Sprout Safety and Solutions from Japan

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Sprouts are grown hydrophonically, are usually eaten raw:

-in Salads

-in Sandwiches

or

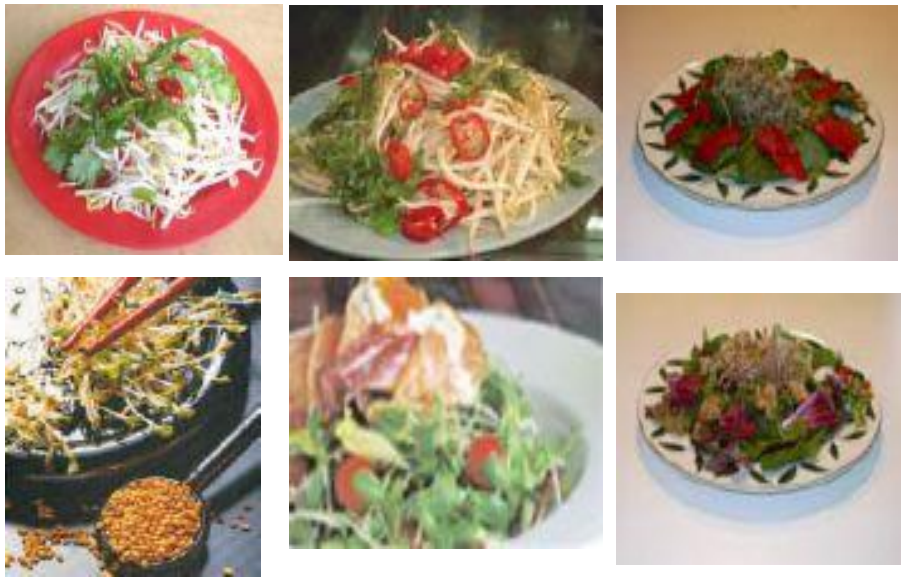
Can be cooked as oriental-style meal

Mung bean sprout

Radish sprout

Broccoli sprout

Alfalfa sprout



Delicious cuisine with sprouts

- Mung bean sprouts
- Alfalfa
- Soy bean

- Anti-oxidants
- Anti-carcinogens
- Anti-cholesterol

Sprouts

- United States

  Alfalfa

- Canada and Europe

  Mung bean sprouts: lightly cooked (stir fry)

  Soy bean sprouts

Japan

Mung bean sprouts: lightly cooked (stir fry)

Radish sprout

More exotic sprouts appearing (broccoli, buckwheat, onion, cabbage, rice)

Bean Sprouts Market Size

- USA $200-250m (300, 000 tons per year)
- Japan $ 450-500m (360,000 tons per year)

- Expanding market

- Small/domestic producers

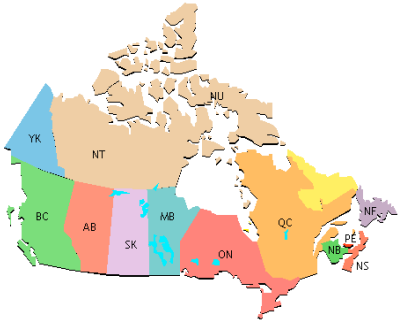
- Health benefits

Reported International Sprout Outbreaks, 2003-2009

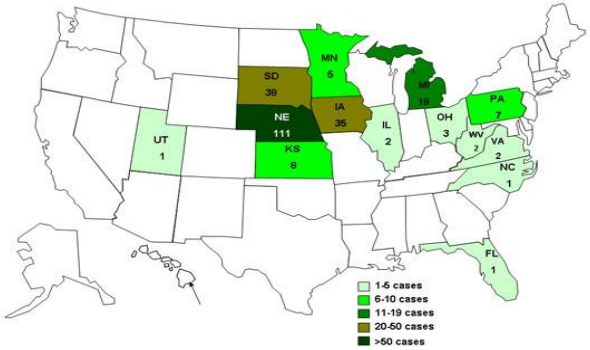
Year	Pathogen	No of cases	Outbreak location	Sprout Type	Sources	References
2003	*S.* Saintpaul	8	2 US states	Alfalfa	Seed	CDC 2003
2003	*E. coli* O157:NM	13	2 US states	Alfalfa	Seed	Ferguson and others 2005
2003	*E. coli* O157:H7	6	1 US state	Alfalfa	Seed	CDC 2003
2003	*S.* Chester/Sandiego	20	1 US state	Alfalfa	Seed	CDC 2003; SproutNet
2004	S. Bovismorbificans	33	3 US states	Alfalfa	Seed	CDC 2004
2004	*E. coli* O157:NM	3	1 US state	Alfalfa	Seed	CDC 2004
2005	*Salmonella* spp	648	Canada	Mung bean	Seed/sprouter	CFIA 2005
2005	*S.* Montevideo,	12	Japan	Radish	Seed/sprouter	Saito and others 2006; Watanabe and others 2006
2006	*S.* Oranienburg	110	Australia	Mixed	Recall	OzFoodNet, 2006
2007	*S.* Weltevreden	45	Sweden, Finland, Denmark	Alfalfa	Seed	(Emberland *et al.*, 2007)
2008	*S.* typhimurium	13	1 US state	alfalfa	seeds	Michigan Department of Agriculture 2008
2009	*S.* Saintpaul	235	14 US states	alfalfa	seeds	CDC 2009a
2009	*L. monocytogenes*	31	6 US states	alfalfa	seeds	CDC 2009b

Recent outbreaks

Canada:
In August 2009, multi state outbreak of salmonellosis linked to onion/alfalfa sprouts occurred.



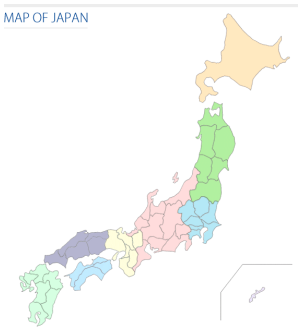
USA:
As of May 07,2009
Cases Infected with the Outbreak Strain of *Salmonella* Saintpaul. Collaborative investigative efforts of many local, state, and federal public health, agriculture and regulatory agencies led to the implication of alfalfa sprouts.



Japan:

In August, 2005, Salmonella Montevideo infection cases were reported in Miyagi Prefecture linked to white radish sprouts.

However, Till Today, There is no reported cases of illness caused by mung bean sprouts.



