



Improving ready-to-eat meat safety: Evaluating the bacterial-inactivation efficacy of microplasma-based far-UVC light treatment of food-contact surfaces and deli turkey breast

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ABSTRACT

The safety of ready-to-eat (RTE) deli meats, especially those sliced in retail establishments, may be improved by light-based surface decontamination. Conventional 254 nm ultraviolet-C (UVC) systems have strong germicidal effects but pose human-health hazards that make them unsuitable for retail use. This study therefore explores the efficacy of microplasma-based 222 nm far-UVC lamps as a safer alternative for decontaminating liquid buffer, two common food-contact surfaces (polyethylene terephthalate and stainless steel), and RTE turkey breast. In all three non-meat cases, the system achieved approximately 5-log reductions of both *Listeria monocytogenes* and *Salmonella* Typhimurium. The system also caused a 1.3-log reduction of *L. monocytogenes* and a 1-log reduction of *S. Typhimurium* on turkey breast at the highest tested dose of 786.3 mJ/cm². Color is a key quality indicator for RTE meat consumers, and treatment caused no significant change in L*, a*, or b* color values ($p > 0.05$) until doses reached 224.7 mJ/cm². However, higher doses could lead to statistically significant color changes. Given that far-UVC light has been deemed human-safe by other studies, the proposed system has considerable potential to improve RTE food-related safety in retail establishments, even when consumers and workers are present.

1. Introduction

In the United States, more than 90% of cases of listeriosis – a food-borne illness with high mortality (20–30%) and hospitalization rates (94%) caused by *Listeria monocytogenes* (*L. monocytogenes*; FDA, 2020a, b) – have been attributed to ready-to-eat (RTE) deli meats (Sampedro et al., 2022). Those RTE meats sliced and processed in retail establishments account for an estimated 83% of deli meat-associated cases of listeriosis (Kurpas et al., 2018). This preponderance is largely due to their greater susceptibility to cross contamination than their counterparts in manufacturing facilities, which in part arises from additional processing steps including slicing and repackaging (Tsaloumi et al., 2021). Moreover, *L. monocytogenes* is prevalent in retail deli establishments (Assisi et al., 2021). In one study, 9.5% of 4503 samples obtained from food and non-food contact surfaces in 30 deli establishments in various U.S. states tested positive for it (Simmons et al., 2014). Additionally, RTE meat products have frequently been the source of *Salmonella* spp. infections (Neri et al., 2019; Osaili et al., 2014), including

some very serious outbreaks (CDC, 2021). To mitigate the food-safety risks associated with RTE deli meats, therefore, it is critical to develop novel technologies and/or practices for microbial control.

As traditional thermal methods may cause undesirable changes to foods' nutritional and/or flavor attributes (Kaavya et al., 2021), various non-thermal approaches to RTE-meat decontamination have been proposed, including cold plasma (Yadav et al., 2019) and high-pressure processing (Hygreeva and Pandey, 2016), among others. Though such technologies appear effective at inactivating bacteria, they have disadvantages such as high equipment prices, high energy consumption, and/or special personnel-training requirements (Singh et al., 2021; Varalakshmi, 2021), making them suitable only for manufacturing settings (Morris et al., 2007).

One cost-effective alternative comprises light-based technologies, which are easy to operate yet still effective at bacterial inactivation (Belloli et al., 2022). Notably, microorganism control using ultraviolet light (UV), mainly at 254 nm, has been approved by the U.S. Food and Drug Administration and Health Canada, among other regulators

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(Koutchma et al., 2016). Of the various specific wavelengths that have been used to decontaminate RTE meat products, UVC (200–280 nm) has been found to have a better decontamination effect than either UVA (320–400 nm) or UVB (280–320 nm) (Delorme et al., 2020; Kim et al., 2016).

Existing UVC disinfection systems typically rely on low-pressure mercury lamps (Bintsis et al., 2000) with a peak emission of 254 nm, which is close to the maximum absorbance of nucleic acids, i.e., 260 nm (Shen, 2023). Thus, the absorption of UVC light's photons can cause the formation of photoproducts and/or disrupt DNA bonds, resulting in DNA damage (Tavares et al., 2023). While this mechanism can effectively inactivate pathogens, it poses considerable risks to humans, specifically to their skin and eyes (FDA, 2020a,b). Therefore, 254 nm UVC is not suitable for use in retail settings where workers and consumers are present.

On the other hand, far-UVC light (200–230 nm) is human-safe even at high doses, and still effective at pathogen inactivation (Eadie et al., 2021). Far-UVC wavelengths can be generated by microplasma-based krypton chloride (KrCl) excimer lamps, which have the additional benefit of not incorporating toxic mercury (Kim and Kang, 2020). Specifically, 222 nm UVC light cannot penetrate either the outermost dead-cell skin layer or the ocular tear layer, and thus, it poses no threat to human skin or eyes (Fukui et al., 2020; Hickerson et al., 2021). As such, far-UVC technology has excellent potential for addressing RTE food-related safety issues. Nevertheless, little research has previously explored such potential.

Accordingly, in this study, we constructed a microplasma-based KrCl excimer far-UVC system and tested its decontamination efficacy against *L. monocytogenes* and *Salmonella* Typhimurium, in liquid buffer in the first instance. This was necessary because, although it has previously been reported that far-UVC systems can effectively decontaminate buffers inoculated with bacteria, different efficacies may result from variations in light systems, their physical arrangements, experimental setups, and/or experimental operations (Fernández et al., 2020; Koutchma, 2020). Then, we probed far-UVC's potential inactivation mechanisms by investigating damage to the target bacteria's cell-membrane integrity and their generation of reactive oxygen species (ROS), either of which could potentially cause their inactivation (Kang et al., 2018).

Food-contact surfaces are the main loci of cross contamination in retail settings due to handling actions such as cutting, slicing, and packaging (Sheen and Hwang, 2011). Therefore, we next investigated the decontamination efficacy of our far-UVC system against the same two bacteria on stainless steel (SS) and polyethylene terephthalate (PET), two of the most used contact surfaces for RTE meat in such settings (De Cort et al., 2017).

Then, to evaluate our system's real-world potential for bacterial inactivation, we tested it on RTE turkey breast inoculated with the same two pathogens. This RTE deli meat was selected because the growth of *L. monocytogenes* on turkey breast, as modeled by Pradhan et al. (2009), was faster than on deli beef or ham products.

As color is one of the most important factors that consumers use to assess the quality of meat products, strongly affecting their purchase decisions and product acceptance (Tomasevic et al., 2021), we further evaluated if and how far-UVC irradiation affected this property. To the best of our knowledge, this is the first study to investigate far-UVC's inactivation efficacies against *Listeria* or *Salmonella* on real RTE meat samples and how such treatment might impact such samples' color. As this technology is already known to be human-safe, our findings can potentially pave the way for its use to decontaminate food and food-contact surfaces not only at deli counters and similar retail establishments but also in consumers' homes and public dining facilities such as food courts and restaurants, thus greatly enhancing food safety.

