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Enhancing food safety: Employing ultraviolet-C light emitting diodes for water, leaf, and surface disinfection

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ABSTRACT

This study aimed to explore the use of ultraviolet-C light emitting diodes that emit light at 255 nm and 265 nm, to disinfect bacteria present at occurrence levels and spiked in water matrices, salads and stainless-steel surfaces. The UV-C LEDs effectively inactivated bacteria associated with food outbreaks (*Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*) as well as a cocktail of bacteria isolated from packaged salads. Combining the wavelengths did not enhance disinfection. Even low UV fluences of 4 mJ/cm^2 achieved a significant 6-log reduction of bacteria spiked in water at high initial concentrations of approximately 10^8 CFU/mL. Exposure of salad (lettuce and arugula) leaves (110 mJ/cm^2) and contaminated stainless-steel surfaces (11 mJ/cm^2) to three small LEDs that emit light at 265 nm reduced the pathogenic bacteria by 3 and 2-logs, respectively. The results obtained show that this disinfection technology could be promising for the food industry to guarantee effective inactivation of bacteria associated with foodborne diseases present in water, food and surfaces.

Industrial relevance: This study demonstrated the potential of ultraviolet-C light emitting diodes, emitting at 255 nm and 265 nm, to provide an effective and sustainable disinfection solution for the food industry, ensuring the inactivation of bacteria associated with foodborne diseases on water, food, and surfaces, thereby enhancing food safety. Additionally, this technology holds potential for extending product shelf-life, further benefiting the food industry. The results highlighted the effectiveness of UV-C LEDs even at low fluences, making them a practical choice for modern disinfection needs.

1. Introduction

Fresh fruits and vegetables are essential components of a healthy diet (International Food Policy Research Institute, 2024). A diet rich in these foods has been linked to a reduced risk of heart disease, stroke, and diabetes, as well as lower weight, blood pressure, and incidence of cancer. Additionally, it may help to maintain gastrointestinal health and lower the risk of certain eye diseases, dementia, and osteoporosis (Boeing et al., 2012). Over the past decade, there has been a growing demand for fresh fruits and vegetables due to national and international campaigns aimed at increasing consumer awareness about the importance of incorporating these foods into daily diets (International Food Policy Research Institute, 2024).

In households, canteens, or restaurants, fruit consumption typically involves direct washing or peeling before consumption. Similarly, the consumption of both fruits and raw vegetables often entails minimal washing and slight disinfection. These products encompass various fresh fruits and vegetables that undergo specific post-harvest procedures and subsequent packaging in sealed containers, making them readily available for purchase and consumption, either raw or cooked (FAO and Ministry of Social Development and Family of Chile, 2021).

However, due to the minimal processing and frequent consumption in their raw state, ready-to-eat fresh vegetables and fruits can contain food-spoiling bacteria, yeasts, and fungi, and may also be a source of pathogens that can lead to foodborne disease outbreaks (Pandey et al., 2024; Sequino et al., 2022; Taibi et al., 2022; Zhang et al., 2020). In 2010, the World Health Organization estimated that foodborne diseases were responsible for the loss of 33 million healthy life years (Havelaar et al., 2015). In the last decade, *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* have been associated with most of the bacterial outbreaks of foodborne illness associated with fresh products (Thomas et al., 2024). Consequently, the presence of pathogenic and spoilage

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microorganisms on ready-to-eat fresh vegetables and fruits is a significant concern for the food industry (Castro-Ibáñez et al., 2017).

Disinfection is recognized as a crucial processing step to ensure the quality, safety, and shelf-life of fresh fruits and vegetables. It involves the inactivation of microorganisms present in water used in food washing processes, food, and surfaces. Disinfection processes in the food industry are additionally challenged by bacteria that besides growing in a sessile form can also be a part of a biofilm. Biofilm growth offers protection to microorganisms against various environmental stress conditions and renders them more resistant to removal and disinfection (Yuan et al., 2021).

Chlorine has been widely used for disinfection due to its efficacy, relatively low cost, and ease of application. However, it is known to react with natural organic matter, leading to the formation of hazardous disinfection by-products, such as trihalomethanes and haloacetic acids (Gadelha et al., 2019; Li et al., 2022).

Various biological, chemical, and physical disinfection methods have been proposed as alternatives to chlorine (Meireles et al., 2016). Recent studies have suggested that UV light could serve as a promising alternative disinfection method for the food industry (Kebbi et al., 2020; Prasad et al., 2020), given its effectiveness in inactivating a wide range of microorganisms (Kim et al., 2017; Oguma et al., 2002, 2013; Oliveira et al., 2013; Oliveira et al., 2021; Rattanakul & Oguma, 2018).

UV-C light emitting diodes (LEDs) appear to be a promising disinfection alternative compared to the widely used mercury lamps due to their sustainability, longer lifetimes, lower costs, energy consumption and maintenance as well as compact size. Small UV LED modules can be easily incorporated into various food processing devices (Kim et al., 2016; Kim et al., 2017; Murashita et al., 2017; Nyhan et al., 2021; Shin et al., 2016). Another advantage of LEDs is their wavelength diversity. LEDs emitting light at different UV-C wavelengths can be acquired and used for disinfection purposes. Low pressure mercury lamps that emit primarily monochromatic light at 254 nm are commonly used for disinfection of wastewater and drinking water. LEDs emitting light at wavelengths such as 255 nm (close to the wavelength emitted by conventional low pressure mercury lamps) and 265 nm (which overlaps better with DNA absorbance, with a maximum peak around 260 nm) have been investigated for their inactivation efficiency. Studies by Oliveira et al. (2020, 2021) have indicated that LEDs emitting light at 265 nm were more effective than those emitting light at 255 nm in inactivating various *Aspergillus* species spiked in real surface water samples.

In this study, the efficiency of UV-C LEDs emitting light at different wavelengths (255 nm and 265 nm) was evaluated in terms of their ability to inactivate different microorganisms (bacteria, filamentous fungi, and yeasts) present in washing water and salad leaf samples obtained from a fresh food industry. The washing water and salad leaf samples were also spiked with different species of bacteria associated with foodborne outbreaks (*S. enterica*, *L. monocytogenes*, and *E. coli*), as well as bacteria isolated from packaged salads (*Pseudomonas baetica*, *Pseudomonas korrensis*, *Pseudomonas rhodesiae*, *Pseudomonas flavescens*, *Pseudomonas putida, Pantoea autrophlia*, *Pantoea ananatis, Pantoea agglomerans*, *Exiguobacterium artemiae* and *Erwinia persicina*), to assess the potential of UV-C LEDs as a food disinfection technique. Furthermore, UV-C LEDs emitting light at 265 nm were tested for their ability to disinfect stainless-steel discs contaminated with *L. monocytogenes*.

