions were centrifuged at 4000 ×*g* for 20 min at 4 °C. Spore pellets were washed three times in sterile distilled water, corresponding to approximately 10^7 to 10^8 spores/mL and then stored at −20 °C under sterile conditions until use and calculated as log CFU/g in samples.

2.2. Sample inoculation

Fresh produce was purchased from a local grocery store (Seoul, Korea) and stored at 4 °C on the day before the experiment. For iceberg lettuce samples, two to three layers of outer leaves were removed and discarded. Intact inner leaves were removed and cut into 10 by 10 cm pieces. For other types of produce (carrots, spinach, potatoes, cucumbers and apples) outer peelings of the same dimensions were used. Samples were placed on sterile aluminum foil in a laminar flow hood with the fan running. One tenth of a milliliter of suspension was applied as 15 droplets onto the surface of prepared samples using a pipettor. The inoculated samples were dried for 2 h and used immediately for further experiments.

2.3. Preparation of surfactant

Tween 20, 40, 60, 80, 85, and Span 20, 80, 85 (Sigma, St. Louis, MO, USA) were used in this experiment. Various concentrations of surfactant such as 0.03, 0.05, 0.07, 0.1, and 0.2% (w/v) were prepared using sterile distilled water. The hydrophile–lipophile balances (HLB) of surfactants is listed in Table 1.

Table 1
Hydrophile–lipophile balance (HLB) of surfactant used in this study.

Surfactant	HLB	Chemical designation
Span 85	1.8	Sorbitan trioleate
Span 20	8.6	Sorbitan monolaurate
Tween 85	11	Polyoxyethylene sorbitan trioleate
Tween 80	15	Polyoxyethylene sorbitan monooleate
Tween 60	14.9	Polyoxyethylene sorbitan monostearate
Tween 40	15.6	Polyoxyethylene sorbitan monopalmitate
Tween 20	16.7	Polyoxyethylene sorbitan monolaurate

2.4. Procedures for treating produce

For the chlorine treatment, inoculated samples (25 g) were immersed in 500 mL of 0 (distilled water treatment) and 200 ppm chlorinated water at ambient temperature (20 °C) for 5 min with mild agitation. For ultrasound treatment alone and the combination treatment with surfactants, a 5 L ultrasound tank (UC-05, Lab Companion Ltd., Seoul, Korea) was filled with 3 L of distilled water and used at an operating frequency of 40 kHz, a nominal power setting of up to 30 W/L and at the maximum amplitude of 40 μm. A glass beaker (1 L) was placed in the ultrasound tank and filled with 500 mL of various concentrations from 0 (ultrasound treatment alone) and 0.03 to 0.3% of surfactant (Tween 20, 40, 60, 80, 85, and Span 20, 80, 85) solution and treated for 5 min.

2.5. Bacterial enumeration

For enumeration of *B. cereus* spores, each treated sample was transferred into a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 100 mL of 0.1% peptone water and then homogenized in a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. Homogenized samples were heat-shocked at 90 °C for 10 min then cooled immediately in a crushed ice/water mixture. Samples were then tenfold serially diluted in 9 mL blanks of 0.1% peptone water, and 100 μL of appropriate dilutions spread-plated onto *B. cereus* selective medium (Mannitol Eggyolk Polymyxin agar; MYP, Difco). All plates were incubated at 37 °C for 24–48 h before counting.

2.6. Microscopic observation

Lettuce leaves were subjected to primary fixation using 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C for 4 h and washed with 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C for 10 min. Then the specimens were post-fixed using 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C for 2 h and washed with distilled water. After en bloc staining with 0.5% uranyl acetate at 4 °C overnight, specimens were dehydrated in a series consisting of two rinses of 100% tetramethylsilane for 15 min each followed by two rinses of 100% isoamyl acetate for 15 min each then dried in a critical point dryer (CPD 030, BAL-TEC, UK). A thin layer of gold was applied to dried samples with a sputter coater (SCD 005, BAL-TEC, Switzerland). Digital images were collected using a personal computer integrated with a field-emission scanning electron microscope (FE-SEM; AURIGA, Carl Zeiss, Germany) operated in the secondary electron imaging mode at 2 kV with a magnification factor of 5000 times.

2.7. Statistical analysis

All experiments were repeated three times and converted to units of log CFU/g from each duplicate plate count. Data was analyzed using the ANOVA procedure of SAS (Version 8.1. SAS Institute Inc., Cary, NC, USA) for a completely randomized design. The significant ($P \leq 0.05$) means were separated using Duncan's multiple range test.

