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

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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

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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

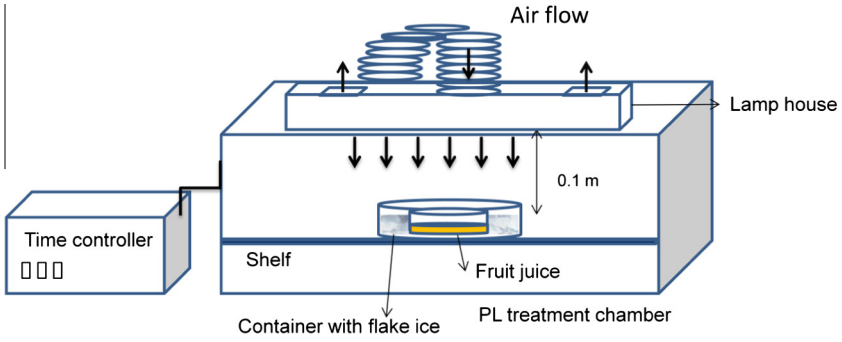

Fig. 1. Schematic diagram of the pulsed light processing system.

dilution aliquots were surface plated by duplicate onto TSAYE for *E. coli*; *S.* Enteritidis and *L. innocua* or PDA for *S. cerevisiae* using a spiral plater (Autoplate 4000, Spiral Biotech, USA). When PL irradiation treatment resulted in low counts (longer treatment times), up to 3-mL of fruit juice was directly pour plated into each Petri dish. Plates were incubated for 72 h at 37 °C (bacteria) and 27 °C (yeast) respectively. A counting grid was used for enumeration of colonies in the case of spiral plating. Survival curves were generated from experimental data by plotting $\log N/N_0$ (where N is the number of CFU/mL at a given time and N_0 the initial number of CFU/mL) versus treatment time.

2.5. UV transparency

Absorbance spectrum of 0.5% $^{V}/_{V}$ dilution of each sample was determined before PL treatment in 1 cm-path quartz cuvettes between 200 and 1100 nm using a UV–VIS spectrophotometer (Jasco V-630, Tokio, Japan) in order to determine the transparency of different juices to light. For this study, uninoculated juice samples were used.

2.6. Mathematical modeling

Microbial inactivation data were fitted with the cumulative form of a Weibull type distribution of resistances (Peleg and Cole, 1998):

$$S(t) = \log\left(\frac{N}{N_0}\right) = -b \cdot t^n \tag{1}$$

where $S(t)$ is the fraction of survivors at a given time and b and n are the scale and the shape parameters, respectively. The b value in the Weibull distribution function represents the rate of inactivation of the cells, while n indicates the concavity of the survival curve ($n > 1$ indicates a downward concavity and $n < 1$, an upward concavity. A log linear shape is a special case when $n = 1$). The values of b and n were then used to generate the resistance frequency curves using the following equation:

$$\frac{d\phi}{dt_c} = bnt_c^{n-1}\exp(-bt_c^n) \tag{2}$$

where t_c is a measure of the organism's resistance or sensitivity and $d\phi/dt_c$ is the Weibull distribution corresponding to t_c. Other statistical parameters which better explain the observed frequencies (distribution mode, t_{cm}; mean, $\bar{t}_c$; variance, $\sigma_t c^2$; and coefficient of "skewness", υ_1) were calculated from the following equations (Peleg and Cole, 1998):

$$t_{cm} = [(n-1)/nb]^{1/n} \tag{3}$$

$$t_c = \{\Gamma[(n+1)/n]\}/b^{1/n} \tag{4}$$

$$\sigma_{tc}^2 = \left\{\Gamma[(n+2)/n] - (\Gamma[(n+1)/n])^2\right\}/b^{2/n} \tag{5}$$

$$\upsilon_1 = \frac{\lfloor\Gamma(n+3/n)/b^{3/n}\rfloor}{\left[\Gamma(n+2/n)/b^{2/n}\right]^{3/2}} \tag{6}$$

where Γ is the gamma function. The distribution mode, t_{cm}, represents the treatment time at which the majority of population dies or is inactivated. The mean, t_c, corresponds to the inactivation time on average with its variance, σ_{tc}^2. The "skewness" coefficient, υ_1, represents the skew of the distribution.

Inactivation curves were also fitted by the biphasic model proposed by Cerf (1977), which can be formulated as follows,

$$\log_{10}\left(\frac{N}{N_0}\right) = \log_{10}(f \cdot e^{-kmax_1 \cdot t} + (1-f) \cdot e^{-kmax_2 \cdot t}) \tag{7}$$

herein f is the fraction of the initial population corresponding to the subpopulation more sensitive to the treatment, $(1-f)$ is the fraction of the initial population corresponding to the subpopulation more resistant to the treatment and $kmax_1$ and $kmax_2$ are the specific inactivation rates of the two populations, respectively.

A modified 4-parameter version of the model proposed by Coroller et al. (2006) based on two mixed Weibullian distributions of bacterial resistances was also applied:

$$\log_{10}\left(\frac{N}{N_0}\right) = \frac{1}{1+10^{\alpha}}\left[10^{-\left(\frac{t}{\delta_1}\right)^{p+\alpha}} + 10^{-\left(\frac{t}{\delta_2}\right)^{p}}\right] \tag{8}$$

where p is a shape parameter, α is the log proportion between the sensitive fraction (f) and the resistant one $(1-f)$, δ_1 and δ_2 are the time for the first decimal reduction of the subpopulation 1 and subpopulation 2, respectively.