Routine Surveillance on Pathogens in fruits and vegetables Products (FY2004-2006)

Samples		Number of Samples			Percentage of Positive Samples								
					E.coli			Salmonella			Enterohemorrhagic *E. coli* O157		
		FY2004	2005	2006	FY2004	2005	2006	FY2004	2005	2006	FY2004	2005	2006
Vegetables	White radish sprouts	121	114	97	7.4%	8.8%	11.3%	-	-	-	-	-	-
	Alfalfa	20	35	22	5.0%	-	18.2%	-	-	-	-	-	-
	Lettuce	123	116	110	4.9%	6.0%	2.7%	0.8%	-	-	-	-	-
	Japanese honewort	95	92	66	26.3%	26.1%	34.8%	1.1%	-	-	-	-	-
	Bean sprouts	147	122	109	27.9%	27.0%	33.0%	-	-	-	-	-	-
	Cucumbers	125	124	101	3.2%	8.9%	5.0%	-	1.6%	-	-	-	-
	Pre-cut vegetables	177	137	160	4.5%	9.5%	6.9%	-	-	-	-	-	-

Homepage of Ministry of Health, Labour and Welfare of Japan
http://www.mhlw.go.jp/topics/syokuchu/index.html

Sources of Seed contamination

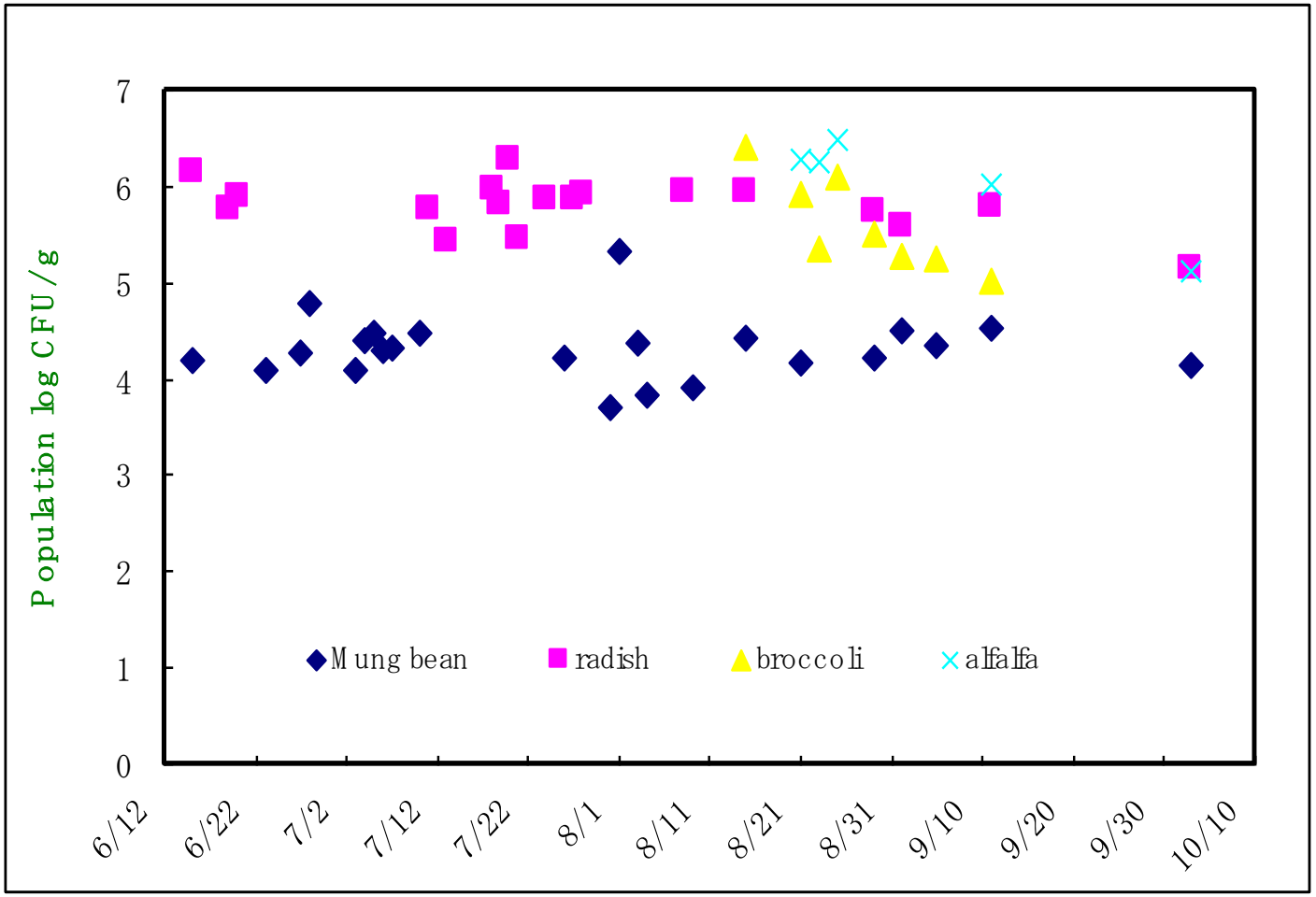
- Contaminated irrigation water

