



# Inactivation of *Listeria monocytogenes* and *Salmonella* Typhimurium in beef broth and on diced beef using an ultraviolet light emitting diode (UV-LED) system

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## ABSTRACT

Ultraviolet Light-Emitting Diode (UV-LED) is a potential decontamination technology for reducing bacterial loads on meat. This study investigated the efficacy of UV-LED technology to reduce *Listeria monocytogenes*, *Salmonella* Typhimurium, total viable counts (mesophiles (TVCM) and psychophiles (TVCP)) and total *Enterobacteriaceae* counts (TEC) when suspended in beef broth and after inoculation onto diced beef. Inoculated samples ( $10^7$  CFU/mL) were treated with UV light using single (255, 265, 285 nm) and combined (255 and 265 nm, 255 and 285 nm, 265 and 285 nm) wavelengths, exposed for 2, 30 or 60 min. Significant ( $p < 0.05$ ) reductions in all of the target bacteria were achieved after 2 min with almost complete elimination after 60 min for all the individual and combinations of wavelengths tested, with the exception of 255 nm. On the diced beef, significant ( $p < 0.05$ ) reductions in *L. monocytogenes*, TVCM and TEC were achieved using 285 nm and all of the combined wavelength treatments after 60 min. It was concluded that UV-LED technologies have potential application for the decontamination of beef products and validation in a commercial plant should be undertaken to facilitate the transfer of this technology to the meat sector.

## 1. Introduction

Traditionally, ultraviolet (UV) light irradiation has been used to disinfect air, solid surface worktops and water but may also be used for liquid foods and decontaminating the surfaces of solid foods (Gayán et al., 2014). Interest in the application of UV light treatments in the food industry has increased in recent years due to its ability to decontaminate without adversely affecting the organoleptic quality. It also reduces the need to use chemicals to disinfect food contact surfaces, many of which are no longer acceptable to consumers (Gayán et al., 2014; Hinds et al., 2019). Moreover, the recent development of ultraviolet light-emitting diodes (UV-LED), eliminates the requirement for mercury UV lamps (Kebbi et al., 2020) and hence do not require toxic substances (harmful to human health and the environment). They also require less energy, are more economical and last up to 100 000 h (Chevremont et al., 2012; Akgün & Ünlütürk, 2017; Song et al., 2019). Modern UV-LED units are designed to be shock-resistant, robust and very compact which allows them to be used to disinfect a range of surfaces in industry settings (Hinds et al., 2019; Khan et al., 2005).

Another potential application of UV-LED technologies is the decontamination of meat and meat products (Cao et al., 2003; D'Souza et al., 2015). Current UV-LED technologies are capable of emitting multiple wavelengths at the same time thereby increasing their effectiveness in killing bacteria (Green et al., 2018). UV-LEDs have been widely investigated for water treatment (Baykuş et al., 2021). However, more research is needed on the applicability of this technology for the decontamination of foods such as meat products targeting commonly found pathogens in meat processing plants. *Listeria* and *Salmonella* spp. outbreaks are frequently associated with foodborne illness linked to the consumption of undercooked meat products (Heredia & García, 2018). These pathogens are excreted in the faeces of cattle, contaminating beef hides, and are readily transferred to the carcasses during dehiding. While several studies have investigated their application in the decontamination of food plant surfaces (Uesugi et al., 2007; Rajkovic et al., 2010; Jean et al., 2011; Haughton et al., 2012), there are very few studies investigating the use of UV-LED to decontaminate food directly.

The objective of this study was to investigate the application of UV-LED treatments using single (255 nm, 265 nm and 285 nm) and

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combinations (255/265 nm, 255/285 nm and 265/285 nm) of wavelengths to reduce or eliminate *L. monocytogenes* and *S. Typhimurium* in liquid beef and solid beef products over short (2 min), medium (30 min) and long (60 min) treatment times.

## 2. Method and materials

### 2.1. Bacterial strains and preparation of cultures

*Listeria monocytogenes* (T1093) and *Salmonella* Typhimurium (SARB63) strains, originally isolated from beef, were obtained from the Teagasc culture collection, Ashtown, Dublin, Ireland. To prepare the inoculum, a cryoprotective culture bead from frozen storage was streaked on tryptone soya agar (TSA, Oxoid Ltd., Basingstoke, U.K., CM0131) and incubated at 37 °C for 24 h. A single colony was then selected to inoculate 20 mL of tryptone soya broth TSB, (Oxoid, CM0129) and incubated overnight in a shaking incubator set at 37 °C. The culture obtained was centrifuged at 7500×g at 4 °C for 10 min and washed 3 times with phosphate buffered saline (PBS; Oxoid, BR0014), before resuspension in 20 mL PBS and serial dilutions prepared to obtain a cell concentration of approximately 10<sup>7</sup> CFU/mL.

