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Short communication

UV-C light inactivation and modeling kinetics of *Alicyclobacillus acidoterrestris* spores in white grape and apple juices

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ABSTRACT

In the present study, the effect of short wave ultraviolet light (UV-C) on the inactivation of *Alicyclobacillus acidoterrestris* DSM 3922 spores in commercial pasteurized white grape and apple juices was investigated. The inactivation of *A. acidoterrestris* spores in juices was examined by evaluating the effects of UV light intensity (1.31, 0.71 and 0.38 mW/cm^2) and exposure time (0, 3, 5, 7, 10, 12 and 15 min) at constant depth (0.15 cm). The best reduction (5.5-log) was achieved in grape juice when the UV intensity was 1.31 mW/cm^2. The maximum inactivation was approximately 2-log CFU/mL in apple juice under the same conditions. The results showed that first-order kinetics were not suitable for the estimation of spore inactivation in grape juice treated with UV-light. Since tailing was observed in the survival curves, the log-linear plus tail and Weibull models were compared. The results showed that the log-linear plus tail model was satisfactorily fitted to estimate the reductions. As a non-thermal technology, UV-C treatment could be an alternative to thermal treatment for grape juices or combined with other preservation methods for the pasteurization of apple juice.

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1. Introduction

Alicyclobacillus acidoterrestris is a thermoacidophilic, non-pathogenic, rod-shaped spore-forming bacterium with a central, subterminal, or terminal oval spore and grows at pH values ranging from 2.5 to 6.0 at temperatures of 25 to 60 °C (Yamazaki et al., 1996). ω-alicyclic fatty acids are the major lipid components of *A. acidoterrestris* membranes and are associated with the resistance of the organism to acidic conditions and high temperatures (Hippchen et al., 1981). These thermo-acidophilic properties constitute the main difficulty in the inactivation of this organism (Bae et al., 2009). Fruit juices are generally treated at temperatures of about 95 °C for 2 min (Komitopoulou et al., 1999). Spores have been shown to survive such heat treatments (Splittstoesser et al., 1998) and surviving spores can germinate and grow at pH <4 in fruit juice, leading to spoilage (Walker and Phillips, 2007). The characteristic spoilage involves the formation of a phenolic or antiseptic odor with or without cloudiness and generally without gas production. Spoilage by *A. acidoterrestris* is difficult to detect; in fact, the spoiled juice appears normal or has light sediment (Walker and Phillips, 2005).

Thermal processing ensures the safety and shelf life of fruit juices, but can result in the loss of sensory and nutritional quality. Consumers demand for fruit juices that have high quality and fewer chemicals. Therefore, fruit-juice industry should take the necessary measures to prevent economical losses due to spoilage. Among the non-thermal methods developed in the last few decades, ultraviolet light (UV) is one of the most promising technologies because it is easy to use and lethal to most of the microorganisms (Bintsis et al., 2000), also it does not generate chemical residues (Guerrero–Beltrán and Barbosa–Cánovas, 2004).

UV light is the region of the electromagnetic spectrum that ranges from 100 to 400 nm. This UV range may be further divided and classified as UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm), and the vacuum UV range (100–200 nm). The UV-C light has germicidal effect on microorganisms such as bacteria, yeasts, molds and viruses (Caminiti et al., 2012). It has also been approved to treat food surfaces and clear fruit juices (US–FDA, 2002). The formation of photoproducts in the DNA is the principal inactivation effect of UV. The most important product is the pyrimidine dimer formed between adjacent pyrimidine molecules on the same strand of DNA. These molecules can interrupt both DNA transcription and translation, resulting in cell death (Franz et al., 2009).

