Microbial Contamination in Sprouts: How Effective Is Seed Disinfection Treatment?

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Abstract: Microbial contamination of sprouts by *Salmonella* and *Escherichia coli* O157 : H7 has been a common cause of foodborne diseases and a continuing challenge to the sprout industry. Seed disinfection treatment has been recommended as a major intervention step in a multihurdle approach to reduce the risk of illness associated with contaminated sprouts. U.S. Food and Drug Administration cited 20000 ppm calcium hypochlorite as an example treatment in its recommendation for seed treatment and this treatment has been considered the reference standard for seed disinfection treatment for over a decade. However, promising new disinfection treatments have emerged in recent years. In this study, we summarized published data and compared the efficacies of different disinfection methods in the reduction of microbial contamination on seeds. Our findings suggest that while biological interventions such as competitive exclusion and certain chemical treatments appear to be similar to 20000 ppm calcium hypochlorite for seed disinfection, physical methods especially high pressure may be more effective than the reference standard regardless of the type of bacteria or seed. The combination of 2 or more treatments, sequentially or simultaneously, may further improve disinfection results. Since treatments with high levels of chemical disinfectants, especially 20000 ppm calcium hypochlorite, can pose environmental and worker safety risks, alternative intervention approaches should be considered. Additional studies to confirm the greater efficacy of certain physical and combined seed disinfection treatments and to identify other effective management strategies are needed to further improve sprout safety.

Keywords: alfalfa, *Escherichia coli* O157 : H7, *food safety*, *pathogens*, *Salmonella*, sprouts

Introduction

Multiple outbreaks have been linked to consumption of raw and lightly cooked sprouts throughout the world (Taormina and others 1999; Ben Chapman 2012; Centers for Disease Control and Prevention Foodborne Outbreak Online Database 2012). Outbreaks have been associated with a wide variety of sprout types, including alfalfa, mung bean, clover, cress, soybean, and radish spouts. The infected population can be as few as single number (Taormina and others 1997) and as large as several thousands including dozens of deaths, as in the catastrophic public health crisis in Japan in 1996 (Ministry of Health and Welfare of Japan 1997) and more recently in Germany (Robert Koch Inst. 2011). As sprouts have been popular due to their nutritive value (Kurtzweil 1999) and other perceived benefits such as anticarcinogenic and anticholesterolemic properties (Donaldson 2004), many consumers could be exposed to disease if the sprouts are contaminated.

Unlike other fresh produce, sprout production involves a unique seed germination process that can support the growth of pathogens, if present. Contamination of sprouts by microbial pathogens is a major concern and poses a challenge to the industry because the optimal conditions for sprout germination are also ideal for bacterial proliferation. A variety of microbes comprise the normal microbial community in sprouts (Loui and others 2008). *Salmonella* and *E. coli* O157 : H7 are the 2 most frequent pathogenic contaminants causing sprout-associated outbreaks in the United States (Figure 1).

To help industry minimize microbial hazards in sprouts, the U.S. Food and Drug Administration (FDA) asked the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) to review the science behind sprout outbreaks and suggest recommendations to enhance sprout safety. In 1999, FDA released guidelines, based largely on the work of NACMCF, which included 5 basic recommendations: (1) growing seed for sprouting using good agricultural practices, (2) conditioning and storing seed under sanitary conditions, (3) following GMPs, as appropriate, at sprouting facilities, (4) applying a disinfection treatment to seed immediately before sprouting, and (5) in-process testing of spent sprout irrigation water for pathogens of concern before finished product enters market channels (U.S. Food and Drug Administration 1989, 1999a,b, 1999; National Advisory Committee on Microbiological Criteria for Foods 1999). As an example, FDA cited 20000 ppm calcium hypochlorite for seed disinfection treatment.

Sprout associated outbreaks appeared to decline after release of FDA's sprout guidelines in 1999 (Figure 1). However, the extent of implementation of these guidelines is a continuing concern (Thomas and others 2003). In addition, the efficacy of the seed disinfection treatment itself has been found to be highly variable (Montville and Schaffner 2004). Since the introduction of these guidelines in 1999, sprout-associated outbreaks have not been satisfactorily controlled (Figure 1). It is important to examine the effectiveness of the different seed disinfection treatments as one of the control strategies. Not only the 20000 ppm calcium hypochlorite, but also other emerging options that might work better for reducing microbial contamination in sprouts should be assessed.

