ELIMINATING *SALMONELLA ENTERICA* IN ALFALFA AND MUNG BEAN SPROUTS BY ORGANIC ACID AND HOT WATER IMMERSIONS

S. PAO[1], A. KALANTARI and M.F. KHALID

Virginia State University
Agricultural Research Station
Petersburg, VA 23806

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ABSTRACT

This study evaluated the efficacy of acid and heat treatments for eliminating Salmonella enterica *in sprouts grown from inoculated seeds*. Salmonella *was detected at 7.6 and 6.9 log cfu/g, respectively, in alfalfa and mung bean sprouts after germination. Immersing the alfalfa and mung bean sprouts, respectively, for 24 and 48 h in 2% acetic acid eliminated* Salmonella *(<1 cell/25 g). In 5% acetic acid, the elimination of* Salmonella *in alfalfa and mung bean sprouts was achieved after 4 and 16 h, respectively. However, similar treatments by citric acid were ineffective. Dipping alfalfa sprouts in hot water at 70, 80, 90 and 100C, respectively, for 10, 5, 3 and 3 s eliminated* Salmonella. *For mung bean sprouts, the elimination was observed at 70 or 80C for 20 s, 90C for 10 s, or 100C for 5 s. This study demonstrated that acetic acid and hot water treatments can be effective in inactivating* Salmonella *for sprout safety.*

PRACTICAL APPLICATIONS

This study showed that *Salmonella* can proliferate in sprouting systems during seed germination despite constant water change. Consumers, when uncertain with the microbial safety of home-grown or purchased raw sprouts, should apply adequate intervention treatments prior to consumption. Soaking raw sprouts in acetic acid solutions may help to reduce *Salmonella* contamination. However, extended treatment times are required. Heat treatment is traditionally used in preparing raw sprouts for Asian cuisines. Our study demonstrates that this practice is very effective for ensuring sprout

[1] Corresponding author. TEL: 804-524-6715; FAX: 804-524-5186; EMAIL: spao@vsu.edu

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safety. The data should be considered for use in consumer and extension services.

INTRODUCTION

Raw seed sprouts have caused numerous foodborne disease outbreaks in recent years (Mohle-Boetani *et al.* 2001; Liu and Schaffner 2006). In many of these outbreaks, contaminated seeds were implicated as the source of bacterial pathogens that multiplied during the sprouting process. Between 1990 and 2005, at least 27 salmonellosis outbreaks were linked to the consumption of alfalfa and mung bean sprouts in North America (Taormina *et al.* 1999; Powell 2005).

A number of seed sanitizing methods including hydrogen peroxide, sodium hypochlorite, calcium hypochlorite, ethanol and organic acids have been tested for their effectiveness in killing pathogens on seeds prior to sprouting (Delaquis *et al.* 1999; Sharma *et al.* 2002). Chlorine treatments in particular have been recommended for treating seeds to enhance the safety of commercially produced sprouts (Beuchat *et al.* 2001; Brooks *et al.* 2001; Proctor *et al.* 2001; Gill *et al.* 2003). Other treatments including heat, ozone and irradiation have been shown to decrease microbial populations on seeds, but no single treatment has been demonstrated to be effective in eliminating pathogens without significantly reducing germination and sprout yield (Taormina *et al.* 1999; Rajkowski *et al.* 2003; Liu and Schaffner 2006).

Since *Salmonella* and other pathogens present on seeds can become internalized during sprouting, subsequent biocidal washing is considered ineffective in sprout disinfection (Warriner *et al.* 2003). A recent report by Singh *et al.* (2005) indicates that *S.* Typhimurium on surface-inoculated cowpea sprouts, but not sprouts grown from contaminated seeds, can be eliminated by treating the sprouts with vinegar for extended periods of time. Also, consumers have been advised to cook sprouts before eating to reduce the risk of foodborne illness (FDA 1999). However, data on required cooking time and temperature are not available. In this study, we evaluated various acid and heat treatments in eliminating *Salmonella enterica* from alfalfa and mung bean sprouts grown from artificially contaminated seeds.

MATERIALS AND METHODS

Inoculum Preparation

Four *S. enterica* cultures (*S.* Enteritidis ATCC 13076, *S.* Montevideo ATCC 8387, *S.* Newport ATCC6962, and *S.* Typhimurium ATCC14028) were

maintained at 4C on tryptic soy agar (TSA) (unless otherwise stated, all media were from Biotrace International, Bothell, WA). The cultures were transferred to tryptic soy broth and incubated at 36C for 24 h, followed by spreading onto TSA (0.2 mL/plate) and incubated at 36C for 24 h to produce bacterial lawns. The lawns were pooled with sterile swabs and blended by a laboratory blender (IUL Instruments, Barcelona, Spain) with 0.1% peptone water to prepare the inoculum for immediate seed inoculation (Pao *et al.* 2004).