### 2.2. Inoculation of beef

Fresh hindquarter beef was obtained from a local commercial beef plant and transported under chilled conditions to the Teagasc Food Research Centre, Ashtown where it was cut into uniform cubes (diced) of approximately 10 g each using an autoclaved knife and cubes were randomly assigned to the control or inoculated (*L. monocytogenes* or *S. Typhimurium*) groups. Control samples were fully immersed in maximum recovery diluent (MRD, Oxoid, CM0733) for 30 s using a sterile tweezers, allowed to drain for a further 30 s and placed on transparent sterile glass Petri dishes (5.5 cm diameter). Beef cubes were also inoculated with *S. Typhimurium* or *L. monocytogenes* by immersion in 100 mL of the prepared inoculum (approximately 10<sup>7</sup> CFU/mL) for 30 s to ensure complete contamination of each cube and drained for 30 s before placing on a sterile glass petri dishes. To prepare beef broth samples, solid meat cubes (10 g) were prepared, left uninoculated or inoculated as above, diluted in 90 mL MRD, stomached for 60 s and 10 mL samples were pipetted into the sterile glass petri dishes.

### 2.3. UV collimated beam unit

A UV-LED collimated Triple Wavelength beam unit (PearlBeam, AquiSense Technologies, Erlanger, Kentucky, United States of America) was used for the study. This system is comprised of a three channel LED controller which enables precise control of LEDs emitting wavelength exposures at 255, 265, 285 nm. The UVinaire (a replaceable LED lamp module) and collimating tube were placed in a biosafety cabinet with a low-density polyethylene black liner covering the glass to prevent outside light sources from interfering with the UV process, while the controller was manually operated via a shutter switch and LED selector from outside of the cabinet. The glass Petri dishes containing samples were placed under the UVinaire beam unit and the desired wavelength (s) were switched on using the controller. Samples were exposed to one of the six UV single or combined wavelength treatments (255, 265, 285, 255/265, 255/285 and 265/285 nm) for assigned treatment times (t = 2, 30 and 60 min) at room temperature. The diced beef samples were treated on both sides, which involved treating the upper surface and then aseptically inverting and transferring to a separate sterile glass Petri dish for further wavelength treatment. The distance between the samples and the UV-LED source was set at 4 cm for all experiments.

### 2.4. Microbiological analysis

Prepared samples (10 g of meat or 10 mL of liquid) were

subsequently diluted tenfold in 90 mL MRD, stomached (solid samples only) before serial dilution in MRD and plating in duplicate onto the appropriate medium. *L. monocytogenes* was enumerated on Brilliance *Listeria* Agar (Oxoid, CM1080) containing Brilliance *Listeria* Selective Supplement (Oxoid, SR0227) and Brilliance *Listeria* Differential Supplement (Oxoid, SR0228) and incubated at 37 °C for 24 h. *S. Typhimurium* was enumerated on X.L.D. Agar (Oxoid, CM0469) and incubated at 37 °C for 24 h. Mesophilic and psychophilic total viable counts (TVC) were enumerated on Plate Count Agar (PCA, Oxoid, CM0325) incubated at 30 °C for 72 h and 6.5 °C for 10 days respectively. Using the pour plate technique Violet Red Bile Glucose agar (VRBGA, Oxoid, CM0485) was used to enumerate TEC and incubated at 37 °C for 24 h.

### 2.5. Irradiance measurements

The emission spectra for individual UV-LED wavelengths was measured using a PM100A Optical Power Meter (Thorlabs Instrumentation, Newton, New Jersey, United States) and a S100C photodiode sensor. For measurements, the photodiode sensor was placed in the centre of the UV light at the same height and location as the surface of the beef broth or diced beef samples and the irradiance for each wavelength (255, 265 and 285 nm) recorded. To determine the irradiance of combined treatments, the total irradiance of individual wavelengths was added together and recorded. From this, the UV dose and Petri factor could be calculated according to Bolton and Linden (2003) using equations (1) and (2):

$$\text{UV dose (mW}\cdot\text{sec/cm}^2 \text{ or MJ/cm}^2 \text{ or J/m}^2\text{)} = I \times T \quad (1)$$

where:

