Author Instructions

▣ Scope of the journal

The Internet Journal of Food Safety (IJFS) is an international scientific journal in the English language published on the website, http://www.internetjfs.org. IJFS is intended for publication of research and review articles on all aspects of food safety such as various hazards and causes related to food spoilage and poisoning, methods for evaluating food safety, food preservation technology, microbial detection and controlling methods, food processing and technology for improving microbial food quality etc.

▣ Preparation of manuscript

1. Types and lengths of papers:
   - Short communication: Limit within 3 pages
   - Full Research paper: Unlimited
   - General interest and review paper: Unlimited

2. Basic format to submit manuscript:
   - Use Micro-office word program.
   - Set up page margin as top and bottom-2.5 cm & right and left- 2.0 cm
   - Use Time New Romans as a font style and 10-font size except for a front page.
     (Use the style guide for a front page including title, the name of author(s) and affiliation).
   - Prepare the manuscript following the format described below (style guide).
     (Use the 'formatting example' as a reference)
   - Number pages.

▣ Style guide

1. Full title: 16 font size, **bold**, Use Title Case (Capitalize every first character of words).
2. The name of author(s): 12 font size, list full names of all authors, Indicate footnote asterisk (*) after the name of the corresponding author and use superscript numbers if authors are affiliated with more than one work places.
3. Affiliation(s) of authors with address(s): 11 font size, *italic.*
4. Abstract: 10 font size, Limit the abstract to 300 words or fewer.
5. Key words: 3 to 8 numbers.
6. Corresponding author: fill up the complete information including mailing address, telephone & fax numbers (including country code), and E-mail account.
7. Main body of manuscript (including Introduction, Materials and Methods, Results, Discussion or Results and Discussion, Acknowledgment, Reference): arrange the information following the format we suggested.
8. Tables and Figures: embed all Tables and Figures within manuscript as you wish.
9. References: list only those references cited in the text. References should be arranged alphabetically by the first author's last name. Use the following format:
   - *Journal articles:*
     Bread OJ, Oltuma ED, Kim JY. 2006. Inhibitory effect of chemical sanitizer against foodborne pathogens. J. Food Sci. 78: 223-229.
   - *Books by author(s) or editor(s):*
     Megi SK, Yakazakii Y. 1998. Analysis methods. 3th ed. New York: Elsevier, pp. 788-790.
   - *Paper or chapter in book:*
     Kim JK, Yun JU. 2005. Chemical composition of plant produces. pp. 34-40. In Kang TG (ed.). Nutritional analysis. Hankang publishing. Seoul, Korea.
   - *Web pages:*
     Jeff HY (or Anonymous if unknown). 2005. Emerging pathogenic outbreaks. Available at: http://www.fda.org/emer/doc/2006.htm (accessed June 22 2006)

▣ Formatting example

Please see below (ex: the manuscript is just used to produce an example and means nothing).

Note: Manuscripts may be editorially rejected, without review, on the basis of poor English or lack of conformity to the standards set forth in these instructions.

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Effect of Chemical Sanitizer on Inhibiting the Growth of *Salmonella thyphimurium* and *Listeria monocytogenes* (16 font, Use Title Case)

(12 font spacing)

Orith Fow[1], Andrea Cheaven[2], Hyun-Jin Kim[2*] (12 font size, full names of all authors)

(8 font spacing)

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Abstract: This study was undertaken to compare the efficacy of chlorous acid, sodium hypochorite, and lactic acid in eliminating total mesophilic microorganisms, *Salmonella* typhimurium, and *Listeria monocytogenes* during post-treatment refrigerated storage. Treatment with sodium hypochlorite for 10 min did not reduce the total aerobic count. However, treatment with lactic acid or chlorous acid for 10 min initially reduced the total aerobic count by 0.7 and 0.9 log CFU/g respectively and maintained the same or lower level of the total aerobic count during storage time. Treatment with chlorous acid reduced *S. typhimurium* from 5.0 log to undetectable levels and remained undetectable during a 9 days storage period. These data suggest that treatment with chlorous acid may be useful in reducing total mesophilic microorganisms, *S. typhimurium*, and *L. monocytogenes* in commercial mungbean sprouts. (limit to 300 words)

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Key words: mungbean sprouts, chemical sanitizer, pathogens, disinfection, microbial safety (3 to 8 words)

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Introduction

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Increases in the consumption of fresh fruits and vegetables in the United State has been paralleled by an increase in the number of foodborne illnesses attributed to fresh produce (Adrenne et al. 2001). Since 1995, sprouts have been increasingly implicated in foodborne outbreaks (Bread 1997). Many microbiological surveys have shown the presence of a variety of foodborne pathogens in sprouts (Adrenne et al. 2001; Bread 1997). For instance, Salmonella spp. and Listeria monocytogenes have been isolated from sprouted seeds, including alfalfa, mungbean, cress, soybean, and mustard (Kim and Bredit 2006).