Seed Inoculation

Alfalfa seeds (*Medicago sativa* L.) and mung beans (*Phaseolus aureus* Roxb.) were obtained from sprouting seed companies and stored at 4C. Before inoculation, seeds were warmed to room temperature (24C). The seeds were then soaked in inoculum with gentle agitation for ~1 min to allow thorough wetting. The inoculated seeds were evenly distributed on weighing dishes and air-dried for 2 h at 25C in a forced air chamber (Model 680A, Lab Line, Dubuque, IA) then left at room temperature for another 22 h before sprouting.

Sprouting Procedures

An automatic sprouter (Freshlife Model 2000, Tribest, Santa Fe Springs, CA) was disassembled and sanitized with 80C hot water. After reassembling, inoculated alfalfa or mung bean seeds (150 g) were placed on the sprouter tray within the sprouter barrel and the water barrel below was filled with approximately 2.5 L of sterilized tap water. The sprouting system used water recycled from the barrels to intermittently sprinkle the sprouter tray for 4 days at 25C. Water in the barrel was replaced daily. Germinated sprouts samples were taken from the sprouter for decontamination treatments.

Sprout Treatments

For acid treatments, acetic and citric acids (Fisher Scientific, Fair lawn, NJ) were diluted by weight to 5% and 2% using sterile deionized water at 24C. Approximately 25 g of alfalfa or mung bean sprouts were placed in a 150-mL sterile bottle. Then, the bottle was filled with either acetic or citric acid solution at 0, 2 or 5% to submerge the sprouts for up to 48 h. Gentle agitation was given periodically to release trapped air in sprouts. For thermal treatments, alfalfa or mung bean sprouts (25 g) were immersed in hot water for up to 30 s at 70, 80, or 90C in a circulator (Ecoline RE120, Brinkmann, Westbury, NY) or at 100C in a glass beaker.

Microbial Testing

For *Salmonella* enumeration, each seed or sprout sample (10 g) was macerated and diluted with 0.1% peptone water. Appropriated sample

dilutions were spread onto XLD (xylose-lysine-deoxycholate) agar plates and incubated for 48 h at 36C before typical colonies were counted (Pao *et al.* 2004). For *Salmonella* detection, each sprout sample (25 g) was macerated with lactose broth (225 mL) for pre-enrichment. Before incubation, the pH of each sample broth was measured by indicator strips (ColorpHast, EMD, Gibbstown, NJ) and neutralized when necessary by droplets of 1 N NaOH. The pre-enrichment at 36C for ~20 h was followed by a second-enrichment in Rappaport-Vassiliadis broth at 42C for ~18 h, and post-enrichment in Mannose (M) broth at 36C for ~7 h before *Salmonella* enzyme immunoassay (EIA) (Tecra, Frenchs Forest, Australia) was performed. The enrichment protocol was established according to an approved AOAC method (998.09, AOAC International, Gaithersburg, MD) for testing raw foods (Tecra 2002). The EIA-positive M broth samples were plated on XLD agar for isolation and confirmation using methods described previously (Pao *et al.* 2005).

RESULTS AND DISCUSSION

Prior to sprouting, the artificially inoculated alfalfa and mung bean seeds had *S. enterica*, respectively, at 7.2 and 6.1 log cfu/g to represent the worst contamination scenario. After seed germination for 4 days, *Salmonella* counts reached to 7.6 log cfu/g in alfalfa and 6.9 log cfu/g in mung bean sprouts despite daily changing of water in the sprouting system. Previous research also indicated that some strains of *Salmonella* have the capability to establish population levels near 7.8 and 6.8 log cfu/g, respectively, in alfalfa and mung bean sprouts during a 2-day seed germination process (Fett 2002; Howard and Hutcheson. 2002).

Data in Table 1 indicate that immersing the contaminated alfalfa and mung bean sprouts in 2% acetic acid, respectively, for 24 and 48 h eliminated *Salmonella* (<1 cell/25 g). With 5% acetic acid, *Salmonella* elimination was achieved after 4 h in alfalfa sprouts and 16 h in mung bean sprouts. Previous investigations have also noted that the reduction of *Salmonella* in artificially contaminated seeds by acetic acid treatments is positively influenced by increased exposure time and acid concentration (Delaquis *et al.* 1999; Pao *et al.* 2006). The results of this current study indicate that alfalfa sprouts require less decontamination time by acetic acid treatments in comparison to mung bean sprouts. The matured sprouts of mung bean are larger than alfalfa sprouts. One possible rationale is that the smaller sprouts contribute greater contact area by weight, allowing more thorough acid infiltration to inactivate *Salmonella* during treatment. Our results corroborate a previous finding by Singh *et al.* (2005) showing vinegar (containing 5% acetic acid) treatments for up to 3 h can significantly reduce, but not eliminate, *Salmonella* on cowpea sprouts grown from contaminated seeds.

