

# Analysis of Published Sprout Seed Sanitization Studies Shows Treatments Are Highly Variable

REBECCA MONTVILLE AND DONALD W. SCHAFFNER\*

Food Risk Analysis Initiative, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901-8520, USA

MS 03-359: Received 21 August 2003/Accepted 5 November 2003

## ABSTRACT

Consumption of raw sprouts has caused many foodborne illness outbreaks in the last decade, and most outbreaks have been linked to contaminated seeds. Many seed sanitization treatments have been studied as a means to reduce the risk of illness associated with sprouts. Published data on seed sanitization were analyzed collectively to identify factors that influenced the efficacy of seed sanitization and to determine the variability associated with various sanitization processes. Temperature and duration of the sanitization treatment were found to produce a negligible effect on log microbial reductions. *Salmonella*, *Escherichia coli* O157:H7, and total aerobic microorganisms were all inactivated at similar rates. Data were fit to triangular or uniform distributions for 16 different chemical treatments. Among the most effective treatments were 8% hydrogen peroxide (uniform distribution [2.5, 4.5]), 20,000 ppm of chlorine (triangular distribution [1, 2.5, 6.5]), and 1% Ca(OH)<sub>2</sub> (triangular distribution [0.5, 4, 5]). Chemical treatments where more published data were available showed more variability.

The consumption of raw sprouts has become the focus of increasing concern during the past decade. Despite increased awareness and guidelines set forth by the Food and Drug Administration (FDA) (17), foodborne disease outbreaks linked to sprouts have continued (8, 27, 50). Sprouts pose a unique challenge to the food industry, because the conditions required to produce the sprouts provide a near-optimal environment for pathogen growth (20).

Contamination of sprouts is generally thought to arise from contaminated seeds (7, 8, 11, 28, 50). Seed quality is difficult to control, because it is not until late in the production process that it is determined whether seed will be used for agriculture or food production (29). The FDA has recommended soaking seeds in 20,000 ppm of chlorine solution before sprouting to eliminate pathogens (17). Alternatively, sprouters may use another sanitization method demonstrated to cause a 5-log CFU reduction (29).

Several outbreaks bring into question the effectiveness of 20,000 ppm of chlorine as a seed treatment. An outbreak of *Salmonella* Kottbus in alfalfa sprouts was linked to seed that underwent a chlorine sanitization step, although records indicate the concentration of chlorine was probably lower than the recommended 20,000 ppm (50). An outbreak of *Salmonella* Typhimurium in clover sprouts was also linked to sanitized seeds. A trace-back investigation indicated that sprouters who had soaked the seeds in 20,000 ppm of chlorine had fewer cases attributed to their sprouts compared with those who did not; however, the sanitization step did not eliminate any incidence of illness (8). An outbreak of *Salmonella* Muenchen in Wisconsin was also attributed to seeds pretreated with calcium hypochlorite (32).

Most research on sprout safety has focused on elimi-

nating pathogens from seed due at least in part to the FDA's recommendation. Yet, there is still no consensus on the comparative efficacy of soaking seeds in various sanitizing solutions. As part of a larger project simulating risks associated with the sprouting process, a survey and analysis of published literature on seed sanitization were completed. The primary objectives of the research presented here are (i) to identify factors that influence the efficacy of seed sanitization and (ii) to determine the variability associated with the seed sanitization processes based on a comprehensive analysis of the scientific literature.

## MATERIALS AND METHODS

Scientific and medical indexes were used to locate all relevant literature on methods of seed sanitization (3, 5, 6, 9, 10, 12, 14, 15, 18, 19, 22–24, 30, 31, 38, 40, 42, 44, 47–49). Epidemiological literature was reviewed to assemble a comprehensive understanding of primary risk factors in the sprouting process (7, 8, 11, 25, 27, 28, 32, 33, 45, 46, 50). Processes described by only a single research publication, fewer than five observations of which would not be practical for small or medium sprouting operations, were not included in our analysis (12, 13, 16, 18, 26, 30, 34–39, 47, 49). The excluded studies included treatments using irradiation, 10% ethanol, 1 to 2% Na<sub>3</sub>PO<sub>4</sub>, lactic acid, acetic acid, citric acid, Fit, ozonated water, sodium chlorite, calcinated calcium, ozone, gaseous acetic acid, allyl isothiocyanate, thymol, and cinnamic aldehyde.

Graphic data were converted to numerical data using SigmaScan Pro (SPSS, Chicago, Ill.) where necessary. Data were combined where appropriate, and histograms were created using Excel (Microsoft, Redmond, Wash.). If an initial inoculum level was not provided, the log microbial reduction was calculated by substituting the amount recovered by a control water wash for initial inoculum. The one reference that provides both initial inoculum level and a control treatment verifies that these values are similar (22). Where seeds were soaked before sanitization and

\* Author for correspondence. Tel: 732-932-9611, Ext 214; Fax: 732-932-6776; E-mail: Schaffner@aesop.rutgers.edu.

TABLE 1. Results of ANOVA analysis to determine significance of factors involved in seed sanitization