$$I = \text{Intensity (mW/cm}^2 \text{ or W/m}^2\text{)}$$

$$T = \text{time (seconds)}$$

Petri factor (PF) indicates the area of even distribution of irradiated light on a Petri dish and was calculated as follows:

$$\text{PF} = \frac{\sum \frac{E_n}{E_0}}{n} \quad (2)$$

where:

$$E_n = \text{incident irradiance measured at various distances from the centre of the petri dish (measured every 5 mm) (mW/cm}^2\text{)}$$

$$E_0 = \text{incident irradiance measured at the centre of the petri dish (mW/cm}^2\text{)}$$

$$n = \text{number of measurements taken}$$

The Petri factor was 0.9 or greater for all treatments.

### 2.6. Statistical analysis

Microbial counts were converted to log<sub>10</sub> CFU/g values with a limit of detection of 0.70 log<sub>10</sub> CFU/g or CFU/mL for *L. monocytogenes*, *S. Typhimurium*, TVCm and TVCp and 0.30 log<sub>10</sub> CFU/g or CFU/mL for *Enterobacteriaceae* counts. In this study, samples were prepared in duplicate for the control, *L. monocytogenes* and *S. Typhimurium* (n = 6). There was a total of n = 7 different treatments, exposing samples to time points 2, 30 and 60 min. This was performed in triplicate through three independent experiments (n = 3), for each treatment (beef broth and diced beef (n = 2)). In total, 756 pieces of diced beef were analysed. Data from the experiment was analysed using a two-way ANOVA with wavelength and treatment time as different variables. Tukey's multiple comparisons *post-hoc* test was determined with significance defined at p < 0.05. Statistical analysis was performed using GraphPad Prism 7.02.

### 3. Results

#### 3.1. Beef broth

Six different treatments were applied as follows; 255 nm, 265 nm, 285 nm, 255/265 nm, 255/285 nm and 265/285 nm for 2, 30 and 60 min (UV dosages Table 1) and the effect on *L. monocytogenes*, *S. Typhimurium*, TVCm, TVCp and TEC in a beef broth were investigated (Table 2). An exposure time of 2 min was sufficient to achieve significant ( $p < 0.05$ ) reductions in *L. monocytogenes* (all treatments). Furthermore, the counts achieved after treatment at 255/285 nm and 265/285 nm (1.8 and 2.7 log<sub>10</sub> CFU/mL, respectively) were significantly ( $p < 0.05$ ) lower than those obtained with 285 nm and 255/265 nm treatments which were statistically similar to the counts at 265 nm but significantly ( $p < 0.05$ ) lower than the *L. monocytogenes* count (6.0 log<sub>10</sub> CFU/mL) obtained after exposure to 255 nm. Exposure for 2 min was also sufficient to achieve significant ( $p < 0.05$ ) reductions in *S. Typhimurium* with all treatments except 255 nm, with 285 nm, 255/285 nm and 265/285 nm being the most effective treatments (3.0–3.8 log<sub>10</sub> CFU/mL residual count). Significantly ( $p < 0.05$ ) lower counts were obtained for TVCm after 2 min using 285 nm and the 3 combination treatments with 255/285 nm and 265/285 nm achieving significantly ( $p < 0.05$ ) lower counts than 285 nm or 255/265 nm, a pattern that was repeated for TVCp. All treatments, except 255 nm, achieved significantly ( $p < 0.05$ ) lower TEC, with 255/285 nm and 265/285 nm followed by 285 nm being the most effective treatments.

Increasing exposure to 30 min resulted in reductions in *L. monocytogenes* that ranged from 3.9 to 7.1 log<sub>10</sub> CFU/mL. All counts were significantly reduced with residual levels of <0.9 log<sub>10</sub> CFU/mL, except at 255 nm. A similar pattern was obtained after 60 min with reductions of 7.4 log<sub>10</sub> CFU/mL with all treatments except 255 nm (4.6 log<sub>10</sub> CFU/mL). In contrast maximum reductions of *S. Typhimurium* were obtained after 30 min only with 285 nm, 255–285 nm and 265 285 nm, with the counts obtained being significantly ( $p < 0.05$ ) lower than those at 265 nm and 265 - 255 nm, both of which were significantly ( $p < 0.05$ ) lower than 255 nm (which was statistically similar to the control). After 60 min the *S. Typhimurium* counts for all treatments were below the limit of detection, except 255 nm (4.8 log<sub>10</sub> CFU/mL).

