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THE EFFICACY OF ATP MONITORING DEVICES AT MEASURING ORGANIC MATTER ON POSTHARVEST SURFACES

A Thesis Presented

by

KRISTIN MARIE LANE

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Food Science

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ORGANIC MATTER ON POSTHARVEST SURFACES

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ABSTRACT

THE EFFICACY OF ATP MONITORING DEVICES AT MEASURING ORGANIC
MATTER ON POSTHARVEST SURFACES

SEPTEMBER 2019

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The Food Safety Modernization Act (FSMA), specifically the Produce Safety
Rule (PSR), requires growers to clean and sanitize food-contact surfaces to protect
against produce contamination. The ATP monitoring device is a potential sanitation tool
to monitor the efficacy of an on-farm cleaning and sanitation program that could help
growers meet regulatory expectations mandated by PSR. The ATP device uses
bioluminescence to detect all ATP (found in bacteria and produce matter cells) from a
swabbed surface. Little work has been done to test the efficacy of these tools under
postharvest conditions. The present study evaluated ATP measurement for postharvest
surface cleanliness evaluation. Concentrations of leafy greens (spinach, romaine, red
cabbage) (with/without *L. innocua*) were used as organic matter inocula onto stainless
steel, HDPE plastic, and bamboo wood coupons to represent postharvest surfaces. The
ATP levels on the coupons were measured using swabs and ATP monitoring device.
Results showed that the concentration of *L. innocua* and leafy greens on a surface had a
highly significant effect on the ATP device reading (P<0.0001). There was a limit of
detection of *L. innocua* at 4.5 log CFU/coupon where the ATP device could no longer
detect ATP from *L. innocua*. The type of leafy green on a food-contact surface did not
affect the ATP reading (P=0.88). Leafy greens with *L. innocua* had a higher ATP reading

when compared to saline and *L. innocua*, demonstrating the presence of leafy green matter contributes to ATP reading when combined with *L. innocua*. The different food-contact surfaces had different ATP readings (P=0.03) and the ATP device did not detect bacterial or leafy green ATP from bamboo wood surfaces (P=0.16). Based upon our results, ATP measurement is an appropriate tool to measure produce or bacterial contamination on stainless steel or HDPE plastic surfaces, however it is not recommended for wood surfaces.

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CHAPTER 1

LITERATURE REVIEW

Introduction

The Food Safety Modernization Act (FSMA) created stricter science-based standards in food safety in 2011 to prevent foodborne illness in the United States *(18)*. Within FSMA, the Produce Safety Rule (PSR) created new practices that minimize the risk of human illness by preventing foreseeable biological hazards that could adulterate raw agricultural commodities (RAC) on farms *(20)*(21 CFR 112). 21 CFR 112.123(d)(1) requires covered farms to "sanitize all food contact surfaces of equipment and tools used in covered activities as frequently as reasonably necessary to protect against contamination of covered produce" *(20)*. PSR compliance can be difficult on farms because materials used as food-contact surfaces, such as wood, can be difficult to clean and sanitize and can harbor bacteria *(1, 35, 38, 56, 64)*. Improper worker hygiene and training, wild animals, farm visitors, contaminated irrigation water, and improper fertilizers increase the risk of food-contact surface contamination on farms *(5, 36, 37, 53, 60, 64)*. Needs assessment studies have shown that growers would like more technical support and trainings on how to be compliant not only to FSMA, but also food safety regulations set by buyers *(17, 57)*. Many growers that are not covered under PSR still must comply with food safety regulations set by buyers and private retailers in the food industry *(57)*. Increased education on food safety regulations was reported as a top need of growers and will continue to increase in the coming years *(17, 57)*.

The ATP monitoring device is a potential tool that could help growers in PSR compliance by determining the cleanliness of postharvest surfaces. The ATP monitoring

device is a sanitation tool that detects ATP from the cells of organic matter (which can include produce and bacteria) after a surface has been cleaned and sanitized. The ATP device can tell the grower if the cleaning and sanitation plan in place effectively removed all organic debris from a surface *(2, 59)*. The ATP device works in real-time so that a re-clean of a surface can be performed immediately if needed *(2, 34, 44)*.

The ATP device has been used in food processing centers *(42)*, food service establishments *(13, 49)*, breweries *(16, 44)*, and hospital kitchens *(2)* to verify if cleaning and sanitation procedures are effective. Despite success in other food environments *(31, 42, 46)*, it is important to test the efficacy of the ATP device in an on-farm environment due to different contamination risks *(5, 60)*, different prevalence of food matrices *(20, 60)*(21 CFR 112.1), and different food-contact surfaces *(56)*. Farms use wood surfaces, due to convenience, that can be more difficult to clean than the smooth surfaces (such as stainless steel) required by regulation in food processing centers *(6, 55, 56)*. Products on farms are RACs, which do not have the same level of microbial kill steps that products in food processing centers have, creating an increased concern for contamination *(20, 54, 60)*(21 CFR 112.1). Farms have an increased risk of surface contamination from wild and domesticated animals, farm visitors, irrigation water, and fertilizers that are not present in food processing environments *(5, 20, 37, 64)*(21 CFR 112.81, 112.33).

The ATP device differed in results when different types of bacteria *(11, 59, 61)*, surfaces *(1, 35)*, and food matrices *(31, 40, 62)* were tested. A gram-negative bacteria was more difficult to detect by the ATP device than a gram-positive bacteria *(40, 59, 61)*. Although not previously tested with the ATP device, wood surfaces have been demonstrated to be more difficult to detect bacteria from than plastic *(1)*. Food matrices

higher in protein have been shown to be more difficult to detect by the ATP device than food matrices lower in protein *(31, 40, 61)*. The differences in ATP device results based on different environments suggests that the ATP device should be confirmed for use on-farm before it is recommended to growers.

The Food Safety Modernization Act

Major concerns about foodborne illness and cross-contamination in the United States led to the enactment of the Food Safety Modernization Act, or FSMA, to take preventive, rather than reactive, action to reduce the risks and causes of foodborne illness *(18)*(21 CFR). The law's goal is to create a safer food supply and cut back bacterial food contamination in the food industry *(18)*(21 CFR). FSMA is broken into seven main rules *(18)*(21 CFR). One of the main rules is the Produce Safety Rule (PSR) *(20)*(21 CFR 112). PSR created new produce safety practices to minimize adverse health risks associated with biological hazards that could adulterate produce *(20)*(21 CFR 112). Unless covered under an exemption, all farms that have RACs must comply to the standards *(20)*(21 CFR 112.1). PSR created regulatory guidelines for eight specific areas on farms: personnel qualifications and training, health and hygiene, agricultural water, biological soil amendments, domesticated and wild animals, growing, harvesting, packing and holding activities, equipment, tools, buildings, and sanitation, and sprouts *(20)*(21 CFR 112). Farms are required to document compliance to PSR by keeping records on environmental monitoring plans and analytical methods used within the environmental monitoring plan *(20)*(21 CFR 112.150). A farm is covered under PSR if the average value of produce sold over the last 3 years was more than $25,000, after accounting for inflation *(20)*(21 CFR 112.4). It is possible for a farm to be exempt from the rule and have modified

requirements to follow *(20)*(21 CFR 112.5,6). However, even if a farm is exempt from the rule, there are food safety policies set by buyers and private retailers which require documentation of GAPs (good agricultural practices) to sell a grower's produce *(57)*. The documentation required across buyers can differ, making it more complicated for growers to keep track of compliance to both buyer demands and federal regulations *(57)*. Growers have reported a desire for increased education on federal regulations and technical support *(17, 57)*. Identifying sanitation tools, such as ATP monitoring devices, can help growers with their request for food safety support.

Foodborne Illness

1 in 6 people are affected by foodborne illness each year in the United States *(18, 52)*. Foodborne illness comes from the consumption of a food product that has been contaminated with pathogenic bacteria *(47)*. On farms, pathogenic bacteria can come from sources such as manure, processing equipment, insects, irrigation water, and human workers *(45, 60, 64)*. Cross-contamination can occur if workers are not properly trained on how to follow GMPs (good manufacturing practices) such as proper hand washing technique *(36, 53)*, or proper cleaning and sanitation procedures. On farms, cross-contamination is more difficult to control because commonly used surfaces such as wood can be more difficult to clean *(35, 38, 56, 64)*.

Produce-Associated Foodborne Illness

Over the years, there has been an increase in contamination of produce by pathogenic bacteria on farms *(45)*. Contamination of produce is a concern because produce is commonly consumed raw and does not receive additional processing to kill

bacteria from its surface *(7, 45, 54)*. Between 1998-2008, 46% of foodborne illnesses were caused by the consumption of raw produce *(47)*. Contaminated produce has been estimated to cause 20 million illnesses and cost 38 billion dollars in the United States each year *(45)*. A goal of FSMA is to reduce the number of foodborne illnesses, and specifically produce, through PSR *(18, 20)*(21 CFR 112.1).

Leafy Green-Associated Foodborne Illness

Leafy green vegetables, in particular, are a major source of foodborne illness outbreaks in the United States (22%) *(27, 47)*. When comparing vegetable row crops, leafy greens were responsible for 98% of the foodborne illness outbreaks between 1998 and 2013 *(5)*. Spinach, lettuce, and cabbage were in the top seven most commonly contaminated leafy greens in California from 1996-2016, with spinach and lettuce being the top two *(60)*. Leafy greens can have pathogens in their natural bacterial microflora that if not cleaned effectively can cause illness *(21, 37)*. Leafy greens are most commonly contaminated with the pathogenic bacteria *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes (37)*. In 2006, spinach contaminated with *E. coli* was the cause of a major foodborne illness outbreak in the U.S and Canada *(45)*. In 2010, romaine lettuce contaminated with *E. coli* caused a major foodborne illness outbreak in the U.S. *(45)*. In 1981, 42 cases of listeriosis were caused by cabbage contaminated by untreated manure *(41)*. Leafy greens are among the RACs covered under PSR *(20)*(21 CFR 112.1).

