Enhancing the safety of Ready-To-Eat meats by Ultraviolet light intervention against *L. monocytogenes* and its influence on product quality

by

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ABSTRACT

The efficacy of UV light was determined against *Listeria monocytogenes* at different growth phases (log and stationary) and growth temperatures (37 and 4°C). Different UV parameters such as intensity and exposure times were evaluated to optimize this post lethality treatment as an intervention strategy in the post processing environments.

Listeria monocytogenes serotype 4b was cultured in Brain Heart Infusion (BHI) broth at 37°C. Cells were harvested during their log and stationary phase and subjected to low (3 – 4 mW/ sq. cm) and high (7-8 mW/ sq. cm) intensity of UV light. *Listeria monocytogenes* in growth media (BHI) was subjected to UV for 0,10,30,50,70,90 and 110s while for bologna, *L. monocytogenes* was spray inoculated followed by 30 min of attachment time and exposed under UV every 30s for up to 300s. Cells were recovered on Modified Oxford agar (MOX) after 36h of incubation at 37°C. Additionally, shelf life along with the quality attributes such as color and lipid oxidation due to UV radiation was assessed over a period of 8 weeks on the bologna stored at 0 and 4°C under vacuum.

UV treatment was effective ($p<0.05$) in reducing the growth of *L. monocytogenes* both in the growth medium and on bologna. Populations of *L. monocytogenes* were significantly reduced ($p<0.05$) after 10 and 30s of exposure times in growth media irrespective of the growth temperature and UV light intensities. On bologna, 180s of UV exposure significantly reduced

($p<0.05$) *L. monocytogenes* populations irrespective of UV intensities. Irrespective of the UV light intensity and exposure times, significantly higher ($p<0.05$) reductions were observed in the log phase cells as compared to the stationary phase cells. Furthermore, application of UV affected ($p<0.05$) the lightness (L) and redness (a) of the meat but did not cause any lipid oxidation on the Ready-to-Eat meat irrespective of the storage temperatures. The data suggests the potential use of UV light as a possible post process intervention in the food processing plants.

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LIST OF ABBREVIATIONS

UV	Ultraviolet Light
RTE	Ready – to – eat
L. monocytogenes	*Listeria monocytogenes*
FDA	Food and Drug Administration
USDA	United States Department of Agriculture
FSIS	Food Safety Inspection Services
CDC	Center for Disease Control and Prevention
CFR	Code of Federal Regulations

CHAPTER 1

GENERAL INTRODUCTION

Listeria monocytogenes is a Gram-positive, non-spore forming, facultative intracellular foodborne pathogen capable of causing serious complications in pregnant women, neonates and immunocompromised adults. *Listeria monocytogenes* is widely distributed in the environment and frequently found in plants, soil, animal, water, dirt, dust, and silage (Farber and Peterkin, 1991; Beresford *et al.,* 2001). *Listeria monocytogenes* may also be present in slaughter animals and subsequently in raw meat and poultry which can be introduced into the processing environment thereby cross-contaminating the food contact surfaces, equipment, floors, drains, standing water, and employees (Fenlon et al., 1996). In addition, *L. monocytogenes* can establish niches in damp environments and form biofilms in the processing environment that are difficult to eliminate during cleaning and sanitation (Giovannacci *et al.,* 1999). These factors coupled with the other major characteristics of *L. monocytogenes* such as its heat and salt tolerance and its ability to grow at refrigeration temperatures and survive at freezing temperatures (McClure *et al.,* 1997) makes it a formidable pathogen to control.

Listeria monocytogenes contributes significantly to food related illnesses and deaths in the United States (Scallan *et al.,* 2011) with total cost of illnesses estimated to be $2.04 billion in 2010 (Schraff, 2012). Cases and outbreaks of human listeriosis are often due to the consumption of *Listeria*-contaminated foods, which include any Ready-to eat (RTE) foods such as meat, cheese, milk, produce etc. *Listeria monocytogenes* emerged as a problem in processed meat and poultry products such as deli-meats and hot dogs in the 1980's and several outbreaks of foodborne illness implicating RTE meat and poultry products as the source of *L. monocytogenes* were and continue to be reported. Since 1989, the United States Department of Agriculture and

Food Safety Inspection Service (USDA-FSIS) randomly sampled and tested RTE meat and poultry products produced in federally inspected establishments for presence of *L. monocytogenes* (FSIS, 2009). At the same time, FSIS established a "zero tolerance" (e.g., no detectable level of viable pathogens permitted) for *L. monocytogenes* in RTE meat and poultry products, thereby declaring RTE products testing positive for *L. monocytogenes* as "adulterated" under the Federal Meat Inspection Act (FMIA) or the Poultry Products Inspection Act (PPIA) (FSIS, 2011).

The regulation published in the Code of Federal Regulations (CFR, 2010) states that "establishments must comply with at least one of the three alternatives to provide an intervention if a RTE product is produced and exposed post-lethality to the processing environment." If an establishment chooses Alternative 1, they must use a post-lethality treatment that reduces or eliminates microorganisms on the product and an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*. If an establishment chooses Alternative 2, they can use either the post-lethality treatment of product or an antimicrobial agent or process that suppresses or limits growth. If an establishment selects Alternative 1 or Alternative 2, they must also have a sanitation program that addresses the testing of food contact surfaces. For Alternative 3, an establishment can choose to use sanitation as their intervention strategy.

The effectiveness of post-process decontamination technologies such as high pressure processing, and pre/post-package surface pasteurization have been studied as possible intervention strategies for controlling *L. monocytogenes* in RTE meat and poultry products. Other common approaches include the formulation of products with antimicrobial additives such as lactates and diacetates, and organic acids to control *L. monocytogenes* in RTE meat and poultry products. As the major aim of the food industry is to ensure safety of its products while

maintaining quality, alternative treatments like non-thermal processing have become increasingly common and explored for lowering foodborne pathogen level. New technologies that are rapid cheaper, and less disruptive to product quality than traditional thermal processing are being validated for controlling *L. monocytogenes* in the food industry.

One of the emerging non-thermal technologies is ultraviolet (UV) radiation to mitigate *L. monocytogenes* on RTE meats. The FDA and USDA have concluded that the use of UV irradiation is safe and in 2000, the FDA approved UV-light as alternative treatment to thermal pasteurization of fresh juice products (FDA, 2000). The performance criterion defined by FDA for fruit and vegetable juice processing is a 5-$\log_{10}$ reduction in the number of target pathogen of concern (FDA, 2000). Use of UV energy in the food industry has several advantages over chemical disinfections since it leaves no residue, does not moisten or alter the temperature of treated materials, can be used without legal restrictions, are cost effective, quick and effective (Yousef and Marth, 1988). Several UV source types, such as continuous UV low-pressure and medium-pressure mercury lamps, pulsed UV, and excimer lamp technologies have been developed that can be applied to foods (Wolfe, 1990). However, the efficacy and specific characteristics of these UV light sources used today for water treatment have not been evaluated for food applications. Currently, the traditional low-pressure mercury UV lamps at 254 nm are used for disinfection of food surfaces and liquid food treatments. In our study a panel of UV light bulbs emitting germicidal UV-C wavelength at 254nm were used on both growth media and RTE meat (bologna) against *L. monocytogenes*.

Previous studies have reported UV treatment to be beneficial in reducing bacterial content of a variety of liquids such as brines, recycled water, poultry chill water juices, etc., as well as in certain food matrices when used individually and in combination. More information is required

on the potential of UV radiation against *L. monocytogenes* depending on their age and temperature of growth as these factors can significantly impact food safety. Moreover, an ideal technology would be one that has no impact on the quality and shelf life of the product during storage. Research is required to determine the listericidal activity of UV radiation in growth media and food matrix. This research study aims to explore the potential of UV energy against log and stationary *L. monocytogenes* grown at 37 and 4°C along with its impact on quality attributes. Thus, providing yet another intervention strategy for the food industry. The overall hypothesis was \ UV light has the ability to reduce *L. monocytogenes* population irrespective of growth temperature, phases on growth medium and food matrix without compromising the food quality.

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CHAPTER 2

LITERATURE REVIEW

HISTORY

The knowledge of reported history of *Listeria monocytogenes* dates back to the year 1926. It was in the animal breeding facility of the Department of Pathology at Cambridge, when Murray and colleagues observed six sudden death cases of young rabbits. It was initially named as *Bacterium monocytogenes* because of its prominent production of large mononuclear leukocytes called monocytosis (Murray *et al.,* 1926). Later, it was renamed as *Listerella hepatolytica*, and *Listerella monocytogenes* in order to commemorate Lord Lister in bacteriological nomenclature. In 1940, Pirie proposed the name *Listeria monocytogenes,* as the generic name *Listerella* was previously used for a mycetozoan (Pirie, 1940). The increasing mortality and the probing characteristics presented by the disease intrigued further investigation and research about *Listeria monocytogenes.*

TAXONOMY

Listeria has long been associated with Coryneform bacteria based on their morphological resemblances – Gram positive, non-spore forming rods (Bousfield, 1972). But due to the uncertainty in its phylogentic position better clarification through improved taxonomical methods were required. A numerical taxonomic survey of *L. monocytogenes* and related bacteria was conducted by Wilkinson and Jones in 1977 and classified *Listeria* under the family Lactobacillaceae as they were closely related to *Lactobacillus* and *Streptococcus*. The chemotaxonomic markers indicated that *L. monocytogenes* belonged to the low G+C percent (36- 42%) group of Gram-positive bacteria (Feresu and Jones, 1988). The presence of particular lipoteichoic acids in *Listeria* distinguished its relatedness with *Bacillus, Streptococcus* and

Lactobacillus from Coryneform bacteria (Ruhland and Fiedler, 1987). The 16S and 23S rRNA sequencing (Collins *et al*., 1991) along with complete genome sequencing (Glaser *et al*., 2001) confirmed the close relationship of *Listeria* with *Bacillus* and *Staphylococcus* and its remoteness with *Lactobacillus*, thus forming a separate family, the *Listeria* monocytogenesceae (*Listeria monocytogenes – Brochothrix* subline). This demonstrates *L. monocytogenes* as a defined taxon and not associated with Cornyeform bacteria and its nearest neighbor being the *Brochothrix*.

Focusing on the taxonomy of the Genus *Listeria*, the core phylogeny consists of six different species: *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. grayii* and *L. ivanovii* (Wiedmann, 2002) and *L. murrayi* being the subspecies within *L.grayii* (Rocourt *et al.*, 1992). The DNA/DNA hybridization, 16S and 13S rRNA sequencing and protein mapping confirmed that the genus *Listeria* has two closely related but different lines of lineage. *Listeria grayii* is categorized as a distinct descent and *L. monocytognes, L. innocua, L. seeligeri, L. welshimeri* and *L. ivanovii* as a separate descent. Moreover, the second line of descent is further divided into 2 groups: Group 1 – *L. monocytogenes* and *L. innocua* and group 2 - *L. seeligeri, L. welshimeri* and *L. ivanovii*. Thus the taxonomy of *Listeria* is still complicated and requires detailed research and insight to describe its taxonomic position.

MORPHOLOGY

Listeria monocytogenes is Gram positive with rounded ends measuring 0.5 µm in diameter and 1 - 2 µm in length. When incubated at 20 – 25°C a tumbling motility is observed because of the presence of peritrichous flagella while at 37°C they display slower motility (Peel *et al.*, 1988). An umbrella or inverted pine tree appearance is observed in semisolid stab cultures due to their microaerophilic nature (Roberts *et al.*, 2009). Gray and Killinger in the year 1966 reviewed the cultural characteristic of *L. monocytogenes* on plate medium such as nutrient agar. *Listeria*

monocytogenes formed smooth, translucent, slightly raised 0.2-0.8 mm in diameter colonies with entire margin after 24h of incubation. The growth rate increases by the presence of glucose i.e. fermentable sugar. In a liquid medium, turbidity is observed after 8-24h of incubation at 37°C. *Listeria monocytogenes* has the ability to grow across a wide range of temperatures 1-2°C (termed as psychrotrophs) to 45°C (mesophile) and pH between 4.5 and 9.2. Moreover, they also grow and survive at high salt (NaCl) concentration up to 20% w/v and at very low water activity below 0.93 (Petran and Zottola, 1989).

BIOCHEMISTRY

Listeria monocytogenes is facultatively anaerobic, catalase positive, oxidase negative and homo-fermentative. However, they also can grow in aerobic and microaerophilic conditions. They are also Methyl Red and Voges-Proskauer test positive. The additional biochemical tests which are useful for identification of different *Listeria* species includes acid production from D-xylose, L-rhamnose, α-methyl-D-mannoside, and D-mannitol. The hemolytic activity of the three species, *L. monocytogenes*, *L. seeligeri*, and *L. ivanovii* are regularly demonstrated by using the CAMP (Christie, Atkins, and Munch-Petersen, 1944) test in . *Listeria monocytogenes* lyses red blood cells that can be observed using horse or sheep blood-containing agar plates. Because of the difficulty to read from blood agar plates and to differentiate between *L. monocytogenes* (pathogenic) and *L. innocua* (non-pathogenic), the API-*Listeria* (BioMérieux, Marcy-l'Etoile, France) - patented 'DIM' test was specifically designed. This test is based on the absence or presence of arylamidase, which helps to distinguish between *L. monocytogenes* and *L. innocua*. The scheme of identification of *Listeria monocytogenes* spp. is given in Table 1

Table 1: Identification of *Listeria monocytogenes* spp. (Low, J.C and Donachie, W, 1997)

Organisms	**DIM[1] test**	**Haemolysis of horse blood**	**CAMP test**		**Production of acid from:**		
			Staphylococcus aureus	***Rhodococcus equi***	**L-rhamnose**	**D-xylose**	**Alpha-methyl D-mannoside**
Listeria monocytogenes	-[2]	+	+	(+)[3]	+	-	+
L. ivanovii	+	+++	-	+	-	+	-
L. innocua	+	-	-	-	v	-	+
L. welshimeri	+	-	-	-	v	+	+
L. seeligeri	+	v	-	-	v	+	v

+, Positive; -, negative; v, variable reaction; +++ strong positive.
[1]DIM, Differentiation of *L. innocua* and *L. monocytogenes.*
[2]*All L. monocytogenes* isolates are DIM negative but occasional isolates of the other species may be negative.
[3]Enhanced haemolysis may be apparent.

Serological studies on *L. monocytogenes* were initiated by Seastone in 1935 by using agglutination and absorption experiments. Paterson in the year 1939-40 divided the genus *L. monocytogenes* into four serological types on the basis of Somatic (0) and Flagellar (H) antigens. The extended serovar scheme with more antigenic variants is the Seeliger/Donker-Voet scheme that is currently used (Donker-Voet, J., 1972; Seeliger, H. P. R., 1976; Table 2). This classification has been used to study the phenotypic characteristics associated with virulence of the 12 antigenic profiles (serotypes) which have been distinguished (Seeliger, H. P. R. and Jones,D.,1984).

Table 2: Serovars of the genus *Listeria monocytogenes* (adapted from Sutherland and Porrit, 1997).

Serovar	**O-antigens**													**H-antigens**							
1/2a	I	II	(III)													A	B				
1/2b	I	II	(III)													A	B	C			
1/2c	I	II	(III)														B		D		
3a		II	(III)	IV												A	B				
3b		II	(III)	IV								(XII)	(XIII)			A	B	C			
3c		II	(III)	IV								(XII)	(XIII)				B		D		
4a			(III)		(V)		VII		IX							A	B	C			
4ab			(III)		V	VI	VII		IX	X						A	B	C			
4b			(III)		V	VI										A	B	C			
4c			(III)		V		VII									A	B	C			
4d			(III)		(V)	VI		VIII								A	B	C			
4e			(III)		V	VI		(VIII)	(IX)							A	B	C			
5[a]			(III)		(V)	VI		(VIII)		X						A	B	C			
6a[a]			(III)		V	(VI)	(VII)		(IX)						XV	A	B	C			
6b[a]			(III)		(V)	(VI)	(VII)		IX	X	XI					A	B	C			
7			(III)									XII	XIII			A	B	C			
L. grayi			(III)									XII		XIV						E	

Listeria monocytogenes is currently divided into three phylogenetic lineages I, II and III, based on molecular typing by combinational comparison of both the ribotype pattern, and RFLP analysis of the haemolysin (*hly*), internalin A (*inlA*) and Actin A (*ActA*) genes (Wiedmann *et al.,* 1997). The lineage I cluster includes *L. monocytogenes* serotypes, most strains of serotype 4b and 1/2b, 3b, 4d which are significantly more common from human clinical sources. Serotypes 1/2a, 1/2c, 3a and 3C cluster within lineage II and are more common from food sources, and the lineage III cluster includes serotypes 4a, 4c and some serotype 4b strains that are rarely recovered from either food or humans (Call *et al.,* 2003; Gray *et al.,* 2004). It is usually serotype 4b (lineage I) and Serotype 1/2a (lineage II) that are implicated in a large number of human listeriosis cases.

LISTERIA MONOCYTOGENES AS A FOOD-BORNE PATHOGEN

Listeriosis was initially recognized as a disease of animals and it was not until the early 1980's that *L. monocytogenes* was first recognized as a food-borne pathogen. Most listeriosis cases are common in neonates, the elderly, pregnant women, or otherwise immunocompromised individuals (under treatment with chemotherapy or immuno-suppressant drugs) and usually transmitted through the consumption of contaminated foods (Mead *et al.,* 1999) causing life threatening diseases. The great concern to pregnant women is the possible onset of abortion of the fetus posing a 12-fold increased risk in comparison with the normal population, while the immunocompromised individuals (AIDS patients) have an estimated 100-500 fold increased risk over the normal population to acquire Listeriosis after consumption of contaminated food (Hof, 2003). Moreover, L. monocytogenes can multiply in refrigeration temperature, thereby, making this trait an important implication both to the food industry and to the consumer via disease transmission.

LISTERIOSIS:

A total of 37.2 million food-borne illness occur each year out of which 3.6 million (9.7%) are caused by bacteria, and *Listeria monocytogenes* is one of the leading bacterial pathogens, causing 19% of deaths due to consumption of contaminated food (Scallan *et al.*, 2011). *Listeria monocytogenes* causes listeriosis in animals and humans encompassing a wide variety of disease symptoms that are similar in animals and humans. The incidence of listeriosis in humans is not known, but the route of transmission may be either direct contact with infected animals or indirectly via milk, cheese, meat, eggs, or vegetables or as a source of contamination of the pasteurized product with raw product (Cooper and Walker, 1998). According to Gerald *et al*., 1971, 5-10% of humans may be asymptomatic carriers, harboring *L. monocytogenes* spp. in their feces or vagina. This is called non-invasive listeriosis, which usually occurs in healthy adults. It generally amounts to gastrointestinal illness called febrile gastroenteritis, fever, vomiting and diarrhea, where the degree of severity is dependent on the characteristics of the host and the organism's environment. (Schlech, 1997). The more severe form of listeriosis is invasive listeriosis, which is common in neonates, pregnant women and their fetuses, the elderly and the immunocompromised. The clinical features manifested by this form of listeriosis usually range from mild influenza-like symptoms to meningitis and/or meningoencephalitis. In serious cases, meningitis is frequently accompanied by septicemia, (Schlech, 2000). Particularly, in pregnant women, even though the most usual symptom is a mild influenza-like illness without meningitis, the infection of the fetus is extremely common and can lead to abortion, stillbirth, or delivery of an acutely ill infant (Mylonakis *et al.*, 2002). The detailed risk of listeriosis in different populations along with their clinical presentation is tabulated in Table 3.

Table 3: Clinical Syndromes Associated with *Listeria monocytogenes* Infection (Ryser and Marth, 2007).

POPULATION	CLINICAL PRESENTATION
Pregnant women	Fever ± myalgia ± diarrhea Preterm delivery Abortion Stillbirth
Newborns: <7 days old ≥ 7 days old	 Sepsis, pneumonia Sepsis, meningitis
Nonpregnant adults (Immunosuppressed)	Sepsis, meningitis, focal infections
Healthy adults	Diarrhea and fever

The infective dose of listeriosis is not yet clearly determined but it may take less than 1000 cells to cause infection. However, this is dependent on the immune status of the infected individual and the strain of the organism. The incubation period can range from a few days to three weeks and may be preceded by gastrointestinal symptoms, which manifest after approximately 12h incubation (Mead *et al.,* 1999). Being considered as an important foodborne pathogen and accounting for several billion dollars for medical treatments, effective precautions and surveillances are required to prevent contamination and decrease the rise of listeriosis per year.

PREVALANCE AND OUTBREAKS

Listeriosis outbreaks are linked to foods, which are heat-treated and receive a high degree of processing and generally designed as read-to-eat (RTE) foods (McLauchlin, 1996). The presence of *L. monocytogenes* in RTE foods is due to its ability to survive post process (cooking) procedures rather than being able to survival during processing itself. Due to their ubiquitous and saprophyte nature, *L. monocytogenes* can enter the processing environment at low intensities and subsequently establish itself in wet places such as conveyor belts, floors, drains, and other transporting equipment. Thereby these niches act as a suitable source for dissemination of *L.*

monocytogenes on cooked food (CDC, 2002). Over the years, several worldwide sporadic and epidemic outbreaks implicating *L. monocytogenes* in contaminated foods were reported. The first significant outbreak occurred in the year 1981 at Maritime Canada, involving 41 cases, 18 deaths (mostly in pregnant women and neonates) due to contaminated coleslaw (Schlech, 1983). Common food items that have been linked to outbreak of listeriosis include dairy, soft cheeses, coleslaw; meat products such as deli meats, RTE hot dogs and poultry seafood; and vegetable based products (Elliot and Kvenberg, 2000; CDC, 2002). A recent multistate outbreak of listeriosis was linked to imported Frescolina Marte brand ricotta salata cheese. As of October 25th, 2012, a total case count of 22 persons across 14 states with 20 being hospitalized and four deaths, including one fetal loss has been reported (CDC, 2012). FDA's investigation process is still examining the causes that have led to this outbreak. Another significant multistate outbreak was in early September 2011, due to consumption of contaminated cantaloupe produced by Jensen Farms in Colorado infecting 146 people and causing 30 deaths in 28 states (CDC, 2011). The deaths primarily occurred in individuals aged 48-96. This outbreak is termed as the second deadliest *L. monocytogenes* outbreak in US (Neuman, 2011) after the 1985 California *L. monocytogenes* outbreak in Mexican style soft cheese, with 52 deaths, including 19 stillbirths and 10 infant deaths (CDC, 1985). FDA, after conducting an environmental inspection at the cantaloupe farm, after the 2011 outbreak, isolated outbreak strains of *L. monocytogenes* from the packing and cold storage facility containing whole cantaloupes. This was the first documented listeriosis outbreak associated with fresh, whole cantaloupe in the United States (FDA, 2012).