Chemical treatment	Concentration		Concentration	Significance of factors <sup>a</sup>				
	Minimum	Maximum		Soak time	Organism	Temperature	Seed type	Inoculum size <sup>b</sup>
Water alone	—	—	—	<b>0.0226</b>	0.0887	0.1022	<b>0.0004</b>	< <b>0.001</b>
Chlorine compounds								
Ca(OCl) <sub>2</sub>	900 ppm	2,000 ppm	<b>0.0060</b>	<b>0.0413</b>	<b>0.0302</b>	0.6366	<b>0.0017</b>	
Ca(OCl) <sub>2</sub>	9,500 ppm	20,000 ppm	0.1907	<b>0.0050</b>	0.0804	<b>0.0232</b>	<b>0.0104</b>	< <b>0.0001</b>
NaOCl	100 ppm	500 ppm	0.6195	<b>0.0044</b>	<b>0.0139</b>	0.1278	0.1278	
NaOCl	1,000 ppm	2,040 ppm	0.1353	<b>0.0005</b>	<b>0.0013</b>	<b>0.0535</b>	<b>0.0535</b>	
Acidified NaOCl <sub>2</sub> /ClO <sub>2</sub>	200 ppm	1,200 ppm	0.7689	<b>0.0057</b>	0.2577	0.7080	0.9930	<b>0.0021</b>
Other								
Ca(OH) <sub>2</sub>	0.5%	1%	0.2296	<b>0.0031</b>	0.3939	0.2192	—	< <b>0.0001</b>
EtOH (30%)	30%	30%	—	0.1098	<b>0.0443</b>	<b>0.0443</b>	<b>0.0443</b>	
EtOH (70%)	70%	80%	0.7930	0.8748	0.3462	0.1430	0.1430	
H <sub>2</sub> O <sub>2</sub>	0.1%	0.2%	0.4500	0.1407	<b>0.0489</b>	—	—	
H <sub>2</sub> O <sub>2</sub>	1%	4%	0.5633	0.2830	0.2138	—	0.9355	
H <sub>2</sub> O <sub>2</sub>	6%	10%	0.1243	0.1101	<b>0.0016</b>	—	—	0.3070
Na <sub>3</sub> PO <sub>4</sub>	4%	15%	0.6313	< <b>0.0001</b>	0.6452	<b>0.0021</b>	—	
Tsunami at 23°C	40 ppm	1,060 ppm	0.5855	<b>0.0155</b>	0.2841	0.1031	—	
Tsunami at 55°C	40 ppm	160 ppm		0.0106	0.1120	0.3154	—	< <b>0.0001</b>
Tween at 23°C	0.01%	2%	0.7112	0.5801	0.1180	0.4295	0.7112	
Vegi-clean	10,000 ppm	20,000 ppm	0.7036	0.0503	—	—	—	

<sup>a</sup> The significance is represented by ANOVA *P* value. *P* values judged to be significant (i.e., close to or less than 0.05) are shown in bold.

<sup>b</sup> The effect of inoculum size was determined only for treatments with more than 25 observations. The inoculum was rounded to the nearest 0.5 log CFU before analysis.

results showed the effect to be negligible, the soaked and unsoaked data were pooled. Significance of all factors was determined using single-factor analysis of variance (ANOVA) and Duncan's multiple range test using SAS statistical software version 8.0 (SAS Institute Inc, Cary, N.C.). Data were fit to distributions with the aid of BestFit (Palisades Corporation, Newfield, N.Y.).

## RESULTS

Data on the efficacy of seed sanitization included many different chemical agents tested at varying concentrations and temperatures (generally 21 or 55°C), for different durations (0.5 to 60 min), on different types of seed (alfalfa, mung bean, and rice), and using different test organisms (most frequently *Escherichia coli* O157:H7 and various *Salmonella* serovars).

Commercial produce washes Tsunami and Vortexx (both with active ingredient peroxyacetic acid) were combined, because their efficacy was not significantly different (*P* = 0.0696). Acidified NaOCl<sub>2</sub> and acidified ClO<sub>2</sub> were also not significantly different (*P* = 0.7434) and were combined. The lower and upper limits for concentration to be included in each sanitizing agent category are shown in Table 1. Data using 100 ppm of acidified chlorite (three observations) were excluded because they were statistically different from all other data (data not shown). The only grouping that showed a significant effect of concentration on efficacy was 2,000 ppm of Ca(OCl)<sub>2</sub>. Duncan's multiple range test showed 1,000 and 1,900 ppm to have significantly lower efficacy than 900, 1,800, and 2,000 ppm (data

not shown), an indicator that the differences in microbial reductions are likely due to a methodological difference not concentration.

The duration of sanitization had a varying effect on log microbial reductions (Table 1). Although the effect of soaking time on effectiveness of 20,000 ppm of Ca(OCl)<sub>2</sub> was statistically significant (*P* = 0.005), no clear linear trend with soak time is apparent (Fig. 1). Analysis showed that soaking 3 min in 20,000 ppm of Ca(OCl)<sub>2</sub> was statistically different from 5 and 15 min but not from 10 and 30 min.

The effect of soaking time was similarly ambiguous across a variety of treatments. Analysis of water-only wash duration (Table 1) showed 60 min and 5 min to be similar but 4 min and 5 min to be different (details not shown). For the 2,000-ppm Ca(OCl)<sub>2</sub> treatment, a 20-min soak was similar to 3- and 10-min soaks but not similar to a 15-min soak. Analysis of 200 ppm of NaOCl revealed that soaking for 30 s was similar to 60, 45, and 30 min but dissimilar to 3 min. When seeds were treated with 2,000 ppm of NaOCl, similar results were achieved at 30 s and 60 min but not 5 min. The only sanitizer that showed a clear linear trend with soak time was acidified chlorite, which showed steadily decreasing log reductions with increasing soak times. Despite apparent statistical significance (i.e., low *P* values) in some cases, almost all of the data show nonsensical trends. This apparent statistical significance is most likely due to methodological differences among publications and not any real influence of soak time on treatment effectiveness.