2. Material and methods

2.1. Salad and water samples collection and characterization

Samples were collected in a producer of fourth range fresh fruit and vegetable products. Three types of samples were chosen: (i) water samples collected from two different leaf washing tanks (one with and other without chlorine disinfection); (ii) a mixture of baby green lettuce and arugula leaves collected before the washing step; (iii) a mixture of lettuce and arugula leaves collected after the packaging step. The samples were stored at 4 °C and analyzed within 18 h of collection. The water samples collected were characterized in the laboratory in terms of pH, total organic carbon, chemical oxygen demand, total solids and total suspended solids using standard methods (APHA, 1995).

2.2. Inactivation experiments using ultraviolet-C light emitting diodes

A PearlLab Beam™ LED reactor (AquiSense Technologies, USA) was used to test the inactivation of different microorganisms in water and salad samples as well as the disinfection of contaminated surfaces (Fig. 1). Stainless-steel discs (10 mm diameter) with an area of 79 mm^2 were used since this material is highly used by the food industry.

The inactivation experiments were performed in a Class II biological safety cabinet (NuAire). All the inactivation experiments were performed at cold temperature to mimic the processing conditions in industry. To ensure temperature control, the samples were placed in a refrigerated double walled Petri dish with internal circulation of cold water maintained at 4 °C (using a B. Braun Thermomix BU water bath). A magnetic stirrer was used to ensure homogeneous mixing of the liquid samples placed in the refrigerated double walled Petri dish. The PearlLab Beam™ LED reactor contains three small UV-C LEDs that emit light at different wavelengths, a UV homogenizing collimating tube, and a wavelength selector. The wavelengths tested in this study were 255 nm, 265 nm and the combination of both wavelengths (3 LEDs that emit at 255 nm and 3 LEDs that emit at 265 nm). The wavelengths 255 nm and 265 nm were tested due to their overlap with the wavelength emitted by the conventionally used low pressure mercury lamps (that emit monochromatic light at 254 nm) and DNA absorbance (with a maximum peak around 260 nm). The average light intensity was measured using an ILT 950 UV Spectroradiometer (Massachusetts, USA) at 4 cm, the same height used to perform the inactivation experiments. The average light intensity values measured were: (i) 93.5 μW/cm^2 for the 3 LEDs that emit at 255 nm; (ii) 203.9 μW/cm^2 for the 3 LEDs that emit at 265 nm; and (iii) 293.1 μW/cm^2, for the combination of both wavelengths. Fig. S1 shows the emission spectra of UV-C LEDs that emit light at 255 nm and 265 nm when illuminated separately and combined. The LEDs that emit light at 265 nm have a much higher irradiance than the LEDs that emit light at 255 nm. To account for this difference in irradiance and enable the comparison of inactivation experiments conducted with other setups, the UV fluence (mJ/cm^2) was determined as the product of the fluence rate (irradiance) and the exposure time in seconds. The average irradiance values were determined considering different correction factors (reflection, petri dish, water, and divergence) as described by Bolton and Linden (2003). The following correction factors were used: 0.98 for the reflection factor, 0.90 for the Petri factor and 0.64 for the divergence factor. The water factor, that considers the sample absorbance at the wavelengths of interest, was determined for the liquid samples. The absorbance of all the cell suspensions tested were similar at the wavelengths emitted by the LED system (Fig. S2).

Inactivation experiments with spiked lettuce and arugula leaves from packaged ready-to-eat salads were also performed on a tank with 10 L capacity and two submerged custom-made LED panels that emit light at 265 nm to test the efficiency of inactivation at a larger scale (Fig. 2). The reactor used was previously described by Fraga et al. (2019) and Marques et al. (2022). The light intensity measured in each of the LED panels was 254 ± 34 μW/cm^2 and 279 ± 7 μW/cm^2. The distance from the light source to the water sample was 4 cm. A chiller (Hailea® Model HC-1000 A) was used to maintain the samples at a constant temperature (4 °C). The tank was totally covered during the experiments to guarantee personal protection from the light. The inactivation experiments were performed in duplicate.

2.2.1. UV-C LED inactivation of microorganisms present at occurrence levels in water and salad samples

Inactivation experiments using the PearlLab Beam™ LED reactor (depicted in Fig. 1) were conducted using unspiked water samples from

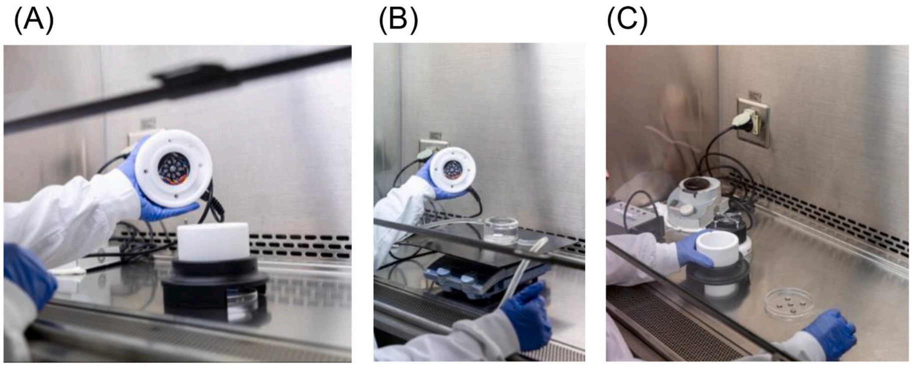


Fig. 1. Inactivation experiments illustration. (A) PearlLab Beam reactor (AquiSense Technologies, USA) used in the inactivation assays. (B) Water inactivation assay representation. (C) Stainless-steel discs disinfection assay.

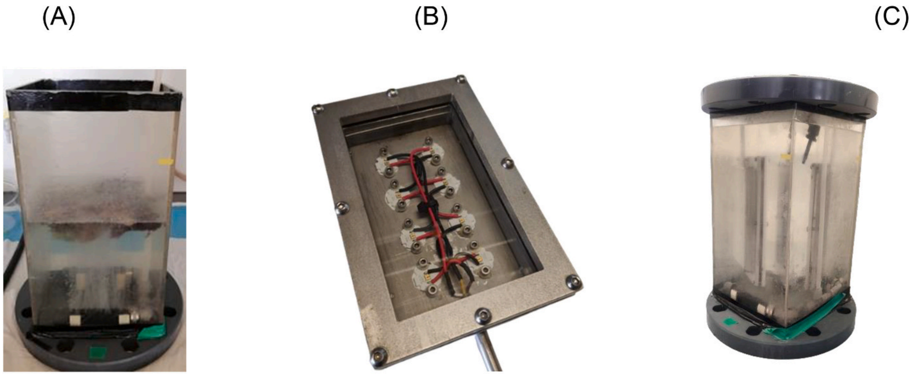


Fig. 2. Setup used for larger scale inactivation assays (A) tank with water and leaves; (B) custom-made LED panel that emits light at 265 nm; (C) tank with two LED panels.

washing tanks (a washing tank without chlorine added and a washing tank with added chlorine), salad leaf samples of lettuce and arugula collected before the washing process as well as after processing and packaging.