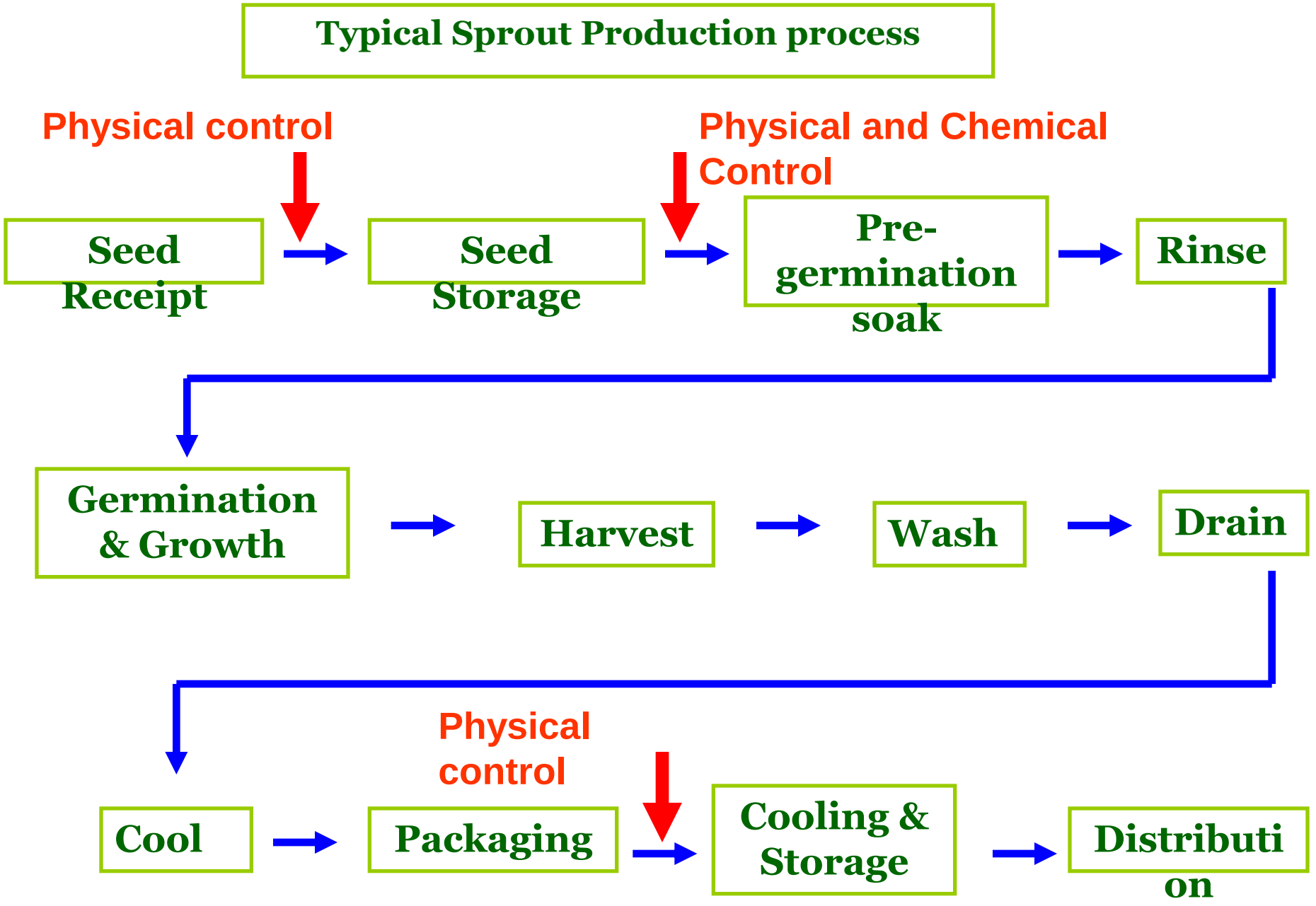
- Grazing animals

- Manure

- Equipment

Survival of *Escherichia coli* O157:H7 in different seed sample stored at 4C for 4 months.




Typical Sprout Production process
Physical control
Physical and Chemical Control
Seed Receipt
Seed Storage
Pre-germination soak
Rinse
Germination & Growth
Harvest
Wash
Drain
Physical control
Cool
Packaging
Cooling & Storage
Distribution

Seed Decontamination

Industry

- Eliminate pathogens
- Maintain seed viability
- Low cost and practical

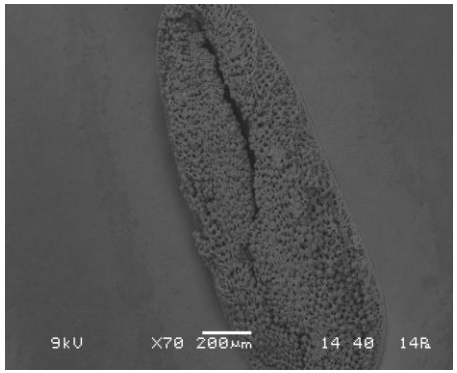
FDA

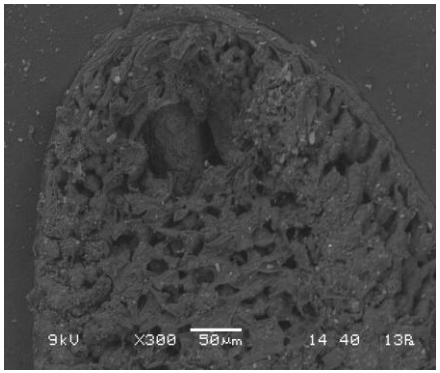
- 5 log reduction required
- 20, 000 ppm Calcium hypochlorite

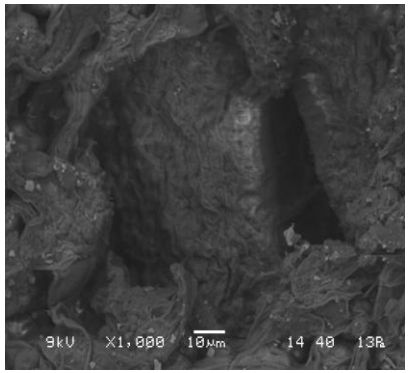
Problems

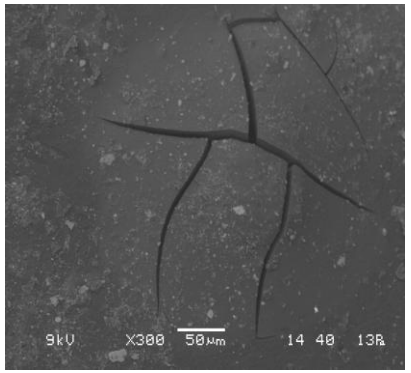
- Not totally effective
- Worker safety
- Incompatible with organic production
- No other sanitizer listed

