NONTHERMAL PLASMA TREATMENT OF PACKAGED BROILER BREAST FILLETS TO REDUCE NATURAL MICROFLORA AND *CAMPYLOBACTER JEJUNI*

by

TAYLOR GRACE KRONN

(Under the Direction of Yao-wen Huang)

ABSTRACT

Spoilage organisms and pathogens are commonly associated with fresh broiler meat. A nonthermal dielectric barrier discharge (DBD) plasma system was developed as an antimicrobial treatment for fresh broiler breast fillets (BBF). Experiments were designed and conducted to determine the ability of the DBD plasma system to reduce spoilage organisms and *Campylobacter jejuni*. After log cycle reductions were achieved, a test was run to determine the effect of the log cycle reduction on the shelf life of the BBF.

A treatment voltage of 75 kV was administered for 3 or 5 min depending on the particular experiment. The DBD plasma system was able to produce a log cycle reduction of ~2.00 cfu/ml for both spoilage organisms and C. jejuni. Treatment with the DBD plasma system was able to maintain the microbial quality of the fresh BBF for 14 d (4°C) which is ~7 d longer than typical shelf life. This system proves that it has the ability to improve safety and quality of fresh BBF.

INDEX WORDS: Nonthermal plasma, broiler breast fillets, antimicrobial treatment, ozone, packaging

NONTHERMAL PLASMA TREATMENT OF PACKAGED BROILER BREAST FILLETS TO REDUCE NATURAL MICROFLORA AND *CAMPYLOBACTER JEJUNI*

by

TAYLOR GRACE KRONN

B.S., University of Kentucky, Lexington, KY, 2009

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

© 2013

Taylor Grace Kronn

All Rights Reserved

NONTHERMAL PLASMA TREATMENT OF PACKAGED BROILER BREAST FILLETS TO REDUCE NATURAL MICROFLORA AND *CAMPYLOBACTER JEJUNI*

by

TAYLOR GRACE KRONN

Major Professor: Yao-wen Huang

Committee: Kurt Lawrence
Hong Zhaung

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

ACKNOWLEDGEMENTS

I would like to acknowledge the patience and dedication of my supervisor Dr. Kurt Lawrence, for his guidance, encouragement, and support throughout my Master's program. I would also like to acknowledge the other advisors who helped me through this process, Dr. Hong Zhuang, Dr. Yao-wen Huang, Dr. Kelli Hiett, Dr. Michael Rothrock, and Dr. Kevin Keener. Thank you to all the members of the Quality and Safety Assessment Research Unit and Latoya Wiggins at Richard B. Russell Research Building for your support and willingness to help me through this challenging endeavor.

Sincerest thanks,

Taylor Kronn

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER

1 INTRODUCTION 1

REFERENCES 6

2 LITERATURE REVIEW 9

Spoilage of poultry 9

Poultry and pathogens 10

Nonthermal inactivation of food borne pathogens 11

Mechanisms of bacterial destruction 16

Production of plasma 18

Plasma treatment of food 20

Ozone gas for treatment of food 22

Conclusions 24

REFERENCES 25

3 NONTHERMAL PLASMA SYSTEM FOR EXTENDING SHELF LIFE OF POULTRY BREAST FILLETS 32

REFERENCES 49

4 NONTHERMAL PLASMA TREATMENT OF PACKAGED INOCULATED POULTRY BREAST FILLETS FOR THE REDUCTION OF SPOILAGE BACTERIA AND ZOONOTIC PATHOGENS 60

REFERENCES 75

5 SUMMARY AND CONCLUSION 84

LIST OF TABLES

Page

Table 3.1: Combined data for triplicate samples, plasma treated BBF with natural background microflora. 59

Table 4.1: Experimental details of three studies to test efficacy of nonthermal plasma generation system 81

Table 4.2: Recovery of total cfu/ml of microflora inoculated on the surface of BBF 82

Table 4.3: Recovery of total cfu/ml of *C. jejuni* inoculated on the surface of BBF 83

LIST OF FIGURES

Page

Figure 3.1: Entire plasma generation system with control box, high-voltage transformer, electrodes, and dielectric barriers. 53

Figure 3.2: Outlines on dielectric barriers used to accomplish parallel placement of electrodes. 54

Figure 3.3: Diagram of plasma treatment station..55

Figure 3.4: Filling station with inlet MA gas and outlet vacuum for flushing sample. 56

Figure 3.5: (Top) Direct sample placement. (Bottom) Indirect sample placement. 57

Figure 3.6: Microbial growth of natural background microflora over 14 day storage period at 4°C packaged in modified atmosphere (MA) after treatment with ARS-plasma generation system. Values and standard deviation based on triplicate experiments. Area inside dashed lines indicates minimum growth range to declare product spoiled...58

Figure 4.1: Diagram of plasma treatment station..79

Figure 4.2: Microbial growth of natural background microflora over 14 day storage period at 4°C packaged in modified atmosphere (MA) after treatment with ARS-plasma generation system. Values and standard deviation based on triplicate experiments. Area inside dashed lines indicates minimum growth range to declare product spoiled...80

CHAPTER 1

INTRODUCTION

Food safety and food quality are of key importance in the food industry. Every year approximately 9 billion chickens are processed in the United States, and of these, 80% are marketed as fresh product. Of these 7.2 billion fresh chickens, 2% to 4% are estimated to be lost as a result of spoilage (Russell, 2009). The spoilage of fresh poultry products is associated with the growth of certain psychrotrophic bacteria when the product is stored aerobically under chill conditions. Most commonly theses bacteria are predominantly pigmented and non-pigmented strains of *Pseudomonas* spp. and produce the characteristic putrid or 'off' odors when the organisms reach population levels of 10^8 cfu/ml (Pooni and Mead, 1984); some literature states that poultry is considered microbiologically spoiled at levels of 10^6 or 10^7 cfu/ml (Hinton Jr et al., 2004; Owens, 2010). Shelf life under chill conditions depends on the temperature at which the poultry is held (Ikeme et al., 1982).

The most common foodborne organisms associated with poultry meat are *Salmonella* Enteritidis, *Staphylococcus arueus*, *Campylobacter jejuni*, and *Listeria monocytogenes* according to the USDA chicken fact sheet (Food Safety and Inspection Service, 2011a) and Cunningham (1987). *Salmonella* Enteritidis may be found in the intestinal tract of poultry as well as other warm blooded animals. The above mentioned strain is only one of about 2,000 associated with poultry and egg shells. One of the most common microorganisms on poultry meat is *C. jejuni* which is a usual cause of diarrheal

illness in humans; this microorganism is mostly spread through cross contamination. Poultry is commonly known to be contaminated with *Campylobacter* at a rate of 71 to 91% and *Salmonella* at a rate of 88 to 89% (Dufrenne et al., 2001; Parveen et al., 2007). The occurrence of disease caused by *Salmonella* and *Campylobacter* is 15.19 and 13.02 per 100,000 population, respectively based on the Center for Disease Control and Prevention FoodNet estimates (Vugia et al., 2009).

There are a number of treatment procedures used to treat poultry meat, but many of the methods have disadvantages. Systems that require high pressure degrade the sensory quality of chicken meat (Kruk et al., 2011). Irradiation is mistrusted by consumers and the product must carry the 'Radura' symbol (Food Safety and Inspection Service, 2011b) which makes this an unappealing processing technique for producers. Ultraviolet light treatment is ineffective because of the irregular nature of poultry surfaces and shadows that can be created on the meat (Bolder, 1997). High intensity pulsed electric fields have shown limited success in the broiler industry because the meat would require direct contact of electrodes to the food (Dincer and Baysal, 2004). Treatments that occur before packaging, such as chlorine rinses, leave the chicken meat susceptible to cross contamination from workers, contact surfaces, or aerosolized pathogens during the remainder of the processing procedure (Hecer et al., 2007). Finally, processes that require heat in order to eliminate microbial hazards can alter the characteristics consumers expect in a raw, fresh chicken. In addition to the processing obstacles mentioned above, none of the treatments allow for post-packaging disinfection. Technology that can treat a food product after packaging would greatly improve the safety and reduce the chances of cross contamination.

Work with plasma has grown in popularity in the past decade as producers and consumers desire ways to process and sanitize foods with economical and convenient techniques and without using chemical additives. Simply stated, plasma is generated when enough energy is supplied to a neutral gas to cause charge production. The phenomenon is achieved by ionization or photoionization when the electrons or photons with sufficient energy collide with the neutral gas atoms or other molecules (Gaunt et al., 2006). A major component of plasma, ozone, has been used in the treatment and disinfection of water, a popular practice for over 150 years (Jindal et al., 1995). Ozone is a strong antimicrobial due to its high reactivity, penetrability, and spontaneous decomposition to a non-toxic product (O_2). In 2001, ozone received the status of generally recognized as safe (GRAS) for food usage in the United States (Department of Health and Human Services, 2001). In 2002 the USDA issued FSIS directive 7120.1 which named ozone as a Safe and Suitable Ingredient Used in the Production of Meat and Poultry Products.

Plasma, specifically nonthermal dielectric barrier plasma, is an appealing nonthermal treatment for poultry because of the simple operation, lack of chemical additives, and ability to treat the sample after packaging. The basic principle of nonthermal DBD plasma generation involves the administration of high voltages of electricity between two electrodes (at least one electrode insulated by a dielectric barrier) in order to ionize the atmosphere between them (Gaunt et al., 2006). The radical gas particles generated will react with and kill the microorganisms on the surface of the food product. During 24 h storage the radical particles will dissociate and return to the

ambient gas particles that existed after packaging/before treatment (Klockow and Keener, 2009).

Plasma work has been applied more widely in the field non-animal tissue foods, Petri plates, and spore strips with success. Niemira and others (2012) reduced *Salmonella* Stanley by 2.9 to 3.7 log cfu/ml and *E. coli* O157:H7 by 3.4 to 3.6 log cfu/ml on golden delicious apples with a 40 l/min flow rate of cold plasma. Gas plasma has been used to reduce *Salmonella* Enteritidis on the surface of egg shells by up to 2.5 log cfu/eggshell and 4.5 log cfu/eggshell (Ragni et al., 2010). Dirks and others (2012) stated that reducing the background microflora of raw poultry has the potential to extend the shelf life of the product as well as make it safer, potentially making plasmas an appealing intervention for poultry processing.

There are numerous ways to produce gas plasmas depending on the product or surface being treated and the intended use of plasma treatment (Critzer et al., 2007; Jung and Moon, 2008; Rowan et al., 2007). Keener and Klockow (2009) developed a novel DBD plasma system that was first used on spinach leaves. The effectiveness of this PK-1 system showed the potential for this set-up to antimicrobially treat other food products. Keener and others (2012) later used this system and a modified version (PK-2) to treat *Bacillus subtilis* spore strips. During these tests, it was determined that measuring ozone concentrations was a good indicator of the plasma strength; therefore this measurement along with others, were recorded to monitor the performance of the PK-1 and PK-2 systems. Depending on the experiment, various voltages, electrode separations, samples placements, and fill gases were used optimize the antimicrobial potential of the plasma

system. Based on these experiments and settings, the following research built upon the observations and successes of these previously used systems.

Because nonthermal plasma treatment of packaged food has not been widely tested, especially with meat tissue, research must be conducted in this area. The objectives of this project are 1) to establish system parameters for effective treatment of poultry with a high voltage DBD plasma device so that further research can be continued in this novel field 2) and reduce microorganisms on the surface of packaged broiler breast meat with the nonthermal DBD plasma system. It is hypothesized that treatment with nonthermal plasma will reduce microorganisms on the surface of the meat and extend the shelf life of packaged broiler breast fillets.

REFERENCES

1. Bolder, N. M. 1997. Decontamination of meat and poultry carcasses. *Trends in Food Science & Technology*. 8:221-227.

2. Critzer, F. J., K. Kelly-Wintenberg, S. L. South, and D. A. Golden. 2007. Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces. *Journal of Food Protection®*. 70:2290-2296.

3. Cunningham, F. 1987. Types of microorganisms associated with poultry carcasses.

4. Department of Health and Human Services, F. 2001. Secondary Direct Food Additives Permitted in Food for Human Consumption. p. 33829-33830. *In* F.a.D. Administration (ed.), vol. 66. Food and Drug Administration.

5. Dincer, A. H., and T. Baysal. 2004. Decontamination techniques of pathogen bacteria in meat and poultry. *Critical Reviews in Microbiology*. 30:197-204.

6. Dirks, B. P., D. Dobrynin, G. Fridman, Y. Mukhin, A. Fridman, and J. J. Quinlan. 2012. Treatment of Raw Poultry with Nonthermal Dielectric Barrier Discharge Plasma To Reduce Campylobacter jejuni and Salmonella enterica. *Journal of Food Protection*. 75:22-28.

7. Dufrenne, J., W. Ritmeester, E. D. Asch, F. Van Leusden, and R. De Jonge. 2001. Quantification of the contamination of chicken and chicken products in the Netherlands with Salmonella and Campylobacter. *Journal of Food Protection®*. 64:538-541.

8. Food Safety and Inspection Service. Date, 2011, Poultry preparation: Chicken from farm to table: Foodborne organisms associated with chicken. Available at: http://www.fsis.usda.gov/fact_sheets/chicken_from_farm_to_table/index.asp. Accessed January 30, 2012.

9. Food Safety and Inspection Service. Date, 2011, Poultry preparation: Chicken from farm to table: Irradiation of poultry. Available at: http://www.fsis.usda.gov/fact_sheets/chicken_from_farm_to_table/index.asp. Accessed January 30, 2012.

10. Gaunt, L. F., C. B. Beggs, and G. E. Georghiou. 2006. Bactericidal action of the reactive species produced by gas-discharge nonthermal plasma at atmospheric pressure: A review. *Ieee Transactions on Plasma Science*. 34:1257-1269.

11. Hecer, C., F. Balci, and C. D. Udum. 2007. The effects of ozone and chlorine applications on microbiological quality of chickens during processing. *J. Biol. Environ. Sci*. 1:131-138.

12. Hinton Jr, A., J. A. Cason, and K. D. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. *International Journal of Food Microbiology*. 91:155-165.

13. Ikeme, A., B. Swaminathan, M. Cousin, and W. Stadelman. 1982. Extending the shelf-life of chicken broiler meat. *Poultry Science*. 61:2200-2207.

14. Jindal, V., A. L. Waldroup, R. H. Forsythe, and M. J. Miller. 1995. Ozone and Improvement of Quality and Shelf Life of Poultry Products. *The Journal of Applied Poultry Research*. 4:239-248.

15. Jung, J. S., and J. D. Moon. 2008. Corona discharge and ozone generation characteristics of a wire-plate discharge system with a glass-fiber layer. *Journal of Electrostatics*. 66:335-341.

16. Keener, K. M., J. Jensen, V. Valdramidis, E. Byrne, J. Connolly, J. Mosnier, and P. Cullen. 2012. Decontamination of Bacillus subtilis spores in a sealed package using a non-thermal plasma system. *Plasma for Bio-Decontamination, Medicine and Food Security*:445-455.

17. Klockow, P. A., and K. M. Keener. 2009. Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *Lwt-Food Science and Technology*. 42:1047-1053.

18. Kruk, Z. A., H. Yun, D. L. Rutley, E. J. Lee, Y. J. Kim, and C. Jo. 2011. The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. *Food Control*. 22:6-12.

19. Niemira, B. A. 2012. Cold Plasma Reduction of Salmonella and Escherichia coli O157:H7 on Almonds Using Ambient Pressure Gases. *Journal of Food Science*. 77:M171-M175.

20. Owens, C. M., Alvarado, C., Sams, A.R. 2010. Poultry Meat Processing, Second Edition. CRC Press.

21. Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry. *Journal of Food Protection®*. 70:2466-2472.