2.7. Statistical analysis

Statistical analyses were carried out using InfoStat 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina). Significance level was set at $p < 0.05$. Multivariate outliers were detected by Mahalanobis distance and removed from data set. Model performance was evaluated using the root mean square error (RMSE) (Alzamora et al., 2005); the Akaike information criterion (AIC) (Akaike, 1973) and the Bayesian Schwarz criterion (BIC) (Quinn and Keough, 2002):

$$\mathrm{RMSE} = \sqrt{\frac{\sum\left(\mu_{observed} - \mu_{predicted}\right)^2}{n}} \tag{9}$$

$$\mathrm{AIC} = N\left[\ln\left(\frac{2\pi\sigma^2}{N}\right) + 1\right] + 2 \tag{10}$$

$$\mathrm{BIC} = N\left[\ln\left(\frac{2\pi\sigma^2}{N}\right) + 1\right] + P \cdot \ln(N) \tag{11}$$

where N is the number of observations; μ is the response value; P is the number of parameters of the model and σ^2 is the variance calculated from the mean square error (MSE).

The root mean square error (RMSE), which measures the average deviation between the observed and the fitted values, was used to evaluate the performance of models. The other criteria were used to detect model overfitting. According to Akaike's and Bayesiańs theories, the most accurate and parsimonious model yields the smallest AIC and BIC values (Quinn and Keough, 2002). Both criteria are closely related and can measure the efficiency of the parameterized model in terms of predicting the data but the BIC criterion is a bit more conservative because the penalty term is larger in BIC than in AIC.

Principal component analysis (PCA) was applied to illustrate the relationship among tested juices and Weibull or biphasic model parameters. The Cophenetic Correlation Coefficient (CCC) was obtained as a measure of how faithfully the analysis preserves the original Euclidean distances among data points. A good PCA analysis corresponds to a CCC value close to 1.0.

3. Results and discussion

3.1. Juice temperature

During PL treatments, temperature of juices increased with time as a consequence of the absorption of light but according to the adopted process design (pre-refrigerated sample + container with ice flakes, initial temperature ~2 ± 1 °C), the temperature value was always below 20 °C. Fig. 2 shows temperature evolution during PL treatment in natural fruit juices in a Petri dish which was submerged in ice flakes. On average, the temperature of samples treated for 60 s increased between 7.4 and 16.8 °C, depending on the type of fruit juice. Strawberry juice yielded the highest temperature increase (16.7 °C) and orange juice, the smallest one (10 °C). Apple and melon juices exhibited similar increase (12.6 °C and 10.5 °C, respectively) and temperature profile. Temperature profiles corresponding to commercial apple and orange juices were respectively overlapped with those corresponding to melon and natural apple juices (data not shown).

3.2. Absorbance spectra of untreated and treated PL systems

Fig. 3shows spectra corresponding to untreated juices. Absorbance spectra of natural (Fig. 3A) and commercial (Fig. 3B) juices were determined in a range of 200–1100 nm in order to examine the dependence of the efficiency of PL treatment on wavelength. All juices absorbed mainly in the UV range, with negligible absorption in the visible or near infrared. Strawberry juice exhibited the highest absorptivity in the UV range, followed by orange (with an absorption peak in 263 nm), melon and natural apple juice. Commercial orange and apple juices exhibited similar spectra to those corresponding to natural ones. In order to achieve inactivation by PL, contact between photons and microorganisms should occur, therefore any body between the light source and the microorganism that absorbs light will impair the disinfection process (Gómez-López et al., 2007). Wang et al. (2005) reported a maximum PL inactivation of *E. coli* at 270 nm (0.43 log per mJ/cm^2), while above 300 nm no inactivation occurred. They suggested that UV absorption by the pyrimidine dimers in DNA induces covalent joining and inhibits the cell replication, which is the major cause of microorganism inactivation with UV radiation. Woodling and Moraru (2007) observed that although the entire UV range seemed to contribute to the inactivation of *L. innocua*, the effect of the UV-B and UV-C ranges were stronger than the effect of UV-A range.

3.3. Evaluation of PL effectiveness

Survival curves of *E. coli*, *S. cerevisiae*, *L. innocua* and *S.* Enteritidis in commercial apple and orange juices and natural apple, melon, orange or strawberry juices processed by PL at different doses are shown in Fig. 4. In general, inactivation curves exhibited a marked upward concavity, reaching after 60 s (71.6 J/cm^2) of PL treatment between 0.6 and 6.2 log-reduction cycles in the case of commercial juices (Fig. 4A) and between 0.3 and 6.9 log-reduction cycles for natural ones (Fig. 4B). Exceptionally, when *S. cerevisae* was inoculated in commercial apple (Fig. 4AII) and natural melon (Fig. 4BII) juices almost sigmoidal inactivation curves were obtained (Fig. 4). All inactivation curves were characterized by a more pronounced decrease during the first 10 s of treatment (fluence $\leqslant$12 J/cm^2) and then, the number of survivors decreased slowly as the treatment time increased (Fig. 4). Changes more or less abrupt in the inactivation curve shape could be associated to different sensitivities in the population even leading to the presence of two subpopulations with different resistance to stress for some microorganisms assayed. The upward concavity, which indicates that process became less effective for higher doses, led to the presence of tail in several survival curves. The occurrence of tailing could be attributed, among others, to the existence of more resistant members in the population (low *f* value) (Table 1), and/or high absorption of samples in the UV region (Fig. 3), as the presence of suspended solids impairs the disinfection process. Pataro et al. (2011) also reported the presence of tail in PL inactivation curves of *E. coli* and *L. innocua* in orange juice but not in apple juice. None

