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MINI-REVIEW
Potential for Dry Thermal Treatments to Eliminate Foodborne Pathogens on Sprout Seeds

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ABSTRACT

Consumption of raw sprouts have been associated with several outbreaks of foodborne diseases. Contaminated seeds used to produce sprouts are the main source of pathogenic microorganisms that multiply during the sprouting process, which is favored by high moisture contents and temperatures in the optimal range for microbial growth. The current intervention recommended by the Food and Drug Administration to decrease seeds' microbial load is the use of 20,000-ppm calcium hypochlorite, which produces a reduction of around 3-logarithmic cycles for *Escherichia coli* and *Salmonella*. Therefore, there is a need for new procedures to further reduce or eliminate microorganisms in seeds used for the preparation of sprouts. One potential treatment is the application of dry thermal treatments which have been used for decades to reduce plant pathogens from seeds to eliminate pathogenic *E. coli* and *Salmonella* in seeds used for sprout production while preserving seed vigor and viability. This review will discuss the potential for dry heat treatment of seeds from the *Brassicaceae* and *Leguminosae* families to reduce contaminated pathogenic microorganisms.

Keywords: Pathogens, dry heat, sprout seeds

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INTRODUCTION

oodborne disease outbreaks associated with vegetables and vegetable processing continue to be one of major sources of public health and economic concerns associated with food systems (Hedberg *et al.*, 1999; Sewell and Farber, 2001; Sivavapalasingham *et al.*, 2004; Hanning *et al.*, 2008; 2009). Although most of the primary bacterial pathogens and viruses responsible for the majority of the foodborne illnesses have been historically identified with a particular animal food source, such as poultry for *Salmonella* and *Campylobacter* (Ricke, 2003b; Park *et al.*, 2008; Dunkley *et al.*, 2009; Horrocks *et al.*, 2009; Foley *et al.*, 2011; Finstad *et al.*, 2012; Ricke *et al*, 2013), beef for pathogenic *E. coli* (Anderson *et al.*, 2009; Callaway *et al.*, 2013), and retail deli meats for

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Listeria (Lungu *et al*., 2010; Crandall *et al*., 2011; Milillo *et al*, 2012) most of these same pathogens can contaminate vegetable crops as well. The fact that many vegetable products are consumed raw further increases the risk as pathogens are capable of growing on these raw products (Escartin *et al*., 1989; Asplund and Nurmi, 1991; Abdul-Raouf *et al*., 1993; Hedberg *et al*., 1999; Nutt *et al*., 2003a,b). It has also been shown that supernatants derived from centrifugation of some raw vegetables after mechanical stomaching will not only support growth but increase virulence in pathogens such as *Salmonella* (2003a,b; 2004).

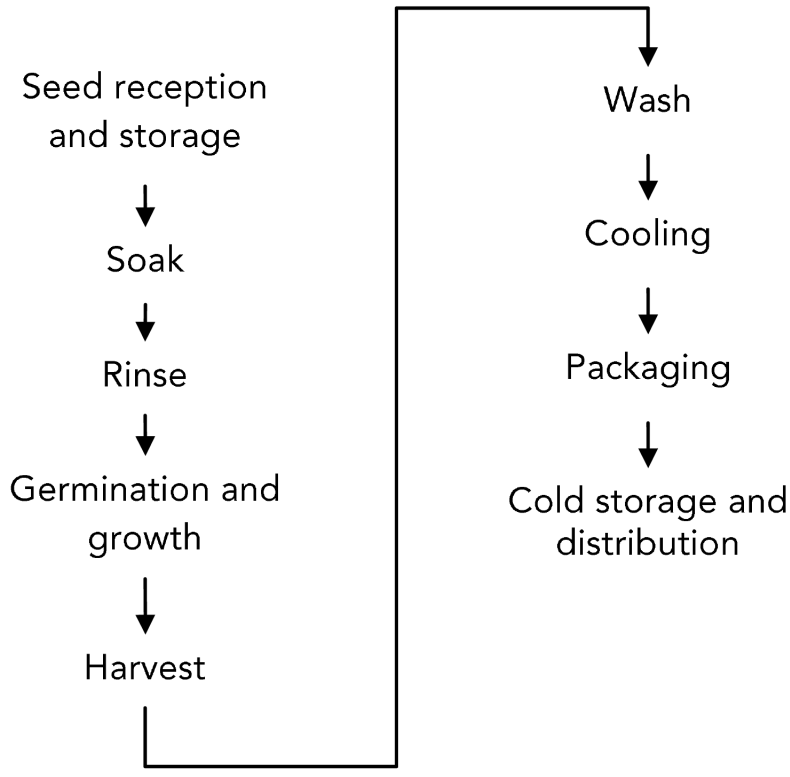
Part of the difficulty in assessing the impact of potential foodborne pathogen contamination is the multitude of routes and sources that these pathogens can originate from at the production as well as the retail side (Sagoo *et al*., 2003; Gibson and Ricke, 2012; Neal *et al*., 2012b). Routes and sources of contamination include manure used to fertilize fields, aerosols from contaminated wastes, contaminated irrigation water, contaminated wash water used during processing, and sick humans, who handle the produce, just to name the more extensively studied sources (Beuchat and Ryu, 1997; Pillai and Ricke, 2002; Islam *et al*., 2004; Fonseca and Ravishankar, 2007; Jay *et al*., 2007). Raw sprouts represent one aspect of vegetable commodity production that has proven to be somewhat difficult to construct consistently effective and comprehensive food safety protocols for their application during production. Sprouts are produced from seeds that are germinated in high moisture environments at temperatures that are optimal for the development of pathogenic bacteria. Contaminated seeds are the main source of microorganisms during sprouts production, and certainly, the fact that sprouts can be consumed raw is a primary issue that promotes food borne illnesses. However, there are limited interventions available that have been shown effective to eliminate pathogens while retaining seeds vigor and germination rate. This review will offer discussion on raw sprouts as a source of foodborne pathogens, current control measures, and the potential for dry heat as an alternative treatment of seeds.