Listeria-Associated Foodborne Illness

Listeria species are a concern in the food environment because the pathogen *L. monocytogenes* causes the serious disease listeriosis in susceptible populations *(23, 43, 50, 58)*. *L. monocytogenes* causes 1,600 foodborne illnesses each year in the United

States *(60)*. Listeriosis has a 20% mortality rate when developed in susceptible populations, making *L. monocytogenes* a pathogen of major concern *(23, 50, 58)*. The high-risk factor of *L. monocytogenes* has caused the United States to create a zero-tolerance policy *of L. monocytogenes* in 25g of RTE foods *(50, 58)*.

Listeria species are a concern on farms due to their persistent growth conditions and ubiquity in the environment *(43, 50, 58)*. *Listeria* commonly live in soil, manure, water, humans, animals, or reconstituted pesticides on farms, but can also live outside of a host *(21, 45)*. *Listeria* species can grow and survive in a pH range of 4.7 to 9.2, high salt concentrations, reduced water activity, and refrigeration temperatures (0.5-9.3°C) *(43, 54)*. *Listeria* species form biofilms on food-contact surfaces in difficult to reach places by attaching to the surface, often with other food particles *(50, 54, 58)*. The type of food particle on the surface can affect the biofilm attachment *(62)*. For example, absorbed proteins on a surface can hinder biofilm formation *(50)*. The species in the biofilms do not require as many nutrients to survive and can be very difficult to remove from a food-contact surface, especially in the presence of food particles *(50)*.

L. monocytogenes is most commonly found in dairy, seafood, vegetables, fruits and RTE foods *(23, 43, 54, 58)*. Between 1996 and 2016, *Listeria* species were the cause of 27 incidents of leafy green contamination in California *(60)*. In 2016, *L. monocytogenes* illnesses were most often linked to dairy and fruit products *(19)*. Specifically, between 2013 and 2016, there was a 9.1% increase in *Listeria* illnesses in the United States due to a multi-state outbreak of *Listeria* found in prepackaged lettuce in 2015 *(19)*. PSR requires the creation of a written environmental monitoring plan to detect for the presence of *Listeria* species on-farm *(20)*(21 CFR 112.145). The ATP monitoring

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device may be a viable tool to be incorporated into an on-farm environmental monitoring plan for *Listeria*.

Cleaning and Sanitation

Cleaning and sanitation is a process that 1.) removes debris and soil from the surface (cleaning) and 2.) reduces microorganisms to a safe level (sanitation) *(13, 29, 56)*. During the cleaning step, the surface is wet with potable water to initially remove debris. The surface is scrubbed with soap and water to mechanically remove debris. The surface is rinsed again to remove soap. During the sanitation step, the sanitizer is applied to the surface and allowed to remain on the surface according to manufacturer's instructions *(29, 56)*.

21 CFR 112.123(d)(1) of the PSR states that food-contact surfaces must be cleaned and sanitized a reasonable amount to ensure microbial hazards do not contaminate RACs *(20)*. It can be a challenge to control contamination of surfaces on farms due to animal presence, worker hygiene, agricultural water, and difficult to clean food-contact surfaces *(5, 37, 56)*. Many farms use wood food-contact surfaces that are difficult to clean and require additional surface scrubbing with a rough bristled brush to remove debris *(56)*. Farms have large pieces of machinery such as conveyors, wash tanks, and storage bins that can have hard to reach places to clean and sanitize that could harbor bacteria *(56, 58)*. Growers have requested technical tools and guidance to comply with new regulations for cleaning and sanitation *(17, 57)*.

Environmental Monitoring

Environmental monitoring (EMP) is a commonly used procedure in processing facilities that continuously evaluates an area to make sure procedures are working properly. EMPs are used in food facilities to continuously monitor identified biological (i.e. bacteria), chemical, and physical hazards in the environment that could contribute to food contamination. Monitoring sites should include locations in the plant where food is exposed to or areas known to harbor bacteria *(58)*. Most monitoring should include Zone 1 (food-contact surfaces) and Zone 2 (area surrounding food-contact surface) *(15)*.

PSR states that all equipment and tools, both food-contact and non-food-contact, must be monitored to prevent contamination of covered produce *(20)*(21 CFR 112.123). One way to monitor biological hazards on a farm is to swab a surface and ship the swab sample to a laboratory for microbial plate count analysis to determine bacterial presence *(3, 26)*. 21 CFR 112.124 requires record keeping of sanitizer efficacy and concentrations to ensure sanitizer concentration is not above an unsafe level, or so low the sanitizer does not work, as a way of monitoring chemical hazards *(20)*. Physical hazard monitoring on farms can be the inspection of tools such as forklifts and tractors for function of pieces that can contact produce *(20)*(21 CFR 112.124). Continual education of growers on how they can contribute to a safer food supply can include supplying them with tools on how to reduce microbial contaminations *(57)*.

Microbial Plate Counts

The focus of the present work is to determine the efficacy of sanitation tools to monitor the biological hazards of food-contact surfaces on farms. The approved method

to monitor biological hazards of food-contact surfaces in food processing centers is

microbial plate counts *(3, 20, 26, 34)*(21 CFR 112.152). Microbial plate counts require

the swabbing of a food-contact surface to retrieve a sample, sample preparation, transfer

onto microbial agar plates, and 24-48 hour incubation period before results can be

received *(10, 14, 24, 34, 40, 42, 44, 46)*. The results received by microbial plate counts

report the presence or absence of bacteria on a food-contact surface *(46)*.

Microbial plate counts can be used as a tool to determine cleaning and sanitation

efficacy by detecting residual bacteria on a food-contact surface *(34)*. PSR states food-

contact surfaces must be sanitized "as frequently as reasonably necessary to protect

against contamination of covered produce" *(20)*(21 CFR 112.123(d)(1)). It can be

difficult to determine how often to clean and sanitize food-contact surfaces since it takes

48 hours to receive microbial results with traditional microbial plate counts. Traditional

microbial plate count analysis cannot be performed on-site *(10)*. The sample must be

shipped to a laboratory with the appropriate tools such as an incubator and agar plates.

The shipment of the sample to the lab can add time to receive results *(10)*. It can be

inconvenient and expensive to ship samples to a laboratory *(10)*. The delay in results does

not allow for corrective cleaning action to be taken immediately *(40)*. A farm or

processing plant cannot hold production while waiting for results *(44)*. While waiting for

results, unclean surfaces can accumulate more debris and bacteria, creating a higher risk

of produce contamination *(40)*. Cleanliness of a surface is especially important if the

surface is Zone 1 (food-contact-surface) *(15)*. There is a risk of foodborne illness for the

consumer if bacteria that develops in Zone 1 is pathogenic and contaminates produce *(34,*

42). Food-contact surfaces must be cleaned and sanitized in a reasonable time period to prevent the spread of contamination of one food product to the other *(58)*.

A benefit of microbial plate counts is its ability to differentiate between bacterial presence and organic matter on a soiled surface by use of differential media tests *(34, 46)*. For example, Lahou and Uyttendaele used three swabs (sponge stick, environmental swab, foam spatula) on three food-contact surfaces (stainless-steel, neoprene rubber, HDPE plastic) to detect *L. monocytogenes (32)*. Differential microbial plate counts were used to determine 2 log CFU of *L. monocytogenes* was on the surfaces *(32)*.

ATP Monitoring Device

A possible sanitation tool to be used as a compatible method with microbial plate counts is the ATP monitoring device. The ATP monitoring device is a handheld unit that detects ATP from a swab sample. ATP is found in all living cells so it can be used to detect the presence of bacteria or organic matter from a food-contact surface *(9, 13, 34, 40)*. The ATP device is a rapid method because it can detect ATP in 15 seconds on-site *(13, 33, 34, 40, 44)*. The ATP device is used after a surface has been cleaned and sanitized. The ATP device can evaluate the efficacy of the cleaning and sanitation procedure by detecting residual ATP on the surface *(2, 10, 13, 14, 34, 40, 59)*.

A benefit of the ATP monitoring device is its ability to give results in real-time, allowing corrective action of unclean surfaces immediately *(13, 34, 40, 44)*. The ATP device detects ATP from both bacteria and organic residues on a surface *(13, 34, 40, 49)*. The presence of any organic residue points to ineffective cleaning of a surface *(34, 40, 44)*. Organic matter creates a favorable environment and nutrient availability for bacterial growth and adhesion to a surface *(13, 34, 40)*. The ATP device can be used to track

sanitation of surfaces over time since it is used on-site, and determine locations where residues are harbored *(40, 44)*. The ATP monitoring device has an initially high cost investment, but an inexpensive price per swab (about 2 dollars) *(13, 28, 40, 44)*. The ease of device use (requires only four steps) frees workers for other tasks and increases efficiency *(28, 44)*.

A limitation of the ATP monitoring device is its inability to differentiate between microbial ATP and food ATP *(33, 34)*. Knowledge of the type of bacteria present on a surface is useful if the ATP is from pathogenic bacteria, and should be a concern to the user *(34)*. The ATP device has limits of detection for detecting ATP from both bacteria and food residue. The limit of detection was found to be between 2 and 4 log CFU when bacteria were inoculated onto a surface *(14, 34, 40, 59)*. Differences in limits of detection among studies was seen due to differences among devices used and differences in inoculated bacterial cell structures *(31, 40, 59)*. The limit of detection for different food matrices inoculated onto food-contact surfaces was found to be between a 1:10 dilution and 1:1000 dilution *(59)*. The differences in food limits of detection was due to differences in food nutrition profiles *(62)*. Despite the limits of detection, the ATP device has been recommended in other works as a sanitation tool to determine if a surface needs to be re-cleaned and re-sanitized *(10, 13, 31, 44, 49, 59)*.

