Comprehensive REVIEWS in Food Science and Food Safety

Overview of Recent Events in the Microbiological Safety of Sprouts and New Intervention Technologies

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Abstract: There has been an increasing trend in consumption of sprouts worldwide due to their widespread availability and high nutrient content. However, microbial contamination of sprouts readily occurs due to the presence of pathogenic bacteria in seeds; and the germination and sprouting process provide optimal conditions for bacterial growth. In recent years, there has been a rise in the number of outbreaks associated with sprouts. These outbreaks occurred mainly in the US, Canada, UK, as well as Europe. More recently in 2011, there were 4 sprout-related outbreaks, with the *Escherichia coli* O104:H4 outbreak in Germany causing around 50 deaths and 4000 illnesses reported. On top of pathogenic *E. coli*, *Salmonella* spp. are often associated with sprout-related foodborne disease outbreaks. The contamination of sprouts has become a worldwide food safety concern. Hence, this review paper covers the outbreaks associated with sprouts, prevalence and characteristics of pathogens contaminating sprouts, their survival and growth, and the source of these pathogens. Physical, biological, and chemical interventions utilized to minimize microbial risks in sprouts are also discussed.

Introduction

Sprouts mainly originate from the *Leguminosae* family and there are different varieties of sprouts existing in the market, such as the alfalfa, mung bean, radish, and soy sprouts (Robertson and others 2002). Increasingly, consumers are looking for natural, "healthy," inexpensive, and convenient food. Sprouts fall into this category and are gaining popularity across the world as they are a good source of various nutrients that include proteins, carbohydrates, minerals, and vitamins (Robertson and others 2002; Gabriel and others 2007; Martínez-Villaluenga and others 2008; Peñas and others 2009). In addition, several epidemiological studies have been conducted and concluded that consumption of sprouts may help protect against certain chronic diseases and cancers. This is due to the high content of phytochemicals known as glucosinolates present in the sprouts. Glucosinolates function as antioxidants and aid in cancer prevention. When glucosinolates are hydrolyzed the isothiocynates formed also have antioxidant properties and can induce death of cancer cells (Bellostas and others 2007; Martínez-Villaluenga and others 2008). Thus, consumption of sprouts can bring about a host of health benefits.

During the germination of seeds, macromolecules such as lipids and proteins are broken down to form nutrients that are more easily digested and absorbed (Peñas and others 2009). Seeds are first soaked in water before being placed under warm and humid conditions. These conditions are ideal and optimal for bacterial proliferation. The germination step is the main source of contamination in sprouts as bacteria present in the seeds may become internalized during the sprouting (US FDA 1999). In various studies, the microbial loads in seeds were found to be between 3.0 and 6.0 log CFU/g; with sprouts having counts that were 2 or 3 logs greater (Thompson and Powell 2000; Martínez-Villaluenga and others 2008; Ren and others 2009).

Other than germination, there are many potential sources of contamination during the production of sprouts. These are categorized as preharvest or postharvest contamination. Some potential sources of preharvest contamination include the type of fertilizer used, irrigation water, or soil quality (Guan and Holley 2003; Pachepsky and others 2011). Postharvest contamination of sprouts may occur during the transportation, handling, and storage of the sprouts. Sprouts are usually consumed in the raw form in western countries so that the nutrients and other components that confer health benefits are not lost. Hence, there is a high risk involved in sprout consumption because there are no steps taken before consumption to eliminate the pathogenic bacteria that may be present.

The U.S. Food and Drug Administration (FDA) has published several recommendations to consumers regarding consumption of sprouts (US FDA 1999, 2012). As washing the sprouts before consumption is ineffective to reduce microbial load, consumers are recommended to cook the sprouts thoroughly to inhibit or eliminate the bacteria present. Also, intervention methods aimed at reducing or eliminating bacterial populations following preharvest and postharvest contamination in sprouts have been studied.

MS 20121310 Submitted 9/24/2012, Accepted 12/11/2012. *Authors Yang, Meier, Ann Lo, Yuan, Lee Pei Sze, and Yuk are with the Food Science & Technology Programme, Dept. of Chemistry, National Univ. of Singapore, 3 Science Drive 3, Singapore 117543. Author Chung is with the Dept. of Food and Nutrition, Inha Univ., Incheon 402–751, Korea. Direct inquiries to author Yuk (E-mail: chmyukhg@nus.edu.sg).*

doi: 10.1111/1541-4337.12010

Table 1–Summary of outbreaks associated with sprouts from 2000 to 2011.

Etiology[a]	Year of occurrence	Number of outbreaks	Number of illnesses	Source	Country
Salmonella Enteritidis	2000	5	204	Bean sprouts, mung bean sprouts, alfalfa sprouts, spicy sprouts	US, Canada, Netherlands
Salmonella spp.	2000	5	814	Alfalfa sprouts, mung bean sprouts, clover sprouts	US, Canada
Salmonella Kottbus	2001	1	32	Alfalfa sprouts	US
Salmonella Saintpaul	2003	4	115	Alfalfa sprouts	US
Salmonella Chester	2003	1	26	Alfalfa sprouts	US
Salmonella Oranienburg	2005	1	125	Alfalfa sprouts	Australia
Salmonella Braenderup	2006	1	4	Bean sprouts	US
Salmonella Stanley	2007	1	44	Alfalfa sprouts	Sweden
Salmonella Weltevreden	2007	1	45	Alfalfa sprouts	Denmark, Norway, Finland
Salmonella Typhimurium	2008	1	24	Alfalfa sprouts	US
Salmonella Bovismorbificans	2009	1	42	Alfalfa sprouts	Finland
Salmonella Cubana	2009	1	14	Alfalfa sprouts	Canada
Salmonella Bareilly	2010	1	190	Bean sprouts	UK
Salmonella Newport	2010	1	28	Alfalfa sprouts	US
E.coli O157:H7	2002	3	15	Alfalfa sprouts	US
E.coli O157:NM	2003	1	13	Alfalfa sprouts	US
E.coli O104:H4	2011	1	3842 (53 deaths, 855 HUS cases)	Fenugreek sprouts	Germany

[a]Data compiled from CDC website on foodborne outbreak surveillance (http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx), outbreak database http://www.outbreakdatabase.com/ and Kansas State University (http://bites.ksu.edu/sprouts-associated-outbreaks).

These methods include physical, biological, as well as chemical interventions. Physical intervention methods include the use of heat (Jaquette and others 1996), chilling (Tian and others 2012), high pressure (Neetoo and Chen a,b), irradiation (Saroj and others 2007), as well as supercritical carbon dioxide (Jung and others 2009). Biological interventions are composed of antagonistic microorganisms (Liao 2008), antimicrobial metabolites (Nandiwada and others 2004), and bacteriophages (Ye and others 2010). Chemical interventions are comprised of disinfectants and sanitizers such as ozone (Singla and others 2011), chlorine (Bang and others 2011), as well as electrolyzed water (Zhang and others 2011).

The first topic addressed in this review is a summary of the etiology of sprout-related outbreaks occurring worldwide from 2000 to 2011. The prevalence and characteristics of the common foodborne pathogens associated with sprouts are then evaluated, followed by a short discussion on the sources of contamination including the pre- and postharvest sources. Survival and growth of the specific pathogens are addressed, along with factors and conditions that influence their ability to persist in the environment. Finally, the physical, biological, and chemical intervention methods aimed at reducing or eliminating bacteria that were introduced during preharvest and postharvest treatments in the sprouts are reviewed and evaluated. The ultimate objective of this review is to provide the sprout industry some thoughts on developing effective strategies and interventions that can produce safe sprouts to the consumers.

Incidences Associated with Sprouts

The number of sprout-related foodborne outbreaks has been on the rise from 2000 to 2011. When evaluating the food safety of sprouts it is crucial that the prevalence of foodborne pathogens as well as the associated outbreaks are analyzed.

In the list of outbreaks from 2000 to 2011, summarized in Table 1, it is observed that alfalfa sprouts were implicated in most of the outbreaks, followed by a small number attributed to bean sprouts. Alfalfa sprouts are the most common sprouts available; hence, their likelihood of causing an outbreak is higher. The bacterial pathogens attributed to these outbreaks are largely *Salmonella* spp., *Escherichia coli* O157, and *E. coli* O104: H4, the latter being attributed as the cause of one of the worst foodborne outbreaks in Europe. The Robert Koch Inst.—Germany's disease control agency—reported 3842 illnesses as a result of the outbreak, during which 855 people acquired hemolytic uremic syndrome (HUS) with 35 deaths and 2987 people acquired enterohemorrhagic *E. coli* (EHEC) gastroenteritidis with 18 deaths. The outbreak was linked to fresh sprouts from a farm in Lower Saxony, Germany. Possible routes of contamination of the sprouts were from the fenugreek seeds (RKI, 2011).

Outbreaks due to consumption of sprouts contaminated with *Salmonella* have been frequently reported throughout the world since the year 1998. While microbiological studies carried out in Norway, Spain, and Korea were not able to isolate *Salmonella* from the sampled sprouts (Robertson and others 2002; Abadias and others 2008; Kim and others 2009), surveys in USA and India reported prevalence of *Salmonella* in sprouts as high as 7% and 19.4%, respectively (Samadpour and others 2006; Saroj and others 2006).

