EVALUATION OF THE EFFECTS OF ELECTRODE CONFIGURATION AND POWER PULSE REGIME ON THE INACTIVATION EFFICIENCY OF SALMONELLA ENTERICA BY ATMOSPHERIC COLD PLASMA

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Abstract: Effective food decontamination method is critical in safeguard food safety in fresh produce worldwide. While there are several commercially available methods for food decontamination many are unsustainable or lack consumer approval. Our research team has constructed a novel ACP device based on surface dielectric barrier discharge (SDBD) for fresh produce decontamination, however, there many variables related to device design and operational conditions can be further evaluated to improve foodborne pathogen inactivation by the device. Therefore, this thesis aimed to evaluate the effect of electrode configuration and power pulse regime on the inactivation efficiency of foodborne pathogen *Salmonella enterica* by the ACP device. Glass coverslips spot-inoculated with a five-strain mixture of *S. enterica* strains were used as ACP treatment subjects initially to evaluate the performance of the device under variable electrode width, electrode gaps, and power pulsing regime. Native pecans were used as ACP treatment targets under the selected best performance conditions. The results showed that electrode width at 0.5 cm (A3) & electrode gaps at 0.1 (A1 and A2) or 0.5 mm (A3) had the best inactivation rates of *S. enterica* cells on glass coverslips. Additionally, A3 had the lowest average final treatment temperature. Taken all together, A3 was selected to evaluate the power pulsing regime. Among the power pulsing regime tested, 2cm distance with 30 seconds of treatment with 5 second off pulse following 20 or 30 second off pulse for 4.5 minutes was acceptable with log reduction & final temperature. Finally, inactivation studies using *S. enterica* inoculated native pecans revealed that ACP treatment can reduce the recoverable colony forming units from the native pecans. Unfortunately, the actuator used in the native pecan studies were not in full function, therefore, the ACP tests using inoculated native pecans will be further carried out in the near future. In conclusion, in this study we evaluated the design of the actuators, physical parameters, length of treatment & output of plasma in CAP on the inactivation efficiency of *Salmonella* enterica on native pecans and found there to be an effect. This finding is helpful for advancing the use of CAP in food decontamination.

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CHAPTER I

INTRODUCTION

Introduction

Fresh produce is an important part of a healthy human diet all around the world, adding rich sources of nutrients such as mineral, fiber, vitamins, proteins and carbohydrates[1]. [1]. Most families can buy fresh food, which allows them to achieve this healthy goal that the world is trending towards without exerting significant effort[1]. Unfortunately, the number of foodborne illnesses linked to fresh produced are increasing in parallel with the increase in consumption[1].In fact, every year as many as 1 in 6 American contracts a food borne illness after consuming a food product with foodborne pathogens[2]. Fresh produce is the leading cause of foodborne illness[3-4]. In the United States, fresh produce is thought to be responsible for 26% of the overall economic loss caused by foodborne illnesses, costing $39 billion per year[1].

A significant challenge to the decreasing the risk of foodborne illness from fresh produce, is the fresh produce can be contaminated with foodborne pathogen at any phase of the production or processing[5], emphasizing the importance of processing fresh produce to lessen the chance of foodborne illness. However, fresh produce is often minimally processed[1]. There are several methods available to achieve decontamination on fresh produce but with limitations.

Chlorine wash is a commonly used decontamination method for produce, however, it requires a large amount of water and produces toxic by-products[6-8]. While irradiation is effective against microbial population and parasites, consumers have concerns of the level of safety of ingesting irradiated food[9]. For consumer acceptance, the decontamination method used to reduce the microbial population on fresh produce should have minimum impact on the color or texture of the fresh produce. Overall, a decontamination method which incorporates the perspectives of the consumers, is unharmful to the health of workers & consumers and is environmentally & economically sustainable is needed.

Atmospheric Cold Plasma could fill this niche as no chemicals are added to the system beside using atmospheric air[10]. The Atmospheric Cold Plasma device uses electricity and an actuator to generate reactive oxidative species through the means of exciting the atmospheric air[11-12]. The actuator used in Surface Dielectric Barrier Discharge (SDBD) Atmospheric Cold Plasma system is composed of asymmetrically placed electrodes on both side of a sheet of dielectric material [13]. An additional believed strength of the Atmospheric Cold Plasma treatment is no residue left after treatment[14]. While it is known Atmospheric Cold Plasma can inactivate micro-organisms on many types of surfaces including foods[11, 14]. However, very few studies have investigated the unification of physical parameters which leads to the highest reduction of micro-organisms. The physical parameters known to influence microbial inactivation are treatment time, the rate at which the reactive species are produced, the energy supplied to the actuator, actuator design & distance of the item from the actuator. The aim of this study was to optimize the physical parameters to achieve a more thorough decontamination of foods. The preliminary objectives were evaluated with glass coverslips inoculated with a 5-strain mixture of *Salmonella enterica* after analysis, native pecans inoculated with the same 5-strain mixture were used as a

model. These results of this basic investigation can help to understand the microbial inactivation abilities of Atmospheric Cold Plasma for the food industry.

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CHAPTER II

REVIEW OF LITERATURE

Food Contamination, Foodborne Illness & Outbreaks

The Centers for Disease Control & Prevention estimates that "1 in 6 Americans get sick from contaminated foods or beverages each year and 3,000 die"[1]. The U.S. Department of Agriculture estimates that foodborne illnesses cost more than $15.6 billion each year [1]. The World Health Organization found the global finical burden of food borne illnesses to be comparable to major infectious diseases, such as HIV/AIDS, malaria & tuberculosis[2]. In the lastest report by World Health Organization (WHO) in 2016, the global burden of foodborne illnesses was 33 million Disability Adjusted Life Years (DALYS, one DALY represents the loss of year of full health) and children under 5 years old bore 40% of this burden[2]. It was found only 31 foodborne hazards caused 600 million foodborne illnesses & 420,000 deaths in 2010 globally[2]. Foodborne illness outbreaks can be difficult to identify thus outbreaks often go uninvestigated, unreported, and unrecognized; outbreaks may only be identified if there is a major economic or public health impact[2].

These illnesses are mostly associated with eating contaminated foods, especially these fresh and minimally processed, such as vegetables and fruits [3]. The types of foodborne contaminants are numerous, they include allergens, toxins, chemicals, parasites, bacteria & viruses[2]. The bacterial pathogens, including *Salmonella enterica* and Shiga toxin-producing. *Esherichia coli*, alone cause two-thirds of foodborne illness worldwide with high burden in developing countries[4]. Additionally, most of these microbes are of zoonotic importance resulting in significant impact on both economic & public health sectors[4].

It's important to note the consumer demand for fruit and vegetables has increased within the recent years[5]. In fact, United States vegetable consumption raised 24% from 1977 to 1999[5]. The increase in vegetable consumption is likely due the nutrition research showing consumption ensure an adequate intake of vitamins, minerals, fibers and antioxidants[6]. There has also been an increase in demand & market share for fresh ready-to-eat vegetables[6]. Unfortunately, there have been many recalls of fresh produce due to foodborne illness outbreaks, one way to combat this is to improve food safety. The benefits of improving food safety is a safer food supply, more consumer confidence, less stress on the healthcare system due to improved health, improved productivity, reduced loss of income & health care costs for the affected individuals[7]. As a result, postharvest decontamination treatment becomes a critical step in reducing foodborne illness risks associated with produce.

Food Decontamination Technologies for Produce

There are serval methods of post-harvest decontamination for fresh produce to help lower the foodborne illness outbreaks. Each method has its own attributes and limitations. The process of the method, application and efficacy will be discussed further in this literature review for

chlorine wash, microwave, irradiation, chlorine dioxide gas, electrolyzed oxidizing water, organic acids, pulsed light, and ozone.

Chlorine Wash

In the fresh produce industry, chlorine - based sanitizers are commonly utilized in processing[8]. However, the wash uses large wash basins and large amounts of water[8], which has an impact on the environment. Sodium hypochlorite is used to deliver chlorine to the wash water, the pH is often at 6.5[9]. The World Health Organization determined a concentration of 50 - 200ppm of chlorine at a pH less than 8 with a wash time over a minute is necessary for ensure effectiveness[10]. Unfortunately, Goodburn & Wallace found even complying to the recommend concentration, pH and wash time results in typical log reduction of less than 2 logs[9]. This is the theorized result of the chlorine reacting with the organic matter released from the fresh produce thus the amount of free chlorine for disinfection is reduced[8, 11-12]. Processing plants routinely try to avoid this by adding extra chlorine to wash water with a high organic content, this practice produces noxious chlorine by-product & chlorine off-gassing putting employees at risk[13-14]. Additionally, chlorine wash was found to not reduce populations of internalized microbes in leafy vegetables[15].

Chlorine Dioxide Gas

Chlorine dioxide is a polar gas which is easily dissolvable in water. The decontamination (antimicrobial) effect is due to its capacity to oxidize and chlorinate many organic compounds[16]. Thus, non-specific oxidative damage to microbial cell results in the loss of permeability control is identified as the primary lethal effect. In addition to controlling microbial populations, chlorine

dioxide is effective in controlling viral & fungal contamination of fresh produce[17-18]. Chlorine dioxide can disinfect irregular surfaces and lengthen the shelf life of produce[19]. The treatment is performed by injecting a stream of chlorine gas into the water [19]. The concentration of chlorine dioxide increases when injected but afterwards decreases due to reaction with the present vegetable during the remaining time[19]. The efficacy of chlorine dioxide wash, is dependent on the chlorine dioxide concentration, pH and treatment time, could reach 6 logs reduction at 30 min contact time[9]. However, the gas for the treatment needs to be made on site, it cannot be shipped due to the unstableness of the gas[10]. Additionally, chlorine dioxide gas can be explosive when concentrated and too high of an concentration of the gas can result in a bleaching of the foods color[10, 19]. Additionally, there usually is no sensory differences between treated and non-treated food[19]. The Food & Drug Administration has approved the use of chlorine dioxide gas for disinfection of fresh produce & food processing industries, due to its efficacy & saftey[20]. The United State implements a maximum 5ppm concentration of chlorine dioxide for application the disinfection of whole fresh produce[10]. Furthermore, this treatment also produces small amounts of toxic by-products[19].

Electrolyzed Oxidizing Water

Electrolyzed oxidizing (EO) water can be used as treatment on vegetables including sprouts[9]. The process of producing EO water is through electrolysis of dilute salt solutions in a way that separates the electrolysis chamber into cathode and anode through use of a diaphragm[21]. Thus, the major advantage to using electrolyzed oxidizing water as a treatment is, it can be performed on site[22]. The treatment is not corrosive to skin, mucous membrane, or organic material[22]. A benefit for those carrying out the treatment is the lack of need to store chemicals however deionized water is needed[22]. Conversely, the if the EO water is not continuously

supplied with H^+, HOCL and Cl_2 by electrolysis the antimicrobial activity is rapidly lost, which could be troublesome if the Device breaks down[22]. Furthermore, EO water is corrosive, excluding stainless steel, metal corrosion can happen[22]. Interestingly, the mechanism of decontamination is similar to chlorine dioxide due to the generation of chlorine ions during the EO water generation process which emits chlorine gas[22].

Organic Acids

The organic acid decontamination method often applies lactic, acetic & citric acids[23]. These acids are generally recognized as safe (GRAS) and are known for their ability to act on a wide range of bacteria while rapidly inhibiting microbial cells present in food[24-27]. When applying organic acid via a electrostatic spray, the log reduction is better than or comparable to chlorine, chlorine dioxide and ozone[9]. The results of a treatment of organic acids is very dependent on the concentration, pH of environment and dissociation of each acid[28]. It is important to remember with this method, if the treatment is not completely lethal then we could create resistance[28]. Application of organic acid has been used on meat products for some time[28]. However, when applied to beef there was a color change thus a mixture of acids may be more appropriate to use on beef [28]. When chicken was treated with organic acids a color change occurred again, the skin was yellowed or darkened after treatment[29]. Temperature is an important parameter to a successful treatment; Anderson & Marshall showed the most effective treatment of sanitizing beef semitendinosus muscle was application of acetic acid at 70^{c}[30]. Organic acids can also be used as sanitizer on ready to eat foods, due to organic acids being considered as generally recognized as safe (GRAS) compund[23]. A electrostatic spray system was use to perform organic acid method on spinach & lettuce[31]. The produce was sprayed 3 to 4 time for 5 second each

spray[31]. This treatment affects microbes in 2 ways cytoplasmic acidification and accumulation of acid to toxic levels[31].

Irradiation

The irradiation process for fresh produce is carried out by applying rays of irradiation, the permitted sources to produce gamma-rays are caesium-137 & cobalt-60[32].The Food & Drug Administration has approved 1-10 kilogray (unit of radiation absorbed dose) to extend shelf life of produce[32]. In order for treatment for to be effective, it needs to be completed soon after harvest[33]. The mechanism works based on one of two ways; one exposed to radiation the microbial body generates energy transfer within their body, resulting in the destruction of chemical & molecular bonds or water molecules can become ionized thus producing free radicals resulting in microbial death[34]. The reduction of microbes depends on the type of food, oxygen presence, water content, types of microorganism radiation and storage temperature[9, 35-40]. It is important to note a study found *Salmonella* to grow after irradiation treatment, suggesting it cannot be used in isolation[9, 41]. However, too long of treatment can result in discolored meat along with an off flavor & off odor[34, 42]. One major advantage of using radiation for microbial decontamination is the treatment can be done to fresh, frozen, and packaged food[34]. Irradiation can be performed on poultry, red meat, egg products, fish products & dry ingredients[34]. Additionally, the treatment has low effects on the environment & can control parasite infection[34]. The largest drawback to utilizing radiation is the consumer concern of the level of safety of ingesting the food.

Pulsed-Light System

Pulsed – light system uses high intensity flashes of light to kill microbes[43]. To perform this treatment a power supply, pulse configuration device and lamp is needed[43]. Additionally, it is known for the greater the light intensity of the pulse the greater the kill of microbes[43]. Limitations of this method is only the surface of the food is decontaminated and can only be used on clear packages[43]. Furthermore, if a certain microbe needs a longer treatment time it is possible for the food to get too hot and affect the quality[43].The geometry configuration for this treatment starts with energy being stored in a high power capacitor for a fraction of a second[43].Then that energy is released to a xenon lamp unit in a shorter time than the capacitor and the lamp lets out an intense pulse of light that last a few hundred microseconds[43]. Pulsed-light has been shown to reduce E. coli O157:H7 on romaine lettuce both in- package and direct treatment with average log reduction of 2.33 and 2.68 respectively[44].

