International Journal of Food Microbiology 447 (2026) 111567

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Decontamination of black peppercorns using UV-LED technology and its effect on cell viability☆

Víctor Arcos-Limiñana [a,*], Marie Polet [b], Bavo Verhaegen [b], Arturo B. Soro [c], Brijesh K. Tiwari [d], Soledad Prats-Moya [a], Salvador Maestre-Pérez [a], Koenraad Van Hoorde [b]

[a] Department of Analytical Chemistry, Nutrition and Food Sciences, University of Alicante, Spain
[b] Foodborne Pathogens Unit, Sciensano, Belgium
[c] Area of Food Technology, Departament de Ciència Animal I dels Aliments, facultat de Veterinària, Universitat Autònoma de Barcelona, Spain
[d] Teagasc Ashtown Food Research Centre, Teagasc, Ireland

ARTICLE INFO

Keywords:
VBNC
Cell viability
Spores
V-PCR
ABNC
Food safety
Disinfection
Inactivation

ABSTRACT

Ultraviolet light-emitting diodes (UV-LEDs) are being investigated for potential use in food disinfection due to their customisable wavelength and high energy efficiency. The objective of this study was to examine the impact of UV-LED treatments, for up to 30 min, at wavelengths of 280, 300, and 365 nm, as well as simultaneous irradiation with 280 and 300 nm, on the culturability of *Escherichia coli*, *Bacillus cereus* spores, and *Salmonella enterica* in black peppercorns. It resulted in a reduction of up to 1.65, 1.35 and 1 log colony-forming units per gram (CFU/g) for *E. coli*, *S. enterica*, and *B. cereus* spores, respectively. The viability of *S. enterica* was also evaluated in black pepper using a viability PCR method with DyeTox13. No significant differences were observed between active and inactive non-culturable states. This finding suggests that UV light did not cause substantial lethal damage to the bacteria, but instead rendered them non-culturable, potentially leading to an underestimation of the food safety risk. These findings are encouraging concerning the potential applications of UV-LEDs in the spice industry.

1. Introduction

The production of black pepper is a complex, multi-stage process involving numerous microbiological risks which must be carefully assessed and controlled. The majority of black pepper harvested is dried in the open air, where contact with dust, insects and livestock can easily contaminate the peppercorns (Shango et al., 2021). Furthermore, black pepper is mainly produced in developing countries (FAOSTAT, 2022), where preventive hygienic measures have yet to be fully implemented, especially in small-scale productions. Moreover, the consumption of black pepper is frequently raw, thereby increasing the risk of infection even at low concentrations. According to the Rapid Alert System for Food and Feed (RASFF), the most reported pathogen in black pepper in the EU is *Salmonella* spp. (RASFF, 2024). However, *Bacillus cereus* is also a prevalent pathogen in black pepper (Mathot et al., 2021) and has the ability to form spores, which are highly resistant to disinfection treatments (Gayán et al., 2013).

The application of heat treatments has been demonstrated to be an effective method for food disinfection. However, the primary value of black pepper lies in its volatile and flavour compounds, which are susceptible to degradation when subjected to high temperatures (Shango et al., 2021). Consequently, non-thermal treatments have been receiving increased attention as means of ensuring the safety of spices without compromising their quality. Low water activity, non-thermal food disinfection has been mainly performed on grains, and few of research has been conducted on spices in general and black pepper in particular (Arcos-Limiñana et al., 2025a; Deng et al., 2020). Although each non-thermal disinfection method has its own limitations, ultraviolet (UV) radiation is a promising candidate for the disinfection of black pepper since it is an energy-efficient technology, that can be easily implemented in production lines, and there is evidence proving that it is safe (Erdoğdu and Ekiz, 2013; Gabriel et al., 2020). Furthermore, UV radiation has been observed to enhance some of the organoleptic qualities of black pepper (Collings et al., 2018; Hinds et al., 2021).

Most studies have employed conventional mercury UV-C lamps with a peak wavelength of 254 nm, yielding generally positive results (Bang

☆ This article is part of a Special issue entitled: 'FoodMicro2024' published in International Journal of Food Microbiology.

* Corresponding author at: Analytical Chemistry, Nutrition and Food Science Department, University of Alicante, P.O. Box 99, E-03080, Alicante, Spain.
E-mail address: victor.arcos@ua.es (V. Arcos-Limiñana).

https://doi.org/10.1016/j.ijfoodmicro.2025.111567
Received 12 May 2025; Received in revised form 3 September 2025; Accepted 3 December 2025
Available online 4 December 2025

et al., 2020; El Darra et al., 2021; Park et al., 2019, 2020), but there is a trend worldwide following the Minamata Convention to gradually reduce the use of mercury due to its ecotoxicity (EPA, 2014; EU, 2024). Moreover, the possibility of adjusting the wavelength of LEDs allows for the optimisation of their efficiency in terms of disinfection and the mitigation of their impact on quality (Soro et al., 2021a), thereby rendering them more suitable as UV sources. Although UV-LEDs have shown promising efficacy, the number of studies investigating them is limited (Hinds et al., 2021; Jiang et al., 2020).

However, some studies suggest that UV treatments do not inactivate bacteria but render them viable but nonculturable instead (Chiang et al., 2022; Xu et al., 2018). To test viability, a DNA-intercalating photoreactive agent is typically used. These agents become permanently bonded to free DNA and DNA from membrane-compromised bacteria when exposed to light, preventing its replication (dos Reis Lemos and Maisonnave Arisi, 2024). However, unlike other disinfectant agents such as heat, chemicals, and cold plasma, UV light does not affect the integrity of the cell membranes (Lee and Bae, 2018). Consequently, traditional intercalating agents are unable to access the DNA, even if the bacteria become inactive (Nocker et al., 2007; Zhang et al., 2015), since the primary mechanism of action of UV light is the alteration of the DNA structure. DyeTox13 was developed as an intercalating and membrane-permeable agent that binds only to DNA from metabolically inactive cells, rendering it more suitable for estimating the effect on viability of UV-treated bacteria (Chiang et al., 2022; Lee and Bae, 2018; L. Li et al., 2022).