After 30 min, the same pattern was observed with TVCm and TVCp and again after 60 min in which all treatments achieved significantly ( $p < 0.05$ ) lower counts than the control and 285 nm, 255/285 nm and 265/285 nm > 265 nm and 255/265 nm > 255 nm (30 min) and 285 nm, 255/285 nm, 265/285 nm and 255/265 nm > 265 nm > 255 nm (60 min). After 30 min all treatments except 255 nm were below the limit of detection. At this wavelength it took another 30 min to achieve a significantly ( $p < 0.05$ ) lower count (>0.7 log<sub>10</sub> CFU/mL) as compared to the control (3.1 log<sub>10</sub> CFU/mL).

#### 3.2. Diced beef

The same UV treatments and UV dosages were applied targeting the same bacteria as for the diced beef (Table 3). Although 4 treatments

**Table 1**

UV dose values at the different UV treatments for 2 min, 30 min and 60 min.

| UV Treatment (nm) | Treatment time (mins) |                    |                    |
|-------------------|-----------------------|--------------------|--------------------|
|                   | 2                     | 30                 | 60                 |
|                   | mJ/cm <sup>2</sup>    | mJ/cm <sup>2</sup> | mJ/cm <sup>2</sup> |
| 255               | 0.6                   | 9.7                | 19.4               |
| 265               | 3.6                   | 54.1               | 108.2              |
| 285               | 6.4                   | 96.6               | 193.2              |
| 255/265           | 4.3                   | 63.8               | 127.5              |
| 255/285           | 7.1                   | 106.3              | 212.5              |
| 265/285           | 10.0                  | 150.7              | 301.3              |

nm = wavelength.

**Table 2**

*L. monocytogenes*, *S. Typhimurium*, total mesophilic counts (TVCm), total psychrophilic counts (TVCp) and total *Enterobacteriaceae* counts (TEC) (log<sub>10</sub> CFU/mL) in beef broth exposed to different UV treatments for 2 min, 30 min and 60 min.

