International Journal of Food Microbiology 364 (2022) 109535

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Inactivation of *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* on apple peel and apple juice by ultraviolet C light treatments with two irradiation devices

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ARTICLE INFO

Keywords:
Modelisation
Microorganisms
Pathogens
Survival
Irradiation
Inactivation model
Weibull
Biphasic

ABSTRACT

Following the market trends, the consumption of fresh and cold-pressed juice in Europe is increasing. However, a primary concern – particularly in apple juice – is the related outbreaks caused by food-borne pathogens. One of the challenges is to find methods able to reduce pathogenic loads while avoiding deterioration of nutritional properties and bioactive compounds that occur in thermal pasteurization processes. In this study, the inactivation of *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* was evaluated under different ultraviolet C (UVC_{254nm}) light treatments (up to 10,665.9 ± 28.1 mJ/cm^2), in two different steps of the production chain (before and after juice processing): on apple peel discs and in apple juice. The systems proposed were a horizontal chamber with UVC_{254nm} emitting lamps treating the product disposed at a distance of 12 cm, and a tank containing UVC_{254nm} lamps and in which the product is immersed and agitated. Final reductions ranged from 3.3 ± 0.5 to 5.3 ± 0.4 logarithmic units, depending on the microorganism, matrix and used device. The survival curves were adjusted to Weibull and biphasic models (R^2-adj ≥ 0.852), and UVC doses needed for the first decimal reduction were calculated, being lower for the apple peel discs (0.20 to 83.83 mJ/cm^2) than they were for apple juice (174.60 to 1273.31 mJ/cm^2), probably for the low transmittance of the apple juice compared to the surface treatment occurring on the peels. Within the treatments evaluated, the UVC_{254nm} irradiation of apple peels immersed in water was the best option as it resulted in a reduction of the tested microorganisms of ca. 2–3 log units at lower UVC_{254nm} doses (< 500 mJ/cm^2) when compared to those occurring in apple peel treated with the UVC chamber and in juice. As contamination can proceed from apples, the sanitization of these fruit prior to juice production may be helpful in reducing the safety risks of the final product, reducing the drawbacks related to the poor transmittance of the fruit juices.

1. Introduction

Juices, as an alternative way for fruit and vegetable intake, are a good approach to easily increase consumption of such products associated with health benefits (WHO, 2004). Nowadays, trends in this sector are transferred to products that are perceived as healthier, fresher and more natural options: fresh juices and cold-pressed juices, which consumption is expected to increase 7 % each year (AIJN, 2019).

However, fresh fruit and vegetable juices have caused several outbreaks in the last years (2005–2020), especially apple juice (16 outbreaks) and orange juice (2 outbreaks) (Krug et al., 2020). Among the cases of apple juice of known etiology, 11 were related with the presence of *Escherichia coli* (being 7 of them the verotoxigenic strain O157:H7), 4 of them associated with *Cryptosporidium parvum*, and 1 of them related to *Salmonella enterica* serovar Typhimurium (CDC, 2021), being all of them in unpasteurized products. In this regard, the European legislation (CE (EU) 2073/2005, (Comission Regulation, 2019) establishes limits for three selected microorganisms in non-pasteurized fruit and vegetable juices. The first is *E. coli*, whose maximum permitted load is <10^3 CFU/g in 2/5 samples, and it is used as a hygiene index, and as safety parameters, *Salmonella* spp. population must be undetected in 25 g in 5/5 samples, and *Listeria monocytogenes* populations must be <10^2 CFU/g in

Abbreviations: BPW, buffered peptone water; cUVC, chamber UVC device; SCS, *Salmonella* Chromo Select; SP, saline peptone; TSA, tryptone soy agar; TSB, tryptone soy broth; tUVC, tank UVC device; UVC, ultraviolet C; XLD, xylose lysine desoxycholate.

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https://doi.org/10.1016/j.ijfoodmicro.2022.109535
Received 29 July 2021; Received in revised form 16 December 2021; Accepted 6 January 2022
Available online 10 January 2022
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5/5 samples.

The contamination of the apples in the field and during their processing represents the main cause of presence of foodborne pathogens in the derived juices, so it is key to implement and enforce include good agricultural practices (GAP) and good manufacturing practices (GMF) (Reij and Den Aantrekker, 2004). However, strategies conducted in handling may not be enough to assure safety of those products, as subsequent contamination or cross-contamination can occur, so additional steps need to be added (e.g. disinfection of the fruits used, disinfection of the washing water of the cleaning step of fruits in fruit industry, or decontamination of the juice). Alternatives to thermal pasteurization, with the purpose to reduce pathogenic microorganisms and simultaneously, preventing the drawbacks that the application of this technology may originate in apple juices (e.g. deterioration of the quality of the product during processing and storage degradative reactions including quality depletion because of phenolic changes, vitamin destruction and flavor component damage (Achir et al., 2016; Petruzzi et al., 2017)) are being investigated (Wibowo et al., 2019).

One substitute that has acquired commercial status is the application of high hydrostatic pressures, but the high investment needed, the maintenance level and other technical issues make it an expensive option (Elamin et al., 2015). Other alternative treatments that are currently being investigated include ultrasound, pulsed light, ozone, high electric pulsed fields and ultraviolet light (Gouma et al., 2020; Yildiz et al., 2019). Among them, ultraviolet (UV) radiation has gained interest due to the absence of toxic by-products generated during the treatment, no production of off-tastes and off-odors of the treated products, and the lower requirement of energy when compared to high hydrostatic pressure processes (Riganakos et al., 2017). It involves the irradiation of the product with the electromagnetic spectrum that ranges from 100 to 400 nm, being the part comprised between 200 and 280 nm (part C) the most germicidal. As this absorbance coincides with the highest absorbance of DNA (254 nm), DNA replication is blocked compromising the cell function of microorganisms (Guerrero-Beltrán and Barbosa-Cánovas, 2004).

Ultraviolet C (UVC) light irradiation is considered safe for milk disinfection by the European Food Safety Authority (EFSA, 2016) and its use is now allowed for certain products in European Union (mushrooms, bread, bread's yeast *Saccharomyces cerevisiae*, and milk) (EU 2017/2470 (European Union, 2017)). Moreover, its usage is permitted in the United States as a method to reduce human pathogens and other microorganisms in juices (21CFR179.39) and has been already evaluated in apple juices and cider for its effect in natural occurring microbiota and artificially inoculated microorganisms, using different lab- and pilot-scale devices (Adhikari et al., 2015; Gouma et al., 2015). However, juice treatment with UVC has some limitations related to the penetration of light in the product. Parameters such as color, organic matter suspensions and fibers can affect transmittance of the media, limiting its efficacy to the surface of the product. As an example, Fenoglio et al. (2020) used a pilot-scale equipment to treat juices of different fruits and concluded that the UVC dosage needed to reduce 1 log unit the tested microorganisms (*E. coli*, *Lactobacillus plantarum* and *Saccharomyces cerevisiae*) increased with decreasing transmittances and increasing turbidities.

A strategy to avoid this penetration drawback is to irradiate fruits prior to the juice processing. In this regard, Colás-Medà et al. (2021) proposed the irradiation of orange peels to inactivate alterative spore-forming bacteria as a method to reduce microbial load in orange juices, resulting in higher reductions compared to the treatment applied directly to the juice. However, efficacy of UVC light irradiation is also correlated with fruit surface roughness, contact angle and surface energy, being less hydrophobic fruits with smooth surfaces (pears or apples) more susceptible to UVC irradiation than hydrophobic and rough surface fruits (cantaloupe or strawberry) (Adhikari et al., 2015).

For the aforementioned casuistry, the present study aims to evaluate UVC_{254nm} irradiation via two different devices to reduce final loads of the selected microorganisms of interest (*E. coli*, *S. enterica* and *L. monocytogenes*) in apple juices. The application of UVC_{254nm} has been evaluated in two scenarios entailing different steps of the juice production process: the irradiation of apple fruit prior to juice production in order to reduce microbial load of the prime material; or the irradiation of apple juice as a final treatment to prevent any further contamination prior to the packaging. In addition, two different devices have been compared in order to elucidate the best approach for each scenario.

