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REVIEW

Development of Effective Seed Decontamination Technology to Inactivate Pathogens on Mung Bean Seeds and Its Practical Application in Japan

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Abstract

The majority of seed sprout-related outbreaks has been associated with *Escherichia coli* O157:H7 and *Salmonella*. Therefore, it is necessary to find an effective method to inactivate possible pathogenic bacterial populations on the seeds prior to sprouting. In general, sanitizing is more effective in reducing contamination on seeds than on sprouts. A successful seed decontamination treatment must inactivate microbial pathogens while preserving seed viability, germination, and vigor. Seeds vary in sensitivity to antimicrobial agents and other treatments, which determine how well they germinate and grow after treatment. In addition, a treatment that is effective for one type of seed may not be applicable to all types of seeds. Seeds vary in surface features, which may influence how well an antimicrobial agent can access and inactivate pathogens on or in the seed. The use of a number of physical, non-thermal processing technologies, alone or in combination with antimicrobial chemicals, could be useful for seed decontamination. Until now, hot water treatment at 85°C for 40 seconds followed by cooling in cold water for 30 sec and soaking in chlorine water was found effective in inactivating pathogens while preserving seed viability, germination, and vigor. Therefore, hot water treatment could be an effective seed decontamination method for mung bean seeds intended for sprout production.

Discipline: Food
Additional key words: Mung bean, sprout, pathogen inactivation, hot water treatment, practical application

Introduction

The sprouting of seeds as food for human consumption originated in the Far East and because of their nutritive value, sprouts have gained popularity worldwide. Sprouts are low in calories and fat and provide substantial amounts of key nutrients, such as vitamins, minerals, proteins, enzymes, folate, and fiber[1,24,33]. They are usually eaten raw as ingredients of salads and sandwiches, or slightly cooked in various dishes. There are different types of sprouts, namely, adzuki bean (*Phaseolus angularis*), alfalfa (*Medicago sativa*), beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef.), broccoli (*Brassica oleracea* convar. *botrytis*), buckwheat (*Fagopyrum esculentum*), chickpea (*Cicer arietinum* L.), cress (*Lepidium sativum*), lentil (*Lens culinaris*), flax (*Linum usitatissimum*), mung bean (*Phaseolus aureus*), mustard (*Sinapis alba*), green and yellow pea (*Pisum sativum*), onion (*Allium cepa*), quinoa (*Chenopodium quinoa*), radish (*Raphanus sativus*), red cabbage (*Brassica oleracea* var. *capitata* f. rubra), rice (*Oryza sativa* L.), rye (*Secale cereale*), sesame (*Sesamum indicum*), soy (*Glycine max* L. *Merr.*), spelt (*Triticum spelta*), sunflower (*Helianthus annuus*) and wheat (*Triticum aestivum*). However, there has been an increase in consumer demand for mung bean,

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radish, alfalfa, broccoli, rice, wheat, and other seed sprouts that are prepared either commercially or at home. In the United States, EU, and Japan, alfalfa, broccoli, radish, clove, onion, and bean sprouts have been available in grocery stores and restaurants for quite a number of years. However, with the recent shift in consumer lifestyle towards "healthy living and healthier foods," the consumption of raw sprouts, mostly in salads and sandwiches has also increased. Generally, the following three kinds of sprouts are found in the market according to production quantity, production method, and consumption style:

1. Bean Sprouts

These sprouts are widely produced and consumed around the world. They are sprouted from relatively large legume seeds. There are three types of seeds: mung beans, black matpe, and soy beans. These sprouts are usually produced in a dark growing room within 7–10 days, and the annual worldwide production is estimated to be approximately 40 million tons (9 billion Lbs). The production/consumption quantity of bean sprouts is larger in Asian countries than in Western countries. However, the consumption quantity is currently on the rise in Europe and Japan. In Europe, there are approximately 10 large-scale factories producing 30 to 100 tons per day. Sprouts categorized as bean sprouts are mostly eaten cooked and stir fried in both Western and Asian countries. However, raw consumption makes up only a small percentage (< 1 %) of the total consumption worldwide. Soaking bean sprouts for a short time (10 – 20 sec) will improve the taste of the sprouts, while keeping their crisp texture.