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To that end, we summarized and compared the efficacies of different seed disinfection treatments that have been reported in the literature (Table 1).

Chemical Disinfection

As a classical tool to combat microbial contamination, chemicals have been widely studied for disinfection treatment of sprout seeds. Although their efficacy could be limited by the degree of exposure of the pathogens to chemical agents and additional approaches to ensure an effective exposure such as presoak, optimized chemical-to-seed ratio, stirring, and vacuum might be critical, the convenience and affordability of chemical treatments promote them as the primary choice for sprout seed disinfection. A representative chemical treatment, calcium hypochlorite used at a concentration of 20000 ppm, is cited by the FDA in its guidance recommending seed disinfection (U.S. Food and Drug Administration 1999a). However, the sprout industry has not fully welcomed this treatment, largely due to concerns about worker safety and its unfavorable impact on the environment. In fact, some argue that the referenced 20000 ppm calcium hypochlorite disinfection treatment for sprout seeds is not consistent with the production of "organic" sprouts and that its use is not allowed in some countries, such as Germany (Weiss and others 2007). Some sprout firms and countries are adopting alternative treatments. For example, in Japan, one of the major sprout-consuming countries, a heat treatment for disinfection of mung beans is used (Bari and others 2010a).

20000 ppm calcium hypochlorite

Cited by the FDA and considered as the reference standard for seed disinfection, 20000 ppm calcium hypochlorite has been reported to effectively reduce the microbial load on seeds. Seed treatment with 20000 ppm calcium hypochlorite at room temperature for 10 to 15 min produces an average of 3.08 log CFU/g reduction in bacterial population (Figure 2). However, there is a high level of variability in the efficacy reported in the literature (Table 1). Increasing the treatment time did not improve the level of microbial reduction but did decrease the germination rate of the seeds (Kim and others 2003). Although the variation in efficacy could be attributed to many factors, including different experimental protocols, the inability of the chlorine to reach the bacteria in contaminated seeds could be a major cause of the inconsistency. Greater microbial reductions were observed on smooth seeds than on scarified seeds (Holliday and others 2001) and different seed surface coating conditions and topology were likely the key contributors to the variation of accessibility to the pathogens (Fransisca and Feng 2012). This treatment, under typical conditions (10 to 15 min), does not significantly affect the germination rate, and the disinfection effects are similar on different types of bacteria.

Other chemicals

The need to decontaminate seeds in a way that is efficacious, cost-effective, safe, and environmentally friendly, without a negative impact on seed germination, has prompted researchers to continue testing alternative seed treatments. Many other commonly used chemical agents such as H_2O_2, ethanol, lactic acid, peroxyacetic acid, and fatty acid have been tested for seed disinfection (Beuchat 1997; Lang and others 2000; Kim and others 2003; Liao 2009; Buchholz and Matthews 2010; Chang and others 2010) but their efficacies vary (Table 1). In addition, few of these treatments have been the subject of repeated studies, making an accurate estimate of efficacy of individual treatments very difficult. Although the average efficacy of some of these chemical treatments may be similar to that of 20000 ppm calcium hypochlorite (Figure 2), and the maximal reported efficacy could reach a microbial reduction as high as 7.11 log CFU/g, the majority of these treatments can only achieve a moderate bacterial reduction (<3.50 log CFU/g). The used treatment conditions also vary significantly between different chemicals. The treatment time could be as short as several seconds and as long as days at either ambient or elevated temperatures. Because of the inconvenience of the applications due to the varying treatment conditions, inadequate validation of efficacies, as well as the influence of FDA guidance for seed disinfection, these treatments are less adopted than the 20000 ppm calcium hypochlorite.

Physical Inactivation

Physical methods, especially heat and high-pressure treatments, have been used for microbial inactivation in applications by other food industry groups, for example, juice processing. The recent adoption of this strategy for sprouts appears promising. Unlike chemical treatments, which may be limited by inaccessibility to pathogens sheltered in scarified surfaces and the interior of the seeds, physical treatments have better penetration characteristics for reaching bacteria in those places. In addition, physical methods such as heat and high pressure are more environmentally friendly and have been actively promoted by researchers in countries where the 20000 ppm calcium hypochlorite treatment is not an option.