Seeds are generally recognized as the source of the microbial menagerie present in the final product (Hook et al. 2005). Foodborne pathogens including *Salmonella* spp. and *L. monocytogenes* have been isolated from seeds. Most research with sprouts dealt with disinfection procedures to eliminate pathogens while preserving germinability of the seed. Treatment of seeds with 20,000 ㎍/ml (ppm) calcium hypochlorite has been recommended to reduce the size of the population (Will and Grace 2003), although in some cases, this treatment was unable to completely prevent regrowth of *E. coli* O157:H7 during sprouting of inoculated seeds (Will and Grace 2003). It is possible that the pathogens were protected from the disinfectant in some way, perhaps by their location in or on the seeds. Besides, seed sprouting provides an excellent environment for the growth of many types of microorganisms. If foodborne pathogens are present on or in the seed, they are likely to grow to significant population levels in the finished sprout (Bread 1997). Therefore, antimicrobial treatments after sprouting offer the best promise for reducing several foodborne pathogens in commercial mungbean sprouts in the final products.

Chlorous acid ($HClO_2$) is a newly developed disinfectant (Alcide Co. Redmond, WA) and has been approved by the FDA for use on raw agricultural commodities. However, to date, no research studies have compared chlorous acid with other widely used disinfectants on sprouts. Thus, this study was undertaken to compare the efficacies of chlorous acid,

sodium hypochorite and lactic acid in eliminating total mesophilic microorganisms, *S. typhimurium* and *L. monocytogenes* on commercial mungbean sprouts.
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Materials and Methods

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Cultures and cell suspension. *Salmonella typhimurium* (ATCC 19585, ATCC 14028 and DT104 Killercow) and *Listeria monocytogenes* cultures (ATCC 19114, ATCC 19113 and ATCC 7644) were used to inoculate mungbean sprouts. Each strain of *S. typhimurium* or *L. monocytogenes* was cultured in Tryptic Soy Broth (TSB: Difco laboratory. Detroit, MI) at 37ºC for 24 h, harvested by centrifugation at 4.000×g for 20 min at 4ºC and washed three times with buffered peptone water.
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Sample preparation. Commercial mungbean sprouts were purchased from local stores and inoculated with *S. typhimurium* or *L. monocytogenes* as follows: Prepared culture cocktails of *S. typhimurium* or *L. monocytogenes* were diluted in 5 L sterile deionized water to a concentration of $10^{5\text{-}6}$ CFU/ml. Two 500 g bunches of mungbean sprouts were immersed in 5 L of aqueous suspension containing *S. typhimurium* or *L. monocytogenes* for 20 min at room temperature, and then dried in a laminar flow biosafety hood for 30 min with the fan running.
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Chemical treatments and storage. Each 500 g bunch of inoculated mungbean sprouts was submerged in 5 L of 200 ppm sodium hypochorite (Food Science of America Inc., Seattle, WA), 2% lactic acid (pH 2.0, FisherScientific, Pittsburgh, PA), or 268 ppm chlorous acid (pH 2.5, working strength "Sanova"; Alcide corporation, WA) for 10 min at room temperature (22°C). Sterile deionized water was used as a control. Following treatments, 500 g of inoculated and treated sprouts were placed in UV sterile plastic zip lock bags (G. T. Bag company, Novato, CA) and stored at 4ºC for further experiments.
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Bacterial enumeration. Inoculated and chemically treated mungbean sprouts (25 g) were placed in a stomacher bag containing 50 ml buffered peptone water and homogenized for 2 min using a model 400 circulator Seward stomacher (Seward, London, UK). After homogenization, the sample was serially 10-fold diluted with 9 ml sterile buffered peptone water. Total mesophilic microorganisms were enumerated after spread plating 100 µl onto duplicate plates of Plate Count Agar (PCA: Difco), incubated at 32ºC for 48 h. Xylose Lysine Desoxycholate Agar (XLD: Difco) and Oxford Agar Base (OAB: Difo) were used as selective media for enumeration of *S. typhimurium* and *L. monocytogenes*, respectively, and incubated at 37ºC for 24 h.
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Statistical analysis. All experiments were repeated three times with duplicate samples. Data were analyzed by analysis of variance using the ANOVA procedure of SAS (SAS Institute, Cary, NC) for a completely randomized design (treatment, storage time and treatment×storage). When the effect was significant ($P < 0.05$), means were separated using Duncan's multiple range test.
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Table 1. Populations (Log_{10}CFU/ml[a]) of total mesophilic microorganisms following chemical treatments on mungbean sprouts stored at 4ºC.

