PULSED LIGHT AS A RISK MITIGATION TECHNOLOGY FOR DECONTAMINATION OF HARD-COOKED EGGS AND CONVEYOR BELT SURFACES

By

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To my family and friends

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AEB	American Egg Board
AMS	Agricultural Marketing Service
APHIS	Animal and Plant Health Inspection
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
EQAP	Egg quality assurance program
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug administration
FSIS	Food Safety Inspection Service
MAP	Modified atmosphere packaging
PL	Pulsed light
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
WHO	World Health Organization

Abstract of Thesis Presented to the Graduate School
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Ready-to-eat (RTE) products including hard-cooked eggs are potential vehicles of foodborne illness as they are susceptible to recontamination post processing. To date, there are limited alternatives to control post-processing contamination in RTE products. A novel technology referred as to pulsed light (PL) has found ever-increasing applications for decontamination of foods and food contact surfaces.

In the present study, the efficacy of PL for inactivation of *E. coli* K12 on hard-cooked eggs and conveyor belt surfaces was investigated.

Hard-cooked eggs and surface coupons were inoculated with *E. coli* K12. Samples were positioned 5.5 cm, 9.5 cm and 14 cm away from the quartz window of a prototype PL system and treated from 0.4 s to 30.0 s. Initial and survivor counts (log CFU/egg) were determined by: standard plate count on antibiotic-added media and the most probable number (MPN) for counts falling below the limit of detection (1.7 log CFU/egg; 2 log CFU/coupon). The temperature (°C) was also monitored during PL treatment. The effect of PL on selected quality parameters was assessed by changes in color, texture and microstructure of the hard-cooked egg-white. Results revealed significant reduction

($P<0.05$) in *E. coli* K-12 populations after PL exposure. Maximum inactivation was attained in hard-cooked eggs (6.40 ± 0.00 log CFU/egg) and coupons (7.06 ± 0.00 log CFU/coupon) after 30 s and 6 s of PL treatment at 5.5 cm and 9.5 cm from the quartz window respectively. Likewise, temperature increased significantly ($P<0.05$) uniquely in hard-cooked eggs (5.60 ± 0.60°C - 8.80 ± 0.90°C) at the maximum time tested (30 s). Conversely, no significant differences ($P>0.05$) were observed in color, texture and microstructure post PL treatment.

In conclusion, PL may be a practical step to enhance the safety of hard-cooked eggs and contribute to minimize the risk associated with RTE foods, which may have a positive impact in the food industry and public health.

CHAPTER 1
INTRODUCTION

Salmonellosis accounts for approximately 1 million cases of human illness and represents a major economic burden for the US (Scallan and others 2011). According to the Centers for Disease Control (CDC) and Prevention, the most up-to-date statistical data indicates that this foodborne infection have resulted in numerous cases of morbidity (2,290 hospitalizations) and mortality (29 deaths) in the US among the laboratory-confirmed bacterial cases per a population of 100,000 individuals (CDC 2011). The consumption of eggs and egg products (246 per capita) has been identified as the major agent implicated with its etiology (USDA 2011c), contributing to medical expenditures estimated at $365 million annually (CDC 2011).

Schoeni and others (1995) reported that several harmful pathogens constitute the primary serotypes implicated in salmonellosis outbreaks in the US (Howard and others 2011). Among the salmonella species, the most prominent include *Salmonella* Typhimurium and *Salmonella* Enteritidis, both of which are capable to grow and penetrate through the shell of the egg facilitating their ability to induce both external and internal contamination pertaining to the egg white (albumin) and egg yolk respectively.

In response to the high incidence of the egg-associated salmonellosis, multiple regulatory agencies have combined their efforts to improve the safety of the egg industry. These are namely the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA). In 1990, the FDA categorized eggs as a “potentially hazardous food” in the Food Code (Braden 2006). Subsequently, the FDA collaborated with the USDA Food Safety and Inspection Service (FSIS), to develop a “farm-to-table” risk assessment of *S*. Enteriditis in

shelled eggs, which provided guidance for the federal and state Egg Safety Action Plan (Braden 2006). Currently, the USDA FSIS regulates the safety of egg products, such as pasteurized liquid and dried-egg products, mandated by the Egg Products Inspection Act (EPIA) enacted in 1970 (Braden 2006).

Proper cooking is considered a critical step to assure egg safety. Other microbial populations other than *Salmonella*, may propagate in manufacturing facilities and promote recontamination (Tauxe 1997). Egg products such as hard-cooked eggs treated under adequate thermal conditions (90°C) may seem to be a negligible microbial hazard (FDA 2011b). Nonetheless, exposure to post-processing contamination from equipment, food handlers or airborne during preparation and packaging procedures commonly occur (Kim and others 2008). In fact, the persistence of microorganisms, namely *Pseudomonas* aeruginosa, *Listeria monocytogenes* and *Bacillus cereus* on food-contact surfaces as well as in food-processing environments has been well documented (Kusumaningrum and others 2003; Musgrove and others 2004).

Factors including moisture, the removal of the eggshells and the pH have been reported to initiate bacterial infection causing further deterioration of the eggs. Stadelman and Cotterill (1995) and Hierro and others (2009) reported that humid conditions on the egg surface as well as the removal of its protective barrier (shell), increases the likelihood of bacteria colonization, consequently resulting in rotten eggs. According to Schoeni and others (1995), the pH of the albumin ranging from 7.6 to 9.7 facilitates the growth of *Salmonella*. Moreover, internal quality degeneration and color changes (e.g., black, green, pink colorations in the egg yolk) may be indicative of microbial growth such as

Pseudomonas spp., *Listeria* and *Aeromonas* respectively (Claire and others 2004; Mead 2007).

Mitigation of the microbial load on eggs and associative products resultant from post-processing practices may be possible through the application of novel technologies such as pulsed light (PL) (Anderson and others 2000). Pulsed light has been defined as a preservation method involving the emission of intense and intermittent pulses of broad-spectrum (100-1100 nm) light, which may be implicated in the inactivation of both pathogenic and spoilage microorganisms (Oms-Oliu and others 2010). To illustrate, PL has demonstrated to be effective for the decontamination of shell eggs and food-contact surfaces (Guerrero-Beltrán and Barbosa-Cánovas 2004). Studies by Lasagabaster and others (2011) and Haughton and others (2011), observed nearly 5 log unit reductions of *S.* Typhimurium and *S.* Enteritidis on shell eggs and contact surfaces (i.e., polyethylene) treated with PL respectively.

Justification of the Study

The risk of egg-borne salmonellosis may be prevented by the application of adequate heat treatment; however, recontamination by ubiquitous pathogens and spoilage microorganisms namely *L. monocytogenes* and *B. cereus* may occur. Therefore, the inactivation of microbial hazards in eggs and egg-processing facilities continues to be a major safety concern, promoting to some extent, the need for development and assessment of alternative technologies (Musgrove and others 2004).

A promising alternative to conventional treatments for enhancing the quality and safety of food products is PL. Unlike most thermal methods (e.g., heating), pulsed light provides minimally processed foods without adverse

changes on quality or functionality (e.g., nutrients loss, rheological properties) (Oms-Oliu and others 2010). Pulsed light has been reported to be effective in reducing microbial contamination of foods (e.g., eggshell) and food-contact surfaces in addition to be environmental friendly (e.g., energy efficient, chemical free) (Elmnasser and others 2007). Hence, PL can be considered a suitable alternative to be implemented on a large scale in the egg industry and avoid potential costly recalls.

The present study was conducted to evaluate the effectiveness of PL on the inactivation of microbial populations on hard-cooked eggs and food-contact surfaces used on a commercial scale.

Objectives

The overall objective of this study was to evaluate the effectiveness of PL as a microbial mitigation technology for hard-cooked eggs and conveyor belt surfaces. However, the following specific objectives were met in this study:

First, the effect of PL on microbial load and surface temperature of hard cooked was estimated in terms of inactivation levels and temperature rise during the treatment.

Second, the effect of PL on microbial populations on conveyor belt surfaces was assessed based on the level of inactivation.

Third, the effects of PL on the quality of hard-cooked eggs was assessed by egg white texture, color and SEM.

CHAPTER 2
LITERATURE REVIEW

The Egg Industry and its Significance

Eggs are considered one of the most commonly consumed agricultural commodities throughout the world. In 2007, the worldwide egg output topped a volume of 59 million tons of eggs, corresponding to a threefold increase since 1970 (20 million tons) (Food and Agriculture Organization, [FAO] 2009). China and US are by far the largest world producers accounting for approximately 37% (23.8 million tons) (Figure 2-1) and 9% of the global egg output (5.3 million tons) respectively. Other main egg producers are India, Japan and Mexico, which together contribute to about 13% of the world market (Figure 2-1)(FAO 2009). Similarly, the egg industry has witnessed a rise in the per capita consumption up to 349 eggs in industrialized countries such as China, which has also been regarded as the biggest consumer (FAO 2009).

The US egg production has added important value to the economy and diet of the American households. In 2010, the egg production estimated in 91.4 billions, generated over 6 billion dollars in revenues (USDA 2012). Therefore, eggs have been ranked tenth among the major commodities in the US and valued in $4.48 billion in the international market (FAO 2012). In 2011, Americans consumed 246 eggs or egg products (e.g., liquid eggs, hard boiled eggs) combined per capita as an excellent and inexpensive ($1.23-1.27 per dozen large eggs) source of protein (American Egg Board, [AEB] 2010a; USDA 2011a).

Within US, the production of eggs is concentrated in five major states: California, Indiana, Iowa, Ohio and Pennsylvania. These together, account for about 45% of the national egg output (USDA 2012). According to the most up-

to-date statistics on chicken and eggs published, Iowa is the major egg producer, holding 15 % of the domestic output (1.11 billion eggs) (USDA 2012). While California, Indiana, Ohio and Pennsylvania generate 6.1, 7.1, 8.2 and 8.1% share respectively (USDA 2012).

The American egg industry involves the production of table and hatching eggs as well as eggs products from in-line breaking facilities. In 2009, the production of eggs totaled 91.9 billion of which 57% were table eggs, 14% hatching eggs and 30% egg products (USDA 2011b). Specialized in-line breaking plants transform eggs into derivative products such as liquid, frozen, dried eggs and other specialty forms (Stadelman and Cotterill 1995).

Processing of Eggs and Derivative Products

Shell Eggs

Upon collection, eggs are subjected to sorting, washing, grading, packaging and cooling operations to be either commercialized as shell eggs or be further processed in egg-breaking facilities. Prior to washing, eggs are visually inspected to identify defects such as cracks, meat and blood spots (Meunier and Latour 2000).

Washing aims to minimize the percentage of dirty eggs and likelihood of bacterial penetration through the shell. In commercial facilities, eggs are transported on conveyor rollers, brushed and sprayed with water solution containing a sanitizer. The adequacy of this step depends on several factors such as temperature and quality of the water solution. To illustrate, the temperature of the washing solution must be at least 11.1°C warmer than the internal contents of the eggs to prevent contraction (USDA 2006). While, the quality of the water is maintained through the application of alkaline detergents (50-200 ppm chlorine or similar strength) aimed to sanitize and control the

bacterial load by keeping the pH above 10 (Knape and others 2002). Due to the deterioration of the cuticle during washing, food grade mineral oil is used to reduce the loss of carbon dioxide and moisture from internal contents (USDA 2006).

Grading and packaging operations are oftentimes conducted simultaneously. Grading is attained by sorting eggs according external and internal quality factors through the use of candling equipment. Clean and resistant packaging materials are required to protect the integrity of eggs during grading, distribution and retailing (USDA 2006).

Following packaging, eggs are transported to a room cooler until distribution (Meunier and Latour 2000). Eggs should be ideally maintained between 70-85% relative humidity and at adequate temperature conditions to prevent “sweating”. Unprocessed eggs must be held at least at 15.6°C ambient temperature, while processed eggs at 7.2°C or lower temperatures (USDA 2011c).

Egg Products

Eggs used for the elaboration of derivatives products should preferably be of high quality. To illustrate, the internal contents of eggs can become mixed and watery over time. To delay these structural changes, eggs are refrigerated between 7 and 10 days prior processing (AEB 2010b).

After storage, eggs are broken and their contents separated and mixed with other ingredients (Figure 2-2). Automated equipment enables visual inspection and cracking operations at production rates as high as 162,000 eggs/h (AEB 2010b). Whole and separated eggs (e.g., egg white) are blended and filtered for removal of shell residues and membranes (e.g., chalazae).

Ingredients such as sodium chloride (for control of gelation), sucrose (for lower-temperature pasteurization) and sodium lauryl sulfate (whipping agent) are added to obtain certain desirable properties (Stadelman and Cotterill 1995).

Followed separation, eggs are pasteurized to be either marketed as liquid or frozen products otherwise further processed in the form of dried egg products. Pasteurization is an effective mean to eliminate any viable pathogens especially *Salmonella* spp. as demanded by The Egg Products Inspection Act of 1970 (Latimer and others 2008). The conditions of commercial pasteurization differ among products and must abide by temperature and holding time requirements stipulated by the FSIS (USDA 2005a). Upon pasteurization, liquid eggs are promptly cooled and stored either at refrigeration (7.2°C egg white, <4.4°C whole egg or egg yolk) or freezing temperatures (< -23.3°C) (Stadelman and Cotterill 1995; AEB 2010b; Jaekel and others 2008). Instead, dried egg products are desugarized previous to heat treatment to prevent associated Maillard reactions.

Hard-cooked eggs are high value-added products due their many applications by consumers and food establishments. Nowadays, hard-cooked eggs are commercially available in the form of individual ready-to-eat (RTE) products or pickled eggs containing brine or pickling materials (e.g., vinegar, red-beet juice). Moreover, hard-cooked eggs bulk packaged in modified atmospheres (MAP) serve as ingredients in foods provided by catering services, restaurants and other food establishments (Kim and others 2008).

According to Stadelman and Cotterill (1995), hard-cooked eggs in shells should meet certain criteria to be considered of high quality. These include: minimum cracking during cooking, easy of peelability and centered yolk with

absence of dark rings all influenced by several factors such as pH and temperature. For example, freshly laid eggs are associated with an increased difficulty in the removal of shells due to the low pH of the albumen. Similarly, discoloration of the egg yolk has been attributed to the formation of ferrous sulfide during cooking due to elevated temperatures and prolonged holding times (Stadelman and Cotterill 1995).

In commercial facilities, hard-cooked eggs are prepared by rapid heating and cooling steps. The cooking step is achieved by immersion of eggs in water quickly heated by steam or gas to 90-95°C. Likewise, eggs are cooled rapidly by immersion in sanitary tap water to minimize discoloration of the yolk. Moreover, if eggs are desired to be commercialized in shells, an air-cooling step is incorporated to the process aimed to extent the shelf life (Stadelman and Cotterill 1995).

Microbiology of Eggs

Innate Mechanisms of Defense

As earlier mentioned, fresh eggs are endowed with physical and chemical defenses against microbial invasion. The main physical barriers include the shell, shell membranes (inner and outer) and viscosity of the albumen. The eggshell contains about 10,000 pores, which are covered almost in its totality by a glycoprotein referred as to the cuticle. The shell membranes resemble tangle thread structures that act as bacterial filters impeding any microbial movement in conjunction with the viscous albumen (Board 1980).

Chemical barriers of eggs include the pH of the albumen, the presence of antibodies and other antimicrobial components. The pH of the albumen rising from 7.4 to about 9.2 post-laying and the low availability of nitrogen prevent microbial growth (Mead 2007). The occurrence of antibodies such as

immunoglobulin Y (in the egg yolk) and immunoglobulin M and immunoglobulin A (in the egg albumin) inhibit also the growth of *Lactobacillus* spp., *Micrococcus* spp. and other microflora in the ovary and oviduct prior laying (Yamamoto and others 1997). Moreover, the role of antimicrobial proteins and peptides such as ovotransferrin which binds iron and lysozyme which damage the peptidoglycan cell wall of gram-positive bacteria has also been documented (Ibrahim and others 2000; Anton and others 2006; Silphaduang and others 2006).

Routes of Contamination

Although most eggs (90%) are considered innocuous at ovoposition, bacterial contamination may occur through two major transmission routes: vertical and horizontal (Board 1980). The vertical transmission route encompasses mainly trans-ovarian and trans-oviductal infections, which arise during the formation of the yolk in the ovarium and the vitelline and albumin in the oviduct respectively. While, the horizontal transmission route involves trans-shell infection post laying and contamination of egg products as they exposed to the surrounding environment (e.g., feces, nests, food contact surfaces) (Stadelman and Cotterill 1995).

Vertical contamination

Trans-ovarian infection and trans-oviductal have shown to be important routes of bacterial colonization in microorganisms such as *Salmonella* species. Harry (1963) isolated *Pasteurella haemolytica*, *Lactobacillus* spp., *Micrococcus* spp. from the ovarium of flocks. Similarly, numerous studies have supported the colonization of *Salmonella* strains in the reproductive organs of hens (Okamura and others 2001; Gast and others 2007). Miyamoto and others (1997) found a high incidence of *Salmonella* Enteritidis in eggs (3 of 6 eggs) situated in the vagina of flocks. Moreover, Okamura and others (2001) reported the ability of

six different serovars of *Salmonella* including *S.* Enteritidis and *S.* Typhimurium to colonize the ovarium and the oviduct of intravenously inoculated flocks. Recovery studies in the egg yolk, ovary and oviductal regions of hens were the highest for *S.* Enteritidis than other serotypes suggesting the high affinity of this microorganism to cause infection (Okamura and others 2001; Gantois and others 2009).