2. Materials and methods

2.1. Bacterial strains and culture preparation

S. enterica subsp. *enterica* serovar Typhimurium (ATCC 14028) and *L. monocytogenes* serotype 2 (ATCC, 19112) were preserved by freezing them in glycerol solutions at -80°C before our experiments. Working cultures were first streaked onto brain heart infusion (BHI) agar (Sigma-Aldrich, St. Louis, MO, USA), incubated at 37°C for 24 h, and stored at 4°C . A single colony of each bacterial strain was aseptically transferred to a sterile culture tube, containing 5 mL of BHI broth. Tubes were then incubated at 37°C with shaking for 18 h and 20 h, respectively (Jin et al., 2023). A cell pellet of each strain was obtained by centrifugation at $3000 \times g$ for 5 min at 4°C and washed twice with sterile phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA).

2.2. Microplasma-based 222 nm far-UVC treatment system

The decontamination system we built (Fig. 1a) consisted of an acrylic frame containing a microplasma-based far-UVC lamp ($5.08 \text{ cm} \times 5.08 \text{ cm}$, Eden Park Illumination Inc., Champaign, IL, USA) and a lab jack on which samples could be placed at various controllable distances from the lamp. Prior to plugging it into a standard power outlet, we connected the lamp to a ballast that transforms commercial electricity into high-voltage electricity. The latter type of electricity is needed for excitation of the gas mixture, which leads to the formation of KrCl* excimer microplasma that can emit light at 222 nm. The system's irradiation intensity was measured with a fiber-optic spectrometer (AvaSpec-ULS2048, Avantes, Lafayette, CO, USA) calibrated to a range of 200–250 nm. The UV dose (fluence, mJ/cm^2) was calculated by multiplying the measured irradiation-intensity values (irradiance, mW/cm^2) by the treatment time (in seconds).

2.3. Evaluating far-UVC radiation's decontamination efficacy of liquid buffer

The pelleted cells of each bacteria mentioned in section 2.1 were first re-suspended in PBS and diluted to our desired concentration of approximately 10^6 to 10^7 colony-forming units per milliliter (CFU/mL). To evaluate the decontamination efficacy of the far-UVC lamp, we transferred two successive 5 mL samples of inoculated PBS into a sterile Petri dish that was then positioned directly under the lamp at a distance of 4 cm. The depth of the liquid in the Petri dish was around 0.5 cm. These inoculated liquid samples were stirred throughout treatment using a stir plate to improve the uniformity of irradiation (Singh et al., 2021), with 100 μL subsamples of each being drawn periodically. Those subsamples were either directly plated or subjected to serial dilution, followed by plating onto BHI agar plates that were incubated for 24 h at 37°C before bacterial enumeration. Samples were diluted to a range that yielded below 250 colonies per plate before visual enumeration. The limit of detection (LOD) for liquid buffer was calculated as 1 log CFU/mL, and the results are reported as CFU per mL of suspension. Log reduction was calculated using Eq. (1):

$$\text{Log reduction} = \text{Log} (N / N_0) \quad (1)$$

where N_0 and N are respectively the before-treatment and after-treatment bacterial counts (in CFU/mL, in the case of the liquid-buffer experiments).

2.4. Bacterial-inactivation mechanisms

To investigate cell-membrane damage to the target bacteria, we used propidium iodide (PI) dye (Sigma Aldrich, St. Louis, MO, USA). Bacterial suspensions of *S. Typhimurium* and *L. monocytogenes* with concentrations of around 10^8 CFU/mL were first prepared using PBS solution, and

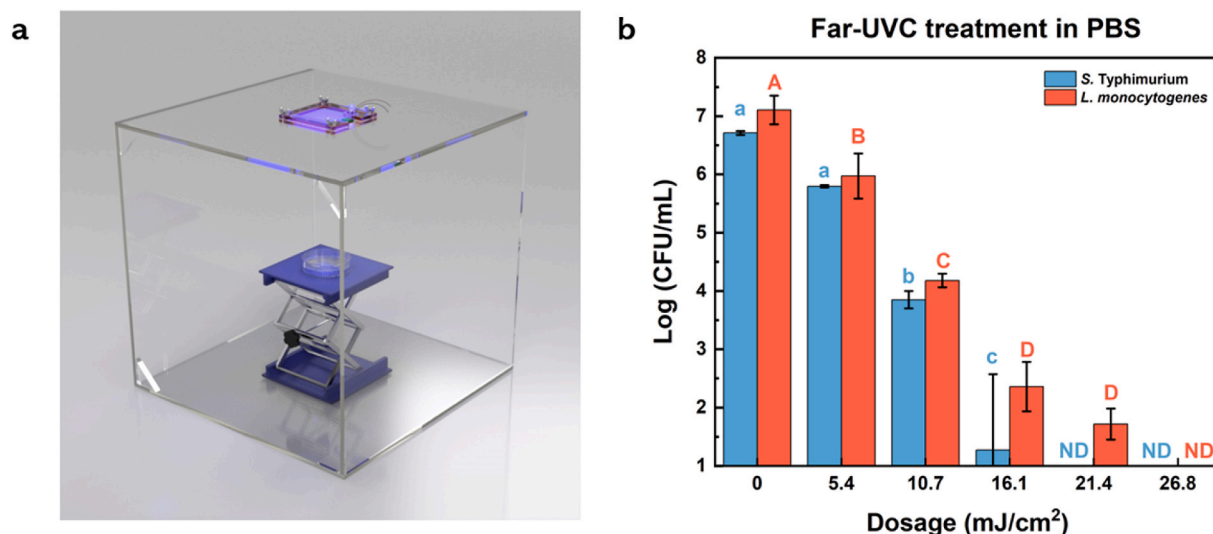


Fig. 1. a) The developed 222 nm far-ultraviolet C (far-UVC) light system for decontaminating liquid buffer and food-contact surfaces. b) Average log population in colony-forming units per milliliter (CFU/mL) of *Salmonella* Typhimurium (blue) and *Listeria monocytogenes* (orange) in phosphate-buffered saline (PBS) solution after various far-UVC light doses, with error bars indicating standard deviations. Same-lettered intervals indicate no significant difference at 0.05 significance level ($p > 0.05$). ND = not detected; the limit of detection (LOD) was 1 log CFU/mL, and values below LOD were substituted with half of LOD. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

samples treated with the far-UVC light at 321.7 mJ/cm², along with untreated ones, were collected for PI staining with a dye concentration of 10 μM. The stained samples were then incubated in the dark for 30 min at room temperature and subsequently washed with PBS. Observations were made using a confocal microscope (SP8, Leica, Wetzlar, Germany) at an excitation wavelength of 514 nm and an emission wavelength of 617 nm.

To assess oxidative stress and detect total ROS in bacterial cells, we employed 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA; Sigma Aldrich, St. Louis, MO, USA). Following the same staining procedure as with the PI, both untreated and treated samples with a concentration of 10 μM were incubated in the dark for 30 min at room temperature and washed with PBS. Subsequent observation was conducted with the same confocal microscope mentioned above, but at excitation and emission wavelengths of 488 nm and 535 nm, respectively.

2.5. Evaluating far-UVC radiation's decontamination of food-contact surfaces

SS (Type 304, Mirror-Like #8; McMaster-Carr, Elmhurst, IL, USA) was cut into 4.7 cm × 4.7 cm coupons that were washed and autoclaved at 121 °C for 15 min before use. Samples of PET film (McMaster-Carr, Elmhurst, IL, USA), measuring 1 cm × 4.2 cm, were soaked in 70% ethyl alcohol for 10 min for decontamination and dried in a biosafety cabinet (BSC) a day before the experiments. Sterile forceps were used for all handling of SS coupons and PET films.