Total microorganisms, total coliforms, *E. coli,* enterococci, filamentous fungi, and yeasts were quantified in the collected samples. Before and after the inactivation experiments, serial ten-fold dilutions of the samples were prepared and analyzed following different methods for the different target microorganisms. Lettuce and arugula leaves collected before washing and from ready-to-eat salad packages were analyzed for total microorganisms at 30 °C according to standard ISO 4833-1:2013 and ISO 4833-1:2013/Amd 1:2022. Water samples from washing tanks, without and with chlorine, were analyzed for total microorganisms at 36 °C according to standard ISO 6222:1999. Quantification of total coliforms, *E. coli* and enterococci was performed by the most probable number (MPN) using the Colilert-18 and Enterolert-E Quanti-Tray 2000 assays (IDEXX, USA) according to ISO 9308-2:2012 and ISO 7899-1:1998. Yeasts and molds were enumerated by spreading 0.1 mL of sample into malt extract agar (Oxoid, UK). The medium was supplemented with 10 mg/L of chloramphenicol (Oxoid, UK) to inhibit bacteria growth. The plates were incubated at 25 °C for 5 days.

The unspiked samples were then exposed to the UV-C LEDs that emit light at 255 and 265 nm, during 5 min (water samples) and 10 min (lettuce and arugula leaves). Samples were analyzed before UV exposure and after exposure. In the experiments conducted with the salads, one leaf of lettuce and one leaf of arugula (~2.5 g) were placed in a refrigerated double walled Petri dish and exposed to UV-C LEDs on both sides of the leaf (Fig. 1).

2.2.2. UV-C LED inactivation of bacteria spiked in water samples

The inactivation assays conducted with the PearlLab Beam™ LED reactor (depicted in Fig. 1) were performed with the samples described above (microorganisms present in water and leaves at occurrence levels) as well as the same matrices spiked with different bacterial strains.

The spiked bacterial strains included isolates from a packaged ready-to-eat salad of lettuce and arugula (*Pseudomonas baetica*, *Pseudomonas korrensis*, *Pseudomonas rhodesiae*, *Pseudomonas flavescens, Pseudomonas putida, Pantoea autrophlia*, *Pantoea ananatis, Pantoea agglomerans, Exiguobacterium artemiae* and *Erwinia persicina*), as well as pathogens associated with associated with the contamination of fruits and vegetables such as *S. enterica* serovars Typhimurium, Wernigerode and Derby, *L. monocytogenes* and *E. coli*, regularly tested in ready to eat foods (Commission Regulation (EC) No 2073/2005—EN - EUR-Lex, 2020). All the isolates were identified (by 16S rRNA sequencing) and provided by the Food & Nutrition Group from Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal. All the bacterial strains were preserved on tryptic soy broth (VWR, USA) and glycerol at −80 °*C. Prior* to use, each strain was streaked on tryptic soy agar (VWR, USA). *S. enterica* and *E. coli* strains were grown at 37 °C for 24 h, *L. monocytogenes* was grown at 30 °C for 24 h, and the bacteria isolated from packaged salad were grown at 22 ± 2 °C for 48 h. The bacterial strains were then grown in 100 mL tryptic soy broth (VWR, USA) at 37 °C for 24 h (*S. enterica* and *E. coli* strains), at 30 °C for 24 h (*L. monocytogenes*) and at 22 °C for 48 h (bacteria isolated from packaged salad).

After bacterial grow, the cells were harvested by centrifugation at 6000 ×*g* for 10 min at 4 °C and washed twice with phosphate buffered saline solution (PBS). The final pellets were resuspended in PBS as well as in the water samples collected from the washing tank. The real water samples were filtered (using nitrocellulose membranes with a pore size of 0.2 μm PALL, NY, USA) and autoclaved (121 °C for 15 min) prior to being used to resuspend the cells to remove any microorganisms that might be present in the water. The two different procedures were followed because filtration might remove solids and natural organic matter that may protect the microorganisms from inactivation whereas autoclaving the samples may alter the natural organic components. Cell suspensions were enumerated by spectrophotometry and colony forming unit counting and diluted to an initial spiked concentration of approximately 10^8 CFU/mL. The absorption spectrum of these suspensions was measured for each bacterium using a Biochrom Ultrospec 2100 Pro UV/Vis spectrophotometer in quartz cuvettes with a 1 cm path length (Fig. S2). High cell densities were used to maximize the resolving power of disinfection measurements. Bacterial cocktails were prepared by mixing equal amounts of each bacterial strain.

Each sample was analyzed at the beginning (time zero) and after different exposure times (2 min, 5 min and 10 min) to each LED wavelength (255 nm and 265 nm) as well as their combination. A control, not exposed to UV-C LEDs, was performed in all the inactivation experiments to verify if the concentration of microorganisms remained stable during the experimental time. The concentration of the different isolates in the control experiments (samples not exposed to UV-C LEDs) was stable throughout the inactivation experimental time.

2.2.3. UV-C LED inactivation of bacteria spiked in salad samples

In these experiments, lettuce and arugula leaves were spiked with a bacterial cocktail of *S. enterica* Typhimurium, *L. monocytogenes* and *E. coli* by immersing the leaves in a cell suspension at a concentration of 10^8 CFU/mL for 30 min followed by air drying for 45 min. The concentration of the bacteria measured on the leaves after drying was 10^5 CFU/g. The spiked leaves (~50 g) were placed in the large container (laboratory washing tank) with 6 L of sterile distilled water and two submerged custom-made UV-C LED panels that emit light at 265 nm (Fig. 2) to predict if disinfection can be expected when the leaves are placed in the large food industry washing tanks. After 5 min and 10 min of exposure to UV-C LED panels the leaves and the water were analyzed for the enumeration of *S. enterica* Typhimurium, *L. monocytogenes* and *E. coli* using chromogenic media (Rapid'Salmonella plates, Rapid'l. *mono* plates and Rapid'E.coli2 plates, respectively) from Bio-Rad (U.S.A).