| UV Treatment (nm)       | Treatment time (mins)            |      |                                  |      |                                  |      |
|-------------------------|----------------------------------|------|----------------------------------|------|----------------------------------|------|
|                         | 2                                |      | 30                               |      | 60                               |      |
|                         | Count (log <sub>10</sub> CFU/mL) | SEM  | Count (log <sub>10</sub> CFU/mL) | SEM  | Count (log <sub>10</sub> CFU/mL) | SEM  |
| <i>L. monocytogenes</i> |                                  |      |                                  |      |                                  |      |
| Control <sup>1</sup>    | 7.5 <sup>a</sup>                 | 0.16 | 7.2 <sup>a</sup>                 | 0.03 | 7.5 <sup>a</sup>                 | 0.08 |
| 255                     | 6.0 <sup>b</sup>                 | 0.04 | 4.3 <sup>b</sup>                 | 0.35 | 2.9 <sup>b</sup>                 | 0.40 |
| 265                     | 5.1 <sup>bc</sup>                | 0.52 | 0.4 <sup>c</sup>                 | 0.37 | 0.1 <sup>c</sup>                 | 0.00 |
| 285                     | 4.0 <sup>c</sup>                 | 0.37 | 0.1 <sup>c</sup>                 | 0.00 | 0.1 <sup>c</sup>                 | 0.00 |
| 255/265                 | 4.2 <sup>c</sup>                 | 0.56 | 0.9 <sup>c</sup>                 | 0.53 | 0.1 <sup>c</sup>                 | 0.00 |
| 255/285                 | 1.8 <sup>d</sup>                 | 0.86 | 0.1 <sup>c</sup>                 | 0.00 | 0.1 <sup>c</sup>                 | 0.00 |
| 265/285                 | 2.7 <sup>d</sup>                 | 0.45 | 0.1 <sup>c</sup>                 | 0.00 | 0.1 <sup>c</sup>                 | 0.00 |
| <i>S. Typhimurium</i>   |                                  |      |                                  |      |                                  |      |
| Control                 | 7.4 <sup>a</sup>                 | 0.15 | 7.5 <sup>a</sup>                 | 0.05 | 7.5 <sup>a</sup>                 | 0.24 |
| 255                     | 6.7 <sup>a</sup>                 | 0.19 | 6.5 <sup>a</sup>                 | 0.24 | 4.8 <sup>b</sup>                 | 0.30 |
| 265                     | 5.2 <sup>b</sup>                 | 0.42 | 3.5 <sup>b</sup>                 | 0.31 | 0.1 <sup>c</sup>                 | 0.00 |
| 285                     | 3.8 <sup>cd</sup>                | 0.39 | 0.9 <sup>c</sup>                 | 0.88 | 0.1 <sup>c</sup>                 | 0.00 |
| 255/265                 | 5.0 <sup>bc</sup>                | 0.45 | 3.6 <sup>b</sup>                 | 0.13 | 0.1 <sup>c</sup>                 | 0.00 |
| 255/285                 | 3.4 <sup>d</sup>                 | 0.25 | 0.1 <sup>c</sup>                 | 0.00 | 0.1 <sup>c</sup>                 | 0.00 |
| 265/285                 | 3.0 <sup>d</sup>                 | 0.18 | 0.1 <sup>c</sup>                 | 0.00 | 0.1 <sup>c</sup>                 | 0.00 |
| TVCm                    |                                  |      |                                  |      |                                  |      |
| Control                 | 6.4 <sup>a</sup>                 | 0.16 | 6.4 <sup>a</sup>                 | 0.31 | 6.6 <sup>a</sup>                 | 0.11 |
| 255                     | 6.7 <sup>a</sup>                 | 0.03 | 4.7 <sup>b</sup>                 | 0.04 | 4.1 <sup>b</sup>                 | 0.39 |
| 265                     | 5.7 <sup>a</sup>                 | 0.12 | 1.8 <sup>c</sup>                 | 0.40 | 1.8 <sup>c</sup>                 | 0.15 |
| 285                     | 3.9 <sup>b</sup>                 | 0.12 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>d</sup>                 | 0.00 |
| 255/265                 | 4.5 <sup>b</sup>                 | 0.16 | 2.0 <sup>c</sup>                 | 0.18 | 0.1 <sup>d</sup>                 | 0.00 |
| 255/285                 | 2.8 <sup>c</sup>                 | 0.17 | 0.3 <sup>d</sup>                 | 0.27 | 0.1 <sup>d</sup>                 | 0.00 |
| 265/285                 | 2.5 <sup>c</sup>                 | 0.25 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>d</sup>                 | 0.00 |
| TVCp                    |                                  |      |                                  |      |                                  |      |
| Control                 | 6.3 <sup>a</sup>                 | 0.14 | 6.3 <sup>a</sup>                 | 0.29 | 6.6 <sup>a</sup>                 | 0.13 |
| 255                     | 6.6 <sup>a</sup>                 | 0.14 | 5.4 <sup>b</sup>                 | 0.08 | 5.4 <sup>b</sup>                 | 0.08 |
| 265                     | 6.5 <sup>a</sup>                 | 0.20 | 3.6 <sup>c</sup>                 | 0.21 | 2.7 <sup>c</sup>                 | 0.31 |
| 285                     | 5.0 <sup>b</sup>                 | 0.12 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>d</sup>                 | 0.00 |
| 255/265                 | 5.3 <sup>b</sup>                 | 0.10 | 3.2 <sup>c</sup>                 | 0.14 | 0.1 <sup>d</sup>                 | 0.00 |
| 255/285                 | 2.8 <sup>c</sup>                 | 0.11 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>d</sup>                 | 0.00 |
| 265/285                 | 2.3 <sup>c</sup>                 | 0.15 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>d</sup>                 | 0.00 |
| TEC                     |                                  |      |                                  |      |                                  |      |
| Control                 | 2.9 <sup>a</sup>                 | 0.35 | 2.8 <sup>a</sup>                 | 0.27 | 3.1 <sup>a</sup>                 | 0.31 |
| 255                     | 2.4 <sup>ab</sup>                | 0.14 | 2.4 <sup>a</sup>                 | 0.22 | 0.5 <sup>b</sup>                 | 0.12 |
| 265                     | 2.2 <sup>b</sup>                 | 0.07 | 0.1 <sup>b</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 |
| 285                     | 1.2 <sup>c</sup>                 | 0.11 | 0.1 <sup>b</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 |
| 255/265                 | 2.0 <sup>b</sup>                 | 0.05 | 0.1 <sup>b</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 |
| 255/285                 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 |
| 265/285                 | 0.1 <sup>d</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 | 0.1 <sup>b</sup>                 | 0.00 |

<sup>1</sup>The values represent the average counts and standard error of the mean (SEM). Numbers with different superscript letters (a,b,c,d) within the same column indicate statistical significance ( $p < 0.05$ ) between UV treatments. nm = wavelength.