We spot-inoculated 100 μL of resuspended cell-pellet cultures of each bacteria strain (as described in section 2.1) separately onto one side of each of the target food-contact surfaces. In the case of the PET film, the suspensions were diluted for spot inoculation to an initial concentration of 10⁵–10⁶ CFU/cm². In the case of SS, the suspensions were directly inoculated without dilution, because bacterial concentrations on that material are drastically reduced after air-drying (Fuster-Valls et al., 2008).

Following inoculation, the PET film samples were treated with our far-UVC lamp for various amounts of time ranging from 2 min to 8 min. After treatment, they were placed in sterile tubes containing 9 × m_0 mL PBS solution and vortexed for 5 min (m_0 being the weight of an individual sample). Again, 100 μL subsamples of the resulting suspensions were drawn, followed by serial dilution, plating, and incubation under

the same conditions mentioned above; and then the remaining bacteria were enumerated. The bacteria on inoculated but untreated control samples of PET were also enumerated in the same way.

The SS coupons were also treated with our lamp system for varying amounts of time, in this case ranging from 20 s to 2 min 20 s. After far-UVC treatment, each treated SS coupon was placed in a sterilized bag with 1 × m_0 mL PBS solution (m_0 being the weight of an individual sample), which was then shaken for 3 min using a stomacher (Stomacher 80 Biomaster; Fisher Scientific, Pittsburgh, PA, USA). Next, 100 μL subsamples of the resulting suspensions were drawn, and the above-mentioned enumeration approach again followed; the bacteria on inoculated but untreated (control) coupons were enumerated in the same way. For both contact-surface types, enumeration results are reported as log CFU/cm². The LOD values for PET and SS were 0.377 log CFU/cm² and 0.702 log CFU/cm², respectively. Log reduction was determined using Eq. (1), but with the unit changed to CFU/cm² for the food-contact surface experiments.

2.6. Evaluating far-UVC radiation's decontamination of RTE turkey breast

RTE sliced turkey breast was purchased from a local supermarket (Champaign, IL, USA) and kept in a freezer before our experiments. Because meat samples are three-dimensional structures, evaluation of our far-UVC system's ability to decontaminate them was conducted in a different treatment chamber: i.e., one equipped with a stage made of quartz glass (10 cm × 10 cm × 0.1 cm, Eden Park Illumination Inc., Champaign, IL, USA), which far-UVC radiation can penetrate, and two far-UVC lamps, one located 2 cm above the stage, and the other, 2 cm below. The two lamps were adjusted to provide the same irradiation intensity, as measured by the same fiber-optic spectrometer mentioned above; and accordingly, we report UV doses (fluence, mJ/cm²) from one side of the meat only.

The turkey breast was cut into circles 10 mm in diameter. The washed cell pellets described in section 2.1 were diluted to our desired concentration of approximately 10⁷ CFU/g, and each sample was spot-inoculated on one side with bacterial suspension. Next, the inoculated meat was allowed to dry in the BSC before being divided into a control and a treatment group. The treatment group was then exposed to far-UVC radiation for a total of 7 min in 1 min increments separated by

cool-down intervals of 90 s to avoid the samples' temperature becoming elevated. Both the untreated and treated samples were then vortexed with $9 \times m_0$ mL PBS solution in a microcentrifuge tube for 5 min (m_0 being the weight of an individual sample). Lastly, 100 μ L samples of the resulting suspensions were diluted and plated (or plated without dilution) onto BHI agar and incubated at 37 °C for 24 h before bacterial enumeration, reported as log CFU/g. The log reduction on RTE turkey breast was calculated using Eq. (1), with the unit being CFU/g for the real-food experiments.

2.7. Evaluation of the color of RTE turkey breast after far-UVC treatment

To evaluate whether and how much RTE turkey-breast color changed due to far-UVC treatment, we used a colorimeter (Minolta CR-400, Konica Minolta, NJ, USA) before and after such treatment. This device was calibrated with a white tile before use, and the measurement was performed on three randomly selected locations on each side of each sample. Each such color measurement was conducted with three independent samples. For subsequent statistical analysis, the resulting total of nine measurements were averaged. In the $L^*a^*b^*$ color system, L^* represents darkness to lightness, a^* indicates green to red (where -a points to green and +a to red), and b^* signifies blue to yellow (where -b points to blue and +b to yellow).

2.8. Modeling far-UVC's bacterial-inactivation data

Log-linear and Weibull approaches were both utilized to model our treatments' inactivation curves. We employed the model-fitting tools in the 'ggplot2' and 'dplyr' packages in the statistical software RStudio (R Foundation for Statistical Computing, version 2023.12.1–402).

Log-linear modeling has been widely adopted to describe microbial inactivation resulting from both thermal and non-thermal processes. It follows the first-order kinetics assumption given in Eq. (2),

$$\log \frac{N}{N_0} = -kd \quad (2)$$

where N is the number of surviving microorganisms, following irradiation dosage d ; N_0 is the initial number of microorganisms; and k is a first-order inactivation rate constant (cm^2/mJ) (Bialka et al., 2008; Rockey et al., 2021).

However, many microbial-inactivation curves are non-linear. Therefore, Weibull modeling, which describes bacterial inactivation as a more complex phenomenon (van Boekel, 2002), was also used for analyzing our data. The Weibull model is computed as

$$\log \frac{N}{N_0} = - \left(\frac{d}{\alpha} \right)^\beta \quad (3)$$

where α describes the scale parameter (mJ/cm^2) that indicates the dose for the first decimal reduction (Nicolau-Lapeña et al., 2022), and β is a shape parameter that represents the upward ($\beta < 1$) or downward concavity ($\beta > 1$) of the curve (Bialka et al., 2008).

Following Soro et al. (2021) and Zhu et al. (2021), we used the standard statistical parameter R^2 along with two information-theory criteria, the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), to evaluate whether the log-linear or the Weibull model was a better fit for our data.

2.9. Statistical analysis

All our experiments were replicated three times independently and are reported in average \pm standard deviation (SD). Statistical analysis was performed with one-way analysis of variance (ANOVA) using SPSS software (IBM Corp., Armonk, NY, USA), and Tukey's paired-comparison tests were used to evaluate significant differences among means at $p < 0.05$.

3. Results and discussion

3.1. Far-UVC irradiation's efficacy at decontaminating liquid buffer

Cross-contamination is a major cause of the spread of pathogens in production and distribution chains. Specifically, liquids, such as water used for washing foodstuffs, have been identified among the most common sources of bacterial transmission (Ebert, 2018; Ravishankar et al., 2010) during both food processing and handling (Possas and Pérez-Rodríguez, 2023). Therefore, it is important to include liquids in studies of the efficacy of decontamination.

Additionally, in sterilization research, pH-change-resistant buffer solutions have been commonly used to study bacterial-inactivation efficacy and the inactivation mechanisms of various technologies such as UV (Gabriel and Nakano, 2009; Kim et al., 2002; Schenk et al., 2011). In part, this could be because results obtained from real food samples can be confounded by food products' pH and/or nutrient content, both of which can impact the growth and survival of microbial cells (Jeon and Ha, 2018; Reinders et al., 2001). Therefore, we first studied the decontamination efficacy of our far-UVC system against *S. Typhimurium* and *L. monocytogenes* in a liquid PBS buffer.

