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Study of the inactivation of some microorganisms in turbid carrot-orange juice blend processed by ultraviolet light assisted by mild heat treatment

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ABSTRACT

The aim of this study was to evaluate the effect of short wave ultraviolet light (UV-C, 0–10.6 kJ/m^2) assisted by mild heat treatment (UV-C/H; 40, 45 or 50 °C) on the inactivation of *Escherichia coli*, *Saccharomyces cerevisiae* and *Pseudomonas fluorescens* in freshly squeezed carrot-orange juice blend. In addition, the suitability of three conceptually different models was analyzed to characterize the inactivation kinetics. All treatments provoked moderate to high microbial inactivation depending on temperature and microorganism (2.6–6.0 log reductions). The use of UV-C assisted by mild heat treatment notably improved inactivation compared to single UV-C. Synergistic inactivation effects on *E. coli* and *P. fluorescens* were observed at combined UV-C/H (45 and 50 °C). Gompertz and Geeraerd models allowed a better fit and more accurate parameter estimation compared to the Weibull model.

1. Introduction

Consumer demand towards fresh-like, ready-to drink and healthier fruit juices has increased in the last decades mainly due to the presence of antioxidants, vitamins and minerals. These compounds play an important role in the prevention of heart diseases, cancer and diabetes (Matthews, 2006). In particular, carrot juice is rich in most of the natural antioxidants, including carotenoids, phenolics, vitamin C and tocopherol (Sharma et al., 2012). It is the main natural source of β-carotene, which protects against the free radicals generated endogenously through normal diet and metabolic activity, as well as from environmental sources. β-carotene provides this protection by acting as a strong quencher of singlet molecular oxygen and peroxyl radical scavengers (Schafer et al., 2002). Orange juice is a popular product representing a substantial source of vitamin C (Polydera et al., 2003). The antioxidant effect of vitamin C has been the focus of many research studies. It has been concluded that vitamin C helps in the prevention of cancer (Byers and Perry, 1992; Wittes, 1985).

Although heat pasteurization is the most commonly used technique for fruit processing, as it ensures safety and long product shelf life, it is well-known that traditional thermal processes cause significant damage on organoleptic, nutritional and physicochemical properties of fluid foods (Elmnasser et al., 2008). In order to prevent those undesirable effects, a wide range of emerging technologies has been investigated in the last decades for alternative processing of fruit and vegetable juices. The list encompasses technologies with different levels of development, going from those already implemented in industry such as high hydrostatic pressures (Dede et al., 2007; Buzrul et al., 2008), those approved by the FDA and under implementation, like ultraviolet light (Koutchma et al., 2007; Unluturk et al., 2010) ozone (Patil et al., 2009; García Loredo et al., 2015), and pulsed light (Pataro et al., 2011; Ferrario et al., 2013, 2015); to those that are less developed such as ultrasound (Char et al., 2010a; Ferrante et al., 2007).

Short-wave ultraviolet light (UV-C) is one of the most promising low-cost and energy efficient non-thermal technologies, used for decontamination of freshly squeezed juices. It encompasses the UV spectrum range from 200 to 280 nm, being lethal to a large variety of microorganisms, without generating chemical residues (Baysal

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et al., 2013). In particular, exposure to UV light results in the cross-linking of neighboring pyrimidine nucleotide bases in the same deoxyribonucleic acid (DNA) strand, eventually causing cell death (Gabriel, 2012). UV-C pasteurization is affected by many different factors such as UV light source choice, reactor design, flow rate, type of liquid, viscosity, density, UV-C light absorptivity, presence of soluble and insoluble solids, and particle size (Koutchma, 2009). Until recently, the implementation of UV-C as a decontamination technique has been limited to clear liquid foods and beverages. It has been successfully applied, achieving more than the 5 log reductions required by the US Food and Drug Administration (FDA, 2000), for the inactivation of *E. coli* in clear apple juice encompassing a broad range of UV-C doses from 18.7 to 531 kJ/m^2 (Keyser et al., 2008; Franz et al., 2009; Char et al., 2010a; Caminiti et al., 2012). Moreover, more than 5 log reductions of *Salmonella* Typhimurium were also achieved in pineapple juice applying an UV-C dose of 137.5 kJ/m^2 (Mansor et al., 2014), and *Alicyclobacillus acidoterrestris* spores in white grape juice (1.31 mW/cm^2) (Baysal et al., 2013), among others. UV-C possesses notoriously less effectiveness in highly turbid juices due to the presence of large amounts of UV-absorbing compounds and suspended particles. For instance, Char et al. (2010a) obtained only 1.5 log reductions of *Escherichia coli* in UV-C treated freshly squeezed orange juice (18.7 kJ/m^2). Authors explained this remarkable low efficiency of UV-C disinfection due to the presence of colored compounds and pulp particles which caused poor UV-C light transmission. Baysal et al. (2013) observed only up to 2 log reductions of *A. acidoterrestris* spores in UV-C treated apple juice (1.31 mW/cm^2), which possessed higher turbidity and absorption coefficient than the white grape juice used in the same study. These particles reduce UV light transmittance, thereby impairing the disinfection process (Gayán et al., 2013). Large suspended particles may also block the incidence of light on the microbial load (Guerrero-Beltrán and Barbosa-Cánovas, 2004). To overcome this limitation, combined processes have been recently designed by applying UV light with the assistance of other processing techniques to achieve maximal benefits in microbial reduction and retention of juice quality (Shah et al., 2016). The combination could be between UV-C technology and heat, other nonthermal technologies, or the addition of chemicals and preservatives. For example, higher inactivation compared to individual treatments, was achieved when applying UV-C light (0.011 kJ/m^2) combined with ultrasound (20 kHz, 95 μm-wave amplitude) for *S. cerevisiae* in apple juice (López-Malo et al., 2005), and *E. coli* in orange juice (Char et al., 2010a). In addition, UV-C (25 kJ/m^2) combined with 50 ppm of citral and 1500 of vanillin in orange juice delayed *Zygosaccharomyces bailii*, *E. coli* and *Listeria innocua* recovery during 13 days of refrigerated storage (Ferrario et al., 2011). Moreover, the combination of UV-C (203 kJ/m^2) and addition of sodium benzoate (250, 500, 1000 and 2000 ppm) for peach nectar processing demonstrated a synergistic inactivation effect on *Aspergilllus niger* and *Aspergillus flavus* (Flores-Cervantes et al., 2013). Tan (2012) observed a 5 log reduction of *Listeria innocua* in green guava juice treated by UV-C (0.035 kJ/m^2) followed by mild heat treatment (55 °C, 60 s). In this context, the use of ultraviolet light assisted by mild heat treatment to reach the desired inactivation effect, represents an alternative for the development of minimally processed turbid juices.

Microbial inactivation by UV-C in liquid media has been extensively characterized in literature. Few authors have reported linear behavior, such as Ochoa-Velasco and Beltrán (2013). However, most authors have reported non-linear survival curves after UV-C processing. Some of them have reported the presence of tail (Baysal et al., 2013; Unluturk and Atilgan, 2014), shoulder (Gayán et al., 2012a) or both (Quintero-Ramos et al., 2004). Several authors have successfully applied the Weibull model, which is based on the hypothesis that there is a cumulative form of a temporal distribution of lethal events. Therefore, each microbial cell dies or is inactivated at a specific time. Weibull model has shown good applicability for the characterization of microbial inactivation in orange juice or peach nectar (Taze et al., 2015; Flores-Cervantes et al., 2013) and solid matrices like pear slices (Schenk et al., 2008). Other authors have used predictive models which take into account shoulder and/or tail, such as the modified version of Gompertz and Geeraerd models. For general predictive purposes, the Gompertz and Geeraerd models have an important practical advantage over most other models. All the parameters derived from these models have a clear biological and/or graphical meaning, and the three phases of the inactivation curve (shoulder, log-linear phase and tailing region) are easily recognizable. Geeraerd model successfully characterized survival data of pathogenic microorganisms in solid and liquid food matrices subjected to different treatments, such as mild thermal treatment (Geeraerd et al., 2000) and UV-C assisted or not by mild heat (Gayán et al., 2013). Whereas, Gompertz model adequately characterized inactivation curves during a mild thermal treatment combined with vanillin plus citral (Char et al., 2010b), and during ultrasound treatment combined with low weight chitosan addition (Guerrero et al., 2005), among others. Nevertheless, suitability and comparison of these models has not been deeply analyzed in turbid juices treated by UV-C.

The aim of this research was to investigate the effect of UV-C treatment assisted by mild heat treatment (UV-C/H) on the response of some microorganisms of concern inoculated in a carrot-orange juice blend. The suitability of Weibull, Gompertz and Geeraerd models was analyzed to characterize single and combined UV-C processing inactivation kinetics for a range of temperatures and microorganisms.