2. Materials and methods

2.1. Preparation of the apple matrices: peel discs and juice

Apples cv. 'Golden' with no post-harvest treatments were kindly provided by a local producer. Apples were washed with cold tap water and discs of 2.54 cm^2 were cut with a cork borer sterilized by flamed ethanol. The peel discs were used for the first scenario studied, consisting in the treatment of raw fruit prior to processing in order to decrease the microbial impact in the processed juice. The use of apple juice was related to the second proposed scenario, consisting in the treatment of the final product to reduce its microbial load, regardless its origin. The apple juice was prepared from a multi-varietal concentrate (70 % soluble solids) by adding tap water to reach a final concentration of 11.2 % soluble solids and a density of 1045.0 g/L (*CD 2001/112/EC* (Council Directive, 2001)). Soluble solids were measured using a handheld refractometer PAL-1 (Atago, Japan) and density was calculated using Eq. (1) and using a 25 mL-pycnometer to determine the mass of the solutions.

$$d_j\ (g/L) = \left(\frac{m_w - m_0}{m_j - m_0}\right) d_w \quad (1)$$

where d_j is the density of the juice (in g/L), m_0 is the mass of the empty pycnometer (g), m_w is the mass of the pycnometer with water (g), m_j is the mass of the pycnometer with juice, and d_w is the density of water (g/L) at the working temperature.

The juice transmittance (% T) was calculated from the measured absorbance (A) at 254 nm using a spectrophotometer (UV-1600PC, VWR International, USA) and using the following Eq. (2):

$$\mathrm{A} = -log10\left(\frac{\%T}{100}\right) \quad (2)$$

2.2. Microbial culture conditions and inoculation in apple matrices

Prior to the experimentation, all strains were stored in 20 % glycerol at −20 °C. For the inoculation of apple matrices (peel discs and juice), an inoculum of three microorganisms was prepared. It consisted on a cocktail of: one foodborne bacterium used as an indicator of the hygiene of the process – *Escherichia coli* (from *Colección Española de Cultivos Tipo* (CECT): strains CECT-101, CECT-515, CECT-516 and CECT-543) – and two foodborne pathogens – *Salmonella enterica* (strains CECT-4300 (serovar Enteritidis), and strains ATCC ® BAA-707 (serovar Agona), ATCC ® BAA-710 (serovar Montevideo), and ATCC ® BAA-711 (serovar Gaminara) from the *American Type Culture Collection* (ATCC)) and *Listeria monocytogenes* (strains CECT-933 (serovar 3a), CECT-940 (serovar 4d), CECT-4031 (serovar 1/2) and CECT-4031 (serovar 4b))–. In preliminary experiments (data not shown), interspecies UV_{254nm} sensitivity differences were found. For this, four strains of each microorganism were used, in order to ensure representativity of the different resistances to UV_{254nm} light that industries could encounter in case of a contamination of the produce.

The inoculum was prepared using 10 mL of culture of each strain incubated at 37 °C overnight, in tryptone soy broth (TSB, Biokar, France) for *E. coli* or *S. enterica* and TSB supplemented with 6 g/L yeast extract (Biokar) for *L. monocytogenes*. Suspensions were centrifuged at

8,900 ×g for 10 min at 20 °C (Hettich-Universal 320 R, Tuttlingen, Germany), supernatant was discarded and the pellet was resuspended in half the initial volume of saline solution (8.5 g/L NaCl). Concentrations of each microorganism in the inoculum were checked by decimal dilutions in saline peptone (SP, 8.5 g/L NaCl (VWR Chemicals, USA), and 1 g/L peptone (Biokar)) plated on tryptone soy agar (TSA, Biokar) and *Salmonella* chromo select agar (SCS, Sigma-Aldrich) for *E. coli*, onto TSA and xylose lysine deoxycholate agar (XLD, Biokar) for *S. enterica*, and onto TSA supplemented with yeast extract (6 g/L) and Palcam agar (Biokar) with a selective supplement for Palcam (Biokar) for *L. monocytogenes*. Plates were incubated at 37 °C for 24 ± 2 h for *E. coli* and *S. enterica* and for 48 ± 2 h for *L. monocytogenes*. To determine the concentration of each bacterium in the inoculum, decimal dilutions were plated onto XLD agar that is selective for *S. enterica* and Palcam agar that is selective for *L. monocytogenes*. When the inoculum was plated on SCS agar, the colonies of *S. enterica* and *E. coli* could not be distinguished one from another. For this, *E. coli* population in this work was calculated by Eq. (3):

$$E.coli \text{ population (CFU/mL)} = \text{Population in SCS agar (CFU/mL)} - \text{Population in XLD agar (CFU/mL)} \quad (3)$$

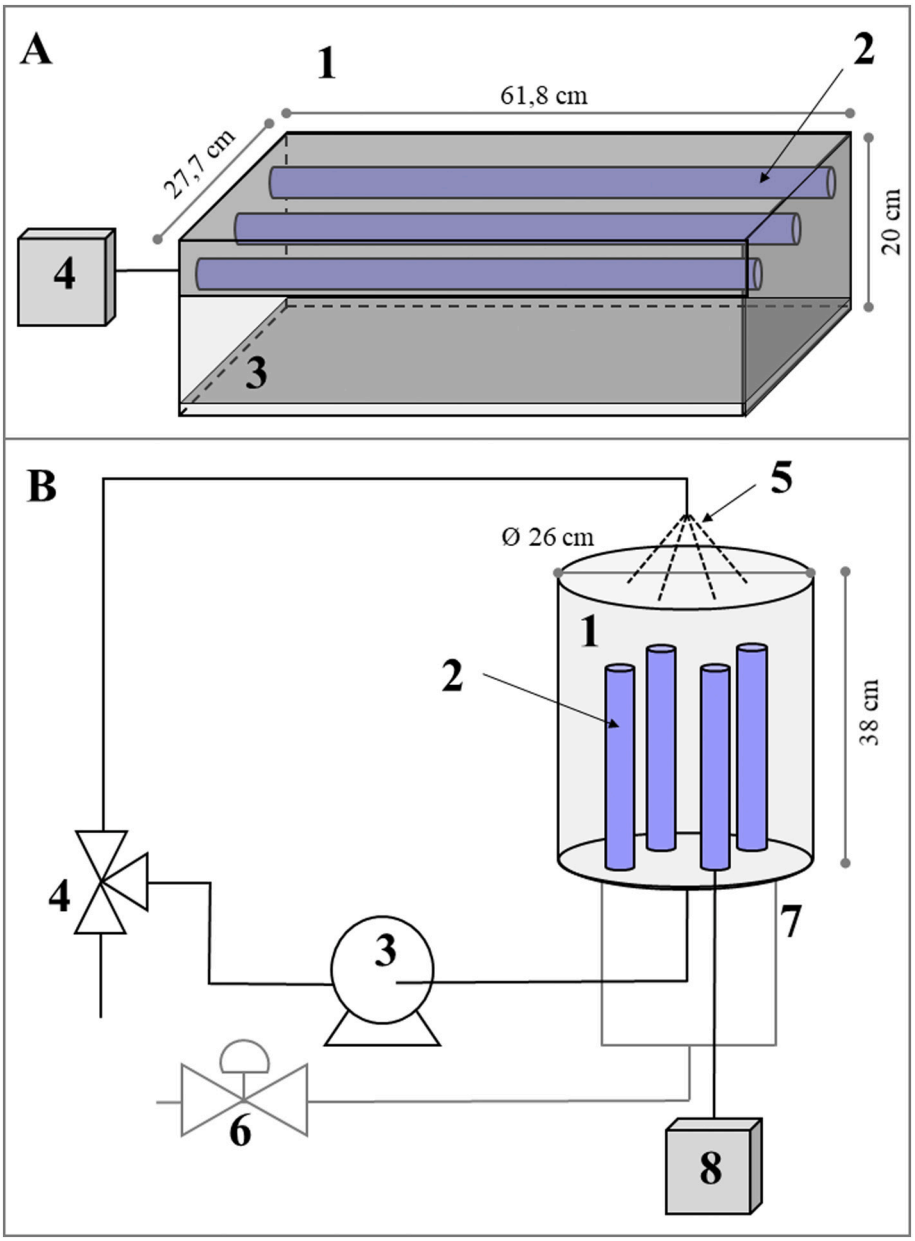

Fig. 1. Scheme of (A) the chamber UVC_{254nm} device (cUVC): enclosure (1), UVC lamps (2), sample platter (3), and power supply for lamps (4); and (B) the tank UVC_{254nm} device (tUVC): tank (1), UVC lamps (2), water pump (3), water circuit valve (4), water recirculation (5), air regulator valve (6), air inlet (7), and power supply for lamps (8).

Inoculation was performed as follows. The apple peel discs were spot inoculated pipetting 50 μL of the prepared concentrate to reach a concentration of 5×10^5 CFU/cm^2, and dried for 2 h at 25 °C. The apple juice was inoculated with an adequate volume of the prepared concentrate to reach a concentration of 5×10^5 CFU/mL.