There have been Although the number of reported cases of listeriosis is relatively low compared to *Camplyobacter* and *Salmonella*, the rate of mortality is very high thereby making *L. monocytogenes* one of the most significant food pathogens (Cope *et al.*, 2010). A summary of

the outbreaks from 2005-2010 related to consumption of *L. monocytogenes* contaminated foods in different states along with the illnesses and fatalities is shown in Table 4.

TABLE 4: *Listeria monocytogenes* outbreaks (2005-2010) related to consumption of contaminated foods (CDC - Foodborne Outbreak Online Database).

YEAR	STATE	ILLNESS	HOSPITALIZATION	DEATHS	FOOD ASSOCIATED
2005	Multistate	13	13	1	Deli meat, sliced turkey
2005	Texas	12	12	0	queso fresco, unpasteurized
2005	New York	3	3	0	chicken, grilled
2006	Oregon	3	2	1	other cheese, pasteurized
2006	Minnesota	2	0	0	taco or nacho salad
2007	Massachusetts	5	5	3	other milk, pasteurized; skim milk, pasteurized
2008	New York	5	5	3	tuna salad
2008	Multistate	20	16	0	sprouts
2008	Multistate	8	4	0	cheese, Mexican-style, pasteurized
2009	Multistate	8	3	0	mexican style cheese
2009	Washington	2	2	0	cheese
2010	Louisiana	14	7	2	hog head

A total of 12 outbreaks occurred from 2005-2011, leading to a total of 95 illnesses, 72 hospitalizations and 10 deaths. Of the 12 outbreaks, 3 occurred in 2005 & 2008, 2 in 2006 & 2009 and 1 each in 2007 & 2010. Consumption of implicated products was mostly in private homes or restaurants. In the last 7 years there have been 6 multistate outbreaks with several deaths indicating the vulnerability of elderly individuals, immune-compromised and infants to listeriosis. This has led the USDA-FSIS and FDA to issue stringent *L. monocytogenes* regulations and also to issue mandatory/voluntary recalls of foods contaminated with/suspected to be contaminated with *L. monocytogenes*.

REGULATIONS AND POLICIES

The prevalence of *L. monocytogenes* is usually tried to be reduced by the implementation of regulations by the USDA and FDA on RTE foods. As per the Federal Register Interim Final Rule 9 CFR Part 430, *L. monocytogenes* has been mentioned as a post-process contaminant i.e can contaminate RTE products after a lethality treatment. This mandates a post-lethality treatment to control *Listeria monocytogenes.* It is also categorized as a hazard in a Hazard Analysis Critical Control Points (HACCP) plan which requires a proper pre-requisite program Standard Operating Procedure (SOP) or Sanitation Standard Operating Procedure (SSOP) to control the organism in an establishment. Along with a valid HACCP and sanitation program, cleanliness of equipment and storage facilities on a regular basis with emphasis on hard to reach locations within the plant where pathogens are likely to survive and grow is imperative. Use of sanitizers must be effective in eliminating microflora as well as provide no detrimental effects on the equipment and the end food products. Separation of raw products and finished RTE products is essential in the processing plant to eliminate the chances of cross contamination. Another significant move made by the US FDA was by classifying *L. monocytogenes* as an adulterant

which established that the presence of *L. monocytogenes* on food or food contact surface would automatically trigger a product recall. Due to various recalls, fatalities and being categorized as an adulterant, by the USDA-FSIS and FDA has implemented a strict "zero tolerance policy" on *L. monocytogenes* contamination of RTE products in 1989.

To comply with the above rule, FSIS has given three alternatives for a RTE establishment/manufacturer to control *L. monocytogenes* in post-lethality exposed RTE products. The manufacturer is expected to follow either one of the three alternatives for their post lethality control program.

ALTERNATIVE 1: It combines a post process intervention with the formulation hurdles that suppresses or limits the growth of the organism (Illustration 1).

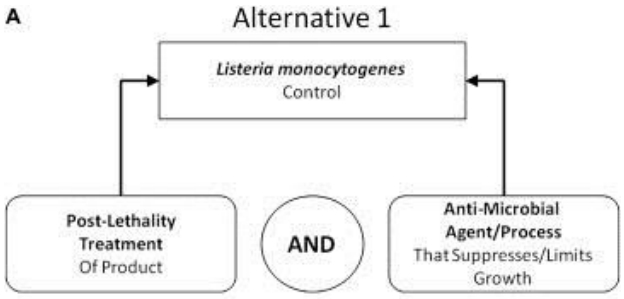

Illustration 1: Alternative 1 for *Listeria monocytogenes* post lethality program

ALTERNATIVE 2: Provides an option of either the intervention process or the formulation hurdle combined with a verified sanitation plan (Illustration 2).

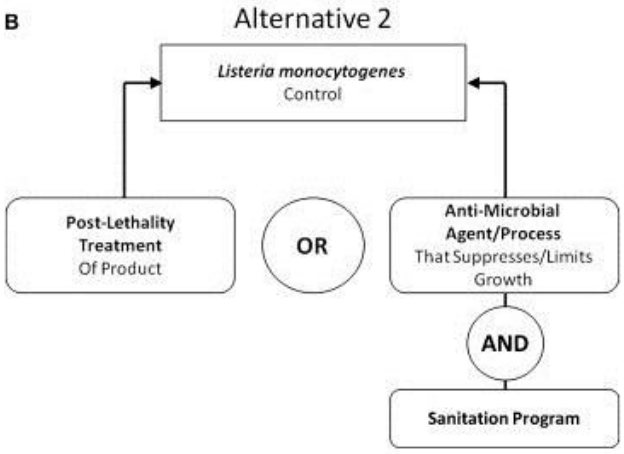

Illustration 2: Alternative 2 for *Listeria monocytogenes* post lethality program

ALTERNATIVE 3: Depending on an effective sanitation program along with end product testing (Illustration 3).

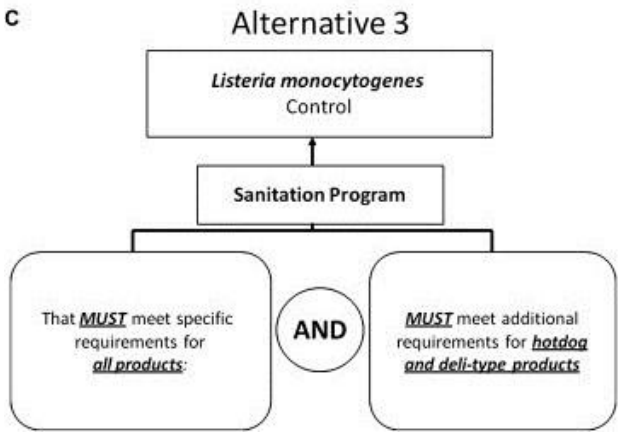

Illustration 3: Alternative 3 for *Listeria monocytogenes* post lethality program

Alternative 1 and 2 are recommended (by FSIS) to include the post-lethality step in their HACCP plan while the antimicrobial agent/process to suppress/limit the growth of *L. monocytogenes* can be part of a prerequisite program (SSOP). A number of thermal and non-

thermal post-lethality treatments can be included but are not limited to steam/hot water pasteurization, pre-packaged/post-package surface pasteurization, high hydrostatic pressure processing, ozone, radiation and organic acids. If Alternatives 2 and 3 are chosen by the establishments, food contact surfaces must be tested to ensure sanitary conditions for control of *L. monocytogenes* in post-lethality processing environments. The USDA-FSIS requires such establishments to have a plan ready for hold-and-test scenario wherein the food contact surface tests positive for *L. monocytogenes*, stating the sampling size, location and frequency. Sampling frequencies are usually justified based on history and trends of *L. monocytogenes* occurrence in a plant, features of the plant, type of product and volume, plant layout and product flow. In addition, establishments choosing alternative 3 producing hotdog or deli products must always verify corrective actions taken. Reduction/elimination of *L. monocytogenes* in establishments completely dependent on alternative 3 (sanitation programs) are subjected to more frequent verification testing by FSIS followed by the alternative 2 and 1 establishments (Table 5).

Implementation of these alternatives and regulations by the 9 CFR 430 could possibly reduce the occurrence of *L. monocytogenes* in RTE products because of stringent control procedures, improved interventions and increased sampling. Results from FSIS testing for *L. monocytogenes* in RTE products indicate that percent *L. monocytogenes* positive samples from 4.61% (1990) declined to 0.38% (2010) and also 38% lower incidence of the pathogen in 2010 (USDA-FSIS, 2004). The data showed decreased incidences per 100,000 cases from 0.47 case rate in 1996 to 0.30 in 2010. *Listeria monocytogenes* is a major emphasis in Healthy people 2020 initiative and it aims at an objective of 0.2 cases on average of 100,000 cases with a 25% improvement from the baseline report of 0.3 cases per 100,000 cases.

Table 5: Alternatives to control *Listeria monocytogenes* in food processing facilities as recommended by the US FDA (Ryser and Marth, 2007).

Alternative	Post - Lethality Treatment	Formulation Hurdles	Food Contact Surface Monitoring	Verification Frequency
3	No	No	Weekly	High
2	No	Yes	3 months	Medium-High
2	Yes	No	3 months	Medium-Low
1	Yes	Yes	6 months	Low

A revised version of 9 CFR 430 was released in September 2012 (USDA, 2012). Though the information has not changed significantly since the May 2006 version of the Compliance Guideline, FSIS recommends that establishments should review this information to ensure that they are in compliance with the regulation. The revised *L. monocytogenes* guideline replaces previous versions of the guideline and Q&A's (last updated in 2006), and provides recommendations that establishments producing post-lethality ready-to-eat (RTE) products can follow to meet the requirements of 9 CFR Part 430 also known as the *Listeria monocytogenes* Rule. Although, revised guideline have been reformatted and contains additional information to assist establishments in complying with the *Listeria monocytogenes* Rule.

One major change in the guideline includes the increase in the number of product samples under routine risk based L. m (RLm) and Intensified Verification Testing (IVT) programs. It intends to increase from 3 to 5 samples per sampling unit and to five 25 g product samples, thereby increasing the analytical test portion from 25 g to 125 g and increasing the possibility of finding positive *L. monocytogenes*. New and updated information on developing a *Listeria monocytogenes* control program to test for *L. monocytogenes* or an indicator organism on Food

Contact Surfaces (FCS) is also available in the revised version. It is now vital that establishments must take time to review and reassess their *L. monocytogenes* control programs to ensure possible detection, and elimination of the pathogen in post-lethality environments.

PATHOGENESIS

Given the risk and prevalence of *L. monocytogenes* as a causative agent of human listeriosis it is integral to understand the complexity of the disease and the confrontation of the pathogen with the host immune system, so prevention of the disease can be approached in a different way.

Listeria monocytogenes is a facultative intracellular bacterium that has the capacity to enter, survive and multiply in phagocytic and non-phagocytic host cells along with its ability to cross the host cell wall. Following ingestion of contaminated food, L. monocytogenes reaches the intestinal tract surviving the gastric acid. Endothelial cell invasion follows, which is facilitated by the internalin proteins, or by cell-to-cell spread after the uptake by macrophage (Sleator *et al.,* 2009). Once past the intestinal barrier, *L. monocytogenes* disseminates via the lymph / blood system to lymph nodes, the spleen, or the liver (Vázquez-Boland *et al.,* 2001). Macrophages in the spleen and liver (Kupffer cells) rapidly clear *L. monocytogenes* from the blood stream (Vázquez-Boland *et al.,* 2001). Kupffer cells being a part of the innate immune response inhibit *L. monocytogenes* replication during the first 2-3 days of infection, which induces an acquired immune response (Portnoy *et al.,* 2002). At this point, chemokines secreted by the Kupffer cells attracts neutrophils which adhere to the Kupffer cells and destroy both extracellular *L. monocytogenes* and infected host cells (Conlan, 1997). However, within 6h most of the viable *L. monocytogenes* have been internalized by hepatocytes, which is the preferred site for the intracellular replication (Vázquez-Boland *et al.,* 2001). During autophagy, hepatocytes actively destroy both the internalized *L. monocytogenes* cells and themselves while others rapidly

proliferate and survive until checked by acquired immunity (Portnoy *et al.,* 2002). The cells that survive undergo the physiological shifts like adjustment of metabolic systems, alteration of the cell wall, increased expression of cell surface structures, all of which play a role in adaptation of *L. monocytogenes* to stressful environments (Gray *et al.,* 2006). In order to avoid its exposure to the host's humoral immune system *L. monocytogenes* spread cell-to-cell via actin-based motility (Portnoy *et al.,* 2002). After approximately four days of infection, listeriosis is confined by granuloma where shedding and dissemination of the bacteria occur via the blood stream to other sites within the host, including the uterus (and unborn fetus), central nervous system, brain and other organs (Portnoy *et al.,* 2002). Then the infection completes itself by the activation of a CD4 / CD8 T – cell response, where the infection often becomes systemic resulting in mortality (Sleator *et al.,* 2009; Hiromatsu *et al.,* 1992).

INFECTION CYCLE

One of the amazing host-pathogen interactions of *L. monocytogenes* is its ability to invade and replicate in mammalian host cells. *Listeria monocytogenes* can invade both phagocytic and non-phagocytic cells. The entry into the phagocytic cells like macrophages or dendritic cells are bacterial independent while the entry into non-phagocytic cells such as epithelial cells is induced by the bacterium itself, which is termed as its infection cycle (Figure 4). This type of infection cycle portrays the evolutionary conversion of several bacterial proteins to mimic cellular functions. The entry into non-professional phagocytes involve two major *L. monocytogenes* proteins namely Internalin A (Inl A) and Internalin B (Inl B). They further interact with growth factors like E-cadherin and hepatocyte growth factor (HGF) or Methionine or both to get internalized into the vacuole. By the action of the pore forming toxin Listeriolysin O (LLO) and phospholipases PlcA and PlcB, the internalized bacteria is lysed from the vacuole and released

into the cytoplasm, where they start to multiply and polymerize actin. ActA, bacterial surface protein triggers the polymerization of actin at one of the bacterial pole thereby giving rise to a structure called actin tail or the actin comet. This tail propels the bacterium through cell cytosol penetrating the cytoplasmic membrane, generating protrusions and thus spreading to neighboring cells. Upon entry into the neighboring cell, bacteria present in a double membrane vacuole, escape using PlcB and LLO to perpetuate the cycle. Thus, the cell-to-cell spread of *L. monocytogenes* occurs without being externally exposed, avoiding contact with circulating antibodies and other extracellular bactericidal compounds. This makes *L. monocytogenes* a very potent and invasive pathogen.

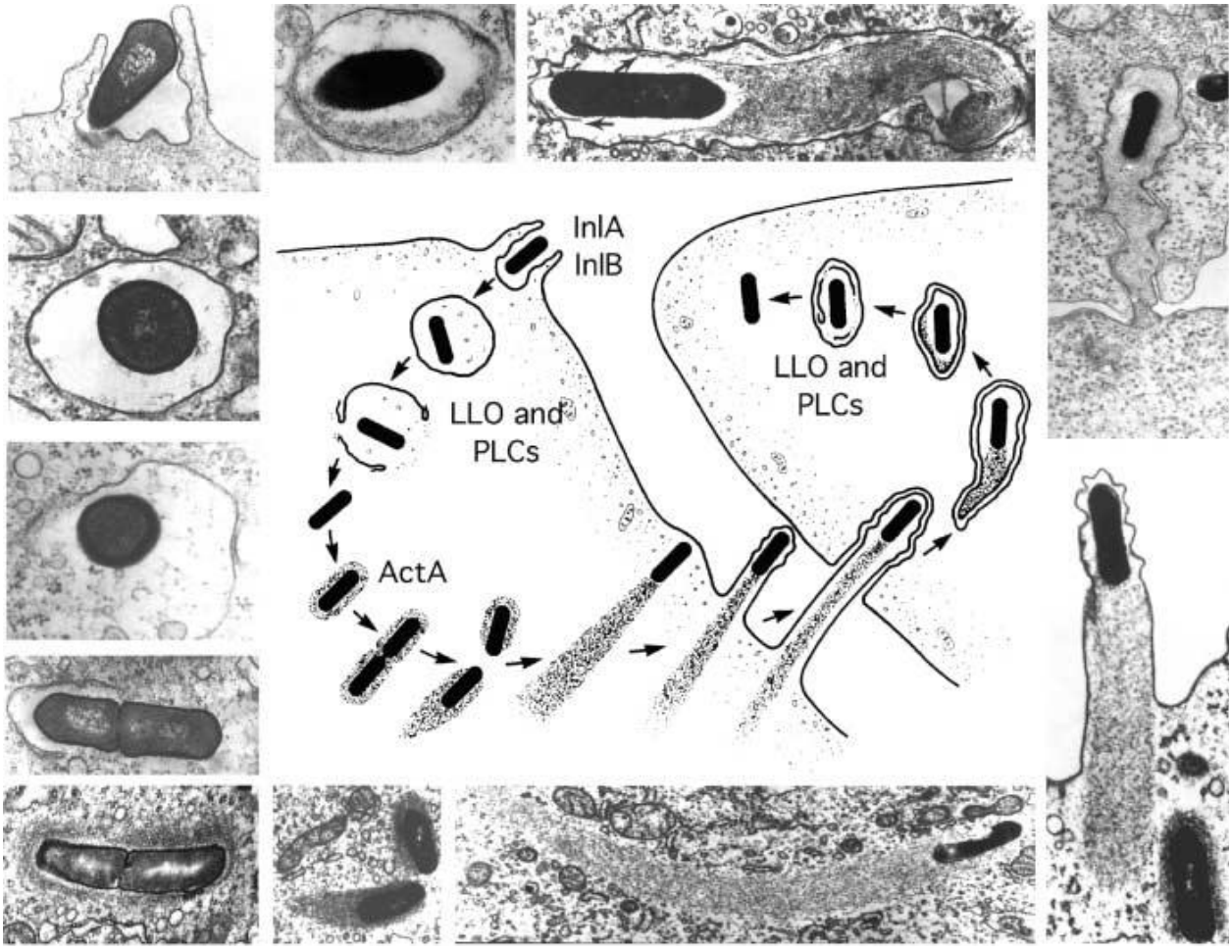

Illustration 4: Infection cycle of *Listeria monocytogenes* **(**Portnoy *et. al*, 2002)

Along with mentioned internalins, several additional internalins have also been described to be involved in the bacterial infection and colonization process *in vivo*. Deletions in the three membrane bound internalins InlG, H and E (InlG/H/E) shows reduced virulence *in vivo* following oral infection (Raffelsbauer *et al.,* 1998) but lead to an upregulation of InlA/B resulting in an hyper-invasive bacterial phenotype (Bergmann *et al.,* 2002).

CONTROL OF LISTERIA MONOCYTOGENES IN RTE FOODS

POST-LETHALITY TREATMENTS

Controlling of *L. monocytogenes* in RTE foods due to post-processing contamination is mandatory. Different methods can be adopted to reduce the risk of illness or death from *L. monocytogenes* in these products. Since it is a post process contaminant, several treatments such as High Pressure Processing (HPP), post-pasteurization or bactericidal surface treatments, and / or antimicrobial agents, such as lactates and diacetates, can be applied to reduce the presence and /or limit the outgrowth of *L. monocytogenes* on the meat product. Thus, a lethality treatment that is applied or is effective after post-lethality exposure to the final or sealed package of product in order to reduce or eliminate the level of pathogens resulting from contamination is described as post lethality treatment. The food industry wants to ensure safety of its products while maintaining quality. Alternative treatments for lowering foodborne pathogen level are being explored and today, new technologies that are faster, cheaper, and less disruptive to quality than traditional thermal processing have become increasingly common. Several non-thermal processing technologies are in use and include high-pressure processing, pulsed electric fields, active packing, electronic pasteurization, ultraviolet light, and irradiation.

HIGH-PRESSURE PROCESSING

High Pressure Processing (HPP) is a process to inactivate pathogenic microorganisms by using

pressure rather than heat to effect pasteurization. Pressure of about 400–600MPa or 58,000–87,000psi is used either at chilled or mild process temperatures (<45°C), allowing most foods to be preserved with minimal effects on taste, texture, appearance, or nutritional value (Balasubramaniam and Farkas, 2008). For example, HPP is used in products like deli meats to be pasteurized after slicing and packaging, thus mitigating the risk of contamination from the manufacturing environment. The USDA-FSIS had recognized HPP as an acceptable food safety intervention for eliminating *L. monocytogenes* in processed meat products (Hayman *et al.*, 2004). HPP is also used to effectively inactivate *E. coli*, *Salmonella*, and *Vibrio*, as well as many yeasts, molds, and bacteria that are responsible for food spoilage (He *et al.*, 2002). Margosch *et al* in 2006 studied the synergies of heat and pressure to sterilize pathogenic spores such as *Clostridium botulinum* and spoilage spores such as *Bacillus* and *Clostridia* and eliminated it from the food system. However, the limitation with this process is that the equipment is very expensive compared to the traditional heat transfer methods, possible meat color or texture changes may be observed.