TABLE 2. Summary of published data where inactivation of *Salmonella* and *Escherichia coli* O157:H7 on seeds was compared in the same study

Reference	Organisms	Greater reduction (no. of conditions)	Mean greater reduction <sup>a</sup>	Initial inocula	Treatments
5	<i>Salmonella</i> cocktail (Montevideo, Infantis, Anatum, Cubana, Stanley) and <i>E. coli</i> O157:H7 5-strain cocktail	<i>Salmonella</i> (24)	1.24 log CFU of <i>Salmonella</i>	<i>E. coli</i> : 3.2 log CFU, <i>Salmonella</i> : 4.8 log CFU	Water, Ca(OH) <sub>2</sub> , Tsunami 200
37	<i>Salmonella</i> cocktail (Montevideo, Infantis, Anatum, Cubana, Stanley) and <i>E. coli</i> O157:H7 5-strain cocktail	<i>Salmonella</i> (15), <i>E. coli</i> (3)	1.29 log CFU of <i>Salmonella</i>	<i>E. coli</i> : 3.31 log CFU, <i>Salmonella</i> : 4.25 log CFU	Water, Ca(OH) <sub>2</sub> , Tsunami 200 (with heat and ultrasound)
15	<i>Salmonella</i> cocktail (Anatum, Infantis, Newport, and Stanley) and <i>E. coli</i> O157:H7 cocktail	<i>Salmonella</i> (15)	1 log CFU of <i>Salmonella</i>	<i>E. coli</i> : 4.1 log CFU, <i>Salmonella</i> : 6.8 log CFU	Water, buffer, Ca(OCl) <sub>2</sub>
6	<i>Salmonella</i> cocktail (Agona, Enteritidis, Gam-inara, Michigan, Montevideo, Typhimurium) and <i>E. coli</i> O157:H7 5-strain cocktail	<i>Salmonella</i> (8), <i>E. coli</i> (4)	0.32 log CFU of <i>E. coli</i>	<i>E. coli</i> : 5.12 log CFU, <i>Salmonella</i> : 6.54 log CFU	Water, DE broth, Ca(OCl) <sub>2</sub> , Fit
14	<i>Salmonella</i> cocktail (Anatum, Infantis, Newport, Stanley) and <i>E. coli</i> O157:H7 3-strain cocktail	<i>E. coli</i> (1)	0.6 log CFU of <i>E. coli</i>	<i>E. coli</i> : 6.5 log CFU, <i>Salmonella</i> : 8 log CFU	Buffered chlorine
19	<i>Salmonella</i> cocktail (Montevideo, Infantis, Anatum, Cubana, Stanley) and <i>E. coli</i> O157:H7 5-strain cocktail	<i>Salmonella</i> (37), <i>E. coli</i> (15)	0.32 log CFU of <i>Salmonella</i>	<i>E. coli</i> : 4 log CFU, <i>Salmonella</i> : 4.2 log CFU	Water, Ca(OCl) <sub>2</sub> , H <sub>2</sub> O <sub>2</sub> , Ca(OH) <sub>2</sub>

<sup>a</sup> Mean greater reduction was calculated by determining the difference between log microbial reductions for *Salmonella* and *E. coli* for each treatment, then taking the average of the differences.

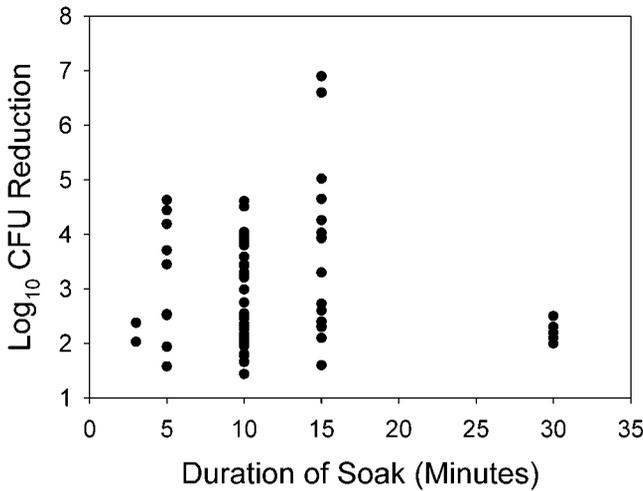


FIGURE 1. The effect of soak time on pathogen reduction for 20,000 ppm of chlorine on alfalfa seeds. A total of 58 observations from six publications are included.

Despite some research that shows differences in their attachment to sprouts (2), *E. coli* and *Salmonella* were removed from seed at similar rates for all chemical treatments. A summary of the literature that directly compares removal of the two pathogens from seed is given in Table 2. In three of six studies, the average difference of removal was greater for *Salmonella* by approximately 1 log CFU. However, the inoculum size for *Salmonella* was greater than for *E. coli* in all studies, making it difficult to determine which effects were due to the organism and which were due to the inoculum size. Comparison of treatment effectiveness on *Salmonella* and *E. coli* over all the conditions shown in Table 2 indicates no clear trend. ANOVA analysis found effectiveness of sanitizers against different pathogens to significantly differ for 2,000 ppm of Ca(OCl)<sub>2</sub>, 200 and 2,000 ppm of NaOCl, 0.1% H<sub>2</sub>O<sub>2</sub>, 8% H<sub>2</sub>O<sub>2</sub>, and 30% EtOH.

The mean reductions and statistically significant differences (Duncan's multiple range) for the effectiveness of different treatments on *E. coli* O157:H7, *Salmonella*, and total plate counts across all relevant studies are presented in Table 3. As with the within-study analysis shown in Table 2, there is no consistent trend indicating which organism was inactivated most easily or which organisms were statistically similar and different. For example, 2,000 ppm of CaOCl<sub>2</sub> showed a 1-log CFU greater reduction for *Salmonella* than *E. coli*, but acidified chlorite showed almost a 1-log CFU greater reduction for *E. coli*. NaOCl at a concentration of 200 ppm showed *E. coli* significantly different from *Salmonella* and total plate count, but at 2,000 ppm of *E. coli* and *Salmonella* were similar but significantly different from total plate count.