Recent studies have shown that UV-C technology is one of the most common technologies used to preserve liquid food products including fruit juices such as orange juice (Tran and Farid, 2004; Keyser et al., 2008), apple juice (Keyser et al., 2008; Franz et al., 2009; Caminiti et al., 2012), grape, cranberry and grapefruit juices (Guerrero–Beltrán et al., 2009), pomegranate juice (Pala and Toklucu, 2011), and liquid egg white (Unluturk et al., 2010). However, very few studies focus on the effects of UV-C application to bacterial spores. Since *A.acidoterrestris* is an emerging food spoilage organism in the fruit juice and fruit juice products in the industry (Walker and Phillips, 2007), the spores of this bacterium should be eliminated from these products. Therefore, this study was conducted to examine the efficiency of UV-C radiation on

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the inactivation of *A. acidoterrestris* (DSM 3922) spores in white grape and apple juices at constant depth.

2. Material and methods

2.1. Test microorganism

A. acidoterrestris type strain DSM 3922 used in this study was kindly provided by Dr. Karl Poralla (Fakultät für Biologie, Eberhard–Karls–Universität Tübingen, Tübingen, Germany). Cultures were grown for 2 days at 43 °C on *Bacillus acidocaldarius* medium (BAM, 0.25 g $CaCl_2$. $2H_2O$, 0.5 g $MgSO_4.7H_2O$, 0.2 g $(NH_4)_2SO_4$, 3.0 g KH_2PO_4, 1 g yeast extract, 5 g glucose, 1 mL trace element solution, 1 l deionized water, pH 4.3) and then stored at 4 °C as stock cultures. Trace element solution contains 0.28 g $FeSO_4.7H_2O$ (Merck), 1.25 g $MgCl_2.4H_2O$ (Merck), 0.48 g $ZnSO_4.7H_2O$ (Merck) and 1 l deionized water (Baysal and Icier, 2010).

2.2. Fruit juice samples

Alicyclobacillus-free commercial pasteurized apple and white grape juices were provided from a local market. pH values of the juices were measured by a pH meter (Metrohm, Switzerland) while soluble solid content (°Brix) was determined by a refractometer (Mettler, Toledo). Additionally, the turbidity of each sample was determined by using a turbidimeter (2100AN, HACH Company, CO, USA). The pH, °Brix and turbidity values were approximately 3.2, 16.6 and 5.5 NTU for white grape juice, and 3.8, 11 and 10 NTU for apple juice, respectively.

2.3. Preparation of spore suspensions

To induce sporulation, cells grown at 43 °C for 2 days on BAM medium were spread onto BAM agar (pH 4.3) and incubated at 43 °C for seven days until at least 80% of cells sporulated, as determined by the phase contrast microscopy. Firstly, spores were harvested by depositing approximately 5 mL portions of sterile water onto the surfaces of BAM agar and then were dislodged by gentle rubbing with a sterile swab. Next, the pooled suspensions from plates containing spores were centrifuged (5000 ×*g*, 15 min at 4 °C), and this was followed by resuspension in sterile distilled water and centrifugation (5000 ×*g*, 10 min at 4 °C). This procedure was repeated for four times. Finally, the final pellets were resuspended in the sterile distilled water and heated (80 °C for 10 min) to kill vegetative cells and then stored at 4 °C until use.

2.4. UV-C treatments

2.4.1. UV-C irradiation equipment

UV-C irradiation of samples was conducted using a collimated beam apparatus as described by others (Bolton and Linden, 2003; Unluturk et al., 2010). The apparatus consisted of a low mercury UV lamp (UVP XX–15, UVP Inc., CA, USA) with peak radiation at 254 nm wavelength. The UV radiation was collimated with a flat black painted tube which was in the same size with a Petri dish. The samples were placed in 6 cm diameter Petri dishes directly below the collimated UV beam and stirred continuously during the irradiation with a vortex mixer (IKA, Yellowline TTS 2, IKA® Werke GmbH & Co. KG, Germany). The irradiance I_0 of the lamp was measured by a UV–VIS radiometer supplied with UVX–25 sensor (UVX, UVP Inc., CA, USA) placed at the same distance from the UV lamp as the plates. The UV lamp was switched on for about 30 min prior to UV treatment in order to minimize fluctuations in intensity.