2. Materials and methods

2.1. Strains and preparation of inocula

Experiments were performed using *Escherichia coli* ATCC 35218, *Saccharomyces cerevisiae* KE 162 and *Pseudomonas fluorescens* ATCC 49838. Initial *E. coli* inoculum was prepared by transferring a loopful of Trypticase Soy Agar plus 0.6% w/v Yeast Extract (TSAYE, Biokar Diagnostics, Beauvais, France) slant stock culture to a 20 mL of Trypticase Soy Broth supplemented with 0.6% w/v 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 *S. cerevisiae* and *P. fluorescens*, for which the initial inocula were prepared by transferring a loopful of fresh stock cultures maintained in Sabouraud Dextrose Agar (SAB, Biokar Diagnostics, Beauvois, France) or Nutrient agar (NA, Biokar Diagnostics, Beauvois, France) to 20 mL of Sabouraud Dextrose Broth (SAB Broth, Biokar Diagnostics, Beauvois, France) or Nutrient broth (NB, Biokar Diagnostics, Beauvois, France), respectively. Incubation was performed at 27 °C for 24 h. All inocula were harvested by centrifugation (1475 *g*, 5 min) (Labnet, Edison, New Jersey, USA), washed twice with peptone water to obtain a cell density of 10^7–10^9 CFU mL^{-1}. For the inoculation, 5 mL of the microbial suspension was added to 745 mL of carrot-orange juice prior to UV-C treatment.

2.2. Carrot-orange juice blend preparation

Fresh carrot juice was manually obtained under aseptic conditions by pressing carrots (*Daucus carota*, var. Chantenay). A household juicer (Ju655, Moulinex, Taipéi, Taiwan, China) was sanitized with 70% (v/v) ethanol and exposed to UV-C for 10 min. Similarly, fresh orange juice (*Citrus sinensis*, var. Valencia) was

obtained by using a household squeezer (Arno, São Paulo, São Paulo, Brazil). Carrots and oranges were previously rinsed with 5% sodium hypochlorite and sterile water to eliminate surface microbial load. Juices were filtered with a double sterile muslin cloth (carrot) or centrifuged (157 *g*, 20 °C, 10 min) in order to reduce pulp amounts (orange). Both juices were subsequently mixed to obtain a 50:50 ratio (v/v) juice blend. For reducing native flora, the resultant blend was thermally treated during 13 min using a double jacket coil tube connected to a thermostatically controlled water bath (HAAKE, Mess-Technik, Karlsruhe, Baden-Wurtemberg, Germany), to attain 70 ± 1 °C. During thermal treatment, temperature was monitored using a T-type thermocouple connected to a data logger Digi-Sense model 69202-30 (Barnant Company Division, Barrington, Illinois, USA). After treatment, the blend was collected in amber glass bottles and stored at −80 °C until use.

2.3. UV-C treatment

The short-wave ultraviolet light (UV-C) device (Fig. 1) used for juice blend treatment consisted of two serially connected UV-C lamps (TUV-30 W, 253.7 nm, Philips, Amsterdam, Netherlands), each one inside a 0.87 m-long glass tube leaving an annular flow space (outer diameter = 0.031 m; inner diameter = 0.024 m, volume = 0.22 L), established as the irradiation chamber. Inlet and outlet of juice to the UV-C lamps was carried out by autoclavable flexible hoses (L/S 24, Cole-Parmer, Masterflex, Barrington, Illinois, USA), which discharged into a double jacket vessel, connected to the water bath to attain 20, 40, 45 or 50 °C. UV-C performed at 20 °C was considered as a single treatment (UV-C). UV-C performed at 40, 45 or 50 °C corresponded to combined treatments, involving the assistance by mild heat to the UV-C process (UV-C/H). In addition, single thermal treatments (H) were performed as controls in the same way but with lamps turned off.

Before starting the treatments, UV-C lamps were turned on during 15 min in order to stabilize them and to sterilize the irradiation chamber. Firstly, juice blend (745 mL) was added to the vessel and recirculated at 1.6 L/min through the device using a peristaltic pump (CPX-400, Cole Parmer, Vernon Hills, Illinois, USA). Secondly, after the elapsed time required for juice tempering was achieved (30 min), the UV-C lamps were turned off and the 5-mL

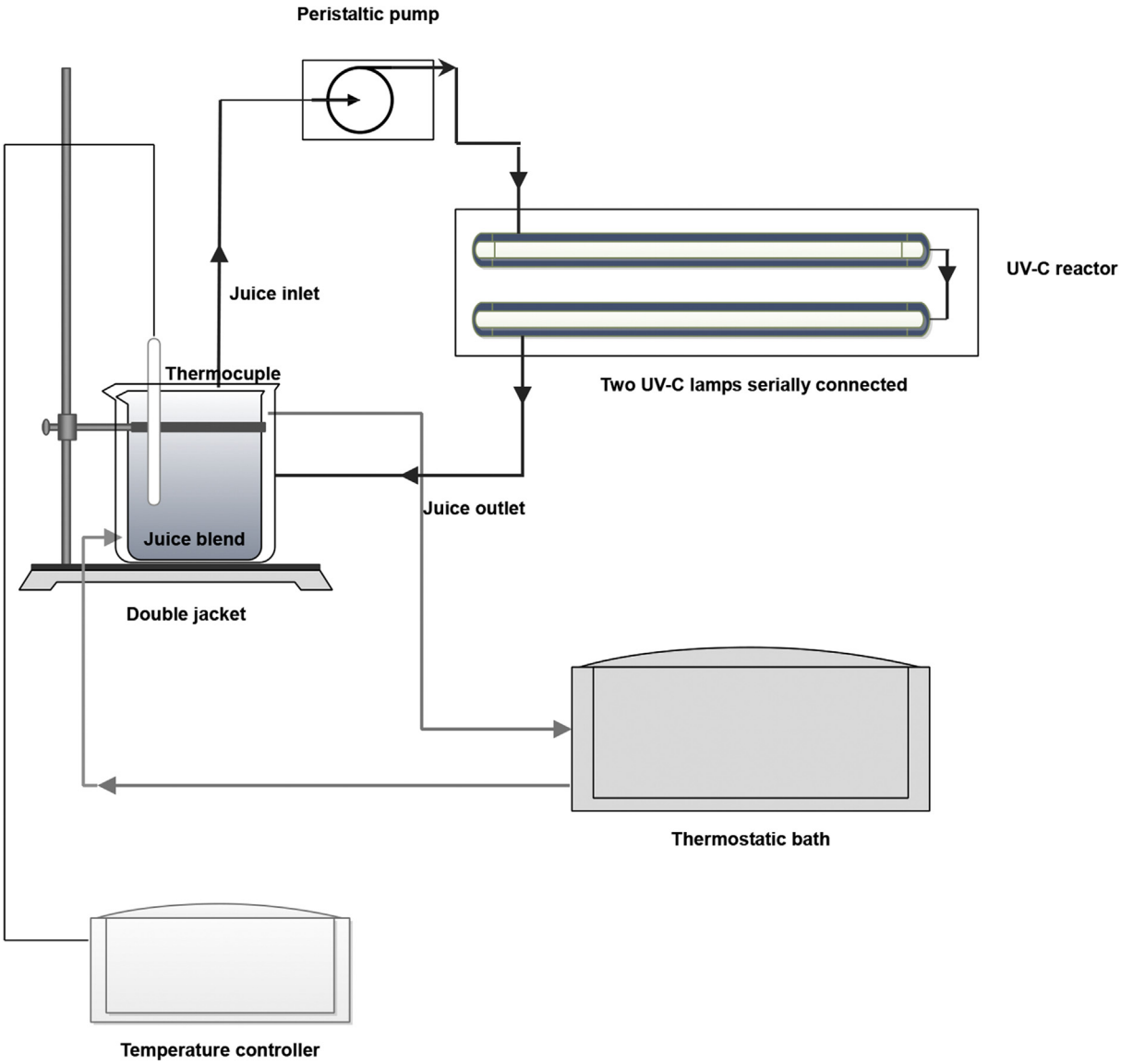

Fig. 1. Diagram showing the UV-C device used in this study.

inoculum was added to reach a final volume of 750 mL. The inoculated juice was recirculated through the UV-C device for 2 min in order to ensure a proper mixing of the system. Samples were taken at preset time intervals (0, 1, 2, 3, 5, 7, 9, 11, 13 and 15 min) achieving at the end of treatment a maximum dose of 10.6 kJ/m^2 (0.01232 J/mL). They were immediately stored in amber glass 10 mL bottles under refrigeration, until their analysis. Experiences were carried out in triplicate. The UV-C dose emitted from the lamps was determined by using a radiometer (Melles Griot, 13 PEM 001 model, Colorado, USA). Total UV-C dose was calculated as the average of both lamps.

