EFFECT OF ULTRAVIOLET LIGHT AGAINST SPOILAGE BACTERIA AND *LISTERIA MONOCYTOGENES* ON READY-TO-EAT BOLOGNA

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Abstract – *Listeria monocytogenes* is an important foodborne pathogen and a serious public health issue due to its severity of infection and high fatality rate. Efficacy of ultraviolet (UV) light was evaluated at various intensities and exposure times against *L. monocytogenes* on bologna and its impact on spoilage bacteria was determined. *Listeria monocytogenes* in log and stationary phase were subjected to low (3-4 mW/ sq. cm) and high (7-8 mW/ sq. cm) intensity of UV light in laboratory media and surface inoculated bologna for up to 300 s. Cells were recovered on Modified Oxford agar (MOX) after 24h of incubation at 37°C and microbiological shelf life was assessed over a period of 8 weeks on bologna stored at 0 and 4°C. Populations of *L. monocytogenes* were reduced ($p<0.05$) after 180s of UV exposure irrespective of UV intensities. Higher ($p<0.05$) reductions were observed in log phase as compared to stationary phase cells. Reduction in log and stationary cells of *L. monocytogenes* without affecting quality attributes suggests the potential use of UV light as a post process intervention in food processing plants.

Key Words – *Listeria monocytogenes,* Post-process recontamination, Ready-To-Eat meat

- INTRODUCTION

Listeria monocytogenes is widely distributed in the environment and frequently found in plants, soil, animal, water, dirt, dust, and silage (Farber and Peterkin, 1991). It's presence in slaughter animals and in raw meat and poultry can be introduced into the processing environment thereby cross-contaminating food contact surfaces, equipment, floors, drains, standing water, and employees (Fenlon and others, 1996). *L. monocytogenes* can establish a niche in damp environments and form biofilms that are difficult to eliminate during cleaning and sanitation (Giovannacci *et al.,* 1999).

Listeria monocytogenes contributes significantly to foodborne illnesses and deaths in the U.S. (Scallan *et al.,* 2011) with an estimated cost of illnesses of about $2.04 billion in 2010 (Schraff, 2012). These incidences are due to consumption of *Listeria*-contaminated foods, which include any Ready-to eat (RTE) meat, cheese, milk, produce etc. The FSIS established a "zero tolerance" for *L. monocytogenes* in RTE meat and poultry products, thereby declaring RTE products positive for *L. monocytogenes* as "adulterated" under Federal Meat Inspection Act or Poultry Products Inspection Act (FSIS, 2011). The Code of Federal Regulations (CFR, 2010) states "establishments must comply with one of three alternatives to provide intervention if a RTE product is produced and exposed post-lethality to processing environment." Alternative 1 refers to use of post-lethality treatment that reduces/ eliminates microorganisms on the product and an antimicrobial agent or process that suppresses growth of *L. monocytogenes*. Alternative 2 involves either post-lethality treatment or an antimicrobial agent or process that suppresses growth. Alternatives 1 or 2 are used in conjunction with a sanitation program that addresses testing of food contact surfaces. For Alternative 3, an establishment can choose to use sanitation as their intervention.

Effectiveness of decontamination technologies such as high pressure processing, and pre/post-package surface pasteurization have been studied as possible strategies for controlling *L. monocytogenes* in RTE meat and poultry products. Other common approaches include formulation of products with antimicrobials such as lactates and diacetates, and organic acids

to control *L. monocytogenes* in RTE meat products. To ensure safety of food products while maintaining its' quality, alternative treatments like non-thermal processing are being currently explored for lowering foodborne pathogen level.

One of the emerging non-thermal technologies is Ultraviolet (UV) light to mitigate *L. monocytogenes* on RTE meats. The FDA and USDA have concluded that the use of UV irradiation is safe and use of UV energy in the food industry has several advantages over chemical disinfections as it leaves no residue, does not moisten or alter temperature of treated materials, rapid, and cost 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 for food application (Wolfe, 1990). However, the efficacy and specific characteristics of UV light sources used for water treatment have not been evaluated for food applications.