PULSE ELECTRIC FIELD TECHNOLOGY

Pulsed Electric Field (PEF) processing is application of pulses of high voltage (typically 20 - 80 kV/cm) to foods placed between 2 electrodes, under ambient temperature for less than 1s. Due to this process the energy loss due to heating of foods is minimized hereby extending the shelf life without sacrificing the food quality attributes (Qin *et al.*, 1995). Although studies have concluded that PEF preserves the nutritional components of the food, effects of PEF on chemical and nutritional aspects of foods must be better understood before it is used in food processing. PEF treatment has lethal effects on various vegetative bacteria, mold, and yeast but restricted in processing liquid and semi-liquid food products such as juices, milk, yogurt, soups, and liquid

eggs (Barbosa-Cánovas *et al.*, 1999). Raso and others (1998) reported that apple juice from concentrate treated with PEF at 50kV/cm, 10 pulses, and pulse width of 2µs and processing temperature of 45°C had a shelf-life of 28d compared to a shelf-life of 21d for fresh-squeezed apple juice. Fernandez-Molina and others (1999) studied the shelf-life of raw skim milk (0.2% milk fat), treated with PEF at 40kV/cm, 30 pulses, and treatment time of 2µs and observed that the shelf-life of the milk increased to 2 weeks when stored at 4°C. Inactivation of *L. monocytogenes* Scott A in pasteurized whole, 2%, and skim milk was observed when exposed with PEF. Approximately 1 – 3 log reduction was seen at an ambient temperature of 25°C and 4-log at 50°C (Reina *et al.*, 1998).

ACTIVE PACKAGING

Also known as 'intelligent packaging' has attributes beyond the basic physical barrier property. They are specifically designed to change the condition of packaged food to give extended shelf life, improved safety, and desirable quality (Ahvenainen and Hurme, 1997). In food safety, active packaging is usually used for the inhibition of pathogen and spoilage microorganisms. It is done by extending lag phase of the microbial growth, which thereby reduces its growth rate and maximum growth number (Han, 2003). In 2000, a commercially available product, Toxin Guard™ by Toxin Alert Inc. (Ontario, Canada) was designed by Bodenhamer. It is a polyethylene-based packaging material system, which detects the presence of pathogenic bacteria (*Salmonella* spp., *Campylobacter* spp., *Escherichia coli* O157 and *Listeria monocytogenes*) with the aid of immobilized antibodies. When the analyte (i.e. toxin or microorganism comes in contact with the material) it first binds to a specific, labeled antibody and then to a capturing antibody printed as a certain pattern. This allows the indication of

spoilage of that food product. This system can also be applied for the detection of pesticide residues or proteins resulting from genetic modifications.

ULTRAVIOLET RADIATION

With the growing negative public reaction over chemicals added to foods, and the flavor and quality deteriorating in both thermal and non-thermal processing, UV light in food processing may be a promising application for the food industry. As a physical preservation method, UV irradiation has a positive consumer image. The FDA and USDA have concluded that the use of UV irradiation is safe. In 2000, the FDA approved UV-light as alternative treatment to thermal pasteurization of fresh juice products with a performance criterion of 5-log reduction in the number of target pathogen (FDA, 2000). The germicidal effects of UV radiation have been known since the 1800s. It is an established technology for water treatment, air disinfection and surface decontamination (Yousef and Marth, 1988).

UV radiation waves are between the X-ray and visible light spectrums usually in the range of electromagnetic waves 100 to 400nm long. The division of UV radiation is classified as Vacuum UV (100-200nm), UV-C (200-280nm), UV-B (280-315nm) and UV-A (315-400nm). UV disinfection utilizes either: low-pressure lamps that emit maximum energy output at a wavelength of 253.7nm; medium pressure lamps that emit energy at wavelengths from 180 to 370nm; or lamps that emit at other wavelengths in a high intensity "pulsed" manner. The germicidal effects are optimum in the UV range of between 245 and 285 nm (Yaun et al., 2003). Thus the section of interest is the UVC (200 to 280 nm) as this is the wavelength that is most effective in inactivating bacteria and viruses. It is usually between 254nm, the DNA mutations are induced through UVC absorption by the nucleic acids (Center for Food Safety and Applied Nutrition, 2000). Antimicrobial effect of UV radiation occurs due to the photochemical changes

that take place in proteins and nucleic acids when the UV radiation is absorbed. The DNA absorption of the UVC light causes crosslinking between neighboring pyrimidine bases on the same DNA strand, and thus formation of hydrogen bonds with the purine bases on the other strand is impaired thereby blocking the DNA transcription and translation, and leading to cell death due to mutations (Miller *et al.*, 1999). According to the EPA (1999), the destruction or inactivation of microorganisms by UV radiation is directly related to the UV dose.

The calculation of the UV dosage is: $\mathbf{D = I \cdot t}$

Where: D = UV Dose, mW×s/cm2

I = Intensity, mW/cm2

t = Exposure time, s.

The above calculation describes that a constant fraction of the living population is inactivated during each progressive increment in time. This dose-response relationship for germicidal effect indicates that high intensity UV energy over a short period of time would provide the same kill as lower intensity UV energy at a proportionally longer period of time (Tchobanoglous, 1997). Generally, UV radiation is susceptible to most microorganisms with varying UV dosage. Viruses and molds require a much higher dose for inactivation than bacteria (Morgan, 1989). Moreover, the susceptibility to UV also depends on the species, age, cell wall and presence of spores. Chang *et al* in 1985 described that Gram-positive bacteria tend to be more resistant than Gram-negative organisms and spore formers are more resistant then non-spore formers. Vegetative bacteria tend to be most resistant to UV radiation just prior to active cell division, during the lag phase. It has also been found that a given dose will become less effective with increase in the number of cells. The absence of oxygen will also increase microbial resistance to UV irradiation (Jay, 2000). The most critical factor for UV radiation in liquids is the transmissivity of the

material being sterilized, as even small amounts of solutes or particulates will attenuate and scatter UV light, resulting in a lower measure of microbial inactivation. Also of importance is the thickness of the radiation path through the liquid because attenuation of the UV light is increased with the length of passage (Center for Food Safety and Applied Nutrition, 2000).

APPLICATION OF UV

Various foods and beverages have been treated with UV radiation to decrease bacterial content and eliminate pathogens such as *L. monocytogenes.* Kim *et al* (2006) studied the effect of UV radiation on *Listeria monocytogenes* present in peptone water and reported that intensities between 250 to 600μW/cm^2 reduced all suspended cells in peptone water by 5 log after 2min while after 3min *L. monocytogenes* was completely reduced with a reduction of 8.4 log. Yousef and Marth (1988) exposed *L. monocytogenes* to short-wave UV energy (100μW/cm^2) for a time period of 0.5 to 10min. Matak et *al* (2005) studied the efficacy of UV light in the reduction of *Listeria monocytogenes* in goat's milk. About 10^7 cells of *L. monocytogenes* were exposed to UV light with a cumulative UV dose of 15.8mj/cm^2. More than 5 log reduction was achieved indicating that UV radiation could be used to reduce *L. monocytogenes* contamination of non-transparent (colloidal) fluids. An UV radiation research was conducted by Kissinger and Willits (1966) reported that 99% of microorganisms were reduced in maple sap. Though, the germicidal use of UV in food is restricted due to the fact that UV acts only on transparent liquids literature shows the ability of UV light to disinfect surfaces of meat products. This demonstrates that UV light has the potential to reduce bacterial contamination on food surfaces and can be used as post lethality treatment to control *L. monocytogenes* and other pathogens of concern (Bachmann, R, 1975). Study by Wong *et al* (1998) evaluated the use of UV irradiation to reduce levels of *E. coli* and *Salmonella* on pork skin and muscle. Other studies including, *L. monocytogenes* on chicken

meat (Kim *et al*., 2002), and *Salmonella* Typhimurium on poultry carcasses (Wallner-Pendelton *et al.*, 1994) have shown significant reduction after UV exposure. Other applications of UV include its effectiveness on shell eggs (Kuo *et al*., 1997), extension of shelf- life of fresh mackerel fish by 7 days over untreated fish (Huang and Toledo, 1982). Ultraviolet radiation is also used to treat air and surfaces in hospitals and laboratories where aseptic facilities are required (Collins, 1979). It is also used on the packaging materials for aseptic packaging (Farkas, 2001). UV-C radiation along with ozone has become one of the alternative sanitizing methods, as the combination has accounted for reducing microbial flora of fresh cut onion, escarole, carrot and spinach wash waters collected from food manufacturing operations (Selma *et al.*, 2008). Sommers *et al* in 2010 used UV-C in combination with 3 antimicrobials (Generally Regarded as Safe antimicrobials, GRAS) and found it to be very effective, with 3.6–4.1 log of the three pathogens (*L. monocytogenes*, *Salmonella*, and *Staphylococcus aureus*) being inactivated by the end of 12 week of storage period with no impact on the color or texture of the frankfurters.

EFFICIENCY OF ULTRAVIOLET RADIATION

The efficiency of UV radiation in reducing levels of *L. monocytogenes* depends on several factors such as the type of strain; food source the organism is present on, opacity of the fluid and the presence of suspended solids. Its unique advantages including lower cost as compared to chlorination; lack of residues vs chemical antimicrobials; and no legal restrictions on its dosage (Yousef and Marth, 1988) has made it superior to the other technologies. UV rays are not capable of penetrating solid foods but it can act as very potent surface decontaminant for most of the food products with smooth surfaces (Stermer *et al*., 1987). Its ability to penetrate liquids makes it one of the most used non-thermal intervention strategy in liquid and water processing/ treatment plants. The common concern of irradiation affecting the quality attributes of the food

products is overcome by reports proving that there were no significant effects on oxidative rancidity i.e. thiobarbituric acid (TBA), pH, color, sensory or odor (Sommers, 2010). Fu *et al* (1995a and b) reported no effects on quality attributes on pre-cooked pork chops, cured ham and steak when irradiated either at low or high-energy dose.

Thus the recent advances in science and engineering have made UV light irradiation a viable option for commercial application in food processing. As a non-thermal alternative to traditional thermal processing, UV light has a potential to be used for pasteurization of juices and beverages, as well as a post lethality treatment in controlling microbial contamination on meats and shell eggs surfaces, and as a means for the shelf life extension of various products. Therefore, UV light processing can improve safety of selected solid and liquid foods without appreciable loss in quality or nutrient content.

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CHAPTER 3

GROWTH BEHAVIOR OF COLD ADAPTED AND NON- COLD ADAPTED *LISTERIA MONOCYTOGENES* IN LABORATORY MEDIA AND MEAT EXTRACT

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ABSTRACT

The ubiquitous nature of *Listeria monocytogenes* and its ability to grow at refrigerated temperature has made *L. monocytogenes* a significant threat to the safety of ready-to-eat (RTE) products. Studying the growth physiology of *L. monocytogenes* in cold adapted and non-adapted conditions aids our ability to prevent and reduce *L. monocytogenes* growth in refrigerated RTE foods and to reduce listeriosis. *Listeria monocytogenes* serotype 4b was cultured in Brain Heart Infusion (BHI) broth and meat extract at 37 and 4°C. A cold adapted *L. monocytogenes* was also cultured by reducing the temperature by 5°C in a stepwise manner until the strain has reached 4°C. Twenty four hour and 24d growth curves were conducted on cold adapted and non-adapted *L. monocytogenes* grown in BHI and meat extract at 37 and 4°C respectively. The cells were recovered on plate count agar (PCA) and modified oxford agar (MOX) after 24h of incubation. From each growth curve, the growth rate constant as well as the mean generation times were calculated. Data were analyzed using ANOVA to find significant differences ($p<0.05$) between the means of cold adapted and non-adapted cells. Significant differences ($p<0.05$) were observed between the cold adapted and non-adapted *L. monocytogenes* irrespective of growth medium. Approximately a 1 log CFU/mL difference was observed for the cells grown in BHI and a 1.5 log CFU/mL difference on the cells grown in meat extract. Significant differences ($p<0.05$) was also observed in growth rate and mean generation time. Identification of *L. monocytogenes*

growth behaviors provide insight that will be needed to mitigate risk of this organism in the further processing areas.

INTRODUCTION:

Listeria monocytogenes has been associated with foodborne illnesses for over two decades and classified as a foodborne pathogen causing major worldwide economic and public impact. It primarily affects pregnant women and their neonates as well as patients who are immuno-compromised. The majority of the large listeriosis outbreaks detected in the United States have been associated with the consumption of ready-to-eat (RTE) meats such as, frankfurters and deli meats, milk and dairy products, and raw produce (CDC, 2012). A recent multistate listeriosis outbreak associated with cantaloupe consumption that involved a total of 146 invasive illnesses, 30 deaths and one miscarriage is a clear example of foodborne listeriosis (CDC, 2011). *Listeria monocytogenes* and accounts for 19% of a total 1351 food-borne fatality episodes each year (Scallan *et al.*, 2011). In order to reduce overall cases of food-borne illnesses, listeriosis is a major focus in the "Healthy People 2020" initiative with an objective of 0.2 cases on average of laboratory-confirmed *L. monocytogenes* infections per 100,000 cases, which equates to a 25% improvement from the baseline report of 0.3 cases per 100,000 cases (CDC, 2010). These large outbreaks have also prompted the U.S. Food and Drug Administration (FDA) and the U.S Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) to establish a "zero tolerance policy" under which ready-to-eat (RTE) foods contaminated with a detectable level *L. monocytogenes* are deemed adulterated.

Listeria monocytogenes is a Gram-positive, non-spore forming, facultative anaerobic pathogen, which is ubiquitous in the environment. Its ability to grow at temperatures ranging from 2 to 45°C is one of the many significant characteristics of this pathogenic bacterium, thus allowing refrigeration temperature to act as an effective enrichment for the organism. Therefore, it is considered a cold tolerant organism as its optimum growth temperature is in the range of 30 to

37°C, while it has the ability to grow at temperatures < 15°C (Walker and Stringer, 2003). Gray *et al.* in 1948 described that *L. monocytogenes* can grow at 4°C after storage for 3 days to 3 months and furthermore, Fenlon (1999) reported *L. monocytogenes* could survive in soil, cattle feces, pond water, and animal silage for years. Moreover, the U.S. Department of Health and Human Services - U.S. Department of Agriculture, (2003) cited that *L. monocytogenes* can proliferate in many refrigerated RTE foods, such as deli meats, smoked fish, and milk.

Growth of *L. monocytogenes* at 4°C is generally very slow with doubling times of 12 to > 50 h along with a longer lag phase (Lou and Yousef, 1999). By this time, the bacteria generally have adjusted to the cold environment, with characteristics of higher rate of survival (and growth) in chilled and frozen products, thereby, decreasing the shelf life of the product. This is because of their ability to adapt, and gradually develop tolerance or resistance to cold stress (Beales, 2004), therefore, when adapting to adverse conditions, changes in gene expression along with induction of specific proteins and changes in membrane composition of the bacteria are vividly observed. The cold adaptation of bacteria is usually divided into three phases: (i) initial cold shock, (ii) acclimation, and (iii) cold-adapted (Thieringer *et al.*, 1998). Cold shock is the rapid change from bacterium's optimum growth temperature (35°C) to a lower temperature (4°C) leading to the synthesis of cold shock proteins (CSPs), which are at low levels at 37°C, and increases dramatically in this acclimation phase. These CSPs can facilitate translation initiation by acting as RNA chaperones, binding to single-stranded RNA until the ribosome can initiate translation (Hunger *et al.*, 2006) thus assisting in cold adaptation. Research findings by Zhu *et al.*, in 2005 indicated modifications in the lipid composition of *L. monocytogenes* cultured at 30°C (non-adapted) or at 5°C (cold-adapted), where the cold adaptation of *L. monocytogenes* is mediated by an increase in the content of neutral lipid classes and in the a-15:0/a-17:0 fatty acid ratio. For

example, in a further processing plant, this bacterial pathogen can be present in environment for a very long time experiencing cold conditions, leading to cold adaptation thereby enhancing its survival in RTE products.

Along with CSP's and cold adaptation strategies, the role of alternative Sigma factors (σ) plays a major role in the survival of *L. monocytogenes* under adverse environmental conditions, particularly to cold temperature. The role of general stress Sigma (σ) factors is to provide a mechanism to the bacteria for rapid cellular responses to changing environmental conditions by associating to a class of essential dissociable subunits of prokaryotic RNA polymerase. The alternate sigma factor Sigma B (σ^B) is usually identified in Gram-positive bacteria and this factor is activated in response to temperature down shock, due to the accumulation of cryoprotectents by Sigma B during growth of bacteria at low temperatures (Becker *et al.*, 2000).

The versatility of *L. monocytogenes* to adapt and survive in refrigerated temperatures poses a major risk to a wide variety of further processed products. Therefore, the focus of the current study is to determine growth patterns of non- cold adapted and cold adapted *L. monocytogenes* at 37°C (optimum growth temperature) and 4°C (its psychrotolerant temperature) in both laboratory media (BHI) and meat extract. Understanding growth mechanisms of *L. monocytogenes* in cold adapted and non-adapted conditions will be critical to improve our ability to prevent and reduce *L. monocytogenes* growth in refrigerated RTE foods and to further reduce listeriosis.

MATERIALS AND METHODS:

Preparation of bacterial culture: *L. monocytogenes* serotype 4b (ATCC # 19115) strain was used for the study. The culture was started from three separate lyophilized beads representing three distinct colonies by inoculating into three sterile Brain Heart Infusion (BHI, Acumedia,

MI) broth (10 mL) at 37°C for 24h. In order to check the purity of the culture, the bacterial cultures from three BHI tubes were streaked onto three separate Modified Oxford Agar (MOX) plates (Acumedia, MI) with Modified Oxford *Listeria* supplement (Acumedia, MI) and incubated at 37°C for 24h. After the purity of the culture was determined, an isolated and distinct colony from each of the MOX plate was selected and inoculated into three BHI tubes and incubated for 24h at 37°C. The resulting bacterial culture (0.1 mL) was transferred and grown in three separate BHI tubes (10 mL) for 24h at 37°C and repeated twice to obtain three independent bacterial populations to achieve approximately 9 $\log_{10}$ CFU/ml. These cultures were then serially diluted in 0.1% peptone water (Acumedia, MI) to get a population of ~4 $\log_{10}$ CFU/mL from which 1 mL was introduced into three separate BHI and meat extract bottles (500 mL) and incubated at 4°C.

Preparation of cold adapted bacterial culture: A cold adapted strain of *L. monocytogenes* serotype 4b was prepared and used for the study. After three generations of growth of *L. monocytogenes* was obtained with approximately 9 $\log_{10}$ CFU/mL in three separate BHI at 37°C, 0.1 mL of the culture from the BHI tubes were transferred into three sterile BHI tubes and incubated at 32°C. Caution was taken to maintain the fresh BHI tubes in the previous growth temperature in order to avoid temperature shock of the growing *L. monocytogenes* when transferred from higher to lower temperature. Every 24h, the cultures were serially diluted in 0.1% peptone water and plated onto Plate Count Agar (PCA; Acumedia, MI) to determine if the culture has reached approximately 9 $\log_{10}$ CFU/ml before transferring the culture to the next temperature. Similarly, *L. monocytogenes* was adapted to 4°C by a stepwise 5°C decrease in temperature. The strain which was adapted to 4°C was termed as cold adapted *L. monocytogenes*. The culture obtained was transferred into three sterile BHI tubes and incubated at 4°C for

approximately 6 days and repeated twice to obtain three independent bacterial populations of approximately 9 $\log_{10}$ CFU/ml. These cultures were further serially diluted in 0.1% peptone water to get a population of ~4 $\log_{10}$ CFU/mL from which 1 mL was introduced into three separate BHI and meat extract bottles (500mL) and incubated at 4°C.

Growth curve in BHI: A 20 day growth curve was performed on non-cold and cold adapted *L. monocytogenes* inoculated in BHI at 4°C. Samples were taken every 24h by collecting 1 mL sample from each bottle using a pipette, serially diluted in 0.1% peptone water, and spread-plating the appropriate dilution onto PCA; and incubated at 37°C for 24h. The number of Colony Forming Units per mL (CFU/mL) along with the spectrophotometric measurements (Barnstead Turner SP 830+, Dubuque, IA) at 629 nm (Optical Density) of the sample was determined every sampling period.

Growth curve in meat extract: The growth patterns of non-cold and cold adapted *L. monocytogenes* grown in meat extract at 4°C was also conducted. The meat extract was prepared by blending commercially manufactured RTE turkey frankfurters with peptone water in 1:1 V/V ratio. The blended meat was then centrifuged (Thermo Scientific Sorvall Legend RT+, Germany) at 6792 x g for 10 min, and the supernatant was used as the meat extract. Sampling for the growth curve was performed every 24h over a period of 24 days by collecting 1 mL of sample from each bottle followed by serial dilution in 0.1% peptone water and spread plating onto on PCA and incubated at 37°C for 24h. The number of Colony Forming Units per mL (CFU/mL) along with the absorbance measurements (Optical Density) at 629 nm of the sample was determined every sampling period.

Data Analysis: All the experiments were conducted in triplicate. With the obtained data, graphs were constructed with microbiological populations on the Y-axis and the time of sampling on the X-axis**.** Data was analyzed using SAS 9.2 (SAS Institute, Cary, NC) Proc GLM to determine significant differences between growth patterns of non-cold adapted and cold adapted *L. monocytogenes* at $P<0.05$.

Calculation of Growth rate (μ**) and Mean Generation Time (g):** The growth rate along with their mean generation time was calculated on all the growth curves. The growth rate constant (μ) is defined as the increase in the number of cells during a certain time period, and it was calculated by using the formula:

$$\mu = ((\log_{10} N_t - \log_{10} N_0)\ 2.303) / (t - t_0)$$

Where;

N_t = the number of cells at the stationary phase (t)

N_0 **=** the number of cells at the start of the log phase (t0)

2.303 = the growth rate constant

$t - t_0$ **=** the time interval. (Stationary phase – log phase)

This provides the growth rate of *L. monocytogenes* per day for each of the conducted growth curve. The mean generation time or "doubling time" **(g)** is the average time required for the culture to double. This is calculated from the following equation:

$$g = (\log_{10} N_t - \log_{10} N_0) / \log_{10} 2$$

where;

N_t = the number of cells at the stationary phase (t)

N_0 **=** the number of cells at the start of the log phase (t0)

Log 2 = 0.301.

This provides the mean generation time of *L. monocytogenes* in hours for all the growth curves. The data between the non-adapted and cold adapted *L. monocytogenes* on their growth constant and generation time was analyzed using Student *t* test.