3. Results

3.1. Microscopic observation

FE-SEM micrographs of lettuce leaf surface structures are shown in Fig. 1. Untreated and ultrasound treated (5 min) samples did not show any surface damage (Fig. 1a,b). However, injured surfaces were observed for samples treated with ultrasound for ≥10 min. The degree of damage increased with increasing treatment time. These results indicate that 5 min is an adequate ultrasound treatment time for reducing levels of *B. cereus* spores without causing deterioration of quality. Thus, further studies utilizing ultrasound treatment were conducted for 5 min.

3.2. Ultrasound treatment alone on produce

We observed a <0.7 log reduction when inoculated produce was treated with distilled water (0 ppm chlorinated water) for 5 min. The reduction with 200 ppm chlorine treatment was not significantly different from that of the control ($P<0.05$). However, the viability of spores treated with ultrasound was significantly reduced ($P<0.05$). Ultrasound treatment alone can achieve an additional 1 log reduction compared to 200 ppm chlorine treatment. When various types of produce were treated with ultrasound alone, the ones most susceptible to control were cucumbers and apples, showing reductions of 1.87 and 1.86 CFU/g, respectively (Fig. 2).

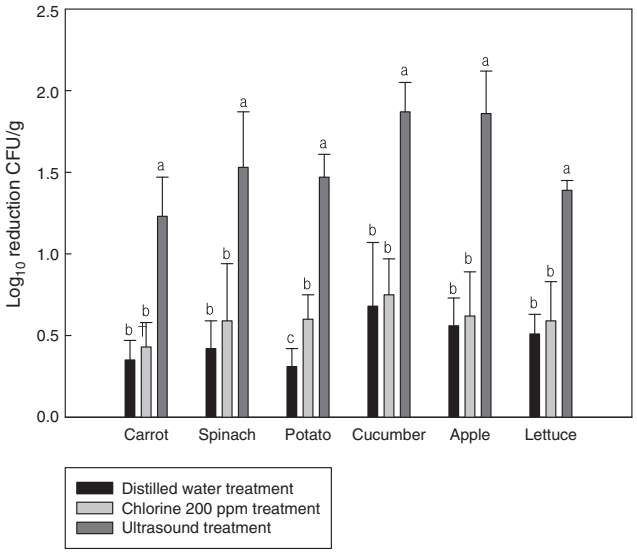

Fig. 2. Reduction levels of *Bacillus cereus* spores on produce following 5 min treatments with distilled water, 200 ppm chlorine, and ultrasound alone. †Different letters within a treatment indicate significant differences ($P<0.05$).

3.3. Combination of ultrasound and surfactants on lettuce and carrot

Figs. 3 and 4 show that all combinations of ultrasound and surfactant treatments, except for those containing span 85, significantly reduced numbers of cells remaining on surfaces of lettuce and carrots compared to ultrasound treatment alone ($P<0.05$). The effect of detachment was enhanced by increasing the hydrophile and lipophile balance (HLB) value of surfactants. Tween 20 and Tween 40 solutions were the most effective surfactants for detaching *B. cereus* spores from lettuce leaf surfaces. The maximum reductions were 2.49 and 2.44 log CFU/g, respectively, on lettuce (Fig. 3). The combination of ultrasound and surfactant treatment on carrots produced results similar to those of lettuce. Consequently, we found that 0.1% Tween 20 was the most effective surfactant for reducing levels of *B. cereus* spores when combined with ultrasound, resulting in a 2.22 log reduction (Fig. 4).

3.4. Concentration of surfactants on lettuce and carrots

To determine the best concentration of surfactant (Tween 20) in combination with ultrasound, the concentration was increased from 0 to 0.3%. As shown in Figs. 5 and 6, efficacy of reduction against *B. cereus* spores significantly increased as surfactant concentration rose to 0.1%, though there was no significant difference between using 0.1 to 0.3% surfactant ($P<0.05$). This combination of ultrasound and Tween 20 achieved an additional approximately 1 log reduction compared to ultrasound treatment alone, and more than 2 log greater reduction than immersing in 200 ppm chlorine for the same treatment time of 5 min. Based on these data, it was concluded that 0.1%