2.3.1. Electric energy per order estimation

Electric energy per order ($\boldsymbol{E_{EO}}$) is defined as the electric energy in kilowatt hours [kWh] required to reduce microbial load by one order of magnitude in 1 m^3 of contaminated sample. In our study, it was calculated to determine the involved energy delivered to the UV-C treatment and its efficiency. The E_{Eo} values were estimated according to the equations proposed by Bolton et al. (2001) for flow-through operations in electric-energy-driven systems.

2.4. Microbial enumeration

Each sample was serially diluted in 0.1% (w/v) peptone water and surface plated by duplicate onto TSAYE for *E. coli*, NA for *P. fluorescens* or SAB agar for *S. cerevisiae* using a spiral plater (Autoplate 4000, Spiral Biotech, Norwood, Massachusetts, USA). When treatment resulted in low counts (longer treatment times), up to 3-mL of juice blend was directly pour plated into each Petri dish. Plates were incubated for 48 h at 37 °C (*E. coli*) and 27 °C (*S. cerevisiae* and *P. fluorescens*), 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$_0$ is the initial number of CFU/mL) versus treatment time.

2.5. Physicochemical characterization of juices

For the physicochemical characterization of juices uninoculated and untreated fresh juice samples were used. Particle size of juice samples was measured in the range from 0.1 to 1000 μm by static light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, Worcestershire, UK). Pump speed was set at 1800 rpm, and a refractive index (RI) corresponding to 1.35 and absorption parameter of 0.1 were used, according to the specifications for colored samples (Malvern Instruments, 2004). The weight-average sizes volume-surface diameter (D$_{3,2}$) and volume-weighted mean diameter (D$_{4,3}$) were expressed as, $D_{3,2} = \sum n_i d_i^3 / \sum n_i d_i^2$, and $D_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of particles of diameter d_i (Corredig et al., 2001).

In order to obtain the UV absorption coefficient of the juice, several dilutions of the juice blend in distilled water were prepared. Absorbance at 254 nm was determined in 1-cm light path quartz cuvettes on a UV-VIS spectrophotometer (V-630, Jasco, Tokio, Japan). The slope of the regression line obtained by plotting absorbance vs. sample concentration (%v/v) was considered as the absorption coefficient. It is important to remark that this coefficient does not correspond to the molar absorptivity, since concentration is not expressed as mol/L (Oteiza et al., 2010).

Turbidity was determined by a turbidimeter (LaMotte 2020we, Chestertown, Maryland, USA) using AMCO Primary Turbidity (100 NTU) and Formazin standards (4000 NTU). All measurements were performed in triplicate.

2.6. Mathematical modeling

An alternative description of survival curves was detailed by Peleg and Cole (1998) using a Weibull-type distribution. This model considers that each organism in a population dies or is inactivated at a specific time. Accordingly, there is a spectrum of death resistances in the population and the shape of the survival curve will be determined by the shape distribution having different distribution parameters. 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. These parameters were derived using non-linear regression, and then were used to generate the resistances frequency curves using the following equation:

$$\frac{d\varphi}{dtc} = bntc^{n-1}\exp(-btc^n) \tag{2}$$

where t_c is a measure of the organism's resistance or sensitivity and $d\varphi/dt_c$ 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, σ^2_{tc}; and coefficient of "skewness", υ_1) were calculated from the following equations (Peleg and Cole, 1998):

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

$$\bar{t}_c = \{\Gamma[(n+1)/n]\}\Big/ b^{1/n} \tag{4}$$

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

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

where Γ is the gamma function. The distribution mode, t_{cm}, represents the treatment time at which the majority of population dies or inactivates. The mean, $\bar{t}_c$, corresponds to the inactivation time on average with its variance, σ^2_{tc}.The "skewness" coefficient, υ_1, represents the skew of the distribution.

Inactivation curves were also fitted by the Gompertz equation (Linton et al., 1995):

$$Log(N/N_0) = C.e^{-e(A+B.t)} - C.e^{-e(A)} \tag{7}$$

herein, the estimated parameters (A, B and C) represent the different regions in the survival curve: (A), the initial shoulder [min]; (B), the maximum death rate [min^{-1}] and (C), the overall change in number of survivors [−]. This equation has been demonstrated to be particularly suitable for sigmoid survival curves (an initial shoulder followed by an exponential phase and a tailing region) and survival curves with tail or shoulder (Alzamora et al., 2009).

The Geeraerd model, which is a 4-parameter model taking into account shoulder length and/or tailing, was also applied (Geeraerd et al., 2000):

$$\log(N) = log\left[\left(10^{log(N_0)} - 10^{\log(N_{res})}\right).e^{-k_{max}.t.}\left(\frac{e^{k_{max}.SL}}{1+\left(e^{k_{max}.SL}-1\right).e^{-k_{max}.t}}\right) + 10^{log(N_{res})}\right] \quad (8)$$

where, k_{max} is the death rate [min^{-1}]; SL, the shoulder length duration; and LR, the relation between the initial population ($LogN_0$) and survivor population ($LogN_{res}$) determined after treatment. The different versions of this model correspond to the cases of $N_{res} = 0$ (log-linear with shoulder), or the condition of $SL = 0$ (log-linear with tail). It is important to highlight, that Geeraerd model is an extension of the log-linear model to account for shoulder and/or tail presence. This model is capable of simulating independently a smooth initiation (shoulder phase) and/or saturation (tail phase) during exposure to a treatment, and offers a possible interpretation about microbial survival and tailing phenomenon (Gonzalez Barron, 2012).

2.7. Statistical analysis

Statistical analyses were carried out using InfoStat 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina). Significance level was set at $p < 0.01$. 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):

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

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

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

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 over-fitting. According to Akaike's and Bayesian's 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 a given parameterized model in terms of data prediction. However, the BIC criterion is a bit more conservative because the penalty term is more severe than in AIC.

Additionally, principal component analysis (PCA) was applied to illustrate the spatial relationship among tested strains in carrot orange juice blend and Weibull, Gompertz or Geeraerd 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 (Balzarini et al., 2008). A good PCA analysis corresponds to a CCC value close to 1.0.

3. Results and discussion

3.1. Physicochemical characterization of juices

Orange juice (pH: 3.5 ± 0.1, 11.10 ± 0.1 °Brix) was characterized by higher average diameter, $D_{3,2}$ (12.60 ± 0.40 μm), and presence of aggregates, $D_{4,3}$ (271.00 ± 2.00 μm), but fewer suspended particles (turbidity: 3723.00 ± 9.00 NTU); whilst carrot juice (pH: 6.5 ± 0.1; 7.10 ± 0.10°Brix) showed lower $D_{3,2}$ (4.60 ± 0.40 μm) and $D_{4,3}$ (29.30 ± 4.40 μm) with many suspended particles (turbidity: 7167.00 ± 5.00 NTU). Consequently, orange juice exhibited higher penetration of light (Absorption coefficient: 0.23 cm^{-1}) compared to carrot juice (Absorption coefficient: 0.26 cm^{-1}). Carrot-orange juice blend (pH: 3.8 ± 0.1, 10.60 ± 0.20° Brix) was characterized by intermediate $D_{3,2}$ (5.10 ± 0.10 μm) and $D_{4,3}$ (60.80 ± 8.70 μm) values and the highest turbidity observed (7667.00 ± 15.00 NTU); therefore, exhibiting the lowest penetration of light at the most germicidal UV fraction (absorption coefficient: 0.32 cm^{-1}).

3.2. Microbial inactivation

Inactivation curves corresponding to single mild heat treatment (H) at 40 and 45 °C did not exhibit changes along treatment time for all studied microorganisms. Whereas, H treatment performed at 50 °C (Fig. 2I) only reduced *E. coli* and *S. cerevisiae* by 0.6 log reductions. While *P. fluorescens* treated with H at 50 °C showed a decay of 1.6 log reductions. Fig. 2.II illustrates survival curves of *E. coli* ATCC 35218, *S. cerevisiae* KE 162 and *P. fluorescens* ATCC 49838 after exposure to UV-C and to UV-C/H treatments at 40, 45 and 50 °C in carrot-orange juice. Overall, inactivation curves exhibited a slight shoulder and absence of tail for single UV-C; whereas, for combined treatments, absence of shoulder and presence of tailing was observed (Fig. 2.II, i-iii). The occurrence of tailing could be attributed, among others, to the existence of more resistant members in the population, and/or high absorption of samples in the UV region stemmed from the presence of suspended solids. Three explanations are possible for the occurrence of a shoulder in an inactivation curve: the existence of clumps of microbial cells, a period during which cells are able to resynthesize a vital component faster than the rate of cellular destruction, and the presence of a large number of cell constituents that need to be inactivated (single-hit multiple-target phenomenon). In the last case, the type of damage is cumulative rather than instantly lethal (Geeraerd et al., 2000).