Furthermore, few studies have also suggested that eggs are infected as soon as they become in contact with the cloaca possibly contaminated with *Salmonella* occurring in the feces (Barrow and Lovell 1991). Keller and others (1995) suggested that eggs are exposed to *S.* Enteritidis as they pass through the heavily infected cloacal tissues of chickens (50-80%). However, Miyamoto and others (1997) inoculated intracloacally hens with *S.* Enteritidis obtaining low recoveries on the outer surface of eggs, which suggests a limited diffusion into egg contents. Similarly, de Buck (2004) and others maintained that the likelihood of fecal contamination during oviposition is minimum in healthy hens as the vagina is oriented beyond the gastrointestinal tract.

The possible infection of eggs with *Campylobacter* spp. remains controversial given the few studies investigating their role as egg invaders. Jacobs-Reitsma (1995) isolated 19 strains of *Campylobacter* in 29 of the total 43 flock layers sampled. However, serotyping results demonstrated that *Campylobacter* spp. do not represent a risk for egg infection (Jacobs-Reitsma 1995). Sahin and others (2003) artificially contaminated egg yolk and egg white with *C. jejuni* and sampled 1,500 eggs obtained from broiler-breeder from commercial flocks and hatchery. Although *C. jejuni* was detected in artificially contaminated eggs until 14 days of storage at 18°C, no recovery was positive in

those from commercial flocks or from hatchery. Newell and Fearnley (2003) stated that though previous studies have reported the survival of *C. jejuni* in about 1% of eggs from colonized hens, the prevalence of this microorganism cannot be further confirmed due to the limited experimental data.

Horizontal contamination

Trans-shell contamination may initially occur due to the presence of feces and other contaminants (e.g., soil, nesting materials) in the environment of chicken houses. Hen feces are regarded as bacterial reservoirs. Schoeni and others (1995) inoculated eggs with feces containing three different serotypes of *Salmonella* (*S.* Enteritidis*, S.* Typhimurum*, S.* Heidelberg) and observed increases in bacterial counts (3-5 logs) after 1-3 days of storage (25°C).

The extent of bacterial penetration may be favored by highly contaminated hen houses, storage conditions (temperature and humidity) and state of the cuticle and the shell after laying. Padron (1990) and Cox and others (2000) highlighted that heavily contaminated nests and dirty hen house floors increase the likelihood of bacterial contamination. Similarly, De Reu and others (2008a) noted a positive correlation between the initial contamination of shells and the total aerobic counts in poultry houses. Berrang (1999) outlined that a high differential of temperature between the freshly laid egg and the environment causes contraction allowing bacteria to be drawn into the egg especially under humid conditions. As indicated by Howard and others (2011), egg surface contamination is also more probable immediately after ovoposition before the cuticle dries out and seals the pores of the shell.

Others sources of bacteria include water, food contact surfaces and food handlers during processing. During washing, a buildup of manure, fungi and

bacteria are removed from the eggshell and deposit into the recycled water. As an illustration, Knape and others (2002) estimated total aerobic counts of eggs in off-line and in-line facilities. Samples collected from the conveyor belt system and recirculated water yielded the maximum counts corresponding to 4.62 and 3.34 log CFU/mL for in-line facilities and 5.47 and 4.43 log CFU/mL for off-line facilities. Hope and others (2002) indicated that egg products receiving a minimum thermal treatment such as liquid egg products may harbor microorganisms spread on the work surfaces. Claire and others (2004) demonstrated that specialty products such as hard-boiled eggs can be cross-contaminated after thermal treatments with persistent microorganisms namely *Listeria monocytogenes*. These researchers reported the survival of this pathogen in artificially contaminated egg products at refrigeration temperatures (4-12°C) yet under modified atmosphere packaging (MAP) (up to 80% CO_2).

Numerous studies have been undertaken to determine the microflora associated with eggs and their spoilage (Mayes and others 1983; de Reu and others 2008b). Gram-positive have been identified as the main bacteria found on the eggshell varying qualitatively and quantitatively according geographical location (de Reu and others 2008b). Their prevalence may be attributed to their ability to adapt to dry conditions (Stadelman and Cotterill 1995). In particular, *Micrococcus* represents the largest genus occurring on shell eggs although *Enterobacteriacea* spp., *Streptococcus* spp. and *Staphylococcus* spp. remain also important (Seviour and Board 1972; Bruce and Johnson 1978; de Reu and others 2006).

Recent studies have detected *Enterobacteriacea*, yeast, molds and *Pseudomonas* on the surface of eggs during rinsing or in-line washing operation

of commercial egg facilities. Jones and others (2004) found moderate counts of *Enterobacteriacea* and *Pseudomonas* (<1 log CFU/mL rinsed water), and even higher counts of molds and yeasts (1.5 log CFU/mL rinsed water). Musgrove and others (2004) characterized the *Enterobacteriacea* genera most commonly distributed in egg washing facilities in the US through the collection of rinsed water from nine different processing plants.

Conversely, the invasion of spoilage microorganism into the egg contents depends more on their capability to overcome the defense mechanisms innate to eggs (e.g., ovotransferrin, lysosyme). For example, rotten eggs contain mostly gram-negative bacteria including *Alcaligenes, Pseudomonas, Escherichia, Proteus* and *Aeromonas* given their low nutritional requirements and ability to survive at low temperatures (Stadelman and Cotterill 1995). After diffusion through the shell, these microorganisms grow rapidly to cause characteristic colorations such as green, black and pink in the case of *Pseudomonas* spp. (Mead 2007). In the same way, molds and yeasts (e.g., *Cladosporium*, also known as “whiskers”) can spread into the egg contents generating off odors (Board and Fuller 1994).

Salmonella **Enteritidis and Egg-borne Poisoning**

S. Enteritidis has been ranked number 1 or 2 among the most common serotypes isolated from salmonellosis infections worldwide (Braden 2006). During the early 1980s, *S.* Enteritidis emerged simultaneously in the UK, Europe and North America causing alarming outbreaks. Epidemiological studies have long linked eggs and egg products as the major responsible of this pandemic (Tauxe 2002; Cogan and Humphrey 2003; Mumma and others 2004).

The incidence of *S.* Enteritidis in the US aroused dramatically in the late 1970s and spread rapidly from coast to coast by the mid-1980s (Braden 2006). During this period, the CDC investigated the roots of *S.* Enteritidis infections caused in the American Northeast region. A total number of 65 outbreaks causing 2,119 reported cases, 257 hospitalization and 11 deaths were scrutinized. Particularly, a large outbreak involving the consumption of stuffed pasta with raw eggs labeled as “fully cooked” and affecting 3,000 persons in seven states, led to the main conclusion that *S.* Enteritidis was present in shell eggs (Tauxe 2002).

Since 1980, the numerous foodborne outbreaks caused by this pathogen have tracked back to eggs as the major food vehicle. Between 1985 and 2003, a total number of 997 outbreaks were reported involving 33,687 illnesses, 3,281 hospitalizations and 82 deaths (Braden 2006). In particular, the number of outbreaks increased sharply from 26 in 1985 to a peak of 85 in 1990 and decreased subsequently until 2003 although the infection rates attributed to the consumption of egg or egg products remained constant (Braden 2006). An extraordinary case, was the outbreak of 1994 caused by an ice cream brand cross contaminated with raw liquid eggs, which resulted in an estimated of 4,000 reported cases and 250,000 illnesses in the entire nation (Hennessy and others 1996).

Sporadic and massive egg-borne infections often result from breakdown in cooking practices. Hedberg and others (1993) noted that patients with sporadic infections had five times higher probability to have consumed raw or undercooked eggs three days before illnesses compared to the control subjects. A review of *S.* Enteritidis outbreaks (1985-1999) indicated that 62% of the

reported cases corresponded to commercial establishments including restaurants (Vugia and others 1993; Patrick and others 2004).

The frequency of contamination of individual eggs may result in an underestimation of the human exposures. Ebel and Schlosser (2000) calculated this frequency in 1 every 20,000 laid eggs within the US. Considering the large production of this commodity, estimated in at least 65 billion annually, even a low incidence of *S.* Enteritidis as such, would contribute to a significant number of exposures (approximately 50,000-11,0000 infections each year) (Ebel and Schlosser 2000).

Control of *Salmonella* Enteritidis and other Associative Pathogens

Regulatory Agencies and Major Interventions

At present, the USDA and FDA are the two major federal agencies responsible to assure the safety and quality of shell eggs and egg products. The USDA Food Safety and Inspection Service (FSIS) regulate the safety of egg products including liquid eggs and dried egg products in accordance with the Egg Products Inspection Act emitted in 1970. Similarly, the USDA Animal and Plant Health Inspection (APHIS) administers the National Poultry Improvement Plan (NPIP) aimed at enhancing the health of poultry and safety of poultry products. The USDA Agricultural Marketing Service (AMS) monitors shell egg grading, handling and importation operations in terms of proper quality, labeling and packaging under the Regulations Governing the inspection of eggs (7 Code of Federal Regulations [CFR] Part 57) (USDA 2008; USDA 2011c).

On the other hand, the FDA collaborates in the control of *S.* Enteritidis and conducts inspections to egg producers. The FDA works together with FSIS towards the elaboration of new means intended to halt salmonellosis incidence such as the amendment of the FDA Food Code to include shell eggs as

“potentially hazardous foods”. Other example is the Egg Safety Rule, which went into effect in July 2010 for medium and large (with 50,000 or more laying hens) producers and became mandatory for small producers (with at least 3,000 laying hens) in July 2012 (FDA 2011a). In addition, FDA coordinates audits to egg producers since 1990, and works closely with the CDC to trace back egg-borne outbreaks and establish opportune and preventive measures intended to reduce associated human illnesses (Braden 2006).

Other government agencies include state and local agriculture and health departments. While grading for quality is voluntary, state agriculture departments inspect for compliance with official US standards, grades and weight classes of processors that do not contract out USDA/AMS grading service. State and local health departments monitor retail food and foodservice establishments for conformity with their requirements such as the consumption of pasteurized egg products in health care facilities or hospitals (USDA 2011c).

Major preventive and control measures implemented towards the safety and quality of eggs and egg products include the Egg Products Inspection Act, the adoption of on-farm and on-egg facilities quality-assurance programs, the requisite of refrigerated systems for distribution and storage of eggs and the education of consumers and food workers about the associative risks with improper cooking and handling practices (Patrick and others 2004).

The EPIA of 1970 essentially eliminated the primary cause of salmonellosis attributed to broken shells contaminated with dirt or foreign materials (Holt and others 2011). Provision of this act requires all egg products be Salmonella free by the application of approved pasteurization methods. Cracked or dirty eggs are diverted to breaking facilities to be rapidly heated and

held at the temperature and time validated by scientifically based considerations (USDA 2005a).

Egg quality assurance programs (EQAPs) are structured plans practiced among states to reduce egg contamination from farm to consumer. These voluntary plans, intended primarily to minimize the likelihood of *Salmonella* in hen houses and processing facilities, are similar as they had been designed in mutual cooperation (Mumma and others 2004). However, differences may be encountered depending on the state or the program adopted. For instance, Pennsylvania's EQAP is considered the most costly, rigorous and comprehensive. In contrast, a self-certification program developed by United Egg Producers (5-Star) is very flexible in terms of microbiological testing (Mumma and others 2004). Some of the requisites contemplated by the Pennsylvania's EQAP include the acquisition of chicks from negative-*Salmonella* breeders, rodent control and biosecurity programs, testing of manure, feed and egg pool, cleaning and sanitation of hen houses and egg processing plants (USDA 2007).

Requirements concerning refrigerated storage have also been a key contribution in counteracting *S.* Enteritidis proliferation. A regulation imposed by the FSIS requires shell eggs to be stored and transported at ambient temperature below 7.2°C within 36 hr and after being packaged for ultimate consumer use (FDA 2011b). Prompt refrigeration of eggs has a favorable effect on the estimated number of human illness from 130,000 to 89,000 as reported in a risk assessment conducted by the FSIS (USDA 2005b). Similarly, quick cooling and refrigeration of eggs have a combined effect with pasteurization in reducing the viability of any surviving bacteria (USDA 2005a). Programs

destined to educate food workers and consumers regarding the importance of handling and cooking eggs adequately have also contributed to reduce *S.* Enteritidis infection. In 1996, the USDA FSIS in cooperation with the FDA, pioneered a “farm-to-table” risk assessment which provided a starting point for the federal and state Egg Safety Action Plan. This program comprises guidelines on how to handle, store and cook eggs safely (FDA 2011b). Similarly, the Partnership for Food Safety Education (FSE), initiated different campaigns (e.g., “BAC Down!”) designed to instruct consumers and food workers in proper handling practices (Partnership for Food Safety Education 2010).

Methods for Decontamination of Eggs and Egg Products

Although the incidence of egg-borne infections has fallen significantly as a result of the many interventions towards public safety, new means are required to ensure the most the safety of eggs and egg products. In particular, EQAPs were recognized to decrease the morbidity and mortality rates of *S.* Enteritidis by 50% and 100% respectively in 1999 (Patrick and others 2004). Similarly, a survey of egg-producing farms and flocks in Pennsylvania (90% of flocks are affiliated to the Pennsylvania EQAP) found that the percentage of *S.* Enteritidis contaminated environments (48% vs 10%) and flocks (26% vs 2%) decreased dramatically from 1990 to 1998 (Braden 2006). Despite these facts, egg-borne salmonellosis continues to be a vexing problem for public health and hence demands new control measures (Braden 2006).

Washing and sanitization

Washing of table eggs prior consumption is commonly practiced in the US for surface decontamination despite the lack of mandatory standard procedures to execute this step. The main goal of washing is the reduction of 1 to 6 log

values of the external microbial load of shell eggs although damage of the cuticle favors bacterial penetration (Hierro and others 2009). Currently, most egg processors follow the Regulations Governing the Voluntary Grading of Shell Eggs stipulated by the USDA AMS (7 CFR Part 56) (USDA 2008).

Pasteurization

In addition to pasteurization of liquid and dried egg products, various methods have been proposed for in shell-pasteurization. According to a risk assessment of *S.* Enteritidis on eggs and egg products, a 3 log unit reduction in shell eggs would decrease the human illness associated with this microorganism by 70% (USDA 2005b). Several studies have been published assessing the applicability of hot air, hot water, infrared and other alternatives for whole egg pasteurization (Barbour and others 2001). James and others (2002) constructed temperature profile curves for the surface and internal contents of eggs when subjected to the foregoing technologies. The temperatures attained during treatment were sufficient as to produce considerable microbial reduction without causing a significant raise on the inside contents. Hou and others (1996) investigated the effect of water bath and hot air ovens on microbial reduction and functional properties of eggs. Water bath (57°C, 25 min), hot air (55°C, 180 m) and a combination of both methods (water-bath at 57°C for 25 min, hot-air at 55°C for 60 min) produced 3, 5 and 7 log reductions respectively without affecting the overall functionality of eggs.

Edible coatings

Several studies have reported the use of edible coatings to prolong the shelf life and enhance nutritional and quality properties of eggs. To illustrate, Caner and Cansiz (2008) investigated the benefits of chitosan-based coatings formulated with organic acids (e.g., acetic, lactic and propionic) on shelf life of

shell eggs. Results derived from coated eggs demonstrated lower weight loss rates, improved quality attributes and mineral preservation in the yolk (e.g., calcium, iron) as compared to uncoated eggs after 4 weeks of storage. Similar findings were reported by Suppakul and others (2010) who evaluated the effects of cellulose-based coatings (methylcellulose, hydroxypropyl methylcellulose) on stability of shell eggs over time. Moreover, Kim and others (2008) assessed assorted coatings including chitosan-lysozyme, whey protein isolate and bake sheen in terms of quality retention and microbial control. While all the coatings, reduced weight losses, pH variation and changes in color, only the chitosan-lysozyme coating was effective in controlling the growth of *S.* Enteritidis.

Modified atmospheres

The use of modified atmospheres involves a recent method for bacterial control whose application results moderately complicated in eggs due to their complex composition. Németh and others (2012) stored eggs under vacuum, MAP) (50-50 N_2 and CO_2) and refrigeration (4°C) with an acidified brine solution (citric acid 4.5%). It was found that the color and taste of peeled hard-cooked eggs was the most adequate until week 2 under modified atmospheres. However, after this period, large deterioration of quality was observed as eggs became microbiologically unsafe. Similarly, Claire and others (2004) determined the shelf life and safety of hard-cooked eggs packaged in MAP. They concluded that MAP cannot be considered adequate barriers for microbial control as they observed growth of *L. monocytogenes* even under harsh conditions (80% CO_2, 4°C).

Non-thermal technologies

Thermal applications for pasteurization of shell eggs have proven to be effective on the inactivation of *S.* Enteritidis. However, it has also been suggested that these methods may produce cracked and poor quality eggs (Lasagabaster and others 2011). For example, changes in protein conformation, gel network formation and other rheological changes can reduce the acceptability among consumers (Kiosseoglou and Paraskevopoulou 2005; Lasagabaster and others 2011).