2.3. UVC_{254nm} devices

The use of two laboratory-scale devices (UV - Consulting Peschl España) with different UVC_{254nm} lamps configurations was explored in this investigation. The first (Fig. 1A) consisted of a horizontal UVC chamber (61.8 × 27.7 × 20 cm) equipped with three monochromatic UVC lamps (254 nm, 30 W) (cUVC). The UVC_{254nm} intensity during treatments was monitored by a UV-sensor Easy HW (Peschl Ultraviolet, Germany) radiometer, that was placed in the same position than the samples. The second device (Fig. 1B) consisted of a 15 L (38 × 26 Ø cm) tank with four UVC lamps (254 nm, 17.2 W) (tUVC) distributed vertically in its basis. The tank is provided with a closed system and a pump that permits the recirculation of the liquid inside. Prior to the treatments, the devices were switched on for 30 min to pre-heat the lamps and ensure light stability. Irradiation dose was calculated using Eq. (4) (Kowalski, 2009):

$$\text{Dose}\left(\text{mJ}/\text{cm}^2\right) = \text{Intensity}\left(\text{W}/\text{m}^2\right) \times \text{time (s)}/10 \quad (4)$$

2.4. Microbial inactivation by UVC_{254nm} irradiation

2.4.1. Sample disposition in the UVC_{254nm} devices and irradiation treatments

Both matrices (apple peel discs and apple juice) were submitted to irradiation treatments in the two UVC_{254nm} devices (cUVC and tUVC). Irradiation doses at which samples were subjected were established by preliminary studies and are shown in Table 1. Also, the bacterial populations in inoculated but untreated samples were monitored at each treatment times to evaluate the effect that matrices could have on their survival.

In the cUVC device, samples were positioned on a tray at a 12 cm distance from the lamps, and light was transmitted through air. Inoculated apple peel discs were distributed directly in the tray, and at each sampling time, 5 discs per replicate (3 replicates) were introduced in a sterile filter bag (BagPage®, Internscience BagSystem, France). For the trials using inoculated apple juice, 12-well plates distributed on the tray (Falcon, USA) were used containing 1.2 mL of sample per well (4 mm depth). One milliliter of juice was collected per replicate (3 replicates) at each evaluated dose.

In the tUVC device, inoculated apple discs were immersed in 14 L of cold tap water, that was recirculated at a flow rate of 17.5 L/min. At each sampling time, 5 discs per replicate (3 replicates) were placed in a sterile filter bag (BagPage®) and 1 mL per replicate (3 replicates) of washing water was also collected to evaluate the remaining bacteria in the water due to their physical removal from the peel surface. For the trials using inoculated apple juice, 14 L of the prepared juice were set in the tank and aliquots of one milliliter were collected per replicate (3 replicates) at each evaluation dose.

Table 1
UVC_{254nm} irradiation doses (mJ/cm^2) at which apple peel discs or juice were subjected depending on the device: the chamber (cUVC) or the tank (tUVC).

Apple peel discs		Apple juice	
cUVC device	tUVC device	cUVC device	tUVC device
602.4 (6.7)	96.0 (1.0)	95.2 (1.9)	600.0 (1.0)
1473.9 (1.0)	192.0 (1.0)	603.7 (4.9)	904.0 (1.0)
2375.1 (1.5)	304.0 (1.0)	889.5 (1.0)	1200.0 (1.0)
3290.6 (1.0)	600.0 (1.0)	1195.1 (10.5)	1504.0 (1.0)
4504.3 (1.0)	904.0 (1.0)	1517.9 (45.3)	1800.0 (1.0)
6100.5 (102.7)	1200.0 (1.0)	1799.1 (21.1)	2704.0 (1.0)
7687.1 (129.3)	1504.0 (1.0)	2096.3 (33.9)	3000.0 (1.0)
9128.3 (39.6)	3304.0 (1.0)	2432.3 (7.8)	3600.0 (1.0)
10,665.9 (28.1)	4504.0 (1.0)	3056.3 (37.4)	4800.0 (1.0)
	7504.0 (1.0)	3644.1 (1.0)	6000.0 (1.0)

Values are the mean and values in brackets express the standard error.
cUVC, chamber UVC_{254nm} device; tUVC, tank UVC_{254nm} device.

2.4.2. Microbiological analysis

To determine bacterial populations on apple peel discs, 5 mL buffered peptone water (BPW, Biokar) was added to the 5-disc pool and the mix was homogenized in a paddle blender (IUL, Spain) for 90 s (250 impact/min), decimally diluted in SP, spread onto agar plates (XLD for *S. enterica* counts, SCS for colony count of *S. enterica* and *E. coli*, and Palcam for *L. monocytogenes* counts) and incubated at 37 °C for 24 or 48 h. Adequate growth on selective media of the tested microorganisms was checked and compared to growth on nutritive media after different UV_{254nm} irradiation doses applied to a pure culture in order to avoid underestimation, that could occur if too sub-lethally damaged cells could not effectively grow on selective media (data not shown). Given the compositing of 5 discs with a total surface of 12.7 cm^2 and diluted in 5 mL BPW, a quantification limit of 10 CFU/cm^2 was established. When populations were below the detection limit, and presence was confirmed by plating in the adequate media the incubated BPW homogenate suspension (37 °C for 24 ± 2 h), an arbitrary value of ½ quantification limit was established for further data treatment.

To determine bacterial populations in liquid samples (apple juice or washing water in peel discs trials using tUVC), samples were decimally diluted in SP, spread onto agar plates and incubated at the same conditions described above. Quantification limit was 25 CFU/mL, and when counts were below and pathogen presence was confirmed, an arbitrary value of ½ quantification limit was considered for further data treatment.

2.4.3. Microbiological inactivation models

To avoid small differences in initial concentrations between experiments, microbiological data were expressed as the survival fraction N/N_0 where N is the population measured for each replica and N_0 is the mean of the initial population of each experiment in the untreated samples ($n = 3$). Survival curves were obtained by plotting the logarithm of the survival fraction ($\log N/N_0$) in front of treatment doses (mJ/cm^2), including data from at least two trials ($n = 6$). The kinetic parameters of the microbial inactivation under UVC light exposure were obtained using the GInaFit complement for Excel (Geeraerd et al., 2005). According to the behavior of the survival curve, the inactivation data were fitted to two possible inactivation kinetic models: (i) the Weibull model (Eq. (5)), that is a model that typically fits upward and downward concavity in curves and uses a δ parameter, which is close to the classical concept of the D value, established for sterilization processes (Albert and Mafart, 2005), and (ii) the biphasic model (Eq. (6)), that assumes one initially major population that is more sensitive to stress (initial decline) and one minor subpopulation that is more resistant to stress (tail, smoother constant decline) (Cerf, 1977):

$$\text{Weibull model}: \text{Log}\frac{N}{N_0} = -\left(\frac{d}{\delta}\right)^p \quad (5)$$

where d (mJ/cm^2) is the applied UV dose, δ (mJ/cm^2) is a scale parameter that indicates the dose for the first decimal reduction, and p (dimensionless) is a shape parameter describing upward or downward concavity of the curve, and

$$\text{Biphasic model}: \text{Log}\frac{N}{N_0} = log\left(f^{(-k_{max1}d)} + (1-f)^{(-k_{max2}d)}\right) \quad (6)$$

where f is the fraction of the initial major subpopulation, k_{max1} and k_{max2}

(cm^2/mJ) are the first-order inactivation rate constants for the initially major (*1*) and minor (*2*) populations, and *d* (mJ/cm^2) is the applied UV dose.

The model performance was compared by means of the adjusted correlation coefficient R^2 (R^2-adj) and the root mean square error (RMSE).

2.4.4. Statistical analysis

Data obtained from experimentation was collected as follows. Experiments in the different apple matrices and devices were performed in different days, and performed at least twice (2 replicates in 2 different days), with three samplings each time (3 repetitions). For each sampling, the effect of UVC_{254nm} light was evaluated simultaneously on the three microorganisms by plating them in different selective media. A repetition in apple peel disc experiments consisted on 5 different discs taken randomly from the chamber or the tank. A repetition in apple juice experiments consisted on 1 mL of juice, taken randomly from the 12-well plates or from the tank. Models were obtained as described in Section 2.4.3 ($n = 6$). For experiments in the tUVC device, 1 mL per repetition (3 repetitions, 2 replicates, n = 6) were aliquoted to check microbial load in washing water after the treatments. Data in washing water were checked for significant differences between treatments by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied.