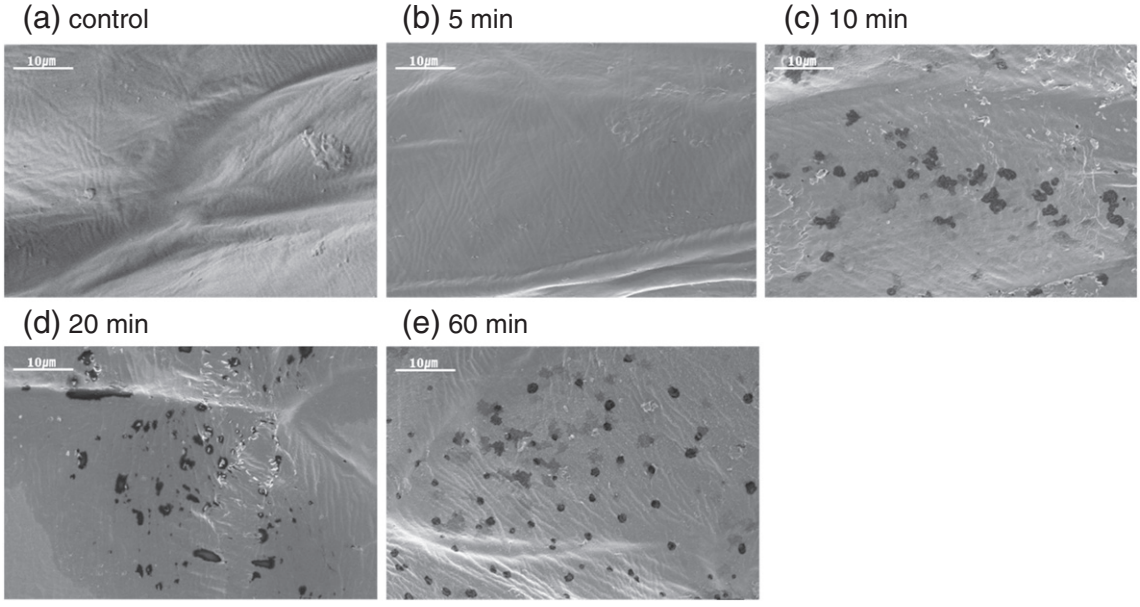

Fig. 1. Field-emission scanning electron micrographs of iceberg lettuce leaf pieces after ultrasound treatment.

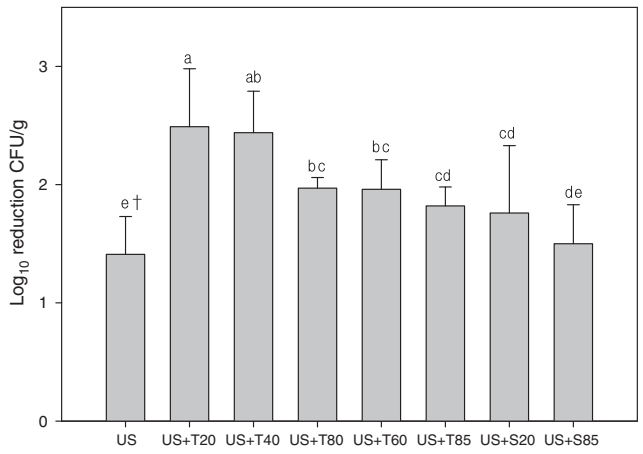

Fig. 3. Reduction levels of *Bacillus cereus* spores on iceberg lettuce following 5 min treatments with ultrasound alone, or the combination of ultrasound and surfactant. †Different letters within a treatment indicate significant differences ($P<0.05$). *US: ultrasound (frequency: 40 kHz, power: 30 W/L). T20: Tween 20; T40: Tween 40; T60: Tween 60; T80: Tween80; T85: Tween 85; S20: Span 20; S85: Span 85.

