Aquaponics

Food Safety Hazards Associated with Smooth-Textured Leafy Greens Produced in Aquaponic, Hydroponic, and Soil-Based Systems With and Without Roots at Retail

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EXECUTIVE SUMMARY

Much confusion exists about the foodborne hazard potential of different agricultural systems. This experiment examined the food safety hazard potential of smooth-textured leafy greens (STLG) produced in aquaponic, hydroponic, and soil-based agricultural systems, both with and without an attached root at grocery stores. Of these six treatments, there were three samples collected from five representative sources (e.g. grocery stores or producers). Samples were analyzed for aerobic plate count (APC), enterobacteriaceae, coliforms, non-pathogenic *E. coli,* and listeria. The results indicate that there is no significant difference between treatments in APC (p=0.166), enterobacteriaceae (p=0.328), non-pathogenic *E. coli,* and listeria (p=.866), indicating that aquaponic production systems have the same hazard potential as hydroponic and soil-based production systems. Even though there was no statistical significance regarding listeria, all the samples did contain listeria. The presence of listeria is concerning, especially because 40% of samples of hydroponic produce without roots contained listeria. There is a significant difference between treatments for coliforms (p=0.015); however, the means were classified in the same group due to the high variance in data. The presence of an attached root was found to have no correlation with contamination potential at grocery stores in all tests. Given that aquaponics is a new and small industry compared to soil-based produce production, a foodborne illness outbreak could greatly decrease consumer trust. It is recommended that good agricultural practices (GAP's) be developed and implemented for aquaponic systems to reduce the risk of bacterial contamination and protect consumer health.

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INTRODUCTION

Issue Description

The hazards encountered by consumers purchasing smooth-textured leafy greens (STLG) grown aquaponically, hydroponically and in-soil, both with and without attached roots at grocery stores are addressed in this study. With various food production techniques in use today, questions have arisen about the food safety hazards associated with these techniques. STLG generally fall into the class of foods described as ready-to-eat (RTE), meaning the product will usually be consumed raw. Cooking can remove bacterial contamination; therefore, foods that do not get cooked before consumption can pose a greater food safety risk. Another factor affecting contamination may be the presence of roots at retail, which are occasionally left intact as an indicator of freshness or 'natural production'. It is known that roots in aquaponics, like other production systems, are a common place for pathogen formation when analyzed at the site of production, due to the wet environment and large surface area (personal communication with Nick Phelps and Michele Schermann, 13 Feb. 2015). However, it is not known if bacterial contamination is more likely in produce at grocery stores that is sold with an attached root.

Literature Review

The association between foodborne pathogens and the food production technique used, as well as the presence of an attached root at retail, needs further research and exploration to be fully understood by professionals and consumers. The growth of pathogens on food grown in soil-based systems that use manure additions as the main source of fertility, are a well known concern in RTE foods (Leifert et al., 2008). Zoonotic pathogens like *Escherichia coli* (*E. coli)* can survive in manure for months (WHO, 2011). These pathogens will grow on the exterior and interior of contaminated plants, potentially causing foodborne illness when consumed (Teplitski et al., 2009). Contamination can often be traced to untreated irrigation water, contaminated soil, uncomposted manure treatments, and human touch. Contamination varies depending on the plant species, but persists on the plant longer when the produce is damaged by harvest (Critzer & Doyle, 2010).

The assumption that common pathogens need a warm-blooded host to thrive causes some to believe that harmful pathogens are not able to survive in aquaponic systems, which use cold-blooded fish (Bernstein, 2011). Nina Fedoroff (2013) has proposed aquaponics as a solution to pathogen contamination that is spread from manure-fertilized soil to the foods grown on that soil. Similarly, Michelle Menken, a certified organic food inspector with the Minnesota Crop Improvement Association, supports the idea that aquaponic systems pose minimal food safety risk due to the lack of warm-blooded animal manure (personal communication, 3 Feb. 2015). However, others believe that adding fish to an agricultural system could add additional food safety concerns, including increased risk of Salmonella and *E. coli* (Klinger and Naylor, 2012).