2.2.4. UV-C LED inactivation of bacteria spiked on stainless-steel surfaces

Three small LEDs that emit light at 265 nm (PearlLab Beam™ LED reactor depicted in Fig. 1) were also tested in terms of the potential inactivation of bacteria on contaminated stainless-steel surfaces. The stainless-steel discs (10 mm diameter; 79 mm^2) were degreased overnight in a 96 % ethanol solution, then rinsed with distilled water, and sterilized in an autoclave for 15 min at 121 °C before use.

The stainless-steel surfaces were tested unmodified and modified. The modification procedure was based on a solvent free method proposed by Huertas et al. (2019). The stainless-steel discs were contaminated by spiking with *L. monocytogenes* as well as used to produce *L. monocytogenes* biofilms. In the spiked assays, the discs were immersed in the bacteria suspensions (10^8 CFU/mL) for 10 min and then they were left to dry in a sterile Petri dish. On the other hand, the biofilm production and quantification for *L. monocytogenes* was optimized in a previous study (Santos, 2022). The optimal temperature and incubation time were evaluated, and the results indicated that a higher biofilm production was obtained at 4 °C and room temperature after 7 incubation days. To produce the L. *monocytogenes* biofilm for the inactivation studies, 20 mL of the bacteria suspension (10^8 CFU/mL) were transferred to two sterile Petri dishes with 5 stainless-steel discs placed in each Petri dish: one suspension with 5 discs was incubated at room temperature and the other suspension with 5 discs left at 4 °C for 7 days.

The contaminated stainless-steel discs were placed in a Petri dish on top of ice to maintain a cold temperature during the exposure to the three small LEDs that emit light at 265 nm for 2.5 min (27 mJ/cm^2) and 5 min (55 mJ/cm^2). The contaminated discs (without biofilms) were exposed during 1 min (11 mJ/cm^2), 3 min (33 mJ/cm^2) and 5 min (55 mJ/cm^2). Five discs were not subject to UV-C LED light to quantify the initial CFU/mL. To recover the cells that were attached to the surface, the stainless-steel discs were transferred to Falcon tubes containing 5 mL of PBS and were vortexed for 10 min. From this initial cell suspension, serial dilutions were prepared and 100 μL of each dilution were plated in tryptic soy agar. The tryptic soy agar Petri dishes were incubated overnight at 37 °C, and the colonies were counted. To evaluate the efficacy of this method of disinfection, the log reduction was calculated based on the ratio of the initial bacteria concentration and the bacteria concentration after the exposure to the UV-C LEDs (expressed in colony forming units/area).

The untreated and treated stainless-steel surfaces were also analyzed by scanning electron microscopy (SEM) to evaluate possible cell morphology damages after photolysis. To prepare the samples for SEM analysis, a protocol previously described by Oliveira et al., (2020) that was adapted from Panngom et al. (2014) was followed. Briefly, the samples were washed with phosphate buffer (pH 7) for 5 min and fixed in Karnovsky's Fixative (Polysciences Inc., Germany) overnight following the protocol's recommendations (2.5 % (*v*/v) of glutaraldehyde, 2.0 % (v/v) of paraformaldehyde and 0.1 M of PBS solution. Samples were then washed three times with PBS for 10 min and centrifuged at 10000 *g* for 5 min. Pellet was fixed with osmium tetroxide (1.0 % v/v) for 2 h protected from light. Samples were then dehydrated using several ethanol solutions with a gradient increase in concentration (30, 50, 70, 80, 90, 95 and 100 %) for 10 min being only the last dehydration step (100 %) performed twice for 15 min. Lastly, the samples were freeze dried for 30 min and mounted in carbon conductive tape. The SEM analysis was done using a JSM 7001F microscope after sputtering the samples with an Au/Pd thin film using a Quorum Technologies Q150T ES model device.

2.2.5. Determination of logarithmic reductions and inactivation rate constants

The disinfection efficiencies were experimentally evaluated by monitoring the reduction in the viable bacteria concentrations (measured in terms of colony forming units per milliliter of sample or most probable number per 100 mL, depending on the target microorganisms) as a function of the UV fluence applied. The direct photolysis inactivation rate constants (k_f) were determined in the different inactivation assays as the slope of the following linear regression:

$$log\left(C_0/C\right) = k_f \times \text{UV fluence}$$

where the log-reduction, $\log(C_0/C)$ represents the logarithm of the ratio of the concentration of viable microorganisms present in the samples before LED exposure (C_0) and the concentration of viable microorganisms present in the samples after UV-C LEDs exposure (C), respectively.

3. Results and discussion

3.1. UV-C LED inactivation of microorganisms present at occurrence level in water and salad samples

UV-C LEDs that emit light at 255 nm and 265 nm were tested in terms of their efficiency to inactivate microorganisms present at occurrence levels in different samples collected in a fresh produce industry: (a) water samples collected from two washing tanks (with and without disinfectant); (b) salad leaves (mixture of lettuce and arugula) collected before washing process; (c) salad leaves collected after packaging.

The water samples presented the following characteristics: pH = 7.05; total organic carbon = 6.13 mg/L; chemical oxygen demand = 7.68 mg O_2/L; total solids = 0.29 g/L; total suspended solids = 61.74 mg/L.

The target microorganisms evaluated before and after UV exposure were total microorganisms, total coliforms, *E. coli*, enterococci , and fungi (filamentous fungi and yeasts).

The results obtained showed that total coliforms, *E. coli*, enterococci and fungi were below the detection limit of the used methods in the water and leaf samples tested. The water samples collected in the washing tank without disinfectant presented an average of 286 ± 7 CFU/mL of total microorganisms grown after incubation at 36 °C that were inactivated to levels below detection after 5 min exposure to three single small LEDs that emit at 255 and 265 nm. The water samples collected in the washing tank with chlorine were totally free from contamination of the target microorganisms.

Leaves collected before the washing process and package salad samples presented a concentration of $1.14 \times 10^8 \pm 2.6 \times 10^6$ CFU/g and $2.4 \times 10^7 \pm 2.8 \times 10^6$ CFU/g of total microorganisms grown at 30 °C, respectively. After 10 min of exposure to the LEDs that emit light at 255 nm (44 mJ/cm^2), log reductions of 1.02 ± 0.003 and 0.76 ± 0.03 were obtained in the assays conducted with the samples of leaves collected before the washing process and the packaged salad samples, respectively. After the same exposure time (10 min that correspond to a UV fluence of 110 mJ/cm^2), three single small LEDs that emit light at 265 nm achieved log reductions of 1.99 ± 0.019 and 1.48 ± 0.04 in the assays conducted with the samples of leaves collected before the washing process and the packaged salad samples, respectively.