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*. Moreover, an ideal technology would be one that has no impact on quality and shelf life of the product during storage. This study aims to explore the potential of UV light against *L. monocytogenes* and its impact on microbiological shelf life.

- MATERIALS AND METHODS

Preparation of Bacterial culture and inoculum:

Listeria monocytogenes serotype 4b strain was used for this study. The purity of the culture was confirmed by streaking on to Modified Oxford Agar (MOX) with *Listeria* supplement and incubated at 37°C for 24h. The resulting bacterial culture (0.1mL) was transferred and grown in three separate BHI tubes (10mL) for 24h at 37°C to obtain three independent bacterial populations to achieve approximately 9 $\log_{10}$ CFU/mL. Cultures were then serially diluted in 0.1% peptone water (PW) to get a population of ~4 $\log_{10}$ CFU/mL from which 1mL was introduced in 3 separate BHI broths and incubated at 37 and 4°C. Log and stationary phase of *L. monocytogenes* grown at 37°C was reached at 12 and 18h, respectively, whereas the log and stationary phase of *L. monocytogenes* grown at 4°C was reached at 10 and 20d, respectively. Bacterial suspensions of each phase and temperature were centrifuged at 1294.3 x g for 10 min at 4°C and the supernatant was decanted. Bacterial count of the suspension was enumerated by serial dilution and spread plating 100µl on MOX plates. Separate suspensions were made for log and stationary phase cells at 37 and 4°C.

Excision sampling:

Sampling of bologna was done using a template measuring 5 by 5 sq.cm. After UV exposure, surface of the product was excised and transferred into a sterile sampling bag (4 x 6 inches). 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 for 1 min prior to further analysis.

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 sterile petriplate. Suspensions were subjected to UV radiation of 254 nm at low and high intensity for 0, 10, 30, 50, 70, 90 and 110s. The exposed cell

suspension was serially diluted in 100µl of 0.1% PW and appropriate dilutions were spread plated onto MOX plates; incubated for 36h at 37°C, and colonies were counted and reported as $\log_{10}$ CFU/mL.

Slicing and UV treatment of bologna:

Beef bologna was purchased and used for the study. 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. *Listeria monocytogenes* suspensions (log and stationary phases separately) were collected in sterile spray bottles and 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 bologna was then transferred from food trays onto sterile petriplates and exposed to UV light. Two intensities (low- 3.45 mW/sq.cm and high- 7.22 mW/sq.cm) and 11 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. The samples were 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/ sq. cm.

Packaging of bologna:

After UV exposure bologna was placed into nylon/PE vacuum bags (standard barrier, 20.3 x 25.4 cm, 3 mil) and vacuum packaged at -30kPa and 5% vacuum and stored at 0 and 4°C for 8 weeks. Samples were analyzed weekly.

Microbiological shelf life analysis:

Microbial analysis for shelf life was done on bologna for 8 weeks. Sample from each parameter (intensity, exposure time and storage temperature) was drawn randomly and used for analysis weekly for 8 weeks. Samples were excised and spread plated (100µL) onto PCA, TSA+0.6% YE, and VRB agar for 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 d.

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 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

Effect of UV light against L. monocytogenes in laboratory media:
When exposed to low intensity (3.45 mW/sq.cm), significant difference ($p<0.05$) was observed at 10 and 30s exposure between the log and stationary cells of *L. monocytogenes* irrespective of growth temperatures. Reductions ($p<0.05$) were observed in log phase cells at 10s where approximately 4 and 3 $\log_{10}$ reduction in populations of *L. monocytogenes* grown at 37 and 4°C, respectively were observed. A 2-$\log_{10}$ reduction was observed at 37°C and a 4-$\log_{10}$ reduction at 4°C at 30s UV exposure time was observed for stationary cells. Thus, log phase cells were more susceptible to UV radiation compared to stationary phase cells suggesting the resistance of *L. monocytogenes* during their stationary or starved phase. This increases the resistance to inactivation during 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 ($p<0.05$) 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. 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. From previous experiments, it was concluded that log phase *L. monocytogenes* were significantly reduced within 10s of UV exposure irrespective of intensity. In order to 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 subsequent significant reduction ($p<0.05$) was at 8s. At high intensity, significant reduction was observed at 2 and 4s with a decrease in population of 3.5 and 5-$\log_{10}$, respectively. However, stationary phase *L. monocytogenes* did not show similar trends.