Hydroponic food production systems are free from animal manure and animals in general, suggesting that they may pose less of a food safety concern to consumers. One study comparing the incidence of internalized *E. coli* O157:H7 on hydroponic, and soil-grown spinach, found that soil-grown spinach had a higher likelihood of internalizing the contaminant than hydroponic, though *E. coli* internalization does occur in hydroponic systems. These researchers proposed that this was because internalization of contaminants increases when roots are damaged, which occurs more often in soil-based systems than hydroponic systems (Macarisin et al. 2014). Although, high levels of *E. coli* have also occurred on lettuce roots grown hydroponically when exposed to contaminated water (Wachtel et al. 2002).

Literature describing the contamination potential of produce sold with an attached root is extremely limited, and in need of further research. Roots contain a large surface area, and exist in a wet environment. These conditions allow the roots of plants to be a common place for pathogen formation (personal communication with Nick Phelps and Michele Schermann, 13 Feb. 2015). Selling plants with the root attached would provide an ideal environment for those pathogens to cross-contaminate the edible portion of the food. Handling techniques during and after production can also affect the pathogens found on the food (Sirsat and Neal, 2013). Any contact the roots have with the edible portion of food during handling and in the marketplace could lead to an increased foodborne illness hazard for food sold with the roots intact (Wachtel et al. 2002).

Pathogens of Concern

A variety of tests were performed to identify indicator-bacteria and pathogens of concern, including aerobic plate count, coliforms, enterobacteriaceae, listeria, and non-pathogenic *E. coli*.

Aerobic plate count (APC) is a count of all the bacteria in a sample that is able to to be cultured in the presence of oxygen. Most of these bacteria are not a food safety concern, but they can show the suitability of the plant matter as a growing media for harmful bacteria.

Coliforms reflect fecal contamination from warm-blooded animals, including humans, but are not an accurate indicator of fish manure contamination. For example, coliforms can be introduced into an aquaponic system by a person in contact with the system who has not properly washed their hands.

Enterobacteriaceae is a group of gram negative, rod-shaped bacteria. These bacteria can be found in soil, marine environments, vegetable matter, and the intestinal tract of humans. Although many enterobacteriaceae are not harmful, some serious human pathogens such as *E. coli* and *Salmonella* are enterobacteriaceae. High numbers of enterobacteriaceae can indicate the growth potential for pathogenic organisms.

Listeria is a genus of bacteria, but only one of them, *Listeria monocytogenes*, is of concern for food safety. The USDA threshold for *L. monocytogenes* is zero tolerance

(Chen et al., 2003). Presence or absence of Listeria spp. in a sample, as it was measured for this study, indicates that there is a suitable environment and exposure risk for *L. monocytogenes*.

E. coli proliferate in the lower intestine of warm-blooded organisms, and their presence on RTE foods show a lack of proper hygiene. Most strains are relatively harmless, but *E. coli* 0157:H7 is a serious human pathogen. Like with the listeria test, presence of *E. coli* does not necessarily mean that the harmful strain is present. It does, however, indicate the suitability of the environment and exposure risk for *E. coli* 0157:H7.

Series Goals

A comprehensive, informational library is compiled through student research as part of an ongoing research project at the University of Minnesota in HORT 4601: Aquaponics: Integrated Fish and Plant Food Systems. The series mission is to supply interested parties with valuable information regarding aquaponics to expand the field and help provide sustainable, safe, healthy, and reliable products. These products may be physical, educational, or value based.

Chapter Goals

The goal of this research is to better understand the varying food safety hazards associated with aquaponic, hydroponic, and soil-grown STLG available with and without attached roots at grocery stores in Minnesota.

Objectives

1. Collect STLG samples at grocery stores produced in aquaponic, hydroponic, and soil-based systems with and without attached roots.
2. Quantify and compare bacterial contamination of STLG samples collected in Objective 1.

METHODS

Site Description

The following site description contains the conditions under which a group of students assessing food safety hazards conducted research. These students were part of a larger collaboration of students all in the 2015 University of Minnesota HORT 4601 spring semester course. The location of the aquaponics research was in the Minneapolis/Saint Paul Metropolitan area and the Duluth area, State of Minnesota, U. S. A. Smooth textured leafy green produce samples were collected at grocery stores within 48 km (30 mi) of the cities Minneapolis, St. Paul, and Duluth. The site description of specific retailers and produces was kept anonymous, as the goal of the research was to assess the difference between production methods, not specific producers or retailers. All samples were analyzed at Market Fresh Lab in Minneapolis, MN.