Therefore, the implementation of alternative practices for decontamination of eggs and quality retention has drawn significant attention. In this case, non-thermal methods seem a promising approach for producing safe and minimally processed foods. Some of these non-thermal technologies include electrolyzed water, ozone, ionization irradiation and UV radiation. Davies and Breslin (2003) reported not significant reductions in eggshell bacterial populations after ozone or herbal treatment. Additionally, the insoluble powdery residue of herbs and the undesirable smell of ozone do not justify the use of these methods by any means. Bialka (2004) detected comparable log reductions of *S.* Enteritidis with electrolyzed water (2.3 CFU/g) and commercial detergent (2 CFU/g). However, the cuticle was adversely affected by both treatments although no significant effects on the albumen height and eggshell strength were observed (Bialka 2004). Similarly, Mészáros and others (2006) identified changes in flow behavior and functional properties (e.g., foaming and whipping) of irradiated eggs at doses ranging from 0.5 to 3.0 kGy. In contrast, Pinto and others (2004) suggested that the sanitation dose is not sufficiently severe as to impair egg functionality.

To date, the most promising approach for microbial disinfection of eggs is perhaps the application of UV radiation. Literature has extensively reviewed the effectiveness of UV radiation on inactivation microorganism in a variety of surfaces including eggshells. To illustrate, Kuo and others (1997) demonstrated that UV radiation considerably reduced total aerobics, molds and *S.* Typhimurium inoculated on shell eggs. Moreover, the application of Pulsed Light which combines the effects of UV, visible and infrared lights has been regarded more effective than UV radiation alone. For example, PL has demonstrated to disinfect eggs artificially contaminated with *S.* Enteritidis within short exposure times (Keklik and others 2010).

Pulsed Light Technology

Pulsed Light Generation

Pulsed light is a non-thermal preservation method with potential applications in surface decontamination of food, equipment and packages. The use of PL in the processing of foods was approved by the FDA in 1996 (FDA 2012c). Its effect on microbial inactivation has been extensively investigated as an alternative and/or complement technology to conventional methods namely thermal (e.g., sterilization) and chemical treatments (e.g., hydrogen peroxide) that may cause undesirable effects on quality of foods. Pulsed light has proven to be efficient for disinfection on the surface of foods (e.g., shell eggs, blueberries), food contact materials (e.g., stain steel surfaces) and packages (e.g., polyethylene terephthalate [PET]) (Ozen and Floros 2001; Woodling and Moraru 2005; Elmnasser and others 2007).

Pulsed light generation involves the use of a system designed to magnify and convert electromagnetic energy into short, intense and intermittent light pulses (Oms-Oliu and others 2010). This system comprises three major

components, which include: the power supply, lamp and a high voltage connection (Elmnasser and others 2007).

The light emitted by the system consists of a broad-spectrum wavelengths varying from UV to near infrared, which elicit microbial inactivation. The wavelength spectrum of PL ranges from 100-1100 nm and includes: UV-C (100-280 nm), UV-B (280-320 nm), UV-A (320-400 nm), visible light (400-700 nm) and infrared (700-1100 nm) (Guerrero-Beltrán and Barbosa-Cánovas 2004; Oms-Oliu and others 2010). Rowan and others (1999) attributed most of the germicidal effect to the UV region wherein UV-C accounts for about 50%. It was observed that the inactivation of food related microorganisms such as *S.* Enteritidis and *Listeria monocytogenes* increased 3-fold using a UV-C flash. In contrast, Takeshita and others (2003) maintained that the entire wavelength spectrum act synergistically and accounts for an enhanced lethality as observed on yeast cells of *Saccharomyces cerevisiae.*

Mechanisms of Action of PL

The lethality of the effect of PL on microbial inactivation can be attributed to three major mechanisms: photochemical, photothermal and photophysical. Most of the germicidal effect of PL has been attributed to the deoxyribonucleic acid (DNA) damage caused by photochemical mechanisms, which include the formation of dimmers and other photoproducts (Krishnamurthy and others 2010). In contrast it has been also suggested that these DNA injuries may be reversible through repair mechanisms in the absence or presence of light involving the action of DNA photolyases (Sinha and Häder 2002; Hierro and others 2009).

The contribution of photothermal mechanisms varies depending on the treatment time, thermal absorption difference between the food matrix and bacteria and PL wavelength. Krishnamurthy and others (2010) observed an increased temperature in *S. aureus* during exposure times greater than 5 s. Wekhof and others (2001); Takeshita and others (2003) attributed this effect to the differences of light absorption between bacteria and the surrounding food media, which leads to cell death as water vaporizes and disrupts bacterial membrane.

Finally, photophysical effects have been also reported on macromolecular components such as proteins and other cellular organelles. Takeshita and others (2003) presumed that the lethal effect of PL was not only accounted by DNA injuries but also by physical damage induced in vital organelles. Indeed, transmission electron micrographs (TEM) revealed higher levels of eluted protein, distorted cell membranes, and expanded vacuoles of SC cells in the case of PL as compared to UV light treatment (control) as reported by Takeshita and others (2003).

Potential Applications for Decontamination of Eggs and Contact Surfaces

The application of PL on traditional food commodities such as eggs and egg products may minimize their associated risk. On average, Americans consumed a total of 246 eggs or egg derivatives (e.g., liquid eggs) combined per person in 2011 (USDA 2011a). Due to their widespread consumption, the safety of these products is crucial. Shell eggs may harbor food pathogens, mainly *S.* Enteritidis that has been extensively reported in the literature (Howard and others 2011). Infection of the outer surface of specialty egg products with *L. monocytogenes* may also occur due to improper handling practices or contact

with contaminated work surfaces in commercial facilities (Claire and others 2004).

Pulsed light seems to outperform current preservation methods by enhancing the safety of eggs without compromising sensory and nutritional quality. Previous studies have investigated the impact of PL on shell eggs contaminated with *S.* Enteritidis. Lasagabaster and others (2011) attained maximum inactivation (5.0±0.3 log CFU/shell egg) within the limit of detection ($5x10^2$ CFU/shell egg) at low fluencies (2.1 J/cm^2). Whereas, Keklik and others (2010) obtained total inactivation (4.0±0.3 log CFU/cm^2) with approximately 17 J/cm^2 energy applied during the treatment. In contrast, Hierro and others (2009) achieved the highest reduction (1.94±0.7) at 12 J/cm^2. Overall, temperature fluctuation and changes in quality (e.g., eggshell strength, cuticle, rheological properties) of shell eggs were not encountered. In addition, the nature of the light, the initial bacterial level and others factors (e.g., temperature) may be accountable for differences in inactivation kinetics of different PL systems (Keklik and others 2010).

Similarly, PL has shown to be effective on inactivation of food contact surfaces as illustrated in the literature Levy and others (2012) investigated the efficiency of PL on reducing microbial populations of spore-forming microorganisms on assorted food contact materials. To illustrate, high microbial inactivation (5 log) was observed on polystyrene with uniquely 0.6 J/cm^2 and 0.8 J/cm^2 for *Bacillus subtilis* and *Aspergillus niger* (Levy and others 2012). Woodling and others (2005); Haughton and others (2011) achieved reductions of *E. coli* and *L. innocua* in the 4 log order using different energy levels on stainless-steel surfaces with diverse finishes (electropolished, milled, glass

bead blast finish and aluminum oxide treated). Similarly, Rajkovic and others (2010) effectively eliminated *L. monocytogenes* and *B. subtilis* populations (6.5 log CFU/side of the knife) from the surface of a meat-slicing knife (stainless steel material) with low energy exposure (3 J/cm^2). In agreement with Woodling and others (2005), Rajkovic and others (2010) outlined that the efficiency of PL largely depends on the surface topography, the time interval between treatment and contamination and the food composition contacting the surface.

Major Considerations and Limitations

The efficiency of PL mainly depends on the fluence received by the food or contact surface during the treatment. Fluence is the energy released by the lamp per unit area of sample expressed in J/cm^2 (Gómez-López and others 2007). This in turn is a function of critical process factors such as treatment time, distance from the PL source, thickness, food composition, sensitivity of target microorganism and others, which require to be taken into consideration prior implementation in the food industry.

Overall, long exposure time, short distances and low thickness enable greater disinfection. These parameters were confirmed by Hillegas and Demirci (2003) and Sharma and Demirci (2003) who attained maximum reductions of *Clostridium sporogenes* (5.47 log CFU/g in clover honey) and *E. coli* O157:H7 (4.89 log CFU/g in alfalfa seeds) under the foregoing conditions. The effect of time, distance and thickness can be explained in terms of number of pulses (increases as a function of time), energy intensity (decreases as a function of distance) poor penetrability (2 µm in opaque media) (decreases as a function of thickness) of PL respectively (Wallen and others 2001; Hillegas and Demirci 2003; Gómez-López and others 2005a).

The degree of microbial inactivation exerted by PL depends also on food composition attributed to carbohydrates, water, protein and fats. Krishnamurthy and others (2004) reported that PL is more suitable for solids surfaces than liquids (e.g., wine, fresh juices) in which the process differs as affected by solids concentration closely related to absorbance. Gómez-López and others (2005b) attempted to decontaminate vegetables (e.g., spinach, soybean sprouts) and agar plates supplemented with starch, water, protein and oil at various concentrations. These authors failed to inactivate *Candida lambica* in samples containing either 10% oil or 10% casein. Thus, they concluded that the treatment efficiency is affected by the capacity of these components to absorb within the UV wavelength of PL (Gómez-López and others 2005b). This was also observed by Ozer and Demirci (2006) who only attained 1 log unit reduction of *E. coli* O157:H7 or *L. monocytogenes* in salmon fillets with no adverse effect on quality. Conversely, foods rich in carbohydrates and water such as fruits and vegetables seem more suitable for PL treatment. However, the development of off-odors and decrease of shelf life have also been reported in vegetables such as white cabbage and lettuce (Gómez-López and others 2005b).

The shadowing effect and sensitivity to PL are also major limitations influenced by heavily contaminated surfaces and irregular surfaces and possibly the type of microorganism. Microbial populations situated in the upper layers of foods overlap those in the lower layers making their inactivation difficult (Gomez-Lopez and others 2005a). Likewise, certain microorganisms such as *E. coli* O157:H7 and *S.* Enteritidis may penetrate through irregularities of foods surfaces (e.g., pores) such as cut lettuce and eggs (Seo and Frank 1999;

Lagunas-Solar and others 2006; Hierro and others 2009). To date, no general patterns about the response of different microorganisms to PL have been established. However, cell wall composition and repair mechanisms have been outlined as the main factors determining their resistance or susceptibility to the treatment (Gómez-López and others 2007; Oms-Oliu and others 2010). Some researchers have proposed the following decreasing order of sensitivity: Gram-negative, Gram-positive and fungal spores (Rowan and others 1999). While others, have not noted significant differences among inactivation of different microflora (Gómez-López and others 2007).

Finally, the temperature build up is perhaps the principal drawback implicated with this technology. Fine and Gervais (2004) failed to decontaminate wheat flour and black pepper due to overheating of samples which resulted in alteration of color and flavor profiles. Similarly, Wambura and Verghese (2011) reached a maximum increase of 68°C on sliced ham treated for 120 s and at 4.5 from PL strobe. Some investigators have highlighted the incorporation of cooling systems and the limited use of the infrared spectrum as possible approaches to minimize overheating (Demirci and Panico 2008). However, this does not necessarily impede the manifestation of this effect as observed by Jun and others (2003) that attained a considerable increase in final temperature of corn meal (120°C).

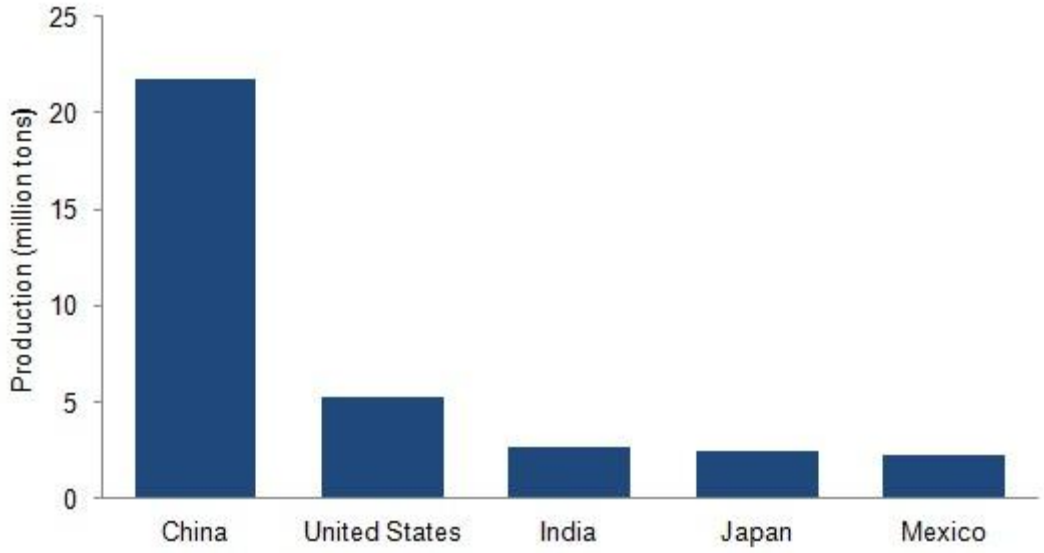

Figure 2-1. Hen egg production in selected countries. Adapted from: FAO (2009)

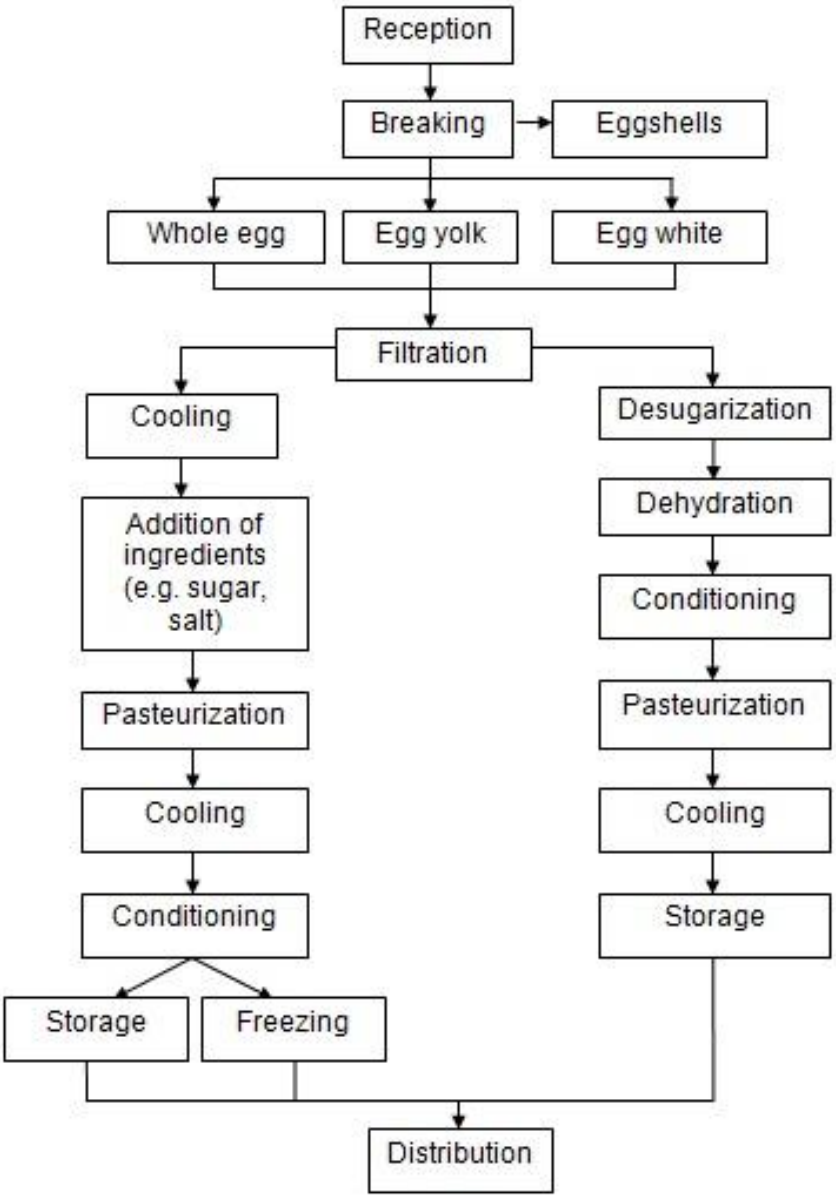

Figure 2-2. Elaboration of egg products in breaking facilities. Adapted from: Stadelman and Cotterill (1995)

CHAPTER 3
EFFICIENCY OF PULSED LIGHT FOR MICROBIAL INACTIVATION ON HARD-COOKED SHELLED EGGS

Background

As alluded to previously, hard-cooked eggs are prepared by rapid heating (90-95°C) and cooling operations that induce total microbial inactivation. Nevertheless, recontamination may occur as a result of inadequate manipulation by food handlers or exposure to airborne and infected surfaces.