TABLE 1.
EFFECTS OF ACID TREATMENTS ON ELIMINATING *SALMONELLA* IN SPROUTS*

Soaking time (h)	Number of samples positive† after treatment							
	Alfalfa				Mung bean			
	Acetic acid		Citric acid		Acetic acid		Citric acid	
	2%	5%	2%	5%	2%	5%	2%	5%
0	3	3	3	3	3	3	3	3
1	3	3	3	3	3	3	3	3
2	3	2	3	3	3	3	3	3
4	3	0	3	3	3	3	3	3
8	3	0	3	3	3	3	3	3
16	1	0	3	3	3	0	3	3
24	0	0	3	3	3	0	3	3
48	0	0	3	3	0	0	3	3

* Alfalfa and mung bean sprouts were germinated from artificially inoculated seeds and had *Salmonella* contamination at 7.6 and 6.9 log cfu/g, respectively, before treatment.
† Of three 25-g samples enriched and tested by the *Salmonella* enzyme immunoassay.

Data in Table 1 also indicate that citric acid treatments were ineffective in eliminating *Salmonella* from alfalfa and mung bean sprouts. Immersing alfalfa and mung bean sprouts in 5% citric acid for up to 48 h failed to achieve elimination. Citric acid is widely used in food processing because of its favorable flavoring characteristics. However, it is less effective as an antimicrobial acid or preservative when compared with acetic acid (Banwart 1989; Pao *et al.* 2006).

Brief dipping of sprouts in hot water was effective in inactivating *Salmonella* on the contaminated sprouts (Table 2). Treating alfalfa sprouts in hot water at 70, 80, 90 and 100C, respectively, for 10, 5, 3 and 3 s eliminated *Salmonella*. For mung bean sprouts, the elimination was achieved by immersion in hot water at 70 or 80C for 20 s, 90C for 10 s or 100C for 5 s. Again, the smaller alfalfa sprouts likely contributed greater contact area by weight, allowing faster thermal transfer to inactivate *Salmonella* during treatment. In many Asian produce markets, unpackaged sprouts are sold directly to consumers. Although this practice is vulnerable to additional microbial contamination, it is understood that a brief cooking by the consumer is required to prevent foodborne illness.

In conclusion, bacterial pathogens such as *Salmonella* can proliferate in sprouting systems during seed germination despite constant water change. Consumers, when uncertain with the microbial safety of home grown or purchased raw sprouts, should apply adequate intervention treatments prior to

TABLE 2.
EFFECTS OF HOT WATER TREATMENTS ON ELIMINATING *SALMONELLA* IN SPROUTS*

Soaking time (s)	Number of samples positive† after treatment							
	Alfalfa				Mung bean			
	70C	80C	90C	100C	70C	80C	90C	100C
0	3	3	3	3	3	3	3	3
1	3	3	2	1	3	3	3	3
3	3	1	0	0	3	3	3	2
5	1	0	0	0	3	2	1	0
10	0	0	0	0	1	2	0	0
20	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0

* Alfalfa and mung bean sprouts were germinated from artificially inoculated seeds and had *Salmonella* contamination at 7.6 and 6.9 log cfu/g, respectively, before treatment.
† Of three 25-g samples enriched and tested by the *Salmonella* enzyme immunoassay.

consumption. Soaking raw sprouts in acetic acid solutions may help to reduce *Salmonella* contamination. However, extended treatment times are required, particularly when treating larger sprouts. Not all organic acids provide the same killing effect. For example, citric acid is not effective in decontaminating *Salmomella* in sprouts. Heat treatment is traditionally used in preparing raw sprouts for Asian cuisines. The current study demonstrates that this practice is very effective for ensuring sprout safety. Dipping alfalfa and mung bean sprouts in boiling water respectively for 3 and 5 s can eliminate *Salmonella*. This study demonstrated that acetic acid and hot water treatments, when properly applied, can be effective in inactivating *Salmonella* for sprout safety. The potential impacts of these treatments on the sensory acceptance of sprout dishes should be considered in practical applications and/or future studies.

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