Seed Scan Electron Microscopy



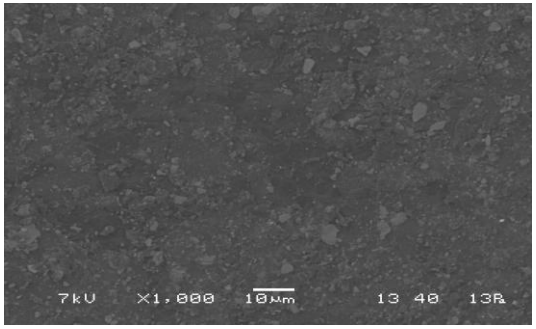


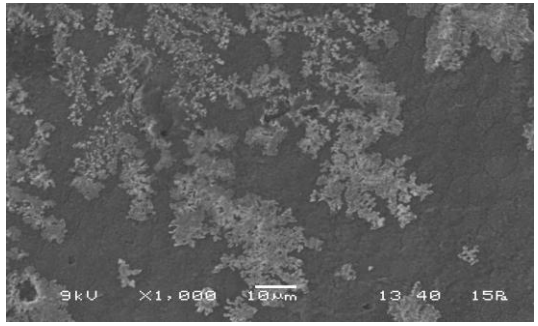


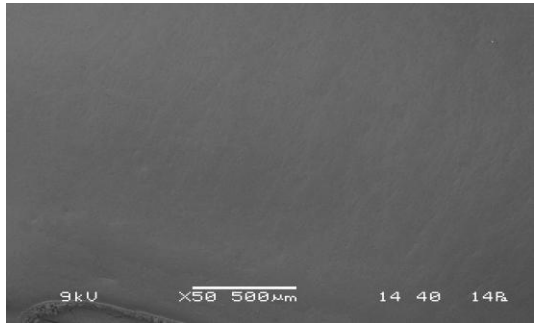


Stem scar porous is enough to penetrate bacteria in the seed

Cracks and crevices







Seed Surface with natural contaminants

Seed surface with *E.coli* O157:H7

Seed surface after AcEW wash

Chemical Interventions - Seed

$Ca(OCl)_2$, NaOCl, ClO_2, acidified ClO_2, acidified $NaClO_2$, $Ca(OH)_2$, calcinated calcium, H_2O_2, acidic electrolyzed water, ethanol, sulfuric acid, lactic acid, citric acid, acetic acid, thyme oil, ozone, trisodium phosphate, colicin type E-2, Tsunami^R, Vortexx^{TM}, Vegi-Clean^{TM}, Fit^R, Calcifresh-S^{TM}, Citrobio^{TM}, Citricidal^{TM}, Environne^{TM}, Citrex^{TM}

- Gas phase treatments
  - Acetic acid vapor, allyl isothiocyanate, trans-anethole, carvacrol, cinnamic aldehyde, thymol, ammonia

Physical Interventions - Seed

- Dry heat
- Hot water
- Irradiation (gamma radiation, pulsed UV)
- Hydrostatic pressure
- Radio frequency dielectric heating

Biological Interventions - Seed

- Antagonistic bacteria
  - Lactic acid bacteria
  - Fluorescent pseudomonads
  - Whole bacterial communities

- Bacteriophage vs. *Salmonella*

Combinations – Mung Bean Seed

Dry heat (50^{o}C, 1 h) followed by gamma irradiation (2.0 kGy)

→ 4.6 log reduction of *E. coli* O157:H7 (no survivors), no effect on germination, reduced sprout growth rate

Bari et al. 2003. J. Food Prot. 66: 767-774.

Combinations – alfalfa, broccoli, radish, and mung bean Seed

Dry heat (50^{o}C, 17 h) followed by gamma irradiation (1.0 kGy)

→ 5.0 log reduction of *E. coli* O157:H7 (no survivors), no effect on germination, reduced sprout growth of mung bean.

Bari et al. 2009. J. Food Prot. 72(3): 631-636.

Successful Seed Decontamination Methods

Dry Heat – Mung Bean Seed

Dry heat (55^{o}C 4-7 days)

- Eliminates *Salmonella* and *E. coli* O157

  - No effect on mung bean germination

  - Alfalfa viability reduced

Hu, et al. 2004. J. Food Prot. 67: 1257-1260.

Hot Water – Mung Bean Seed

- Hot water (5g seed/250 ml)
  - 55^{o}C/20 min → 5 log reduction of *Salmonella*
  - 60^{o}C/10 min → 5 log reduction of *Salmonella*
  - 70^{o}C/5 min → 5 log reduction of *Salmonella*
  - 80^{o}C/2 min) → 6 log reduction of *Salmonella*

  - No effect on seed germination

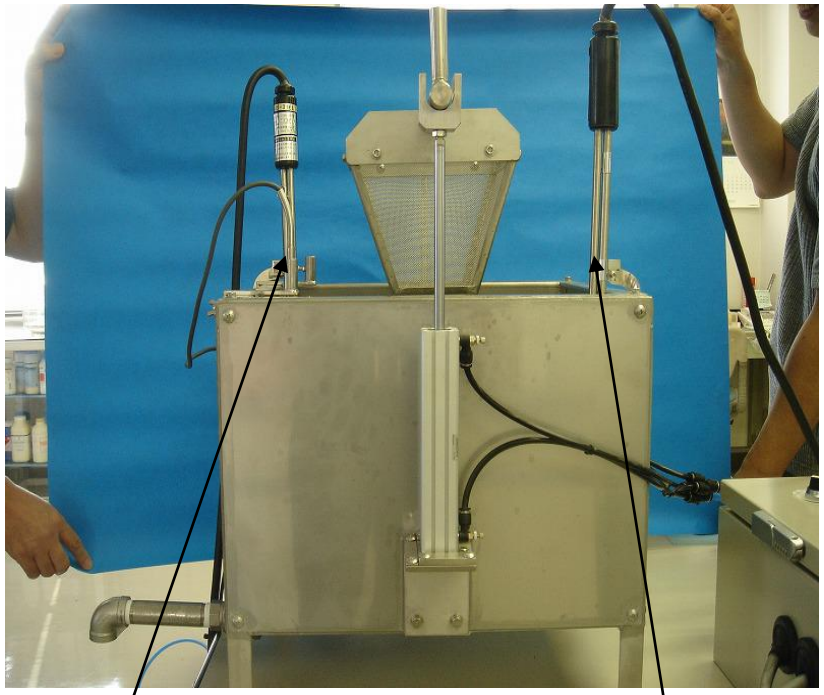
Weiss et al, 2005. *Eur Food Res Technol.* 221:187- 191.

Hot Water – Mung Bean Seed

- Hot water (5g seed/250 ml)
  - 90^{o}C/1.5 min → 5 log reduction of *Salmonella*
  - 90^{o}C/1.5 min → 6 log reduction of *E. coli* O157:H7
  - No viable pathogen.
  - No survivor after enrichment.
  - Not much effect on seed germination.

Bari, et al. (2008). *Journal of Food Protection*, 71, 830-834.

