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# PULSED UV LIGHT FOR THE DESTRUCTION OF PATHOGENS ON CHICKEN

A Thesis in

Animal Science

by

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# ABSTRACT

As the world population continues to increase, so does the occurrence of foodborne illness outbreaks. Globally, one out of ten individuals are infected and become ill after consuming contaminated food. A growing global population has resulted in an increased requirement for high quality, dietary protein. In response, the demand and consumption of poultry has increased since the 1960's, with anticipated growth to continue through 2030. Currently, poultry products are associated with over 16% of all foodborne illness outbreaks in the United States. Despite the constant efforts to reduce pathogens in poultry products, contamination of poultry products by various pathogens still occurs.

Antimicrobial interventions aim to reduce the presence of pathogens on poultry products. Pulsed ultraviolet (PUV) light, a novel technology to the food industry, has emerged as a potential alternative to current microbial reduction interventions. A depth of research has shown the ability of PUV light to reduce the presence of pathogens on food. The USDA Food Safety and Inspection Service has established performance standards for chicken parts with a maximum acceptable presence for *Salmonella* and *Campylobacter*. A non-thermal and non-chemical process, PUV light may be a more effective alternative to current interventions. Therefore, this research was undertaken to evaluate PUV light treatment for the decontamination of the surface of chicken products.

In the first phase of this research, the effectiveness of PUV light as a surface decontamination process on chicken thighs was evaluated using a static PUV light system. Skinless and skin-on chicken thighs were surface inoculated with 6-7 $\log_{10}$

cfu/cm$^2$ of *Escherichia coli*, *Salmonella* and *Campylobacter*, in separate trials. PUV light treatment variables included the distance from the quartz window of the PUV light (8 and 13 cm) and time of exposure (0, 5, 15, 30, and 45 seconds) which provided total energy between 0 and 62.2 J/cm². Microbial reductions were evaluated by comparing treated samples to untreated controls. Increased exposure to PUV light resulted in an increased ($p<0.05$) reduction of *E. coli*, *Campylobacter* and *Salmonella*. Treatment by PUV light for 5 and 45 seconds on lean surface thighs resulted in $\log_{10}$ cfu/cm$^2$ reductions of 1.2 and 2.0 for *E.coli*, 1.5 and 2.2 for *Campylobacter*, and 1.6 and 2.4 for *Salmonella*, respectively. Skin-on chicken thighs treated by PUV light for 5 and 45 seconds resulted in $\log_{10}$ cfu/cm$^2$ reductions of 1.2 and 2.0 for *E.coli*, 1.1 and 1.9 for *Campylobacter*, and 0.9 and 1.8 for *Salmonella*, respectively.

In the second phase of this project, a pilot scale pulsed UV system was used to evaluate the effectiveness of PUV light as an antimicrobial intervention on various chicken parts. The system consisted of two PUV light chambers mounted above an adjustable conveyor belt. Prior to treatment, boneless/skinless (B/S) chicken breasts, B/S thighs and bone-in/skin-on thighs were inoculated with ~8 $\log_{10}$ cfu/cm$^2$ concentration of *E. coli*. Total energy (J/cm$^2$) delivered to the surface of the chicken parts was considered the main treatment variable leading to microbial reductions. The conveyor was set at 10 cm below the quartz window of the two PUV light units and total energy values (5, 10, 20, 30 J/cm$^2$) were achieved by adjusting conveyor speed. Two passes under the PUV lights, one conveyor pass for the top and one for the bottom were utilized for each of the three types of chicken parts tested. Treated samples were evaluated against untreated samples to quantify microbial reduction as a result of PUV light. Increased total energy

of PUV light resulted in increased ($p<0.05$) reductions of *E. coli* on all parts. Exposure to PUV light at 5 and 30 J/cm$^2$ resulted in $\log_{10}$ cfu/cm$^2$ reductions of 0.34 and 0.94 for B/S breasts, 0.29 and 1.04 for B/S thighs and 0.10 and 0.62 for bone-in/skin-on thighs, respectively.

The last phase of this research, using the same pilot scale PUV light system, evaluated quality attributes of chicken parts before and after treatment. B/S chicken breasts, B/S thighs and bone-in/skin-on thighs were treated with 30 J/cm$^2$ of PUV light. Lipid and protein oxidation were measured and recorded at 0, 24, 48 and 120 h after the pulsed light treatment. Concentrations of malonaldehyde (MDA) were used as the indicator for lipid oxidation. PUV light treatment did not increase ($p>0.05$) the concentration of MDA in chicken breast of thigh samples. Time was significant ($p<0.05$) in contributing to the development of MDA in both treated and untreated samples. Treated and untreated chicken parts averaged 3.20 to 5.17 ug of MDA per 10 g for B/S breasts, 2.60 to 4.26 ug of MDA per 10 g for B/S thighs and 3.73 to 8.86 ug of MDA per 10 g for bone-in/skin-on thighs, at 0 and 120 h respectively. When evaluating products for protein oxidation, carbonyl content was used as the indicator for oxidation. PUV light did not increase ($p>0.05$) the occurrence of protein oxidation (free carbonyl concentration) in chicken breast or thigh samples. CIELAB color space, L*, a*, b* parameters were used to evaluate the color changes of samples after PUV light treatment. L*, a*, and b* values of B/S breasts, B/S thighs and bone-in/skin-on thighs did not significantly ($p>0.05$) change as a result of PUV light treatment.

In conclusion the results of this research, demonstrate that PUV light has the capability to reduce the presence of pathogenic microorganisms on the surface of raw

chicken without contributing to adverse quality effects. More research is needed to evaluate the effect that PUV light has on sensory quality and shelf life. Constraints, such as, surface temperature rise of the product needs to be addressed prior to commercial application.

**Key Words:** chicken, pulsed ultraviolet light, *Salmonella*, *E. coli*, *Campylobacter*

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# Chapter 1

## Introduction

The world population is estimated to peak at 9 billion individuals by the year 2042 (U.S. Census Bureau, 2016). According to the Centers for Disease Control and Prevention (CDC, 2015), 15% of the U.S. population becomes infected by and experiences a foodborne illness after consuming contaminated food or beverages annually. Globally, approximately 600 million people get ill and 500,000 die every year after consuming food products contaminated with pathogens. Despite constantly improving methods to reduce pathogens, there is still an occurrence of foodborne illness outbreaks (Scallan et al., 2011; Demirci and Ngali, 2012).

The consumption of poultry has continued to increase since the 1960's with anticipated growth to continue through 2030 (Keklik, 2009; PoultryTrends, 2017). In 2015, the United States produced 8.69 billion broilers at a value of $28.7 billion (NASS, 2016; PoultryTrends, 2017). Increased production and consumption of poultry is a result of a combination of low production costs, efficient production time and overall nutritional value.

Manipulation of the carcass during processing creates the opportunity for the product to become contaminated with bacteria, including pathogens. Product contact surfaces and cross contamination increases the risk for raw products to contain large concentrations of pathogenic bacteria. There are a number of pathogens that are associated with raw poultry, but *Campylobacter* and *Salmonella* account for the majority of poultry-associated foodborne illness outbreaks (FDA, 2001)

Government agencies oversee compliance standards and assurance programs to regulate the presence of pathogenic contamination on the product. In 2014, the CDC identified *Salmonella* and *Campylobacter* as two of the most prevalent contributors to both illness and hospitalization of all foodborne pathogens. Summarized by a CDC morbidity and mortality weekly report, foodborne illness outbreaks in poultry accounted for 16% of total outbreaks (CDC, 2015). In response to the risk associated with these pathogens, the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) has developed a set of standards to limit the prevalence of *Salmonella* and *Campylobacter* on poultry products.

Performance standards for *Campylobacter* and *Salmonella* define a maximum acceptable percent positive occurrence for the pathogens on raw poultry. For chicken parts a maximum of 7.7% positive sampling is allowed for *Campylobacter* and total of 15.4 % positive for *Salmonella* (USDA-FSIS, 2016; USDA-FSIS, 2018).

To meet compliance for these standards, poultry processors implement decontamination interventions. Traditionally, these microbial reduction interventions are achieved through processes including chemical or thermal treatments. Recently, alternative processes of decontamination have been evaluated. Processes including: ultrasound, hydrostatic pressure, electric fields and pulsed ultra-violet (UV) light all have potentials for food decontamination (Bolder, 1997; Demirci & Ngadi, 2012; Zhi-Hong et al., 2018).

Pulsed ultraviolet (PUV) light, also known as pulsed light, includes a spectrum of light wavelengths from 100-1100 nm. This spectral range includes the full UV light spectrum of 100-400 nm, the visible light spectrum (400-700 nm) and infrared spectrum

(700-1000 nm). Pulsed light inactivates bacteria and viruses by disrupting DNA in living cells. Germicidal, UV-C (100-280 nm) wavelengths contribute to the photochemical, photothermal and photophysical disturbances that result in rapid inactivation of microorganisms (Gomez-Lopez et al., 2007; Elmnasser et al., 2007; XENON, 2015). UV-C wavelengths account for over 54% of the total energy delivered by PUV light (Kristnamurthy et al., 2010). The infrared spectrum provides localized heat, which causes lyses of the microorganism's cell wall (Kristnamurthy et al., 2010; Demirci & Ngali, 2012). Pulsed light has been proven to effectively reduce both *Salmonella* (Paskeviciute et al., 2010; Keklik et al., 2010) and *Campylobacter* (Haughton et al., 2011) on boneless, skinless chicken breast. Similarly to UV light, PUV light is regulated by the Food and Drug Administration (FDA) and the Department for Health and Human Services (DHHS) (FDA, 2015).

# Chapter 2

## Literature Review

The current world population is 7.5 billion with expectations to peak at 9 billion by the year 2042 (U.S. Census Bureau, 2016; OECD, 2016). In the United States, it is estimated that 9.4 million individuals become ill after consuming food or beverages contaminated with pathogens (CDC, 2015). An estimated 600 million people become ill after eating contaminated food and over 500,000 die every year globally from foodborne illnesses (WHO, 2018). Current estimates establish that poultry accounts for approximately 27% of the world's total meat consumption. Furthermore and as seen in Figure 2-1, poultry meat accounts for over 35% of all meat produced globally (FAO, 2017; Capita et al., 2002; PoultryTrends, 2017). Second to fish, poultry products are the next leading cause of foodborne illness outbreaks, accounting for 16% of all outbreaks in the United States (CDC, 2015). The Organization for Economic Cooperation and Development (OECD, 2016) and the Food and Agriculture Organization (FAO, 2015) project that poultry consumption will increase 10% per capita (Poultry Trends, 2017) by 2027. Considering those figures, it becomes crucial to ensure that food safety practices continue to improve, in general and specific to poultry processing, to reduce the occurrence of human illness and reduce economic loss.

| | Million metric tons 2015 | 2016* | 2017** | % change 2017 over 2016 |
|---|---|---|---|---|
| **Total production** | **320.5** | **321** | **322** | **0.3** |
| Bovine meat | 67.6 | 68.3 | 69.6 | 1.9 |
| Poultry meat | 116.9 | 117.2 | 117.7 | 0.4 |
| Pig meat | 116.1 | 115.6 | 114.7 | -0.8 |
| Ovine meat | 14.4 | 14.4 | 14.5 | 0.6 |
| **Total trade** | **29.9** | **31.2** | **32** | **2.5** |
| Bovine meat | 9.2 | 8.9 | 9 | 0.8 |
| Poultry meat | 12.2 | 12.8 | 13.2 | 2.9 |
| Pig meat | 7.2 | 8.3 | 8.6 | 4.1 |
| Ovine meat | 1 | 0.9 | 0.9 | -2 |
| **Supply and demand indicators** | | | | |
| World per capita food consumption (kg/year) | 43.5 | 43.1 | 42.7 | -0.9 |
| **FAO meat price index (2002-04=100)** | **2015** | **2016** | **2017 (Jan-May)** | **% change: Jan-May 2016 over Jan-May 2015** |
| | 168 | 156 | 165 | 11.4 |

**Figure 2-1**. World balance of meat types (Poultry Trends, 2017).

## Government regulation

Meat and poultry and egg products and processors are regulated by the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS). This includes any food products that contain more than 3% meat or 2% poultry products and all egg products. Under the office of DHHS, the Centers for Disease Control and Prevention (CDC), monitors, researches and investigates any outbreak of foodborne diseases.

In 1997, the National Advisory Committee on Microbiological Criteria for Foods, chartered under the USDA, developed and implemented a food safety management system under the Pathogen Reduction Act of 1996; known as, hazard analysis of critical control point (HACCP). HACCP involves a number of regulatory steps to be implemented by food processors including critical control point (CCP) identification and

establishment of critical limits, monitoring procedures, corrective actions, safety outcome verification procedures, and documentation (FDA, 2014). By identifying, monitoring and controlling critical control points, actions can be taken to ensure physical, chemical and microbial hazards are avoided or eliminated at these processing steps. Hazard specific interventions reduce the risk of these hazards reaching the consumer. In 2012, the Food Safety and Inspection Service reassessed the national standard for HACCP plans specific to not-ready-to-eat (NRTE) poultry products. Establishments producing NRTE chicken and turkey parts and comminuted products had to reassess HACCP plans to control for *Salmonella* and *Campylobacter* (Federal Register, 2012). HACCP plans are specific to establishments and reviewed by FSIS on-site inspectors. FSIS does not have authority to initiate a recall but can detain products that are a risk to consumers. Ultimately, the FSIS works with state and local meat producers to address food safety risks to detect and prevent potential foodborne illness (USDA, 2017).

## Foodborne diseases

In the United States, between 6.6 and 12.7 million individual cases of foodborne illness occur every year due to 31 major pathogens. *Salmonella* and *Campylobacter* are the two most frequently associated foodborne pathogens with poultry products. For its relevance to this research *Salmonella*, *Campylobacter* as well as *Escherichia coli* will be reviewed in depth. It is important to recognize the occurrence of other pathogens that have been associated with chicken and poultry products. Less common pathogens associated with poultry, in order of frequency, include; *Shigella*, *Clostridium* species,

*Listeria monocytogenes*, and *Staphylococcus aureus* (Bolder, 1997; Mead et al., 1999; Capita et al., 2002; Demirci & Ngadi, 2012; Ebel et al., 2016).

### *Salmonella*

*Salmonella* are motile rod shaped, non-spore forming bacteria that are Gram-negative and facultative anaerobes (Coburn et al., 2007; FDA, 2012). *Salmonella* was first discovered by Daniel E. Salmon in 1885. There are two specific species of *Salmonella; bongori* and *enterica.* There are six subspecies of *Salmonella enterica* including; *enterica, salamae, arizonae, diarizonae, houtenae, and indica.* Each subspecies can be further differentiated by serotype, which is identified by O and H antigens specific to each serotype located on cell membrane surface (Keklik et al., 2009; FDA, 2012; CDC, 2018a).

Currently over 2,500 serovars of *Salmonella* have been isolated. Two serotypes of *Salmonella enterica,* Enteritidis and Typhimurium are the most frequent *Salmonella* serotypes associated with foodborne illness outbreaks. Other strains of *Salmonella* can cause illness, though *Salmonella* Enteritidis and Typhimurium combined account for over 30% of all *Salmonella* outbreaks (CDC, 2017a). Infection with *Salmonella* Typhimurium results in Typhoid fever with symptoms that manifest within 1 to 14 days. Infected individuals symptoms include fever, malaise, abdominal pain, headache, nausea, anorexia and constipation. More common than *Salmonella*. Typhimurium is infection associated with *Salmonella* ser. Enteritidis which is commonly known as salmonellosis or entercolitis. Symptom onset of salmonellosis occurs within 8-72 hours resulting in fever,

nausea and abdominal pains associated with vomiting and diarrhea (Coburn et al., 2007; FDA, 2012).

The duration of the illness can last as long as 4 to 7 days and lead to further complications if not treated with antibiotics (Coburn et al., 2007; Keklik et al., 2009). The total number of cases of salmonellosis varies greatly due to inconsistent diagnosis and reporting, but estimates suggest that there are 200 million to 1.3 billion cases of entercolitis annually worldwide, resulting in 3 million deaths (Coburn et al., 2007). In the United States, *Salmonella* accounts for greater than 6% of all foodborne associated outbreaks (Ebel et al., 2016; CDC, 2017a). Associated outbreaks correlate to a total of 10% of all foodborne illness, 25% of hospitalizations and 30% of foodborne associated deaths in the United States (Scallan et al., 2011, Mead et al., 1999). Treatment is accomplished by the administration of antibiotics (CDC, 2018a)

Common in most cooked and uncooked food products, *Salmonella* is a major concern in poultry and meat processing. Performance standards established by the FSIS now limit the occurrence of this species present in NRTE raw products. In a 52-piece sampling, that translates to 8 positive samples of Salmonella or 15.4% occurrence. Sampling beyond this standard would render the facility out of compliance and halt production (USDA-FSIS, 2016; Federal Register, 2016).

### *Campylobacter*

*Campylobacter jejuni* is a motile, rod shaped bacterium that is Gram-negative and microaerophilic, requiring a limited oxygen environment. Theodore Escherich first described *Campylobacter* in 1880 deriving its name from the Greek word “kampylos”

which translates to curvy, describing the bacteria's shape (Keener, et al. 2004). *Campylobacter* is frequently associated with raw dairy products, poultry, meat, eggs and water., This infectious pathogen is the leading cause of diarrheal disease globally (Haughton et al., 2011; FDA, 2012).

*Campylobacter jejuni* and *coli* are the two most common strains associated with foodborne illness outbreaks, with the overwhelming majority specific to *C. jejuni*. Compared to other foodborne pathogens, *Campylobacter* only accounts for 0.5% of total outbreaks in the United States (CDC, 2017b; Ebel et al., 2016). *Campylobacter* accounts for almost 15% of the total individual foodborne illness occurrence, 17% of hospitalizations and nearly 6% of foodborne associated deaths in the United States (Mead et al., 1999; Scallan et al., 2011).

The disease associated with the infection of *Campylobacter*, is known as campylobacteriosis. Symptoms onset is usually 2 to 5 days and can persist for about a week. Symptoms include diarrhea, fever and cramps that can last up to a week. Complications are associated with a weakened immune system (FDA, 2012; Keener et al., 2004; CDC, 2017b). Recovery is typically self-limiting, meaning the infected individual can recover without the need for medication.

Common in most cooked and uncooked food products, *Campylobacter jejuni* is a major concern in poultry and meat processing. Performance standards established by the FSIS now limit the occurrence of this strain present in ready-to-ship raw product. In a 52-piece sampling, that translates to 4 positive samples of *Campylobacter* or 7.7% occurrence. Sampling beyond this standard would render the facility out of compliance and halt production (USDA-FSIS, 2016; Federal Register, 2012).

***Escherichia coli***

*Escherichia coli* is Gram negative bacterium that can be found in the intestinal tract of nearly all living organisms. Theodor Escherich discovered the bacteria in 1885 and acknowledged *Escherichia coli* with his namesake. *E. coli* is generally recognized as a harmless bacteria common in the gastrointestinal tract of humans and animals (FDA, 2012).

However, *E. coli* has some serotypes with a variety that are pathogenic. The seven most common *E. coli* pathotypes are the following;  enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), Shigella/enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and enterotoxigenic *E. coli* (ETEC), and adherent invasive *E. coli* (AIEC) among others (Croxen, et al. 2013). Individual serotypes are distinguished by the cells O and H antigens on the membrane surface. With respect to the occurrence of foodborne illness outbreak and illness, these pathotypes are typically identified as either STEC O157 or STEC non-O157. *E. coli* O157:H7 produces a strong toxin and is the main pathotype of concern in food processing (CDC, 2018b).

An individual infected with a pathogenic strain of *E. coli* can expect symptoms to include fever, abdominal cramps, diarrhea and vomiting. Symptom onset is usually 3 to 4 days after infection but can range from 1 to 10 days and can last 5 to 7 days. For the majority of infected individuals *E. coli* is self limiting; recovery without the need of medication (FDA, 2012; CDC, 2018b). In less common circumstances (5-10%) individuals infected with shiga-toxin producing *E. coli* can develop hemolytic uremic

syndrome (HUS). Without medical attention HUS can lead to kidney failure and death (Keklik, 2009; Croxen et al., 2013; CDC, 2018b).

In the United States, *E.coli* accounts for nearly 20% of all foodborne illness outbreaks annually (Ebel, et al. 2016; CDC 2018b). Regardless of having such a high prevalence of outbreaks *E.coli* only accounts for less than 1.5% of the total foodborne illness occurrences, 4.5% of hospitalizations and nearly 4.5% of foodborne associated deaths in the United States (Mead et al., 1999; Scallan et al., 2011).

**Other noteworthy pathogens**

*Shigella* is identified as a Gram-negative, rod-shaped bacterium that has been associated with products such as chicken salad and other prepared foods. Typically not associated with raw poultry products, contamination is spread through unsanitary preparation environments or infected individuals (Keklik, 2009; FDA, 2012). Consumption of small numbers of cells (10-200) can result in symptom onset within 8 hours and typically lasts 5-7 days. Symptoms include cramps, diarrhea, fever; and vomiting (FDA, 2012). *Shigella* is associated with roughly 0.5% of all foodborne illness outbreaks in the U.S. and results in a hospitalization rate of roughly 24% (Mead et al., 1999; Scallan et al., 2007, CDC, 2017a).