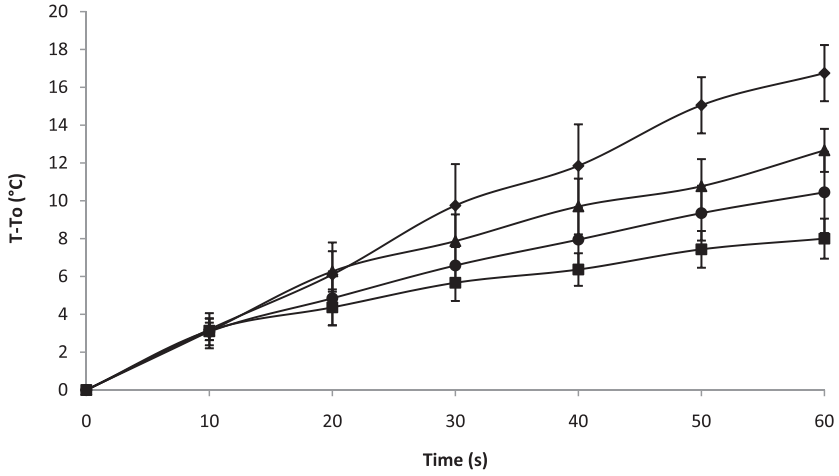

Fig. 2. Temperature profiles of apple (▲), melon (●), orange (■) and strawberry (♦) natural juices refrigerated with flake ice during treatment with PL at 10 cm from the lamp, *T*: juice temperature at a given time of treatment, To: initial juice temperature. (*I*) standard deviation.

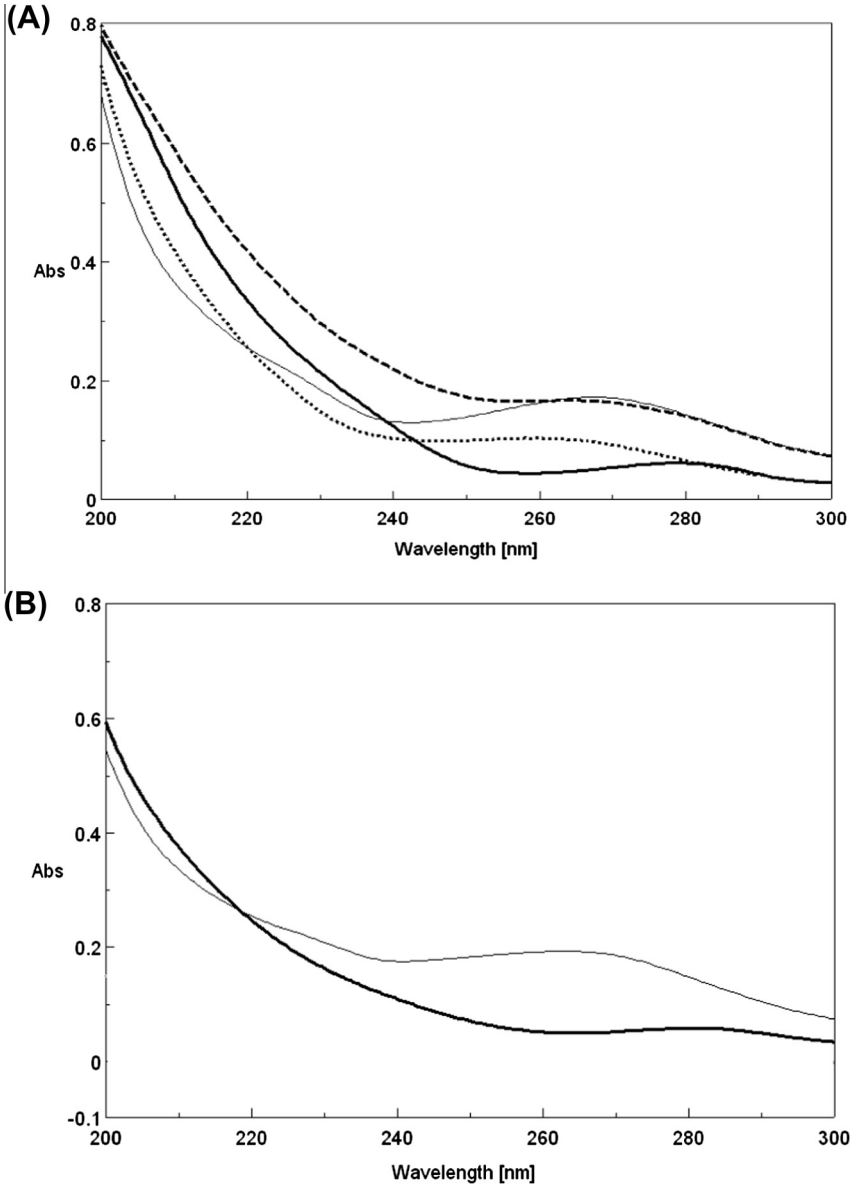

Fig. 3. Absorbance spectrum of natural (A) or commercial (B) juices of apple (▬), melon (·····) orange (—) and strawberry (– – –).

of the survival curves exhibited shoulder, in agreement with findings of other authors (Choi et al., 2010; Izquier and Gómez-López, 2011; Gómez et al., in press).