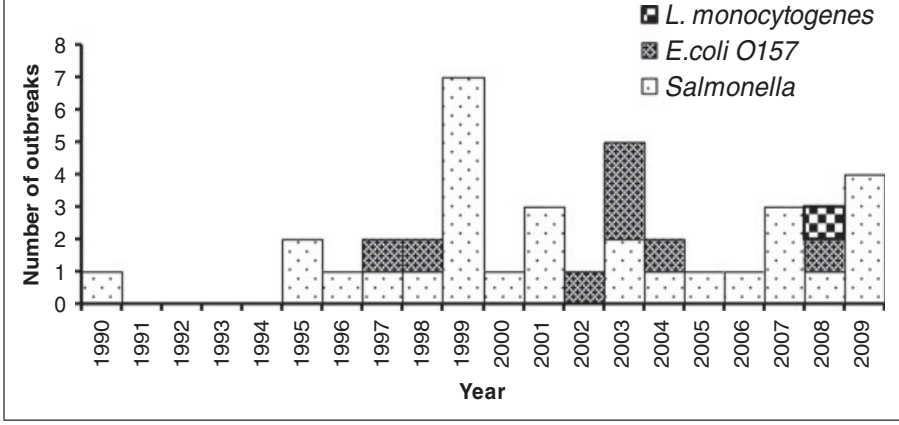


Figure 1–Sprouts-associated outbreaks in the U.S.A. Data represent the confirmed outbreaks by Centers for Disease Control and Prevention.

Table 1–Studies of disinfection treatments on sprout seeds.

Chemical			Physical and biological treatment			Combination		
Author, Year	Disinfection condition†	Reduction (log CFU/g)	Author, Year	Disinfection condition†	Reduction (log CFU/g)	Author, Year	Disinfection condition†	Reduction (log CFU/g)
20000 ppm $Ca(OCL)_2$			**Heat**			**Chemical + Chemical**		
Bari, 2010a	20 min	2.50	Bari, 2003	50 C, 1 h	1.00	Bari, 2010a	20000 ppm $Ca(OCL)_2$ + Chlorine (2000 ppm)	2.91
Bari, 2010a	20 min	2.70	Bari, 2003	50 C, 1 h	0.90	Bari, 2010a	20000 ppm $Ca(OCL)_2$ + Chlorine (2000 ppm)	3.21
Buchholz, 2010	15 min	1.13	Bari, 2003	50 C, 1 h	1.73	Fransisca, 2012a	Malic acid (10%) + Thiamine dilauryl sulfate (1%)	3.41
Charkowski, 2001	15 min	2.50	Bari, 2008	90 C + 0 C, 90 s + 30 s	4.34	Fransisca, 2012b	Malic acid (10%) + Thiamine dilauryl sulfate (1%)	3.02
Fransisca, 2012	20 min	3.41	Bari, 2008	90 C + 0 C, 90 s + 30 s	5.08	Fransisca, 2012b	Malic acid (10%) + Thiamine dilauryl sulfate (1%)	1.88
Fransisca, 2012	20 min	1.36	Bari, 2009a	50 C, 24 h	3.50	Holiday, 2001	$Ca(OH)_2$ (1%) + Tween 80 (1%)	3.96
Fransisca, 2012	20 min	2.16	Bari, 2009a	50 C, 24 h	5.00	Holiday, 2001	$Ca(OH)_2$ (1%) + Tween 80 (1%)	3.39
Gandhi, 2003	10 min	4.98	Bari, 2009a	50 C, 24 h	5.00	Holiday, 2001	$Ca(OH)_2$ (1%) + Span 20 (1%)	3.12
Holiday, 2001	10 min	2.96	Bari,2009a	50C,24h	2.50	Holiday, 2001	$Ca(OH)_2$ (1%) + Span 20 (1%)	2.99
Holiday, 2001	10 min	3.19	Bari, 2009b	75 C + 0 C, 20 s + 20 s	4.38	Lang, 2000	Lactic acid (2.5%) + Chlorine (2000 ppm)	3.70
Lang, 2000	15 min	6.90	Bari, 2009b	75 C + 0 C, 20 s + 20 s	5.80	Lang, 2000	Lactic acid (5%) + Chlorine (2000 ppm)	3.40
Liao, 2009	45 min	2.80	Bari, 2010a	85 C, 10 s	2.82	Kim, 2010	$Ca(OCL)_2$ (200 μg/mL) + Drying (24 h)	1.50
Kim, 2003	10 min	4.75	Bari, 2010a	85 C, 10 s	3.29	Kim, 2010	CLO_2 (200 μg/mL) + Drying (24 h)	3.80
Kumar, 2006	20 min	1.14	Bari, 2010a	85 C, 40 s	3.69	Pierre, 2006	Emery 658 + Glycerol monolaurate	4.60
Kumar, 2006	20 min	0.51	Bari, 2010a	85 C, 40 s	3.84	Pierre, 2006	Emery 658 + Glycerol monolaurate	6.90
Rajkowski, 2009	20 min	0.00	Feng ,2007	55 C, 8 d	1.17	Zhao, 2010	Levulinic acid (0.5%) + SDS (0.05%)	6.40
Zhao, 2010	20 min	6.30	Feng, 2007	55 C, 8 d	7.75	Zhao, 2010	Levulinic acid (0.5%) + SDS (0.05%)	5.60
Zhao, 2010	20 min	6.30	Hu, 2004	55 C, 4 d	5.00	**Chemical + Heat**		
Other chemicals			Hu, 2004	55 C, 5 d	3.00	Bang, 2011	ClO2(500μg/mL) + drying (25 C, 2 h) + Heat (55 C, 36 h)	4.80
Beuchat, 1997	NaOCl, 2,000 ppm, 30s	3.90	Neetoo, 2011	65 C, 10 d	4.70	Bari, 2003	50 C + Electrolyzed oxidizing water	4.02
Beuchat, 1997	H_2O_2, 6%, 30 s	3.57	Neetoo, 2011	65 C, 10 d	4.50	Bari, 2003	50 C + Electrolyzed oxidizing water	1.52
Beuchat, 1997	Ethanol, 80%, 30 s	3.76	**High pressure**			Bari, 2003	50 C + Electrolyzed oxidizing water	2.64
Buchholz, 2010	Peroxyacetic acid, 1%	1.77	Neetoo, 2008	600 MPa, 2 min	5.70	Bari, 2009a	50 C + Sanitizer, 17 h	5.00
Buchholz, 2010	Peroxyacetic acid, 3%	1.34	Neetoo, 2008	500 MPa, 2 min	3.50	Bari, 2009a	50 C + Sanitizer, 17 h	5.00