Both UV LEDs were able to reduce the microbial load of the fresh salads measured in terms of total microorganisms that grow at 30 °C. The higher inactivation levels achieved by the LEDs that emit light at 265 nm may be explained due to the higher intensity emitted by these LEDs compared to the LEDs that emit light at 255 nm (Fig. S1 of the supplementary material section). The lettuce and arugula leaves tested presented the same visual aspect before and after 10 min of exposure to the UV-C LEDs that emit at 255 nm and 265 nm.

Green et al. (2020) used low pressure mercury lamps that emit at 254 nm and UVC LEDs that emit at 277 nm to inactivate *E. coli* on lettuce leaves and *L. monocytogenes* on apple skin. Similar log reductions were achieved in this study and reported by the authors on apple skin and lettuce leaves.

3.2. *UV-C LED inactivation of bacteria spiked in water samples*

Since the real water samples collected at the food industry had low levels of microorganisms (section 3.1), inactivation experiments were also performed using bacteria spiked in phosphate-buffered saline solution (Figs. S3, S4 and Table S1) as well as filtered and autoclaved water collected in the washing tank.

Real water samples were also tested in the inactivation assays to understand the effect of the matrix composition in the inactivation efficiency, since natural mater and turbidity, present in the washing tank water, may scavenge the light, and protect the microorganisms from inactivation as well as react with the UV light to produce reactive free radicals.

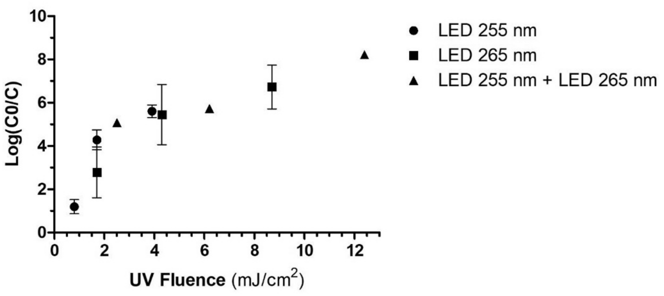
Fig. 3 and Fig. 4 display the inactivation results obtained when real water samples were spiked with a mixture of bacteria associated with foodborne pathogenic outbreaks (*S. enterica* serovars Typhimurium, Wernigerode and Derby, *L. monocytogenes* and *E. coli*) as well as bacteria isolated from packaged ready to eat salad, respectively.

The relatively low UV fluences needed to achieve high levels of inactivation of the two bacteria cocktails spiked at high concentrations in real water samples show that the LEDs that emit light at 265 nm and 255 nm are effective to cope with contamination of washing water of the food industry.

Table 1 presents the inactivation rate constants (k) and the correspondent coefficient of determination obtained when the different bacteria cocktails were spiked into filtered and autoclaved water samples and exposed to LEDs that emit light at different wavelengths.

The fluence based inactivation rate constants (k_f) obtained may be used to evaluate the efficiency of LEDs that emit light at different wavelengths to disinfect different bacteria cocktails spiked in different water matrices. The coefficient of determination (R^2) values obtained, ranging from 0.80 to 0.98, indicate a reasonably good fit between the inactivation data obtained and the linear regression model.

(A) Filtered water



(B) Autoclaved water

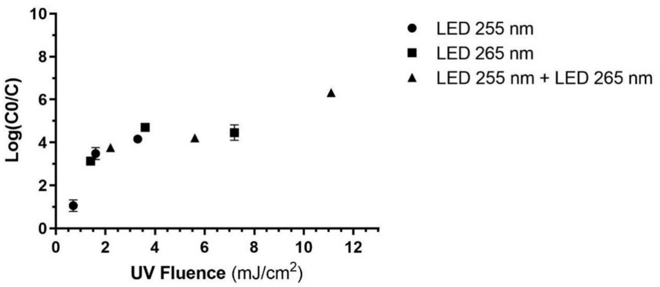


Fig. 3. Inactivation results in log (C_0 /C) of a cocktail of *S. enterica* serovars Typhimurium, Wernigerode and Derby, *L. monocytogenes* and *E. coli* spiked in filtered (a) and autoclaved (b) water after exposure to UV-C LEDs that emit light at two different wavelengths (255 and 265 nm) and their combination. Data are expressed as mean of two independent experiments. Error bars represent duplicate results.

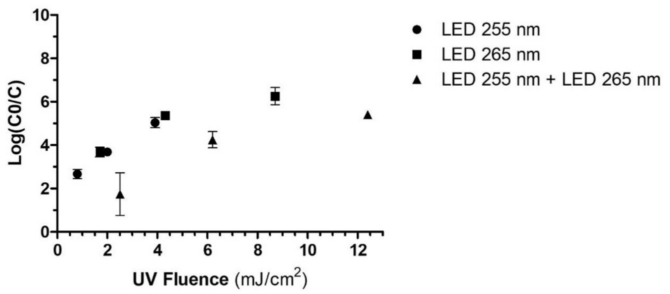
Across the tested conditions, the inactivation rate constants did not vary considerably, ranging from a minimum of 0.86 cm^2/mJ and maximum of 2.36 cm^2/mJ.

When considering the water matrix, the data suggest that the filtered water generally yielded slightly higher inactivation rate constants compared to the autoclaved water. This observation could be attributed to differences in the water quality composition between the two matrices since the filtered water contains lower levels of suspended solids that may protect the microorganisms from inactivation. Suspended solids have been described to hinder disinfection by: (i) reducing the available concentration of disinfectant and, thus, leading to a lower exposure dose and, (ii) shielding the microorganisms against the action of the disinfectant (Freitas et al., 2024; Henao et al., 2018; Yoon et al., 2022).

Using the combination of wavelengths was not beneficial compared to using the individual wavelengths. Higher inactivation rate constant (k_f) values were obtained using the LEDs that emit light at 255 nm for the inactivation of the cocktail of *S. enterica* Typhimurium, Wernigerode and Derby, *L. monocytogenes* and *E. coli* spiked on filtered and autoclaved water. On the other hand, the LEDs that emit light at 265 nm were more effective to inactivate the bacteria cocktail with salad isolates (Table 1) as well as most of the isolated bacteria spiked into phosphate-buffered saline solution (Fig. S3, Fig. S4 and Table S1).