For all microorganisms investigated, more inactivation was observed in commercial apple and orange juices (Fig. 4A) compared with natural ones (Fig. 4B), being PL treatment more effective in apple juice. PL treatment in natural strawberry and orange juices lacked of effectiveness as less than 1 log-reduction cycle was achieved for all microorganisms studied. These results suggest that the level of inactivation was limited mostly by the absorption of light in the UV range. Both natural orange and strawberry juices showed high absorbance of light in the range of wavelengths from 200 to 400 nm (Fig. 3A and B). This is in agreement with the results reported by other authors who observed that the PL inactivation was lower in systems with greater absorption in the UV-region (Sauer and Moraru, 2009; Koutchma and Parisi, 2004). Other sample variables like pH and °Brix, did not appear to affect inactivation rate, as treatment in the less acidic juice (melon juice) exhibited the highest inactivation level, while solid content of natural squeezed juice was lower than natural apple juice but showed less inactivation. These findings are in agreement with Sauer and Moraru (2009), who reported that PL treatment in model solutions with low absorbance of UV light and high pH values was more efficient than in apple juice and cider. Chaine et al. (2012) also suggested that the lower microbial inactivation obtained in sugar syrup respect to distilled water could be attributed to differences in light transmission in the UV-C region. Murakami et al. (2006) and Koutchma et al. (2004) showed that solutions with different degree of soluble solids (°Brix) did not affect inactivation rates during continuous UV treatment. On the other hand, Koutchma et al. (2004) reported that the presence of suspended particles (which increases the turbidity of the system) can negatively impact the disinfection efficacy due to additional absorbance, scattering and or blocking of UV light. Different microbial responses to PL treatments could not only be due to differences in microorganism susceptibility to the UV region but to the broad spectrum. Takeshita et al. (2003) observed that yeasts exposed to PL (3.5 J/cm^2) provoked elution of protein caused by membrane disruption as well as structural changes like expanded vacuoles which were absent in treatment with UV-C alone. The high doses applied in this work

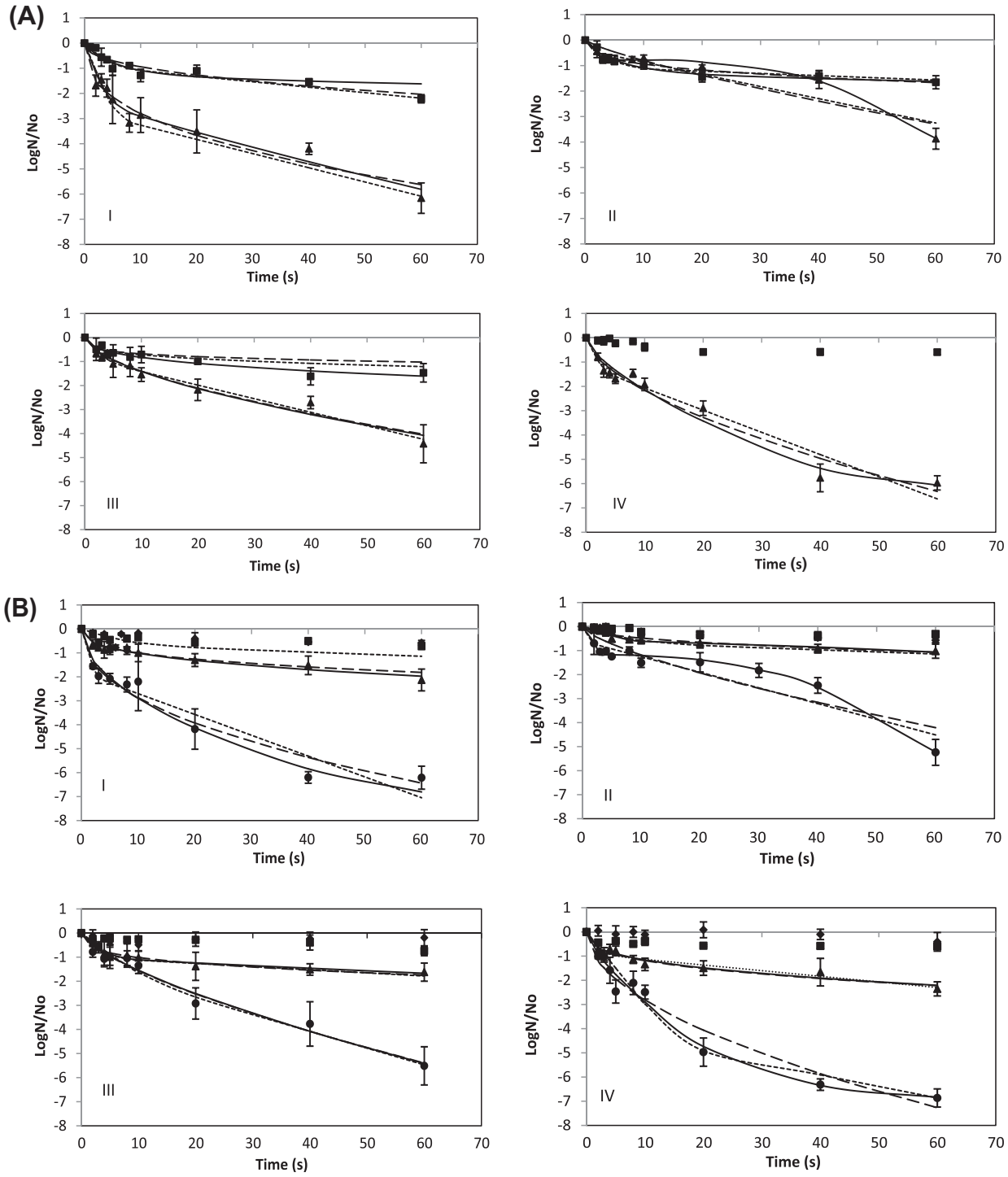

Fig. 4. Experimental survival curves (points) and fitted values derived from Weibull (dashed line), biphasic (dotted line) and Coroller (solid line) models for *E. coli* (I), *S. cerevisiae* (II), *L. innocua* (III) and *S.* Enteritidis (IV) in apple (▲); melon (●); orange (■) and strawberry (♦) juices treated with PL. (A) Commercial juices; (B) natural juices.

would suggest that the photophysical effect would have played a major role in the inactivation of microorganisms, so the differences in susceptibility within the microorganisms assayed may be also attributed to different photophysical resistances.