Nowadays, hard-cooked eggs are mainly bulk packaged in MAP and distributed for consumption. Regarding this, Claire and others (2004) maintained that MAP shall not be considered adequate barrier for microbial inhibition as it allows the growth of *L. monocytogenes* even under extreme conditions (80% CO_2, 5°C). Another effort to control microbial growth on hard-cooked eggs involved edible coatings formulated with known antimicrobial agents (e.g., chitosan-lysozyme), which also resulted unsuccessful (Kim and others 2008).

A recent recall involving hard-cooked eggs in brine has further questioned the safety of these RTE products and has called for alternative methods to mitigate the impact of post-processing contamination (FDA 2012a). In this study, the effects of PL on microbial populations and temperature variation of artificially contaminated hard-cooked eggs were investigated. The efficiency of PL was assessed based on the level of microbial reduction as described by Uesugi and others (2007). Traditionally, the egg industry has relied on Coliforms, especially *E. coli,* as biological indicators of unsanitary conditions (Downes and Ito 2001; Musgrove and others 2004). Therefore, *E. coli* K12 was selected as the target microorganism since it is non-pathogenic hence can be safely used for pilot plant challenge studies.

Materials and Methods

Sample Preparation

Dry-packed shelled hard-cooked eggs were provided by Michael Foods Inc. (Minnetonka, MN, USA) and stored at refrigerated conditions (5°C). Prior to experimentation, the eggs were sorted according size and washed with sterile water. The eggs were then dried on stainless steel screens under a laminar flow hood and adjusted to room temperature (25°C) for approximately 1 h.

Acquisition and Maintenance of Bacterial Culture

A laboratory stock strain of *E. coli* K12 (MDD 333) was obtained from the University of Tennessee (Knoxville, TN, USA) and subsequently adapted to rifampicin (Rif) (Fisher Scientific International, Fair Lawn, NJ, USA) at the University of Florida (Gainesville, FL, USA) to suppress any background flora.

Rifampicin is an antibiotic capable of affecting the bacterial RNA polymerase and interfering with transcription processes (Jun Jin and Gross 1988). Throughout the experiments, stock solutions of this antibiotic were prepared, stored under refrigeration (5°C) and covered with aluminum foil given its thermal and light sensitivity. The preparation of stock solutions [10000 µg/mL] involved dissolving 0.4 g of the antibiotic in 40 mL of high performance liquid chromatography (HPLC) grade methanol, and then filter-sterilizing (0.2 µm) (Fisher Scientific International, Fair Lawn, NJ, USA).

The antibiotic-resistant cultures were prepared according to protocols outlined by Lindeman and Suslow (1987) with slight modifications. For this purpose, a thawed culture of *E. coli* K12 was grown in 10 mL of tryptic soy broth (TSB) (Becton Dickinson, Franklin Lakes, NJ, USA) for 24±2 h at 37°C. Subsequently, 1 mL of this preculture was spread-plated onto tryptic soy agar (TSA) (Becton Dickinson) supplemented with Rif (200 µg/mL). Spontaneous Rif

mutants were maintained at -80°C in 30% glycerol-TSB solution and at 5°C on TSA-Rif agar which was subcultured biweekly to ensure viability. For microbial enumeration, TSA-Rif media was used at a lower concentration (80 µg/mL) to allow for selective growth while reducing the stress on bacterial cells.

Growth Studies

Growth curves were performed to ensure that bacterial cells were in the stationary phase given its significance to food microbiology. As described by Rees and others (1995), as cells reach stationary phase, they undergo a large number of phenotypic and genotypic changes, which increase their resistance to a range of stresses (e.g., thermal, oxidative, starvation). With respect to *E. coli*, cells shrink, the cytoplasm becomes condensed, the cell membrane altered and the chromosomal DNA more compactly packed (Rees and others 1995).

Isolated colonies of *E. coli* K12 were transferred from TSA-Rif plates to 10 mL TSB-Rif tubes (200 µg/L) which were incubated in a static incubator 24±2 h at 37°C (VWR International, Batavia, IL, USA). The cultures were successively transferred for two days in 10 mL TSB-Rif tubes and incubated under the same conditions to obtain uniform cell type (Beuchat and others 2003). On the day of the experiment, the cultures were serially diluted (1:10) in (0.1%) peptone water (PW) (Becton Dickinson) yielding cell populations of approximately 10^2 CFU/mL and then grown into 100 mL TSB-Rif flasks (200 µg/L).

Bacterial growth was monitored at 1h intervals by direct and viable cell counts methods. In the first case, a cuvette containing 3 mL of the inoculated broth was placed into a spectrophotometer and set for hourly readings at a wavelength of 570nm (Shimadzu Scientific Instruments, Inc., Model UV-1201, Japan). Growth curves were finalized after three consecutive readings as

indicated bacteria entering the stationary phase. Viable cell counts were conducted simultaneously by serial dilutions of the inoculated broth in PW (0.1%). Appropriate dilutions were pour-plated into TSA-Rif, incubated 24 h at 37°C, enumerated by standard plate counting (SPC) and expressed as CFU/mL. Growth curves were built by creating scatter plots of counts (CFU/mL) versus time (h).

Preparation of Inoculum

The inoculum was prepared by performing culture transfers three days before experimentation. Isolated colonies were grown either in 10 mL tubes or 250 mL flasks of TSB-Rif (200 µg/L) and incubated 17±2 h at 37°C.

Cells were harvested by centrifugation three times (4,000 x g, 10 min at 25°C), washed and finally resuspended in either 2.5mL or 250 mL of PW (0.1%) to reach variable cell density. The initial bacterial populations yielded $4x10^9$ CFU/mL and 10^9 CFU/mL for spot-inoculation and whole-inoculation studies respectively. This was verified by SPC as previously described.

Inoculation of Eggs

Two separated methods were used to inoculate egg surfaces. The first was spot inoculation that consisted in placing on the equator of each egg five spots of approximately 5 µL to yield a total inoculation of 25 µL. The second was whole inoculation that is more representative of a real-case scenario in which a food product is completely contaminated. For this purpose, an approximate area of 4 cm^2 in the end opposite to the air cell of each egg was delineated. Each egg was subsequently immersed 15 s in a beaker containing 250 mL of the inoculum with exception to the marked area (Kim and others 2008). Immediately after contamination, the samples were air-dried under a laminar flow hood at room temperature. The drying time was experimentally determined and

corresponded to 90 min and 150 min for spot-inoculated eggs and whole-inoculated eggs respectively.

Pulsed Light Treatment

The treatments were conducted at room temperature using a prototype continuous PL system (LH840-LMP-HSG, Xenon Corp., Wilmington, MA, USA) (Figure 3-1). The system comprised a controller unit, a hydraulic conveyor belt and a treatment chamber housing two adjustable Xenon flash lamps. This unit generated a broad-spectrum light (100-1100 nm) at a pulse rate of three pulses/s and a pulse width of 360 µs. Values of energy intensity (J/cm^2) were adopted from measurements conducted by Krishnamurthy (2006) in a PL batch system of similar characteristics (Figure 3-2).

Spot-inoculated eggs were placed on sterile aluminum discs (Fisher Scientific International) and treated under static condition. Conversely, whole-inoculated eggs were inserted about two-thirds to the end of a sterile rod coupled to a rotational setup (Figure 3-3). This system included a gear motor (Dayton DC motor, Electric Motor Warehouse, MI, USA) set at 60 revolutions/min (RPM) by adjusting a speed controller (UK1225-7A, Canakit, BC, CAN) and taking frequent RPM readings with a non-contact tachometer (Neiko 20713A, USA). This specific RPM was selected to simulate conveyor-belting conditions in which eggs rotate moderately fast.

Exposure time and distance from the PL quartz window were established as the experimental variables. Samples were individually centered and treated for 1, 3, 5, 10, 15, 20 and 30 s at 5.5 cm, 9.5 cm and beneath the quartz window. All PL treatments were performed on *E. coli* K12 in stationary growth stage.

Temperature Measurements

To monitor temperature variation on the surface of eggs during the treatment, a non-contact infrared thermometer (Omega OS423-LS, Omega Technologies, Stamford, CT, USA) was used. Temperature measurements were performed before and after the PL treatment. To assure that all measurements were taken at the same distance, the sensing head of the thermometer was placed directly on the surface of eggs.

Recovery and Microbial Enumeration

Untreated and treated samples were transferred to sterile stomacher bags containing 25 mL and 50 mL of PW (0.1%) in the case of spot inoculated and whole inoculated eggs respectively. These volumes were selected as immersed the inoculated areas in the bags. Cells were recovered by vigorous hand shaking for 1 min as described by Keklik and others (2010). Rinse solutions were then serially diluted in PW (0.1% w/v) and pour-plated in TSA-Rif and statically incubated 24 h at 37°C. Additionally, negative controls for TSA-Rif were also poured to determine if contamination had occurred in any stage of the experiment. Results were expresses as log CFU/egg being the limit of detection (LOD) 1.7 log CFU/egg.

Most Probable Number

When the counts fell below 25 CFU/mL for the lowest dilution, the survivors were enumerated by the most probable number (MPN) method with three-tube sampling for *E. coli* as described by the Bacteriological Analytical Manual (BAM) with slight modifications (FDA 2012b). In short, 10-fold dilutions of the inoculum in the rinse solutions (1/10, 1/100, 1/1000) were prepared initially. Then, 1 mL portions of each dilution were transferred to 9mL-tubes of Lauryl tryptose broth (LST), (Becton Dickinson) and incubated 24±2 h at 35°C.

Next, a loopful of each gassing tube was transferred to EC broth (Becton Dickinson) and incubated at 45.5°C. Each gassing EC tube was streaked on TSA-Rif to eliminate possible growth of ubiquitous *E. coli.* Isolated colonies were finally transferred to Levine's eosin-methylene blue plates (L-EMB, Becton Dickinson), incubated 18-24 h at 35°C and examined for the presence of suspicious colonies (dark centered and flat, with or without metallic sheen).

Evaluation of PL Efficiency

Inoculated and untreated eggs served as control samples to account for losses corresponding to unrecoverable and non-viable cells post drying. The level of microbial inactivation (Log [N/No]) was calculated by subtracting the survivor counts (N) resultant from PL treatment from the initial counts (N_o) represented by the control samples. Results were expressed in log CFU/egg. Additionally, survivor curves were built by creating scatter plots of log survivor counts (N) versus treatment time (t).

Statistical Analysis

The data resulted from each experiment was tabulated as the average of three replicates ± standard error mean (SEM). Inoculated eggs that were not treated served as controls. Differences among treatments were analyzed using the general linear model (GLM) procedure in MINITAB version 15. Multiple comparisons of the mean values were determined using the Tukey's method with a confidence interval of 95%.

Results and Discussion

Growth Studies

Results from these preliminary studies (Figure 3-4) assured that consistent inoculum suspensions of *E. coli* K12 in the stationary could be obtained throughout the experiments. This also contributed to decrease the variability

among PL challenge studies as microbial cells exhibit variable resilience to inactivation according to their growth stage (Rees and others 1995). As illustrated in the Figure 3-4, *E. coli* K12 cells entered the early stationary phase after 15 h of incubation approximately. For this reason, the incubation time of the inoculum destined for the inactivation studies was set to 16±2 h.

Temperature Measurements

Pulsed light has been considered a non-thermal technology when applied for short times. However, prolonged exposure may produce increases temperature as energy accumulates in foods products.

Hence, temperature changes on the surface of hard-cooked eggs during PL treatment at different distances were recorded and depicted in Figure 3-5.

According to the measurements undertaken with the infrared noncontact thermometer, the average initial temperature of samples was 19.7±0.5°C. In general, the surface of the eggs experienced increases in temperature up to ~9°C and ~6°C at 5.5 cm and 9.5 cm from the quartz window respectively (Figure 3-5).

In accordance with the literature, as the distance from the lamp source decreased and the treatment time increased, the food samples receive a more intense treatment (Gómez-López and others 2005a). Therefore, higher temperatures could be expected on the surfaces as illustrated in Figure 3-5. The temperature buildup triggered by PL in this study is moderately small, especially during the first seconds of exposure (~2°C).

Literature has considered that the temperature rise during PL treatment is strongly dependant on the color of food products (Oms-Oliu and others 2010). Thus, dark products are associated with higher energy absorption contributing

to a more prominent rise in temperature (Oms-Oliu and others 2010). To illustrate, it has been demonstrated that dark fish samples absorb significantly more energy leading consequently to rises in temperature (Oms-Oliu and others 2010). Fine and Gervais (2004) observed an overheating effect, more dominant in black pepper than wheat flour treated with a fluence of 31.12 J/cm^2 at 2 cm from the xenon lamp. On the contrary, Keklik and others (2012) recorded temperature variations of 7.8°C and 13.3°C on shell eggs at 14 cm and 9.5 cm from the UV lamp respectively. Therefore, in this study is reasonable to obtain a minimal temperature rise since peeled hard-cooked are products of white or light appearance.

Another possible explanation to temperature changes during PL treatment is the fluence threshold, a term adopted by Wekhof (2000). As alluded to previously, fluence is referred as to the energy delivered to the sample by the system during PL exposure. Regarding this, Wekhof (2000) identified an energy level beyond which *E. coli* cells on a polymeric surface increased rapidly their temperature above 120°C. However, this threshold may be dependent on the type of food and the extent in which energy is absorbed. Given the limited absorption of energy in light food products, it is probable that samples did not reach a fluence threshold in any of the treatments.

Finally, the variations in temperature attained in this study are comparable to those reported in the literature using PL. Hierro and others (2009) reported maximum differences on temperature readings of 3°C on shells eggs as fluence reached 12 J/cm^2.

Similarly, Lasagabaster and others (2011) recorded temperature gradients on shell eggs in a range of 2.1°C and 4.2°C after treatment energy of 2.1 J/cm^2

and 10.5 J/cm^2. These correspond to treatments of 3-10 s at 5.5 from the quartz window in which temperature fluctuated 1-3°C above the initial temperature of the samples.

Evaluation of PL Efficiency in Spot-inoculated Eggs

As previously outlined in the review of literature, PL comprises three major mechanisms of inactivation. These include: photochemical, photothermal and photophysical which may have induced a sequent of detrimental reactions in *E. coli* cells, namely the loss of motility as outlined in the literature (Barbosa-Cánovas and others 2000). In this study, the photochemical mechanism could be mainly attributed to the wavelength range of 200-300 nm, reportedly the wmost lethal for *E. coli*, according Wang and others (2005). Particularly, UV radiation at 254 nm triggers the formation of pyrimidine dimmers, distorting the DNA helix and preventing cell replication. Additionally, UV light causes cross-linking of aromatic amino acids (e.g., tyrosine, tryptophan) at their carbon-carbon double bonds to ultimately contribute to membrane depolarization and irregular ionic flow (Lado and Yousef 2002).

Other photochemical mechanisms attributed to the UV spectrum of PL are the production of peroxides and ozone. At long-wave UV (320-400 nm), hydro peroxide radicals are generated in unsaturated fatty acids constituting the membrane and resulting in permeability changes. While, at short-wave UV (180-240 nm) oxygen is absorbed and converted into ozone. However, the production of ozone may be greater in the lamp unit itself and may not play a significant role in the killing mechanism of PL as described by Nordhauser and Olson (1998).

In contrast, the photothermal and photophysical mechanisms have been commonly associated with overheating during the treatment (Oms-Oliu and others 2010). The temperature profile conducted (Figure 3-5), suggest an absence of these effects. However, some researchers have sustained that the lack of changes in temperatures shall not be misinterpreted, as overheating may be instantaneous and localized in bacterial cells inducing considerable damage (Takeshita and others 2003).

Survivor curves of *E. coli* K12 treated for up to 30 s at 5.5 cm and 9.5 cm from the quartz window are presented in Figure 3-6 respectively. Almost the totality of data points were determined by SPC with the exception of the counts of 20 s and 30 s at 5.5 cm and at 9.5 cm from the quartz window respectively. These corresponded to MPN estimates.

As illustrated, survivor curves were characterized by the absence of shoulders, an upward concavity and a prominent tailing effect. The lack of shoulders denotes a prompt inactivation of microorganisms as energy is readily available at the beginning of the PL treatment. In contrast, UV light survivor curves exhibit a shoulder caused by a delayed response of injured microorganisms to the treatment (Sastry and others 2000). An upward concavity indicated that the treatment efficiency decreased gradually as emphasized by Izquier and Gómez-López (2011). In the case of survivor curves at 9.5 cm, the concavity effect was less evident. A sound explanation of this pattern is that fluencies delivered at 9.5 cm are lowered that those at 5.5 cm as described in the Figure 3-2. Finally, tailing effects are presumably attributed to microbial cells shielding, clustering or resisting to PL as explained by Sastry and others (2000) in UV light technology.

Similar findings have been repeatedly reported in previous studies with *E. coli* and other microorganisms on liquid and solid substrates. Bialka and others (2008a) observed non-linear curves with tailing off survivors of *E. coli* O157:H7 and *Salmonella enterica* treated with almost 65 J/cm^2 of PL. Moreover, Bialka and others (2008b) identified similar survivor curves with concavity (upward and downward) and tailing effects when modeling the PL inactivation of *E. coli* K12 on solid substrates (e.g., agar, whey protein). Rowan and others (1999) described comparable patterns in cultures of *E. coli,* and *L. monocytogenes* cultures seeded on agar surfaces and inactivated with pulsed light emissions of high or low UV spectrum. Uesugui and others (2007) and Woodling and Moraru (2007) also obtained similar trends on survivors of *L. innocua* suspended or inoculated in liquid and solid substrates and exposed to ~13 J/cm^2. Therefore, it can be concluded that the previously described effects are typical microbial responses to PL. The degree of concavity and tailing may be dictated by the susceptibility of the microorganism throughout the treatment as influenced by experimental variables (e.g., energy absorbed by the sample) and complex interactions with the substrate.