There are no standards for what is considered a "passed" or "failed" surface across the different brands of ATP devices, making it difficult to determine differences among ATP device brands *(9, 34, 49)*. Users can set the standards of "pass" and "fail" surfaces following manufacturer's instructions for the device brand of choice *(10, 28)*. Carrick et. al. found inconsistent results in microbial detection potentially due to the

inability to extract the ATP from microbial cells, absorption of ATP into the swab, or the

swab interfering with the bioluminescence detection *(9)*. Despite Carrick et. al.'s finding

of variability in ATP device readings *(9)*, other works *(2, 10, 13, 31, 34, 46)* have found

linear correlations between relative light units (RLU)(unit of ATP device) and colony

forming units (CFU)(unit of microbial plate counts) and recommend ATP device use.

How the ATP Device Works

The ATP monitoring device detects ATP, the energy source found in all living

cells *(9, 13, 34, 40)*. ATP is present in bacterial and organic food cells, meaning the ATP

device should detect both bacterial and produce residues on a surface *(13, 34, 40, 49)*.

The ATP device works via the luciferase reaction (Fig. 1) *(8)*, the enzyme derived from

fireflies *(24, 59, 66)*. Luciferase is in solution in the swab bulb, so that when the bulb is

broken, the reaction between ATP on the swab and luciferin is triggered *(66)*. Luciferin

and ATP react in the presence of oxygen to create bioluminescence as a byproduct *(66)*.

The bioluminescence is measured by the ATP device in relative light units (RLU) *(66)*.

The luciferase reaction occurs quickly so once the swab bulb is broken, the swab should

be inserted into the ATP device immediately to get an accurate reading *(66)*. The RLU

reading of the ATP device can be directly correlated to the amount of ATP on the swab

(49, 66).

The ATP monitoring device is simple to use because it only requires the swabbing

of the surface, breaking of the swab bulb, and insertion into the handheld device *(28)*. To

retrieve a sample, a grower swabs a $100cm^2$ postharvest surface in the vertical, diagonal,

and horizontal direction after the surface has been cleaned and sanitized *(28)*. The top of

the swab (swab bulb) is snapped, the swab is shaken for 5 seconds, and the swab is inserted immediately into the device *(28)*. The device used for the present study gives a reading in 15 seconds *(28)*. The ATP device notifies the user if the surface is clean, denoted by a "check mark", or dirty, denoted by an "X" *(28)*. Standards of clean and dirty surfaces are set by the user prior to use *(28)*. The ATP device gives readings on a scale of 0-9999 RLU. There are many brands of the ATP device on the market, but all brands use a similar testing procedure *(31)*.

Locations of ATP Device Use

The ATP monitoring device has been used in food processing facilities to support sanitation programs. For example, in dairy processing, the ATP device was used to create a qualitative "cut off" value for raw milk that was safe for human consumption *(4)*. Aycicek et. al. used the ATP device to detect cleanliness of various surfaces in a kitchen hospital and found the device to be comparable to microbial culture methods ($K=0.249$; $p<0.001$) *(2)*. The ATP device was used by non-specialized staff in hospital kitchens, and educated the staff on where hygiene practices on kitchen surfaces needed to be improved based on ATP results *(2)*. Two previous studies determined that the ATP device was useful in food retail premises because the ATP device was able to detect discernable differences in results before and after surface cleaning *(13, 49)*. Niksic et. al. recommended the ATP device be used with microbial plate counts after cleaning and sanitation took place because the ATP device could not determine the quantitative number of bacterial ATP on a surface *(42)*. Turner et. al. used the ATP device in animal facilities and determined the device was a valuable tool to be used with microbial plate counts to ensure the surface of interest was sterile since there was a limit of detection of

bacteria (2 and 4 log CFU) with the ATP device *(59)*. Ogden used the ATP device to determine food-contact surface cleanliness in a brewery after a cleaning and sanitation procedure *(44)*. Ogden tracked the pass/fail rate of the locations swabbed to determine where cleaning and sanitation had to be improved within the brewery *(44)*. Ehrenfeld et. al. demonstrated the ATP device was a useful tool to determine surface cleanliness by performing field swabbing in a brewery and directly inoculating beer samples onto the swab tip *(16)*. The ATP device was able to detect 1-10μl beer samples and did not have interference from disinfectants when disinfectants and ATP were inoculated directly onto the swab tip *(16)*. Omidbakhsh et. al. did not recommend the ATP device for use in healthcare settings because results showed interference from disinfectants on healthcare surfaces that gave false positive (clean) results *(46)*.

There has been widespread use of the ATP device in different facilities, however no studies have investigated the use of the ATP device in an on-farm environment. The on-farm environment can differ from food processing facilities in many ways. The on-farm environment can have increased risk of produce and surface contamination due to wild animals, farm visitors, manure, soil, worker hygiene, irrigation water, and fertilizer *(5, 37, 45)*. Surfaces used on-farm such as wood can be more difficult to clean than commonly used stainless steel surfaces in processing plants *(35, 38, 56, 64)*. Wood surfaces can harbor bacteria that seep beneath the surface and are difficult to remove and detect by microbial plate counts *(1, 22)*. Farms have wash tanks and storage bins that can harbor bacteria and that are not commonly present in food processing plants *(56, 58)*.

Effect of Inoculation Method on the ATP Device

Previous research differed in the recovery method of the sample onto the swab. There are two methods used to test how well an ATP device is reading the swab: direct swab inoculation or swabbing of an inoculated surface. In the direct swab inoculation, the sample is pipetted onto the swab in a laboratory setting. The direct swab inoculation can be used to make relationships between ATP and APC (aerobic plate count) response because a known amount of bacteria is being put onto the swab and there is no ATP interference from a surface *(46)*. If a correlation is found between ATP and APC methods, the direct swab inoculation can be used to make comparisons between different ATP monitors *(2, 10, 13, 31, 34, 46, 61)*. The second method is the swabbing of a food-contact surface *(11, 34, 40)*. Surface swabbing can be done in a laboratory with a surface inoculated with bacteria *(34)* or in a field study *(2, 10, 13, 33)*. Studies have shown that the direct swab inoculation has a lower limit of ATP detection by the ATP device than the limit of detection of swabbing an inoculated surface *(34, 40, 46)*. The results show that the swab does not retrieve as much ATP from a surface when compared to direct inoculation. In application, the ATP device would be used to detect ATP from a swabbed surface.

Effect of Bacteria on the ATP Device

The cellular origin of bacterial ATP impacts how well ATP is detected by the ATP device. Turner et. al. reported a different limit of detection of the ATP device for gram-negative bacteria compared to gram-positive bacteria inoculated directly onto a swab *(59)*. Specifically, *S. aureus* had a limit of detection of 2 log CFU, while *E. coli* had a limit of detection of 4 log CFU under the same environmental conditions *(59)*. Viator

et. al. found that *P. aeruginosa* (gram-negative) had a limit of detection of 5 log CFU and *S. aureus* (gram-positive) had a limit of detection of 4 log CFU when inoculated onto stainless steel *(61)*. Leon and Albrecht found a limit of detection to be 4 log CFU when *L. rhamnosus*, a gram-positive bacteria, was inoculated onto plastic *(34)*. Turner et. al. suggested that different cellular structures impact the overall ATP response *(59)*. The previous studies demonstrated an increased difficulty by the ATP device in detecting gram-negative bacteria than gram-positive bacteria *(34, 59, 61)*, potentially due to incomplete cell lysis of gram-negative bacterial cells *(59)*. Previous studies have also researched *E. coli (11, 31, 40, 59)*, *S. aureus (31, 59, 61, 63)*, *P. aeruginosa (31, 61, 63)*, *S. cerevisiae (9, 31, 61)*, *Lactobacillus (31, 34)*, and *Salmonella (31)*.

Listeria species have not been frequently used to test the efficacy of the ATP device. One study found a lower RLU reading for *L. monocytogenes* in cheese than for *L. monocytogenes* in fish extract *(62)*. *L. monocytogenes* was embedded in the cheese, making L. monocytogenes ATP potentially more difficult for the ATP device to detect *(62)*. 21 CFR 112.145 requires an environmental monitoring program specifically for the testing of the farm environment for *L. monocytogenes* and *Listeria* species *(20)*. *Listeria* is a gram-positive, facultative anaerobe, and it is important to test if the limit of detection is the same as other gram-positive bacteria *(43, 50, 58)*. The prevalence of *Listeria* contamination in produce on farms makes it a concern for detection by the ATP device *(5, 21, 45, 60)*.

Effect of Food Matrices on the ATP Device

Different types of food matrices can result in different readings by the ATP device. The detection of food debris on a food-contact surface by the ATP device is

important because food debris can enhance, or change, the attachment of bacteria onto a surface *(40, 62)*. In a study by Moore and Griffith, raw tomatoes (low protein) (0.001 dilution factor) were detected at a lower concentration by the ATP device than milk (high protein) (0.1 dilution factor) and raw chicken (high protein)(0.01 dilution factor) when inoculated onto stainless steel *(40)*. The ATP swab used in the present study (Hygiena SuperSnap™ High-Sensitivity ATP swab) detected lower concentrations of orange juice (low protein) (0.0001dilution factor) and mixed greens (low protein) (0.0001 dilution factor) than raw beef (high protein) (0.001 dilution factor) and milk (high protein) (0.01 dilution factor) when inoculated directly onto the swab *(31)*. Viator et. al. found orange juice and yogurt to be detected by the ATP device at the lowest dilution (0.00001 dilution factor), ground beef and deli turkey in the middle (0.0001 dilution factor) and flour (0.001 dilution factor) at the highest when inoculated onto stainless steel *(61)*. Viator et. al. suggested the flour was the most difficult to detect by the ATP device due to the high amount of processing it receives that removes ATP *(61)*. Whitehead et. al. found that the ATP device was better at detecting complex food matrices (meat, fish extract, and cheese) than simple matrices (cholesterol, fish oil, fatty acids, glycogen, starch, lactose, BSA, fish protein, casein) *(62)*. The studies suggest that the ATP device can detect low protein foods such as mixed greens, orange juice, and tomatoes at a lower dilution level than high protein foods such as milk and meat products *(31, 40, 61)*. The data suggests that the ATP device may be able to detect leafy greens and produce on farms at a low dilution level.