Sauer and Moraru (2009) obtained 2.5–2.7 maximum log cycle reductions with a fluence of 13 J/cm^2 for *E. coli* ATCC 25922 and *E.* O157:H7 respectively, in commercial type clarified apple cider under static conditions. Similar inactivation of *E. coli* (2.9 log red; 12 J/cm^2; 10 s) was achieved in this work. Palgan et al. (2011) reported 5 log cycle reductions in commercial apple juice and 1 log cycle reduction in commercial orange juice applying a PL fluence of 28 J/cm^2 for *E. coli* K12 DSM 1607 also in static conditions. In this work, 20 s of PL treatment (24 J/cm^2) provoked similar inactivation of *E. coli* in commercial orange juice but somewhat less inactivation in commercial apple juice (3.5 log red.).

3.4. Mathematical models and their assessment

Data corresponding to inactivation of all microorganisms in natural orange and strawberry juices and *S.* Enteritidis in commercial

Table 1
Estimated parameters of Weibull, biphasic and modified Coroller models corresponding to *E. coli*, *S. cerevisiae*, *L. innocua* and *S.* Enteritidis survival in natural and commercial juices treated with PL during 60 s.

Juice/microorganism		b (s^{-n})	n (–)	f (–)	$kmax_1$ (1/s)	$kmax_2$ (1/s)	α (–)	δ_1 (s)	p (–)	δ_2 (s)
Commercial apple	*E. coli*	1.14***(0.17)	0.39***(0.04)	0.995***(0.00)	1.25***(0.19)	0.14***(0.02)	2.15**(0.61)	1.64**(1.64)	0.88**(0.88)	13.70^{ns}(13.73)
	S. cerevisiae	0.13 ^{ns}0.08)	0.79***(0.16)	0.585***(0.15)	2.42^{ns}(29.05)	0.11***(0.01)	0.69***(0.04)	2.33***(0.18)	3.22***(0.27)	42.30***(1.25)
	L. innocua	0.36***(0.06)	0.59***(0.05)	0.855***(0.06)	0.77**(0.28)	0.13***(0.01)	1.92 ^{ns}(++)	5.55*(++)	0.59 ^{ns}(0.27)	5.55 ^{ns}(++)
	S. Enteritidis	0.55***(0.11)	0.60***(0.05)	0.927***(0.05)	1.28 ^{ns}(0.74)	0.21***(0.02)	0.67*(0.39)	2.40*(0.76)	2.52***(0.16)	39.46**(3.3)
Commercial orange	*E. coli*	0.35***(0.07)	0.43***(0.06)	0.868***(0.05)	0.60***(0.14)	0.05***(0.01)	4.87 ^{ns}(++)	11.01^{ns}(++)	0.43 ^{ns}(0.87)	17.30^{ns}(++)
	S. cerevisiae	0.45***(0.07)	0.32***(0.05)	0.910***(0.03)	0.53***(0.02)	0.02**(0.01)	1.13*(0.46)	5.60*(2.33)	0.66*(0.31)	195.20^{ns}(++)
	L. innocua	0.37***(0.07)	0.29**(0.06)	0.792**(0.06)	0.61**(0.28)	0.04***(0.01)	0.36 ^{ns}(18)	8.79 ^{ns}(++)	0.43 ^{ns}(0.98)	42.16^{ns}(++)
Natural melon	*E. coli*	0.98***(0.16)	0.47***(0.05)	0.985***(0.00)	1.99 ^{ns}(0.24)	0.19***(0.05)	6.46 ^{ns}(++)	1.24**(0.45)	0.51***(0.09)	193.89^{ns}(++)
	S. cerevisiae	0.23*(0.12)	0.71***(0.14)	0.749***(0.15)	2.92^{ns}(57.1)	0.15***(0.14)	1.14***(0.08)	2.17***(0.23)	2.68***(0.27)	35.60***(1.91)
	L. innocua	0.32***(0.06)	0.69***(0.05)	0.947***(0.07)	0.43***(0.43)	0.16***(0.03)	0.91 ^{ns}(++)	5.37 ^{ns}(++)	0.70*(0.33)	5.37^{ns}(++)
	S. Enteritidis	0.83***(0.15)	0.53***(0.04)	0.999**(0.00)	0.69***(0.08)	0.11**(0.04)	4.94***(1.93)	2.28***(0.52)	0.72***(0.1)	24.70^{ns}(39)
Natural apple	*E. coli*	0.44***(0.05)	0.37***(0.04)	0.798***(0.03)	1.5*(0.72)	0.05**(0.00)	1.34^{ns}(++)	9.55^{ns}(++)	0.37^{ns}(0.45)	9.55^{ns}(++)
	S. cerevisiae	0.17***(0.04)	0.45***(0.06)	0.765***(0.07)	0.24***(0.06)	0.02**(0.01)	0.48***(0.09)	6.85***(1.27)	1.51***(0.41)	100.4***(22.04)
	L. innocua	0.51***(0.06)	0.30***(0.04)	0.901***(0.02)	0.63***(0.09)	0.03***(0.01)	0.95***(0.13)	3.75***(0.65)	0.92***(0.31)	92.1***(28)
	S. Enteritidis	0.53***(0.06)	0.35***(0.04)	0.875***(0.05)	0.97***(0.09)	0.05***(0.04)	1.24^{ns}(++)	6.63^{ns}(++)	0.36^{ns}(0.45)	6.64^{ns}(++)

(value) Standard error of each parameter.
(++) Standard error value greater than 50.
^{ns} Non-significant.
* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

orange juice were not modeled since the inactivation was scarce. Fig. 4 shows the fitting of experimental inactivation data using the cumulative Weibull distribution function (Eq. (1)); the biphasic model (Eq. (7)) and the modified version of the model proposed by Coroller et al. (2006) (Eq. (8)). Table 1 displays the estimated parameters obtained from fitting these models to experimental data. In particular, Table 2 enumerates the specific statistics related to the Weibullian distribution calculated according Eqs. (2)-(6). In order to compare the goodness of fit of the three models, Table 3 displays RMSE, AIC and BIC values associated to the predicted PL survival curves.

