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030 10 JUL 15 1999

December 15, 1999

FDA / CFSAN / OPDFB
Mrs Michelle Smith
200 C Street, S.W., Rm 4133
Washington DC20204
USA

Dear Mrs Michelle Smith,

It has been nice meeting you recently again at the Sprouts Summit at the NCFST in Chicago.

In our brief conversation, I have mentioned to you about our research and practical experience on seed sanitizing. We made our comments also on last years public meeting in Washington. As mentioned, I believe that there is an urgent need for a recommended alternative to the chemical treatment, and heat as a hot water flash treatment seem to me to be the ideal alternative, also after hearing the reports presented by all speakers at the recent Sprouts Summit.

We have stated our views in the written comments which have been submitted. For reference, I have included a copy.

Also you will find enclosed our most recent report on heat treatment, and our presentation which we have prepared for last years public meeting in Washington.

We would certainly appreciate to hear your views and any comments. Let me mention again that our company is approaching the seed sanitation from a practical point. As a supplier we can only recommend methods, which can be successfully practiced in a sprouting operation - under the quite difficult conditions.

Our next step is to install a fully automatic seed pasteurizing system for test purposes at Fuji Natural Foods in California. As this will be a test under actual operating condition we believe that the studies and results will be of highest value. Fuji Natural Foods shares with us the opinion that, specially now during this difficult situation, everybody has to work together in order to find a practical solution quickly to prevent further outbreaks.

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We are inviting the FDA and other organizations working on the subject to take this opportunity and look closer at the seed pasteurizing system, since there is an urgent need for an effective alternative to the chemical treatment.

For us as a company, which has been engaged in the sprouts industry for 25 years, we are convinced that sprouts are the best vegetables for human beings in the future. We have to break through this very hard and most difficult time the sprouts industry came across. To expand this wonderful food material in the world, we are making our effort as much as possible with you and those who recognize the value of the sprout - a "Natures Treasure"!

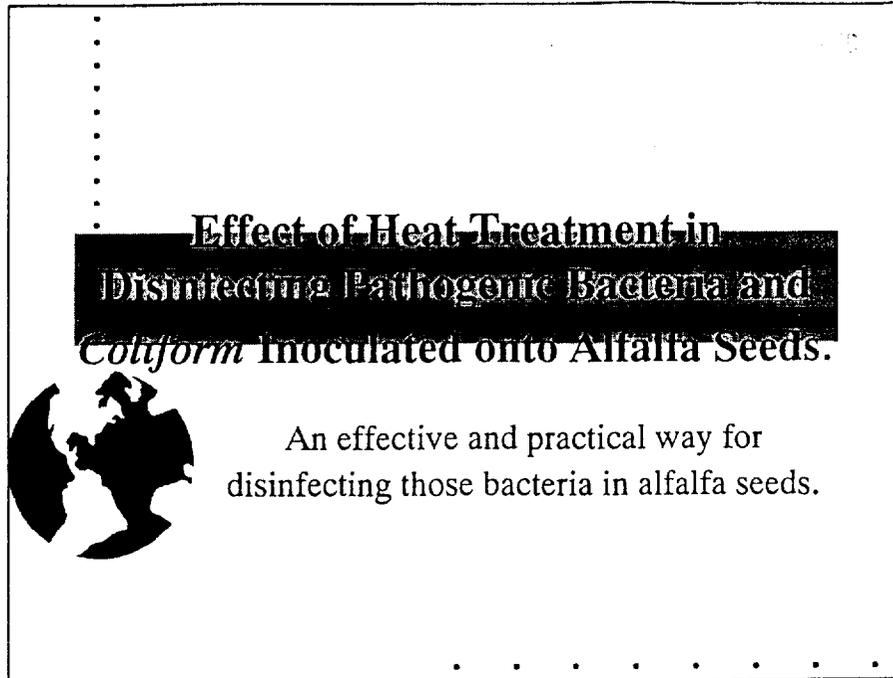
May I kindly ask you to look over the enclosed reports. As you have been spending a lot of efforts and time to look at the sprouts in the USA, your comments would be greatly appreciated. Please do not hesitate to contact me if you would like to get more details about the enclosed material.

We are very much looking forward hearing from you soon.

Yours sincerely,

German Regli,
Daisey Machinery CO.,LTD..

September 1998



**Effect of Heat Treatment in
Disinfecting Pathogenic Bacteria and
Coliform Inoculated onto Alfalfa Seeds.**



An effective and practical way for
disinfecting those bacteria in alfalfa seeds.

INTROUCTION

Our company has done research since many years to find a **practical solution** for seed sanitizing, in particular for mung beans.