Other foodborne pathogens have also been isolated from sprouts. These include *Aeromonas hydrophila, Bacillus cereus, Listeria monocytogenes*, and *Staphylococcus aureus* (Beuchat 1995; Thompson and Powell 2000). The first and only outbreak of *B. cereus* associated with sprouts dates back to the year 1973 (Portnoy and others 1976). Not many studies have been carried out regarding the prevalence of *B. cereus* in vegetable sprouts. Nevertheless, a Danish study reported that the microorganism was detected in all 40 sprout samples tested. However, none of the sprout samples contained the number of *B. cereus* required to induce diarrheal illness. In fact, all of the samples acquired were found to contain a much lower number of the foodborne pathogen—which was less than 10^3 CFU/g—as compared to the infective dose (Rosenquist and others 2005). The other foodborne pathogens have not been involved in any sprout-related outbreaks.

Sources of Contamination

There are various sources of contamination of sprouts, which can be classified as pre- or postharvest sources (Figure 1). Contaminated seeds have been the source of most sprout-associated foodborne illnesses and are considered to be the most common source of contamination. As illustrated by several studies

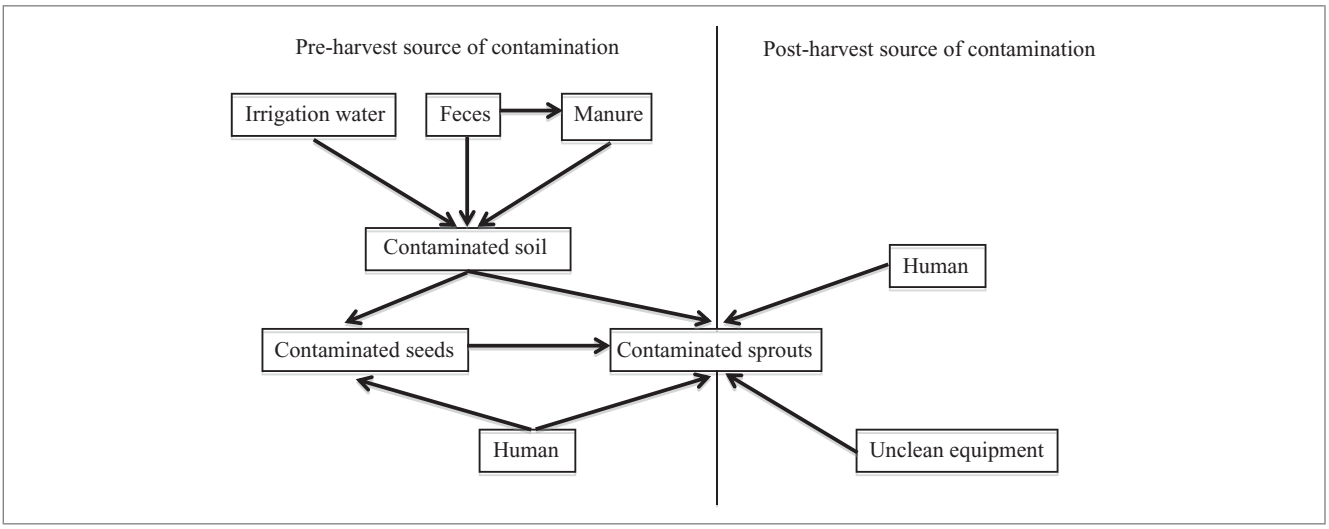

Figure 1–Overview of preharvest and postharvest sources of contamination of sprouts.

(Prokopowich and Blank 1991; Robertson and others 2002), seeds can harbor high levels of bacteria, ranging between 10^3 and 10^6 CFU/g. For this reason, this part focuses not only on contamination sources of sprouts, but also those of seeds.

Preharvest Sources of Contamination

The pathogen load and the quality of the seeds are the main factors influencing contamination of the final product. The harvested seeds are either destined for agricultural use or for sprouting, with the latter accounting for only a small proportion. The decision for use is often not made until harvest. Due to the fact that seeds are treated as a raw agricultural product, rather than as a food product, some seed growers do not follow good agricultural practices (GAP) the way they should (NACMCF 1999; Robertson and others 2002).

Pathogens are introduced to seeds in several ways. One of these sources includes the use of manure as a fertilizer (CFIA 2007). The spreading of animal waste on agricultural land is a cheap and practical solution for improving soil quality. Animal manure can contain pathogenic bacteria, such as *Campylobacter* spp. and *Salmonella* spp. (Östling and Lindgren 1991; Guan and Holley 2003). Hence, the use of manure may result in the introduction of these pathogens in the soil. Afterwards, the pathogens can migrate and attach to the produce. Attachment of foodborne pathogens is diverse and may occur through the fimbriae, flagella, and biofilms. Jeter and Matthysse (2005) illustrated that diarrheagenic *E. coli* strains, including serovar O157:H7, were able to attach to alfalfa sprouts, whereas other strains did not. In their study, it was suggested that more than one mechanism for binding to plant surfaces is needed. Thus, it is important to study the attachment mechanism of pathogens to prevent colonization of pathogens on seeds and sprouts. Furthermore, cracks and cavities in the seed may support survival of pathogenic bacteria, suggesting that damage of the seeds needs to be prevented.

The growing location is also considered as one of the potential sources of contamination. Fields on which domestic or wild animals have grazed are more likely to be contaminated by pathogenic bacteria due to remains of feces from infected animals (Brackett 1999). Pathogens, such as *Salmonella* spp. and *E. coli* O157:H7 can survive in feces or/and soils for several months (Scott and others 2006; O'Neill and others 2011). Therefore, once the fields were contaminated, the fields would be a source of these pathogens for a long period and thus would contaminate the sprouts grown on the fields. Another key source of contamination is the type of irrigation system used and source of irrigation water. Low-quality water is often used due to the lack of availability of good-quality water. Sources of irrigation water may be potable or rain water, groundwater, surface water, and raw or inadequately treated wastewater (Pachepsky and others 2011). Use of the latter exhibits the highest risk of contamination with pathogens. The microbial load of water was reported in several studies. A study by Cross (1997) reviewed the quality of irrigation district water and reported that 8% of the irrigation water samples collected in Alberta, Canada, contained more than 100 fecal coliforms per 100 mL of sample. Another study revealed that *E. coli* O157 was present in 2 of 96 water samples from a river in northern Nigeria used for large-scale irrigation (Chigor and others 2010). Johnson and others (2003) also reported that the prevalence of *E. coli* O157:H7 and *Salmonella* spp. in water samples of southern Alberta was at levels of 0.9% and 6.2%, respectively. Thus, these studies illustrate the presence of pathogens in irrigation water, which can lead to contamination of the cultured product.

Finally, seeds may become contaminated with foodborne pathogens by direct contact with infected farm workers. Therefore, clean and sanitary toilets and proper hand-washing facilities must be available to workers in the field where they work. In addition, workers must receive adequate training in hygiene for their job.

Postharvest Sources of Contamination

During harvesting, seeds and sprouts are exposed to dirt and debris, making contamination likely during this stage. Furthermore, they are not sorted until delivery. Consequently, unclean harvesting equipment, storage area, and transportation vessels are other factors that have to be considered when evaluating the contamination source. In addition, harvesting and postharvest processing involve considerable human contact, making poor personal hygiene of workers a potential source of contamination. Knowledge about fundamental food safety is often insufficient. In a report about safety evaluations (NACMCF 1999), it was observed that 23 out of the 45 evaluated firms did not have hot water for cleaning.

Once the product leaves the sprouting facility, the sprouts are exposed to other sources of contamination. For instance, contamination of sprouts can further occur at food service outlets, such as restaurants and delis, or at the consumer's home. Improper storage conditions facilitate growth of pathogens. Furthermore, pathogens can be introduced to food by inadequate hygiene of food establishments or through infected food handlers.

All these factors illustrate that proper harvesting and handling of the seeds and sprouts are important to avoid bacterial contamination. It can be concluded that a comprehensive approach, based on good manufacturing practices and principles of HACCP, can reduce the risk of sprout-associated disease.

Factors Affecting the Survival and Growth of Pathogens

The significance of contamination of a product is mainly dependent on the pathogen's infectious dose and its survival during storage. Several intrinsic and extrinsic factors have an impact on bacterial growth in sprouts during storage. Storage temperature is one of these factors. Castro-Rosas and Escartín (2000) investigated how viability of pathogens was affected under refrigerator conditions. They observed that refrigeration resulted in a 50% reduction in the number of viable *S.* Typhi and a 90% reduction to 2.1 log CFU/g of *Vibrio cholera* on alfalfa sprouts after 15 d of storage. Another study reported similar findings in bean sprouts (Francis and O'Beirne 2001). Although *L. monocytogenes* is capable of growth at low temperatures, reduced temperature led to a reduced growth rate. Numbers of *L. monocytogenes* on soybean sprouts decreased by 1.5 log cycles when samples were stored at 4 °C, whereas no significant change in numbers was observable during storage at 8 °C. From these results, it can be concluded that storage of sprouts in the refrigerator can inhibit growth of foodborne pathogens.