The inactivation rate constants obtained can be used to determine the UV fluences needed to achieve a certain level of inactivation (e.g. 6-log reduction). For the inactivation of the *S. enterica* Typhimurium, Wernigerode, and Derby, *L. monocytogenes* and *E. coli* cocktail spiked in filtered water, a 6-log reduction could be achieved using UV-C LEDs emitting light at 255 nm with a UV fluence of 2.7 mJ/cm^2. In contrast, UV-C LEDs emitting light at 265 nm would require a UV fluence of 3.8

(A) Filtered water



(B) Autoclaved water

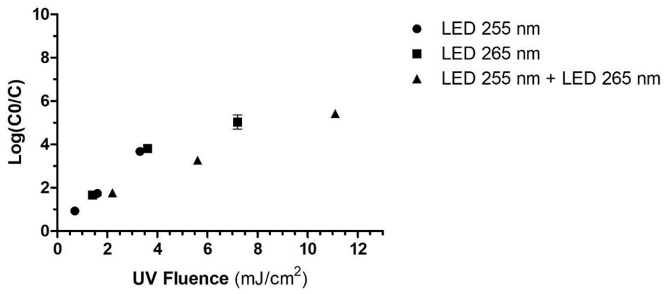


Fig. 4. Inactivation results in log (C_0/C) of a mixture of bacteria isolated from packaged salad spiked in filtered (A) and autoclaved (B) water after exposure to LEDs that emit light at two different wavelengths (255 and 265 nm) and their combination. Data are expressed as mean of two independent experiments. Error bars represent duplicate results.

mJ/cm^2 to attain the same 6-log reduction. For the cocktail of bacteria isolated from packaged salad, to achieve the same level of inactivation (6-log), a UV fluence of 2.8 and 4.3 would be required using the LEDs emitting light at 265 nm and 255 nm, respectively.

The germicidal efficiency at different wavelengths for several species of microorganisms was demonstrated to be between 220 nm and 270 nm (Bolton, 2017), with DNA damage predominantly at 265 nm (Sun et al., 2023). According with Oliveira et al. (2020, 2021), UV-C LEDs that emit at 255 nm and 265 nm led to morphology changes in the spores of *Aspergillus* sp., formation of cyclobutene pyrimidine dimers, effects on the proteome, membrane permeability and enzymatic activity. Green et al. (2018) compared the inactivation efficacy and performance of UV-LEDs emitting at 259 nm, 268 nm, 275 nm, 289 nm, and 370 nm for the foodborne pathogens *E. coli*, *Listeria* and *Salmonella*. The study shows that UV-LEDs emitting at 259 nm and 268 nm achieved the highest log count reductions out of the tested wavelengths.

3.3. UV-C LED inactivation of bacteria spiked in salad samples

Inactivation experiments were also performed in a larger 10 L reactor (Fig. 2) with lettuce and arugula leaves spiked with a cocktail of *S. enterica* Typhimurium, *L. monocytogenes,* and *E. coli*. Two submerged UV-C LED custom-made panels that emit light at 265 nm were tested in a container with 6 L of sterile distilled water as well as lettuce and arugula leaves spiked at a concentration of 10^5 CFU/g. Fig. 5 shows the log reduction results obtained after 5 and 10 min of exposure to the UV-C LED panels. The decision to use a 265 nm wavelength in our experiments was grounded in the insights gained from bacterial strains spiked into a phosphate-buffered saline solution. These findings robustly substantiate the effectiveness of UV-C LEDs emitting light at 265 nm in reliably inactivating a broad spectrum of bacterial species.

Table 1
Inactivation rate constants (k_f) and the correspondent coefficient of determination (R^2) obtained for the different bacteria cocktails after inactivation with LEDs emitting at 255 nm, 265 nm, and the combination of both wavelengths. Square brackets indicate the UV fluence limits of the linear regression. The k_f value represents the mean values and standard deviation of the inactivation rate constants obtained in two independent assays.

Bacteria cocktail	Matrix	Wavelength (nm)	k_f (cm^2/mJ)	R^2
S. enterica Typhimurium, Wernigerode and Derby; *L. monocytogenes;* *E. coli*	Filtered water	255 [0–1.96 mJ/cm^2]	2.22 ± 0.32	0.92
		265 [0–1.74 mJ/cm^2]	1.60 ± 0.96	0.98
		255|265 [0–2.48 mJ/cm^2]	2.05 ± 0.09	0.80
	Autoclaved water	255 [0–1.62 mJ/cm^2]	2.36 ± 0.45	0.85
		265 [0–1.44 mJ/cm^2]	2.16 ± 0.18	0.90
		255|265 [0–2.06 mJ/cm^2]	1.84 ± 0.32	0.95
Bacteria isolated from packaged salad	Filtered water	255 [0–1.96 mJ/cm^2]	1.40 ± 0.45	0.85
		265 [0–1.74 mJ/cm^2]	2.12 ± 0.18	0.90
		255|265 [0–2.48 mJ/cm^2]	0.87 ± 0.32	0.95
	Autoclaved water	255 [0–1.62 mJ/cm^2]	1.05 ± 0.12	0.98
		265 [0–1.44 mJ/cm^2]	1.16 ± 0.02	0.92
		255|265 [0–2.06 mJ/cm^2]	0.86 ± 0.05	0.98

Five minutes of exposure to UV-C LEDs that emit light at 265 nm allowed a colony forming units log reduction of 1.9, 2.3 and 2.2 for *S. enterica* Typhimurium, *L. monocytogenes* and *E. coli*, respectively. After 10 min of exposure a colony forming units log reduction of 3.0, 3.4 and 3.4 was obtained for *S. enterica* Typhimurium, *L. monocytogenes* and *E. coli*, respectively.

The water from the container where the lettuce and arugula leaves were washed before exposure to the UV-C LEDs presented concentrations around 10^3 CFU/mL, whereas after 5 min of UV exposure *S. enterica* Typhimurium, *L. monocytogenes, E. coli* were not detected.

Previous studies already used UV light to disinfect lettuce and other leafy greens. Artés-Hernández et al. (2009) showed that UV disinfection using low pressure mercury lamps was effective to reduce mesophilic counts on minimally processed spinach and effectively extended its shelf-life. The authors tested UV fluences from 454 to 1135 mJ/cm^2 and reported that low to moderate levels of UV-C radiation can be considered an effective alternative to chlorine for sanitising minimally processed spinach leaves and preserving their quality. The higher doses applied were reported to induce tissue damage in spinach leaves, making nutrients available for microbial growth. In our study, the leaves presented the same visual aspect before and after exposure to the different LED systems tested. Allende et al. (2006) have shown the efficacy of UV-C treatment on Red-Oak-Leaf lettuce, showing that growth of pathogens such as *S. enterica* Typhimurium and spoilage organisms such as *Erwinia* spp. could be delayed on leaves when treated with UV