Effect of Food-Contact Surfaces on the ATP Device

The type of contact surfaces may influence the efficacy of the ATP response. Lahou and Uyttendaele compared the efficacy of three swabbing methods (environmental swab, sponge stick, and foam spatula) when *L. monocytogenes* was inoculated onto three food contact surfaces (stainless steel, polyethylene, and rubber) *(32)*. Lahou and Uyttendaele found a small significant difference among the surface types ($p = 0.026$) *(32)*. *L. monocytogenes* was always detected on rubber, but not always on stainless steel, suggesting the surface material may influence the survival of the bacterial cells *(32)*. There was a decreased viability of *L. monocytogenes* on stainless steel when compared to HDPE plastic, again suggesting surface material may influence viability of *L. monocytogenes* cells *(32, 54)*.

Stainless steel has been the standard for ATP monitoring device testing *(11, 31, 40, 61, 62)* because it is most commonly recommended for use in food processing due to its smooth, nonporous surface *(35, 56)*. Moore and Griffith recommended that the ATP device be used with visual assessment when using the ATP device to determine cleanliness of stainless steel *(40)*. Viator et. al. found the ATP device compatible for use with stainless steel *(61)*. Whitehead et. al. found the ATP device useful as a screening method for stainless steel cleaning and sanitation to determine hard to clean areas *(62)*. Leon and Albrecht tested the ATP device when *Lactobacillus rhamnosus* was inoculated onto plastic and found the device to be recommendable to measure surface sanitation in food processing centers *(34)*. The results of previous studies suggest the ATP device could be compatible with stainless steel and plastic used on farms *(34, 40, 61, 62)*.

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Wood surfaces are commonly used on farms due to preference or necessity, but they are more difficult to clean because they are porous and rough *(56)*. Wood surfaces require an additional step of scrubbing with a bristled brush and soap to ensure cleanliness *(56)*. Gilbert and Watson found wood surfaces to be difficult to clean and harbors of *Salmonella (22).* Ak et. al. found increased difficulty in detecting bacteria contaminated onto wood than bacteria contaminated onto plastic *(1)*. Lücke and Skowyrska showed no significant difference in the amount of bacteria detected after inoculated beechwood, maple, and polyethylene were cleaned *(35)*. However, Lücke and Skowyrska did change the cleaning methods used depending on the surface material *(35)*. Lücke and Skowyrska recommended that beechwood, maple, and plastic are viable surface materials as long as the cleaning method is changed for each surface *(35)*. Despite the difficulty in bacterial detection from wood, Milling et. al. found that bacterial levels from chicken manure decreased more quickly on pine wood-sawdust when compared to larch, maple, and plastic, suggesting antimicrobial properties of pine wood-saw dust *(39)*. Milling et. al. found that gram-negative bacteria were more affected by the antimicrobial properties of the wood than gram-positive bacteria *(39)*. Two studies determined that contamination of polyethylene and wooden cutting boards with bacteria did not yield a significant difference in microbial counts between surface types *(12, 38)*. Miller et. al. suggested that differences between bacterial detection on wood could be due to differences in fat, nutrient, and moisture level present on the surface *(38)*. Wood has not been tested for use with the ATP device. The different reports on the effectiveness of wood as a food-contact surface demonstrate that wood should be tested with the ATP device.

The ATP device may retrieve bacterial ATP differently from different surfaces. *Listeria* adherence to different types of surfaces *(54)* may affect the ATP device retrieval of *Listeria* cells. For example, *L. monocytogenes* was found to adhere the least to polypropylene plastic surfaces and the most to granite and marble *(54)*. *L. monocytogenes* adhered the most to granite and marble due to the surfaces' thresholds between hydrophobic and hydrophilic, surface electron acceptor values, and many contact points for *L. monocytogenes* attachment *(54)*. *L. monocytogenes* is less viable on stainless steel, but more viable on polypropylene plastic *(54)*. Surface roughness did not correlate to differences among *Listeria* surface adhesion *(51, 54)*. The proven effect surface type has on *Listeria* viability and attachment makes it important to test whether the ATP device can accurately retrieve *Listeria* from a postharvest contact surface.

Conclusion

The goal of this research was to determine if the ATP monitoring device was a suitable sanitation tool for use on postharvest surfaces to monitor cleaning and sanitation procedures. The ATP device can be a tool that is used on-site to compliment microbial plate counts that do not give results for 24-48 hours. Current research has determined the efficacy of ATP devices at monitoring cleaning and sanitation programs in locations such as hospitals, food retail establishments, and food processing centers. The ATP device should be tested for use on-farm due to challenges in cleaning and sanitation such as contaminated irrigation water, wild animals, farm visitors, and worker hygiene. The ATP device ability to detect bacteria can be affected by differences in bacterial cell walls. The ATP device better detects food matrices on a surface that are low in protein. There have

been foodborne illness outbreaks in the United States due to *Listeria* contamination of leafy green produce on farms. The ATP device has not been commonly tested with leafy greens and *Listeria*. The ATP device has been proven effective with stainless steel and plastic food-contact surfaces, but has not been tested with wood surfaces, a common postharvest contact surface on farms. ATP monitoring could help growers who are looking for tools to display an effective cleaning and sanitation plan and are compliant to PSR guidelines. Confirmation that the device can be used on farms can support the implementation of an effective cleaning and sanitation plan that will improve produce food safety.

CHAPTER 2

RESEARCH

<u>**Introduction**</u>

The Food Safety Modernization Act (FSMA), and specifically the Produce Safety

Rule (PSR), implemented stricter regulations on-farm to help reduce incidence of

foodborne illness *(18)*(21 CFR). Post-harvest cleaning and sanitation is one of the

sections within the regulation itemized within the law *(20)*(21 CFR 112). 21 CFR

112.123(d)(1) requires compliant farms to "sanitize all food contact surfaces of

equipment and tools used in covered activities as frequently as reasonably necessary to

protect against contamination of covered produce" *(20)*. Cleaning and sanitation can be

difficult on farms due to increased sources of contamination such as irrigation water, wild

animals, worker handling, and inappropriate fertilizers *(5, 37)*. Growers have requested

technical support and guidance on compliance with FSMA regulations *(17, 57)*.

Microbial plate counts have been used as a sanitation tool to monitor cleaning and

sanitation programs and detect bacteria from surfaces *(32)*. However, microbial plate

counts require the swabbing of a food-contact surface to retrieve a sample, sample

preparation, transfer onto microbial agar plates, and 24-48 hour incubation period before

results can be received *(10, 14, 34, 44, 46)*. ATP monitoring devices have been used as a

sanitation tool to compliment microbial plate counts in hospital kitchens, retail food

establishments, dairy processing plants, and breweries *(2, 3, 44, 49)*. The ATP device

gives sanitation results in real-time by detecting the residual amount of ATP, found in all

living cells, on a surface *(13, 34, 40, 44)*.

The readings of the ATP monitoring device can be affected by the environment. ATP device readings can differ based on the type of bacteria present on a surface, specifically if the bacteria is gram positive or gram negative *(11, 59, 61)*. The type of food matrix present on a surface, such as the amount of protein the food contains, can affect the ATP reading *(31, 40, 61, 62)*. The type of food-contact surface can affect how well bacteria are retrieved from a surface using a swab and bacterial viability on the surface, but not many surface types have been tested with the ATP device *(1, 32, 54)*.

Differences seen in ATP device readings in past work demonstrate the need for the device to be tested for suitable use for postharvest farm. On-farm has risks of contamination from irrigation water, wild animals, worker handling, and inappropriate fertilizers *(5, 37)* that is not seen in food processing centers. The number of foodborne illness outbreaks of leafy greens contaminated with *Listeria* on-farm have increased and should be tested for detection by the ATP device *(19, 41)*. Between 1996 and 2016, *Listeria* species were the cause of 27 incidents of leafy green contamination in California *(60)*. Specifically, spinach, lettuce, and cabbage were in the top seven most commonly contaminated leafy greens in California from 1996 to 2016 *(60)*. In 1981, 42 cases of listeriosis were caused by cabbage contaminated by untreated manure *(41)*. Between 2013 and 2016, there was a 9.1% increase in Listeria illnesses in the United States due to a multi-state outbreak of Listeria found in prepackaged lettuce *(19)*. Leafy greens should be tested with *Listeria* due to the ability of the type of food matrix to affect bacterial surface attachment *(40, 62)*. The severity of listeriosis in susceptible populations, *Listeria's* ability to form biofilms in a wide range of environments, and *Listeria*'s gram-positive nature makes *Listeria* an important bacteria to test for ATP device detection *(23,*

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50, 58). Wood is a common food-contact surface on farms, but wood can be difficult to

clean and has been shown to harbor bacteria in its surface so it should be tested for

compatibility with the ATP device *(1, 22, 56).* Stainless steel has most commonly been

tested for use with the ATP device, and can be used as a standard to compare with wood

and plastic surfaces *(11, 31, 40, 61, 62). L. monocytogenes* is more viable on plastic than

on stainless steel *(32, 54)* which is a reason to include HDPE plastic as a surface

comparison. Two studies suggested surface material may influence viability of *L.*

monocytogenes cells *(32, 54).* The goal of the presented research is to test the efficacy of

the ATP monitoring device as a sanitation tool for the postharvest farm environment.

Materials and Methods

Bacterial Inoculum Preparation

Listeria innocua (ATCC #33090, Manassas, VA) was used to inoculate surfaces.