Furthermore, relative humidity and composition of the gas atmosphere have an influence on growth of pathogens. In some cases, sprouts are distributed and offered unpacked to consumers in public markets. The sale of sprouts in this manner is not ideal, as contamination may occur from the air or infected consumers. Choosing the right packaging conditions is important to maintain product quality and to retain microbiological safety. Sprouts respire actively during the respiration process and oxygen (O_2) will be consumed, producing CO_2 and H_2O. Oxygen is required to produce enough energy by aerobic respiration to maintain sprout quality (Gorris and Peppelenbos 1992). The effect of moderate vacuum packaging (MVP), a type of modified atmosphere packaging (MAP), on mung bean sprouts was evaluated in one study (Gorris and others 1994). In the MVP system, the sprouts were packed in a rigid airtight container under 400 mbar pressure and afterwards stored at 4 °C. A low initial O_2 content also slowed down the metabolic activity of the product and growth of spoilage organism. In this study, MVP was found to improve the microbial quality and the sensory quality of the mung bean sprouts, whereas results for the alfalfa sprouts showed worse sensory quality and constant microbial quality. Of more importance were the results of the pathogen challenge test. It was observed that strains of *Y. enterocolitica*, *S.* Typhimurium, *B. cereus*, and *L. monocytogenes* did not survive the applied storage conditions of mung bean sprouts. Accordingly, they concluded that MVP might be useful for storage of mung bean sprouts. However, there are only a few studies about ideal packaging conditions of sprouts. Hence, further investigations are needed.

Nutrient availability is a further key factor influencing survival and growth of pathogens. Hamilton and Vanderstoep (1979) illustrated that sprouting resulted in a large increase in water content, which is a favored condition for bacterial growth. Furthermore, they reported that alfalfa seeds and sprouts contain more proteins and carbohydrates than other vegetables, such as cabbage or lettuce. In another study it was reported that mung bean sprouts had the highest protein and starch contents of the analyzed Chinese vegetables (Wills and others 1984). A nearly neutral pH and the above-mentioned factors make sprout an ideal substrate for bacterial growth.

Lastly, the presence of competitive bacteria and antimicrobial compounds, as well as the formation of biofilm can influence bacterial growth on sprouts. Francis and O'Beirne (2001) reported that the analyzed soybean sprouts did not support growth of *L. monocytogenes* due to competition from the high numbers (10^8 CFU/g) of background microflora. However, sprouts are generally low in antimicrobial compounds as illustrated by Wills and others (1984) who reported that mung bean sprouts are fairly low in organic acids, such as malic or citric acids, which can have an inhibitory effect on bacterial growth. Finally, Fett (2000) found that naturally occurring biofilm on sprouts allows protected colonization sites for human pathogens, such as *Salmonella* spp. and *E. coli* O157:H7.

Thus, different factors may influence survival and growth of foodborne pathogens on sprouts. Control of these factors to suppress growth of pathogens is needed to minimize the risk of foodborne disease associated with sprout consumption.

Intervention Technologies

Physical intervention technologies

Recent studies have employed physical treatments, such as refrigeration, dry heat/hot water, high hydrostatic pressure (HHP), irradiation, and supercritical carbon dioxide (SC-CO_2) treatment, to reduce or eliminate foodborne pathogens on seeds or sprouts. This section will review the effectiveness of these physical intervention technologies. The intervention methods, which can achieve a 5-log reduction of foodborne pathogens when used alone, are summarized in Table 2.

Temperature. Temperature plays an important role in affecting survival of pathogens on sprouts. Refrigeration has been reported to have an inhibitory effect on the growth of 4 foodborne pathogens (*S.* Typhimurium, *S. aureus*, *L. monocytogenes*, and *E. coli* O157:H7) on sprouts since populations of these pathogens did not significantly increase after storage at 4 °C for 15 d (Tian and others 2012). Also, Weiss and Hammes (2005) found that the population of *S.* Senftenberg W775, *S.* Bovismorbificans, and *E. coli* O157:H- remained unchanged during storage at 2 °C for 8 wk. However, cold storage, which can prevent the growth of these pathogens, can hardly eliminate or reduce these pathogens on sprouts.

Compared to cold storage, heat treatment is a more effective way to enhance the microbial safety of sprouts due to its high effectiveness in reducing pathogens in seeds and sprouts. The effects of 2 heat treatments, dry heat and hot water, on inactivation of food pathogens and germination of the seeds have been investigated in the last decade. For dry heat treatment, the time and temperature needed to eliminate foodborne pathogens on sprout seeds varies with the type of sprouts. Dry heat treatment (50 °C) for 17 or 24 h in radish, broccoli, and alfalfa seeds effectively reduced *E. coli* O157:H7 below the detection limit, but the effect was not pronounced in mung bean seeds (Bari and others 2009a). Dry heat treatment of mung bean seeds at 55 °C successfully eliminated *E. coli* O157:H7 (10^6 CFU/g) and *Salmonella*

Table 2–The efficacy of physical intervention technologies to inactivate foodborne pathogens.

Method	Seed/sprout type	Treatment conditions	Pathogens	Log reduction (CFU/g)	Reference
Dry heat	Alfalfa and broccoli/radish seeds	50 °C/17 or 24 h	*E. coli* O157:H7	5	Bari and others (2009a)
	Mung bean seeds	55 °C/4 d	*E. coli* O157:H7	6	Hu and others (2004)
	Alfalfa seeds	55 °C/6 d	*E. coli* O157:H7	8	Feng and others (2007)
Hot water	Mung bean seeds	55 to 80 °C/2 to 20 min	*E. coli* O157:H- and *Salmonella* spp.	>5	Weiss and Hammes (2005)
	Radish and alfalfa seeds	53 to 64 °C/0.5 to 8 min			
	Mung bean seeds	90 °C for 90 s followed by dipping in chilled water for 30 s	*E. coli* O157:H7/ *Salmonella* spp.	6.08/5.34	Bari and others (2008)
	Alfalfa sprouts	70, 80, 90 and 100 °C for 10, 5, 3, and 3 s	*Salmonella* spp.	7.6	Pao and others (2008)
	Mung bean sprouts	70, 80, 90, and 100 °C for 20, 20, 10, and 5 s		6.9	
HHP	Garden cress seeds	300 MPa/15 min/4 °C	*S.* Typhimurium, *E. coli* MG1655, and *L. innocua*	6	Wuytack and others (2003)
	Alfalfa seeds	650 MPa/15 min/20 °C	*E. coli* O157:H7	>5	Neetoo and others (2008)
	Alfalfa seeds	550 MPa/2 min/40 °C	*E. coli* O157:H7	>5	Neetoo and others (2009a)
Irradiation	Alfalfa sprouts	3.3 kGy	*L. monocytogenes*	6	Schoeller and others (2002)

(10^4 CFU/g) to undetectable levels (<10 CFU/g) without affecting the germination rate (Hu and others 2004). Feng and others (2007) reported similarly that no *Salmonella* (2-log reduction) and *E. coli* O157:H7 (8-log reduction) were observed on alfalfa seeds after dry heating at 55 °C for 6 d and there was no significant decrease in the percentage of germination.

Alternatively, hot water treatment also has high bactericidal activity against foodborne pathogens. Hot water treatment was first applied to reduce populations of pathogenic bacteria on sprout seeds by Jaquette and others (1996). They found that treatment at 57 or 60 °C for 5 min could effectively kill 2.5 log CFU/g of *S.* Stanley inoculated on alfalfa seeds with no significant loss of germination rate; however, germination rate was greatly reduced when slightly higher temperature or longer treatment time was used. Weiss and Hammes (2005) showed that by using thermal treatment, greater than 5-log reduction in *E. coli* O157:H- and *Salmonella* spp. (*S.* Senftenberg W775 and *S.* Bovismorbificans) was achieved in mung bean seeds (55 to 80 °C for 2 to 20 min), radish and alfalfa seeds (53 to 64 °C for 0.5 to 8 min), while maintaining the sprouting rate to be greater than 95%. In addition, less time was required to inactivate pathogens when a higher temperature was employed. For example, when *E. coli* O157:H7 and *Salmonella* spp. were treated with hot water at 85 °C for 40 s followed by dipping in cold water for 30 s and soaking in chlorine water (2000 ppm) for 2 h or treated at 90 °C for 90 s followed by dipping in chilled water for 30 s, no viable pathogens were found in the enrichment medium and during the sprouting process and no appreciable reduction in the germination yield of the mung bean seed was observed (Bari and others 2008, 2010).

Although most studies applied heat treatment to seed decontamination, heat treatment could be an effective decontamination method for sprouts as well. Pao and others (2008) examined the effectiveness of hot water treatment in killing 4 *Salmonella enterica* cultures (*S.* Enteritidis, *S.* Montevideo, *S.* Newport, and *S.* Typhimurium) on alfalfa and mung bean sprouts. By immersing alfalfa sprouts in hot water at 70, 80, 90, and 100 °C for 10, 5, 3, and 3 s as well as treating mung bean sprouts at the same temperatures for 20, 20, 10, and 5 s, 7.6 and 6.9 log CFU/g reductions of *S. enterica* were attained for alfalfa and mung bean sprouts, respectively.