Weibull type model was appropriate for representing survival data, except for *S. cerevisiae* response in commercial apple (Fig. 4A, II) and natural melon (Fig. 4B, II) juices in which the estimated parameters were not significant. High R^2_{adj} values were obtained; showing that between 82.5% and 98.5% of the variation in the experimental data could be explained by the selected model (data not shown). All systems processed with PL exhibited n values <1 (Table 1), as expected according to the notorious upward concavity. For *E. coli*, the b parameter, which represents the inactivation rate of cells, varied between 0.35 (commercial orange juice) and 1.14 (commercial apple juice) and for *S. cerevisiae*, it varied between 0.17 (natural apple juice) and 0.45 (commercial orange juice) (Table 1), indicating that the inactivation rate of the microorganisms was strongly influenced by the matrix in which they were. The b and n parameters were used to generate the frequency distribution of resistances (data not shown) and to calculate the associated statistics: mode, mean, variance and coefficient of skewness for obtaining a better explanation on the effect of PL in the inactivation of microorganisms investigated. All distributions of resistances lacked of mode and were strongly skewed to the right, showing that the majority of the microorganisms in the population were sensitive to PL treatment at very low doses (data not shown). Frequency distributions corresponding to natural melon and commercial apple juices exhibited lower mean and variance values than orange and natural apple juice (Table 2). In general, *E. coli* cells displayed the highest sensitivity to treatment followed by *S.* Enteritidis; *L. innocua* and *S. cerevisiae*. These findings are in agreement with Anderson et al. (2000) who also reported that Gram-negative bacteria were more sensitive than the Gram-positive ones. In contrast, Gómez-López et al. (2005) did not find a sensitivity pattern among different groups when they studied sensitivity of PL in an extensive variety of microorganisms. On the other hand, several studies have reported the dependence between the PL inactivation achieved and the matrix employed as well as the type of microorganism evaluated (Pataro et al., 2011; Palgan et al., 2011; Gómez et al., in press). The high variance values obtained (Table 2), even for frequency distribution corresponding to melon juice in which PL treatment was significantly effective, suggested that this model did not provide an adequate fitting to the experimental data and/or the heterogeneity of the response was important as it was reflected by the tails of the distributions. In contrast with our findings, several studies reported that the Weibull model could quantitatively describe microbial inactivation by PL in both liquid (Uesugui et al., 2007; Sauer and Moraru, 2009), and solid substrates (Bialka et al., 2008; Izquier and Gómez-López, 2011).

The biphasic linear model was appropriate for representing survival data as shown by the high R^2_{adj} values obtained, ranging between 88.6% and 99.7% (data non-shown) and low RMSE values (Table 3). The value f described in Eq. (7) represents the fraction of PL sensitive population after PL treatment. This fraction varied for *E. coli*, between 0.798 (natural apple juice) and 0.995 (commercial apple juice); for *S. cerevisiae*, between 0.585 (commercial apple juice) and 0.910 (commercial orange juice); for *L. innocua*, between 0.792 (commercial orange juice) and 0.947 (melon juice) and for *S.*

Table 2
Weibull model related statistics [a,b] corresponding to *E. coli*, *S. cerevisiae*, *L. innocua* and *S.* Enteritidis survival in juices treated with PL for 60 s.

Juice/microorganism		$\overline{t_c}$ (s)	σ^2_{tc} (s^2)	v_1 (–)
Commercial apple	*E. coli*	2.6	70	11.5
	S. cerevisiae	15.1	374	2.8
	L. innocua	8.7	244	4.4
	S. Enteriditis	4.1	51	4.3
Commercial orange	*E. coli*	31.6	+++	8.9
	S. cerevisiae	85.3	+++	21.7
	L. innocua	333.8	+++	31.3
Natural melon	*E. coli*	2.3	33	7.1
	S. cerevisiae	9.9	203	3.2
	L. innocua	6.7	99	3.4
	S. Enteriditis	2.6	28	5.4
Natural apple	*E. coli*	38.5	+++	13.4
	S. cerevisiae	127.2	+++	7.8
	L. innocua	87.4	+++	27.5
	S. Enteriditis	30.9	+++	16.0

[a] Statistics of Weibullian model, $\overline{t_c}$ distribution's mean, σ^2_{tc} variance, v_1 coefficient of skewness.
[b] +++ Value of the statistic greater than 1000.

Enteritidis, between 0.875 (natural apple juice) and 0.999 (melon juice) (Table 1). PL treatment in commercial apple juice inoculated with *S. cerevisiae* presented the lowest *f* value ($f = 0.585$, Table 1), fact indicating the presence of an important resistant subpopulation of the yeast to the applied treatment compared with the other evaluated microorganisms and juices. In contrast, *S.* Enteritidis in melon juice and *E. coli* in commercial apple juice exhibited the highest values for f parameter (0.999 and 0.995, respectively) underlying the presence of a huge proportion of the sensitive fraction. The kinetic parameter $kmax_1$ was always significantly greater than parameter $kmax_2$, indicating very high inactivation rate during the first seconds of treatment. For example, *S. cerevisiae* showed little $kmax_2$ values in commercial orange and natural apple juices, suggesting the presence of tail (i.e. refractary population to PL). Therefore, in these cases, PL treatment beyond 10 s did not further contribute to improve the inactivation.