Model machine for P2 level room (1/２０ size)　300g seeds



Start button

Auto control monitor

Temperature sensor

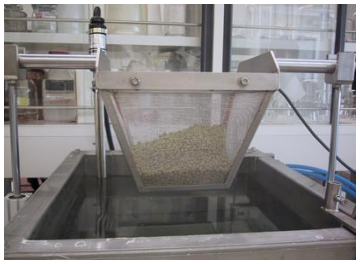
Heater

Flow diagram of scale up study:

Seed inoculation

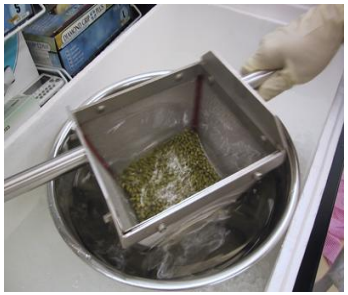
Drying at RT

Pack and store at 4C

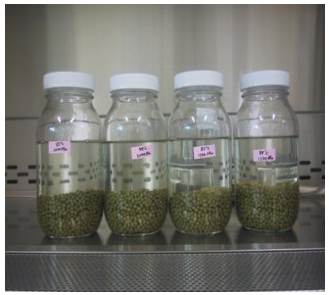


Hot water machine

Dip in hot water



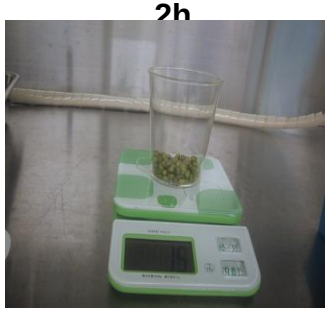
Dip in chill water



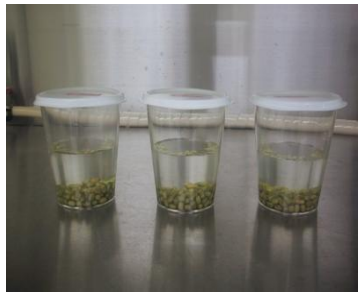
Soak in 2000ppm chlorine water for 2h

Drain off and weight

Add TSB for enrichment study



Drain off and weight



Add water for germination study

Scale up study / *E.coli* O157

Treatment Condition	*E. coli* O157:H7		
	Population recovered (log CFU/g)[a]		
	Treatment day	24h Enrichment positive/total[b]	72 h after germination positive/total[c]
Control	5.69 ± 0.55	nd	nd
85^{0}C, 10sec	2.87 ± 0.20	nd	nd
85^{0}C, 20sec	1.39 ± 0.16	nd	nd
85^{0}C, 30sec	*	9/9	9/9
85^{0}C, 40sec	*	9/9	9/9
85^{0}C, 10sec + 2000 ppm , 2 h	2.16 ± 0.18	nd	nd
85^{0}C, 20sec + 2000 ppm, 2 h	*	9/9	9/9
85^{0}C, 30sec + 2000 ppm , 2 h	*	9/9	5/9
85^{0}C, 40sec + 2000 ppm , 2 h	*	0/9	0/9
Control +20,000 ppm , 20min	3.19 ± 0.20	9/9	9/9
Control +20,000 ppm, 20min +2000 ppm, 2 h	2.78 ± 0.10	9/9	9/9

Scale up study / *Salmonella*

Treatment Condition	*Salmonella*		
	Population recovered (log CFU/g)[a]		
	Treatment day	24h Enrichment positive/total[b]	72 h after germination positive/total[c]
Control	5.84 ± 0.77	nd	nd
85^{0}C, 10sec	2.55± 0.12	nd	nd
85^{0}C, 20sec	*	9/9	9/9
85^{0}C, 30sec	*	9/9	9/9
85^{0}C, 40sec	*	9/9	9/9
85^{0}C, 10sec + 2000 ppm , 2 h	2.45 ± 0.15	9/9	9/9
85^{0}C, 20sec + 2000 ppm, 2 h	*	9/9	9/9
85^{0}C, 30sec + 2000 ppm , 2 h	*	9/9	4/9
85^{0}C, 40sec + 2000 ppm , 2 h	*	0/9	0/9
Control +20,000 ppm , 20min	3.14 ± 0.18	9/9	9/9
Control +20,000 ppm, 20min +2000 ppm, 2 h	2.63 ± 0.16	9/9	9/9

Scale up study / Germination

Table : Germination yield for non-inoculated mung bean seeds after hot water treatment at 85℃ (185F) for different time spans. (day 4 at 30℃)

Temp. ℃	Time sec	Germination %±S.D.	Growth %±S.D.
Control		99.3±0.9	96.4±3.0
85	10	100±0.0	98.5±0.7
	20	99.9±0.2	98.4±0.5
	30	99.7±0.6	98.8±0.4
	40	99.5±0.4	96.2±1.0
	60	97.2±2.0	82.7±4.8
	90	65.3±8.8	19.8±5.4

Scale up Trial Conclusions

Hot water treatment at 85^{0}C followed by dipping in chilled water for 30 sec and soaking into chlorine water (2000ppm) for 2 h could successfully inactivated *E. coli* O157:H7 and *Salmonella* in mung bean seeds.

No viable pathogens were found, no survivors were found in the enrichment medium and during the 72 h sprouting process.

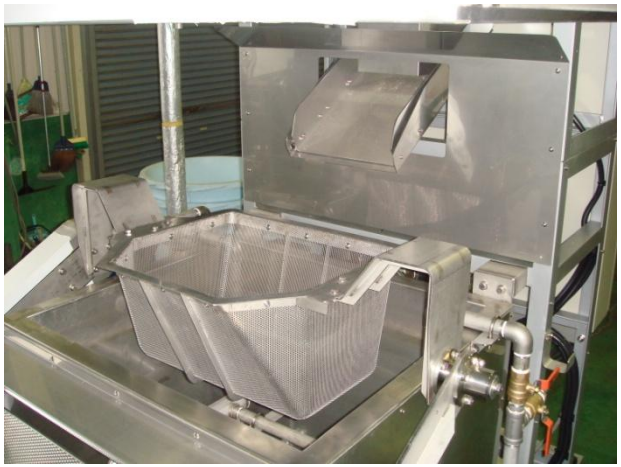
The germination yield of the seed was not affected significantly. Therefore, these treatments could be used in the sprout production industry as an effective seed decontamination method for mung bean seeds intended for sprout production.