High hydrostatic pressure. High hydrostatic pressure processing (HHP) is a novel nonthermal method which can inactivate foodborne pathogens while maintaining the organoleptic properties and nutritional values of the food product (Rendueles and others 2011). Wuytack and others (2003) and Ariefdjohan and others (2004) made the initial studies in the application of HHP to seed decontamination to improve microbial safety and quality of seeds and showed that the effect of HHP varied depending on strains. Treatment of 300 MPa (15 min, 4 °C) on garden cress seeds caused 6-log reductions of *S.* Typhimurium, *E. coli* MG1655, and *L. innocua*, more than 4-log reductions of *Shigella flexneri* and pressure-resistant *E. coli* LMM1010, and a 2-log reduction of *S. aureus* (Wuytack and others 2003). Ariefdjohan and others (2004) also found that *L. monocytogenes* was more resistant to the bactericidal effects of HHP than *E. coli* O157 on alfalfa seeds. In addition, the decontamination efficacy of HHP is also a function of temperature, pressure, exposure time, presoaking treatment, and type of seeds. It was demonstrated that treatment at 650 MPa, 20 °C, for 15 min was equally effective with treatment at 550 MPa, 40 °C, for 2 min in *E. coli* O157:H7 elimination without sacrificing the germination rate (Neetoo and others 2008, 2009a). Soaking seeds prior to pressure treatment is conducive to enhance pressure inactivation of *E. coli* O157:H7 and *Salmonella* spp., but it was usually at the cost of seed viability (Neetoo and others 2009b; Neetoo and Chen a,b). Later, Neetoo and Chen (2011) proposed that applying a combination of dry heating (60 °C for 24 h or 65 °C for 12 h) with pressure treatment of 600 MPa, 35 °C, for 2 min to eliminate *Salmonella* and *E. coli* O157:H7 on alfalfa seeds did not significantly affect the germination rate, although the sprouting yield was reduced. Similar to the fact that the resistance of pathogenic bacteria to HHP was highly variable, the variation in pressure tolerance was also observed in different seeds and the order of pressure tolerance was red clover > crimson clover ≈ broccoli > radish seeds, as reported by Neetoo and Chen (2010b). Therefore, only presoaking red clover seeds for 60 min at 4 °C followed by HHP at 600 MPa, 20 °C, for 5 min could inactivate *Salmonella* and *E. coli* O157:H7 without reducing germination percentages of the seed and sprout yield (Neetoo and Chen 2010b). In addition, antimicrobial compounds, like hypochlorite and carvacrol, also had a positive effect on HHP, with higher reductions of microbial

populations on alfalfa and mung bean sprouts (Peñas and others 2009, 2010).

Irradiation. Ionizing radiation as a nonthermal process can be effectively used to improve microbial safety as well as to extend shelf life of a wide variety of food products (Song and others 2009). Ionizing radiation for the treatment of foods includes gamma rays from cobalt 60 or cesium 137, electrons generated from machine sources (e-beam), and x-rays (Komolprasert 2007). The U.S. FDA has approved usage of irradiation treatment for sprouting seeds with maximum doses of 8 kGy (Code of Federal Regulations Title 21 2011b); however, there is no specific irradiation dose for sprouts. Irradiation has been shown to be effective in decreasing pathogen populations on sprouts as well as seeds intended for sprout production. Rajkowski and Thayer (2000) reported that *Salmonella* spp. were not recovered from alfalfa sprout that was grown from *S. enterica* Mbandaka-contaminated alfalfa seeds after gamma-irradiation with a dose of 0.5 kGy or greater. Schoeller and others (2002) determined that irradiation of alfalfa sprout with beta-radiation at 3.3 kGy produced a 6 log reduction of *L. monocytogenes* without any noticeable changes in appearance or odor. In broccoli and mung bean sprouts inoculated with *L. monocytogenes*, low-dose (1 kGy) gamma-irradiation reduced *L. monocytogenes* by 4.88 and 4.57 log CFU/g, respectively (Bari and others 2005). Similarly, a 2-kGy radiation dose could completely eliminate 4 log CFU/g *S.* Typhimurium on seeds (Saroj and others 2007).

Gamma-ray-irradiation was reported to be more effective than e-beam-irradiation in decreasing *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and *B. cereus* populations on broccoli and red radish sprouts, but not on their seeds (Waje and others 2009). The shelf life of alfalfa sprouts and broccoli sprouts was increased by 10 d with a low-dose (2 kGy) irradiation (Rajkowski and Thayer 2001; Rajkowski and others 2003). Although the current allowable radiation dose for seed decontamination in the USA is 8 kGy, a high dose of irradiation has adverse effects on germination percentage, yield ratio, length, and thickness of sprouts (Rajkowski and Thayer 2001; Rajkowski and others 2003). Contrarily, doses of 1 and 2 kGy have been shown to reduce or eliminate foodborne pathogens without affecting the nutritional, textural, and sensory qualities in most cases (Bari and others 2005; Hajare and others 2007; Nagar and others 2012). Combination of low-dose irradiation (0.75 and 1.5 kGy) with acidified sodium chlorite (ASC) was more effective than either treatment alone in reducing *E. coli* O157:H7 to below the detection limit (<1 log CFU/g) on mung bean seeds. However, *E. coli* O157:H7 was detected in most samples in the enrichment and after germination, and the yield and sprout length were decreased by gamma-irradiation (Nei and others 2010). Saroj and others (2007) also observed the same phenomenon that the survived *S.* Typhimurium (1.8 log CFU/g) reached 8 log CFU/g after 48 h of sprouting on seeds treated with a 1-kGy radiation dose and recommended that sprouts rather than seeds should be irradiated to prevent contamination from posttreatment handling. In addition to ASC, heat treatment was also used in combination with irradiation to suppress the growth of *E. coli* O157:H7. The result showed that heat treatment at 50 °C for 17 h followed by a 1.0-kGy dose of irradiation successfully reduced *E. coli* O157:H7 numbers to below the detectable levels in mung bean seeds, which was not achieved by heat treatment at 50 °C alone for 24 h (Bari and others 2009a). Thus, hurdle technology could greatly enhance the microbiological safety of sprout products.

Supercritical carbon dioxide. Supercritical carbon dioxide treatment is one of the most promising cold-pasteurization techniques which can be potentially used to preserve various foods, such as pork (Choi and others 2009), dehydrated powdered infant formula (Kim and others 2010), apple cider (Yuk and others 2010), and spinach leaves (Zhong and others 2008). The bactericidal effects of SC-CO_2 are probably due to the inactivation of key enzymes for cell metabolism by decreased intracellular pH, bacterial disruption by an increase in internal pressure, the modification of cell membranes, and the extraction of intracellular substances (Spilimbergo and Bertucco 2003).

The effect of supercritical carbon dioxide treatments for microbial inactivation on seeds for sprout production has been examined. Generally, greater microbial reductions were achieved by treating the seeds with higher SC-CO_2 pressure, temperature, or longer treatment time. For example, as the pressure was increased from 2000 psi (13.8 MPa) to 4000 psi (27.6 MPa), at 50 °C for 15 min, the reduction of *E. coli* K12 increased from 26.6% to 81.3%, while as the time was extended from 15 min up to 60 min at 4000 psi and 50 °C, the reduction of *E. coli* K12 increased from 81.3% to 92.8%. In addition, the maximum reduction levels of aerobic microorganisms and *E. coli* K12 were 85.6% (<4 log CFU/g reduction) and 92.8% (<4 log CFU/g reduction), respectively, at 4000 psi and 50 °C for 60 min and the germination characteristics of alfalfa seeds were unaffected (Mazzoni and others 2001). Also, the survival count of *E. coli* O157:H7 was reduced by 3.51 log CFU/g with SC-CO_2 (99.5% purity) treatment at 15 MPa and 35 °C for 10 min, whereas those of *L. monocytogenes* and *S.* Typhimurium were reduced by 2.65 and 2.48 log CFU/g with treatment at 10 MPa and 45 °C for 5 min (Jung and others 2009). Although treatment at 20 MPa and 45 °C for 15 min led to more than 7 log CFU/g reduction in these 3 pathogens, the seed germination capability was impaired under this condition (Jung and others 2009). Based on these results, SC-CO_2 treatment alone cannot be able to achieve a 5-log reduction of foodborne pathogens on seeds used for sprout production as recommended by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) to minimize the risk of food poisoning (NACMCF 1999).

Biological intervention technologies

The strategies for food biopreservation are generally based on the use of antagonistic microorganisms, antimicrobial metabolites, and bacteriophages. The efficacy of different biocontrol strategies is summarized in Table 3.

Nonpathogenic microorganisms that can compete with pathogens for physical space and nutrients have been applied to reduce foodborne pathogens in food products. Several studies pointed out that *Pseudomonas* spp. were some of the bacteria that could be used to inhibit the growth of *Salmonella* spp. on sprouts or seeds (Matos and Garland 2005; Fett 2006; Weiss and others 2007; Liao 2008). Matos and Garland (2005) tested the antimicrobial activity of *P. fluorescens* 2–79 strain against a cocktail of 4 serovars of *Salmonella* (*S.* Anatum, *S.* Infantis, *S.* Newport, and *S.* Stanley) on alfalfa sprouts. *P. fluorescens* 2–79 exhibited highest inhibitory effect on *Salmonella* on the first day of alfalfa growth with reduction of 4.22 log/CFU, but the effect was limited as the time increased. Fett (2006) also described the high inhibitory activity of *P. fluorescens* 2–79 against the same *S. enterica* serovars (*S.* Anatum, *S.* Infantis, *S.* Newport, and *S.* Stanley) on sprouting alfalfa seeds, which resulted in an average reduction of 5 log CFU/g at 6 d of sprouting without affecting sprout yield and appearance.