2. Young Sprouts or Mini Sprouts

"Young sprouts" refer to those sprouts grown in rotary drums or containers in darkened rooms for a relatively short amount of time. This kind of sprout includes alfalfa, broccoli, radish, clover, etc., sprouted from relatively small-sized seeds. Alfalfa and broccoli sprouts were the first sprouts to gain popularity in the United States. They are high in nutritional value and continue to be popular in Western countries. These sprouts were first introduced into the Japanese market about 20 years ago, and now they are produced and consumed in various Asian countries. There are two ways to grow these sprouts: (a) in rotary drums or containers, and (b) in trays (using germinated seeds) in darkened rooms (sometimes using light to green them). In terms of processing (a) involves a washing-dehulling-dewatering process before packing, while (b) involves no processing (Table 1). These sprouts are generally eaten raw in any country.

3. Green sprouts or Micro Greens

"Green sprouts" or "micro greens" refer to those sprouts grown in greenhouses. These sprouts are generally grown in trays in a greenhouse under sunlight and grown longer than "young sprouts" or "mini sprouts". This kind of sprouts includes radish, broccoli, cress, sunflower, cabbage, etc., and is generally eaten raw in any country. Table 1 shows the production methods of the

Table 1. The production methods and consumption style of three types of sprouts

Item	Classification			
	A	B		C
	Bean Sprouts	Young or Mini Sprouts		Green Sprouts or Micro Greens
Seed	Mung bean, Soya bean, Black matpe	Alfalfa, Broccoli, Radish, Clover, etc.		Radish (Kaiware daikon), Broccoli, Cabbage, Cress, Sunflower, etc.
Growing Method	Darkened Rooms	Darkened Rooms sometimes using light to green them		Green House
	Container growing or Floor growing	(a) Rotary drum or container growing	(b) Tray growing (germinated seeds)	Tray growing
Processing	Dehulling–(Root Removing) –Washing–Dewatering	Dehulling -Washing -(Disinfecting) -Dewatering	No processing	No processing
Main packaging Style	Mostly film bag (sealed) Some plastic tray pack	Plastic tray pack	Plastic tray pack	Plastic tray pack
Main style of consumption	Cooked	Raw	Raw	Raw

above mentioned three types of sprouts.

Outbreaks of disease associated with sprouts

Despite being a popular health food, multiple outbreaks of disease linked to the consumption of raw sprouts have occurred in a great number of countries. The first recorded outbreak of foodborne disease from the consumption of raw, sprouted seeds was in 1973 and this was from soy, mustard, and cress grown in home-sprouting packs that were contaminated with *Bacillus cereus*. In 1988, there were large outbreaks of food poisoning in both the United Kingdom and Sweden from eating raw mung bean sprouts. Five different *Salmonella* serotypes were associated with the outbreaks, and three of these serotypes were detected in bags of mung bean seeds, which had come from Australia.

In the following year, cress sprouts contaminated with *S.* Gold-Coast were implicated in another outbreak in the United Kingdom. In the early 1990s, three outbreaks of salmonellosis from sprouts were identified in Finland. One outbreak of over 490 cases, in both Finland and Sweden, was due to *S.* Bovismorbificans in alfalfa sprouts, and these seeds also had been imported from Australia. Several other outbreaks were reported in the 1990s, in which salmonellae were implicated and alfalfa sprouts were the usual vehicles.

In the world's largest reported outbreak of *Escherchia coli* O157:H7 infections, which occurred in Japan in 1996, white (daikon) radish sprouts were epidemiologically linked to approximately 6,000 of the nearly 10,000 cases reported[70]. The pathogen was not detected in the cultures of implicated seeds. In the following year, white radish sprouts were again implicated in an outbreak of *E. coli* O157:H7 infection affecting 126 people in Japan[67].

In June 1998, a cluster of *E. coli* O157: NM infections in Northern California and Arizona were associated with eating an alfalfa and clover sprout mixture produced by the same sprouter implicated in the *S.* Senftenberg outbreak[22]. *E. coli* O157: NM isolates from the patients had indistinguishable PFGE patterns.

In 1999, there were five reported outbreaks in the United States and Canada associated with various *Salmonella* serotypes. Alfalfa and clover sprouts were implicated in these outbreaks (Table 2). In the United States, there was an outbreak that occurred in 2009 with 235 reported cases from a 14-state outbreak due to consuming alfalfa sprouts contaminated with *S.* Saintpaul (Table 2). From January 2000 until now, there have been over 22 reported outbreaks all over the world associated with sprouts from commercial growers, 16 of which were due to various *Salmonella* serotypes, six due to *E. coli* O157:H7, and one due to *Listeria monocytogenes*. Mung bean, alfalfa, radish, clover, and mixed sprouts have been implicated as the likely source of these outbreaks (Table 2).