2.4.2. UV transparency

The transparency of juices to UV-light was determined by measuring the absorbance in 1 cm-path quartz cuvette using an UV–VIS spectrophotometer (Cary 100 Bio, Varian Inc., CA, USA) set at 254 nm. Absorbance coefficient A_e was calculated by measuring the absorbance of dilutions of the juices and determining the slope of absorbance against concentration (Caminiti et al., 2012).

2.4.3. UV-C inactivation treatments

Spores (1×10^6 CFU/mL) were inoculated into fruit juices and then exposed to UV-C radiation of known intensity levels (1.31, 0.71 and 0.38 mW/cm^2). The average UV intensity (average irradiance or fluence rate) in the stirred sample (I_{avg}) was calculated by an integration of Beer–Lambert law (Eq. (1)) over the sample depth (Morowitz, 1950):

$$I_{avg} = I_o\left(1-e^{-A_eL}\right)/A_eL \quad (1)$$

where I_0 is the incident intensity (mW/cm^2), A_e is the absorbance coefficient (cm^{-1}) at 254 nm wavelength and L is the path length (cm).

The radiant exposure (D; mW s/cm^2 or mJ/cm^2) is defined as the energy delivered per unit area of the UV reactor and calculated using the Eq. (2):

$$D = I_{avg}\ t \quad (2)$$

where t is the exposure time. The UV-doses were within the range of 0–489 mJ/cm^2 for grape fruit juice and 0–539 mJ/cm^2 for apple juice based on the exposure times of 0, 3, 5, 7, 10, 12 and 15 min.

2.4.4. Microbiological analysis

Following UV-C irradiation, spore counts were determined by spread plating the diluted samples onto BAM agar (pH 4.3). The plates were incubated at 43 °C for 2–5 days. Microbial count determinations were performed in two replications and expressed as CFU/mL.

2.5. Modeling of inactivation data

Survival curves were obtained by plotting the logarithm of the survival fractions (N/N_0) versus the treatment doses, expressed in mJ/cm^2. N is the spore counts after UV-treatment and N_0 is the initial number of spores before treatment. The GInaFiT was used for testing nonlinear survival curves (Geeraerd et al., 2005). As survival curves showed tails, the log-linear plus tail (Geeraerd et al., 2000) and Weibull models (Mafart et al., 2002) were used and compared for each inactivation curve.

The Weibull model was used with Eq. (3) (Izguier and Gómez–López, 2011);

$$logN = logN_o - (d/\delta)^p \quad (3)$$

Where d is the applied dose for UV (mJ/cm^2), δ is the scale parameter and p is the shape parameter. δ (mJ/cm^2) is the UV dose for the first decimal reduction, p (dimensionless) is a shape parameter describing concavity or convexity of the curve. Curves with downward concavity are obtained if $p > 1$ and curves with upward concavity are obtained if $p < 1$ (Fröhling et al., 2012).

The log-linear plus tail model was used with Eq. (4);

$$logN = log\left(\left(10^{logN_o} - 10^{logN_{res}}\right)e^{(-k_{max}d)} + 10^{logN_{res}}\right) \quad (4)$$

Where N_{res} is the residual population density (log CFU/mL) and k_{max} is the inactivation rate of the log-linear part of the curve (cm^2/mJ) (Izguier and Gómez–López, 2011);

2.6. Statistical analysis

Data are averages ± standard deviations of three independent UV inactivation experiments for three independent spore batches. The mean and standard deviation of the treatments were calculated using Microsoft Excel. A one-way analysis of variance (ANOVA) and F-test

for comparisons were analyzed to determine significant differences between treatment means. Differences at $p < 0.05$ were considered significant. The values of mean square (MSE), root mean square error (RMSE), correlation coefficient (R^2) and adjusted R^2 (adj-R^2) values of models were calculated and compared by GInaFiT program. The model with the smallest RMSE was considered to be the best fit for the inactivation curve (Berney et al., 2006).