Temperature was determined to be an insignificant variable for almost all treatments (Table 1). For most treatments, most data were collected at 21°C. For example, information on the efficacy of a 20,000-ppm Ca(OCl)<sub>2</sub> soak included 76 observations between 21 and 23°C and only 2 at 55°C. Despite an apparently statistically significant difference (*P* = 0.0232), Duncan's multiple range test showed

TABLE 3. Mean log CFU reduction of various organisms by different sanitizers

Sanitizer type	Mean log CFU reduction <sup>a</sup>		
	<i>E. coli</i>	<i>Salmonella</i> spp.	Total aerobes
Water	0.99 A	1.58 A	0.69 A
Ca(OCl) <sub>2</sub> (2,000 ppm)	2.01 A	3.03 A	3.42 A
Ca(OCl) <sub>2</sub> (20,000 ppm)	2.81 A	3.21 A	— <sup>b</sup>
NaOCl (200 ppm)	0.23 A	0.88 B	1.10 B
NaOCl (2,000 ppm)	0.70 A	1.08 A	2.71 B
Acidified chlorite	2.03 A	1.35 A	1.97 A
H <sub>2</sub> O <sub>2</sub> (0.2%)	1.14 A	0.41 B	0.68 AB
H <sub>2</sub> O <sub>2</sub> (1%)	2.33 A	1.52 A	2.01 A
H <sub>2</sub> O <sub>2</sub> (8%)	2.89 A	3.49 AB	4.04 B
EtOH (30%)	2.07 A	—	2.98 B
EtOH (70%)	2.64 A	3.28 A	3.11 A
Ca(OH) <sub>2</sub>	2.97 A	3.22 A	—
Na <sub>3</sub> PO <sub>4</sub>	2.10 A	1.99 A	—
Tsunami at 23°C	1.20 A	1.42 A	—
Tsunami at 55°C	1.97 A	2.52 A	—
Tween	0.84 A	0.36 A	0.49 A

<sup>a</sup> Mean log reductions within the same row followed by the same letter are not statistically different.

<sup>b</sup> No data were available for analysis.

no difference between the conditions, and the mean reduction at 55°C was lower (2.03 log CFU) than the reduction at 21°C (3.11 log CFU).

The treatment with the most varied temperatures was water washing, where data were available at 21, 22, 30, 54, 55, 57, and 58°C. Both visual inspection and correlation analysis showed little effect of temperature on reduction (*P* = 0.1022). Sanitization at 21 and 30°C was significantly different for 2,000 ppm of NaOCl but not for 200 ppm of NaOCl. The effect of temperature on Na<sub>3</sub>PO<sub>4</sub> was also found to be significant (*P* = 0.0021); however, the mean for 55°C (1.73 log CFU) was again lower than for 21°C (2.15 log CFU). Two treatments showed a highly significant temperature effect, Tween (*P* = 0.0017) and Tsunami and Vortexx (*P* < 0.0001), but when limited data (three observations) on efficacy of Tween at 55°C were excluded from this analysis, the temperature dependency effect was eliminated (*P* = 0.4295). Data on efficacy of Tsunami and Vortexx at 55°C were considered separately from data at lower temperatures.

Most data available were on the efficacy of sanitizing alfalfa seeds. However, some data were available on sanitizing mung beans and rice seeds. For the only treatment where data were available for all three seed types (water wash), the effect of seed type was significant (*P* = 0.0004). The ability of water to remove bacteria from mung beans was statistically significantly different than its ability to remove bacteria from rice or alfalfa seed. The sanitizers that were significantly affected by seed type were 2,000 and 20,000 ppm of Ca(OCl)<sub>2</sub>. In both cases, an approximately 1-log CFU higher reduction was observed on mung beans versus alfalfa seeds. However, the inoculum level on mung

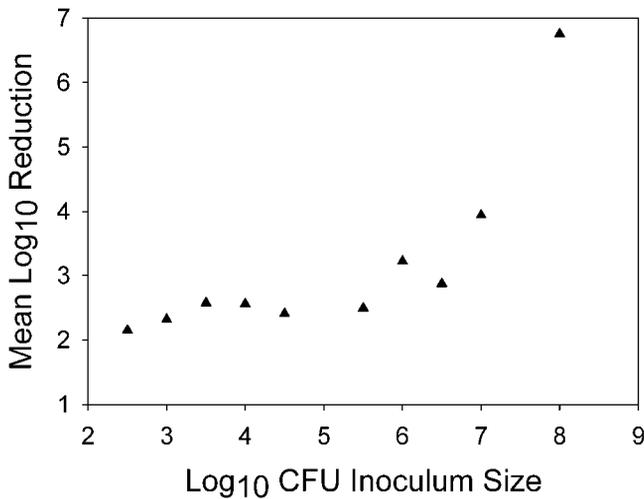


FIGURE 2. The effect of inoculum size on log microbial reductions on alfalfa seeds. Data shown are inoculum sizes rounded to the nearest 0.5 log CFU and the corresponding mean log reductions for 20,000 ppm of  $\text{Ca}(\text{OCl})_2$ .

beans was much higher than on alfalfa seeds, so differences may not be due to seed type.

Inoculum size effect was examined only for conditions with more than 25 observations (water wash, 20,000 ppm of  $\text{Ca}(\text{OCl})_2$ , acidified chlorite, Tsunami and Vortexx at 23°C, 8%  $\text{H}_2\text{O}_2$ , and 1%  $\text{Ca}(\text{OH})_2$ ). A significant effect was observed for all except 8%  $\text{H}_2\text{O}_2$ . The effect of inoculum size on mean log reduction for 20,000 ppm of  $\text{Ca}(\text{OCl})_2$  is shown in Figure 2, and a similar linear trend was observed for the other sanitizing agents.