SPROUT PRODUCTION AND FOODBORNE DISEASE

Sprouts are primarily consumed raw in the United States and are derived from numerous types of seeds, including beans, radishes, and alfalfa. Nutritionally, they are considered a good source of amino acids, oligopeptides, fiber, vitamins, trace elements and minerals as well as phytochemicals with purported health benefits (Marton, *et al*., 2010). In the United States, sprouts are produced commercially and by individuals using home sprouters. As the popularity of sprouts has increased, the outbreaks of foodborne illness related to pathogenic microorganisms have presented significant challenges to the sprout industry. Recommendations were introduced in 1999 by the Food and Drug Administration to treat sprout seeds with bleach (calcium hypochlorite) to reduce pathogenic loads; however, the recommended treatment has limited effectiveness (Brooks *et al*,. 2001; Fett, 2002; Montville and Schaffner, 2004).

In addition, gastrointestinal illnesses from the consumption of raw sprouts continue. The Centers for Disease Control and Prevention (2012) reported a very recent multi-state outbreak of a Shiga-toxin producing strain of *Escherichia coli* (STEC) associated with clover sprouts with a hospitalization rate of more than 25% of those reportedly affected. This most recent outbreak did not result in any known cases of hemolytic uremic syndrome (HUS) or deaths. However, another strain of STEC on fenugreek sprouts in Germany (in 2011) was responsible for approximately 50 deaths and 850 cases of HUS. The increasing popularity of sprouts and the sprouting conditions that permit a single pathogenic cell to grow to an infective dose is very problematic, particularly for immune-compromised individuals that are highly susceptible to severe complications from foodborne illness. There is no single treatment method, including the Food and Drug Administration -recommended chemical treatment that completely destroys all *Salmonella* and *E. coli* on contaminated seeds (Montville and Schaffner, 2005) therefore, the purpose of this review is to discuss safe, non-chemical treatments of sprout seeds that could be made commer-

Figure 1. Sprout Production Process (Adapted from NACMCF, 1999)



cially-feasible and would potentially alleviate health risks associated with sprout consumption.

PATHOGENIC BACTERIA ON SPROUTS

Although sprout-related illnesses are predominantly attributed to *Salmonella* spp. and enterohemorrhagic *E. coli* (serotype O157:H7), there have been outbreaks involving other STECs, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Aeromonas hydrophila* (Bari *et al.*, 2010). The seeds are considered the primary source of contamination with exponential growth of the microorganisms throughout sprouting due to the conditions (temperature and moisture) maintained during the sprouting process (Figure 1), which can permit a single viable pathogen cell to grow to an infective dose of 2 to 3 log colony forming units (CFU)/g in only 2 days (Hu *et al.*, 2004). Although contamination during sprouting, irrigation, and post-harvesting is possible, the majority of cases of foodborne-illness are attributed to contaminated seeds which is likely from the use of contaminated irrigation water or fertilizer, fecal contamination from animals (residing in neighboring fields or wild animals with access to the seed fields), or inadequate hygiene practices during seed collection (National Advisory Committee on Microbiological Criteria for Foods (NACMCF) 1999). Additionally, treatment of the sprouts after germination is impractical due to the delicate nature of the sprouts. Treatment of seeds is considered more effective due to the lower microbial load and potential problems with bacteria located within the sprout tissues (Penas *et al.*, 2010) therefore elimination of the pathogens on the seed and the maintenance of sterilized conditions for growth are the most favorable preventative methods.