3. Results and discussion

In the present study, UV intensities were 1.31, 0.71 and 0.38 mW/cm^2 and exposure times were between 0 and 15 min. UV light only penetrates a very short depth inside the liquid food surface. The absorption coefficient of the tested grape juice was lower (5.82 cm^{-1}) than that of the apple juice (12 cm^{-1}). Also, grape juice was less turbid (5.49 NTU) than apple juice (10 NTU). UV-C light sensitivity of *A. acidoterrestris* (DSM 3922) spores was tested at the 0.15 cm depth for both types of fruit juice. Fruit juice was added to 6 cm standard Petri dish to obtain a sample depth of 0.15 cm. The depth was calculated from the ratio of the sample volume and the surface area of a Petri dish. An adequate stirring was applied during treatment in order to ensure equal distribution of UV dose through the sample. The edge effects caused by stirring are avoided by using the smallest possible sample volume (Bolton and Linden, 2003).

For modeling, to avoid small differences in initial concentrations between experiments inactivation data were expressed as log (N/N_0) versus UV doses (Hereu et al., 2012). UV doses were calculated by multiplying the UV intensities (mW/cm^2) by the exposure times and were within the range of 0–489 mJ/cm^2 for grape fruit juice and 0–539 mJ/cm^2 for apple juice based on the exposure times of 0, 3, 5, 7, 10, 12 and 15 min. ANOVA analysis showed that there was no statistical difference between the treatment means ($p > 0.05$). As seen in the Figs. 1 and 2, the reduction in the population of spores depended on the UV dose and fruit juice type. The increased dose of UV-C decreased the spore populations inoculated into fruit juices ($p < 0.001$). The type and physical properties of juice medium are very important for UV-C

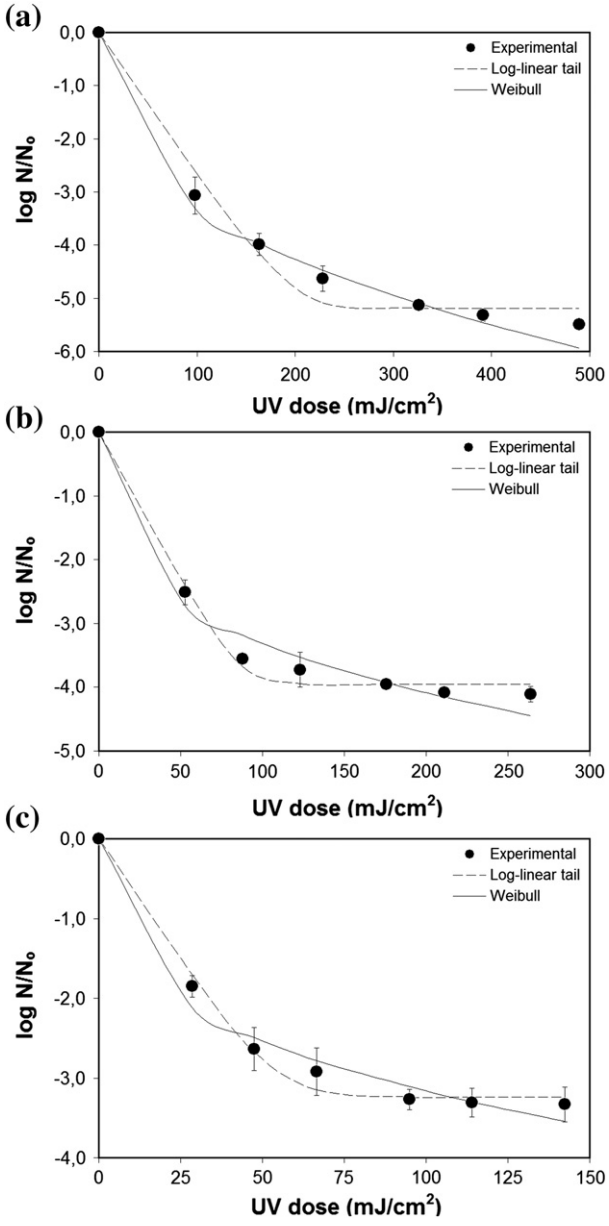

Fig. 1. Survival data (mean and standard deviation) of *Alicyclobacillus acidoterrestris* DSM 3922 spores in white grape juice fitted log-linear plus tail (dashed line) and Weibull (solid line) models at UV intensity of 1.31 (a), 0.71 (b) and 0.38 mW/cm^2 (c).