During the MPN procedure, it was observed that microbial populations increased suggesting that sublethally injured cells were able to recover. To date, there are only a few studies reporting repairing mechanisms post PL treatment. For example, Gómez-López and others (2005a) reported reactivation mechanisms in a wide range of microorganism including *E. coli* with specially when exposed to light. Hierro and others (2009) identified that *S.* Enteritidis had also the capability to photoreactivate. Added to these studies, Rincon and

Pulgarin (2003) demonstrated that *E. coli* K12 recovered to its initial population after illumination without a photocatalyst and exposure for 3 h in the dark.

Literature has described that *E. coli* can potentially remediate the lethal effects of pyrimidine dimers during UV treatment by both photoreactivation and excision repair mechanisms (Witkin 1976). The first is achieved by "photoreactivating enzyme" which selectively acts on pyrimidine dimmers binding them in the dark and monomerizing them in light between 310 nm and 400 nm wavelength. The second is mainly accomplished by DNA polymerase I, which replaces the pyrimidine dimers by repair replication using the intact DNA region as a template (Witkin 1976). Therefore, repair of damaged cells could have occurred during MPN procedures.

As alluded to previously, the reported microbial reduction (Table 3-1) considered the recovery losses resultant from inoculation and drying steps. These losses were found to be less than 0.1 log CFU per egg with standard deviation of 0.1 log CFU. Moreover, the survivor counts obtained by MPN were not considered for the calculation of levels of inactivation as this a probabilistic approach for bacterial enumeration.

To determine the effect of treatment time and distance on the levels of microbial reduction, pairwise comparisons of the inactivation means were obtained by Tukey (Table 3-1). Bacterial counts were dramatically reduced at the first PL exposure ($P<0.05$) and consistently decreased with increasing time and output energy. Microbial populations were reduced between 2.20±0.36 and 6.40±0.00 log orders (CFU/egg) during PL treatment performed at 5.5 cm from the quartz window. Exposure to PL light during 1 s and 3 s were significantly different to the rest of the treatments ($P<0.05$). Treatment duration of 5 and 10

s, 10 and 15 s, and 20 and 30 s yielded similar inactivation (P>0.05). Log reductions were in the range of 1.83±0.29 and 6.40±0.00 log CFU/egg at 9.5 cm from the quartz window. The application of PL illumination for 1 s produced microbial inactivation significantly different from all other treatments (P<0.05). Treatment time had little effect on microbial populations after 3, 5 and 10 s (P>0.05). Whereas, exposure equivalent or longer than 15 s reduced significantly *E. coli* K12 populations (P<0.05). Distance from the quartz window did not influence significantly on *E. coli* K12 reduction (P>0.05), except at 15, 20, and 30 s.

Under these experimental conditions, the optimum treatment condition was 20 s at 5.5 cm from the quartz window. However, this would not be necessarily practical for food applications as the maximum cumulative treatment cannot exceed 12 J/cm^2 as stipulated by the FDA (FDA 2012c).

As outlined in the literature, the lethality of PL may be strongly influenced by the substrate and features of the PL system (e.g., pulse width). Ozer and Demirci (2006) achieved scarcely 1 log reduction on *E. coli* O157:H7 inoculated on salmon and treated 30 s at 5 cm from the quartz window. Bialka and others (2008) investigated the effect of PL on the same microorganism in contact with the surface of strawberries. These researchers achieved 2 log unit inactivation after 30 J/cm^2 of PL treatment, and presumed that part of the bacterial population remain protected in the crevices of the fruit. In the same year, Bialka and others (2008b) estimated the inactivation of *E. coli* K12 in solids models. It was found that ~70J/cm^2 PL exposure were required to induce almost 4 log reduction on microbial cells added to 4% agar. Sharma and Demirci (2003) achieved a population reduction of *E. coli* O157:H7 of 4.8 log CFU per gram of

alfalfa seeds illuminated at 8 cm from the quartz window for up to 90 s. A previous study by Rowan and others (1999) attained high reductions (6 log units) of *E. coli* O157:H7 *in vitro* in less than 1 s as explained by the high frequency of the pulses (85 nanoseconds width).

The findings of this study suggest that peeled hard-cooked eggs are suitable substrates for PL treatment by allowing for a high degree of inactivation. However, it is also possible that some fraction of microorganisms may have slightly internalized into the surface, survived the PL treatment and recovered during MPN protocols as previously described. Keklik and others (2010) demonstrated that shell eggs inoculated with *S*. Enteritidis can be totally decontaminated after PL treatment at ~4 cm from the quartz window. These authors reported slightly lower reductions as compared to our study, attributed mainly to differences in microbial sensitivity as elucidated by Rowan and others (1999). Another study by Uesugui and Moraru (2009) found that PL reduced by 1.4 log CFU *L. innocua* on Vienna sausages after exposure to 9.4 J/cm^2. In our experiment, this energy level is nearly equivalent to ~10 s of treatment at 5.5 cm from the quartz window, in which, reductions of approximately 4.5 log CFU were attained. These results vastly differ from this PL study as *L. innocua* was utilized as a surrogate of *L. monocytogenes* that is reportedly the most resistant bacteria to PL (Oms-Oliu and others 2010).

Evaluation of PL efficiency in whole-inoculated eggs

Survivor curves of *E. coli* K12 in whole-inoculated eggs treated for up to 20 s at 5.5 cm and 9.5 cm from the quartz window are presented in Figure 3-7 respectively. The shape of the curves was similar to those earlier described in spot-inoculated eggs. However, the degree of concavity was less pronounced

and the tailing effect more predominant as the microbial populations leveled off at around 15 s and reached a plateau (9.5 cm distance). This indicated that the treatment efficiency rapidly decreased over time. Izquier and Gómez-López (2011) observed similar patterns in survival counts of aerobics on Iceberg lettuce and attributed these effects to the irregularity of the vegetables and the heterogeneity of microorganisms.

Microbial reductions took into account losses attributed mainly to drying procedures. These losses were estimated in 0.2 ± 0.1 log CFU. At a distance of 5.5 cm and 9.5 cm from the PL quartz window, the level of inactivation ranged from 1.3 to 3.4 log CFU/egg and 0.9 to 2.0 log CFU/egg respectively (Table 3-1). At 5.5 cm distance, the log reductions of *E. coli* K12 were comparable after exposure from 1 to 10 s ($P>0.05$). Treatments from 3 to 15 s were not significantly different ($P>0.05$) while those from 15 to 20 s were also similar in terms of inactivation ($P>0.05$). At 9.5 cm distance, the log reductions did not vary much despite of the treatment time ($P>0.05$). Overall, treatments at 5.5 cm and 9.5 cm exhibited similar trends ($P>0.05$) except at 20 s.

After exposures for 20 s and 10 s at 5.5 cm and 9.5 cm respectively, the number of survivors remained relatively constant. Since the scope of this study was to determine levels of inactivation within short times, the samples were not subjected to further treatment.

A possible explanation that may warrant the higher residual counts obtained in this experiment is the inoculum size. For example, some authors sustained that inactivation processes perform uniformLy regardless the inoculum size (Uesugi and others 2007). While others, like Molinari and others (2004) maintained in some cases the inoculum size correlates to the treatment

efficiency as observed in *Saccharomyces cerevisiae* inactivation by pulsed electric fields. For PL treatment, it is possible that microbial populations located on the upper layers of eggs overlap those in the lower layers (Gómez-López and others 2005b). In this way, only the susceptible cells may have been inactivated while the hidden ones may have survived the treatment. This hypothesis is substantiated by the poor degree of penetration in PL technology, uniquely two micrometers in opaque foods (Oms-Oliu and others 2010). One feasible approach to overcome this could be the utilization of treatment at higher energy levels as declared in the literature (Elmnasser and others 2007).

Additionally, the spreading of the inoculum across the egg surface and inherent features of the PL system may have limited the extent of microbial inactivation. Smooth surfaces do not necessarily guarantee a uniform distribution of microbial cells as observed by Hierro and others (2009) in agar versus eggs with an intact cuticle. These authors obtained similar log reductions of *S.* Enteritidis (2.5 CFU/egg) on unwashed eggs exposed up to 12 J/cm^2 on a PL system endowed with polished-metal treatment chamber and two xenon lamps. The configuration of this equipment could have allowed for utmost illumination during the treatment. Lasagabaster and others (2011) estimated *S.* Typhimurium counts reduction on the surface of eggshells of almost 5 log following PL fluencies of ~10 J/cm^2. The PL setup utilized by these researchers comprised also two xenon lamps in addition to reflective surfaces in the upper and bottom to provide multidirectional exposure. The findings in our study suggest that microbial populations presumably concentrated on the poles of the eggs, were not inactivated as illumination was restricted by the light dissipation and the absence of reflectors in the PL system.

Table 3-1. Inactivation of *E. coli* K12 on hard-cooked eggs

Distance from quartz window (cm)	Treatment time (s)	Log reduction (CFU/egg)[a]	Log reduction (CFU/egg)[b]
5.5	1	2.20 ± 0.36 A	1.28 ± 0.35 A
5.5	3	3.28 ± 0.21 B	1.94 ± 0.48 AB
5.5	5	3.51 ± 0.37 C	2.22 ± 0.53 AC
5.5	10	4.58 ± 0.09 CD	2.21 ± 0.67 AC
5.5	15	5.02 ± 0.25 D	2.98 ± 0.72 BC
5.5	20	≥6.40 ± 0.00 E[c]	3.36 ± 0.88 C
5.5	30	≥6.40 ± 0.00 EG[c]	NA
9.5	1	1.83 ± 0.29 A	0.88 ± 0.25 A
9.5	3	2.98 ± 0.34 BC	1.60 ± 0.02 A
9.5	5	3.10 ± 0.36 C	1.91 ± 0.17 A
9.5	10	3.41 ± 0.23 C	2.06 ± 0.05 A
9.5	15	4.18 ± 0.34 E	2.00 ± 0.16 AB
9.5	20	4.89 ± 0.45 F	1.90 ± 0.21 A
9.5	30	≥6.40 ± 0.00 G[c]	NA

Means in the same column followed by the same capital letters were not significantly different (P> 0.05).
[a]Log reductions of spot-inoculated eggs.
[b]Log reductions of whole-inoculated eggs.
[c]MPN enrichments were positive and the LOD of SPC (1.7 log CFU/egg) was subtracted from sthe initial population to determine the log reduction.
NA Not applicable

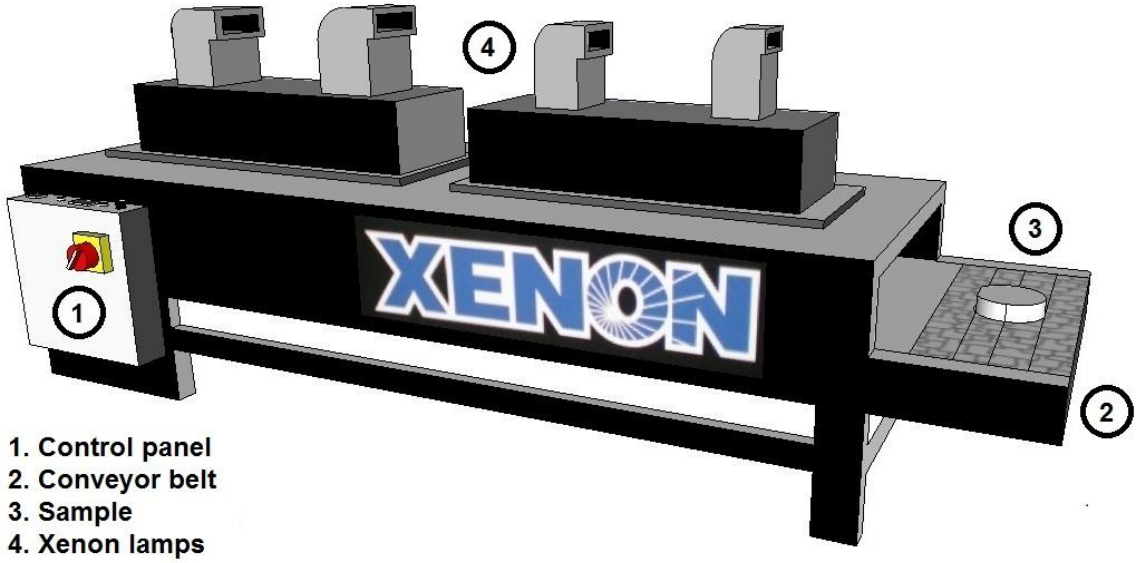

Figure 3-1. Schematic diagram of continuous PL system (LH840-LMP-HSG).

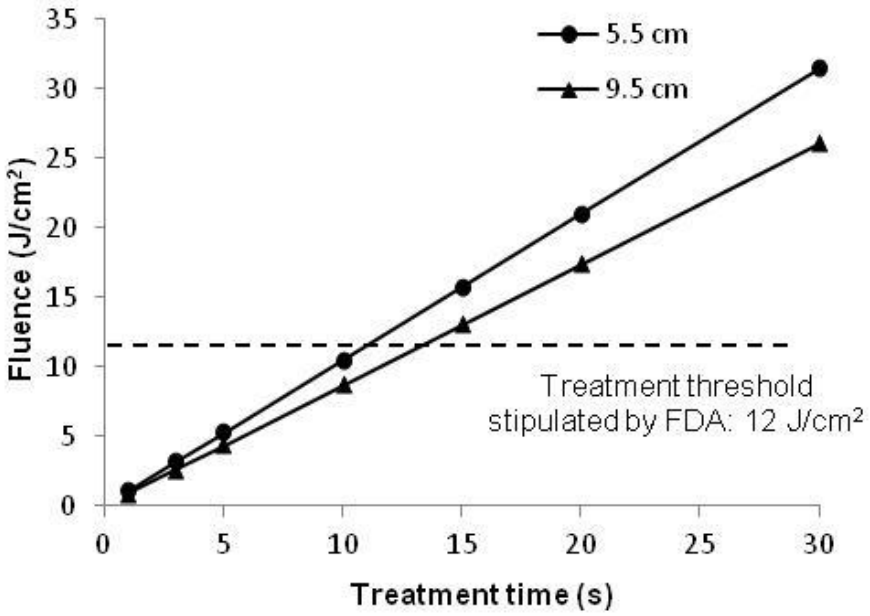

Figure 3-2. Energy intensity (fluence) during PL treatment as measured by a radiometer (Adapted from Krishnamurthy 2006).

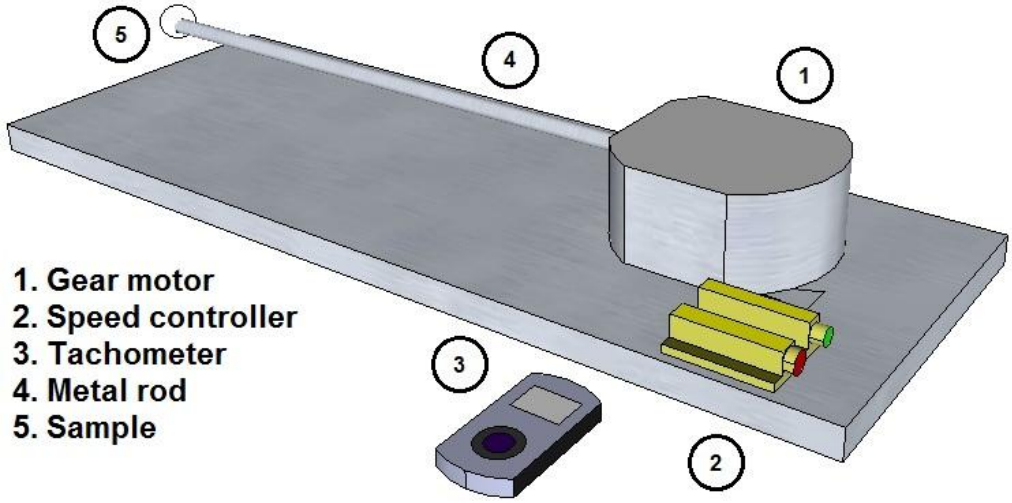

Figure 3-3. Rotational setup for treating whole-inoculated eggs during PL treatment.