3. Results

3.1. Modeling of the inactivation curves

The data from the survival curves under different irradiation doses of UVC light of *E. coli*, *S. enterica* and *L. monocytogenes* artificially inoculated on apple peel discs and in apple juice were adjusted to two mathematical models: Weibull and biphasic. To evaluate the goodness of fit of the models, two parameters were used (Table 2): (i) R^2-adj values, expressing the goodness of fit of a dataset to a model adjusted to the number of variables, and (ii) RMSE, that expresses the average deviation between the observed and the fitted values. Both models were considered to accurately describe the inactivation curves of the microorganisms on apple peel discs and in apple juice, as the lowest R^2-adj values were 0.868 and 0.852 for Weibull and biphasic models both on apple peel discs at tUVC device, respectively, and the highest RMSE values were 0.355 and 0.391 for Weibull and biphasic models for apple peel discs and apple juice at tUVC device, respectively. As Weibull and biphasic models showed similar fit values and give different biological information of the inactivation rates, both were used to explain inactivation curves in this paper.

3.2. UVC_{254nm} inactivation of bacteria in apple peel discs

The initial populations of *E. coli*, *S. enterica* and *L. monocytogenes* on apple peel discs were 5.4 ± 0.1, 5.8 ± 0.4 and 5.6 ± 0.3 log units, respectively. Population survival in apple peel for the duration of the experiment was confirmed in untreated samples, as no significant reductions ($p > 0.05$) were observed (data not shown). The reduction curves of the bacteria artificially inoculated on apple peel discs and exposed to UVC_{254nm} were assessed (Fig. 2). A significant reduction ($p < 0.001$) of *E. coli*, *S. enterica* and *L. monocytogenes* populations was observed after 602.4 ± 6.7 mJ/cm^2 when using the cUVC system, and 904.0 ± 1.0 (Fig. 2A), 304.0 ± 1.0 (Fig. 2B) and 96.0 ± 1.0 mJ/cm^2 (Fig. 2C) for each microorganism, respectively, when using the tUVC system.

The kinetic profile was different depending on the target microorganism and on the device used for the irradiation. However, a common trend could be detected: curves showed a quick reduction of the population at lower irradiation doses followed by a less pronounced decrease in microbial cells. This behavior is well described by the Weibull and biphasic model parameters (Table 3).

Given by the Weibull model, the radiation dose for the first decimal reduction (δ) is an adequate parameter to compare methods for their efficacy, having a meaning close to the classical concept of the D-value (Albert and Mafart, 2005). The models showed differences between cUVC and tUVC formats, being the δ-values higher for the former (83.83 ± 7.56 mJ/cm^2, *E. coli*) than they were for the latter (27.30 ± 42.61 mJ/cm^2, *E. coli*). Mentioned values are for *E. coli* but comparison between both formats can be extended to the other microorganisms in this case. Differences were also observed between microorganisms, being the higher δ-values corresponding to *E. coli* inactivation. In contrast, *S. enterica* and *L. monocytogenes* were more sensitive, needing lower irradiation doses to achieve the first decimal reduction (81.14 ± 50.71 and 3.63 ± 5.28 mJ/cm^2 for *S. enterica* in cUVC and tUVC systems, respectively, and 9.95 ± 8.66 and 0.20 ± 0.14 mJ/cm^2 for *L. monocytogenes* in the same devices, respectively). After that and until the irradiation with 10,665.9 ± 28.1 or 7504.4 ± 1.0 mJ/cm^2 in the cUVC or the tUVC devices, population of *E. coli*, *S. enterica* and *L. monocytogenes* had decreased 3.6 ± 0.6 and 3.8 ± 0.9, 3.3 ± 0.5 and 3.5 ± 0.3, and 3.4 ± 0.3 and 3.9 ± 0.5 log units (Fig. 2), respectively. Given also by the Weibull model, the type of the shape is assumed by the *p* parameter, being in all the cases <1 for the models obtained: from 0.14 ± 0.03 (*L. monocytogenes*, tUVC) to 0.51 ± 0.15 (*E. coli*, cUVC), indicating downward concavity. In the biphasic models, the values of $kmax_1$ and $kmax_2$ are related with the variation in the reduction slopes, being *f* the fraction of the least resistant subpopulation that decreases according to $kmax_1$. The *f*-value ranged between 0.97 and 0.99, indicating that the majority of the population had low resistance to the UVC_{254nm} irradiation in these systems. These first and least resistant subpopulations had higher inactivation rates ($kmax_1$), ranging from 7.93 ± 4.13 (*E. coli*) to 3.92 ± 1.78 (*L. monocytogenes*) $cm^2/10^3$·mJ when working with the cUVC device, and ranging from 21.26 ± 4.28 (*S. enterica*) to 13.66 ± 5.02 (*E. coli*) $cm^2/10^3$·mJ when using the tUVC device. In contrast, the most resistant population showed a lower inactivation rate ($kmax_2$), ranging from 0.14 ± 0.11 (*L. monocytogenes*) to

Table 2
The statistical indices indicating the goodness of fit for Weibull and biphasic models describing the inactivation kinetics of *E. coli*, *S. enterica* and *L. monocytogenes* artificially inoculated on apple peel discs and apple juice under UVC_{254nm} light irradiation.

Microorganism	Parameter	Apple peel discs				Apple juice			
		cUVC device		tUVC device		cUVC device		tUVC device	
		Weibull	Biphasic	Weibull	Biphasic	Weibull	Biphasic	Weibull	Biphasic
E. coli	R^2-adj	0.968	0.931	0.868	0.852	0.956	0.983	0.988	0.988
	RMSE	0.217	0.226	0.315	0.333	0.267	0.174	0.154	0.138
S. enterica	R^2-adj	0.978	0.953	0.935	0.941	0.993	0.989	0.918	0.989
	RMSE	0.145	0.214	0.269	0.257	0.148	0.185	0.355	0.130
L. monocytogenes	R^2-adj	0.984	0.907	0.932	0.887	0.988	0.981	0.954	0.984
	RMSE	0.127	0.371	0.304	0.393	0.178	0.228	0.310	0.185

cUVC, chamber UVC_{254nm} device; tUVC, tank UVC_{254nm} device; R^2-adj, R^2 adjusted; RMSE, root mean standard error.