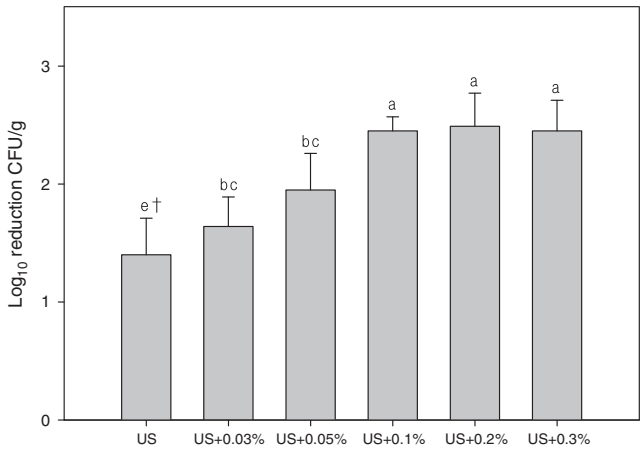

Fig. 5. Reduction levels of *Bacillus cereus* spores on iceberg lettuce following 5 min treatments with the combination of ultrasound and various concentrations of Tween 20 treatment. †Different letters within a treatment indicate significant differences ($P<0.05$). *US: ultrasound (frequency: 40 kHz, power: 30 W/L).

Tween 20 was the optimum concentration of surfactant for combination with ultrasound. The highest reductions of spores, 2.45 log on lettuce and 2.28 log on carrots, were obtained using this concentration.

4. Discussion

Seymour et al. (2002) reported that the combination of ultrasound and 100 ppm chlorine treatment for 10 min did not lead to subjective breakdown of fruit and vegetable tissues. Only parsley leaves showed any notable change in appearance caused by ultrasound treatment in a tank. In our preliminary studies (Sagong et al., 2011), iceberg lettuce leaves treated with ultrasound for 5 min were evaluated by colorimetric and texture analysis. No significant influences on the color and texture of lettuce were observed immediately after treatments and after 7 days storage. To further elucidate these data, we observed damage to lettuce treated with ultrasound after 0, 5, 10, 20 and 60 min through observation with FE-SEM (Fig. 1). Also, it is known that temperature of the tank continuously increases during ultrasound treatment. However, it required 60 min to reach approximately 60 °C (data not shown). Conversely, temperature rose to less than 30 °C when ultrasound treatment was applied for 5 min. These results demonstrate that ultrasound treatment for 5 min provides adequate control without causing degradation of quality or increasing the temperature in the ultrasound tank.

The bacterial spore consists of a complicated structure and chemistry including dipicolinic acid and calcium ions. These characteristics give it higher resistance to pressure, heat, and other agents such as acids and chemical sanitizers (Adriano et al., 2007). Arroyo et al. (1997), investigating hydrostatic high pressure (HHP) as a control intervention, demonstrated that the decrease in the initial number of *B. cereus* spores was not large, less than 1 log CFU/mL, even when applied at a pressure of 400 MPa at 10 °C for 20 min. However, our results show that ultrasound treatment could reduce *B. cereus* spores by more than 1 log CFU/g ($p<0.05$) during a shorter treatment time. This is due to a physical phenomenon called cavitation which causes the reduction of microorganisms during ultrasound treatment (Frizzell, 1988; Mahvi et al., 2005). This cavitation causes detachment and inactivation of spores from produce surfaces (Dehghani, 2005; Russell, 2002). Therefore, ultrasound treatment might be more effective than HHP processing for controlling *B. cereus* spores.

In our experiments, water treatment (0 ppm chlorinated water) alone could not effectively reduce *B. cereus* spores (Fig. 2), owing to

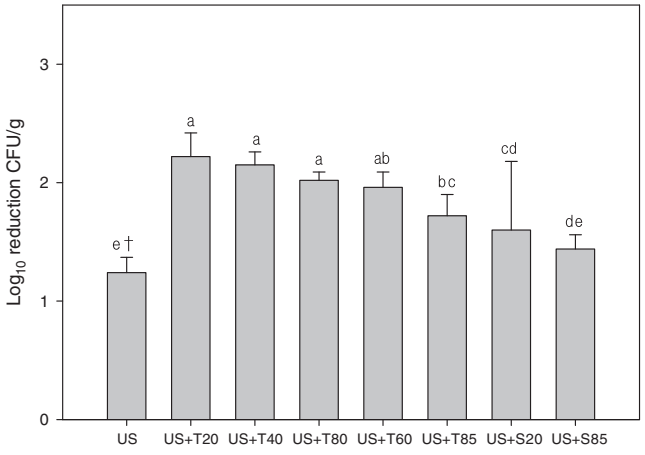

Fig. 4. Reduction levels of *Bacillus cereus* spores on carrots following 5 min treatments with ultrasound alone, or the combination of ultrasound and surfactant. †Different letters within a treatment indicate significant differences ($P<0.05$). *US: ultrasound (frequency: 40 kHz, power: 30 W/L). T20: Tween 20; T40: Tween 40; T60: Tween 60; T80: Tween80; T85: Tween 85; S20: Span 20; S85: Span 85.