The objective of this study was to evaluate the efficacy of UV light irradiation at different wavelengths (280, 300 and 365 nm) and a combination of them (280 + 300 nm) using UV-LEDs in the microbial inactivation of *Salmonella enterica*, *E. coli* and *B. cereus* spores in black peppercorns. The impact of these treatments on the viability of *S. enterica* was also investigated using digital PCR (dPCR).

2. Methodology

2.1. Bacterial strains and culture conditions

S. enterica serovar Weltevreden (strain S23FP03082; originally isolated from coriander seeds from a 2023 outbreak), *B. cereus* (strain TIAC 4992; originally isolated from oregano leaves from a 2019 outbreak), and *E. coli* (strain TIAC 2481) were obtained from the culture collection of Sciensano, Belgium. The bacterial species identity was confirmed using MALDI-TOF-MS (Microflex, Bruker, Germany).

The stock bacteria were resuscitated on nutrient agar (Oxoid, United Kingdom) and incubated aerobically at 37 °C for 24 h for *E. coli* and *S. enterica*, and at 30 °C for 48 h for *B. cereus*. To prevent cell damage, fresh bacterial cultures were prepared daily from an isolated colony on brain heart infusion (BHI, Oxoid, United Kingdom). For *B. cereus*, bacterial suspensions were aerobically incubated at 30 °C for 3 days to promote sporulation (Freire et al., 2024).

2.2. Sample preparation, microbial inoculation and UV-LED treatment

A cocktail inoculum was prepared by mixing the *E. coli*, *S. enterica* and *B. cereus* spores' suspensions to have a final concentration of 7–8 log CFU/mL of each. Prior to mixing, the *B. cereus* suspension was centrifuged at 4000 ×*g* for 10 min, subsequently the supernatant was discarded, and the pellet resuspended to achieve the appropriate concentration. This suspension was then heated at 80 °C for 15 min in a heat block to inactivate vegetative cells and induce the formation of spores, according to the method described by Freire et al. (2024). A volume of 300 µL of the microbial cocktail was added to a sterile 50 mL falcon tube containing 13.5 g of black peppercorns. The tube was vigorously shaken until the peppercorns were dry. A final concentration of *E. coli* and *S. enterica* of 5 to 6 log CFU/g, and 4 to 5 log CFU/g for *B. cereus* in black peppercorns were obtained.

A single unprocessed raw batch of black peppercorns from Vietnam was purchased from a local market in Brussels, Belgium, in March 2024. Black peppercorn samples were subjected to UV treatment using a UV-LED device (PearlLab Beam, Aquisense Technologies, NC, USA) operating at wavelengths of 280, 300 and 365 nm, and a combination of 280 and 300 nm, as previously described by Soro et al. (2021a). The experimental setup comprised a 90-mm petri dish with the sample placed on top of a vibrating shaker that promoted the movement and rotation of the peppercorns during irradiation, emulating a vibrating conveyor, and a UV-LED lamp placed 5 cm above the sample surface, as shown in Fig. 1. The UV irradiance was measured using a Opticalmeter, model ILT2400 radiometer (International Light Technologies, USA). Irradiance and doses are shown in Table 1. The experimental setup was enclosed in a box during irradiation to ensure safety and dark conditions. At 0, 10, 20, and 30 min of irradiation time, a pepper aliquot of 2 g was taken from the petri dish, and decimal dilutions were prepared using buffered peptone water (BPW, Neogen, UK). The experiments were conducted using individual wavelengths of 280 nm, 300 nm and 365 nm, as well as a combination of 280 and 300 nm. Experiments were performed in triplicate.

2.3. Microbiological analysis

Two grams of positive (inoculated, untreated sample) and negative (not inoculated, untreated sample) controls, as well as treated samples, were suspended in 20 mL of buffered peptone water (BPW, Oxoid, United Kingdom) and shaken at 450 rpm for 5 min. Ten-fold dilutions were subsequently prepared from these suspensions, and a 0.1 mL aliquot of each dilution was spread plated in duplicate onto MacConkey (Oxoid, United Kingdom), mannitol egg yolk polymyxin (MYP, Bio-Rad, France), and xylose lysine deoxycholate (XLD, Bio-Rad, France) agars for the enumeration of *E. coli*, *B. cereus*, and *S. enterica*, respectively. After incubating XLD and MacConkey at 37 °C for 24 h and MYP at 30 °C for 24 h, colonies of a round, red-pink appearance on MacConkey agar were identified as *E. coli*, pink colonies with a precipitation circle were enumerated as *B. cereus* in MYP agar, and colonies with a black centre and a lightly transparent aura were enumerated as *S. enterica* in XLD agar. Identity confirmation of bacteria was done by MALDI-TOF-MS.

2.4. DyeTox13 viability treatment

For each experiment a 500-µL aliquot of cell suspension was taken and treated with 50 µM DyeTox13 (Setareh Biotech, OR, USA) following the procedure as described previously (Chiang et al., 2022; Lee and Bae, 2018; L. Li et al., 2022). After the addition of the dye, the cell suspensions were mixed thoroughly by vortexing and then incubated in the dark at room temperature for 10 min. Subsequently, samples were exposed to intense blue light for 15 min using a PhAST blue

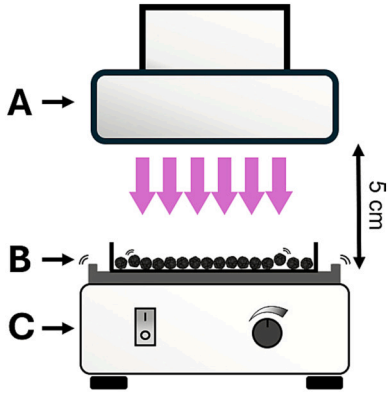

Fig. 1. Experimental setup composed of the UV lamp (A), a petri dish containing the inoculated sample (B), and the shaker (C).

Table 1
UV irradiance and dosage depending on the wavelength and the time of exposure at a 5 cm distance.