After initial analysis, data were fit to triangular and uniform distributions (Table 4). The triangular distribution is a three-parameter distribution with a minimum value,

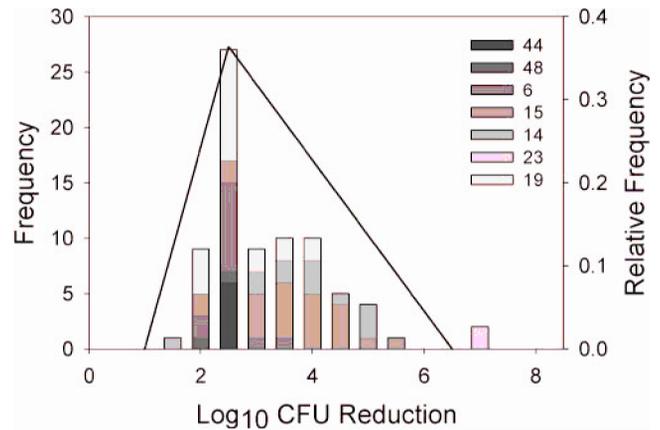


FIGURE 3. Triangular distribution (1.0, 2.5, 6.0) fit to literature data for log CFU reduction caused by 20,000 ppm of chlorine. A total of 78 observations taken from seven publications are included. Data from each publication are represented by a different shade.

most likely value, and maximum value. The uniform distribution is a two-parameter distribution with a minimum value and a maximum value that assumes equal probability of all values within the range. Although both of these distributions are simplistic, the data were generally crude and sparse; describing them with greater complexity may be misleading. An example of a triangular distribution with parameters 1, 2.5, and 6.5 fit to literature data for a 20,000-ppm  $\text{Ca}(\text{OCl})_2$  seed soak is given in Figure 3.

Not surprisingly, there is a shift in range and modal values as the concentration of chlorine increases (Fig. 4). For 200 ppm of  $\text{NaOCl}$  and 2,000 ppm of chlorine (regardless of source), the minimum "reduction" was actually a slight increase of 0.5 log CFU. A 200-ppm soak was

TABLE 4. Statistical distributions selected to describe efficacy of pathogen removal from seed

Treatment	Distribution	Parameters			No. of observations	Reference(s)
		Minimum	Mode	Maximum		
Water alone	Triangular	-1.0	1.0	4.5	45	5, 15, 22, 31, 38, 43
Chlorine compounds						
$\text{Ca}(\text{OCl})_2$ (2,000 ppm)	Triangular	-0.5	3.5	5.0	17	3, 14, 15, 43, 44, 48
$\text{Ca}(\text{OCl})_2$ (20,000 ppm)	Triangular	1.0	2.5	6.5	78	6, 14, 15, 19, 23, 44, 48
$\text{NaOCl}$ (200 ppm)	Triangular	-0.5	0.5	2.5	21	3, 22, 31, 43, 44, 48
$\text{NaOCl}$ (2,000 ppm)	Triangular	-0.5	0.5	5.0	17	3, 22, 31, 43, 44, 48
Acidified chlorite	Triangular	0.5	2.0	3.5	32	31, 43, 44, 48
Other						
$\text{Ca}(\text{OH})_2$	Triangular	0.5	4.0	5.0	35	5, 19, 48
EtOH (30%)	Triangular	1.0	3.0	4.0	7	31, 44
EtOH (70%)	Triangular	2.0	3.5	4.5	9	3, 31, 44
Hydrogen peroxide (0.2%)	Triangular	0.0	1.0	2.0	6	3, 44, 48
Hydrogen peroxide (2%)	Triangular	0.0	2.0	4.0	22	3, 31, 43, 44, 48
Hydrogen peroxide (8%)	Uniform	2.5	—	4.5	27	3, 19, 44, 48
$\text{Na}_3\text{PO}_4$ (>4)	Uniform	2.0	—	2.5	13	44, 48
Tsunami and Vortexx at 23°C	Triangular	-0.5	2.0	3.0	30	5, 43, 44, 48
Tsunami and Vortexx at 55°C	Triangular	0.5	2.0	4.0	18	5
Tween at 23°C	Triangular	-0.5	1.0	1.5	9	5, 31, 44, 48
Vegi-clean	Triangular	0.5	2.0	3.0	5	43, 44

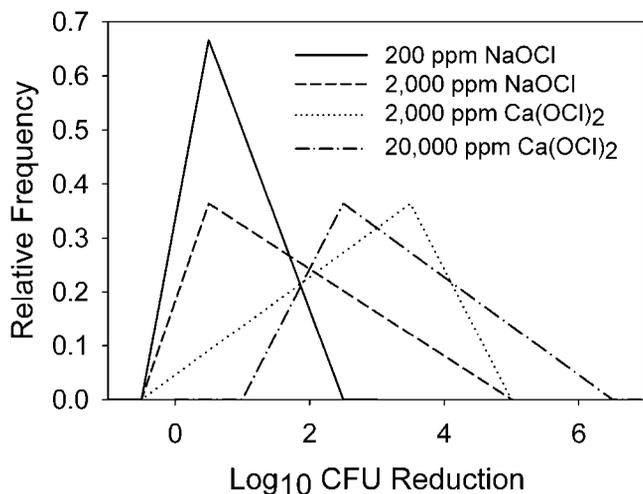


FIGURE 4. Comparison of triangular distributions fit to literature data for effectiveness of 200 ppm of NaOCl, 2,000 ppm of NaOCl and Ca(OCl)<sub>2</sub>, and 20,000 ppm of Ca(OCl)<sub>2</sub> in reducing bacterial concentration on sprout seeds.

shown to provide a maximum of a 2.5-log CFU reduction, whereas 2,000 ppm was shown to reduce bacteria up to 5 log CFU. The corresponding range for a 20,000-ppm Ca(OCl)<sub>2</sub> soak was 1 to 6.5 log CFU. The modal reduction for Ca(OCl)<sub>2</sub> was 3.5 log CFU. The only other treatment shown to have a mode higher than 2.5 log CFU was 70% EtOH (Fig. 5). However, EtOH has been researched much less thoroughly than chlorine, and it is possible that further experimentation would not show such favorable results. The effect of a water soak on bacterial populations was highly variable. Some publications reported an almost 1-log CFU increase (43), whereas others reported a more than 4-log decrease (15). The effectiveness of Tsunami and Vortex at 55°C had a similarly large range (0.5 to 4 log CFU) with a mode of 2 log CFU. Acidified chlorite was similarly peaked but with a considerably smaller range.