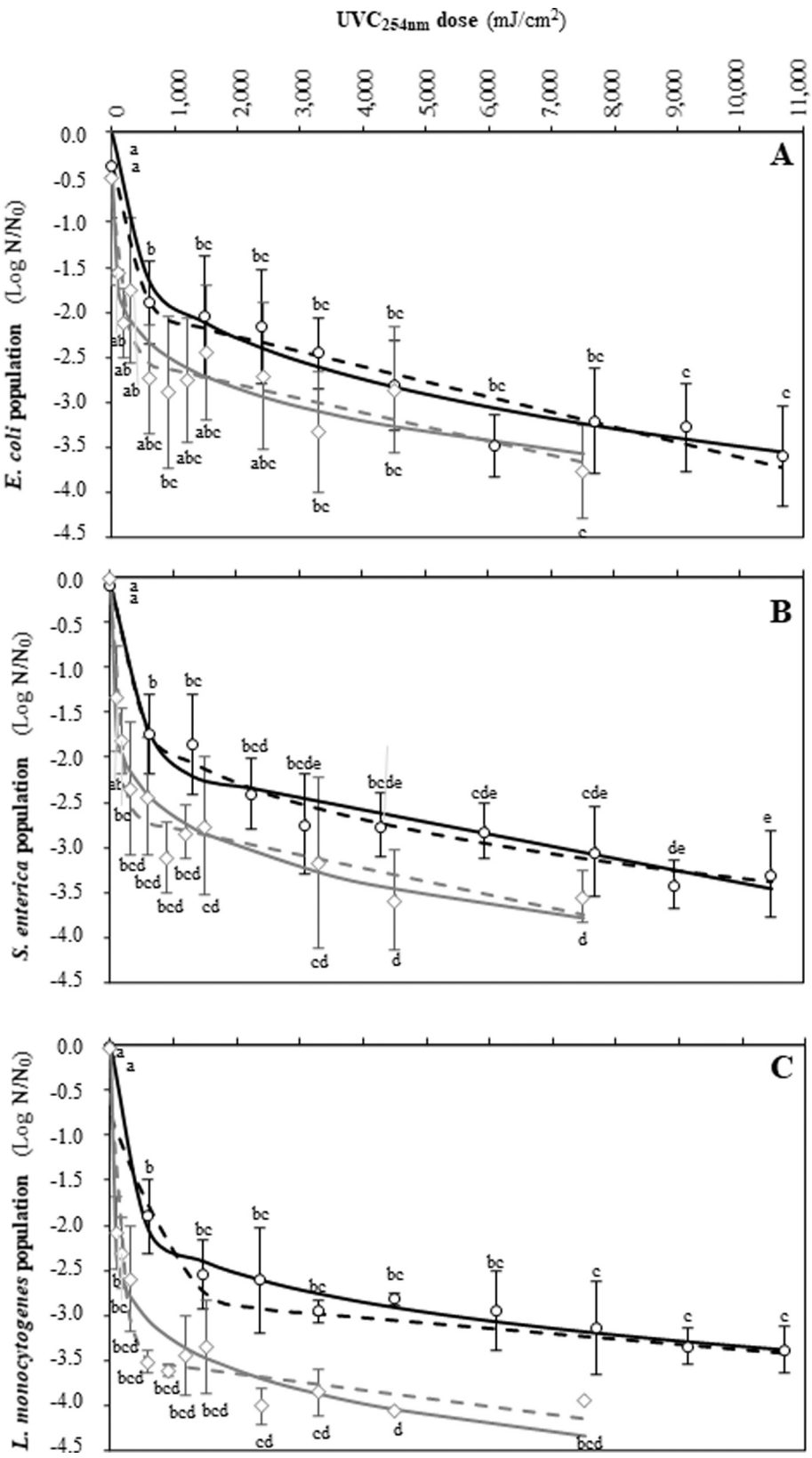

Fig. 2. Survival curve of *E. coli* (A), *S. enterica* (B) and *L. monocytogenes* (C) artificially inoculated on apple peel discs at the evaluated UVC_{254nm} doses. Represented are the data corresponding to the use of the horizontal UVC_{254nm} device (cUVC) (black) or the tank UVC_{254nm} device (tUVC) (grey). The dots represent experimental data when using the cUVC device (circles) or the tUVC device (diamonds), expressed by the mean, and deviation bars show the standard error ($n = 6$). The lines represent fitted data predicted values, according to the Weibull model (solid line) and the biphasic model (discontinuous line). Different letters indicate significant differences ($p < 0.05$) among bacterial surviving populations at different UVC_{254nm} doses, according to Tukey's test.

0.39 ± 0.07 (*E. coli*) $cm^2/10^3 \cdot mJ$ via the cUVC system, and ranging from 0.21 ± 0.15 (*L. monocytogenes*) to 0.36 ± 0.12 (*E. coli*) $cm^2/10^3 \cdot mJ$ with the tUVC system.

The population of the three selected microorganisms that remained in washing water during the UVC_{254nm} treatments in the tUVC device was monitored (Fig. 3). The population after the first UVC_{254nm} irradiation dose (96.0 ± 0.1 mJ/cm^2) was 2.8 ± 0.3, 2.0 ± 0.3 and 1.3 ± 0.2 log CFU/mL for *E. coli*, *S. enterica* and *L. monocytogenes*, respectively. Populations significantly decreased ($p < 0.05$) with increasing irradiation doses, and achieved undetectable levels (<1.1 log units) after

Table 3
UVC_{254nm}-resistance parameters obtained from the fitting of Weibull and biphasic models to the inactivation curves of *E. coli*, *S. enterica* and *L. monocytogenes* artificially inoculated on apple peel discs and apple juice under UVC_{254nm} light irradiation.

			Apple peel discs		Apple juice	
Model	Microorganism	Kinetic parameters	cUVC device	tUVC device	cUVC device	tUVC device
Weibull	*E. coli*	δ	83.83 (7.56)	27.30 (42.61)	331.30 (132.82)	1273.31 (202.79)
		p	0.51 (0.15)	0.20 (0.05)	0.60 (0.09)	0.90 (0.08)
	S. enterica	δ	81.14 (50.71)	3.63 (5.28)	174.60 (37.44)	567.76 (291.90)
		p	0.24 (0.03)	0.17 (0.03)	0.55 (0.04)	0.61 (0.12)
	L. monocytogenes	δ	9.95 (8.66)	0.20 (0.41)	198.23 (51.75)	539.36 (215.78)
		p	0.17 (0.02)	0.14 (0.03)	0.55 (0.05)	0.66 (0.10)
Biphasic	*E. coli*	f	0.97 (0.02)	0.98 (0.01)	0.99 (0.01)	0.99 (0.04)
		k_{max1}	7.93 (4.13)	13.66 (5.02)	3.63 (0.30)	2.46 (0.16)
		k_{max2}	0.39 (0.07)	0.36 (0.12)	0.34 (0.60)	0.00 (0.23)
	S. enterica	f	0.98 (0.01)	0.99 (0.02)	0.99 (0.01)	0.99 (0.01)
		k_{max1}	6.95 (1.61)	21.26 (4.28)	5.31 (0.51)	2.16 (0.25)
		k_{max2}	0.31 (0.06)	0.34 (0.09)	0.17 (0.32)	0.00 (0.61)
	L. monocytogenes	f	0.98 (0.01)	0.99 (0.01)	0.98 (0.01)	0.99 (0.01)
		k_{max1}	3.92 (1.78)	20.13 (4.93)	24.91 (8.34)	2.44 (0.18)
		k_{max2}	0.14 (0.11)	0.21 (0.15)	2.12 (0.18)	0.17 (0.48)

Values in brackets represent the standard error of each parameter. δ, UVC_{254nm} dose for the first decimal reduction (mJ/cm^2); p, shape parameter (dimensionless), f, fraction of initial major subpopulation (the least resistant); k_{max1} and k_{max2}, inactivation rates ($cm^2/10^3 \cdot mJ$).

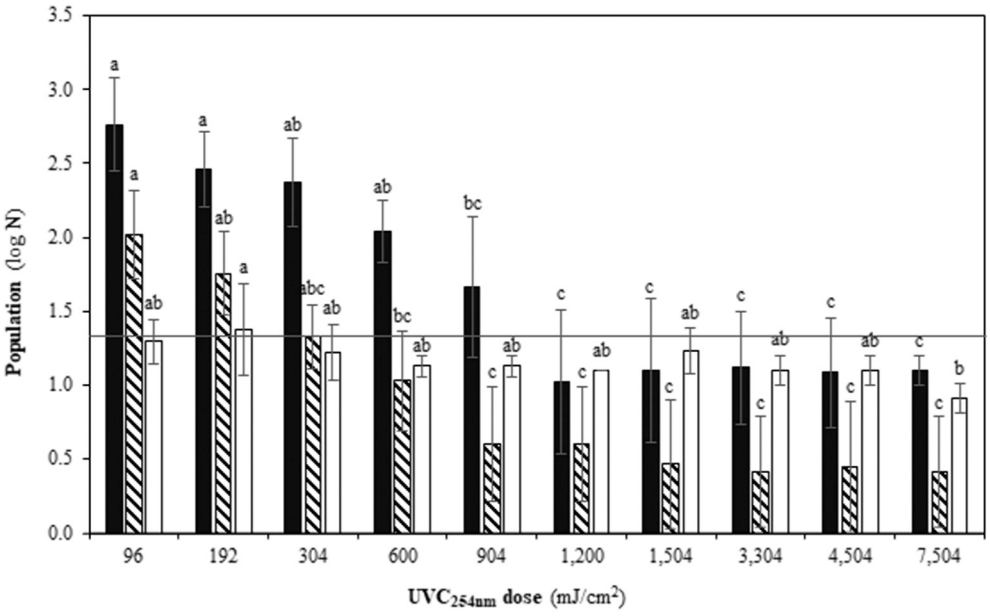

Fig. 3. Remaining populations in washing water corresponding to the treatments with the tank UVC_{254nm} device (tUVC) of artificially inoculated apple peel discs with *E. coli* (black bars), *S. enterica* (strapped bars) and *L. monocytogenes* (white bars). Data is expressed as the mean ± standard error (n = 6), and different letters indicate significant differences ($p < 0.05$) among remaining populations for each bacterium at different UVC_{254nm} doses, according to Tukey's test. Continuous line represents the quantification limit (1.39 log units, 25 CFU/mL).

4504.0 ± 1.0, 600.0 ± 1.0, and 3304.0 ± 0.1 mJ/cm^2, for each microorganism, respectively. At the end of the study, all counts were below the limit of detection.

3.3. UVC_{254nm} inactivation of bacteria in apple juice

The apple juice from concentrate used for the experiments had a pH of 3.60 ± 0.04, a total soluble solid content of 12.0 ± 0.3 %, its density was 1045.0 ± 1.0 g/ L and the transmittance was <0.01 %.