Ozone

Ozone, or triatomic oxygen O_3, is an unstable allotrope of oxygen with a high oxidation potential. Ozone is generated by either ultraviolet radiation or corona discharge methods, with corona discharge being the choice of most commercial applications[45].There are many suggested applications of ozone in the food industry such as sanitation of food plant equipment, food surface hygiene, reuse of wastewater, chemical oxygen demand of food plant waste and lowering biological oxygen demand[46-49]. It was found when fruits and vegetables treated with ozone the self-life increased[46, 50]. The use of ozone as a produce decontaminate treatment, the log reduction can be viewed equal to chlorine wash[9]. A study showed *E.coli* O157:H7 and *Salmonella* sp. Treated with ozone typically had a 2-3 log reduction per fruit for 22 days of storage and no negative effect on fruit texture or color[51]. However, there are some areas in which ozone should

not be used such as cleansing of shellfish[52], conversion of green tea to black tea[53], disinfection of poultry carcasses and chill water in the poultry industry[54-57]. This is likely due to ozone not reacting with water however it does slowly decompose in the water[45, 58]. For this reason, if ozone is to be used in water there needs to be continual production of it since it is being decomposed[59-60].

Due to the ozone having strong oxidizing properties it was found concentrations 0.2ppm and higher of ozone along with length of exposure can cause varying degrees of damage to the respiratory tract[45, 61]. Thus, it is important to monitor the health of people who might have contact with ozone[62]. The symptoms of ozone toxicity include headache, dizziness, a burning sensation in the eyes and throat, sharp taste, sharp smell and cough[62]. Chronic toxicity symptoms are more severe and can be weakness, decreased memory, increased muscular excitability and increased prevalence of bronchitis[62]. Ozone is known to be corrosive and could reduce the life of processing equipment, lessening the practically of this method for industry[9].

Atmospheric Cold Plasma Technology

Overview of Cold Plasma

The fourth state matter, plasma, can be used in food decontamination[63]. There are many different methods to produce plasma from gases, they can differ from energy source, gasses utilized, pressure of gasses utilized and temperature differences[63]. The temperature difference in plasma can be characterized into thermal and nonthermal plasma[63]. Nonthermal plasma also known as cold plasma differs because it does not generate the same level of heat as thermal plasma[63]. Atmospheric plasma is produced with gasses at atmospheric pressure level where as vacuum plasm is produced at pressure less than atmospheric pressure level[63]. Cold plasma does not use additional chemicals, it only excites the electrons and atoms in gas which results in the

formation of active species of gasses with antimicrobial activity[45, 64-66]. Further, current knowledge shows that cold plasma does not leave residue on items treated[67]. The mechanisms of cold plasma action, types of cold plasma, potential application for food decontamination, and the challenges in developing cold plasma for food decontamination applications, will be discuss in the following section.

Mechanisms of Cold Plasma Action

The ions and electrons from the plasma are generated at an electrode by means of a dielectric barrier discharge (DBD) power source, radiofrequency (RF) or microwave (MW), and a biasing power source is applied to another electrode creating a significant ion bombardment component during plasma treatment[68-69]. The cold plasma treatment is a simultaneous deposition/removing process in where loosely "deposited species" over planar or topographical surfaces are sputtered off by radicals and reactive ion during deposition[69-70]. There are many variables to the efficacy of cold plasma including varying electrode geometries, relative simplicity, employment of different gases, reduced gas flow rate, and uniform discharge ignition over serval meters[70]. The oxygen species created in the plasma react with organic contaminants to form lower molecular weight hydrocarbons & H_2O, CO, CO_2[68]. When using the ultra-violet geometries, the VUV photons affect the plasma chemical process in the discharge [71-72]. Due to the high energy range the photons are able to break chemical bonds and induce photoionization[71-72]. Most of the by-products from the treatment are small quantities of gases such as carbon dioxide, and water vapor with trace amounts of carbon monoxide & other hydrocarbons[68, 73]. Through inactivation kinetic studies it was found that the inactivation of the bacteria is a complex process[74]. In addition to the etching effect of cold plasma treatment there are oxidation of lipid membranes, nucleic acids, and proteins thus leading to destruction of cell membrane, disruption

of the cell metabolism and eventually cell death[75-78]. Many factors can affect the inactivation process such as, the type of medium in/on which the cells are seeded, the type of bacteria, the number of cell layers in the sample, the type of exposure, operating gas mixture, contribution of UV or lack-thereof[74]. It is more often that UV does not play a role in air plasma, although in a few cases this was not the case[74]. When UV does play a role, the observed survivor curves tend to have a rapid phase first which is followed by a slower second phase[74]. Through plasma medicine, it has been found that VUV photons can inactivate microorganisms[72, 79-80], but might also alter the DNA of cells as proteins and DNA absorb UV radiation[72, 81].

Potential Application for Food Industry

Decontamination Utilizing Cold Plasma

Cold plasma is able to effectively treat contaminated foods with a treatment time of 3 – 120 seconds depending on the item being treated and the conditions of the enivronment[63]. The treatment is able to inactivate microbes on fruits, vegetables, poultry and meats [63]. A study using atmosphere cold plasma on cherry tomatoes found a 3.1, 3.6 and 6.7 log reduction of *Salmonella, E. coli and L. monocytogenes* respectively[82]. However cold plasma treatment can have negative effects on food quality such as loss of vitamins, accelerated lipid oxidation and sensory characteristics (color & texture)[83]. These effects can occur during treatment or later in storage, it hypothesized optimization of treatment may lessen the negative effects[83].

Cold Plasma treatments are thought be able to treat the packing surrounding the food. Cold plasma treatments can perform inactivation of many micro-organisms (bacterial cells, spores, yeasts & molds) of the packaging by adhering to the surfaces within short treatment times[67-68]. The polymer surfaces materials include plastic bottle, lids and films which can be

rapidly sterilized using cold plasma, without affecting their properties or depositing residues[67-68]. Additionally, in vacuum ultraviolet geometry, it was found VUV and UV photons can produce cross-linking in polymer material[72, 84]. It was also discovered, cold plasma can be used for degradation of pesticide residue in food products[70]. However, they saw the firmness decrease over storage time due to surface softening and collapse of internal structures[70]. One study with strawberries that contained fludioxonil, azoxystrobin, pyriproxyfen and cyprodinil, showed that cold plasma had a removal efficiency of 45-71%[85-86]. Cold plasma can also enhance cooking properties, decrease cooking time, and positively influence the surface characteristics of grains[70]. Sarangapani Yamuna Devi, et al. showed a significant reduction of cooking time of black gram after low pressure plasma treatment[70, 87]. Additionally, they found increased water absorption capacity with plasma treatment after 4 hours of soaking due to formation of simple sugars, such as glucose, dextrin, maltose[70, 87]. Chizoba-Ekezie, hypothesized that cold plasma could have granular corrosion/etching abilities which could induce modifications via molecular degradation[70]. Lee et al., found cold plasma etching and surface depolymerization to increase α-amylase activity up to 1.21 folds and water absorption in brown rice[88]. Plasma can also be used to sterilize the packaging of foods resulting in a safer food and enhanced self-life[74]. A study looking at the protein rich isolates from grain pea found a 191% rise in solubility & 113% rise in water uptake in the protein isolates after cold plasma treatment[70, 89].

Limitations of Cold Plasma

A study done by Liang et al., showed that high concentration of bacteria cluster, reduces the penetration capacity of reactive species and bacterial spores are more resistant to plasma treatment than vegetative cells [70, 90]. They also showed fungi with cell walls composed chiefly of chitin to exhibit stronger resistance than bacteria due to the intricate rigidity imparted [70, 90].

Another study done by Fernandez, Noriega & Thompson, on the treatment of eggshells showed a decreased interaction between reactive species, a result of stacking of microbes, considering the relatively unsmooth surface[70, 91]. The nature of the matrix also influences the efficiency of the treatment, a agar plate or filter membrane can be effectively decontaminated, compared to the matrix present on a cheese slice or surface of a fruit due to location of microorganism from the outer to inner tissues[70, 92].

Types of Cold Plasma (based on methods of cold plasma generation)

Vacuum Ultraviolet

Biozone Scientific developed a new process to generate cold oxygen plasma by subjecting air to high-energy deep-UV light with an effective radiation spectrum between 180 and 270 nm[75]. The wavelength range of vacuum ultraviolet photons fits with in the ultraviolet range of 100-400 nm, thus the ultraviolet aspect[72]. The highly energetic photons produced are able to travel several tens of millimeters along noble gas channels which allows for photo chemistry reactions on the surface of the of the item being treated[72]. Additionally, the highly energetic photons can have a strong impact on the energy transport in the discharge[72]. There is a large energy gap which allows us to see UV wavelength resonant transitions to the ground[72]. These resonant photons have the ability to be easily reabsorbed in the plasma due to the high number of neutral particles in APP, they also carry a lot of energy[72]. This cold gas plasma is composed of several species besides UV photons like free radical molecules, electrons, negative and positive ions, and ozone[75, 93]. This geometry is very effective the breaking most organic bonds of surface contaminants[68].

Capacity Couple Plasma

Another way to generate cold plasma is a capacity couple plasma (CCP) source, it is a very common type of technological plasma sources[68, 94]. This system is placed in a chamber and consists of two metal electrodes separated by a small distance[94]. The gas pressure in the chamber can be equal or lower to atmospheric pressure[68]. The system is propelled by a single RF power supply, often at 13.56 MHz[68]. Only one of the electrodes is connected to the power supply, and then other electrode is grounded[68]. The plasma specifically formed with this method has many applications including deposition, sputtering and cleaning[68, 95].

Inductive Couple Plasma

Another configuration to generate cold plasma is inductive coupled plasma (ICP). Where the energy source is derived from the electrical currents which are produced by electromagnetic induction, that is, by time-varying magnetic fields[68, 96]. There are two types of geometry for ICP, planar & cylindrical[68]. In planar geometry, the electrode is a coil of flat metal wound into the shape of a spiral[68]. In cylindrical geometry, the electrode is shaped like a helical spring[68]. In both geometries, a time varying electric current is passed through the coil creating a time varying magnetic field around it, thus induces azimuthal electric currents in the rarefied gas, leading to formation and break down of plasma[68]. A benefit to this configuration is it is relatively free of contamination due to the electrodes placed completely outside the reaction chamber[68]. This inductive couple plasma generation has a better efficiency in destroying biological matter[74, 97].

Electron Cyclotron Resonance

An electron cyclotron resonance (ECR) plasma configuration has a microwave input at 2.45 GHz and a magnetron which generates plasma[68, 98]. The mechanism for plasma generation is the electrons moving spirally & vertically along the magnetic field lines[68]. The "electrons travel far enough to gain sufficient energy to strike gas molecules and cause ionization"[69]. Electron density (ion flux) is over ten times higher than CCP or ICP plasma configurations, and therefore ECR may be more efficient for surface treatments of packaging[68].

Gliding Arc Discharge

Gliding arc discharges (GAD) configuration is created in a reactor in open air conditions, containing two or more diverging metallic electrodes operating at a high potential difference of 9kV and 100mA[70]. The system works by pumping in a "inlet gas consisting of humid air into the discharge gap between the electrode, allowing an arc to form in between the narrowest inter-electrode area, which is subsequently blown away by the inlet gas into the diverging area"[70]. The plasma produced can be thermal or non-thermal depending on the conditions, thus GAD possess excellent adaptability for both liquid and surface treatments[70].

Atmospheric Pressure Plasma Jet

Another way to generate plasma is through a plasma pen or jet, in which a stream of plasma can be directed at the object to be treated[75]. A common device configuration for atmospheric pressure plasma jet (APPJ) is a quartz reactor tube containing dual-pin parallel

tungsten electrodes which are positioned 12mm apart of each other[99]. It is important to note the cells treated are not permeabilized instantly with this configuration[99]. It is thought a gradual effect takes place on the cells; thus, some cells could receive a mild enough treatment that they could repair themselves if they are un the correct environment[99]. The cells which receive significant treatment begin the process of cell lysis[99] thus resulting in cell death. A study conducted on *Pseudomonas aeruginosa* biofilms on stainless steel showed an "exposure of the cells to atmospheric plasma jet for 15 seconds resulted in viable population reductions of as high as 5 to 6 log cycles"[78] thus a successful treatment does not have to mean a long treatment time. Another study showed APPJ's ability to reduce the level of human norovirus GII in salmon sashimi with air, O_2 or N_2[100]. The treatment did not affect the quality of the fish however, more extensive research on seafoods is needed[100].

Corona Discharge Plasma

Corona discharge plasma (CDP) generation is a diffuse route for plasma ignition which develops around sharp pointed electrodes[70, 99]. The diffuse route contains a substantial electric field for expediting the ionization energy of produced electrons to the surrounding environment of gas atoms or molecules[70, 99]. CDP occurs predominantly on one electrode and generated at high voltage[70]. Additionally, CDP configuration is simple to implement and inexpensive, however, it is restricted to non-homogeneous small areas[70].

Radio Frequency Plasma

Radio frequency cold plasma configuration uses of rapidly cycling electrical impulses, operating at various power & voltage setting to ionize gases[63]. This is usually achieved by placing

a gas within an oscillation electromagnetic field[70]. The electromagnetic field is produced by an distant electrodes kept outside the reactor or induction coil[70]. There are several different configurations within radio frequency plasma, the food being treated can even be placed between the electrodes, making an electrode contact system[63]. Another geometry is the food being treated can be below the electrodes with the plasma being blown on to the surface of the food, like a cold plasma jet[63]. It's important to know, the composition of the initial gas has a significant impact on the antimicrobial power of the plasma produced, before choosing the gas[101-102]. Bol'shakov found oxygen to be the best gas source to achieve sanitization for RFP[97].

Microwave Powered Plasma

Microwave powered (MP) plasma is "generated at low pressure, driven by electromagnetic waves at frequencies over hundreds of MHz"[70]. This method generated the plasma via a "magnetron that supplies microwaves into a process chamber guided by coaxial cable"[70]. The irradiation produced is then absorbed by the process gas, thus heat is produced, causing ionization reactions through inelastic collisions[70]. One advantage to MP is it does not use electrodes and can be easily enkindled in air[70]. Additionally, MP has low gas requirements and typically large quantities of reactive species are released[70]. However, if MP is to be used for a large area it needs an array of discharges comparable to the arrangement of plasma[70].