We are using HEAT TREATMENT as method of Sanitizing which has the following advantages:

1. Easy and safe handling
2. Natural method of sanitizing
3. Very effective to kill human pathogens and seed borne diseases
4. Practical solution for ever day use in a sprouting factory

We developed a fully automatic system for pasteurizing seed, which is successfully in use in Japan since several years. This system is patented in the USA. After the good results on mung beans we have extended our research recently to Alfalfa seed.

We will introduce first our most recent results on Alfalfa. Second we will show you a section of a video which is showing the system in operation.

Inoculation of bacteria

- *Klebsiella pneumoniae* ssp *pneumoniae*
- *Salmonella enteritidis*
- *Escherichia coli* (ATCC 25922)
- *Listeria*

Tested in F&S
research center
of the B.M.L.
corporation

- following the method of C.B.JAQUETTE et al.
APPL.ENVIRON.MICROBIOL.V.62,1996.

- Heat treatment of seeds

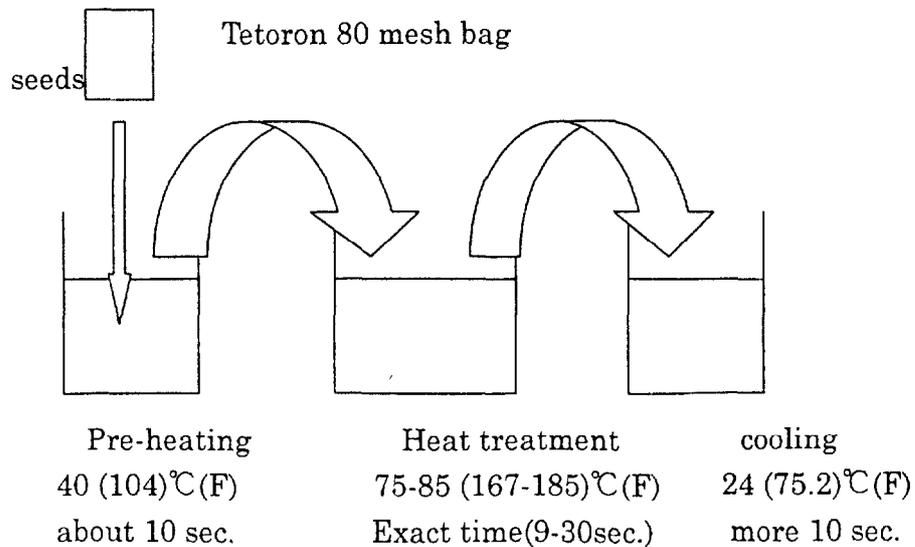


Table 2 Effect of heat treatment in killing *Salmonella enteritidis* on alfalfa seeds

Heat treatment		Survival of <i>S.</i> -	
Water temp °C(F)	Treatment time (sec.)	(CFU/g of seed)*	% Disinfection
40 (104)	9	830000000	-33.87096774
75 (167)	9	3700000	99.40322581
	20	56000	99.99096774
	30	44000	99.99290323
77 (170.6)	9	0	100
80 (176)	9	0	100

* initial population of inoculated seeds was 6.2×10^8

PATENT US005615518A

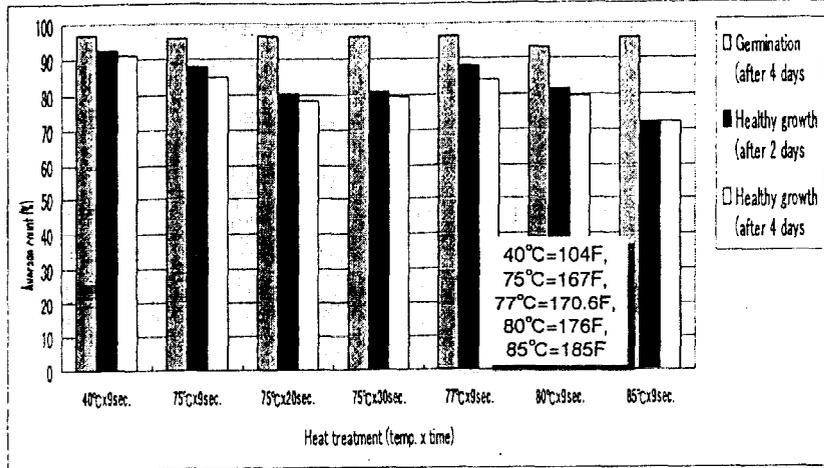
Table 3 Effect of heat treatment in killing *E.coli* on alfalfa seeds