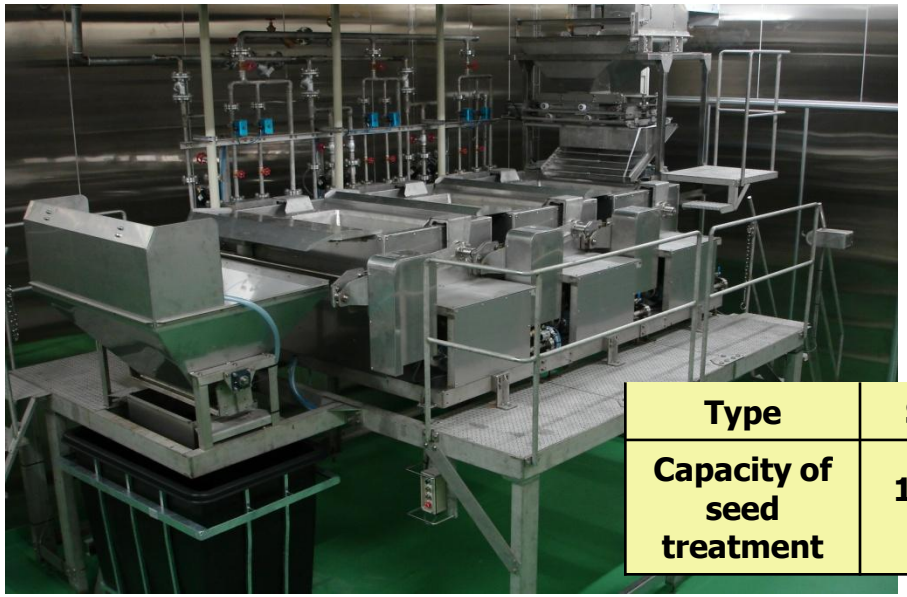
Questions

Can 500g of seed really be considered as a semi-commercial batch? While industry, usually 25-75kg of seeds using/ batch

Practical study of Hot water treatment

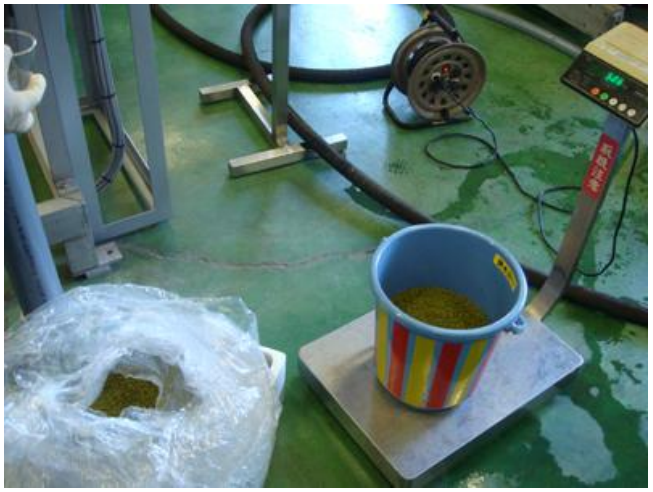
Type	SBS1300
Capacity of seed treatment	300[kg/h]

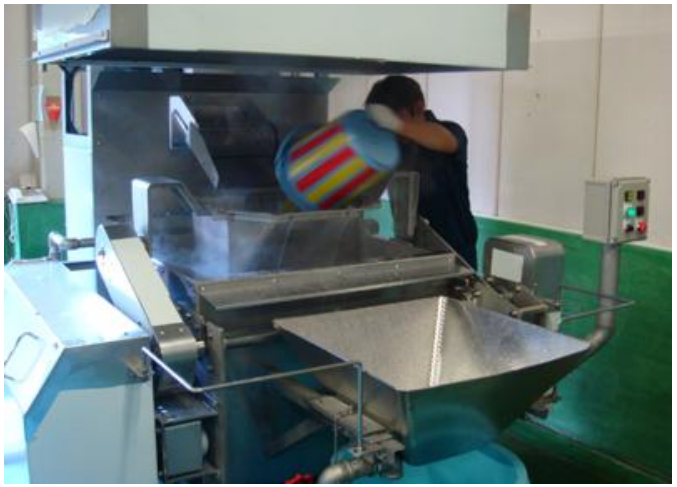




Type	SBS1500
Capacity of seed treatment	1500 [kg/h]

Practical test / Pasteurization machine





Inoculated seed weighted

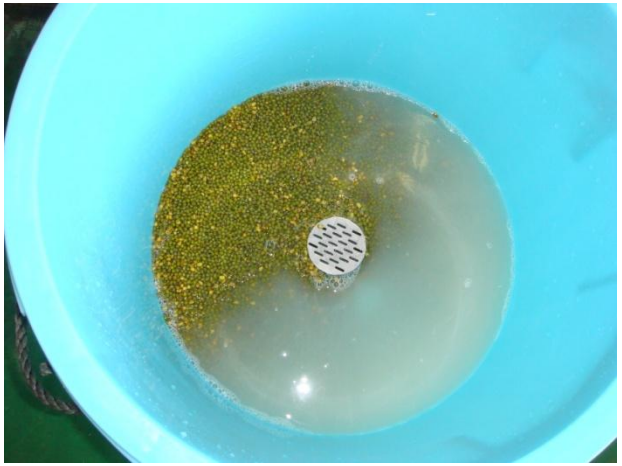
Hot water treatment 85C, 40 sec

Practical test / Pasteurization machine

Treated seeds were transferred in bin

Cool immediately with cold water

Practical test / 20,000ppm Ca-chlorite



Practical test / 20,000ppm Ca-chlorite + 2,000ppm Na-chlorite soak

Practical test / 2,000ppm Na-chlorite soak

Soak in sanitizers for 2h

Multiple location samples (n=10) were tested for Bacteria.

Practical test/germination

Mung bean sprouted in bins (10kg x 3 lots)

Spent irrigation water testing at 48h

Practical test/germination

Sprouts after 72h of germination

Multiple location samples (n=10) were tested for Bacteria.

Practical test / Yield

Table : The harvest yield of sprouts grown from non-inoculated mung bean seeds after hot water treatment both with and without the combination of chlorine soaking.