Table 3–The efficacy of antagonistic microorganism-, antimicrobial metabolite-, and bacteriophage-based strategies to inactivate foodborne pathogens.

Method	Seed/sprout type	Treatment conditions	Pathogens	Log reduction (CFU/g)	Reference
Antagonistic microorganisms	Alfalfa seeds	Alfalfa seeds inoculated with *Salmonella* spp. (10^3 CFU/g) were treated with *P. fluorescens* 2 to 79 (10^8 CFU/mL) for 2 h	*Salmonella* spp.	5	Fett (2006)
	Mung bean seeds	Mung bean seeds were preinoculated with *P.* jessenii (10^8 CFU/g) at day 0 and *S.* Senftenberg (1 to 10 CFU/g) was added at day 1	*S.* Senftenberg	7	Weiss and others (2007)
	Mung bean seeds	Mung bean seeds were soaked in *Salmonella* cocktail suspension (10^6 CFU/mL) for 20 min and then transferred to *E. asburiae* JX1 suspension (10^6 CFU/mL) for 20 min	*Salmonella* spp.	5.56	Ye and others (2010)
Antimicrobial metabolites	Mungbean sprouts	Mungbeans sprouts were dipped in mundticin (200 BU/mL) for 30 min before inoculated with *L. monocytogens* (10^4 CFU/g)	*L. monocytogens*	2	Bennik and others (1999)
	Alfalfa seeds	Alfalfa seeds contaminated with *E. coli* O157:H7 were soaked in a colicin solution (10000 AU/g or 204000 AU/g) at 37 °C for 35 min	*E. coli* O157:H7	3 to 5	Nandiwada and others (2004)
Bacteriophages	Broccoli seeds	Broccoli seeds inoculated with *Salmonella* (10^2 to 10^3 CFU/g) were treated with Phage-A and Phage-B (10^6 PFU/mL) at 25 °C for 24 h	*Salmonella* spp.	1.5	Pao and others (2004)
	Mung bean seeds	Mung bean seeds were soaked in *Salmonella* cocktail suspension (10^6 CFU/mL) for 20 min and then transferred to bacteriophage cocktail suspension (10^6 PFU/mL) for 20 min	*Salmonella* spp.	3.41	Ye and others (2010)

Liao (2008) found that a combination of sanitizer and *P. fluorescens* 2–79 was possible to achieve a 5-log reduction of *Salmonella*. In addition to *P. fluorescens* 2–79, *P. jenssenii* also has great potential as a biocontrol agent on sprouts. It has been reported that preinoculation with *P. jenssenii* on seeds caused a 7-log reduction of *S.* Senftenberg after 7 d of sprouting (Weiss and others 2007). Alternatively, an *Enterobacter asburiae* strain (labeled "JX1") isolated from mung bean sprouts and tomatoes has also shown its great potential for controlling growth of *Salmonella* on mung beans. It reduced the levels of *Salmonella* (*S.* Agona, *S.* Berta, *S.* Enteritidis, *S.* Hadar, *S.* Heidelberg, *S.* Javiana, *S.* Montevideo, *S.* Muenchen, *S.* Newport, *S.* Saintpaul, and *S.* Typhimurium) from 6.72 ± 0.78 log CFU/g to 1.16 ± 2.14 log CFU/g after 4 d of sprouting when coinoculated with *Salmonella* on mung beans (Ye and others 2010).

The use of bacteriocin, one kind of antimicrobial metabolites, for decontamination of sprouts and seeds is another biological intervention method. Mundticin, a bacteriocin produced by *Enterococcus mundtii*, reduced the population of *L. monocytogenes* by 2 log CFU/g if mung bean sprouts were first dipped in a solution of mundticin (200 U/mL) (Bennik and others 1999). A E2-type colicin produced by *E. coli* strain Hu194 resulted in significant reduction of *E. coli* O157:H7 on alfalfa seeds (Nandiwada and others 2004), while nisin inhibited growth of psychrotrophic microorganisms, anaerobic microorganisms, and *B. cereus* on sprouts stored at 10 °C (Kim and others 2008). Enterocin AS-48 (25 μg/mL), which had no effect on *S. enterica* CECT 4300 when used alone, significantly reduced or prevented growth of *S. enterica, E. coli* O157:H7, *Shigella* spp., *Enterobacter aerogenes*, *Y. enterocolitica, A. hydrophila*, and *P. fluorescens* in soybean sprouts stored at 6 and 15 °C when used with 0.1% to 2.0% polyphosphoric acid in washing treatments on artificially contaminated soybean sprouts (Cobo Molinos and others 2008).

Application of bacteriophages to sprouts or seeds to control the foodborne pathogens also represents a promising biological intervention method. Pao and others (2004) reported that addition of lytic Phages-A (targeting *S.* Typhimurium and *S.* Enteritidis) in mustard seeds achieved a 1.37-log reduction of *S.* Typhimurium and *S.* Enteritidis, while application of the mixture of Phage-A and Phage-B (specific for *S.* Montevideo) in the soaking water of broccoli seeds caused a 1.50 log reduction of *Salmonella* spp. (*S.* Typhimurium, *S.* Enteritidis, and *S.* Montevideo). A recent study demonstrated that coinoculating lytic bacteriophage with a cocktail of *Salmonella* spp. (*S.* Agona, *S.* Berta, *S.* Enteritidis, *S.* Hadar, *S.* Heidelberg, *S.* Javiana, *S.* Montevideo, *S.* Muenchen, *S.* Newport, *S.* Saintpaul, and *S.* Typhimurium) on mung bean sprouts caused the reduction of *Salmonella* levels from 6.72 ± 0.78 log CFU/g to 3.31 ± 2.48 log CFU/g after 4 d of sprouting. Moreover, the inactivation efficiency could be further enhanced if a combination of *E. asburiae* JX1 and bacteriophages was used since no *Salmonella* inoculated on alfalfa sprout was recovered from the enrichment medium after 4 d of sprouting (Ye and others 2010). This suggested a combination of *E. asburiae* JX1 and bacteriophages could be an effective way to inhibit growth of *Salmonella* on sprouting seeds.

Chemical intervention technologies

A number of chemical decontamination methods have been developed using various chemical sanitizers including chlorine, electrolyzed water, ozone, and other acidic compounds.

Chlorine and chlorine dioxide treatment. Chlorine is the most commonly used sanitizing agent for treatment of fresh produce products in the United States. The U.S. Food and Drug Administration (FDA) recommends sanitizing seeds with 20000 ppm active

chlorine, as $Ca(OCl)_2$, for 15 min, and to monitor the bacteria count in spent irrigation water as a means to control risk of foodborne illness associated with sprouts (Federal Register 1999).

Some promising results have been reported for chlorine treatment in eliminating pathogens on sprouts and their seeds (Table 4). For example, Tornuk and others (2011) reported that soaking wheat seeds with NaOCl (100, 200, or 400 ppm) at room temperature for 30 min, followed by 3 min rinsing with tap water, successfully reduced the native *E. coli* load to below the detection level (<1 log CFU/g) under all treatment conditions, with no significant negative impact on seed germination capacity. Bang and others (2011) reported a 5.9-log reduction in the *E. coli* O157:H7 population on radish seeds when treated with 500 μg/mL ClO_2 for 5 min, followed by 24 h drying at 45 °C and 23% RH, then treated with dry heating for 48 h at 70 °C and 23% RH.

However, most studies have reported that chlorine is ineffective in the complete eradication of pathogens from vegetable surfaces, particularly if the initial bacterial load is high. Singh and others (2003) showed that for alfalfa seeds inoculated with an initial level of 6 log CFU/g of *E. coli* O157:H7, washing treatments using 10 to 50 mg/L ClO_2 for 3 to 10 min at 21 °C only achieved a maximum microbial reduction of 1.24 log CFU/g. It was also observed that the *E. coli* O157:H7 population quickly increased to a level similar to control (7.8 to 8.2 log CFU/g) during sprouting of the treated seeds. Fransisca and others (2011) reported a maximum of 1.65 log CFU/mL reduction in the nonpathogenic *E. coli* O157:H7 87-23 count of radish seeds treated with 200 to 20000 ppm NaOCl for 20 min followed by spraying with 0.04% calcinated calcium solution. However, the cell count significantly increased to a level higher than that of the control during sprouting. Beuchat and others (2001) showed that treating alfalfa seeds inoculated with *Salmonella* and *E. coli* O157:H7 (5 to 6 log CFU/g initial level) with 20000 ppm $Ca(OCl)_2$ for 30 min only resulted in microbial reductions of 2.3 to 2.5 and 2.0 to 2.1 log CFU/g, respectively. Contrarily, Gandhi and Matthews (2003) showed that treatment of alfalfa seeds with 20000 ppm $Ca(OCl)_2$ for 10 min at room temperature resulted in more than a 5 log reduction in the inoculated *S.* Stanley population and reduced the microbial load to a nondetectable level. However, rapid microbial growth was still observed during sprouting of the treated seeds, and the microbial count reached 7 log CFU/g in 2-d-old sprouts. Continuous treatment of the growing sprouts with 100 ppm chlorine has been shown to be ineffective in inhibiting microbial growth. Lang and others (2000) showed that treatment with 20000 ppm chlorine for 15 min at 25 °C significantly reduced *E. coli* O157:H7 in alfalfa seeds to a nondetectable level when plated on selective sorbitol MacConkey agar (SMAC) and nonselective BHI agar. However, cell counts increased to 7 to 8 log CFU/g during sprouting, indicating that the treatment was unable to completely remove pathogens.