22. Pooni, G., and G. Mead. 1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. *Food Microbiology*. 1:67-78.

23. Ragni, L., A. Berardinelli, L. Vannini, C. Montanari, F. Sirri, M. E. Guerzoni, and A. Guarnieri. 2010. Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *Journal of Food Engineering*. 100:125-132.

24. Rowan, N. J., S. Espie, J. Harrower, J. G. Anderson, L. Marsili, and S. J. MacGregor. 2007. Pulsed-plasma gas-discharge inactivation of microbial pathogens in chilled poultry wash water. *Journal of Food Protection*. 70:2805-2810.

25. Russell, S. M. Date, 2009, Understanding poultry products spoilage. Available at: http://www.wattagnet.com/Understanding_poultry_products_spoilage.html. Accessed July 10, 2012, 2012.

26. Vugia, D., A. Cronquist, M. Cartter, M. Tobin-D'Angelo, D. Blythe, K. Smith, S. Lathrop, D. Morse, P. Cieslak, and J. Dunn. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 states, 2008. *Morbidity and Mortality Weekly Report*. 58:333-337.

CHAPTER 2

LITERATURE REVIEW

Spoilage of poultry

The safety and quality of poultry products is crucial to the success of the poultry industry. Assessing spoilage of poultry meat and working to extend the shelf life of broiler meat and other poultry products is of key importance. Primary causes of spoilage in poultry products include prolonged distribution or storage time, inappropriate storage temperature, high initial bacterial counts, and high post-rigor meat pH *(43).*

It is well established that the spoilage of raw poultry products is associated with the growth of certain psychrotrophic bacteria when the product is stored aerobically under chill conditions; notably theses bacteria are mainly pigmented and non-pigmented strains of *Pseudomonas* spp. and produce the characteristic putrid odors when the organisms reach population levels of 10^8 $\log_{10}$ cfu/ml *(40).* Research has shown the levels are bacteria that are present on chicken carcasses immediately after slaughter are not the organisms which spoil poultry under refrigeration conditions. The bacteria that are found on a spoiled carcass are challenging to find at the time of processing *(43).* The spoilage bacteria are initially very low after processing, but they rapidly multiply to cause spoilage odor and slime *(43).*

Spoilage bacteria on chicken can come from a number of different sources such as feathers and feet of live birds, the water supply in the processing plant, the chill tanks, and the processing equipment; these sources are linked to spoilage bacteria on the carcass

immediately after processing *(16, 43)*. Spoilage defects are caused when bacteria utilize nutrients on the surface of the meat tissue which leads to an accumulation of metabolic by-products. The action of extracellular enzymes produced by psychrotrophic spoilage organisms that multiply on poultry surfaces at refrigeration temperatures is also a cause of defects *(18, 43)*. As spoilage progresses, slime develops and exudes an unpleasing, ester-like smell. This odor is soon followed by slime formation which come in the form of small, translucent, moist colonies; these colonies will later increase in size and coalesce to form a slime coating on the meat *(43)*.

Poultry and pathogens

The most common foodborne organisms associated with poultry meat are *Salmonella* Enteritidis, *Staphylococcus arueus*, *Campylobacter jejuni*, and *Listeria momocytogenes* according to the USDA chicken fact sheet *(9)* and Cunningham *(3)*. *Salmonella* Enteritidis may be found in the intestinal tract of poultry as well as other warm blooded animals. The above mentioned strain is only one of about 2,000 associated with poultry and egg shells. *Staphylococcus arueus* is carried by humans and is most commonly a concern on improperly refrigerated foods. One of the most common microorganisms on poultry meat is *C. jejuni* which is a usual cause of diarrheal illness in humans; this microorganism is mostly spread through cross contamination. *Listeria momocytogenes* is killed by cooking but can be re-introduced by poor hygiene or abusive temperature treatment *(9, 47)*. Reducing or eliminating the above mentioned pathogens from poultry is crucial to maintaining a safe food supply, especially before these organisms have the opportunity to form biofilms.

Formation of biofilms on the surface of food tissue is a concern because these three dimensional networks of polysaccharides that attach microorganisms to surfaces and protect them from external stressors *(32)*. Microorganisms can attach to the surface of a food where they extract nutrients from the food and proliferate on the surface to form a biofilm *(48)*. In work done with bell peppers, atmospheric pressure glow discharges (He-O_2) was an effective bacterial inactivation agent that did not cause unacceptable discoloration of the peppers; this process was shown to be more successful than the use of low-pressure ultraviolet sources *(48)*. Although there has been effective work with regards to biofilms, it has not been proven if atmospheric pressure plasmas can penetrate and kill microorganisms imbedded in animal tissue biofilms.

Nonthermal inactivation of food borne pathogens

Numerous nonthermal techniques exist to inactivate microorganisms on food including chlorine, high pressure processing, irradiation, pulsed electric fields, ultraviolet radiation and ozone/plasmas. The following section covers the techniques and the effectiveness of the methods and highlights the advantages associated with nonthermal plasmas.

Chlorine

Use of chlorine is becoming more scrutinized because of toxicity issues and disinfection by-products *(42)*. Chlorine washes inhibit glucose oxidation in bacteria and show a bactericidal effect, but the use of excess chlorine forms toxic and carcinogenic compounds called tri-halo methane by reacting with the meat *(14, 20)*. The process of decontamination with chlorine requires large consumption of water. Chlorine washed

chicken must also come into contact with other processing surfaces before packaging which could be a source for secondary contamination *(14)*.

High Pressure Processing

The capacity of high pressure processing (HPP) to eradicate microorganisms made this technology appealing as safe and consumer friendly because it is effective regardless of geometry of the product and without the formation of detrimental heat damage changes or use of preservatives/additives *(31)*. The primary antimicrobial action of HPP is related to the effect on the cell envelope. Cellular morphology is altered by pressure, and cell division slows with increased pressure application *(17)*. Research done on various meats concluded that more investigation was needed because of the different responses of microorganisms on different meat products; factors such as pressure, temperature, and time of exposure can all effect the effectiveness of this process *(8)*. In a study conducted by Kruk, chicken breast fillets were treated with three different pathogens: *Salmonella* Typhimurium, *Escherichia coli*, and *Listeria monocytogenes (31)*. The samples were treated at pressures of 300, 450, and 600MPa for 5 min. Increases in hydrostatic pressure had an effect on all three pathogens that were inoculated. The most prominent effects were observed with 450 and 600 MPa which completely inactivated *E. Coli* and *L. monocytogenes.* Pressure of 600 MPa reduced bacterial count by 6-8 $\log_{10}$ (cfu/g) which improved the shelf life for 7-14 days. Increased pressure did negatively affect sensory characteristics of chicken breast fillets. Flavor, aroma, strength and juiciness were the major characteristics affected *(31)*. Increases in meat hardness, cohesiveness, gumminess, and chewiness were also seen. Although this antimicrobial processing treatment does improve the safety and freshness of the meat, the sensory

qualities are sacrificed. HPP is also limited by the factors of enzymatic and oxidative spoilage which requires HPP to be used with other processing techniques *(46)*.

Irradiation

Irradiation denotes any process which involves the application of ionizing radiation *(22)*. Ionizing radiation utilizes either gamma rays from radionuclides such as cobalt 60 or cesium 137 or high energy electron produced by machine sources *(5)*. Decontamination of poultry carcasses by ionizing radiation has been approved in the US, but its commercial use is currently limited. Pathogens are affected by irradiation mainly by hydrogen peroxide production that results from the generation of free radicals during irradiation. The hydrogen peroxide produced acts as a potent antimicrobial and can eventually result in the production of long-lived hypochlorite which is very toxic to pathogens *(34)*. Application of irradiation technology has been very limited because of consumer distrust of any process based on the nuclear industry. Because irradiated chicken must carry the international ‘Radura’ symbol along with “treated with irradiation”, it is easy for consumers to identify these products *(9)*. Also, consumers lack sufficient knowledge of foodborne illnesses and the advantageous ability of irradiation to penetrate whole poultry or large pieces of meat.

Pulsed electric field

High intensity pulsed electric field (PEF) processing involves the application of pulses of high voltage (typically 20-80kV/cm) to foods placed between two electrodes *(5)*. Like other non-thermal treatments for food, PEF greatly reduces detrimental effects to the sensory and physical properties of the food. Some studies have proven that PEF preserves the nutritional components of food, but there is not enough knowledge of PEF’s

effects on the chemical and nutritional aspects of food to declare its efficacy. As with other novel processing and sanitation techniques, it is not completely understood how PEF causes microbial inactivation. Several theories have been proposed and the general consensus focuses on electrical breakdown and electroporation or disruption of cell membranes *(5)*. Research has shown that PEF treatment causes reduction in bacterial counts and extends the lag phase of bacterial growth. Hermawan and others *(15)* were able to produce a 4.3 log_{10} cfu/mL in liquid eggs, but only 1 log_{10} cfu/mL was in response to the PEF; the addition log reductions came from a mild heat treatment. Citrus juices have been successfully treated with PEF to pasteurize the product *(19)*, but this treatment method has showed little promise outside of liquid food products. Applications of electrical stimulation have shown limited success in the broiler industry for pathogen/spoilage reductions and treatment of meat would require direct contact of electrodes to the food *(5)*.

Ultraviolet radiation

UV light treatment has limited use in the area of meat and poultry processing. Continuous UV light exposure has the ability to reduce bacterial load in processing areas, but this bacterial reduction is only seen in the atmosphere *(1)*. Sumner and others *(44)* had limited success with complete elimination of *Salmonella typhimurium* on the surface of poultry skin with UV radiation. Because of the irregular nature of poultry skin surfaces, shadows are created and UV light is an ineffective decontamination method *(1)*.

Ozone

The use of ozone in the treatment and disinfection of water has been a popular practice for over 150 years *(20)*. Use of ozonated water for chilling poultry has

demonstrated there were no visual or sensory off-flavors produced by the water. However, there were minimal bacterial reductions (<1 log) for both total counts and psychrotrophs *(1)*. It has been found that ozone is more effective and efficient at lower concentrations and treatment times than standard sanitizers including chlorine *(25, 27)*. Gomella reported that ozone showed stronger and more rapid antimicrobial action against spores, fecal and pathogenic microorganisms, and viruses when compared to chlorine *(12, 25)*. The study of gas discharge as a disinfection technology is gaining more recognition as scientists strive to improve safety processing practices. The main emphasis in the resurgence of ozone technology is using non-thermal plasma at atmospheric pressure *(10)*. During the production of the non-thermal plasma, bactericidal agents are generated in the form of reactive oxygen species (ROS), ultraviolet (UV) radiation, energetic ions, and charged particles. Ozone, atomic oxygen, superoxide, peroxide, and hydroxyl radicals are included in the reactive oxygen species reported in the air *(10)*.

Nonthermal plasma

Research has been conducted on common food borne pathogens on various non-food media to determine plasma's effectiveness on these microorganisms. This technique has allowed researchers to verify plasma's effect on specific organisms without having the interference of food products. Ziuzina and others have proven that atmospheric cold plasma has the ability to inactivate *E. coli* in liquid media in a sealed package *(50)*. This work successfully inactivated *E. coli* in two different liquid media while also testing the efficacy of other processing variables such as exposure time, sample placement, and storage time after treatment. Nonthermal plasma was able to decontaminate spore strips

containing *Bacillus subtilis* spores in a sealed package *(23)*. The higher ionization voltages in this work allowed for shorter treatment times to achieve complete elimination of the spores. When nonthermal plasma has been used to treat food products, it has occurred before packaging *(24, 37, 38)*. As shown by the above research, work with nonthermal plasma either focuses on elimination of microorganisms in an isolated environment on media or on food before it has been packaged, leaving it susceptible to cross contamination. There is a lack of research that explores the benefits of nonthermal plasma treatment on a packaged food product to improve the microbial quality.

Mechanisms of bacterial destruction

The mode of bacterial destruction by ozone and reactive species is a topic that scientists cannot agree upon *(20)*. There are a number of different mechanisms that can cause the death of bacterial cells, but no single mechanism has been recognized as the primary cause. Oxidative damage can cause great damage to bio-macromolecules by oxygen radicals, but the damage that leads to cell death is unclear. Consequences of oxidative damage include the following: [1] Cell adaptation by up-regulation of defense systems, [2] injury to cell involving damage to molecular targets such as lipids, DNA, protein, and carbohydrates, and [3] cell death that arises through excessive unrepaired damages which triggers cell death *(10)*. Bacterial destruction can also vary among Gram negative, Gram positive, and spore forming organisms.

The cell wall structure plays an integral role in the differences between Gram-positive and Gram-negative bacteria when discussing plasma destruction of the cells. The cell wall of Gram-positive cells has a thick peptidoglycan layer that is 20 to 80 nm thick *(45)*. The peptidoglycan layer contains acidic polysaccharides which include

techoic and lipotechoic acids that are tightly bound together. These acidic polysaccharides are responsible for the characteristic positive charge on the surface of the cell. The periplasmic space between the cell membrane and the peptidoglycan layer varies with bacteria *(45)*.

Gram-negative cell walls are slightly more complicated because they contains multiple layers which include and outer membrane, a thin peptidoglycan layer, and periplasmic space between the peptidoglycan and cell membrane *(45)*. The outer membrane is similar to the cell wall structure in that it is composed of a bilayer which contains a unique composition of polysaccharides and proteins. The outermost layer contains lipopolysaccharides (LPS); these are fragments of polysaccharides which are integrated into membrane lipids. The innermost layer is comprised of a lipid layer which is attached to the thin peptidoglycan layer below by proteins. The outermost membrane acts as a protective barrier by only allowing relatively small particles to penetrate. Porin proteins create special membrane channels which allow molecules to transverse the entire width of the outer membrane. The peptidoglycan layer gives some rigidity to the cell, but it is only 1 to 3 nm thick. This thin peptidoglycan layer allows for more flexibility and makes the Gram-negative bacteria more sensitive to lysis as compared to Gram positive *(21)*. In work done with ozonated wash water for poultry, Gram negative test bacteria were more susceptible to the lethal action of pulsed plasma gas discharge which may be due to the complex nature of Gram negative cell envelopes *(42)*.

Despite many years of research, the mechanism of ozone disinfection is still not completely understood. It is known that ozone is a powerful oxidizing agent which readily forms reactive OH and HOO radicals in aqueous solution *(10)*. Among the most

likely targets for gaseous ozone are cell wall targets such as fatty acids and peptides. Also, oxidation by ozone can stimulate lipid peroxidation which results in the reduction of membrane fluidity *(10)*.

Production of plasma

Producing and maintaining plasma constitutes one of the greatest challenges in the area of plasma technology. Simply stated, plasma is generated when enough energy is supplied to a neutral gas to cause charge production. The phenomenon is achieved by ionization or photoionization when the electron or photons with sufficient energy collide with the neutral gas atoms or other molecules *(10)*. The design and transmission rates of plasma producing systems are based on the items, surfaces, or spaces being treated. The most commonly used method for plasma generation utilizes the electrical breakdown of a neutral gas in the presence of an electric field *(2)*. In the case of electrical breakdown, electrons and other charged particles accelerate under the externally applied electric field. This in turn transfers energy through inelastic collisions to other particles and atoms.

In addition to the method of plasma generation, the feed gas is a determining factor in the types of reactions that the plasma can initiate because some chemical species are more reactive than others *(36)*. Because gas particles have different ionization energies and properties, the gas used for plasma production can influence the antimicrobial properties of the plasma generated.

In the past, non-thermal plasma discharges have been operated at low pressures, but recent research is developing technology that operates these systems at atmospheric pressure and temperatures *(33)*. This new method would allow for elimination or replacement of expensive vacuum systems with simpler ones. There are a number of

operating systems and discharge modes that can be constructed to produce non-thermal plasma discharges depending on space or item that needs to be treated. The gas mixture, dielectric surface properties, and operating conditions influence the different modes which range from filamentary to completely diffuse barrier discharges *(28)*.