## DISCUSSION

Our analysis showed that temperature and duration of soaking did not affect the ability of various chemicals to reduce bacteria from seeds. Taormina and Beuchat (44) found that increased temperature affected only control treatments in reducing *E. coli* O157:H7 and that Ca(OCl)<sub>2</sub>, acidified NaClO<sub>2</sub> and ClO<sub>2</sub>, hydrogen peroxide, and trisodium phosphate were similarly effective at 21 and 55°C. Beuchat and Scouten (5), however, found that chemical treatments were more effective in reducing *Salmonella* from seed at 55 than at 21°C. There were similar findings when the use of heat and ultrasound were combined (37). Jaquette et al. (22) showed that a water soak at 54°C is significantly more effective than a water soak at 21°C.

Duration of soaking has been suspected to be a significant factor, because seeds imbibe more water as they are soaked longer, thereby releasing bacteria. Taormina and Beuchat (44) showed the effect of soak duration to be highly variable. For many treatments, a 10-min soak had no advantage over a 3-min soak. However, inoculum sizes were relatively low and, in many cases, population levels

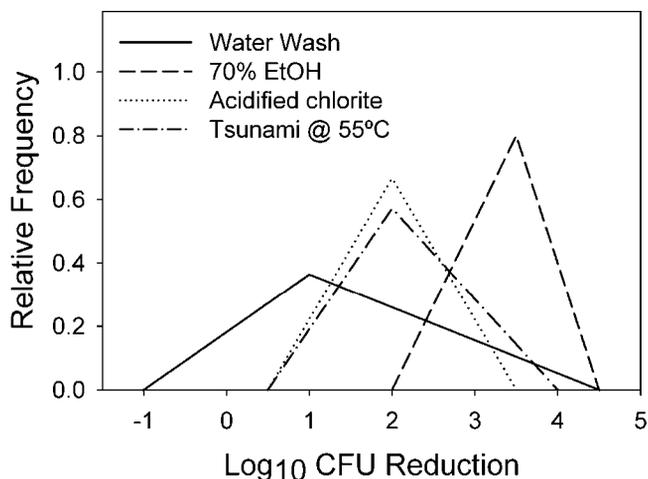


FIGURE 5. Comparison of triangular distributions for effectiveness of a wash with water alone, 70% EtOH, Tsunami and Vortex at 55°C, and acidified chlorite.

were reduced below the level of detection. It has been demonstrated that the activity of chlorine steadily decreases over time (most significantly at lower initial concentrations) (14). Scouten and Beuchat (5, 37) also found variable results on the effect of soaking time; in some cases, longer soaking times decreased efficacy; in other cases, efficacy increased. Jaquette et al. (22) also showed differences between 5- and 10-min soaks to be insignificant in reducing *Salmonella* Stanley.

The difference in the removal of various pathogens at varying concentrations was difficult to determine. Our analysis showed that *Salmonella*, *E. coli*, and aerobic mesophiles were removed at similar rates. Barak et al. (2) examined the differences in attachment between *E. coli* O157:H7 and *Salmonella enterica* serovars on sprouts and found that *Salmonella* had better attachment; however, this study used full-grown sprouts inoculated by a 4-h adhesion assay. Studies directly comparing removal of the two organisms from seed have shown no consistent trend (5, 6, 14, 15, 19, 37).

Our analysis showed a linear effect between inoculum size and log microbial reduction. Wrinkled alfalfa seeds have been shown to have larger populations of aerobic bacteria than smooth seeds; there is evidence that these bacteria are harder to remove (10). Holliday et al. (19) found removal of bacteria from nonscarified and scarified seed to be variable, however. In one seed lot, bacteria were removed less effectively from the scarified seed, and in the other lot, log microbial reductions were not significantly different from controls.

Overall, there was a larger range for treatments where more data were available. This is further evidence that seed sanitization is a highly variable process. No sanitization technique reviewed here was able to completely eliminate pathogens from seed. In fact, our analysis shows the scientific literature indicates that the recommended 20,000-ppm CaOCl<sub>2</sub> treatment most often produces a 2.5-log reduction and produces equal to or greater than a 5-log reduction only approximately 9% of the time (Fig. 3). In

some cases, pathogens were reduced to below the level of detection, but when enrichment steps were performed, bacteria were recovered. Studies using naturally contaminated seeds and 20,000-ppm CaOCl<sub>2</sub>, however, showed complete removal of *Salmonella* Mbandaka and Muenchen from seed (14, 42). All studies, even studies using naturally contaminated seed, performed sanitization of seeds on a relatively small scale (100 g or less). There is no evidence indicating how seeds would fare on a commercial scale, although data with apples indicate that treatments that are effective in the laboratory are not as effective on a commercial scale (1).

The results of this analysis are dependent on the quantity and quality of the data currently available in the published literature and, in many cases, the number of replicates was low. Inoculation procedure, inoculum size, and sanitization technique are also all likely to influence the efficacy of sanitization (4). No published research to date has been able to remove pathogens from seed effectively enough to ensure complete removal in a commercial production facility. Even if pathogens on seed are reduced to low levels, the sprouting process allows multiplication to occur up to dangerous levels. In fact, seeds implicated in *Salmonella* outbreaks have been found to have microbial loads of less than 1 CFU/g (21, 41). Sprout safety might be best improved by methods other than seed sanitization, which appears to be of limited and highly variable effectiveness.