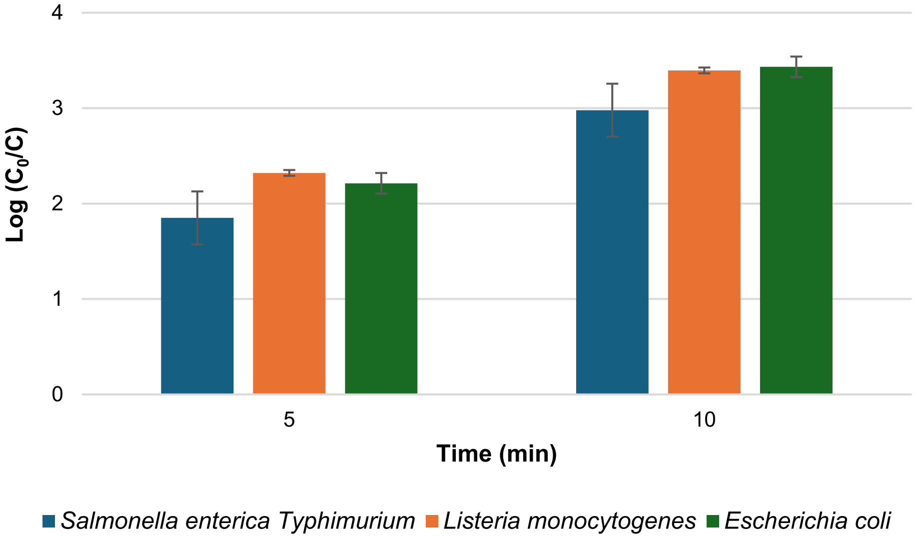


Fig. 5. Inactivation results (log C_0/C) of lettuce and arugula leaves spiked with a cocktail of *S. enterica* Typhimurium, *L. monocytogenes* and *E. coli* after exposure to two UV-C LED panels that emit light at 265 nm. Data are expressed as mean of two independent experiments. Error bars represent duplicate results.

light and then subsequently storing leaves under modified atmosphere packaging conditions. The authors report that the use of two-sided UV-C radiation is effective in reducing the natural microflora and extending the shelf-life of minimally processed 'Red Oak Leaf' lettuce. The UV-C radiation did not affect the sugars and organic acids of the lettuces significantly. Green et al. (2020) showed that a low-pressure mercury lamp emitting light at 254 nm was more effective in the dry treatment of *E. coli* O157:H7 on lettuce, while UV-LEDs at 277 nm were more effective against *L. monocytogenes* on apples. Both light sources achieved log reductions similar to an aqueous sodium hypochlorite wash. UV-C treatment did not significantly affect chlorophyll content and enzyme activity. However, exposure to UV-C increased browning of romaine lettuce leaves over the course of storage. This study shows that LEDs that emit light at 265 nm may be an effective disinfection alternative to low pressure mercury lamps for the disinfection of leaves and washing water. Future studies should address the effect of LED treatments on the chemical quality and shelf life of the products.

3.4. UV-C LED inactivation of bacteria spiked on stainless-steel surfaces

To explore the effectiveness of UV-C LED light that emit light at 265 nm in the disinfection of stainless-steel surfaces, stainless-steel discs were contaminated with *L. monocytogenes*. The inactivation results obtained are presented in Fig. 6.

A log reduction of approximately 2 was obtained after exposure to an UV fluence of 11, 33 and 55 mJ/cm^2 of *L. monocytogenes* to the three UV-C LEDs that emit light at 265 nm.

The effect of UV-C LEDs in the inactivation of *L. monocytogenes* in several surfaces including stainless-steel was previously studied (Kim & Kang, 2020). A log reduction of 0.5 to 0.9 was achieved for this bacterium depending on the material used with a wavelength of 280 nm and a UV fluence of 0.5 mJ/cm^2 to 3 mJ/cm^2. Kim and Kang (2020) concluded that the efficacy of the inactivation is dependent on the surface where the bacteria adhere. Depending on the hydrophobicity and roughness of the material the log reductions achieved are different. An irradiation of 265 nm of UV-C LED was used to decontaminate a surface of polyethylene contaminated with bacteria, including *Campylobacter jejuni* (Moazzami et al., 2021). After 1 min (20 mJ/cm^2) of exposure to UV-C LED, a log reduction of 2.0, similar to what was obtained in this study, was reported.

L. monocytogenes biofilms were also produced at 4 °C, a refrigerated temperature highly used in the food industry plants, as well as at room temperature (approximately 22 °C) for 7 days in stainless-steel discs. The discs were then exposed to UV-C LEDs that emit light at 265 nm for 2.5 min (27 mJ/cm^2) and 5 min (55 mJ/cm^2). The initial concentration in CFU/area obtained for the biofilms produced at 4 °C (3.5×10^4 CFU/mm^2 ± 1.5×10^4 CFU/mm^2) was higher than the initial concentration obtained for room temperature (2.1×10^3 CFU/mm^2 ± 8.5×10^2 CFU/mm^2). Moltz and Martin (2005) studied the influence of temperature in *L. monocytogenes* biofilm formation and reported oposite results. After an incubation of 20 h, cells growing at 20 °C and 37 °C reached a cell density of 10^6 CFU per piece whereas, after 4 days at 4 °C, it reached a cell density of 10^5 CFU per disc. The values of log reduction obtained for these inactivation conditions are presented in Fig. 7.

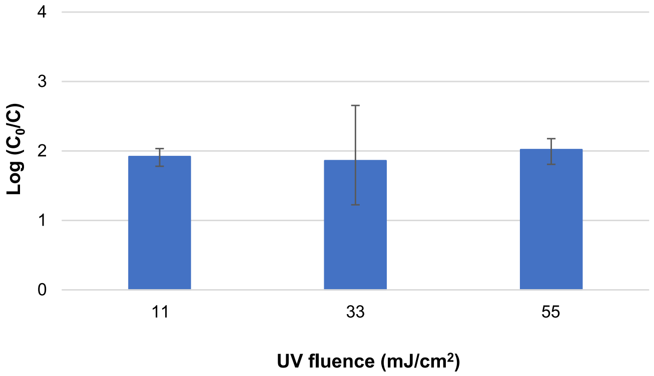


Fig. 6. Values of log reduction (log C_0/C) obtained for the inactivation of *L. monocytogenes* spiked in stainless-steel discs after exposure to light emitting diodes that emit light at 265 nm with an UV fluence of 11, 33 and 55 mJ/cm^2. Error bars correspond to the results obtained in two replicate experiments where 5 discs were analyzed in terms of the colony forming units of *L. monocytogenes* before and after exposure to the LEDs.