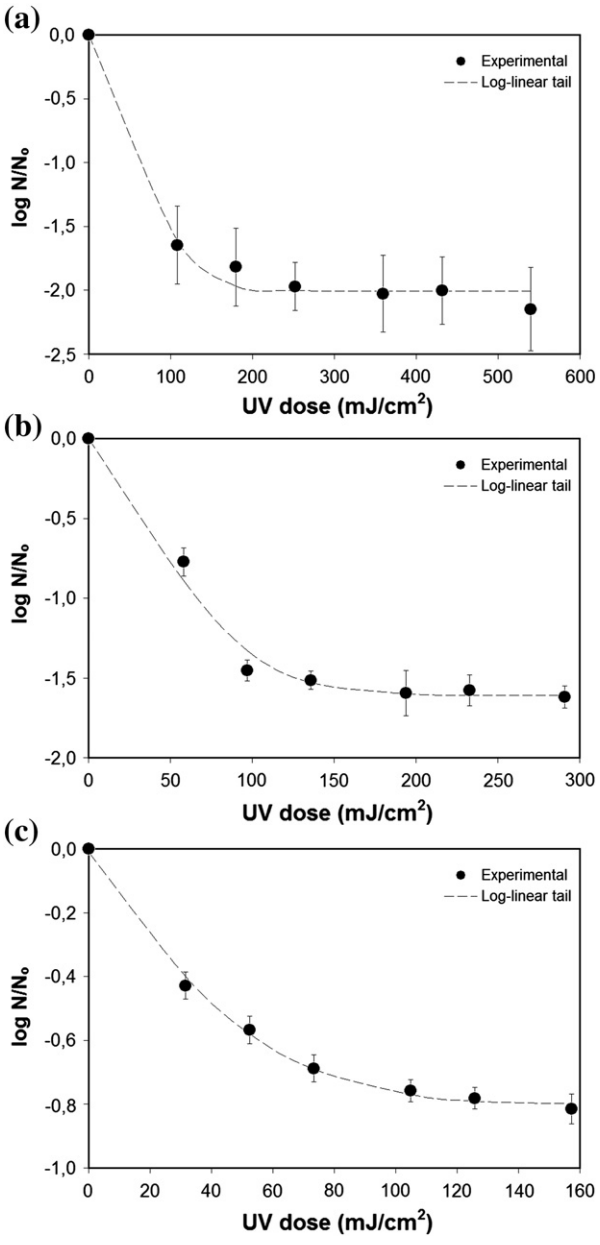

Fig. 2. Survival data (mean and standard deviation) of *Alicyclobacillus acidoterrestris* DSM 3922 spores in apple juice fitted log-linear plus tail model (dashed line) at UV intensity of 1.31 (a), 0.71 (b) and 0.38 mW/cm^2 (c).

Table 1
Log-linear plus tail and Weibull parameters for UV-C inactivation kinetics of *Alicyclobacillus acidoterrestris* spores in white grape and apple juices.