*Clostridium* species are rod shaped, spore forming, Gram-positive anaerobic bacterium that are naturally present in the human and animal intestines. The presence of these bacteria on undercooked poultry or meat can lead to infections causing cramps, diarrhea and dehydration (FDA, 2012). *C. perfringens* has an insignificant rate of hospitalization and rarely results in mortality. This bacterium is typically under reported

and is estimated to account for a larger number of presumptive illnesses than what is reported (Mead et al., 1999; Scallan et al., 2011). With a large ingestion ($10^6$ cells), symptom onset can be observed within 16 hours and usually lasts 12 to 24 hours (FDA, 2012).

*Listeria monocytogenes* is a motile, non-spore forming, Gram-positive facultative anaerobic bacterium. This bacterium is commonly present in the food and soil and contaminates raw product during processing. *L. monocytogenes* is commonly found in a variety of foods including, dairy products, raw and cooked poultry and meats (Mead, et al. 1999; Keklik, 2009). The pathogen can lead to listeriosis; a life threatening disease with common symptoms including vomiting, diarrhea and nausea. Onset is observed within a few hours to 2-3 days. *L. monocytogenes* accounts for over 5% of all foodborne illness outbreaks in the U.S. and can last days to weeks resulting in a 94% rate of hospitalization and 15% mortality (Scallan et al., 2011; FDA, 2012; CDC, 2017a). Poultry can be a vector of *L. monocytogenes* but associated infection is usually a result of cross contamination post-processing (Henning & Cutter, 2001; Keklik et al., 2009).

*Staphylococcus aureus* are Gram-positive, spherical bacterium and are ubiquitous, meaning they are found almost everywhere. Poultry products are reported to be a vector of the bacterium, though cross contamination with the environment or infected carriers is more common post-processing. Infection of *S. aureus* leads to rapid intoxication within 1 to 7 hours with symptoms lasting only a few hours resulting in nausea, abdominal cramping, vomiting and diarrhea (FDA, 2012). *S. aureus* has a relatively low accountability of total outbreaks in the U.S, but roughly 6% of intoxications do result in hospitalization (Scallan et al., 2011).

## Performance standards

In 1996, the FSIS implemented performance standards for both *Salmonella* and *Campylobacter* specific to poultry production. The Food Safety Inspection Service (FSIS), an agency of the United States Department of Agriculture is responsible for enforcing regulations and policies to ensure the safety and wholesomeness of the country's meat, poultry and certain egg products supply (USDA, 2017).

In January 2015, the FSIS released a notice that addressed the need for updating the then current performance standards for not-ready-to-eat (NRTE) poultry products. Current poultry production and processing practices do not lend themselves to completely eliminating bacterial contamination on raw products. A combination of effective sanitary procedures and the inclusion of disinfection interventions can minimize raw product contamination (Federal Register, 2015).

Previous performance standards were set in 1996 specific to whole chicken and comminuted turkey. These standards did not address the presence of *Salmonella* or *Campylobacter* allowance values for chicken parts. Currently, 80% of raw chicken processed is in the form of individual parts. Without appropriate performance standards there is lack of control parameters to reduce pathogens. Furthermore, higher concentrations of pathogens create an increased opportunity of cross-contamination, when contamination spreads from the raw product to an individual, preparation area or other foods (Federal Register, 2016).

After acknowledging the need to update the performance standards, FSIS implemented the Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Baseline Survey. From January 2012 to August 2012, this survey

established the percent positive sampling of *Salmonella* and *Campylobacter* of various raw chicken parts (USDA, 2012).

In January 2015, the FSIS prepared the proposal that reported the data collected from the baseline survey. The new performance standards (Table 2-1) also factored what reduction of *Salmonella* and *Campylobacter* was necessary to reach the Healthy People 2020 goals. The Healthy People 2020 goals are a science-based set of objectives that the Office of Disease Prevention and Health Promotion (ODPHP) published with the aim to improve the overall health of the United States. The new performance standards will achieve a predicted 33% and 19% reduction of foodborne illness rate specific to *Salmonella* and *Campylobacter*, respectively (ODPHP, 2018; Federal Register, 2015).

**Table 2-1.** Current performance standards specific to poultry products

| Product | Maximum Acceptable % Positive | | Performance Standard | | Minimum # of Samples to Assess Product Control | |
|---|---|---|---|---|---|---|
| | *Salmonella* | *Campylobacter* | *Salmonella* | *Campylobacter* | *Salmonella* | *Campylobacter* |
| Broiler Carcass | 9.8 | 15.7 | 5 of 51 | 8 of 51 | 11 | 10 |
| Turkey Carcass | 7.1 | 5.4 | 4 of 56 | 3 of 56 | 14 | 19 |
| Communited Chicken | 25 | 1.9 | 13 of 52 | 1 of 52 | 10 | 52 |
| Communited Turkey | 13.5 | 1.9 | 7 of 52 | 1 of 52 | 10 | 52 |
| Chicken Parts | 15.4 | 7.7 | 8 of 52 | 4 of 52 | 10 | 13 |

USDA, 2018; Federal Register, 2016

## Pathogen monitoring during production

Using routine, random sampling, the FSIS and individual establishments monitor the presence of *Salmonella* and *Campylobacter* prevalence on poultry products. Collaboration with the CDC on illness data, FSIS will be able to evaluate the poultry

industry's progression in reducing the occurrence of product contamination and foodborne illness. FSIS began assessing whether establishments met the new pathogen reduction performance standards on May 11, 2016 (FSIS, 2016).

Every establishment has designated FSIS inspection program personnel (IPP). This/these individual(s) sample product using an ongoing schedule over a moving one year window. This means annual reports are generated from results obtained within one year of the report. Table 2-1 provides the number of samples required to assess product controls (USDA-FSIS, 2016).

When sampling, pre-chilled buffered peptone water (BPW) is used as a rinsing solution and samples are collected in large sterile plastic bags. Chicken carcasses are sampled using whole carcasses rinsed with BPW and 100 mL of the rinsate is collected to analyze for *Salmonella* and *Campylobacter*. When sampling chicken parts, approximately 120 mL of rinsate is collected from 4 lbs. ± 10% of the eligible parts. Both chicken carcasses and part rinsates are transferred to separate sterile specimen jars and evaluated at the establishment's microbiology or quality lab (USDA-FSIS, 2016).

The presence and/or absence of *Salmonella* and *Campylobacter* in commercial poultry processing is assessed using real-time polymerase chain reactions (PCR) that are specific for identifying each pathogen. Commercial processors use on-site PCR systems or outsource to third party PCR capable labs. Real time PCR generates a report within 50-75 minutes (Whyte et al., 2002; Hong et al., 2003). The IPP records the results of sampling. In addition to IPP sampling and record keeping of presence/absence of pathogens, USDA FSIS inspectors take samples once a week. The performance standards are only evaluated using the samples collected by USDA. An establishment is in

compliance if within a moving one year window products are below the maximum acceptable percent positive. If an establishment does not meet the performance standards it will receive either a warning or corrective action depending on the severity of positive sampling over the one year moving window. Non-compliance results in a reassessment of an establishments HACCP system and modification where necessary (FSIS, 2016).

## Poultry processing

Poultry is the general term associated with the meat that is harvested from a variety of poultry fowl including but not limited to, chicken, turkey, goose, duck, guinea, pheasant, and quail (Keklik, 2009; Maragoni et al., 2015). The meat from these animals provides a large variety of nutrients needed for a healthy life. Poultry products supply high amounts of digestible proteins, saturated and unsaturated fatty acids, B-group vitamins and minerals including, iron, zinc and copper. The UN Food and Agricultural Organization (FAO) have recognized poultry meat as an inexpensive food that is crucial in meeting essential nutrient shortfalls in developing and underdeveloped countries (Maragoni et al., 2015).

Growing populations of developing countries that require protein in their diets have contributed to the increased demand of poultry meat. Projections by the Organization for Economic Cooperation and Development (OECD, 2016) and Food and Agriculture Organization (FAO, 2015) of the United Nations estimate that poultry consumption will increase 10% within the next ten years (Figure 2-2). To ensure that poultry meat can be produced in an efficient and cost effective manner, the commercial

industry has established a process (Figure 2-3) that incorporates both automation and manual labor to process chicken.

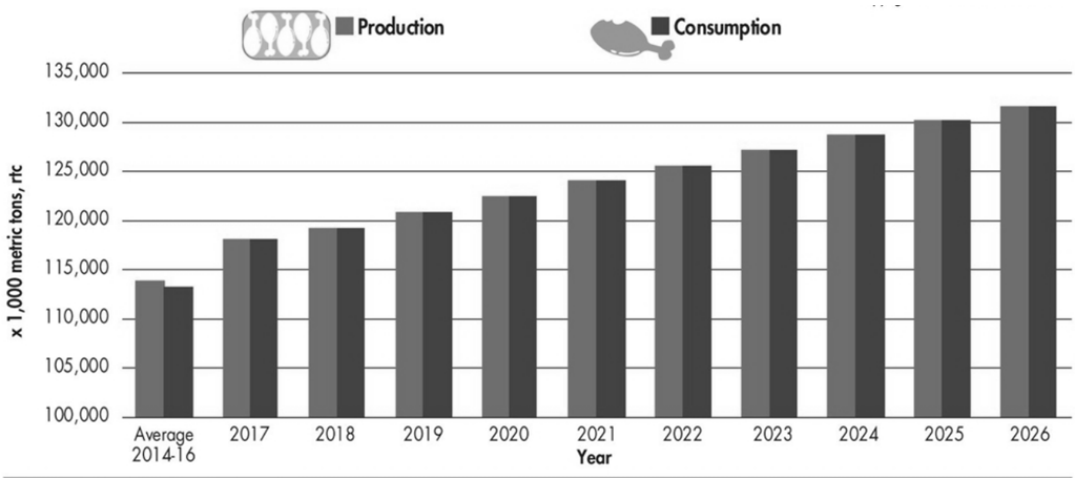


**Figure 2-2**. World poultry meat production and consumption projections (PoultyTrends, 2017).

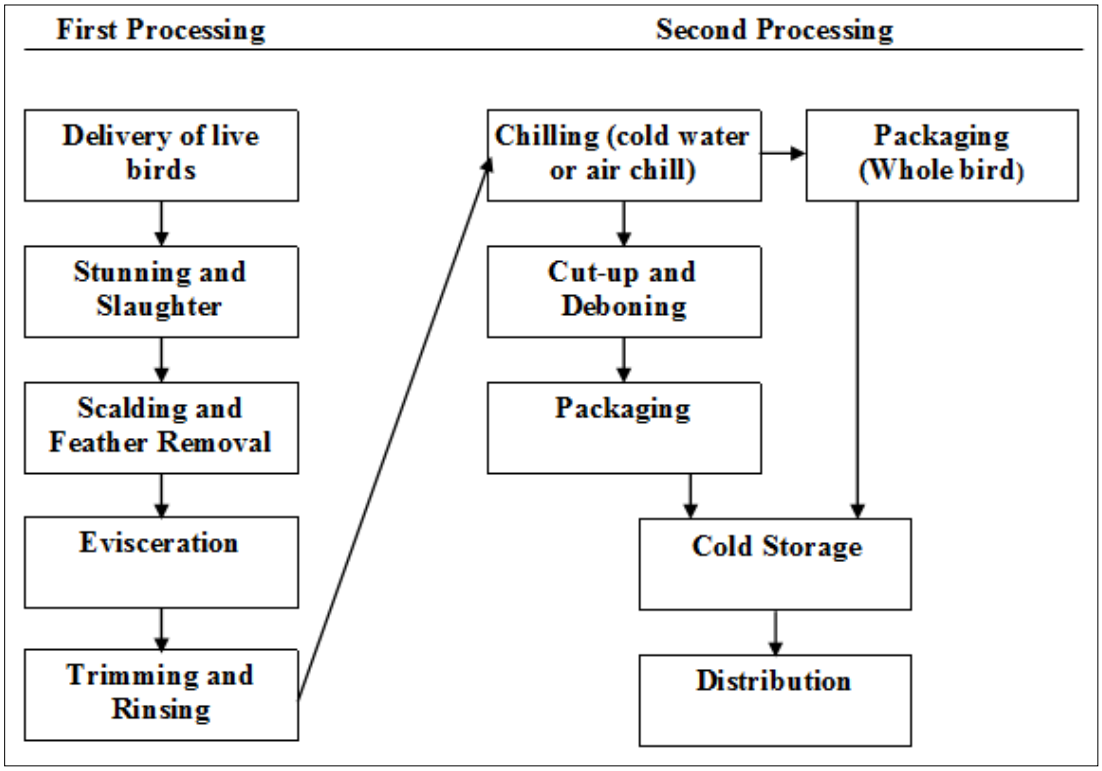


**Figure 2-3**. Poultry processing steps.

Once live birds reach mature weight they are transported to slaughter and processing plants. At the plant, birds are unloaded from the trucks and stunned prior to slaughter. In the United States there are currently only two methods of stunning; electric water bath stunning or controlled atmosphere stunning (CAS). Electric water bath stunning requires birds to be hung in shackles prior to stunning. The birds' heads are submersed in a water bath and met with an electric current lasting 10–12 seconds. Electric water bath stunners require 10 to 28 volts to deliver 10 to 45 mA per bird. Controlled atmosphere stunning is a progression system that stuns the live animal over an extended period of time by reducing concentrations of oxygen and increasing concentrations of other gases. Carbon dioxide ($CO_2$), inert gases (such as, argon or nitrogen), or a mixture of these gases are commonly used to replacement oxygen. (Sheilds & Raj, 2010). This process has been labeled by animal activist groups as a more humane system, acknowledging that after a period of time the birds will wake back up after being removed from the gas system. With CAS systems birds are hung by shackles after being stunned (Keener et al., 2004).

Following stunning and exsanguinations birds hanging from shackles pass through a series of hot water (50-60°C) baths that loosen feather follicles for ease of removal. Feather picking is accomplished using stiff rubber fingers that rotate in a series of alternating directions effectively removing feathers (Salvik et al., 1995; Keener et al., 2004).

In large scale commercial processing, a series of automated machines make the necessary incisions to effectively remove the evisera (including, the gastrointestinal and reproductive tract, heart, liver, and lungs). Before exiting evisceration, chicken carcasses

are inspected. USDA inspectors observe each bird with its corresponding evisera to identify and remove any carcasses that may pose a risk to food safety of consumer health. Carcasses go through multiple rinses cycles with the aim to reduce the presence of any solid contamination (Keener et al., 2004; USDA, 2013).

After evisceration, chicken carcasses are chilled. The USDA mandates that chicken carcasses must reach a certain chilled temperature. The United States requires carcasses to be chilled to 4.4°C or lower in 4, 6, or 8 hours for carcasses weighing less than 4, 4-8, or over 8 pounds, respectively prior to any further manipulation or packaging of the carcasses (CFR, 1992, James et al., 2006).

In the United States, there are two common forms of whole carcass chilling, including ice water bath submersion and air chill. Water bath submersion involves a large bulk tank filled with ice water with an inclusion of an antimicrobial. Chlorine diluted to a 50 ppm concentration is an example of a commonly applied antimicrobial (Bolder, 1997; Demirci & Ngadi, 2012). An auger is utilized to move birds in a time that is appropriate, for the antimicrobial to be effective and necessary chilling. Air chilling is an alternative chilling method that is usually paired with organic acid (1-2% concentrations) or chlorine rinses to obtain similar antimicrobial affects as water bath chilling. Once the carcass has been chilled to a temperature of roughly 4°C it is considered to be ready for packaging and further product manipulation (James et al., 2006). Prior to the performance standards updated in 2016 only whole carcasses need to be monitored and controlled for pathogens. The update now requires similar control for deboned and parted out products to be included in the standards (USDA-FSIS, 2016).

The inclusion of chicken parts in the performance standards resulted in poultry processors ensuring that these products had the necessary low levels of both *Salmonella* and *Campylobacter*. To guarantee that the chicken parts would meet compliance with performance standards, secondary microbial reduction interventions were incorporated in the production lines. Similar to whole carcass interventions, chicken parts are predominantly treated with chlorine or organic acid rinses (USDA-FSIS, 2016).

**Raw chicken decontamination interventions**

As regulated by the FSIS, processed poultry must meet performance standards that were established over a 7 year identification process. The current standards dictate a maximum percent acceptability for *Salmonella* and *Campylobacter.* The predominant interventions currently used in decontamination of NRTE poultry products are variations of organic acid or chlorine dilution rinses (Bolder, 1997; Killinger et al., 2010; Demirci & Ngadi, 2012). In the United States, whole carcasses first microbial reduction intervention occurs at chilling. With the updated performance standards now including a maximum percent positive sampling of parts, many processors have incorporated addition al interventions. With variation across companies, cut-up and deboning steps now include secondary decontamination interventions. The following sections will introduce a variety of both established and novel decontamination techniques that have potential application in poultry processing.

### i) Chemical interventions

Numerous chemical treatments are proven to be effective microbial reduction interventions in poultry processing. As previously mentioned, chlorine and organic acid rinses are two treatments that are commonly used. Other chemical treatments including inorganic phosphates (trisodium phosphate and polyphosphates), oxidizers (hydrogen peroxide, ozone) can also be alternatives.

Chlorine dissolves in water forming either hypochlorous acid or hypochorlite ions, which both act as antimicrobials (Bolder, 1997; Demirci & Ngadi, 2012). Chlorine is applied on poultry carcasses at or less than 50 ppm for roughly 1 minute which results in a 1 $\log_{10}$ reduction (Bolder, 1997). Another inclusion of chlorine is in the form of acidified sodium chlorite ($NaClO_2$) derived from chlorous acid and chlorine dioxide. Monochloramine ($NH_2Cl$) and cetylpyridinium chloride (CPC) are both generated through interactions between chlorine and ammonia. These two compounds have been reported to reduce *Salmonella* by 2 $\log_{10}$ (Demirci & Ngadi, 2012). Chlorine and associated compounds result in glucose oxidation and disruption of protein synthesis disrupting cellular function (Bolder, 1997).

Differing from chlorine based solutions, is the use of inorganic phosphates in the form of trisodium phosphate (TSP) that uses high pH and ionic strength to cause bacterial autolysis (cell death). The FDA assigned a generally recognized as safe (GRAS) status to TSP as used as a food antimicrobial. TSP concentrations of 5-10% in solution result in a reduction of 1-2 $\log_{10}$ dependent on time of exposure (Bolder, 1997; Capita, et al. 2002; Demirci & Ngadi, 2012).

The application of organic acids has become more common for the treatment of meat surfaces with the FDA designation of generally recognized as safe or GRAS. Acids such as, acetic, citric, lactic, malic, propionic, and tartaric are cheap and efficiently result in reductions of 1 $\log_{10}$. Microbial reduction is a result of cell membrane acidification which disruptions energy production and regulation of the bacteria (Mani-Lopez et al., 2012). Hydrogen peroxides and ozone have both been reported to be effective antimicrobials but are not as efficient as the above mentioned chemical treatments (Bolder, 1997).

**ii) Thermal interventions**

Thermal interventions used for microbial decontamination are hot water in the form of liquid or steam and chilling or freezing. Water is the predominant intervention used to remove visible debris from the surface of the chicken carcasses. The use of water as a rinse removes dirt and the heat incorporated acts a lethal effect for bacteria. At a temperature greater than 95°C significant reduction of microflora was obtained and at 65°C only 1 $\log_{10}$ reductions were reported (Bolder, 1997). An alternative application of hot water is in the form of steam. Limited data is established acknowledging the effectiveness, but extended exposure can result in carcass deterioration (Demirci & Ngadi, 2012). Both steam and hot water are effective for sanitation purposes but not for food decontamination due to effects on quality.

Immersion chilling results in a significant reduction of microbes on individual carcasses. Rinsing and spraying with chilled water can be effective but water pressure tends to not have an effect on microbial reduction. Freezing and long term chilling has a

limited effect on microbial reduction but do result in significantly reducing the growth of microbes (Keklik, 2009; Demirci & Ngadi, 2012).

### iii) Physical interventions

With increasing poultry production and consumption, continued research in food safety has led to the development of a number of novel technologies that all provide potential as alternative microbial reduction interventions for poultry processing. These non-thermal interventions are vacuum/gas packing, irradiation, high pressure processing, pulsed electric field, ultrasonic energy and both ultraviolet and pulsed ultraviolet light (Bolder, 1997; Demirci & Ngadi, 2012).

Irradiation is a process that uses ionizing energy to inactivate microorganisms. Ionizing energy is produced from radioactive isotopes; machine generated accelerated electrons or x-rays which all result in high lethality of pathogens. Specific to the energy source, treatment time is seconds to minutes of exposure to obtain 90% microbial reduction. Currently, industry's use of irradiation is limited due to consumer perception and overall cost associated with the treatment (Demirci & Ngadi, 2012; Zhi-Hong et al., 2018).