A DBD is the gas discharge between two electrodes which are separated by one or more dielectric layer and a gas-filled gap *(10, 28)*. The dielectric barrier discharges operates at ambient temperatures and atmospheric pressure driven by pulsed or sinusoidal voltages at frequencies from 50 Hz up to several tens of kHz and at least one of the electrodes has an insulating layer *(26)*. The plasma will remain cold because of the presence of a dielectric barrier helps to ensure that the current is limited so that the discharge does not transit to a spark or arc *(11, 29)*.

Dielectric barrier discharges operating at atmospheric pressure typically produce miniature filamentary discharges known as microdischarges *(28)*. When using controlled operating conditions, stable, uniform, non-filamentary, or glow discharges are produced, and these are more effective for surface modification compared to filamentary discharges *(7)*. Several mixes of gas can be treated to create the so-called atmospheric pressure glow (APG) discharge which is a uniform mode of barrier discharge; the diffuse and uniform discharge has temporal features that are reproducible and stable. Although differences exist, filamentary and diffuse discharges have common characteristics which include the generation of cold nonequilibrium plasmas at atmospheric pressure and the strong influence of local field distortions caused by space-charge accumulation *(10)*.

Literature has established that relative humidity plays an important role in species generated during ionization and these effects can influence the bactericidal efficacy of

plasma treatments *(23)*. Air humidity has a strong influence on the reduction rate of bacteria. Higher killing rates of microorganisms are seen when there are higher concentrations of water vapor in the processing gas *(13)* and increased moisture content of the feed gas improves the efficacy of remote cold plasma treatment systems *(41)*. Based on a study done with *Bacillus* spores, no reduction was found when dry air was used, but 70% relative humidity deactivated all spores in 2.5 and 7.5 minute treatments *(13)*. Researchers believe that OH is the most important species in humid environments because OH is formed directly from water by electron impact *(7, 13)*.

Plasma is composed of numerous different ionized species that exist for varying lengths of time depending on their individual properties. Many of the species are immeasurable because of their reactive nature and brief half-life. Ozone is one component of plasma that is highly reactive but exists long enough to be measured and quantified. For this reason, experiments involving plasma treatments of food often record ozone concentrations to indicate the strength of the plasma *(27)*. Dielectric barrier discharge (DBD) which produces atmospheric plasma was first developed as a large-scale means of producing ozone for industrial purposes *(30)* indicating that the two have been closely related for sanitizing and antimicrobial applications.

Plasma treatment of food

Plasma work has been applied more widely in the field non-animal tissue foods with success. But very little research has been conducted on the effectiveness of plasma treatment of poultry meat. One study carried out by researchers at Drexel University used DBD plasma in a limited way to disinfect a small segment of chicken breasts and chicken skin surfaces. A 2.5-cm diameter electrode probe was attached to a power

supply, and the probe was manually operated by hand and held at a distance of 1 to 2 mm from the chicken surface for treatment *(6)*.

Chicken breasts and chicken thigh skin were both inoculated with *Salmonella enteric* and *Campylobacter jejuni* and treated for this experiment. *S. enteric* and *C. jejuni* were unable to be detected after 5 s of plasma treatment on chicken breast samples. On all chicken breast samples, reduction of pathogens compared to controls were found to be statistically significant ($P < 0.05$) at all time-points and all three inoculum levels (10^2, 10^3, and 10^4 cfu/ml) *(6)*. Smaller log reductions were seen on the chicken thigh skin due to the fatty nature of the skin which would promote adherence of the pathogens. Also the pores and feather follicles of the skin made it less easily treated by DBD plasma *(6)*.

Less successful results were seen for log reductions of the natural micro flora of chicken samples. Background microflora of chicken breasts were only reduced by 0.84 and 0.85 $\log_{10}$ cfu/ml after 15 and 30 s of DBD plasma exposure. Log reductions of chicken thigh skin was even less at 0.33 and 0.21 $\log_{10}$ cfu/ml following 15 and 30 s of exposure. It was hypothesized that the greater resistance of the background microflora could be due to the fact that it was a mixed population of Gram negative and Gram positive microorganisms including *Pseudomonas*, lactic acid bacteria, *Enterobacteriaceae*, and bacilli *(6)*. *S. enteric* and *C. jejuni* may be more susceptible to plasma treatment because they were introduced in higher concentrations than the natural microflora and they are not in their ideal environment for survival.

Ozone gas for treatment of food

Ozone is one of the major components of gas plasmas, and it has a relatively long half compared to the other reactive oxygen species (approximately 7 h at 24°C and 87%

RH *(35)*) The half-life of ozone can vary depending on air temperature, air movement, relative humidity, and other factors. Ozone concentrations are often measured as an indicator of the strength of a plasma because of the relative ease with which is can be tested *(27)*. Ozone itself has been studied independently and in conjunction with plasma, and the following information covers the uses and effectiveness of ozone treatments for food.

Ozone is a strong antimicrobial due to its high reactivity, penetrability, and spontaneous decomposition to a non-toxic product (O_2). In 2001, ozone received the status of generally recognized as safe (GRAS) for food usage in the United States *(4)* . And in 2002 the USDA issued FSIS directive 7120.1 which names ozone as a Safe and Suitable Ingredient Used in the Production of Meat and Poultry Products (FSIS 2002). Because relatively low concentrations of ozone and short contact times are sufficient to inactivate bacteria, molds, yeasts, parasites, and viruses, ozone is an excellent candidate for the treatment of foods. The effects of ozone on particular microorganisms will vary with the physiological state of the culture, pH of the medium, temperature, humidity, and presence of additives (e.g., acids, surfactants, and sugars). Some precautions must be taken when using this reactive gas because excessive use of ozone may cause oxidation of some ingredients on food surfaces *(25)*.

When comparing aqueous versus gaseous ozone, there have been several studies that prove the gaseous form of ozone is more effective in reducing the microbial populations on food samples. One of these reasons is the longer half-life of gaseous ozone; gaseous ozone has a half-life of approximately 12 hours while aqueous ozone is reduced to 20-30 minutes *(39)*. Microbial cultures associated with poultry have been

tested by the spread plate method on open Petri plates that were exposed to an ozone gas treatment. The said treatment decreased microbial populations by >4 to 7 logs for bacteria and >4 logs in the case of fungi *(25)*. Another experiment conducted by Yang and Chen treated broiler parts and chicken necks with gaseous ozone for 5 to 9 minutes respectively, and again the microbial counts for broiler and microbial suspensions decreased 1.0 (broiler parts), 0.6 (fresh chicken necks), and 3.0 (spoiled chicken necks) logs *(49)*.

Keener acknowledges the potential benefit of reactive oxygen species, other than ozone, in his work on the decontamination of *Bacillus subtilis* spores in an in-package, nonthermal plasma system. In this work Keener not only measures ozone, but also carbon monoxide and nitrous oxide compounds *(23)*. Because work with food and nonthermal plasma is in its infancy, the mode of decontamination is not completely understood. It has been shown that ozone production reaches a plateau at a certain time of treatment, and longer exposure to nonthermal plasma can have positive benefits past maximum ozone generation *(23)*. Niemira also states that the mode of action for the reduction of pathogens may rely on chemistries resulting from the recombination of some of the species produced during ionization rather than the short lived plasma products *(37)*.

Conclusions

Spoilage of fresh broiler meat as well as food borne illness associated this product are common problems that affect the industry and consumers who purchase this product. With a demand for safe, chemical-free antimicrobial processes, there is a great need to reduce microorganisms on the surface of broiler meat without affecting the quality and without introducing harmful by-products. Nonthermal plasma has shown promise in

regards to its decontamination method through the reactions of radical oxygen species. Because the initial and final components of nonthermal plasma treatment are ambient gas (mainly O_2), this processing technique has great potential in the area of food antimicrobials.

The existing literature on nonthermal plasma outlines many varying systems used for plasma generation, but each of these technologies is specific to the medium being treated. This research must be adapted to effectively reduce microorganisms on broiler meat. The limited work done with in-package, nonthermal plasma generation systems must also be combined with general nonthermal plasma generation technology to optimize post-packaging applications.

REFERENCES

1. Bolder, N. M. 1997. Decontamination of meat and poultry carcasses. *Trends in Food Science & Technology*. 8:221-227.

2. Conrads, H., and M. Schmidt. 2000. Plasma generation and plasma sources. *Plasma Sources Science and Technology*. 9:441.

3. Cunningham, F. 1987. Types of microorganisms associated with poultry carcasses.

4. Department of Health and Human Services, F. 2001. Secondary Direct Food Additives Permitted in Food for Human Consumption. p. 33829-33830. *In* F.a.D. Administration (ed.), vol. 66. Food and Drug Administration.

5. Dincer, A. H., and T. Baysal. 2004. Decontamination techniques of pathogen bacteria in meat and poultry. *Critical Reviews in Microbiology*. 30:197-204.

6. Dirks, B. P., D. Dobrynin, G. Fridman, Y. Mukhin, A. Fridman, and J. J. Quinlan. 2012. Treatment of Raw Poultry with Nonthermal Dielectric Barrier Discharge Plasma To Reduce Campylobacter jejuni and Salmonella enterica. *Journal of Food Protection*. 75:22-28.

7. Dorai, R., and M. J. Kushner. 2003. A model for plasma modification of polypropylene using atmospheric pressure discharges. *Journal of Physics D: Applied Physics*. 36:666.

8. Fonberg-Broczek, M., B. Windyga, J. Szczawinski, M. Szczawinska, D. Pietrzak, and G. Prestamo. 2005. High pressure processing for food safety. *ACTA BIOCHIMICA POLONICA-ENGLISH EDITION-*. 52:721.

9. Food Safety and Inspection Service. Date, 2011, Poultry preparation: Chicken from farm to table: Foodborne organisms associated with chicken. Available at: http://www.fsis.usda.gov/fact_sheets/chicken_from_farm_to_table/index.asp. Accessed January 30, 2012.

10. Gaunt, L. F., C. B. Beggs, and G. E. Georghiou. 2006. Bactericidal action of the reactive species produced by gas-discharge nonthermal plasma at atmospheric pressure: A review. *Ieee Transactions on Plasma Science*. 34:1257-1269.

11. Georghiou, G., A. Papadakis, R. Morrow, and A. Metaxas. 2005. Numerical modelling of atmospheric pressure gas discharges leading to plasma production. *Journal of Physics D: Applied Physics*. 38:R303.

12. Gomella, C. 1972. Ozone practices in France. *Journal (American Water Works Association)*:39-45.

13. Hähnel, M., T. von Woedtke, and K.-D. Weltmann. 2010. Influence of the Air Humidity on the Reduction of Bacillus Spores in a Defined Environment at Atmospheric Pressure Using a Dielectric Barrier Surface Discharge. *Plasma Processes and Polymers*. 7:244-249.

14. Hecer, C., F. Balci, and C. D. Udum. 2007. The effects of ozone and chlorine applications on microbiological quality of chickens during processing. *J. Biol. Environ. Sci*. 1:131-138.

15. Hermawan, N., G. A. EVRENDILEK, W. Dantzer, Q. Zhang, and E. Richter. 2007. Pulsed electric field treatment of liquid whole egg inoculated with Salmonella enteritidis. *Journal of Food Safety*. 24:71-85.

16. Hinton Jr, A., J. A. Cason, and K. D. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. *International Journal of Food Microbiology*. 91:155-165.

17. Hoover, D. G. 2001. Microbial inactivation by high hydrostatic pressure. p. 419-449. *In* V.K. Juneja, and J.N. Sofos (ed.), Control of foodborne microorganisms Marcel Dekker, New York.

18. Ikeme, A., B. Swaminathan, M. Cousin, and W. Stadelman. 1982. Extending the shelf-life of chicken broiler meat. *Poultry Science*. 61:2200-2207.

19. Jia, M., Q. Howard Zhang, and D. B. Min. 1999. Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice. *Food Chemistry*. 65:445-451.

20. Jindal, V., A. L. Waldroup, R. H. Forsythe, and M. J. Miller. 1995. Ozone and Improvement of Quality and Shelf Life of Poultry Products. *The Journal of Applied Poultry Research*. 4:239-248.

21. Johnson, F. 2004. Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces. p. 61. *In*, Food Science and Technology, vol. Master's Degree. University of Tennessee.

22. Juneja, V. K., and J. N. Sofos (ed.). 2001. Control of foodborne microorganisms. Marcel Dekker, New York.

23. Keener, K. M., J. Jensen, V. Valdramidis, E. Byrne, J. Connolly, J. Mosnier, and P. Cullen. 2012. Decontamination of Bacillus subtilis spores in a sealed package using a non-thermal plasma system. *Plasma for Bio-Decontamination, Medicine and Food Security*:445-455.

24. Kim, B., H. Yun, S. Jung, Y. Jung, H. Jung, W. Choe, and C. Jo. 2011. Effect of atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two different gas compositions. *Food microbiology*. 28:9-13.

25. Kim, J. G., A. E. Yousef, and S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: A review. *Journal of Food Protection*. 62:1071-1087.

26. Kim, S. J., T. H. Chung, S. H. Bae, and S. H. Leem. 2009. Characterization of Atmospheric Pressure Microplasma Jet Source and its Application to Bacterial Inactivation. *Plasma Processes and Polymers*. 6:676-685.

27. Klockow, P. A., and K. M. Keener. 2009. Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *Lwt-Food Science and Technology*. 42:1047-1053.

28. Kogelschatz, U. 2002. Filamentary, patterned, and diffuse barrier discharges. *Ieee Transactions on Plasma Science*. 30:1400-1408.

29. Kogelschatz, U. 2002. Industrial innovation based on fundamental physics. *Plasma Sources Science and Technology*. 11:A1.

30. Kogelschatz, U. 2003. Dielectric-barrier discharges: their history, discharge physics, and industrial applications. *Plasma chemistry and plasma processing*. 23:1-46.

31. Kruk, Z. A., H. Yun, D. L. Rutley, E. J. Lee, Y. J. Kim, and C. Jo. 2011. The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. *Food Control*. 22:6-12.

32. Kumar, C. G., and S. K. Anand. 1998. Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology*. 42:9-27.

33. Leipold, F., Y. Kusano, F. Hansen, and T. Jacobsen. 2010. Decontamination of a rotating cutting tool during operation by means of atmospheric pressure plasmas. *Food Control*. 21:1194-1198.

34. Lewis, S. J., A. Velasquez, S. L. Cuppett, and S. R. McKee. 2002. Effect of electron beam irradiation on poultry meat safety and quality. *Poultry Science*. 81:896-903.

35. McClurkin, J., and D. Maier. 2010. Half-life time of ozone as a function of air conditions and movement. *Julius-Kühn-Archiv*:S. 381.

36. Niemira, B. A. 2012. Cold Plasma Decontamination of Foods. p. 125-142. *In* M.P. Doyle, and T.R. Klaenhammer (ed.), Annual Review of Food Science and Technology, Vol 3, vol. 3.

37. Niemira, B. A. 2012. Cold Plasma Reduction of Salmonella and Escherichia coli O157:H7 on Almonds Using Ambient Pressure Gases. *Journal of Food Science*. 77:M171-M175.

38. Oner, M. E., P. N. Walker, and A. Demirci. 2011. . Effect of in-package gaseous ozone treatment on shelf life of blanched potato strips during refrigerated storage. *International Journal of Food Science and Technology*. 46:406-412.

39. Perry, J. J., and A. E. Yousef. 2011. Decontamination of Raw Foods Using Ozone-Based Sanitization Techniques. *Annual Review of Food Science and Technology*. 2:281-298.

40. Pooni, G., and G. Mead. 1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. *Food Microbiology*. 1:67-78.

41. Ragni, L., A. Berardinelli, L. Vannini, C. Montanari, F. Sirri, M. E. Guerzoni, and A. Guarnieri. 2010. Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *Journal of Food Engineering*. 100:125-132.

42. Rowan, N. J., S. Espie, J. Harrower, J. G. Anderson, L. Marsili, and S. J. MacGregor. 2007. Pulsed-plasma gas-discharge inactivation of microbial pathogens in chilled poultry wash water. *Journal of Food Protection*. 70:2805-2810.

