PRELIMINARY REPORT SUBMITTED TO THE FOOD AND DRUG ADMINISTRATION UPON REQUEST FOR INFORMATION:

% MOISTURE OF ALFALFA SEEDS BEFORE AND AFTER ARTIFICIAL INOCULATION AND VISUAL IMPACT OF INOCULATION

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At the May 17, 2005 Food and Drug Administration public meeting on the current science related to foodborne illness associated with the consumption of sprouts, Donald Schaffner and Kathleen T. Rajkowski gave presentations. Schaffner presented his analysis of the published data on seed sanitization to identify which factors affect the efficacy and variability of the different sanitization protocols to remove bacterial pathogen from seeds used for sprouts. They found that the time and temperature of treatment did not affect the log microbial reduction. However, Schaffner did report that there was more variability for the chemical treatment when there in the published data. Rajkowski presented data on the efficacy of a reformulated sanitizer along with the sanitizing treatment most studied (Ca hypochlorite). As a result of this study, the reformulated sanitizer was consistently effective, whereas, the hypochlorite treatment was not.

Since naturally contaminated seeds carry a low pathogen level of less than 1 CFU/g, investigators used artificially inoculated seeds in they studies with a know bacterial count to determine the sanitization efficacy. Schaffner’s report indicated that there was also variability in the procedure used to obtain inoculated seeds. There was a question as to whether the various inoculating protocol affect the seeds and the study results. A study was done to compare these inoculation procedures as it impacts the % moisture following the published inoculation procedure, drying time and storing conditions and to examine the inoculated seed during the procedure.

**Method and Materials**

**Seed Inoculation.** Alfalfa seeds were supplied by both Caudill Seed Co. (Louisville, KY) and International Specialty Supplies (Cookeville, TN). Table 1 lists the inoculation liquid, contact and drying times, drying procedure and storage conditions. The inoculation procedures were followed as reported for each protocol (15 total) using 100 gm samples. Each procedure was done twice.

**Seed Moisture Content.** The % moisture analysis was done according to the determination listed for alfalfa seeds by the International Seed Testing Association using the constant high temperature (130 ± 3 ° C) oven method 9.5.9 (11).
Microscopic imaging.

A single layer of seeds was poured into a glass petri dish for microscopic examination of the seed coats. Violet fluorescent light was used to distinguish cracked/broken seed coats from intact seeds. The broken or cracked seeds were set aside. The cracked seeds along with a control (seed coat intact) were placed on a double-sided adhesive circle attached to a glass slide. The seeds were photographed then hydrated in 0.1 % peptone water for 1 min, plotted to remove the liquid and photographed. The seeds were left to dry in a biological hood for 24 h and photographed.

Alfalfa seeds were viewed with a model MZ FLIII stereo fluorescence microscope (Leica Microsystems, Bannockburn, IL) used in two modes; reflected illumination from a model KL1500 (Schott NA, Inc., Auburn, NY) 150 W halogen lamp house equipped with two fiber optic guides, and alternately, blue (>475 nm) epifluorescence from excitation with violet light from a 50 W Mercury lamp house. Digital images were collected with a model DC100 charge coupled device camera (Leica Microsystems, Bannockburn, IL). The width of the field of view was 13 millimeters.

Results

Table 2 lists the moisture content. The average % moisture of the control alfalfa seeds was 6.6 ± 0.47 %. Immediately after inoculation, the moisture content increased two fold and by the time the stored seed were used the moisture content was close to the initial level.

Figure 1 shows the alfalfa seeds under the violet fluorescent light. The exposed cotyledon was easily distinguishable from the seed coat (fluoresced). Using this characteristic it was easy to separate intact seeds from those with broken seed coat. In Figure 2 are a series of photomicrographs showing the seeds after hydration and drying. The seed coat expanded and separated from the cotyledon leaving a gap where liquid was able to enter. Upon drying this gap did close but to the original position.
Conclusion

1. In answer to the question raised at the May 17, 2005 public meeting, the inoculation procedures that were used by the various researchers did not affect the moisture content of the artificially inoculated alfalfa seeds.

2. In answer to the question, “What concepts or underlying principles should guide efforts to improve the safety of sprouts?”, the quality of the seeds is most important. Broken seed coats do provide a haven for pathogens to become lodge between the coat and cotyledon. Since the process of artificially breaking the seed coat, scarification, is sometime used by producers, the use of such seeds should be avoided by sprout growers.

Reference List


sanitization studies shows treatments are highly variable. J. Food Prot. 67:758-765.


monocytogenes on alfalfa seeds and sprouts and effects on sensory quality of sprouts. J. Food Prot. 66:44-51.


Figure 1. Alfalfa seeds as viewed with a violet fluorescence light (425 nm barrier filter)
Figure 2. Alfalfa seeds before, after hydration and drying as viewed using white light luminescence.