A 1 to 2 log reduction of pathogens during seed treatments has little practical significance because proliferation of surviving pathogens is expected during sprouting, which provides a favorable environment for microbial growth (Jin and Lee 2007). As a result, some studies have investigated the effect of direct treatment on sprouts. However, these treatments have also been demonstrated to have limited efficacy. Kim and others (2009a) demonstrated that for broccoli sprouts inoculated with 7 to 8 log CFU/g *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, treatment by dipping into 50 ppm ClO_2 at room temperature for 5 min achieved approximately 1.24 to 1.66 log CFU/g reduction for the 3 types of pathogenic bacteria. Slight improvement was observed in combined treatment using 50 ppm ClO_2 and 0.5% (w/v) fumaric acid, which produced an overall reduction of 2.39 to 2.74 log CFU/g. Similar results were reported in a subsequent study on inoculated alfalfa sprouts (Kim and others 2009b), which showed that a 10-min treatment with 50 ppm ClO_2 reduced the pathogenic bacterial cell count by 2.23 to 2.37 log CFU/g, whereas 50 ppm ClO_2 + 0.5% fumaric acid resulted in a 3.57 to 4.06 log CFU/g reduction. Jin and Lee (2007) showed that treating mung bean sprouts with 100 ppm chlorine for 5 min reduced inoculated *S.* Typhimurium and *L. monocytogenes* cell counts by 3.0 and 1.5 log CFU/g from an initial level of 4.6 and 5.6 log CFU/g, respectively. Daily spray application of a 100 ppm chlorine solution on growing sprouts was only marginally effective, achieving an approximately 1.5 reduction in population of *Salmonella* as compared to control, but total microbial count is still 6 log CFU/g.

Generally, chlorine treatment of seeds prior to germination and during sprouting (treatment on sprouts) has been demonstrated to have limited efficacy on pathogen reduction. The average microbial reduction by chlorine treatment was reported to be about 2.5 log CFU/g (Montville and Schaffner 2004), which is much lower than the 5-log reduction recommended by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF 1999). Moreover, there is a concern on the formation of chlorine chemical residues, which could adversely affect human health and the environment. For example, Li and others (1996) have demonstrated that certain organic substances in water can combine with chlorine to form potentially carcinogenic by-products such as trihalomethanes (THMs). In addition, treatment using 20000 ppm chlorine may impair the germination ability of seeds or quality of sprout products (Fransisca and others 2011).

In consideration of these limitations, studies have been done to explore other potential treatments, such as the use of electrolyzed water, ozonated water, and acid compounds, to improve the conventional decontamination procedures.

Acid compounds. The antimicrobial effect of organic acids (Table 5) has been attributed to the undissociated form of acid molecules that are able to move across the cell membrane and release a proton when they are inside cell cytoplasm. This would ultimately reduce the intracellular pH, which can lead to inactivation of intracellular enzymes, inhibition of nutrient transport, and elimination of the electrochemical proton gradient across the cell membrane resulting in depletion of the energy reservoir within the cell (Booth 1985). Hence, fruits and vegetables that possess large amounts of natural organic acids (such as acetic, benzoic, citric, and malic acids) would be more resistant to bacterial contamination.

Nei and others (2011) reported that treating radish and alfalfa seeds with 8.7% (v/v) gaseous acetic acid at 55 °C for 2 to 3 h resulted in a >5-log reduction in *E. coli* O157:H7 and *Salmonella* cell counts in both seed types, while a 48-h treatment reduced *E. coli* to undetectable levels in both seed types even after enrichment step. However, no treatment successfully eliminated *Salmonella* in treated seeds. The authors have also reported that this acetic acid treatment is more efficient than the recommended 20-min, 20000 ppm chlorine treatment, which only produced 2.0 to 3.0-log reduction for both species in both seed types. Singh and others (2005) showed that treatment using 5% (v/v) acetic acid for 30 min reduced the *S.* Typhimurium population by 7.8 log in cowpea seeds. This was found to be more efficient than the 1 h, 20000 ppm, chlorine treatment, which showed only a 3.5-log reduction in treated seeds. However, regardless of treatment, presence of pathogens was re-detected in sprouts grown from treated seeds. On the other hand, for cowpea sprouts inoculated with *S.* Typhimurium, treatment with 5% acetic acid

Table 4–Pathogen reduction by chlorine and chlorine dioxide interventions applied to sprouts and seeds.

Seed/sprout type	Treatment conditions	Pathogen	Log reduction (CFU/g)	Reference
Alfalfa seeds	Treat inoculated seeds with aqueous chlorine diosice (ClO_2) (10, 25, and 50 mg/L) for 3, 5, and 10 min at 120 rpm and at 21 °C	*E. coli* O157:H7	0.01 to 1.24	Singh and others (2003)
Wheat seeds and sprouts	Treat wheat seeds with sodium hypochlorite (NaOCl) (100, 200, and 400 ppm) at room temperature for 30 min, followed by rinse with tap water at approximately 10 °C for 3 min	*E. coli*	3.36 to 5.15	Tornuk and others (2011)
Radish seeds	Immerse the *E. coli*-inoculated seeds in ClO_2 (500 μg/mL, 5 min), followed by drying (45 °C, 23% RH, 24 h) and subsequently dry heating (70 °C, 23% RH, 48 h)	*E. coli* O157:H7	5.9	Bang and others (2011)
Radish seeds	Immerse inoculated seeds in sodium hypochlorite (NaOCl) (200 and 20000 ppm) solution for 20 min at room temperature with stirring (600 rpm), followed by rinse in distilled water for 1 h with stirring (600 rpm)	Nonpathogenic *E. coli* O157:H7 87–23	0.39 to 1.65	Fransisca and others (2011)
Alfalfa seeds	Treat inoculated seeds with chlorine solution (200 or 20000 ppm, as $Ca(OCl)_2$) at 22 °C for 15 or 30 min with shaking (100 rpm)	*Salmonella* *E. coli* O157:H7	2.3 to 2.5 2.0 to 2.1	Beuchat and others (2001)
Alfalfa seeds	Treat inoculated seeds with 20000 ppm $Ca(OCl)_2$ at room temperature for 10 min, followed by rinse with water Treated seeds were germinated and sprouts were sprayed with 100 ppm chlorine at 1 and 2-d of growth	*Salmonella*	4.98 (seed) approximately 1.5 (sprout)	Gandhi and Matthews (2003)
Alfalfa seeds	Treat inoculated seeds with 20000 ppm chlorine for 15 min at 25 °C	*E. coli* O157:H7	6.9	Lang and others (2000)
Alfalfa seeds	15 min of 20000 ppm $Ca(OCl)_2$ treatment on 5 kg inoculated seeds with agitation every 5 min (industry method), on 5 kg inoculated seeds in a commercial air washer (washer method), or on 25 g inoculated seeds with continuous shaking at 100 rpm (laboratory method)	*S.* Stanley	1.55 (industry) 1.13 (washer) 0.24 (laboratory)	Buchholz and Matthews (2010)
Alfalfa seeds	Treat inoculated seeds with 20000 ppm $Ca(OCl)_2$ for 20 min at room temperature at 600 rpm	*E. coli* O157:H7	4.41	Fransisca and others (2011)
Alfalfa seeds Radish seeds	Treat inoculated seeds with chlorine solution (200 and 20000 ppm, $Ca(ClO)_2$) for 20 min Treatment same as alfafa seeds	*E. coli* O157:H7; *Salmonella* *E. coli* O157:H7; *Salmonella*	0.7 (200 ppm); 2.7 (20000ppm); 1.5 (200 ppm); 2.7 (20000ppm); 1.3 (200 ppm); 2.0 (20000ppm); 1.4 (200 ppm); 2.1 (20000ppm)	Nei and others (2011)
Mungbean seeds	Treat inoculated seeds with 20000 ppm $Ca(ClO)_2$ at 28 °C for 20 min	*E. coli* O157:H7 *Salmonella*	1.14 0.53	Kumar and others (2006)
Cowpea seeds and sprouts	Treat inoculated seeds with 20000 ppm chlorine for 1 h, or treat inoculated sprouts with 20000 ppm chlorine for 15, 30, 45, 60, 90, 120, or 180 min, at 30 °C and 50 rpm	*S.* Typhimurium	3.5 (seed) 1.6 to 5.4 (sprout)	Singh and others (2005)
Broccoli sprouts	Treated inoculated sprouts with 50 ppm ClO_2, 0.5% fumaric acid (FA), and a combination of 0.5% fumaric acid followed by 50 ppm ClO_2 and (FA + ClO_2) for 5 min	*E. coli* O157:H7 *S.* Typhimurium *L. monocytogenes*	1.66 (ClO_2); 2.29 (FA); 2.39 (FA + ClO_2) 1.64 (ClO_2); 2.02 (FA); 2.74 (FA + ClO_2); 1.24 (ClO_2); 2.26 (FA); 2.65 (FA + ClO_2)	Kim and others (2009a)
Alfalfa sprouts	Treated inoculated sprouts with 50 ppm ClO_2, 0.5% fumaric acid (FA), and a combination of 0.5% fumaric acid followed by 50 ppm ClO_2 and (FA + ClO_2) for 1, 3, 5, and 10 min	*E. coli* O157:H7 *S.* Typhimurium *L. monocytogenes*	1.73 to 2.37 (ClO_2); 2.46 to 3.45 (FA); 2.99 to 4.06 (FA + ClO_2) 1.71 to 2.23 (ClO_2); 2.35 to 3.11 (FA); 2.63 to 3.57 (FA + ClO_2) 1.72 to 2.36 (ClO_2); 2.36 to 2.84 (FA); 2.44 to 3.69 (FA + ClO_2)	Kim and others (2009b)
Mungbean sprouts	Treat inoculated sprouts with 100 ppm chlorine dioxide (ClO_2) for 5 min at 22 °C	*S.* Typhimurium *L. monocytogenes*	3.0 1.5	Jin and Lee (2007)
Radish sprouts	Treat inoculated sprouts in NaOCl (100 ppm) solution at 500 rpm for 5 min	*E. coli* *Salmonella* spp.	2.79 2.86	Issa-Zacharia and others (2011)

Table 5–Pathogen reduction by organic acid antimicrobial interventions applied to sprouts and seeds.