## REFERENCES

- Annous, B. A., G. M. Sapers, A. M. Mattrazzo, and D. C. R. Rordán. 2001. Efficacy of washing with a commercial flatbed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. *J. Food Prot.* 64:159–163.
- Barak, J. D., L. C. Whitehand, and A. O. Charkowski. 2002. Differences in attachment of *Salmonella enterica* serovars and *Escherichia coli* O157:H7 to alfalfa sprouts. *Appl. Environ. Microbiol.* 68:4758–4763.
- Beuchat, L. R. 1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. *Int. J. Food Microbiol.* 34:329–333.
- Beuchat, L. R., J. M. Farber, E. H. Garrett, L. J. Harris, M. E. Parish, T. V. Suslow, and F. F. Busta. 2001. Standardization of a method to determine the efficacy of sanitizers in inactivating human pathogenic microorganisms on raw fruits and vegetables. *J. Food Prot.* 64:1079–1084.
- Beuchat, L. R., and A. J. Scouten. 2002. Combined effects of water activity, temperature and chemical treatments on the survival of *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J. Appl. Microbiol.* 92:382–395.
- Beuchat, L. R., T. E. Ward, and C. A. Pettigrew. 2001. Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food Prot.* 64:152–158.
- Breuer, T., D. H. Benkel, R. L. Shapiro, W. N. Hall, M. M. Winnett, M. J. Linn, J. Neimann, T. J. Barrett, S. Dietrich, F. P. Downes, D. M. Toney, J. L. Pearson, H. Rolka, L. Slutsker, and P. M. Griffin. 2001. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. *Emerg. Infect. Dis.* 7:977–982.
- Brooks, J. T., S. Y. Rowe, P. Shillam, D. M. Heltzel, S. B. Hunter, L. Slutsker, R. M. Hoekstra, and S. P. Luby. 2001. *Salmonella* Typhimurium infections transmitted by chlorine-pretreated clover sprout seeds. *Am. J. Epidemiol.* 154:1020–1028.
- Castro-Rosas, J., and E. F. Escartin. 1999. Incidence and germicide sensitivity of *Salmonella typhi* and *Vibrio cholerae* O1 in alfalfa sprouts. *J. Food Safety* 19:137–146.
- Charkowski, A. O., C. Z. Sarreal, and R. E. Mandrell. 2001. Wrinkled alfalfa seeds harbor more aerobic bacteria and are more difficult to sanitize than smooth seeds. *J. Food Prot.* 64:1292–1298.
- Como-Sabetti, K., S. Allaire, K. Parrott, C. M. Simonds, S. Hrabowy, B. Ritter, W. Hall, J. Altamirano, R. Martin, F. Downes, G. Jennings, R. Barrie, M. F. Dorman, N. Keon, M. Kucab, A. Al Shab, B. Robinson-Dunn, S. Dietrich, L. Moshur, L. Reese, J. Smith, K. Wilcox, J. Tilden, G. Wojtala, J. D. Park, M. Winnett, L. Petrilack, L. Vasquez, S. Jenkins, E. Barrett, M. Linn, D. Woolard, R. Hackler, H. Martin, D. McWilliams, B. Rouse, S. Willis, J. Rullan, G. Miller, Jr., S. Henderson, J. Pearson, J. Beers, R. Davis, and D. Saunders. 1997. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts—Michigan and Virginia, June–July 1997. *Morb. Mortal. Wkly. Rep.* 46:741–744.
- Delaquis, P. J., P. L. Sholberg, and K. Stanich. 1999. Disinfection of mung bean seed with gaseous acetic acid. *J. Food Prot.* 62:953–957.
- Fan, X. T., and D. W. Thayer. 2001. Quality of irradiated alfalfa sprouts. *J. Food Prot.* 64:1574–1578.
- Fett, W. F. 2002. Factors affecting the efficacy of chlorine against *Escherichia coli* O157:H7 and *Salmonella* on alfalfa seed. *Food Microbiol.* 19:135–149.
- Fett, W. F. 2002. Reduction of *Escherichia coli* O157:H7 and *Salmonella* spp. on laboratory-inoculated Mung bean seed by chlorine treatment. *J. Food Prot.* 65:848–852.
- Fett, W. F. 2002. Reduction of the native microflora on alfalfa sprouts during propagation by addition of antimicrobial compounds to the irrigation water. *Int. J. Food Microbiol.* 72:13–18.
- Food and Drug Administration. 1999. Guidance for industry: reducing microbial food safety hazards for sprouted seeds and guidance for industry: sampling and microbial testing of spent irrigation water during sprout production. *Fed. Regist.* 64:57893–57902.
- Himathongkham, S., S. Nuanualsuwan, H. Riemann, and D. O. Cliver. 2001. Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in artificially contaminated alfalfa seeds and mung beans by fumigation with ammonia. *J. Food Prot.* 64:1817–1819.
- Holliday, S. L., A. J. Scouten, and L. R. Beuchat. 2001. Efficacy of chemical treatments in eliminating *Salmonella* and *Escherichia coli* O157:H7 on scarified and polished alfalfa seeds. *J. Food Prot.* 64:1489–1495.
- Howard, M. B., and S. W. Hutcheson. 2003. Growth dynamics of *Salmonella enterica* strains on alfalfa sprouts and in waste seed irrigation water. *Appl. Environ. Microbiol.* 69:548–553.
- Inami, G. B., S. M. C. Lee, R. W. Hogue, and R. A. Brenden. 2001. Two processing methods for the isolation of *Salmonella* from naturally contaminated alfalfa seeds. *J. Food Prot.* 64:1240–1243.
- Jaquette, C. B., L. R. Beuchat, and B. E. Mahon. 1996. Efficacy of chlorine and heat treatment in killing *Salmonella stanley* inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. *Appl. Environ. Microbiol.* 62:2212–2215.
- Lang, M. M., B. H. Ingham, and S. C. Ingham. 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. *Int. J. Food Microbiol.* 58:73–82.
- Lee, S. Y., K. M. Yun, J. Fellman, and D. H. Kang. 2002. Inhibition of *Salmonella* Typhimurium and *Listeria monocytogenes* in Mung bean sprouts by chemical treatment. *J. Food Prot.* 65:1088–1092.
- Mahon, B. E., A. Ponka, W. N. Hall, K. K. Komatsu, S. E. Dietrich, A. Siitonen, G. Cage, P. S. Hayes, M. A. Lambert-Fair, N. H. Bean, P. M. Griffin, and L. Slutsker. 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. *J. Infect. Dis.* 175:876–882.
- Mazzoni, A. M., R. R. Sharma, A. Demirci, and J. R. Ziegler. 2001. Supercritical carbon dioxide treatment to inactivate aerobic microorganisms on alfalfa seeds. *J. Food Safety* 21:215–223.
- Mohle-Boetani, J., B. Werner, and M. Polumbo. 2002. Outbreak of *Salmonella* serotype Kottbus infections associated with eating alfalfa sprouts—Arizona, California, Colorado, and New Mexico, February–April 2001. *Morb. Mortal. Wkly. Rep.* 51:7–9.