The initial populations of *E. coli*, *S. enterica* and *L. monocytogenes* in the apple juice were 5.5 ± 0.2, 5.3 ± 0.2 and 5.6 ± 0.2 log units, respectively. Population survival in apple juice for the duration of the experiment was confirmed in untreated samples, as no significant reductions ($p > 0.05$) were observed (data not shown). The reduction curves of the bacteria artificially inoculated in apple juice and exposed to UVC_{254nm} were assessed (Fig. 4). Populations significantly decreased ($p < 0.001$) after 603.7 ± 4.9 (*E. coli*) and 95.2 ± 1.2 mJ/cm^2 (*S. enterica* and *L. monocytogenes*) when the cUVC device was used. With the tUVC device, *E. coli* and *S. enterica* populations in apple juice were significantly reduced ($p < 0.001$) after UVC_{254nm} treatment with a dose of 904.0 ± 1.0 mJ/cm^2, while 1200.0 ± 1.0 mJ/cm^2 were needed to significantly reduce ($p < 0.001$) *L. monocytogenes* populations.

As observed for apple peel, the kinetic profile depended on the target microorganism and on the device used for the irradiation. In this case, a common trend could be detected (a non-linear but gradual decrease in the bacterial population was observed with increased irradiation dose), behavior that is well described by the Weibull and biphasic model parameters (Table 3).

The radiation dose for the first decimal reduction (δ) of the microorganisms in the apple juice obtained with the Weibull model was higher in the tUVC experiments, ranging from 1273.31 ± 202.79 mJ/cm^2 for *E. coli* to 539.36 ± 215.78 mJ/cm^2 for *L. monocytogenes*. Contrarily, such doses ranged from 331.30 ± 132.82 (*E. coli*) to 174.60 ± 37.44 (*S. enterica*) mJ/cm^2 when the cUVC device was used. In both

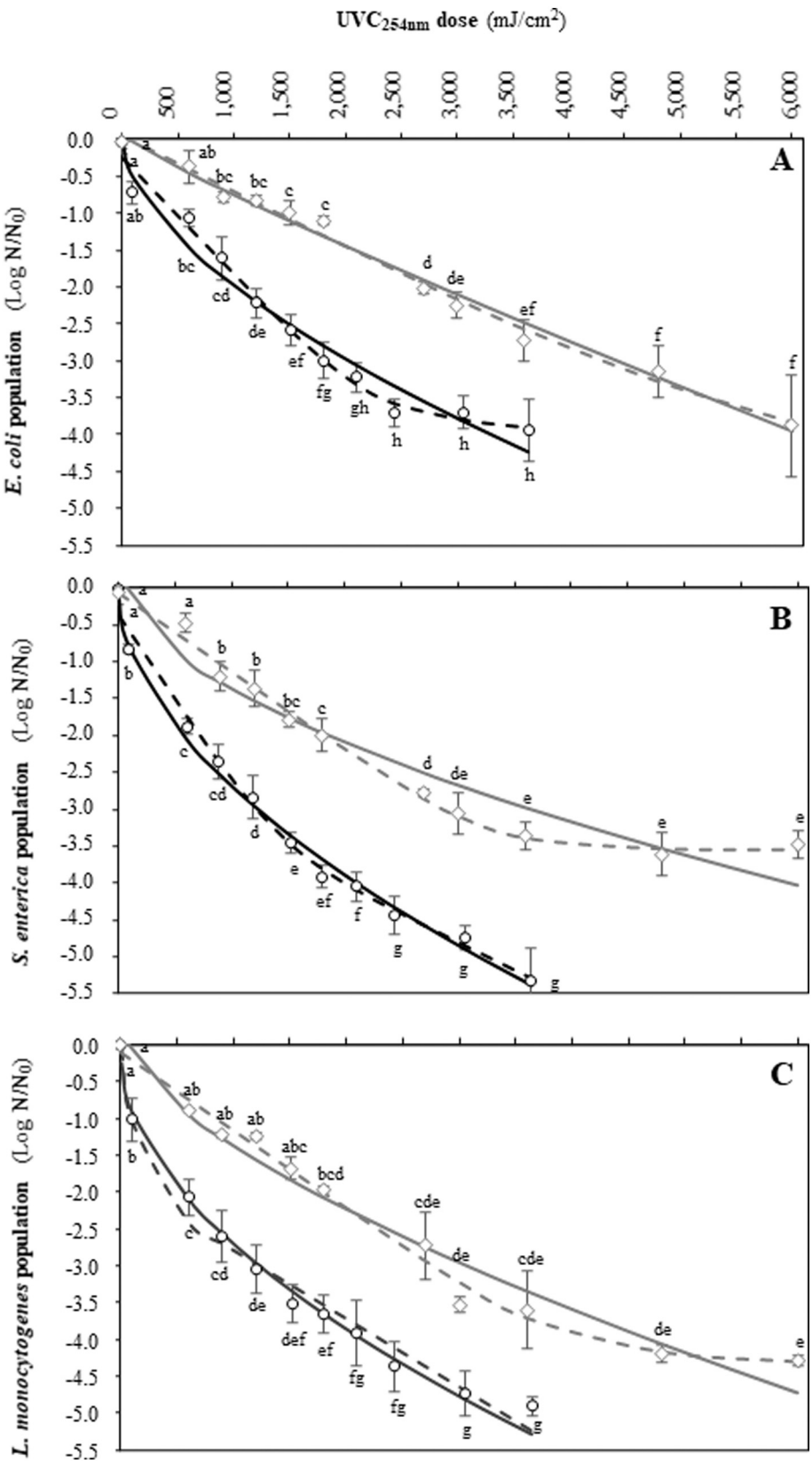

Fig. 4. Survival curve of *E. coli* (A), *S. enterica* (B) and *L. monocytogenes* (C) artificially inoculated in apple juice at the evaluated UVC_{254nm} doses. Represented are the data corresponding to the use of the horizontal UVC_{254nm} device (cUVC) (black) or the tank UVC_{254nm} device (tUVC) (grey). The dots represent experimental data when using the cUVC device (circles) or the tUVC device (diamonds), expressed by the mean, and deviation bars show the standard error (n = 6). The lines represent fitted data predicted values, according to the Weibull model (solid line) and the biphasic model (discontinuous line). Different letters indicate significant differences ($p < 0.05$) among bacterial surviving populations at different UVC_{254nm} doses, according to Tukey's test.

cases, *E. coli* was the most resistant, needing almost 2-fold higher irradiation to be reduced 1 logarithmic unit. After the UVC_{254nm} treatments, populations of *E. coli*, *S. enterica* and *L. monocytogenes* were reduced by 3.9 ± 0.4, 5.3 ± 0.4 and 4.9 ± 0.1 log units with the cUVC system (10,665.9 ± 28.1 mJ/cm^2) and by 3.9 ± 0.7, 3.5 ± 0.2 and 4.3 ± 0.1 log units with the tUVC system (7504.0 ± 1.0 mJ/cm^2). Regarding the shape of the curve (*p*), except for the case of the model describing the inactivation of *E. coli* after irradiation with tUVC device (in which this

parameter was approximated as 1– 0.90 ± 0.08 – indicating more linearity), in the other cases the *p* parameter had values ranging from 0.55 ± 0.05 (*S. enterica* and *L. monocytogenes*, cUVC) to 0.66 ± 0.10 (*L. monocytogenes*, tUVC), indicating downward concavity. These values (< 1) indicate that the remaining cells may have the ability to adapt to the applied stress (Van Boekel, 2002). Given by the biphasic model, the values of $kmax_1$ and $kmax_2$ were higher when the cUVC device was applied in comparison to the use of tUVC device. The most sensitive population (*f*) was represented by a fraction of 0.98–0.99 of the microorganisms, whose $kmax_1$ values ranged from 24.91 ± 8.34 (*L. monocytogenes*, cUVC) to 3.63 ± 0.30 (*E. coli*, cUVC) $cm^2/10^3 \cdot mJ$. Although *L. monocytogenes* was the most sensitive microorganism when subjected to UVC_{254nm} irradiation with the cUVC system, $kmax_1$ values were similar for the three microorganisms when the tUVC system was used, ranging from 2.16 ± 0.25 (*S. enterica*) to 2.46 ± 0.16 (*E. coli*) $cm^2/10^3 \cdot mJ$.