Fig. 1a shows the first of our two far-UVC systems described above, and Fig. 1b presents the results of our buffer experiments. After inoculation, the samples' initial concentrations of *S. Typhimurium* and *L. monocytogenes* were found to be 6.71 ± 0.03 and 7.11 ± 0.25 CFU/mL, respectively. As can be seen from Fig. 1b, as UV doses increased from 5.4 mJ/cm^2 (equivalent to 10 s of treatment) to 10.7 mJ/cm^2 (20 s), and 16.1 mJ/cm^2 (30 s), the concentrations of *L. monocytogenes* decreased significantly ($p < 0.05$). The population of *S. Typhimurium* started to exhibit a significant difference after 10.7 mJ/cm^2 (20 s) of treatment ($p < 0.05$).

S. Typhimurium samples exhibited a 5.44-log reduction after 16.1 mJ/cm^2 of treatment and reached an undetectable level (limit of detection, LOD: 1 log CFU/mL) after 21.4 mJ/cm^2 of treatment (40 s). *L. monocytogenes* concentrations underwent a slightly lower decrease, i. e., 5.39-logs after 21.4 mJ/cm^2 of treatment; and reducing them to below the LOD also required a higher irradiance: 26.8 mJ/cm^2 . These results indicate *L. monocytogenes*' greater resistance to UV treatment than *S. Typhimurium*'s in liquid media. This may be because *Listeria* spp., as gram-positive bacteria, have thicker peptidoglycan walls surrounding their cytoplasmic membranes; and such walls may endow them with greater rigidity, and thus higher resistance to mechanical and photochemical damage (Ha et al., 2017; Jeon and Ha, 2018; Virto et al., 2005). As Fig. S1 shows, the absorbance of the PBS buffer was similar to that of the water, with neither showing any peak around 222 nm. This suggests that the buffer itself had little effect on the far-UVC treatment.

The above results are comparable with those of many previous studies that utilized 222 nm UV radiation to inactivate pathogens in liquid buffer. For instance, Narita et al. (2020) showed that *S. Typhimurium* can be reduced by 2–3 logs at 12 mJ/cm^2 , and by > 7 logs, i. e., to an undetectable level, at 36 mJ/cm^2 . They also tested their system against a different gram-negative bacteria, *Escherichia coli* (*E. coli*), which was reduced by 2–3 log by a 6 mJ/cm^2 dose and reached an undetectable level when subjected to a dose of 24 mJ/cm^2 . Kang et al. (2018) also showed that far-UVC light could achieve over 5 log reductions against *Staphylococcus aureus* (*S. aureus*), *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 in buffer at lower doses of 5–6 mJ/cm^2 . Variations in lamp characteristics, experimental setups, bacterial strains, and/or procedures may account for these observed differences (Singh et al., 2021).

Our results also suggest that 222 nm UVC can be as effective for bacterial inactivation in liquid as the conventional 254 nm UVC utilized in many other studies. For example, Kim et al. (2002) used 254 nm UVC on *S. Typhimurium*, *E. coli* O157:H7, and *L. monocytogenes* in peptone water, and reported 5-log reductions at doses between 30 and 60 mJ/cm^2 . Our results therefore suggest 222 nm far-UVC light's excellent

potential as an alternative to conventional 254 nm UVC light for addressing the safety of liquids in a broad range of food-relevant scenarios.

Table S1 displays the fitting results of our far-UVC bacterial-inactivation data for buffer. To determine whether a log-linear or Weibull model has a better fit, the use of R^2 is insufficient (Buzrul, 2022; Serment-Moreno et al., 2015). Therefore, we also used AIC and BIC, which measure the information lost when a model's probability distribution is used to approximate the "true" distribution of data (Serment-Moreno et al., 2015). The smaller the AIC and BIC values, the better the model fits the data (Inguglia et al., 2018).

For our liquid-buffer inactivation data, in the case of both bacteria, the Weibull model had higher AIC and BIC values than the log-linear model did (Table S2). This suggests that the log-linear model was better than the Weibull one (Fig. S2) for estimating far-UVC's impact on bacteria in liquids. Additionally, the R^2 values for both pathogens were >0.97 in the log-linear model, implying a highly linear fitting. Such results suggest that bacterial inactivation in buffer solution using our far-UVC lamp system followed first-order kinetics, potentially because the bacteria in constantly stirred liquid buffer were uniformly treated.

Additionally, our log-linear models' k or slope values (Table S1) were respectively $0.317 \text{ cm}^2/\text{mJ}$ and $0.243 \text{ cm}^2/\text{mJ}$ for *S. Typhimurium* and *L. monocytogenes* in buffer. The reciprocal of k can be used to estimate the dosage required for a 1-log reduction in a microbial population (Unluturk et al., 2010), meaning that $3.16 \text{ mJ}/\text{cm}^2$ and $4.12 \text{ mJ}/\text{cm}^2$ would be needed to achieve 1-log reduction of each of our target pathogens in this medium; and therefore, based on their highly linear fitting results, the dosages needed for 5-log reductions can be estimated as $15.8 \text{ mJ}/\text{cm}^2$ for *S. Typhimurium* and $20.6 \text{ mJ}/\text{cm}^2$ for *L. monocytogenes*.

3.2. Potential mechanisms of bacterial inactivation by 222 nm far-UVC light

Next, we studied the potential mechanisms whereby 222 nm UV treatment was able to inactivate bacteria. Because the PI dye cannot permeate intact membranes, it has been widely adopted for the measurement of bacteria's cell-membrane integrity (Davey and Hexley,

2011). Conversely, when membranes are damaged, PI can penetrate them and bind to nucleic acids within the bacterial cell, at which point it becomes excited by 514-nm light and emits red fluorescence (Crowley et al., 2016). As shown in Fig. 2, which includes bright-field and fluorescent confocal-microscope images, far-UVC-treated samples of both *S. Typhimurium* and *L. monocytogenes* demonstrated higher red-fluorescence intensity than untreated control samples did, indicating loss of membrane integrity and thus the probable death of bacterial cells due to far-UVC treatment (Crowley et al., 2016). This was observed at a lower magnification as displayed in Fig. S3. These observations indicate that far-UVC irradiation could have damaged the cell membrane, contributing to bacterial inactivation. Such an effect was also previously found when far-UVC was used to inactivate fungi (*Aspergillus flavus* and *Fusarium graminearum*) (Jin and Wang, 2024).

Similarly, we used H_2DCFDA dye to study levels of intracellular ROS, buildup of which is another common pathway to bacterial inactivation (Santos et al., 2013). From a cell's perspective, ROSs are undesirable by-products that appear in conjunction with oxidative damage to cell components and potentially can cause cellular injury or even death (Negre-Salvayre et al., 2002). H_2DCFDA can penetrate cell membranes freely, and upon encountering ROS oxidation, it becomes excited by 488-nm light and emits green fluorescence. This phenomenon occurs because H_2DCFDA becomes hydrolyzed in the cell to form the dichloro-fluorescein carboxylate anion, and converts to highly fluorescent 2', 7'-dichlorofluorescein upon ROS oxidation (Kalyanaraman et al., 2012). Therefore, an increase in the intensity of green fluorescence coming from far-UVC-treated samples of either *S. Typhimurium* or *L. monocytogenes* dyed with H_2DCFDA would tend to indicate that such treatment raised ROS (Fig. 3 and S4, which also includes the bright-field and fluorescent confocal-microscope images at high and low magnifications, respectively). Prior research also suggests that ROS damage the molecules making up nucleic acids, proteins, and cytoplasmic membranes (Jin et al., 2023; Kang et al., 2018). As in our above-mentioned observations of cell-membrane integrity, higher ROS levels in both tested samples may have led to damage.