43. Russell, S. M. Date, 2009, Understanding poultry products spoilage. Available at: http://www.wattagnet.com/Understanding_poultry_products_spoilage.html. Accessed July 10, 2012, 2012.

44. Sumner, S. S., E. A. Wallner-Pendleton, G. W. Froning, and L. V. E. Stetson. 1996. Inhibition of Salmonella typhimurium on agar medium and poultry skin by ultraviolet energy. *Journal of Food Protection®*. 59:319-321.

45. Talaro, K. P., and B. Chess. 2011. Foundations in microbiology. *Recherche*. 67:02.

46. Thakur, B., and P. Nelson. 1998. High-pressure processing and preservation of food. *Food Reviews International*. 14:427-447.

47. Uyttendaele, M., P. De Troy, and J. Debevere. 1999. Incidence of Salmonella, Campylobacter jejuni, Campylobacter coli, and Listeria monocytogenes in poultry carcasses and different types of poultry products for sale on the Belgian retail market. *Journal of Food Protection®*. 62:735-740.

48. Vleugels, M., G. Shama, X. T. Deng, E. Greenacre, T. Brocklehurst, and M. G. Kong. 2005. Atmospheric plasma inactivation of biofilm-forming bacteria for food safety control. *Ieee Transactions on Plasma Science*. 33:824-828.

49. Yang, P., and T. Chen. 1979. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. *Journal of Food Science*. 44:501-504.

50. Ziuzina, D., S. Patil, P. Cullen, K. Keener, and P. Bourke. 2013. Atmospheric cold plasma inactivation of Escherichia coli in liquid media inside a sealed package. *Journal of Applied Microbiology*.

CHAPTER 3

NONTHERMAL PLASMA SYSTEM FOR EXTENDING SHELF LIFE OF RAW BROILER BREAST FILLETS

---

[1]Taylor G. Kronn, Kurt C. Lawrence, Hong Zhuang, Kelli L. Hiett, Michael J. Rothrock, Yao-wen Huang, Kevin M. Keener. To be submitted to *Applied Engineering in Agriculture*.

ABSTRACT.

A nonthermal dielectric barrier discharge (DBD) plasma system was developed and enhanced to treat broiler breast fillets (BBF) in order to improve the microbial quality of the meat. The system consisted of a high-voltage source and two parallel, round-aluminum electrodes separated by three semi-rigid polypropylene barriers extending well beyond the electrodes. The broiler sample was packaged in sealed polyolefin plastic bags to allow for adequate gas volume in the package. A modified atmosphere (MA) blend of gas (65% O_2, 30% CO_2, 5% N_2) was used to enhance the generation of reactive oxygen species during treatment. Since numerous variables affect consistent and reproducible ionization within the plastic bag, the dielectric barrier and electrode assembly were designed such that the electrodes were parallel, the separation was fixed, and the plastic bag maintained uniform contact with the dielectric barriers throughout the ionization field. Voltage, current, and power were monitored throughout treatment to maintain consistent settings. This research investigates the ability of this plasma system to extend shelf life of BBF by reducing the number of spoilage bacteria. The system was tested on BBF and compared to triplicate untreated controls. Samples were treated outside the plasma generation field at ambient air temperature and pressure for 3 min at 75 kV then stored at 4°C. Surviving microbes were recovered on days 1, 3, 7, 10, and 14 via rinsing and plating on nutrient agar. There was a mean log reduction of 1.53 $\log_{10}$ cfu/ml after 24 hours. After 14 days of storage, the treated samples had a mean log of 5.56 $\log_{10}$ cfu/ml which was 2.43 cfu/ml lower than the control packaged in air and 1.52 $\log_{10}$ cfu/ml lower than the control packaged in MA. A level of 5.56 $\log_{10}$ cfu/ml falls below the generally accepted spoilage limit of 10^7. Results demonstrate the ability of

nonthermal DBD system to reduce natural microflora on the surface of BBF and its applications in food safety and shelf-life extension.

Keywords.

Antimicrobial treatment, broiler fillets, modified atmosphere, nonthermal plasma, ozone, packaging

Food safety and food quality are of key importance in the food industry. Every year approximately 9 billion chickens are processed in the United States, and of these, 80% are marketed as fresh product. Of these 7.2 billion fresh chickens, 2% to 4% are estimated to be lost as a result of spoilage (Russell, 2009). In addition to spoilage, poultry is commonly known to be contaminated with *Campylobacter* at a rate of 71 to 91% and *Salmonella* at a rate of 88 to 89% (Dufrenne et al., 2001) (Parveen et al., 2007). The occurrence of human disease caused by *Salmonella* and *Campylobacter* is 15.19 and 13.02 per 100,000 population, respectively based on the Center for Disease Control and Prevention FoodNet estimates (Vugia et al., 2009).

There are a number of antimicrobial procedures used to treat broiler meat, but many of the methods have disadvantages. Systems that require high pressure degrade the sensory quality of poultry meat (Kruk et al., 2011). Irradiation is mistrusted by consumers and the product must carry the ‘Radura’ symbol (Food Safety and Inspection Service, 2011) which makes this an unappealing processing technique for producers. Ultraviolet-light treatment is ineffective because of the irregular nature of poultry surfaces and shadows that can be created on the meat (Bolder, 1997). High-intensity pulsed-electric fields have shown limited success in the broiler industry because the meat would require direct contact with the electrodes (Dincer and Baysal, 2004). Treatments that occur before packaging are effective, such as chlorine rinses, but leave the poultry meat susceptible to cross contamination from workers or contact surfaces during the remainder of the processing procedure (Hecer et al., 2007). Finally, processes that require heat in order to eliminate microbial hazards can alter the characteristics consumers expect in a raw, fresh chicken. In addition to processing obstacles with the

above mentioned techniques, none of these methods sanitize the food product after it has been packaged. A method that occurred post-packaging would eliminate the risk of cross contamination and ensure the product was safe upon exiting the processing facility.

Work with plasma has grown in popularity in the past decade as producers and consumers desire ways to process and sanitize foods with economical and convenient techniques and without using chemical additives. A major component of plasma, ozone, has been used in the treatment and disinfection of water, a popular practice for over 150 years (Jindal et al., 1995). Ozone is a strong antimicrobial due to its high reactivity, penetrability, and spontaneous decomposition to a non-toxic product (O_2). In 2001, ozone received the status of generally recognized as safe (GRAS) for food usage in the United States (Department of Health and Human Services, 2001). In 2002 the USDA issued FSIS directive 7120.1 which named ozone as a Safe and Suitable Ingredient Used in the Production of Meat and Poultry Products (FSIS 2002).

Plasma work has been applied more widely and successfully in the field of non-animal tissue foods. Niemira et al. (2012) reduced *Salmonella* Stanley by 2.9 to 3.7 log colony forming units (cfu) per ml and *E. coli* O157:H7 by 3.4 to 3.6 log_{10} cfu/ml on golden delicious apples with a 40 liter/min flow rate of cold plasma. Gas plasma has been used to reduce *Salmonella* Enteritidis on the surface of egg shells by up to 2.5 log_{10} cfu/eggshell and 4.5 log_{10} cfu/eggshell (Ragni et al., 2010). Dirks et al. (2012) stated that reducing the background microflora of raw poultry has the potential to extend the shelf life of the product as well as make it safer, potentially making plasma an appealing intervention for poultry processing. For these reasons, this article explores the methods used to develop a nonthermal plasma treatment system for animal tissue, specifically

broiler breast fillets, to make a safer product for producers and consumers without degrading the quality. Also, this research explores the feasibility of applying a plasma treatment that occurs after the broiler meat has been packaged.

System Design

A nonthermal dielectric barrier discharge (DBD) plasma system was developed based on previous work and designs used to treat spinach leaves and *Bacillus subtilis* spore strips (Keener et al., 2012; Klockow and Keener, 2009). Their two systems, PK-1 and PK-2, served as the basis for the plasma system used in this research. The plasma generation system consisted of an AC Dielectric Test Set (BK 130, Phenix Technologies, Accident, MD), high voltage wires, aluminum electrodes, and dielectric barriers as shown in figure 3.1. Poultry samples were packaged in sealed bags before treatment and filled with a modified atmosphere gas to enhance ionization in the package. Ozone levels were measured after treatment to monitor the concentration of O_3 to ensure that the system was producing consistent plasma levels. The advantages of this system for treatment of broiler meat include the ease of operation, absence of chemical additives, lack of byproducts, and post-packaging applications.

Electrodes and high-voltage transformer

The BK-130 AC Dielectric Test Set consisted of a high-voltage transformer, power supply, and control system. Two 15.24-cm (6-in.) diameter spun-aluminum electrodes (Phenix Technologies, Accident, MD) were connected to the high-voltage transformer. The two electrodes were arranged parallel to one another on the top and bottom of the sample. The electrodes were isolated from the sample package by

dielectric barriers (described below) and insulated from the bench with a yellow high-voltage electrical blanket (Salisbury Standard Rubber Insulating Blanket with Velcro, 36 X 36 Class 0 Type 1, Lab Safety Supply, Chicago, IL). The top electrode was connected to the 130 kV tap of the high-voltage transformer with a 1.09 m (3.58 ft) high-voltage spark plug wire (MSD 8.5 mm Super Conductor Spark Plug Wire, MSD, El Paso, TX). For additional insulation, the high-voltage wire was fed through a length of 1.09 m (3.58 ft) Tygon tubing (1.27 cm/0.5 in. diameter). The bottom electrode was connected to the return terminal of transformer with the supplied ground wire and a jumper connected the ground and guard terminals. Exact parallel alignment of the electrodes was crucial to creating a uniform and repeatable plasma-generation field. To accomplish this, placement locations for the electrodes and spacers were traced on the dielectric barriers to accomplish consistent assembly of the system. Figure 3.2 shows the method used to accurately place the electrodes.

The control box was positioned approximately 2.44 m (8 ft) from the transformer which was just outside the fume hood. This particular test set had an adjustable voltage range from 0 to 130 kV with a maximum current of 46 mA. A maximum of 75 kV was used for this experiment because this was sufficient to create complete ionization in the sample bag. All experiments were conducted in a fume hood to reduce the potential for any dangerous ionized gases, namely ozone, from entering the lab atmosphere.

The total system power was measured with a power meter (Kill-a-watt, Intertek, Hong Kong). A safe zone of 3.05 m (10 ft) was maintained around the non-thermal plasma system at all times during operation. An SM-70 ozone monitor (Ozone Solutions,

Hull, IA) was placed in the middle of the lab to ensure that ozone levels did not exceed 0.5 ppm for worker safety.

Dielectric barriers

Numerous preliminary experiments were conducted to determine the ideal materials and specifications for the dielectric barriers. Figure 3.3 shows the configuration of the electrodes, dielectric barriers and polyolefin sample bag. The top electrode was surrounded by a 30.48 x 30.48 x 3.18-cm (12 x 12 x 1.25-in.) ultra-high molecular weight (UHMW) Poly-E plate (U.S. Plastics Corp., Lima, Ohio) plastic block. This plastic block insulated the electrode and concentrated the electrical charge toward the packaged BBF while also weighting the system to eliminate air gaps between dielectric layers. Directly underneath the upper electrode, there was a 35.56 x 27.94 x 0.24-cm (14 x 11 x 0.095-in.) polypropylene cutting mat (Cuisinart, East Windsor, NJ) and then a 40.64 x 40.64 x 0.158-cm (16 x 16 x 1/16-in.) polypropylene (PP) sheet (U.S. Plastics Corp., Lima, Ohio). The PP sheet was in contact with the polyolefin bag containing the sample. Likewise, underneath the plastic sample bag, a 60.96 x 60.96 x 0.318-cm (24 x 24 x 1/8-in.) PP sheet isolated the bottom electrode.

Since it is well known that the applied voltage and electrode separation play key roles in ionization of gas particles, great lengths were taken to ensure that the electrodes remained parallel and at a fixed distance while positioned around the flexible polyolefin sample bag containing the poultry breast fillet. Preliminary experiments determined that a sample-bag height of 3.81 cm (1.5 in.) was adequate to accommodate thick breast fillets while still minimizing the electrode separation. Therefore, polypropylene rods (3.81-cm tall and 3.81-cm diameter), the same thickness as the filled sample bag, were used as

spacers to support the corners of the top barrier to help keep the dielectric barriers parallel. The corners of the bottom dielectric barrier were also supported by spacers (2.54-cm tall and 3.81-cm diameter) to prevent the lower barrier from sagging which could cause an air-gap over the bottom electrode. The overall electrode gap, including the plastic bag and three dielectric barriers was 4.32 cm (1.7 in.) while the polyolefin sample bag gap was 3.81 cm (1.5 in.).

For the electrode configuration described above, a voltage of 75 kV was needed to maintain constant plasma generation. Refer to figure 3.3 for a detailed diagram of the treatment system. The DBD plasma sample holder sat on top of a Teflon base to further insulate the system from the hood. Additional insulation was added by draping the hood with a rubber high-voltage insulating blanket. A small section of PVC was used to elevate the high-voltage wire away from potential grounds.

Depending on system use, the dielectric barriers had to be changed every few weeks once the plastic begins to warp. When the area between the electrodes became 'sticky' and produced a residue, the plastic was cleaned with 70% EtOH. After several cleanings, the PP sheet was retired. Based on the shape and the texture of the barrier, sound judgment was used to determine time of replacement for the PP sheets.

Sample packaging

Although the ultimate goal of this method is to treat BBF in commercial tray packs, these measurements were carried out with polyolefin bags to better control the ionization response within the contained atmosphere of the package and assess the potential of the system while minimizing variables associated with various tray packs. Before packaging, the fillets were placed in a weigh boat (140 x 140 x 22 mm) (Standard

boat LG white, Heathrow Scientific, Vernon Hills, IL), skin side up. A Cryovac polyolefin bag (22.86 x 35.56 x 3.81 cm) (L3340, Cryovac Sealed Air Corp., Duncan, SC) was sealed and used to package the broiler samples. Care was taken to keep the bag from touching the fillet surface to prevent removing microorganisms. The plastic bag was then sealed with an Uline Tabletop Poly Bag Sealer - Impulse (Uline, Pleasant Prairie, WI). A modified atmosphere (MA) gas blend (65% O_2, 30% CO_2, 5% N_2) (Praxair, Danbury, CT) was used to fill the bags with a needle through a rubber septa. To ensure pure MA gas in the bag, the system was flushed with a tube and needle attached to the bench-top vacuum. The inlet MA gas and the vacuum were run simultaneously for ~20 s to flush the bag. During the filling process the bag was placed in a structure fixed at 3.81 cm (1.50 in.) to ensure that all bags were filled to the same height. Figure 3.4 shows the bag-filling station and fill method used. Ensuring equal height of all samples and maintaining a parallel plasma system was crucial to obtain consistent treatments.

Treatment of samples

After the BBF was packaged according to the previously described specifications, the bag was placed in the nonthermal plasma system. For direct treatments, the package and sample were placed directly over the electrode. For indirect treatments, the BBF in the weigh boat was placed to the side of the bag so that only the gas space in the bag was between the electrodes. Figure 3.5 shows the locations of direct and indirect sample placement. Once the bag was placed, the top dielectric barriers were positioned over the bag, and finally the electrode and electrode insulator were placed on top of the barriers.

When stacking elements of the plasma system, parallel placement of all layers was necessary. If there were any air gaps between elements, this would cause a loss of

ionization in the plastic bag; the electrical charge would ionize gas in the gap spaces and not inside the bag containing the sample. Preliminary experiments showed lower ozone levels when close contact was not maintained between all levels of the system.