High pressure processing, is the usage of high pressure (~70,000 psi) to reduce microorganisms. Typically a food source is packaged and placed in a pressure tank surrounded by water. High pressure is applied and results in conformational changes in cellular membranes and organelles resulting in irreversible cell damage. Limitations to this technology are the high cost associated with treatment and that it is not effective against spore forming organisms (Demirci & Ngadi, 2012; Zhi-Hong et al., 2018).

Pulsed electric field (PEF) is a process that applies short durations of high voltage electric shocks across a food source. Currently, this process is limited to liquid food treatment and is limited due to high cost of operation (energy requirements), limit capacity of treatment and sensory changes to food products (Demirci & Ngadi, 2012, Zhi-Hong et al., 2018).

Ultrasonic treatment of poultry carcasses has been investigated and requires carcasses to be fully submersed and then exposed to the sonification which results in cell disruption. This technology is amplified when paired with low pH and increased temperature (Demirci & Ngadi, 2012; Zhi-Hong et al., 2018).

Vacuum and gas packaging are more of a microbial inhibitor than a reduction treatment, but has been reported in reducing aerobic dependent microorganisms (Demirci & Ngadi, 2012). The use of ultraviolet light and pulsed ultraviolet light as a food decontamination intervention will be discussed in more detail in the following sections.

**iv) Ultraviolet light**

Usage of Ultra-Violet (UV) light is a newer technology to the food industry that has been identified as an effective microbial reduction method. With the potential of the application, the FDA has derived a set of parameters that regulate the usage of UV (FDA, 2011).

UV light is a portion of the electromagnetic spectrum that falls below that of the visible light spectrum. UV is emitted using a mercury arc bulb consisting of a mixture of mercury and argon vapor (Guerrero-Beltr et al., 2004; Demirci and Ngadi, 2012). UV light has a cumulative wavelength of 100 nm to 400 nm and is differentiated into 4

specific ranges. The 4 ranges are Vacuum-UV (100-200 nm), UV-C (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). In humans, UV-A is commonly associated with sun tanning and UV-B results in sun burns. UV-C's wavelength is absorbed by DNA and can lead to mutations, this is the common UV light associated with cancer. Vacuum-UV is absorbed by water and air but only under vacuum conditions (Demirci & Ngadi, 2012).

Ultraviolet photons within UV-C's wavelength are particularly energetic and when absorbance by DNA causes bonds to break by nature of photochemistry. These manipulations in DNA by means of photo-hydration, photo-splitting and photo-dimerization allow them to have germicidal effects against bacteria, including foodborne pathogens (Demirci & Ngadi, 2012). UV light is the portion of the electromagnetic spectrum that comes before the portion of visible light (Table 2-2). Ranging from 100-400 nm, UV light consists of roughly 10% of the total radiation produced by the earth's sun. (Demirci & Ngadi, 2012) Each sub-portion of the UV spectra, vacuum-UV, UV-C, UV-B, and UV-A have the following photon energy ranges (eV); 12.4-120, 12.4-4.43, 4.43-3.94 and 3.94-3.10, respectively. Photon energy derived from each sub-portion of the UV region correlates to differing bimolecular reactions, as seen in Table 2-2.

**Table 2-2**. UV type and characteristics

| Type | Wavelength | Range (nm) | Characteristics |
|---|---|---|---|
| UV-A | Long | 320-400 | Changes in human skin (tanning) |
| UV-B | Medium | 280-320 | Skin burning (cancer) |
| UV-C | Short | 200-280 | Germicidal effects (DNA) |
| UV-V | Very Short | 100-200 | Vacuum UV range |

(Guerrero-Beltr, et al. 2004)

Photochemical interactions can be either beneficial or adverse for living organisms. Longer wavelengths, produced by the UV-A portion of the spectra, are correlated to skin tanning and can enhance the production of vitamin D synthesis.

Inversely, shorter wavelengths in the UV spectra can cause skin damage, which can lead to cancer. UV-C wavelengths produce a germicidal effect through the formation of thymine dimers in a living cells DNA. These altercations in the DNA lead to cellular inactivation (Keklik et al., 2009; Kristnamurthy et al., 2010; Keklik et al., 2011; Demirci & Ngadi, 2012).

The germicidal effects of UV light were first acknowledged in 1877 by Arthur Downes and T.P. Blunt, two English scientists, who discovered the ability of the sun to prevent microbial growth (Demirci & Ngadi, 2012). The germicidal effect of UV light was later correlated to dosage (intensity × time), wavelength of radiation and sensitivity of the specific microorganism. In 1930, Frederick Gates published the first analytical bactericidal experiment that presented peak effectiveness at 265 nm. Throughout the rest of the $20^{th}$ century, research investigating the germicidal effect of UV light was limited (Reed, 2010). In recent decades, increasing interest has sparked in the application of UV light as a microbial reduction technology for aspects of the medical fields and for food safety. With renewed interest in the bactericidal effects of UV light, varying technologies have been developed to produce UV light.

Chun et al. (2009) tested the efficacy of UV-C on *Campylobacter jejuni, Listeria monocytogenes,* and *Salmonella enterica* inoculated at a 6-7 $\log_{10}$ cfu/gram on chicken breasts. In this study UV-C irradiation was administered at 5 $kJ/m^2$. There was a reduction of the initial populations of *C. jejuni, L. monocytogenes, and S.* Typhimurium by 1.26, 1.29, and 1.19 $\log_{10}$ cfu/g, respectively (Chun et al. 2009). Wallner-Pendleton, et al. (1994) inoculated broiler carcasses with *Salmonella* Typhimurium and exposed them

to UV-C at a wavelength of 253.7 nm for 5 minutes. This exposure resulted in a 61% reduction of total *S.* Typhimurium.

The current allowance of UV treatment of food products is established as a regulation under the Code of Federal Regulations (FDA, 2011). The approved radiation source of UV delivery is low pressure mercury lamps which emit 90% of the total energy at a wavelength of 253.7 nm (2,537 angstroms). For the purpose of surface microbial control of food and food products, UV light can be used without production ozone. High fat-content food must be treated in a vacuum or inert gas atmosphere. The total intensity of radiation that can be applied is 1 watt (of 253.7 nm) per 5 to 10 $ft^2$. With the intent to sterilize water, UV can be used without ozone production and a total coefficient of absorption of 0.19 per cm or less. Flow rate of water cannot exceed 100 gal/h per 1 watt (253.7 nm) at a depth of 1 cm or less. The UV lamp-operating temperature can range from 36 to 46°C. Decontamination of juices by UV light follows similar specifications as water (FDA, 2011).

**Pulsed ultraviolet light**

Usage of pulsed ultraviolet (PUV) light or pulsed light is a discovery through the application of conventional UV light that has resulted in a more effective microbial reduction method. With the potential of application, the FDA has derived a set of parameters that regulate the usage of pulsed light (FDA, 2015).

Pulsed light includes all of the conventional UV spectrum, visible light spectrum and part of the Infrared spectrum. Pulsed light has a cumulative wavelength of 100 nm to 1100 nm. With the inclusion of this expanded spectrum, the wavelengths beyond UV

travel at a higher intensity with greater penetration strength. The higher wavelengths carry the UV light, specifically UV-C, at a higher intensity. Pulsed light is emitted using a Xenon gas bulb and is applied through an adjustable pulsation. The addition of the Infrared spectrum introduces an additional microbial decontamination application. Infrared light creates localized heat generation on the cell wall and causes the cell to lyses and die (Gomez-Lopez et al., 2007; Demirci & Ngadi, 2012; Mcleod et al., 2018; Xenon, 2018).

Pulsed UV (PUV) light, also associated as pulsed light, is a novel UV technology and process that amplifies the interactions produced by continuous UV light. PUV light originates from an inert gas (e.g. xenon) flashlamp that produces a broad spectrum of electromagnetic radiation ranging from 100-1100 nm (UV, visible and infrared wavelengths). It has been quantified that 54% of the energy emitted is produced from the UV spectrum, justifying the term pulsed "UV" light. Flashes or pulsation of the light can range from 1-5 pulses per second and the duration of each pulse can be reduced to a few hundred microseconds. The high energy density ($J/cm^2$) of each pulse is produced by storing electrical energy in an electrical storage capacitor. The stored energy released at peak power is used to excite the gas within the flashlamp resulting in short releases of high intensity light pulses. The system delivers energy that is 20,000 times more intense than that of the sun on the earth's surface (Elmnasser et al., 2007; Krishnamurthy et al., 2010; Demirci & Ngadi, 2012). PUV light creates a unique interaction that results in an increased germicidal effectiveness compared to continuous UV light.

PUV lamps consist of an inert gas (e.g. Xe, Ar, Kr), and are characterized by the release of extremely intense, short time, and broad spectrum flashes. Flashlamps and

surface discharge (SD) lamps are the two sources for pulsed light. Typical flashlamps are activated by releasing energy between two electrodes confined to a small envelope of inert gas. The UV efficiency of a flashlamp is ~9% and produces an intensity of 600 $W/cm^2$. Under these conditions, a xenon flashbulb has a lifespan of about 1 month. Compared to flashlamps, SD lamps have a high efficiency (17%) and higher intensity (30,000 $W/cm^2$). SD lamps produce plasma by discharging high power electricity along the surface of a dielectric substrate (e.g. fused silica tube) inside an envelope containing xenon gas (Demirci & Ngadi, 2012).

In one study, *C. jejuni, E. coli, and Salmonella* Enteritidis inoculated chicken skin had $\log_{10}$ reductions of 1.22, 1.69, and 1.27, respectively with the application of pulsed light for 2 seconds (Haughton et al., 2011). Keklik, et al. (2011), inoculated the surface of chicken carcasses with *Escherichia coli* K12 and exposed carcasses to PUV light at approximately 5 cm. There were effective $\log_{10}$ reductions ranging from 0.87 to 1.43 cfu/mL after 30 and 180 seconds of treatment.

When comparing similar studies using UV light or pulsed light, pulsed light is more effective in the decontamination and inactivation of bacteria, including foodborne pathogens. The time needed for the same effective log reductions is much lower using pulsed light than conventional UV light. This attribute allows for the easier application of pulsed light in current processing procedures that process at an accelerated speed (Demirci & Ngadi, 2012).

**Microbial inactivation mechanisms by UV and PUV light**

The germicidal wavelengths associated with UV and PUV lights are a result of predominantly photochemical changes that disrupt cellular DNA structure. It is known that photons with wavelengths in the range of 240 to 280 nm are effectively absorbed by DNA in living cells. After absorbing the UV light, DNA is damaged, ultimately impeding replication and causing cell death. Additional mechanisms are hypothesized to contribute to microbial reduction by pulsed UV light (Kristnamurthy et al., 2010). Ultimately, it has been confirmed that broad spectrum (e.g. PUV light) has no germicidal effect when wavelengths lower than 320 nm are filtered out (Elmnasser et al., 2007). It does appear that a combination of visible and infrared wavelengths do contribute towards microbial reduction when paired with the high peak power delivery of pulsed UV light.

When UV wavelengths penetrate a cell wall, the germicidal UV-C wavelengths create disruptions in cellular DNA, which inhibits a microorganism's ability to reproduce. UV light results in the formation of photo-dimerization in pyrimidine bases and photo-hydration of cytosine (Figure 2-4). The bonds formed by these disruptions result in the inability for DNA to unzip for replication effectively inhibiting cellular reproduction. If a cell lacks the ability to repair such DNA disruptions, cell death will occur due to mutations and impaired replication and DNA transcription (Elmnasser et al., 2007; Kristnamurthy et al., 2010).

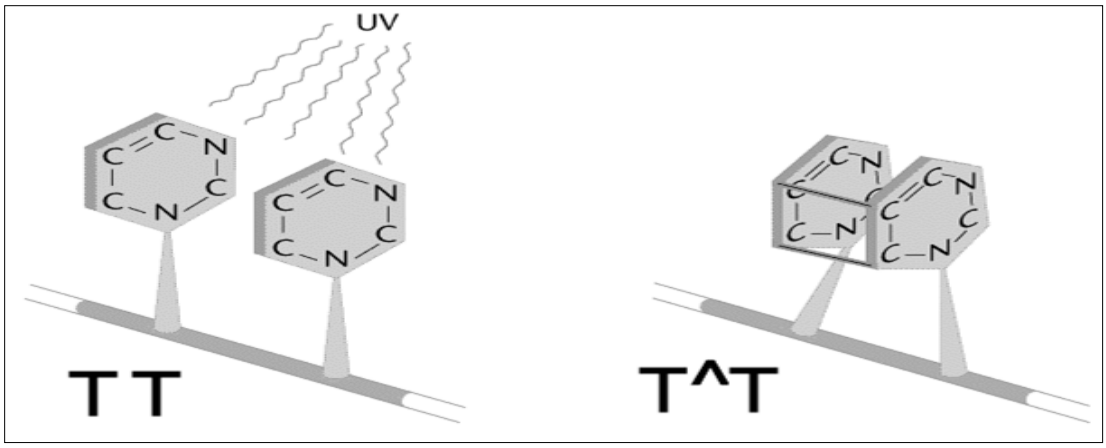


**Figure 2-4.** Depiction of Thymine dimer formation after exposure to UV light (Carr, 2011)

UV light mainly inactivates cellular DNA, which can be repaired under certain experimental conditions. Pulsed UV light damages DNA too severely to be repaired by enzymes. It is hypothesized that the intensity of the energy delivery damages the DNA repair enzymes, effectively inactivating their function (Elmnasser et al., 2007; Demirci & Ngadi, 2012).

With context to this chapter, photothermic mechanisms associated with microbial reduction is specific to pulsed UV light. Pulsed UV light emits a broad spectrum that includes visible and infrared wavelengths. High power pulses create an opportunity for localized heating to contribute to cell wall drying and cellular destruction. The water content of the microorganism becomes vaporized which generates a steam flow at the cell membrane evacuating intercellular content. At high dosage and over prolonged times of exposure, temperature significantly increases. It is reasonable to infer that at low levels, heat has a minimal contribution to microbial reduction compared to UV lights germicidal effects (Elmnasser et al., 2007; Demirci & Ngadi, 2012).

Similar to photothermal effects, physical altercation of a microorganism is another phenomenon of pulsed UV light. Studies have reported that under microscopic

views that pulsed UV light causes physical changes of the microorganisms. Other studies have reported complete cell lyses or destruction beyond the DNA interactions. It is hypothesized that the broad spectrum wavelength emitted in short powerful bursts could contribute to intense micro-vibrations on the cell membranes that lead to distortion and lyses (Figure 2-5).

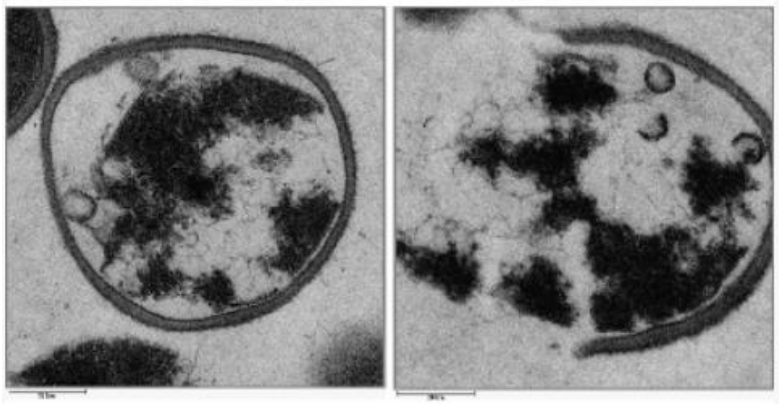

**Figure 2-5.** Example of cell membrane collapse after exposure to PUV light (Kristnamurthy et al., 2010).

### i) Effects of PUV light on quality

The use of UV light as an effective microbial reduction intervention in food processing is only beneficial if there are no other quality changes associated with treatment. Both UV and PUV light are advantageous because of the exclusion of chemicals that may contribute to changes in flavors or aromas of the food products. However, the wavelengths (radiation energy) associated with UV and PUV light have the potential to promote photochemical interactions with biomolecules in food products. The following sections will acknowledge these interactions and the effect of UV and PUV light on quality changes in food (Koutchma, 2009; Keklik, et al, 2010)

The factors that determine the effect if any on photochemical changes is dependent on the absorption of photons and an excessive delivery of energy to the

product surface. At 253.7 nm, radiant energy is 112.8 kcal/Einstein (1 Einstein equals 1 mol of photons), which has the potential to disrupt O-H, C-C, C-H, C-N, H-N and S-S bonds (Spikes, 1981). Furthermore, food sensitivity to UV wavelengths is correlated to nutrients that lend themselves to be "light sensitive." These nutrients include; vitamin A, carotenes, cyanobalamin (vitamin $B_{12}$) vitamin D, folic acid, vitamin K, riboflavin (vitamin $B_2$), tocopherols (vitamin E) tryptophan, and unsaturated fatty acids. The sensitivity of the prior mentioned nutrients differs by the interaction of sensitivity to specific wavelengths. It can be concluded that the effect of PUV light on photochemical interactions would be greater acknowledging the broad spectrum associated with the technology. It is reported that vitamin D is the specifically altered by UV wavelengths. Carbohydrates tend to be unaffected by light (Spikes, 1981).

### ii) Effect on lipid oxidation by PUV light

Lipid peroxides are produced in many foods when exposed to irradiation by UV or visible light. Reactive lipid free radicals can furthermore react with other nutrients such as amino acids and carotenoids. The lifetime of the free radicals is dependent on the water activity of the food (Spikes, 1981). The most common technique to assess lipid oxidation in meat products is an assay developed by Tarladgis et al., (1960) using 2-thiobarbituric acid (TBA) to liberate malonaldehyde from unsaturated fatty acids as an indicator of oxidation.

Pulsed light includes the visible light and infrared spectrum which produce heat as a byproduct of application. Additional heat can cause undesirable attributes including potential increase in microbial load if a cooling step is not to follow. UV light also serves

as an activation agent of lipid peroxidation. Oxidation in food products can lead to an off flavor and increased rancidity. A study using pulsed light as a decontamination intervention in frankfurters noted high amounts of lipid peroxidation at close distances (Keklik, et al. 2009). If applied appropriately, negative effects can be avoided when using pulsed UV light.

UV light induces oxidation processes in meat which affects the sensorial properties of the product. This disadvantage is also observed with pulsed UV light but is limited due to the short pulsation. As reported by Paskeviciute et al., (2011) lipid oxidation varied between 0.204 and 1.019 mg of malonaldehyde per kilogram of meat depending on the total dosage. In the same study, after 5.1 J/cm$^2$ exposure to PUV light there was no detection of raw meat smell, chicken broth flavor/taste or cooked meat flavor/taste changes when compared with control. Furthermore, there was no visual color change in raw chicken after PUV light treatment.

### iii) Effect on protein oxidation by PUV light

Protein oxidation is identified as one of the leading causes to quality deterioration in meat, including poultry. The progression of protein oxidation stems from the presence of high concentrations of unsaturated lipids; heme pigments metal catalysts and other free oxidizing radicals. The sensory changes associated with protein oxidation in meat and poultry include deterioration of flavor, discoloration, nutrient destruction and formation of toxic compounds. Oxidation of both lipids and proteins has been correlated to freezing of meat and tissue hardening. After freezing, these products exhibited reduced texture-forming ability and water-holding capacity. It has been suggested that oxidation of

proteins is the mechanism that is the ultimate contributor to reduced quality and functionality in meat products over time and after freezing (Xiong, 2000).

### iv) Effect on color by PUV light

Color is one of three (flavor and texture, also) quality attributes of food that determine the overall acceptability of a food product in the eyes of a consumer. With respect to meat and poultry, discoloration can be an indicator that the product is spoiled. Discoloration in food products is a natural chemical change that occurs over time. Processing interventions can contribute to the overall rate of color change (Wroslstad & Smith, 2010). Color is measured quantitatively using one of two specific methods; Hunter color or CIE L*a*b* color scales which both have chromameters. These methods use three dimensional spaces that consist of luminous reflectance, lightness and chromacity coordinates, which was developed by the Commission International del'Eclairage or International Commission on Illumination in 1931. Color parameters are identified as L, a, and b for the Hunter color scale and L*, a*, and b* for CIE L*a*b* color scale. The number associated with L/L* indicate the lightness of a sample. The numbers associated with a/a* and b/b* are the chromaticity coordinates. Chromatity can be expressed as –a, +a, -b, and +b values indicating green, red, blue, and yellow color, respectively. (Sapan et al., 1999; Bialka, 2006; Wroslstad & Smith, 2010). Determination of color parameters of poultry products are used to evaluate the effect, if any after treatment interventions that could have a potential.

## Summary of Literature Review

Poultry products are one of the most common sources of protein worldwide. As global population increases, the total consumption of these products continues to grow as well. Poultry products continue to be one of the largest contributors of foodborne illness outbreaks in both the United States and globally. Food safety innovation is necessary to produce a safe product for consumption. Establishing alternative interventions of microbial reduction will continue to ensure food safety regulations are obtained using the most cost effective and efficient techniques. The 2016 Food Safety and Inspection Service amendments to the poultry performance standards will reduce the occurrence of foodborne illness outbreaks as they relate to poultry products. Furthermore, these standards also provide a challenge for the poultry industry to innovate and implement the necessary interventions to meet these new establishments.