Treatment (mW/cm^2)	Log-linear plus tail model			Weibull model		
White grape juice						
	k_{max}[a] mean (SE)[e]	$logN_{res}$[b] mean (SE)	4D mean (SE)	δ[c] mean (SE)	p[d] mean (SE)	4D mean (SE)
0.383	0.14 ± 0.01	2.9 ± 0.1	nc[f]	2.84 ± 0.81	0.32 ± 0.02	nd
0.709	0.11 ± 0.01	2.0 ± 0.0	nc	2.04 ± 0.68	0.30 ± 0.02	219.8 ± 4.0
1.315	0.06 ± 0.1	0.7 ± 0.0	172.8 ± 19.8	9.55 ± 3.54	0.43 ± 0.05	229.9 ± 12.9
Apple juice						
	k_{max} mean (SE)	$logN_{res}$ mean (SE)	4D	δ	p	4D
0.383	0.04 ± 0.0	4.3 ± 0.0	nc	–[g]		
0.709	0.04 ± 0.0	3.3 ± 0.1	nc	–		
1.315	0.04 ± 0.01	3.1 ± 0.2	nc	–		

[a] k_{max}: Specific inactivation rate (cm^2/mJ).
[b] $logN_{res}$: Residual population density (log CFU/mL).
[c] δ: Dose for the first decimal reduction (mJ/cm^2).
[d] p: Shape parameter (dimensionless).
[e] : Standard error.
[f] : Not calculated by GInaFiT program.
[g] : Not applicable.

inactivation. Koutchma et al. (2004) studied the effects of pH and °Brix on the UV-treatment of apple juice and cider and concluded that the °Brix and pH did not affect the efficiency of UV-light inactivation. On the other hand, the UV-C efficiency is related to the presence of suspended solids in juice medium and its transparency (Koutchma et al., 2002; Murakami et al., 2006). The absorption coefficient of the tested grape juice was lower (5.82 cm^{-1}) than that of the apple juice (12 cm^{-1}). Also, grape juice was less turbid (5.49 NTU) than apple juice (10 NTU). Therefore, the limited transparency and turbidity of the apple juice in this study could reduce the penetration of the UV light into the juice and decrease the efficiency of the spore inactivation.

The results showed that the highest intensity level significanly reduced the spore numbers in white grape juice (5.5-log reduction) (Fig. 1a). At the UV intensity levels of 1.31, 0.71 and 0.38 mW/cm^2, only 2.1 ± 0.3, 1.6 ± 0.1, and 0.8 ± 0.1 log reductions were obtained after 15-min of treatment in apple juice, respectively (Fig. 2).

In the present study, spore reductions did not fit for the first-order kinetics models due to a tailing effect (Figs. 1 and 2). Therefore, log-linear plus tail (Geeraerd et al., 2000) and the Weibull models (Mafart et al., 2002) were compared statistically for the treatment of juices based on the data points. Both models fitted the experimental data of the white grape. The inactivation data of the apple juice could also fit with the log-linear plus tail model but Weibull model was unable to fit the inactivation data by the GInaFiT program. Therefore, this model was not included in the modeling of UV-C inactivation. Previously, the inactivation of organisms under UV-light has been shown to follow the first-order kinetics in solution (EPA, 2003), but it has also reported that they can exhibit a sigmoidal shape with shoulder and/or a tail (CFSAN–FDA, 2006). Related literature also showed the survival curves of UV light treatment with tails and shoulders (Donahue et al., 2004; Keyser et al., 2008; Matak et al., 2007; Sastry et al., 2000) similar to our findings. If the end of the survival curve corresponds to a tailing phase in which the inactivation rate decreases, this may be related to the solids in suspension that block the UV-light through the system, nonhomogenous treatments, aggregation of microorganisms or the presence of resistant subpopulation (Hijnen et al., 2006; Hoyer, 1998; Kuo et al., 2003; Sastry et al., 2000).

Table 1 represents the parameters by applying these models in grape and apple juices. Inactivation rates (k_{max}) of log-linear plus tail in grape juice decreased as the UV intensities increased and were found as 0.14, 0.11 and 0.06 cm^2/mJ for 0.38, 0.71 and 1.31 mW/cm^2, respectively. Also, it was found that inactivation rates for the inactivation data in apple juice were the same for three intensity levels but there were differences among the residual populations resulting in different UV resistances. Log-linear plus tail has the advantage because this model estimates the logarithm of the residual population (log N_{res}) that characterizes the tailing of inactivation kinetics (Hereu et al., 2012). If it is desired to reach minimal counts, it is unnecessary to prolong a treatment beyond the indicated fluences because no additional inactivation would be achieved. Also, there is a risk that samples get deteriorated by the treatment (Izguier and Gómez–López, 2011). To determine predicted residual populations, logarithms of the survivors were plotted against UV dose. As the UV-intensity increased, the residual population density was reduced. These values were calculated as 2.9, 2.0 and 0.7 log CFU/mL corresponding to respective UV intensities in grape juice (Table 1). On the other hand, predicted residual counts were