For the biofilms produced at 4 °C a log reduction of 1.1 and 1.2 was obtained after 2.5 min and 5 min exposure to UV-C LEDs, respectively. For the biofilms produced at room temperature, a log reduction of 1.5 was obtained after 2.5 min exposure to the LEDs and a higher log reduction of 2.3 was measured after 5 min exposure. As expected, as the time of exposure increases the biofilm inactivation increases. Comparing the log reduction values obtained for the biofilms produced at 4 °C and at room temperature, we can see that the log reductions obtained for the biofilms produced at 4 °C are lower than the ones produced at room temperature. Low temperature has been reported to adversely affect the

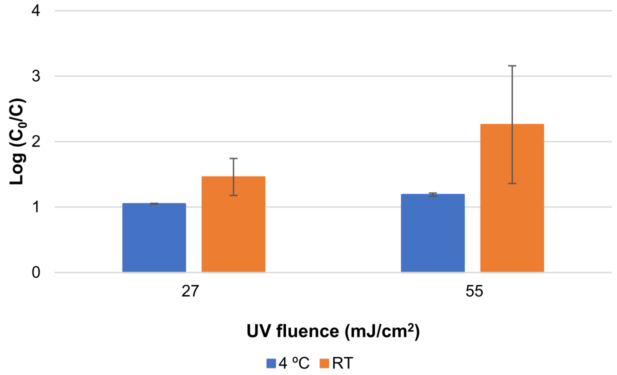


Fig. 7. Log reduction (log C_0/C) values obtained after *L. monocytogenes* biofilms exposure to ultraviolet light emitting diodes that emit light at 265 nm with an UV fluence of 27 and 55 mJ/cm^2. The biofilms were grown for 7 days at 4 °C and at room temperature. The error bars correspond to the results obtained in two replicate experiments. 5 discs were analyzed in each experiment.

inactivation efficiency. As an example, Chen et al. (2016) reported lower inactivation of *Enterococcus faecalis* rates at 4 °C compared to the inactivation rates measured at 25 °C, despite the UV dose applied.

To test the biofilm inactivation using photocatalytic surfaces, the modified discs of stainless steel with titanium dioxide (TiO_2) were immersed in a *L. monocytogenes* suspension for 7 days at room temperature. After the incubation time, some discs were exposed to UV LED at 265 nm for 5 min (55 mJ/cm^2). The other discs were not exposed to UV LED light to assess if the presence of TiO_2 could inactivate bacteria cells. A log reduction of 0.8 was obtained after exposure to TiO_2 and UV-LED radiation at 265 nm for 5 min. A smaller reduction of the biofilm cells was observed compared with direct photolysis. Chorianopoulos et al. (2011) studied the efficacy of photocatalytic treatment on *L. monocytogenes* biofilms. They used TiO_2 modified stainless steel and the surfaces were exposed to UV-A that emit light at 365 nm for 90 min. They concluded that the treatment without irradiation did not affect the biofilm population, as our results also demonstrate. On the other hand, they report a log reduction of 3 after 90 min UV-A irradiation which is much higher than the log reduction reported in this study. A longer exposure time of the modified discs to UV LED light could lead to a higher log reduction. However, compared with the direct photolysis results obtained, the development of photocatalytic surfaces in this case does not bring benefit in terms of inactivation and pollution control.

Fig. 8 shows the SEM images of *L. monocytogenes* before and after 10 min exposure (110 mJ/cm^2) to the three small UV-C LEDs that emit light at 265 nm.

The SEM images show that some bacterial cells exposed to the LEDs have morphological damages marked by the white circles (Fig. 8B). Hinds et al. (2020) had already reported altered surface morphologies in *Bacillus subtilis* cells treated with UV LEDs that emit light at a different wavelength (285 nm).

Our study shows that LEDs that emit light at 265 nm are a promising disinfection alternative to low pressure mercury lamps that can be used by the food industry to disinfect surfaces.

4. Conclusions

This study highlighted the potential of UV-C LEDs as a promising disinfection method for the food industry. This technology effectively inactivated bacteria associated with the spoilage of ready-to-eat salads and foodborne diseases, positioning it as a viable alternative to low-pressure mercury lamps and chemical treatments. Additionally, UV-C LEDs demonstrated significant efficacy in disinfecting stainless steel surfaces contaminated with *L. monocytogenes*. The findings support the potential of UV-C LED technology as an effective and sustainable approach for microbial inactivation, with implications for enhancing safety and hygiene practices across various industries. Further research is needed to explore the underlying mechanisms of inactivation, pilot-scale applications, and economic viability of this technology.

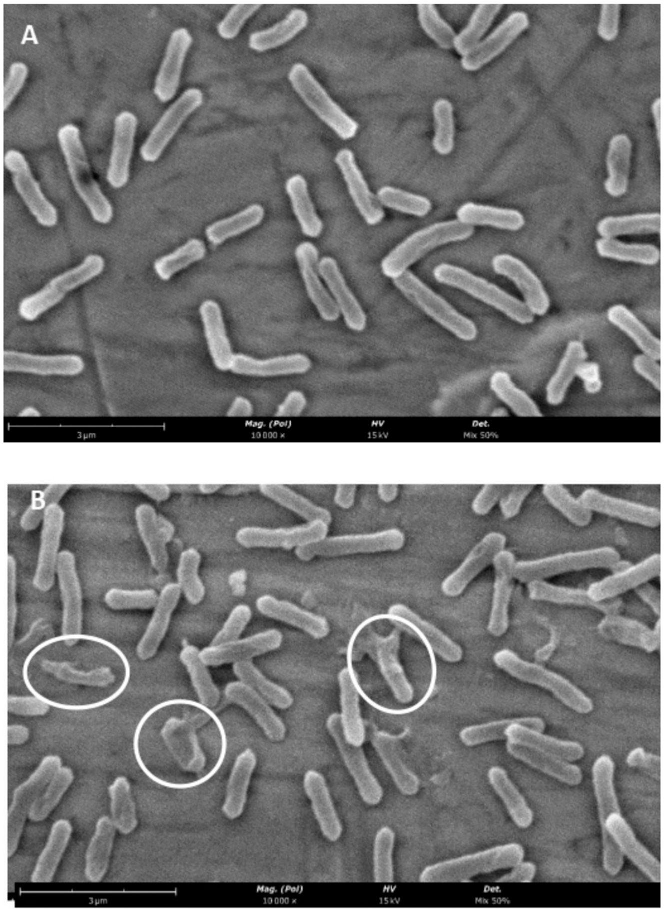


Fig. 8. Scanning electron microscopy images of *L. monocytogenes* not exposed (A) and after 10 min (110 mJ/cm^2) of exposure (B) to ultraviolet light emitting diodes that emit light at 265 nm.

CRediT authorship contribution statement

Ana Paula Marques: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Carolina Santos:** Writing – review & editing, Writing – original draft, Formal analysis. **João Sério:** Writing – review & editing, Writing – original draft, Formal analysis. **Maria Teresa Barreto Crespo:** Writing – review & editing, Resources, Funding acquisition. **Vanessa Jorge Pereira:** Writing – review & editing, Writing – original draft, Resources, Methodology, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2024.103848.

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