Potential source of contamination

There are some reports that seeds for sprouts can be contaminated by bacterial pathogens, but they are not conclusive. In the field, seeds may be contaminated by birds and animal excretion, by runoff from animal production facilities, or through the use of untreated agricultural water or improperly composted manure. During harvesting, seeds can be contaminated by dirty equipment. Harvested seeds from various fields are mixed and a small amount of contaminated seeds can pollute a large amount of seeds. During the scarification process, where seeds are rubbed against hard surfaces to facilitate germination, bacteria can enter the seed and survive there for several months. During transportation to the processing facility and through distribution systems to grocery stores and homes, there are many occasions for the seeds and sprouts to become contaminated. Once present on or in the seed, pathogens are likely to survive for extended periods of time. Studies have shown that *Salmonella* can survive for months under dry conditions such as those used to store alfalfa seeds[39]. The natural microflora on rice seed showed long-term viability, decreasing by less than 0.5 log after 260 days of refrigerated storage and approximately two logs after 277 days at ambient conditions[49]. *Erwinia herbicola*, a common saprophyte of alfalfa seed, could be isolated for at least three years after treatment of seeds with 1% sodium hypochlorite[40]. In mung bean seeds, *E. coli* O157:H7 and S*almonella* can survive for at least a year under refrigerated storage[5].

Sprouted seeds represent a unique microbial food safety concern due to the potential for certain pathogenic bacteria (i.e. *Salmonella, E. coli* O157:H7, *Listeria monocytogenes, Bacillus cereus, Yersinia enterocolitica, Shigella* spp., *Kliebsella*) to grow rapidly during the germination and sprouting of the seeds. For sprouts contaminated by pathogens that do not grow during sprouting (protozoa, viruses), the risks of adverse public health consequences are similar to those already noted for fresh produce[45]. In fact, the risks associated with those microorganisms might be reduced due to the extensive washing that sprouts receive during their production. Pathogenic bacteria that either cannot grow under the conditions encountered during sprout production (e.g., *Campylobacter jejuni*) or that are not likely to be competitive enough to reach the levels needed to have an adverse public health impact (e.g., *Staphylococcus aureus*) are not considered to be an increased risk in sprouts compared to other fresh produce.

Seed sprouting process and the point of treatment

Seed sprouting is an easy process that can be done at home or on a commercial scale (Fig. 1). All that is required is soaking dried viable seeds to allow for germination and to maintain high levels of moisture. Sprouts usually emerge in 2–7 days, depending on seed type.