One of these emerging technologies, pulsed UV light has been reported to reduce microbial contamination including, *Salmonella* and *Campylobacter.* Various studies already support the effectiveness of pulsed UV light as a microbial decontamination intervention. Therefore, this research has been undertaken to evaluate the efficacy of pulsed UV light's ability to reduce *Salmonella*, *Campylobacter* and *E. coli.* Quality parameters have also been addressed to better understand PUV lights treatment effects on poultry products. The results from this research will help establish the necessary parameters for the potential application of this intervention in commercial poultry processing.

# Chapter 3

## Decontamination of skin and lean surface chicken thigh meat by pulsed ultraviolet light using a static treatment system


### Abstract

With continued incidences of foodborne illness outbreaks associated with raw chicken, there is a need to explore different microbial interventions to reduce pathogens. The effectiveness of Pulsed Ultra-Violet (PUV) light for destruction of *Salmonella, E. coli* and *Campylobacter* on the surface of chicken thigh meat was investigated. Lean and skin surface chicken thighs were inoculated to a 6-7 log CFU/cm$^2$ before exposure to PUV light in separate trials for each of the above mentioned pathogens. Treatment variables included the distance from the quartz window of the PUV light (8 and 13 cm) and treatment time (0, 5, 15, 30, and 45 seconds). Comparison of treated samples to control (0 sec) samples allowed for quantification of microbial reduction due to PUV light treatment. For lean and skin surface samples, the distance by treatment time interaction was not significant ($P>0.05$) for reduction of *E. coli*, *Campylobacter,* or *Salmonella* counts. Distance from the PUV light did not affect lean surface microbial reduction for *E. coli*, *Campylobacter,* or *Salmonella* ($p>0.05$). Lean surface microbial reduction increased ($p<0.05$) with PUV light exposure time for *E. coli*, *Campylobacter,* and *Salmonella*. Exposure to PUV light for 5, 15, 30 and 45 seconds on lean surface thighs resulted in $\log_{10}$ reductions of 1.2, 1.5, 1.8 and 2.0 for *E.coli,* 1.5, 1.7, 1.8 and 2.2 for *Campylobacter*, and 1.6, 1.8, 2.0 and 2.4 for *Salmonella*, respectively. Distance from the PUV light did not affect skin surface microbial reduction for *Campylobacter* ($p>0.05$). Skin surface microbial reduction for *E. coli* and *Salmonella* was increased ($p<0.05$) with

closer proximity to the PUV light source. Skin surface microbial reduction increased ($p<0.05$) with PUV light exposure time for *E. coli, Campylobacter,* and *Salmonella*. PUV light exposure for 5, 15, 30 and 45 seconds on skin surface thighs resulted in $\log_{10}$ reductions of 1.2, 1.4,1.7, and 2.0 for *E.coli,* 1.1, 1.3, 1.6, and 1.9 for *Campylobacter*, and 0.9, 1.3, 1.6, and 1.8 for *Salmonella,* respectively. Results indicate the potential of PUV light as a microbial reduction application for the surface of raw chicken meat.

**Key Words:** chicken thigh, pulsed ultraviolet light, *Salmonella*, *E. coli*, *Campylobacter*

## Introduction

Since the 1960's, consumption of poultry has increased due to low production cost, high nutritional value and increased demand. The average annual per capita consumption of chicken has increased from 7.7 kg in 1990 to 13.8 kg in 2015 (Henchion et al., 2014; FAO, 2015). In 2016, the Organization for Economic Cooperation and Development (OECD)/ Food and Agriculture Organization (FAO) of the United Nations projected that global poultry consumption would increase by 10% over the next decade (Poultry Trends, 2017). As consumption of poultry products continues to increase, the microbial risks associated with these products increases as well. In 1990, there were 7 foodborne illness outbreaks associated with chicken, accounting for 1.6% of all foodborne illness outbreaks in the United States (CDC, 1996). In 2015, there were 22 chicken associated illnesses, which accounted for 11% of all foodborne illness outbreaks in the United States (CDC, 2017a). Increasing microbial safety of chicken products is important to both consumers and processors (McLeod, et al. 2018, Keklik, et al. 2010). Industry efforts to address this need include a variety of antimicrobial interventions applied during chicken processing.

*Salmonella* spp., *Escherichia coli* O157:H7 and *Campylobacter* represent some of the most prevalent foodborne pathogens associated with raw chicken (USDA, 2012; Haughton et al., 2011; McLeod et al., 2018). The report by the Foodborne Disease Active Surveillance Network indicated that the number of foodborne illness outbreaks caused by *Salmonella*, *E. coli*, and *Campylobacter* was 535, 99 and 23, respectively (CDC, 2015). Chicken provides all of the necessary conditions needed to harbor and grow these pathogens, and if consumed, without adequate heating or by cross contamination, can

lead to gastroenteritis and other negative symptoms (Sarjit and Dykes, 2016; Keener et al., 2004; Keklik et al., 2011; Coburn et al., 2007).

In 2016, the Food Safety Inspection Service (FSIS) within the United States Department of Agriculture (USDA) implemented performance standards for Salmonella spp. and Campylobacter. This new protocol set the maximum acceptable percent positive for chicken parts to 15.4% and 7.7% for *Salmonella* spp. and *Campylobacter*, respectively. The intention of this protocol is to reduce the occurrence of foodborne illness outbreaks associated with raw poultry (USDA, 2012; Federal Register, 2016).

Currently, the poultry industry commonly uses chlorine or organic acid solutions as antimicrobial interventions. Less common are other chemical and physical treatments such as, trisodium phosphate, high hydrostatic pressure, irradiation, pulsed-field electricity and ultraviolet light (UV) (Bolder, 1997; Demirci & Ngadi, 2012; Zhi-Hong et al., 2018). Pulsed Ultra-Violet (PUV) light is an emerging technology that utilizes light with a broader spectrum than UV only, and it also uses a higher intensity than conventional UV systems. Conventional UV wavelengths include a spectrum of 100 – 400 nm. The germicidal, UV-C wavelengths fall between 100 – 280 nm with the maximum germicidal effect at 254 nm. PUV light uses a xenon bulb to produce a broad wavelength spectrum, 100 – 1100 nm, with over 50% of the energy deriving from the UV region. Unlike the continuous, low intensity output of conventional UV light, PUV light is emitted in short bursts of very high intensity light (Bialka et al., 2008; Demirci & Ngadi, 2012; Keklik et al., 2010). In a review by Gomez-Lopez et al., (2007), a continuous UV lamp delivered 3.9 $mW/cm^2$ and two types of xenon pulsed lamps delivered in excess of 60 $W/cm^2$ in the fluence range of 0-200 $mJ/cm^2$. In 1996, the Food

and Drug Administration approved the use of pulsed light (up to 12.0 J/cm$^2$) for microbial inactivation on foods (FDA, 2015).

The mechanisms of microbial inactivation by PUV light are of photochemical (disruption of DNA structure through creation of thymine dimers), photothermal (localized heating at the surface of the food product) and photophysical (micro-vibrations created at the surface of the cell by high-energy pulses) (Krishnamurthy et al., 2010; Elmnasser et al., 2007; Gomez-Lopez et al., 2007).

Further research is needed to investigate the microbial reduction potential and quality effects of PUV light across different chicken parts both with and without the presence of skin. Current research has not investigated samples with higher fat content, differing meat chemistry and varying muscle orientation. In this study, the effectiveness of PUV light's ability to inactivate *Salmonella* spp., *E. coli*, and *Campylobacter* on the surface of raw chicken thighs was evaluated.

## Materials and Methods

### Bacterial strains

*Salmonella enterica* subsp. *enterica* ser. Typhimurium (ATCC 13311) was obtained from the American Type Culture Collection (Manassas, VA). *Escherichia coli* K12 was obtained from the *E. coli* Reference Center at the Pennsylvania State University. Antibiotic resistant cultures (*Salmonella* Typhimurium and *E. coli* K12) were prepared as described by Catalano and Knabel (1994), to allow for the use of antibiotic specific media. Nalidixic acid (ARCOS Organics, Geel, Belgium) and streptomycin sulfate

(Fisher BioReagents, Fair lawn, NJ) resistant (NSR) cultures of *S.* ser. Typhimurium and *E. coli* K12 were obtained from frozen culture stock of Keklik et al. (2010 and 2011), respectively. Cultures were maintained separately in tryptic soy broth (BD-Difco, Franklin Lakes, NJ) supplemented with 0.6% yeast extract and 100 µg/mL of each nalidixic acid and streptomycin sulfate (TSBYE-NS). *S.* ser. Typhimurium and *E. coli* K12 were plated on tryptic soy agar (BD-Difco, Franklin Lakes, NJ) supplemented with 0.6% yeast extract and 100 µg/mL of each nalidixic acid and streptomycin sulfate (TSAYE-NS). Colonies of *S.* ser. Typhimurium NSR and *E. coli* K12 NSR were both isolated from TSAYE-NS and were used to prepare separate stock cultures stored in 20% glycerol and kept at -80ºC. Working cultures were sub-cultured every 14 days and were maintained in TSBYE-NS at 4ºC.

*Campylobacter jejuni* (ATCC 29428) stock culture was stored in 20% glycerol and kept at -80ºC. *C. jejuni* stock culture (1 mL) was transferred to 10 mL of Brucella Broth (Criterion, Santa Maria, CA) and incubated at 37ºC for 24-72 hours under anaerobic conditions (<0.1% O2 and >16% CO2) using GasPak Anaerobic System (BD, Franklin Lakes, NJ). A loopful of the Brucella Broth was plated on Charcoal Cefoperazone Deoxycholate agar (CCDA-Preston; Oxoid, Hampshire, UK) and incubated at 37ºC for 24-72 hours under microaerophillic conditions. Presumptive colonies representing morphology of *C. jejuni* were confirmed using the Microgen *Campylobacter* Latex Agglutination Kit (Microgen Bioproducts Ltd., Camberley, UK). Working cultures sub-cultured every 3 to 5 days and were maintained on CCDA under anaerobic conditions at 37±2 ºC.

**Pulsed ultraviolet light static system**

Pulsed Ultraviolet light was generated using a SteriPulse-XL 3000 Pulsed Light System (Xenon Corporation, Wilmington, MA). The system, represented in Figure 3-1, had an energy output of 1.27 J/cm2 per pulse at 1.8 cm below the xenon bulb. The xenon bulb pulsed 3 times per second, with each pulse lasting 360 μsec. A polychromatic burst was produced with each pulse emitting wavelengths ranging from 100 to 1,100 nm. Over 54% of the emitted energy was in the UV broad-spectrum (100-400 nm) (Bialka et al., 2008; Kristnamurthy et al., 2010).

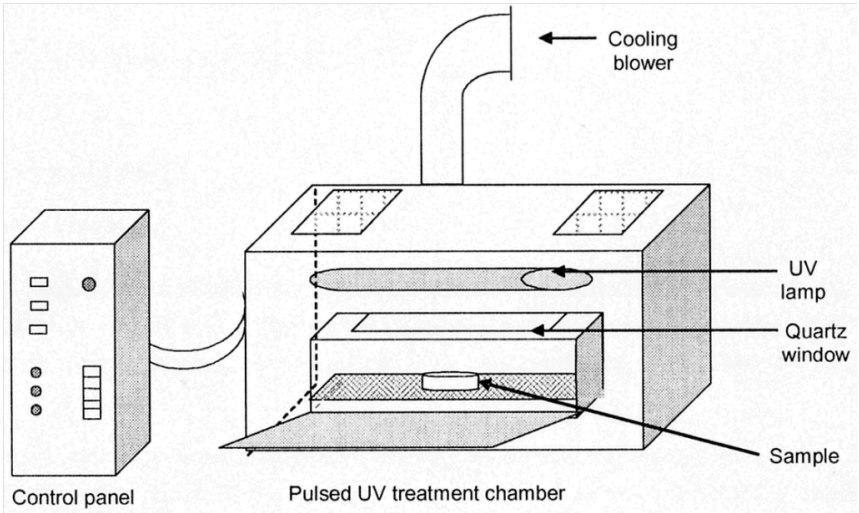


**Figure 3-1.** Representation of SteriPulse -XL 3000 Pulsed UV-Light System (Bialka et al., 2008).

**Inoculum preparation**

In separate trials, 1 mL of NSR *S.* ser. Typhimurium NSR or *E. coli* K12 NSR working cultures were transferred to 100 mL of TSBYE-NS and incubated at 37ºC for 24 hours and then centrifuged (30 minutes at 3,300 × g and 10ºC). After incubation the supernatant was removed and sterile 0.1% peptone water (BD, Franklin Lakes, NJ) was used to resuspend the cells. The suspension was centrifuged under the same conditions.

After the supernatant was removed, the pellet was re-suspended with 50 mL of sterile 0.1% peptone water, yielding 108-109 cfu/mL.

*C. jejuni* colonies were isolated on CCDA as previously described, transferred to 10 mL of Brucella Broth and incubated under anaerobic conditions at 37ºC for 48 hours. After incubation the solution was centrifuged (5 minutes at 3,300 × g at 10ºC) and the supernatant was discarded. The pellet was then rinsed with sterile 0.1% peptone water and re-centrifuged under the same conditions. Cells were re-suspended with 5mL of sterile 0.1% peptone water, yielding $10^6$-$10^7$ cfu/mL.

**Chicken thigh meat preparation**

Chicken thigh meat was obtained from a local poultry processing plant and kept frozen (ca. -17ºC) until use. Chicken meat was transferred to a refrigerator (ca. 2ºC) to thaw 48 hours prior to each trial. Thighs were removed from the refrigerator and allowed to warm to ambient temperature (~21ºC) 2 hours before the trial. Lean or skin surface of chicken thighs were cut to standard sized pieces (5 × 5 × 1 cm).

**Inoculation of chicken thigh meat**

In individual trials, NSR *S.* ser. Typhimurium NSR, *E. coli* K12 NSR or *C. jejuni* were applied to the top surface of the chicken thigh samples (5 × 5 × 1 cm). To obtain $10^5$-$10^6$ cfu/cm$^2$ on the surface, 0.1 mL of inoculum solution was applied and spread evenly to the top surface of each chicken sample (n = 9). Following inoculation, NSR *S.* ser. Typhimurium NSR and *E. coli* K12 NSR samples were held for 1 hour at ambient temperature (~21ºC) to allow for bacterial attachment. To reduce the death of the

microaerophilic *C. jejuni*, inoculated samples were held for a shorter period of time, 30 minutes.

**Pulsed ultraviolet light treatment**

Inoculated thigh samples (n = 9) were individually subjected to PUV light treatment. Treatment variables included the distance from the quartz window of the PUV light unit (8 and 13 cm) and treatment time (5, 15, 30, and 45 seconds; 3 pulses per second). Nine replications were used for each treatment combination.

**Microbial analysis**

After treatment with pulsed UV light, chicken samples were transferred to a filtered stomacher bag (Classic 400, Seward Ltd., Worthing, UK) with 25 mL of buffered peptone water (BPW; Oxoid, Hampshire, UK). Samples were then homogenized (Model 400, Seward Ltd., Worthing, UK) for 2 minutes at 260 rpm. Solutions filtered out of the samples were serially diluted in 9 ml of BPW. NSR *S.* ser. Typhimurium NSR, *E. coli* K12 NSR samples were auto-plated on TSAYE-NS plates using an autoplater (Autoplate 4000, Spiral Biotech; San Diego, CA) and incubated at 37ºC for 24 hours. *C. jejuni* samples were manually plated since the charcoal based agar did not allow for enumeration using the Autocount system. *C. jejuni* was plated on CCDA plates and incubated at 37ºC for 48 hours. TSAYE-NS plates were enumerated using an autocounter (Q-Count, Version 2.1, Spiral Biotech; San Diego, CA). CCDA plates were visually enumerated. For both enumeration methods, counts were expressed as cfu/$cm^2$. $Log_{10}$ reductions were determined by comparing plate counts of treated samples to the plate

counts of controls (untreated samples). *S.* ser. Typhimurium NSR colonies were confirmed using Microgen *Salmonella* Latex Agglutination Kit (Microgen Bioproducts Ltd., Camberley, UK). C. jejuni colonies were confirmed using Microgen *Campylobacter* Latex Agglutination Kit (Microgen Bioproducts Ltd., Camberley, UK). *E. coli* colonies were visually confirmed by Gram staining.

**Temperature measurements**

Temperature profile during the pulsed UV light treatment was determined using a type K thermocouple (Omegaette HH306, Omega Engineering Inc, Stamford, CT) with a15 cm long thermocouple probe placed 2 mm under the surface of the chicken thigh sample. The 1 x 1 mm tip measured the fluctuation in temperature. During exposure to PUV light, temperatures were recorded at 5 second intervals for up to 45 seconds at 8 and 13 cm from the quartz window of the PUV light unit.

**Energy measurements**

Using a Nova Laser Power/Energy Monitor (OPHIR Optronics Ltd., Wilmington, MA) energy delivered to the location of the sample surface was recorded at 8 and 13 cm from the quartz window of the PUV light unit. Literature references that energy emitted from xenon flashlamps consists of 54% of the UV spectra (Bialka, et al. 2008). The energy monitor reported total energy delivered. Energy recordings were averaged over 30 pulses and then calculated according to exposure duration to assess total energy (joules/$cm^2$) delivered to the sample.

energy (J/pulse) / area of Nova Laser monitor (15.9$cm^2$) = J/ pulse/$cm^2$

J/pulse/$cm^2$ * 3 pulses/second = J/$cm^2$/second

$J/cm^2$/second * durations of exposure (seconds) = $J/cm^2$ delivered to sample

**Statistical analysis**

Complete experimental design was replicated 9 times. Statistical Analysis Software 9.4 (SAS, Cary, NC) was used as the statistical platform for analysis. An overall 2-way ANOVA with predicator variables proximity and duration was used to assess the microbial reduction associated with proximity, duration and their interaction. When needed, Tukey multiple comparison test was used to establish a significant confidence interval for treatment conditions at 95%.

## Results and Discussion

$Log_{10}$ reductions due to PUV light exposure for *Campylobacter jejuni*, *E. coli* K12 NSR and *Salmonella* ser. Typhimurium NSR were evaluated and compared for both skin and lean surface chicken thigh. Furthermore, temperature and energy profiles were assessed for each treatment parameter during the pulsed UV light treatment.

**Microbial reduction**

The $log_{10}$ reduction of *Campylobacter jejuni*, *E. coli* K12 NSR and *Salmonella* ser. Typhimurium NSR on the surface of raw chicken thighs after treatment by pulsed UV light was assessed at treatment times of 5, 15, 30 and 45 seconds and at 8 or 13 cm from the quartz window of the PUV unit.

**i) Lean surface chicken thigh**

The distance by treatment time interaction for lean surface samples was not significant ($P>0.05$) for microbial reduction of *E. coli*, *Campylobacter* or *Salmonella*.

The evaluation of each treatment variable, while holding the other variable constant, showed no significant difference ($p>0.05$) due to proximity to the PUV light. Microbial reduction on the surface of chicken thighs after 45 seconds of PUV light exposure was significantly greater ($p\leq0.05$) when compared to 5 seconds of exposure. Lean surface microbial reduction increased ($p<0.05$) with PUV light exposure time for *E. coli*, *Campylobacter* and *Salmonella*. Exposure to PUV light for 5, 15, 30 and 45 seconds on lean surface thighs resulted in $\log_{10}$ reductions of 1.2, 1.5, 1.8 and 2.0 for *E. coli*, 1.5, 1.7, 1.8 and 2.2 for *Campylobacter*, and 1.6, 1.8, 2.0 and 2.4 for *Salmonella*, respectively (Table 3-1).

Similar results were reported by Keklik et al., (2010) who studied the effect of PUV light for the reduction of *Salmonella* ser. Typhimurium on the surface of boneless skinless chicken breast. They reported $\log_{10}$ reductions of *Salmonella* (cfu/cm$^2$) ranging from 1.2 to 2.4 after a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively. Chun et al., (2009) tested the efficacy of UV-C for reduction of *Campylobacter jejuni*, *Listeria monocytogenes*, and *Salmonella* typhimurium. Chicken breasts samples were inoculated with 6-7 $\log_{10}$ cfu/gram of the prior mentioned pathogens, and exposed to 5 kJ/m$^2$ of UV-C (254 nm) irradiation. There was a reduction of the initial populations of *C. jejuni*, *L. monocytogenes*, and *S.* typhimurium by 1.26, 1.29, and 1.19 log10 CFU/g, respectively. In another study, McLeod et al., (2017) subjected boneless skinless chicken breast fillets inoculated with pathogenic bacteria to pulsed UV light. Pulsed UV light exposure with fluences ranging from 1.25 to 18 J/cm$^2$ resulted in average reductions from 0.9 to 3.0 $\log_{10}$ (cfu/cm$^2$) of *S.* Enteritidis, *L.*

*monocytogenes*, *S. aureus*, *E. coli* EHEC, *E. coli* ESBL, *Pseudomonas* spp., *B thermospacta* and *C. divergens*.