As observed in Fig. 2.II, when the maximum dose was applied (10.6 kJ/m^2), UV treatment provoked between 2.9 and 6.0 log reductions for *E. coli*; 2.5 and 4.2 log reductions for *S. cerevisiae* and 2.9 and more than 6.0 log reductions for *P. fluorescens*, depending on the temperature selected to assist UV-C processing. Our results are in agreement with those reported by Schenk et al. (2008), who reported inactivation curves without shoulder and with tailing for *Listeria innocua*, *Listeria monocytogenes*, *Escherichia coli*, and *Zygosaccharomyces bailli* inoculated in pear slices and processed by UV-C (0–87 kJ/m^2). Curves without shoulders but possessing heavy tails were also observed by Taze et al. (2015), for the inactivation of native microflora in UV-treated (0–1.0842 kJ/m^2) orange juice.

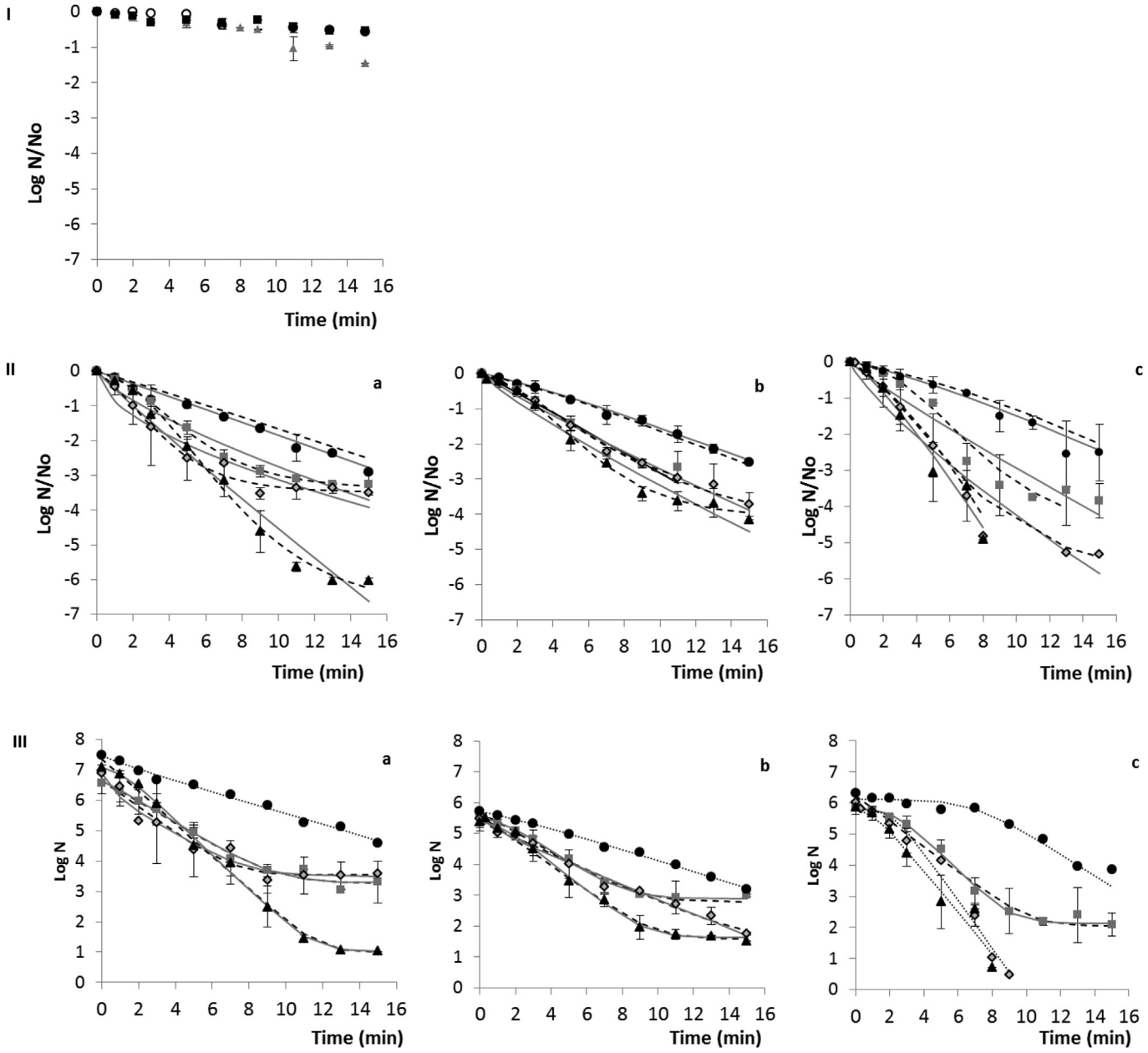

Fig. 2. Microbial inactivation curves in carrot- orange juice blend processed by single and combined UV-C/H treatments (maximum dose: 10.6 kJ/m^2). 2.I) Single H (50 °C): *E. coli* (▲), *S. cerevisiae* (■) and *P. fluorescens* (○); 2.II) Single UV-C (●) or combined UV-C/H at 40 °C (■), 45 °C (♦) or 50 °C(▲). Experimental inactivation data (symbol) and fitted values derived from Weibull (▬) and Gompertz (▬ ▬) models; III) Single UV-C (●) or combined UV-C/H at 40 °C (■), 45 °C (♦) or 50 °C (▲). Experimental inactivation data (symbol) and fitted values derived from Geeraerd model with shoulder and tailing (▬), with shoulder (…), and with tailing (▬ ▬). *E. coli* (a), *S. cerevisiae* (b) and *P. fluorescens* (c). Experimental microbial inactivation curves (symbols) corresponding to single H treatment (15 min) at 50 °C of *E. coli* (▲), *S. cerevisiae* (■) and *P. fluorescens* (○) in carrot- orange juice blend (I). Experimental microbial inactivation curves (symbols) fitted with the Weibull (▬) and Gompertz (▬ ▬) models (II); and experimental microbial inactivation curves (symbols) fitted with the Geeraerd model in its versions with shoulder and tailing (▬), with shoulder and no tailing (…), and with tailing without shoulder (▬ ▬) (III) of *E. coli* (a), *S. cerevisiae* (b) and *P. fluorescens* (c) in carrot- orange juice blend, treated with single UV-C light for 15 min (maximum dose: 10.6 kJ/m^2) (●), or combined UV-C/H at 40 °C (■), 45 °C (♦) or 50 °C(▲).

In our study, curves with shoulder and no tailing were only observed for *P. fluorescens*, when UV-C/H treatment was performed at 45 and 50 °C (Fig. 2.IIc and 2.IIIc). Similarly, Gayán et al. (2012b) observed inactivation curves with shoulders and no tailing effect for *E. coli* in orange juice (pH: 2.8, 10 °Brix, 4460 NTU, α: 81.10 cm^{-1}) treated with UV-C light (25 °C, 13.6 J/mL). Additionally, for the combined UV-C/H treatments at 40 °C and 50 °C, they obtained inactivation curves with shoulder and without tail (0.25–0.84 log reductions). Gouma et al. (2015) also detected inactivation curves with shoulders and no tails for apple juice (pH: 3.6, 2.4 NTU, α: 24.9 cm^{-1}) inoculated with *S. cerevisiae* and treated with UV-C at different temperatures (25, 45 and 50 °C). They observed less than one log reduction of *S. cerevisiae* using a UV dose of 3.7 J/mL, while in our study 2.5 to 4.2 log reductions for *S. cerevisiae* KE 162 were achieved. On the same fashion, Gayán et al. (2012a) also obtained survival curves with an initial lag phase followed by an exponential inactivation rate of *Salmonella* Typhimurium in Mc Ilvaine buffer added with tartrazine (pH 7.0 added; ε = 23.7 cm^{-1}) after UV-C exposure (25 J/mL) at 25 °C or combined with mild temperatures in the range of 50–55 °C. Similarly, Gayán et al. (2013), treated

E. coli with UV light at different temperatures (25–55 °C) in Mc Ilvaine buffer ($\varepsilon = 23.6\ cm^{-1}$) and also reported survival curves possessing an initial lag phase followed by an exponential inactivation rate.

Results obtained in the present study showed that *E. coli* and *P. fluorescens* were more sensitive to single and combined UV-C treatments than *S. cerevisiae* (Fig. 2.II and 2.III). These findings are in agreement with those reported by Guerrero-Beltrán and Barbosa-Cánovas (2005), who observed that *S. cerevisiae* was more resistant to UV-C treatment (75–450 kJ/m^2) than *L. innocua* and *E. coli* in apple juice (11.8° Brix, pH: 3.8). Similarly, Franz et al. (2009) reported that *E. coli* and *Lactobacillus brevis* were more sensitive to UV-C light (0.06 $kJ/s.m^2$) than *S. cerevisiae* in cloudy apple juice.