RESULTS AND DISCUSSION:

The growth pattern of microorganisms in food is a three stage process and categorized into lag, exponential and stationary phases. The lag phase allows the bacteria to adjust to its new environment before the start of the exponential phase, during which bacterial cells actively divide and multiply in a logarithmic manner. The bacterial population reaches its maximum density during the stationary phase and equilibrium is established in bacterial growth. Quantification of bacterial growth at various temperatures is essential for the determination of the length of the lag phase and the rate of bacterial growth. This information is critical in the food industry in order to evaluate the microbial safety of foods along with their shelf life.

Growth pattern of non-cold adapted *L. monocytogenes* in BHI at 37°C: The optimal growth temperature of *L. monocytogenes* is 37°C. Illustration 1 shows the growth pattern of *L. monocytogenes* grown at 37°C in Brain Heart Infusion broth (BHI). The initial inoculum (N_0) was 1000 CFU/mL with lag phase of 2h, and exponential growth phase of 14h. The strains reached the stationary phase after 20-22h; with an N_t of about 9-10 log CFU/mL. The growth rate constant of *L. monoctogenes* at 37°C was $\mu = 0.77$ per hour and the time taken for 1 generation is g= 54.54 minutes, which was calculated by using the formulae mentioned previously. This growth curve serves as the baseline to understand complete growth physiology of *L. monocytogenes* under normal conditions.

Growth pattern of non-cold adapted and cold adapted *L. monocytogenes* in BHI at 4°C: Most microorganisms accommodate to a variety of changing and stressful environments in order to survive and multiply. *Listeria monocytogenes* is one such organism to strive the cold stress by adapting and proliferating at 4°C. Illustration 2 depicts the growth curve patterns of both the non-cold adapted and cold adapted *L. monocytogenes* grown at 4°C in BHI. The bacterial population of the adapted cells versus the non-adapted cells shows significant difference ($P<0.05$) from day 4 indicating the early onset of exponential phase of the adapted *L. monocytogenes*. This can be related to their respective growth rate and generation time per day. The growth rate (μ) and the generation time (g) per day of the cold adapted *L. monocytogenes* was 0.86 and 24h respectively, while for the non-cold adapted *L. monocytogenes* $\mu = 0.81$ and g= 29h 28m.

Growth pattern of non-cold adapted and cold adapted *L. monocytogenes* in meat extract at 4°C: The growth patterns of *L. monocytogenes* inoculated in meat extract and incubated at 4°C was also studied (Illustration 3). Significant differences ($P<0.05$) were observed in the growth of adapted and non-adapted *Listeria* population starting day 1. The adapted strain reached the population of 5-log CFU/mL while the non-adapted strain reached 3.5-log CFU/mL after 24d of incubation. The growth constant of adapted strain ($\mu=0.26$) was higher ($P<0.05$) than the non-adapted strain ($\mu=0.21$) with generation time of 2.5d and 3.2d respectively.

When the bacterium transitions from optimum growth temperature to cold environment, they respond by altering their membrane composition and gene expressions (Lui *et al.,* 2002). The bacterium undergoes cold shock, acclimation and adaptation, which were observed in the adapted strain of *L. monocytogenes* in our study. Thus during this temperature downshift proteins like Cold Shock Proteins (CSP's) and Cold Acclimation Proteins (CAP's) were induced in *L.*

monocytogenes and these proteins are also present in a wide range of other bacterial pathogens including, *Bacillus cereus, Yersinia enterocolitica, Vibrio vulnificus, Vibrio cholerae, Salmonella enteritidis, Staphylococcus aureus,* and *Aeromonas hydrophila* (Chan and Wiedmann, 2008). In a two dimensional protein gel electrophoresis study by Phan-Thanh and Gormon (1995), 38 CSP's were reported to be induced in *L. monocytogenes* which were grown in modified chemically defined medium at 4°C as compared to 25°C.

Along with gene expression, a reversible conversion of the lipid membrane from crystalline to gel facilitates an ideal membrane lipid state for the enhanced survival of the adapted *L. monocytogenes* (Russell, 1990) compared to the non-cold adapted strain. Approximately, 95% of *L. monocytogenes* membrane when grown at 37°C is composed of anteiso form of branched chain fatty acid, at 4°C membrane fatty acid profile of *L. monocytogenes* changes from iso to anteiso thus increasing branching and mediating normal cellular functions similar to the cells at 37°C (Annous *et al.,* 1997). Moreover, the enzymes in the cold adapted bacteria are evolved toward enhanced conformational flexibility compared to the mesophilic bacteria thereby being more efficient and active at low temperatures (Feller and Gerday, 1997).

Sigma B (σ^B), stress response regulator is very vital for *L. monocytogenes* survival and growth at low temperatures. Its activity appears within 20 – 30 m of the cold shock stress signal being induced from *L. monocytogenes* (Becker *et al.,* 2000). Role of σ^B has been studied for many years as they are responsible for transcription of cold stress genes (Chan *et al.,* 2007), growth phase dependent adaptation (Becker *et al.,* 2000), and also in transport of osmoprotectants, metabolism, and virulence (Kazmierczak *et al.,* 2003). For a pre-adapted *L. monocytogenes* used in this study, all these factors may already be unregulated and could possibly enable better growth of *L. monocytogenes* in BHI and meat extract. The versatility of this adapted bacterium

to grow faster in food matrix may be due to its cold tolerance and resistance absorbed during its cold adaptation period. Comparing the growth patterns of adapted versus the non-adapted strain has provided insight to the growth strategies that might be followed by *L. monocytogenes* harboring in cold environment for long period of time. Though the research warrant more details on start of the lag and log phases, an estimation of growth behavior of *L. monocytogenes* in different scenarios have been reported. Calculation of growth rate and generation time of each growth curve has strongly indicated the differences between adapted and non-adapted *L. monocytogenes*. The practical approach of conducting the growth curve on a real food matrix and using a pre-adapted strain is a useful resource for the food industry to understand the growth physiology of *L. monocytogenes*. Continued research is needed for a better understanding of the mechanisms *L. monocytogenes* uses to adapt to cold temperatures and growth at temperatures as low as 0°C. This will be critical to improve our ability to prevent spoilage and safety problem in refrigerated RTE foods by designing effective methods of preservation and thus reduce human listeriosis infections.

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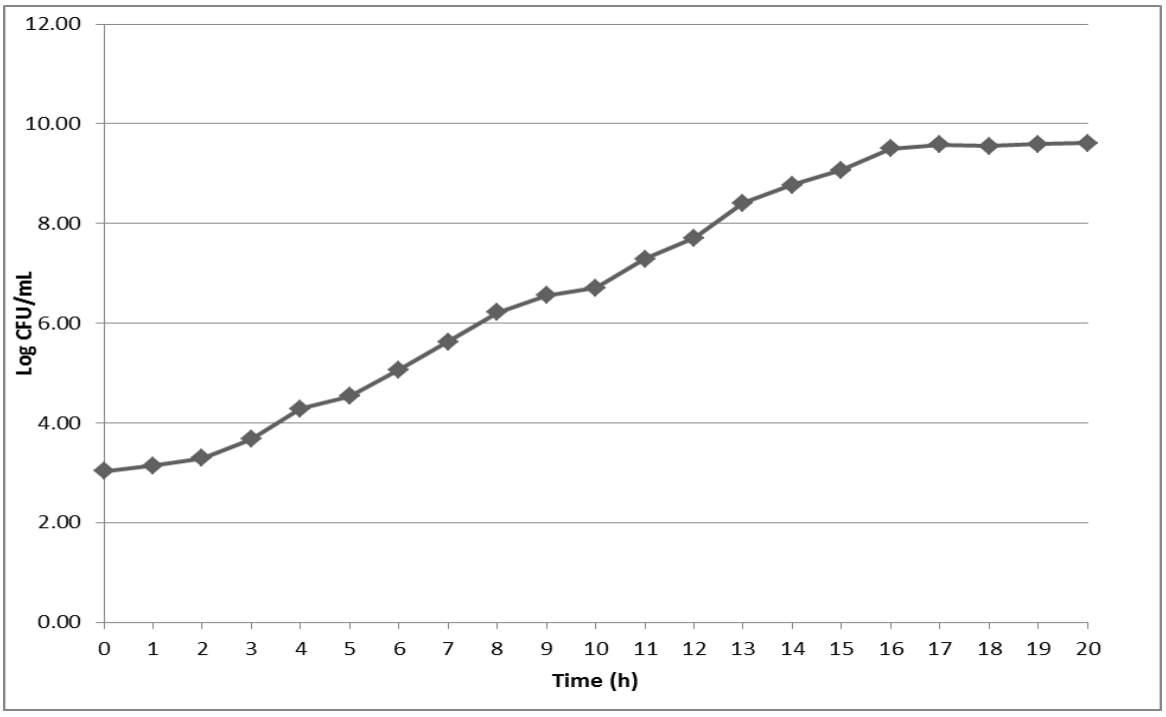
LIST OF ILLUSTRATIONS

ILLUSTRATION 1



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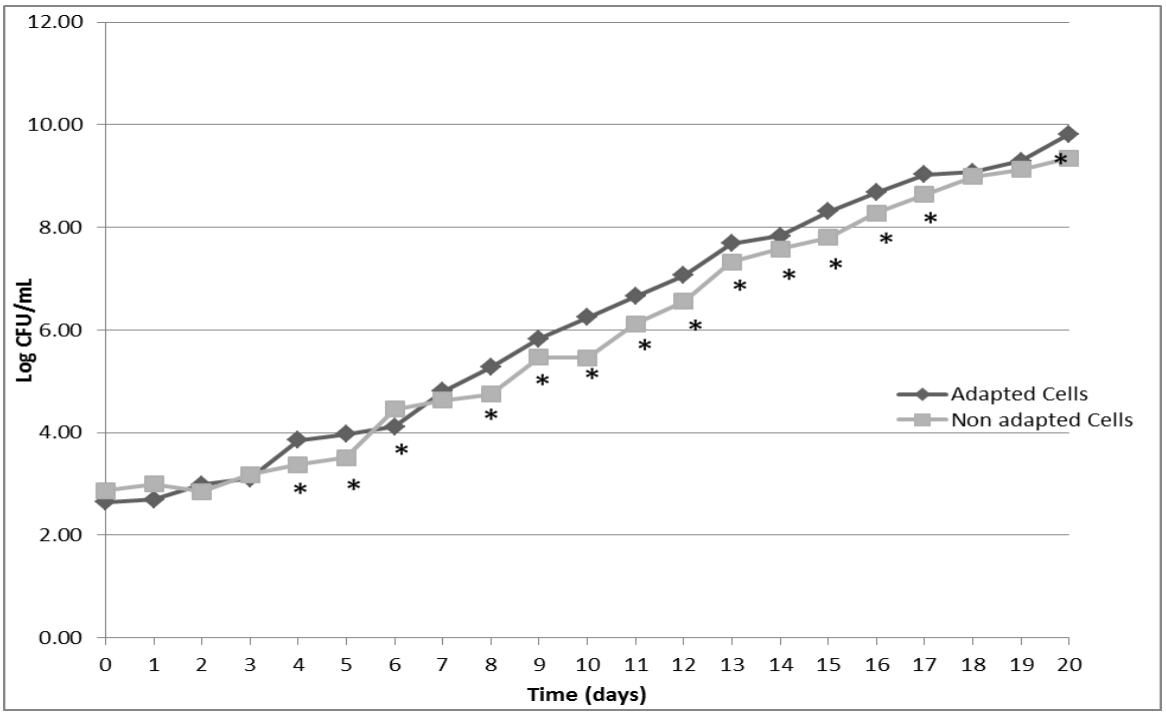
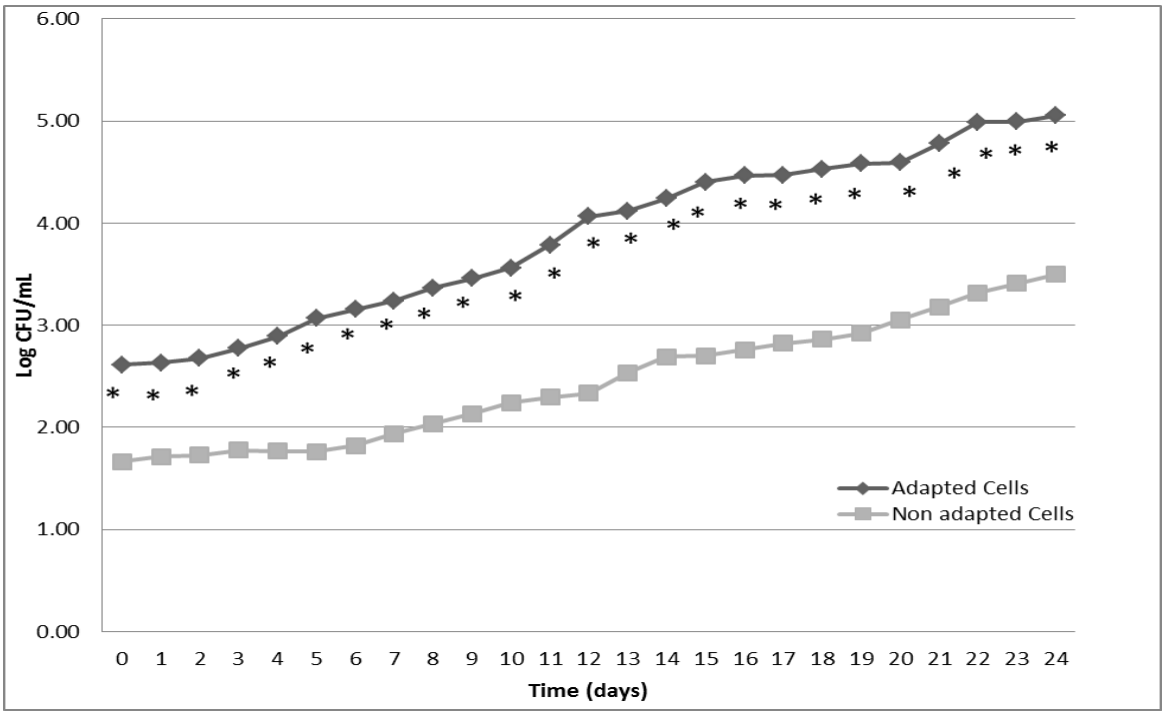


ILLUSTRATION 3



CHAPTER 4

ULTRAVIOLET LIGHT AS A POST-LETHALITY TREATMENT AGAINST *LISTERIA MONOCYTOGENES* ON BOLOGNA AND ITS' IMPACT ON QUALITY ATTRIBUTES

Manuscript prepared for sumbission to Journal of Food Protection

ABSTRACT

Listeria monocytogenes is an important foodborne pathogen and a serious public health issue due to its severity of infection and high fatality rate. These high incidences are due to the consumption of *Listeria*-contaminated foods especially in Ready-To–Eat (RTE) food, which does not require any additional heating/cooking after processing. This categorizes *L.monocytogenes* as a post process re-contamination threat to the food processing industry mandating post-lethality treatment to mitigate the organism in the industry. The efficacy of ultraviolet light was evauated at different intensities and exposure time against log and stationary phases of *Listeria monocytogenes* on growth medium and RTE meat and its impact on quality and shelf life.

Listeria monocytogenes serotype 4b was cultured in brain heart infusion (BHI) broth at 37°C. Cells were harvested during their log and stationary phase and subjected to low (3 – 4 mW/ sq. cm) and high (7-8 mW/ sq. cm) intensity of UV. *L. monocytogenes* was spray inoculated onto RTE product - bologna followed by 30m of attachment time. Inoculated bologna was then subjected to UV radiation every 30s from 0 to 300s. Cells were recovered on Modified Oxford agar (MOX) after 24h of incubation at 37°C. Additionally, shelf life along with the quality attributes such as color and lipid oxidation due to UV radiation was assessed over a period of 8 weeks on the RTE meat stored at 0°C and 4°C under vacuum.

Overall, populations of *L. monocytogenes* were significantly reduced ($p<0.05$) after 180s of UV exposure and further significant ($p<0.05$) reductions were observed after 150s irrespective of UV intensities. Moreover, significantly higher ($p<0.05$) reductions were observed in the log phase cells as compared to the stationary phase cells. Furthermore, application of UV slightly affected ($p>0.05$) the 'a' and 'l' values of color but no lipid oxidation on the RTE meat was observed irrespective of the storage temperatures and UV intensities. Reduction in *L. monocytogenes* population between the log and stationary phase without significantly affecting the quality attributes suggests the potential use of UV light as a possible post process intervention in the food processing plants.

INTRODUCTION:

Listeria monocytogenes has been associated with food and categorized as a foodborne pathogen for the past two decades. The Centers for Disease Control and Prevention (CDC) has estimated 1600 cases of listeriosis and about 260 deaths annually in the United States (CDC reference and year). The estimated cost of this illness in 2010 was $2.04 billion (Schraff, 2012). Schraff in 2012 also stated that listeriosis is considered a burden, as it contributes to both human morbidity and mortality as well as to health care costs because it involves newborn, pregnant women, elderly and immune-compromised in the risk group.

The contamination of *L. monocytogenes* is common in RTE meats during post-processing steps (Beresford *et. al*, 2001). This is of a major food safety concern because the RTE cooked meats have longer shelf life and are consumed without further cooking/heating. Moreover, these meats are stored at refrigeration temperature (4°C) and since *L. monocytogenes* can proliferate and grow at this temperature, it poses additional problems. (Lou and Yousef, 1999). Due to these factors, there have been a number of listeriosis outbreaks that have been linked to RTE foods (FSIS, 2003). The RTE foods usually become contaminated with *L. monocytogenes* due to cross contamination or physical contact with contaminated raw foods (Lou and Yousef, 1999). In 2003, the Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS) completed a risk assessment identifying RTE foods which pose the greatest risk of listeriosis. Out of twenty three RTE food categories studied, meat and poultry products especially the deli meats were found to pose the greatest risk for listeriosis (FDA and FSIS, 2003). Studies by Endrikat *et al*. (2010) and Pradhan *et al*. (2010) indicate the highest per annum risk of illness and death from *L. monocytogenes* is from deli meat consumption, and about 83% were attributed to deli meats sliced and packaged at retail facilities while the remainder was from

the prepackaged deli meat. Thus the number of reports indicating the association of RTE meat products with listeriosis and recall of tons of products has led the USDA to establish a "zero tolerance" policy for *L. monocytogenes* in RTE meat products, to prevent the contamination of *L. monocytogenes* in RTE meat products.

Due to their ubiquitous nature and also the capability to grow over different temperatures and the uncertainty of age of the organism it becomes mandatory to minimize the incidence and number of cells of the organism during product shelf life up to the point of consumption. Moreover, the realization that recontamination is an important source of *L. monocytogenes* in commercially prepared RTE foods, various control methods in the meat and poultry-processing environment to mitigate listeriosis become critical. Some of the common approaches include food contact surface testing, sanitation, post-processing lethality treatment, and product formulation with microbial growth inhibitors. In order to effectively control *L. monocytogenes* in RTE foods, the processing industries are recommended to use any one of the three alternatives that are stipulated by the FSIS; Alternative 1— Use of post-lethality treatment and a growth inhibitor against *Listeria* on RTE products. The FSIS verification activity focuses on the effectiveness of the post-lethality treatment along with sanitation. Alternative 2 uses either a post-lethality treatment or a growth inhibitor for *Listeria* on RTE products and alternative 3 uses sanitation measures only. Establishments following alternative 3 are the most frequent targets for the FSIS verification activity (FSIS, 2006). Therefore, it depends on the manufacturer to take proper measurements for reducing the contamination of *L. monocytogenes* in food.

Alternative processing technologies or non-thermal processing are now gaining more attention and are preferred as they can effectively destroy microorganisms, without compromising on the quality and storage-stability of foods. Moreover, the alternative processing technologies have

minimal process-induced changes in sensory and nutritional characteristics of the foods compared to the traditional processing technologies (Ahvenainen, 1996).

One of the non-thermal technologies includes the non-ionizing radiation - Ultraviolet radiation, which is proven to be extremely effective at reducing various pathogens. The FDA and USDA have concluded that the use of UV irradiation is safe and in 2000, the FDA approved UV-light as alternative treatment for thermal pasteurization of fresh juice products with a performance criterion of 5-log reduction in the number of the target pathogen of concern (FDA, 2000). Many studies have reported the ability of UV light to disinfect surfaces of meat products. Wong *et al.,* in 1998 evaluated the use of UV irradiation to reduce levels of *Escherichia coli* and *Salmonella* on pork skin and muscle while Kim *et al.,* 2002 studied the reduction of *Listeria monocytogenes* on chicken meat. In another study by Lyon *et al.,* in 2007, approximately 2-log reduction of *L. monocytogenes* was observed on UV treated broiler breast fillets. These studies establish the potential of UV light to be used as a post lethality treatment to control *L. monocytogenes* because of the presence of anti-listerial activity (Clifford, 1991).

Ultraviolet light involves the use of radiation from the ultraviolet region of the electromagnetic spectrum for decontamination purposes. The germicidal properties are reported in the range between 100-400nm. This range is again divided into UV-A (from 315-400nm), UV-B (from 280-315nm), UV-C (from 200-280nm), and vacuum UV range. However, the highest germicidal effect is obtained between 250 to 270nm and it decreases as the wavelength increases (Bachmann, 1975). Therefore, UV-C at wavelength of 254nm is used for disinfection of surfaces, water, and foods.

The inactivation mechanism of UV irradiation involves the photochemical damage to RNA and DNA within the microorganism, as nucleic acids are the most important absorbers of light energy between wavelengths of 240 to 280 nm (Jagger, 1967.) When the nucleic acids absorb UV light, crosslinking between the neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand occurs. This mutation impairs the formation of the hydrogen bonds to the purines bases on the opposite strand, thereby blocking the DNA transcription and replication. This mechanism compromises the cell function and eventually leads to cell death (Miller *et al.*, 1999). Although radiation is very effective in controlling food-borne pathogens in meat, it is known to generate free radicals that cause lipid peroxidation and other chemical changes that might influence the quality of meat (Hashim *et al.*, 1995). Furthermore, radiation can also induce color change in the meat depending upon irradiation dose, animal species, muscle type, pH and packaging type. It is usually observed that after radiation the light meat produces pink color whereas dark meat becomes brown or gray (Nam and Ahn, 2003). Demanding the importance of product quality by all manufacturers due to radiation.