Listeria was chosen due to its prevalence in fruit and vegetable related foodborne illness

outbreaks and the severity of listeriosis it can cause in susceptible populations *(19, 41,*

60). L. innocua was chosen so that it may be used in field trials since it is a BSL 1

organism and an indicator organism for the pathogen *L. monocytogenes*. Frozen stock

culture was prepared in sterile vials containing 30% glycerol and stored at -80°C. Prior to

experiments, a loop of *L. innocua* from brain-heart infusion (BHI) slant (BBL, Becton

Dickinson, Franklin Lakes, NJ) was used to inoculate 9 mL BHI broth (BBL, Becton

Dickinson). The broth was incubated at 37°C for 18 h with continuous shaking at 130

rpm to reach a target inoculum level of 9 log CFU/mL. *L. innocua* concentration was

determined by plating serial dilutions and counting colonies in the range 25-250 on

Oxford agar (Oxoid, Thermo Scientific Remel, Waltham, MA) with Oxford *Listeria*

selective supplement (Oxoid) incubated at 37°C for 24 ± 1 h or Petrifilm™

Environmental Listeria Plates (3M Co., St. Paul, MN) incubated at 37°C for 26 ± 1 h.

Postharvest Contact Surfaces

Coupons (96 cm^2) of stainless steel flat SS316L sheets with type #2B finish

(McMaster-Carr, Cleveland, OH), high-density polyethylene (HDPE) plastic (Thirteen

Chefs, Redmond, WA), and bamboo wood (Lestaven, China) were used to mimic

traditional postharvest produce contact surfaces *(56)*. Stainless steel, HDPE plastic, and

wood have been shown in previous studies to affect bacterial retrieval by swabs and

bacteria viability differently *(1, 22, 32, 54)*. The stainless steel coupons had a smooth,

shiny texture. The HDPE plastic coupons were slightly rigid in a crisscross pattern. The

bamboo coupons were smooth, with a few small cracks. All postharvest surface materials

were cleaned and autoclaved at 121°C for 20 min.

Inoculation of Postharvest Contact Surfaces

Before each trial, coupons were aseptically unwrapped of aluminum foil and

spaced evenly throughout a sterile biosafety cabinet. Each coupon was inoculated with

0.2 µL of specified inoculum (bacteria and/or produce matter) in five locations to make

an "X" pattern and total inoculum level of 10 µL. The inoculum was dried for at least 30

min before being swabbed. Coupons were inoculated directly to mimic the application

use of the ATP device on-farm.

Swabbing of Postharvest Contact Surfaces with ATP Swabs

Swabbing took place once inoculated surfaces were visibly dry and no sooner

than 30 min after inoculation. ATP swabs (SuperSnap™ High-Sensitivity ATP swab,

Hygiena, LLC, Camarillo, CA) were stored at 4°C and removed to room temperature (22°C) at least 10 min before swabbing. Each coupon was swabbed once in the horizontal, vertical, and diagonal direction across the 96 cm^2 surface, according to manufacturer's instructions (Hygiena, LLC). After each surface was swabbed, the ATP level was measured immediately by placing the swab into the ATP monitoring device (EnSure™ Monitoring System, Hygiena, LLC). Results were reported in 15 sec and recorded in relative light units (RLU) in the system device range of 0-9999 (0 log RLU to 4 log RLU). A blank swab control was always read before each trial.

Swabbing of Postharvest Contact Surfaces with Environmental Swabs

Swabbing took place once the postharvest contact surfaces were determined completely dry by sight and no sooner than 30 min after inoculation. Environmental swabs (3M™ Quick Swab, 3M Co.) were stored at 4°C and removed to room temperature (22°C) at least 10 min before swabbing. Each coupon was swabbed once in the horizontal, vertical, and diagonal direction across the 96 cm^2 surface, according to manufacturer's instructions (3M Co.). After the surface was swabbed, Letheen neutralizing buffer was released onto the swab. The sample was serially diluted and plated on Petrifilm™ Environmental Listeria Plates (3M Co.) and incubated at 37°C for 26 ± 1 h.

Produce Matrices Preparation

Spinach (Fresh Express, Big Y, Amherst, MA), romaine (Fresh Express, Big Y, Amherst, MA) and red cabbage (Big Y, Amherst, MA) were chosen as three leafy green matrices. The leafy greens were chosen due to their prevalence in farm-related foodborne illness outbreaks *(19, 41, 60)*. The produce have different chlorophyll levels, which was

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hypothesized to affect ATP device response *(30, 65)*. Spinach and romaine were purchased bagged and pre-washed. A full head of red cabbage was purchased and rinsed with DI water and dried before use. The leafy greens were not used for longer than 3 days after being opened and were stored at 4°C when not being used. Each leafy green was made into a 10% solution by blending in a blender (Coolife HS-167, Guangdong, China) prepared from 10 g leafy green with 90 mL sterile DI water. The solution was blended on "medium" speed for 45 sec then strained with a cheesecloth (Imperial, Worcester, MA). The 10% solution was prepared fresh daily. Sterile 0.85% saline (Fisher Bioreagents, Fair Lawn, NJ) solution was used as a control inoculum. For the comparison of the three produce contact surfaces, a 0.4% spinach solution (prepared by blending 0.36 g spinach with 90 mL sterile DI water) was created so that it could be combined with a high and low concentration of *L. innocua* without exceeding the maximum detection level of the ATP device. The solution was blended and measured in the same way done for the 10% leafy green solutions.

Dose Response Curve of *L. innocua* on Stainless Steel

Serial dilutions of 10μL of *L. innocua* in sterile 0.85% saline were inoculated onto 96cm^2 stainless steel coupons. Each coupon was inoculated with 0.2 µL of specified inoculum in five locations to make an "X" pattern and total inoculum level of 10 µL. The final concentrations of *L. innocua* on the stainless steel coupons ranged from 2 to 7 log CFU/coupon. Sterile 0.85% saline was used as a control and graphed as 0 in results. Each concentration was repeated for three replicates on three separate stainless steel coupons per trial.

Dose Response Curve of Spinach on Stainless Steel

A 10% spinach solution was made by blending 10 g spinach with 90 mL sterile DI water for 45 sec. The 10% spinach solution was strained to remove solids with a cheesecloth. The 10% spinach solution was diluted in 1 to 5 dilutions in sterile 0.85% saline to create five spinach concentrations of 1 (10% spinach solution) to 0.0016 (0.0016 dilution level). Sterile 0.85% saline solution was used as a control and graphed as 0 in the results. Three experimental trials were performed with three replicates on separate stainless steel coupons per trial.

Inoculation of *L. innocua* and Leafy Greens on Stainless Steel

L. innocua was diluted in 1 to 2 dilutions in 10% solutions of spinach, romaine, red cabbage, or sterile 0.85% saline to create five concentrations of *L. innocua*. The five concentrations of *L. innocua* in sterile 0.85% saline were performed with each trial as a control. *L. innocua* were inoculated onto stainless steel coupons as described previously. Each *L. innocua* concentration was inoculated on two coupons per trial. Each 10% leafy green solution was run as a separate trial.

Inoculation of *L. innocua* and Spinach on Different Surfaces

L. innocua was diluted in 0.4% spinach solution and sterile 0.85% saline to create two concentrations of *L. innocua*. The concentrations of *L. innocua* were 3.4 and 5.4 log CFU/coupon to represent a high and low level of bacteria. A sterile 0.85% saline solution was used as a control. The five combinations of *L. innocua,* spinach, and saline (saline, saline + 3.4 log CFU/coupon, saline + 5.4 log CFU/coupon, spinach + 3.4 log CFU/coupon, spinach + 5.4 log CFU/coupon) were individually inoculated onto stainless steel, HDPE plastic, and bamboo wood coupons. Each inoculum combination was

inoculated on two coupons per trial, per surface type. The 0.85% saline control was subtracted from each of the *L. innocua* average values to give an adjusted value that was graphed.

Statistical Analysis

Every experiment was repeated in three trials. Each trial had at least one blank control coupon per surface type. Reported values from the ATP monitoring device were converted to $\log_{10}$ prior to statistical analysis. Log CFU values recorded for *L. innocua* were based on 10 µL inoculated per coupon. Spinach concentrations were recorded as the starting solution as 1 multiplied by serial dilution factor, with sterile 0.85% saline solution as 0. Sterile 0.85% saline controls were combined and averaged for each surface type across the experiments to determine the starting ATP device reading of sterile 0.85% saline without leafy greens or *L. innocua*. Statistical tests PROC GLM, ANOVA, IML, REG, SORT, DUNCAN, CONTRASTS, and LSMEANS were performed using SAS Statistical Software (version 9.4, SAS Institute Inc., Cary, NC). Significance was determined at the level of P=0.05. Analysis of variance, regression, mean separation, and orthogonal polynomial comparison were used to characterize lines and determine differences among means and interactions. Standard error of mean, means, and graphs produced using Microsoft Office 365 2019 Excel Spreadsheet (Microsoft Corp., Redmond, WA).