Wavelength (nm)	Irradiance (W/cm^2)	Dose 10 min (J/cm^2)	Dose 20 min (J/cm^2)	Dose 30 min (J/cm^2)
280	0.068	40.8	81.6	122.4
300	0.049	29.4	58.8	88.2
365	0.043	25.8	51.6	77.4
280 + 300	0.112	67.2	134.4	201.6

photoactivation system (GenIUL, Barcelona, Spain).

2.5. DNA extraction and d-PCR conditions

According to the manufacturer's instructions, DNA was extracted from the 500-μL dye-treated and non-treated samples using a Maxwell RSC cultured cells DNA Kit on the Maxwell RSC 16 device (Promega Corporation, Madison, USA). Since *Salmonella* was already tested in previous studies with DyeTox13 (L. Li et al., 2022), *S. enterica* was selected for the DyeTox13 assays, using the single-copy invA gene (invasion protein A) for its quantification due to its high specificity and sensitivity (Ceyssens et al., 2024). *E. coli* pUC18 was used as an internal control.

The primer sequences are provided in Table 2. dPCR assays for DNA amplification were performed in a QIAcuity 24-well Nanoplate 8.5 k (Qiagen, Hilden, Germany) using a QIAcuity Digital PCR System (Qiagen, Hilden, Germany). Each of the 12-μL dPCR reactions comprised 3 μL of DNA, 0.06 μL of the 50 μM probe dilution (IDT, Leuven, Belgium), 3 μL of the 4× Probe PCR Master mix Qiagen, Hilden, Germany), and 0.06 μL of each 100 μM forward and reverse primer dilution (IDT, Leuven, Belgium). The probes and primers mentioned above were used to quantify the DNA. The dPCR assays were conducted under the following conditions: the temperature was held at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of denaturation (95 °C for 15 s) and annealing (60 °C for 60 s). Every DyeTox13-treated sample was coupled with untreated twin samples to measure the number of active (viable) and total cells, respectively. A calibration curve from 8 to 3 log CFU/mL was prepared, and different concentrations of inoculum were used as intercalating agent controls. The number of active but non-culturable (ABNC) cells was determined by subtracting the plated CFU from the total active cell count. Inactive cells were calculated by subtracting the active count from the total cell count.

2.6. Statistical analysis

The experiments were conducted three times independently, with duplicate measurements taken in each case. To address the significance of the effect of wavelengths and treatment time on microbial inactivation, the average and standard deviation of logarithmic reductions (|initial log CFU/g – log CFU/g after treatment|) were used to express the results, and an analysis of variance (ANOVA) and Tukey's multiple comparison test were conducted for all tests. The statistical analysis was performed using SPSS v26 (IBM, NY, USA), and statistical significance was tested at $p < 0.05$.

Table 2
Sequences and melting temperatures (Tm) of the primers and probe used for conducting the d-PCR.

Name	Sequence	Tm
invA-F	5'-CACCGAAATACCGCCAAT AAAG-3'	54.3 °C
invA-R	5'-AGCGTACTGGAAAGGGAAAG-3'	54.7 °C
invA-Probe	5′–/56-FAM/ATCGCACCD/ZEN/TCAAAGGAACCGTAA/3IABkFQ/–3'	60.8 °C

Notes: Both primers and probe were pruchased from ThermoFisher, Netherlands.

3. Results and discussion

3.1. Effectiveness of UV-LED to inactivate E. coli, B. cereus spores and S. enterica

The inactivation potential of UV-LED's irradiation on *E. coli*, *B. cereus* spores and *S. enterica* was tested for three different wavelengths (280, 300 and 365 nm) and a combination of two of them (280 + 300 nm), placing the sample in a shaker to irradiate the whole surface of the peppercorns. Variability in the effectiveness of the different UV-LED treatments was observed across the tested bacteria, treatment times, and UV wavelength.

E. coli was the most sensitive bacterium to UV light, exhibiting a reduction of 1.6 log CFU/g in the inoculated black peppercorns after the best conditions: 30 min at the combined 280 + 300 nm treatment (Fig. 2A). In contrast, the independent irradiation with 280 and 300 nm wavelengths resulted in a lower reduction - up to 1.35 Log CFU/g after 30 min - than their combination. However, both wavelengths exhibited a similar disinfection kinetic pattern. UV-A irradiation (365 nm), conversely, resulted in less than half the disinfection achieved by the other wavelengths, with a reduction of only 0.7 Log CFU/g.

The load of *B. cereus* spores in black peppercorns achieved the highest reduction upon 30 min of treatment with both 280 nm and 280 + 300 nm treatments, as shown in Fig. 2B. In contrast to the observations made in *E. coli*, the combined wavelength treatment did not result in a higher reduction than the 280 nm treatment alone. Nevertheless, the 280 nm and the combined wavelength treatments were markedly more effective than 300 and 365 nm, exhibiting a remarkable higher reduction. Lastly, a negligible reduction was observed using the 365 nm wavelength. While the bacterial reduction after 10 min of treatment with 300 and 365 nm was similar, the outcome of 300 nm irradiation increased significantly with longer treatment times, whereas that of 365 nm remained unchanged.

As presented in Fig. 2C, a reduction of 1.35 log CFU/g was attained following the 30-min UV-LED treatment of *Salmonella* inoculated peppercorns. Irradiation with a 300 nm wavelength for 30 min resulted in the highest bacterial reduction, although it did not differ significantly from irradiation with 280 nm or the combined wavelengths. Nevertheless, the steadily increasing disinfection rate could suggest that the disinfection process has not yet reached a plateau, and that better results could be achievable through extended treatment times.

Among all the factors affecting the reduction of the bacterial load, treatment time has been identified as the most significant across all bacteria. While most of the log reduction was usually achieved within the initial 10 min of treatment, a significant increase in the bacterial reduction was observed after 20 or 30 min of treatment for all bacteria, irrespective of the conditions. During the first 10 min, bacteria that are most exposed to the UV light would quickly receive a lethal dose, resulting in a rapid decrease in microbial load. However, in the case of bacteria that are allocated in areas where only a fraction of the light reaches, a lethal dose would gradually be achieved, resulting in a decline in the bacterial reduction rate, but a steady overall reduction (Arcos-Limiñana et al., 2025a).