Pathogen decontamination overview

The assurance of the absence of pathogens on seeds

Table 2. Reported sprout-associated outbreaks 1996-2009

Year	Pathogen	No. of cases	Outbreak location	Sprout Type	Sources	References
1996	*S.* Montevideo, and *S.* Meleagridis	>500	2 U.S. states	Alfalfa	Seed and/or sprouter	43
1996	*E. coli* O157:H7	>6,000	Japan	Radish	Seed	36
1997	*E. coli* O157:H7	126	Japan	Radish	Seed	26
1997	*S.* Meleagridis	78	Canada	Alfalfa	Seed	8
1997	*S.* Infantis and *S.* Anatum	109	2 U.S. states	Alfalfa, Mung bean, others	Seed	25
1997	*E. coli* O157:H7	85	4 U.S. states	Alfalfa	Seed	11
1997-1998	*S.* Senftenberg	52	2 U.S. states	Clover, Alfalfa	Seed and/or sprouter	22
1998	*E. coli* O157:NM	8	2 U.S. states	Clover, Alfalfa	Seed and/or sprouter	22
1998	*S.* Havana	14	2 U.S. States	Alfalfa	Seed	2
1998	*S.* Havana, *S.* Cubana, and *S.* Tennessee	34	5 U.S. states	Alfalfa	Seed and/or sprouter	62
1999	*S.* Mbandaka	75	4 U.S. states	Alfalfa	Seed	32
1999	*Salmonella* spp	34	1 US state	Alfalfa	Seed	65
1999	*S.* Muenchen	99	3 US States	Alfalfa	Seed	50
1999	*S.* Saintpaul	36	1 US state	Clover	Seed	66
1999	*S.* Paratyphi B var. Java	46	4 Canada states	Alfalfa	Seed	9
2000	*S.* Enteritidis	75/27	1 US state/Nether land	Mung bean	Seed	17, 66
2001	*S.* Enteritidis	84	3 Canada States	Mung bean	Seed	10, 27
2001	*S.* Kottbus	32	4 U.S. States	Alfalfa	Seed	12, 74
2001	*S.* Enteritidis	30/84	1 US State/Canada	Mung bean	Seed	10, 58
2002	*E. coli* O157:NM	5	1 US state	Alfalfa	Seed	58
2002	*S.* Abony	13	Finland	Mung bean	Seed	38
2003	*S.* Saintpaul	8	2 US states	Alfalfa	Seed	13
2003	*E. coli* O157:NM	13	2 US states	Alfalfa	Seed	23
2003	*E. coli* O157:H7	6	1 US state	Alfalfa	Seed	13
2003	*S.* Chester/Sandiego	20	1 US state	Alfalfa	Seed	13, 58
2004	*S.* Bovismorbificans	33	3 US states	Alfalfa	Seed	14
2004	*E. coli* O157:NM	3	1 US state	Alfalfa	Seed	14
2005	*Salmonella* spp	648	Canada	Mung bean	Seed/sprouter	18
2005	*S.* Montevideo,	12	Japan	Radish	Seed/sprouter	52, 69
2006	*S.* Oranienburg	110	Australia	Mixed	Recall	46
2007	*S.* Weltevreden	45	Sweden, Finland, Denmark	Alfalfa	Seed	20
2008	*S.* Typhimurium	13	1 US state	alfalfa	seeds	37
2009	*S.* Saintpaul	235	14 US states	alfalfa	seeds	15
2009	*L. monocytogenes*	31	6 US states	alfalfa	seeds	16

can be regarded as the critical control point, as defined by the Codex Alimentarius Commission. Many studies have been performed to decontaminate seeds. Seeds have been soaked, dipped, sprayed, and fumigated with a wide range of chemical compounds with and without heat. Chlorine, as 20,000 ppm calcium hypochlorite, has been extensively tested[42,51]. Many other chemical agents were studied to decontaminate the sprout seeds. Some examples of interventions studied are: heat[30], combination of heat and chemical[31,53,60], NaOCl, acidified NaOCl, $Ca(OCl)_2$, acidified $Ca(OCl)_2$, Na_3PO_4, Tsunami™, Vortexx™, H_2O_2 [62], calcinated calcium[71], gaseous acetic acid[19], fatty organic acid[34], allyl isothiocyanate[48], ammonia fumigation[28], organic acid[34], CO_2[35], ozone[44,68], electrolyzed water[54], ozonated water[55,56], acidified electrolyzed water[59], peroxyacetic acid[51], scarification[29,47], ultra sound[53], pressure[56], competitive inhibition[73], and packaging modification[57].

The use of gamma irradiation in combination with chemical treatments (e.g., 20,000 ppm calcium hypochlorite) was investigated, but still does not guarantee the required 5 log reduction[63]. Bari et al.[7] treated alfalfa, radish and mung bean seeds with dry heat (50 °C for 1 hr) followed by gamma irradiation from a Co^{60} gamma source to reduce *E. coli* O157:H7. At an irradiation dose of 2.0 kGy plus the heat treatment, *E. coli* O157:H7 was completely eliminated from the alfalfa and mung bean seeds whereas a dose of 2.5 kGy plus heat was needed to eliminate the pathogen from the radish seeds. The combined treatments did not affect the germination percentage, but the resulting sprout length decreased. When Bari et al.[5] increased the dry heat treatment from 1–17 h followed by irradiation doses 0 to 1 kGy, they reported that the heat treatment followed by 1 kGy dose eliminated the *E. coli* O157:H7 from the radish, broccoli, and alfalfa seeds without affecting germination or sprout length. However, for the mung bean seeds, this treatment (heat plus irradiation) did affect the sprout length, but did eliminate the pathogen.

It has been recommended by the National Advisory Committee on Microbiological Criteria for Foods[45] to achieve a 5-log reduction of pathogens on seeds used for sprout production, and it had been shown that treatment with 20,000 ppm calcium hypochlorite is adequate since a sprout outbreak did occur when the seeds were washed in the calcium hypochlorite[50]. It has, however, been observed that this method may not always be sufficiently effective in reducing the numbers of pathogens from laboratory-inoculated seeds[41]. The application of chlorine or other disinfectants for the production of organic food is not accepted in many countries in Europe. In addition, high levels of chlorine discharged into municipal wastewater treatment facilities present a large burden for these facilities[30]. Therefore, physical or biological alternative treatments have to be developed to improve the safety of these ready-to-eat products.