Results

Dose Response Curve of *L. innocua* on Stainless Steel

Different concentrations of *L. innocua* on a stainless steel coupon were used to
determine if a change in the concentration of bacteria caused a change in the ATP device
reading. *L. innocua* was used to see if there was a relationship between *L. innocua*
concentration and RLU reading, and if the device could detect *L. innocua* at all
concentrations tested. Analysis of variance was used to determine if a change in *L.
innocua* concentration on stainless steel affected the RLU reading (Fig. 2). There was a
highly significant difference (P<0.0001) among the RLU readings as *L. innocua*
concentration/coupon increased or decreased, indicating the RLU response was sensitive
to changes in *L. innocua* concentration. Orthogonal polynomial comparison and
regression analysis were performed to determine the relationship between the change in
L. innocua concentration and RLU reading (Fig. 2). There was a quadratic relationship
between the *L. innocua* concentration and RLU reading. In the range 4.5 to 7.5 log
CFU/coupon, as *L. innocua* concentration increased 1 log CFU/coupon, the RLU value
increased 1 log RLU/coupon, showing a direct correspondence between log CFU/coupon
and log RLU/coupon. At 3.5 log CFU/coupon *L. innocua* and below, the log
RLU/coupon reading was below 0.27. The average log RLU/coupon of 0.85% saline
control on stainless steel was 0.36 (Fig. 2). The lower RLU reading of *L. innocua*
compared to 0.85% saline demonstrated that the ATP device does not detect *L. innocua*
ATP at 3.5 log CFU/coupon and below. The lower RLU reading of *L. innocua* compared
to 0.85% saline demonstrated a limit of detection of the ATP device at 4.5 log
CFU/coupon of *L. innocua*. The R^2 value of the relationship was 0.99, demonstrating it
was a strong fit to the data.

Dose Response Curve of Spinach on Stainless Steel

The response of the ATP device to different concentrations of spinach solution on a stainless steel coupon was determined. Different concentrations of spinach were used to determine if a change in the concentration of organic produce matter on a stainless steel surface caused a change in the ATP device reading. There was a highly significant difference ($P<0.0001$) in the RLU reading as the relative spinach concentration/coupon increased or decreased, demonstrating that the RLU reading was sensitive to changes in spinach concentration. Orthogonal polynomial comparison and regression analysis were performed to determine the relationship between the change in relative spinach concentration and RLU reading (Fig. 3). There was a cubic relationship that demonstrated as the relative spinach concentration/coupon decreased, the log RLU/coupon decreased. The R^2 value of the relationship was 0.99, demonstrating it was a strong fit to the data.

Inoculation of *L. innocua* and Leafy Greens on Stainless Steel

Concentrations of *L. innocua* were combined with three types of leafy greens (spinach, romaine, red cabbage) and 0.85% saline on stainless steel to determine if the combination of different types of organic matter and bacteria on a postharvest contact surface impacted the ATP device reading. These results are presented in Fig. 4. There was not a significant difference in RLU reading among the three types of leafy greens combined with *L. innocua* on stainless steel ($P=0.88$), indicating the ATP device does not detect different types of leafy greens differently (Fig. 4). Since there was no significant difference among leafy green types, the RLU readings of the leafy greens were averaged for each *L. innocua* concentration and compared against those of 0.85% saline with *L. innocua* (Fig. 5). The slope of the line of leafy greens with *L. innocua* was 0.7175. The

slope of the line of 0.85% saline with *L. innocua* was 1.1071. The different slopes show the dose response was different when leafy greens were present. There was a highly significant difference (P=0.0009) between the RLU readings of leafy greens when compared to the RLU readings of 0.85% saline. There was a non-significant interaction between leafy green type and *L. innocua* concentration, leafy green type and 0.85% saline, and leafy green type, 0.85% saline and *L. innocua* concentration (Fig. 4). There was a significant interaction between *L. innocua* concentration and 0.85% saline (P=0.03) (Fig. 5). The results showed the presence of organic matter with *L. innocua* on stainless steel did have an additive effect on RLU reading (Fig. 5). The *L. innocua* concentrations in both organic matter and 0.85% saline had a highly significant impact on the RLU reading (P<0.0001). The results showed the ATP device can detect the change in *L. innocua* concentration despite the presence of organic matter.

Inoculation of *L. innocua* on Different Surfaces

7.4 log CFU/coupon was inoculated onto stainless steel, HDPE plastic, and bamboo wood to determine *L. innocua* retrieval from different postharvest contact surfaces by an environmental swab (Table 1). There was a highly significant difference in *L. innocua* retrieval from the different surfaces (P=0.0017). Specifically, there was not a significant difference among the average *L. innocua* retrieval from stainless steel and HDPE plastic. There was a significant difference among the average *L. innocua* retrieval from stainless steel and HDPE plastic compared to bamboo wood.

Inoculation of *L. innocua* and Spinach on Different Surfaces

A high (5 log CFU/coupon) and low (3 log CFU/coupon) dose of *L. innocua* was combined with 0.4% spinach or 0.85% saline and inoculated onto stainless steel, HDPE

plastic, and bamboo wood. The study was done to determine if there were differences in RLU readings between different postharvest produce contact surfaces. The different types of produce contact surfaces had significantly different ATP responses (P=0.03). The interaction between inocula type and surface type was highly significant (P<0.0001). The combinations of high and low dose *L. innocua* with spinach or saline had a highly significant impact on the RLU reading among the surfaces (P<0.0001) (Fig. 6). The RLU reading for bamboo was not significantly different among the different inoculum (P=0.16), suggesting the ATP device did not detect the spinach or *L. innocua* ATP on the bamboo. Among all surface types, there was not a significant difference in RLU reading between the saline control and low dose *L. innocua*, suggesting the ATP device does not detect bacterial ATP below 3 log CFU/coupon. There was not a significant difference between the surface type RLU readings when low dose *L. innocua* was on the surface (P=0.09) (Fig. 6). The results suggest that stainless steel and HDPE plastic had a higher recovery of ATP than bamboo.

Discussion

The goal of this research was to determine if the ATP monitoring device is a suitable sanitation tool for postharvest surfaces. Previous work demonstrated the ATP monitoring device could potentially give different readings for different types of bacteria *(11, 31, 59, 61)*, food matrices *(31, 40, 61, 62)*, and food contact-surfaces *(32, 39, 54)*. The farm environment has different sources of bacterial contamination from wild animals, fertilizer, irrigation water, and workers *(5, 37)*, RACs such as leafy greens

(20)(21 CFR 112.1), and wood surfaces (56) that were tested for use with the ATP device that had not been previously tested.

The ATP monitoring device correspondingly detected changes in *L. innocua* concentration on a stainless steel at 4.5 to 7.5 log CFU/coupon (Fig. 2). In the range of 4.5 to 7.5 log CFU/coupon, the RLU reading corresponded linearly to the *L. innocua* concentration, showing the ATP device can detect the different concentrations of *L. innocua* on stainless steel. Other works have found linearity between log CFU and log RLU testing bacteria other than *Listeria (31, 34, 46, 59)*. Kupski et. al. determined strong linearity for the ATP device when *E. coli*, *L. plantarum*, *P. aeruginosa*, *S. cerevisiae*, *S. typhimurium*, and *S. aureus* were serially diluted and pipetted directly onto the swab *(31)*. Omidbakhsh et. al. reported a correlation between serial dilutions of *S. aureus* log CFU and log RLU readings when 10μL *S. aureus* was inoculated directly onto the swab *(46)*. Leon and Albrecht found a linear correlation between *L. rhamnosus* log CFU and log RLU inoculated onto plastic cutting boards *(34)*. Carrick et. al. reported initial linearity when 10μL of serial dilutions of *P. damnosus* were inoculated directly onto the swab, but the linearity was not consistently replicated *(9)*.

The present study showed that *L. innocua* concentration below 4.5 log CFU/coupon on stainless steel did not have a higher RLU reading than the 0.85% saline control (Fig. 2). The lack of a higher RLU reading of *L. innocua* compared to 0.85% saline demonstrated the limit of detection of the ATP device to be 4.5 log CFU/coupon (Fig. 2). The ATP device did not detect the increase in the amount of ATP on stainless steel due to the presence of *L. innocua* compared to 0.85% saline without *L. innocua* (seen by dotted line threshold) (Fig. 2). When 3.4 log CFU/coupon of *L. innocua* was

inoculated onto stainless steel, HDPE plastic, and bamboo wood, there was not a significant difference between the 3.4 log CFU/coupon of *L. innocua* and the saline control (Fig. 6).

Limits of detection by ATP devices have been seen when used with bacterial types other than *Listeria (11, 31, 34, 59, 61)*. There have been reported differences in limits of detection based on the cellular structure of the bacteria *(11, 59, 61)*. The limit of detection of *S. aureus*, a gram-positive bacteria, has been reported as 2 log CFU when inoculated directly onto the swab *(46, 59)*. *E. coli*, a gram-negative bacteria, has been reported to have a higher limit of detection of 4 log CFU when inoculated directly onto the swab, potentially due to inability of swab reagents to break down the cell *(59)*.

Inoculation of bacteria onto a surface has shown higher limits of detection than inoculation of bacteria onto a swab. Viator et. al. found that *P. aeruginosa* (gram-negative) had a limit of detection of 5 log CFU and *S. aureus* (gram-positive) had a limit of detection of 4 log CFU on stainless steel *(61)*. Leon and Albrecht also found a limit of detection to be 4 log CFU when *L. rhamnosus*, a gram-positive bacteria, was inoculated onto plastic *(34)*. Moore and Griffith found the limit of detection of *E. coli* on stainless steel to be 4 log CFU, which could be a difference attributed to the type of ATP device used *(40)*. The results of other studies *(34, 46, 59, 61)* showed gram-positive bacteria had a lower limit of detection than gram-negative bacteria. The higher limit of detection of *S. aureus* (4 log CFU) when inoculated onto stainless steel versus direct swab inoculation (2 log CFU) showed that the swab does not as effectively detect bacteria from a surface as it does from a swab *(59)*. The limit of detection of *L. innocua* determined by the present study (4 log CFU/coupon) agrees closely with other limits of detection of gram-positive

bacteria inoculated onto stainless steel and plastic *(34, 61)*, however wood has not been previously tested for use with the ATP device.

