Journal of Food Engineering 118 (2013) 302–311

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

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

Inactivation kinetics of some microorganisms in apple, melon, orange and strawberry juices by high intensity light pulses

Mariana Ferrario [a,c], Stella Maris Alzamora [a,b], Sandra Guerrero [a,b,*]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 CABA, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, Argentina
[c] Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina, Argentina

ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form 8 April 2013
Accepted 13 April 2013
Available online 24 April 2013

Keywords:
Pulsed light
Weibull model
Biphasic model
Coroller model
Fruit juices
Microorganism inactivation

ABSTRACT

The suitability of some models was analyzed to characterize the Pulsed Light (PL) inactivation kinetics for *Escherichia coli* ATCC 35218, *Listeria innocua* ATCC 33090, *Salmonella* Enteritidis MA44 and *Saccharomyces cerevisiae* KE162 in commercial juices and fresh squeezed juices. A negative relationship was found between the absorbance of juices and PL effectiveness. PL treatment (2.4–71.6 J/cm^2) was ineffective in natural strawberry and orange juices. In general, inactivation curves exhibited a marked upward concavity, reaching after 60 s-PL treatment to 0.3–6.9 log-reduction cycles. Nonlinear semilogarithmic survival curves were fitted by conceptually different models: the Weibull model, the biphasic model and a modified version of the Coroller model. Biphasic and Weibull models compared to the modified Coroller model allowed a better fit and more accurate estimation of parameters. A multivariate approach to data analysis by principal components (PCA) showed relevant spatial relationships among estimated model parameters, revealing PL treatment efficacy in the different juices.

1. Introduction

Consumer demands towards fresh-like, ready-to drink and healthier fruit juices have increased in the last decades mainly due to the content of antioxidants, vitamins and minerals which play an important role in the prevention of heart diseases, cancer and diabetes (Matthews, 2006). This fact has led to the emergence of "nonthermal" technologies since it is well-known that traditional thermal processes cause significant damage on organoleptic, nutritional and physicochemical properties of fluid foods (Elmnasser et al., 2008).

Pulsed Light (PL) is a technology to decontaminate surfaces by killing microorganisms using short time pulses (100–400 μs) of an intense broad spectrum between 100 and 1100 nm with 54% of emitted energy in the ultraviolet range (Gómez-López et al., 2007; Oms-Oliu et al., 2010). PL used for food processing applications typically emits 1–20 flashes per second at an energy density in the range of about 0.01 to 50 J/cm^2 at the surface. PL has potential applications for the treatment of foods that require a rapid disinfection. Other advantages of PL are the lack of residual compounds and the absence of applied chemicals disinfectants and preservatives (Oms-Oliu et al., 2010). It has, comparatively to continuous UV light, higher penetration depth and emission power (Krishnamurthy et al., 2007). Nevertheless, when light intensity or treatment duration is relatively high, the increase in the product temperature may be greater than desirable, causing burning of surface layers of food if no cooling system is implemented (Elmnasser et al., 2008).

Several studies have shown different effectiveness of the pulsed-light process in the inactivation of vegetative cells and spores (Jun et al., 2003; Krishnamurthy et al., 2007; Choi et al., 2010; Gómez et al., in press). Many questions about the nature of microbial inactivation by PL still remain unanswered. Nevertheless, PL efficacy has been mainly attributed to microbial DNA damages by thymine dimmer formation (photochemical effect) Wekhof, 2000) and/or to localized overheating of microbial cells (photothermal effect) (Wekhof, 2000) and/or to structural damage caused by the pulsing effect (photophysical effect) (Krishnamurthy et al., 2008). It is possible that all these mechanisms coexist, and the relative importance of each one would depend on the fluence imparted to the food and target microorganism (Gómez-López et al., 2007).