(*Continued*)

Table 1–Continued

Chemical			Physical and biological treatment			Combination		
Author, Year	**Disinfection condition†**	**Reduction (log CFU/g)**	**Author, Year**	**Disinfection condition†**	**Reduction (log CFU/g)**	**Author, Year**	**Disinfection condition†**	**Reduction (log CFU/g)**
Chang, 2010	Monocaprylin, 75 mM, 10 min	7.05	Neetoo, 2009a	600 MPa, 2 min, 20 C	3.70	Bari, 2009a	50 C + Sanitizer, 17 h	4.50
Chang, 2010	Monocaprylin, 75 mM, 10 min	7.11	Neetoo, 2009a	Presoaked + 600 MPa	5.00	Bari, 2009a	50 C + Sanitizer, 17 h	5.00
Chang, 2010	Caprylic acid, 75 mM, 10 min	7.05	Neetoo, 2009b	550 MPa, 2 min, 40 C	4.90	Bari, 2010a	85 C (10 s) + Chlorine (2000 ppm, 2 h)	3.53
Chang, 2010	Caprylic acid, 75 mM, 10 min	7.11	Neetoo, 2010	500 MPa, 2 min, 45 C	5.80	Bari, 2010a	85 C (10 s) + Chlorine (2000 ppm, 2 h)	3.29
Himathongkham, 2001	Ammonia, 180 ppm, 22 h	3.00	Neetoo, 2010	500 MPa, 2 min, 45 C	5.20	Bari, 2010a	85 C (40 s) + Chlorine (2000 ppm, 2 h)	3.69
Himathongkham, 2001	Ammonia, 180 ppm, 22 h	2.00	Wuytack, 2003	300 MPa, 15 min	6.00	Bari, 2010a	85 C (40 s) + Chlorine (2000 ppm, 2 h)	3.84
Himathongkham, 2001	Ammonia, 180 ppm, 22 h	5.50	Wuytack, 2003	300 MPa, 15 min	6.00	Bari, 2010b	85 C (40 s) + Chlorine (2000 ppm, 2 h)	4.69
Himathongkham, 2001	Ammonia, 180 ppm, 22 h	5.00	**Irradiation**			Bari, 2010b	85 C (40 s) + Chlorine (2000 ppm, 2 h)	4.84
Holiday, 2001	H_2O_2, 8%, 10 min	3.48	Nei, 2010	1.5 kGy	2.80	**Heat + Irradiation**		4.56
Holiday, 2001	H_2O_2,8%,10min	3.25	Saroj, 2007	2 kGy, on ice	4.60	Bari, 2003	50 C + Irradiation (2.0 kGy)	
Holiday, 2001	$Ca(OH)_2$, 1%, 10 min	3.65	Thayer, 2003	2 kGy	2.00	Bari, 2003	50 C + Irradiation (1.5 kGy)	4.85
Holiday, 2001	$Ca(OH)_2$, 1%, 10 min	3.15	Thayer, 2003	2 kGy	3.30	Bari, 2003	50 C + Irradiation (2.0 kGy)	5.49
