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Inactivation kinetics of *Alicyclobacillus acidoterrestris* in apple juice submitted to ultraviolet radiation

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ABSTRACT

Ultraviolet-C radiation (UV-C) is widely used as an alternative strategy to control microorganism in food products. *Alicyclobacillus acidoterrestris* is a thermo-acidophilic, non-pathogenic and spore-forming bacterium, able to grow at low pH and high temperatures. It is a concern, particularly in apple juice thermal pasteurization, because it is responsible for quality degradation.

The main objective of this work was to study the influence of UV-C radiation treatments with seven different intensities (0.34, 0.86, 2.59, 5.59, 8.45, 11.50 and 13.44 W/m^2) on *A. acidoterrestris* inactivation in apple juices. Treatments were carried out in a camera (75 × 70 × 45 cm^3) with four germicidal UV lamps with peak emission at 254 nm. Commercial juices were artificially inoculated with bacterium, with initial loads around 10^7 CFU/mL and then exposed to UV-C radiation and the treatment impact on microbial loads was assessed throughout exposure times. The UV transmittance at 254 nm of the juice was 58%. Results showed that the log-survival of *A. acidoterrestris* decreased linearly with treatment time, for all intensities tested. When the most severe intensity was used, the number of spores decreased drastically (around 5-log reduction) after 8 min of treatment. Overall it can be concluded that UV-C radiation is a promising treatment with a drastic impact on the loads of *A. acidoterrestris* in apple juices, especially when high intensities are used.

1. Introduction

Heat processing is the most widely used treatment for microorganisms and enzymes inactivation, thereby extending products shelf life. However, adverse effects on sensory and nutritional characteristics of the foods may occur (Braddock, 1999).

In this context, and particularly for the beverages branch, non-thermal technologies have received increasing attention due to their potential for inactivating spoilage and pathogenic microorganisms. In addition, they can help minimizing quality losses in terms of flavour, colour and nutritional compounds (Baysal, Molva, & Unluturk, 2013; Lu et al., 2010; Noci et al., 2008).

Ultraviolet radiation is an alternative non-thermal strategy to control microorganisms in food products (Baysal et al., 2013; Shama & Alderson, 2005). Short-wave ultraviolet light (UV-C) is a radiation in the range 200–280 nm in the UV spectrum (Char, Mitilinaki, Guerrero, & Alzamora, 2010). Microorganisms that are exposed to UV-C light are affected at the DNA (deoxyribonucleic acid) level (Perkins-Veazie, Collins, & Howard, 2008; Terry & Joyce, 2004), which compromises their survival.

UV-C is a non-ionizing radiation and does not generate chemical residues or undesirable by-products that may negatively affect sensory characteristics (Guerrero-Beltran & Barbosa-Canovas, 2004). However, UV-C radiation has a limited penetration depth and consequently its efficiency depends on the length of the food to be treated (Bintsis, Litopoulou-Tzanetaki, & Richard, 2000). Since FDA has approved the use of UV-C light for fruit juices pasteurization, this technology has been applied to liquid foods and beverages (Koutchma, 2009; Unluturk, Atilgan, Baysal, & Tari, 2008).

One particular concern in hot-fill fruit and fruit juices, which uses temperatures around 82–85 °C (Bahçeci & Acar, 2007), is the thermo-acidophilic bacteria *Alicyclobacillus acidoterrestris*. As a producer of off-flavours, this bacteria is considered to be an important target in the quality control of acidic beverages and to be used in the design of adequate pasteurization processes

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(Murakami, Tedzuka, & Yamasaki, 1998). They produce spores that can survive to pasteurization processes even in acidic environments, and are able to germinate and multiply during storage of the products at room temperature (Bevilacqua, Sinigaglia, & Corbo, 2008; Silva & Gibbs, 2001; Torlak, 2014). Many studies indicate that species of *Alicyclobacillus* are frequently isolated in the productive process of apple juice, which creates a great risk for their development and consequent deterioration (Salomão, 2009; Splittstoesser, Churey, & Lee, 1994). Moreover, in the juice heat-treated, thermoresistant spores of *Alicyclobacillus* hardly have competitors since yeasts and other bacteria, which might be present in the feedstock, can be inactivated due to adverse temperature conditions. Currently, 17 species of *Alicyclobacillus* are recognized, and one of the most reported on fruit products is *A. acidoterrestris* (Jensen & Whitfield, 2003).

Some authors approached the impact of UV-C treatments in fruit juices, such as apple (Keyser, Műller, Cilliers, Nel, & Gouws, 2008; Lu et al., 2010), orange and guava-and-pineapple (Keyser et al., 2008) and pineapple juice (Shamsudin, Noranizan, Yap, & Atikah, 2014). However, mathematical modelling of inactivation kinetics due to radiation effect is still scarce and requires investigation.