The shape of PL inactivation curves is generally described as sigmoid with presence of tail. Tailing is associated to many phenomena as lack of homogeneous population (Xiong et al., 1999), multihit phenomena (Yousef and Marth, 1988), presence of suspended solids (FDA, 2000), use of multiple strains that may vary in their

* Corresponding author at: Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 CABA, Argentina. Tel.: +54 (0)11 45763366.
E-mail address: sguerrero@di.fcen.uba.ar (S. Guerrero).

0260-8774/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jfoodeng.2013.04.007

susceptibility to UV-C, varying abilities of cells to repair DNA mutations (EPA, 1999), sample topography, and shading effect that may have been originated by the edge of the Petri dishes used in some experiments (Gómez-López et al., 2007; Yaun et al., 2003, 2004).

There are relatively few quantitative data on PL inactivation. Some authors found complete inactivation of microorganisms and absence of tailing (Otaki et al., 2003; Krishnamurthy et al., 2007; Wang et al., 2005).

As regards mathematical models used to describe inactivation curves, Weibull model has been extensively used to characterize the inactivation of pure cultures inoculated in liquid and solid fruit derivates processed by nonthermal technologies (Guerrero et al., 2005; Ferrante et al., 2007; Schenk et al., 2008). This model is based on the hypothesis that resistance to stress of population follows a Weibull distribution. Peleg and Cole (1998) proposed that nonlinear survival curves were unlikely the result of mixed populations; but were due to the cumulative form of a temporal distribution of lethal events. According to this concept each individual organism dies or is inactivated at a specific time. Because there is a spectrum of resistances in the population, the shape of the survival curve is determined by its distribution properties. Modified versions of Weibull model could be versatile describing many shapes of inactivation curves often observed in nonthermal processes. Nevertheless, this model overestimated PL effectiveness in some of studied cases (Uesugui et al., 2007; Sauer and Moraru, 2009; Izquier and Gómez-López, 2011). The study carried out by Uesugui et al. (2007) demonstrated that PL level of inactivation was influenced by inoculum size when the treatment was applied to surfaces that allowed the hiding of microbial cells. According to these authors, the Weibull model is adequate to accurately predict microbial inactivation in clear liquids, but it fails for products where the influence of various substrate properties on inactivation is significant.

Other models, like the biphasic one, are based on the hypothesis that two subgroups having very different levels of resistance to stresses could coexist in a microbial population describing a biphasic log-linear decrease in the population (Coroller et al., 2006). A general primary model based on mixed Weibull distribution characterizing two subpopulations with different levels of resistance to stress was proposed by Coroller et al. (2006). This flexible model, has demonstrated to describe various shapes of inactivation curves having parameters with biological significance, good fit and accurate prediction ability.

This research aimed to investigate the effect of PL treatment on the response of some microorganisms of concern inoculated in different fruit juices. Additionally, the suitability of Weibull, biphasic and modified Coroller models was analyzed to characterize PL inactivation kinetics for a range of fruit juices and microorganisms.

2. Materials and methods

2.1. Strains and preparation of inocula

Experiments were performed using *Eschericchia coli* ATCC 35218; *Listeria innocua* ATCC 33090, *Salmonella* Enteritidis MA44 and *Saccharomyces cerevisiae* KE162. Initial bacterial inoculum was prepared by transferring a loopful of Trypticase Soy Agar plus 0.6% w/w Yeast Extract (TSAYE, Biokar Diagnostics, Beauvais, France) slant stock culture to a 20 mL Erlenmeyer-flask of Trypticase Soy Broth supplemented with 0.6% w/w Yeast Extract (TSBYE; Biokar Diagnostics, Beauvais, France). It was incubated at 37 °C under agitation for 18 h until it reached stationary phase. A similar procedure was repeated for the yeast culture, where the initial inoculum was prepared by transferring a loopful of a fresh stock culture maintained in Potato Dextrose Agar (PDA; Britania, Buenos Aires, Argentina) to a Erlenmeyer-flask containing 20 mL of Sabouraud Dextrose Broth (Sab; Britania, Buenos Aires, Argentina). Incubation was performed at 27 °C for 24 h. All inocula were harvested by centrifugation (5000 rpm, 5 min) (Labnet, USA), washed twice with saline and re-suspended in peptone water to give a cell density of 10^8–10^9 CFU/mL. For the inoculation, 100 μL of the microbial suspension was added to 4.9 mL fruit juice prior to PL treatment.