Plasma-Activated Water

Plasma activated water (PAW) can also be called plasma activated liquids, plasma acid and nutrient broths, it mainly contains reactive species from the plasma[58, 102-105]. The plasma-activated water is a solution which contains significant portion of reactive oxygen species (ROS)

compared to control facilitates microbial inactivation, regarding its high oxidation-reduction potential[70, 106-107]. Like other forms of cold plasma, the type and concentration of the reactive species that are present are dependent on the gases and liquids chosen to generate plasma[58, 108].Reactive oxygen nitrogen species (RONS) production strongly depends on different parameters, including the type of feeding gas, plasma power supply, applied voltage, treatment time, discharge frequency, electrode configuration, the distance between the electrode and liquid surface and volume of the solution[109-113]. In PAW, direct plasma discharge in aqueous solution, gas-phase plasma discharge over the surface of the aqueous solution and multiphase discharge, where plasma is ignited in bubbles or ignites in gaseous phase but mixed with water droplets[113]. The different configurations involve different energy transfer, chemical reaction and diffusion process[111, 113-115], thus selection of the correct configuration is important. The use of nitrogen, oxygen and water as parent molecules for the production of PAW will result in the formation of a number of primary species, including atomic oxygen, singlet oxygen, superoxide, ozone, hydroxyl radicals, excited and atomic nitrogen, which will then continue to react and form secondary species, including hydrogen peroxide, peroxynitrite, nitric oxide, nitrates and nitrite ions[58, 106, 108].

Dielectric Barrier Discharge

One configuration to produce to plasma is with a dielectric barrier discharge (DBD)[68-69]. DBD is the production of electrical discharge between two electrodes separated by insulating dielectric barrier[68, 76]. The DBD method uses a high voltage alternating current, often at lower RF frequencies[68], which flows through the electrodes. There are many configurations for the electrodes some are cylindrical, using coaxial plates with a dielectric tube between them, most are typically planar, using parallel plates separated by a dielectric barrier[68]. Commonly in a coaxial

configuration, the common fluorescent tubing and dielectric are shaped in the same form[68]. The inside of the container is filled at atmospheric pressure with a rare gas halide mix, a rare gas with the glass walls acting as the dielectric barrier[68] or atmospheric air. This process requires high energy levels to be sustained due to the atmospheric pressure level of the gas[68]. Materials commonly used for dielectrics aspect are glass, quartz, ceramics, and polymers[68, 116].

Surface Dielectric Barrier Discharge

Surface Dielectric Barrier Discharge (SDBD) is a unique type of DBD plasma generation, through use of actuator which are, asymmetrically places electrodes on both sides of a sheet of dielectric material[117]. One of the electrodes on the actuator is left exposed to the atmospheric has while the other is insulated preventing contact[118]. Plasma develops adjacent to the uncovered electrode and varies in forms from diffuse discharges with low glow to distinct streamers based on the amplitudes of supplied AC voltage & applied frequency[118]. The actuators used in SDBD can generate induced near wall zero net mass flux jet which traps the atmospheric gas as well as, drives the fluid trapped in the plasma towards the surface being treated[117]. SDBD can be used to treat a large surface due to these mechanics of the design.

Prior research has shown the results of microbial inactivation through SDBD treatment to be dependent on the physical parameters of application. During formation of the plasma, feed gases such as nitrogen, oxygen, helium, argon and carbon dioxide have an impact on the effectiveness of bacterial inactivation[103]. Another important parameter is the treatment time, has a significant impact on the concentration of ozone produced during the treatment[119]. It is important to note, when the relative humidity level is low, and oxygen is utilized as a feed gas or if there is a high concentration of oxygen in the feed gas relationship between ozone & treatment time is

proportional[120]. This is attributed to quenching resulting from an increase in water dissociation and the ozone-water direct reaction[121-123]. Equally important, the power/voltage is directly proportional to microbial inhibition caused by the increase in the production of reactive species as the voltage rises, and these reactive species are primarily responsible for the microbial inhibition[120].

There is little information available combining the effects of all impactful parameter to attempt to achieve a higher bacterial inactivation. The results of a study, expanding on these effects, could allow the SDBD ACP to be used in processing of foods in the future. The goals of this study were to evaluate the physical parameters that would increase the effectiveness of the SDBD ACP treatment on bacterial inactivation on foods. In chapters three & four the effects of the actuator design, treatment time and distance of item from actuator are investigated. Further, chapter 5 quantifies the rate at which ozone is produced and is detected at various distances from the actuator. In chapter 6, the actuator is narrowed to a single design and the effect of different pulse regimes on bacterial inactivation is investigated. Lastly, in chapter 7 the glass coverslips are replaced with native pecans as a model of the combined polished parameters on bacterial inactivation.

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CHAPTER III

BACTERIAL INACTIVATION STUDY OF ACP DEVICE 1.0

1. Materials and Methods

1.1. **Atmospheric cold plasma actuator & device construction.** Surface dielectric barrier discharge (SDBD) actuators were constructed using a Teflon F4B dielectric barrier sheet, 267.2 x 177 x 1.6mm and asymmetrically arranged copper electrodes (Hua Xing PCBA Limited, P. R. China). A total of five types of actuators (A1-A5) were constructed with electrode width of 0.1 cm, 0.5 cm, or 1.0 cm and electrode gap (distance between opposite electrodes) of 0.1 mm, 0.5mm, or 1.0 mm (Table 1). The treatment chamber holding the actuator was a pelican 1450 protector case composed of polypropylene and had ports with removable plugs to allow sensors to take measurements during the treatments. The case two latches were composed of ABS which created a tight seal once latched

Actuator	Electrode Width	Electrode Gap
A1	0.1 cm	0.5 mm
A2	0.5 cm	0.1 mm
A3	0.5 cm	0.5 mm
A4	0.5 cm	1 mm
A5	1 cm	0.5 mm

Table 1: Actuators arrangement of electrode width & gap

SDBD actuators were given input power that ensured full glow of actuator (Table 2). A pulsing power circuit was utilized to reduce heat buildup in inside of the airtight chamber. Unless stated otherwise, the pulse intervals for all actuators were 5 seconds with plasma output and 3 seconds off.

Actuator, distance & time	Avg. Watts
A1 2cm 5min	239
A2 2cm 5min	221
A3 2cm 5min	300
A4 2cm 5min	392
A5 2cm 5min	208
A1 5cm 2min	266.3333333
A2 5cm 2min	250.3333333
A3 5cm 2min	276
A4 5cm 2min	358
A5 5cm 2min	292
A1 2cm 2min	241.3333333
A2 2cm 2min	301.6666667
A3 2cm 2min	326
A4 2cm 2min	395.6666667
A5 2cm 2min	363.3333333

Table 2: Average input power(watts) per actuator device 1.0, determine by the unform full glow of the actuator and watts screen on the device (n=24).

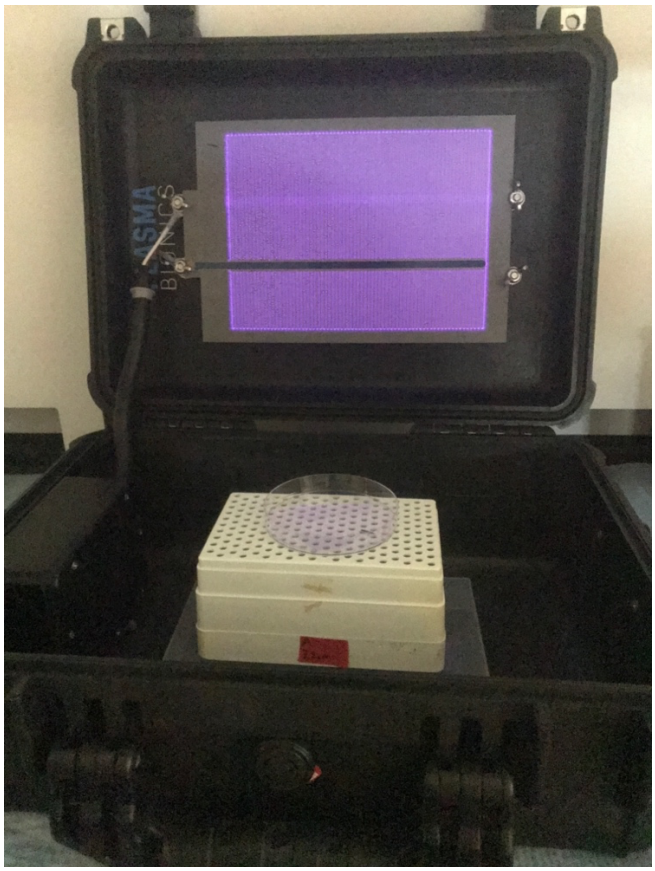

Figure 1: Picture of ACP Device 1.0 with actuator A1 operating at an uniform full glow. The actuator was attached via wing nuts, which allowed the switching of actuators. The lid was closed and latched to ensure a close system when the treatment was performed.

1.2. **Preparation of bacterial culture & inoculum.** A five-strain mixture of *Salmonella enterica* subsp. *enterica* serovars Enteritidis, Typhimurium, Javiana, Oranienberg, and Agona was used in all the experiments. The five- strain mixture was utilized to represent the range of sensitivity to the treatment[1]. Each strain was grown individually in 5ml of tropic soy broth (TSB) at 37 ºC with shaking (250 rpm) for 19 hours. Once the growth period was complete each culture was vortexed and 1ml was collected and combined with all strains grown. Then 1ml of the combined culture was vortexed before centrifuging at 12,000rpm for 3min. The pellet was res-suspended in 0.1% sterile peptone water. A serial dilution of 1:10 of the suspension in sterile peptone water was performed to determine cell concentration and for inoculum of coverslips. The highest

dilutions (-8, -7, and -6) were plated on a tropic soy agar (TSA) plate by drop plating and the plates were incubated at 37 ºC for 24 hours before enumeration.

1.3. **Inoculation of coverslips.** Glass coverslips 22x22mm (Eisco Labs) were placed in a glass petri dish and autoclaved prior to the experiment. For inoculation, 3 coverslips were placed in individual sterile plastic petri dishes labeled for each individual actuator and one control group. A total of 50ul of the -2 dilution of inoculum was dropped on each coverslip as evenly as possible. The coverslips were then left to dry in the biosafety cabinet for 60 minutes.

1.4. **Treatment.** Boxes were used inside the treatment chamber to set the coverslips at a distance of 2cm or 5cm from the actuator. The selected distances were inspired by earlier works of this lab, which show a log reduction was achieved at a 5cm distance[2]. To achieve the treatment distances, the distance from the actuator sheet to the bottom of the inside of the chamber was measured. Then a empty pipette box &/or index cards were stacked the height of chamber minus 2cm or 5cm. Before each treatment, the actuator matching the labeled periti dish was installed in the chamber. Then the matching periti dish was placed inside the chamber and the Device was turned on till the actuator had uniform glow before closing the chamber. Using a timer, we ran the Device for 2 or 5 minutes. In each trial, three replicate coverslips were used for each treatment (actuator, time, and distance) and the controls (untreated).

1.5. **Microbial recovery and log reduction calculations.** Overall, the methods for microbial recovery & log reduction calculations were like earlier works of this lab with minor modifications [1]. Immediately after treatment, each coverslip (including controls) was placed in individual sterile 50ml conical tubes containing 10ml of sterile 0.1% peptone

water. Once all actuators were done, all the wash tubes were vortex for 15 seconds, rested for 10 seconds repeated 3 times for a total of 1:05 minute per wash tube. The wash fluid was diluted by 1:10 serial in sterile 0.1% peptone water and drop-plated onto TSA plates. The plate counts of cells (colony forming units, CFU) from each wash fluid for each replicate were transformed to log CFU/coverslip. The log reductions due to cold plasma treatment were calculated by subtraction log CFU/coverslip of the treated coverslips from their corresponding average log CFU/coverslip of controls.

1.6. **Statistical analysis.** One-way Analysis of variance (ANOVA) was performed to calculate statistical differences among the treatments using data from two independent trials with three replicates in each trial for each treatment. Following the ANOVA, Hsu's MCB test was performed to rank the treatments based on their mean log reduction. Then depending on if the data was parametric or nonparametric a student's t-test or wilixcon test was performed to see the p-values between the treatments with the higher log reductions from the Hsu's MCB test. The significant difference was defined as $p < 0.05$.

2. **Results.** In each trial, three replicate coverslips were used for each treatment (actuator, time, and distance) and the controls (untreated). The performance (log reductions of *Salmonella enterica*) of actuators at treatment time (2 and 5 min) and distance (2 cm and 5 cm) is summarized in Figure 2. A3 & A4 was found to have the highest *Salmonella enterica* log reduction at a treatment time of 5 minutes & 2 cm distance.

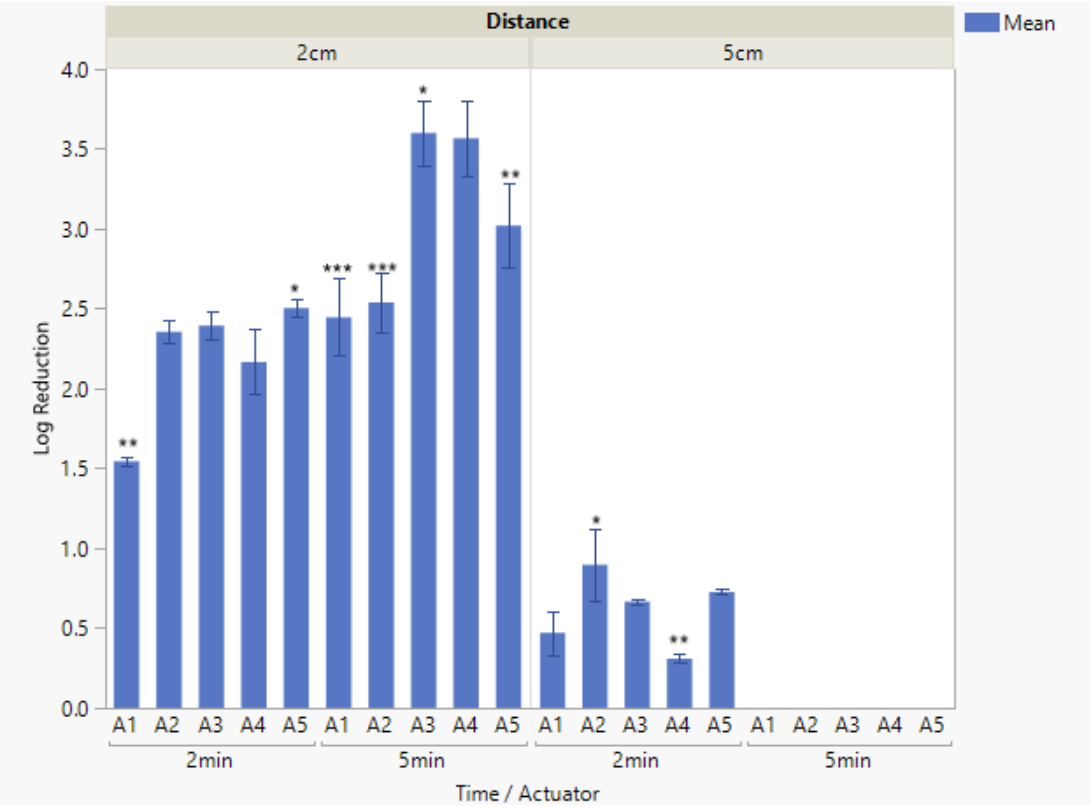

Figure 2: Reduction of *Salmonella enterica* on glass coverslips as a function of CAP device 1.0 treatment distance and time regarding actuator design. Standard error bars are constructed using 1 standard error from the mean. Asterisk depicts the differences in means with 0.05 significance.