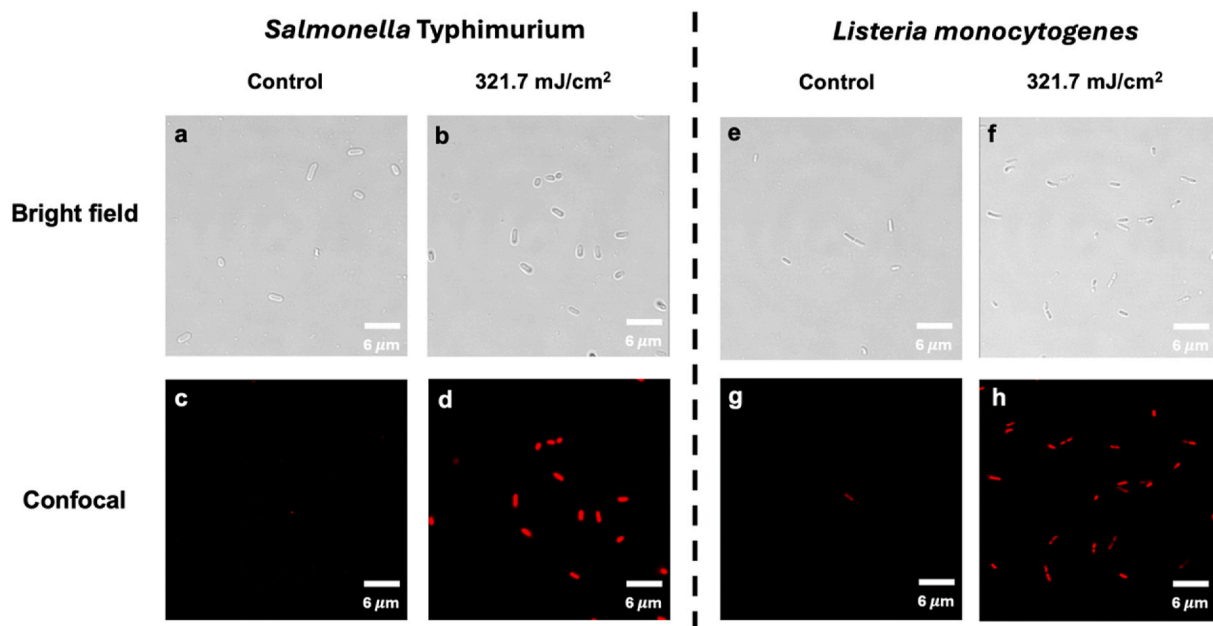


Fig. 2. Results of cell-membrane integrity assay before and after 222 nm far-UVC treatment. (a–d) Bright-field (a,b) and confocal microscope fluorescent images (c,d) of propidium iodide-dyed *Salmonella Typhimurium* that was not treated (control) and treated with a $321.7 \text{ mJ}/\text{cm}^2$ dose of 222 nm far-UVC radiation. (e–h) Bright-field (e,f) and confocal microscope fluorescent images (g,h) of propidium iodide-dyed *Listeria monocytogenes* that was not treated (control) and treated with a $321.7 \text{ mJ}/\text{cm}^2$ dose of 222 nm far-UVC radiation.

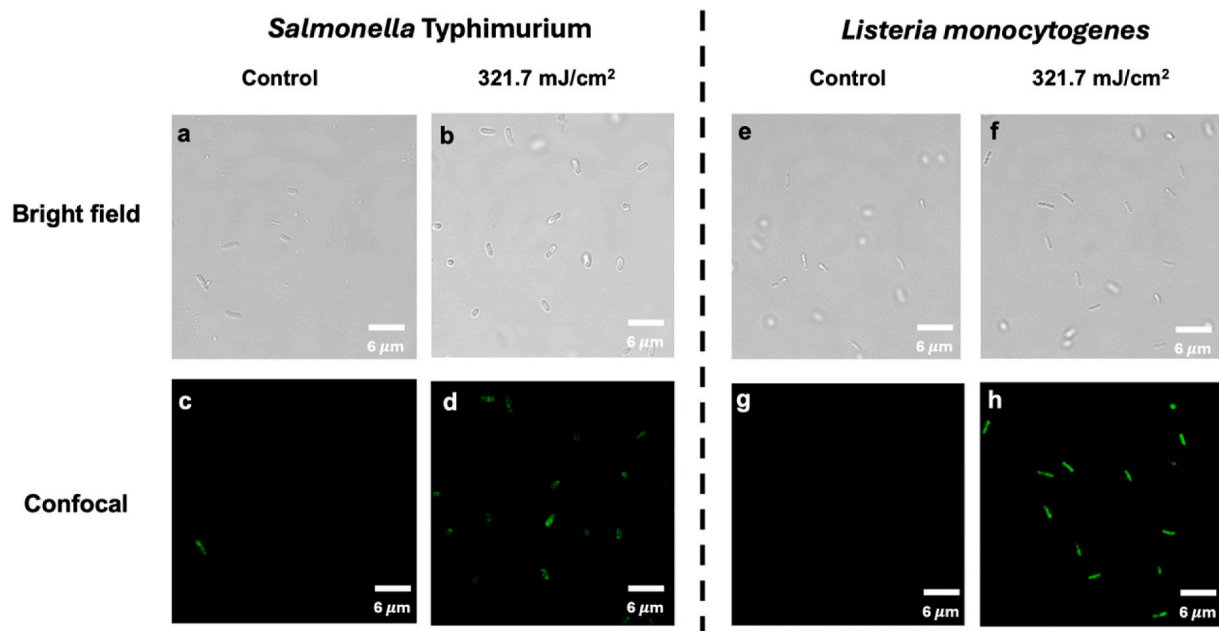


Fig. 3. Results of assays for intracellular reactive oxygen species accumulation before and after 222 nm far-UVC treatment. (a–d) Bright-field (a,b) and confocal microscope fluorescent images (c,d) of H₂DCFDA-dyed *Salmonella* Typhimurium that was not treated (control) and treated with a 321.7 mJ/cm² dose of 222 nm far-UVC radiation. (e–h) Bright-field (e,f) and confocal microscope fluorescent images (g,h) of H₂DCFDA-dyed *Listeria monocytogenes* that was not treated (control) and treated with a 321.7 mJ/cm² dose of 222 nm far-UVC radiation.