Numerous studies have observed significant variations in treatment effectiveness between different wavelength irradiation, showing a strong relationship with the targeted bacteria (Ha and Kang, 2013; Hinds et al., 2021; Li et al., 2017; Li et al., 2019; MacIsaac et al., 2023; Martín-Sómer et al., 2023; Park et al., 2019, 2020). In fact, despite both being gram negative, *S. enterica* exhibited a somewhat similar reduction to all wavelengths, whereas *E. coli* was mainly affected by the shorter wavelengths. A similar outcome was reported in a study in which *Salmonella Typhimurium* was equally affected by both 254 and 352 nm irradiation in black pepper powder, whereas *E. coli* was more sensitive to the 254 nm irradiation (Park et al., 2020). However, in a prior study, a similar method was employed to reduce the load of *E. coli* in black pepper, but a 254 nm conventional UV lamp was used (Arcos-Limiñana

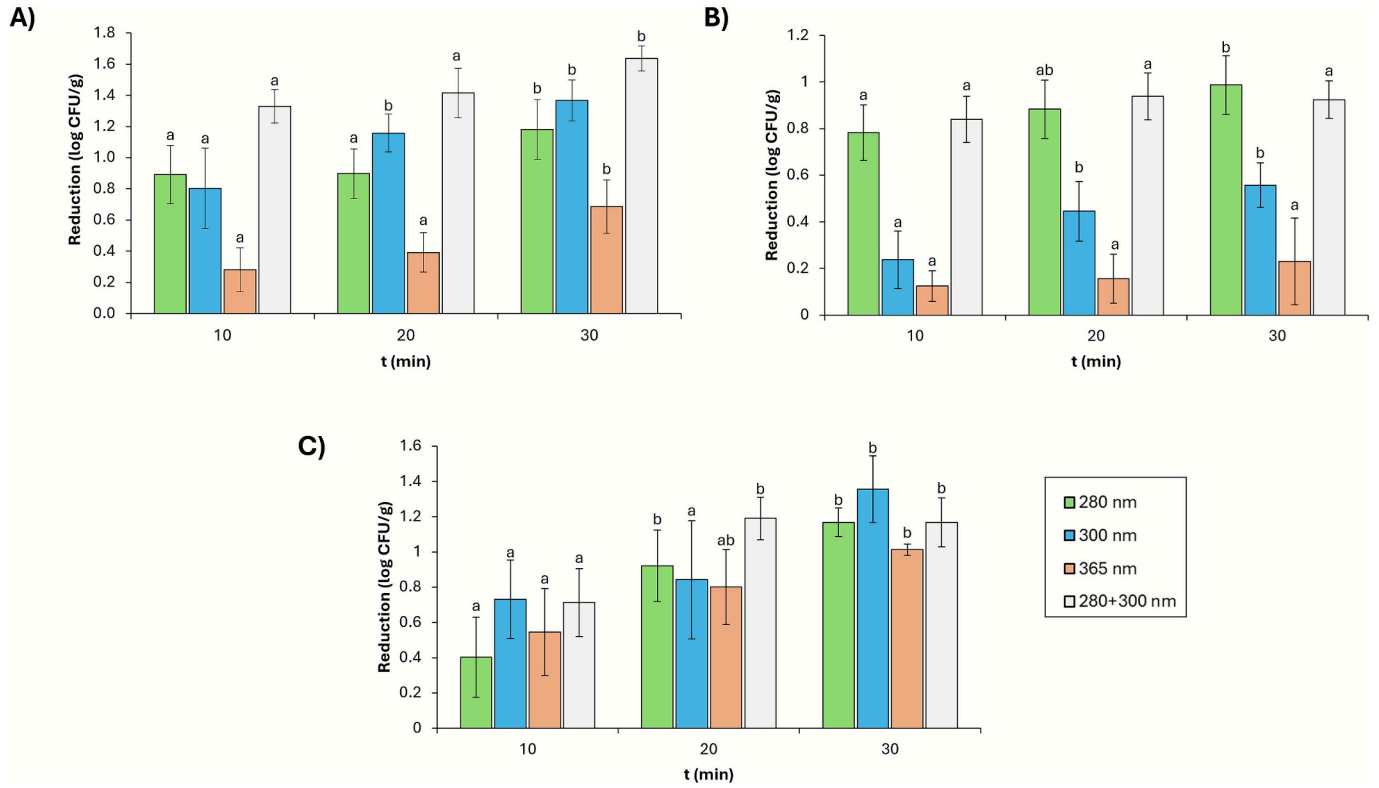

Fig. 2. Mean log CFU/g reduction and standard deviation of *Escherichia coli* (A), *Bacillus cereus* spores (B) and *Salmonella enterica* serovar Weltevreden (C) in black peppercorns after different UV-LED wavelength irradiation and treatment times (min). Notes: Different letters represent significant differences ($p < 0.05$) between treatment times per wavelength.

et al., 2025b). To obtain a similar bacterial inactivation level as the 280 + 300 nm treatment, it was necessary to double the treatment time and increase the irradiance. It was only after a 90-min treatment period that a higher reduction was observed, reaching a 2.4 log CFU/g reduction with the same inoculum starting concentration. This finding supports the hypothesis that achieving a higher disinfection level is possible with extended treatment times.

Among the studied bacteria, *B. cereus* spores showed the greatest resistance. The study of spores is a prominent area of research in food microbiology, largely due to their resistance to disinfection treatments, including heat and chemical treatments, as well as their occurrence in spices (Mathot et al., 2021). This occurrence is due to the contamination of the spice during production and improper storage conditions, with *B. cereus* as one of the most common spore-generating species and black pepper as the main driver (Mathot et al., 2021). In the present work, the 280 and 280 + 300 nm wavelength were the most effective in inactivating *B. cereus* spores, with a notable difference. Hinds et al. (2021) showed that *Bacillus subtilis* was more effectively inactivated in black peppercorns by the 280 nm wavelength than by 300 or 365 nm (Hinds et al., 2021), indicating a higher susceptibility of *Bacillus* spp. to the 280 nm wavelength. This wavelength is closer to the maximum DNA absorption of most bacteria, including in the case of spores (Martín-Sómer et al., 2023). Consequently, it has the potential to produce a more pronounced impact on the DNA, thereby increasing the lethality of the treatment. While the wavelength can be tuned to effectively damage the DNA of specific bacteria, the 280 nm wavelength has been observed to also repress photoreactivation and dark repair by damaging DNA repair enzymes (Li et al., 2017), resulting in a higher lethality.