Knowing the potential of UV radiation, research was conducted to determine the ability of UV-C light to inactivate *L. monocytogenes* grown at different temperatures (37 and 4°C) and growth phases (log and stationary), inoculated in growth media (BHI) and food matrix (bologna) and exposed at different intensities and times. Additionally, the quality attributes such as microbial shelf-life, color, and lipid oxidation due to UV radiation was assessed over a period of 8 weeks on bologna stored at 0 and 4°C under vacuum.

MATERIALS AND METHODS:

Preparation of Bacterial culture: *Listeria monocytogenes* serotype 4b strain was used for this study. The culture was started from three separate lyophilized beads representing 3 distinct

colonies by inoculating into three sterile brain heart infusion (BHI, Acumedia, MD) broth (10mL) at 37°C for 24h. The purity of the culture was confirmed by streaking on to Modified Oxford Agar (MOX) plates (MOX, Acumedia, MD) with *Listeria* supplement (Dalynn Biologicals, AB, Canada) and incubating at 37°C for 24h. Following this, an isolated and distinct colony from the MOX plates was selected and inoculated into three BHI tubes and incubated for 24h at 37°C. The resulting bacterial culture (0.1mL) was transferred and grown in three separate BHI tubes (10mL) for 24h at 37°C and repeated twice to obtain three independent bacterial populations to achieve approximately 9 $\log_{10}$ CFU/mL. These cultures were then serially diluted in 0.1% peptone water (PW; Acumedia, MI) to get a population of ~4 $\log_{10}$ CFU/mL from which 1mL was introduced in three separate BHI bottles (500 mL) and incubated at 37 and 4°C.

Sample Preparation: The log and stationary phases of *L. monocytogenes* grown at 37 and 4°C in BHI were determined from the growth curves conducted previously in chapter 3. The log and stationary phase of *L. monocytogenes* grown at 37°C reached at 12h and 18h, respectively. The log and stationary phase of *L. monocytogenes* grown at 4°C reached at 10d and 20d, respectively. Bacterial suspensions of each phase and temperature were prepared, and 25mL BHI tubes with bacterial suspension were centrifuged (Sorvall Legent RT+ Centrifuge, Thermo Scientific, Thermo Electron Corp., Germany) at 1294.3 x g for 10 min at 4°C and the supernatant was decanted. The obtained pellets were again re-suspended in 10 mL of sterile 0.1% PW followed by centrifugation for another 10 min at 1294.3 x g. Finally, the pellet was re-suspended in 10 mL of sterile PW and vortexed. Similarly, multiple suspensions were made and pooled together in a sterile 50 mL centrifuge tube. Bacterial count of each suspension was enumerated by serial dilution and spread plating 100µl on MOX plates. Thus separate suspensions were made for log and stationary phase cells at 37 and 4°C.

Excision sampling: Sampling of the product was done using a template measuring 5 x 5 sq.cm. After UV exposure, the surface of the product was excised and then transferred into a sterile sampling bag (10.16 x 15.24cm, 4oz, A&R Belley Inc., Quebec, Canada). The sample was then weighed and equivalent amount of sterile 0.1% PW was added (1:1 wt/vol), followed by homogenizing the sample in a stomacher (Seward stomacher 400 circulator, UK) for 1 min prior to further analysis.

UV chamber: The chamber measures 44.5 x 30.5 x 24 inches in dimension with a panel consisting of 10 UV bulbs emitting at 254nm (RGF Environmental systems, FL). The panel is hung in a metal-framed box with a plexi-glass sheet placed horizontally at the bottom on which the sample is placed. The intensity of the UV light is measured by adjusting the height of the sample. Low intensity is the farthest distance from the UV light while high intensity is closest to the UV light. The intensity of the UV light is measured using a UVX Radiometer (UVP, CA). The intensities were thus set at 3.45mW/sq.cm for low and 7.22mW/sq.cm for high. The UV chamber is pre-warmed by turning on the UV bulbs an hour before the start of the experiment.

UV treatment of bacterial suspension in growth media (BHI): Cells were harvested during their log and stationary phase and 1.5 mL of the cell suspension (3 mm in depth) was taken in a 3 cm sterile petriplate. All suspensions were subjected to UV radiation of 254 nm at low and high intensity for various exposure times - 0, 10, 30, 50, 70, 90 and 110s. The exposed cell suspension was serially diluted in 100µl of 0.1% PW and the appropriate dilutions were spread plated onto MOX plates. Plates were incubated for 36h at 37°C, and colonies were counted and reported as $\log_{10}$ CFU/mL.

Slicing of bologna: Commercially packed Ready-To-Eat (RTE) beef bologna was purchased and used for the study. The bologna was sliced using a sterile knife to obtain 1-inch thick slices and

were placed on sterile food trays. The sliced bologna was then allowed to dry for 15 min under a biosafety cabinet.

UV treatment of bacterial suspension on food matrix (bologna): *Listeria monocytogenes* suspensions (log and stationary phases separately) were collected in sterile spray bottles of 100 mL capacity and was used for inoculating bologna. Approximately 1 mL of the bacterial sample was spray inoculated and spread uniformly on the sliced bologna using a sterile spread stick. This was followed by an attachment time for 30 min and the bologna was then transferred from the food trays onto sterile 150 x 15 mm petriplates and exposed to UV light. Two intensities (low – 3.45 mW/sq.cm and high – 7.22 mW/sq.cm) and eleven exposures times of 0, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300s were used in the study. After UV exposure, sampling of the product was done by excision as described previously. The sample was then serially diluted in sterile PW and spread plated on MOX, incubated for 36h at 37°C, and colonies were counted and reported as $\log_{10}$ CFU/mL.

Packaging of bologna for shelf-life and quality assessments: After UV exposure the bologna was placed into nylon/PE vacuum bags (standard barrier, 20.3 x 25.4 cm, 3 mil; Prime Source Vacuum Pouches, Koch Supplies, Inc., MO) and vacuum packaged at -30kPa and 5% vacuum (Koch Ultravac, Kansas City, MO) and stored at 0 and 4°C for a period of 8 weeks. Samples were removed from the coolers weekly for analysis.

Microbiological analysis: General microbial analysis for shelf life was done on the bologna for 8 weeks. One sample from each parameter (intensity, exposure time and storage temperature) was drawn randomly and used for analysis weekly for 8 weeks. The samples were excised and directly spread plated (100µL) onto Plate Count Agar (PCA, Acumedia, MI), Tryptic Soy Agar (TSA, Acumedia, MI) with 0.6% Yeast Extract (YE, Acumedia, MI), and Violet Red Bile (VRB,

Acumedia, MI) agar for the estimation of total aerobic plate count, yeast and molds, and total coliforms, respectively. The PCA and VRB plates were incubated at 37°C for 24h while TSA+ YE was incubated at room temperature for 5 days.

Color evaluation: The surface of the UV treated bologna was tested for color change over the period of 8 weeks. The surface color was determined using a reflectance colorimeter (Konica Minolta chroma meter, model CR-300, Minolta Co. Ltd., Ramsey, NJ) using Hunter's color space system of lightness (L), redness (a) and yellowness (b). The colorimeter was calibrated and the surface color was read at 2 different locations in duplicates.

Thiobartituric acid analysis (TBARS): TBARS values were measured using the extraction procedure of Guillen and Guzman, (1998) with some modifications. Samples (10 g of meat) were homogenized with 30 mL of DW (Deionized water) for 2 min and 2 mL of homogenate was mixed with 4 mL of TCA/TBA (Trichloroacitic acid/Thiobarbituric acid) reagent [15% TCA (w/v) and 20 mM TBA] and 100 µl BHA (Butylated hydroxyanisole). Solution was heated for 15 min in boiling water, cooled for 10 min in cold water, and centrifuged at 6037.2 x g for 10 min. Supernatant was used to read the absorbance (Barnstead Turner SP 830+, Dubuque, IA) at 531 nm against a blank that contained all reagents minus the sample. The absorbance was calculated from a calibration curve prepared using 1,1,3,3- tetramethoxypropane (TEP) as a standard. The TBARS was expressed as mg of malonaldehyde (MDA) per kg of meat (mg MDA/kg).

Statistical analysis: Experiments were conducted in triplicate and data was analyzed using SAS 9.2 software (SAS Institute, Cary, N.C.). Analysis of variance, significant differences in the growth phases and temperature, intensities, exposure times and storage temperature were determined with Tukeys' LSD test at $p \leq 0.05$ using the PROC GLM procedures.

RESULTS AND DISCUSSION:

The efficacy of UV light was studied against *L. monocytogenes* at different growth phases (log and stationary) and temperatures (37 and 4°C). Different parameters such as intensity and exposure timings were evaluated to optimize UV light treatment as an intervention in the post processing environments.

Effect of UV light against *L. monocytogenes* in growth media: Survival of log and stationary *L. monocytogenes* grown at 37 and 4°C was studied. When exposed to low intensity (3.45 mW/sq.cm), significant difference ($p<0.05$) was observed at 10 and 30s exposure times between the log and stationary cells of *L. monocytogenes* irrespective of the growth temperatures (Illustration 1A and B). Reductions ($p<0.05$) were observed in the log phase cells at 10s where approximately 4 $\log_{10}$ reduction in the populations of *L. monocytogenes* grown at 37 and 4°C, respectively were observed. A 3 $\log_{10}$ reduction was observed at 37°C and at 4°C of 30s UV exposure for the stationary cells. This infers that log phase cells were more susceptible to UV radiation compared to the stationary phase cells suggesting the resistance of *L. monocytogenes* during their stationary phase or starved phase while the resistance is not encountered during their doubling (growth) period. This increases the resistance to inactivation during any food processing and also leads to increased virulence due to the activation of signal for the expression of several virulence factors (Lee *et al.*, 1995). Further UV exposures reduced ($p<0.05$) the populations of *L. monocytogenes* irrespective of phases and temperature of growth.

When *L. monocytogenes* were exposed to high intensity (7.22 mW/sq.cm) of UV radiation, significant differences ($p<0.05$) were observed between the log and stationary cells at 10, 30 and 50s of UV exposure when grown at 37°C, while at 4°C significant differences ($p<0.05$) were observed only at 10s of UV exposure (Illustration 2A and B). Substantial decline were seen in

the survival of *L. monocytogenes* as the exposure time increased, thereby reaching its detection limit irrespective of growth temperatures and phases. Though intensity did not play a significant role, it indicated as a constant fraction of the living population being inactivated during each progressive increment in exposure time (dose-response relationship). Thus the germicidal effect indicates that high intensity UV energy over a short period of time or lower intensity UV energy over a longer period of time would provide the same kill (Charles *et al.,* 1999) being an added advantage to food processors.

No significant differences ($p>0.05$) were detected comparing the growth temperatures – 37 and 4°C of *L. monocytogenes* (Illustration 3A and B, Illustration 4A and B). Though, several studies have identified that *L. monocytogenes* growing at low temperatures are differentially expressed relative to those growing at 37°C (Bayles *et al.,* 1999), the UV exposure on bacterial survival at two different temperatures rendered non-significant results in our study.

From the previous experiments, it was concluded that log phase *L. monocytogenes* showed significant kill within 10s of UV exposure irrespective of intensity. In order to visualize and determine the exact survival curve, a 10s UV exposure study was conducted at 37°C against *L. monocytogenes*. At low intensity, significant reduction ($p<0.05$) of up to 2.5-$\log_{10}$ was observed at 4s while the subsequent significant reduction ($p<0.05$) was at 8s (Illustration 5A). At high intensity, the significant reduction was observed at 2 and 4s with a decrease in population of 3.5-$\log_{10}$ and 5-$\log_{10}$ respectively (Illustration 5B). But the stationary phase *L. monocytogenes* did not show the similar trend.

The inactivation of *L. monocytogenes* by UV energy is depends on the age of the culture and intensity of the radiation. Along with these factors, the transmittance of the sample is an important factor for the effective microbicidal action of UV radiation. UV radiation cannot

penetrate into food surfaces but can be used as a surface decontaminant. Typically, it can be used for the treatment of drinking water and surface treatments; thus, transmittance and turbidity of the solution are important factors that affect the efficiency of the treatment (Waldroup *et al.,* 1993). This is a major reason for the use of UV light to reduce most pathogens in brines, recycled water, juices etc. Quintero-Ramos *et al.* (2004) observed greater than 5-log reduction of *E. coli* ATCC 25922 in apple cider using an average UV dose between 7 and 18 mJ/cm^2. *Shigella sonnei, Salmonella typhi, Streptococcus faecalis* and *Staphylococcus aureus* when suspended in water and exposed to UV light demonstrated similar susceptibility to UV light, and achieved a 3-log reduction of the microorganisms (Chang *et al.,* 1985). In brines, the *L. monocytogenes* reductions were seen in the following descending order: water, fresh brine, 5% spent brine, 35% spent brine, 55% spent brine and undiluted spent brine. In water and fresh brine *L. monocytogenes* population reduced below detection limit (1 log CFU/ml) and no significant reductions were observed in any of the spent brines (McKinney *et al.,* 2009). Parikh *et al* in 2011, found combinations of UV + 0.5% Citric acid and UV + 500-ppm Dimethyl dicarbonate to be most effective in reducing *L. monocytogenes* to undetectable levels after 45 and 60 min of treatment, respectively in chill brines. Thus suggesting the use of UV application to treat variety of liquids that maybe used in the food processing industry.

Effect of UV light against *L. monocytogenes* on food matrix: Due to its performance in reducing the survival of most pathogens, the use of UV technology as a bactericidal food safety process for surface decontamination on conveyor belts, stainless steel, fresh meats, meat surfaces etc., has been well documented. Morey *et al.,* (2010) studied the efficacy of UV on various conveyor belts and showed that bacterial counts were significantly reduced ($p<0.05$) on all belt types irrespective of UV light intensities and times of exposure. In order to minimize the entry of

L. monocytogenes into poultry further processing plants, Lyon *et al.,* (2010) exposed UV onto the surface of chicken meat and observed approximately 2-$\log_{10}$ CFU/breast reduction. Studies have been conducted on fully cooked meat surfaces but in conjunction with another post lethality treatment such as use of antimicrobials (Sommers *et al.,* 2009). In the current study, the sole use of UV radiation was examined as a surface decontamination on RTE meat against log and stationary *L. monocytogenes* cells at two different UV intensities. Significant reductions ($p<0.05$) in *L. monocytogenes* were observed from 180s irrespective of phases and intensity (Illustration 6A and B). Though the time of exposure appears to be very high it is not likely that contamination containing high levels of *L. monocytogenes* similar to that used in this study will occur in any food industry. Moreover, studies have shown that UV light is more effective at killing bacteria on smooth surfaces (Kuo *et al.,* 1997) rather than the rough surfaces which might contain bacteria hidden in pores.

Quality assessment of UV radiated meat: The color, lipid oxidation and general microbiology for shelf life was conducted on vacuum packaged meat stored for 8 weeks at 0 and 4°C. Color data indicates decrease ($p<0.05$) in the redness (a) of the meat from ~16 – 13 (Table 3A and B, Table 4A and B) which correlates with an increase ($p<0.05$) in the lightness (L) of the meat from ~53 – 55 (Table 1A and B, Table 2A and B). The change in the color of the meat is directly proportional to the UV absorbed, which might be due to the formation of heme pigments and curing ingredients - metmyoglobin and nitrosylhemochrome respectively (Brewer, 2004). This change was only observed after 210s after UV exposure and was not visually observed. The increase in the lightness of the meat after UV radiation is due to the breakdown of the chemical bonds causing the fading of the color which is referred to as the bleaching effect (Nassau, 2001). The dose of radiation did not have any impact ($p>0.05$) on the thiobarbituric acid reactive

substances (TBARS) value thus indicating no formation of free radicals due to lipid oxidation. The shelf life of UV exposed and non-exposed bologna remained unaffected after 8 weeks of storage under vacuum as results showed that bacterial and yeast and mold counts were below 1-$\log_{10}$ CFU/sq. cm for all the samples throughout the study irrespective of the treatment. The UV treatment, additives and preservatives in the meat and vacuum packaging are the major factors that might have contributed towards the intact shelf life of the bologna.

Based on the results, it can be concluded that UV radiation can be used as a potential post lethality treatment in the further processing industry. This treatment is a potential alternative to many antimicrobials such as chlorine, organic acids as UV is cheaper and does not leave residues on foods or surfaces. Moreover, UV is easy to install, can operate under any food processing environmental conditions; is environmental friendly and can effectively and quickly mitigate *L. monocytogenes*. Thus this non-thermal technology can be included either in Alternative 1 or 2 in the *L. monocytogenes* sanitation programs where they can either be used solely or in combination with other post lethality treatments to enhance pathogen control in the food industry.

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LIST OF ILLUSTRATIONS

No significant difference ($p<0.05$) was observed in stationary *L. monocytogenes* counts between the temperatures at a particular exposure time.

Illustration 4A: Effect of high intensity UV exposure on survival of log *L. monocytogenes* grown at 37°C and 4°C.

No significant difference ($p<0.05$) was observed in log *L. monocytogenes* counts between the temperatures at a particular exposure time.

Illustration 4B: Effect of high intensity UV exposure on survival of stationary *L. monocytogenes* grown at 37°C and 4°C.

No significant difference ($p<0.05$) was observed in log *L. monocytogenes* counts between the temperatures at a particular exposure time.

Illustration 5A: Effect of low intensity uv exposure (10s) on survival of log and stationary phase *L. monocytogenes* at 37°C.

The letters (a, b, c, d) indicates difference ($p<0.05$) in log *L. monocytogenes* counts compared to control at a particular exposure time.

Illustration 5B: Effect of high intensity UV exposure (10s) on survival of log and stationary phase *L. monocytogenes* at 37°C.

The letters (a, b, c) indicates difference ($p<0.05$) in stationary *L. monocytogenes* counts compared to control at a particular exposure time.

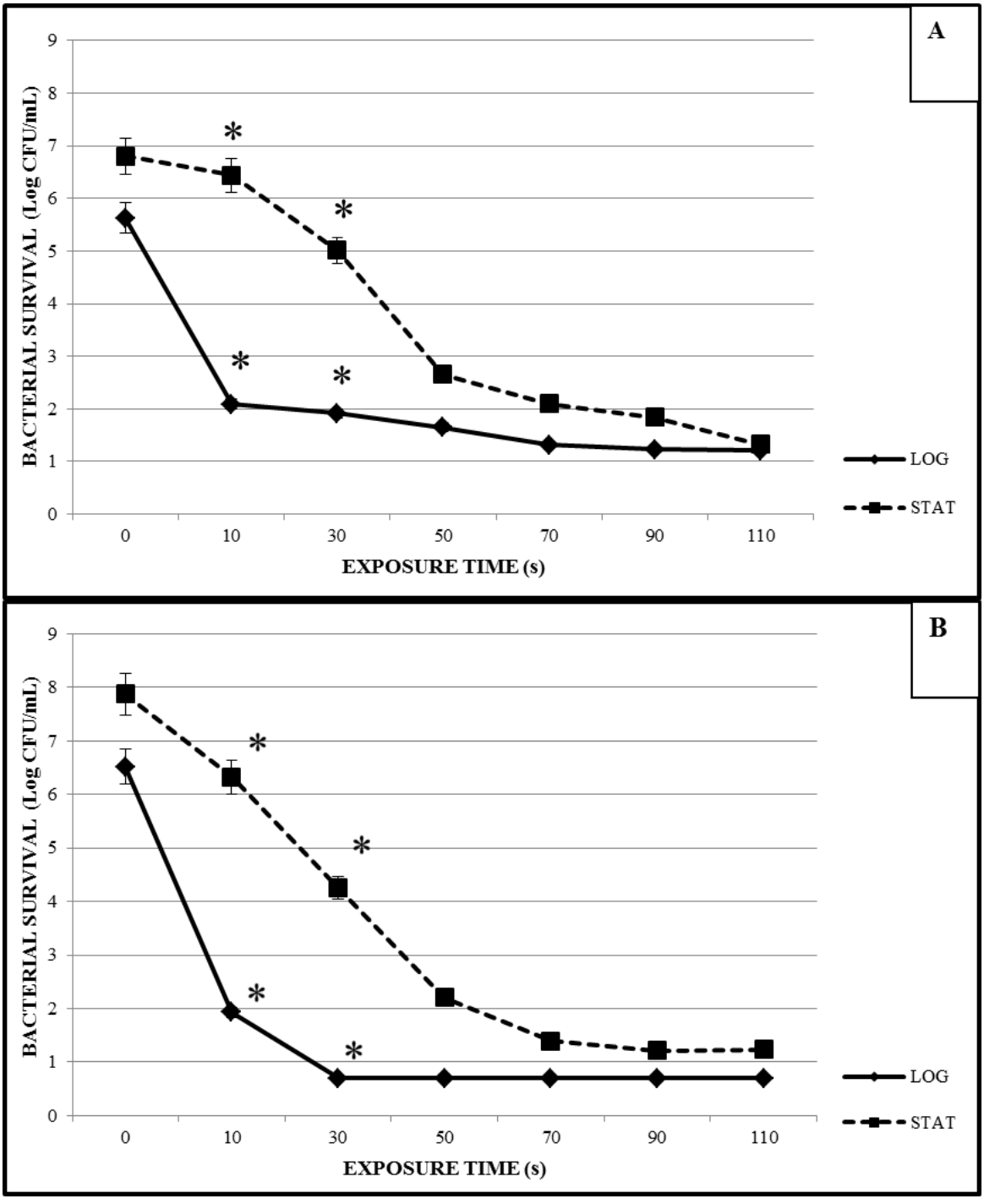
Illustration 6A: Effect of low intensity UV exposure on survival of *L. monocytogenes* on bologna.

The letters (a, b) indicate difference ($p<0.05$) in log and stationary

Illustration 6B: Effect of high intensity UV exposure on survival of *L. monocytogenes* on bologna.