The inactivation degree obtained after UV-C or UV-C/H treatments in carrot- orange juice blend, and the corresponding significant differences among treatments obtained by the Tukey Test are shown in Fig. 3. The *Treatment*temperature* interaction parameter was statistically significant predicting log N/No with high F-value (p-value< 0.001). The adjusted R-squared statistic (determination coefficient) indicated that the model explained the observed variability in a range of 99.0% (data not shown). Exposure to single H treatment provoked up to 0.6–1.5 log reductions for all tested strains, and no significant differences were observed with temperature increase (Fig. 3). On the other hand, a significant higher inactivation compared to single H treatment was observed after exposure to UV-C, reaching up to 2.5–2.9 log reductions at 15 min, with no differences detected among strains (Fig. 3). Synergistic effects were observed for *P. fluorescens* inactivation, as 5.3 and more than 6.0 log reductions were obtained at 15 min of combined UV-C/H treatment when temperature was set at 45 and 50 °C, respectively (Fig. 3). Moreover, synergism was also detected for *E. coli* inactivation after UV-C/H at 50 °C, reaching up to 6.0 log reductions after 15 min of treatment (Fig. 3). Results shown in the present study are in agreement with Gayán et al. (2012b), who reported a synergistic effect on the inactivation of *E. coli* STCC 4201 when UV-C/H was performed at 50 °C.

Regarding *S. cerevisiae* inactivation, UV-C/H at 45 and 50 °C increased the inactivation observed compared to UV-C, as 3.4–3.5 log reductions were observed (Fig. 3). Only *P. fluorescens* exhibited additive effect for combined UV-C/H treatment at 40 °C condition; whereas, indifferent effects were observed for the remaining strains for these UV-C/H combination. In contrast, Gouma et al. (2015) observed that a temperature of 50 °C, hardly affected the UV-C inactivation *of S. cerevisiae* in apple juice. However, when the combined UV-C and mild heat treatment was performed at higher temperatures (55.0 and 57.5 °C), a synergistic inactivation effect was clearly observed.

The $\boldsymbol{E_{EO}}$ values calculated for *E. coli*, *S. cerevisiae* and *P. fluorescens* decontamination in carrot-orange juice blend by single UV-C and combined UV-C/H treatments were in the range from 0.35 to 0.83 kW^*h/m^3/order. Unfortunately, there is little information regarding $\boldsymbol{E}_{EO}$ estimation for reducing microbial load in juices or other food matrixes processed by UV-C light for comparison purposes. In previous studies, Ferrario and Guerrero (2016) evaluated pulsed light decontamination efficacy of *Escherichia coli*, *Salmonella* Enteritidis and *Saccharomyces cerevisiae* inoculated in commercial (pH: 3.5, 11.1 °Brix) and freshly squeezed (pH: 3.5, 12.6 °Brix) apple juices treated by single pulsed light (0.0175 J/mL, T < 25 °C, flow rate: 155 mL/min) in continuous flow mode operation. The ***E*EO** values obtained in that study were significantly higher, in the range of 1.8^*10^3- 4.1^*10^3 kW^*h/m^3/order,

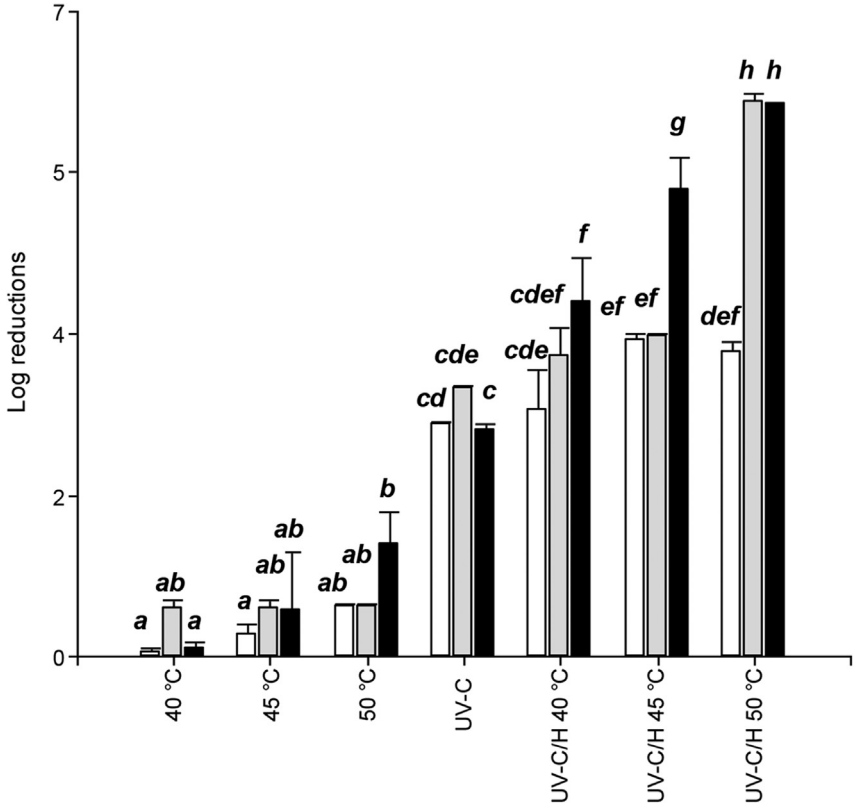

Fig. 3. Log-reductions and significant differences corresponding to (■) *E. coli* ATCC 35218, (▩) *P. fluorescens* ATCC 49838, (□) *S. cerevisiae* KE 162 in carrot-orange juice blend treated with single UV-C, or H and combined UV-C/H. Different letters above the bars represent significant differences ($p < 0.05$) among treatments according to the Tukey Test.

Table 1
Estimated parameters of Geeraerd, Weibull and Gompertz models corresponding to *E. coli*, *S. cerevisiae and P. fluorescens* survival in carrot-orange juice blend treated with single UV-C or combined UV-C/H at different temperatures for 15 min.

Treatment	Microorganism	Geeraerd										Weibull			Gompertz			
		Log-linear tail			Shoulder log-linear			Shoulder log-linear tail										
		LG	*k* (min^{-1})	R^2_{aj}	*SL* (min)	*k* (min^{-1})	R^2_{aj}	*LG*	*SL* (min)	*k* (min^{-1})	R^2_{aj}	*b* (min^{-n})	*n* (−)	R^2_{aj}	*A* (min)	*B* (min^{-1})	*C* (−)	R^2_{aj}
UV-C	*E. coli*	–	–	–	−0.75 (0.01)	0.41 (0.02)	98.7	–	–	–	–	0.18 (0.02)	1.01(0.03)	99.4	0.43 (0.38)	−0.04 (0.08)	−11.93 (20.00)	99.2
	S. cerevisiae	–	–	–	1.00 (0.01)	0.41 (0.01)	99.6	–	–	–	–	0.11 (0.01)	1.15 (0.05)	98.9	0.82 (0.14)	−0.07 (0.05)	−7.40 (5.07)	99.1
	P. fluorescens	–	–	–	6.92 (0.03)	0.79 (0.04)	98.1	–	–	–	–	0.09 (0.02)	1.22 (0.08)	97.5	1.10 (0.31)	−0.05 (0.08)	−11,78 (27.23)	97.5
UV-C/H 40 °C	*E. coli*	3.36	0.80	98.2	−2,88 (0.05)	0.50 (0.05)	93.2	3.35	0.23 (0.06)	0.83 (0.51)	97.9	0.41 (0.06)	0.83 (0.06)	97.2	0.96 (0.16)	−0.25 (0.03)	−4.07 (0,28)	99.2
	S. cerevisiae	0.89	0.75 (0.24)	99.0	−0.77 (0.03)	0.58 (0.04)	96.4	2.67	1.24 (0.02)	0.89 (0.12)	99.6	0.27 (0.03)	1.02 (0.05)	98.7	0.92 (0.19)	−0.21 (0.05)	−4.23 (0.72)	99.1
	P. fluorescens	4.20	0.94 (0.79)	97.5	–	–	–	3.70	2.35 (0.06)	1.26 (0.23)	99.5	0.25 (0.05)	1.09 (0.09)	97.7	1.48 (0.13)	−0.25 (0.03)	−4.88 (0.27)	99.4
UV-C/H 45 °C	*E. coli*	3.12	1.00 (0.61)	93.6	–	–	–	3.42	−1.99 (0.09)	0.78 (0.81)	94.3	1.30 (0.20)	0.40 (0.07)	92.6	0.66 (0.64)	−0.54 (0.16)	−3.97 (0.88)	97.1
	S. cerevisiae	3.82	0.59 (0.72)	98.3	–	–	–	4.29	−1.14 (0.04)	0.54 (0.79)	98.2	0.34 (0.04)	0.90 (0.05)	98.5	0.82 (0.17)	−0.19 (0.03)	−4.84 (0.51)	99.3
	P. fluorescens	5.41	1.32 (1.44)	96.0	−1.53 (0.21)	0.84 (0.11)	86.9	4.95	2.04 (0.29)	1.71 (0.86)	97.5	0.56 (0.13)	0.86 (0.10)	92.1	1.28 (0.33)	−0.30 (0.07)	−5.76 (0.59)	95.1
UV-C/H 50 °C	*E. coli*	6.39	1.23 (0.88)	99.2	−0.72 (0.17)	1.02 (0.07)	96.4	6.09	0.96 (0.10)	1.32 (0.66)	99.5	0.55 (0.11)	0.92 (0.08)	96.5	1.40 (0.15)	−0.27 (0.03)	−6.77 (0.29)	99.2
	S. cerevisiae	4.00	0.93 (1.57)	96.0	–	–	–	3.79	1.11 (0.22)	1.07 (1.28)	96.3	0.40 (0.05)	0.91 (0.05)	97.2	1.17 (0.10)	−0.26 (0.02)	−4.63 (0.20)	99.4
	P. fluorescens	–	–	–	0.94 (0.20)	1.52 (0.13)	94.9	–	–	–	–	0.30 (0.05)	1.30 (0.09)	96.8	1.21 (0.77)	−0.08 (0.21)	−31.89 (140.75)	96.5