Furthermore, 280 nm UV light is effectively absorbed by aromatic bonds, including amino acids like tryptophan and tyrosine. This process promotes porosity in the membrane through amino acid photodissociation (Li et al., 2019). Additionally, it has been observed that the particle shielding effect of the matrix can be reduced due to the higher propensity of 280 nm to reach bacteria that are attached to particles or partially hidden in the matrix, and to inactivate aggregated bacteria more effectively (MacIsaac et al., 2023). Using a reactor is another effective method for reducing shadows and increasing the light's reach, as evidenced by the findings of previous studies (Arcos-Limiñana et al., 2025b; Ha and Kang, 2013; Park et al., 2019, 2020).

The lack of an additive effect of the combined 280 + 300 nm treatment suggests that either one or both wavelengths have a diminished capacity to reach the partially hidden bacteria after inactivating the most vulnerable ones. In this scenario, the limit of inactivation is being reached, and light cannot reach its target; therefore, no further disinfection would be achieved even with higher irradiances or increased treatment times. However, other studies have used longer treatment times or higher doses and have observed a further disinfection than in the present study (Arcos-Limiñana et al., 2025b). In one study where a combined wavelength (254 + 352 nm) treatment was applied, a similar result was obtained as with individual wavelengths, particularly in the case of *E. coli* (Park et al., 2020). This study also observed a similar cell injury rate in the individual and combined treatments. Nevertheless, in another study that used the same wavelength combination but disinfected *Campylobacter jejuni*, an increased disinfection rate was observed (Soro et al., 2021b), but the irradiation was not carried out on a food matrix. Further investigation is required to verify or refute this hypothesis by testing longer irradiation times.

In light of the aforementioned findings and the results of previous research, the 280 nm and the combined 280 + 300 nm wavelengths were observed to be the most effective treatments due to their effectiveness in damaging DNA and preventing self-repair mechanisms, although in the case of *E. coli*, the combined 280 + 300 nm treatment significantly improved the bacterial reduction. However, the treatment effectiveness of UV-LED technology should be assessed individually for each bacterium and sample.

3.2. Effect of UV-LED on the viability of S. enterica

After the disinfection treatment, an aliquot of *S. enterica* was taken to extract the DNA to estimate the number of metabolically active and inactive cells resulting from it by adding DyeTox13 and using a PCR method. Although culturability was strongly correlated with treatment time and wavelength, no clear trends regarding cell state could be identified. The results of the dPCR, as illustrated in Fig. 3, show that no significant difference could be discerned between the active and inactive states of *S. enterica* in response to any of the UV wavelength treatments.

The findings observed in this study suggest that UV light may not directly inactivate bacteria, but rather induce the ABNC state among active cells, although high variations do not allow for small changes to be detected. The ABNC state is defined as the bacteria that are not culturable but whose metabolism is still active enough to eject the DyeTox13 out of the cell membrane. This state is induced by severe injuries or stress that prevents the cell from replicating permanently or temporarily and has previously been observed to occur when exposed to UV irradiation (Liu et al., 2023; Xu et al., 2018). It has been observed that bacteria can recover from this state when in favourable and nutrient-rich environments, such as some types of food. Furthermore, viable but non-culturable pathogens have been observed to preserve some recovery capacity and infectivity (Liu et al., 2023; Zhang et al., 2015). However, relatively low doses of UV-C light have been observed to inhibit the DNA damage repair function, while higher doses produced observable DNA lesions (Xu et al., 2018). Nevertheless, it remains unclear whether different wavelengths and food matrices could lead to a decreased or enhanced recovery rate, since different behaviours have been observed among a range of species and environments (Ramamurthy et al., 2014).

Previously, the loss of culturability caused by UV light was observed using traditional methods (Nocker et al., 2007; Zhang et al., 2015), which prevented an accurate measurement of all viable bacteria. This is the first time of DyeTox13 v-PCR being conducted in a complex matrix, and a high variability was observed in the results, preventing the identification of significant differences between cell states. A number of potential sources of uncertainty may have contributed to these results. As this particular dye has never been tested on such a complex matrix, it is reasonable to consider that a matrix effect has played a substantial role in the variability observed. The interference of molecules from black pepper may have hindered the ability of the dye to reach the target and perform its function effectively. Other sources of uncertainty might be the multi-step sample preparation procedure, which could have promoted the non-culturable state among the active bacteria; the presence of a high number of dead cells; and high levels of biomass in the dilution (Fittipaldi et al., 2012).

In addition to food safety concerns, alterations in spice quality represent a significant issue when disinfection treatments are employed. Previous studies have demonstrated that UV-LED treatments can promote both positive and negative changes in spices and other foods (Fan et al., 2021; Ghasemzadeh et al., 2016; Hinds et al., 2021; Jiang et al., 2020), but despite the relevance of this topic, there is still a lack of studies in this area, and further research is required to advance in the understanding the strengths and weaknesses of UV-LED in the food industry.