(1.5kg seeds were used for each of the cases)

hot water	combination	Yield (kg)	Yield *
no	no	16.1	10.7
	chlorine soaking	16.2	10.8
85℃40sec	no	15.2	10.1
	chlorine soaking	15.3	10.2

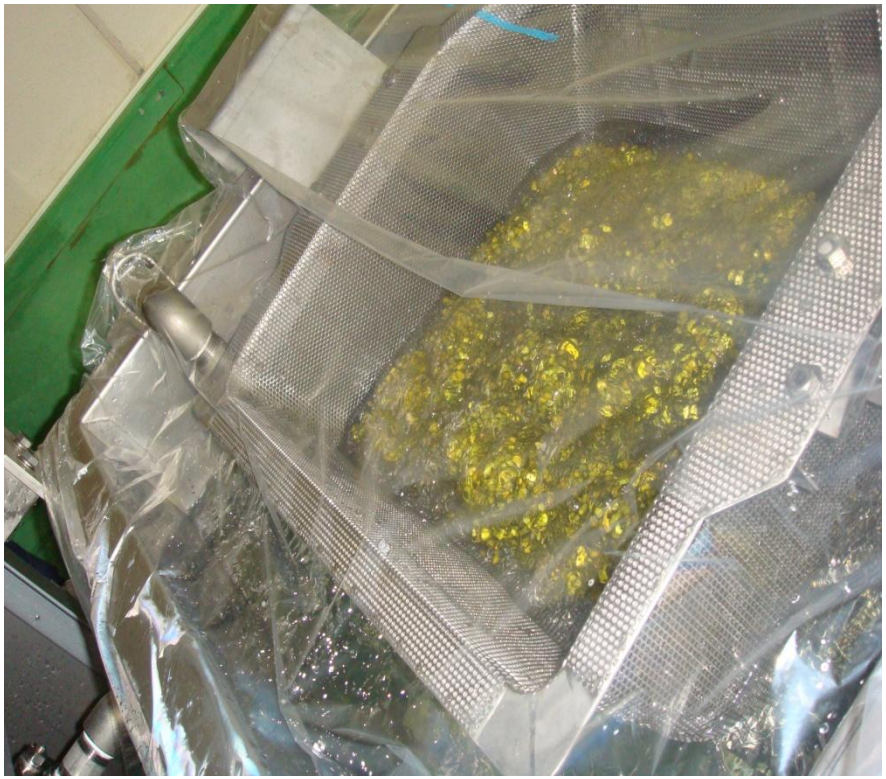
*: Yield / seeds

Table: Practical scale (3kg) test of hot water treatment (85^{0}C) 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]		
	Treatment day	24h Enrichment positive/total[b]	72 h after germination positive/total[c]
Control	5.85 ± 0.35	nd	nd
85^{0}C, 10sec	3.59 ± 0.15	10/10	10/10
85^{0}C, 10sec + 2000 ppm , 2 h	2.48 ± 0.17	10/10	10/10
85^{0}C, 40sec	*	9/10	9/10
85^{0}C, 40sec + 2000 ppm , 2 h	*	4/10	3/10
Control +20,000 ppm , 20min	4.17 ± 0.13	10/10	10/10
Control +20,000 ppm, 20min +2000 ppm, 2 h	2.78 ± 0.25	10/10	10/10

[a]Data represent average values along with the standard deviation of three different experiments.
Asterisk indicates counts below detectable level of <1 log CFU/g.
[b]no of positive results out of total tests after enrichment.
[c]no of positive results out of total tests after germination.
nd, not done

Practical test / improving chlorine soaking



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Scale-Up Seed Decontamination Process to Inactivate *Escherichia coli* O157:H7 and *Salmonella* Enteritidis on Mung Bean Seeds

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Abstract

A majority of the seed sprout–related outbreaks have been associated with *Escherichia coli* O157:H7 and *Salmonella* spp. Therefore, it is necessary to find an effective method to inactivate these microorganisms on the seeds before sprouting. When treatment with hot water at 85°C for 40 sec followed by dipping in cold water for 30 sec and soaking into chlorine water (2000 ppm) for 2 h was performed, no viable pathogens were found in the enrichment medium and during the sprouting process. The germination yield of the seed was not affected significantly ($p > 0.05$). Therefore, these treatments could be useful for the decontamination method of mung bean seeds intended for sprout production.

Introduction

nated with *Salmonella* Enteritidis phage type 13 occurred in

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Research Note

Practical Evaluation of Mung Bean Seed Pasteurization Method in Japan

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ABSTRACT

The majority of the seed sprout–related outbreaks have been associated with *Escherichia coli* O157:H7 and *Salmonella*. Therefore, an effective method for inactivating these organisms on the seeds before sprouting is needed. The current pasteurization method for mung beans in Japan (hot water treatment at 85°C for 10 s) was more effective for disinfecting inoculated *E. coli* O157:H7, *Salmonella*, and nonpathogenic *E. coli* on mung bean seeds than was the calcium hypochlorite treatment (20,000 ppm for 20 min) recommended by the U.S. Food and Drug Administration. Hot water treatment at 85°C for 40 s followed by dipping in cold water for 30 s and soaking in chlorine water (2,000 ppm) for 2 h reduced the pathogens to undetectable levels, and no viable pathogens were found in a 25-g enrichment culture and during the sprouting process. Practical tests using a working pasteurization machine with nonpathogenic *E. coli* as a surrogate produced similar results. The harvest yield of the treated seed was within the acceptable range. These treatments could be a viable alternative to the presently recommended 20,000-ppm chlorine treatment for mung bean seeds.

Pasteurization of other seeds

Black matpe

Temp. ℃	Time sec	Germination 1 %±S.D.	Germination 2 %±S.D.
control		99.2±0.5	96.3±0.4
88	20	99.8±0.4	98.2±1.3
	30	99.2±0.2	96.8±0.9
	40	98.6±0.5	95.1±1.9
	50	98.1±0.3	93.7±1.4
	60	96.5±0.4	86.4±1.7

Alfalfa

Temp. ℃	Time sec	Germination 1 %±S.D.	Germination 2 %±S.D.
control		82.9±1.6	78.0±1.8
80	10	83.7±2.6	81.6±2.4
85	10	77.8±3.7	71.1±4.7
88	10	67.2±4.3	52.2±4.5
	30	27.7±1.9	20.4±1.9

Soy bean

Temp. ℃	Time sec	Germination 1 %±S.D.	Germination 2 %±S.D.
control		99.7±0.6	77.1±2.6
70	5	95.9±3.4	76.3±5.1
	10	82.2±4.5	59.6±2.8
	15	80.2±2.3	55.4±11.7
	20	70.0±2.0	35.7±22.2
	30	65.9±5.1	27.3±5.1