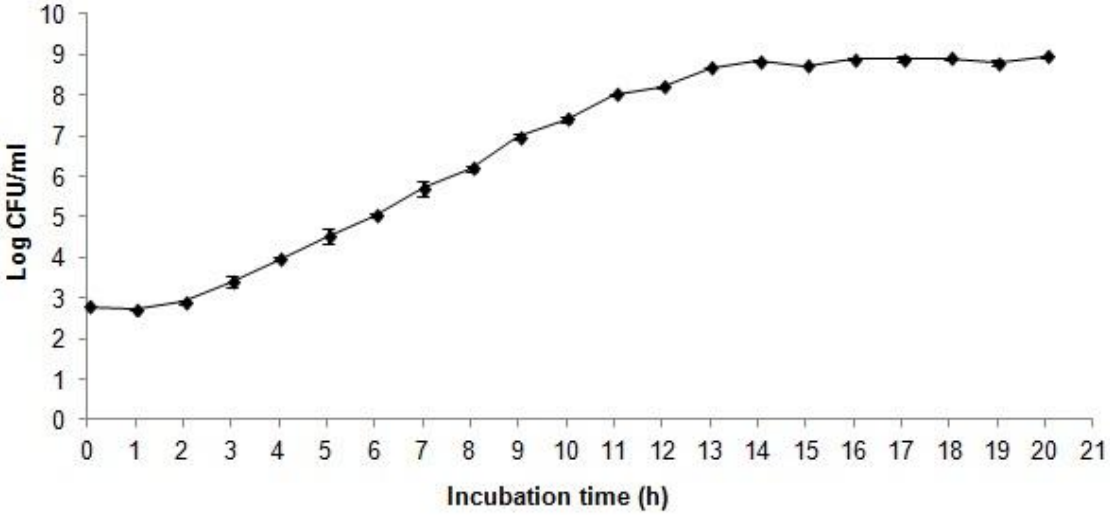

Figure 3-4. Average log10 CFU/mL growth curve of *E. coli* K12 over a 20 h incubation

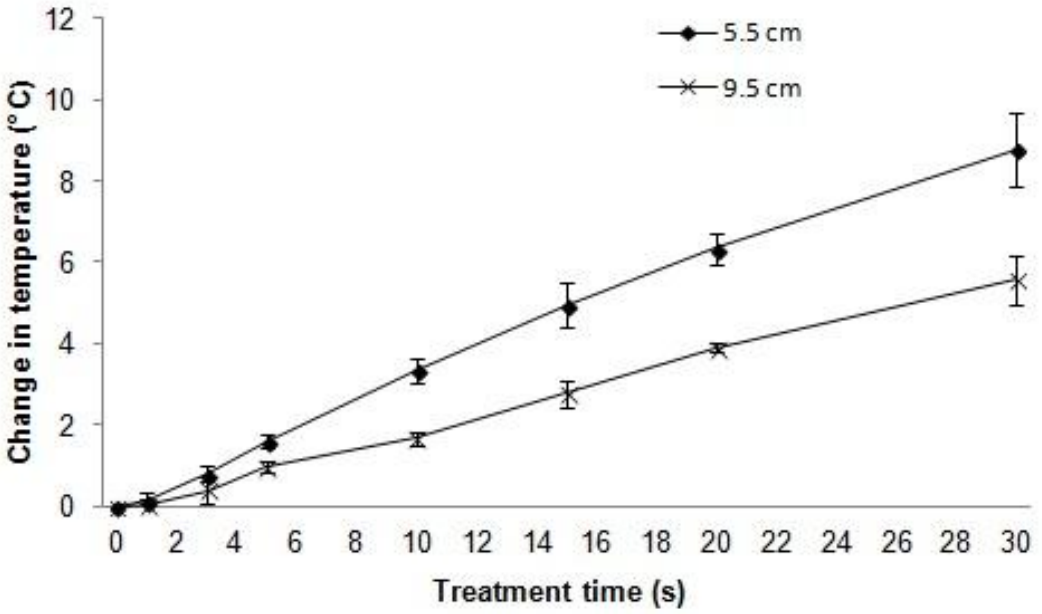

Figure 3-5. Surface heating of peeled hard-cooked after PL treatment (n=3). Initial temperature of the eggs: 19.7±0.5°C.

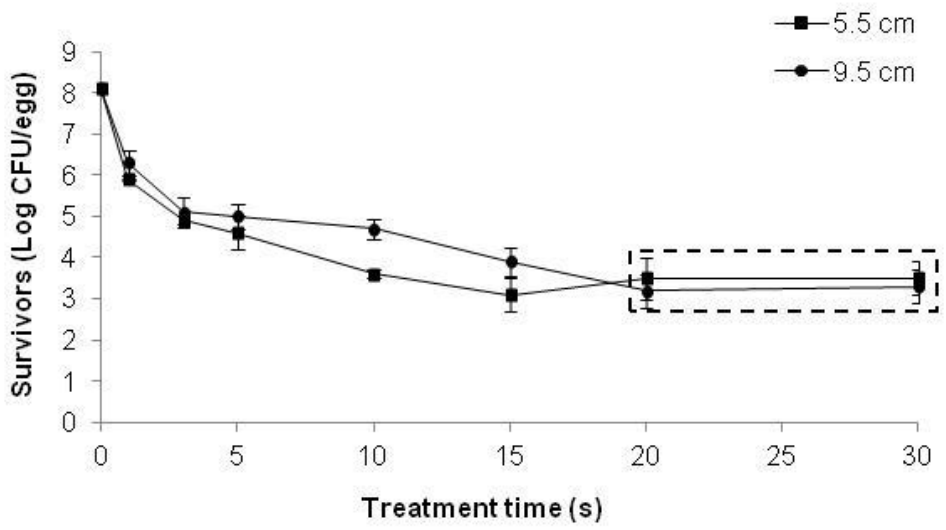

Figure 3-6. Survivor curves for spot-inoculated eggs with *E. coli* K12 treated with PL at distances of 5.5 cm and 9.5 cm from the quartz window (n=5). *Note:* (i) the data points in the dotted rectangle represent survivors determined by MPN except from 20 s at 9.5 cm, as SPC resulted in less than 25 CFU/mL.

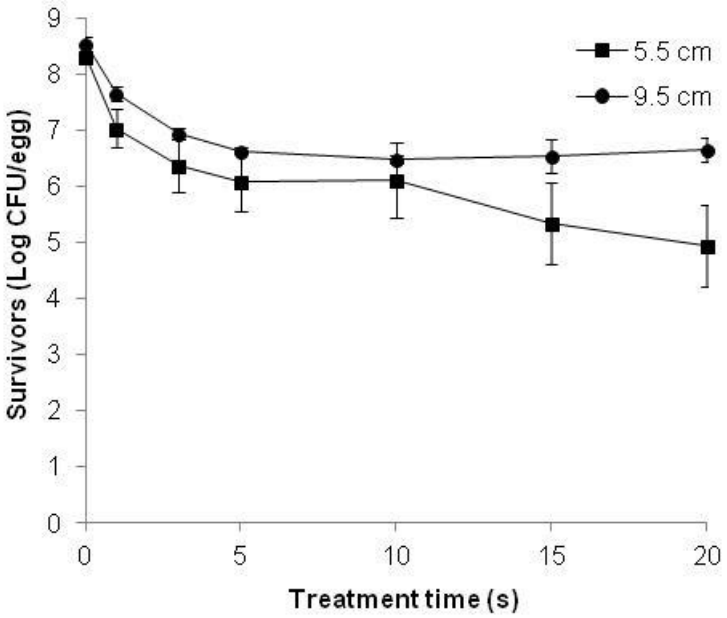

Figure 3-7. Survivor curves for whole-inoculated eggs with *E. coli* K12 treated with PL at distances of 5.5 cm and 9.5 cm from the quartz window (n=5).

CHAPTER 4
EFFICIENCY OF PULSED LIGHT FOR MICROBIAL INACTIVATION ON CONVEYOR BELT SURFACES

Background

Contaminated food contact surfaces are major vehicles for the introduction of pathogens to foods. The prevalence of various bacteria, namely *E. coli* and *L. monocytogenes* in the processing environment (e.g., transfer belts) has been extensively reported in the literature (Kusumaningrum and others 2003). Therefore, disinfection of contact surfaces is of crucial importance to minimize recontamination of RTE foods and potential outbreaks.

In this study, the efficiency of PL in reducing the microbial populations on surfaces was evaluated. High density polyethylene (HDPE) was selected to simulate conveyor belt materials encountered in egg facilities. Intact and worn HDPE coupons respectively, were used to examine differences in treatment efficiency based on microbial reduction and observations regarding the microbial distribution on the coupons.

Materials and Methods

Sample Preparation

High density polyethylene sheets in compliance with FDA regulations were used for this experiment. The HDPE sheets were cut into rectangular coupons (5.8 cm x 7.6 cm) and prepared with two different types of surface finish: smooth and sand blast (Figure 4-1). Before the treatments, the coupons were washed 10 min in a an ultrasonic bath (Fisher Scientific, Fair Lawn, NJ, USA) containing Versa-Clean solution diluted up to 1:30 with water, then immersed in 20% ethanol for 10 min and afterwards rinsed three times with tap water.

Inoculation of Coupons

Coupons were housed in plastic containers previously sanitized and then inoculated by placing 10 spots of 10 µL of the inoculum (~10^9 CFU/mL) around the center of each coupon. Once inoculated, the coupons were allowed to dry under a laminar flow hood for about 60 min, in which the time was derived experimentally. Complete air-drying created a worst-case scenario in which bacteria are localized on solid surfaces rather than suspended in a liquid media and thus they become more difficult to kill (Woodling and Moraru 2007).

Recovery and Microbial Enumeration

Treated and untreated coupons were placed in sterile stomacher bags containing 50 mL of PW (0.1%), rubbed on the inoculated area and shaken for 1 min as described by Woodling and Moraru (2007). The rinse solutions were then serially diluted in PW (0.1%), pour-plated in TSA-Rif (80 µg/L) and statically incubated 24 h at 37°C. Negative controls for TSA-Rif were also poured to disregard contamination during the execution of the experiments. Results were expresses as log CFU/coupon being the LOD 2.0 log CFU/coupon.

Most Probable Number

When the PL treatment yielded counts below 25 CFU/mL for the lowest dilution, bacterial enumeration was performed using the MPN Completed test for *E. coli* as described by BAM (FDA 2012b).

Pulsed Light Treatment

Each inoculated coupon was individually centered and treated in the PL system at 9.5 cm and 14 cm beneath the quartz window. Treatment time ranged from 0.4 s to 6 s.

Evaluation of PL Efficiency

The level of microbial inactivation (Log [N/No]) was calculated by subtracting the survivor counts (N) from the initial counts (No) that were expressed in log CFU/coupon. In addition, survivor curves were built by creating log-linear plots of survivor counts (N) versus treatment time (t).

Statistical Analysis

The data resulted from each experiment was tabulated as the average of three replicates ± standard error mean (SEM). Inoculated eggs that were not treated served as controls. Differences among treatments were analyzed using the general linear model (GLM) procedure in MINITAB version 15. Multiple comparisons of the mean values were determined using the Tukey's method with a confidence interval of 95%.

Results and Discussion

Evaluation of PL Efficiency Inoculated HDPE Surfaces

As illustrated by the survival curves in Figure 4-2 and 4-3 respectively, the population of *E. coli* K12 treated with PL diminished in a non-linear fashion. After 2-3 s of PL exposure, data points were determined by MPN since survivor counts were below 25 CFU/plate and hence not considered for calculating levels of inactivation.

Survivor curves exhibited similar profile to those previously described in the inactivation of spot-inoculated eggs. However, the concavity effect was more prominent in smooth surfaces (Figure 4-3) whereas the tailing effect in rough surfaces (Figure 4-2). The underlying reason for this phenomenon is that microbial cells are more evenly distributed on smooth surfaces. While on rough surfaces, imperfections allowed bacteria to hide and shield from the effect of PL treatment that is consistent with previous studies by Woodling and Moraru

(2007). They achieved lower inactivation of *L. innocua* of ~4 logs on aluminum oxide surfaces versus ~5.5 logs on those with a mill finish at the same fluence (~13 J/cm^2).

Table 4-2 summarizes the log reductions of *E. coli* K12 achieved on smooth and rough surfaces that took into account recovery losses. These losses were estimated in 1.0 ± 0.05 log CFU for both surfaces. The effects of distance and time on PL efficiency were also investigated.

Overall, treatments at 9.5 cm and 14.5 were not significantly different ($P>0.05$). For rough surfaces, the level of inactivation ranged from 1.25±0.05 to 7.04±0.00 log CFU/coupon and 1.18±0.05 and 7.04±0.00 log CFU/coupon at 9.5 cm and 14 cm distance respectively. At 9.5 cm, the log reductions of *E. coli* K12 were significantly different ($P<0.05$) with exception to the treatments for 2 s and 3 s. Instead, at 14 cm bacterial inactivation was only comparable for 1 s and 2 s treatments. For smooth surfaces log reductions ranged from 2.08±0.29 to 7.06±0.00 log CFU/coupon and 1.52±0.36 to 7.06±0.00 log CFU/coupon at 9.5 cm and 14 cm distance. Additionally, all treatment times were significantly different ($P>0.05$).

Moreover, the optimal treatment condition was 6 s and 3 s for rough and smooth surfaces respectively, as bacterial counts fell below the LOD for MPN (1.18 log CFU/coupon). Particularly in the case of rough surfaces, microbial populations reached a plateu between 2 and 3 s and consequently decreased. This can be explained by the presence of cells concealed in the crevices of the surfaces that may have survived and repaired post treatment during the MPN procedures. However, no clear pattern on mechanisms contributing to cell repair was identified. These observations coincide with those by Woodling and

Moraru (2007) that also identified recovery of microbial cells beyond the enrichment process in some instances.

As alluded to previously, for the 2 distances evaluated there was not considerable differences in terms of inactivation levels. This may be due to that the energy delivered by the system in both cases was sufficiently high as to induce similar inactivation, a trend that was more evident in smooth surfaces. Similar effects were noted by Haughton and others (2011) regarding the inactivation of *E. coli* on polyethylene cutting board that was comparable despite of the intensity applied. These researchers obtained approximately 3 and 4 log unit reduction after treatments with the same duration but different fluence at 11.5 cm (6 J/cm^2) and 14 cm (4.5 J/cm^2) from the quartz window. Morey and others (2010) also found that the survival populations of *L. monocytogenes* on conveyor belts significantly reduced independent of UV light intensities used.

In another study by Ringus and Moraru (2013), *L. innocua* was reduced 7.1 and 7.2 log CFU on HDPE and low-density polyethylene (LDPE) surfaces at the maximum fluence tested (~8 J/cm^2). A comparison of these results with the inactivation of *E. coli* K12 on smooth surfaces (~7 log CFU/ coupon after 3 J/cm^2) suggests that *L. innocua* is more resistant to PL treatment as supported by earlier findings. For example, Rowan and others (1999) estimated survivors of *E. coli* to be approximately as less as 1.5 log CFU than *L. monocytogenes* after equal PL treatment. The natural resistance of this microorganisms to PL exposure reported in others studies may be attributable to its gram-positive morphology.

Another factor that could have influenced the results in our study was the orientation of the coupons towards the xenon lamp during treatment. Considering the close time intervals of exposure, this may have played an important role particularly in samples not alienated in the middle of the PL lamp. Gómez-López and others (2005a) identified an additional effect on PL lethality determined by the position of samples with respect to the xenon lamp. These researchers found that petri dishes with *L. monocytogenes* located peripherally in a PL shelf resulted in lower inactivation (0-2 log CFU/cm^2) than those situated directly below the xenon lamp (3.2-6 log CFU/cm^2). Added to these findings, Hsu and Moraru (2011) noted that fluence decays not only with increasing vertical distances from the lamp, but also with the target samples shifting away from the focal point in horizontal and diagonal directions. These researchers revealed that the spatial distribution of the fluence in air and liquid media along a PL chamber is well described by a Gaussian model in a three-dimensional plane (x-y-z).

Finally, the surface heating on coupons during treatment were considered negligible and thus were not reported in this study. After the coupons were subjected to the maximum time (6 s, ~6 J/cm^2) conducive to total microbial inactivation in both surfaces, the temperature barely rose 2°C. This may be attributed to the fact that HDPE possess a low thermal conductivity and thus its physical properties (e.g., hydrophobicity) may not be altered during PL treatment. In agreement with these results, Ringus and Moraru (2013) reported temperature increases of 2-3°C after 8 J/cm^2 in LDPE and HDPE substrates.

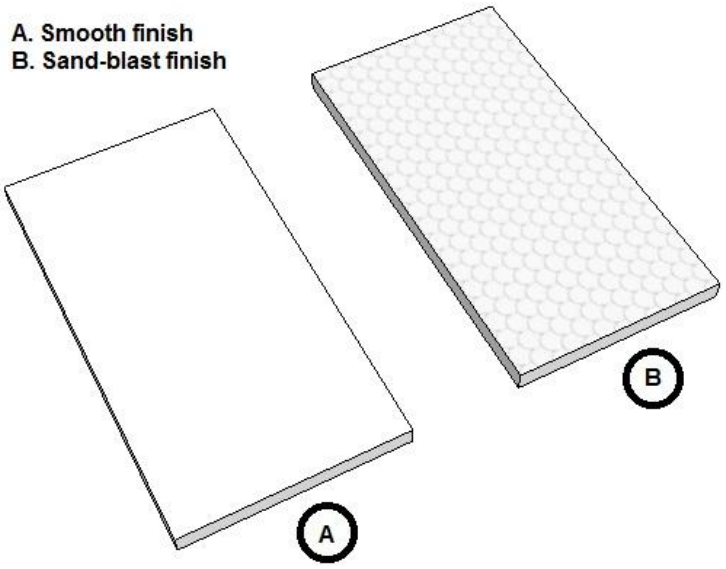

Figure 4-1. Types of coupons utilized for PL inactivation studies (n=3).

