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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.

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