Limits of detection are important to note, especially for pathogenic bacteria, because 2 log CFU of *L. monocytogenes* in 100 g food can make a susceptible person sick with listeriosis *(48)*. The United States has a zero-tolerance policy of *L. monocytogenes* in ready-to-eat foods *(50, 58)*. *L. monocytogenes* most often contaminates fruits, but there has been an increase in *Listeria* contamination of vegetable row crops due to a large multistate outbreak of prepackaged lettuce *(19)*. 21 CFR 112.145 requires an environmental monitoring program specifically for the testing of the farm environment for *L. monocytogenes* and *Listeria* species *(20)*. The ATP monitoring device may be a good sanitation tool for the environmental monitoring of *Listeria* on farms. However, due to the *Listeria* limit of detection by the ATP device, the ATP device is recommended for use with microbial plate counts because microbial plate counts can detect 2 log CFU of *Listeria* from stainless steel, plastic, and rubber surfaces *(32)*.

The ATP device in the present study detected changes in spinach concentration on stainless steel at all concentrations tested (Fig. 3). In the range 1 to 0.0016 relative spinach concentration/coupon, the RLU reading increased as the relative spinach concentration increased. All log RLU/coupon readings for the spinach concentrations tested were above the 0.85% saline control RLU/coupon reading (Fig. 3). The higher log RLU/coupon readings of the spinach concentrations compared to 0.85% saline showed that the ATP device could detect the additional ATP present from spinach at relative spinach concentrations/coupon of 0.0016 and higher.

Previous works have found the ATP device has limits of detection for types of mixed greens. Kupski et. al. found the limit of detection of mixed greens directly inoculated on a swab to be 0.0001 dilution factor *(31)*. The low limit of detection seen by Kupski et. al. (0.0001) may be due to the difference in cell retrieval when a surface is swabbed versus when the swab is directly inoculated *(31)*. The difference between direct swab inoculation and surface swabbing limits of detection was seen for other food matrices as well. The limit of detection of milk inoculated onto stainless steel was 0.1 *(40)*, compared to 0.01 when directly inoculated onto a swab *(31)*.

Three leafy greens (spinach, romaine, red cabbage) were chosen for comparison in the present study due to the potential of differences in chlorophyll level in the leafy greens to affect the amount of ATP the cells had, and therefore affect the RLU reading *(30, 65)*. All three leafy greens have been implicated in major foodborne illness outbreaks on farms *(19, 41, 45)*. Between 1996 and 2016, *Listeria* species were the cause of 27 incidents of leafy green contamination in California *(60)*. Spinach, lettuce, and cabbage were in the top seven most commonly contaminated leafy greens in California in 1996 to 2016 *(60)*.

The present study showed there was not a significant difference in the RLU readings of the three types of leafy greens inoculated on stainless steel (P=0.88). The lack of difference between the leafy greens could be due to their similar nutritional makeup *(25)*. The effect of chlorophyll level on the ATP device reading was not investigated further due to the lack of difference in RLU readings among the leafy greens.

The type of food matrix present on a surface has been shown to affect bacterial retrieval by a swab *(40, 62)*. For example, *L. monocytogenes* cells embedded in cheese on

stainless steel had a lower RLU reading than L. monocytogenes cells not embedded in

fish extract on stainless steel *(62)*. The present study combined the three leafy greens

with concentrations of *L. innocua* to determine if the type of leafy green would interfere

with the detection of *L. innocua* or have an additive effect on the RLU reading. The

significant difference between RLU reading of *L. innocua* in saline and RLU reading of

L. innocua in leafy greens showed the leafy green ATP was able to be detected by the

ATP device in the presence of *L. innocua* (Fig. 5) (P=0.0009).

The efficacy of the ATP device to detect *L. innocua* and spinach from stainless

steel, HDPE plastic, and bamboo wood was tested in the present study. Spinach was

chosen as the leafy green food matrix because there was no difference in RLU readings

of spinach, romaine, and red cabbage (Fig. 4). Stainless steel is the most commonly used

surface for the testing of the ATP device due to its prevalence in food processing centers

and smooth, non-porous surface *(11, 31, 35, 40, 56, 61, 62)*. Leon and Albrecht tested the

ATP device when *Lactobacillus rhamnosus* was inoculated onto plastic and found the

device to be recommendable, but plastic has not been tested with the ATP device when

both organic matter and bacteria are present *(34)*. Wood is a contact surface often used on

farms due to necessity or preference, but it has not been studied for use with the ATP

device *(56)*.

The present study showed a significant difference in RLU reading among all three

surface types (P=0.03). The present study demonstrated a highly significant difference in

L. innocua retrieval among the three surface types (P=0.0017). *L. innocua* was retrieved

at a lower amount from bamboo wood than from stainless steel and plastic by the

environmental swab. The lower retrieval of *L. innocua* cells from wood tells why the

ATP device detected low levels of ATP from bamboo wood, potentially due to *L. innocua* cells seeping beneath the wood surface.

There was not a significant difference in RLU readings for the bamboo wood across the different combinations of inoculum (P=0.16), suggesting the ATP device did not detect the presence of ATP from *L. innocua* or spinach on the bamboo surface. Previous works using traditional microbial plate counts have also reported increased difficulty in detecting bacteria from contaminated wood than bacteria from contaminated plastic *(1)*. Gilbert and Watson found wood surfaces to be difficult to clean and harbors of *Salmonella (22)*. Ak et al. suggested that increased difficulty in retrieval of bacteria from wood surfaces could be due to the bacteria being absorbed into the wood surface *(1)*. Two studies determined that contamination of polyethylene plastic and wood cutting boards with bacteria did not yield a significant difference in microbial counts between surface types *(12, 38)*. Miller et. al. suggested that differences between bacterial detection on wood could be due to differences in fat, nutrient, and moisture level present on the surface *(38)*. Lücke and Skowyrska suggested that proper cleaning specific for wood surfaces can eliminate bacterial harboring within wood and make wood just as efficient as polyethylene plastic *(35)*. The differences in results among surface types suggests that more research should be done to determine the cause of difficulty in retrieval of bacteria from wood.

The present study tested the ATP monitoring device to determine if it was a suitable sanitation tool for postharvest conditions. The ATP device has been shown to accurately detect *L. innocua* at 4 log CFU/coupon and above on stainless steel. The ATP device can accurately detect spinach at 0.0016 spinach concentration/coupon and above

on stainless steel. Different types of leafy greens will not have different ATP device readings. The ATP from leafy greens will have an additive effect on RLU reading when combined with *L. innocua*. The ATP device can detect different amounts of ATP depending on the type of surface being swabbed. The ATP device is ineffective at detecting ATP from *L. innocua* and spinach on bamboo wood and should be studied further. The ATP monitoring device can be used to determine the efficacy of cleaning and sanitation procedures at removing higher amounts of organic matter, but microbial plate counts can be used to determine if lower levels of organic matter not detectable by the ATP device were effectively removed.

CHAPTER 3

FUTURE WORK

The results of the present study confirmed the ATP monitoring device as an effective tool for growers to monitor their cleaning and sanitation plan in real-time if used with microbial plate counts. The experiments in the present study were performed in a controlled laboratory. A study should be performed to test the efficacy of the ATP device on multiple farms in a field study. The farm postharvest contact surfaces would be inoculated with a biosafety level 1 microorganism combined with different produce found on the farm. Swabbing of the surfaces would be performed on-site prior to inoculation, post-inoculation, and post-cleaning and sanitation. The study would verify the efficacy of the ATP device because it would account for more variables found on a farm environment.

The study could be better supported and have a larger scope if it was repeated with other brands of ATP devices on the market. Every grower will not use the same brand of ATP device, so it is important that brand options are compared. Kupski et. al. compared five brands of ATP devices and found differences between them in a laboratory setting *(31)*. A study that repeated the experiments conducted in the present study would allow for more brand options and suggestions for growers to use.

A study comparing more types of bacteria, food matrices, and surfaces would strengthen the current results and account for more variability in environments seen across farms. Specifically, more research should look at the effect spinach has on *Listeria* attachment to surfaces and wood as a suitable postharvest contact surface. More studies with the ATP device would delineate ATP device efficacies and limitations.

APPENDIX A

TABLES

Surface type		
Stainless steel	HDPE plastic	Bamboo wood
4.6 ± 0.24	4.5 ± 0.12	1.6 ± 0.52

Table 1: Log CFU/coupon retrieval of 10µL *L. innocua* by environmental swabs from stainless steel, HDPE plastic and bamboo wood

FIGURES

$$ATP + D\text{-luciferin} + O_2 \xrightarrow{\text{luciferase}} AMP + oxyluciferin +$$
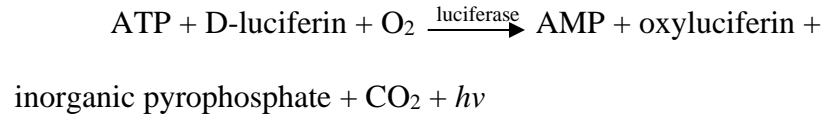
inorganic pyrophosphate $+ CO_2 + hv$