4. Conclusion

The present study has demonstrated the efficacy of UV-LED coupled to an emulated vibration conveyor for the disinfection of black peppercorns, using different wavelengths. The disinfection of *E. coli* and *S. enterica* resulted in a reduction of up to 1.65 and 1.35 log CFU/g, respectively. Additionally, the UV-LED treatment of *B. cereus* spores resulted in a reduction of up to 1 log CFU/g. The combined 280 + 300 nm treatment was observed to be the most effective, or equally effective as, other wavelengths; however, the simultaneous irradiation of both wavelengths did not improve the outcome. Although this method

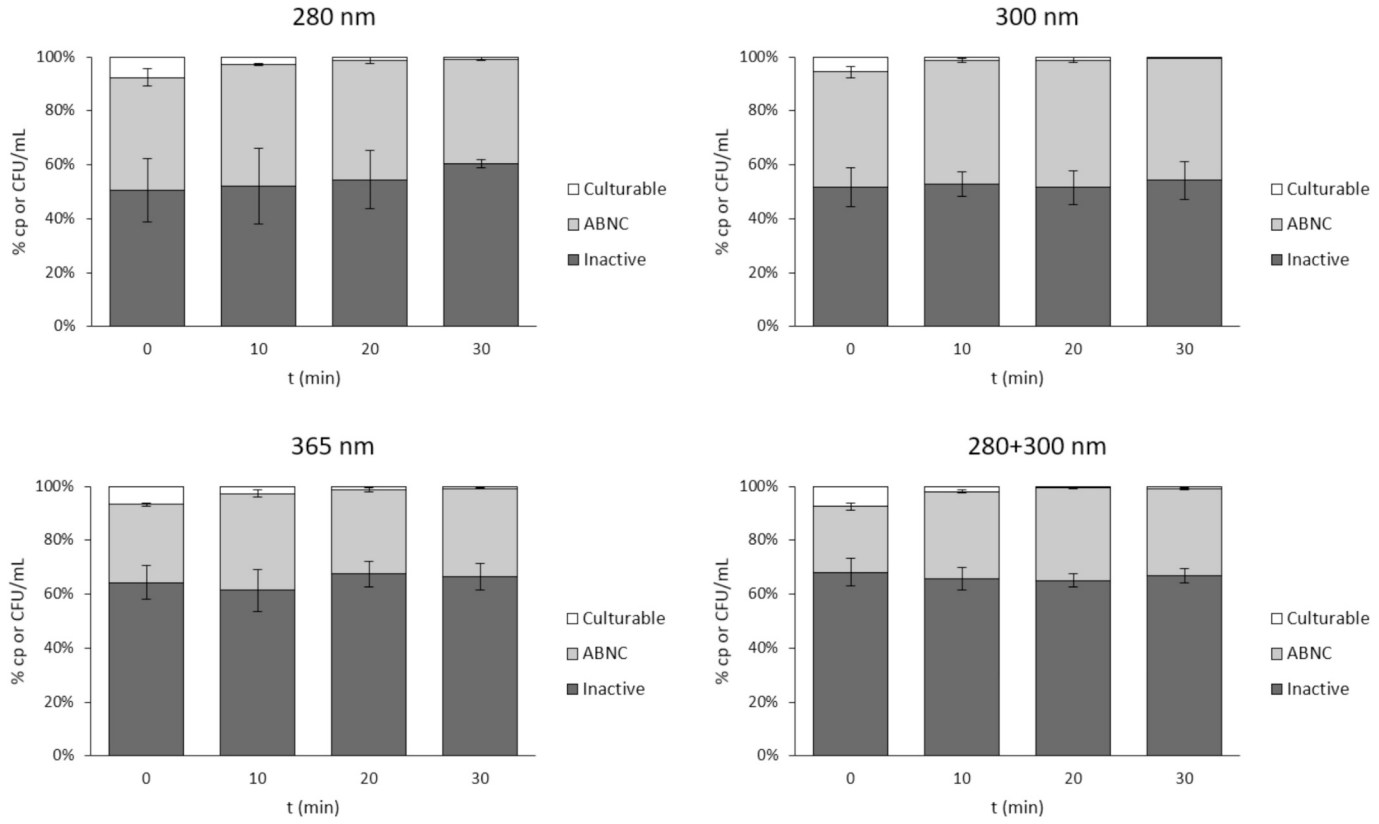

Fig. 3. Mean cell state distribution (%) and standard deviation between metabolically inactive, active but non-culturable (ABNC) and culturable *Salmonella enterica* serovar Weltevreden after different UV wavelengths and treatment times.

provided effective disinfection, the food industry often requires a higher level of disinfection. This can be achieved by increasing treatment times or irradiance, or by combining this method with other non-thermal disinfecting methods, such as cold plasma or ozone.

The lack of a significant reduction in the viable cell counts of *S. enterica* Weltevreden, despite a loss of culturability, suggests that the UV treatment may render a substantial proportion of cells non-culturable (ABNC) rather than lethally inactivating them. This highlights a potential risk of underestimation using culture-based methods alone.

However, this same lack of significance could be due to the high variability observed. Several alternative approaches could be taken to enhance the measurement precision: to develop a more matrix-independent intercalating agent; to integrate it with complementary assays such as flow cytometry assays, metabolic activity measurements, SEM observations, or mRNA qPCR; to use an anionic surfactant to increase the permeability of dead cells without damaging the active cells; to use chemical agents to eliminate molecule interferents selectively; or to optimize the biomass, amount of DNA and the number of rounds of DyeTox13 for a specific matrix (Codony et al., 2020; dos Reis Lemos and Maisonnave Arisi, 2024; Xu et al., 2018). A better understanding of the nonculturable states in food matrices is necessary after non-thermal disinfections and would help to assess the real burden of pathogens in a nonculturable state in the food industry, especially after UV treatments (Xu et al., 2018).

CRediT authorship contribution statement

Víctor Arcos-Limiñana: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Marie Polet:** Writing – review & editing, Supervision, Methodology. **Bavo Verhaegen:** Writing – review & editing, Supervision, Methodology. **Arturo B. Soro:** Writing – review & editing, Methodology, Conceptualization. **Brijesh K. Tiwari:** Writing – review & editing. **Soledad Prats-Moya:** Writing – review & editing, Conceptualization. **Salvador Maestre-Pérez:** Writing – review & editing. **Koenraad Van Hoorde:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We want to thank all the technicians of the Foodborne Pathogens unit of Sciensano for their invaluable support. We are also grateful to the University of Alicante for their financial support through a predoctoral contract of the Vicerrectorado de Investigación y Transferencia de Conocimiento of the University of Alicante (UAFPU21-05 and UAEEBB23-02).