3.3. Far-UVC irradiation's efficacy at decontaminating food-contact surfaces

In the case of PET film (Fig. 4a), log reduction of surface bacteria was greater at higher irradiation doses. Specifically, as doses increased, the density of *S. Typhimurium* decreased significantly from an initial level of 6.22 ± 0.01 ($p < 0.05$): to 1.53 ± 0.16 log CFU/cm² at a dose of 64.3 mJ/cm², and to 0.78 ± 0.67 log CFU/cm² at a dose of 128.7 mJ/cm², the latter being a 5.44-log reduction. Further increasing the dose to 193.0 mJ/cm² (or higher) resulted in bacterial density falling below the LOD, which in this instance was 0.377 CFU/cm². In the case of *L. monocytogenes*, as the treatment dose increased from 64.3, to 128.7, to

193.0 mJ/cm², the bacterial concentration underwent reductions of 4.13, 4.28, and 5.07 log CFU/cm²; and at a dose of 257.4 mJ/cm², the density of this strain fell below the same LOD.

Our results for SS coupons were similar. When exposed to dosages of 10.7–21.4 mJ/cm² and 32.2–42.9 mJ/cm², the density of *S. Typhimurium* significantly decreased ($p < 0.05$; Fig. 4b). *S. Typhimurium*'s log reductions ranged from 2.73–2.87 and 4.26–4.87 for the two respective above-mentioned intervals. Further increasing the dose to 53.6 mJ/cm² (or above) resulted in its bacterial density falling below the LOD (0.702 CFU/cm²). In the case of *L. monocytogenes*, log reductions were 1.69–2.13 and 2.90–2.95 for those same intervals. At 53.6 mJ/cm², a 3.74-log reduction was achieved; and at dosage of 64.3 mJ/cm² (or

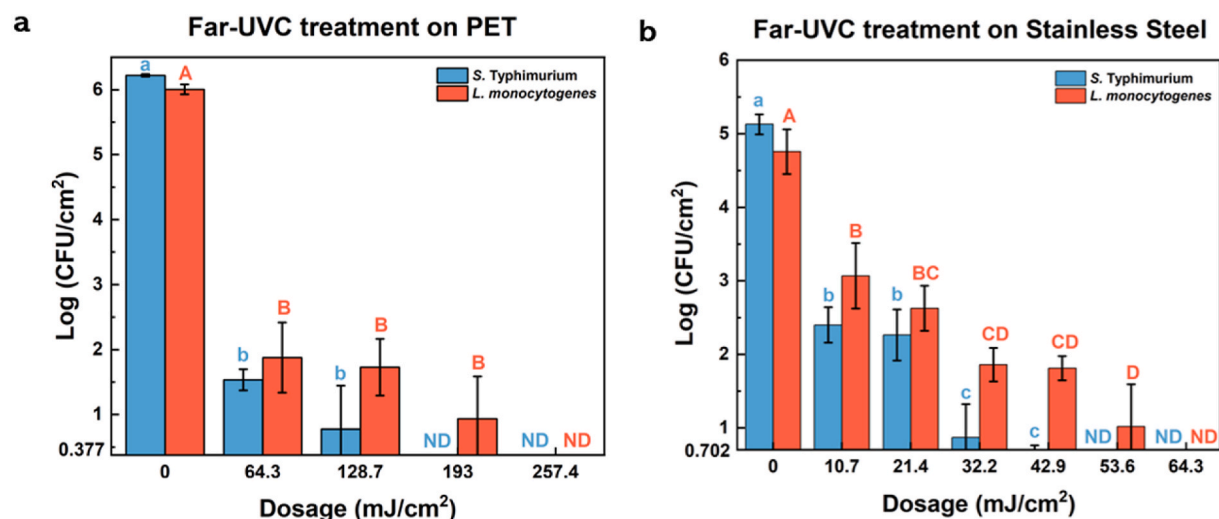


Fig. 4. Average log population in colony-forming units per square centimeter (CFU/cm²) of *Salmonella* Typhimurium and *Listeria monocytogenes* on a) polyethylene terephthalate (PET) film and b) stainless steel after 222 nm far-ultraviolet C (far-UVC) light treatments of various doses, with error bars representing standard deviations. Adjacent treatment-interval results marked with the same letters do not differ significantly, whereas those marked with discrepant letters differ at the 0.05 significance level ($p < 0.05$). ND = not detected; the limits of detection were 0.377 log CFU/cm² for PET and 0.702 log CFU/cm² for stainless steel, and values below each LOD were substituted with half of LOD.

above), the density of *L. monocytogenes* fell below its LOD, which was the same as *S. Typhimurium*'s. Ning et al. (2023) examined the effect of 222 nm far-UVC light on SS inoculated with three different target pathogens – *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* – and achieved log reductions of 2.93–3.25 after exposure of 27–54 mJ/cm². The same study's highest exposure level, 108 mJ/cm², resulted in log reductions ranging from 3.41 to 3.45. However, our results demonstrate higher efficacies at even lower dosages. These differences may have resulted from different bacterial strains being tested, different experimental setups and procedures, lamps, and/or types of surfaces.

These results are also better than those reported in previous studies that used 254 nm light for bacterial decontamination of SS. For example, Sommers et al. (2010) achieved > 5-log reduction in *Salmonella* spp. and *L. monocytogenes* on electroplated and bead-blasted stainless-steel coupons using 400 mJ/cm² of 254 nm UV light. Their study's various dosages – 50, 100, 200, and 400 mJ/cm² – were not precisely comparable to ours, which ranged from 10 to 75 mJ/cm². However, at a nearly equivalent dosage (i.e., theirs: 50 mJ/cm², ours: 53.6 mJ/cm²), Sommers et al. achieved reductions of just 2.5 log for *Salmonella* spp. and of 2.2 log for *L. monocytogenes*, as against our 5.13- and 3.75-log reductions. Similarly, Kim et al. (2002) observed 4.5–4.8 log reductions in *S. Typhimurium* and 3.8–4.0 log reductions in *L. monocytogenes* with 60–90 mJ/cm² doses of traditional UVC, as against the 5.1- log reductions in *S. Typhimurium* and 4.7 log reductions in *L. monocytogenes* we recorded with a similar dosage (i.e., 64.3 mJ/cm²) of far-UVC. Our system's effectiveness was also dramatically better than that of Bae and Lee's (2012) 254 nm UVC system, which achieved 3.06- and 2.08-log reductions for *S. Typhimurium* and *L. monocytogenes*, respectively, at the immensely higher dose of 2548 mJ/cm². Although different experimental setups, operations, and/or procedures may have affected these differences, they are of such magnitude that far-UVC light could be fundamentally more able than traditional UVC to inactivate bacteria on SS.

As compared to their counterparts on SS, pathogens on PET film were more resistant to our far-UVC treatment: i.e., higher doses or longer exposure times were required to effectively control them. This difference may be due to these two materials' distinct surface characteristics and other properties. Such observations align well with results previously reported by Haughton et al. (2011): that when tested on multiple food-contact surfaces including SS, polyethylene-polypropylene films, and aluminum, 254 nm UVC light was least effective on the films. Previous research has also shown that macro patterns on plastic surfaces, such as ridges and hinges, provide environments in which bacteria could hide (Wang et al., 2015). This may explain the higher dosage required for bacterial inactivation on PET than on SS in the present study.