The letters (a, b) indicate difference ($p<0.05$) in log and stationary compared to control at a particular exposure time.

ILLUSTRATION 1



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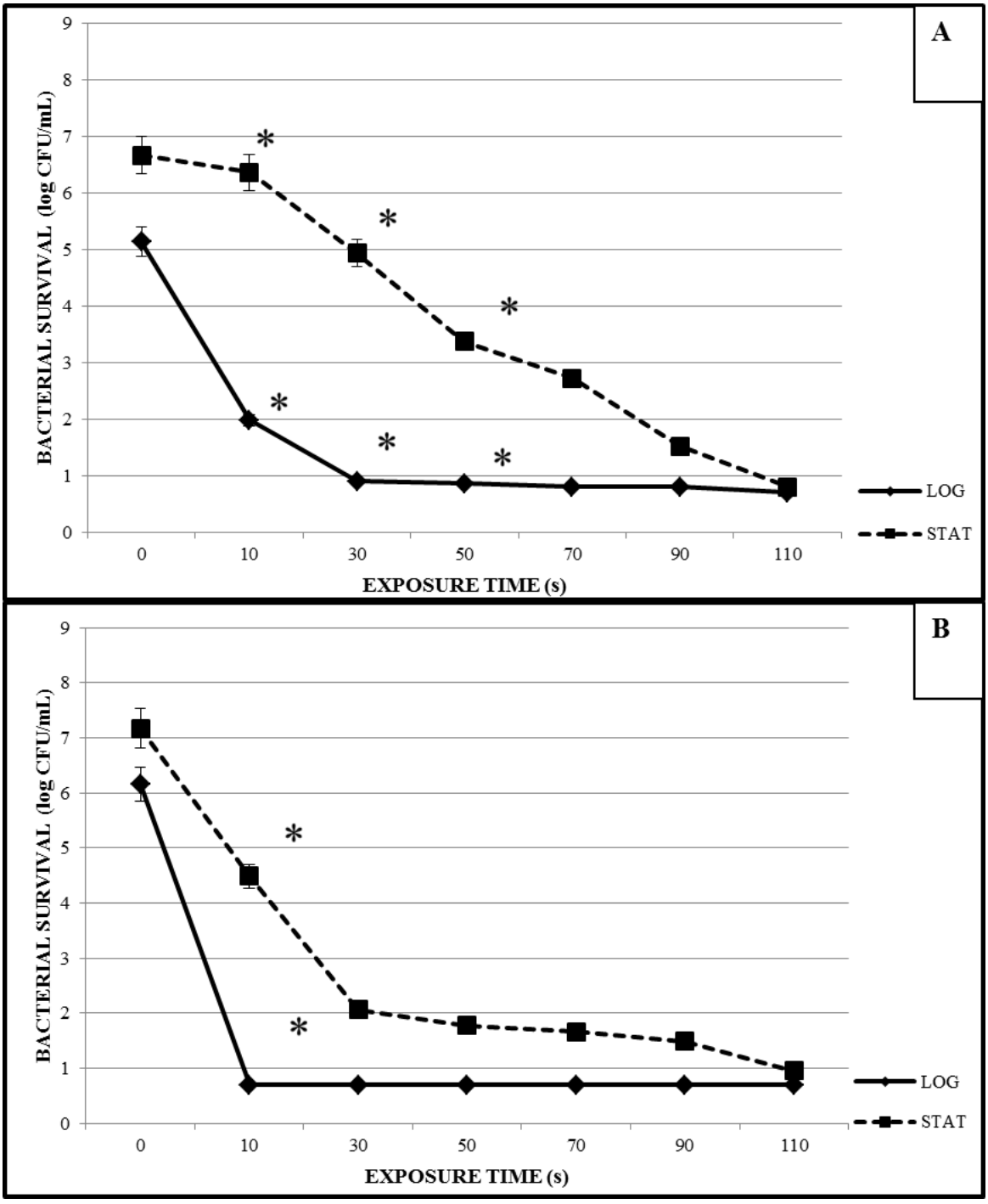

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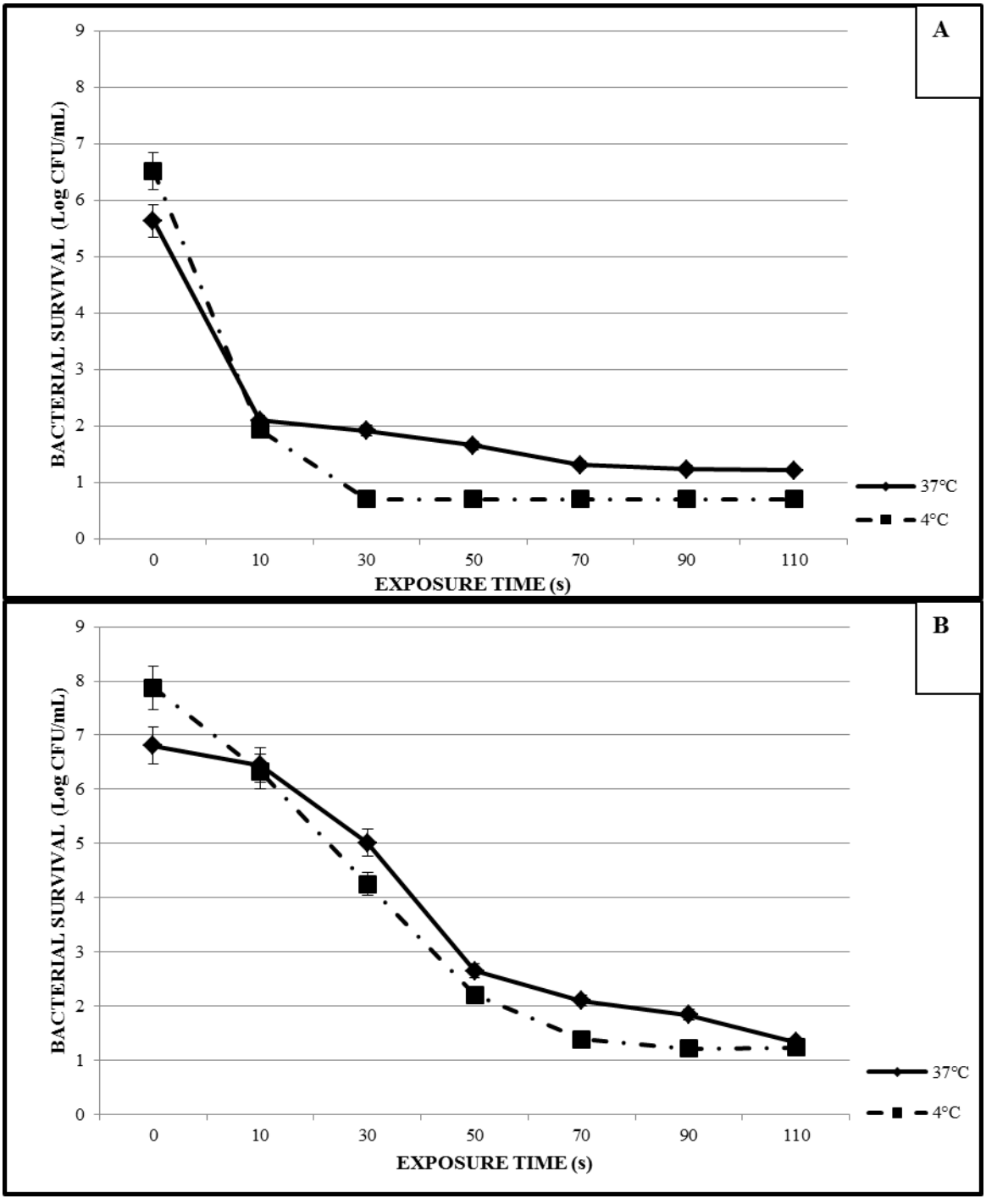

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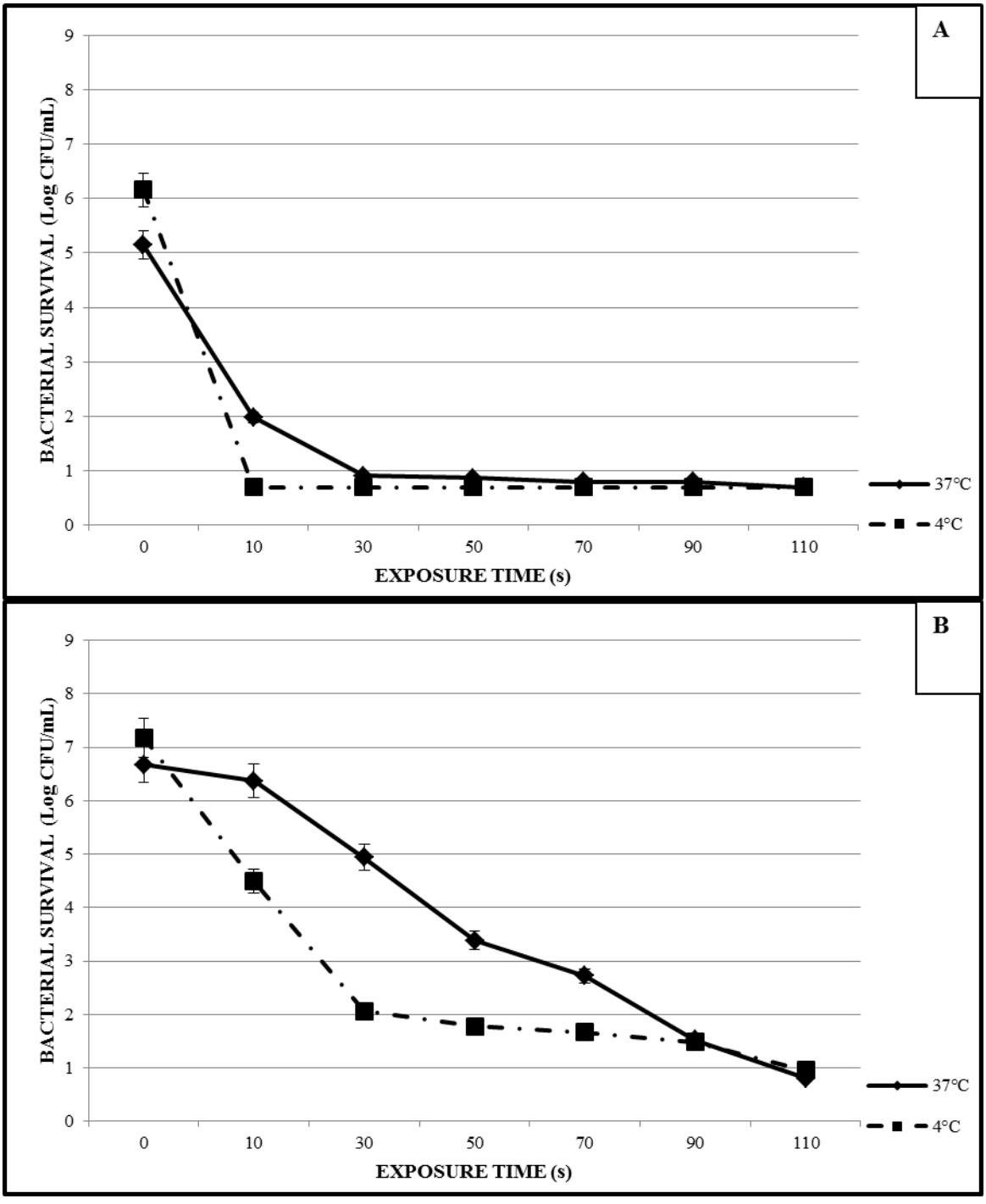

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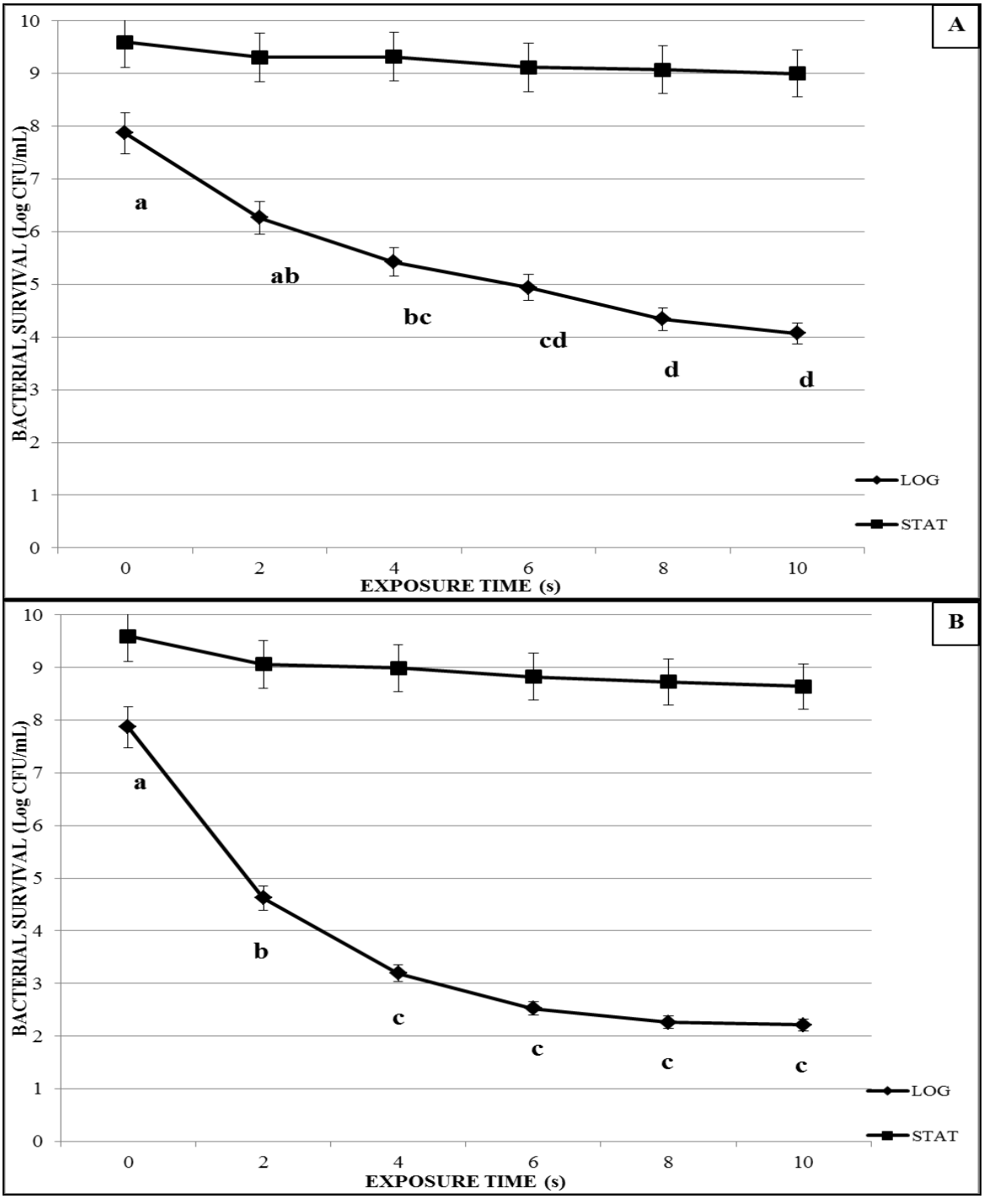

ILLUSTRATION 6

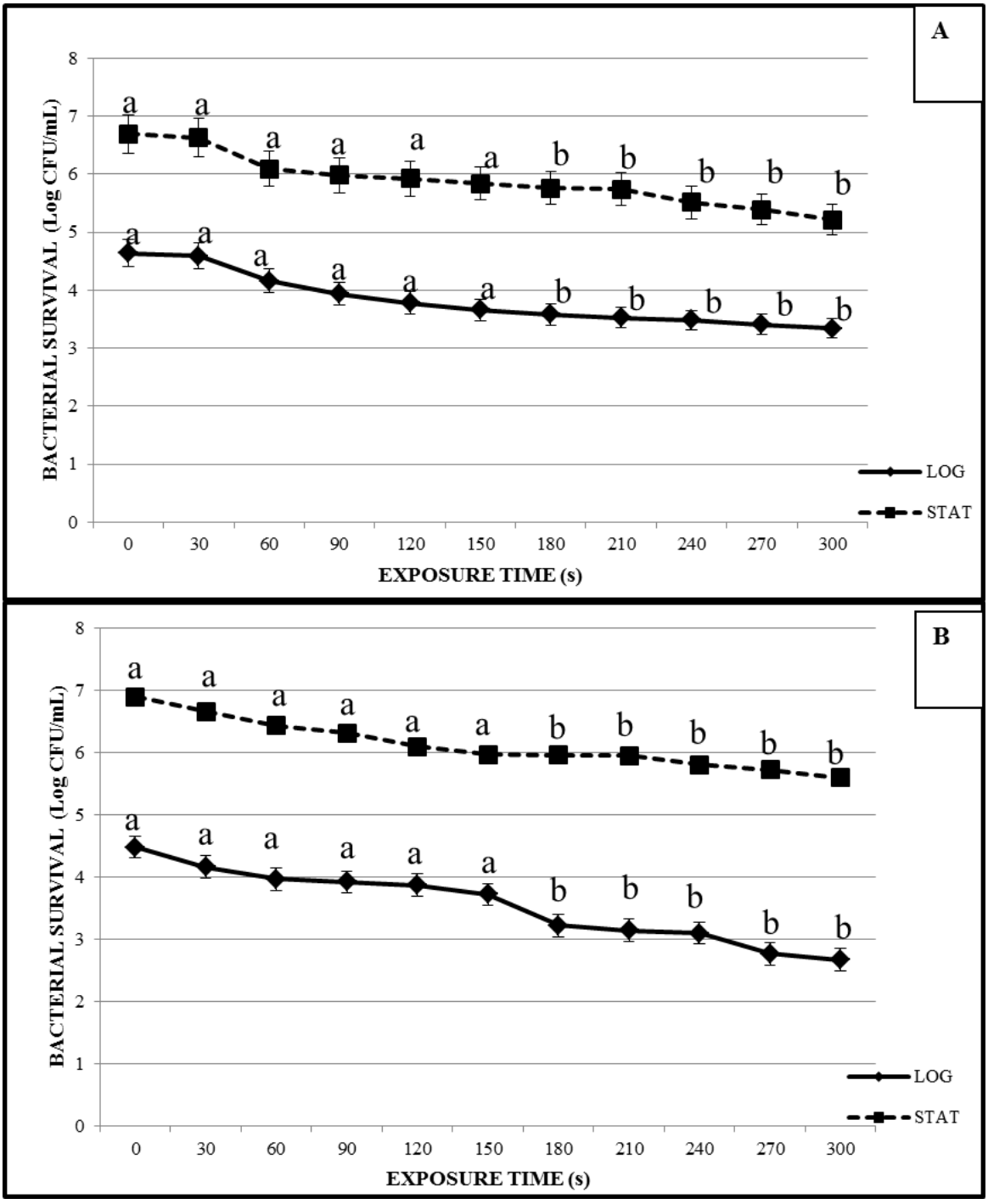

Table 1A: Lightness **(**L-value; mean ± std. error) of bologna exposed to low intensity (3.45 mW/ sq. cm) UV light and stored at 0°C.

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	50.85± 1.04^{x}	53.37 ± 1.48^{x}	54.32 ± 0.38^{x}	54.34 ± 0.48^{x}	55.33 ± 0.98^{x}	54.50 ± 0.71^{x}	55.60 ± 0.91^{x}	54.93 ± 0.88^{x}	55.55 ± 1.24^{x}
30	51.87 ± 1.03^{x}	53.82 ± 1.67^{x}	55.19 ± 0.58^{x}	54.71 ± 1.21^{x}	54.85 ± 1.13^{x}	55.21 ± 0.60^{x}	54.66 ± 0.60^{x}	55.09 ± 0.95^{x}	55.66 ± 1.19^{x}
60	50.96 ± 1.37^{x}	53.97 ± 1.61^{x}	56.04 ± 0.28^{x}	55.40 ± 1.43^{x}	55.33 ± 1.56^{x}	54.57 ± 1.23^{x}	53.34 ± 1.38^{x}	55.18 ± 1.12^{x}	55.82 ± 1.30^{x}
90	52.63 ± 0.45^{x}	54.22 ± 0.89^{x}	55.34 ± 0.32^{x}	55.63 ± 0.68^{x}	55.66 ± 0.65^{x}	55.61 ± 1.03^{x}	55.44 ± 1.19^{x}	56.48 ± 1.75^{x}	55.91 ± 0.89^{x}
120	51.09 ± 0.32^{x}	53.42 ± 0.15^{x}	54.04 ± 0.77^{x}	54.31 ± 1.00^{x}	54.18 ± 0.79^{x}	54.37 ± 0.85^{x}	54.41 ± 0.59^{x}	54.92 ± 1.91^{x}	54.88 ± 1.17^{x}
150	50.53 ± 1.04^{x}	54.28 ± 1.71^{x}	55.17 ± 1.23^{x}	55.31 ± 0.55^{x}	54.46 ± 1.10^{x}	55.24 ± 1.15^{x}	55.23 ± 0.96^{x}	55.50 ± 0.72^{x}	54.97 ± 1.33^{x}
180	51.41 ± 0.19^{x}	53.54 ± 1.67^{x}	55.45 ± 0.89^{x}	55.52 ± 1.12^{x}	55.28 ± 0.64^{x}	54.83 ± 0.73^{x}	54.91 ± 0.67^{x}	55.57 ± 0.86^{x}	56.18 ± 1.35^{x}
210	50.75 ± 2.24^{x}	53.87 ± 1.80^{x}	53.52 ± 0.83^{x}	55.42 ± 1.05^{x}	54.42 ± 1.30^{x}	53.33 ± 0.49^{x}	55.85 ± 1.23^{x}	55.67 ± 0.91^{x}	56.20 ± 1.67^{x}
240	50.89 ± 0.24^{x}	54.47 ± 0.89^{x}	54.70 ± 1.13^{x}	55.10 ± 0.98^{x}	55.89 ± 0.54^{x}	55.54 ± 0.19^{x}	56.04 ± 1.15^{x}	55.63 ± 1.03^{x}	55.41 ± 0.72^{x}
270	50.93 ± 0.28^{x}	54.79 ± 0.78^{x}	54.62 ± 0.15^{x}	55.35 ± 0.92^{x}	55.30 ± 0.56^{x}	55.34 ± 0.88^{x}	55.63 ± 0.60^{x}	55.08 ± 0.65^{x}	55.69 ± 1.53^{x}
300	49.78 ± 0.85^{x}	53.40 ± 0.86^{x}	52.49 ± 0.65^{y}	52.39 ± 0.67^{y}	53.54 ± 0.58^{y}	53.05 ± 0.53^{y}	53.00 ± 0.26^{y}	53.19 ± 0.83^{y}	53.99 ± 0.15^{y}

$^{x, y}$ indicates difference ($p \leq 0.05$) in L-value compared to control within each week.