This work is framed within the doctoral thesis of Víctor Arcos Limiñana.

References

Arcos-Limiñana, V., Maestre-Pérez, S., Prats-Moya, M.S., 2025a. A comprehensive review on ultraviolet disinfection of spices and culinary seeds and its effect on quality. Compr. Rev. Food Sci. Food Saf. 24 (1). https://doi.org/10.1111/1541-4337.70076.

Arcos-Limiñana, V., Brunet, C., Prats-Moya, M.S., Maestre-Pérez, S., 2025b. UV-C disinfection and impact on the quality of black, white, green, and pink peppercorns using a scalable reactor. Food Bioprocess Technol. 1–12. https://doi.org/10.1007/S11947-025-03768-Y/FIGURES/6.

Bang, I.H., Kim, Y.E., Lee, S.Y., Min, S.C., 2020. Microbial decontamination of black peppercorns by simultaneous treatment with cold plasma and ultraviolet C. Innov. Food Sci. Emerg. Technol. 63, 102392. https://doi.org/10.1016/j.ifset.2020.102392.

Ceyssens, P.J., Mueller-Doblies, D., Mattheus, W., 2024. Design and validation of RT-PCR assays to differentiate *Salmonella* vaccine strains from wild-type field isolates. Vet. Sci. 11 (3), 120. https://doi.org/10.3390/VETSCI11030120/S1.

Chiang, E.L.C., Lee, S., Medriano, C.A., Li, L., Bae, S., 2022. Assessment of physiological responses of bacteria to chlorine and UV disinfection using a plate count method, flow cytometry and viability PCR. J. Appl. Microbiol. 132 (3), 1788–1801. https://doi.org/10.1111/jam.15325.

Codony, F., Dinh-Thanh, M., Agustí, G., 2020. Key factors for removing bias in viability PCR-based methods: a review. Curr. Microbiol. 77 (4), 682–687. https://doi.org/10.1007/S00284-019-01829-Y/METRICS.

Collings, E.R., Alamar Gavidia, M.C., Cools, K., Redfern, S., Terry, L.A., 2018. Effect of UV-C on the physiology and biochemical profile of fresh Piper nigrum berries. Postharvest Biol. Technol. 136, 161–165. https://doi.org/10.1016/j.postharvbio.2017.11.007.

Deng, L.Z., Tao, Y., Mujumdar, A.S., Pan, Z., Chen, C., Yang, X.H., Liu, Z.L., Wang, H., Xiao, H.W., 2020. Recent advances in non-thermal decontamination technologies for microorganisms and mycotoxins in low-moisture foods. Trends Food Sci. Technol. 106, 104–112. https://doi.org/10.1016/J.TIFS.2020.10.012.

El Darra, N., Xie, F., Kamble, P., Khan, Z., Watson, I., 2021. Decontamination of Escherichia coli on dried onion flakes and black pepper using infra-red, ultraviolet and ozone hurdle technologies. Heliyon 7 (6). https://doi.org/10.1016/j.heliyon.2021.e07259.

EPA, 2014. EPA Strategy to Address Mercury-Containing Products.

Erdoğdu, S.B., Ekiz, H.İ., 2013. Far infrared and ultraviolet radiation as a combined method for surface pasteurization of black pepper seeds. J. Food Eng. 116 (2), 310–314. https://doi.org/10.1016/j.jfoodeng.2012.12.026.

Fan, L., Liu, X., Dong, X., Dong, S., Xiang, Q., Bai, Y., 2021. Effects of UVC light-emitting diodes on microbial safety and quality attributes of raw tuna fillets. LWT 139, 110553. https://doi.org/10.1016/J.LWT.2020.110553.

FAOSTAT, 2022. Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL.

Fittipaldi, M., Nocker, A., Codony, F., 2012. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. J. Microbiol. Methods 91 (2), 276–289. https://doi.org/10.1016/J.MIMET.2012.08.007.

Freire, V., Condón, S., Gayán, E., 2024. Impact of sporulation temperature on germination of Bacillus subtilis spores under optimal and adverse environmental conditions. Food Res. Int. 182, 114064. https://doi.org/10.1016/J.FOODRES.2024.114064.

Gabriel, A., David, M., Elpa, M., Michelena, J., 2020. Decontamination of dried whole black peppercorns using ultraviolet-c irradiation. Food Microbiol. 88. https://doi.org/10.1016/J.FM.2019.103401.

Gayán, E., Álvarez, I., Condón, S., 2013. Inactivation of bacterial spores by UV-C light. Innovative Food Sci. Emerg. Technol. 19, 140–145. https://doi.org/10.1016/J.IFSET.2013.04.007.

Ghasemzadeh, A., Ashkani, S., Baghdadi, A., Pazoki, A., Jaafar, H., Rahmat, A., 2016. Improvement in flavonoids and phenolic acids production and pharmaceutical quality of sweet basil (Ocimum basilicum L.) by ultraviolet-B irradiation. Molecules 21 (9), 1203. https://doi.org/10.3390/molecules21091203.

Ha, J.-W., Kang, D.-H., 2013. Simultaneous near-infrared radiant heating and UV radiation for inactivating *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red pepper (*Capsicum annuum* L.). Appl. Environ. Microbiol. 79 (21), 6568–6575. https://doi.org/10.1128/AEM.02249-13.

Hinds, L.M., Guclu, G., Kelebek, H., Selli, S., O'Donnell, C.P., Tiwari, B.K., 2021. Effect of ultraviolet light emitting diode treatments on microbial load, phenolic and volatile profile of black peppercorns. LWT Food Sci. Technol. 152, 112133. https://doi.org/10.1016/j.lwt.2021.112133.

Jiang, Y., Ai, C., Liao, X., Liu, D., Ding, T., 2020. Effect of slightly acidic electrolyzed water (SAEW) and ultraviolet light illumination pretreatment on microflora inactivation of coriander. LWT Food Sci. Technol. 132, 109898. https://doi.org/10.1016/j.lwt.2020.109898.