The double Weibull simplified model described in Eq. (8) proposed by Coroller et al. (2006) did not accurately represent all survival curves. Regarding most of the survival curves, α parameter representing the log relationship between the most sensible population proportion (f) and the most resistant population proportion ($1 - f$), was near or greater than 1, indicating that the majority of population was the sensitive one which died in the first seconds of PL treatment since the time for the first decimal reduction (δ_1) value varied between 1.24 s (*E. coli*; melon juice) and 11.01 s (*E. coli*, commercial orange juice) (Table 1). Time to first log reduction values corresponding to the second subpopulation (δ_2) were not significant in many cases and greatly varied among systems and microorganisms (5.37–195.20 s) (Table 1). Situations in which high δ_2 values (greater than 60 s) were obtained, would imply that this model based in the existence of two subpopulations did not well characterize PL inactivation in the period of time studied. This result is in concordance with the very low $kmax_2$ values obtained from biphasic model fitting to these survival curves (for instance, in Table 1, *E. coli*/natural melon juice; *S. cerevisiae*/commercial orange juice; *S. cerevisiae*/natural apple juice and *L. innocua*/natural apple juice). Most of survival curves were described by a notoriously decelerated second period of inactivation which could be linear or nonlinear. The modified double Weibull model adopted in this work did not seem to adequately fit this type of curves. However, for inactivation patterns in which the first decay was followed by a plateau and a second marked decay (sigmoidal-type curves), this model gave a good quality of fit, as for example, in the case of survival curves corresponding to inactivation of *S. cerevisiae* in commercial apple and melon juices (Fig. 4 and Table 1).

3.5. Comparison of the models

Based on the RMSE values, the modified Coroller model and the biphasic model presented equivalent qualities of fit. The biphasic model showed the best performance with 7 smaller RSME values of 15 evaluated kinetics followed by the modified Coroller model with 6 smaller ones. Specially, in melon juice, there were great differences in the RSME values in favor of modified Coroller model (Table 3). However, this model had poor predictive performance in most cases according to the AIC and the BIC, which take both, fit and parsimony, into account (Coroller et al., 2006). It is probable that this model may result in overfitting and the AIC and BIC criteria penalize the number of parameters in the model making a balance between the fit and the parsimony of the model. Only in three exceptional cases, there were great differences among the three AIC or BIC in favor of the modified Coroller model. In these cases corresponding to *S. cerevisiae* inactivation kinetics in melon and commercial apple juices and *S.* Enteritidis in commercial apple juice, this model provided very small AIC and BIC values compared to Weibull and biphasic model values. It was just commented that

Table 3
Minimum RSME; AIC and BIC values[a] for the survival curves of the assayed microorganisms in PL treated fruit juices.

Juice/microorganism		RMSE			AIC			BIC		
		Weibull	Coroller	Biphasic	Weibull	Coroller	Biphasic	Weibull	Coroller	Biphasic
Commercial apple	*E. coli*	0.41	0.39	**0.37**	−6.19	−5.37	**−8.72**	−5.80	−4.16	**−7.81**
	S. cerevisiae	0.45	**0.08**	0.31	−6.64	**−36.24**	−12.31	−6.04	**−35.03**	−11.41
	L. innocua	0.25	0.28	**0.23**	**−31.49**	−11.84	−18.21	**−30.89**	−10.63	−17.31
	S. Enteritidis	0.41	**0.21**	0.46	−8.34	**−17.64**	−4.21	−7.33	**−16.43**	−3.21
Commercial orange	*E. coli*	0.22	0.24	**0.14**	−24.95	−18.18	**−34.15**	−24.15	−16.59	**−33.25**
	S. cerevisiae	0.16	**0.14**	0.17	−23.36	−21.03	**−23.44**	**−22.97**	−20.24	−22.85
	L. innocua	**0.15**	0.16	0.17	**−28.07**	−23.00	−24.25	**−27.46**	−21.79	−23.34
Natural melon	*E. coli*	0.45	**0.44**	0.60	**−16.74**	−6.39	2.67	**−15.94**	−5.17	3.26
	S. cerevisiae	0.60	**0.16**	0.51	−2.39	**−27.27**	−4.12	−1.6	**−25.68**	−2.93
	L. innocua	**0.26**	0.30	0.31	**−17.62**	−10.75	−12.36	**−17.02**	−9.54	−11.45
	S. Enteritidis	0.50	**0.12**	0.49	**−7.16**	−5.55	−2.86	**−6.76**	−4.34	−1.95
Natural apple	*E. coli*	0.13	0.15	**0.10**	−31.49	−24.47	**−34.15**	−30.88	−23.26	**−33.25**
	S. cerevisiae	0.11	**0.08**	**0.08**	−35.32	−38.08	**−39.88**	−34.72	−36.87	**−39.98**
	L. innocua	0.13	0.11	**0.10**	−31.49	−30.73	**−34.26**	−30.89	−29.52	**−33.25**
	S. Enteritidis	**0.15**	0.16	0.17	**−28.78**	−23.17	−24.10	**−28.12**	−21.96	−23.2

[a] Boldface RSME; AIC or BIC value is the best value in the row for model comparison.