Mung bean eating habits

Mung bean sprouts are usually eaten raw as compo-

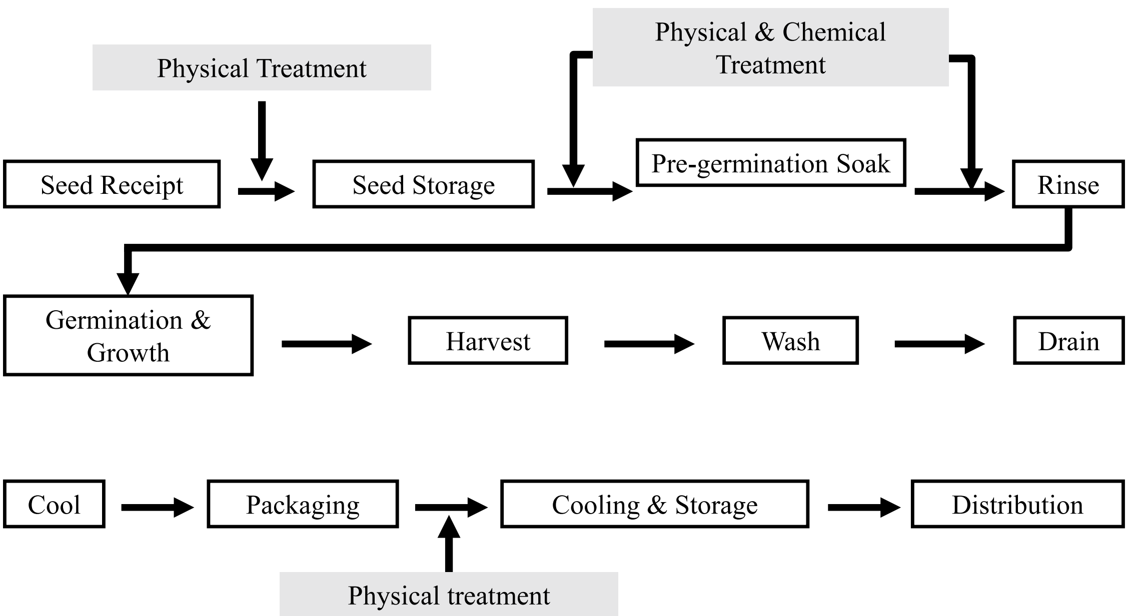

Fig. 1. Typical sprout production process and disinfection treatments

nents of salads, or slightly cooked in various dishes. Mung bean sprouts are the most common bean sprouts in most Asian countries including Japan. These sprouts are stir-fried as a vegetable accompaniment to a meal, usually with ingredients such as garlic, ginger, spring onions, or pieces of salted dried fish to add flavor. Uncooked bean sprouts are used in filling for Vietnamese spring rolls, and in a variety of Malaysian and Thai dishes. In Korea, slightly cooked mung bean sprouts, called *sukju-namul*, are often served as a side dish. In Japan, "bean sprouts," known as *moyashi,* are usually eaten with slightly cooked or stir fried in various dishes. About 400,000 tons/year of bean sprouts are consumed in Japan. However, there have been no reported outbreaks of illness from locally produced and retailed bean sprouts. The reason for this may be the eating habits of Japanese consumers, who usually consume cooked or stir-fried sprouts in home-style dishes. On the other hand, Japanese sprout producers have long been disinfecting mung bean seeds with hot water prior to cultivation. The primary purpose of these hot water treatments prior to sprouting is to disinfect the seeds of their microbiological loads (mold and bacteria), which may contribute to possible rotting during cultivation[21]. Other purposes include: softening hard seeds that are occasionally present in seed lots; enhancing germination; and improving the color of the bean sprouts. Until now, many mung bean sprout producers in Europe and the United States have introduced hot water treatment machines for disinfection.

Successful seed decontamination method

In Japan, sprout safety, especially for mung bean sprouts, has not been given much attention because sprouts are commonly consumed cooked or stir-fried in many home-style dishes, and there have not been any reported outbreaks of locally produced and retailed bean sprouts. However, sprout producers voluntarily use a hot water disinfection process (85°C for 10 seconds) as an effective treatment for reducing seed-borne microorganisms related to spoilage during sprout cultivation without reducing their germination rate[21]. Mung bean seeds possess thick seed coats and are thus very resistant to hot water. Therefore, hot water treatment for seed disinfection is thought to be an ideal method for mung beans.