Table 1. Classifications and common names of sprout seeds (Adapted from Sproutpeople, n.d.)

Family	Common Names
Amaranthaceae	Amaranth
Amaryllidaceae	Garlic chive, leek, onion
Brassicaceae/Cruciferae	Broccoli, cabbage, mustard, tatsoi, arugula, mizuna, garden cress, radish
Chenopodiaceae	Quinoa
Compositae	Sunflower
Cucurbitaceae	Pumpkin
Gramineae/Poaceae	Oats, barley, millet, rye, wheat, spelt, triticale, corn
Leguminosae	Peanut, garbanzo bean, soybean, lentil, alfalfa, black bean, pinto bean, pea, clover, fenugreek, adzuki bean, mung bean
Linaceae	Flax
Pedaliaceae	Sesame
Polygonacea	Buckwheat
Rosaceae	Almond
Umbelliferae	Celery, dill

SOURCES OF SPROUTS

Sprouts are derived from a wide variety of seeds including grains, beans, nuts, and various brassica vegetables (Table 1). Although alfalfa and mung bean sprouts are among the more well-known varieties, there are numerous other legumes, such as peanuts, soybeans, lentils, and peas that are consumed as sprouts (Sproutpeople, n.d.). Other seed sources are eclectic and range from onion to almond to sunflower; thus, sprout consumption is diverse which generates additional challenges in seed treatments since some seeds may be more susceptible to certain treatments. For example, mung bean seed coats are tough and considered relatively heat resistant thus less affected by heat treatment than other seeds (Bari *et al*., 2010). Consequently, germination rates of treated seeds should be evaluated on a case-by-case basis (Bari *et al*., 2010).

CURRENT ANTIMICROBIAL TREATMENTS

The current recommendations by the Food and Drug Administration for reducing pathogenic loads on sprouts is to treat the seeds (prior to sprouting) with 20,000 ppm $Ca(OCl)_2$ or other approved antimicrobial agents (Food and Drug Administration, 1999). The recommended treatment does not completely eliminate the pathogenic load with a mean reduction of only 2.81 log CFU/g and 3.21 log CFU/g for *E. coli* and *Salmonella*, respectively (Fett, 2002; Food and Drug Administration, 1999; Montville, and Schaffner 2004). Based on a case study of an outbreak of

Salmonella Typhimurium on treated and untreated sprout seeds, Brooks *et al*. (2001) concluded that the risk of infection is only reduced but not completely eliminated with the Food and Drug Administration -recommended treatment. In addition, there is significant waste generated with chlorinated water posing ecological and economic challenges for sprout producers (Bari *et al*., 2010). Lastly, the majority of raw sprout consumers are demographically classified as health-conscience individuals that are generally averse to chemically-treated products; thus, there is significant interest in effective, safe, and natural approaches to the removal of *E. coli* and *Salmonella*.

Since simply washing the seeds or the sprouts with sterilized water is insufficient to remove the pathogens, antimicrobial treatment is the only mechanism to reduce the microbial load. Various physical and chemical treatments have been applied to seeds to enhance the chlorine efficacy, reduce the effective chlorine dose, or to provide an alternative to the use of chlorine altogether, but the results have been inconsistent. This may be partly attributed to bacteria embedded deep in crevices, naturally occurring on the surface of certain seeds or generated by damage during handling that are not readily removed with traditional antimicrobial washes (Takeuchi and Frank, 2000; 2001; Enomoto *et al*. 2002; Solomon *et al*., 2002; Takeuchi *et al*., 2002; Wachtel *et al*., 2002; Feng *et al*., 2007; Brandl, 2008; Gomes *et al*., 2009; Kroupitski *et al*., 2009; Erickson, 2012; Neal *et al*., 2012a). Gandhi *et al*., (2001) used a green fluorescent protein expressing *Salmonella* Stanley to demonstrate this microorganism's ability to penetrate alfalfa sprout tissue. In addition, extreme conditions can dramatically reduce seed viability so germination rates limit the extent of treatment. Sodium bicarbonate, trisodium phosphate, acetic acid, hydrogen peroxide, ethanol, commercial peroxyacetate solutions, and acidic electrolyzed water (a solution comprised of less than 80 mg/L free chlorine with a high oxidation-reduction potential and a pH range of 2.3 to 2.7) are some of the chemical treatments evaluated over the past decade with varying results but no single method is able to achieve the Food and Drug Administration recommended 5-logrithmic reduction of all pathogens (Kim *et al*., 2006, Nei, *et al*. 2011, Pandrangi *et al*., 2003). There are other potential chemical antimicrobial treatments that have been evaluated in other food model systems or against pure cultures (Aldrich *et al*., 2011; Ganesh *et al*., 2012; Muralli *et al*., 2012; Neal *et al*., 2012c). Irradiation, ultrasound, and pressure have also been examined under a variety of conditions for various food systems but were generally inadequate methods to eradicate inoculated pathogens without combining with other treatments (Penas *et al*., 2010; Kim *et al*., 2006; O'Bryan *et al*., 2008).