Disinfection of Alfalfa seeds

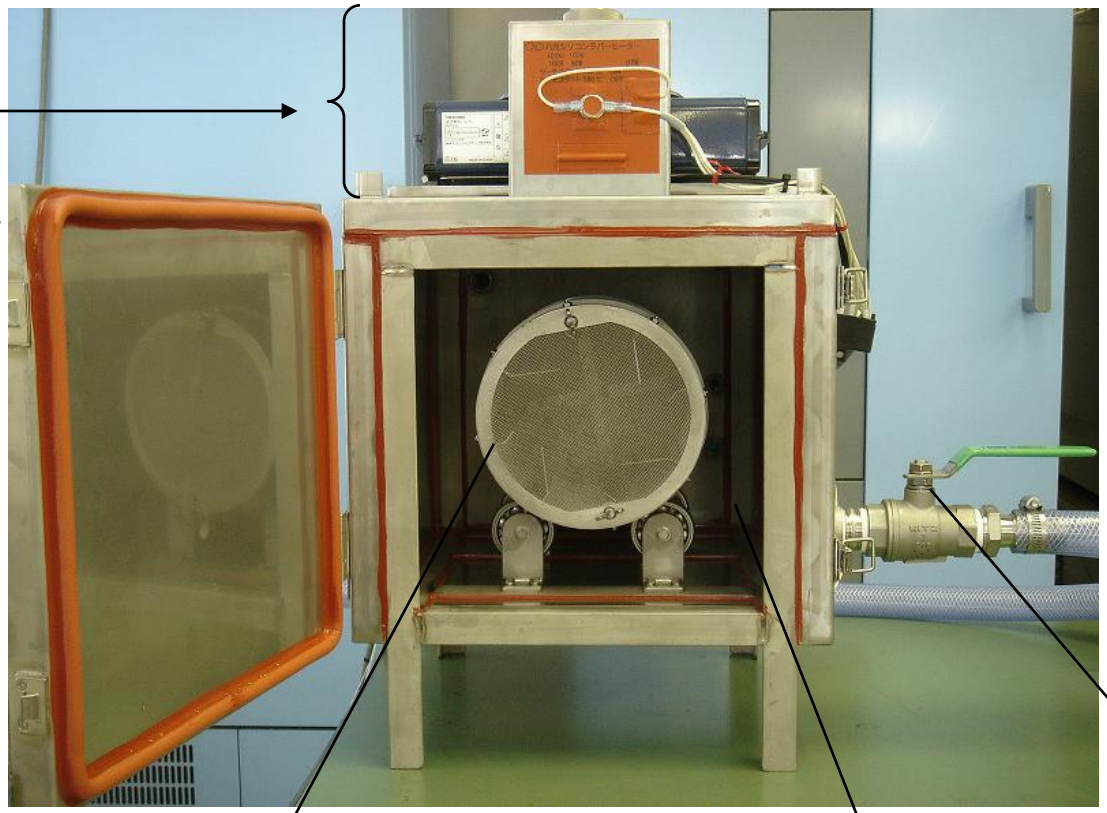
- Acetic Acid Gas Fumigation

  Delaquis et al. 1999. J. Food Prot. 62: 953-957. (Mung bean seed)

- Acetic Acid Gas Fumigation Machine (2001)

Acetic acid fumigation machine (1/20 size)

Heating for evaporation of the acetic acid



Exhaust

Rotary Drum
(1.5 cpm)

Chamber
(Temperature controlled at 55℃)

Alfalfa

Broccoli

Clover

Radish

Acetic acid gas fumigation condition

- Temp. 45⇨55℃

  after Feng et al J.Food Prot.70 (2007)

  dry heat at 55℃ for up to 6 days

- Gas concentration

  saturation 45℃=5.1%, 55℃=8.7%

Table : Treatment of alfalfa seeds with gaseous acetic acid followed by soaking in chlorine water.

Treatment Condition	*E. coli* O157:H7			***Non-pathogenic E.coli***		
	Population recovered (log CFU/g)[a]			Population recovered (log CFU/g)[a]		
	Treatment day	24h Enrichment	72 h after germination	Treatment day	24h Enrichment	72 h after germination
Control	4.80 ± 0.45	ND	ND	4.84 ± 0.37	ND	ND
Acetic acid 0.5 h	*	+	+	*	+	+
+2000ppm soaking for 2h	*	+	+	*	+	+
Acetic acid 6h	*	+	+	*	+	+
+2000ppm soaking for 2h	*	+	+	*	+	+
Acetic acid 12h	*	+	+	*	+	+
+2000ppm soaking for 2h	*	+	+	*	+	+
Acetic acid 17h	*	+/-	-	*	+/-	-
+2000ppm soaking for 2h	*	-	-	*	-	-
Acetic acid 24h	*	-	-	*	-	-
+2000ppm soaking for 2h	*	-	-	*	-	-

[a]Data represent average values along with the standard deviation of three different experiments.
Asterisk indicates counts below detectable level of <1 log CFU/g.
+, indicates positive after enrichment.
-, indicates negative after enrichment.
+/-, indicates seldom positive

Acetic Acid Gas Fumigation and Enrichment

Treatment	*E. coli* O157:H7		*E. coli*	
	Population recovered	24h enrichment	Population recovered	24h enrichment
	log CFU/g	Negative / total	log CFU/g	Negative / total
Acetic acid gas 30 min.	<2	0 / 6	<2	0 / 6
Acetic acid gas 30 min. + 2,000ppm chlorine soak	<2	1 / 6	<2	1 / 6
Acetic acid gas 17 h.	<2	17 / 18	<2	6 / 6
Acetic acid gas 17 h. + 2,000ppm chlorine soak	<2	18 / 18	<2	6 / 6

USA seeds

Acetic Acid Gas Fumigation and Germination

Treatment	USA seeds		CAN seeds	
	Germination %	Growth %	Germination %	Growth %
No (control)	91.5 ± 1.7	91.5 ± 1.7	79.1 ± 2.6	77.9 ± 2.5
Acetic acid gas 30 min.	94.7 ± 2.1	93.3 ± 1.7	83.2 ± 1.5	82.0 ±0.6
Acetic acid gas 30 min. + 2,000ppm chlorine soak	92.6 ± 2.5	90.1 ± 2.7		
Acetic acid gas 24 h.			73.6 ± 6.3	72.4 ± 6.3
Acetic acid gas 24 h. + 2,000ppm chlorine soak			75.7± 1.2	73.2 ± 0.2
Acetic acid gas 48 h.	92.2 ± 2.0	91.0 ± 1.5		

Variety of alfalfa seeds

AUSTRALIA

CANADA

ITALY

USA

CONCLUSIONS

- Hot water treatment at 85^0C 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.

- On the other hand, acetic acid fumigation for 17 h followed by soaking in 2000ppm chlorine water could achieve complete elimination of pathogens in alfalfa seeds.

- These treatments could be a positive alternative to presently recommended 20,000-ppm chlorine treatment for sprouted seeds.

- Therefore, seed specific treatments need to be recommended for the safety of seeds intended for sprout production.

Enjoy safe
Pork roll with
Bean Sprout !

Thank you for your attention!

Questions!!