ABSTRACT – Studies have shown that sprout is a nutritious food for the human diet; however, it is also an appropriate medium for bacterial growth. Microbiological testing has been indicated as part of an overall strategy to enhance the safety of sprouts. Alfalfa and broccoli seeds were run in bioreactors built in 3D printer in conjunction with distilled water and fertilizer. The sprouting medium was tested daily during sprouting for total counts by Pour Plate Technique for 4 days, on agar medium, at 37°C. The effect of chlorine on the sprouts showed an efficient method to reduce the microorganism growth, whereas the probiotics presented a different colony forming in the analysis controlling the development of previous microorganism.

KEYWORDS: food safety; alfalfa; broccoli; microbiological tests.

1. INTRODUCTION

The consumption of sprouting seeds has been a common practice in many cultures for years. This is a fast and easy process, and can be done at home or at commercial scale. Sprouts usually emerge in 2-7 days, depending on seed type; in addition, high level of moisture is required for the germination of the seeds.

Sprout is a nutritious food for the human diet because it is a good source of carbohydrates, fats, proteins, vitamins and minerals. Nevertheless, it is also an appropriate medium for bacterial growth (Feng, 1997).

Because outbreaks of foodborne illness have been associated with the consumption of raw sprouts recently, microbiological testing of sprout medium has been indicated as part of an overall strategy to enhance the safety of sprouts (Fu et al., 2000).

The most causative agent has been Salmonella, although Escherichia coli O157:H7 and Bacillus cereus have also been linked with the outbreaks. Studies have indicated that pathogens can exceed 10⁷ per gram of sprouts produced from inoculated seeds during sprout production without adversely affecting the appearance (Taormina, 1999).

Effective treatments of sanitizing sprouting seed are needed due to foodborne outbreaks related to the consumption of contaminated sprouts such as alfalfa and broccoli. The capability of
chlorinated water to eliminate bacterial human pathogens from mung seeds was determined by treatment of seeds with high levels of chlorine (16,000 to 18,000 ppm). Populations of *Salmonella* and *E. coli O157:H7* were able to reduce by up 99.99% without harming germination of the seed; therefore, treatment of sprouting seed with chlorinated water will help to ensure the microbial safety of sprout (Fett et al., 2001).

Probiotics can be considered functional foods because they provide health benefits in addition to the traditional nutrition function. Most probiotic products contain lactic-acid-producing bacteria such as *Lactobacillus* and *Bifidobacterium*.

Studies suggest some beneficial effects of probiotics such as enhancement of the intestinal microflora capacity to block the invasion of potential pathogens, relief of symptoms of irritable bowel syndrome, prevention of colon cancer, inhibition of Helicobacter pylori, and reduction of blood cholesterol level. An adequate level of viable bacteria in a probiotic product is critical to achieve a health benefit. Probiotics could be promoted as a beneficial food supplement because they are not linked to pathogenic microorganisms (Lin, 2003).

This research project involves the production and safety assessment of green sprouts in a novel device – a bioreactor developed by research students from the Illinois Institute of Technology (IIT) and printed by a 3D printer.

2. MATERIALS AND METHODS

The bioreactors started running using 2.5 grams of broccoli and alfalfa seeds, 0.05 grams of fertilizer (*Miracle Gro*), and distilled water. In order to have a distributed flow, it was regulated the pump air through the reactor, as shown in figure 1. In the first week, the reactor received only the components listed above, and in the second and third week, it received chlorine and probiotic, respectively. During the experiments with chlorine, two methods had the efficiency tested: the application of chlorine drops every day and once in the beginning of the reaction. In the probiotic experiment, a capsule of commercial product containing it was diluted in distilled water, homogenized, and warmed to a better dissolution and then applied to the reactor with seeds and fertilizer.

Figure 1 – Bioreactor with seeds in a distributed air flow.
Samples of sprouting water medium were taken every day for 4 days and microbiologically analyzed with the Pour Plate Technique. This method consists of diluting the samples several times until the bacteria colonies count is possible. The diluted solutions were placed in Petri dishes, where agar nutrient was added later. The Petri dish was gently mixed to the solution, and stored in an incubator at 37ºC, then after 24 hours the counting was done.

3. RESULTS

The counting of Colony-Forming Unit (CFU) was made in triplicates for more accuracy, the results were organized in figure 2 and 3 for broccoli, and alfalfa sprouts.

Figure 2 – CFU counts of sprouting medium of broccoli seeds for various protocols.

Figure 3 – CFU counts of sprouting medium of alfalfa seeds for various protocols.
The control experiment presented a high bacteria count, as expected because of the absence of any component besides the fertilizer. However, the reactors that received chlorine had a lower CFU number and the experiment with daily chlorine addition showed a better result if compared to the once addition of it. The figures 2 and 3 show the best performance of the experiment with chlorine.

The reactions with probiotic bacteria presented a high CFU count due the presence of various strains in the compost, but it was possible to notice that the colony formed had a different shape and size from the previous ones of control and chlorine experiments (figure 4 and 5), proving that the probiotic bacteria prevented the growth of other microorganisms.

In comparison with previous studies of Fu et al. (2000) the reactor sprouting had a 3 to 4 log lower.

Figure 4 – Pour plate test results of regular reactor sprouting at $10^{-2}$ dilution.

Figure 5 – Pour plate test results of reactor sprouting with probiotics at $10^{-2}$ dilution.

The sprouts were ready to be harvest after 4 days and the wet weights were 18.6 grams and 13.4 grams, for alfalfa and broccoli respectively. The figure 6 shows the harvested sprouts.
4. CONCLUSION

According to the results, the chlorine was effective to keep the bacterial count low. On the other hand, the samples containing probiotic bacteria presented a different culture formation, showing that the presence of probiotic microorganism effectively prevented the growth of the other organism.

In future work, composition analysis and microbiological tests should be done for identification of pathogen bacteria, to ensure that the sprout product is not only safe for human consumption, but also delivers nutrition and probiotic function.

5. REFERENCES


6. ACKNOWLEDGES

The authors gratefully acknowledge the financial support of Coordination of Superior Level Staff Improvement (CAPES) and Institute of International Education (IIE).