Heat treatment		Survival of <i>E.coli</i>	
Water temp °C(F)	Treatment time (sec.)	(CFU/g of seed)	% Disinfection
40 (104)	9	780000000	18.75
75 (167)	9	42000000	95.625
	20	460000	99.95208333
	30	2300	99.99976042
77 (170.6)	9	210	99.99997813
80 (176)	9	0	100

* initial population of inoculated seeds was 9.6×10^8

PATENT US005615518A

Fig.1 Influence of heat treatment on germination and growth of alfalfa seeds. (tested under laboratory condition)



PATENT US005615518A

Table 5 Influence of seed heat treatment on yield (tested under growing plant condition)

Treatment of seeds	Amount of harvest*
Heat treatment 80 (176)°C(F)	11.31±0.1**
Control	11.65±0.4

*1kg seeds were used for each container

** mean ± S.E.

PATENT US005615518A

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Conditions for using heat treatment as an effective and practical method to sanitize alfalfa seeds:

- ① Hot water temperature: 75-85(167-185)°C(F) depending on bacterial contamination
- ② Treatment time: 9-30sec. (exactly equal time for each seed is very important)
- ③ 3 step cycle: PREHEAT & WASHING, HEAT TREATMENT and COOLING (as patented method)
- ④ The contact of each individual seed with the hot water need to be exactly the same.
- ⑤ Using O₃ water or chlorine in cooling stage can be a further advantage.

PATENT US005615518A

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December 4, 1999

Effect of heat treatment in disinfecting inoculated bacteria in seeds.

Introduction

A comparison study was performed to show the effect of disinfecting inoculated bacteria in alfalfa seeds. In this test, two inoculated seeds samples were used to represent seeds heavily contaminated by pathogenic bacteria and seeds with a lower level of contamination. Some trials for promoting seed germination were also done.

Materials and methods

Plant materials

I Table of alfalfa seed samples

Harvest	Location	<i>E.coli</i> test results	Scarification	% injured seed
1996	California	Negative	No	2.5±0.4

Strain and preparation of inoculum

II Table of Inoculation

Inoculated seed group	Inoculated bacteria	Inoculation way	Initial CFU/g of seeds
L	<i>E.coli</i> (ATCC25922)	Dip in the suspension	1.7×10^4
N	<i>E.coli</i> (from rodent feces)	Spray the suspension	2.5×10^3

The method of the inoculated seed group L was used following the method of Jaquette C.B. et.al. (1). A suspension culture of *E.coli* was diluted with the ratio of 1 to 1000 in 0.1% peptone water. Seeds were dipped in this solution for 30 seconds and dried after the solution was discarded. The seeds of the inoculated seed group N were sprayed with the solution equally until the seeds were wet.

Heat treatment of seeds Five grams of seeds were put in a stainless steel net (tea ball, inside diameter 75mm) and dipped in hot water of 50-70°C for about 9-10 seconds as a pre-heat process, then drawn out and dipped again in hot water of the exact treatment temperature (between exact temperature and +1°C temperature) for the exact time (between exact seconds and +1 seconds) as the heat treatment. Immediately, the bag was dipped in tap water (about



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15°C) for about nine seconds for cooling after drawing it out again. The time for drawing out and dipping again was about three to five seconds. The hot water of a hot water bath and from a faucet of regulated hot tap water were used and the amounts of water were more than three liters. Three samples were tested for each treatment.

Pre-soaking of seeds

Seeds were pre-soaked for 30 minutes in water of 25°C and kept at 25°C.

Calcium hypochlorite treatment

The inoculated seeds were also treated with 3% calcium hypochlorite (20,000ppm as chlorine) as a method according to FDA recommendations. Five grams of seeds were put in a 100ml solution of 3% calcium hypochlorite and stirred for 10 minutes. The solution was stirred for 20 to 30 minutes previously. After that, the solutions were discarded and the seeds were rinsed four times with autoclaved distilled water.

Seeds activity test

Twenty uninoculated heat treated seeds were placed on moist paper towels in petri plates. The percentage of seeds that germinated after four days was determined. Five petri plates were used for each treatment and at least three replicates were done.

Microbiological methods

Five grams of each type of treated seeds were put in sterilized bags and mixed with 10ml of 1% peptone and ground by a roller for 30 seconds. Undiluted samples (200 μ l and or 20 μ l) were plated on Chromocult coliform agar (Merck) for the detection of an *E.coli* colony. All plates were incubated at 34°C for 48 hours. The peptone solution with the seeds was also incubated at 34 °C overnight and the solutions were checked for the detection of *E.coli* on the above mentioned medium following the case of the previous plate counts being zero.