3. **Discussion & Conclusion.** The results suggested a 5-minute treatment time with a 2 cm distance is optimal for actuators A3, A4 & A5. When the mean log reductions from this study were compared to previous studies, the log reduction was considerably lower and more variable. It was theorized, the Device was malfunctioning as these results did not coincide with previous studies. For this reason, a treatment time of 5 minutes at 5 cm distance was not tested. The Device was evaluated by engineers and was determined the Device to be arcing inside the chamber during treatment thus loss of power to produce cold plasma. The power available to the actuator was lessened due to the uninsulated gap between a bolt connected to the input power & the actuator.

CHAPTER IV

EVALUATION OF THE EFFECT OF ELECTRODE ARRANGMENT ON THE INACTIVATION OF SALMONELLA ENTERICA USING ACP DEVICE 2.0

1. **Methods and materials**

1.1. Adjustments made to ACP device (therefore, ACP 2.0). ACP 2.0 was almost identical to ACP 1.0 except that the bolts used to attach the actuators were shortened to prevent the arcing that occurred previously along with additional insulation of the electrical elements.

1.2 Preparation of bacterial culture & inoculum. A five-strain mixture of *Salmonella enterica* subsp. *enterica* serovars Enteritidis, Typhimurium, Javiana, Oranienberg, and Agona was used in all the experiments. The five- strain mixture was utilized to represent the range of sensitivity to the treatment [1]. Each strain was grown individually in 5ml of tropic soy broth (TSB) at 37 ºC with shaking (250 rpm) for 19 hours. Once the growth period was complete each culture was vortexed and 1ml was collected and combined with all strains grown. Then 1ml of the combined culture was vortexed before centrifuging at 12,000rpm for 3min. The pellet was res-suspended in 0.1% sterile peptone water.

A serial dilution of 1:10 of the suspension in sterile peptone water was performed to determine cell concentration and for inoculum of coverslips. The highest dilutions (-8, -7, and -6) were plated on a tropic soy agar (TSA) plate by drop plating and the plates were incubated at 37 ºC for 24 hours before enumeration.

1.2. **Inoculation of coverslips.** Glass coverslips 22x22mm (Supply info) were placed in a glass periti dish and autoclaved prior to the experiment. For inoculation, 3 coverslips were placed in individual sterile plastic petri dishes labeled for each individual actuator and one control group. A total of 50ul of the -1 dilution of inoculum was dropped on each coverslip as evenly as possible. The coverslips were then left to dry in the biosafety cabinet for 60 minutes.

3.1. **Treatment**. Boxes were used inside the treatment chamber to set the coverslips at a distance of 2cm or 5cm from the actuator. The selected distances were inspired by earlier works of this lab, which show a log reduction was achieved at a 5cm distance[2]. To achieve the treatment distances, the distance from the actuator sheet to the bottom of the inside of the chamber was measured. Then a empty pipette box &/or index cards were stacked the height of chamber minus 2cm or 5cm. Before each treatment, the actuator matching the labeled periti dish was installed in the chamber. Then the matching periti dish was placed inside the chamber and the Device was turned on till the actuator had uniform glow before closing the chamber. Using a timer, we ran the Device for 2 or 5 minutes. In each trial, three replicate coverslips were used for each treatment (actuator, time, and distance) and the controls (untreated). The temperature of the treatment was measured at the same time. A hydrometer & thermometer from Radio Shack was used. The probe was taped next to the periti dish to portray the temperature on the coverslips.

3.2. **Microbial recovery and log reduction calculations.** Overall, the methods for microbial recovery & log reduction calculations were like earlier works of this lab with minor modifications [1]. Immediately after treatment, each coverslip (including controls) was placed in individual sterile 50ml conical tubes containing 10ml of sterile 0.1% peptone water. Once all actuators were done, all the wash tubes were vortex for 15 seconds, rested for 10 seconds repeated 3 times for a total of 1:05 minute per wash tube. The wash fluid was diluted by 1:10 serial in sterile 0.1% peptone water and drop-plated onto TSA plates. Spread-plating (100 ul or 250 ul) was carried out for treatments that couldn't be enumerated by drop-plating (5 min at 2 cm) due to low cell recovery. The plates were incubated overnight at 37 ºC before enumeration. The plate counts of cells (colony forming units, CFU) from each wash fluid for each replicate were transformed to log CFU/coverslip. The log reductions due to cold plasma treatment were calculated by subtraction log CFU/coverslip of the treated coverslips from their corresponding average log CFU/coverslip of controls.

3.3. **Statistical analysis.** One-way Analysis of variance (ANOVA) was performed to calculate statistical differences among the treatments using data from two independent trials with three replicates in each trial for each treatment. Following the ANOVA, Hsu's MCB test was performed to rank the treatments based on their mean log reduction. Then depending on if the data was parametric or nonparametric a student's t-test or wilixcon test was performed to see the p-values between the treatments with the higher log reductions from the Hsu's MCB test. The significant difference was defined as $p < 0.05$.

2. Results

2.1. Effect of treatment time and distance on the performance of ACP device in inactivation of *S. enterica.*

The overall performance of the ACP device 2.0 as influenced by treatment time/distance is shown in Figures 3 & 4. Actuators A1, A2 & A3 at 5-minute treatment time and 2cm distance, had the 3 highest log reductions of *S. enterica* respectively (n=6). It is worth noting, actuator A1 at 5-minute treatment time & 2 cm distance only had a slightly difference when compared to A2 at 5 minute treatment time & 2cm distance. The actuators A1 & A5 was found to have the lowest log reduction when comparing treatments at a treatment time of 2 minutes & 5 cm distance. However, when comparing treatments of 5 minutes and 5 cm distance, actuators A4 & A5 had the highest log reduction, yet there was no significant difference between the two (figure 3). Additionally, this comparison showed actuator A1 to have a significantly less log reduction. Furthermore, there was a significant difference in mean log reduction of A1 treatment time of 5min at 2cm distance to A4 &A5 at a treatment time of 5 minutes at 5 cm distance. A comparison of treatments of 2 minutes at 2cm distance only had slight differences between actuators, with the highest log reduction from actuator A4 (n=6) (figure 4).

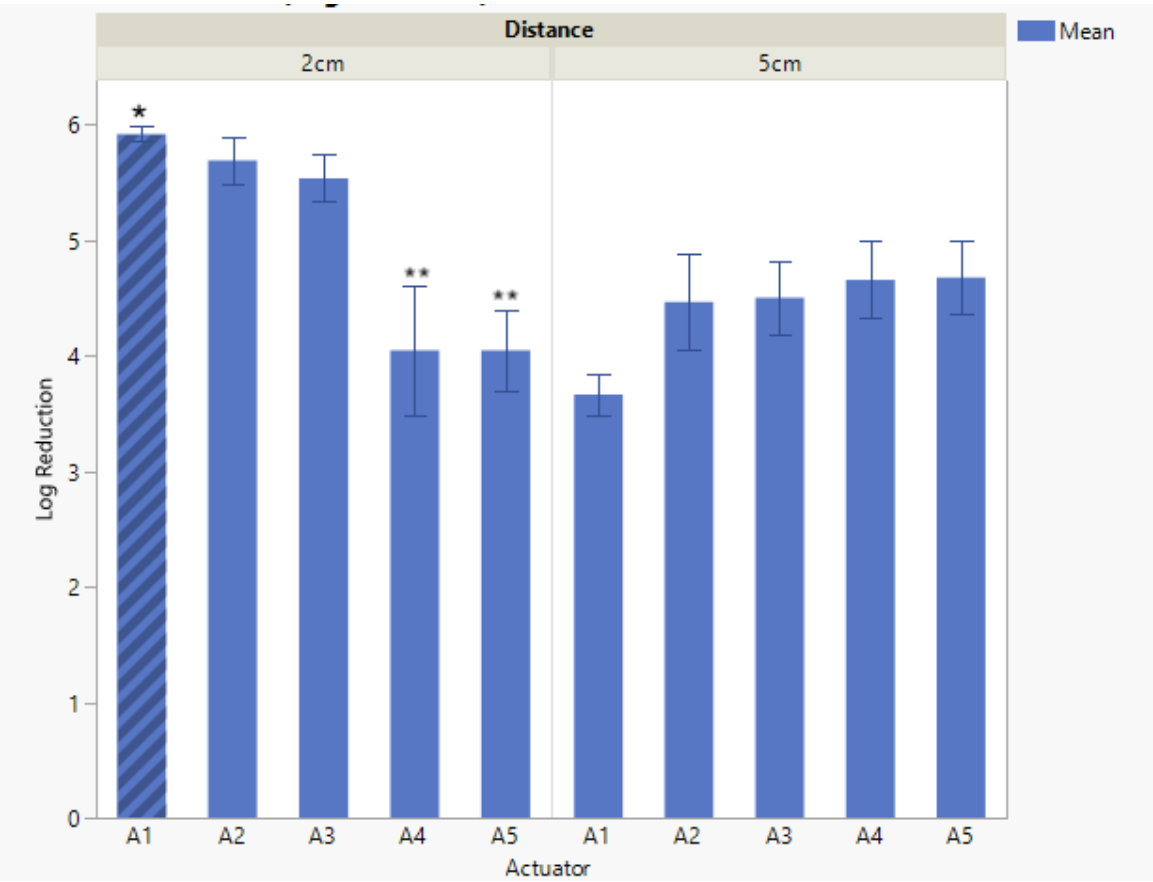

Figure 3: Reduction of *Salmonella enterica* on glass coverslips after 5 min treatment by atmospheric cold plasma (CAP device 2.0) with actuators varying in electrode width and gaps Error bars represent standard errors (n =6). Asterisk depicts the differences in means with 0.05 significance.

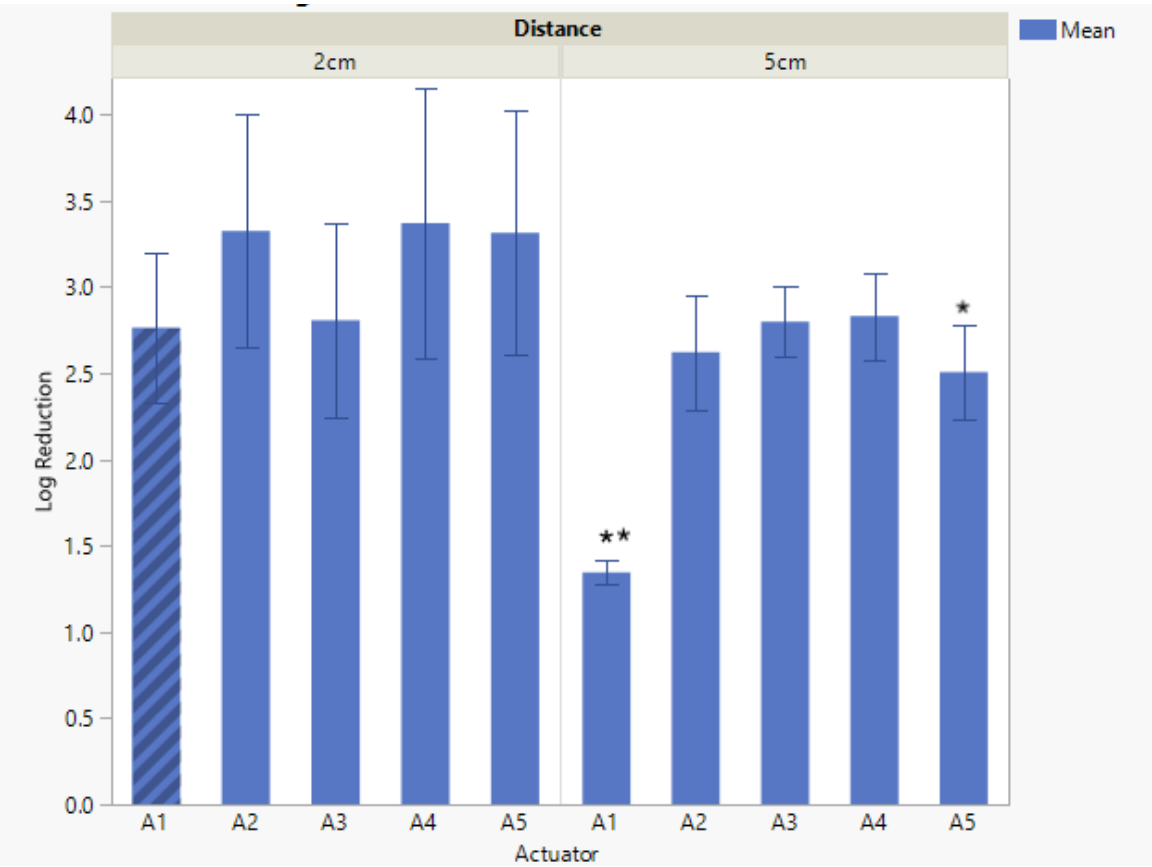

Figure 4: Reduction of *Salmonella enterica* on glass coverslips after 2 min treatment by atmospheric cold plasma (CAP device 2.0) with actuators varying in electrode width and gaps. Error bars represent standard errors (n =6). Asterisk depicts the differences in means with 0.05 significance.

2.2. Effect of electrode width on the performance of ACP device in inactivation of *S. enterica* .The overall performance of the ACP device 2.0 as influenced by electrode width is shown in Figure 5. When comparing the electrode width to average log reduction, including all treatment times & distances, an electrode width of 0.1 cm had the highest average log reduction with a treatment time of 5 minutes at 2cm distance (n=6). However, the log reduction of electrode width of 0.1cm for 2min at 5cm distance & electrode width of 1cm for 5min at 2cm was statistically lower when compared to the other widths. The treatment with the second highest average log reduction was 0.5 cm electrode width for 5minutes at 2cm distance, however there was only a slight difference

between the highest average log treatment. Taken all together, distance and treatment times, electrode width at 0.5 cm (A3) performed the best among all tested.