Jung, 2009	SC-CO_2, 10 MPa, 5 min, 45 C	2.48	**Biological**			Bari, 2003	50 C + Irradiation (1.5 kGy)	4.43
Jung, 2009	SC-CO_2, 15 MPa, 10 min, 35 C	3.51	Fett, 2006	Pseudomonads 2 to 79	4.98	Bari, 2003	50 C + Irradiation (2.0 kGy)	5.69
Kim, 2003	Electrolyzed oxidizing water	1.66	Kocharunchitt, 2009	Phage	1.00	Bari, 2009a	50 C + Irradiation (1.0 kGy), 17 h	5.00
Kim, 2003	Deionized water, 10 min	0.57	Matos, 2005	Pseudomonads 2 to 79	4.22	Bari, 2009a	50C + Irradiation (1.0 kGy), 17 h	4.50
Kim, 2003	Chlorine water, 10 min	1.70	Nandiwada, 2004	Bacteriocin (colicin)	3.00	Bari, 2009a	50 C + Irradiation (0.25 kGy), 17 h	5.00
Kumar, 2006	NaOCl, 200 ppm, 28 C	3.50	Ye, 2010	E.asburiae	5.56	Bari, 2009a	50 C + irradiation (0.25 kGy), 17 h	5.00
Kumar, 2006	NaOCl, 200 ppm, 28 C	3.50	Ye, 2010	Phage	3.41	Bari, 2009a	50 C + Irradiation (0.25 kGy), 17 h	5.00
Lang, 2000	Lactic acid, 5%, 10 min, 42 C	3.00				**Others**		
Lang, 2000	Acetic acid, 5%, 10 min, 42 C	2.40				Neetoo, 2011	65 C (12 h) + 600 MPa (2 min, 35 C)	5.00
Liao, 2009	Acidified sodium chlorite, 800 ppm	4.00				Neetoo, 2011	65 C (12 h) + 600 MPa (2 min, 35 C)	4.40
Nei, 2010	Acidified chlorite, 200 to 1200 ppm	2.90				Nei, 2010	Acidified chlorite + Irradiation (1.5 kGy)	4.20
Pierre, 2006	Fatty-acid-based sanitizer	5.45				Rajkowski, 2009	Ozone + 1% Peroxyacetic acid	4.12
Pierre, 2006	Fatty-acid-based sanitizer	5.62				Ye, 2010	E.asburiae + Phage	6.72
Sharma, 2003	Electrolyzed oxidizing water	1.56				Ye, 2010	E.asburiae + Phage	7.62
Stan, 2003	Electrolyzed oxidizing	2.04						

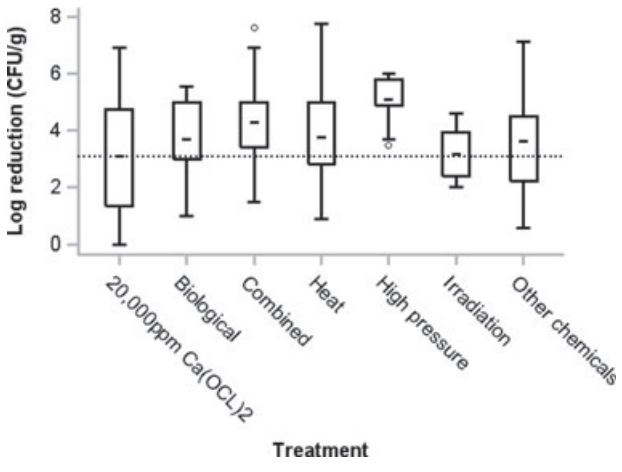
C: Temperature (°C), s: second, min: minute, h: hour, d: day.
† Disinfection treatments for different pathogens or seeds under same conditions were listed separately.