Table 1B: Lightness **(**L-value; mean ± std. error) of bologna exposed to low intensity (3.45 mW/ sq. cm) UV light and stored at 4°C.

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	51.56 ± 0.42^{x}	53.85 ± 0.97^{x}	55.19 ± 2.09^{x}	54.94 ± 1.37^{x}	54.94 ± 1.48^{x}	54.76 ± 0.74^{x}	55.31 ± 1.41^{x}	55.06 ± 1.71^{x}	55.55 ± 1.57^{x}
30	52.51 ± 0.94^{x}	52.70 ± 3.13^{x}	55.06 ± 0.70^{x}	55.07 ± 0.56^{x}	54.73 ± 0.53^{x}	54.95 ± 0.89^{x}	54.91 ± 1.12^{x}	55.29 ± 0.53^{x}	55.98 ± 0.53^{x}
60	50.56 ± 1.30^{x}	53.31 ± 1.28^{x}	54.85 ± 0.73^{x}	54.10 ± 0.66^{x}	53.89 ± 0.62^{x}	54.14 ± 0.10^{x}	54.81 ± 1.13^{x}	55.13 ± 0.78^{x}	54.17 ± 0.41^{x}
90	52.47 ± 0.36^{x}	54.07 ± 0.40^{x}	55.24 ± 0.78^{x}	55.13 ± 1.08^{x}	55.56 ± 1.03^{x}	54.83 ± 1.10^{x}	55.35 ± 1.28^{x}	56.14 ± 1.39^{x}	55.78 ± 1.12^{x}
120	51.34 ± 1.25^{x}	52.50 ± 0.66^{x}	54.85 ± 0.79^{x}	54.40 ± 1.24^{x}	53.09 ± 0.40^{x}	53.19 ± 0.37^{x}	53.93 ± 1.35^{x}	53.42 ± 0.21^{x}	54.27 ± 1.00^{x}
150	51.70 ± 1.16^{x}	54.10 ± 1.39^{x}	55.04 ± 1.67^{x}	55.86 ± 1.96^{x}	55.85 ± 1.78^{x}	55.62 ± 1.71^{x}	56.04 ± 1.96^{x}	56.10 ± 1,64^{x}	56.33 ± 1.84^{x}
180	50.36 ± 0.58^{x}	54.00 ± 0.75^{x}	54.80 ± 0.49^{x}	55.07 ± 0.91^{x}	54.94 ± 0.67^{x}	56.06 ± 0.28^{x}	55.52 ± 0.56^{x}	55.99 ± 0.84^{x}	56.25 ± 0.69^{x}
210	51.64 ± 0.37^{x}	54.05 ± 1.11^{x}	54.51 ± 0.26^{x}	54.79 ± 0.82^{x}	54.88 ± 0.74^{x}	54.99 ± 0.63^{x}	55.30 ± 0.38^{x}	55.43 ± 0.40^{x}	55.48 ± 0.39^{x}
240	51.19 ± 0.70^{x}	54.05 ± 0.88^{x}	54.52 ± 1.02^{x}	55.14 ± 0.47^{x}	54.55 ± 1.28^{x}	55.31 ± 0.73^{x}	54.47 ± 1.33^{x}	55.53 ± 0.32^{x}	55.51 ± 0.93^{x}
270	51.27 ± 0.97^{x}	54.03 ± 0.94^{x}	54.57 ± 1.41^{x}	54.73 ± 1.55^{x}	54.33 ± 1.06^{x}	55.02 ± 1.42^{x}	54.77 ± 1.51^{x}	54.61 ± 1.14^{x}	54.92 ± 1.59^{x}
300	51.09 ± 0.41^{x}	53.89 ± 1.41^{x}	54.20 ± 1.97^{x}	53.77 ± 0.79^{x}	55.18 ± 1.89^{x}	54.72 ± 0.41^{x}	54.78 ± 2.17^{x}	55.26 ± 0.43^{x}	54.28 ± 1.17^{x}

$^{x, y}$ indicates difference ($p \leq 0.05$) in L-value compared to control within each week.

Table 2A: Lightness **(**L-value; mean ± std. error) of bologna exposed to high intensity (7.22mW/ sq. cm) UV light and stored at 0°C.

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	49.84 ± 0.89^{x}	52.94 ± 1.17^{x}	54.30 ± 1.06^{x}	53.36 ± 0.17^{x}	54.41 ± 1.00^{x}	54.63 ± 2.31^{x}	53.50 ± 0.63^{x}	53.86 ± 0.53^{x}	54.32 ± 0.47^{x}
30	52.42 ± 1.24^{x}	54.86 ± 0.54^{x}	54.49 ± 1.36^{x}	56.15 ± 0.71^{x}	54.34 ± 1.81^{x}	54.31 ± 1.44^{x}	54.67 ± 0.86^{x}	55.33 ± 1.08^{x}	56.49 ± 0.56^{x}
60	50.35 ± 1.91^{x}	53.72 ± 1.27^{x}	53.98 ± 1.89^{x}	55.56 ± 0.46^{x}	54.83 ± 0.69^{x}	54.34 ± 1.15^{x}	54.88 ± 0.86^{x}	55.26 ± 0.85^{x}	55.43 ± 0.56^{x}
90	52.61 ± 0.64^{x}	54.26 ± 0.76^{x}	55.14 ± 1.36^{x}	55.57 ± 0.67^{x}	55.56 ± 1.19^{x}	55.72 ± 1.36^{x}	55.84 ± 1.13^{x}	55.81 ± 1.12^{x}	56.26 ± 0.79^{x}
120	50.90 ± 0.73^{x}	52.71 ± 1.24^{x}	53.49 ± 1.48^{x}	54.17 ± 0.43^{x}	54.66 ± 0.80^{x}	54.78 ± 0.26^{x}	54.69 ± 0.83^{x}	54.35 ± 1.06^{x}	55.47 ± 0.76^{x}
150	51.05 ± 1.12^{x}	54.32 ± 1.10^{x}	54.73 ± 1.67^{x}	54.66 ± 0.75^{x}	55.44 ± 1.07^{x}	54.87 ± 0.77^{x}	55.68 ± 0.10^{x}	55.46 ± 0.58^{x}	56.09 ± 0.90^{x}
180	51.07 ± 0.10^{x}	53.56 ± 0.80^{x}	54.32 ± 1.58^{x}	56.07 ± 1.00^{x}	56.17 ± 1.15^{x}	55.11 ± 0.81^{x}	54.95 ± 0.74^{x}	55.85 ± 1.25^{x}	56.78 ± 0.78^{x}
210	51.42 ± 0.71^{x}	55.05 ± 0.91^{x}	55.34 ± 1.11^{x}	56.21 ± 0.66^{x}	55.37 ± 1.71^{x}	55.94 ± 1.32^{x}	55.92 ± 1.47^{x}	56.41 ± 1.32^{x}	56.29 ± 1.14^{x}
240	51.56 ± 0.35^{x}	54.32 ± 1.25^{x}	54.77 ± 1.43^{x}	55.80 ± 0.85^{x}	55.54 ± 0.81^{x}	54.88 ± 0.95^{x}	55.20 ± 1.05^{x}	56.29 ± 0.89^{x}	56.44 ± 0.88^{x}
270	50.05 ± 0.64^{x}	54.74 ± 0.64^{x}	54.63 ± 0.81^{x}	56.16 ± 0.53^{x}	56.26 ± 0.36^{x}	55.63 ± 0.89^{x}	55.30 ± 0.65^{x}	56.29 ± 1.10^{x}	55.98 ± 0.86^{x}
300	49.70 ± 0.48^{x}	53.20 ± 0.88^{x}	52.96 ± 1.34^{x}	53.10 ± 1.18^{x}	53.86 ± 0.67^{x}	52.98 ± 0.42^{x}	53.12 ± 1.09^{x}	53.56 ± 0.37^{x}	54.33 ± 0.64^{x}

$^{x, y}$ indicates difference ($p \leq 0.05$) in L-value compared to control within each week.

Table 2B: Lightness **(**L-value; mean ± std. error) of bologna exposed to high intensity (7.22mW/ sq. cm) UV light and stored at 4°C.

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	50.22 ± 2.59^{x}	53.34 ± 1.74^{x}	54.42 ± 2.62^{x}	55.12 ± 2.19^{x}	54.12 ± 1.79^{x}	54.57 ± 2.13^{x}	53.16 ± 1.51^{x}	53.20 ± 1.09^{x}	53.30 ± 1.39^{x}
30	51.56 ± 1.40^{x}	52.92 ± 1.26^{x}	54.56 ± 0.10^{x}	54.88 ± 0.29^{x}	54.70 ± 0.25^{x}	54.61 ± 0.55^{x}	55.21 ± 0.31^{x}	54.15 ± 0.49^{x}	54.55 ± 0.51^{x}
60	50.96 ± 0.74^{x}	52.47 ± 1.01^{x}	54.57 ± 0.68^{x}	54.42 ± 1.42^{x}	54.18 ± 0.60^{x}	54.40 ± 0.78^{x}	54.58 ± 0.70^{x}	54.72 ± 1.15^{x}	54.96 ± 0.88^{x}
90	51.52 ± 0.80^{x}	54.37 ± 0.66^{x}	54.74 ± 0.47^{x}	55.41 ± 1.12^{x}	55.80 ± 1.20^{x}	55.33 ± 1.10^{x}	55.34 ± 0.88^{x}	54.37 ± 0.94^{x}	54.47 ± 1.05^{x}
120	50.85 ± 1.08^{x}	53.86 ± 1.28^{x}	53.47 ± 0.19^{x}	53.93 ± 1.61^{x}	53.75 ± 1.33^{x}	54.74 ± 0.48^{x}	52.84 ± 0.65^{x}	55.46 ± 0.83^{y}	55.92 ± 1.11^{y}
150	51.86 ± 1.35^{x}	53.61 ± 1.16^{x}	54.66 ± 1.36^{x}	55.00 ± 2.09^{x}	57.19 ± 1.44^{x}	55.36 ± 0.88^{x}	56.18 ± 1.32^{x}	55.26 ± 1.19^{y}	56.82 ± 1.34^{y}
180	51.10 ± 0.68^{x}	53.50 ± 0.54^{x}	54.58 ± 0.97^{x}	55.38 ± 1.10^{x}	53.27 ± 0.50^{x}	54.65 ± 1.52^{x}	55.87 ± 0.86^{x}	55.85 ± 1.05^{y}	55.32 ± 1.35^{y}
210	51.24 ± 0.26^{x}	54.06 ± 0.72^{x}	55.17 ± 0.46^{x}	55.27 ± 0.42^{x}	54.75 ± 0.49^{x}	54.69 ± 1.07^{x}	55.93 ± 1.02^{x}	55.23 ± 1.06^{y}	55.35 ± 0.34^{y}
240	50.71 ± 0.62^{x}	53.48 ± 1.64^{x}	54.56 ± 1.57^{x}	55.07 ± 0.52^{x}	54.96 ± 0.58^{x}	55.36 ± 0.79^{x}	54.74 ± 1.89^{x}	55.56 ± 0.71^{y}	55.53 ± 0.74^{y}
270	51.12 ± 1.15^{x}	53.17 ± 0.95^{x}	54.96 ± 0.71^{x}	55.32 ± 1.31^{x}	54.53 ± 1.55^{x}	54.50 ± 1.10^{x}	55.18 ± 1.42^{x}	55.81 ± 0.67^{y}	55.31 ± 1.48^{y}
300	51.63 ± 1.61^{x}	54.28 ± 0.88^{x}	53.39 ± 1.14^{x}	55.68 ± 0.62^{x}	54.42 ± 1.55^{x}	55.15 ± 2.18^{x}	54.66 ± 1.14^{x}	55.54 ± 0.23^{y}	55.02 ± 1.54^{y}

$^{x, y}$ indicates difference ($p \leq 0.05$) in L-value compared to control within each week.

Table 3A: Redness **(**a-value; mean ± std. error) of bologna exposed to low intensity (3.45 mW/ sq. cm) UV light and stored at 0°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	16.34 ± 0.49^{x}	16.07 ± 0.85^{x}	15.99 ± 1.66^{x}	16.28 ± 0.20^{x}	15.63 ± 0.28^{x}	15.72 ± 0.19^{x}	15.22 ± 0.49^{x}	15.12 ± 0.23^{x}	14.42 ± 0.13^{x}
30	16.06 ± 0.68^{x}	16.14 ± 0.63^{x}	15.96 ± 0.54^{x}	15.99 ± 0.79^{x}	15.48 ± 0.42^{x}	15.02 ± 0.30^{x}	15.13 ± 0.66^{x}	14.60 ± 0.34^{x}	14.10 ± 0.90^{x}
60	16.18 ± 0.79^{x}	16.10 ± 0.26^{x}	15.55 ± 0.35^{x}	15.56 ± 0.36^{x}	15.13 ± 0.71^{x}	15.18 ± 0.16^{x}	14.85 ± 0.13^{x}	14.29 ± 0.21^{x}	14.57 ± 0.56^{x}
90	16.61 ± 0.49 ^{x}	16.51 ± 0.31^{x}	15.62 ± 0.49^{x}	15.59 ± 0.45^{x}	15.10 ± 0.62^{x}	14.25 ± 0.78^{y}	14.94 ± 0.88^{x}	14.81 ± 1.35^{x}	14.25 ± 1.27^{x}
120	16.63 ± 1.04^{x}	16.02 ± 0.10^{x}	15.32 ± 0.37^{x}	15.65 ± 0.35^{x}	15.36 ± 0.41^{x}	14.97 ± 0.38^{xy}	14.47 ± 0.73^{x}	14.01 ± 0.90^{x}	14.07 ± 0.46^{x}
150	16.32 ± 0.67^{x}	15.60 ± 0.40^{y}	15.60 ± 0.65^{x}	15.63 ± 0.16^{x}	14.80 ± 0.57^{x}	14.39 ± 0.29^{y}	14.55 ± 0.43^{x}	14.84 ± 0.37^{x}	14.10 ± 0.66^{x}
180	16.07 ± 0.49^{x}	15.80 ± 0.17^{y}	15.57 ± 0.22^{x}	15.39 ± 0.14^{x}	15.02 ± 0.23^{x}	14.76 ± 0.09^{y}	14.58 ± 0.09^{x}	14.70 ± 0.68^{x}	14.09 ± 0.72^{x}
210	15.96 ± 0.73^{y}	15.21 ± 0.38^{y}	15.87 ± 0.18^{x}	15.36 ± 0.78^{x}	15.01 ± 0.75^{x}	14.88 ± 0.45^{xy}	13.53 ± 0.34^{y}	13.62 ± 1.05^{y}	13.87 ± 1.04^{y}
240	15.99 ± 0.40^{y}	15.37 ± 0.19^{y}	15.45 ± 0.28^{x}	15.27 ± 0.48^{y}	14.25 ± 0.47^{y}	14.49 ± 0.49^{y}	13.73 ± 1.15^{y}	13.56 ± 0.72^{y}	13.06 ± 0.47^{y}
270	15.41 ± 0.61^{y}	15.08 ± 0.44^{y}	15.20 ± 0.15^{y}	15.09 ± 0.30^{y}	14.26 ± 0.55^{y}	14.08 ± 0.71^{y}	13.62 ± 0.33^{y}	13.69 ± 0.84^{y}	13.01 ± 0.50^{y}
300	15.06 ± 0.51^{y}	15.08 ± 0.60^{y}	15.28 ± 0.60^{y}	15.05 ± 0.75^{y}	14.03 ± 0.17^{y}	14.03 ± 0.37^{y}	13.86 ± 1.50^{y}	13.55 ± 0.35^{y}	13.61 ± 0.46^{y}

$^{x, y}$ indicates difference ($p \leq 0.05$) in a-value compared to control within each week.

Table 3B: Redness **(**a-value; mean ± std. error) of bologna exposed to low intensity (3.45 mW/ sq. cm) UV light and stored at 4°C.

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	16.17 ± 0.44^{x}	16.53 ± 0.67^{x}	15.46 ± 1.58^{x}	16.36 ± 1.18^{x}	16.67 ± 0.59^{x}	16.71 ± 0.35^{x}	16.26 ± 0.75^{x}	16.71 ± 0.83^{x}	16.59 ± 0.57^{x}
30	15.79 ± 0.38^{x}	16.02 ± 0.38^{x}	15.86 ± 0.25^{x}	15.87 ± 0.37^{x}	16.03 ± 0.38^{x}	16.20 ± 0.48^{x}	16.04 ± 0.39^{x}	16.19 ± 0.24^{x}	16.05 ± 0.16^{x}
60	15.38 ± 0.74^{x}	16.11 ± 0.25^{x}	15.14 ± 0.43^{x}	16.49 ± 0.28^{x}	16.25 ± 0.13^{x}	16.35 ± 0.19^{x}	16.06 ± 0.43^{x}	16.12 ± 0.46^{x}	16.40 ± 0.10^{x}
90	15.72 ± 0.29^{x}	15.88 ± 0.80^{x}	15.96 ± 0.66^{x}	15.89 ± 0.16^{x}	16.92 ± 0.53^{x}	16.09 ± 0.53^{x}	15.92 ± 0.59^{x}	15.80 ± 0.69^{x}	15.93 ± 0.53^{x}
120	15.45 ± 0.20^{x}	15.78 ± 0.59^{x}	15.96 ± 0.66^{x}	15.99 ± 0.68^{x}	16.50 ± 1.76^{x}	16.50 ± 0.17^{x}	16.23 ± 0.75^{x}	16.23 ± 0.38^{x}	16.09 ± 0.49^{x}
150	14.89 ± 0.64^{x}	15.51 ± 0.97^{x}	15.53 ± 0.62^{x}	15.64 ± 0.72^{x}	15.39 ± 0.81^{x}	16.60 ± 0.74^{x}	15.51 ± 1.04^{x}	15.46 ± 0.65^{x}	16.57 ± 0.51^{x}
180	15.45 ± 0.37^{x}	15.85 ± 1.46^{x}	15.89 ± 0.19^{x}	15.44 ± 0.03^{x}	15.69 ± 0.58^{x}	16.48 ± 0.13^{x}	15.10 ± 0.57^{x}	16.39 ± 0.21^{x}	16.30 ± 0.10^{x}
210	15.52 ± 0.35^{x}	15.82 ± 0.33^{x}	15.80 ± 0.22^{x}	15.83 ± 0.34^{x}	15.83 ± 0.33^{x}	15.99 ± 0.20^{x}	15.63 ± 0.21^{x}	15.72 ± 0.23^{x}	15.83 ± 0.10^{x}
240	14.38 ± 0.31^{y}	15.21 ± 0.16^{y}	15.40 ± 0.26^{x}	15.49 ± 0.10^{x}	15.76 ± 0.19^{x}	15.21 ± 0.45^{y}	15.58 ± 0.31^{x}	15.23 ± 0.18^{y}	15.35 ± 0.10^{y}
270	14.41 ± 0.40^{y}	15.24 ± 0.58^{y}	15.58 ± 0.19^{x}	15.44 ± 0.31^{x}	15.66 ± 0.31^{y}	15.62 ± 0.49^{y}	15.70 ± 0.37^{x}	15.60 ± 0.26^{y}	15.45 ± 0.49^{y}
300	14.83 ± 0.49^{y}	15.46 ± 0.51^{y}	15.15 ± 1.13^{x}	14.00 ± 1.25^{y}	14.58 ± 0.68^{y}	14.37 ± 1.71^{y}	13.88 ± 2.28^{y}	15.37 ± 0.12^{y}	15.54 ± 0.49^{y}

$^{x, y}$ indicates difference ($p \leq 0.05$) in a-value compared to control within each week.