Lee, S., Bae, S., 2018. Evaluating the newly developed dye, DyeTox13 Green C-2 Azide, and comparing it with existing EMA and PMA for the differentiation of viable and nonviable bacteria. J. Microbiol. Methods 148, 33–39. https://doi.org/10.1016/J.MIMET.2018.03.018.

Li, G.-Q., Wang, W.-L., Huo, Z.-Y., Lu, Y., Hu, H.-Y., 2017. Comparison of UV-LED and low pressure UV for water disinfection: photoreactivation and dark repair of Escherichia coli. Water Res. 126, 134–143. https://doi.org/10.1016/J.WATRES.2017.09.030.

Li, R., Dhankhar, D., Chen, J., Cesario, T.C., Rentzepis, P.M., 2019. A tryptophan synchronous and normal fluorescence study on bacteria inactivation mechanism. Proc. Natl. Acad. Sci. U. S. A. 116 (38), 18822–18826. https://doi.org/10.1073/PNAS.1909722116/ASSET/2E0F2FE4-1584-4A4E-8BB3-1C6DDABB03FA/ASSETS/GRAPHIC/PNAS.1909722116FIG 05.JPEG.

Li, L., Fu, J., Bae, S., 2022. Changes in physiological states of Salmonella typhimurium measured by qPCR with PMA and DyeTox13 Green Azide after pasteurization and UV treatment. Appl. Microbiol. Biotechnol. 106 (7), 2739–2750. https://doi.org/10.1007/s00253-022-11850-0.

Liu, J., Yang, L., Kjellerup, B.V., Xu, Z., 2023. Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. Trends Microbiol. 31 (10), 1013–1023. https://doi.org/10.1016/J.TIM.2023.04.009.

MacIsaac, S.A., Rauch, K.D., Prest, T., Simons, R.M., Gagnon, G.A., Stoddart, A.K., 2023. Improved disinfection performance for 280 nm LEDs over 254 nm low-pressure UV lamps in community wastewater. Sci. Rep. 13 (1), 1–9. https://doi.org/10.1038/s41598-023-34633-7.

Martín-Sómer, M., Pablos, C., Adán, C., van Grieken, R., Marugán, J., 2023. A review on LED technology in water photodisinfection. Sci. Total Environ. 885, 163963. https://doi.org/10.1016/J.SCITOTENV.2023.163963.

Mathot, A.G., Postollec, F., Leguerinel, I., 2021. Bacterial spores in spices and dried herbs: the risks for processed food. Compr. Rev. Food Sci. Food Saf. 20 (1), 840–862. https://doi.org/10.1111/1541-4337.12690.

Nocker, A., Sossa, K.E., Camper, A.K., 2007. Molecular monitoring of disinfection efficacy using propidium monoazide in combination with quantitative PCR. J. Microbiol. Methods 70 (2), 252–260. https://doi.org/10.1016/J.MIMET.2007.04.014.

Park, M.-J., Kim, J.-H., Oh, S.-W., 2019. Inactivation effect of UV-C and mild heat treatment against Salmonella typhimurium and Escherichia coli O157:H7 on black pepper powder. Food Sci. Biotechnol. 28 (2), 599–607. https://doi.org/10.1007/s10068-018-0480-4.

Park, M.-J., Kim, J.-H., Oh, S.-W., 2020. Inactivation of Salmonella typhimurium and Escherichia coli O157:H7 on black pepper powder using UV-C, UV-A and TiO2 coating. Food Sci. Biotechnol. 29 (2), 283–291. https://doi.org/10.1007/s10068-019-00651-3.

Ramamurthy, T., Ghosh, A., Pazhani, G.P., Shinoda, S., 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. Front. Public Health 2 (JUL). https://doi.org/10.3389/FPUBH.2014.00103.

RASFF, 2024. Rapid Alert System for Food and Feed Window. https://webgate.ec.europa.eu/rasff-window/screen/search.

Regulation (EU) 2017/852 of the European Parliament and of the Council of 17 May 2017 on Mercury, and Repealing Regulation (EC) No 1102/2008. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02017R0852-20240730, 2024.

dos Reis Lemos, L.M., Maisonnave Arisi, A.C., 2024. Viability dyes quantitative PCR (vPCR) assays targeting foodborne pathogens - scientific prospecting (2010−2022). Microchem. J. 197, 109769. https://doi.org/10.1016/J.MICROC.2023.109769.

Shango, A.J., Mkojera, B.T., Majubwa, R.O., Mamiro, D.P., Maerere, A.P., 2021. Pre- and postharvest factors affecting quality and safety of pepper (*Piper nigrum* L.). CAB Rev.: Perspect. Agric., Vet. Sci., Nutr. Nat. Resour. 16 (31). https://doi.org/10.1079/PAVSNNR202116031.

Soro, A.B., Whyte, P., Bolton, D.J., Tiwari, B.K., 2021a. Application of a LED-UV based light technology for decontamination of chicken breast fillets: impact on microbiota and quality attributes. LWT 145, 111297. https://doi.org/10.1016/J.LWT.2021.111297.

Soro, A.B., Whyte, P., Bolton, D.J., Tiwari, B.K., 2021b. Modelling the effect of UV light at different wavelengths and treatment combinations on the inactivation of campylobacter jejuni. Innov. Food Sci. Emerg. Technol. 69, 102626. https://doi.org/10.1016/J.IFSET.2021.102626.

Xu, L., Zhang, C., Xu, P., Wang, X.C., 2018. Mechanisms of ultraviolet disinfection and chlorination of Escherichia coli: culturability, membrane permeability, metabolism, and genetic damage. J. Environ. Sci. (China) 65, 356–366. https://doi.org/10.1016/J.JES.2017.07.006.

Zhang, S., Ye, C., Lin, H., Lv, L., Yu, X., 2015. UV disinfection induces a vbnc state in *escherichia coli* and *pseudomonas aeruginosa*. Environ. Sci. Tech. 49 (3), 1721–1728. https://doi.org/10.1021/ES505211E/SUPPL_FILE/ES505211E_SI_001.PDF.