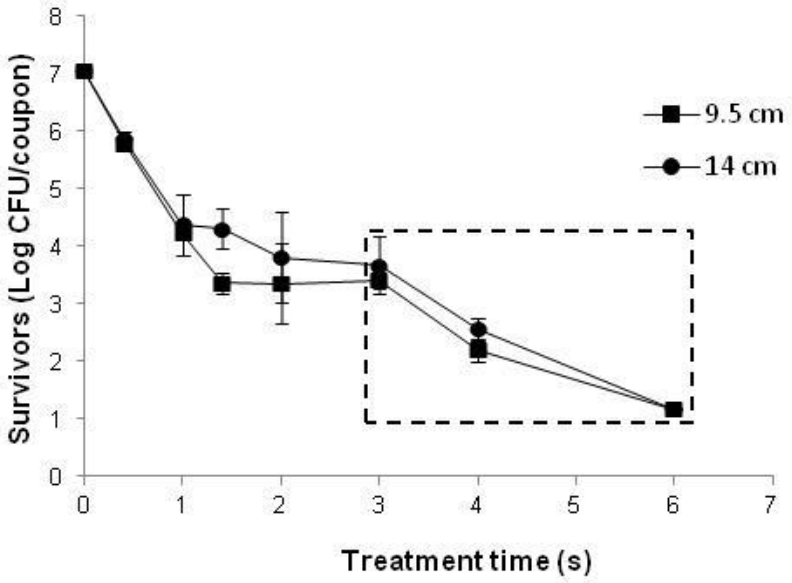

Figure 4-2. Survivor curves of *E. coli* K12 on surfaces with a sand-blast finish treated with PL at distances of 5.5 cm and 9.5 cm from the quartz window (n=3). Note: (i) the data points in the dotted rectangle corresponded to MPN estimates of survivors, as SPC resulted in less than 25 CFU/mL.

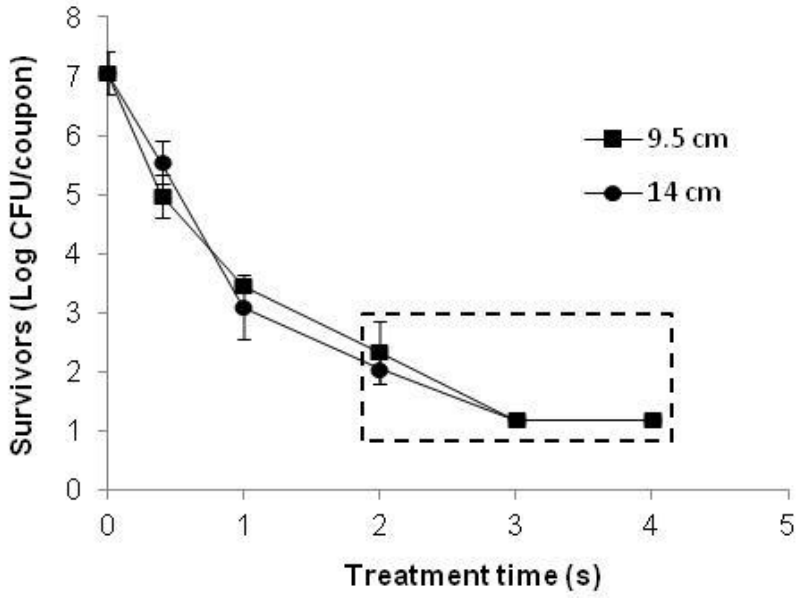

Figure 4-3. Survivor curves of *E. coli* K12 on surfaces with a smooth finish treated with PL at distances of 5.5 cm and 9.5 cm from the quartz window (n=3). Note: (i) the data points in the dotted rectangle corresponded to MPN estimates of survivors as SPC resulted in less than 25 CFU/mL.

Table 4-1. Inactivation of *E. coli* K12 on HDPE surfaces

Distance from quartz window (cm)	Treatment time (s)	Log reduction (CFU/coupon)[a]	Log reduction (CFU/coupon)[b]
9.5	0.4	1.25 ± 0.02A	2.08 ± 0.29A
9.5	1.0	2.81 ± 0.11B	3.61 ± 0.04B
9.5	2.0	3.69 ± 0.70C	≥5.10 ± 0.00C[c]
9.5	3.0	≥5.04 ± 0.00CD[c]	7.06 ± 0.00D[d]
9.5	4.0	≥5.04 ± 0.00CD[c]	NA
9.5	6.0	7.04 ± 0.00E[d]	NA
14.0	0.4	1.18 ± 0.05A	1.52 ± 0.36A
14.0	1.0	2.67 ± 0.10B	3.98 ± 0.54B
14.0	2.0	3.23 ± 0.29BC	≥5.10 ± 0.00C[c]
14.0	3.0	≥5.04 ± 0.00D[c]	7.06 ± 0.00D[d]
14.0	4.0	≥5.04 ± 0.00D[c]	NA
14.0	6.0	7.04 ± 0.00E[d]	NA

Means in the same column followed by the same capital letters were not significantly different (P> 0.05).
[a]Log reductions on coupons with a sand-blast finish.
[b]Log reductions on coupons with a smooth finish.
[c]MPN enrichments were positive and the LOD of SPC (2 log CFU/coupon) was subtracted from the initial population to determine the log reduction
[d]The MPN enrichments were negative. Results were expressed as total reduction equal to the initial inoculum level.

CHAPTER 5
EFFECT OF PULSED LIGHT TREATMENT ON SELECTED QUALITY ATTRIBUTES OF HARD-COOKED EGGS

Background

Color is considered one of the main factors influencing the purchasing decision and overall acceptability of food products (Caner 2005). Color values of lightness (L^*), greenness-redness (a^*) and blueness-yellowness can objectively evaluate the appearance of hard-cooked eggs. Additionally, total color difference (ΔE^*), recognized as a unit of acceptable commercial color difference, allows for detection in color changes as a result of a treatment.

Texture is referred as to an important quality attribute defined by a range of parameters that denote freshness and adequate preparation methods in food products. In the case of semi-solid foods (e.g., egg-white gels), these mechanical parameters can be determined by texture profile analysis (TPA). Texture profile analysis involves a dual-compression cycle and quantification of specific attributes (e.g., hardness) derived from force-deformation curves generated during the analysis (Szczesniak 2002). Additionally, the texture and color of foods are directly correlated with their microstructure as described by Handa and others (1998). In this study, color, texture and microstructure were evaluated to better understand how PL influences overall quality of hard-cooked egg whites.

Materials and Methods

Color of Shelled Hard-cooked Eggs

The color of the egg white was determined using a color machine vision system (CMVS). The equipment consists of a Nikon D200 digital color camera (Nikon Corp, Japan), housed in a light box [42.5 cm (W) x 61.0 cm (L) x 78.1(H)] (Wallat and others 2002). The camera (focal light, 35 mm; polarization,

18.44 mm) connected to a computer, was used to capture the images prior to color analysis. A software referred to as LensEye (Engineering and Cybersolutions Inc. Gainesville, FL) analyzed color based L, a* and b* values. The camera was calibrated with a standard white tile (L*: 97.349; a*: -0.065, b*: 1.952) (Labsphere, North Sutton, NH, USA). All samples were aligned in the center of the light box prior to acquiring the images. The total color difference (ΔE^*), was calculated using the following equation:

$$\Delta E^* = ((L^{'} - L^{'}_{ref})^2 + (a^{'} - a^{'}_{ref})^2 + (b^{'} - b^{'}_{ref})^2)^{1/2} \quad (5\text{-}1)$$

L^*, a^*, and b^*values of untreated were used as color references in the calculation of ΔE^*.

Texture Profile Analysis

The procedure was adapted from the method of Min and others (2005) with slight modifications. Texture was performed at ambient temperature (25°C) using a TA.XT Plus Texture analyzer (Texture Technologies Corporation, Scarsdale, NY, USA) with a 2 kg load cell and a cylinder probe (76 mm diameter, TA 30). Egg white gels were prepared in form of cylinders of 10 mm diameter × 10 mm length. Each cylinder was axially subjected to a 60% compression two times at a speed of 1 mm/s and rebounding time of 6 s between cycles.

Hardness, cohesiveness, springiness, chewiness, gumminess and adhesiveness were obtained from the TPA curves recorded after each trial (Figure 5-1). Hardness was determined by the maximum force (H) required achieving the first compression. Cohesiveness was defined by the relation between the area under the curve of the second (A_2) and first compressions (A_1) respectively springiness was calculated by the ratio of the rates at which the

deformed material regenerates to its initial form after the second and first compressions respectively (X_2/X_1). Chewiness was represented by the product of hardness, cohesiveness and springiness. Gumminess was obtained by the product of hardness and cohesiveness. Finally, adhesiveness was denoted by the negative area under the curve obtained between compression cycles (A_3).

Microstructure of Hard-Cooked Eggs

Samples were prepared according to the protocols described by Handa and others (1998). Circular segments (10 mm diameter, 2 mm thickness) of hard-cooked egg white were fixed overnight at room temperature (25°C) in 2.5% glutaraldehyde (0.1M cacodylate buffer, pH 7.3) and rinsed in 0.1M cacodylate buffer (pH 7.3) 3 times. Next, the samples were fixed in 1% Osmium Tetroxide (0.1M cacodylate buffer, pH 7.3) for 1 h and rinsed in distilled water three times. All samples were then dehydrated respectively using acetone series (30, 50, 70, 80, 90, and 100% 3 times) and critical point dried. Once dried, the samples were outgassed under vacuum, sputter-coated with gold and mounted onto aluminum studs and examined using a Field Emission Scanning Electron Microscope (JSM-633OF, JEOL, Tokyo, Japan) (University of Florida, Particle Science Engineering Department, Gainesville, FL, USA).

Statistical Analysis

Quality parameters with exception to scanning electron micrographs were tabulated as the average of three replicates ± Standard Error Mean (SEM). Untreated samples were used as controls. Differences among treatments were analyzed using the general linear model (GLM) procedure in MINITAB version 15. Multiple comparisons of the mean values were determined using the Tukey's method with a confidence interval of 95%.

Results and Discussion

Color of Shelled Hard-cooked Eggs

As alluded to previously, color is a major visual characteristic associated with quality in the food industry and market leading to the acceptability of a food product. A widely accepted approach to quantify color in foods is according to the chromaticity values L^*, a^*, b^* of the CIELAB system as defined by the Commission Internationale deI'Eclairage (CIE) (Mendoza and others 2006). Additionally, color changes induced by a treatment can be determined by ΔE^* (Mc Bride and others 2004).

CIELAB color parameters on the surface of hard-cooked eggs before and after PL treatments are summarized in Table 5-1. The L^* values ranged from 92.9±0.02 to 94.5±0.58 for eggs exposed 5 s at 5.5 cm and 20 s at 9.5 cm from the xenon lamp respectively, indicating light-color eggs. The a^* values ranged from -1.94±0.18 to -2.39±0.11 for eggs treated 20 s and 10 s at 5.5 cm from the xenon lamp, suggesting a greenness coloration. Lastly, b^* values varied from 9.97±1.13 to 11±0.60 in samples subjected to 5 s at 9.5 cm PL treatment and control samples, indicating a yellow pattern on the surfaces of eggs. Overall, L^*, a^*, b^* values were relatively consistent and not significantly different ($P>0.05$) regardless of the treatment. All color values were in close agreement with those shown in a study by Ibarz and others (1999), researchers that obtained mean values of 90.85, -2,98 and 9,04 for L^* , a^* and b^* respectively on eggs boiled during 10 min . Similarly, comparable L^* values and a^* and were reported by Aggarwal (2008); Kim and others (2008) and Hope (1999) in hard-cooked egg white treated with MAP, edible coatings and at natural pH conditions (~9.11) respectively. In the same way, Handa and others (1998) and Hope (1999) obtained approximated chromaticity values. Slight differences can be mainly

attributed to the severity of the heat treatment during sample preparation (Hall and others 1977; Vadera and others 2006). As the heating time of hard-cooked eggs increases, the albumen is perceived as white rather than brilliant or transparent and thus L^* values tend more to 100 as described by Ibarz and others (1999). Additionally, this can result in greater b^* values, as the surfaces of peeled hard-cooked turn slightly yellow.

Furthermore, the recorded L^*, a^*, b^* values were adapted to total color difference units. According to literature, ΔE^* values lower than 3, indicate that color differences cannot be detected by naked human eye (Hong and others 2004). In this study, the ΔE^* values were below 3 and were not significantly different ($P>0.05$) as determined by Tukey's pairwise comparisons. However, when samples means were compared uniquely in terms of treatment levels (5.5 cm and 9.5 cm), there was a significant effect of distance from the xenon lamp ($P<0.05$) on ΔE^* values. As described by the literature, as the distance between samples and the light source decrease, the treatment becomes more "severe" (Oms-Oliu and others 2010). Therefore, PL may be capable to induce certain changes in appearance of peeled hard-cooked, not necessarily noticeable to the human eye.

According to Gómez-López and others (2007), there are many factors inducing color changes by PL in foods during exposure including treatment conditions, food attributes and the associated overheating. It has been outlined that moderately short treatments retain color of food samples (Demirci and Panico 2008). For example, Dun and others (1989) observed that potato slices and dry cottage cheese illuminated with a fluence of 3 to 4 J/cm^2 retained their color. A study by Shuwais and others (2000) detected not changes in Hunter

color values in catfish fillets treated with 2-4 flashes of 2.5-5 J/cm^2. Comparatively, Keklik and others (2011) proved that PL preserved the cuticle of shell eggs as determined by CIELAB color space on samples exposed 20 s at 9.5 cm from the xenon lamp.

Additionally, it has been established that colored and high-protein foods absorb more energy during the PL treatment, which may cause. In this case, Ozer and Demirci (2006) reported that salmon fillets changed significantly their color after treatments for up to 45 s at 3-5 cm distance. Also, Fine and Gervais (2004) elaborated that color changes in black pepper and wheat flour may be attributed to overheating and UV absorption that in more pronounced in dark food products.

Results obtained in this study revealed no significant differences in color between treatment groups ($P>0.05$) as earlier mentioned. Although, the treatment conditions were to some extent “severe” at 5.5 cm from the xenon lamp, the integrity of hard-cooked was maintained. Unlike colored foods, hard-cooked eggs are light products and thus absorb light energy to a limited amount (Fine and Gervais 2004). Accordingly, the temperature build up exhibited in other food products (e.g., meat fillets, flours) was not observed.

Texture Profile Analysis of Peeled Hard-cooked Eggs

Szczesniak (2002) defined texture as “multiparameter attribute” comprised by a range of characteristics that can be uniquely perceived by the human senses of touch and pressure. Hence, results derived from texture testing instruments, must be correlated in terms of sensory perception. It is well know that the textural properties relevant to solids and semisolid foods namely egg white gels can be well described by TPA (Szczesniak 2002). Table 5-2

summarizes the TPA of egg-white gel before and after PL treatment. The extent of compression (60%) was selected at 60% to avoid fracture that cannot be precisely determined by TPA given the unclear breaking points (Handa and others 1998). TPA curves, depicted in Figure 5-1, were asymmetric due to the incapability of the gels to promptly recover.

In general, similar profiles were obtained suggesting no clear relationship between treatment conditions and egg-white gel texture ($P>0.05$). Five parameters were recorded from each TPA curve (Figure 5-1). These included hardness (N), adhesiveness, springiness, cohesiveness, gumminess (N).

Hardness is considered as to the force necessary to bite down a food sample for the first time (Min and others 2005). Highest and lowest hardness values corresponded to the samples exposed at 9.5 cm for 10 s and 5.5 cm for 5 s with the xenon lamp. Studies by Woodward and Cotterill (1985) and Ibarz and others (1999) demonstrated similar hardness in egg white gels boiled and cooked at 95°C for 10 min respectively. Comparatively, Toledo (2006) reported approximately that 15 N were necessary to achieve the first compression (hardness) on TPA performed on plain liquid-egg water combinations (71.6%, 28.4%) retorted at 121,11 °C and 103,42 kPa. In contrast, Handa and others (1998) estimated hardness of egg white between 41 and 102 N after being heat-induced at 80°C for 40 min at pH ranging from 3 to 11. Gel hardness is determined by a gamut of factors including temperature, time, disulfide bonds and pH (~9.7 after egg storage) (Holt and others 1984; Stadelman and Cotterill 1995; Handa and others 1998). Therefore, consideration of these factors during sample preparation may contribute to discrepancies when measuring gel strength.

Adhesiveness involves the force required to remove a food material after has been adhered to the palate during eating (Szczesniak 2002).For the most part, adhesiveness was constant with exception to samples treated for 5 and 20 s at 9.5 cm from the xenon lamp. In much the same way, a study conducted by Tabilo-Munizaga and Barbosa-Cánovas (2004) achieved adhesiveness of -7±1 on heat-induced surimi gels formulated without additives. However, it was also mentioned that prediction of adhesiveness by texture apparatus may result in an overestimation as gels naturally tend to stick to the probes (Tabilo-Munizaga and others 2004).