To treat the BBF, the dial on the control box was quickly advanced from 0 to 75 kV. Power and current were monitored during the process and the beginning and end values (W and mA) were recorded in ensure consistent treatments. To stop the system, the dial was turned down rapidly and the kill switch was used before removal of the sample. Samples were treated for a specified time at 75 kV because this voltage was sufficient to achieve complete ionization in the package. Temperature and humidity of the lab were recorded during every replicate of the experiment because these factors, especially humidity, can effect gas ionization (Keener et al., 2012), (Hähnel et al., 2010).

Sampling of ozone gas

Ozone measurements were taken from inside the sample bag after every treatment with Draeger Short Term Detector Tubes (Draeger Safety AG & Co., Lubeck, Germany). These tubes were selected for ease of use and upon the recommendation of researchers conducting similar projects (Keener et al., 2012). In order to determine ozone values when measuring very high concentrations, smaller gas sample volumes of 3 mL were collected with a 3 ml syringe. The syringe was connected to the detection tube via a small length (2 cm, .787 in) of flexible tubing; the detection tube was inserted into the Drager Accuro Detector Pump (Draeger Safety AG & Co., Lubeck, Germany). The syringe volume was expelled into the detection tube and then removed allowing total flow volume of 100 ml to occur (volume required based on manufacturer's instructions). The

observed gas concentration was then multiplied by the volume ratio of the detection tube volume over the syringe volume (Keener et al., 2012).

Shelf life experiment

Broiler breast fillet meat for this experiment was collected from a local processing facility in Athens, GA. The samples were collected in a large bag and immediately transported on ice back to the laboratory. Triplicate samples were packaged with the methods listed above. All samples were treated for 3 min. at 75 kV with a 4.32-cm (1.7-in.) electrode gap outside the plasma generation field (indirect treatment). Controls were packaged in the same way and remained on the counter for 3 min. Ozone measurements were collected for all treated samples since ozone is a reactive oxygen species that has a long enough half life to be measured after treatment. This step allowed for verification of consistent system performance. After treatments, samples were stored at 4°C until the specified sampling days.

The BBF were sampled on days 1, 3, 7, 10, and 14. For microbiological recovery, the fillets were removed from the bags and the top surface of the fillet was cut off to isolate the treated portion of the meat. The microbes were recovered via rinsing with phosphate buffered saline (PBS) (Gibco by Life Technologies, Grand Island, NY) and plating on nutrient agar (Becton Dickinson, Franklin Lakes, NJ) (Beuchat et al., 1998). The plates were incubated for 24 h at 37°C. This experiment was replicated three times.

Results and Discussion

Development and modifications to the system required great attention to detail in order to produce consistent results. Since numerous variables affect consistent and

reproducible ionization within the polyolefin bag, the dielectric barrier and electrode assembly were designed such that the electrodes were parallel, the separation was fixed, and the plastic bag maintained uniform contact with the dielectric barriers throughout the ionization field. Air gaps between layers of the dielectric barriers had to be eliminated to minimize energy loss to the surrounding atmosphere. Energy loss was also addressed by insulating the high-voltage wire that led to the top electrode and insulating the top electrode with the UHMW Poly-E plate block of plastic. Polypropylene was found to be the best plastic for the dielectric barriers, and by minimizing the thickness of the PP sheets, the aluminum electrodes could be positioned closer together.

Initially, atmospheric air was used as the fill gas for the packages because of availability and ease of use. After producing relatively low concentrations of ozone (~ 1600 ppm), compared to other plasma experiments (~ 6000 ppm), the fill gas was changed to a mixed modified atmosphere. Based on success with a 65% O_2, 30% CO_2, 5% N_2 blend (Keener et al., 2012), this gas was used for future experiments. The higher concentration of oxygen in the MA allows for the generation of more reactive oxygen species (ROS) which interact with and presumably play a key role in killing the microorganisms on the surface of the broiler meat. A set of controls packaged with air was used to separate the effect of MA and plasma treatment with MA compared to the typical spoilage rate of packaged broiler fillets.

Treatment times were explored by starting at 5 min (75 kV), the longest time in similar studies (Keener et al., 2012). From this point, time was reduced to see if the same ozone concentrations could be achieved during a 3 min (75 kV) treatment. The plasma system was able to produce equally high ozone levels (~6000 ppm) at 3 min with the MA

gas. Experiments were conducted to ensure that all ozone within the polyolefin bag had dissociated after 24 h. Samples were treated for 3 min at 75 kV then placed in storage at 4 °C. The ozone measurement process was used after 24 h. No ozone was detected with the low concentration (0.05 – 1.4 ppm) Draeger tubes.

Direct and indirect placement of the samples was tested to determine if a certain placement was more beneficial than the other. The difference between the two methods was not significant, and future experiments used indirect positioning. This set-up was chosen because the thickness and shape of the poultry sample may be a variable if the BBF is placed between the two electrodes; using indirect placement allows for only gas to be in the ionization field. Also, arcing on and around the BBF sample may cause harmful reactions on the surface of the sample. Because indirect placement allows for more consistent control of the system, this method was used for later experiments.

Broiler breast fillet samples treated by the nonthermal DBD plasma system had a mean log reduction of 1.53 $\log_{10}$ cfu/ml after 24 h of storage. After 14 days of storage following treatment with the nonthermal plasma system, the treated samples had a mean population of 5.56 $\log_{10}$ cfu/ml which was 1.52 $\log_{10}$ cfu/ml lower than the control sealed with MA. Results of the studies are shown in table 3.1. The microbial reduction differences between the treatment and control groups were statistically significant, and a microbial population of 5.56 $\log_{10}$ cfu/ml falls below the generally accepted spoilage limit of 10^7 cfu/ml (Ikeme et al., 1982; Owens, 2010) therefore; this processing method has the ability to initially reduce the microorganisms on the surface of the BBF in a sealed package and retain the microbial quality of broiler fillets for 14 days of storage.

Figure 3.6 shows the changes in the microbial population of the fillets over the 14 day storage period.

To verify the rate of spoilage of fresh broiler fillets, a preliminary experiment was conducted with the specific BBF and packaging used in this research. Results from this experiment supported the findings cited in literature stating that accepted spoilage levels fall between 10^6 and 10^8 cfu/ml (Hinton Jr et al., 2004; Owens, 2010; Russell, 2009; Wimpfheimer et al., 1990).

Summary

A nonthermal DBD plasma system was developed and modified to treat BBF packaged in polyolefin plastic bags with a modified atmosphere. System specifications and equipment selections were enhanced based on previous work with spinach leaves and *Bacillus subtilis* spores (Klockow and Keener, 2009) (Keener et al., 2012). The system utilized high voltage alternating current to ionize the atmosphere in a sealed package containing a BBF. Sheets of polypropylene plastic were used as the dielectric barriers and a polyolefin plastic bag was used to package the sample. Distance of electrode separation and voltage settings were strictly monitored to optimize ionization in the sample package. Ozone concentrations were measured to record system performance and consistency. Microbial recovery was used to measure the effectiveness of the system and record the microbial quality after a specified storage time.

The system was tested on fresh BBF to see if the antimicrobial treatment could reduce the natural flora of the broiler meat and improve the shelf life at 4°C. Analysis of triplicate experiments resulted in mean log of 5.56 $\log_{10}$ cfu/ml after 14 days of storage which was 1.52 $\log_{10}$ cfu/ml lower than MA control; the plasma treatment produced a

significant log reduction compared to the control groups. A level of 5.56 log_{10} cfu/ml falls below the generally accepted spoilage limit of 10^7 cfu/ml (Owens, 2010) (Ikeme et al., 1982). Therefore this system is capable of reducing the initial, native microflora of BBF and in turn, extending the shelf life of the product. These results are significant because the treatment occurs after packaging which eliminates chance of cross contamination.

As apparent in Figure 3.6, solely packaging the poultry fillets in MA might help to lengthen the shelf life, and DBD plasma treatment with MA further improved the shelf life. The BBF spoiled most quickly when packed with atmospheric air. Although no analytical tests were conducted, it was readily apparent that the samples packaged in air were spoiled after 7 days of storage because of the putrid smell that was emitted when the package was opened. As the sampling days increased, translucent colonies became visible and the odor given off by the samples became stronger as the experiment reached 14 days.

Overall, the ARS - nonthermal plasma treatment system was able to produce consistent results under the specified packaging and treatment conditions established in the system design. The system developed was successful in reducing background microorganisms on the surface of BBF and in turn, extending the shelf life of the product. Operation of the system of the system is relatively simple, and the set-up allows for a post-packaging antimicrobial treatment that prevents the BBF from being cross contaminated after plasma exposure. This research provides a foundation for expansion and improvement in the realm of nonthermal plasma treatment of foods susceptible to rapid spoilage and food borne pathogens. There is great opportunity for further research

related to this system and other food products that have the potential to respond to plasma treatment. A more rigid and structured food package would help to improve the uniformity and stability of the system. Different MA blends could be explored for ionization potential as well as quality retention in the meat. Also, experiments investigating the meat quality after treatment need to quantify color, pH, and texture changes that may occur during plasma treatment or storage.

Acknowledgements

The authors would like to thank Latoya Wiggins and Candace Betts USDA, ARS for assistance in this work. We would also like to thank Jerry Heitschmidt USDA, ARS for his assistance in imaging and recording experimental procedures.

REFERENCES

Beuchat, L., F. Copeland, M. Curiale, T. Danisavich, V. Gangar, B. King, T. Lawlis, R. Likin, J. Okwusoa, and C. Smith. 1998. Comparison of the SimPlate total plate count method with Petrifilm, Redigel, and conventional pour-plate methods for enumerating aerobic microorganisms in foods. *Journal of Food Protection®* 61(1):14-18.

Bolder, N. M. 1997. Decontamination of meat and poultry carcasses. *Trends in Food Science & Technology* 8(7):221-227.

Department of Health and Human Services, F. 2001. Secondary Direct Food Additives Permitted in Food for Human Consumption. 33829-33830. F. a. D. Administration, ed: Food and Drug Administration.

Dincer, A. H., and T. Baysal. 2004. Decontamination techniques of pathogen bacteria in meat and poultry. *Critical Reviews in Microbiology* 30(3):197-204.

Dirks, B. P., D. Dobrynin, G. Fridman, Y. Mukhin, A. Fridman, and J. J. Quinlan. 2012. Treatment of Raw Poultry with Nonthermal Dielectric Barrier Discharge Plasma To Reduce Campylobacter jejuni and Salmonella enterica. *Journal of food protection* 75(1):22-28.

Dufrenne, J., W. Ritmeester, E. D. Asch, F. Van Leusden, and R. De Jonge. 2001. Quantification of the contamination of chicken and chicken products in the Netherlands with Salmonella and Campylobacter. *Journal of Food Protection®* 64(4):538-541.

Food Safety and Inspection Service. 2011. Poultry preparation: Chicken from farm to table: Irradiation of poultry. Beltsville, MD: USDA Food Safety and Insepction Service.

Available at:

http://www.fsis.usda.gov/fact_sheets/chicken_from_farm_to_table/index.asp.

Hähnel, M., T. von Woedtke, and K.-D. Weltmann. 2010. Influence of the Air Humidity on the Reduction of Bacillus Spores in a Defined Environment at Atmospheric Pressure Using a Dielectric Barrier Surface Discharge. *Plasma Processes and Polymers* 7(3-4):244-249.

Hecer, C., F. Balci, and C. D. Udum. 2007. The effects of ozone and chlorine applications on microbiological quality of chickens during processing. *J. Biol. Environ. Sci* 1(3):131-138.

Hinton Jr, A., J. A. Cason, and K. D. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. *International Journal of Food Microbiology* 91(2):155-165.

Ikeme, A., B. Swaminathan, M. Cousin, and W. Stadelman. 1982. Extending the shelf-life of chicken broiler meat. *Poultry Science* 61(11):2200-2207.

Jindal, V., A. L. Waldroup, R. H. Forsythe, and M. J. Miller. 1995. Ozone and Improvement of Quality and Shelf Life of Poultry Products. *The Journal of Applied Poultry Research* 4(3):239-248.

Keener, K. M., J. Jensen, V. Valdramidis, E. Byrne, J. Connolly, J. Mosnier, and P. Cullen. 2012. Decontamination of Bacillus subtilis spores in a sealed package using a non-thermal plasma system. *Plasma for Bio-Decontamination, Medicine and Food Security*:445-455.

Klockow, P. A., and K. M. Keener. 2009. Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *Lwt-Food Science and Technology* 42(6):1047-1053.

Kruk, Z. A., H. Yun, D. L. Rutley, E. J. Lee, Y. J. Kim, and C. Jo. 2011. The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fillet. *Food Control* 22(1):6-12.

Niemira, B. A. 2012. Cold Plasma Reduction of Salmonella and Escherichia coli O157:H7 on Almonds Using Ambient Pressure Gases. *Journal of Food Science* 77(3):M171-M175.

Owens, C. M., Alvarado, C., Sams, A.R. 2010. *Poultry Meat Processing, Second Edition*. CRC Press.

Parveen, S., M. Taabodi, J. G. Schwarz, T. P. Oscar, J. Harter-Dennis, and D. G. White. 2007. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry. *Journal of Food Protection®* 70(11):2466-2472.

Ragni, L., A. Berardinelli, L. Vannini, C. Montanari, F. Sirri, M. E. Guerzoni, and A. Guarnieri. 2010. Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *Journal of Food Engineering* 100(1):125-132.

Russell, S. M. 2009. Understanding poultry products spoilage. WattAgNet.com. Available at: http://www.wattagnet.com/Understanding_poultry_products_spoilage.html.

Vugia, D., A. Cronquist, M. Cartter, M. Tobin-D'Angelo, D. Blythe, K. Smith, S. Lathrop, D. Morse, P. Cieslak, and J. Dunn. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 states, 2008. *Morbidity and Mortality Weekly Report* 58(13):333-337.

Wimpfheimer, L., N. S. Altman, and J. H. Hotchkiss. 1990. Growth of< i> Listeria monocytogenes</i> Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. *International Journal of Food Microbiology* 11(3):205-214.

Figure 3.1: Entire plasma generation system with control box, high-voltage transformer, electrodes, and dielectric barriers.

Figure 3.2: Outlines on dielectric barriers used to accomplish parallel placement of electrodes.

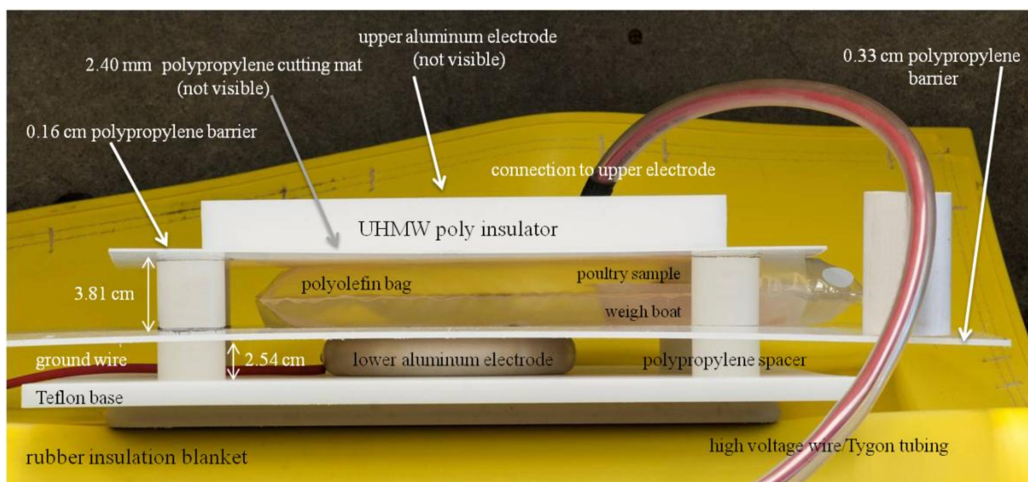

Figure 3.3: Diagram of plasma treatment station.

Figure 3.4: Filling station with inlet MA gas and outlet vacuum for flushing sample.