Table 4A: Redness **(**a-value; mean ± std. error) of bologna exposed to high intensity (7.22 mW/ sq. cm) UV light and stored at 0°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	16.80 ± 0.64^{x}	16.88 ± 1.14^{x}	16.06 ± 1.43^{x}	16.90 ± 0.24^{x}	16.00 ± 0.58^{x}	16.02 ± 0.87^{x}	15.89 ± 0.44^{x}	15.59 ± 0.57^{x}	15.67 ± 0.59^{x}
30	16.69 ± 0.22^{x}	15.76 ± 0.21^{x}	15.87 ± 0.46^{x}	16.21 ± 0.06^{x}	15.55 ± 0.36^{x}	15.44 ± 0.32^{x}	14.60 ± 0.78^{x}	13.96 ± 0.43^{y}	13.35 ± 0.77^{y}
60	16.84 ± 0.66^{x}	15.92 ± 0.09^{x}	15.66 ± 0.33^{x}	16.05 ± 0.11^{x}	14.73 ± 0.36^{x}	14.39 ± 0.11^{x}	13.94 ± 0.76^{x}	13.37 ± 0.59^{y}	13.46 ± 0.26^{y}
90	16.44 ± 0.36^{x}	15.43 ± 0.79^{x}	15.25 ± 1.12^{x}	16.07 ± 1.03^{x}	14.50 ± 0.80^{x}	14.74 ± 0.81^{x}	13.50 ± 1.24^{x}	13.49 ± 1.35^{y}	12.80 ± 1.14^{y}
120	16.04 ± 0.44^{x}	15.10 ± 0.51^{x}	15.48 ± 0.50^{x}	16.12 ± 0.63^{x}	14.73 ± 0.40^{x}	14.10 ± 0.20^{x}	13.80 ± 0.82^{xy}	13.14 ± 1.23^{y}	13.10 ± 0.45^{y}
150	14.45 ± 0.31^{y}	15.03 ± 1.14^{y}	14.86 ± 0.38^{y}	14.77 ± 0.97^{y}	14.23 ± 0.26^{xy}	13.85 ± 0.71^{xy}	13.39 ± 0.21^{xy}	13.06 ± 0.78^{y}	12.56 ± 0.53^{y}
180	14.75 ± 0.07^{y}	15.27 ± 0.11^{y}	14.72 ± 0.31^{y}	14.93 ± 0.35^{y}	14.11 ± 0.38^{y}	14.22 ± 0.41^{xy}	13.91 ± 0.69^{xy}	12.93 ± 0.27^{y}	12.72 ± 0.76^{y}
210	14.93 ± 0.80^{y}	14.60 ± 0.35^{y}	14.63 ± 0.63^{y}	14.40 ± 0.38^{y}	13.86 ± 0.96^{y}	13.30 ± 1.24^{y}	13.00 ± 1.12^{y}	12.42 ± 1.29^{y}	12.43 ± 1.27^{y}
240	13.88 ± 0.15^{y}	14.74 ± 0.47^{y}	14.49 ± 0.66^{y}	14.43 ± 0.31^{y}	13.87 ± 0.47^{y}	13.98 ± 0.82^{y}	13.38 ± 0.81^{y}	12.54 ± 0.81^{y}	12.58 ± 1.12^{y}
270	13.62 ± 0.37^{yz}	14.51 ± 0.22^{y}	14.44 ± 0.35^{y}	13.95 ± 0.23^{y}	13.22 ± 0.68^{y}	13.66 ± 1.01^{y}	13.42 ± 0.74^{y}	12.46 ± 0.92^{y}	12.44 ± 0.99^{y}
300	13.03 ± 0.45^{yz}	14.84 ± 0.36^{y}	14.89 ± 0.25^{y}	14.49 ± 0.51^{y}	13.67 ± 0.44^{y}	13.97 ± 0.47^{y}	13.30 ± 1.10^{y}	12.97 ± 0.14^{y}	12.07 ± 0.98^{y}

$^{x, y}$ indicates difference ($p \leq 0.05$) in a-value compared to control within each week.

Table 4B: Redness **(**a-value; mean ± std. error) of bologna exposed to high intensity (7.22 mW/ sq. cm) UV light and stored at 4°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	15.98 ± 0.59^{x}	16.73 ± 0.67^{x}	16.94 ± 3.75^{x}	16.58 ± 0.77^{x}	16.84 ± 0.83^{x}	16.73 ± 0.57^{x}	16.31 ± 1.84^{x}	17.14 ± 0.46^{x}	17.26 ± 0.46^{x}
30	16.24 ± 0.32^{x}	16.07 ± 0.27^{x}	16.32 ± 0.23^{x}	16.23 ± 0.12^{x}	16.26 ± 0.09^{x}	16.16 ± 0.27^{x}	16.23 ± 0.12^{x}	16.96 ± 0.26^{x}	16.34 ± 0.20^{x}
60	15.03 ± 0.79^{x}	16.08 ± 0.33^{x}	15.96 ± 0.32^{x}	15.97 ± 0.63^{x}	16.23 ± 0.38^{x}	16.04 ± 0.21^{x}	15.82 ± 0.29^{x}	16.04 ± 0.55^{x}	15.96 ± 0.33^{xy}
90	15.39 ± 0.87^{x}	15.35 ± 0.64^{xy}	15.48 ± 0.86^{x}	15.59 ± 0.62^{x}	15.21 ± 0.30^{x}	15.51 ± 0.57^{xy}	15.62 ± 0.45^{x}	15.55 ± 0.54^{xy}	15.48 ± 0.59^{xy}
120	14.98 ± 0.57^{x}	15.58 ± 0.79^{xy}	15.87 ± 0.48^{x}	16.09 ± 0.43^{x}	15.70 ± 0.31^{x}	15.61 ± 0.47^{xy}	15.95 ± 0.09^{x}	15.35 ± 0.52^{xy}	15.44 ± 0.29^{xy}
150	14.36 ± 0.66^{xy}	14.92 ± 0.98^{xy}	15.51 ± 0.33^{xy}	14.74 ± 0.86^{y}	14.68 ± 0.63^{y}	15.06 ± 0.57^{y}	15.06 ± 0.59^{xy}	15.31 ± 0.53^{xy}	15.11 ± 1.00^{xy}
180	14.86 ± 0.41^{xy}	15.09 ± 0.43^{xy}	15.36 ± 0.60^{xy}	14.78 ± 0.21^{y}	14.67 ± 0.17^{y}	15.25 ± 0.79^{y}	15.11 ± 0.41^{xy}	15.65 ± 0.43^{xy}	14.74 ± 1.06^{xy}
210	14.78 ± 0.63^{xy}	15.30 ± 0.19^{xy}	14.75 ± 0.56^{xy}	14.97 ± 0.08^{y}	14.61 ± 0.40^{y}	15.23 ± 0.30^{y}	14.80 ± 0.53^{xy}	15.14 ± 0.27^{xy}	15.19 ± 0.48^{xy}
240	13.69 ± 0.23^{y}	14.42 ± 0.33^{y}	14.51 ± 0.88^{y}	14.45 ± 0.19^{y}	14.74 ± 0.11^{y}	14.89 ± 0.12^{y}	14.98 ± 0.19^{xy}	14.66 ± 0.04^{y}	14.63 ± 0.17^{y}
270	13.88 ± 0.31^{y}	14.79 ± 0.44^{y}	14.82 ± 0.36^{y}	14.17 ± 1.29^{y}	14.61 ± 0.61^{y}	14.97 ± 0.32^{y}	14.06 ± 1.98^{y}	14.57 ± 0.12^{y}	14.27 ± 1.41^{y}
300	13.01 ± 0.62^{y}	14.29 ± 0.27^{y}	14.14 ± 0.61^{y}	14.14 ± 2.15^{y}	14.53 ± 1.72^{y}	14.70 ± 0.96^{y}	14.03 ± 0.49^{y}	14.26 ± 0.39^{y}	14.25 ± 0.62^{y}

$^{x, y}$ indicates difference ($p \leq 0.05$) in a-value compared to control within each week.

Table 5A: Yellowness **(**b-value; mean ± std. error) of bologna exposed to low intensity (3.45 mW/ sq. cm) UV light and stored at 0°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	9.63 ± 0.41	9.67 ± 0.13	10.12 ± 0.51	9.65 ± 0.08	9.64 ± 0.26	9.66 ± 0.24	9.82 ± 0.34	9.77 ± 0.01	9.88 ± 0.21
30	9.85 ± 0.35	9.84 ± 0.43	9.70 ± 0.29	9.77 ± 0.34	9.60 ± 0.28	9.78 ± 0.07	9.71 ± 0.40	9.67 ± 0.25	9.91 ± 0.35
60	9.95 ± 0.35	9.78 ± 0.13	9.91 ± 0.04	9.82 ± 0.13	9.68 ± 0.26	9.62 ± 0.20	9.85 ± 0.43	9.85 ± 0.15	10.05 ± 0.30
90	10.19 ± 0.12	10.09 ± 0.20	10.15 ± 0.13	10.16 ± 0.16	9.91 ± 0.36	9.94 ± 0.26	10.06 ± 0.27	10.46 ± 0.13	10.29 ± 0.52
120	9.82 ± 0.21	9.85 ± 0.36	9.84 ± 0.23	10.06 ± 0.33	9.78 ± 0.33	10.03 ± 0.33	9.94 ± 0.32	10.52 ± 0.02	10.17 ± 0.35
150	9.80 ± 0.29	10.15 ± 0.65	10.05 ± 0.51	10.13 ± 0.56	10.00 ± 0.58	10.15 ± 0.63	10.20 ± 0.68	10.27 ± 0.48	10.03 ± 0.10
180	9.95 ± 0.31	9.74 ± 0.12	9.97 ± 0.25	10.14 ± 0.36	9.69 ± 0.05	9.75 ± 0.10	9.75 ± 0.23	10.05 ± 0.04	10.19 ± 0.38
210	10.20 ± 0.34	10.03 ± 0.19	9.91 ± 0.09	10.09 ± 0.08	9.96 ± 0.13	9.76 ± 0.14	10.13 ± 0.15	10.13 ± 0.27	10.20 ± 0.18
240	9.93 ± 0.40	9.96 ± 0.14	10.04 ± 0.27	10.14 ± 0.36	10.24 ± 0.24	10.20 ± 0.13	10.31 ± 0.17	10.32 ± 0.43	10.26 ± 0.06
270	10.09 ± 0.11	10.18 ± 0.08	10.31 ± 0.29	10.13 ± 0.10	10.29 ± 0.03	10.20 ± 0.09	10.30 ± 0.21	10.36 ± 0.05	10.38 ± 0.23
300	9.55 ± 0.73	9.86 ± 0.40	9.93 ± 0.34	9.84 ± 0.25	9.80 ± 0.25	9.77 ± 0.20	10.38 ± 0.08	10.06 ± 0.24	10.08 ± 0.42

$^{x, y}$ indicates difference ($p \leq 0.05$) in b-value compared to control within each week.

Table 5B: Yellowness **(**b-value; mean ± std. error) of bologna exposed to low intensity (3.45 mW/ sq. cm) UV light and stored at 4°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	9.86 ± 0.27	9.85 ± 0.11	10.08 ± 0.35	9.64 ± 0.32	9.63 ± 0.21	9.65 ± 0.27	9.77 ± 0.61	9.77 ± 0.33	9.71 ± 0.28
30	10.02 ± 0.14	9.97 ± 0.19	9.91 ± 0.09	9.98 ± 0.11	9.93 ± 0.24	9.85 ± 0.15	9.96 ± 0.16	10.16 ± 0.24	9.98 ± 0.19
60	9.98 ± 0.31	9.94 ± 0.15	10.09 ± 0.10	9.85 ± 0.27	9.95 ± 0.11	9.88 ± 0.31	9.95 ± 0.16	10.13 ± 0.05	10.17 ± 0.06
90	10.07 ± 0.12	9.81 ± 0.21	9.91 ± 0.20	9.96 ± 0.14	9.87 ± 0.26	9.80 ± 0.28	9.90 ± 0.33	10.07 ± 0.29	10.04 ± 0.28
120	9.99 ± 0.26	9.72 ± 0.19	9.96 ± 0.29	9.85 ± 0.34	10.06 ± 0.19	9.93 ± 0.32	10.03 ± 0.38	10.11 ± 0.26	10.20 ± 0.35
150	10.01 ± 0.33	9.88 ± 0.23	10.34 ± 0.50	9.86 ± 0.21	10.21 ± 0.65	9.96 ± 0.21	9.96 ± 0.25	10.30 ± 0.43	10.20 ± 0.25
180	9.84 ± 0.38	10.09 ± 0.21	10.01 ± 0.25	10.26 ± 0.42	9.94 ± 0.23	10.17 ± 0.16	10.36 ± 0.33	10.31 ± 0.27	10.42 ± 0.12
210	10.03 ± 0.18	9.74 ± 0.14	9.84 ± 0.13	9.77 ± 0.10	9.79 ± 0.27	9.69 ± 0.14	9.88 ± 0.25	9.91 ± 0.11	9.96 ± 0.08
240	10.21 ± 0.49	10.16 ± 0.15	10.00 ± 0.26	10.25 ± 0.21	9.99 ± 0.22	10.26 ± 0.13	10.20 ± 0.33	10.39 ± 0.10	10.35 ± 0.11
270	10.31 ± 0.04	9.89 ± 0.07	9.98 ± 0.22	9.98 ± 0.24	9.93 ± 0.22	9.90 ± 0.27	10.00 ± 0.36	10.22 ± 0.26	10.30 ± 0.15
300	10.14 ± 0.26	10.05 ± 0.43	10.24 ± 0.48	10.34 ± 0.17	10.18 ± 0.44	10.43 ± 0.19	10.33 ± 0.15	10.52 ± 0.16	10.23 ± 0.58

$^{x, y}$ indicates difference ($p \leq 0.05$) in b-value compared to control within each week.

Table 6A: Yellowness **(**b-value; mean ± std. error) of bologna exposed to high intensity (7.22 mW/ sq.cm) UV light and stored at 0°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	9.44 ± 0.58	9.79 ± 0.30	9.58 ± 0.36	9.44 ± 0.39	9.41 ± 0.30	9.60 ± 0.37	9.53 ± 0.32	9.65 ± 0.40	9.78 ± 0.10
30	10.03 ± 0.11	9.97 ± 0.03	9.70 ± 0.17	9.89 ± 0.10	9.65 ± 0.25	9.61 ± 0.17	9.98 ± 0.56	9.96 ± 0.25	10.12 ± 0.10
60	10.05 ± 0.35	10.05 ± 0.18	9.97 ± 0.08	10.26 ± 0.13	10.01 ± 0.25	10.03 ± 0.17	10.11 ± 0.22	10.29 ± 0.14	10.14 ± 0.17
90	10.29 ± 0.05	10.18 ± 0.56	10.08 ± 0.20	10.15 ± 0.29	10.00 ± 0.44	10.13 ± 0.27	10.22 ± 0.36	10.46 ± 0.36	10.51 ± 0.06
120	10.07 ± 0.43	10.18 ± 0.24	10.01 ± 0.25	10.03 ± 0.31	10.18 ± 0.30	10.14 ± 0.42	9.90 ± 0.16	10.18 ± 0.32	10.57 ± 0.10
150	10.09 ± 0.38	10.31 ± 0.78	10.21 ± 0.70	10.30 ± 0.82	10.21 ± 0.57	10.27 ± 0.12	10.43 ± 0.57	10.35 ± 0.10	10.60 ± 0.56
180	9.98 ± 0.28	10.01 ± 0.06	10.10 ± 0.17	10.24 ± 0.29	10.23 ± 0.34	10.05 ± 0.06	10.08 ± 0.15	10.29 ± 0.19	10.39 ± 0.24
210	10.35 ± 0.18	10.28 ± 0.14	10.27 ± 0.17	10.31 ± 0.17	10.26 ± 0.18	10.40 ± 0.19	10.34 ± 0.29	10.60 ± 0.19	10.60 ± 0.19
240	10.24 ± 0.38	10.31 ± 0.32	10.37 ± 0.25	10.46 ± 0.13	10.46 ± 0.18	10.36 ± 0.45	10.32 ± 0.38	10.76 ± 0.24	10.90 ± 0.22
270	10.17 ± 0.20	10.47 ± 0.01	10.48 ± 0.29	10.53 ± 0.10	10.45 ± 0.03	10.50 ± 0.14	10.63 ± 0.10	10.80 ± 0.09	10.80 ± 0.17
300	10.04 ± 0.34	10.30 ± 0.29	10.07 ± 0.44	10.29 ± 0.13	10.17 ± 0.22	10.07 ± 0.10	10.34 ± 0.14	10.35 ± 0.37	10.38 ± 0.23

$^{x, y}$ indicates difference ($p \leq 0.05$) in b-value compared to control within each week.

Table 6B: Yellowness **(**b-value; mean ± std. error) of bologna exposed to high intensity (7.22 mW/ sq.cm) UV light and stored at 4°C

Exposure Time (s)	Storage Period (Weeks)								
	0	1	2	3	4	5	6	7	8
0	9.43 ± 0.80	9.58 ± 0.40	10.06 ± 0.81	9.65 ± 0.25	9.56 ± 0.20	9.43 ± 0.36	9.50 ± 0.27	9.54 ± 0.39	9.48 ± 0.33
30	9.91 ± 0.40	9.86 ± 0.21	9.83 ± 0.36	9.82 ± 0.31	9.79 ± 0.49	9.79 ± 0.31	9.92 ± 0.32	9.98 ± 0.27	10.04 ± 0.43
60	10.11 ± 0.24	9.74 ± 0.25	9.86 ± 0.28	9.92 ± 0.34	9.77 ± 0.30	9.86 ± 0.12	10.03 ± 0.29	10.08 ± 0.30	10.14 ± 0.17
90	10.47 ± 0.08	10.09 ± 0.38	10.14 ± 0.29	10.07 ± 0.23	10.26 ± 0.10	10.13 ± 0.39	10.10 ± 0.23	10.37 ± 0.46	10.16 ± 0.32
120	10.14 ± 0.30	10.05 ± 0.24	9.96 ± 0.27	10.13 ± 0.15	10.15 ± 0.48	10.01 ± 0.22	10.40 ± 0.36	10.07 ± 0.24	10.26 ± 0.28
150	10.39 ± 0.05	10.41 ± 0.68	10.20 ± 0.28	10.52 ± 0.68	10.19 ± 0.11	10.50 ± 0.52	10.40 ± 0.15	10.39 ± 0.11	10.25 ± 0.34
180	10.09 ± 0.32	10.35 ± 0.21	10.14 ± 0.38	10.37 ± 0.26	9.92 ± 0.16	10.17 ± 0.38	10.45 ± 0.26	10.56 ± 0.24	10.37 ± 0.67
210	10.17 ± 0.22	9.92 ± 0.09	10.25 ± 0.26	10.06 ± 0.16	9.99 ± 0.08	10.07 ± 0.05	10.32 ± 0.35	10.33 ± 0.01	10.27 ± 0.20
240	10.27 ± 0.43	10.38 ± 0.26	10.30 ± 0.44	10.65 ± 0.21	10.45 ± 0.23	10.48 ± 0.11	10.42 ± 0.36	10.70 ± 0.19	10.66 ± 0.18
270	10.25 ± 0.37	10.20 ± 0.28	10.40 ± 0.08	10.34 ± 0.33	10.25 ± 0.34	10.30 ± 0.28	10.56 ± 0.44	10.45 ± 0.11	10.43 ± 0.37
300	10.56 ± 0.43	10.67 ± 0.15	10.40 ± 0.44	10.76 ± 0.21	10.61 ± 0.33	10.45 ± 0.45	10.53 ± 0.49	10.30 ± 0.37	10.63 ± 0.57

$^{x, y}$ indicates difference ($p \leq 0.05$) in b-value compared to control within each week.

CHAPTER 5

CONCLUSIONS AND FURTHER RESEARCH

CONCLUSIONS:

Listeria monocytogenes is a major safety concern for Ready to Eat (RTE) meat and poultry products. *L. monocytogenes* contamination is a hazard that can potentially occur after post-lethality treatment in a processing environment during slicing or packaging of RTE meat products. Based on the experiments conducted in this study the following conclusions can be drawn:

1. The calculation of growth rate and generation time of cold adapted and non-adapted *L. monocytogenes* in Brain Heart Infusion and meat extract aids as a preliminary step to prevent spoilage and safety problem in refrigerated RTE foods along with a better understanding about *L. monocytogenes* growth physiology.

    a. Growth patterns were indicated by their respective growth rate (μ) and the generation time (g) per day

    b. Growth rate constant of *L. monocytogenes* grown at 37°C in BHI was μ = 0.77 per hour and the time taken for 1 generation was g= 54.54 minutes.

    c. Growth patterns of cold adapted and non-cold adapted *L. monocytogenes* in BHI at 4°C was 0.86 and 24h and μ = 0.81 and g= 29h 28m, respectively. The significant difference ($P<0.05$) being observed from day 4 between the adapted versus the non-adapted *L. monocytogenes*.

d. For the adapted and non-adapted *L. monocytogenes* grown in meat extract, the growth constant of adapted strain (μ=0.26) was observed to be significantly higher ($P<0.05$) than the non-adapted strain (μ=0.21) with generation time of 2.5d and 3.2d, respectively.

e. This inferred the versatility of the adapted bacteria to grow more efficiently at 4°C irrespective of the media in which they grow.

2. Post lethality treatments are available to the industry to suppress growth of *L. monocytogenes.* One such intervention strategy is the use of Ultraviolet radiation, which can either be used as a sole intervention or in combination with other interventions against *L. monocytogenes* in ensuring safety to RTE meat and poultry products with minimal quality changes.

    a. Effect of low intensity (3.45 mW/sq.cm) UV light against *L. monocytogenes* in growth media grown at 37 and 4°C indicated significant difference ($p<0.05$) at 10 and 30s between the log and stationary cells of *L. monocytogenes* irrespective of the growth temperatures. Approximately 3 – 4 log reduction of *L. monocytogenes* was observed.

    b. When exposed to high intensity (7.22 mW/sq.cm) of UV energy, significant differences ($p<0.05$) were observed between the log and stationary cells at 10, 30 and 50s when grown at 37°C while at 4°C they were significantly different at 10s.

    c. No significant differences ($p>0.05$) were observed between the growth temperatures – 37 and 4°C of *L. monocytogenes*.

d. Reduction in log phase *L. monocytogenes* was observed under 10s with significant ($p<0.05$) reduction at 4s irrespective of the intensities.

e. Significant reductions ($p<0.05$) were observed from 180s against *L. monocytogenes* irrespective of phases and intensity, when UV light was used as a surface decontamination on bologna.

f. After UV exposure, color changes ($p<0.05$) were observed in the lightness (l) and redness (a) of the RTE meat but no changes were observed in the lipid oxidation as well as their shelf life over a period of 8 weeks.

g. Ultraviolet radiation can either be used as "Alternative 1 or 2" to suppress or limit the growth of *L. monocytogenes* in RTE products.

FURTHER RESEARCH:

As UV treatment is one of the least exploited antimicrobial treatments in the further processing environment more understanding and further exploration is required to prove its efficiency on reduction of pathogens.

- Construction of D-value death curves will be useful to determine the appropriate time of UV exposure required to mitigate *L. monocytogenes* in the food industry.
- To perform in-plant experiments by optimizing the movement of conveyor belts to determine the actual efficiency of UV light on RTE products.
- Using UV light on other types of RTE foods such as cheeses, produce and fresh meat.
- Study *L. monocytogenes* dark-repair mechanism after UV exposure.
- Study the difference at a molecular level to understand its point mutation and difference between the UV exposed and non-exposed *L. monocytogenes*.