In addition, Weiss and Hammes[72] reported that hot water treatment at 80°C for 2 min could reduce >6.0 log CFU/g of *Salmonella* Bovismorbificans and >7.0 log CFU/g of *E. coli* O157:H7 on mung bean. Enomoto et al.[21] showed that hot water treatment (85°C for 9s) was equally or more effective than 20,000ppm calcium hypochlorite treatments in reducing *E. coli* ATCC 25922 in alfalfa seeds. In our laboratory scale study[6] we reported that hot water treatment at 90°C for 90 seconds was effective in eliminating pathogens in artificially inoculated seeds. However, while doing a scale-up study with hot water treatment at 90°C for 90 sec, the germination yield decreased significantly (< 90%) depending on the variety/origin of mung bean seeds and thus was below industry levels. This was followed by a combination study with reduced hot water temperature and time, followed by soaking in chlorine. In that study[4], we reported that hot water treatment at 85°C for 40 seconds followed by dipping in cold water for 30 sec and soaking in 2000-ppm chlorine water could achieve complete elimination of *E. coli* O157:H7 and *Salmonella* Enteritidis in mung bean seeds without affecting the germination yield after the treatment. Finally, we evaluated the scale-up study results[3] with an actual pasteurization machine (SB-1300: Daisey Mechinary Co. Ltd) for disinfecting food poisoning bacteria, *E. coli* O157:H7, *Salmonella,* and non-pathogenic *E. coli* as a surrogate for practical tests. The results of this study suggest that hot water treatments at 85°C for 40 seconds followed by dipping in cold water for 30 sec and soaking into chlorine water (2000 ppm) for 2 h could be an effective method for disinfecting mung bean seeds before sprouting (Table 3). As this pasteurization treatment was performed at pilot scale in a commercial establishment, it could be a positive alternative to the presently recommended 20,000-ppm chlorine treatment for mung bean seeds. This method is easy to implement, and could be used in combination with good manufacturing practices and product testing, allowing processors to supply safe sprouts.

Recommendations

1) There should be an effective seed sanitization method from scientific, practical, economical, and ecological points of view.
2) Seed specific guidelines/ recommendations should be formulated.
3) Need to ensure that food safety guidelines/ regulations should not be a burden for sprout growers/ industry.
4) The hot water pasteurization method for mung beans described in this study is economically feasible, scientifically sound, and environment friendly. Therefore, it can be recommended for practical use.
5) This hot water pasteurization method is widely and voluntarily used by many sprout growers in Japan, and there have been no outbreaks so far with mung bean sprouts.
6) Hot water pasteurization is more effective than the calcium hypochlorite treatment recommended by the

Table 3. Practical scale (3kg) test of hot water treatment (85ºC) followed by dipping in cold water for 30 sec and soaking into chlorine water (2000ppm) for 2 h for mung bean seeds inoculated with non pathogenic *E. coli*

Treatment Condition	Population recovered (log CFU/g)[a]			Germination rate (%)
	Treatment day	24h enrichmrnt positive/total[b]	72h after germination positive/total[c]	
Control	5.85 ± 0.35	nd	nd	98
85ºC, 10sec	3.59 ± 0.15	10/10	10/10	97
85ºC, 10sec + 2,000ppm, 2h	2.48 ± 0.17	10/10	10/10	97
85ºC, 40sec	[d]	9/10	9/10	96
85ºC, 40sec + 2,000ppm, 2h	[d]	4/10	3/10	97
20,000ppm, 20min	4.17 ± 0.13	10/10	10/10	96
20,000ppm, 2h + 2,000ppm, 2h	2.78 ± 0.25	10/10	10/10	95

[a] : Data represent average values along with the standard deviation of three different experiments.
[b] : Number of positive results out of total tests after enrichment.
[c] : Number of positive results out of total tests after germination.
[d] : Counts below the detectable level of <1 log CFU/g

FDA.

7) In Japan, the risk of foodborne illnesses due to mung bean sprouts is close to zero as the sprouts are usually consumed after cooking.
8) Advice should be given to consumers that bean sprouts are better cooked before consumption, and for salads, dipping bean sprouts (mung bean and soy bean) into boiling water for a short time (ca.10 s) reduces the risk of foodborne illnesses.

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