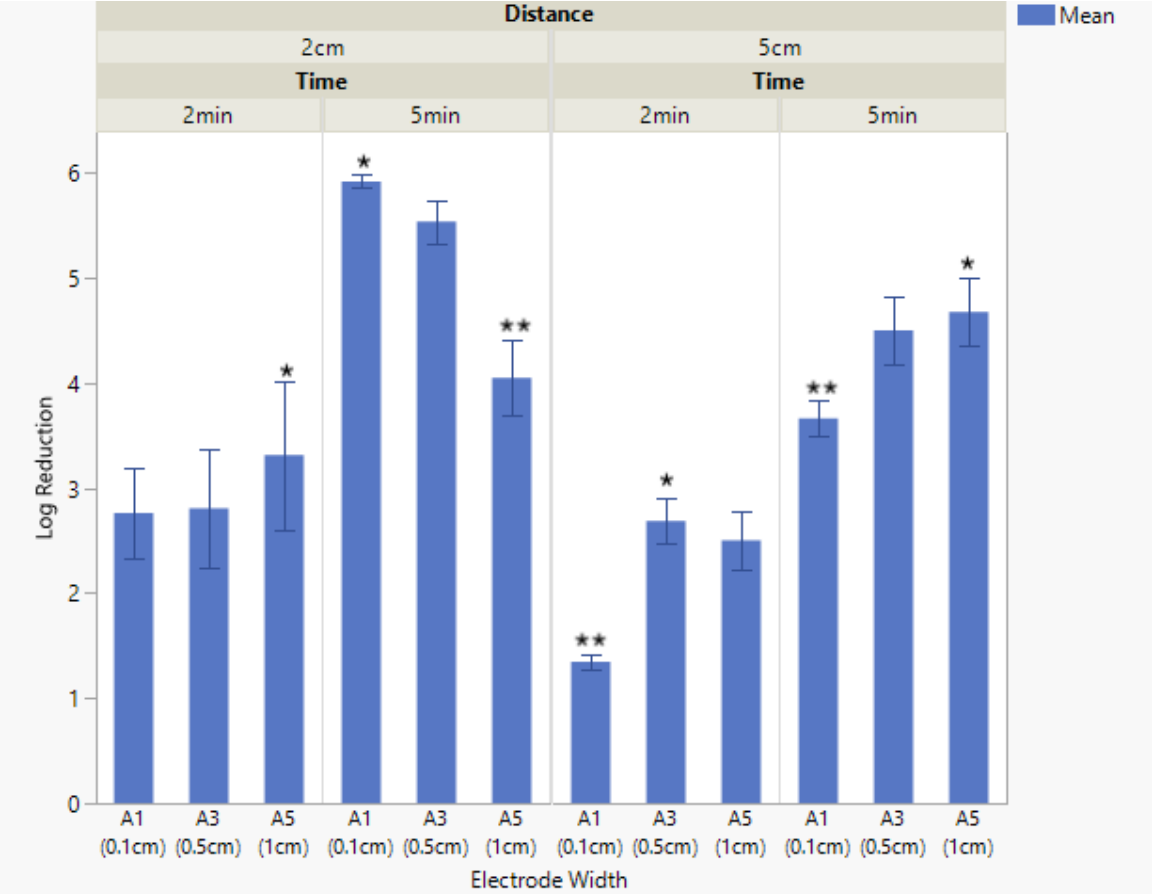

Figure 5: Reduction of *Salmonella enterica* on glass coverslips by atmospheric cold plasma treatment as influenced by the width of the electrodes on the actuator. Error bars represent standard errors (n =6). Asterisk depicts the differences in means with 0.05 significance.

2.3. Effect of electrode gap on the performance of ACP device in inactivation of *S. enterica.* The overall effect of electrode gap on the performance of ACP device 2.0 is shown in Figure 6. When comparing the electrode gap, it was found an electrode gap of 1mm for 5min at a 2cm distance & an electrode gap of 0.5mm for 5min at 2cm distance had a significantly higher log reduction (n=6). However, there was no significant differences between them. Further, the electrode gaps of 0.5 mm & 0.1 mm for 2 minutes at 5 cm distance had a significantly less average log reduction. Taken all

together, distance and treatment times, electrode gaps at 0.1 and 0.5 mm (A2 and A3) performed the best among all tested.

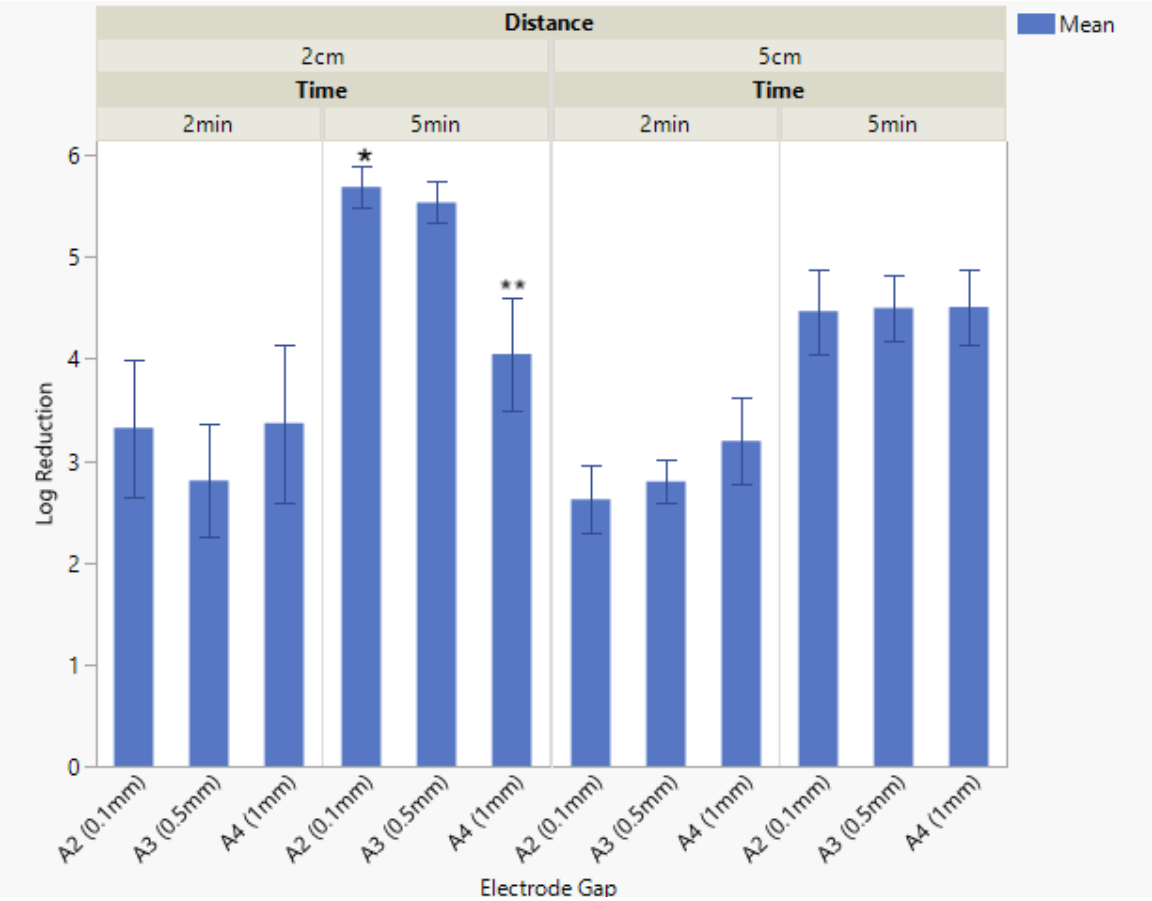

Figure 6: Reduction of *Salmonella enterica* on glass coverslips by atmospheric cold plasma treatment as influenced by the gap between the asymmetrical electrodes on the actuator. Error bars represent standard errors (n =6). Asterisk depicts the differences in means with 0.05 significance.

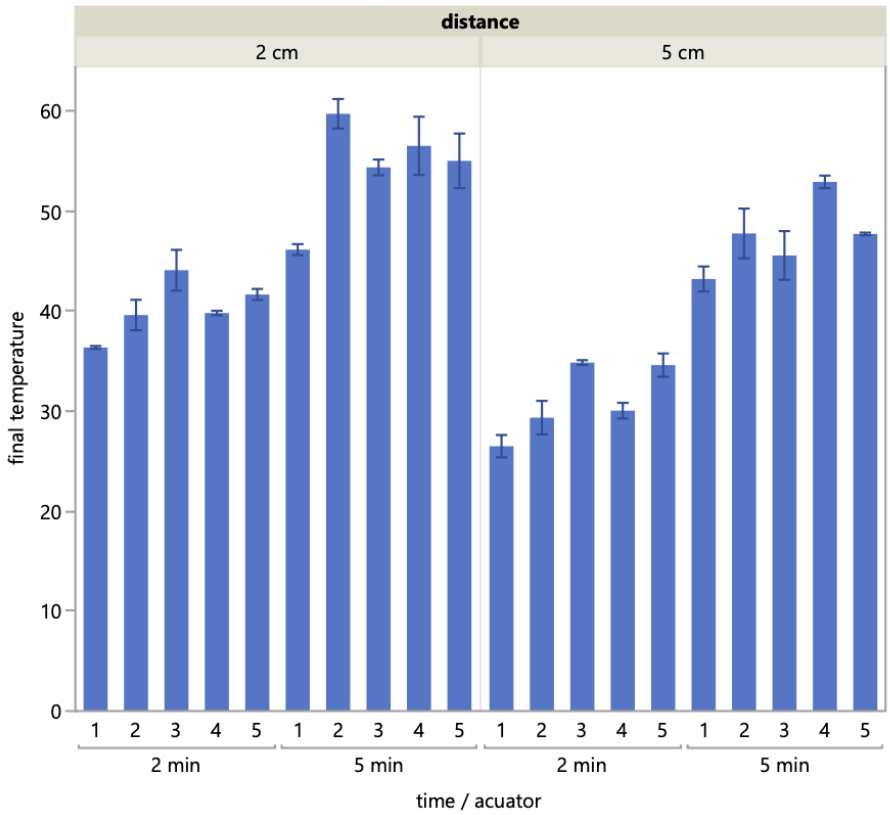

Figure 7: Refence of the average final temperature of treatment per actuator

3. **Discussion & Conclusion.** These findings suggest that ACP treatment at shorter treatment distance (2 cm) and longer treatment time (5 minutes) yield the most inactivation of *Salmonella* enterica and actuator A1 performed the best in such settings. Interestingly, actuators A2 & A3 has the same electrode width but differing gap distance between electrodes yet the log reduction from the treatment of 5 minutes at 2cm distance was not significantly different between the two. When comparing Actuator A1 & A3 at a treatment time of 5 minutes & 2cm distance, A1 had a slightly higher mean log reduction than A3. However, A3 had a slightly higher mean at a treatment time of 5 minutes & 5cm distance than A1 at the same parameters, suggesting A3 as a more versatile actuator. These results showed a 5-minute treatment time & 2 cm distance yielded higher kill rate for the majority of actuators. This finding suggests the treatment time & distance to be an important factor on

mean log reduction. This agrees with previous studies which showed a shorter treatment distance & longer treatment times resulted in a higher inactivation [3].The findings from electrode width & gap suggest an width of 0.1cm & a gap of 0.5mm or 1mm to have the highest average log reduction with a treatment time of 5 minutes at 2cm distance. The effect of SDBD actuator design on *S. enterica* inactivation could be due to an effect on the velocity of induced air flow, thus affecting the flow rate of the plasma [3]. In conclusion, the distance of the space between the actuator and item being treated, length of treatment, electrode width & gap are all important factors which effect the inactivation of *Salmonella* enterica.

CHAPTER V

EVALUATION OF OZONE & TEMPERATURE PRODUCED FROM ACP DEVICE 3.0

1. Methods and Materials

1.1. **Adjustments made to ACP device (therefore, APC 3.0).** The Device then had the frequency tuned for A3, A4 & A5 (A1 & A2 physically could not be tuned reliably) reducing the amount of energy which was turned into heat rather than plasma. This was achieved by changing the high-voltage supply to a more durable unit and setting the frequency to the optimum for each design by monitoring the voltage and current waveforms while tuning. This improves the efficiency and should also help the longevity of actuator life. The box housing the power supply had a knob to select the frequency to actuators A3, A4 & A5. It also had a three-position toggle switch to allow a timed pulse or continuous plasma output. A low-voltage power supply was also placed inside to run the timer circuit & to supply the signal to turn on the high-voltage to activate the actuator to produce plasma. An additional toggle switch was also placed to choose between a 3 second pulse of plasma output with 5 second off or 3 second pulse of plasma output with 10 second off. No changes were made to the chamber portion of the device.