Overall, combining hurdles (or treatments), often a physical and a chemical treatment, is considered more effective than isolated treatments; however, although combinatorial methods can dramatically reduce microbial loads, most are incapable of fully eliminating *Salmonella* and *E. coli* (Penas *et al*., 2010; Beuchat and Scouten, 2002; Ricke, 2003b; Ricke *et al*., 2005; Sirsat *et al*., 2009). Furthermore, selection of antimicrobials must proceed with caution as there is potential for cross protection among different antimicrobials which renders the combinations less effective (Kwon *et al*., 2000; Sirsat *et al*., 2010). This has led to the concept of using genomic screening tools to better predict when cross protection might occur by identifying which gene(s) or gene families are shared for the respective microorganism to successfully resist multiple antimicrobials (Sirsat *et al*., 2010). Genomic analysis based on transcriptome microarrays have been applied to assess potential genetic responses in foodborne pathogens such as *Salmonella* and *Listeria* to either external antimicrobial combinations or intrinsic food properties generated during food processing (Milillo *et al*., 2011, 2012; Sirsat *et al*., 2011; Chalova *et al*., 2012). Combination treatments are also generally cumbersome and, in some cases, expensive so practical usage would be very limited.

THERMAL ANTIMICROBIAL TREATMENTS

The application of heat (also known as "thermotherapy") to eradicate various phytopathogens from seeds is an agronomic practice that has existed for

Table 2. Treatment conditions used in various studies to determine the most effective hot water treatment of seeds

Temp (°C)	Time	References
55	2 d, 5 d, 10 d, 15 d, 20 d	Feng *et al*., 2007; Beuchat and Scouten, 2002; Neetoo and Chen, 2011; Hu *et al*., 2004
60	24 h, 4 d, 8 d, 12 d, 15 d	Neetoo and Chen, 2011; Bang *et al.,* 2011
65	12 h, 24 h, 3 d, 6 d, 12 d	Neetoo and Chen, 2011
70	2 h, 5 h, 10 h, 15 h, 24 h	Neetoo and Chen, 2011; Bang *et al.,* 2011
75	15 min, 60 min, 3 h, 6 h, 12 h	Beuchat and Scouten, 2002; Neetoo and Chen, 2011
80	10 min, 60 min, 2 h, 4 h, 8 h	Beuchat and Scouten, 2002; Neetoo and Chen, 2011

over a century and is used to reduce the losses attributed to diseased plants (Baker, 1962). The basic premise is that the seed can withstand slightly greater thermal treatments than the host pathogens (fungal, bacterial, or viral) thereby eliminating pathogens but retaining germination capabilities of the seed (Jensen, 1888; Grondeau and Samson, 1994). In modern horticulture, treatment of seeds by hot water, dry heat, and hot, moist air is still a practical, ecological alternative to chemical treatments (Gilbert, 2009; Forsberg *et al*., 2005; Miller and Lewis-Ivey, 2005). The use of prolonged dry heat as opposed to hot water or steam to reduce plant pathogen loads has been primarily used over the last 30 years (Luthra, 1953). However, in India, the practice of controlling loose smut (a fungal infection) in wheat by exposure to solar radiation was successfully implemented as early as 1929 (Luthra, 1953). In spite of the lengthier treatment times, the application of dry heat generally causes less damage to the seed than hot water (Grondeau and Samson, 1994; Feng *et al*., 2007).