2.2. Produce samples

Two types of fruit juices (commercial and fresh squeezed) were used in this study with the purpose of comparing microbial responses in matrixes commonly used in this type of research studies. Pasteurized juices, with no declared preservatives, of apple (CEPITA, Coca-Cola, Argentina) (pH: 3.5 ± 0.1; 9.5 ± 3 °Brix) and orange (TROPICANA, PepsiCo Inc, Argentina) (pH: 3.9 ± 0.3; 12.8 ± 1.8 °Brix), and natural squeezed juices of melon (*Cucumis melo*, var. Honeydew; pH: 5.7 ± 0.2; 8.4 ± 2.5 °Brix); orange (*Citrus sinensis*, var. Valencia, pH: 4.3 ± 0.1; 10.4 ± 1.6 °Brix), apple (*Pyrus malus* L., var Granny Smith, pH: 3.5; 12.7 ± 0.1 °Brix) and strawberry (*Fragaria ananassa* var. Duch, pH: 3.6; 9.8 °Brix) were used in this study. Natural juices were aseptically obtained from fruits that were rinsed with 0.02% sodium hypochlorite and sterile water to eliminate surface microbial load and gently dried with a sterile cloth. Juices were obtained under aseptic conditions in a 90% ethanol sanitized and 10 min UV-C exposed household juicer (Black and Decker, JE 1500, China), centrifuged in order to reduce pulp amounts (1000–6000 rpm, 10 min) (Eppendorf, model 5804 R, Hamburg, Germany) and collected for subsequent analysis.

2.3. Pulsed light processing

PL treatments were performed with a RS-3000B Steripulse-XL system (Xenon Corporation, Wilmington, MA, USA), which produce polychromatic radiation in the wavelength range from 200 to 1100 nm. The PL device consisted of an RC-747 power/control module, a treatment chamber that housed a xenon flash lamp (non-toxic, mercury free) and an air cooling system attached to the lamp housing to avoid lamp overheating during operation (Fig. 1). It generated high intensity pulsed light at a pulse rate of 3 pulses per second (pulse magnitude with a peak of ~18 kV) and a pulse width of 360 μs. According to the specifications supplied by the manufacturer, each pulse delivered 1.27 J/cm^2 for an input of 3800 V at 1.9 cm below the quartz window surface of the lamp. The different PL doses were obtained by altering the number of applied pulses. Fluence measurements were taken by a pyroelectric head model ED500 (Gentec Electro-Optics, Québec, Canada) connected to an oscilloscope model TDS 2014 (Tektronix, Beaverton, USA), with an aperture cover of 20.3 cm^2. Measurements were performed in triplicate.

For each PL treatment, 4.9 mL of refrigerated juice (~4 °C) were poured into a 100 mm diameter Petri dish to ensure that the entire dish surface was covered with sample to a depth of 1×10^{-3} m. Inoculum was added and Petri dish was placed at a distance of 0.1 m from the quartz window in a 150 mm Petri dish containing ice flakes to minimize temperature increase of the sample. Inoculated samples were exposed to irradiation for 2–60 s, corresponding to applied fluencies between 2.4 J/cm^2 and 71.6 J/cm^2. Inoculated untreated samples were used as controls. Temperature evolution of juices during PL treatment was monitored using a T-type thermocouple connected to a data logger Digi-Sense model 69202-30 (Barnant Company Division, Barrington, USA).

2.4. Microbial enumeration

To obtain survival curves triplicates corresponding to a given treatment time were collected. Peptone water (0.1% w/v) tenfold