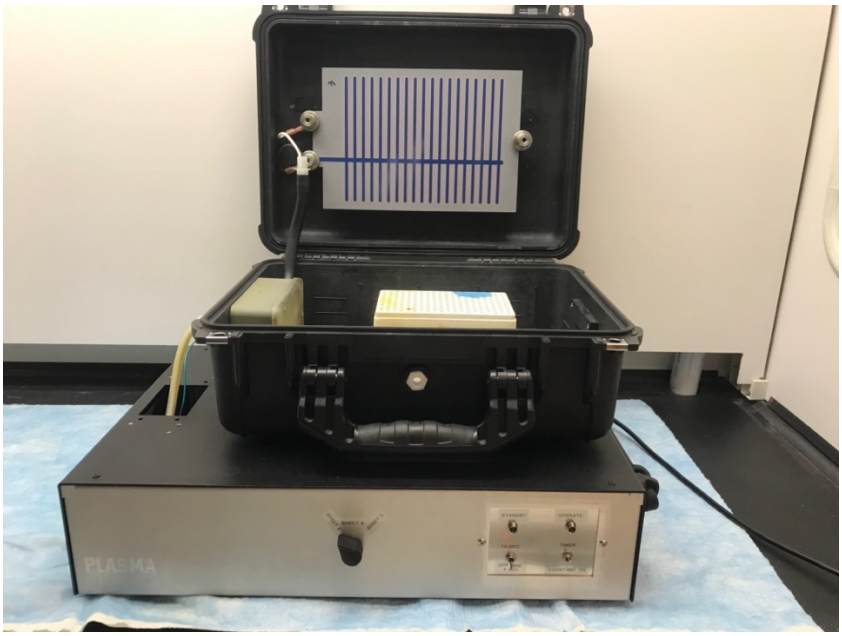

Figure 8: ACP Device 3.0

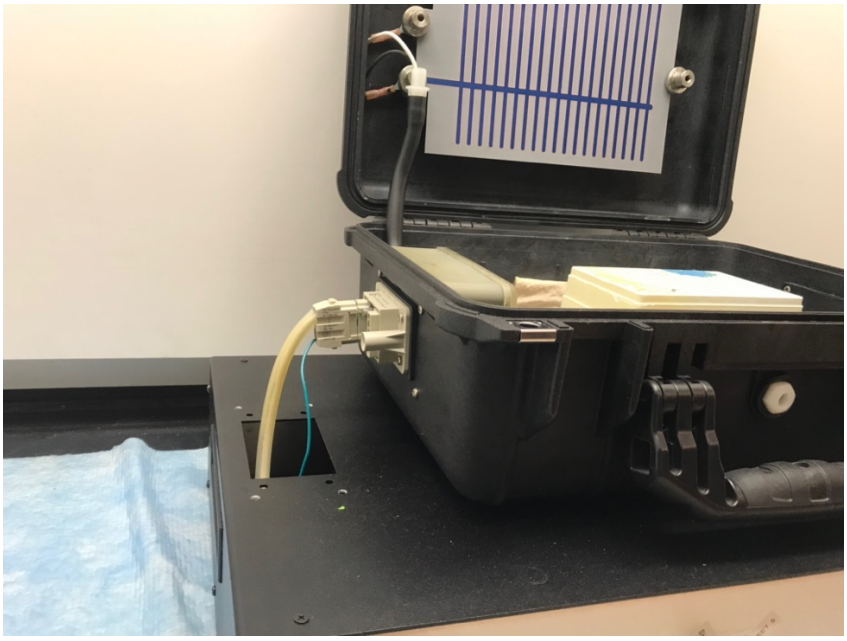

Figure 9: Connection of power supply to actuator & chamber of ACP Device 3.0

Figure 10: The added switches & knobs on the power supply of ACP Device 3.0

1.2. Ozone measurement. PortaSens III Portable Gas Leak Detector was used to monitor the ozone generated inside the device chamber. The sensor used was the O_3 200/1000 PPM with maximum sensing limit of 1200 PPM.

Prior to operating the detector, silicone tubing was fed through the port of the chamber & placed next to the petri dish to ensure the measurements would catch concentration of ozone as the glass coverslips were receiving in earlier studies. Then glass coverslips were inoculated following the same methods as the bacterial inactivation sections (chapter III & IV). Using the log option on the gas detector, measurements for 8 treatments using 8 sets of inoculated glass coverslips were obtained. The same method was repeated with un-inoculated glass coverslips. A t-test was then performed using the highest reading per treatment, no significant difference was found between inoculated and un-inoculated glass coverslips. It was decided to remove the petri dish & glass coverslips to measure the concentration of the ozone along with logging the measurement every 30 seconds throughout the treatment.

1.3. **Experiment.** The ozone concentration was measured using actuators A3, A4 & A5 at distances of 2cm & 5cm for 5 minutes. In addition to the standard 3 seconds of plasma pulse on & 5 seconds of off time, an off time of 10 second, 20 second & 30 seconds were also measured along with a recorded final temperature. The detector was set to log the reading every 30 seconds. An additional set of measurement were taken using actuator A3 at a 2cm distance, the time for plasma pulse to be on stayed at 3 second, the pulse off time was 5 seconds for the first 30 seconds of the treatment, while the rest of 5 minutes had either 20 or 30 seconds off. The detector was set to log the reading every 10 seconds in order to identify when the pulse off time could be extended. The time at which the concentration of ozone reached 800 PPM was used as data points. This was chosen as all actuators did each 800 PPM.

1.4. **Statical analysis.** A one-way anova was performed along with a Hsu's MCB test to rank the treatments based on the mean ozone production. A students' t-test was also performed to analyze the differences between treatments. The significant difference was defined as $p < 0.05$.

2. **Results.**

2.1. **The overall effect actuator design on the production of ozone at a distance of 2cm is shown in Figure 11 and respective final temperatures in Table 3.** Actuator A4 takes the longest to produce 800ppm of ozone while actuator A3 is the quickest to produce 800ppm of ozone. In both actuator A3 & A4, the 10 second off pulse has a significantly less time till 800ppm. Actuator A5, has no difference between 5 second & 10 second off pulse. The treatment using actuator A3 at a 2cm distance with a 10 second pulse off time was found to have lowest time average mean of all whiles having an average final temperature of 45.4°C (n=6). It is worth noting, the 3 lowest average mean treatment were all with actuator A3, however the final temperature was above 50°C for the other two treatments. There was no significant difference

between actuator A3 at 2cm distance with 5, 10 & 20 second pulse off time and actuator A3 at 5cm distance with 5 & 10 second pulse off time. The results for actuator A3 at a 2cm distance with the pulse off time of 5 seconds for the first 30 seconds of the treatment with 4.5 minutes of 20 or 30 seconds off had the highest average mean when compared to all of actuator A3 treatments. However, the final temperature for the actuator A3 at a 2cm distance with the pulse off time of 5 seconds for the first 30 seconds of the treatment with 4.5 minutes of 20 or 30 seconds off were lower than the other pulse arrangements, 40.2°C & 34.9°C respectively

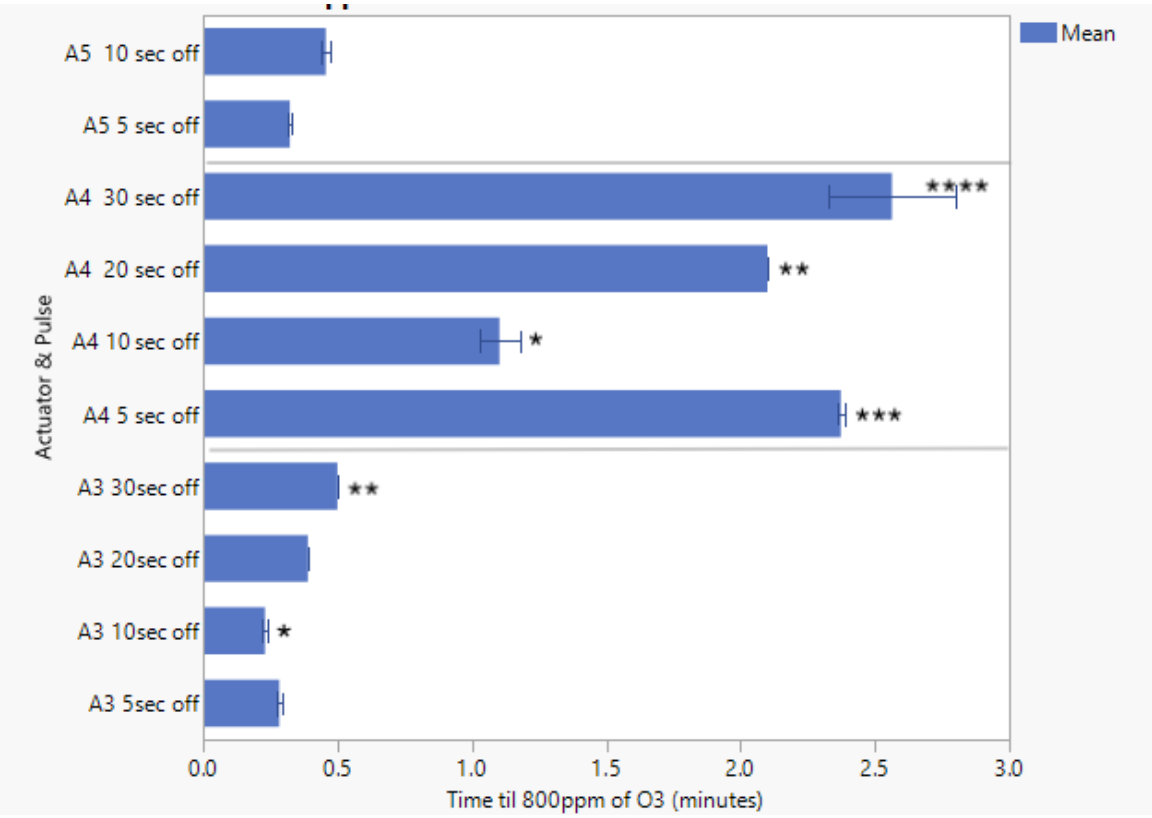

Figure 11: Time when 800ppm ozone concentration is achieved as a function of CAP treatment at 2cm distance regarding the actuator. Standard error bars are constructed using 1 standard error from the mean (n=6). Asterisk depicts the differences in means with 0.05 significance.

2.2. **The overall effect actuator design on the production of ozone at a distance of 5cm is shown in Figure 12 and respective final temperatures in Table 3.** Similar to the results for 2cm distance, actuator A3 is the quickest to 800ppm of ozone. The treatment using actuator A3 at a 5cm distance with a 5 second pulse off time was found to have lowest time average mean (n=6)

of all whiles having an average final temperature of 54.8°C. The actuator A3 treatment with a 30 second pulse off time takes an average of 0.56 minutes to produce 800ppm of ozone the time difference is significantly different from the 5 second pulse off time. However, the temperature for A3 with a 30 second off pulse is 31.7°C. When focusing on the results of actuator A4 at 5cm distance, the 5 second off pulse has a significantly lesser time till 800ppm of ozone. Interestingly, A3 with a 30 second off pulse has a higher average time till 800ppm than A4 with a 5 second off pulse, however A4 with a 5 second off pulse has a high final temperature of 44.9 °C. Additionally, all of the A4 off pulse times at 5cm are significantly different from each other. Whereas there is less differences among A3 off pulse times at 5cm distance.

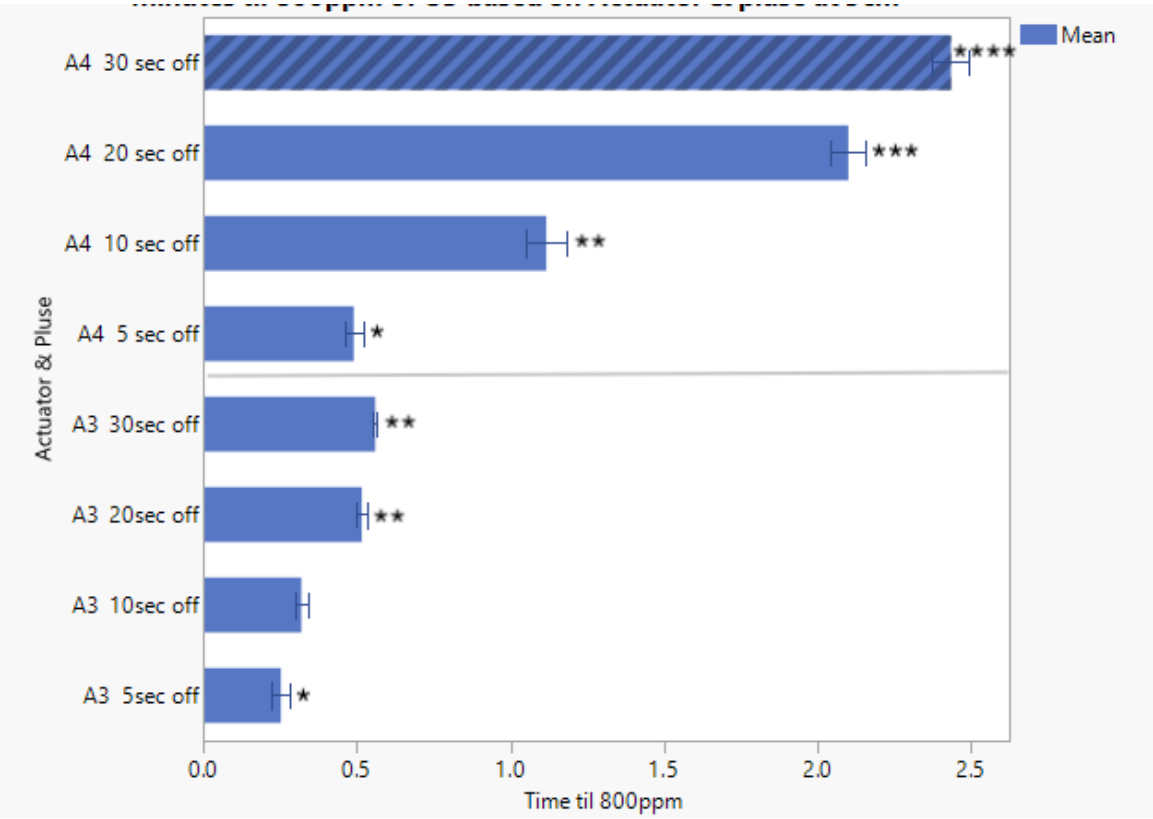

Figure 12: Time when 800ppm ozone concentration is achieved as a function of CAP treatment at 5cm distance regarding the actuator. Standard error bars are constructed using 1 standard error from the mean (n=6). Asterisk depicts the differences in means with 0.05 significance.

Actuator	Final temp.in C
A3 2cm 5min, 5sec off	±63.3
A3 2cm 5min, 10sec off	±45.4
A3 2cm 5min, 20sec off	±31.4
A3 2cm 5min, 30sec off	±31.6
A3 5cm 5min, 5sec off	±54.8
A3 5cm 5min, 10sec off	±39.9
A3 5cm 5min, 20sec off	±35.9
A3 5cm 5min, 30sec off	±31.7
A3 2cm 5min, 5sec:20secoff	±40.2
A3 2cm 5min, 5sec:30secoff	±34.9
A4 2cm 5min, 5 sec off	±75.9
A4 2cm 5min, 10 sec off	±59.2
A4 2cm 5min, 20 sec off	±33.2
A4 2cm 5min, 30 sec off	±30.3
A4 5cm 5min, 5 sec off	±44.9
A4 5cm 5min, 10 sec off	±45.4
A4 5cm 5min, 20 sec off	±33.1
A4 5cm 5min, 30 sec off	±31.5
A5 2cm 5min, 5 sec off	±60.1
A5 2cm 5min, 10 sec off	±46

Table 3: The average final temperature of treatment based on actuator, distance, length & pulse interval time.

3. **Discussion & Conclusion.** These findings suggest actuator A3 to produce ozone quicker than actuators A4 & A5. As theorized, a shorter pulse off time also reached 800 PPM quicker with the expense of a higher average final temperature. While actuator A3 at 2cm distance with pulse off time of 5 seconds for the first 30 seconds of the treatment with 4.5 minutes of 20 or 30 seconds was significantly slower to produce to 800 PPM of ozone, the final temperature was lower. Our findings align with previous studies which highlighted the design & operation conditions of SDBD to have an effect on the discharge chemistry of the plasma[4]. While lesser final temperatures are beneficial to avoid heating the food being treated, this may also affect the average log reduction. A bacterial inactivation trial will need to be performed to discover the balance between final temperature and

average log reduction. In conclusion, actuator A3 is the quickest to produce ozone at both 2cm & 5cm distances but the final temperature can vary.