Springiness, also referred as to elasticity, represents the extent to which a sample goes back to its original shape after bringing the teeth together, without breaking and consequently releasing (Meullenet and Gross 1999). As noted, springiness did not vary considerably among treatment groups. Values of approximately 0.9 indicated that each sample recovered almost 90% after deformation induced by the first compression. In a like manner, Tabilo-Munizaga and others (2004); Min and others (2005) detected similar patterns in hard-cooked eggs irradiated and surimi gels heated at 90°C and pressurized at 400-650 MPa. According to these authors, visco-elastic materials behave similarly in terms of springiness.

Similarly, cohesiveness, gumminess (N) and chewiness (N) attributes were retained despite of treatment conditions. Cohesiveness indicates the degree of compression of a food when situated between the teeth and before breakage (Szczesniak 2002). Gumminess and chewiness define the amount of energy required to chew a solid and semisolid sample to a state fit for swallowing respectively (Meullenet and Gross 1999). Cohesiveness averaged

~0.62 indicating that samples were deformed 40% after the first compression. In no case, gels broke after 60% double-compression cycle. Min and others (2005) achieved lower cohesiveness values as explained by the degree of compression (70%) and the reduced hardness (~4 N). Values of gumminess and chewiness ranged between 5.39-6.75 N and 4.79-5.83 N using 20 s and 5 s of PL illumination at 9.5 cm and 5.5 cm respectively (Table 5-2). Regarding these attributes, Min and others (2005) observed significantly lower values (~1 N) as gumminess and chewiness depend on hardness, cohesiveness and springiness coefficients.

Currently, only a few studies have investigated the effects of PL on texture of foods. Major changes on food quality have been associated with an excessive temperature build up in long-duration treatments (Demirci and Panico 2008). Keklik and others (2010) evaluated the strength of eggshells based on the maximum force required to break the shell and its deformation. Their results suggested not significant difference in failure force or degree of deformation ($P>0.05$) among sample as temperature rise was not significant (3.3-13°C). However, Oms-Oliu and others (2010) determined that the texture of sliced mushrooms was highly affected by the heat buildup (20-100°C) triggered by PL at fluencies of 12 and 28 J/cm^2, but not at 4.8 J/cm^2.

Microstructure

The formation of egg-white gels by heat comprises a mechanism affected by several factors leading together to denaturation and aggregation of the protein structure. During thermal treatment, reactive groups buried in the native protein conformation (e.g., disulfide bonds) become exposed contributing to aggregation mainly ruled by beta-sheet structures (Croguennec and others

2002). As temperature and holding time increases, a higher fraction of egg-white protein becomes denatured and incorporated into the cross-linked gel matrix. As pH rises, the gel network becomes uniformLy and firmLy distributed as water-binding capacity improves due to greater disulfide-bonding interactions.

The main protein fractions implicated in the egg-white gelation include ovoalbumin and ovotransferrin that are in major proportion (together ~70%) (Handa and others 1998). Ovoalbumin is characterized by being more thermo stable (~80°C) and the only egg-white protein formed by free disulfide groups. Its molecules slowly unfold and uncover reactive groups before aggregation adding to a more uniform network. Ovotransferrin is more prone to heat-denaturation (~60°C) and also plays an important role during coagulation of gels.

Micrographs from egg-white gel untreated and treated with PL are shown in Figure 5-2. Overall, no major structural changes were observed in control and treated samples as they appeared quite uniform despite of the treatment applied. In all cases, the gels were characterized by a continuous, tightly packed and dense structure. At higher magnifications (Figure 5-2), a vast number of globules of about 0.2 µm in size along with small-sized pores distributed across the gel network were visualized.

Some reasonable explanations that may justify these observations include the shrinkage that samples underwent during preparation, the pH of the egg-white and thermal treatment. In the present study, the fixed samples experience a drastic shrinkage of at least 50% during supercritical carbon dioxide. This may have caused the structure to become more condensed and difficult to

differentiate between particles and pores. It has also been well established that at alkaline pH (9), the heat-induced egg-white gels comprise relatively small particles due to a high degree of crosslinking and water holding ability. Moreover, high temperatures and/or extended heating times are associated with a continuous gel network as all open spaces in the gel are filled by major protein components such as ovoalbumin. Although the present study did not involve the study of the previously mentioned factors, it can be suggested that sample preparation resulted in the formation of a gel microstructure similar to that reported in previous studies (Woodward and Cotterill 1985).

Furthermore, the findings of this study are supported by the results obtained in color and textural experiments. As emphasized by Handa and others (1998), texture and color of the foods are a result of microstructure. In particular, the strength and other viscoelastic properties (e.g., cohesiveness) may have been highly influenced by the compact and uniform gel matrix. Moreover, since color and texture were not affected by PL treatment, it could have been anticipated that not major differences in the arrangement of egg-white gels would have occurred.

Table 5-1.Effects on color values and color difference of hard-cooked peeled eggs treated with PL (n=6).

Distance from xenon lamp (cm)	Treatment time (s)	L*	a*	b*	ΔE*
Control	0	94.7 ± 0.32 A	-2.36 ± 0.49 A	11.0 ± 0.60 A	_
5.5	5	93.5 ± 0.11 A	-2.28 ± 0.02 A	10.7 ± 0.07 A	1.87 ± 0.45 A
5.5	10	92.9 ± 0.02 A	-2.39 ± 0.11 A	10.0 ± 0.20 A	2.56 ± 0.44 A
5.5	20	94.1± 0.35 A	-1.94 ± 0.18 A	10.4 ± 0.22 A	2.01 ± 0.13 A
9.5	5	94.2 ± 0.67 A	-2.24 ± 0.66 A	9.97 ± 1.13 A	1.57 ± 0.14 A
9.5	10	94.4 ± 0.18 A	-2.19 ± 0.19 A	10.0 ± 1.13 A	1.10 ± 0.06 A
9.5	20	94.5 ± 0.58 A	-2.19 ± 0.38 A	10.5 ± 0.72 A	1.22 ± 0.06 A

Means in the same column followed by the same capital letters were not significantly different (P> 0.05).

Table 5-2. Effects on TPA parameters of hard-cooked egg-white treated with PL (n=6).

Distance from the xenon lamp (cm)	Treatment time (s)	Hardness (N)	Adhesiveness	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
Control	0	9.29 ± 0.87 A	-5.87 ± 2.12 A	0.89 ± 0.03 A	0.62 ± 0.03 A	5.77 ± 0.49 A	5.14 ± 0.48 A
5.5	5	8.73 ± 0.16 A	-5.09 ± 0.60 A	0.89 ± 0.01 A	0.62 ± 0.01 A	5.39 ± 0.04 A	4.79 ± 0.20 A
5.5	10	9.35 ± 0.24 A	-6.36 ± 2.43 A	0.88 ± 0.00 A	0.60 ± 0.01 A	5.65 ± 0.21 A	4.95 ± 0.20 A
5.5	20	9.69 ± 0.29 A	-5.27 ± 0.00 A	0.85 ± 0.02 A	0.64 ± 0.02 A	6.23 ± 0.39 A	5.29 ± 0.21 A
9.5	5	9.72 ± 0.13 A	-3.01 ± 1.69 A	0.88 ± 0.01 A	0.62 ± 0.01 A	6.03 ± 0.13 A	5.30 ± 0.03 A
9.5	10	10.8 ± 0.14 A	-5.27 ± 0.89 A	0.86 ± 0.00 A	0.62 ± 0.00 A	6.75 ± 0.54 A	5.83 ± 0.43 A
9.5	20	9.59 ± 0.51 A	-2.15 ± 0.46 A	0.85 ± 0.03 A	0.62 ± 0.01 A	5.97 ± 0.26 A	5.08 ± 0.38 A

Means in the same column followed by the same capital letters were not significantly different (P>0.05).

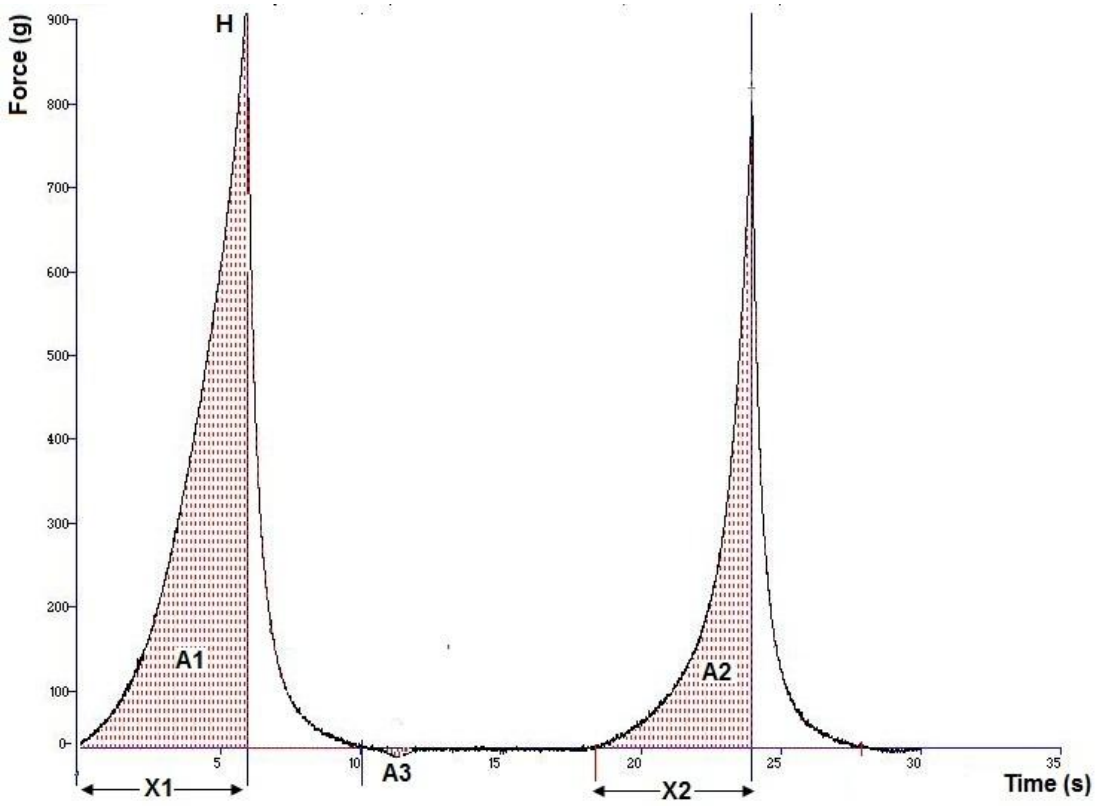

Figure 5-1. Texture profile analysis curve of a control sample of hard-cooked egg gel. H is hardness, A2/A1 is cohesiveness, A3 is adhesiveness and X2/X1 is springiness.

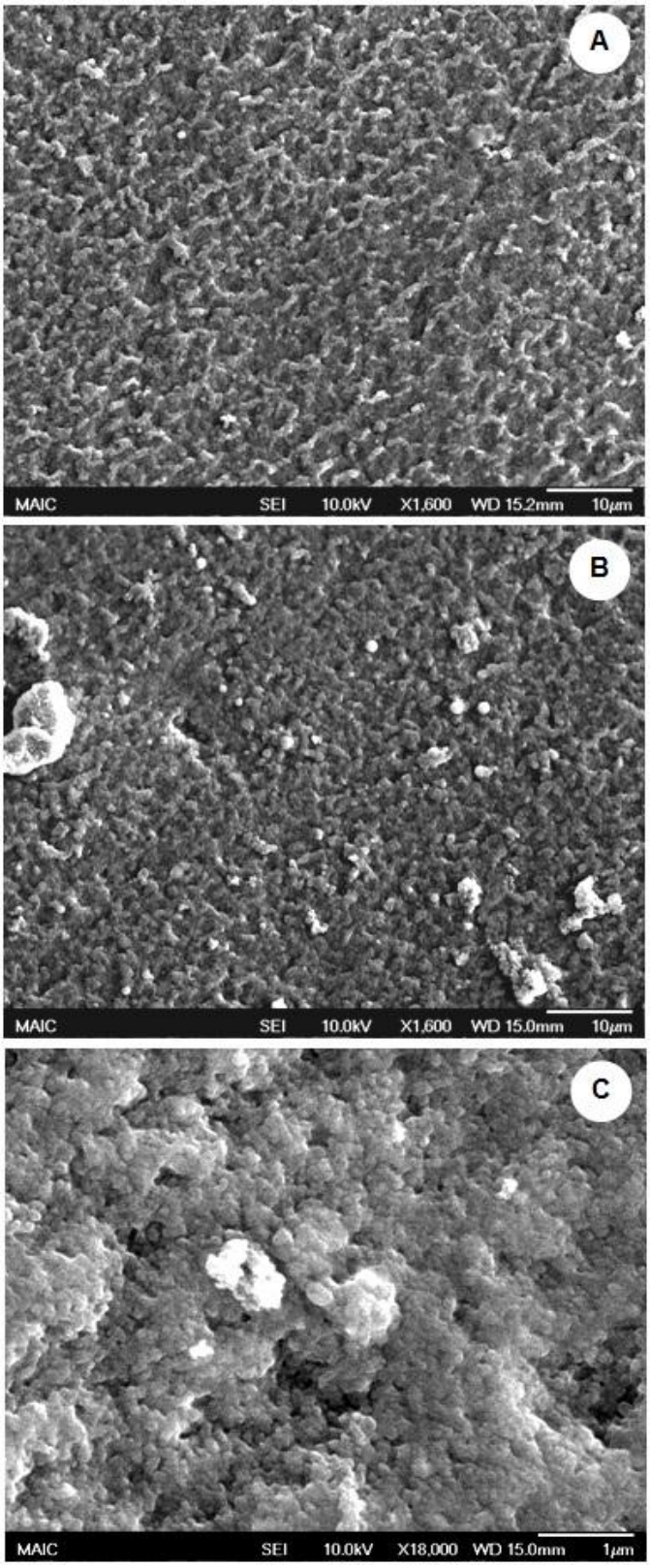

Figure 5-2. Scanning electron micrographs of egg-white gels (n=3).(A) untreated, (B) treated at 5.5 cm from the quartz window for 30 s, (C) treated at 9.5 cm from the quartz window for 30 s.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

In this study, the efficacy of PL as a processing intervention technology for decontamination of hard-cooked eggs and conveyor-belt surfaces was evaluated. According to results obtained, the following conclusions were drawn: Significant inactivation of *E. coli* K12 on spot-inoculated hard-cooked eggs and conveyor belt surfaces was attained after PL treatment. In contrast, the reduction of bacterial populations on whole-inoculated eggs was limited and possibly associated to the configuration of the PL system rather than the distribution of the microorganisms itself. One major disadvantage of PL reported in the literature is the overheating that certain foods experience. However, in this study the temperature buildup was minimal as to compromise the quality of hard-cooked eggs or to induce structural changes on the surfaces. Experimentally, it was also demonstrated that PL maintained the integrity of hard-cooked eggs as determined by color, texture and microstructure of the egg-white.

From an implementation standpoint, PL seems a viable alternative to decontaminate surfaces due to the short time of inactivation and the cumulative energy in compliance with FDA as compared with hard-cooked eggs. However, the usefulness of PL as a disinfection system requires further elucidation of the mechanisms underlying microbial inactivation, energy incident on the food samples and light spectrum accountable for upmost inactivation.

Some other factors that deserve major consideration in this and future studies include initial microbial load, target microorganism, repair mechanisms of microorganisms and their impact on stability of hard-cooked eggs upon storage. The initial population of *E. coli* K12 in this study may not be

representative of a realistic scenario since levels of contamination on foods and surfaces tend to be lower. Similarly, the effectiveness of the treatment may be correlated with the level of contamination on food samples as this contributes to light attenuation. Additionally, under natural conditions, microorganisms may be deposited and attached to surfaces forming biofilms, making their inactivation more difficult. Therefore, manipulation of microbial levels by experimental means could exclude such events.

A serious risk associated with the consumption of RTE products is listeriosis. Therefore, the inactivation of *L. monocytogenes* (or a surrogate microorganism) at optimal conditions would ensure that susceptible flora is eliminated, as this pathogen has been referred as to the most resistant to PL. Finally, the occurrence of repair mechanisms of microorganisms needs to be further assessed under different food substrates and storage conditions (e.g., refrigeration, time) to ultimately determine their impact in food safety and stability.

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BIOGRAPHICAL SKETCH

Braulio Andres Macias-Rodriguez is a native of Ecuador. In the year of 2010, he graduated from the Polytechnic School, Ecuador where he was awarded a bachelor's degree in Food Engineering.

In 2011, he was granted admission to the Department of Food Science and Human Nutrition at the University of Florida, Gainesville, FL to pursue his Masters degree. During his tenure, he served as an ambassador of the Food Science department in the Graduate Student Council, a member of the Food Science Club, and a teaching assistant in the advanced food chemistry course taught at his department.

In addition, he also earned several awards and became actively involved in the Gainesville community. In 2012, he received the award of Outstanding International Student from the College of Agriculture and Life Sciences and a travel grant from the Graduate Student Council to attend the annual Institute of Food Technologist (IFT) conference. Similarly, he engaged in volunteering activities in the Gainesville community, in which, he dedicated his time to prepare meals and interact with the residents at the American Cancer Society Hope Lodge and participated annually in the Alachua County School program as a science fair judge.