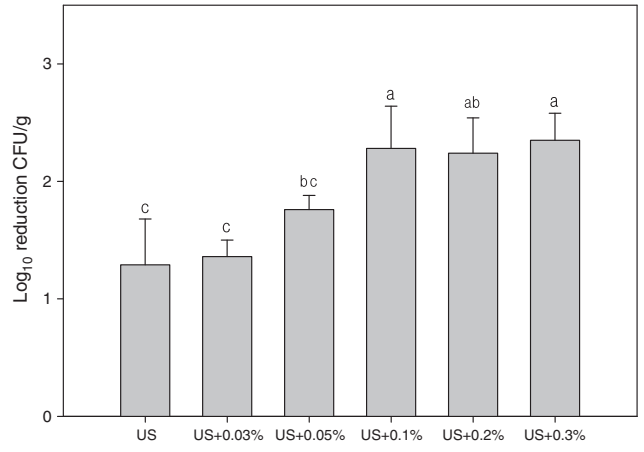

Fig. 6. Reduction levels of *Bacillus cereus* spores on carrots following 5 min treatment with the combination of ultrasound and various concentrations of Tween 20. †Different letters within a treatment indicate significant differences ($P<0.05$). *US: ultrasound (frequency: 40 kHz, power: 30 W/L).

the lack of physical forces (such as the action of surfactants and/or sonication) needed to remove inoculated *B. cereus* spores from sample surfaces during treatment. Furthermore, 200 ppm chlorine treatment could not reduce *B. cereus* spores significantly more than the water control treatment, because, *B. cereus* spores have much higher chemical resistance than their vegetative cell counterparts (Setlow and Setlow, 1993).

It is known that the adhesion properties of bacteria on material surfaces are interrelated with the hydrophobic nature of the cell surface (Boulangé-Petermann et al., 1997; Faille et al., 2002). Many researchers have reported that the entire *B. cereus* group has a hydrophobic character which contributes to strong adhesion of its spores. This is due to the outer spore membrane being composed of a loose balloon-like exosporium composed of proteins, polysaccharides, lipids and ash (Hüsmark and Rönner, 1992; Matz et al., 1970; Rosenberg and Ron, 1999). Similarly, some authors have demonstrated that *B. cereus* spores are highly hydrophobic (Andersson et al., 1998; Hüsmark and Rönner, 1990) which provide attachment ability on food surfaces (Hüsmark and Rönner, 1992) and food processing equipment (Faille et al., 2010).

Based on these theories, we compared adhesion ability of *B. cereus* spores on food surfaces relative to the HLB value of surfactants. As shown in Table. 1, Figs. 3 and 4, the addition of surfactant resulted in increased reduction and the degree of reduction increased with increasing HLB value. But, reduction did not significantly increase at HLB<15. The combination of ultrasound and Tween 20 (16.7 HLB) was the most effective among surfactants tested. From these results, it was demonstrated that *B. cereus* spores could be easily detached from food surfaces by utilizing solutions containing surfactants with high HLB values.

Kerr (2009) observed that the most effective sanitizer for reducing *B. cereus* spores on melon rind was undiluted sodium hypochlorite (6.00% NaOCl). Products containing 1.84–2.40% NaOCl resulted in 2.75 to 3.40 log reduction over 180 min. Furthermore, it was noted that a sanitizer containing 20% HCl caused 3.66 log CFU/cm^2 reduction (Kerr, 2009). However, in our study, we spent only 5 min to achieve a 2.0–2.5 log reduction using a combination of ultrasound and surfactant (0.1% Tween 20). When these combination treatment research studies were compared, our combined treatment (Tween 20/ultrasound) produced greater additional reduction because ultrasound provides the physical effect for detaching spores from food surfaces and addition of surfactant enhances this mechanism.

Consequently, we can draw several conclusions from this study. It is more effective to combine ultrasound with surfactant (0.1% Tween 20) in order to reduce the numbers of *B. cereus* spores than to treat with ultrasound alone or immersing in 200 ppm chlorine alone while at the same time not deteriorating quality. This combination treatment might reduce industrial processing while simultaneously increasing productivity by reducing processing time and eliminating the need for a post-treatment rinse step. Also, consumers can have a fresher food product with better sanitary quality. However, the detaching capacity of surfactants differ according to the type of food microorganisms adhere to. Also, if a processor would like to adapt ultrasound treatment to an industrial environment, cavitation efficiency can decrease owing to additional organic matter, water hardness, and kinds of dissolved gases present in a large plant environment. Therefore, investigations are needed to study adhesion characteristics of spores on food samples or food processing equipment under processing conditions applicable to industry.