Table 2
Evaluation of the two models estimating reductions of spores in white grape and apple juices after UV-C treatment.

Treatment (mW/cm^2)	Statistical indices	Log-linear plus tail model	Weibull model
White grape juice			
0.38	RMSE[a]	0.156	0.213
	R^2[b]	0.989	0.979
	R^2-adj[c]	0.983	0.969
0.71	RMSE	0.171	0.295
	R^2	0.991	0.974
	R^2-adj	0.987	0.960
1.31	RMSE	0.390	0.422
	R^2	0.973	0.968
	R^2-adj	0.959	0.953
Apple juice			
0.38	RMSE	0.019	
	R^2	0.997	
	R^2-adj	0.996	
0.71	RMSE	0.089	
	R^2	0.986	
	R^2-adj	0.980	
1.31	RMSE	0.107	
	R^2	0.987	
	R^2-adj	0.980	

[a] RMSE: Root mean squared error.
[b] R^2: Coefficient of determination.
[c] Adj-R^2: Adjusted coefficient of determination.

higher in apple juice and calculated as 4.2, 3.3 and 3.1 log CFU/mL corresponding to UV intensities of 0.38, 0.71 and 1.31 mW/cm^2 (Table 1).

Also, the Weibull model was applied to the inactivation data by UV-C in grape juice. At all tested intensities, an upward concavity ($p < 1$) was observed. The scale parameter (δ) represents the dose for the first decimal reduction and found as 2.8 ± 0.8, 2.0 ± 0.7 and 9.5 ± 3.5 mJ/cm^2 for 0.38, 0.71 and 1.31 mW/cm^2, respectively. The suggested UV-doses for 4-log reductions at the highest UV intensity in grape juice were 172.8 ± 19.8 for log-linear plus tail and 229.9 ± 12.9 mJ/cm^2 for Weibull model. The required UV-dose to achieve an inactivation of 4 log units was found as 219 ± 4.03 mJ/cm^2 by Weibull model when the UV-intensity was reduced to 0.71 mJ/cm^2.

Table 2 presents the goodness of fit in terms of RMSE, R^2 and adj-R^2. Log-linear plus tail model fitted the experimental data of the white grape juice better as indicated by the lower RMSE values. The goodness-of-fit parameter RMSE of log-linear plus tail was 0.156, 0.171, 0.390 for 0.38, 0.71 and 1.31 mW/cm^2, respectively for inactivation data in grape juice. Also, R^2 values of log-linear plus tail were found as 0.989, 0.991, and 0.973 at intensity levels of 0.38, 0.71, and 1.31 mW/cm^2, respectively. The RMSE values were 0.019, 0.087, 0.107 for 0.38, 0.71 and 1.31 mW/cm^2, respectively for inactivation data in apple juice. R^2 values were 0.997, 0.986 and 0.987 for 0.38, 0.71 and 1.31 mW/cm^2, respectively (Table 2).

To the best of our knowledge, no previous studies have examined the effect of UV-C irradiation on spores of *A.acidoterrestris* in fruit juices. As a conclusion, this study provides the information on the inactivation of *A.acidoterrestris* spores in grape and apple juices by UV-C. This technology could be used as an alternative to thermal treatment for grape juices or combined with other preservation methods for the pasteurization of apple juice. The results showed that log-linear plus tail model could be used to fit the inactivation data in both juices. The future study should investigate the quality characteristics of grape and apple juices during UV-C treatment of spores.

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