**Table 3-1.** Effects of PUV light exposure and proximity on $\log_{10}$ reductions of *Campylobacter jejuni*, *E. coli* K12, and *Salmonella* ser. Typhimurium on raw lean surface chicken thighs

| Parameters | | Microorganism | | |
|---|---|---|---|---|
| | | Campylobacter | Salmonella | E. coli |
| Duration (seconds) | 5 | $1.4\pm0.2^{A}$ | $1.6\pm0.5^{A}$ | $1.2\pm0.3^{A}$ |
| | 15 | $1.7\pm0.2^{B}$ | $1.8\pm0.4^{AB}$ | $1.5\pm0.4^{AB}$ |
| | 30 | $1.8\pm0.2^{B}$ | $1.9\pm0.5^{AB}$ | $1.7\pm0.2^{BC}$ |
| | 45 | $2.1\pm0.3^{C}$ | $2.4\pm0.7^{C}$ | $2.0\pm0.2^{C}$ |
| Proximity[1] (cm) | 8 | $1.8\pm0.2^{A}$ | $1.9\pm0.5^{A}$ | $1.6\pm0.3^{A}$ |
| | 13 | $1.7\pm0.2^{A}$ | $1.9\pm0.5^{A}$ | $1.6\pm0.3^{A}$ |

[1] Distance from the quartz window, which is 5.8cm below the UV strobe.
[A-C]Means within a column and treatment group without a common superscript are significantly different ($p<0.05$).

**ii) Skin surface chicken thigh**

The distance by treatment time interaction for lean and skin surface samples was not significant ($P>0.05$) for microbial reduction of *E. coli*, *Campylobacter* or *Salmonella*. The effect of proximity to the quartz window of the PUV light was inconsistent across microbe strains for skin surface thigh. Proximity to the PUV light source had a significant effect on both *S* Typhimurium and *E. coli* with closer proximity resulting in greater reduction ($p<0.05$). Distance from the PUV light did not affect skin surface microbial reduction for *Campylobacter* ($p>0.05$). Skin surface microbial reduction for *E. coli* and *Salmonella* was increased ($p<0.05$) with closer proximity to the PUV light source. Skin surface microbial reduction increased ($p<0.05$) with PUV light exposure time for *E. coli*, *Campylobacter* and *Salmonella*. PUV light exposure for 5, 15, 30 and 45 seconds on skin surface thighs resulted in $\log_{10}$ reductions of 1.2, 1.4,1.7 and 2.0 for *E. coli*, 1.1, 1.3, 1.6 and 1.9 for *Campylobacter*, and 0.9, 1.3, 1.6 and 1.8 for *Salmonella*, respectively (Table

3-2). A similar study, Haughton et al., (2011) evaluated the reduction of *Campylobacter* spp., *E. coli* and *S.* Enteriditis on boneless skinless chicken breast and chicken skin using high intensity light pulses (3 Hz, 505 J/pulse and pulse duration of 360 μs). After 30 seconds of treatment, inoculated chicken skin had $\log_{10}$ (cfu/g) reductions of 1.22, 1.69 and 1.27 for *C. jejuni*, *E. coli* and *S.* Enteritidis, respectively. Corresponding reductions on inoculated boneless skinless chicken breast had $\log_{10}$ (cfu/g) reductions of 0.96, 1.13 and 1.35 for *C. jejuni*, *E. coli* and *S.* Enteritidis.

**Table 3-2.** Effects of PUV light exposure and proximity on $\log_{10}$ reductions of *Campylobacter jejuni*, *E. coli* K12, and *Salmonella* ser. Typhimurium on raw skin surface chicken thighs

| Parameters | | Microorganism | | |
|---|---|---|---|---|
| | | Campylobacter | Salmonella | E. coli |
| Duration (seconds) | 5 | $1.1\pm0.2^{A}$ | $0.9\pm0.3^{A}$ | $1.2\pm0.3^{A}$ |
| | 15 | $1.3\pm0.2^{AB}$ | $1.3\pm0.2^{B}$ | $1.4\pm0.3^{AB}$ |
| | 30 | $1.5\pm0.2^{B}$ | $1.6\pm0.3^{BC}$ | $1.7\pm0.2^{BC}$ |
| | 45 | $1.9\pm0.2^{C}$ | $1.8\pm0.2^{C}$ | $1.9\pm0.4^{C}$ |
| Proximity[1] (cm) | 8 | $1.4\pm0.2^{A}$ | $1.5\pm0.2^{A}$ | $1.6\pm0.3^{A}$ |
| | 13 | $1.4\pm0.2^{A}$ | $1.3\pm0.3^{B}$ | $1.5\pm0.3^{B}$ |

[1] Distance from the quartz window, which is 5.8cm below the UV strobe.
[A-C]Means within a column and treatment group without a common superscript are significantly different ($p<0.05$).

## Temperature and energy measurements

After warming at room temperature (~21ºC) for two hours, the initial temperature of the raw chicken thigh samples was 22.2 ± 2.2ºC. The fluence (J/cm²) and change in temperature were measured under each treatment condition. Values for energy levels and temperature change are shown in Tables 3-3 and 3-4, respectively. The total energy received by the chicken samples was expressed as Joules / $cm^2$. Energy levels ranged from 3.4 to 62.2 J/$cm^2$ for 5 s at 13 cm and 45 s at 8 cm, respectively. The amount of energy delivered at 8 was significantly greater ($p<0.05$) then the energy delivered at 13

cm. The amount of energy delivered significantly increased ($p<0.05$) with increased treatment time at both 8 and 13 cm form the quartz window the PUV light unit. These results are comparable to data reported by Keklik et al. (2010) who also measured total energy of PUV light delivered by boneless skinless chicken breast to range from 2.9 to 34.8 J/cm$^2$ during a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively.

The change in temperature was not significantly different ($p>0.05$) between skin and lean surface of raw chicken thighs (Table 3-4). The change in surface temperature for lean surface thighs ranged from 2.2 to 22.6ºC for 5 seconds at 13 cm and 45 seconds at 8 cm, respectively. The change in surface temperature for skin surface thighs ranged from 3.4 to 25.1 ºC for 5 seconds at 13 cm and 45 seconds at 8 cm, respectively. Closer proximity to the PUV light and increasing durations of time both significantly increased ($p<0.05$) the rise in temperature at the surface of the chicken thigh. Chicken thigh surface temperature increased during PUV light exposure but lean and skin surface temperatures were not significantly different ($p>0.05$) after PUV light exposure. In a comparable experiment temperature increases ranged from 3.9 to 36.8ºC after a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively (Keklik, et al. 2010). Ozur and Demirci (2006) evaluated the reduction of E. coli and L. monocytogenes on the surface of raw salmon filets after exposure to PUV light. After 60 seconds of exposure to PUV light, surface temperature increased, 91, 68 and 53 ºC at 3, 5 and 8 cm from the quartz window of the PUV lamp, respectively.

**Table 3-3.** Total energy (J/cm$^2$) changes after exposure to pulsed UV light.

| Proximity[1] | Treatment times (s) | | | |
|---|---|---|---|---|
| | 5 | 15 | 30 | 45 |
| 8cm | 6.9±0.1$^{A}$ | 20.7±0.2$^{B}$ | 41.5±0.4$^{C}$ | 62.2±0.6$^{D}$ |
| 13cm | 3.4±0.1$^{A}$ | 10.2±0.2$^{B}$ | 20.3±0.5$^{C}$ | 30.5±0.7$^{D}$ |

$^{A-D}$Means within row without the same letter are significantly different ($p>0.05$).
[1] Distance from the quartz window, which is 5.8cm below the UV strobe.

**Table 3-4.** Chicken thigh surface temperature changes ($T-T_{initial}$) after exposure to pulsed UV light.

| Proximity[1] | Thigh Surface | Treatment times (s) | | | |
|---|---|---|---|---|---|
| | | 5 | 15 | 30 | 45 |
| 8cm | Lean | 4.7±2.2$^{A}$ | 10.3±3.8$^{B}$ | 16.8±5.0$^{C}$ | 22.6±6.3$^{D}$ |
| | Skin | 4.2±1.9$^{A}$ | 9.7±2.3$^{B}$ | 17.4±2.3$^{C}$ | 25.1±3.0$^{D}$ |
| 13cm | Lean | 2.2±0.2$^{A}$ | 5.2±0.7$^{B}$ | 8.8±0.7$^{C}$ | 12.4±0.8$^{D}$ |
| | Skin | 3.4±1.0$^{A}$ | 7.1±1.6$^{B}$ | 11.6±2.3$^{C}$ | 15.2±2.4$^{D}$ |

$^{A-D}$Means within row without the same letter are significantly different ($p>0.05$).
[1] Distance from the quartz window, which is 5.8cm below the UV strobe.

## Conclusion

The results from this study demonstrated that PUV light treatment was effective in reducing *E. coli*, *Campylobacter* and *Salmonella* on the surface of raw chicken thighs. The results further indicated that of the two distances from the quartz window of the PUV light unit, 8 cm was a more effective proximity for microbial reduction. Increased PUV light exposure time led to greater microbial reductions of ~2 $\log_{10}$ (99%) at 45 seconds for all pathogens evaluated for both skin and lean surface chicken thighs. Longer treatment times and closer proximity to the PUV light bulb resulted in a significant ($p<0.05$) increase of surface temperature on the chicken thigh.

The performance standards outlined by FSIS do not provide a numerical requirement for microbial reduction of *Salmonella* and *Campylobacter*. The effectiveness

of PUV light in a commercial poultry processing environment would be dependent on total microbial contamination of the products prior to treatment. This study has provided evidence that PUV light can reduce microbial presence by 90-99%. A commercial operation that requires reduction values within the range of 1-2 $\log_{10}$ could consider PUV light as an alternative microbial reduction intervention for parted products.

The increase in temperature is a negative side-effect of PUV light treatment that needs to be addressed. The increased temperature after extensive exposure of PUV light results in a change of the biochemistry of the chicken parts surface. Similar effects as thermal treatment, the products surface begins to resemble a surface appearance comparable to cooking the product. This change is undesirable for the treatment of a product intended to be maintained as raw.

Continued investigation of PUV technology is needed to assess its efficacy in a commercial poultry processing environment and the effects of PUV light on quality properties of chicken.

## References

Bialka, K. L., & Demirci, A. (2008). Efficacy of pulsed UV-light for the decontamination of Escherichia coli O157: H7 and Salmonella spp. on raspberries and strawberries. *Journal of Food Science, 73*(5), M201-M207. https://doi.org/10.1111/j.1750-3841.2008.00743.x.

Bolder, N. M. (1997). Decontamination of meat and poultry carcasses. *Trends in food science & technology*, 8(7), 221-227. https://doi.org/10.1016/S0924-2244(97)01040-6

Catalano, C. R., & Knabel, S. J. (1994). Destruction of Salmonella enteritidis by high pH and rapid chilling during simulated commercial egg processing. *Journal of food protection*, *57*(7), 592-595. https://doi.org/10.4315/0362-028X-57.7.592

Chun, H. H., Kim, J. Y., Lee, B. D., Yu, D. J., & Song, K. B. (2010). Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. *Food Control*, *21*(3), 276-280. https://doi.org/10.1016/j.foodcont.2009.06.006

Centers for Disease Control and Prevention. (CDC, 1996). Surveillance Summaries, MMWR 1996;45 (No. SS-5).

Centers for Disease Control and Prevention. (CDC, 2015). Surveillance for Foodborne Disease Outbreaks, United States, Annual Report. Atlanta, Georgia: US Department of Health and Human Services. Available at: https://www.cdc.gov/foodsafety/pdfs/2015FoodBorneOutbreaks_508.pdf

Centers for Disease Control and Prevention. (CDC, 2017a). Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet 2015 Surveillance Report (Final Data). *Atlanta, Georgia: US Department of Health and Human Services.* Available at: https://www.cdc.gov/foodnet/reports/annual-reports-2015.html

Coburn, B., Grassl, G. A., & Finlay, B. B. (2007). Salmonella, the host and disease: a brief review. Immunology & Cell Biology, 85(2), 112-118. https://doi.org/10.1038/sj.icb.7100007

Demirci, A., & Ngadi, M. O. (2012). Microbial decontamination in the food industry: novel methods and applications. Oxford: Woodhead Publ.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A., & Federighi, M. (2007). Pulsed-light system as a novel food decontamination technology: a review. Canadian journal of microbiology, 53(7), 813-821. https://doi.org/10.1139/W07-042

Federal Register. (2016). Vol. 81, No. 28. [FR Doc No: 2016-02586]. New Performance Standards for Salmonella and Campylobacter in Not-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Changes to Related Agency Verification Procedures: Response to Comments and Announcement of Implementation Schedule. Available at: https://www.fsis.usda.gov/wps/wcm/connect/03910f0e-48f8-4111-ba52-490b07c25c24/2014-0023.htm?MOD=AJPERES

Food and Agriculture Organization of the United Nations. (FAO, 2015). World agriculture: Towards 2015/2030. An FAO perspective. Economic and Social Development Department. Available at: http://www.fao.org/docrep/005/y4252e/y4252e05b.htm

Food and Drug Administration. (FDA, 2015). Code of Federal Regulations. Pulsed light for the treatment of food: 21CFR179. 41.

Gomez-Lopez, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. *Trends in food science & technology*, *18*(9), 464-473. https://doi.org/10.1016/j.tifs.2007.03.010

Haughton, P. N., Lyng, J. G., Morgan, D. J., Cronin, D. A., Fanning, S., & Whyte, P. (2011). Efficacy of high-intensity pulsed light for the microbiological decontamination of chicken, associated packaging, and contact surfaces. *Foodborne pathogens and disease*, *8*(1), 109-117. http://doi.org/10.1089/fpd.2010.0640

Henchion, M., McCarthy, M., Resconi, V. C., Declan, T. (2014). Meat consumption: Trends and quality matters. *Meat Science,* 98(3), 561-568. https://doi.org/10.1016/j.meatsci.2014.06.007.

Keener, K. M., Bashor, M. P., Curtis, P. A., Sheldon, B. W., & Kathariou, S. (2004). Comprehensive review of Campylobacter and poultry processing. Comprehensive Reviews in Food Science and Food Safety, 3(2), 105-116. https://doi.org/10.1111/j.1541-4337.2004.tb00060.x

Keklik, N. M., Demirci, A., & Puri, V. M. (2010). Decontamination of unpackaged and vacuum-packaged boneless chicken breast with pulsed ultraviolet light. *Poultry science*, *89*(3), 570-581. doi: 10.3382/ps.2008-00476

Keklik, N. M., Demirci, A., & Bock, R. G. (2011). Surface decontamination of whole chicken carcasses using a pilot-scale pulsed UV light system. *Transact. ASABE*, *54*, 993-1000. doi: 10.13031/2013.37083

Krishnamurthy, K., J. C. Tewari, J. Irudayaraj, and A. Demirci. (2010). Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV

light and infrared heating. Food Bioprocess Technol. 3:93–104. https://doi.org/10.1007/s11947-008-0084-8

McLeod, A., Hovde Liland, K., Haugen, J. E., Sørheim, O., Myhrer, K. S., & Holck, A. L. (2018). Chicken fillets subjected to UV-C and pulsed UV light: Reduction of pathogenic and spoilage bacteria, and changes in sensory quality. Journal of Food Safety, 38(1), e12421. https://doi.org/10.1111/jfs.12421

Ozer, N. P., & Demirci, A. (2006). Inactivation of Escherichia coli O157: H7 and Listeria monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment. International journal of food science & technology, 41(4), 354-360. https://doi.org/10.1111/j.1365-2621.2005.01071.x

PoultryTrends. (2017). The Statistical Reference for Poultry Executives. WATT Poultry USA. Available at: http://www.poultrytrends.com/201711/index.php#/1

Sarjit, A., & Dykes, G. A. (2017). Transfer of Campylobacter and Salmonella from Poultry Meat onto Poultry Preparation Surfaces. *Journal of food protection*, *80*(5), 750-757. https://doi.org/10.4315/0362-028X.JFP-16-414

United States Department of Agriculture. (USDA), (2012). Food Safety and Inspection Service. Office of Public Health Science Microbiology Division The Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Survey January 2012–August 2012. https://www.fsis.usda.gov/wps/wcm/connect/a9837fc8-0109-4041-bd0c-729924a79201/Baseline_Data_Raw_Chicken_Parts.pdf?MOD=AJPERES

Zhi-Hong, Z., Lang-Hong, W., Xin-An, Z., Zhong, H., & Charles, B.S. (2018). Non-thermal technologies and its current and future application in the food industry: a review. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.13903

# Chapter 4

## Decontamination of skin and lean surface chicken meat by pulsed ultraviolet light using a conveyor treatment system


### Abstract

The number of foodborne illness outbreaks associated with raw chicken has continued to increase in the recent decades. Emerging antimicrobial technologies can provide interventions to achieve greater microbial reductions and reduce the prevalence of associated pathogens. The effectiveness of pulsed ultraviolet (PUV) light positioned over a conveyor system was evaluated for destruction of *E. coli* on the surface of raw chicken parts. Boneless/skinless (B/S) chicken breasts, B/S chicken thighs and bone-in/skin-on chicken thighs were inoculated with ~8 log cfu/cm$^2$ concentration of *E. coli* before exposure to PUV light. The total energy (J/cm$^2$) of PUV light delivered to the surface of the chicken parts was evaluated based on conveyor speed. The conveyor was set at 10 cm below the quartz window of the PUV light units and total energy (5, 10, 20, 30 J/cm$^2$) was controlled by adjusting conveyor speed. Samples were passed under the PUV lights twice for exposure of both the top and bottom of the products. Comparison of treated samples to untreated samples allowed for quantification of microbial reduction as a result of PUV light treatment. The product type by energy interaction for chicken samples was significantly different ($P<0.05$) for microbial reduction of *E. coli*. Microbial reduction of *E. coli* increased ($p<0.05$) with increased PUV light energy delivery for all chicken parts tested. Exposure to PUV light for 5, 10, 20, and 30 J/cm$^2$ resulted in $\log_{10}$ reductions of

0.34, 0.51, 0.74, and 0.94 for B/S breasts, 0.29, 0.47, 0.59, and 1.04 for B/S thighs and 0.10, 0.28, 0.48 and 0.62 for bone-in/skin-on thighs, respectively.

**Keywords:** chicken, pulsed ultraviolet light, conveyor, continuous treatment, *E. coli*

## Introduction

Raw chicken provides all of the necessary conditions needed to harbor and grow pathogenic microorganisms. The most prevalent foodborne pathogens associated with raw chicken include *Salmonella* spp., *Escherichia coli*, and *Campylobacter* (USDA, 2012; Haughton, et al. 2011; McLeod, et al. 2018). A report by the Foodborne Disease Active Surveillance Network indicated that the number of foodborne illness outbreaks caused by *Salmonella* spp., *E. coli*, and *Campylobacter* was 535, 99 and 23, respectively (CDC, 2015). In 1990, the Centers for Disease Control and Prevention (CDC) reported 7 foodborne illness outbreaks associated with chicken, which accounted for 1.6% of all foodborne illness outbreaks in the United States (CDC, 1996). In 2015, the CDC reported a total of 22 chicken associated foodborne illness outbreaks, which accounted for 11% of all outbreaks in the United States (CDC, 2017a). The increase of associated outbreaks corresponds with continued increase of production and consumption of chicken.

Current intervention steps used throughout poultry processing for the reduction of foodborne pathogens include the application antimicrobial solutions in the form of diluted hypochlorite or organic acid (citric acid, propionic acid, peracetic acid and lactic acid) rinses. Microbial reduction interventions are applied at several different points during poultry processing (Bolder, 1997; Demirci & Nagdi, 2012).

A review by Demirci & Ngadi, (2012) reports that the usage of hypochlorite solutions effectively reduces *Salmonella* and *Camplyobacter* by 0.1-2.4 and 0.2-3.0 logs, respectively. Regardless of its effectiveness, there is overall consumer dissatisfaction associated with the usage of chlorine. The usage of organic acids has become a common

alternative antimicrobial intervention to chlorine rinses that results in similar reductions of pathogens. Benefits of organic acids include its low cost and consumer acceptance. In the review by Demirci & Ngadi (2012), organic acid solutions have been proven in laboratory settings to result in a $\log_{10}$ reduction ranging from 0.6-1.8 for *Salmonella* and 0.2-1.7 for *Camplyobacter*. Killinger et al., (2010) reported over a 2 $\log_{10}$ reduction of aerobic plate counts and coliform levels on carcasses after treatment with 2% lactic acid in a 3 minute rinse. Regardless of its efficacy as an antimicrobial, certain concentrations of organic acid solutions can cause surface discoloration and other quality defects (Demirci & Ngadi, 2012).

With increased production and consumption of poultry, alternative microbial reduction interventions have been investigated, including the application of pulsed ultraviolet (PUV) light. PUV light is a novel process that includes the same germicidal effects associated with UV light. In the UV light spectrum, wavelengths between 100 and 280 nm produce microbial inactivation by altering DNA structure and damaging cellular membranes (Elmnasser et al., 2007; Koutchma, 2009).