28. Mohle-Boetani, J. C., J. A. Farrar, S. B. Werner, D. Minassian, R. Bryant, S. Abbott, L. Slutsker, and D. J. Vugia. 2001. *Escherichia coli* O157 and *Salmonella* infections associated with sprouts in California, 1996–1998. *Ann. Intern. Med.* 135:239–247.
29. National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *Int. J. Food Microbiol.* 52:123–153.
30. Park, C. M., P. J. Taormina, and L. R. Beuchat. 2000. Efficacy of allyl isothiocyanate in killing enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *Int. J. Food Microbiol.* 56:13–20.
31. Piernas, V., and J. P. Guiraud. 1997. Disinfection of rice seeds to sprouting. *J. Food Sci.* 62:611–615.
32. Proctor, M. E., M. Hamacher, M. L. Tortorello, J. R. Archer, and J. P. Davis. 2001. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. *J. Clin. Microbiol.* 39:3461–3465.
33. Puohiniemi, R., T. Heiskanen, and A. Siitonen. 1997. Molecular epidemiology of two international sprout-borne *Salmonella* outbreaks. *J. Clin. Microbiol.* 35:2487–2491.
34. Rajkowski, K. T., and D. W. Thayer. 2000. Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma radiation of inoculated sprouts. *J. Food Prot.* 63:871–875.
35. Rajkowski, K. T., and D. W. Thayer. 2001. Alfalfa seed germination and yield ratio and alfalfa sprout microbial keeping quality following irradiation of seeds and sprouts. *J. Food Prot.* 64:1988–1995.
36. Schoeller, N. P., S. C. Ingham, and B. H. Ingham. 2002. Assessment of the potential for *Listeria monocytogenes* survival and growth during alfalfa sprout production and use of ionizing radiation as a potential intervention treatment. *J. Food Prot.* 65:1259–1266.
37. Scouten, A. J., and L. R. Beuchat. 2002. Combined effects of chemical, heat and ultrasound treatments to kill *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. *J. Appl. Microbiol.* 92:668–674.
38. Sharma, R. R., A. Demirci, L. R. Beuchat, and W. F. Fett. 2002. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. *J. Food Prot.* 65:447–451.
39. Sharma, R. R., A. Demirci, L. R. Beuchat, and W. F. Fett. 2002. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water under pressure. *J. Food Safety* 22:107–119.
40. Soylemez, G., M. M. Brashears, D. A. Smith, and S. L. Cuppett. 2001. Microbial quality of alfalfa seeds and sprouts after a chlorine treatment and packaging modifications. *J. Food Sci.* 66:153–157.
41. Stewart, D. S., K. F. Reineke, J. M. Ulaszek, and M. L. Tortorello. 2001. Growth of *Salmonella* during sprouting of alfalfa seeds associated with salmonellosis outbreaks. *J. Food Prot.* 64:618–622.
42. Suslow, T. V., J. C. Wu, W. F. Fett, and L. J. Harris. 2002. Detection and elimination of *Salmonella* Mbandaka from naturally contaminated alfalfa seed by treatment with heat or calcium hypochlorite. *J. Food Prot.* 65:452–458.
43. Taormina, P. J., and L. R. Beuchat. 1999. Behavior of enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa sprouts during the sprouting process as influenced by treatments with various chemicals. *J. Food Prot.* 62:850–856.
44. Taormina, P. J., and L. R. Beuchat. 1999. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food Prot.* 62:318–324.
45. Van Beneden, C. A., W. E. Keene, R. A. Strang, D. H. Werker, A. S. King, B. E. Mahon, K. Hedberg, A. Bell, M. T. Kelly, V. K. Balan, W. R. MacKenzie, and D. Fleming. 1999. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. *JAMA* 281:158–162.
46. van Duynhoven, Y. T. H. P. 2002. *Salmonella enterica* serotype Enteritidis phage type 4b outbreak associated with bean sprouts. *Emerg. Infect. Dis.* 8:440–443.
47. Wade, W. N., A. J. Scouten, K. H. McWatters, R. L. Wick, A. Demirci, W. F. Fett, and L. R. Beuchat. 2003. Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts and effects on sensory quality of sprouts. *J. Food Prot.* 66:44–51.
48. Weissinger, W. R., and L. R. Beuchat. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. *J. Food Prot.* 63:1475–1482.
49. Weissinger, W. R., K. H. McWatters, and L. R. Beuchat. 2001. Evaluation of volatile chemical treatments for lethality to *Salmonella* on alfalfa seeds and sprouts. *J. Food Prot.* 64:442–450.
50. Winthrop, K. L., M. S. Palumbo, J. A. Farrar, J. C. Mohle-Boetani, S. Abbott, M. E. Beatty, G. Inami, and S. B. Werner. 2003. Alfalfa sprouts and *Salmonella* Kottbus infection: a multistate outbreak following inadequate seed disinfection with heat and chlorine. *J. Food Prot.* 66:13–17.