Study Design

This study uses a quasi-experimental design in which the soil-based system represents the control group, with both the hydroponic and aquaponic production systems representing the variables. Being that the aquaponic industry is so new, there are a limited number of aquaponic producers represented in the collected samples. Ideally, aquaponically produced STLG samples would be obtained from five separate producers. In regards to the hydroponic and soil-based produce that was analyzed, each sample came from a different producer and grocery store. In congruence with the chapter goals, all actions that were taken regarding the retail sites aligned with the possible actions taken by everyday consumers at the grocery stores.

Hypotheses Tested

The hypothesis being tested is that there is no bacterial contamination difference between aquaponic, hydroponic, and soil-based systems at grocery stores. Furthermore, there is no bacterial contamination difference between the STLG's packaged with or without attached roots.

Research Techniques

Collection of Samples

Smooth textured leafy green samples from three production systems with and without attached roots were purchased at grocery stores for a total of six treatments. For the six different treatments five different representative samples were chosen that differ either in the store where the STLG were purchased, or the location where the STLG was produced. Three replications of each representative sample were collected. This sampling scheme resulted in the analysis of 78 samples due to the limited availability of aquaponically produced STLG with roots.

Twenty-five gram samples were purchased from the grocery store. If prepackaged, the sample was not removed from the packaging. If the sample was not prepackaged, researchers obtained a plastic produce bag, and placed one hand on the outside of the bag such that the bag was used as a buffer or glove. These practices ensured that in both methods the lettuce samples never touched the hand of the researcher. Samples were labeled and placed in a cooler with frozen gel packs for same day delivery to the laboratory. Samples were tested at Market Fresh Lab, Minneapolis, MN, within 24 hours of delivery for indicator bacteria including aerobic plate count (APC), coliforms, enterobacteriaceae, non-pathogenic *E. coli,* and listeria (Horowitz and Latimer-Gaithersburg, 2006).

Analysis of Data

The values of indicator bacteria were analyzed based on treatment and condition in order to determine associations in production and handling. Analysis of Variance (ANOVA) was used to analyze if there was a difference in means between the treatments to determine statistical significance ($p \leq 0.05$). A Tukey test was then used to determine what those differences were. A chi-squared test was used to determine if there was a difference with and without attachments of roots and between the treatments when analyzing listeria.

Presentation of Findings

To effectively educate consumers and producers about the findings and recommendations of this study, the research was presented at the 2015 Aquaponics Symposium: Aquaponics in Minnesota, Recent Findings and Best Practices in Food Safety. The poster produced for this presentation can be found on the HORT 4601 aquaponics page (www.aquaponics.umn.edu).

FINDINGS

Aerobic Plate Count (APC)

High levels of aerobic bacteria were cultured from all treatments (Figure 1). The presence of a high level of bacteria could indicate contamination in the production system during production, processing, or at the commercial retailer. The y-axis in Figure 1 is logarithmic, indicating that each line represents a 10-fold increase in amounts of bacteria.

There is no significant difference in APC values between the treatments (p=0.116), resulting in failure to reject the null hypothesis. As seen in Figure 1, there were small differences amongst treatments; however, the large variability in the results may have contributed to the lack of statistical significance.