(value), standard error; $\mathbf{R^2_{aj}}$, determination coefficient.

demonstrating that UV-C was more energy efficient than pulsed light under the tested conditions for reducing microbial load.

Table 1 exhibits the averages and the standard deviations corresponding to the parameters of Weibull, Gompertz and Geeraerd models when single UV-C and combined UV-C/H treatments were applied to the juice blend. Data corresponding to inactivation of all microorganisms subjected to single mild heat treatment were not modeled since the inactivation was scarce. Additionally, Table 2 enumerates the statistics related to the weibullian distribution calculated according Eqs (2)–(6).

For comparison purposes, and in order to analyze the goodness of fit of the three models, Table 3 displays RMSE, AIC and BIC statistics associated to the predicted single UV-C and combined UV-C/H survival responses.

Weibull model was appropriate for representing survival data, exhibiting high R^2_{adj}, which indicates that between 96.5% and 99.5% of variation in the inactivation response could be explained by the model. In general, survival curves derived from applying the combined UV-C/H treatment exhibited n values < 1 (Table 1), as expected according to the notorious upward concavity observed, which indicates that the process became less effective at higher doses. Exceptionally, *P. fluorescens* treated by combined UV-C/H at 50 °C showed downward concavity (n > 1, Table 1); conversely, indicating that the more sensitive members of the population were weakened at lower doses, leaving a large fraction of more resistant members which were affected in much lesser extent. Several studies reported that the Weibull model could quantitatively describe microbial inactivation by UV-C in both liquid (Baysal et al., 2013; Unluturk et al., 2010), and solid matrices (Cheigh et al., 2013). Our findings are in agreement with previous studies that have also reported n values < 1. For instance, Taze et al. (2015) characterized by the Weibull model the survival curve of natural microflora in orange juice exposed to UV-irradiation (1.10 kJ/m^2), reporting n values of less than one for UV-C treated population. In addition, Flores-Cervantes et al. (2013) characterized survival curves of *Aspergillus flavus* and *Aspergillus niger* spores in peach nectar processed by UV-C light alone (203 kJ/m^2) or combined with antimicrobials by Weibull model. They also reported n values < 1 (0.19–0.72) for the inactivation of both microorganisms. Moreover, Schenk et al. (2008) fitted experimental inactivation curves corresponding to *L. innocua*, *L. monocytogenes*, *E. coli* and *Z. bailli* inoculated in pear slices exposed to UV-C light (0 and 87 kJ/m^2), by using the Weibull model. In accordance, they obtained n values lower than 1 for all the inactivation curves, due to the notorious upward concavity observed.

Table 2
Weibull model related statistics [a] corresponding to corresponding to *E. coli*, *S. cerevisiae* and *P. fluorescens* inactivation in carrot-orange juice blend treated with single UV-C or combined UV-C/H at different temperatures.

Microorganism	Treatment		tcm (min)	$\overline{t_c}$ (min)	σ^2_{tc} (min^2)	ν_1 (–)
E. coli	UV-C	20 °C	0.06	5.44	29.00	2.10
	UV-C/-H	40 °C	–	3.23	15.36	2.61
		45 °C	–	1.72	29.34	10.68
		50 °C	–	2.14	4.98	2.21
S. cerevisiae	UV-C	20 °C	1.16	6.49	31.99	1.86
	UV-C/H	40 °C	0.08	3.58	12.32	2.08
		45 °C	–	3.49	15.08	2.37
		50 °C	–	2.86	9.92	2.34
P. fluorescens	UV-C	20 °C	1.89	6.39	26.49	1.74
	UV-C/H	40 °C	0.36	3.45	10.05	1.95
		45 °C	–	1.94	5.37	2.57
		50 °C	0.83	2.31	3.17	1.68

[a] Statistics of Weibullian model, $\overline{t_c}$ distribution's mean, σ^2_{tc} variance, ν_1 coefficient of skewness.

The b and n parameters were used to generate frequency distributions of resistances (Fig. 4) and to calculate the associated statistics: mode, mean, variance and coefficient of skewness for obtaining a better explanation on the effect of single UV-C and combined UV-C/H treatments on the inactivation of the investigated microorganisms (Table 2). Frequency distributions corresponding to all microorganisms assayed and subjected to single UV-C and UV-C/H treatments at 40 °C were flat with considerable spread of data, heavy tail, mode, high mean and variance values, indicating that an important fraction of the microbial population survived after treatment (Fig. 4). Exceptionally, frequency distributions of *E. coli* exposed to UV-C/H at 40 °C lacked a mode (Fig. 4). Whereas, frequency distributions corresponding to UV-C/H treatment at 45 or 50 °C were more skewed to the right, and lacked a mode. In addition, a decrease in mean and variance values was observed with temperature, suggesting that the majority of the population was destroyed in a shorter time compared to lower temperatures (Fig. 4). These results are in agreement with those obtained by Schenk et al. (2008), who observed frequency distributions of resistances with similar right-skewed shapes and considerable spread of death data (large variance values), with tails, without mode. They attributed these frequency shapes to the fact that the majority of the population was destroyed in a short time during UV-C exposure while a fraction survived after treatment.

E. coli was the most sensitive strain to all treatments assayed, exhibiting mean values in a range from 5.5 to 2.1 min, followed by *P. fluorescens* (2.3–6.6 min) and *S. cerevisiae* (2.8–6.6 min) (Table 2). In agreement, Gayán et al. (2016) also reported that Gram-negative bacteria were more sensitive than yeasts to UV-C light.

The Gompertz model was also appropriate for representing inactivation data as shown by the high R^2_{adj} values obtained, ranging between 97.8 and 99.7% (data not shown) and low RMSE values (Table 3). The A parameter, which represents the initial shoulder (min), was low, varying between 0.43 and 1.40 min, 0.78–1.17 min and 1.10–1.48 min for *E. coli*, *S. cerevisiae* and *P. fluorescens*, respectively. Consequently, *P. fluorescens* exhibited the highest A value, indicating its requirement for more than one UV hit to inactivate these strain. No change pattern of A value was observed by increasing temperature, probably due to the absence of a marked shoulder at higher temperatures. Interestingly, the combination of UV-C and H treatments provoked an increase in the global change of the number of survivors (C value), thus indicating better efficacy of the decontamination process (Table 1). Regarding to the maximum death rate, represented by B value, was more affected by the considered strain than on temperature (Table 1). In particular, *E. coli* was the most sensitive strain exhibiting higher B values (−0.25 min^{-1} to −0.54 min^{-1}) for UV-C assisted by mild heat treatment (45 and 50 °C). Inactivation curves of *E. coli* and *P. fluorescens* treated by single UV-C and *P. fluorescens* subjected to UV-C/H at 55 °C lacked of a tail. Although Gompertz model adequately characterized these survival curves, an overestimation of the global change in population (C value) compared to experimental data was observed (Fig. 2, Table 1). An overestimation of C parameter was also reported by Ferrante et al. (2007) who applied the modified Gompertz equation to model *L. monocytogenes* response in sonicated (600 W, 20 kHz, 95.2 μm wave amplitude; 45 °C) fresh squeezed orange juice (pH 3.5) containing vanillin (0; 1000 and 1500 ppm). They described an overestimation of C parameter for the most severe conditions (1000 and 1500 ppm of vanillin addition plus ultrasonic treatment), since the microbial population died fast giving a survival curve without a tailing region and following an almost first order kinetics (Ferrante et al., 2007).