Pulsed UV light uses a xenon flashlamp to produce a spectrum of 100 – 1100 nm, which includes conventional UV light (100 – 400 nm), along with other wavelengths. PUV light is emitted in short bursts of very high intensity, up to 10,000-fold greater than continuous, conventional UV light (Gomez-Lopez et al., 2007; Krishnamurthy et al., 2010). PUV light pulses can be manipulated as per the number and duration of pulses, but current literature references 3 pulses per second with each pulse lasting 360 µs as the most common application (Demirci & Ngadi, 2012).

Various studies have already established PUV light as an effective microbial reduction intervention using a model system in a laboratory.

The prior mentioned studies and previous research using a lab model to apply PUV light supports the ability of the technology to act as a microbial reduction intervention for the surface of raw chicken. In this study, the effectiveness of PUV light for microbial reduction of chicken cuts has been investigated using pulsed UV light chambers positioned over a conveyor system. *E. coli* K12 was be used as an indicator organism to determine the total $\log_{10}$ reductions.

## Materials and Methods

### Dynamic pulsed ultraviolet light conveyor system

Pulsed UV light was generated from the RC-802 Interweave Pulsed Ultraviolet System (Xenon Corporation, Wilmington, MA) which included two 16 inch, linear "C" type xenon flashlamps. The flashlamps were positioned on top of a conveyor (350 cm in length and 38 cm wide) with the ability to adjust light housings from 90° to 180° with respect to the long axis of the conveyor. The conveyor speed ranged from 4.0 to 72.0 feet/minute and proximity to the quartz window of the PUV light housing ranged from 3 to 15 cm. An image of the system is presented in Figure 4-1.

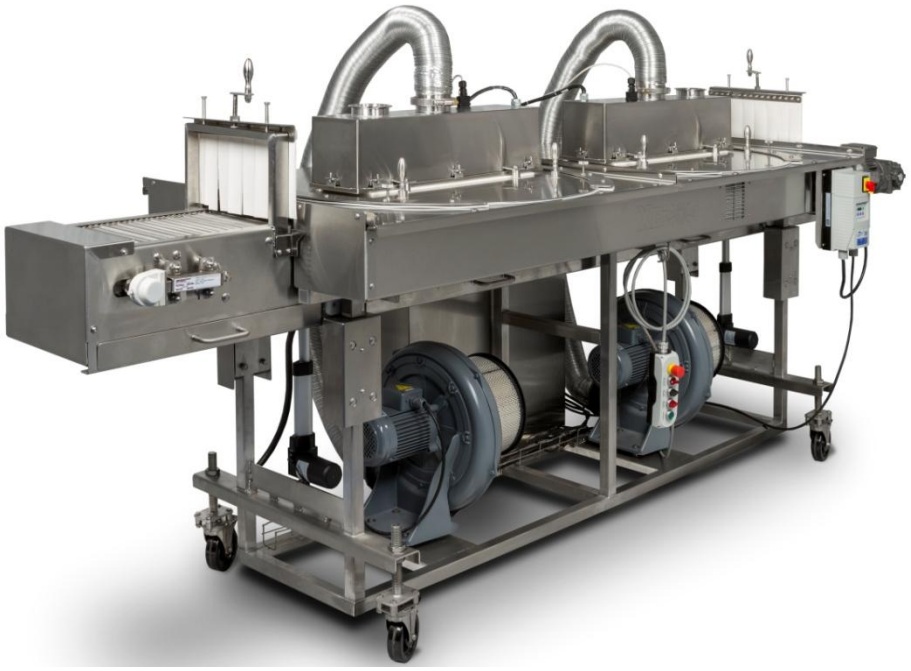

**Figure 4-1.** Image of pulsed UV conveyor system

The system, represented in figure 4-1, produces 6 pulses per second and 505 Joules per pulse from two 16 inch xenon gas flashlamps. Each individual xenon gas bulb pulses 3 times per second, with each pulse lasting 360 μsec. A polychromatic burst is produced from each pulse emitting wavelengths ranging from 100 to 1,100 nm. It was reported that 54% of the energy emitted originates from the UV spectrum (Kristnamurthy, et al. 2010; Bialka, et al. 2008).

**Setting of pulsed ultraviolet light system**

Calibration of the PUV light conveyor system was completed to ensure energy values delivered to the sample were 5, 10, 20 and 30 J/cm$^2$ as used in preliminary research. Energy values for comparison were collected directly beneath the PUV lights. The PUV light chambers were positioned at a 45º to inline with the conveyor system. Additional energy measurements were recorded along the entire length of the conveyor.

The conveyor speeds were recorded to ensure feet/second settings were appropriately represented.

**Microorganism**

Cultures were acquired from the *E. coli* Reference Center at the Pennsylvania State University. For the purpose of this project, Nalidixic acid (ARCOS Organics, Geel, Belgium) and streptomycin sulfate (Fisher BioReagents, Fair lawn, NJ) resistant (NSR) cultures were prepared as described by Catalano and Knabel (1994). Cultures were maintained in tryptic soy broth (TSB; BD-Difco, Franklin Lakes, NJ) supplemented with 0.6% yeast extract. 100 µg/mL of each nalidixic acid and streptomycin sulfate were added to the trypic soy broth with yeast extract (TSBYE-NS) to ensure antibiotic resistant culture growth and eliminate background flora during sampling. *E. coli* K12 was grown on tryptic soy agar (BD-Difco, Franklin Lakes, NJ) supplemented with 0.6% yeast extract and 100 µg/mL of each nalidixic acid and streptomycin sulfate (TSAYE-NS). Isolated colonies from TSAYE-NS were used to produce a stock culture stored in 20% glycerol and stored at -80ºC. Working cultures were sub-cultured from stock cultures and maintained in TSBYE-NS refreshed every 14 days and stored at 4ºC.

**Preparation of inoculum**

For each trial, 1 mL of *E. coli* K12 NSR working culture was transferred to 1000 mL of TSBYE-NS and incubated at 37ºC for 24 hours and then centrifuged (30 min at 3,300 × *g* and 10ºC). After incubation the supernatant was removed and 500 mL of sterile 0.1% peptone water (BD, Franklin Lakes, NJ) was used to resuspend the cells. The

suspension was centrifuged under the same conditions. After the supernatant was removed, the pellet was re-suspended with 1000 mL of sterile buffered peptone water (BPW; Oxoid, Hampshire, UK), yielding $10^8$ cfu/mL.

**Preparation of chicken parts**

Chicken parts were provided by a local poultry processing plant. Parts were kept frozen (ca. -17ºC) until use and transferred to a refrigerator (ca. 2ºC) 48 hours prior to each trial to thaw. Samples were removed from the refrigerator brought to room temperature (~18ºC) 2 hours before trials. Whole parts were weighed and recorded prior to inoculation.

**Inoculation of chicken samples**

In each trial, *E. coli* K12 NSR was applied to the chicken parts by means of total submersion (15 parts per 1000 mL of inoculant). Total submersion was used to achieve even distribution of *E. coli* and obtain ~$10^5$-$10^6$ cfu/$cm^2$ on the surface. Chicken parts were held under submersion for 30 minutes at room temperature (~18ºC) to allow for bacterial attachment of *E. coli* K12 NSR.

**Pulsed ultraviolet light treatment**

Inoculated chicken parts (n = 6) were individually subjected to PUV light treatment. The parts were placed on the conveyor and conveyor speed was adjusted to obtain the desired energy fluence. Total energy was achieved by adjusting the rate of conveyor speed (feet/second) at a fixed proximity of 10 cm below the quartz windows of

the PUV light units. Six replications were evaluated for each treatment. Samples were treated in two passes with 180º top to bottom rotation between passes to obtain exposure of all surfaces to PUV light. Conveyor speeds were adjusted to 25.8, 12.8, 6.4, and 4.3 feet/sec, to obtain fluencies of 5, 10, 20 or 30 J/cm$^2$, respectively at 10 cm below the quartz windows of the PUV light units.

**Microbial analysis**

After treatments of 5, 10, 20 or 30 J/cm$^2$ of PUV light, 25 cm$^2$ was removed from both treated surfaces of the chicken parts using a scalpel for a total of 50 cm$^2$. Surface samples were weighed to ensure a consistent total weight of approximately 50 g from each part. The cut samples of chicken parts were then transferred to a filtered stomacher bag (Classic 400, Seward Ltd., Worthing, UK) with 100 mL of buffered peptone water, (Oxoid, Hampshire, UK) creating a 2:1 dilution of BPW to chicken. Samples were then stomached (Model 400, Seward Ltd., Worthing, UK) for 1 min at 260 rpm. Solutions filtered out of the samples were serially diluted in buffered peptone water. *E. coli* K12 NSR samples were auto-plated on TSAYE-NS plates using an autoplater (Autoplate 4000, Spiral Biotech; San Diego, CA). After incubation at 37ºC for 24 hours, TSAYE-NS plates were enumerated using an autocounter (Q-Count, Version 2.1, Spiral Biotech; San Diego, CA). $Log_{10}$ (cfu/cm$^2$) reductions of bacteria numbers were determined by comparing plate counts of treated samples with the plate counts of controls (untreated samples).

**Temperature measurements**

Temperature profile along the conveyor of the pulsed UV light unit was determined using a type K thermocouple (Omegaette HH306, Omega Engineering Inc, Stamford, CT) with a 15 mm long thermocouple probe 2 mm under the surface of the chicken thigh sample. The probe measurements were derived from the 1 x 1 mm tip of the probe. Temperature measurements for 5, 10, 20 or 30 J/cm$^2$ (obtained by adjusting conveyor speed) were recorded.

**Energy measurements**

Using a Nova Laser Power/Energy Monitor (OPHIR Optronics Ltd., Wilmington, MA) energy received by the samples was determined by recording energy (J/cm$^2$) at stationary 5 cm increments along the length (350 cm) of the conveyor belt. The energy monitor provided an energy profile within the wavelength range of 100-1200 nm which accounts for the total energy delivered by the xenon flashbulbs (100-1100 nm).Energy recordings were averaged over 10 pulses and then calculated according to exposure duration to assess energy (J/cm$^2$) delivered to the sample. By plotting the change of the total energy delivered to the monitor an area under the curve was used to cumulate the total energy at a fixed point. Energy under the curve produced by each xenon flashlamp was summed and conveyor speed was calculated based on the following equations to obtain total energy values of 5, 10, 20, and 30 J/cm$^2$.

energy (J/pulse) / area of Nova Laser monitor (15.9cm$^2$) = J/ pulse/cm$^2$

J/pulse/cm$^2$ * 3 pulses/second = J/cm$^2$/second

J/cm$^2$/second * durations of exposure (seconds) = J/cm$^2$ delivered to sample

### Statistical analysis

Complete experimental design was replicated 6 times for evaluation of microbial reduction. Statistical Analysis Software 9.4 (SAS, Cary, NC) was used as the statistical platform for analysis. An overall 2-way ANOVA with predicator variables product type and energy were used to assess the microbial reduction associated with product type, energy and their interaction. When needed, Tukey multiple comparison test was used to establish a significant confidence interval for treatment conditions at 95%.

## Results and Discussion

$Log_{10}$ reductions due to PUV light exposure *E. coli* K12 NSR was evaluated and compared for B/S thighs, B/S breasts and bone-in/skin on thighs. Furthermore, temperature and energy profiles were recorded and reported for each fluence level during the pulsed UV light treatment.

### Microbial reductions

The $log_{10}$ reduction of *E. coli* K12 NSR on the surface of boneless/skinless (B/S) chicken thigh and breast and skin-on chicken thigh after treatment by the pulsed UV light conveyor system was assessed at energy values of 5, 10, 20 and 30 J/cm².

The product type by energy interaction for chicken samples was significantly different ($P<0.05$) for microbial reduction of *E. coli*. B/S thigh and breast both had significantly ($p<0.05$) greater microbial reduction compared to bone-in/skin-on thigh. As expected from the previous studies, an increase in total energy delivered to the surface of

the chicken resulted in a higher total $\log_{10}$ reduction of microbial cells. Microbial reduction on the surface of chicken parts after exposure of PUV light was significantly greater ($p<0.05$) with increasing energy (J/cm²) delivered. Furthermore, the evaluation of each treatment variable, while holding the other variable constant, was significant ($p<0.05$) due to both product type and energy. Exposure to PUV light for 5, 10, 20 and 30 J/cm² on the surface of B/S breast resulted in $\log_{10}$ reductions of 0.29, 0.47, 0.59, and 1.04, respectively. Exposure to the same energy of PUV light on the surface of B/S thighs resulted in $\log_{10}$ reductions of 0.34, 0.51, 0.74 and 0.94, respectively. PUV light exposure of the same energy values on the surface of skin-on thighs resulted in $\log_{10}$ reductions of 0.10, 0.28, 0.48, and 0.62, respectively (Table 4-1).

Similar microbial reduction values were reported by Keklik et al. (2010), who investigated the effect of PUV light for the reduction of *Salmonella* ser. Typhimurium on the surface of boneless skinless chicken breast. Keklik et al., (2010) reported $\log_{10}$ reductions of *Salmonella* (cfu/cm$^2$) ranging from 1.2 to 2.4 after a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively. McLeod et al., (2017) subjected boneless skinless chicken breast fillets inoculated with pathogenic bacteria to PUV light with fluences ranging from 1.25 to 18 J/cm$^2$ leading to reductions ranging from 0.9 to 3.0 $\log_{10}$ (cfu/cm$^2$) of *S.* Enteritidis, *L. monocytogenes*, *S. aureus*, *E.coli* EHEC, *E. coli* ESBL, *Pseudomonas* spp., *B thermospacta* and *C. divergens*. Haughton et al., (2011) evaluated the reduction of *Campylobacter* spp., *E. coli,* and *S.* Enteriditis on boneless skinless chicken breast and chicken skin using high intensity light pulses (3 Hz, 505 J/pulse and pulse duration of 360 μs). After 30 seconds of treatment, inoculated chicken skin had $\log_{10}$ (cfu/g) reductions of 1.22, 1.69, and 1.27 for *C. jejuni*, *E. coli* and *S.*

Enteritidis, respectively. Corresponding reductions after treatment on inoculated boneless skinless chicken breast resulted in $\log_{10}$ (cfu/g) reductions of 0.96, 1.13, and 1.35.

**Table 4-1.** $\text{Log}_{10}$ reductions of *E. coli* K12 on the surface of B/S thigh and breast and bone-in/skin-on thigh chicken after pulsed UV light treatments

| Chicken Product | Total Energy Delivered (J/cm²) | | | |
|---|---|---|---|---|
| | 5 | 10 | 20 | 30 |
| B/S Thigh | $0.34\pm0.09^{A}$ | $0.51\pm0.09^{B}$ | $0.74\pm0.09^{C}$ | $0.94\pm0.07^{D}$ |
| B/S Breast | $0.29\pm0.07^{A}$ | $0.47\pm0.10^{B}$ | $0.59\pm0.09^{C}$ | $1.04\pm0.08^{D}$ |
| Bone in/ Skin on Thigh | $0.10\pm0.04^{A}$ | $0.28\pm0.11^{A}$ | $0.48\pm0.14^{B}$ | $0.62\pm0.09^{C}$ |

$^{A-C}$Means within row without a common superscript are significantly different ($p<0.05$).

## Temperature and energy measurement

After warming at room temperature (~18º) for two hours, the initial temperature of the raw chicken thigh samples was 17.8 ± 0.6 ºC. At a fixed fluence (J/cm²) change in temperature was measured under each treatment condition. Energy levels used to treat chicken samples were 5, 10, 20, and 30 J/cm$^2$. The total energy delivered to the surface of the chicken samples was within the range of measured energy reported by Keklik et al. (2010) who measured total energy of PUV light received by boneless skinless chicken breast. Energy values in this study ranged from 2.9 to 34.8 J/cm2 during a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively.

The change in temperature was recorded after 5, 10, 20 and 30 J/cm$^2$ exposure of PUV light. Change in temperature was not significantly different ($p>0.05$) between skin and lean surface of raw chicken parts (Table 4-2). The change in surface temperature for chicken parts was 1.4, 3.0, 5.0 and 9.0 ºC after exposure of 5, 10, 20 and 30 J/cm$^2$, respectively. The change in temperature resulted in a final surface temperature for chicken parts of 19.1, 20.8, 22.9, and 26.9 ºC for 5, 10, 20, and 30 J/cm$^2$, respectively.

Energy delivery by PUV light significantly increased ($p<0.05$) the temperature at the surface of the chicken parts. The total change in temperature after exposure to PUV light was influenced by the duration of exposure needed to deliver 5, 10, 20 and 30 J/cm$^2$. It is important to note that altering the total time needed to deliver the prior mentioned energy amounts would influence the temperature fluctuation (±).

In a comparable experiments using a static PUV light system temperature increases ranged from 3.9 to 36.8ºC after a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively (Keklik et al., 2010). Evaluating the reduction of *E. coli* and *L. monocytogenes* on the surface of raw salmon filets after exposure to PUV light, Ozur and Demirci (2006) reported surface temperature increases of 91, 68 and 53 ºC at 3, 5 and 8 cm from the quartz window of the PUV lamp, respectively after 60 seconds of exposure.

**Table 4-2.** Initial and Final temperature of chicken samples after exposure to PUV light

| Temperature (ºC) | Total Energy (J/cm²) | | | |
|---|---|---|---|---|
| | 5 | 10 | 20 | 30 |
| Initial | 17.7±0.8 | 17.8±0.7 | 17.9±0.2 | 17.9±0.7 |
| Final | 19.1±0.6$^{A}$ | 20.8±1.1$^{B}$ | 22.9±0.7$^{C}$ | 26.9±0.6$^{D}$ |
| Change | 1.4±0.7 | 3.0±0.9 | 5.0±0.5 | 9.0±0.6 |

$^{A-C}$Means within row without a common superscript are significantly different ($p<0.05$).

## Conclusions

The results of this study demonstrate that PUV light treatment is effective at reducing *E. coli* K12 on the surface of chicken thighs, breast, and skin. The results indicate that the highest exposure of PUV light results in the greatest reduction of microbes. The conveyor system used in this specific project with two xenon flashbulbs was capable of reducing *E. coli* by a total of 1 $\log_{10}$ (cfu/cm$^2$) at the highest energy value

investigated. Using two xenon flashlamps to deliver 30 J/cm$^2$ required the conveyor speed to be set well below throughput speeds typical in a commercial application. To obtain necessary product throughput on the conveyor more PUV light units would be required to deliver the necessary amount of energy which correlates to microbial reduction. Furthermore, greater delivery of energy (manipulated by proximity and exposure time) resulted in greater temperature rise on the surface of the products. This change is undesirable for the treatment of a product intended to be maintained as raw. Continued investigation using PUV lights over conveyor systems is necessary in order to optimize and increase the potential reduction of microorganisms and evaluate the efficacy of this technology for use in commercial poultry processing.

## References

Bialka, K. L., & Demirci, A. (2008). Efficacy of pulsed UV-light for the decontamination of Escherichia coli O157: H7 and Salmonella spp. on raspberries and strawberries. *Journal of Food Science*, *73*(5), M201-M207. https://doi.org/10.1111/j.1750-3841.2008.00743.x.

Bolder, N. M. (1997). Decontamination of meat and poultry carcasses. *Trends in food science & technology*, 8(7), 221-227. https://doi.org/10.1016/S0924-2244(97)01040-6

Catalano, C. R., & Knabel, S. J. (1994). Destruction of Salmonella enteritidis by high pH and rapid chilling during simulated commercial egg processing. *Journal of food protection*, *57*(7), 592-595. https://doi.org/10.4315/0362-028X-57.7.592

Centers for Disease Control and Prevention. (CDC, 1996). Surveillance Summaries, MMWR 1996;45 (No. SS-5).