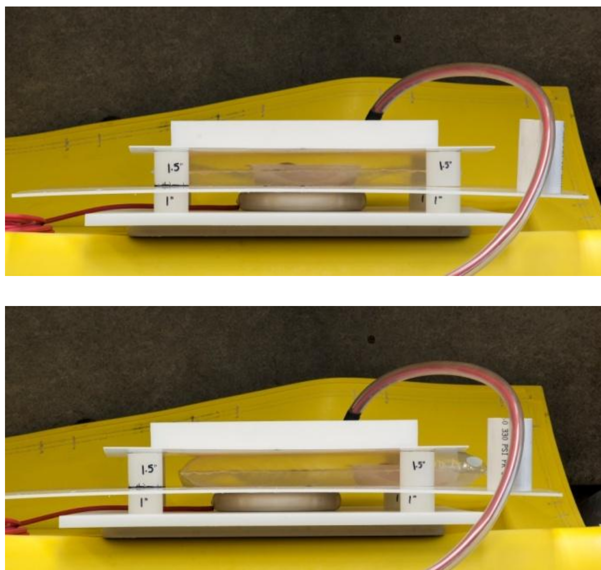

Figure 3.5: (Top) Direct sample placement. (Bottom) Indirect sample placement.

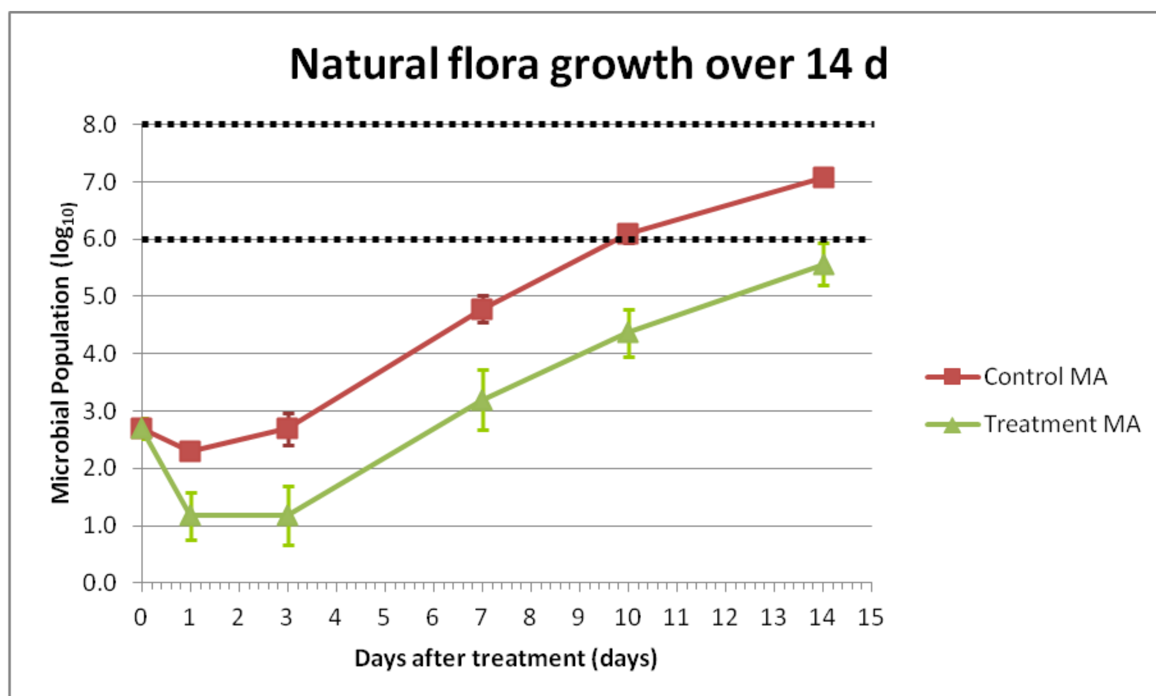

Figure 3.6. Microbial growth of natural background microflora over 14 day storage period at 4°C packaged in modified atmosphere (MA) after treatment with ARS-plasma generation system. Values and standard deviation based on triplicate experiments. Area inside dashed lines indicates minimum growth range to declare product spoiled.

Table 3.1: Combined data for triplicate samples, plasma treated BBF with natural background microflora.

	Rep 1		Rep 2		Rep 3	
	Control	Treatment	Control	Treatment	Control	Treatment
Day 0	2.86±0.23[a]	2.86±0.23	2.39±0.05	2.39±0.05	2.87±0.26	2.87±0.26
Day 1	2.54±0.05	1.43±0.23	2.11±0.16	1.26±0.24	2.23±0.05	0.83±0.75
Day 3	2.60±0.40	1.36±0.39	2.40±0.25	1.40±0.46	3.07±0.18	0.77±0.68
Day 7	4.50±0.18	3.71±0.16	4.51±0.29	2.42±0.46	5.35±0.23	3.47±0.96
Day 10	5.79±0.10	5.88±0.58	5.92±0.05	4.38±0.37	6.56±0.31	4.85±0.28
Day 14	6.93±0.18	5.80±0.47	6.73±0.20	5.25±0.18	7.57±0.04	5.65±0.46

[a] Mean and standard deviation

CHAPTER 4

NONTHERMAL PLASMA TREATMENT OF PACKAGED BROILER BREAST FILLETS TO REDUCE NATURAL MICROFLORA AND *CAMPYLOBACTER JEJUNI*

---

[1]Taylor G. Kronn, Kelli L. Hiett, Michael J. Rothrock, Kurt C. Lawrence, Hong Zhuang, Yao-wen Huang, Kevin M. Keener. To be submitted to *Journal of Food Protection*.

ABSTRACT

Poultry meat is susceptible to rapid spoilage and food-borne pathogens which can lead to losses in profits for producers; therefore research was conducted to examine the effectiveness of nonthermal plasma on the microbial safety and shelf life of broiler breast fillets (BBF). A novel plasma generation system was used as an antimicrobial treatment targeting spoilage organisms and pathogens on raw BBF. The system was tested on uninoculated BBF that were treated for 3 min at 75 kV outside of the plasma generation field and then stored at 4°C for 14 days (experiment 1). Fillets were inoculated with a growth of enriched broiler microflora (10^6 cfu/ml) collected from a composite of fresh fillets and treated for 5 min at 75 kV (experiment 2). Also, fillets were inoculated with *Campylobacter jejuni* (10^6 cfu/ml) and treated for 3 min at 75 kV. All samples were placed in weigh boats and packaged in polyolefin bags for treatment with modified atmosphere 65% O_2, 30% CO_2, and 5% N_2 (experiment 1, 2, 3) or atmospheric air (experiment 3). Samples were treated at ambient air temperature and pressure. The BBF were stored at 4°C and surviving microbes were recovered via rinsing and plating after 24 h for experiments 2 and 3 and on specified sampling days for experiment 1.

Treatment of uninoculated fillets (experiment 1) resulted in a mean of 5.53 $\log_{10}$ cfu/ml at 14 d (2.00 $\log_{10}$ lower than control). This level falls below the generally accepted spoilage limit of 10^7 cfu/ml. For experiment 2 there was a mean log reduction of 2.02 $\log_{10}$ cfu/ml and 1.84 $\log_{10}$ cfu/ml for direct and indirect treatments, respectively. Fillets inoculated with *C. jejuni* (experiment 3) experienced a mean log reduction of 1.30 $\log_{10}$ cfu/ml (air) and 1.57 $\log_{10}$ cfu/ml (MA) compared to untreated controls. Results demonstrate the ability of nonthermal DBD system to reduce microorganisms on the surface of BBF and its applications in food safety and shelf life extension.

Food safety and food quality are of key importance in the food industry. Every year approximately 9 billion chickens are processed in the United States, and of these, 80% are marketed as fresh product. Of these 7.2 billion fresh chickens, 2% to 4% are estimated to be lost as a result of spoilage *(21).* Spoilage bacteria on chicken can come from a number of different sources such as feathers and feet of live birds, the water supply in the processing plant, the chill tanks, and the processing equipment; these sources are linked to spoilage bacteria on the carcass immediately after processing *(10, 21).* The spoilage of fresh poultry products is associated with the growth of certain psychrotrophic bacteria when the product is stored aerobically under refrigerated conditions. Most commonly theses bacteria are predominantly pigmented and non-pigmented strains of *Pseudomonas* spp. and produce the characteristic putrid or 'off' odors when the organisms reach population levels of 10^8 cfu/ml *(19)*; some literature states that poultry is considered microbiologically spoiled at levels of 10^6 or 10^7 cfu/ml *(10, 18).* Shelf life under chill conditions depends on the temperature at which the poultry is held *(11).*

While *Salmonella* Enteritidis, *Staphylococcus aureus*, and *Listeria monocytogenes* are some of the more common foodborne microorganisms associated with poultry meat *(3, 8). Campylobacter jejuni* represents one of the most common poultry meat-associated microorganisms with a 71% - 91% poultry meat contamination rate, *(6). C. jejuni* is the leading bacterial causative agent of gastroenteritis worldwide; this microorganism is mostly spread to the human population through consumption of undercooked chicken or through cross contamination from improper handling of poultry by the consumer *(23).* Antimicrobial treatments that reduce or eliminate *Campylobacter* before consumers have a chance to cross contaminate surfaces would aid in lowering the instance of campylobacteriosis. Post-packaging applications would be advantageous in reducing cases of cross contamination from raw poultry products.

Work with plasma has grown in popularity in the past decade as producers and consumers desire ways to process and sanitize foods with economical and convenient techniques

and without using chemical additives. Plasma is generated when enough energy is supplied to a neutral gas to cause charge production. The phenomenon is achieved by ionization or photoionization when the electrons or photons with sufficient energy collide with the neutral gas atoms or other molecules *(8)*. A major component of plasma, ozone, has been used in the treatment and disinfection of water, a popular practice for over 150 years *(12)*. Ozone is a strong antimicrobial due to its high reactivity, penetrability, and spontaneous decomposition to a non-toxic product (O_2). In 2001, ozone received the status of generally recognized as safe (GRAS) for food usage in the United States *(3)*. In 2002, the USDA issued FSIS directive 7120.1 which named ozone as a Safe and Suitable Ingredient Used in the Production of Meat and Poultry Products *(7)*.

Specifically dielectric barrier discharge (DBD) plasma, which is used in this work, has advantages over other nonthermal processing techniques such as irradiation and high pressure processing which are expensive and require highly specialized equipment *(5, 16)*. Plasma work has been applied more widely in the field of non-animal tissue foods with success. The basis of the following plasma research centers around the system design (PK-1 and PK-2) developed by Klockow and Keener for the treatment of spinach leaves and *Bacillus subtilis* spores strips *(13, 15)*. This design was modified and optimized for the treatment of broiler breast fillets. The goal of this work is to develop and optimize a nonthermal plasma generation system for use on broiler meat. After creating a consistent system, experiments with spoilage organisms and a common foodborne pathogen (*C. jejuni*) were conducted to test its ability to reduce microorganisms on the surface of BBF to make a safer product of higher quality for producers and consumers. This research will expand upon existing work in the field and clearly define the procedures necessary to reduce microorganisms on the surface of broiler meat after it has been packaged.

MATERIAL AND METHODS

Dielectric barrier discharge plasma and treatment station. Broiler breast fillet samples were treated in a nonthermal plasma, dielectric barrier discharge system (ARS-plasma

generation system) in order to disinfect the sample surface. Preliminary experiments were conducted to determine the most effective set-up of the plasma system for BBF treatment (data not shown). The plasma generation system (Fig. 4.1) consisted of an AC Dielectric Test Set (BK 130, Phenix Technologies, Accident, MD), high-voltage wires, aluminum electrodes, and dielectric barriers. All nonthermal plasma treatments were done inside of a fume hood to prevent ionized gases from entering the lab atmosphere. Pure polypropylene (PP) was used for the dielectric barriers. Two 15.24-cm (6 in.) spun aluminum electrodes (Phenix Technologies) were used on the top and bottom of the nonthermal plasma treatment system. The top electrode was surrounded by a 30.48 x 30.48 x 3.180 cm (12.0 x 12.0 x 1.25 in.) ultra high molecular weight (UHMW) Poly-E plate (U.S. Plastics Corp., Lima, Ohio) block of plastic used to insulate the electrode and concentrate the electrical charge toward the packaged poultry. Polypropylene spacers (3.81 cm tall and 3.81 cm in diameter) were used to support the top barrier and keep the dielectric barriers level. Spacers were also used under the bottom electrode to prevent the bottom barrier from sagging over the electrode.

The high voltage-transformer was positioned just outside of the fume hood. A 1.09 m (3.58 ft) high-voltage spark plug wire (MSD 8.50 mm Super Conductor Spark Plug Wire, MSD, El Paso, TX) connected the top electrode to the transformer. The control box (Phenix Technologies) for the nonthermal plasma system was positioned approximately 2.44 m (8.00 ft) from the transformer. The power cord for the system was plugged into a power meter (Kill-a-watt, Intertek, Hong Kong) in order to monitor power throughout the experiment. A SM-70 ozone monitor (Ozone Solutions, Hull, IA) was placed in the middle of the lab to ensure that ozone levels did not exceed 0.50 ppm for worker safety.

Experiments to test system efficacy. Three experiments were conducted to test the microbial log reduction capabilities of the ARS-plasma generation system on BBF. Table 4.1 includes all treatment, storage, and recovery information related to each individual experiment. First (experiment 1) a shelf life study was conducted to determine if the log reduction capabilities

of the plasma system could extend the shelf life of refrigerated (4°C) uninoculated BBF. The fillet samples used in this experiment were not inoculated. Microbiological recovery occurred on specified sampling days during the 14 day period.

Next, a 24 h experiment (experiment 2) was conducted to evaluate the ability of DBD plasma to reduce the microbial population on BBF that were inoculated with an enriched (10^6 cfu/ml) growth of natural flora isolated from fresh BBF. The samples were treated, stored over night at 4°C, and recovered after 24 h to determine the log reduction achieved by the plasma system. Twenty-four hour storage was chosen because all of the ozone and radical particles in the package have dissociated within 24 h and returned to ambient gas. Experiments were conducted to ensure that all ozone within the polyolefin bag had dissociated after 24 h. Samples were treated for 3 min at 75 kV then placed in storage at 4°C. The ozone measurement process was used after 24 h. No ozone was detected with the low concentration (0.05 – 1.4 ppm) Draeger tubes.

The same inoculation and treatment procedures were used to determine the system's effect on BBF inoculated with *C. jejuni* (10^6 cfu/ml) (experiment 3). Samples were inoculated in the same way as the previous experiment and microbiological recovery was conducted after a 24 h storage period at 4°C.

Preparation of microbial inocula. For the shelf life experiment (experiment 1), only the natural microflora of the BBF were analyzed, and no inoculum was required.

For the enriched microflora experiment (experiment 2), natural broiler breast flora was used to inoculate fillet samples. Broiler breast fillets fresh from the processing plant were rinsed in 25 ml of the 1X PBS solution by shaking for 1 min. The broiler rinsate was then diluted 1:10 four times in 1X PBS, 100µl of each dilution was spread plated onto Nutrient Agar (Becton Dickinson, Franklin Lakes, NJ), and the plates were incubated at 37°C for 24 h. Growth from one of the dilutions producing well isolated colonies (~10) was added to 5ml 1X PBS and mixed to reach a level equivalent to a 2.0 McFarland standard. Four ml of this mixture was added to 200

ml Nutrient Broth (Becton Dickinson) in 1 L flasks stopped with cotton gauze and incubated in a shaking incubator at 37°C for 18-24 h. The growth of broiler flora was then diluted in 1X PBS to a final inoculum concentration of 10^6 cfu/ml (determined by culture).