Heat

Heat treatment has been a primary alternative to chemical treatment, especially in Japan. Treating seeds at a relatively warm temperature in the range of 50° C for hours or days have been reported, with varying disinfection efficacies (Bari and others 2003, 2009a, 2010; Hu and others 2004; Feng and others 2007; Neetoo and Chen 2011). A quick treatment at an elevated temperature of 90° C for about 90 s followed by rapid (30 s) cooling to 0° C improved the disinfection compared to the treatments at lower temperatures for longer duration (Bari and others 2008). However, due to the decreased germination rate resulting from the high temperature, this method is not applicable for use on seed for sprouting, although a similar procedure at 75° C appears promising (Bari and others 2009b). Recent work on using heat for disinfection involves treating seeds in hot water at 85° C for 10 s (Bari and others 2010a), which was reported to consistently achieve a bacterial reduction of approximately 3.0 log CFU/g. Increasing the treatment time to 40 s has been shown to further improve treatment efficacy by more than 0.5 log without a significant reduction of germination rate (Bari and others 2010a, 2008b).

High pressure

The application of high pressure for seed disinfection also appears to be very promising (Table 1 and Figure 2). Seed treatment at 500 to 600 MPa for 2 min at room temperature can achieve a reduction in bacterial load of 3.50 log CFU/g or more (Neetoo and others 2008; 2009b). Coupled with presoaking or higher temperature, the high-pressure seed disinfection treatment can be even more effective (Neetoo and others 2009a,b, 2010). Treatment at a medium pressure (300 MPa) for a longer time (15 min) produced similar disinfection results, although a delay in germination was observed (Wuytack and others 2003). On average, the high-pressure treatment can achieve a microbial reduction of 5.09 log CFU/g, which is the most effective method so far among all available seed disinfection treatments and is significantly better than the reference standard of 20000 ppm calcium hypochlorite (Figure 2). The variation in the efficacy of high-pressure treatment between different studies is small, which could be explained by the relatively few number of researchers who have conducted the high-pressure studies. Nevertheless, additional studies should be performed to verify efficacy under different treatment conditions and seed types and to determine if the method is commercially viable.



Treatment	N	Mean	SD	*P* value
20,000 ppm $Ca(OCL)_2$	18	3.08	2.03	Reference
Other chemicals	32	3.61	1.80	0.2658
Biological	6	3.70	1.63	0.4198
Irradiation	4	3.18	1.09	0.9216
Heat	21	3.76	1.71	0.1898
High pressure	9	5.09	0.94	0.0025*
Combined	47	4.29	1.28	0.0072*

Figure 2–Box plot showing comparison of seed disinfection efficacies by various treatments, reported by 44 published articles. Bar in the middle of the box is mean and the dashed line represents the mean reduction of reference treatment, 20000 ppm $Ca(OCl)_2$. Statistical analyses were summarized in the table. General linear model was performed using SAS 9.2 for Windows software. The symbol (*) denotes statistical significance ($P < 0.05$).

Irradiation

Irradiation at different dosages has been tested and approved for seed decontamination by the FDA (U.S. Food and Drug Administration 2000). Although the maximal dose for seed treatment approved by the FDA is 8 kGy (U.S. Food and Drug Administration Memorandum 1999), the majority of studies targeted 2 kGy or below as a generally acceptable practice for the treatments of seeds destined for sprouting with desirable germination and quality. Since there are relatively few studies available, we combined studies conducted at 2.0 kGy with those conducted at 1.5 kGy to summarize treatment efficacy for this review (Rajkowski and Thayer 2001; Schoeller and others 2002; Thayer and others 2003; Bari and others 2004; Saroj and others 2007; Nei and others 2010). Irradiation at this dose range can consistently produce an average of 3.18 log CFU/g reduction in microbial contamination (Figure 2), and it is almost equally effective in seeds and final sprout products (data not shown); however, its impact on length, yield, and appearance of sprouts (Rajkowski and others 2003) and potential nutrient loss (that is, vitamin C, Bari and others 2004) are issues that currently prevent this method from becoming widely acceptable as a disinfection treatment.