Centers for Disease Control and Prevention. (CDC, 2015). Surveillance for Foodborne Disease Outbreaks, United States, Annual Report. Atlanta, Georgia: US Department of Health and Human Services. Available at: https://www.cdc.gov/foodsafety/pdfs/2015FoodBorneOutbreaks_508.pdf

Centers for Disease Control and Prevention. (CDC, 2017a). Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet 2015 Surveillance Report (Final Data). *Atlanta, Georgia: US Department of Health and Human Services.* Available at: https://www.cdc.gov/foodnet/reports/annual-reports-2015.html

Demirci, A., & Ngadi, M. O. (2012). Microbial decontamination in the food industry: novel methods and applications. Oxford: Woodhead Publ.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A., & Federighi, M. (2007). Pulsed-light system as a novel food decontamination technology: a review. Canadian journal of microbiology, 53(7), 813-821. https://doi.org/10.1139/W07-042

Gomez-Lopez, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. *Trends in food science & technology*, *18*(9), 464-473. https://doi.org/10.1016/j.tifs.2007.03.010

Haughton, P. N., Lyng, J. G., Morgan, D. J., Cronin, D. A., Fanning, S., & Whyte, P. (2011). Efficacy of high-intensity pulsed light for the microbiological decontamination of chicken, associated packaging, and contact surfaces. *Foodborne pathogens and disease*, *8*(1), 109-117. http://doi.org/10.1089/fpd.2010.0640

Keklik, N. M., Demirci, A., & Puri, V. M. (2010). Decontamination of unpackaged and vacuum-packaged boneless chicken breast with pulsed ultraviolet light. *Poultry science*, *89*(3), 570-581. doi: 10.3382/ps.2008-00476

Killinger, K. M., Kannan, A., Bary, A. I., & Cogger, C. G. (2010). Validation of a 2 percent lactic acid antimicrobial rinse for mobile poultry slaughter operations. *Journal of food protection*, *73*(11), 2079-2083. https://doi.org/10.4315/0362-028X-73.11.2079

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology*, *2*(2), 138-155. https://doi.org/10.1007/s11947-008-0178-3

Krishnamurthy, K., J. C. Tewari, J. Irudayaraj, and A. Demirci. (2010). Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and infrared heating. Food Bioprocess Technol. 3:93–104. https://doi.org/10.1007/s11947-008-0084-8

McLeod, A., Hovde Liland, K., Haugen, J. E., Sørheim, O., Myhrer, K. S., & Holck, A. L. (2018). Chicken fillets subjected to UV-C and pulsed UV light: Reduction of pathogenic and spoilage bacteria, and changes in sensory quality. Journal of Food Safety, 38(1), e12421. https://doi.org/10.1111/jfs.12421

Ozer, N. P., & Demirci, A. (2006). Inactivation of Escherichia coli O157: H7 and Listeria monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment. International journal of food science & technology, 41(4), 354-360. https://doi.org/10.1111/j.1365-2621.2005.01071.x

United States Department of Agriculture. (USDA), (2012). Food Safety and Inspection Service. Office of Public Health Science Microbiology Division The Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Survey January 2012–August 2012. https://www.fsis.usda.gov/wps/wcm/connect/a9837fc8-0109-4041-bd0c-729924a79201/Baseline_Data_Raw_Chicken_Parts.pdf?MOD=AJPERES

# Chapter 5

## Qualitative analysis of chicken meat treated with pulsed ultraviolet light


### Abstract

With the increasing production and consumption of chicken it is appropriate to investigate the functionality and effectiveness of microbial reduction interventions and the qualitative effects they have on food. The adoption of antimicrobial interventions for food products depends on minimal adverse effects on quality properties. In this project two pulsed ultraviolet (PUV) light units were positioned over a conveyor system and used to treat the surface of raw chicken parts in order to measure the impact on quality properties. Boneless/skinless (B/S) chicken breasts and thighs and bone-in/skin-on thighs were exposure to 30 J/cm$^2$ of PUV light. Individual trials were performed to allow for assessment of lipid oxidation, protein oxidation and changes in color of chicken samples treated with PUV light. Lipid and protein oxidation were measured at, 0, 24, 48 and 120 h after treatment. Malondaldehyde (MDA) concentration was used as the indicator for lipid oxidation. PUV treatment did not produce significant ($p>0.05$) changes in lipid oxidation but, MDA increased during storage ($p<0.05$) for both treated and untreated chicken parts. Average concentrations of MDA were 5.17, 4.26 and 8.86 ug/10 g of meat at 120 hours of storage for B/S breasts, B/S thighs, and bone-in/skin-on thighs, respectively. Free carbonyl content was used as the indicator for protein oxidation. PUV light treatment did not produce significant ($p>0.05$) changes in development of carbonyl's in treated chicken. CIE L*a*b* parameters were used to report color of samples before and after

treatment. L*, a*, and b* values of B/S breasts and thighs and bone-in/skin-on thighs did not change significantly ($p>0.05$) due to treatment with PUV light.

**Keywords:** chicken, lipid oxidation, protein oxidation, color, quality

## Introduction

Chicken consumption is estimated to account for over 25% of the world's total meat protein consumption. With increasing production and consumption, effective decontamination of raw chicken is needed. Traditional interventions, such as chemical treatment have disadvantages such as deterioration of the product quality, safety concerns, high costs or negative sensory changes (Capita et al., 2002; Guerrero-Beltrán and Barbosa-Cánovas, 2004; Lyon et al., 2007; PoultryTrends, 2017).

Recently, the poultry industry has made efforts to develop alternative nonthermal food decontamination interventions. An ideal technology would provide effective microbial reduction, without contributing to nutritional or sensory changes and without leaving chemical residues (Guerrero-Beltrán & Barbosa-Cánovas, 2004).

Pulsed ultraviolet (PUV) light is an emerging technology that decontaminates food surfaces using high intensity pulses of broad spectrum light. The wavelengths emitted include germicidal UV-C that reacts with cellular DNA and membrane structures in varying ways effectively inactivating cellular reproduction and resulting in cellular death. PUV light is produced in high intensity bursts from xenon flashlamps allowing a short exposure time for antimicrobial applications. Previous studies have not reported development of bacterial resistance to PUV application. An important advantage of PUV light compare with chemical antimicrobial treatments is the lack of chemical residue in the treated product. The use of PUV light was approved for treatment of food surfaces in 1999 by the U.S. Food and Drug Administration (Paskeviciute et al., 2011; FDA, 2011).

Studies reported above in chapters 3 and 4 show the effectiveness of pulsed UV light for microbial inactivation, but quality effects were not studied. Therefore, this

project was undertaken to investigate the effects of PUV like on quality attributes. Lipid oxidation, protein oxidation and changes in color were all assessed in order to determine if PUV light caused any adverse effects on chicken.

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## Materials and Methods

Chicken parts were provided by a local poultry processing plant and stored under freezing conditions (ca. -17ºC). Parts were transferred to a refrigerator (ca. 2ºC) to thaw 48 hours before each trial. Samples were brought to room temperature (~18ºC) 2 hours before each trial.

### Treatment by pulsed ultraviolet light

Chicken part samples (boneless/skinless thighs and breasts and bone-in/skin-on thighs) were individually subjected to PUV light treatment. Samples were placed on the conveyor and the complete surface of the products was treated with 30 J/cm$^2$ of PUV light. Energy value of 30 J/cm$^2$ was obtained by adjusting conveyor speed to 4.3 feet/second at a position 10 cm below the quartz windows of the PUV light units.

### Lipid oxidation

Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS), as described by Tarladgis et al., (1960). Boneless/skinless (B/S) chicken thighs and breasts and bone-in/skin-on thighs were all evaluated for lipid oxidation. Treated samples were compared to untreated controls. Whole chicken parts were treated with 30 J/cm$^2$ of PUV light. Lipid oxidation was assessed using a 10 gram sample removed from

the surface of the part then homogenized in a Waring blender (Waring Commercial, Torrington, CT) with 50 mL of distilled (DI) water and 5 mL of both Ethylenediaminetetraacetic acid (EDTA; J.T. Baker, Phillipsburg, NJ) and n-propyl galate (MP Biobedicals, LLC, Solon, OH) for 2 minutes. Both EDTA and n-propyl galate were used as antioxidants, in order to avoid further oxidation during sample preparation. The slurry was then transferred to a distilling flask with side arm and mixed with 47.5 mL of distilled water and 2.5 mL of hydrochloric acid (HCl; VWR Scientific Products, West Chester, PA). The complete solution was then brought to a boil and 50 mL of distillate were collected. Five mL of the distillate were transferred to a glass tube, mixed with 5 mL of thiobarbituric acid (TBA; Sigma Chemical Co.; St. Louis, MO) and heated in boiling water for 35 min to allow for color development. The solution was then transferred to a cuvette and absorbance was measured at 538 nm using an Evolution 201 UV-Visible spectrophotometer (Thermo Scientific, Waltham, MA). The content of malonaldehyde (MDA) per 10 g of meat was calculated using the equation from the standard curve prepared by using serial dilutions of 1, 1, 3, 3-tetraethoxypropane (TEP; Sigma Chemical Co., St. Louis, MO). A modified standard curve was prepared as described by Tarladgis, et al. (1960) and Texas Tech University (TTU, 2018). This procedure was first completed at time 0 and repeated after 24, 48 and 120 hours.

**Protein oxidation**

Protein oxidation was measured using a 2,4-Dinitrophenylhydrazine (DNPH) assay, as described by Ganhão, et al (2010). B/S chicken thighs and breasts and bone-in/skin-on thighs were each evaluated for protein oxidation. Treated samples were compared to untreated controls. Whole parts were treated with 30 J/cm$^2$ of PUV light.

Protein oxidation was assessed using a 1 gram sample of chicken homogenized in a blender (SmartGrind, Black & Decker, New Britain, CT), with 10 mL of sodium phosphate (Sigma Chemical Co.; St. Louis, MO) – NaCl (Fisher Scientific, Fair Lawn, NJ) buffer for 30 seconds. After homogenization, two 0.2 mL aliquots of the mixture were transferred to separate eppendorf tubes, mixed with 10% cold Trichloroacetic acid (TCA; Fisher Science Education, Nazareth, PA) and centrifuged (Microfuge® 20, Beckman Coulter, Brea, CA) for 5 minutes at 4200 g. Once centrifuged, the supernatant was disposed and 1 mL of 2 N HCl werer added to one of the eppendorf tubes and 0.2% DNPH (Sigma-Aldrich, St. Louis, MO) was added to the second eppendorf tube. These tubes will later provide the protein absorbance and DNPH absorbance. These solutions were allowed to incubate for 1 hour at room temperature (~18ºC). After 1 hour, the HCl and DNPH were removed and the pellets were rinsed with 1 mL of 10% cold TCA and washed three times with 1 mL of a 1:1 ethanol:acetate solution to remove any excess DNPH. Pellets were then resuspended in 1.5 mL of sodium phosphate – Guanidine HCl (Dot Scientific Inc., Burton, MI) buffer and centrifuged for 2 minutes at 4200 x g to remove insoluble fragments.

Using Thermo Fischer's Pierce™ BCA protein assay, a working reagent was prepared with 50 parts of BCA reagent A and 1 part of BCA reagent B (Thermo Scientific, Rockford, IL). Using a micropipette, 0.1 mL of the sodium phosphate – Guanidine HCl buffer solution was transferred from each eppendorf tube to separate glass tubes and mixed with 2.0 mL of the BCA working reagent. The mixed solution was incubated at 37ºC for 30 minutes. After incubation solutions were transfered to a cuvette and absorbance was measured at 562 nm as described by Pierce™ BCA Protein Assay

Instructions, using an Evolution 201 UV-Visible spectrophotometer (Thermo Scientific, Waltham, MA). A standard curve was prepared using bovine serum albumin (BSA; Thermo Scientific, Rockford, IL) in dilution amounts as described by Thermo Fischer, Pierce BCA Protein assay. Using Beers Law, protein carbonyl concentration was expressed as ηmol per mg protein using the absorption coefficient for protein hydrazones of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$. The equation below depicts how carbonyl concentration was calculated (K. Barkley, personal communication, September 5, 2018).

slope of standard curve: $y = 1.0227x + 0.0423$

mg protein/mL = ((protein absorbance) – 0.0423) / 1.0227
nmol carbonyls/mL = ((DNPH absorbance) / 21.0) / 1000
nmol carbonyls/mg protein = (mg/protein/mL) / (nmol carbonyls/mL)

**CIELAB color measurement**

Color changes were assessed using a Minolta Chroma Meter (Model CR 310 Minolta Inc., Ramsey, NJ) colorimeter to measure the L*a*b* color space. Using CIEL*a*b* color space, L* is representative of the lightness of the sample, a* and b* are representative chromaticity coordinates. - a* and + a* indicate green and red color, respectively. – b* and + b* indicate blue and yellow color, respectively. When evaluating color of the treated and untreated samples, three random spots were analyzed to provide an averages of L*a*b* values.

**Statistical analysis**

The complete experimental design was replicated 3 times for evaluation of lipid oxidation, protein oxidation and color assessment in independent experiments. Statistical Analysis Software 9.4 (SAS, Cary, NC) was used as the statistical platform for each analysis. The development of lipid oxidation or protein oxidation were evaluated in

separate experiments by an overall 3-way ANOVA with predicator variables product type, treatment and storage time. When needed, Tukey multiple comparison test was used to establish a 95% confidence interval for treatment conditions. Color values were analyzed using an overall 1-way ANONA with treatment as the predictor variable and color value as a response.

## Results and Discussion

### Lipid oxidation

Lipid oxidation was assessed for B/S breasts and thighs and bone-in/skin-on thighs after treatment of 30 J/cm$^2$ of PUV light. Lipid oxidation was reported as ug of malonaldehyde (MDA) per 10 g of meat. Treated and untreated samples were evaluated for MDA concentrations at 0, 24, 48 and 120 h after PUV light treatment.

Product type, treatment and storage time were assessed as predictor variables. The three way interaction of product type by treatment by storage time was not significant ($p>0.05$). Furthermore, treatment by product type and treatment by storage time interactions were not significant ($p>0.05$). The interaction of time by product type was significant ($p<0.05$) in contributing to MDA development. At 120 hours of storage MDA concentration was significantly ($p<0.05$) greater for bone-in/skin-on thighs as compared to B/S thighs and breasts. As a main effect, treatment was not significant ($p>0.05$) in contributing to development of MDA in the chicken samples.

Lipid oxidation (MDA concentration) increased over storage time ($p<0.05$) for all product types (Table 5-1). B/S breasts developed 3.20, 3.71, 3.90, and 5.17 ug of MDA per 10 g of meat at 0, 24, 48, and 120 h, respectively. B/S thighs developed 2.60, 2.82,

3.17, and 4.26 ug of MDA per 10 g of meat at 0, 24, 48, and 120 hours, respectively. Bone-in/skin-on thighs developed 3.73, 4.26, 6.91, and 8.86 ug of MDA per 10 g of meat at 0, 24, 48, and 120 hours, respectively. Thigh meat with skin tended to exhibit the greatest rate of lipid oxidation during storage. That tendency was statistically significant ($p<0.05$) after 120 hours of storage.

A similar study by Keklik et al., (2010), reported the effects of PUV light treatment on lipid oxidation of unpackaged chicken breast. Reported values were 5.87 and 12.43 ug of MDA / 10 g of meat after a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, respectively. Untreated controls were reported to have 5.42 ug of MDA per 10 g of meat. In another study, Paskeviciute et al., (2011) treated the surface of chicken breast with high powered pulsed light (200-1100 nm with pulse duration of 112 µs) and reported 0.204 and 1.019 mg of MDA per kilogram of meat after exposure of 0 and 2.7 J/cm$^2$ of PUV light. In an additional study, Keklik et al., (2009), PUV light treated, unpackaged chicken frankfurters were evaluated for lipid oxidation. After a 5 second treatment at 13 cm and a 60 second treatment at 5 cm, values of 5.60 and 7.65 ug of MDA per 10 g of meat were reported, respectively. This project reported 5.03 ug of MDA per 10 g of meat for untreated unpackaged chicken frankfurters.

**Table 5-1.** Thiobarbituric acid reactive substances increases in chicken parts during storage

| Sample | ug of malonaldehyde / 10 g of meat[1] | | | |
|---|---|---|---|---|
| | 0 hours | 24 hours | 48 hours | 120 hours |
| B/S Breast | 3.20±0.47$^{A}$ | 3.71±0.64$^{AB}$ | 3.90±1.22$^{AB}$ | 5.17±0.49$^{B}$ |
| B/S Thigh | 2.60±0.27$^{A}$ | 2.82±1.02$^{A}$ | 3.17±1.22$^{AB}$ | 4.26±0.49$^{B}$ |
| Bone-in/Skin-on Thigh | 3.73±0.99$^{A}$ | 4.26±1.18$^{A}$ | 6.91±0.83$^{B}$ | 8.86±1.16$^{C}$ |

[1]Represents averages of treated and untreated samples
$^{A-C}$Means within row without a common superscript are significantly different ($p<0.05$).

**Protein oxidation**

Protein oxidation was assessed for B/S breasts and thighs and bone-in/skin-on thighs after treatment of 30 J/cm$^2$ of PUV light. Protein oxidation was reported as nmol free carbonyl per mg of protein (Table 5-2). Treated and untreated samples were evaluated for carbonyl development at 0, 24, 48 and 120 hours after PUV light treatment. Analysis of variance by product type, treatment of PUV light and storage time showed no significant ($p>0.05$) interactions or main effects. A study by Elmnasser et al., (2008) evaluated the effect of PUV light treatment on protein oxidation in milk. Samples were treated with pulsed light at a distance of 4 cm from the xenon lamp and for a total energy fluence of 2.2 J/cm$^2$ per pulse. Samples were treated with 1, 3, 5, 7, and 10 pulses and no significant change in protein oxidation was reported. Soyer et al., (2009), reported protein carbonyl content, measured using DNPH at 370 nm, for chicken leg and breast meat that was stored at -7 ºC. Carbonyl content was reported as 1.78 to 2.88 ηmol/mg protein in leg meat and 1.34 to 2.14 ηmol/mg protein in breast meat after 0 and 6 months of frozen storage, respectively. In addition, Smet et al., (2008) evaluated protein oxidation of chicken meat as influenced by inclusion of dietary antioxidants. Their work showed no change in protein oxidation after 3 and 10 days of refrigerated storage. This supports the resulted reported in this study showing that short duration storage (120 hours or less) does not contribute to protein oxidation.

**Table 5-2.** Carbonyl content in chicken parts during storage

| Sample | Treatment | nmol carbonyls / µg of protein | | | |
|---|---|---|---|---|---|
| | | 0 hours | 24 hours | 48 hours | 120 hours |
| B/S Breast | Untreated | 0.13±0.01 | 0.20±0.06 | 0.14±0.04 | 0.13±0.02 |
| | 30 J/cm² | 0.19±0.06 | 0.16±0.08 | 0.15±0.03 | 0.12±0.01 |
| B/S Thigh | Untreated | 0.18±0.01 | 0.18±0.04 | 0.12±0.03 | 0.16±0.03 |
| | 30 J/cm² | 0.15±0.06 | 0.21±0.06 | 0.16±0.07 | 0.13±0.04 |
| Bone-in/Skin-on Thigh | Untreated | 0.17±0.03 | 0.16±0.06 | 0.14±0.04 | 0.12±0.01 |
| | 30 J/cm² | 0.16±0.07 | 0.16±0.02 | 0.26±0.03 | 0.12±0.00 |

*No significant differences ($p>0.05$) were detected

## CIELAB* color measurement

The color parameters, L*, a* and b* were assessed for B/S breasts and thighs and bone-in/skin-on thighs immediately after treatment of 30 J/cm$^2$ of PUV light. L*, a*, and b* values were reported before and after treatment with PUV light (Table 5-3). Statistically analyzed separately, L*, a*, and b* values of the products did not significantly ($p>0.05$) change after treatment of 30 J/cm$^2$ of PUV light. The results do show a tendency to decrease in lightness (L*) and redness (a*) for all the parts after treatment of 30 J/cm$^2$.

In a similar study, Keklik et al., (2010) reported the change in L*, a*, and b* of B/S chicken breast after treatment with PUV light. Changes in L*, a*, b* values reported after a 5 second treatment at 13 cm, were 0.59, -0.77, and 0.70, respectively. After a 60 second treatment at 5 cm, change in L*, a*, and b* values were reported as 23.43, 3.46, and 7.70, respectively. Isohanni and Lyhs (2009) treated both lean and skin-on chicken fillets with 100 seconds of ultraviolet light and reported L*, a*, b* values over a period of 12 days. After 12 days skinless chicken fillets had reported L*, a*, and b* values of 56.45, 4.49 and 1.66 for untreated product and 53.29, 1.74, and -0.33 for treated products,

respectively. After 12 days skin-on chicken fillets were reported to have L*, a*, and b* values of 70.19, 2.92, and 3.40 for untreated product and 72.86, 4.02, and 7.98 for treated products, respectively. The results were reported as having no significant difference between treated and untreated samples.

**Table 5-3.** Surface color of chicken breast, thigh and skin before and after PUV light treatment

| Product | CIE L*a*b* values | PUV Light Treatment[1] | |
|---|---|---|---|
| | | Before | After |
| Breast | L* | 64.7±1.5 | 62.9±3.1 |
| | a* | 1.0±1.0 | 0.4±0.5 |
| | b* | 12.0±1.1 | 10.7±0.7 |
| Thigh | L* | 54.0±3.3 | 53.5±1.9 |
| | a* | 4.2±1.4 | 3.1±0.9 |
| | b* | 5.3±2.7 | 7.5±0.8 |
| Skin | L* | 75.7±2.1 | 73.4±4.3 |
| | a* | 1.1±0.4 | 0.5±0.3 |
| | b* | 14.5±1.2 | 11.9±3.0 |

[1]Product samples treated with 30 J/cm$^2$
*No significant differences ($p>0.05$) were detected.

## Conclusion

Findings for lipid oxidation, protein oxidation and color in this study indicate that PUV light does not have significant effects on product quality when applied at 30 J/cm$^2$ delivered to the surface of fresh chicken products. This work further supports the idea that PUV light does not adversely affect product quality when used at a fluence rate appropriate for antimicrobial treatment of fresh poultry. More in depth research is necessary to confirm that there is no adverse effect on flavor or sensory attributes of food products when treated by PUV light.

## References

Capita, R., Alonso-Calleja, C., Garcia-Fernandez, M. C., & Moreno, B. (2002). Trisodium phosphate (TSP) treatment for decontamination of poultry. *Food Science and Technology International*, *8*(1), 11-24. https://doi.org/10.1106/108201302023118

Elmnasser, N., Dalgalarrondo, M., Orange, N., Bakhrouf, A., Haertlé, T., Federighi, M., & Chobert, J. M. (2008). Effect of pulsed-light treatment on milk proteins and lipids. *Journal of agricultural and food chemistry*, *56*(6), 1984-1991**.** doi: 10.1021/jf0729964

Food and Drug Administration (FDA, 2014). HACCP Principles & Application Guidelines. https://www.fda.gov/food/guidanceregulation/haccp/ucm2006801.htm#princ.