In the final experiment (experiment 3), the bacterial foodborne pathogen *C. jejuni* was used as the inoculum. To prepare this inoculum, a frozen stock of *C. jejuni* previously isolated from a commercial processing plant broiler carcass rinse was used. The isolate was grown using Campylobacter Cefex agar *(22)*. In short, the isolate was streaked onto Brucella agar (Accumedia, Lansing, MI) supplemented with 5% blood and incubated at 42°C for 18 - 24 h in a microaerobic/blood gas atmosphere (85% N_2, 10% CO_2, 5% O_2). Approximately 10 colonies from the plate were removed and mixed with 5 ml of 1X PBS solution. To grow the *C. jejuni* inoculum to the desired cell density (10^6 cfu/ml), tissue culture plates (Sigma-Aldrich Corp., St. Louis, MO) were prepared with a biphasic growth media containing a combination of 30 ml of Mueller-Hinton broth (Oxoid Limited, Basingstoke, Hampshire, United Kingdom) (top layer) and 45 ml of Mueller-Hinton agar (Oxoid Limited) (bottom layer). One ml of the recovered *C. jejuni*/1X PBS solution was added to the broth (top) layer of the biphasic growth media, and the flasks were incubated under the cultural conditions described above for *C. jejuni*. After incubation, 5 ml of the *C. jejuni* growth was added to 495 ml of 1X PBS to create the 10^6 cfu/ml inoculum. Concentration of this *C. jejuni* inoculum were verified spectrophotometrically (OD_{600}) and culturally via serial dilutions of the cell culture broth using the *C. jejuni* cultural conditions described above.

Broiler breast fillet preparation and inoculation. Fresh, boneless, skinless BBF (95-112 g) were collected from a local commercial processing facility. The breast fillets were pre-cut and sorted by weight in the processing facility. The broiler breast samples were kept on ice during the transportation and preparation steps. Minor trimming was done back in the lab to remove any excess fat. For all three experiments, all BBF were collected at the same time and

transported back to the laboratory in one bag. It was therefore assumed that all breast fillets for an experiment contained the same initial microflora.

No additional preparation was needed for experiments 1; the natural microflora present on the BBF was left, and no inoculum was added. For experiments 2 and 3, the inoculation process began by pipetting 75 ml of inoculum into a plastic food tray (CS979, Cryovac, Duncan, SC). Each individual trimmed BBF was placed skin side down in each tray so that the just the surface was immersed in the liquid; the inoculum did not come over the sides of the fillet. The broiler fillets were left to soak for 30 min to allow the microorganisms to attach to the surface. After soaking, the breast was removed and excess liquid was allowed to drip off of the surface.

Sample packaging. Although the ultimate goal of this method is to treat BBF in commercial tray packs, these measurements were carried out with polyolefin bags to better control the ionization response within the contained atmosphere of the package and assess the potential of the system while minimizing variables associated with various tray packs. Before packaging, the fillets were placed in a weigh boat (140 x 140 x 22.0 mm) (Standard boat LG white, Heathrow Scientific, Vernon Hills, IL), skin side up. A Cryovac polyolefin bag (22.86 x 35.56 x 3.810 cm) (L3340, Cryovac Sealed Air Corp., Duncan, SC) was sealed and used to package the broiler samples. Care was taken to keep the bag from touching the fillet surface to prevent removing microorganisms. The plastic bag was then sealed with an Uline Tabletop Poly Bag Sealer - Impulse (Uline, Pleasant Prairie, WI). A modified atmosphere (MA) gas blend (65% O_2, 30% CO_2, 5% N_2) (Praxair, Danbury, CT) was used to fill the bags with a needle through a rubber septa. To ensure pure MA gas in the bag, the system was flushed with a tube and needle attached to the bench-top vacuum. The inlet MA gas and the vacuum were run simultaneously for ~20 sec. to flush the bag. The same process was used to fill bags with atmospheric air. During the filling process the bag was placed in a structure fixed at 3.81 cm (1.50 in.) to ensure that all bags were filled to the same height and fit the exact height of the treatment station.

Treatment of broiler breast fillets. After broiler fillets were packaged according to the previously described specifications, the bag was placed in the ARS-plasma generation plasma system. The enriched natural flora experiment compared two sample placement scenarios (experiment 2). For direct treatments, the package and fillet were placed directly over the electrode. For indirect treatments, the broiler meat in the weigh boat was placed to the side of the bag so that only the gas space in the bag was between the electrodes. The *C. jejuni* and the background microflora-shelf life experiments (experiments 3 and 1) used indirect sample placement only because it was determined experimentally that there was no difference between direct and indirect placement. Once the bag was placed, the top dielectric barriers were placed over the bag, and finally the electrode and electrode insulator were placed on top of the barriers.

A voltage of 75 kV was used to treat all broiler fillet samples for the three experiments. Power and current were monitored during the process, and the beginning and end values (W and mA) were recorded in ensure consistent treatments. Samples were treated for the time specified in Table 1 at 75 kV because this voltage was sufficient to achieve complete ionization of the atmosphere in the package. Temperature and humidity of the lab were recorded during every replicate of the experiment because these factors, especially humidity, can effect gas ionization *(9, 13)*.

For all experiments, three samples were used for all treatment and control groups. All three of the experiments discussed were run in triplicate as well, resulting in nine data points for each measurement. The control samples were packaged in the same manner as the experimental samples and left on the bench top an equivalent amount of time. Because there was no way to replicate direct and indirect placement of the controls, one set of controls was used to compare to the two treatment groups for the enriched microflora experiment (experiment 2).

Sampling of ozone gas. Ozone measurements were taken after every treatment with Draeger® Short Term Detector Tubes (Draeger Safety AG & Co., Lubeck, Germany). These tubes were selected for ease of use and upon the recommendation of researchers conducting

similar projects *(13)*. In order to determine ozone values when measuring very high concentrations, smaller gas sample volumes of 3 ml were collected with a 3 ml syringe. The syringe was connected to the detection tube via a small length (2 cm) of flexible tubing; the detection tube was inserted into the Drager Accuro Detector Pump (Draeger Safety AG & Co). The syringe volume was expelled into the detection tube and then removed allowing total flow volume of 100 mL to occur. The observed gas concentration was then multiplied by the volume ratio of the detection tube volume to the syringe volume *(13)*.

Sample storage and recovery. The BBF in polyolefin bags were stored at 4°C and removed at the specified sampling time. For microbiological recovery, the bottom of the BBF was removed by cutting to ensure that any surviving microbes on the bottom of the fillet did not interfere with the recovery of the top, treated portion of the sample. Once removed, the broiler breast portion was placed in a plastic bag with 25 ml of 1X PBS and massaged by hand for 1 min. The rinse was then serially diluted to a final dilution of 10^{-3} for experiments 2 and 3 and 10^{-6} for experiment 1 and plated on nutrient agar for experiments 1 and 2 *(2)* and Campy-Cefex agar for experiment 3. The plates were incubated at the temperatures listed in Table 1.

Data Analysis. R statistical software version 2.15.2 (The R Foundation for Statistical Computing) was used for all data analysis. One-way ANOVA tests were used to determine statistical significance and P value of <0.05 was considered statistically significant. A Tukey test was used to determine statistical significance between means ($P<0.05$).

RESULTS AND DISCUSSION

Log reduction and shelf life extension of uninoculated fillets. To verify the rate of spoilage of fresh broiler fillets, an experiment was conducted with the specific BBF and packaging used for all experiments. The fresh BBF reached 10^6 cfu/ml after 7 d of storage (4ºC) which is supported by the findings cited in literature stating that accepted spoilage levels fall between 10^6 - 10^8 cfu/ml *(10, 18, 21, 24)*.

The system was tested on fresh BBF to see if plasma treatment could reduce the natural microflora of the BBF and in turn improve the shelf life at 4°C over a 14 d time period (Fig. 4.2). Broiler samples treated by the nonthermal DBD plasma system had a mean log reduction of 1.53 log_{10} cfu/ml after 24 h of storage compared to the untreated controls. Analysis of triplicate experiments resulted in a total microbial population of 5.56±0.29 log_{10} cfu/ml after 14 days of storage which was 1.52 log_{10} cfu/ml lower than the untreated control packaged in MA. A microbial population of 5.56 log_{10} cfu/ml falls below the generally accepted spoilage limit of 10^6 - 10^8 cfu/ml *(11, 18)*. Dirks and others *(4)* achieved background microflora log reductions of 0.84 and 0.85 log_{10} cfu/ml following 15 and 30 s of exposure to DBD plasma with a handheld device which only covered a 2.50 cm diameter portion of the meat. These lower log reduction numbers could be due to the shorter treatment time or the lack of complete plasma exposure on the broiler surface. The handheld device also could cause inconsistencies in treatment due to fluctuations in electrode-sample separation due to human error. Therefore the ARS-plasma generation system represents an improvement in plasma DFD technology due to its ability to treat the entire sample surface after packaging, allowing for the extension of shelf life by the reduction of BBF native microflora.

Susceptibility of enriched microflora to plasma. In order to control the microflora on the surface of the BBF, a sample of natural broiler microflora was obtained by rinsing fresh BBFs and enriching the collected organisms. This process helped to control the cell density on the samples. The natural microflora was found to be susceptible to plasma treatment of 5 min at 75 kV for both direct and indirect treatment; the log reduction differences between the two sample placement locations were not statistically significant (Table 4.2). The ozone concentration for the treated samples was ~5500 ppm while there was not ozone present in the control samples. The direct treatment yielded a mean log reduction of 2.02±0.31 log_{10} cfu/ml and the indirect treatment yielded a mean log reduction of 1.84±0.14 log_{10} cfu/ml. The slightly higher log reduction in the direct treatment may be due to the increased electrical activity on the surface of the broiler fillet

(Keener, personal communication). Although the microbial reduction is slightly lowers, indirect treatment may be safer and more consistent because the sample is outside of the plasma generation field (Keener, personal conversation), therefore this method of placement was used for all experiments following this determination. Preliminary experiments (data not shown) showed that ionization interactions on the surface of the poultry meat could slightly affect the color of the meat; further quality analysis (pH, texture, sensory) should be conducted to determine the plasma's effect on the meat.

Five minute treatments were originally chosen because this was the longest time in similar experiments *(13)* and it was proven that this amount of time would produce microbial kills. Experiments were conducted to determine if the treatment time could be shortened from 5 min to 3 min in order to improve efficiency. It was determined that there were no changes in ozone concentration (~ 6000 ppm) or microbial reduction when the treatment time was reduced to 3 min. Three minutes was adequate to achieve complete ionization of the atmosphere inside of the package at 75 kV and was therefore used for future experiments (experiments 1 and 3).

In experiment 2, the higher microbial log reductions seen in the inoculated poultry meat compared to the uninoculated meat could be a result of several factors. The higher initial cell density makes more microorganisms susceptible to the reactive plasma species. Also, the meat that was inoculated was moister when treated because it has just been removed from the liquid inoculum. The moisture from the surface of the inoculated fillet could increase the air humidity, and in turn increase the plasma's effectiveness since relative humidity plays an important role in species generated during ionization *(13)*. Air humidity has a strong influence on the reduction rate of bacteria. Higher mean log reductions have been observed when higher concentrations of water vapor are present in the processing gas *(9)* and increased moisture content of the feed gas improves the efficacy of remote nonthermal plasma treatment systems *(20)*.

Susceptibility of *C. jejuni* to plasma. The cell density of *C. jejuni* was controlled in the same manner as listed above by using a diluted liquid inoculum of 10^6 cfu/ml. Both air and MA

gas were used to package the BBF to determine if the higher concentration of O_2 in the MA blend was responsible for reducing the microaerophillic *C. jejuni* population on the surface of the meat. By using both gases, it could be determined whether the oxygen concentration or the nonthermal plasma treatment was responsible for the *C. jejuni* reductions. The *C. jejuni* log reductions produced by the plasma treatment system using either air or MA were statistically higher than the untreated control samples (Table 4.3). After a 3 min treatment at 75 kV and a 24 h storage period, the samples packaged in air were 1.30 $\log_{10}$ cfu/ml lower than the untreated controls; the samples packaged in MA were 1.57 $\log_{10}$ cfu/ml lower than untreated controls. Similar log reductions of pathogens have been achieved in non-animal tissue foods that were treated with nonthermal plasma; Niemira and others *(17)* reduced *Salmonella* Stanley by 2.9 to 3.7 $\log_{10}$ cfu/ml and *E. coli* O157:H7 by 3.4 to 3.6 $\log_{10}$ cfu/ml on golden delicious apples using a 40 l/min flow rate of nonthermal gas plasma. Nonthermal plasma has been used to reduce *Salmonella* Enteritidis on the surface of egg shells by up to 2.5 $\log_{10}$ cfu/eggshell and 4.5 $\log_{10}$ cfu/eggshell *(20)*.

The control fillets (air and MA) experienced a log reduction of ~2.10 $\log_{10}$ cfu/ml during the 24 h storage period due to *C. jejuni's* susceptibility to oxygen rich environments *(1)*. The overall reduction from 10^6 cfu/ml to approximately 10^3 cfu/ml for the treated BBFs is partially due to the inhospitable storage conditions the fillets were held at overnight; the plasma treatment did aid in decreasing the number of microorganisms on the breast fillet surface. There was no statistical difference between the samples (control or treatment) that were packaged with air and those that were packaged with MA indicating that the packaging gas does not influence the antimicrobial potential of the nonthermal plasma system when treating *C. jejuni*. The lack of significant differences between *C. jejuni*-inoculated samples packaged with air and those packaged with MA proves that a special gas blend is not needed to reduce *C. jejuni* populations.

The higher ozone levels generated in the MA package (~5800 ppm) as compared to the air package (~2000 ppm) may account for the slightly higher *C. jejuni* log reduction in MA treatment; ozone levels for controls samples were always 0 ppm. Microbial cultures associated with poultry have been tested by the spread plate method on open petri plates that were exposed to an ozone gas treatment. The said treatment decreased microbial populations by $>10^4$ to 10^7 logs for bacteria and $>10^4$ logs in the case of fungi *(14)*. Another experiment conducted by Yang and Chen treated broiler parts and chicken necks with gaseous ozone for 5 to 9 minutes respectively, and again the microbial counts for broiler and microbial suspensions decreased 1.0 (broiler parts), 0.6 (fresh chicken necks), and 3.0 (spoiled chicken necks) logs *(25)*.

Conclusions. Overall, the ARS - nonthermal plasma treatment system developed was successful in reducing microorganisms (spoilage and pathogenic) on the surface of BBF and in turn, extending the shelf life of the product in the case of spoilage organisms. The system was able to produce consistent results under the specified packaging and treatment conditions of BBF. Operation of the system is relatively simple, and the set-up allows for a post-packaging antimicrobial treatment that prevents the BBF from being cross contaminated after plasma exposure. There is great opportunity for further research related to this system and other food products that have the potential to respond to plasma treatment. A more rigid and structured food package would help to improve the uniformity and stability of the system. Different MA blends could be explored for ionization potential as well as quality retention in the meat. Also, experiments investigating the meat quality after treatment need to quantify color, pH, texture, and sensory changes that may occur during plasma treatment or storage.

This research provides a foundation for expansion and improvement in the realm of nonthermal plasma treatment of foods susceptible to rapid spoilage and foodborne pathogens.

ACKNOWLEDGMENTS

The authors would like to thank Latoya Wiggins and Candace Betts USDA, ARS for assistance in this work. We would also like to thank Jerry Heitschmidt for his assistance in imaging and recording experimental procedures.

REFERENCES

1. Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 1999. Campylobacter jejuni--an emerging foodborne pathogen. *Emerging infectious diseases*. 5:28.

2. Beuchat, L., F. Copeland, M. Curiale, T. Danisavich, V. Gangar, B. King, T. Lawlis, R. Likin, J. Okwusoa, and C. Smith. 1998. Comparison of the SimPlate total plate count method with Petrifilm, Redigel, and conventional pour-plate methods for enumerating aerobic microorganisms in foods. *Journal of Food Protection®*. 61:14-18.

3. Department of Health and Human Services, F. 2001. Secondary Direct Food Additives Permitted in Food for Human Consumption. p. 33829-33830. *In* F.a.D. Administration (ed.), vol. 66. Food and Drug Administration.