The Geeraerd model, in its three versions (log linear plus tail and/or shoulder plus tail), accurately represented all survival curves or with high R^2_{adj} values ranging from 96.0 to 99.6% (data

Table 3
Minimum RSME, AIC and BIC[a] values for the inactivation curves corresponding to *E. coli*, *S. cerevisiae* and *P. fluorescens* inactivation in carrot-orange juice blend treated with single UV-C or combined UV-C/H.

	Microorganism	Log-linear tail			Shoulder log-linear			Shoulder log-linear tail			Weibull			Gompertz		
		RMSE	AIC	BIC	RMSE	AIC	BIC	RMSE	AIC	BIC	RMSE	AIC	BIC	RMSE	AIC	BIC
UV-C	*E . coli*	–	–	–	0.10	−11.34	−10.13	–	–	–	**0.10**	**−12.82**	**−11.91**	0.10	−9.50	−8.29
	S. cerevisiae	–	–	–	0.06	−23.73	−22.52	–	–	–	**0.05**	**−26.39**	**−25.48**	0.05	−25.15	−23.94
	P. fluorescens	–	–	–	**0.17**	**−2.72**	**−1.51**	0.17	−2.23	−0.72	0.26	5.18	6.08	0.26	5.54	6.75
UV-C/H 40 °C	*E . coli*	0.17	−1.80	−0.59	0.35	11.60	12.82	0.20	−0.23	1.28	0.28	7.38	8.29	**0.05**	**−25.20**	**−23.98**
	S. cerevisiae	0.10	−8.68	−8.37	0.20	1.47	1.79	**0.07**	**−15.30**	**−14.91**	0.20	0.61	0.85	0.10	−8.86	−8.54
	P. fluorescens	0.24	5.48	6.69	0.50	19.05	20.26	**0.14**	**−7.11**	**−5.59**	0.49	17.90	18.80	0.22	2.52	3.73
UV-C/H 45 °C	*E. coli*	0.32	10.11	11.32	0.45	16.51	17.72	0.30	9.30	10.81	0.35	11.19	12.10	**0.17**	**−3.42**	**−2.21**
	S. cerevisiae	0.17	−2.81	−1.60	0.17	−3.91	−2.70	0.17	−2.10	−0.59	0.14	−4.92	−4.01	**0.10**	**−11.43**	**−10.22**
	P. fluorescens	0.44	16.20	17.41	0.32	9.53	10.32	0.35	11.79	13.30	0.24	3.75	4.34	**0.22**	**3.00**	**3.78**
UV-C/H 50 °C	*E .coli*	0.22	2.24	3.45	0.48	18.02	19.23	0.17	−1.35	0.16	0.42	14.98	15.88	**0.14**	**−8.99**	**−7.78**
	S. cerevisiae	0.14	−5.94	−4.35	0.41	16.10	17.69	**0.10**	**−11.05**	**−9.06**	0.30	7.92	9.12	0.10	−10.63	−9.04
	P. fluorescens	–	–	–	0.47	13.47	13.26	–	–	–	**0.33**	**8.25**	**8.09**	0.39	10.44	10.22

[a] Boldface RMSE, AIC or BIC value correspond to the best one in the row for model comparison.

not shown). An important increment of k_{max} value was observed as temperature increased (Table 1) for all strains assayed. In particular, *E. coli* exhibited k_{max} values of 0.41 min^{-1} and 1.02 to 1.32 min^{-1} when the temperature was set at 20 and 50 °C, respectively, and according to the model version (Table 1). On the same fashion, an increment in k_{max} values with temperature from 0.41 min^{-1} to 0.93–1.07 min^{-1} and 0.79 min^{-1} to 1.52 min^{-1} was observed for *S. cerevisiae* and *P. fluorescens* at 20 and 50 °C, respectively (Table 1).

Regarding the *SL* parameter, and according to what was observed for *A* values of Gompertz model, no changes in *SL* values were recorded by increasing temperature, suggesting that survival curves did not exhibit a marked shoulder. In fact, the *SL* parameter resulted negative in many cases, indicating absence of shoulder. In addition, a decrease from 1.4–1.9 × 10^3 to 0–10 CFU/mL in N_{res} parameter was observed with temperature increase for *E. coli* and *P. fluorescens* (data not shown).

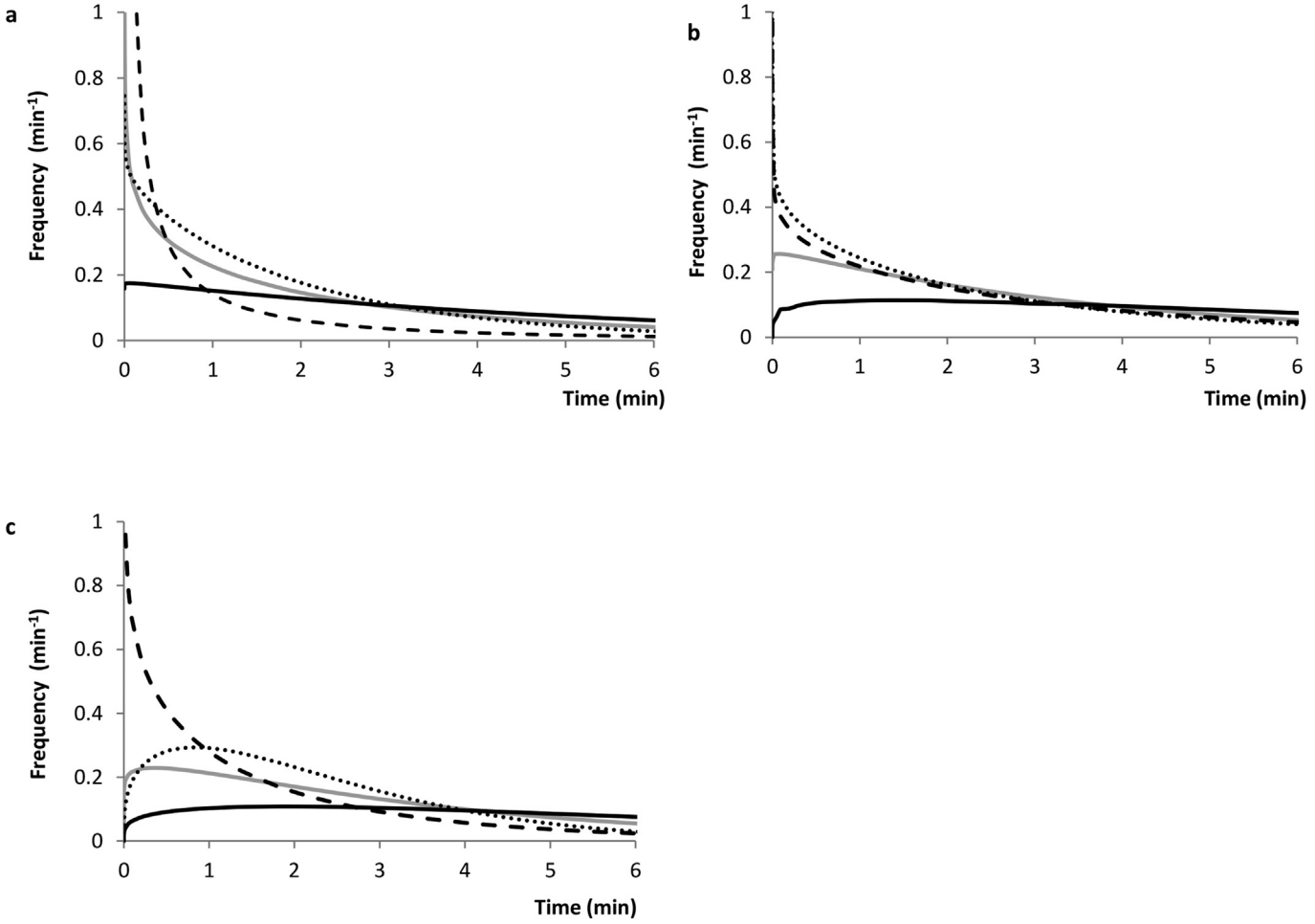

Fig. 4. Weibull frequency distributions of resistances corresponding to microbial inactivation curves in carrot-orange blend juice treated with single UV-C (▬), or combined UV-C/H at 40 (▬), 45 (▬▬) or 50 °C (…) of *E. coli* (a), *S. cerevisiae* (b) and *P. fluorescens* (c).

Gayán et al. (2011) studied the inactivation of *E. coli*, at different growth phase in McIlvaine buffer (pH 7.0) treated with UV-C (0–25 J/mL, 8.5 L/h). These authors characterized survival curves by using the Geeraerd model with shoulder, since they obtained survival curves which lacked of tail but exhibited shoulder. The *SL* parameter of Geeraerd model of logarithmic phase cells was shorter than those of the stationary ones. On the contrary, K_{max} values hardly changed for both cell populations. They related the *SL* to damage and repair mechanisms that are activated during treatment. In contrast, Baysal et al. (2013) investigated the inactivation of *A. acidoterrestris* spores inoculated in apple (pH: 3.8, 11 °Brix and 10 NTU) and white grape (pH 3.2, 16.6 °Brix and 5.5 NTU) juices and treated with by UV-C (0–5 J/cm^2). As survival curves exhibited tail but lacked of shoulder, the Geeraerd model with tail was applied. They observed that k_{max} values in grape juice decreased as the UV intensities increased from 0.0014 to 0.013 kJ/s.m^2. Whereas, no change in k_{max} was detected in apple juice while varying UV intensity. Regarding to N_{res}, a decrease in the parameter was observed as UV dose increased, which was in agreement with the results shown in the present work.