The concept of log-linear behavior of the survivors, first demonstrated by Bigelow in 1921, has been fundamental to the food industry for establishing the concept of decimal reduction time (D). However, some deviations to these linear tendencies frequently occur, depending on the microorganism and the food matrix contaminated (Xiong, Xie, & Edmondson, 1999). Some authors studied the impact of UV-C treatments in fruit juices, using mathematical models to describe kinetics of microbial survival. Baysal et al. (2013) studied the effect of UV-C intensity (3.8, 7.1 and 13.1 W/m^2) and exposure time (0, 3, 5, 7, 10, 12 and 15 min) on the inactivation of *A. acidoterrestris* spores in commercial pasteurized white grape and apple juices. Their results showed a sigmoidal tendency with a tail effect and consequently a first-order kinetics were not suitable for the estimation of spore inactivation in grape juice treated with UV-light. They used a log-linear plus tail and Weibull models to describe survival kinetics. These models had parameters that are related to doses applied, but lack comparisons to D-values. The maximum inactivation they reported for *Alicyclobacillus* in apple juice was around 2-log cycles for the maximum intensity and doses applied. Gouma, Álvarez, Condón, and Gayán (2015) investigated UV lethal effect also combined with a heat (45–60 °C) on yeasts commonly involved in apple juice spoilage (*Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Zygosaccharomyces bailii*, *Dekkera anomala* and *Dekkera bruxellensis*). They reported that *Saccharomyces* spp. showed the greatest UV resistance and that a combination of UV–C light with mild temperatures (50–60 °C) produced a 5-log reduction of *S. cerevisiae* in clarified apple juice with lower UV doses (up to a 89.3% of reduction at 57.5 °C). They present survival curves as a function of doses expressed in energy consumption per unit volume (J/mL). Because survival curves showed initial shoulders, they used a loglinear with shoulder model (Geeraerd, Herremans, & Van Impe, 2000).

The aim of this work was to study the influence of UV-C radiation treatments with different intensities on the inactivation of *A. acidoterrestris* in apple juice, with mathematical modelling of the survival kinetics. It was tested a range of intensities wider than the ones found in literature and D-values were estimated based on the linear inactivation kinetics observed, and seeking comparison of D-values obtained in conventional thermal pasteurization. The effect of radiation intensities on D-values was also assessed.

2. Materials and methods

2.1. Spores suspension

The growth of viable *A. acidoterrestris* CCT 4384 cells was carried out in four slant tubes containing potato dextrose agar (PDA), pH 3.5, incubated at 44 °C for 3 days. The biomass obtained was added to 10 mL AAM broth *(Alicyclobacillus acidocaldarius* medium) formulated according to Murakami et al. (1998) and incubated at 45 °C for 24 h. The enrichment broth was spread over Petri dishes containing AAM medium supplemented with $MnCl_2.4H_2O$ of 0.05% and 1.5% agar (pH 4) and incubated for 10 days at 45 °C. After microscopic confirmation of spores per staining using Malachite Green, 10 mL of water was added to each plate followed by scraping. The spores obtained were centrifuged five times at 3500 rpm (2000 × g) for 15 min. The supernatant was then removed, re-suspended in sterile water and kept in refrigerated conditions until further use.

Spores were enumerated according to methodology reported by Silva, Gibbs, and Silva (2000). The medium was composed by three solutions mixed after sterilization (at 121 °C for 10 min): (i) BAT medium: $CaCl_2.7H_2O$, 0.25 g; $MgSO_4.7H_2O$, 0.5 g; $(NH_4)_2SO_4$, 0.2 g; yeast extract, 2 g; glucose, 5 g; KH_2PO_4, 3 g; and distilled water, 500 mL, adjusted to pH 4.0 with H_2SO_4; (ii) trace elements solution: 1 mL of trace elements solution ($ZnSO_4.7H_2O$, 0.1 g; $MnCl_2.4H_2O$, 0.03 g; H_3BO_3, 0.3 g; $CoCl_2.6H_2O$, 0.2 g; $CuCl_2.2H_2O$, 0.01 g; $NiCl_2.6H_2O$, 0.02 g; $Na_2MoO_4.2H_2O$, 0.03 g; distilled water, 1 L); (iii) agar, 15 g; distilled water, 500 mL.

Incubation of the inoculated plates was at 45 °C for 2 days. Concentration of spores' suspension was 2 × 10^7 CFU/mL.

2.2. Fruit juice samples

Pasteurized apple juice available in the market was used in the experiences. The pH values of the juices were measured by a pH meter (Crison GLP22, Switzerland) and soluble solid content (°Brix) was determined by a refractometer (Atago, China). The juice pH averaged 3.2 and soluble solid content was approximately 10.5 °Brix. The UV transmittance (UVT) at 254 nm of each sample was measured using a spectrophotometer (Specord® S600, Analytic Jena, Germany). It was the ratio of transmitted light energy to incident light energy. Each measurement was performed at least 3 times. The UV transmittance at 254 nm of the juice was 58%.

Each juice sample had a volume of 25 mL of apple juice artificially inoculated with 0.05 mL of spores suspension (2 × 10^7 CFU/mL) placed in a single layer in a Petri dish (90 mm). The samples were then submitted to inactivation treatments.

2.3. Inactivation treatments

UV-C treatments were carried out in a camera (75 × 70 × 45 cm^3) designed by University of Algarve, Portugal. Samples were submitted to a bank of four germicidal UV lamps (TUV 15W/G15 T8, Philips, Holland) with peak emission at 254 nm. Prior to use, UV-C lamps were stabilized by turning them on for 30 min. Juice samples were placed in Petri dishes 30 cm below the lamps. The juice samples had a thickness of 4 mm. A constant stirring (magnetic agitation) was imposed in the juice plate during treatment in order to ensure equal distribution of UV intensity through the sample.

The tested UV-C intensity was measured by a photo-radiometer (DELTA OHM LP9021 UVC, Padova, Italy), giving corresponding intensities of 0.34, 0.86, 2.59, 5.59, 8.45, 11.50 and 13.44 W/m^2. At a given intensity of radiation, samples of 1 mL were taken after 0, 3, 5, 8, 10, 13, 15, 20 and 25 min of exposure.