Acknowledgments

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References

Adriano, O., Henriques, Moran Jr., Charles P., 2007. Structure, assembly, and function of the spore surface layers. Annual Reviews of Microbiology 61, 555–558.
Ahmed, A.H., Moustafa, M.K., Marth, E.H., 1983. Incidence of *Bacillus cereus* in milk and some milk products. Journal of Food Protection 46, 126–128.
Amato, I., 1993. The crusade against chlorine. Science 261, 152–154.
Andersson, A., Granumb, P.E., Rönnera, U., 1998. The adhesion of *Bacillus cereus* spores to epithelial cells might be an additional virulence mechanism. International Journal of Food Microbiology 39, 93–99.
Arroyo, G., Sanz, P.D., Préstamo, G., 1997. Effect of high pressure on the reduction of microbial populations in vegetables. Journal of Applied Microbiology 82, 735–742.
Butz, P., Tauscher, B., 2002. Emerging technologies: chemical aspects. Food Research International 35, 279–284.
Boulangé-Petermann, L., Rault, J., Bellon-Fontaine, Marie-Nöoelle, 1997. Adhesion of *streptococcus thermophilus* to stainless steel with different surface topography and roughness. Biofouling 11, 201–216.
Dehghani, M.H., 2005. Effectiveness of ultrasound on the destruction of *E. coli*. American Journal of Environmental Sciences 1, 187–189.
Dufrenne, J., Soentoro, P., Tatini, S., Day, T., Notermans, S., 1994. Characteristics of *Bacillus cereus* related to safe food production. International Journal of Food Microbiology 23, 99–109.
Faille, C., Jullien, C., Fontaine, F., Marie-Nööelle, B., Slomianny, C., Benezech, T., 2002. Adhesion of *Bacillus* spores and *Escherichia coli* cells to inert surfaces: role of surface hydrophobicity. Canada Journal of Microbiology 48, 728–738.
Faille, C., Sylla, Y., Le Gentil, C., Bénézech, T., Slomianny, C., Lequette, Y., 2010. Viability and surface properties of spores subjected to a cleaning-in-place procedure: consequences on their ability to contaminate surfaces of equipment. Food Microbiology 110, 254–262.
Flint, S., Palmer, J., Bloemen, K., Brooks, J., Crawford, R., 2001. The growth of *Bacillus stearothermophilus* on stainless steel. Journal of Applied Microbiology 90, 151–157.
Frizzell, 1988. Threshold dosages for damage to mammalian liver by high intensity focused ultrasound. IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control 35, 578–581.
García, M.L., Burgos, J., Sanz, B., Ordoñez, J.A., 1989. Effect of heat and ultrasonic waves on the survival of two strains of *Bacillus subtilis*. Journal of Applied Bacteriology 67, 619–628.
Gross, M.J., Logan, B.E., 1995. Influence of different chemical treatments on transport of *Alcaligenes paradoxus* in porous media. Applied and Environmental Microbiology 61, 1750–1756.
Harmon, S.M., Kautter, D.A., 1991. Incidence and growth potential of *Bacillus cereus* in ready to serve foods. Journal of Food Protection 54, 372–374.
Hassan, A.N., Frank, J.F., 2003. Influence of surfactant hydrophobicity on the detachment of *Escherichia coli* O157:H7 from lettuce. International Journal of Food Microbiology 87, 145–152.
Hüsmark, U., Rönner, U., 1990. Forces involved in adhesion of *Bacillus cereus* spores to solid surfaces under different environmental conditions. Journal of Applied Microbiology 69, 557–562.
Hüsmark, U., Rönner, U., 1992. The influence of hydrophobic, electrostatic and morphologic properties on the adhesion of *Bacillus* spores. Biofouling 5, 335–344.
Janes, M.E., Nannapaneni, R., Howard, L., Johnson, M.G., 1999. Sodium chloride and sodium bicarbonate washing solution for removal of enterohemorrhagic *Escherichia coli* O 157:H7 from the surface of chopped lettuce. International Association of Milk, Food and Environmental Sanitarians Annual Meeting, pp. 48–49.
Kaneko, K., Hayashidani, H., Ohtomo, Y., Kosuge, J., Kato, M., Takahashi, K., Shiraki, Y., Ogawa, M., 1999. Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. Journal of Food Protection 62, 644–649.
Kerr, H., 2009. Commercial household disinfectant inactivation of *Bacillus cereus* spores on fruit and vegetable surface. Master's Thesis, University of Tennessee at: http://trace.Tennessee.edu/utk_gradthes/39. Accessed on May, 2010.
Kim, H.U., Goepfert, J.M., 1971. Occurrence of *Bacillus cereus* in selected dry food products. Journal of Milk Food Technology 34, 12–15.
King Jr., A.D., Magnusson, J.A., TöRöK, T., Goodman, N., 1991. Microbial flora and storage quality of partially processed lettuce. Journal of Food Science 56, 459–461.
Mahvi, A.H., Dehghani, H., Kia, E.B., 2005. Inactivation of nematodes by ultrasonic. Journal of Medical Science 5, 75–77.
Matz, L.L., Cabrera Beaman, T., Gerhardt, Philipp, 1970. Chemical composition of exosporium from spores of *Bacillus cereus*. American Society for Microbiology 101, 196–201.
Ordoñez, J.A., Aguilera, M.A., García, M.L., Sanz, B., 1987. Effect of combined ultrasonic and heat treatment (thermoultrasonication) on the survival of a strain of *Staphylococcus aureus*. The Journal of Dairy Research 54, 61–67.
Parkar, S.G., Flint, S.H., Palmer, J.S., Brooks, J.D., 2001. Factors influencing attachment of thermophilic bacilli to stainless steel. Journal of Applied Microbiology 90, 901–908.
Paul, J.H., Jeffrey, W.H., 1985a. Evidence for separate adhesion mechanisms for hydrophilic and hydrophobic surfaces in *Vibrio proteolytica*. Applied and Environmental Microbiology 50, 431–437.
Paul, J.H., Jeffrey, W.H., 1985b. Effect of surfactants on the attachment of estuarine and marine bacteria to surfaces. Canadian Journal of Microbiology 31, 224–228.
Pendurkar, S.H., Kulkarni, P.R., 1989. Heat resistance of *Bacillus spores* exposed to food processing conditions. Die Nahrung 34, 177–180.