3.3. Model performance comparison

The Gompetz model allowed a better fit, exhibiting lower RMSE, AIC and BIC values in 7 from 15 evaluated cases, followed by Geeraerd (5 favourable cases) and Weibull (3 favourable cases) models (Table 3). Geeraerd model had lower predictive performance compared to Gompertz model according to the AIC and the BIC parameters, which consider simultaneously 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. Nevertheless, in 5 exceptional cases, there were great differences among the three AIC or BIC in favour to Geeraerd model (Table 3). In these cases corresponding to *S. cerevisiae* inactivation kinetics at 40 and 50 °C and *P. fluorescens* at 20 and 40 °C, this model provided very low small AIC and BIC values compared to Weibull and Gompertz model values. Interestingly, Weibull model displayed lower BIC and AIC values in the case of single UV-C survival curves, which corresponded to an overestimation of the C parameter by the Gompertz model. This indicates a penalization of the Geeraerd model with shoulder and no tailing effect over the Weibull model, due to its higher number of parameters taken into account. Therefore, the analysis of RMSE, AIC and BIC values determined that, in general, the training of Gompertz models implied better fit, fewer explanatory parameters or both.

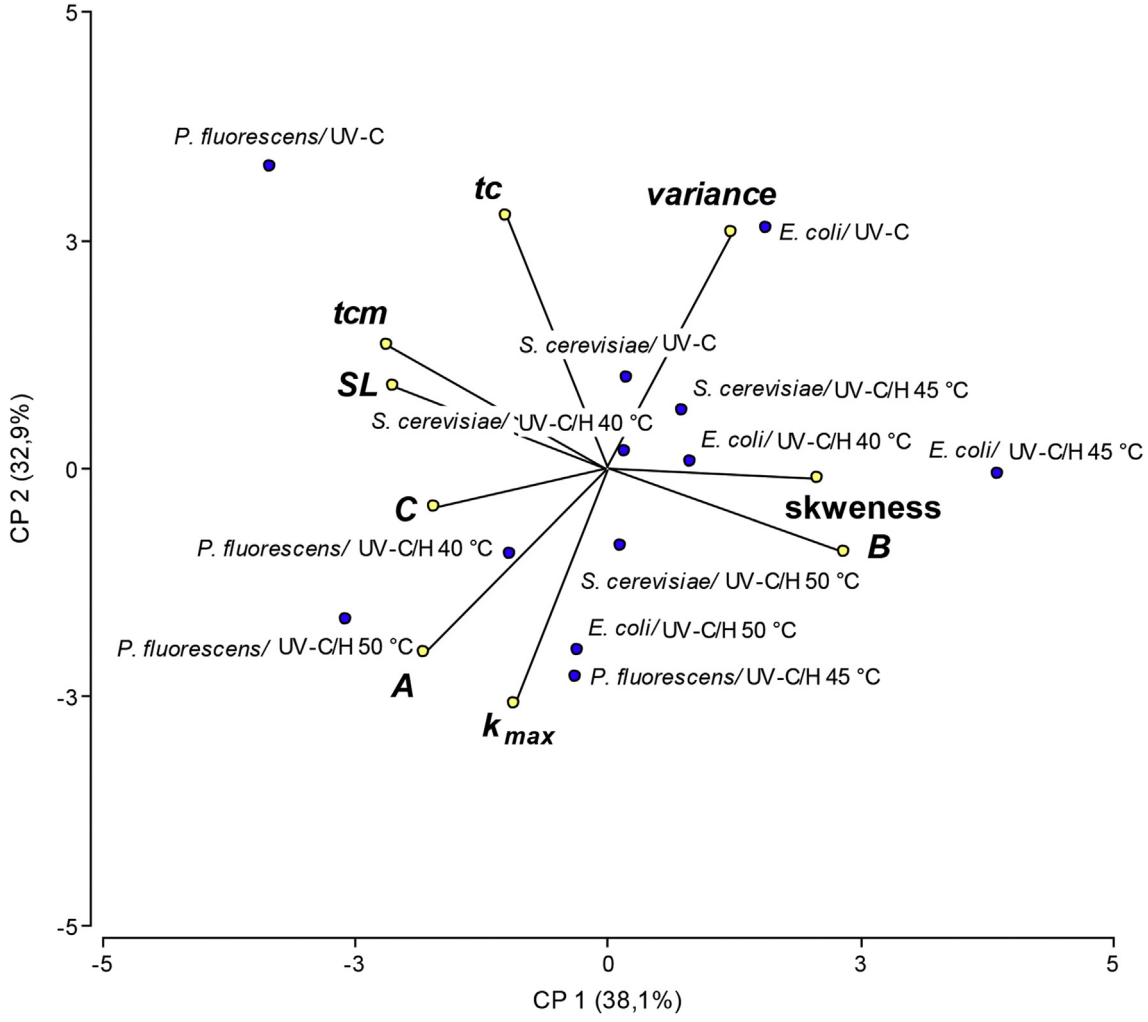

Fig. 5. Principal component analysis (PCA) bi-plots of Weibull, Gompertz and Geeraerd parameters surged from fitting inactivation of *E. coli*, *S. cerevisiae* or *P. fluorescens* in carrot-orange juice blend processed by single UV-C or combined UV-C/H at 40, 45 or 50 °C.

3.4. 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 corresponding to the three evaluated predictive models used to describe microbial inactivation curves corresponding to single UV-C and combined UV-C/H treated carrot juice blend. Two-dimensional representations (PCA bi-plot) of these are presented in Fig. 5 for parameters of the three 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 71% of the total variance. The first two PC (Fig. 5) explained 38.1% and 32.9% of the variance, respectively. The PC_1 separated the mode, shoulder length (*SL*), initial shoulder (A) and the overall change in number of survivors (*C*), which were associated negatively, from *B* and skewness (positively associated). On the other hand, PC_2 was associated positively with mean and variance values, and negatively with initial shoulder (*A*) and the inactivation rate (k_{max}). UV-C in combination with 50 °C resulted the most effective treatment for all strains as these systems were associated with high inactivation rate (k_{max}) and low mean and variance; whereas, single UV-C exhibited the lowest inactivation for all microorganisms assayed as it was highly associated with the highest mean and variance values and the lowest k_{max}. Both bacteria resulted more sensitive to UV-C/H at 50 °C treatment than the yeast, as they were associated to higher k_{max} values. It is important to highlight that *P. fluorescens* showed high and similar k_{max} values for all combined treatments, evidencing its high sensitivity to the combined treatment. Whilst for *E. coli* and *S. cerevisiae*, a gradual increase in k_{max} value was observed with temperature increase.

4. Conclusions

Even though it is a low cost non-thermal preservation method, the application of UV-C for processing liquids is still very much limited due to their low UV transmittance. In this context, the present work demonstrates the potential of UV-C assisted by mild heat treatment (40–50 °C) for enhancement of the inactivation effectiveness in a turbid fresh squeezed juice. Certain combinations of UV-C and mild heat (45 and 50 °C) for carrot-orange juice blend processing provoked synergistic effects on *E. coli* ATCC 35218 and *P. fluorescens* ATCC 49838 inactivation, reaching up to 6 log reductions in population.

Three different mathematical models were used for modeling non-linear survival curves corresponding to different microorganisms in carrot-orange juice blend. Estimated parameters explained, from a different point of view, the influence of single UV-C or combined UV-C/H treatments on microbial inactivation in turbid juice. Weibull model gave additional information regarding the spectrum of resistances within the microbial population. In addition, Gompertz and Geeraerd models gave useful information related to the characterization of the survival curves regarding to the inactivation rate and shoulder length. The Gompertz model exhibited better performance compared to the Geeraerd model, taking into account fit and parsimony. Since our results were rather heterogeneous in terms of the shape of the inactivation curve depending on the temperature applied, the Gompertz model resulted more versatile in applicability since it has only one equation instead of three.

Further studies with other microorganisms of concern should be conducted in the near future. Additionally, the eventual existence of sub-lethal cell damages induced by the combination of the imposed stresses should be evaluated. This would contribute to a better understanding of the microbial response to the proposed combined treatments. Besides, studies will be conducted to assess the effects of UV-C/H treatments on the organoleptic and sensorial properties of carrot-orange juice.

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