Treatment	Before treatment	Storage days			
		0	3	6	9
Water	6.99±0.10A[b]a[c]	6.97±0.10Aa	7.87±0.26Aa	8.03±0.90Aa	9.20±1.06Ba
Sodium hypochlorite	6.96±0.24Aa	6.97±0.45ABb	7.77±0.19BCa	8.18±0.57Ca	9.16±0.60Da
Lactic acid	6.89±0.14Aa	6.27±0.57Bb	5.07±0.59BCb	5.34±1.25BCa	6.36±0.485ACb
Chlorous acid	6.92±0.12Aa	6.07±0.21Bb	5.01±0.95Bb	5.94±2.25Ba	6.62±0.70Bb

[a] Data represent means±standard deviations of three measurements.
[b] Mean with the same letter within a row (following the values) are not significantly different ($P < 0.05$).
[c] Mean with the same letter within a column (following the values) are not significantly different ($P < 0.05$).

Results

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Table 1 shows the populations of total mesophilic microorganisms treated by water or disinfectants (sodium hypochlorite, lactic acid or chlorous acid). Commercial mungbean sprouts had about 10^7 CFU/g of total mesophilic microorganisms which were not reduced by submerging sprouts in distilled water for 10 min (control group). When stored for 9 days at 4ºC, total mesophilic microorganisms increased by more than 2 log and reached more than 10^9 CFU/g. Treatment with sodium hypochlorite for 10 min did not significantly reduce the population of total mesophilic microorganisms ($P > 0.05$). However, when sprouts were treated with lactic acid or chlorous acid for 10 min, levels of total mesophilic microorganisms were statistically minimal ($P < 0.05$).

The effect of sodium hypochlorite, lactic acid and chlorous acid on the survival of *S. typhimurium* and *L. monocytogenes* on sprouts is shown in Tables 2 and 3. Initial levels of each pathogen were around 5 log CFU/g and were not changed by treatment with distilled water (Figure 1). Treatment with 200 ppm sodium hypochlorite decreased both pathogens by 1-2 log and maintained this level of reduction for 9 days (Table 2 and 3).

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Discussion

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To date, there are no research reports evaluating disinfectants on commercial mungbean sprouts after seed sprouting and comparing chlorous acid antimicrobial ctivities with other disinfectants. Most previous studies concerning sprouts have tested seeds before sprouting, particularly alfalfa seeds, and investigated the efficacy of calcium and sodium hypochlorite, hydrogen peroxide, ethanol, and other disinfectants (Kim and Bredit 2006). Treatment with 2040 ppm active chlorine reduced of *S. stanley* on alfalfa seed from 10^{2-3} CFU/g to undetectable levels (< 0.3 log CFU/g). In another study, solutions containing calcium hypochlorite (1800 ppm), sodium hypochorite (2000 ppm), 6% hydrogen peroxide, or 80% ethanol reduced *Salmonella* on alfalfa seed 1000 fold after 10 min (Adrenne et al. 2001). Significant reductions in *Salmonella* populations were observed with most increases in concentration of the test chemical (Adrenne et al. 2001). Our results showed that lactic acid and chlorous acid strongly reduced or eliminated *Salmonella* and preserved mungbean sprouts during storage. In particular, treatment with chlorous acid showed a significant reduction without changing the visual quality of sprouts compared to other treatments (data not shown).

This study shows that chlorous acid is an outstanding food-grade disinfectant because it is highly effective at reducing or eliminating total mesophilic microorganisms, *S. typhimurium* and *L. monocytogenes* in the finished product. Moreover, the potential for pathogen growth during the sprouting process raises the importance of treatments to eliminate pathogens after sprouting because there is no inherent step in raw sprouts to reduce or eliminate pathogens (Will and Grace 2003).

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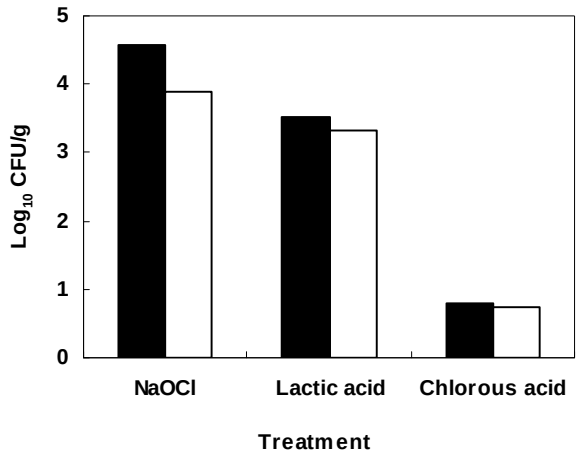

Figure 1. The recovery of injured Listeria monocytogenes using Oxford Agar Base (OAB) both directly and with the Overlay (OV) method. ■: OAB, agar overlay (OV-OAB), □: OAB, direct. Bars with different letters are significantly different ($P < 0.05$).

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