4. Discussion

In this article strategies to minimize prevalence of *E. coli*, *S. enterica* and *L. monocytogenes* in apple juice have been studied in two scenarios, raw fruit (apple peel discs) and final product (apple juice). To the authors' knowledge, this is the first report evaluating and comparing the fate of the three microorganisms (*E. coli*, *S. enterica* and *L. monocytogenes*) both on apple peel discs and in apple juice, in order to apply an UVC irradiation treatment (via two different UVC_{254nm} devices) in two steps of the production chain. Of the possible target microorganisms, *E. coli* and *S. enterica* have been selected as there is sufficient evidence that such enteric bacteria may contaminate the apples from which it is elaborated and may survive for a time in the processed products (Perez-Rodriguez et al., 2014). Moreover, *L. monocytogenes* has been selected as there are studies of the persistent contamination with this pathogen, which is a ubiquitous microorganism, in the apple and apple derivatives production and supply chains (Tan et al., 2019).

The models obtained by fitting the data of surviving populations with increased UVC_{254nm} doses well described the behavior of the selected microorganisms during the treatments, as indicated by the R^2-adj values (≥ 0.852). In fact, the Weibull model has been used by a number of authors to describe microbial inactivation with UVC treatments (Chun et al., 2009; López-Malo et al., 2014; Martínez-Hernández et al., 2015). The Geeraerd's model (Geeraerd et al., 2005) has also been used for this purpose, as it well represents the shoulder that is often observed in inactivation curves of microorganisms under UVC light (Gouma et al., 2015). In the case of the data in the present study, this phenomenon was not observed, indicating that DNA repair systems were not sufficient to repair damage caused by UVC_{254nm} irradiation in the strains and conditions tested, and DNA repair capability was surpassed at the first irradiation doses, being lethal for the microorganisms (López-Malo and Palou, 2005). According to the data obtained, biphasic model was considered adequate, especially for the experiments performed on apple peel discs, as it assumes one initial major subpopulation, that is more sensitive to stress (initial decline) and one minor subpopulation that is more resistant to stress (tail).

As expressed by the first decimal reduction parameter (δ), *E. coli* seemed to be the most resistant bacteria among those studied, as it presented higher δ-values both on apple peel discs and in apple juice, meaning that higher UVC_{254nm} doses were needed to reduce it by 1 log unit. *L. monocytogenes*, in turn, was the most sensitive, as it presented lower δ-values. Typically, higher δ-values are related with lower decrease rates (*kmax*). However, this trend was not observed in all the cases, which depended on the matrix and on the device used. For instance, although *E. coli* needed higher irradiation doses (more than 2-fold) for the first decimal reduction when juice was treated with tUVC device, its decrease rate ($kmax_1$) was similar to that of *S. enterica* and *L. monocytogenes* for the same type of treatment. Noting this, it is worthy to adjust the surviving curves to more than one model in order to fully explain the behavior of microorganisms and possess more information to compare the efficacies of the treatments applied. In this sense, Liao et al. (2017) compared the inactivation rates of *E. coli*, *S. enterica* and *L. monocytogenes* in water during UVC irradiation, and found that the latter was the most resistant of the three. The reported doses for the first decimal reduction were 1.96 ± 0.54, 4.16 ± 0.74 and 16.5 ± 1.7 mJ/cm^2, for each strain, respectively. Contrarily, Graça et al. (2013) found that sensitivity to 50 and 100 mJ/cm^2 of *L. monocytogenes* artificially inoculated on fresh-cut apple was higher than that of *E. coli* and *S. enterica* in the same conditions. Differences in resistances encountered within literature and also within our article may be attributed, among others, to the specific strains tested (Coohill and Sagripanti, 2008). Inter-strain variations in sensitivity to UVC light have been already reviewed (Gayán et al., 2014), and for this reason, in the present study, four strains were used as representatives for each microorganism.

One of the main drawbacks of UVC light is the fact that it does not penetrate the target very deeply. Thus, it is more frequently used for surface sterilization (Guerrero-Beltrán and Barbosa-Cánovas, 2004). In the fruit industry, one of the strategies that can be applied is the treatment of juice conveyed in a thin layer in order to increase the surface area and decrease depth of the product. In this regard, Fenoglio et al. (2020) determined that factors such as high transmittance and turbidity can highly reduce the UVC efficacy for its low penetration ability. They reported that when irradiating juices with 12 lamps emitting at 36 W and after 18 min of treatments *E. coli* populations were reduced by 5 log units in pear juice (transmittance: 89.1 %; turbidity: 21.9 ± 8.8 NTU) and only 3.5 log units in orange-banana-mango-kiwi-strawberry juice blend (transmittance: 42.6 %; turbidity: 1767 ± 3 NTU). The treatment of the juice would be a good solution to decrease microbial load in the final product as it is furtherly packaged and no subsequent contamination should occur. Due to the penetration limitations of the UVC light, another way to reduce microorganisms in the final product would be reducing microbial load in the raw material: the apple fruit. For this reason, this other scenario has been investigated in the present study.

On apple peel discs, reductions ranging between ca. 3.3 to 3.9 log units have been observed at the end of the treatments of 10,665.9 ± 28.1 and 7504.0 mJ/cm^2 with the cUVC and tUVC devices, respectively. However, due to the tailing effect observed, non-significantly different reductions ($p > 0.05$) are achieved with treatments between ca. 1000.0 to 2000.0 mJ/cm^2 in this product. The sharp decrease observed in microbial populations on apple peel discs treated with <1000.0 mJ/cm^2 is attributed to the direct exposure of the microorganisms to the UVC_{254nm} light, for being inoculated on the peel surface. Other authors have reported maximum reductions of *E. coli* and *L. monocytogenes* of 3.0 and 1.6 log units on fresh-cut apple peel (UVC Emitter™ Table-top system, 10 cm distance to the lamps) after irradiation with 200 and 400 mJ/cm^2, respectively (Adhikari et al., 2015). Variances between studies could be attributed to the peel structural differences – including the thickness of the cuticle, the height of the epidermal cells, the smoothness and the presence and direction of microcracks – that occur between different fruits, varieties and even cultivars (Konarska, 2012). Presence of microcracks could explain the tailing effects observed in apple peel by the covering of the microorganisms inside, with the consequent protection from UVC light. The non-homogeneous distribution of such microcracks could also explain the higher variability observed in the apple peel data (standard error: 0.5–0.9) when compared to the data of apple juice (standard error: 0.1–0.5). In fact, depending on the fruit, the surface parameters (roughness, contact angle) differ, which affect in turn the efficacy of UVC_{254nm} light. For instance, Adhikari et al. (2015) observed a reduction of *E. coli* O157:H7 of 2.9 (apples) and 2.0 (strawberries) log CFU/g after 92 mJ/cm^2 and 720 mJ/cm^2, respectively. Reductions of *L. monocytogenes* were also higher in apple (1.6 log CFU/g, 375 mJ/cm^2) than they were in strawberry surface (1.0 log CFU/g, 1190 mJ/cm^2). Taking this into account, each case should be studied in particular, to adjust the irradiation parameters for each type of fruit

surface. To the authors knowledge, there are no more studies evaluating the sanitation efficacy of UVC_{254nm} irradiation on apple peel. The comparison of the performance of the two UVC_{254nm} radiation devices on apple peel discs revealed that initial reductions were ca. 1-log higher when using tUVC device. This was attributed to a physical dragging of the microorganisms from the surface of the apple to the washing water, enhanced by the agitation of it (Collazo et al., 2018; Nicolau-Lapeña et al., 2020). For this reason, lower UVC_{254nm} doses were needed to reduce similar populations, as it is also reflected in δ lower values. A problem that the combined washing-UVC irradiation strategy can present is related to the remaining populations in washing water. Cross-contamination could occur to the following sanitized apples if washing water is recirculated with high microorganism loads (Pablos et al., 2018). For this reason, in this study, the remaining populations of the target microorganisms in washing water during the treatments in this particular scenario have also been investigated. In the present study, 600.0 mJ/cm^2 were needed to reduce *S. enterica* to undetectable levels in water, and 3304.0 and 7504.0 mJ/cm^2 were needed for *L. monocytogenes* and *E. coli*, respectively. Taking into account the application of UVC_{254nm} light to the washing water to be continuous and the lower contamination levels with these microorganisms existing in the fruit industry (Tan et al., 2019), this method could be considered a good approach to maintain safety of washing water. A remark must be done in this regard related to the importance of maintaining turbidity levels of water and organic matter loads low, as these can significantly affect the penetration, and hence, the efficacy, of UVC irradiation (Abadias et al., 2021).