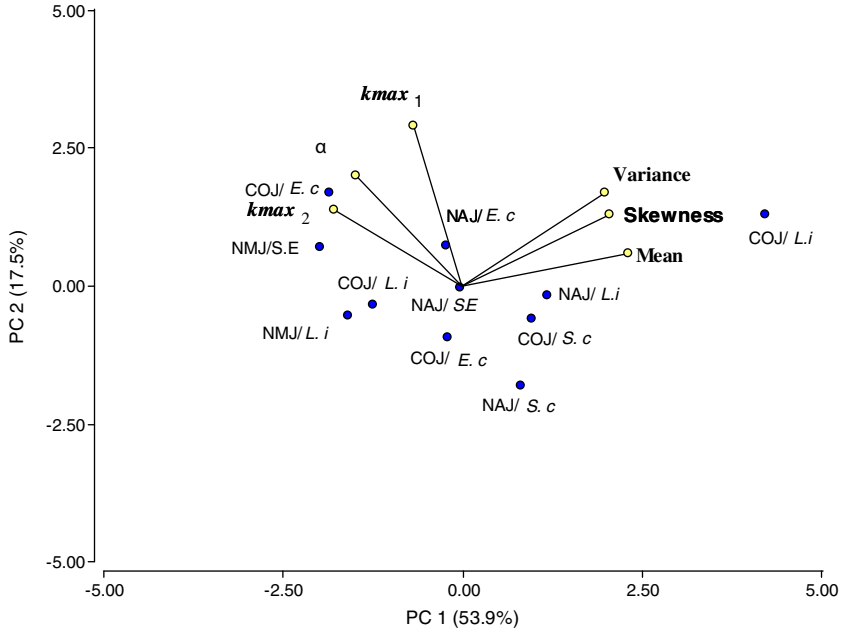

Fig. 5. Principal component analysis (PCA) bi-plots of Weibull and biphasic parameters of commercial (CAJ) and natural squeezed apple juice (NAJ), natural melon juice (NMJ) and commercial orange juice (COJ) inoculated with *E. coli* (*E.c*), *L. innocua* (*L.i*), *S. cerevisiae* (*S.c*) or *S.* Enteritidis (*S.E*).

these survival curves had shapes of sigmoid type, very different from the rest.

Analysis of AIC and BIC values determined that, in general, the training of Weibull model and biphasic model implied better fit, fewer explanatory parameters or both.

3.6. Principal component analysis (PCA) for model parameters

A multivariate approach to data analysis by principal components (PCA) showed the spatial relationships among estimated parameters or statistics surged from Weibull and biphasic models fitting to PL inactivation curves. Because modified Coroller model only adequately fit few experimental data, it was not considered in this analysis. Two-dimensional representations (PCA bi-plot) of these are presented in Fig. 5 for parameters of both models. The CCC obtained was 0.96, indicating that an accurate reduction was achieved with the analysis. Only the first two principal components (PC_1 and PC_2) were retained as they explained more than the 80% of the total variance. The first two PC (Fig. 5) explained 53.9% and 17.5% of the variance, respectively. The PC_1 separated the mean, variance and skewness which were associated positively, from α, and $kmax_2$ (negatively associated). On the other hand, PC_2 was associated positively with $kmax_1$. PL treatment in commercial apple juice inoculated with the Gram-negative bacteria (*S.* Enteritidis and *E. coli*) resulted the most effective as they were associated with a high fraction of the sensitive subpopulation (α) and even the more resistant fraction showed a high inactivation rate ($kmax_2$). In concordance, the lowest values of mean (tc), variance and skewness were obtained. *L. innocua* cells in this matrix exhibited lower sensitivity to the treatment as it presented lower values of the biphasic parameters and higher values of the Weibullian ones, than the Gram-negative bacteria. PL treatment in melon juice resulted very effective too, as high α and $kmax_2$ parameters were obtained, and low tc, skewness and variance. Again, *S.* Enteritidis resulted more sensitive than *L. innocua* cells as it showed higher α and $kmax_2$ values. In natural squeezed apple juice, a great heterogeneity of the response was obtained: the Gram-negative bacteria resulted more sensitive to PL, being *E. coli* more associated with $kmax_1$. *S. cerevisiae* in this matrix was the most resistant as it showed less inactivation rate ($kmax_1$) than *L. innocua*. In commercial orange juice, *E. coli* resulted again the most sensitive strain as it showed lower values of the Weibull parameters, while *L. innocua* was the most resistant.

4. Conclusions

This work bears out that pulsed light processing, a novel non-thermal technology, was capable of inactivating some microorganisms on the different types of fruit juices at low temperature (<20 °C). Significant microbial reductions were reached in very short treatment times (60 s) but the observed inactivation strongly depended on the type of juice and on whether it was commercial or freshly squeezed. Greater juice absorbance values in the UV-C range negatively influenced PL effectiveness.

Different mathematical functions were used for modeling the nonlinear survival curves of the different microorganisms in a variety of fruit juices. The functions considered represent different types of assumptions that can be used regarding differences in population resistance to pulsed-light treatment: (a) a population with a distribution of sensitivities represented by a unique nonlinear behavior (Weibullian model); (b) two subpopulations represented by linear behavior (biphasic model) and (c) two subpopulations associated to a double Weibull distribution of resistances (modified Coroller model). These models constituted good alternatives to quantify microbial response to pulsed light. Estimated parameters explained, from a different point of view, the influence of PL on microbial decline in different juices, as Weibull parameters allowed to know the frequency distributions of microbial inactivation, while the biphasic parameters gave information about inactivation rates of the sensitive and resistant subpopulations. In the near future further studies will be conducted to assess the effects of PL treatments on juice properties besides microbial safety and spoilage under continuous flow for commercial purposes.

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