Since thermotherapy of all types is still used in horticulture to control plant pathogens, it is reasonable that heat treatment of sprout seeds to kill bacteria that are pathogenic to humans is a viable and acceptable alternative to chemicals. Although, outbreaks are not as common in Japan since most sprouts are consumed in cooked dishes hot water treatments of sprout seeds is a relatively common practice in Japan (Bari *et al*., 2010). However, in the United States, sprouts are primarily consumed raw so the efficacy of the method to eliminate pathogenic microorganisms remains under scrutiny (Bari *et al*., 2010). Although several studies have evaluated hot water treatment of seeds as a solitary treatment or in combination with other hurdles (Table 2) (Bari *et al*., 2010; Enomoto *et al*., 2002; Kim *et al*., 2006) there is still limited and conflicting data regarding the use of dry heat on sprout seeds. One of the initial studies demonstrated that dry heat treatment was a highly promising technique for mung bean seeds (Hu *et al*., 2004). Even three days post-sprouting, the authors demonstrated that the levels of inoculated *E. coli* O157:H7 and *Salmonella* remained non-detectible if seeds were treated at 55°C for four and five days, respectively.

Although studies that have attempted to treat alfalfa seeds with dry heat have been confounded by the thermostability of *Salmonella*, there is evidence that the method is still a viable option. Neetoo and Chen (2011) were able to successfully reduce both pathogens to non-detectible levels by treating the seeds at 65°C for 10 days while maintaining germination rates greater than 90%. The lengthy treatment was necessary due to the heat tolerance of *Salmonella*; *E. coli* was eliminated after only 2 days. In contrast to this response, Feng *et al*. (2007) demonstrated that holding alfalfa seeds at 55°C for eight days was insufficient to eliminate *Salmonella* with exponential growth of the pathogen observed during the three-day sprouting period. The same study demonstrated, however, that a six day treatment at 55°C very effectively controlled *E. coli*. The differences in the results of the two studies are likely attributable to the extent of heat treatment (55°C for eight days versus 65°C for 10 days); thus, further studies are warranted to determine optimum conditions. Additionally, there was no evidence presented by Neetoo and Chen (2011) that damaged bacteria would not recover and still grow on the sprouts since they only evaluated the pathogenic load of the seeds. Interestingly, a recent study by Bang *et al*. (2011) demonstrated that humidity control (i.e. RH of 23%) during heating can also permit longer heating times and higher temperatures with minimal effects on germination; therefore, the implementation of humidity control during dry heating may ensure complete elimination of the more recalcitrant bacteria.

Although the use of dry heat alone as a means to control bacterial pathogens has not been extensively evaluated, dry heat has been combined with several different treatments with relatively good outcomes including various chemical (sodium hypochlorite, chlorine dioxide, ethanol, phytic acid, oxalic acid) and physical (radiation, pressure) hurdles (Bang *et al*, 2011; Kim *et al*, 2010; Neetoo and Chen, 2011; Bari *et al*, 2009). In general, the primary interest in using combinatorial techniques is to reduce the length of time necessary to treat the seeds with the dry heat. For example, although Neetoo and Chen (2011) were able to control pathogens with dry heat alone, they also demonstrated that high hydrostatic pressure applied after heat treatment reduced the effective dry heat application time from 10 days to 12 hours. Although the combinatorial technique was effective, the need to implement pressure treatments would be significantly more laborious for seed farmers and would require additional equipment and supplies. Ideally, dry heat treatments alone would be a preferred method, but the efficacy of treatments needs to be systematically evaluated and optimized for each seed type to reduce losses in germination and retain non-detectible levels of pathogens in sprouts.

CONCLUSIONS

Sprouts continue to be a source for foodborne disease outbreaks when consumed raw. Microbial contamination including foodborne pathogens occurs early in sprout production primarily via contaminated seeds which as they sprout, support microbial growth due to the favorable moisture and temperature conditions. To reduce microbial levels on seed the Food and Drug Administration recommends application of 20,000-ppm calcium hypochlorite that will lead to a 3-logarithmic reduction of *Escherichia coli* and *Salmonella*. To further reduce or eliminate microorganisms and foodborne pathogens in seeds that serve as a source of raw sprouts for human consumption will require interventions that are more effective. Dry thermal treatments have been used for decades to reduce plant pathogens from seeds and offer a potential treatment to eliminate pathogenic *E. coli* and *Salmonella* in seeds. However, preservation of seed vigor and viability must be retained and unfortunately some strains of foodborne pathogens can be somewhat heat resistant. Overcoming this will probably require combining dry heat treatment with some additional antimicrobial treatments to achieve synergism in the form of a multiple hurdle intervention approach and thus a more effective reduction in foodborne pathogen levels. Designing optimal multiple hurdle intervention strategies will require not only testing under conditions similar to

the seed environment that the foodborne pathogen is associated with but a better understanding of the biology and genetic responses of the microorganism in the presence of the antimicrobials being used as potential interventions.

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