Chemical agent	Seed/sprout type	Treatment conditions	Pathogen	Log reduction (CFU/g)	Reference
Acetic acid (AA)	Alfalfa seeds	Treat inoculated seeds with 8.7% gaseous AA at 55 °C for 1, 2, and 3 h	*E. coli* O157:H7 *Salmonella*	4.3 to 5.0; 2.5 to 5.1	Nei and others (2011)
	Radish seeds	Treatment same as alfalfa seeds	*E. coli* O157:H7 *Salmonella*	3.4 to 5.2; 3.5 to 5.1	
	Alfalfa seeds	Treat inoculated seeds with 5% AA for 10 min at 42 °C	*E. coli* O157:H7	2.4 (BHI agar) 6.3 (SMAC agar)	Lang and others (2000)
	Cowpea seeds and sprouts	Treat inoculated seeds with 5% AA for 30 min, or treat inoculated sprouts with 5% AA for 15, 30, 45, 60, 90, 120, or 180 min, at 30 °C and 50 rpm	*S.* Typhimurium	7.8 (seed) 2.6 to 7.5 (sprout)	Singh and others (2005)
Peroxyacetic acid (PAA)	Alfalfa seeds	Treat inoculated seeds with 1%, 2%, or 3% of PAA for 5, 10, 15, or 20 min, at room temperature (21 to 23 °C)	*Salmonella* *E. coli* O157:H7	>1 >1	Rajkowski and Ashurst (2009)
	Alfalfa seeds	15 min of 1% or 3% PAA treatment on 5 kg inoculated seeds with agitation every 5 min (industry method), on 5 kg inoculated seeds in a commercial air washer (washer method), or on 25 g inoculated seeds with continuous shaking at 100 rpm (laboratory method)	*S.* Stanley	1.14 (industry) 1.34 to 1.77 (washer) 0.56 to 0.66 (laboratory)	Buchholz and Matthews (2010)
Lactic acid (LA)	Alfalfa seeds	Treat inoculated seedss with 5% LA for 10 min at 42 °C	*E. coli* O157:H7	3.0 (BHI agar) 6.6 (SMAC agar)	Lang and others (2000)
Caprylic acid (CA) and monocaprylin (MC)	Alfalfa seeds	Treat inoculated seeds with CA or MC at 25, 50, and 75 mM concentrations for 30, 60, and 90 min at 4 °C	*E. coli* O157:H7 *Salmonella*	Maximum 1.56 Maximum 2.56	Chang and others (2010)
Phytic acid (PhA) and oxalic acid (OA)	Mung bean seeds Radish seeds	Treat inoculated seeds by dipping in hot (60, 70, and 75 °C) sanitizer (0.05% PhA or 3% OA) for 20 s, followed by dipping in chilled sanitizer (0 °C) for 20 s. A treatment cycle was defined as 20 s in hot sanitizer followed by 20 s in chilled sanitizer, and up to 5 cycles were performed	*E. coli* O157:H7	Maximum 3.0 (PhA, radish) Maximum 5.80 (OA, radish) Maximum 4.38 (PhA and OA, mung bean)	Bari and others (2009b)
Malic acid (MA)	Alfalfa seeds	Treat inoculated seeds using MA (0.1%, 0.5%, 1%, 2%, 4%, 6%, 8%, or 10%) in combination with thiamine dilauryl sulfate (TDS, 0.5% or 1%), at room temperature and 600 rpm for 20 min	*E. coli* O157:H7	2.48 to 4.41	Fransisca and others (2012)

was found to completely eliminate the pathogen within 1 h of soaking, whereas 20000 ppm chlorine treatment could not result in *Salmonella*-free sprouts even after 3 h of soaking. Rajkowski and Ashurst (2009) showed that a 10 to 20-min treatment of alfalfa seeds with 1% peroxyacetic acid (PAA) sanitizer resulted in a >1-log reduction in the level of inoculated *Salmonella* spp. and *E. coli* O157:H7. No additional benefits were observed at higher PAA concentrations (2% to 3%) and contact time did not significantly influence the sanitization results. Another study by Buchholz and Matthews (2010) had shown similar results. In the study, the use of 1% PAA (15 min) was found to be as effective as 20000 ppm $Ca(OCl)_2$, causing a maximum 1-log reduction in a *S.* Stanley population on treated alfalfa seeds, and no additional benefits were observed at 3% PAA concentration.

Similarly, testing on other organic acids generally revealed limited bactericidal efficiency. Lang and others (2000) reported that a treatment using 5% lactic acid (LA) for 10 min at 42 °C caused extensive cell injury of *E. coli* O157:H7 and resulted in no viable cells detected on SMAC agar. However, cell counts still increased to 7 to 8 log during sprouting. Chang and others (2010) tested the efficiency of caprylic acid (CA) and monocaprylin (MC) at 25 to 75 mM concentrations at 4 °C for 30 to 90 min on alfalfa seeds. They reported that a higher chemical concentration and longer treatment time enhanced microbial reduction. Overall, MC was consistently more effective than CA, but the bactericidal effect was limited for both antimicrobials, with maximum pathogen reduction of 1.56 log for *E. coli* O157:H7 and 2.56 log for *Salmonella* spp. (75 mM MC, 90 min). Bari and others (2009b) tested the combined effect of a hot (60 to 75 °C, 20 s)-and-chilling (0 °C, 20 s) cycle with phytic acid (0.05%) or oxalic acid (3%) on *E. coli* O157:H7 inoculated onto mung bean and radish seeds. The authors reported that 5 hot-and-chilling cycles at 75 °C with oxalic acid treatment reduced the pathogen to below the detection limit from initial cell levels of 4 to 5 log CFU/g in both seed types. However, regardless of the extent of reduction, viable cells could still be detected after enrichment. Moreover, although negative impact was found in treated mung bean seeds, significant reduction (>40%) in germination yield was observed in treated radish seeds. Fransisca and others (2012) studied the efficiency of 10% malic acid (MA) in combination with 1% thiamine dilauryl sulfate (TDS) on *E. coli* O157:H7 elimination from alfalfa seeds. The authors reported that 20 min of the 10% MA+1% TDS treatment reduced the pathogen level to below detection from an initial 4 log CFU/g inoculation level, which was similar to results achieved using 20000 ppm $Ca(OCl)_2$ for 20 min. However, surviving cells grew up to 7 to 8 log during sprouting of the treated cells.

Electrolyzed water. Electrolyzed oxidizing water is generated by electrolysis of a NaCl solution. Electrolyzed water can be divided into acidic and alkaline types, depending on the solution pH, and can have other different properties such as oxidation-reduction potential (ORP) and available chlorine concentration (ACC) (Liu and others 2011).

Acidic electrolyzed water (AEW) has been regarded as a novel antimicrobial agent in recent years (Table 6). AEW is characterized by its low pH value (<2.7), high ORP (>1000 mV), and it usually contains a chlorine concentration between 40 and 90 ppm (Zhang and others 2011). Compared with other chemical antimicrobial treatments, AEW has the advantage of having a broad antimicrobial spectrum and causing fewer environmental problems (since NaCl is the only chemical added during AEW production). The use of AEW has been demonstrated to have limited effectiveness in reducing an *E. coli* O157:H7 population in alfalfa seeds (0.22 to 1.56 log reduction) and sprouts (1.05 to 2.72 log reduction), which were treated in AEW (pH 2.6, ORP 1150 mV, ACC 50 ppm) with agitation for 2, 4, 8, 16, 32, and 64 min (Sharma and Demirci 2003). The reduction level is comparable with some treatments using 20000 ppm chlorine (Beuchat and others 2001; Fransisca and others 2011). However, the strongly acidic pH AEW might be detrimental to seeds and sprout viability. Mung bean sprouts have been reported to have a reduced growth rate after AEW treatment (pH 2), as compared to control (water) or other treatments using milder conditions (pH 4 to 6) (Liu and others 2011). Moreover, due to its strong acidity, Cl_2 gas dissolved in AEW solution can be rapidly lost due to volatilization, thus decreasing efficiency of the treatment solution over time, and may also adversely impact on human health and the environment (Len and others 2002). Bari and others (2009b) demonstrated that a combination of hot (70, 75 °C)-and-chilling (0 °C) cycles and electrolyzed acidic water was able to reduce inoculated *E. coli* O157:H7 to an undetectable level (>4 to 5 log reduction) in both mung bean and radish seeds. However, viable cells were still detected after enrichment, indicating the potential risk of bacterial growth during seed germination and sprouting.