Figure 1: Luciferase reaction

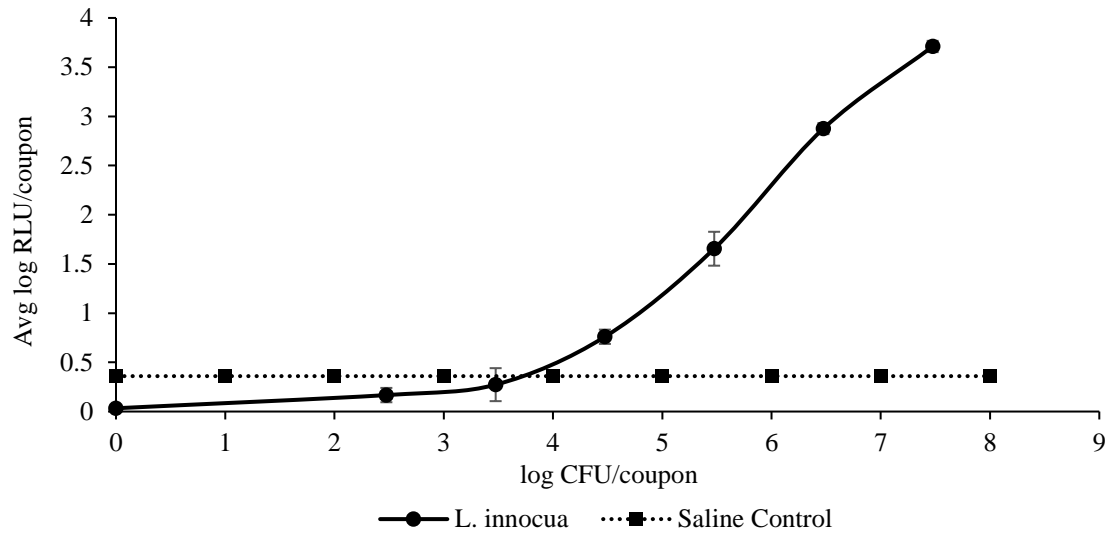

Figure 2: The change in log RLU/coupon when *L. innocua* was diluted to different concentrations in 0.85% saline and 10μL inoculated on stainless steel coupons.
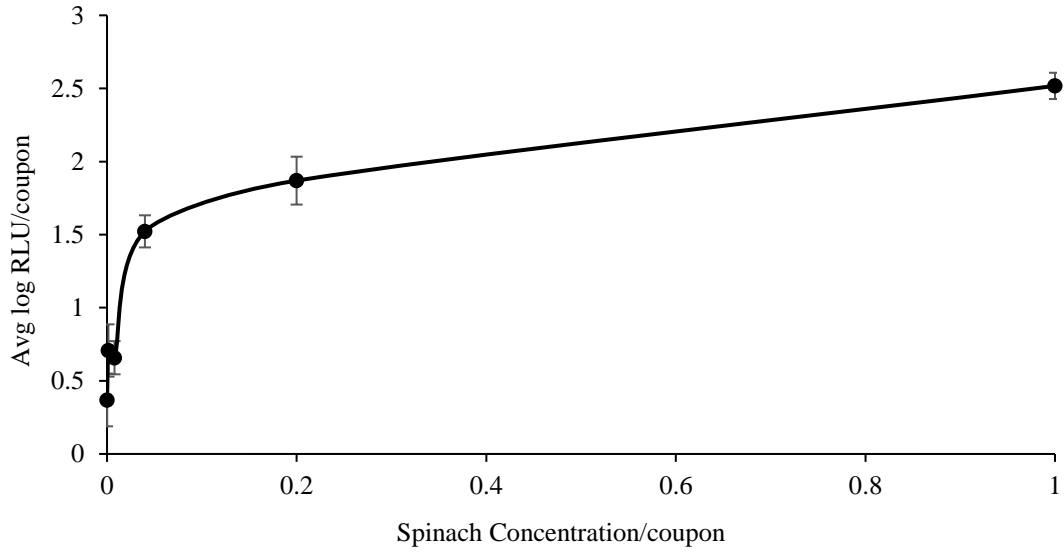
Figure 3: The change in log RLU/coupon when 10% spinach solution was diluted to different concentrations in 0.85% saline and 10µL inoculated on stainless steel coupons.
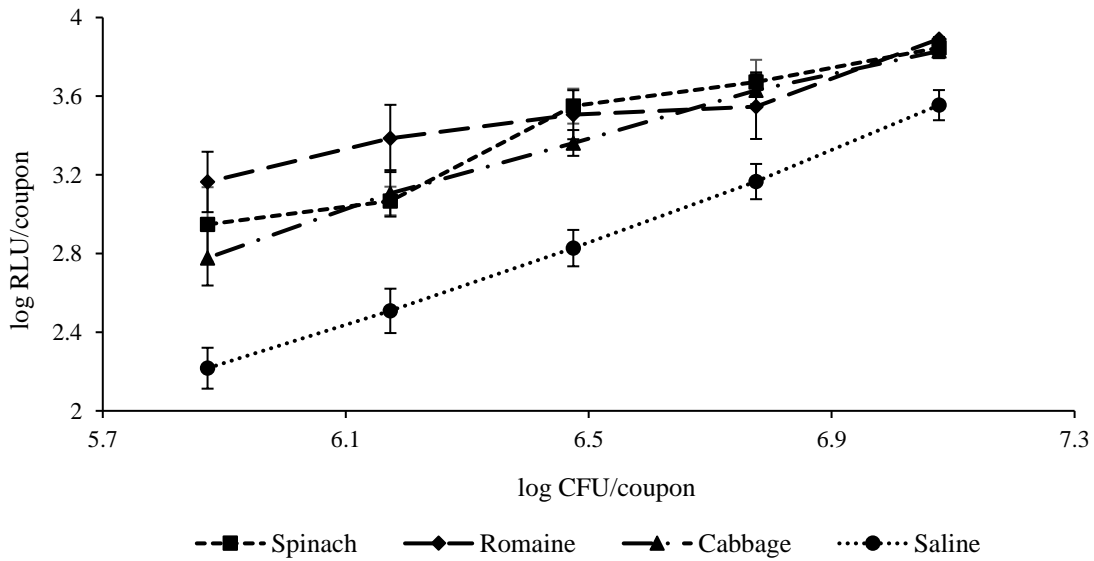

Figure 4: The change in log RLU/coupon when L. innocua was diluted to different concentrations in 10% spinach, romaine lettuce, red cabbage and 0.85% saline and 10µL inoculated on stainless steel coupons.
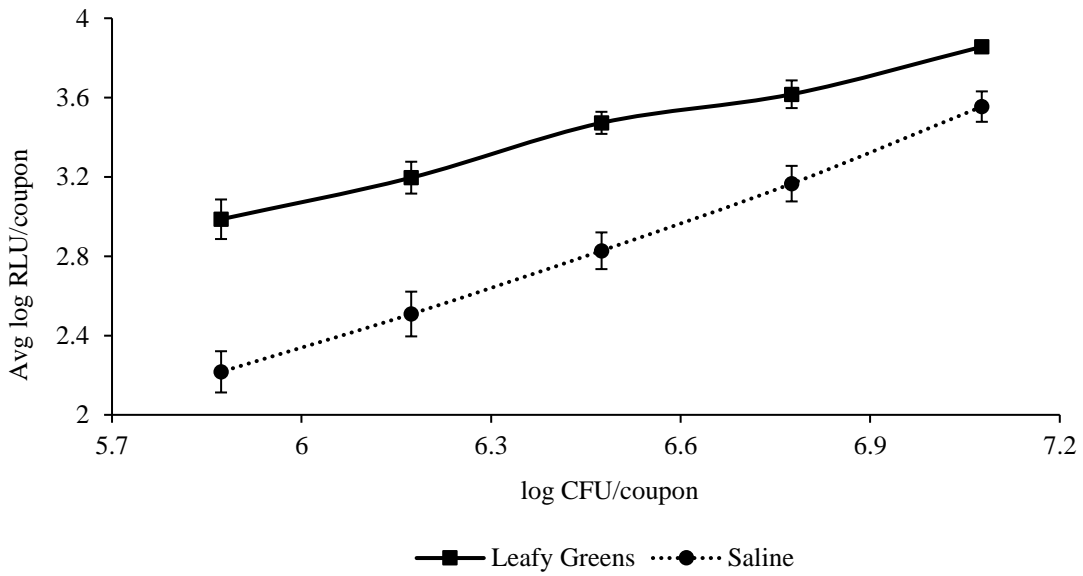
Figure 5: The change in log RLU/coupon when *L. innocua* was diluted to different concentrations in 10% leafy greens (spinach, romaine lettuce, red cabbage) or 0.85% saline and 10µL inoculated on stainless steel coupons.
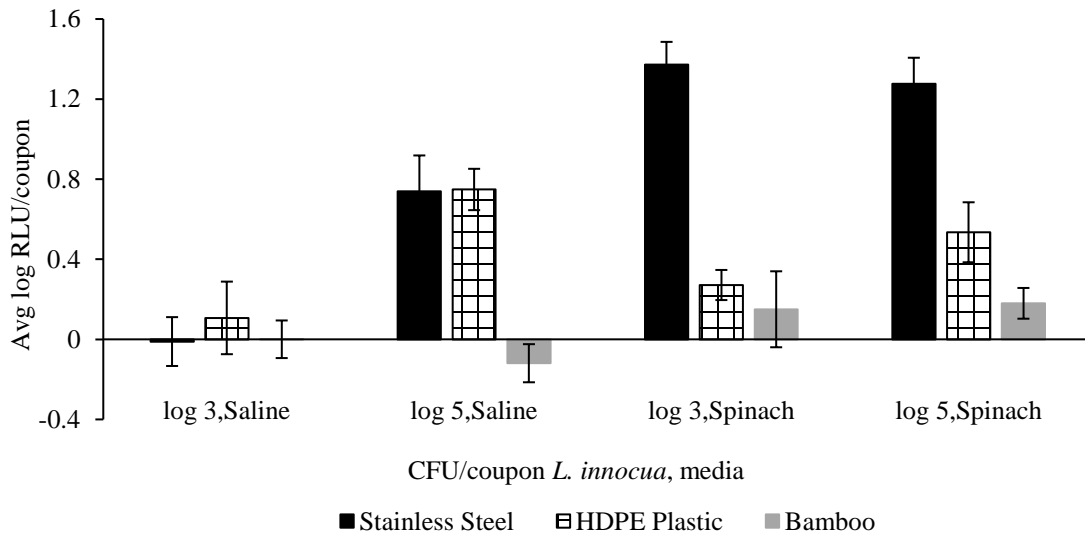

Figure 6: Comparison of log RLU/coupon of low and high dose *L. innocua* with 0.85% saline or 0.4% spinach solution and 10µL inoculated on stainless steel, HDPE plastic, or bamboo wood. Values reported adjusted based on initial saline control.

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