In the apple juice, the decrease of survival fraction of the microorganisms tested was steadier than it was on apple peel discs (except for *L. monocytogenes* when using cUVC), as it is presumed in the $kmax_1$ values indicating a lower decrease rate. This is also observed in the first decimal reduction dose (δ) values, which are higher for juice when compared to peel discs. In contrast to the direct exposure at which microorganisms on the surface of apple peel are subjected, it is assumed that indirect exposure to the UVC_{254nm} light may be the reason for the higher δ-values presented in the juice, as in this case, microorganisms are immersed in a liquid matrix. Transmittance of the juice used in the present study was low (< 0.01 %), challenging the penetration of the UVC_{254nm} light to the matrix and its subsequent effect on microorganisms. There are other studies on artificially inoculated microorganisms' inactivation in apple juice during UVC_{254nm} treatments. For instance Gouma et al. (2015) treated apple juice in a thin layer reactor with UVC lamps and showed reductions of ca. 1.5 log units of *E. coli*, *S. Typhimurium*, *L. monocytogenes* and *Staphylococcus aureus* after 3.7 J/mL irradiation. López-Malo et al. (2014) used a laminar flow device consisting on a 90 cm long glass tube with a UVC lamp connected to a peristaltic pump to treat apple juice. They needed 330 and 528 mJ/cm^2 to reduce L. *monocytogenes* and *Saccharomyces cerevisiae*, respectively. However, comparison between studies is complex because different parameters may affect the results. Some have been mentioned before, such as transmittance and turbidity (Sauceda-Gálvez et al., 2016). Transmittance could be a predictor of the microbial inactivation in low turbid liquids, but fails in foods that present high turbidity values, as large amounts of suspended solids can lead to higher absorbance values due to light scattering from the detector (Koutchma et al., 2004). For instance, *E. coli* artificially inoculated in apple and orange juice which was treated with UVC from 15.1 to 27.1 J/mL (25 °C, 8 W of total power, emitting 90 % of energy, turbulent flow device) was reduced 0.96 ± 0.16 and 0.25 ± 0.04 log CFU/mL in each juice, respectively (Gayán et al., 2012, 2013). In contrast, reduction of *E. coli* in vegetable broth by using the same device and conditions was 3.21 ± 0.19 log CFU/mL, and the authors attributed these variations to the differences in absorbance coefficient and turbidities presented by the three matrices (Gayán et al., 2016). One factor that also affects turbidity of the media is the initial concentration of natural occurring or artificially inoculated microorganisms. When such values are higher than 10^5 or 10^7 CFU/mL (in yeast or bacterial cells, respectively), turbidity of the media increases, hindering the efficacy of the treatments (Murakami et al., 2006). Moreover, depth of the treated sample is another important parameter that, together with transmittance, is related with the proportion of the sample to which irradiation may not be effective. For instance, in the present study, two devices were used: in cUVC device, the sample depth was 4 mm, whereas in the tUVC device, the maximum depth was 8.5 cm due to the distribution of the four UVC emitting lamps inside the tank. The thin layer in the cUVC device enhanced the performance of the UVC_{254nm} treatment, which is well represented by the lower δ-values and higher $kmax_1$-values obtained by the Weibull and biphasic models, respectively. Agitation in the tank was, therefore, essential to facilitate the pass of the juice next to the lamps and avoid lack of irradiation in juice located near the tank walls. However, it did not completely avoid the penetration issue, as shown in the models obtained for the two devices used to treat apple juice, the decrease in survival fractions was steadier and reductions were lower when tUVC system was used in comparison to the cUVC system. In this regard, Guerrero-Beltrán and Barbosa-Cánovas (2005) indicated an indirect correlation between flow rate and inactivation rates, that is dependent on the microorganism, being higher flow rates more effective to decrease microbial populations. For instance, f or *L. monocytogenes* they obtained a linear correlation, for which each increase in flow rate by 3.83 mL/min, the first decimal reduction value (D) decreased by 1 min.

Final populations in apple juice after 3644.1 ± 1.0 and 6000.0 ± 1.0 mJ/cm^2 for cUVC and tUVC devices ranged from ca. 3.5 to 5.3 log units, which are not different ($p > 0.05$) to those achieved in apple peel discs. However, irradiation doses are correlated with the intensity of the lamps but also with irradiation time, which is a key parameter in scaling-up processes, as long treatments times are not feasible for the industry. In the non-thermal pasteurization process with UVC_{254nm} light proposed for apple juice in the present study, the cUVC device has proven to be the most appropriate device in terms of sanitation efficacy. However, it relies on a thin layer to irradiate the maximum surface without penetration losses, which is a possible handicap when scaling-up to the juice industry, in which the surface:depth ratios should be maximized, for instance, with processing belts or transparent tubular coils next to UVC lamps. The tUVC device could be a more adaptable system for industry, but in the present study, when it was applied for apple juice treatments, lower efficacy was observed, mainly attributed to UVC_{254nm} light penetration issues. In this regard, a higher flow rate or a higher number of lamps could be useful in improving its efficacy. For the challenges in juice treatments, in this study the sanitation of apples prior to juice production was also proposed. In this case, a sharper decrease in microbial population was observed at the initial doses (ca. 250 mJ/cm^2) when compared to the effects observed in apple juice, which could be an advantage for industry. Data proved that the immersion of apple in water for their treatment (in the tUVC system) could result in ca. 1- to 1.5-log higher decreases in microbial loads when compared with non-immersed samples (in the cUVC system), making the water-agitation system a good approach for this purpose.

The results of this study highlight the suitability of UVC treatments for microbial inactivation for fruit juices (from apple in this case), being still necessary to optimize the equipment configurations and the escalation to industry levels. However, and as reviewed in Shah et al. (2016), this technology is promising for this type of products. First of all, it can be considered a good alternative for thermal treatment: it can help in maintaining better taste, color profile and ascorbic acid content, giving a product similar to the freshly pressed juice (Choi and Nielsen, 2005). Minimizing microbial risks in apple juices is key to assure safety of this kind of products, as reported in the Introduction section, outbreaks related with food-borne pathogens still occur. When such episodes happen, not only product recalls represent economic losses, but also brand image damages may be unvaluable. Taking into account that energy requirements for UVC treatments are cheaper than those for thermal pasteurization, irradiation appears to be a more cost-efficient technology with similar or better outcomes than thermal treatments

(Tan et al., 2014). For this, UVC treatments are considered a low-cost alternative, especially for small processors (Majchrowicz, 1999). Second, irradiated juices are considered a premium product due to their fresh-like properties and their proven superior quality (Tan et al., 2014). This treatment can be a claim to be positioned in the marked (compared to the large amount of thermally treated juices present on it), as consumers, especially those that are concerned about health, are willing to pay a higher price for *premium* products (Lau et al., 2011). Despite each case should be evaluated individually, considering the needs of the processing industry (production levels, ease to implement the technology within the production chain) and the especifications of UVC-equipment manufacturers (price range, depreciation time) the aforementioned reasons point toward the feasibility of implementing UVC treatments for apple juice production.

5. Conclusions

In this study the efficacy of UVC_{254nm} has been demonstrated against three bacteria of interest (*E. coli*, *S. enterica* and *L. monocytogenes*). To the best of the authors' knowledge, this is the first paper in which UVC_{254nm} efficacy is compared in both scenarios – apple peel discs (raw fruit) and apple juice (final product) – and against the mentioned microorganisms. As contamination of apple can occur prior to the processing of the juice, reduction of contamination in apple peel could, consequently, reduce microbial loads in apple juice, and prevent one of the drawbacks associated to UVC light: its low penetration. For this, two irradiation systems have proved their potential in both scenarios: a horizontal chamber for surface disinfection and a tank in which transmitting lamps are immersed in the liquid media. This paper represents a first approach to minimize microbial loads in fresh apple juices, evaluating the possibility to incorporate UVC_{254nm} irradiation treatments at two different steps during the processing of the juice, especially for small processors (e.g. retail, restoration). Within the options evaluated, the immersion of apples in irradiated water had significantly decreased the bacteria at the lowest UVC_{254nm} tested, representing a good approach to improve safety and maintain quality of such products.

Data availability statement

Data is available under reasonable demand to the corresponding author.

Funding

This research was funded by the Spanish Ministry of Science and Innovation (MICINN), research project QUALISAFEJUICE [PID2019-106645RB-I00], and the Spanish Ministry of Economy, Industry and Competitiveness (MINECO), with a postdoctoral orientation period grant [BES 2017 079779] (I. Nicolau-Lapeña).

Declaration of competing interest

The authors declare no conflict of interests.

Acknowledgements

The authors thank F. Ezzahra Boulal for her technician support. The authors acknowledge Dallan, S.A. Moleva for the contribution with apple juice and UV-Consulting Peschl España for providing the horizontal chamber UVC laboratory scale equipment used in the experiments.

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