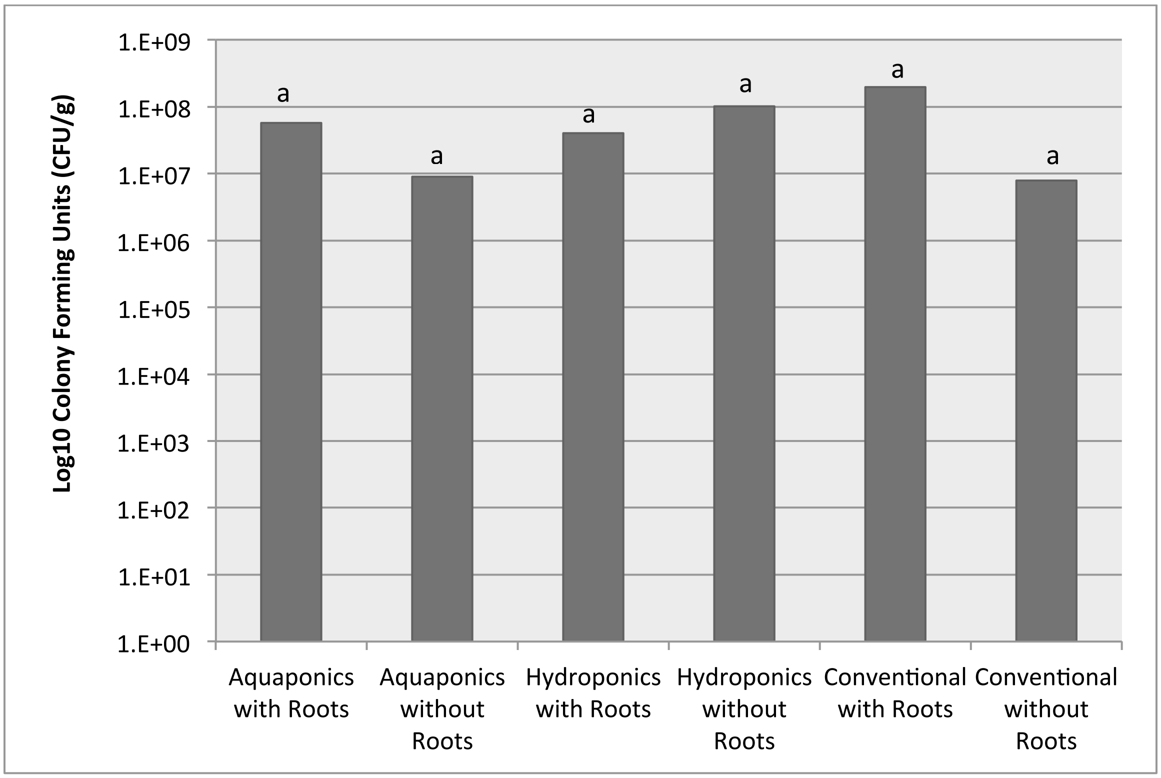

Figure 1: Aerobic Plate Count (APC) of smooth textured leafy greens purchased at grocery stores

Coliforms

Coliforms were present in all treatments, as displayed in Figure 2. These results indicate all treatments were tainted by fecal matter from warm-blooded organisms at some point in production, processing, or retail sale. The y-axis of this graph is logarithmic, indicating that 10-fold variance between treatments.

According to the ANOVA test, there is a significant difference in coliforms between the different treatments (p=0.015), however secondary tests could not determine statistical difference of the means due to the high variance of the data. This indicates that the null hypothesis can be rejected in regards to coliforms, but cannot say which treatments are different from the others.