4. Dirks, B. P., D. Dobrynin, G. Fridman, Y. Mukhin, A. Fridman, and J. J. Quinlan. 2012. Treatment of Raw Poultry with Nonthermal Dielectric Barrier Discharge Plasma To Reduce Campylobacter jejuni and Salmonella enterica. *Journal of Food Protection*. 75:22-28.

5. Fonberg-Broczek, M., B. Windyga, J. Szczawinski, M. Szczawinska, D. Pietrzak, and G. Prestamo. 2005. High pressure processing for food safety. *ACTA BIOCHIMICA POLONICA-ENGLISH EDITION-*. 52:721.

6. Food Safety and Inspection Service. Date, 2011, Poultry preparation: Chicken from farm to table: Foodborne organisms associated with chicken. Available at:

http://www.fsis.usda.gov/fact_sheets/chicken_from_farm_to_table/index.asp. Accessed January 30, 2012.

7. FSIS, U.-. 2002. Safe and suitable ingredients used in the production of meat, poultry, and egg products. . *In* FSIS (ed.).

8. Gaunt, L. F., C. B. Beggs, and G. E. Georghiou. 2006. Bactericidal action of the reactive species produced by gas-discharge nonthermal plasma at atmospheric pressure: A review. *Ieee Transactions on Plasma Science*. 34:1257-1269.

9. Hähnel, M., T. von Woedtke, and K.-D. Weltmann. 2010. Influence of the Air Humidity on the Reduction of Bacillus Spores in a Defined Environment at Atmospheric Pressure Using a Dielectric Barrier Surface Discharge. *Plasma Processes and Polymers*. 7:244-249.

10. Hinton Jr, A., J. A. Cason, and K. D. Ingram. 2004. Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. *International Journal of Food Microbiology*. 91:155-165.

11. Ikeme, A., B. Swaminathan, M. Cousin, and W. Stadelman. 1982. Extending the shelf-life of chicken broiler meat. *Poultry Science*. 61:2200-2207.

12. Jindal, V., A. L. Waldroup, R. H. Forsythe, and M. J. Miller. 1995. Ozone and Improvement of Quality and Shelf Life of Poultry Products. *The Journal of Applied Poultry Research*. 4:239-248.

13. Keener, K. M., J. Jensen, V. Valdramidis, E. Byrne, J. Connolly, J. Mosnier, and P. Cullen. 2012. Decontamination of Bacillus subtilis spores in a sealed package using a non-thermal plasma system. *Plasma for Bio-Decontamination, Medicine and Food Security*:445-455.

14. Kim, J. G., A. E. Yousef, and S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: A review. *Journal of Food Protection*. 62:1071-1087.

15. Klockow, P. A., and K. M. Keener. 2009. Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *Lwt-Food Science and Technology*. 42:1047-1053.

16. Lewis, S. J., A. Velasquez, S. L. Cuppett, and S. R. McKee. 2002. Effect of electron beam irradiation on poultry meat safety and quality. *Poultry Science*. 81:896-903.

17. Niemira, B. A. 2012. Cold Plasma Reduction of Salmonella and Escherichia coli O157:H7 on Almonds Using Ambient Pressure Gases. *Journal of Food Science*. 77:M171-M175.

18. Owens, C. M., Alvarado, C., Sams, A.R. 2010. Poultry Meat Processing, Second Edition. CRC Press.

19. Pooni, G., and G. Mead. 1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. *Food Microbiology*. 1:67-78.

20. Ragni, L., A. Berardinelli, L. Vannini, C. Montanari, F. Sirri, M. E. Guerzoni, and A. Guarnieri. 2010. Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *Journal of Food Engineering*. 100:125-132.

21. Russell, S. M. Date, 2009, Understanding poultry products spoilage. Available at: http://www.wattagnet.com/Understanding_poultry_products_spoilage.html. Accessed July 10, 2012, 2012.

22. Stern, N., B. Wojton, and K. Kwiatek. 1992. A differential-selective medium and dry ice-generated atmosphere for recovery of Campylobacter jejuni. *Journal of food protection*. 55:514.

23. Uyttendaele, M., P. De Troy, and J. Debevere. 1999. Incidence of Salmonella, Campylobacter jejuni, Campylobacter coli, and Listeria monocytogenes in poultry carcasses and different types of poultry products for sale on the Belgian retail market. *Journal of Food Protection®*. 62:735-740.

24. Wimpfheimer, L., N. S. Altman, and J. H. Hotchkiss. 1990. Growth of< i> Listeria monocytogenes</i> Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. *International journal of food microbiology*. 11:205-214.

25. Yang, P., and T. Chen. 1979. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. *Journal of Food Science*. 44:501-504.

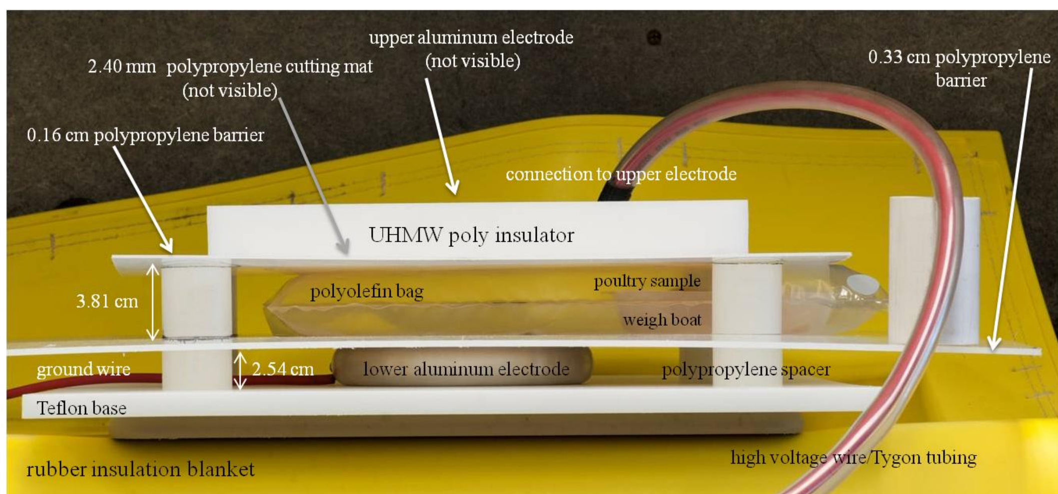

FIGURE 4.1: Diagram of plasma treatment station.

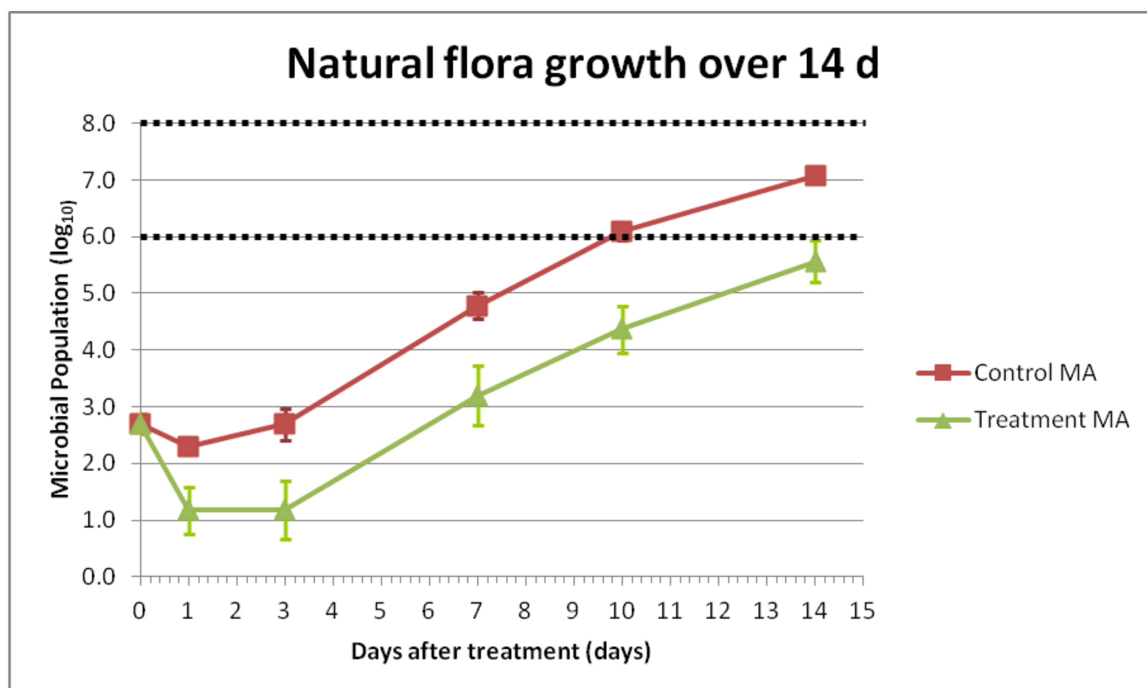

FIGURE 4.2. Microbial growth of natural background microflora over 14 day storage period at 4°C packaged in modified atmosphere (MA) after treatment with ARS-plasma generation system. Values and standard deviation based on triplicate experiments. Area inside dashed lines indicates minimum growth range to declare product spoiled.

TABLE 4.1. Experimental details from three studies to test efficacy of nonthermal plasma generation system.

	Natural background flora (shelf life, experiment 1)	**Enriched microflora (experiment 2)**	***Campylobacter jejuni* (experiment 3)**
Treatment voltage	75 kV	75 kV	75 kV
Treatment time (min)	3 min	5 min	3 min
Storage Temperature (°C)	4°C	4°C	4°C
Packaging gas	MA*	MA*	MA* and air
Sampling day(s)	1, 3,7, 10, and 14	1	1
Agar	Nutrient Agar	Nutrient Agar	Campy-Cefex agar
Incubation Temperature (°C)	37°C	37°C	42°C

*Modified atmosphere (MA) – 65% O_2, 30% CO_2, 5% N_2

TABLE 4.2. Recovery of total cfu/ml of enriched BBF microflora on the surface of BBF.

	Enriched BBF microflora[a]					
Sample placement	Pre-treament (Direct)	Control (Direct)	Post-treatment (Direct)	Pre-treatment (Indirect)	Control (Indirect)	Post-treatment (Indirect)
Rep 1	5.94±0.00	5.24±0.10	3.00±.0.40[b]	7.64±0.00	6.25±0.23	4.57±0.45[b]
Rep 2	7.64±0.00	6.25±0.23	4.58±0.36[b]	6.50±0.00	5.25±0.22	3.40±0.45[b]
Rep 3	6.58±0.00	5.16±0.08	3.02±0.02[b]	6.58±0.00	5.16±0.08	3.19±0.28[b]

[a] Values are total cfu/ml recovered ± standard deviation
[b] Value shows significant reduction ($P<0.05$) from zero time

TABLE 4.3. Recovery of total cfu/ml of *C. jejuni* inoculated on the surface of BBF.

C. jejuni [a]					
Packaging gas		Air		Modified Atmosphere	
	Pre-treatment	Control	Treatment	Control	Treatment
Rep 1	6.73±0.00	4.73±0.09	3.38±.0.19 [b]	4.53±0.32	2.52±0.25 [b]
Rep 2	5.83±0.00	3.75±0.13	2.62±0.09 [b]	3.73±0.11	2.20±0.44 [b]
Rep 3	7.16±0.00	5.05±0.13	3.65±0.17 [b]	5.06±0.14	3.90±0.56 [b]

[a] Values are total cfu/ml recovered ± standard deviation

[b] Value shows significant reduction (P<0.05) from zero time

CHAPTER 5

SUMMARY AND CONCLUSIONS

The objectives of these studies were 1) to develop and modify a nonthermal plasma system, based on previous work done with other food products, that could be used to treat poultry products and 2) to test the plasma system on broiler breast fillets to determine the microbial log reduction capacity of the system and its ability to extend the shelf life of the product.

For the first objective, numerous preliminary experiments were conducted to determine ideal materials and settings for the nonthermal plasma system. The AC Dielectric Test Set - BK 130 (Phenix Technologies, Accident, MD) was used as the high voltage source, and this was attached to two spun aluminum electrodes (Phenix Technologies, Accident, MD) 15.24 cm (6 in.) in diameter. Multiple polypropylene sheets were used as dielectric barriers, and insulation layers were used around the top electrode and high voltage spark plug wire. Atmospheric air and a modified atmosphere (MA) blend (65% O_2, 30% CO_2, 5% N_2) were used as fill gases for the packages. Polyolefin plastic bags were used to package the poultry samples, and a fixed-filling structure was used to fill the bags to the same height. A voltage of 75 kV was used for all experiments in this research, and time varied depending on the treatment scenario. An electrode gap of 4.32 cm (1.7 in.) was consistently used and polypropylene spacers were used to keep the system parallel and stable. Ozone measurements were recorded for all treated samples to monitor the O_3 concentrations and used as an indicator of plasma

strength. Parameters such as voltage, power, current, humidity, and room temperature were recorded during all experiments to ensure consistent treatment. Once all of these parameters were established and the system produced consistent results, experiments were designed to test the antimicrobial efficacy of the plasma generation system.

For the second objective, three different experiments were conducted to determine the microbial log reduction potential of the nonthermal plasma system; inoculated and uninoculated broiler breast fillet samples were used to determine system performance. All samples were packaged the same way for the experiments and filled with either MA or atmospheric air. First, triplicate samples were inoculated with a 10^6 cfu/ml enrichment of natural chicken micro flora. After a 5 min treatment at 75 kV, there was a 2.02 log_{10} cfu/ml mean log reduction for direct treatment and a 1.84 log_{10} cfu/ml mean log reduction for indirect treatment. Second, uninoculated samples were treated for 3 min at 75 kV on the first day of the experiment. Ozone concentrations were recorded and the samples were stored at 4°C until the specified sampling day. Microbiological recovery was conducted on days 1, 3, 7, 10, and 14 via rinsing and plating on Nutrient Agar to record the total plate counts. Initial log reductions of 1.54 log_{10} cfu/ml were seen after 24 h of storage and a mean of 5.56 log_{10} cfu/ml was recorded at day 14. The final log count at day 14 falls below the generally recognized spoilage level of 10^7 cfu/ml. Finally, breast fillets were inoculated with *Campylobacter jejuni* to a level of 10^6 cfu/ml and treated for 3 min at 75 kV. This resulted in a mean log reduction of 1.30 log_{10} cfu/ml for fillets packaged in air and 1.57 log_{10} cfu/ml for fillets packaged in MA.

In conclusion, the nonthermal plasma system was able to function in a stable manner and produce consistent ozone concentrations, indicating the plasma strength was

consistent as well. Through preliminary experiments, small details were observed that helped to better understand intricacies of plasma generation and how small alterations could result in significant changes in the operation of the system. The ease of system operation at atmospheric pressure and lack of chemical additives makes this antimicrobial treatment appealing to both producers and consumers. It was first demonstrated that the plasma system had the ability to reduce the microbial population on the surface of broiler breast fillets; secondly it was demonstrated that these reductions led to an extension of shelf life for the broiler meat product. These results prove that the nonthermal plasma system could have significant impacts in the fields of food safety and food quality.

There is great potential to expand research in this field in relation to poultry products as well as other food products that are susceptible to rapid spoilage and food borne pathogens. More advanced food packaging could be used to improve the stability of the plasma system, and thinner, smaller packaging would allow the electrodes to be placed closer together which would require a lower treatment voltage. The electrode-barrier configuration as well as the barrier materials and thickness could be altered to improve system performance. All experiments in this research removed the top portion of the breast meat, and work must be done to determine the effectiveness of this treatment on the whole surface of a food product. Different MA blends could be explored for ionization potential as well as quality retention in the meat. Also, quality characteristics such as color, pH, texture, and sensory attributes should be investigated to determine the plasma system's effect on the quality of the poultry product.