Slightly acidic electrolyzed water (SAEW), with a milder pH of 5.0 to 6.5, has been reported to be a better alternative to AEW due to its high antimicrobial efficiency, greater stability, cheaper production cost, and less corrosive nature (less corrosion of processing equipment or irritation to hands) (Cui and others 2009). Zhang and others (2011) reported that treatment of mung bean seeds and sprouts with SAEW (pH 5.98 to 6.48, ACC 20 to 120 ppm) for 3 to 15 min resulted in significant microbial reduction in both *E. coli* O157:H7 (1.32 to 1.78 log reductions for seeds, 3.32 to 4.24 log reductions for sprouts) and *Salmonella* Enteritidis (1.27 to 1.76 log reductions for seeds, 3.12 to 4.19 log reductions for sprouts), without significantly affecting seed germination capacity (at ACC of 20 ppm). Issa-Zacharia and others (2011) showed that treating pathogen-inoculated radish sprouts in SAEW (pH 5.6 to 5.8, ACC 20 ppm) for 5 min significantly reduced cell counts of *E. coli* (2.85 log reduction) and *Salmonella* spp. (2.91 log reduction). The authors also reported that this sanitization effect was comparable to treatment using 100 ppm NaOCl and suggested that SAEW may be used as an alternative treatment, which requires less free chlorine, to NaOCl sanitization.

Other chemical treatments. In view of the limited treatment efficacy, some studies have been carried out to evaluate effects of other chemical treatments on pathogen reduction in seeds and sprouts (Table 6).

Compared to conventional chemical interventions, ozone treatment offers several advantages including efficient antimicrobial effect at relatively low concentration and within short contact time, spontaneous decomposition into nontoxic product (O_2), and leaving no safety concerns regarding consumption of chemical residues. Moreover, ozone treatment is a nonthermal process, making it particularly suitable for the decontamination of sensitive products such as sprouts (Kim and others 1999). The use of ozone in gaseous or aqueous form during treatment, storage, and processing of foods has been approved by the U.S. Food and Drug Administration (Code of Federal Regulations Title 21 2011a). Singla and others (2011) demonstrated that the complete immersion of sprouts in 2 ppm ozonated water or spraying 2 ppm ozonated water on sprouts for 5 min. The treated samples stored at 28 °C for 10 d had 1 to 1.5 log and 0.9 to 1.8 log reductions

Table 6–Pathogen reduction by other chemical interventions (acidic electrolyzed water, ozone, SOC sanitizer, and irradiation) applied to sprouts and seeds.

Chemical agent	Seed/Sprout type	Treatment conditions	Pathogen	Log reduction (CFU/g)	Reference
Acidic electrolyzed water (AEW)	Alfalfa seeds and sprouts	Treat inoculated seeds or inoculated sprouts with AEW (pH 2.6, ORP 1150 mV, 50 ppm free chlorine) for 2, 4, 8, 16, 32, and 64 min with agitation	*E. coli* O157:H7	0.22 to 1.56 (seeds) 1.05 to 2.72 (sprouts)	Sharma and Demirci (2003)
	Mung bean seeds Radish seeds	Treat inoculated seeds by dipping in hot (60, 70, and 75 °C) AEW for 20 s, followed by dipping in chilled AEW (0 °C) for 20 s. A treatment cycle was defined as 20 s in hot sanitizer followed by 20 s in chilled sanitizer, and up to 5 cycles were performed	*E. coli* O157:H7	Maximum 5.80 (radish)/maximum 4.38 (mung bean)	Bari and others (2009b)
Slightly acidic electrolyzed water (SAEW)	Mungbean seeds and sprouts	Treat inoculated seeds with SAEW (pH 5.98 to 6.48, ORP 817.5 to 893.8, 20, 40, 60, or 80 ppm free chlorine) at room temperature (20 °C) for 3, 5, 10, and 15 min Treat inoculated sprouts with SAEW (pH 5.98 to 6.48, ORP 817.5 to 893.8, 20, 40, 80, or 120 ppm free chlorine) at room temperature (20 °C) for 3, 5, 10, and 15 min	*E. coli* O157:H7 *Salmonella* Enteritidis	1.32 to 1.78 (seeds); 3.32 to 4.24 (sprouts); 1.27 to 1.76 (seeds); 3.12 to 4.19 (sprouts)	Zhang and others (2011)
	Radish sprouts	Treat inoculated sprouts in SAEW (pH 5.6 to 5.8, 20 ppm free chlorine) at 500 rpm for 5 min	*E. coli* *Salmonella* spp.	2.85/2.91	Issa-Zacharia and others (2011)
Ozonated water	Radish sprouts and mung bean sprouts	Immerse or spray inoculated sprouts with 2 ppm ozonated water for 5 min	*Shigella*	1.5 (immersion);1 (spray)/and 1.8 (immersion); 0.9 (spray)	Singla and others (2011)
	Alfalfa sprouts	Treat inoculated sprouts in water containing 21 ppm ozone for 2, 4, 8, 16, 32, or 64 min at 4 °C, or treat with continuous ozone sparging with and without pressurization (12 psi, 5 min)	*E. coli* O157:H7	0.85 (ozone water)/0.83 to 2.20 (ozone sparging)	Sharma and others (2003)
	Alfalfa seeds	Treat inoculated seeds with gaseous ozone (20 psi ozone pressure, 100% ozone, 100% RH) at 25 °C for 24 h	*Salmonella* spp.	1.5	Rajkowski and Ashurst (2009)
SOC sanitizer	Mungbean seeds	Treat inoculated seeds with 50, 100, 150, and 200 ppm SOC sanitizer at 28 °C for 20 min	*E. coli* O157:H7 *Salmonella*	0.03 to 9.12 0.23 to 9.10	Kumar and others (2006)

in *Shigella* cell counts on radish sprouts and mung bean sprouts, respectively, though a more efficient antimicrobial effect was achieved using 2% malic acid. Combination of 2% malic acid with 2 ppm ozonated water offered the best protection (greatest cell count reduction) on the 2 sprout products without significantly altering their antioxidant status in terms of polyphenols, flavonoids, reducing power, and free radical scavenging activities against DPPH and ABTS. The results suggested a potential commercially applicable intervention strategy for the control of *Shigella* spp. in raw sprouts. In contrast, Sharma and others (2003) found that, regardless of treatment time (2 to 64 min), immersing alfalfa sprouts inoculated with a 5-strain cocktail of *E. coli* O157:H7 in 21 ppm ozonated water did not significantly reduce cell counts as compared to the control. The lack of effect might be attributed to the rapid decrease in ozone concentration upon contact with the sprout surface. Continuous ozone sparging with and without 12 psi hydrostatic pressure, which replenished ozone and increased accessibility of ozone to areas of sprouts where *E. coli* O157:H7 may be hidden, resulted in significantly greater reductions of 0.83 to 2.20 log CFU/g within 4 to 64 min treatment time and caused no detrimental effect on the visible quality of the sprout samples. Moreover, Rajkowski and Ashurst (2009) reported that gamma- and e-beam-irradiation treatment (1 kGy) only reduced *Salmonella* spp. by 1.2 log CFU/g in alfalfa seeds, while a 24-h gaseous ozone treatment (20 psi ozone pressure, 100% ozone and RH) only achieved a 1.5-log reduction.

The use of stabilized oxychloro (SOC) sanitizer has offered some promising results. Hora and others (2007) showed that the use of 200 ppm SOC sanitizer at a seed : sanitizer ratio of 1:5 for 24 h at 28 °C inactivated *E. coli* O157:H7 and *Salmonella* that were naturally present or artificially introduced into mung bean seeds. Kumar and others (2006) treated mung bean seeds with SOC sanitizer (50 to 200 ppm), at 28 °C for 20 min, and reported that *Salmonella* and *E. coli* O157:H7 were completely eliminated at 150 ppm and 50 ppm SOC concentrations, respectively. Germination rate was not affected at tested SOC concentrations and the resulting sprouts were found to be pathogen-free up to 96 h of sprouting. The method was found to be more effective than a 20000 ppm $Ca(OCl)_2$ treatment for 20 min, which failed to eliminate pathogens from seeds and resulted in high (8 log CFU/g) cell counts in the final sprouts. The authors have reported that sanitization efficiency was dependent on treatment time (>8 h optimum) and seed:sanitizer ratio (>1:4 optimal).

Conclusion

The number of sprout-related outbreaks has been on the rise and contamination occurs via preharvest or postharvest sources. In particular, foodborne pathogens such as *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and *B. cereus* have been associated with sprout contaminations. There have been various physical, biological, and chemical intervention technologies studied in an attempt to eliminate or reduce the number of pathogens on seeds or sprouts. Based on the reported data, physical intervention methods such as heat and high hydrostatic pressure are more effective in reducing microbial populations. Chemical interventions were found to have limitations in reducing microbial load. Hurdle technology, which combines the use of these methods, should be utilized to bring about a more effective decontamination of seed and sprout samples.

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