CHAPTER VI

EVALUATION OF THE EFFECT OF PULSING REGIMES ON THE INACTIVATION OF SALMONELLA ENTERICA BY ACP DEVICE 3.0

1. Methods and materials

1.1. Preparation of Bacterial Culture & inoculum.

A five-strain mixture of *Salmonella enterica* subsp. *enterica* serovars Enteritidis, Typhimurium, Javiana, Oranienberg, and Agona was used in all the experiments. The five- strain mixture was utilized to represent the range of sensitivity to the treatment[1]. Each strain was grown individually in 5ml of tropic soy broth (TSB) at 37 ºC with shaking (250 rpm) for 19 hours. Once the growth period was complete each culture was vortexed and 1ml was collected and combined with all strains grown. Then 1ml of the combined culture was vortexed before centrifuging at 12,000rpm for 3min. The pellet was res-suspended in 0.1% sterile peptone water. A serial dilution of 1:10 of the suspension in sterile peptone water was performed to determine cell concentration and for inoculum of coverslips. The highest dilutions (-8, -7, and -6) were plated on a tropic soy agar (TSA) plate by drop plating and the plates were incubated at 37 ºC for 24 hours before enumeration.

1.2. **Inoculation of coverslips.** Glass coverslips 22x22mm were placed in a glass periti dish and autoclaved prior to the experiment. Then 3 coverslips were placed in plastic petri dishes labeled for each individual treatment and one control group. 50ul of the -1 dilution of inoculum was dropped on each coverslip as evenly as possible. The coverslips were then left to dry in the biosafety cabinet for 60 minutes.

1.3. **Treatment.** The inside of the atmospheric cold plasma device 3.0 was measured to ensure the distance from the actuator to the coverslip. The selected distances were inspired by earlier works of this lab, which show a log reduction was achieved at a 5cm distance[2]. Boxes were used inside the Device to set the coverslips a distance of 2cm or 5cm from the actuator. Then actuator, A3 was placed correctly in the Device. The pluses chosen to evaluate were, the first 30 seconds with 5 seconds off followed by 4.5 minutes of 20 seconds off & first 30 seconds with 5 seconds off followed by 4.5 minutes of 30 seconds off, all three had the same on pulse time of 3 seconds. In addition to the combination pulses, a 5, 20 & 30 second off pulse time was also performed. Due to very low humidity, a wet paper towel was added to the chamber resulting in 80-70% humidity

1.4. **Microbial biological recovery.** Overall, the methods for microbial recovery & log reduction calculations were like earlier works of this lab with minor modifications [1]. Immediately, after treatment each coverslip was placed in individual wash tubes, 50ml conical tubes containing 1ml of sterile 0.1% peptone water. The control group was placed in individual wash tubes immediately prior to the first treatment group. Once all

treatments were done, all the wash tubes were vortex for 15 seconds, rested for 10 seconds repeated 3 times for a total of 1:05 minute per wash tube. Then 250 ul of wash fluid from each coverslip was spread plated on to individual tropic soy agar plates. The control treatments wash fluid was diluted in 1ml tubes in 1:10 serial in sterile 0.1% peptone water up to 1000-fold. Each coverslip had a specific tropic soy agar plate, the plate was divided based on the dilutions. Each section received 3 drops of 10ul of the dilution pertaining to it. All plates were incubated overnight at 37 ºC.

1.5. **Log reduction calculations.** The following morning all the tropic soy agar plates including the enumeration & controls were counted. The control plates which had dilutions, the dilution with 30-3 colonies is the dilution we multiplied to get back to the colony forming units per 1ml. The spread plates were also multiplied by a factor to get back to the colony forming units per 1ml. Then the log was taken from the 1ml of colony forming units. Then that log reduction per coverslip was subtracted from the average log reduction from the controls.

3.4. **Statistical Analysis.** A one-way anova was performed following the anova, Hsu's MCB test was performed to rank the treatments based on the mean log reduction along with a students' t-test to evaluate differences in the treatments. The significant difference was defined as $p < 0.05$. In addition, to the data points from this chapter the temperature measurements from the previous chapter are utilized again.

2. **Results. The results of the effect of pulsing regime on the inactivation of *S. enterica* by APC 3.0 at different treatment distance are represented in Figures 13 (2 cm) and 14 (5cm).** At shorter treatment distance (2 cm), **no significance difference were found regarding the inactivation of *S enterica*.** At longer treatment distance, the lowest average (n=6) log reduction was A3 at with the first 30 seconds of 5 second off pulse followed by 20 seconds off pulse for 4.5 minutes with an average final temperature of 34.9 °C (Figure 13). When only comparing the 5cm distance treatments, A3 with 5 seconds off pulse for 5 minutes had the highest significant average (n=6) log reduction with an average final temperature of 54.8°C.

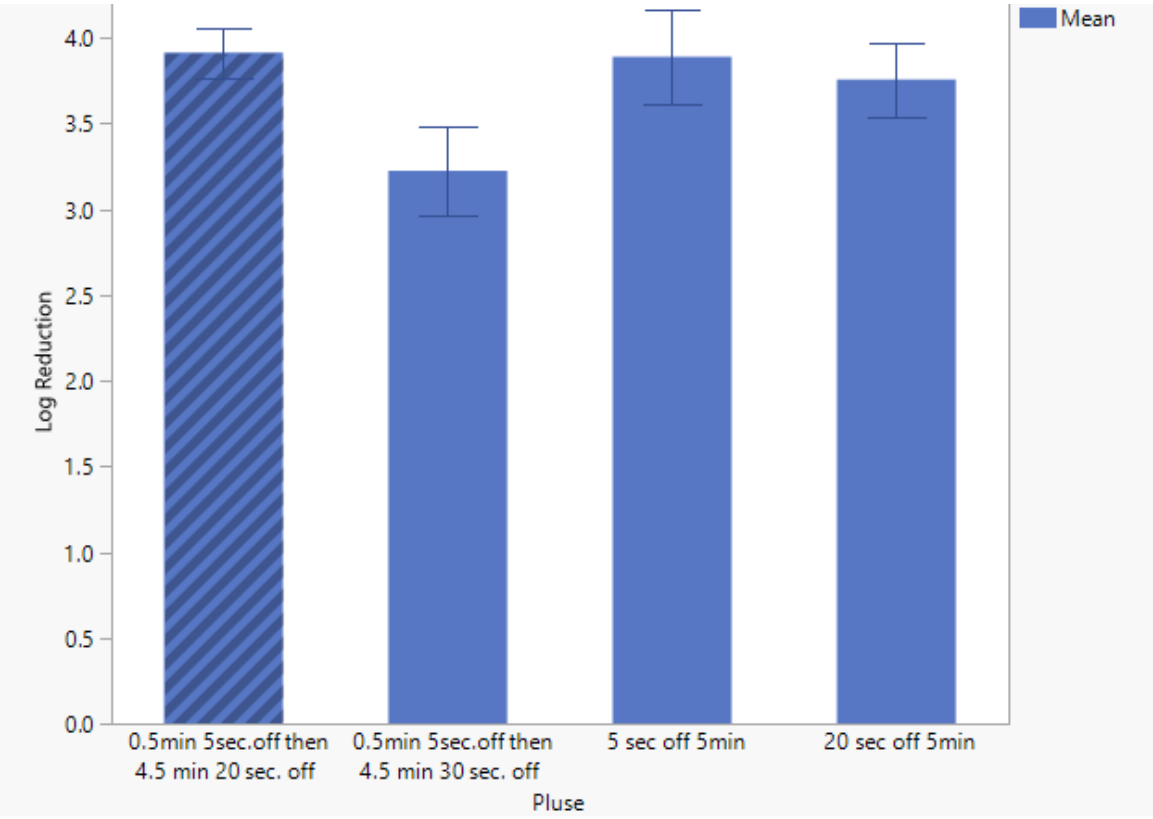

Figure 13: Reduction of *Salmonella enterica* on glass coverslips as a function of ACP treatment using actuator A3 at 2cm for 5 minutes & pulsing time arrangements. Standard

error bars are constructed using the upper & lower quartiles (n=6). Asterisk depicts the differences in means with 0.05 significance.

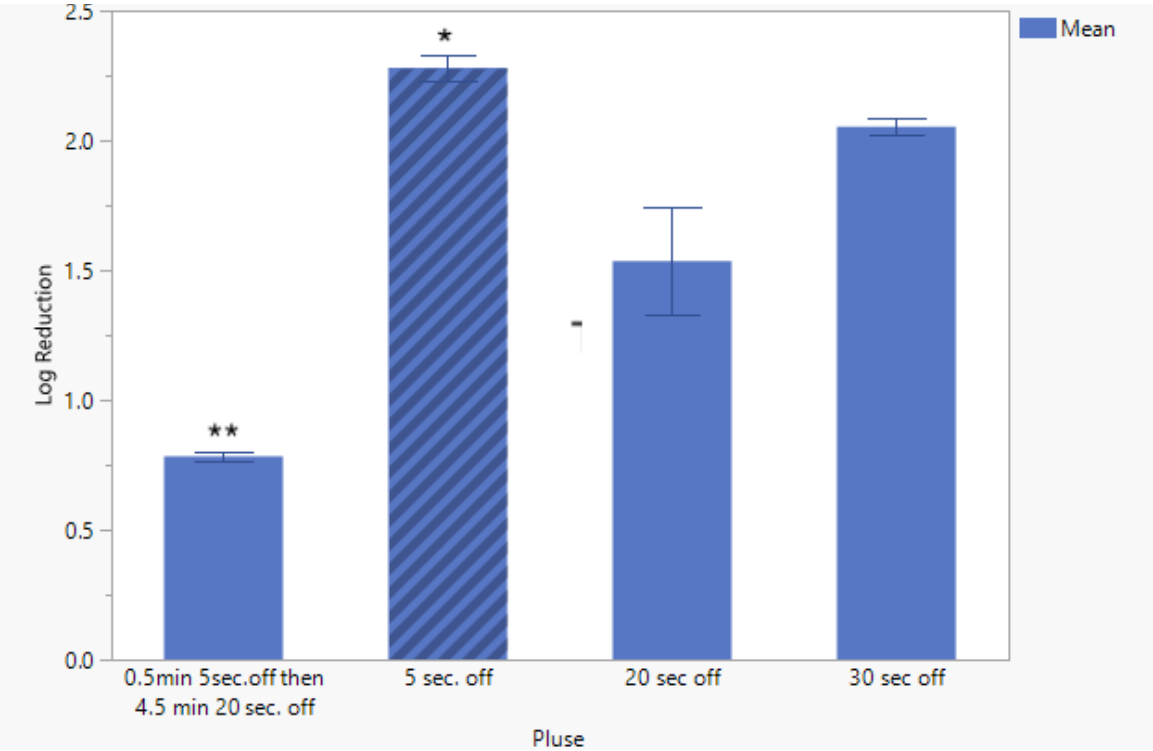

Figure 14: Reduction of *Salmonella enterica* on glass coverslips as a function of ACP treatment using actuator A3 at 5cm for 5 minutes & pulsing time arrangements. Standard error bars are constructed using the upper & lower quartiles (n=6). Asterisk depicts the differences in means with 0.05 significance.

2.1. **Discussion & Conclusion.** These results suggest the use of actuator A3 for 5 minutes at 2cm distance to be the most effective at inactivation of *S. enterica* regardless of the off-pulse time (n=6). Granted, actuator A3 at a 5cm distance did inactivate *S. enterica* but it was significantly lesser than the 2cm distance. The 2cm distance efficacy comes at the cost of a final treatment temperature ranging from 30.1-71.3°C dependent on the pulse regime used. Actuator A3 with 5 seconds of pulse off time at 2cm distance has a average

final temperature of 63.3°C which may not be suitable for certain foods such as fresh produce. Actuator A3 at 2cm distance with the first 30 seconds of treatment with 5 second off pulse following 20 second off pulse for 4.5 minutes & A3 at 2cm distance with the first 30 seconds of treatment with 5 second off pulse following 30 second off pulse for 4.5 minutes final temperatures average 40.2°C & 34.8 °C respectively, while achieving a similar inactivation as the 5 second off time pulse. Thus, the pulse regimes with a combination of 5 seconds off time & a long pulse off time are more applicable on different types of food. Surprisingly, the treatments at 5cm distance had similar final temperatures. Our findings align with previous studies highlighting the effect of pulse duty cycle on the production of heat and reactive species [5]. Overall, when choosing a treatment with high average log reduction of *S. enterica* & more acceptable final temperature, A3 at 2cm distance with the first 30 seconds of treatment with 5 second off pulse following 20 or 30 second off pulse for 4.5 minutes is suggested.

CHAPTER VII

EVALUATION OF COMBINED PARAMETERS ON NATIVE PECANS

1. Methods and materials

1.1. **Preparation of Bacterial Culture & inoculum.** A five-strain mixture of *Salmonella enterica* subsp. *enterica* serovars Enteritidis, Typhimurium, Javiana, Oranienberg, and Agona was used in all the experiments. The five- strain mixture was utilized to represent the range of sensitivity to the treatment[1]. Each strain was grown individually in 5ml of tropic soy broth (TSB) at 37 ºC with shaking (250 rpm) for 19 hours. Once the growth period was complete each culture was vortexed and 1ml was collected and combined with all strains grown. Then 1ml of the combined culture was vortexed before centrifuging at 12,000rpm for 3min. The pellet was res-suspended in 0.1% sterile peptone water. A serial dilution of 1:10 of the suspension in sterile peptone water was performed to determine cell concentration and for inoculum of coverslips. The highest dilutions (-8, -7, and -6) were plated on a tropic soy agar (TSA) plate by drop plating and the plates were incubated at 37 ºC for 24 hours before enumeration.

1.2. **Inoculation of Pecans.** In-shell pecans were removed from 4°C storage and warmed up to room temperature. Then 3 pecans were placed in plastic petri dishes labeled for each individual treatment and control group.

1.3. Then the in-shell pecans were spot inoculated with 50ul of the suspension on the top surface (the surface would be directly facing the actuator sheet during ACP treatment, one-sided placement) or 25ul of the suspension on both the top & bottom surface (two-sided placement). The inoculated in-shell pecan then dried for 30 minutes per side in the biosafety cabinet prior to treatment.