(285 nm, 255/265 nm, 255/285 nm and 265/285 nm) achieved a significant ( $p < 0.05$ ) reduction in TVCm after 2 min, in general exposure for such a short period of time was not sufficient to achieve a significant ( $p < 0.05$ ) reduction in the target bacteria on diced beef. By increasing the exposure time to 30 min, significant ( $p < 0.05$ ) reductions were obtained in *L. monocytogenes* (285 nm, 255/285 nm and 265/285 nm) and TVCm (285 nm, 255/265 nm, 255/285 nm and 265/285 nm) but the other UV-treatment-bacteria combinations were not affected. With the exception of TEC (285 nm, 255/265 nm, 255/285 nm and 265/285 nm), this situation did not change when the exposure time was increased to 60 min.

### 4. Discussion

There is a continuous search for new methods of microbial inactivation to enhance food safety and microbial stability while causing minimal adverse effects to sensory characteristics (Gayán et al., 2011).

**Table 3**

*L. monocytogenes*, *S. Typhimurium*, total mesophilic counts (TVCm), total psychrophilic counts (TVCp) and total *Enterobacteriaceae* counts (TEC) ( $\log_{10}$  CFU/g) on diced beef exposed to different UV treatments for 2 min, 30 min and 60 min.

| UV Treatment (nm)       | Treatment time (mins)      |      |                            |      |                            |      |
|-------------------------|----------------------------|------|----------------------------|------|----------------------------|------|
|                         | 2                          |      | 30                         |      | 60                         |      |
|                         | Count ( $\log_{10}$ CFU/g) | SEM  | Count ( $\log_{10}$ CFU/g) | SEM  | Count ( $\log_{10}$ CFU/g) | SEM  |
| <i>L. monocytogenes</i> |                            |      |                            |      |                            |      |
| Control <sup>1</sup>    | 7.6 <sup>a</sup>           | 0.17 | 7.7 <sup>a</sup>           | 0.02 | 7.6 <sup>a</sup>           | 0.12 |
| 255                     | 7.2 <sup>a</sup>           | 0.15 | 7.2 <sup>ab</sup>          | 0.08 | 7.0 <sup>ab</sup>          | 0.06 |
| 265                     | 7.1 <sup>a</sup>           | 0.10 | 6.8 <sup>ab</sup>          | 0.11 | 6.9 <sup>ab</sup>          | 0.14 |
| 285                     | 6.8 <sup>a</sup>           | 0.37 | 6.5 <sup>b</sup>           | 0.31 | 6.3 <sup>b</sup>           | 0.49 |
| 255/265                 | 7.1 <sup>a</sup>           | 0.15 | 7.0 <sup>ab</sup>          | 0.18 | 6.8 <sup>ab</sup>          | 0.12 |
| 255/285                 | 7.0 <sup>a</sup>           | 0.12 | 6.7 <sup>b</sup>           | 0.20 | 6.6 <sup>b</sup>           | 0.20 |
| 265/285                 | 7.0 <sup>a</sup>           | 0.07 | 6.7 <sup>b</sup>           | 0.12 | 6.7 <sup>b</sup>           | 0.23 |
| <i>S. Typhimurium</i>   |                            |      |                            |      |                            |      |
| Control                 | 7.5 <sup>a</sup>           | 0.20 | 7.3 <sup>a</sup>           | 0.15 | 7.4 <sup>a</sup>           | 0.21 |
| 255                     | 7.3 <sup>a</sup>           | 0.11 | 7.3 <sup>a</sup>           | 0.15 | 7.5 <sup>a</sup>           | 0.19 |
| 265                     | 7.3 <sup>a</sup>           | 0.11 | 7.2 <sup>a</sup>           | 0.15 | 7.2 <sup>a</sup>           | 0.23 |
| 285                     | 7.3 <sup>a</sup>           | 0.19 | 6.8 <sup>a</sup>           | 0.20 | 6.8 <sup>a</sup>           | 0.04 |
| 255/265                 | 7.1 <sup>a</sup>           | 0.14 | 6.8 <sup>a</sup>           | 0.07 | 7.0 <sup>a</sup>           | 0.10 |
| 255/285                 | 7.2 <sup>a</sup>           | 0.13 | 7.1 <sup>a</sup>           | 0.27 | 6.8 <sup>a</sup>           | 0.24 |