Ganhão, R., Morcuende, D., & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. Meat science, 85(3), 402-409. https://doi.org/10.1016/j.meatsci.2010.02.008

Guerrero-Beltran, J. A., & Barbosa-Canovas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food science and technology international*, *10*(3), 137-147. https://doi.org/10.1177/1082013204044359

Isohanni, P. M. I., & Lyhs, U. (2009). Use of ultraviolet irradiation to reduce Campylobacter jejuni on broiler meat. *Poultry science*, *88*(3), 661-668. https://doi.org/10.3382/ps.2008-00259

Barkley, K. (2018, September, 2018). Personal Communication.

Keklik, N. M., Demirci, A., & Puri, V. M. (2009). Inactivation of Listeria monocytogenes on unpackaged and vacuum-packaged chicken frankfurters using pulsed UV-light. *Journal of Food Science*, *74*(8), M431-M439. https://doi.org/10.1111/j.1750-3841.2009.01319.x

Keklik, N. M., Demirci, A., & Puri, V. M. (2010). Decontamination of unpackaged and vacuum-packaged boneless chicken breast with pulsed ultraviolet light. *Poultry science*, *89*(3), 570-581. doi: 10.3382/ps.2008-00476

Lyon, S. A., Fletcher, D. L., & Berrang, M. E. (2007). Germicidal ultraviolet light to lower numbers of Listeria monocytogenes on broiler breast fillets. *Poultry science*, *86*(5), 964-967. https://doi.org/10.1093/ps/86.5.964

Paskeviciute, E., Buchovec, I., & Luksiene, Z. (2011). High-power pulsed light for decontamination of chicken from food pathogens: a study on antimicrobial

efficiency and organoleptic properties. *Journal of Food Safety*, *31*(1), 61-68. https://doi.org/10.1111/j.1745-4565.2010.00267.x

PoultryTrends. (2017). The Statistical Reference for Poultry Exectutuves. WATT Poultry USA. Available at: http://www.poultrytrends.com/201711/index.php#/1

Shacter, E. (2000). Quantification and significance of protein oxidation in biological samples. *Drug metabolism reviews*, *32*(3-4), 307-326. https://doi.org/10.1081/DMR-100102336

Smet, K., Raes, K., Huyghebaert, G., Haak, L., Arnouts, S., & De Smet, S. (2008). Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry Science*, *87*(8), 1682-1688.

Soyer, A., Özalp, B., Dalmış, Ü., & Bilgin, V. (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food chemistry*, *120*(4), 1025-1030. https://doi.org/10.3382/ps.2007-00384

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan Jr, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society*, *37*(1), 44-48. https://doi.org/10.1007/BF02630824

Texas Tech University. (TTU, 2018). Thiobarbituric Acid Analysis (TBA): *Standard Curve Modification* https://www.depts.ttu.edu/meatscience/docs/ThiobarbituricAcidAnalysis.pdf

Thermo Fischer, Pierce™ BCA Protein Assay Instructions Kithttps://tools.thermofisher.com/content/sfs/manuals/MAN0011430_Pierce_BCA_Protein_Asy_UG.pdf

# Chapter 6

## Conclusions and scope of future research

The purpose of this study was to identify and evaluate an alternative surface decontamination intervention to current methods used in commercial poultry processing. Updated Food Safety and Inspection Service (FSIS) performance standards, specific to the acceptable allowances for *Salmonella* and *Campylobacter* on the chicken parts, provided a justification for the following projects.

In this study, pulsed ultraviolet (PUV) light was evaluated as a surface microbial decontamination intervention for raw chicken. In the first project, PUV light was applied to the surface of lean and skin surface chicken thighs that were inoculated with *E. coli*, *Salmonella* and *Campylobacter* in separate trials. For this project a static lab scale system was used to treat samples with PUV light. Samples were treated at two proximities (8 and 13 cm) from the quartz window below the PUV light housing and for 4 durations (5, 15, 30 and 45 seconds). Exposure to PUV light for 45 seconds on lean surface thighs resulted in $\log_{10}$ reductions 2.0 for *E. coli*, 2.2 for *Campylobacter*, and 2.4 for *Salmonella*, respectively. PUV light exposure for 45 seconds on skin surface thighs resulted in $\log_{10}$ reductions of 2.0 for *E. coli*, 1.9 for *Campylobacter*, and 1.8 for *Salmonella*, respectively. Results from this project indicate the potential of PUV light as a microbial reduction application for the surface of raw chicken meat. The effectiveness of this technology warranted scaling up to a commercial system.

Through collaboration with Farmers Pride, dba Bell & Evans (Fredericksburg, PA), and the Xenon Corporation (Wilmington, MA), a conveyor equipped with PUV lights was designed for testing.

The second phase of the study evaluated PUV light treatments applied on a moving conveyor that might be used in a commercial poultry processing plant. The conveyor system combined two pulsed light units which were positioned over a conveyor system with the ability to manipulate proximity to the PUV lights and the speed of the conveyor. Using the data collected from the static system, this project evaluated the overall reduction of *E. coli*, used as an indicatory organism for other pathogens, on the surface of boneless/skinless (B/S) breasts and thighs and bone-in/skin-on thighs. Microbial reduction of *E. coli* increased ($p<0.05$) with increased PUV light energy delivery for all parts. Exposure to PUV light for 30 J/cm$^2$ resulted in $\log_{10}$ reductions of 0.94 for B/S breasts, 1.04 for B/S thighs and 0.62 for bone-in/skin-on thighs, respectively.

Qualitative assessments were also evaluated using the conveyor system. Lipid oxidation, protein oxidation and color are all quality factors that are important to consider after treatment by PUV light. Boneless/skinless (B/S) chicken breasts and thighs and bone-in/skin-on thighs were exposure to 30 J/cm$^2$ of PUV light. Lipid and protein oxidation, measurements were recorded, 0, 24, 48 and 120 hours after treatment. PUV light treatment did not have a significant ($p>0.05$) effect in lipid or protein oxidation. However, MDA concentrations increased in both treated and untreated chicken parts during storage up to 120 hours. Averaged across treated and untreated chicken parts, 5.17, 4.26 and 8.86 ug of MDA per 10 g of meat were recorded after 120 hours for B/S

breasts, B/S thighs, and bone-in/skin-on thighs, respectively. There was no change in color after treatment with PUV light for B/S breasts and thighs and bone-in/skin-on thighs. The results from the qualitative assessment indicate that at 30 J/cm$^2$, or less, treatment of PUV light has no measurable effect on quality for those specific variables.

The overall results of all these projects demonstrate that PUV light can effectively reduce surface microbial contamination without effect other aspects of quality in both lab-scale and pilot-scale assessments. With this in mind there are a number of questions that should be further investigated in preparation for commercial application. The following points of discussion are considerations of future research.

In both the static and conveyor PUV light systems, a significant increase in temperature was associated with increasing delivery of PUV light. The change in temperature on the surface of chicken products is an important constraint of this technology. Alternative applications or controlled cooling should be evaluated with the intention of using PUV light in a commercial setting.

Continued research should be conducted investigating ways to implement PUV light more efficiently. The manipulation of angle, proximity, duration and total energy should be further evaluated to achieve the maximum microbial reduction without introducing any adverse quality effects.

## References

Bialka, K. L. (2006). Decontamination of berries using ozone and pulsed UV-light. Ph.D. dissertation, The Pennsylvania State University, PA.

Bolder, N. M. (1997). Decontamination of meat and poultry carcasses. *Trends in food science & technology*, 8(7), 221-227. https://doi.org/10.1016/S0924-2244(97)01040-6

Capita, R., Alonso-Calleja, C., Garcia-Fernandez, M. C., & Moreno, B. (2002). Trisodium phosphate (TSP) treatment for decontamination of poultry. *Food Science and Technology International*, *8*(1), 11-24. https://doi.org/10.1106/108201302023118

Carr, M. Steven. (2011). Course Materials, Induction of T-T Dimers by UV radiation. Memorial University of Newfoundland. Available at: https://www.mun.ca/biology/scarr/Thymine-Thymine_Dimers.html

Chun, H. H., Kim, J. Y., Lee, B. D., Yu, D. J., & Song, K. B. (2010). Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. *Food Control*, *21*(3), 276-280. https://doi.org/10.1016/j.foodcont.2009.06.006

Centers for Disease Control and Prevention. (CDC, 2015). Surveillance for Foodborne Disease Outbreaks, United States, Annual Report. Atlanta, Georgia: US Department of Health and Human Services. Available at: https://www.cdc.gov/foodsafety/pdfs/2015FoodBorneOutbreaks_508.pdf

Centers for Disease Control and Prevention. (CDC, 2017a). Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet 2015 Surveillance Report (Final Data). *Atlanta, Georgia: US Department of Health and Human Services.* Available at: https://www.cdc.gov/foodnet/reports/annual-reports-2015.html

Centers for Disease Control and Prevention. (CDC, 2017b). Campylobacter (Campylobacteriosis). Available at: https://www.cdc.gov/ecoli/

Centers for Disease Control and Prevention. (CDC, 2018a) Salmonella. Available at: https://www.cdc.gov/salmonella/.

Centers for Disease Control and Prevention. (CDC, 2018b) E. coli (Escherichia coli). Available at: https://www.cdc.gov/campylobacter/.

Coburn, B., Grassl, G. A., & Finlay, B. B. (2007). Salmonella, the host and disease: a brief review. Immunology & Cell Biology, 85(2), 112-118. https://doi.org/10.1038/sj.icb.7100007

Code of Federal Regulations, (CFR, 1992). Temperatures and chilling and freezing requirements, General chilling requirements, Animals and animal products. 9CFR381.66.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic Escherichia coli. *Clinical microbiology reviews*, *26*(4), 822-880. doi: 10.1128/CMR.00022-13

Demirci, A., & Ngadi, M. O. (2012). Microbial decontamination in the food industry: novel methods and applications. Oxford: Woodhead Publ.

Ebel, E. D., Williams, M. S., Cole, D., Travis, C. C., Klontz, K. C., Golden, N. J., & Hoekstra, R. M. (2016). Comparing characteristics of sporadic and outbreak-associated foodborne illnesses, United States, 2004–2011. *Emerging infectious diseases*, *22*(7), 1193. doi: 10.3201/eid2207.150833

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A., & Federighi, M. (2007). Pulsed-light system as a novel food decontamination technology: a review. Canadian journal of microbiology, 53(7), 813-821. https://doi.org/10.1139/W07-042

Federal Register. (2012). Vol. 77, No. 235. [Docket No. FSIS–2012-0007]. HACCP plan reassessment for not-ready-to-eat comminuted poultry products and related agency verification procedures. Available at: https://www.fsis.usda.gov/wps/wcm/connect/0dffacbe-45e8-43ea-8b65-3b7100e19acb/2012-0007.pdf?MOD=AJPERES

Federal Register (2015). Vol.80, No. 16 [Docket No. FSIS–2014–0023]. Changes to the Salmonella and Campylobacter Verification Testing Program: Proposed Performance Standards for Salmonella and Campylobacter in Not-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Related Agency Verification Procedures and Other Changes to Agency Sampling. Available at: https://www.federalregister.gov/documents/2015/01/26/2015-01323/changes-to-the-salmonella-and-campylobacter-verification-testing-program-proposed-performance

Federal Register. (2016). Vol. 81, No. 28. [FR Doc No: 2016-02586]. New Performance Standards for Salmonella and Campylobacter in Not-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Changes to Related Agency Verification Procedures: Response to Comments and Announcement of Implementation Schedule. Available at:

https://www.fsis.usda.gov/wps/wcm/connect/03910f0e-48f8-4111-ba52-490b07c25c24/2014-0023.htm?MOD=AJPERES

Food and Agriculture Organization of the United Nations. (FAO, 2017). Food Outlook. Biannual report on global food markets.

Food and Drug Administration. (FDA, 2011). Code of Federal Regulations. Ultraviolet Radiation for the Processing and Treatment of Food: 21CFR179. 39.

Food and Drug Administration. (FDA, 2012). Bad bug book, foodborne pathogenic microorganisms and natural toxins. Gram-positive bacteria. Second edition. Lampel K, Al-Khaldi S, Cahill S, editors. Silver Spring: Center for Food Safety and Applied Nutrition of the Food and Drug Administration (FDA), US Department of Health and Human Services.

Food and Drug Administration. (FDA, 2015). Code of Federal Regulations. Pulsed light for the treatment of food: 21CFR179. 41.

Gomez-Lopez, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. *Trends in food science & technology*, *18*(9), 464-473. https://doi.org/10.1016/j.tifs.2007.03.010

Guerrero-Beltran, J. A., & Barbosa-Canovas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food science and technology international*, *10*(3), 137-147. https://doi.org/10.1177/1082013204044359

Haughton, P. N., Lyng, J. G., Morgan, D. J., Cronin, D. A., Fanning, S., & Whyte, P. (2011). Efficacy of high-intensity pulsed light for the microbiological decontamination of chicken, associated packaging, and contact surfaces. *Foodborne pathogens and disease*, *8*(1), 109-117. http://doi.org/10.1089/fpd.2010.0640

Henning, W. R., & Cutter, C. (2001). Controlling Listeria monocytogenes in small and very small meat and poultry plants. *The United States Department of Agriculture Food Safety and Inspection Service*.

Hong, Y., Berrang, M. E., Liu, T., Hofacre, C. L., Sanchez, S., Wang, L., & Maurer, J. J. (2003). Rapid detection of Campylobacter coli, C. jejuni, and Salmonella enterica on poultry carcasses by using PCR-enzyme-linked immunosorbent assay. *Applied and environmental microbiology*, *69*(6), 3492-3499. doi: 10.1128/AEM.69.6.3492-3499.2003

James, C., Vincent, C., de Andrade Lima, T. I., & James, S. J. (2006). The primary chilling of poultry carcasses—a review. *International Journal of Refrigeration*, *29*(6), 847-862. https://doi.org/10.1016/j.ijrefrig.2005.08.003

Keener, K. M., Bashor, M. P., Curtis, P. A., Sheldon, B. W., & Kathariou, S. (2004). Comprehensive review of Campylobacter and poultry processing. Comprehensive Reviews in Food Science and Food Safety, 3(2), 105-116. https://doi.org/10.1111/j.1541-4337.2004.tb00060.x

Keklik, N. M., Demirci, A., & Puri, V. M. (2009). Inactivation of Listeria monocytogenes on unpackaged and vacuum-packaged chicken frankfurters using pulsed UV-light. *Journal of Food Science*, *74*(8), M431-M439. https://doi.org/10.1111/j.1750-3841.2009.01319.x

Keklik, N. M. (2009). Decontamination of Poultry Products by Pulsed UV-Light (Doctoral dissertation, The Pennsylvania State University).

Keklik, N. M., Demirci, A., & Puri, V. M. (2010). Decontamination of unpackaged and vacuum-packaged boneless chicken breast with pulsed ultraviolet light. *Poultry science*, *89*(3), 570-581. doi: 10.3382/ps.2008-00476

Killinger, K. M., Kannan, A., Bary, A. I., & Cogger, C. G. (2010). Validation of a 2 percent lactic acid antimicrobial rinse for mobile poultry slaughter operations. *Journal of food protection*, *73*(11), 2079-2083. https://doi.org/10.4315/0362-028X-73.11.2079

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology*, *2*(2), 138-155. https://doi.org/10.1007/s11947-008-0178-3

Krishnamurthy, K., J. C. Tewari, J. Irudayaraj, and A. Demirci. (2010). Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and infrared heating. Food Bioprocess Technol. 3:93–104. https://doi.org/10.1007/s11947-008-0084-8

Mani-Lopez, E., García, H. S., & López-Malo, A. (2012). Organic acids as antimicrobials to control Salmonella in meat and poultry products. *Food Research International*, *45*(2), 713-721. https://doi.org/10.1016/j.foodres.2011.04.043

Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L., & Poli, A. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. *Food & Nutrition Research*, *59*, 10.3402/fnr.v59.27606. http://doi.org/10.3402/fnr.v59.27606

McLeod, A., Hovde Liland, K., Haugen, J. E., Sørheim, O., Myhrer, K. S., & Holck, A. L. (2018). Chicken fillets subjected to UV-C and pulsed UV light: Reduction of pathogenic and spoilage bacteria, and changes in sensory quality. Journal of Food Safety, 38(1), e12421. https://doi.org/10.1111/jfs.12421

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., ... & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging infectious diseases*, *5*(5), 607. doi: 10.3201/eid0505.990502

National Agricultural Statistics Service. (NASS, 2016). Poultry Production and Value 2015 Summary. Available at: http://usda.mannlib.cornell.edu/usda/nass/PoulProdVa//2010s/2016/PoulProdVa-04-28-2016.pdf.

Office of Disease Prevention and Health Promotion. (ODPHP, 2018). Healthy People 2020 Available at: https://www.healthypeople.gov/2020/About-Healthy-People

Organization for Economic Co-operation and Development/Food and Agriculture Organization of the United Nations. (OECD, 2016). OECD-FAO agricultural outlook 2016 –2025. Paris, France: OECD Publishing. Available at: https://doi.org/10.1787/agr_outlook-2016-en

Paskeviciute, E., Buchovec, I., & Luksiene, Z. (2011). High-power pulsed light for decontamination of chicken from food pathogens: a study on antimicrobial efficiency and organoleptic properties. *Journal of Food Safety*, *31*(1), 61-68. https://doi.org/10.1111/j.1745-4565.2010.00267.x

PoultryTrends. (2017). The Statistical Reference for Poultry Exectutuves. WATT Poultry USA. Available at: http://www.poultrytrends.com/201711/index.php#/1

Sapan, C. V., Lundblad, R. L., & Price, N. C. (1999). Colorimetric protein assay techniques. *Biotechnology and applied Biochemistry*, *29*(2), 99-108**.** https://doi.org/10.1111/j.1470-8744.1999.tb00538.x

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... & Griffin, P. M. (2011). Foodborne Illness Acquired in the United States—Major Pathogens. *Infect Dis*. doi: 10.3201/eid1701.P11101

Shields, S. J., & Raj, A. B. M. (2010). A critical review of electrical water-bath stun systems for poultry slaughter and recent developments in alternative technologies. *Journal of Applied Animal Welfare Science*, *13*(4), 281-299. https://doi.org/10.1080/10888705.2010.507119

Slavik, M. F., Kim, J. W., & Walker, J. T. (1995). Reduction of Salmonella and Campylobacter on chicken carcasses by changing scalding temperature. *Journal of Food Protection*, *58*(6), 689-691. https://doi.org/10.4315/0362-028X-58.6.689

Spikes, J. D. (1981). Photodegradation of foods and beverages. In *Photochemical and photobiological reviews* (pp. 39-85). Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-7003-1_2

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan Jr, L. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society*, *37*(1), 44-48. https://doi.org/10.1007/BF02630824

U.S. Census Bureau. (2016). International Programs: World Population: 1950-2050. Available at: https://www.census.gov/population/international/data/worldpop/graph_population.php

United States Department of Agriculture (USDA), (2013). Inspection for Food Safety: The Basics. Available at: https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/production-and-inspection/inspection-for-food-safety-the-basics/inspection-for-food-safety-basics

United States Department of Agriculture . Food Safety Inspection Service. (USDA-FSIS, 2016). Pathogen Reduction – Salmonella and Campylobacter Performance Standards Verification Testing

United States Department of Agriculture. (USDA), (2017). Food Safety and Inspection Service. Available at: https://www.fsis.usda.gov/wps/portal/fsis/home

United States Department of Agriculture. (USDA), (2018) Pathogen Reduction – Salmonella and Campylobacter Performance Standards Verification Testing. https://www.fsis.usda.gov/wps/wcm/connect/b0790997-2e74-48bf-9799-85814bac9ceb/28_IM_PR_Sal_Campy.pdf?MOD=AJPERES

Whyte, P., Mc Gill, K., Collins, J. D., & Gormley, E. (2002). The prevalence and PCR detection of Salmonella contamination in raw poultry. *Veterinary Microbiology*, *89*(1), 53-60. https://doi.org/10.1016/S0378-1135(02)00160-8

World Health Organization. (WHO, 2018). Foodborne diseases. Available at: http://www.who.int/topics/foodborne_diseases/en/

Wrolstad, R. E., & Smith, D. E. (2010). Color analysis. In *Food analysis* (pp. 573-586). Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-1478-1_32

Xenon. (2015). The Incredible Power of Pulsed Light Sterilization. Available at: http://www.xenoncorp.com/markets/sterilization

Xiong, Y. L. (2000). Protein oxidation and implications for muscle food quality. *Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality. New York: Wiley and Sons*, 85-90.

Zhi-Hong, Z., Lang-Hong, W., Xin-An, Z., Zhong, H., & Charles, B.S. (2018). Non-thermal technologies and its current and future application in the food industry: a

review. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.13903