Effect of UV light against L. monocytogenes on bologna:

In the current study, the sole use of UV radiation was examined as a surface decontamination on bologna against log and stationary *L. monocytogenes* cells at two different UV intensities. Significant reductions ($p<0.05$) in *L. monocytogenes* were observed after from 180 s of exposure irrespective of phases and intensity. Though time of exposure appears to be high it is unlikely that high levels of *L. monocytogenes* similar to that used in this study will occur in commercial plants. 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. Morey *et al.,* 2010 studied the efficacy of UV on various conveyor belts and showed that bacterial counts were significantly reduced 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 reductions.

Quality assessment of UV treated meat:

General microbiology for shelf life was evaluated on vacuum packaged meat stored for 8 weeks at 0 and 4°C. Shelf life of UV exposed and non-exposed bologna remained unaffected after 8 weeks of storage as results showed bacterial and yeast and mold counts were below 1-$\log_{10}$ CFU/sq. cm for all samples throughout the study irrespective of treatment. UV treatment, additives and preservatives in the meat are major factors that might have contributed towards the intact shelf life of the bologna.

- CONCLUSION

UV radiation can be used as a potential post lethality treatment as an alternative to many antimicrobials such as chlorine and organic acids. Moreover, UV can effectively mitigate *L. monocytogenes* and 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.

REFERENCES

- Farber, J. M. & Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476−511.
- Giovannacci, I., Ragimbeau, C., Queguiner, S., Salvat, G., Vendeuvre, J. L., Carlier, V. & Ermel, G. (1999). *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. Int. J. Food Microbiology 53: 127−40.
- Fenlon, D. R., Wilson, J. & Donachie, W. (1996). The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. J. Appl. Bacteriol. 81: 641−50.
- Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, & Griffin, P.M. (2011). Food borne illness acquired in the United States-major pathogens. Emer. Infect. Dis. 17: 7-15.
- Schraff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *J*. Food Prot. 75(1): 123-131.
- Code of Federal Regulations [CFR]. (2010). 9 CFR 430.4-Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products.
- Yousef, A. E. & E. H. Marth. (1988), Inactivation of *Listeria monocytogenes* by Ultraviolet Energy. J. Food Sci. 53: 571–573.
- Wolfe, R. L. (1990). "Ultraviolet Disinfection of Potable Water." Environ. Sci. Tech. 24(6): 768-773.
- Lee, I. S., Lin, J., Hall, H. K., Bearson, B. & Foster, J. W. (1995). The stationary-phase sigma factor (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium*. Mol. Microbiol. 17:155–167.
- Charles N. H., Aadithya, T. M., Joan, B. R. & Charles, P. G. (1999). Development and Validation of Dose-Response Relationship for *Listeria monocytogenes*. Quantitat. Microbiol. 1:89-102.
- Kuo F.L., Carey, J. B. & Ricke, S. C. (1997). UV irradiation of shell eggs: effect of populations of aerobes, moulds and inoculated *Salmonella typhimurium*. J. Food Prot. 60:639–643.

- Morey, A., McKee, S. R., Dickson, J. S. &M. Singh. (2010). Efficacy of ultraviolet light exposure against survival of *Listeria monocytogenes* on conveyor belts. Foodborne Pathog. Dis. 7: 737-740.
- Lyon, S. A., Fletcher, D. L. & M. E. Berrang. (2007). Germicidal ultraviolet light to lower numbers of *Listeria monocytogenes* on broiler breast fillets. Poult. Sci. 86: 964–967.