In the case of our food-contact surface data, the log-linear fitting results fell into R² value ranges of 0.76–0.80 for PET (Fig. S5) and 0.82–0.84 for SS (Fig. S6). The AIC and BIC values for the Weibull model of the same data were lower in the cases of both bacteria (Table S2). These results suggest that, for this purpose, the Weibull model is the more accurate of the two (Figs. S5 and S6). This non-linear characteristic could have been because, as briefly noted above, the roughness of the tested food-contact surfaces provided a "shadow effect" that prevented the bacteria on them from being uniformly exposed to far-UVC treatment (Belloli et al., 2022; Haughton et al., 2011). However, none of this is to suggest that a linear model, which is a much simpler model, should not be used in practice to provide a rough estimate. Rather, we are suggesting that the Weibull model, due to its ability to capture both rapid inactivation in the early stage of treatment and a tailing effect at the higher doses, could provide even more accurate information.

Based on the above finding that Weibull modeling is likely to provide better estimation in the case of food-contact surface data, we used it to estimate the dosage needed for the first decimal reduction of each pathogen (Table S3). In the resulting model, α can be used to calculate the dosage needed for the first log reduction (van Boekel, 2002). The relevant dosages for *S. Typhimurium* and *L. monocytogenes* on PET were

16.9 mJ/cm² and 32.2 mJ/cm², respectively; and in the case of SS, 4.9 mJ/cm² and 10.1 mJ/cm², respectively.

3.4. Far-UVC irradiation's efficacy at decontaminating RTE turkey breast

Fig. 5a illustrates our second, two-lamp treatment chamber. As shown in Fig. 5b, the initial level of *S. Typhimurium* was 7.19 ± 0.05 log CFU/g, and our system achieved a 1.01 log reduction at its highest dosage of 786.3 mJ/cm². In the case of *L. monocytogenes*, the initial concentration was 6.98 ± 0.12 log CFU/g and was reduced to 5.63 log CFU/g at the same dosage: a 1.35 log reduction. Both these log reductions were significant ($p < 0.05$). Notably, unlike in our buffer and contact-surface experiments, *L. monocytogenes* showed lower resistance to UV than *S. Typhimurium* did. Some other studies have reported similar observations. For example, Sommers et al. (2010) examined 254 nm UVC's potential for inactivating *Salmonella* spp. and *L. monocytogenes* on the surfaces of boneless pork chops, boneless skinless chicken breasts, and chicken drumsticks, and found that such light's inactivation efficacy was higher for *L. monocytogenes* than for *Salmonella* spp. In another study, Chun et al. (2009) examined UVC's efficacy at decontaminating RTE sliced ham and reported that higher log reductions were achieved for *L. monocytogenes* than *S. Typhimurium*. Additionally, it is worth noting that, because we introduced a cooling period after each 60 s of treatment, the bulk of the bacterial inactivation we observed would have been due to far-UVC irradiation.

Our system's markedly lower ability to inactivate microbes on RTE turkey breast than in liquid buffer or on food-contact surfaces could be due to the food samples' chemical properties and/or their physical ones including surface topography (Chun et al., 2009). Similar results were previously reported by researchers who used far-UVC light to decontaminate cereals (Jin and Wang, 2024) and cheese (Ha et al., 2017). In general, light-based technologies are surface-sterilization methods, i.e., most effective at inactivating bacteria on or very near the surface of the target object (Lyon et al., 2007). Different compositions and surface topographies of food products may therefore critically impact the efficacy of light-based technologies for reducing microbial contamination (Allende et al., 2006; Yaun et al., 2004). For example, the cut edge of meat could provide enough protection to shield bacteria from exposure to UV light (Stermer et al., 1987). Food samples such as meat are naturally porous and irregularly shaped (Adhikari et al., 2015; Yaun et al., 2004), and either or both of those characteristics could also have contributed to the differences in our system's inactivation efficacies across *S. Typhimurium* and *L. monocytogenes*. Nevertheless, >90% reduction for both bacteria (96% for *L. monocytogenes* and 92% for *S. Typhimurium*) demonstrates such technology's potential for contributing to improved food safety.

Our results are also comparable to, and in most cases better than, the previously published results of meat-decontamination experiments with conventional 254 nm UVC light. For example, Kim et al. (2002) found that the highest dosage of 254 nm light they tested, 90 mJ/cm², could inactivate 0.36–1.02 logs of *S. Typhimurium* and 0.46–0.48 logs of *L. monocytogenes* on the surfaces of chicken meat, both with and without skin. Kim et al. (2014) showed that conventional UVC light at 2700 mJ/cm² could reduce *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 on beef by 0.33, 0.21, and 0.27 log, respectively; and Kalchayanand et al. (2020) inoculated beef tissues with *Salmonella* spp. and *L. monocytogenes* and achieved 1.09 and 0.89 log reductions using 590 mJ/cm² of conventional UVC treatment. Our results therefore demonstrate that 222 nm far-UVC lamps can potentially outperform their non-human-safe conventional 254 nm counterparts in terms of overall log reduction.

Efforts towards further improvement could adopt a hurdle approach in which far-UVC irradiation is coupled with one or more other non-thermal decontamination techniques. In a previous example of such an approach, Colejo et al. (2018) evaluated the combination of conventional UVC and non-thermal atmospheric plasma (NTAP) to control

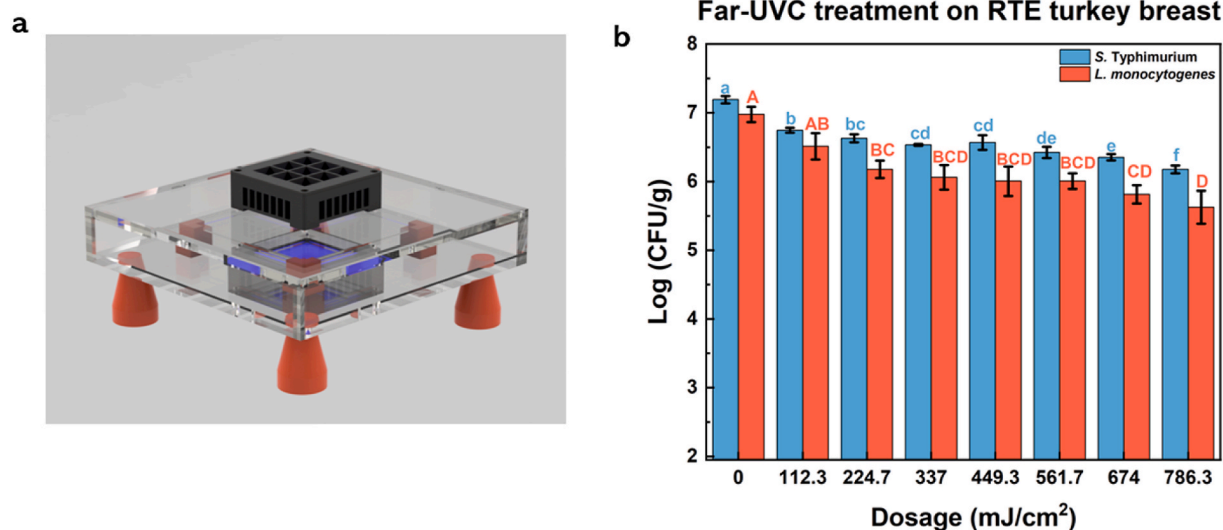


Fig. 5. a) The developed 222 nm far-ultraviolet C (far-UVC) light system for treating ready-to-eat (RTE) turkey breast. b) Average log population in colony-forming units per gram (CFU/g) of *Salmonella* Typhimurium and *Listeria monocytogenes* on RTE turkey breast after far-UVC light treatment at various doses, with error bars representing standard deviations. The limit of detection was 2 log CFU/g. Adjacent treatment-interval results marked with the same letters do not differ significantly, whereas those marked with discrepant letters differ at the 0.05 significance level ($p < 0.05$).

foodborne pathogens on smoked salmon. Specifically, they found that 500 mJ/cm² of 254 nm UVC coupled with 8 min of NTAP resulted in 0.71-log reductions of *L. monocytogenes* and 1.33-log reductions of *S. Typhimurium*. Sommers et al.'s (2009) combination of 254 nm UVC with flash pasteurization attained an even better result, i.e., a greater than 3-log reduction of *Listeria innocua* on frankfurters.