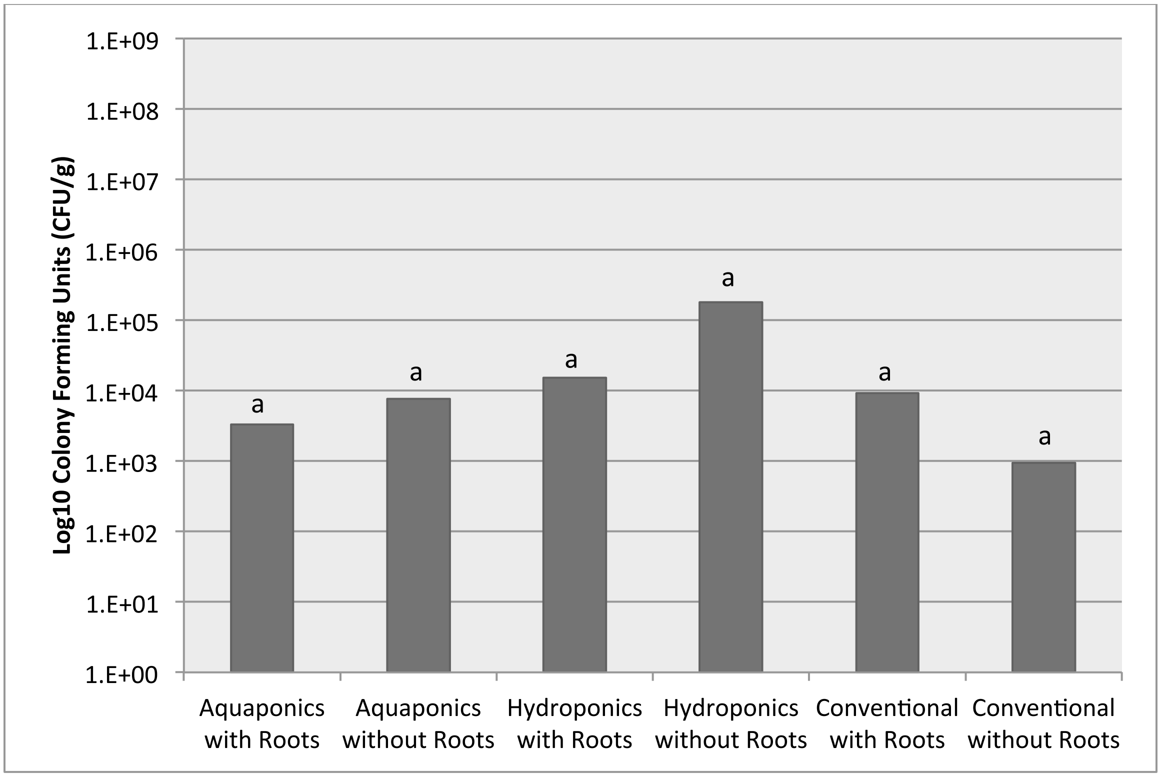

Figure 2: Coliforms of smooth textured leafy greens purchased at grocery stores

Enterobacteriaceae

Figure 3 indicates the level of enterobacteriaceae in the different treatments, suggesting the potential for growth of pathogenic organisms like *E. coli* and *Salmonella* in all production systems. Aquaponic STLG with attached roots had a low number of enterobacteriaceae, but only one source of samples was available. The y-axis of this graph is logarithmic, thus the difference in one line is actually a 10-fold difference.

There is no significant difference in enterobacteriaceae between the treatments (p=0.328). This indicates that there is a failure to reject the null hypothesis.

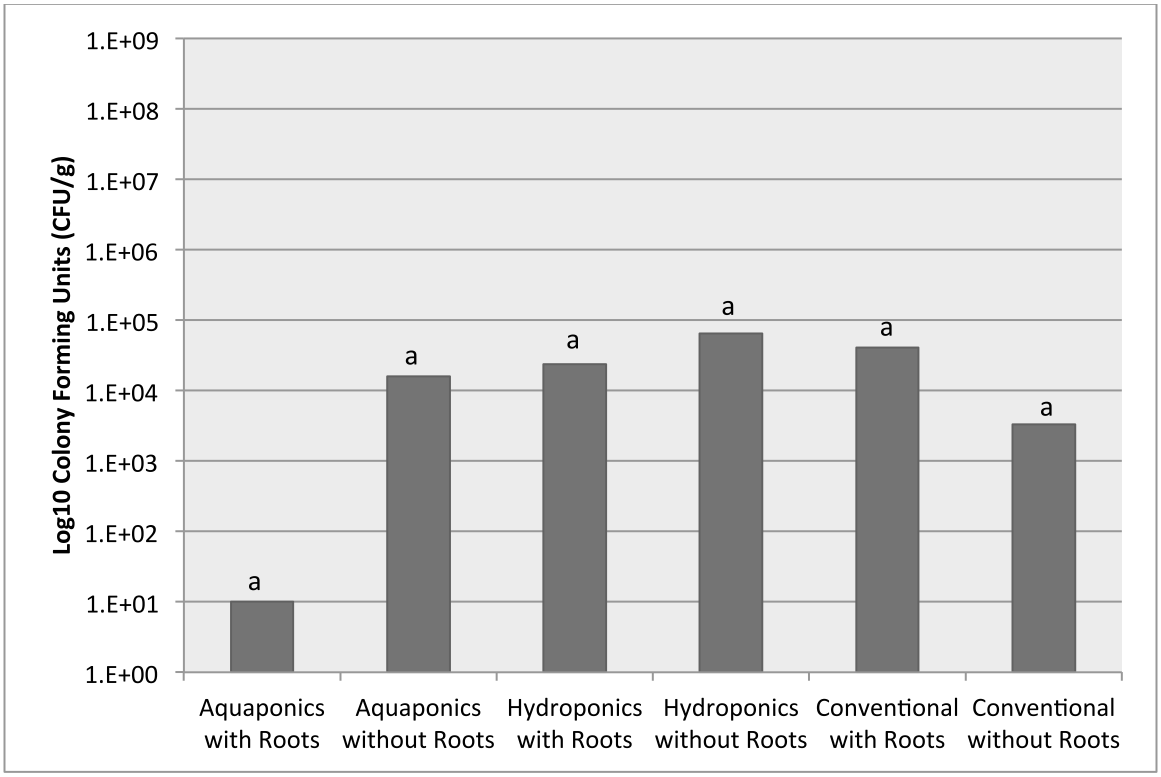

Figure 3: Enterobacteriaceae of smooth textured leafy greens purchased at grocery stores