| 265/285                 | 7.4 <sup>a</sup>           | 0.19 | 6.7 <sup>a</sup>           | 0.19 | 6.8 <sup>a</sup>           | 0.27 |
| TVCm                    |                            |      |                            |      |                            |      |
| Control                 | 7.4 <sup>a</sup>           | 0.22 | 7.4 <sup>a</sup>           | 0.10 | 7.3 <sup>a</sup>           | 0.15 |
| 255                     | 7.3 <sup>a</sup>           | 0.21 | 7.5 <sup>a</sup>           | 0.11 | 7.5 <sup>a</sup>           | 0.10 |
| 265                     | 6.9 <sup>ab</sup>          | 0.03 | 6.9 <sup>ab</sup>          | 0.05 | 7.2 <sup>a</sup>           | 0.13 |
| 285                     | 6.7 <sup>b</sup>           | 0.06 | 6.7 <sup>b</sup>           | 0.23 | 6.0 <sup>b</sup>           | 0.15 |
| 255/265                 | 6.6 <sup>b</sup>           | 0.06 | 6.6 <sup>b</sup>           | 0.29 | 5.8 <sup>b</sup>           | 0.08 |
| 255/285                 | 6.7 <sup>b</sup>           | 0.04 | 6.4 <sup>b</sup>           | 0.26 | 5.7 <sup>b</sup>           | 0.03 |
| 265/285                 | 6.7 <sup>b</sup>           | 0.10 | 6.6 <sup>b</sup>           | 0.15 | 6.2 <sup>b</sup>           | 0.06 |
| TVCp                    |                            |      |                            |      |                            |      |
| Control                 | 7.5 <sup>a</sup>           | 0.12 | 7.5 <sup>a</sup>           | 0.18 | 7.5 <sup>a</sup>           | 0.16 |
| 255                     | 7.7 <sup>a</sup>           | 0.05 | 7.7 <sup>a</sup>           | 0.08 | 7.6 <sup>a</sup>           | 0.04 |
| 265                     | 7.3 <sup>a</sup>           | 0.06 | 7.7 <sup>a</sup>           | 0.15 | 7.5 <sup>a</sup>           | 0.22 |
| 285                     | 7.4 <sup>a</sup>           | 0.09 | 7.3 <sup>a</sup>           | 0.15 | 7.3 <sup>a</sup>           | 0.31 |
| 255/265                 | 7.5 <sup>a</sup>           | 0.19 | 7.7 <sup>a</sup>           | 0.04 | 7.7 <sup>a</sup>           | 0.06 |
| 255/285                 | 7.3 <sup>a</sup>           | 0.21 | 7.4 <sup>a</sup>           | 0.25 | 7.6 <sup>a</sup>           | 0.03 |
| 265/285                 | 7.3 <sup>a</sup>           | 0.06 | 7.4 <sup>a</sup>           | 0.07 | 7.4 <sup>a</sup>           | 0.18 |
| TEC                     |                            |      |                            |      |                            |      |
| Control                 | 3.9 <sup>a</sup>           | 0.19 | 3.7 <sup>a</sup>           | 0.29 | 3.7 <sup>a</sup>           | 0.33 |
| 255                     | 4.3 <sup>a</sup>           | 0.10 | 3.8 <sup>a</sup>           | 0.07 | 4.0 <sup>a</sup>           | 0.13 |
| 265                     | 3.9 <sup>a</sup>           | 0.13 | 3.4 <sup>a</sup>           | 0.16 | 3.8 <sup>a</sup>           | 0.03 |
| 285                     | 3.9 <sup>a</sup>           | 0.06 | 3.9 <sup>a</sup>           | 0.08 | 1.1 <sup>b</sup>           | 0.15 |
| 255/265                 | 3.8 <sup>a</sup>           | 0.04 | 3.5 <sup>a</sup>           | 0.13 | 0.8 <sup>b</sup>           | 0.25 |
| 255/285                 | 3.5 <sup>a</sup>           | 0.19 | 3.6 <sup>a</sup>           | 0.03 | 1.2 <sup>b</sup>           | 0.29 |
| 265/285                 | 3.6 <sup>a</sup>           | 0.23 | 3.8 <sup>a</sup>           | 0.14 | 1.2 <sup>b</sup>           | 0.15 |

<sup>1</sup>The values represent the average counts and standard error of the mean (SEM). Numbers with different superscript letters (<sup>a,b</sup>) within the same column indicate statistical significance ( $p < 0.05$ ) between UV treatments. nm = wavelength.