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Saitama 350-2207, JAPANTel: (+) 81 492 87 2111
Fax: (+)81 492 87 0809e-mail: info@daisey.co.jp
website: www.daisey.co.jp**Results****Effect of disinfecting**

The plate counts of inoculated seeds after each treatment were determined to be as follows:

Treatment	Inoculated seed group	<i>E.coli</i> CFU/g	Negative cases of <i>E.coli</i>
Pre-soak *→Pre-heat(50°C × 9sec.)→Heat(85°C × 9sec.)	L	0	3 / in 3 cases
Pre-soak *→Pre-heat(70°C × 9sec.)→Heat(83°C × 9sec.)		0	3 / in 3 cases
Pre-soak *→Pre-heat(70°C × 9sec.)→Heat(80°C × 9sec.)		0	1 / in 3 cases
Pre-heat(50°C × 9sec.)→Heat(85°C × 9sec.)		600-5760	---
Soaking in 20,000ppm of Calcium hypochlorite for 10min.		1350-2100	---

Pre-soak *→Pre-heat(50°C × 9sec.)→Heat(85°C × 9sec.)	N	0	3 / in 3 cases
Pre-soak *→Pre-heat(70°C × 9sec.)→Heat(80°C × 9sec.)		0	3 / in 3 cases
Pre-heat(50°C × 9sec.)→Heat(85°C × 9sec.)		0-30	---
Soaking in 20,000ppm of Calcium hypochlorite for 10min.		90-135	---

* The seeds were pre-soaked for 30 min. at 25°C previous to heat treatment

The plate counts of the seeds which were treated with pre-soak, pre-heat and heat treatment were zero in both inoculated seed groups. The residue test of *E.coli* (in the peptone water with the treated seeds kept overnight) also tested negative in the case of a higher heat treatment temperature of 85°C in both seed groups and 83°C in seed group L. A lower temperature treatment of 80°C was also effective in eliminating *E.coli* in the case of seed group N but there were some appearances of *E.coli* in seed group L.

The colony counts of the seeds which were treated with calcium hypochlorite showed an equal or higher count than the counts of the seeds after heat treatment not pre-soaked in both seed groups.

Germination of seeds

The germination percentage of the seeds after four days of cultivation were as follows:

Treatment	Germination(%)
Pre-soak *→Pre-heat(50°C × 9sec.)→Heat(85°C × 9sec.)	87.3+4.6
Pre-soak *→Pre-heat(70°C × 9sec.)→Heat(80°C × 9sec.)	88.3+2.1
Pre-heat(50°C × 9sec.)→Heat(85°C × 9sec.)	92.7+1.7
Control	95.0+2.9

* The seeds were pre-soaked for 30 min. at 25°C previous to heat treatment.



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The germination percentage of the pre-soaked and heat treated seeds tended to decrease but some recovery was also observed by decreasing the treatment temperature to 80°C.

Conclusion

The heat treatment of hot water at 85°C for nine seconds appears to be as sufficient in disinfecting inoculated *E.coli* as treating the same seeds with 20,000ppm of calcium hypochlorite for 10 minutes. The pre-soaking before the heat treatment promotes the effect of disinfection but decreases germination. It is necessary to select the suitable pre-soaking and heat treatment condition (according to the level of the seed contamination) for disinfecting the seeds without deeply damaging them as a practical use for this treatment in alfalfa sprouts production.

References

Jaquette, C. B., L. R. Beuchat, and B. E. Mahon. 1996. Efficacy of chlorine and heat treatment in killing Salmonella Stanley inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Applied and Environmental Microbiology, 62: 2212-2215

Reported By:

Daisey Machinery Co., LTD.

Katsuyoshi Enomoto

Master of Science



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Dockets Management Branch (HFA – 305)

5630 Fishers Lane, rm.1061

Rockville, MD 20852

RE: Docket #'s 99D-4488, 99D-4489

December 4, 1999

To whom it might concern:

Our company has been participating actively on the research to find a practical solution to prevent outbreaks of E-coli and Salmonella caused by sprouted products

We can understand the concerns of the FDA very well, specially since there were again several outbreaks during this year.

Our Company, based in Japan, has been in the sprouts business for almost 25 years and over this time we got to know what kind of problems the growers are facing. In Japan the biggest part of the industry is certainly the mung bean sprout, but there are other Green Leaf sprouts - including Radish sprouts - produced as well in, compared with the mung bean sprouts, relatively small quantities.

Accordingly our research and developments were mainly focused on the mung bean. Also in our case, **only solutions which can be adopted in an actual plant - under the sometimes difficult conditions - were acceptable.** Over ten years ago we were starting to look for possibilities to eliminate the organisms which are causing crop diseases and failures.