Listeria

A genus specific test was run to determine the presence of *Listeria spp.* in the plant material samples (Figure 4). Though the *Listeria spp.* test does not necessarily indicate presence of harmful *L. monocytogenes,* which is considered an adulterant and has zero-tolerance by the USDA, it does indicate that listeria bacteria are present. Presence of any listeria species suggests that the environment is suitable for listeria and there is an introduction source. The y-axis shows the number of tests positive for *Listeria spp.* out of the total number of samples.

There is no statistical significance between the food production system and the presence of listeria (p=0.866). Hydroponic STLGs, both with and without roots, have the highest probability of listeria at 27% and 40%, respectively. Even though listeria presence in hydroponics without roots is not statistically significant, it is biologically significant due to the harm listeria can cause in respect to human health. It is plausible, that the high levels of listeria in hydroponic system is due to a lack of perceived risk leading to limited food safety protocols or good agricultural practices. In addition, the lack of other environmental bacteria may allow a single *Listeria spp.* bacteria to proliferate without competition. A chi-squared test also showed that there was no significant difference in listeria contamination between presence and absence of roots (p=0.76), failing to reject the null hypothesis.

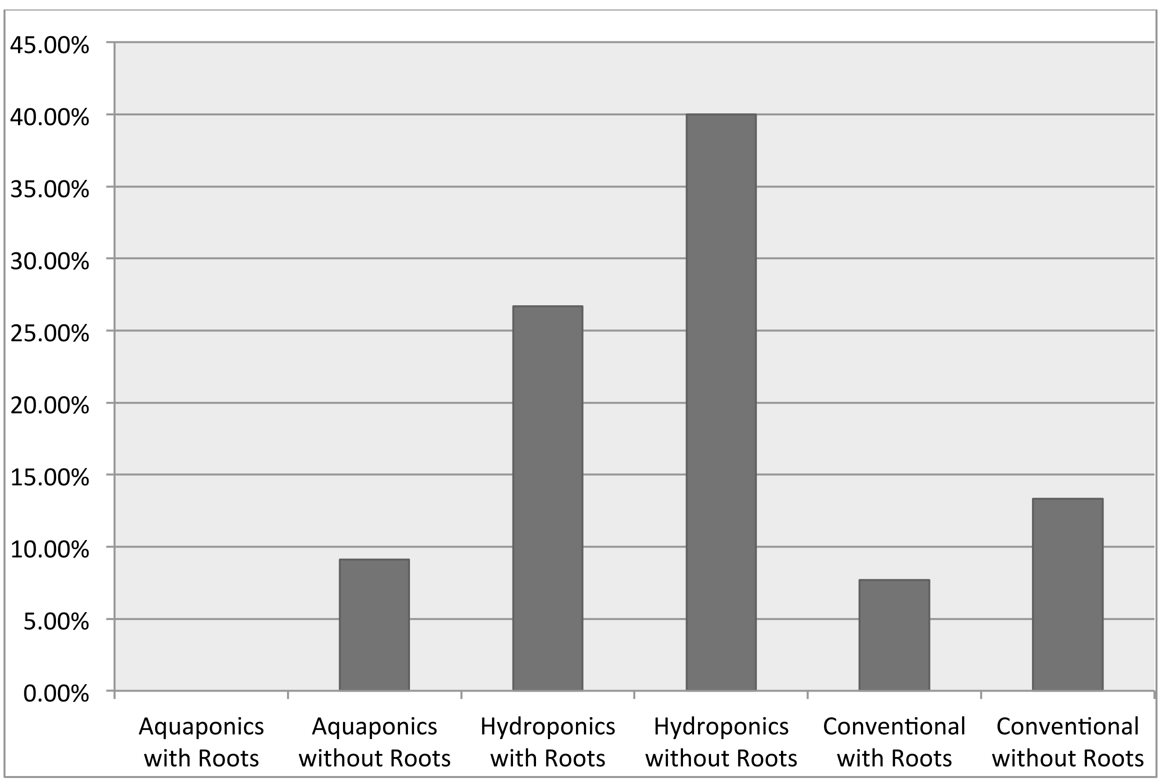

Figure 4: Listeria on smooth textured leafy greens purchased at grocery stores

E. coli

All treatments have a very low value for non-pathogenic *E. coli* at <10 CFU. This means that there is no significant difference in *E. coli* for these treatments. The test indicates fecal contamination and whether the environment is viable for more harmful strains of *E. coli.* In these samples, the presence of *E.coli* was managed appropriately by the practices in the production, processing, and retail site. All systems and RTE STLGs should continue to be monitored to ensure minimized food safety hazard regarding *E. coli.*

Recommendations

Recommendation 1: Avoid human introduction of pathogens

All tests for indicator bacteria and pathogens indicated that bacterial contamination had occurred or the environment was suitable for future contamination in the three production systems. Since manure is not present in aquaponic and hydroponic systems, like in soil-based systems, it is reasonable to infer that the pathogens where introduced by humans or pests. Thus, it is important to limit human interaction to prevent transfer pathogens into the system and strictly monitor for pests. Some possible practices to reduce contamination include limiting visitors to the system, having strict hand washing protocols, and packaging the greens instead of leaving them loose in the grocery store.

Recommendation 2: Inform consumers that the food safety hazards associated with aquaponic RTE STLGs are the same as those for soil-grown and hydroponically grown food.