Roberts, D., Watson, G.N., Gilbert, R.J., 1982. Contamination of food plants and plant products with bacteria of public health significance. Society for Applied Bacteriology Symposium Series 10, 169–195.

Rosenberg, E., Ron, E.Z., 1999. High- and low-molecular-mass microbial surfactants. Applied Microbiology and Biotechnology 52, 154–162.

Russell, A., 2001. Principles of antimicrobial activity and resistance. In: Block, S. (Ed.), Disinfection, Sterilization and Preservation. Lippincott Williams and Wilkins, Philadelphia, PA, pp. 31–57.

Russell, N.J., 2002. Bacterial membranes: the effects of chill storage and food processing. An overview. International Journal of Food Microbiology 79, 27–34.

Sagong, H.G., Lee, S.Y., Chang, P.S., Heu, S.G., Ryu, S.R., Choi, Y.J., Kang, D.H., 2011. Combined effect of ultrasound and organic acids to reduce foodborne pathogens on organic fresh lettuce. International Journal of Food Microbiology 145, 287–292.

Salleh-Mack, S.Z., Roberts, J.S., 2007. Ultrasound pasteurization: the effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. Ultrasonics Sonochemistry 14, 323–329.

Scouten, A.J., Beuchat, L.R., 2002. Combined effects of chemical, heat and ultrasound treatments to kill *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. Journal of Applied Microbiology 92, 668–674.

Setlow, P., 1995. Mechanisms for the prevention of damage to DNA in spores of *Bacillus* species. Annual Review of Microbiology 49, 29–54.

Setlow, B., Setlow, P., 1993. Binding of small, acid-soluble spore proteins to DNA plays a significant role in the resistance of *Bacillus subtilis* spores to hydrogen peroxide. Applied and Environmental Microbiology 59, 3418–3423.

Seymour, I.J., Burfoot, D., Smith, R.L., Cox, L.A., Lockwood, A., 2002. Ultrasound decontamination of minimally processed fruits and vegetables. International Journal of Food Science and Technology 37, 547–557.