Among the non-thermal technologies developed in the last few decades, UV light irradiation is one of the most promising for the meat industry, as it is easy to use and lethal to most types of microorganisms including bacteria and viruses (Bintsis, Litopoulou-Tzanetaki and Robinson, 2000). However, food product characteristics, including their physical state, greatly influence microbial decontamination (Hamidi-Oskouei et al., 2015). In this study, the inactivation of *L. monocytogenes* and *S. Typhimurium* pathogens inoculated into beef broth and diced beef using single (255 nm, 265 nm and 285 nm) and combined (255/265 nm, 255/285 nm, 265/25 nm) UV-LED wavelengths were investigated as was the effect of these treatment on naturally occurring bacterial contamination (TVCm, TVCp and TEC).

Overall, bacteria in the beef broth were more susceptible to UV treatments than when inoculated onto the surface of solid beef. This may be due to the inability of UV treatments to penetrate the exposed outer surface of meat, which has a rough topography providing opportunity for bacterial protection while no such barriers was available in the thin

homogenous layer of broth (Stermer et al., 1987; Hamidi-Oskouei et al., 2015; Midgley & Small, 2006; Reichel et al., 2019). Supporting evidence for this hypothesis is provided by Kim et al. (2014) who also investigated the effect of UV radiation on *L. monocytogenes* and *S. Typhimurium* and found bacteria exposed on the surface of agar decreased significantly after UV treatment while those populations on meat surfaces were maintained.

Combined treatments were more effective than individual UVB or UVC. This was not unexpected as combined wavelengths have an additive effect as previously reported (Beck et al., 2017; Li et al., 2017; Green et al., 2018; Song et al., 2019). *L. monocytogenes* was more UV sensitive than *S. Typhimurium*. Several previous studies have also reported Gram positive bacteria are more sensitive to light treatments, possibly due to Gram negative bacteria producing porphyrins that are less sensitive to photoexcitation, which results in less production of reactive oxygen species which cause oxidative damage and cell death (Szocs et al., 1999; Nitzan et al., 2004; Maclean et al., 2009). It is also possible that *S. Typhimurium* may have a more efficient repair system explaining its enhanced UV resistance as also observed by Gayán et al. (2012).

Throughout the study 255 nm was the least effective single wavelength, even though it is within the UVC-LED spectrum known for its high germicidal effect. Indeed 285 nm was the most effective single UV treatments. Other studies that used UVC treatments reported that they are the most effective wavelength when used at the higher end of the spectrum (270 nm) compared to 255 nm (Waites et al., 1988; Linden et al., 2001). UVC (200–280 nm), known as the germicidal wavelength, can damage the DNA of pathogenic and spoilage microorganisms because maximum DNA absorption occurs in this range (Bolton & Cotton, 2008) and is generally considered to be the most effective light irradiation for microbial inactivation (Gayán et al., 2014). It has previously been reported that bacteria employ repair mechanisms or photoreactivation to UV-damaged DNA due to the photolyase enzyme reversing the dimerization of pyrimidine bases (Schottroff et al., 2018; Cheng et al., 2020). However, wavelengths greater than 280 nm have not only been shown to suppress photoreactivation and dark repair (Li et al., 2017; Nyangaresi et al., 2018) but can also inactivate microorganisms by causing oxidative disturbance to certain biomolecules including lipids and proteins by the production of reactive oxygen species (Argyaki et al., 2016; Brem et al., 2017). The 255/285 nm and 265/285 nm combination treatments used in this study may create an additive effect as a result of alternative inactivation mechanisms occurring (Green et al., 2018).

The data shows that the treatment of raw meat surfaces using specific UV-LED wavelengths can reduce bacterial load, although reduction rates are low. Nevertheless, this study supports Hijnen et al. (2006) in the conclusion that the implementation of UV-LED treatments can effectively inactivate numerous pathogenic and non-pathogenic bacteria.

## 5. Conclusion

This study demonstrated that UV treatment is an effective method for significantly reducing *L. monocytogenes*, TVCm and TEC populations in solid beef products but required the maximum exposure time (60 min) to achieve this. An additive effect was observed in beef when wavelengths were applied in combination. However, minimal reductions were observed for *S. Typhimurium* and TVCp in solid beef even when combined wavelengths were applied. In contrast, UV treatment was more effective for all bacteria in beef broth. In meat broth 285 nm was the most effective single UV treatment but could be combined with 255 nm or 265 nm to enhance the bactericidal effect. Our findings suggest UV technology may find application in the decontamination of solid and liquid meat products.

## CRediT authorship contribution statement

**Siobhán McSharry:** Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Leonard Koolman:** Experimental design, Methodology, Writing – review & editing. **Paul Whyte:** Methodology, Supervision, Writing – review & editing. **Declan Bolton:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare no conflict of interest.

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