1.4. **Treatment.** The inside of the atmospheric cold plasma device 3.0 was measured to ensure the distance from the actuator to the pecans. The selected distances were inspired by earlier works of this lab, which show a log reduction was achieved at a 5cm distance[2]. Boxes were used inside the Device to set the pecans a distance of 2cm or 5cm from the actuator. Then actuator, A3 was placed correctly in the Device. The pluses chosen to evaluate were, 20 seconds off 3 seconds on, the first 30 seconds with 5 seconds off 3 seconds on followed by 4.5 minutes of 20 seconds off 3 seconds on & the first 30 seconds with 5 seconds off 3 seconds on followed by 4.5 minutes of 30 seconds off 3 seconds on. Due to very low humidity, a wet paper towel was added to the chamber resulting in 80-70% humidity.

1.5. **Microbial biological recovery.** Overall, the methods for microbial recovery & log reduction calculations were like earlier works of this lab with minor modifications [1]. Immediately, after treatment each pecan was placed in individual wash tubes (50ml conical tubes) containing 10ml of sterile 0.1% peptone water. The control group was placed in individual wash tubes immediately prior to the first treatment group. After each group of pecans were treated, the corresponding wash tubes were vortex for 15 seconds, rested for 10 seconds and the process was repeated 3 times for a total of 1:05 minute per wash tube. The wash fluid was diluted in 1:10 serial in sterile 0.1% peptone

water. Each coverslip serial dilutions were drop-plated onto Xylose Lysine Deoxycholate agar plate over laid with Tryptic Soy Agar. and all plates were incubated overnight at 37 ºC .

1.6. **Colony forming units' reduction calculations & log reduction calculations.** The following morning all the tropic soy agar plates including the enumeration & controls were counted. For each of the plates, the dilution with 30-3 colonies was multiplied to get back to the colony forming units per 1ml. Then the log was taken from the 1ml of colony forming units. Then for the log reduction, the log of treatment was subtracted from the controls log reduction.

3.5. **Statistical Analysis.** A one-way anova was performed following the anova, Hsu's MCB test was performed to rank the treatments based on the mean reduction of colony forming units or mean log reduction along with a students' t-test to evaluate differences in the treatments. The significant difference was defined as $p < 0.05$.

2. **Results. The results of the combined parameters of ACP device 3.0 on the inactivation of *S. enterica* on native pecans are represented in Figures 15(one-sided), 16 (two-side) and 17 (one-sided verses two-sided).** When comparing the one-sided treatment among each other the 2cm distance with the first 30 seconds 5 seconds off following 4.5 minutes of 20 seconds off had the lowest average mean of recovered colony forming units (n=6)(Figure 15). In addition, it was the only significantly different from the 5cm distance with 20 seconds off time pulse for the one-sided inoculation. The two-sided treatments (Figure 16) also show the 2cm distance with the first 30 seconds 5 seconds off following 4.5 minutes of 20 seconds off had the lowest average mean of recovered colony forming units (n=6). However, it was only significantly different from one other treatment, the 5cm distance with the first 30 seconds 5

seconds off following 4.5 minutes of 20 seconds off. The log reductions of *S. enterica* at 2cm treatment distance with the first 30 seconds 5 seconds off following 4.5 minutes of 20 seconds were compared based on one-sided & two-sided inoculation placement, the two-sided had a statistically higher average log reduction (n=6) (Figure 17).

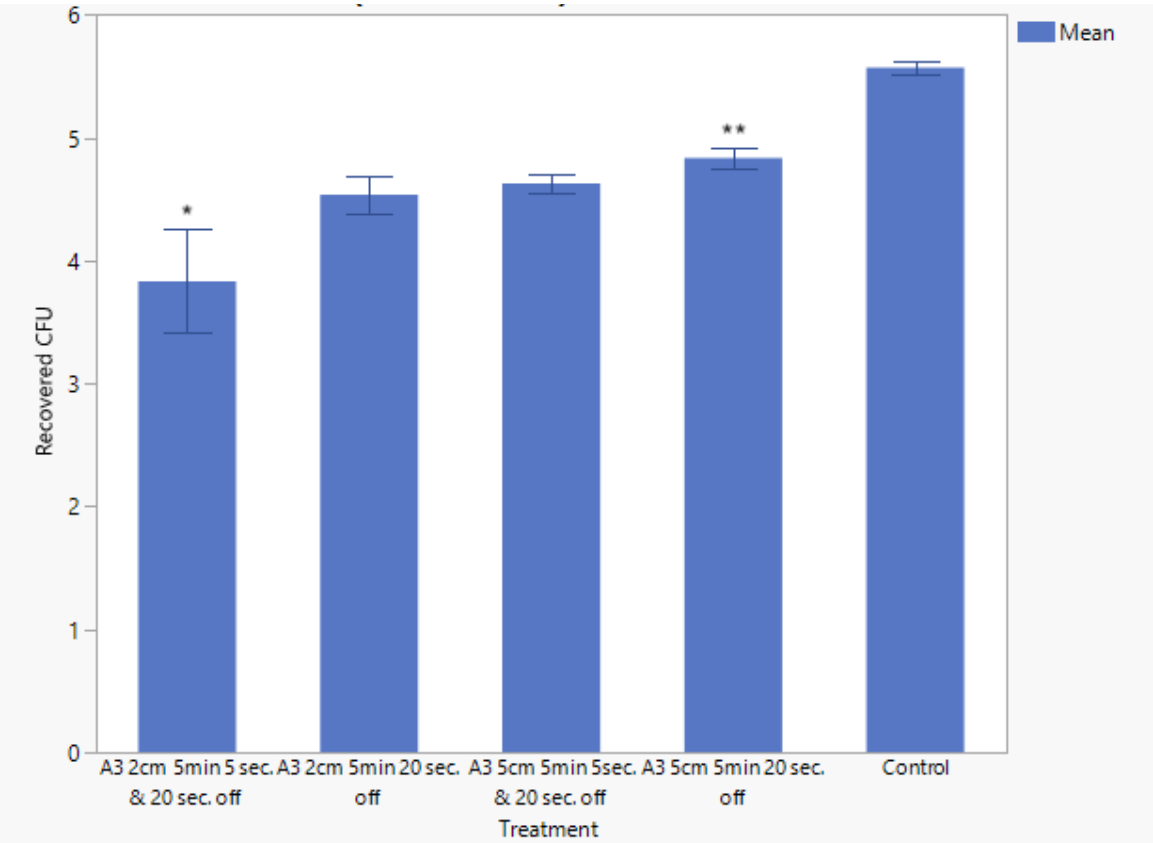

Figure 15: Reduction of *Salmonella enterica* on in-shell pecans (one-sided inoculation) as a function of CAP treatment distance & pulsing time arrangements using actuator A3. Standard error bars are constructed using 1 standard error from the mean (n=6). Asterisk depicts the differences in means with 0.05 significance.

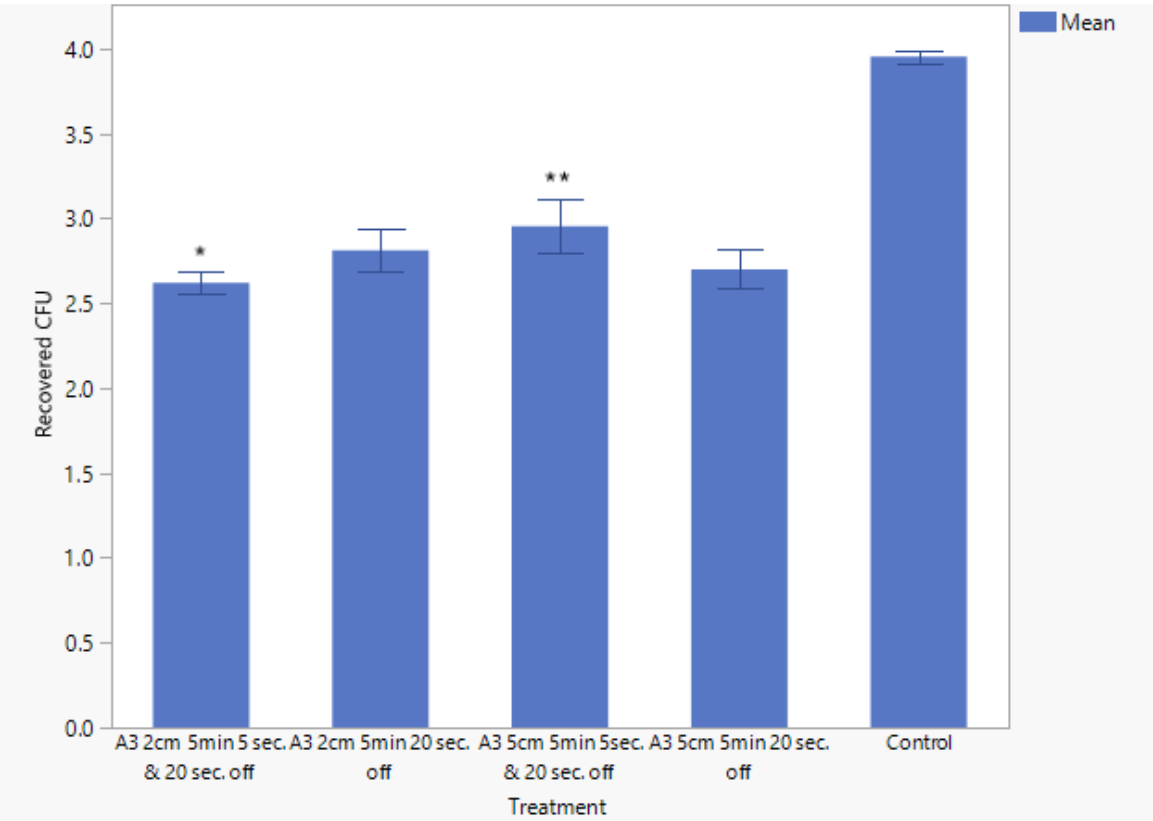

Figure 16: Reduction of *Salmonella enterica* on in-shell pecans (two-sided inoculation) as a function of CAP treatment distance & pulsing time arrangements using actuator A3. Standard error bars are constructed using 1 standard error from the mean (n=6). Asterisk depicts the differences in means with 0.05 significance.

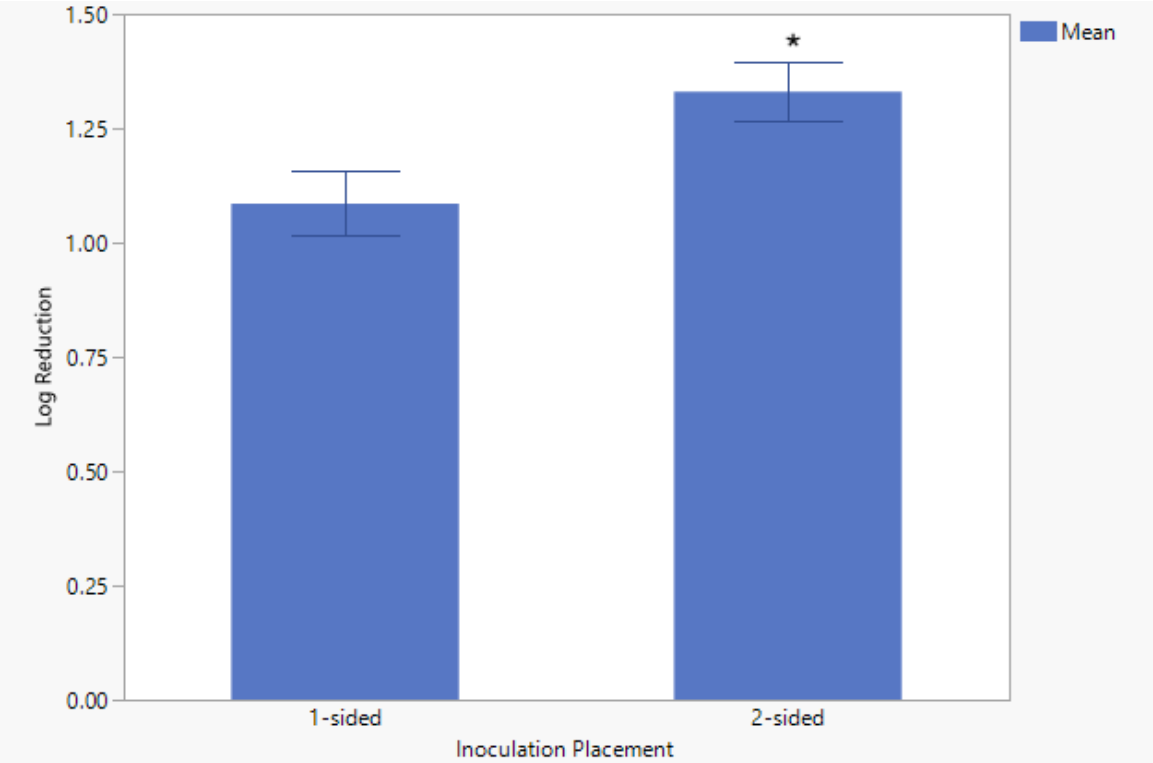

Figure 17: Log reduction of *Salmonella enterica* on in-shell pecans comparison of inoculation placement(s) as a function of CAP treatment using actuator A3 at 2cm distance with the first 30 seconds 5 seconds off following 4.5 minutes of 20 seconds. Standard error bars are constructed using 1 standard error from the mean (n=6). Asterisk depicts the differences in means with 0.05 significance.

2.1. **Discussion & Conclusion.** These results suggest actuator A3 at 2cm distance with the first 30 seconds 5 seconds off following 4.5 minutes of 20 seconds off to have the lowest recovery of inoculated *S. enterica* (highest inactivation of *S. enterica*) in both a one-sided inoculation & two-sided inoculation. In addition, the results also suggest a two-sided inoculation placement to be more effective at bacterial inactivation on native pecans. As expected, the inactivation of *S. enterica* on glass coverslips was higher than the native pecans. A possible explanation is the shell surface are rougher than glass coverslips surface. This algins with previous studies which showed a higher inactivation rate on a smooth surface compared to a rough food surface [6]. It is important to consider

the effect of the use of a new actuator sheet on the one-sided treatments. The actuator most likely did not achieve a full output of plasma due to the bell curve involved in the lifespan & output of an actuator. It was later discovered that the actuator sheet used in the pecan experiment was not fully functional, therefore, the results of pecan experiments will be conducted in a later time. Nonetheless, the results demonstrated that an ACP treatment of 5 minutes can inactivate *S. enterica* on native pecan shells.

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VITA

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Master of Science

Thesis: EVALUATION OF THE EFFECTS OF ELECTRODE CONFIGURATION AND POWER PULSE REGIME ON THE INACTIVATION EFFIECINCY OF SALMONELLA ENTERICA BY ATMOSPHERIC COLD PLASMA

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