Biological Inhibition

Competitive exclusion from normal microbial flora has received some attention for the control of pathogens in food, and this strategy may also work in sprouts to a certain extent. Multiple strains of bacteria, bacteriocins, as well as bacteriophages have been tested to inhibit the growth or multiplication of *Salmonella* or *E. coli* O157 : H7 during sprout production (Nandiwada and others 2004; Matos and Garland 2005; Fett 2006; Kocharunchitt and others 2009; Ye and others 2010). Although 2 strains of lactic acid bacterium have been shown to be able to effectively control (> 6 log CFU/g inhibition) the growth of either *Salmonella* or *E. coli* O157 : H7 in culture broth (Wilderdyke and others 2004), tests on inoculated seeds reveal less satisfactory results. In general, results from the limited number of studies with seeds suggest that the inhibition of microbial contamination by these biological agents could potentially achieve a similar effect as 20000 ppm calcium hypochlorite in reduction of microbial populations. However, due to the complexity of its application, uncertainty about its efficacy on an industrial scale, and the concern of potential adverse health effects, whether this strategy will become a major control option in sprout production remains to be determined.

Combined Treatment

As discussed above, seed disinfection by a single type of treatment can significantly reduce microbial populations; however, combination treatments could be more effective, and synergistic effects may be achieved by applying 2 or more methods, either sequentially or simultaneously.

Different combinations of treatments (Table 1) have been studied to search for better seed disinfection methods (Lang and others 2000; Holliday and others 2001; Bari and others 2003; 2009, 2010a,b; Kim and others 2003, 2010; Pierre and Ryser 2006; Nei and others 2010; Ye and others 2010; Zhao and others 2010; Bang and others 2011; Fransisca and others 2012; Fransisca and Feng 2012). Although some combined treatments are not effective as expected, they usually produce a greater microbial reduction than can be achieved by the individual treatments alone. Not surprisingly, the overall disinfection efficacy of the combined treatments is significantly better than 20000 ppm calcium hypochlorite alone (Figure 2). While identifying an optimal combination might be challenging due to the complexity introduced by the application of multiple treatments and to issues related to practical implementation, combination treatments, once established, could provide the best control strategy.

Conclusions

Seed sanitation is an important part of a multihurdle risk management strategy in the production of sprouts. Numerous studies have evaluated different approaches for pathogen reduction in sprouting seeds. The accumulated evidence is rich so that a fair estimate of the efficacy of different treatments can be generated, as summarized in Table 1. While the majority of the published studies considered here successfully avoided significant losses in seed germination, disinfection efficacies vary. Our results suggest that although 20000 ppm calcium hypochlorite is currently considered to be the reference standard for seed disinfection, it may be equivalent to other approaches, or even less efficient than certain newly emerged treatments (Figure 2). Physical treatments, especially high pressure, show promise for producing better disinfection results than that obtained by 20000 ppm calcium hypochlorite treatment. The improvement may be attributable partially to the greater ability of physical treatments to reach and affect bacteria both inside and outside of seeds. Although irradiation is at least as effective, this treatment has not been accepted by the industry (or sprout consumers) for a number of reasons, including the impact of this treatment on sprout quality and yield. Certain combined treatments also appear to be more effective than 20000 ppm calcium hypochlorite. More studies are needed to confirm the efficacy of physical and combined seed disinfection treatments. The current reference standard may not be the best sanitation treatment for sprout production, because the high levels of chemicals can pose environmental and worker safety risks. Alternative intervention approaches should be considered. Given the "green" nature of physical treatments, further investigations of this type of approach are warranted.

It is worth mentioning that the majority of articles included in this review report studies involving varying experimental designs and procedures and the use of artificially inoculated seeds with significant higher pathogen load than naturally contaminated seeds. In addition, although it appears that seed disinfection could achieve a similar effect on *Salmonella* and *E. coli* O157 : H7, studies on other common or emerging pathogens such as *L. monocytogenes* and *E. coli* O104 : H4 are lacking. Therefore, it is difficult to recommend specific treatments to the sprout industry based on the published literature. Risk reduction measures should be validated for the specific conditions in a production facility. It also should be pointed out that even though satisfactory seed disinfection can be achieved, it is possible that the final products can still be contaminated due to the growth of pathogens survived in the treated seeds during sprouting. It is unlikely that the seed disinfection alone will eliminate the microbial contamination in sprout production, and thus, additional risk management options, such as a microbial sampling and testing program, should also be implemented in order to minimize microbial hazards associated with sprouts. The change of the current industrial practice in sprout production based on new science-based evidence will be necessary to improve sprout safety.

Acknowledgments

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