The fitting results of both model types' inactivation kinetics for both pathogens as inoculated on RTE turkey breast are shown in Fig. S7. In the case of the *S. Typhimurium*-inoculated turkey, the AIC and BIC values for the log-linear and Weibull models were very similar (Table S2). However, in the case of turkey inoculated with *L. monocytogenes*, the Weibull model exhibited slightly lower AIC and BIC values. Thus, it was unclear which model was superior for estimating bacterial inactivation on this type of food sample. This could have been due to a slow decrease in microbial inactivation on the meat samples: Chun et al. (2009) similarly reported, regarding 254 nm light decontamination of an RTE pork product, that neither log-linear nor Weibull models were a good fit for the inactivation of *S. Typhimurium* and *Campylobacter jejuni*, but that the Weibull model showed a better fit for the inactivation of *L. monocytogenes*.

3.5. Evaluation of color changes in RTE turkey breast following far-UVC irradiation

Table 1 summarizes the color effects of treatment with our system, using the CIELAB color space – also referred to as L*, a*, b* – to describe meat samples' colors. Samples that had been treated with far-UVC light at dosages of 112.3, 224.7, 337.0, 449.3, 561.7, 674.0, and 786.3 mJ/cm² were compared against untreated ones, and color differences determined with a significance level set at 0.05. This established that the impacts of far-UVC irradiation on lightness (L*), redness (a*), and yellowness (b*) values were insignificant until dosages reached 449.3 mJ/cm², 674.0 mJ/cm², and 337.0 mJ/cm², respectively.

Our finding that the b* values of RTE turkey breast changed significantly at the third-lowest dosage we tested accords well with Haughton et al.'s (2011): that significant changes occurred for a* and b* values of 254 nm UVC-treated skinless chicken breast at 160 mJ/cm² and 192 mJ/cm², respectively. Such color changes might be associated with the accumulation of metmyoglobin on the surface (Kannan et al., 2001; Wambura and Verghese, 2011). It has also recently been reported that exposing meat to UV light can potentially cause oxidation, which could

Table 1
Color parameters (L*, a*, and b*) of ready-to-eat turkey breast samples following various dosages of far-ultraviolet C treatment.

Dosage (mJ/cm ²)	Color parameters		
	L*	a*	b*
Control (no treatment)	75.30 ± 0.37 ^a	1.60 ± 0.75 ^a	11.85 ± 1.09 ^a
112.3	75.49 ± 0.98 ^a	1.60 ± 0.49 ^a	12.74 ± 0.68 ^a
224.7	74.79 ± 1.33 ^a	1.16 ± 0.20 ^a	12.66 ± 0.67 ^a
337.0	74.55 ± 1.01 ^a	1.50 ± 0.32 ^a	13.34 ± 0.85 ^b
449.3	74.69 ± 0.40 ^b	1.64 ± 0.58 ^a	13.68 ± 0.39 ^b
561.7	74.61 ± 0.58 ^b	1.78 ± 0.27 ^a	14.21 ± 0.85 ^b
674.0	74.56 ± 0.20 ^b	0.91 ± 0.48 ^b	14.40 ± 0.80 ^b
786.3	74.59 ± 0.56 ^b	2.21 ± 0.30 ^b	14.56 ± 0.47 ^b

Note. Values (mean ± standard deviation of three independent treatments) followed by a different superscript letter in the same column represent significant differences ($p < 0.05$).

lead to color changes (Monteiro et al., 2023; Wang et al., 2023). Our color results can be used as a basis for optimizing far-UVC dosages and processes to ensure RTE turkey products' safety without severely compromising their color quality.

Additionally, future research could usefully focus on the combination of inactivation technologies with preservation methods that would help increase their effectiveness: i.e., achieve the desired lethal effect without compromising food products' sensory characteristics and nutritional value. For example, Ha and Kang (2015) treated RTE sliced ham with near-infrared heating and UVC irradiation simultaneously, and reported only a slight increase in a* and b* values and a slight decrease in L* values, with no statistically significant differences, over treatment intervals of up to 70 s. Similarly, Colejo et al. (2018) found that 500 mJ/cm² of 254 nm UVC coupled with 8 min of NTAP resulted in only minor effects on quality properties. Moreover, Sommers et al.'s (2009) combination of 254 nm UVC with flash pasteurization had little effect on color or texture.

4. Conclusion

This study evaluated the antibacterial efficacy of microplasma-based 222 nm far-UVC light against *S. Typhimurium* and *L. monocytogenes* in

buffer, on two common food-contact surfaces, and on RTE turkey breast. Both pathogens were reduced to undetectable levels in buffer and on PET and SS surfaces, demonstrating the strong potential of far-UVC light systems to enhance microbiological safety. All three types of non-meat samples underwent approximately 5-log reductions or more: i.e., $\geq 99.999\%$ of the selected pathogens were inactivated. These findings regarding liquid and food-contact surfaces strongly support the utilization of far-UVC to mitigate cross-contamination concerns. The less impressive log reductions achieved in the case of RTE turkey breast could have been due to the pronounced roughness of the meat's surface. Importantly, however, our results were comparable to previously published work on meat decontamination with conventional UVC. Lastly, our pre-and post-treatment measurements of RTE turkey breast's color were not significantly different when doses were below 224.7 mJ/cm^2 . However, higher doses may lead to statistically significant color change.

In conclusion, our results show the excellent potential of microplasma-based 222 nm far-UVC systems to serve as an alternative to conventional 254 nm UVC ones for enhancing food safety. The non-harmful characteristics of 222 nm far-UVC to human skin and eyes potentially make it more suitable for microorganism control in the presence of consumers and/or workers. Therefore, such technology may make a major contribution to mitigating RTE food-related safety issues in retail establishments, and thus to building a safer and more resilient food system.

CRediT authorship contribution statement

Sei Rim Kim: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Paola Corea Ventura:** Writing – review & editing, Investigation. **Zhenhui Jin:** Writing – review & editing, Visualization, Methodology, Investigation. **Mirai Miura:** Investigation. **Matthew J. Stasiewicz:** Writing – review & editing, Validation, Methodology, Investigation. **Yi-Cheng Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following competing financial interest(s):

Professor Yi-Cheng Wang is concurrently collaborating with the co-founder and chief technology officer of Eden Park Illumination, Inc., Dr. Sung-Jin Park, on a Small Business Innovation Research project sponsored by the U.S. Department of Energy, which is unconnected to the present work. Dr. Park provided technical support with the far-UVC lamps used in the present work, but had no direct involvement in it, and placed no restrictions on any part of its study design; its data collection, analysis, or interpretation; or its publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2024.104674>.

Data availability

Data will be made available on request.

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