Investigating several methods we came to the conclusion that heat, in particular hot water at high temperatures were the most effective and practical solution to successfully disinfect the seed prior sprouting. Since about 8 years the patented system is in use for mung beans in factories producing between 4000 – 200'000 lb bean sprouts per day.

Some years back we became aware of the outbreaks involving human pathogens and as commonly known these pathogens have - generally speaking - very similar characteristics to the organisms causing crop diseases in regard to heat resistance. We decided to intensify our research and extended it to the smaller green leaf sprout seeds such as alfalfa. We have been actively participating in the "Sprouts Task Force" at the National Center for Food Safety and Technology led by Dr Charles Sizer and we have presented our results frequently to the organizations involved.

We were finding that Hot Water Flash Treatment can also be adopted for small size seed – **providing it is done very accurately in regards of contact time as well as temperature.**



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During the time of redesigning our mung bean system we found some hurdles to overcome in order to guaranty that each seed is treated equally. But at this stage we can say that the redesigned system can be adopted for all type of seeds. All in all the priorities are the same: Effectively disinfecting the seed with an absolute minimum reduction of the germination ability. On top of that **a key issue is to have it working under the common condition of a sprouting facility**. In our experience of developing equipment we find very often that the performance can differ very much between the test facility and the actual production facility. Certainly we believe that seed sanitizing is no exemption – rather the most delicate area.

The FDA and all involved organizations have done an amazing job collecting all the information about practices of the industry, documented in the “White Paper” and creating the guidelines – which we are convinced will help the industry prevent outbreaks in the future.

However, we would like add some comments based on experience gained over all the years in the industry in Japan as well as Europe:

- We see an urgent need for an alternative treatment which is practically possible to apply also in a medium to larger scale operation. Heat is the ideal alternative.
- We can understand that it is currently easy to use Calcium Hypo Chlorite immediately also in small operations, but some of our concerns are the environmental aspects. We are sure that you are aware that several countries in Europe have banned the use of Chlorine, or are planning to do so in the not to distant future. Frankly, it sounds somehow strange that the FDA is only recommending the use of high concentrations - but in other parts of the world it is acknowledged that serious problems can arise. In our opinion heat offers an alternative without any impact on the environment.
- Beside the environmental impact and even more important: What about the workers which are exposed to dangerously high levels of chemicals every day. As a highly corrosive chemical it is very important to handle these concentrations very, very carefully. All of us, we are looking for a solution to prevent the damage of peoples health. Handling of high concentration Sodium Hypo Chlorite is in a way a shifting of the problem, which again the Sprouts industry will be exposed to. Heat as a natural disinfectant is again an alternative without risks for the workers in a plant.
- Let me also mention the “organic” aspects of the issue. The health benefits of fresh sprouts have been listed over and over again. Beside the demand for safe food, there is a strong consumer demand for natural, untreated and un-manipulated food. Organic Sprouts are matching these demands perfectly. Certainly such health conscious consumers could not tolerate a highly concentrated chemical treatment. This movement is perhaps stronger in



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Europe, but according to our observations, it is very much increasing in the USA as well. It is in nobodies interest that conscious consumers are finding themselves irritated with an other food item which is not produced according to the principle of producing food naturally. Many businesses are meeting the expectations and this part of the industry needs an effective alternative to the chemical treatment. And again - Heat would be the ideal alternative.

Enclosed you will find some of our recent reports on our research. As you will see, hot water flash treatment using a very short time at a high temperature is already very effective, but with presoaking we found that the disinfecting rate can be further enhanced.

Our team is continuing looking for further improvement and adopting new technologies as well as they become available. Further more we considering also combination treatment methods. The industry needs practical solutions without delay and we are prepared to share our results and experience with other organizations working on the subject in order to ensure that Safe Sprouts, Naturally Produced can be consumed worldwide.

The multiple hurdle strategy of seed disinfection and spend irrigation water testing is a very good approach. However, we hope that the practical side will be observed over a period of time and possible problems will be taken into consideration. Further more we believe that the FDA has sufficient data and experience from related industries which are practicing the testing.

Again out of practical experience, we are somehow concerned how representative the spend irrigation water sample is on mung bean sprouts, since there are vast amounts of water involved. We assume that the recommendations have been based on green leaf sprouts grown on trays or in drums.

We are convinced that the above stated issues are also of concern to the FDA. Let us offer our support and assistance to guide the industry even better. Efficiently working together is most important in order to ensure that everybody can enjoy the benefits of the natural, healthy sprout again.

Yours sincerely,

Daisey Machinery Co., Ltd.
German Regli