Consumers may incorrectly perceive that aquaponic produce in general is safer because it is not grown in soil, it is often grown locally, and it is often more expensive than soil-grown produce. However, this study demonstrates no significant difference between contamination at grocery stores for aquaponic, hydroponic, and soil-grown STLGs. Thus, STLGs from any system need to be appropriately washed, even if they are not in direct contact with animal waste or soil. Unlike soil, which can be detected visually, bacterial contamination is undetectable to the average consumer and illustrates the importance of studies like this to gain consumer awareness about food safety risk between different products. Consumers also need to be aware of the symptoms of various foodborne illnesses and seek medical attention when necessary.

Recommendation 3: Develop and implement a set of good agricultural practices (GAP) to address food safety in all agricultural systems, especially in aquaponics and hydroponics systems that generally don't have GAP's in place yet.

The data indicates there is just as much of a food safety hazard for hydroponic and aquaponic systems as there is for soil-based systems. Thus, it is crucial that similar GAP's or protocols regarding food safety be designed and implemented in all systems. The US Food and Drug Administration should be as concerned about implementing regulations regarding GAP's in food safety for hydroponic and aquaponic production as they are with soil-based agriculture. Even though hydroponics is a well-established production system, there is still minimal data regarding food safety protocols in these systems because there is such a small perceived risk. Data collected through this experiment disproves this perception, indicating that the hazard is not different from soil-based systems that are required to implement GAP's regarding food safety. In addition, the newness and the size of the aquaponic sector make it particularly vulnerable to damage if an outbreak were to be traced back to the sector or a specific aquaponic system. It is vital, therefore, that food safety is seriously considered by aquaponic and hydroponic producers for the good of human health and the viability of the sector.

Conclusions

Contaminated soil and uncomposted manure treatments are potential sources of pathogen accumulation that may apply solely to soil-based systems. However, contamination can also be traced to untreated water and human touch, both of which are critical components of aquaponic and hydroponic systems (Critzer & Doyle, 2010).

The data for this experiment shows no statistical difference for APC, enterobacteriaceae, listeria, and non-pathogenic *E. coli* between treatments, which indicates that there is not a reduced hazard for aquaponics or hydroponics compared to soil-based systems. It is important, therefore, that producers develop their own GAP's, similar to those that soil-based food production systems adhere to.

Aquaponic food production currently does not have required GAPs, but the results provided in this experiment provide enough evidence and incentive for each producer, processer, and retail store to make their own GAP or food safety protocol. This will help protect human health and safety, as well as ensure the viability and continued growth of each business that works with aquaponically produced STLGs.

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