Innovative Food Science and Emerging Technologies 29 (2015) 271–279

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Inactivation kinetics and growth dynamics during cold storage of *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090 and *Saccharomyces cerevisiae* KE162 in peach juice using aqueous ozone

Analia B. Garcia Loredo, Sandra N. Guerrero, Stella M. Alzamora *

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160 (C1428EGA), C.A.B.A., Argentina
Member of Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, Argentina

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 28 January 2015
Accepted 19 February 2015
Available online 8 March 2015

Keywords:
Ozone
Peach juice
Inactivation kinetics
Weibull and Coroller models
Microorganism's recovery

ABSTRACT

The effect of ozone (10 and 18 ppm in the gas supply) on the inactivation of *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090 and *Saccharomyces cerevisiae* KE 162 inoculated in peach juice using a bubble column was investigated. Microorganism growth dynamics in decontaminated juice during 14 days storage (5 ± 1 °C) were also assessed. The highest ozone concentration resulted in lower counts of *E. coli* ATCC 11229 during most part of the treatment; however, after 12 min exposure, coliform counts were reduced by approximately 4.3 log-cycles in peach juice exposed to both ozone levels. *L. innocua* ATCC 33090 counts decreased 3.9 and 4.9 log-cycles after a 12-min exposure using 10 or 18 ppm ozone, respectively. For *S. cerevisiae* KE162, the treatment was less effective and only 1 log-cycle of reduction was achieved regardless of ozone concentration. Nonlinear inactivation curves were successfully fitted with Weibull type and modified Coroller models. Growth dynamics in ozone treated juice during cold storage depended on inoculated microorganism and ozone level applied, but surviving microorganisms faced more difficulties to grow than in unprocessed juice, especially at the highest ozone concentration. Ozone exposure (18 ppm) coupled with low temperature storage conditions seemed to be a good option for preserving peach juice.

Industrial relevance: Ozone processing which can be an alternative pasteurization technology has been studied for obtaining ready-to-drink "fresh-like" juices with a minimum of nutritional, physicochemical, functional or organoleptic changes. Present results indicated that ozone exposure in a bubble column could reduce pathogenic microorganisms' populations and inhibited yeast growth in decontaminated peach juice.

1. Introduction

Fruit juices are an important source of bioactive compounds (such as vitamins, phenolic compounds, anthocyanins and carotenoids) which play an important role in the prevention of heart diseases, cancer and diabetes (Abeysinghe et al., 2007). Thermal pasteurization is the traditional method for preservation of fruit juices. However, thermal processing may cause changes in bioactive compounds present as well as organoleptic changes. Alternative pasteurization technologies have been studied to obtain ready-to-drink "fresh-like" juices with a minimum of nutritional, physicochemical, functional or organoleptic changes (Esteve & Frigola, 2007).

Ozone, a triatomic allotrope of oxygen, is characterized by a high oxidation potential (reacting up 3000 times faster than chlorine with organic material) that conveys bactericidal and viricidal properties (Patil, Valdramidis, Cullen, Frias & Bourke, 2010a). Ozone is extremely reactive with a half-life in the gaseous phase of about 12 h, but in distilled water half-life is reduced to only 20–30 min at 20 °C (Khadre, Yousef & Kim, 2001). The stability of ozone is greatly dependant on the amount of ozone-demanding material present, and on other factors, including temperature and pH (with decreasing stability at increased temperatures and pH) (Kim, Yousef & Khadre, 2003; Perry & Yousef, 2011). Excess ozone auto-decomposes rapidly to produce oxygen, leaving no residues in food (Khadre, Yousef and Kim, 2001). Activated oxygen species resulting from ozone decomposition encompass a number of oxidative radicals, including the superoxide anion radical, the hydroperoxide radical, singlet oxygen, and finally the hydroxyl radical. The last is incredibly reactive and much of the antimicrobial activity of ozone against a broad spectrum of microorganisms has been attributed to the subsequent reaction of its decomposition products (Korycka-Dahl & Richardson, 1978; Perry & Yousef, 2011).

The FDA's approval of ozone as a direct additive to food in 2001 led to the application of ozone for processing of various fruit juices, including apple cider (Steenstrup & Floros, 2004), apple juice (Choi et al., 2012; Patil, Valdramidis, Tiwari, Cullen & Bourke, 2011; Patil et al., 2010a), orange juice (Patil, Bourke, Frías, Tiwari & Cullen, 2009; Patil, Valdramidis, Cullen, Frias & Bourke, 2010b; Tiwari, Muthukumarappan,

* Corresponding author. Tel.: +54 11 45763366.
E-mail address: alzamora@di.fcen.uba.ar (S.M. Alzamora).

http://dx.doi.org/10.1016/j.ifset.2015.02.007
1466-8564/

O'Donnell & Cullen, 2008a,b), blackberry juice (Tiwari, O'Donnell, Brunton & Cullen, 2009a) and tomato juice (Tiwari, O'Donnell, Brunton and Cullen, 2009a). Regarding apple juice and cider, guidance for industry was issued by the FDA (United States Food, Administration Drug & USFDA., 2004) establishing processing and compositional factors needed to be controlled to ensure the efficacy of the ozonation process. In gas or in aqueous phases, ozone has been also applied in water and wastewater treatment, washing and disinfection of fruits and vegetables, and decontamination of meat, poultry, eggs, fish, grains and dry foods (Dubois et al., 2006; Kim et al., 2003; Muthukumarappan, Halaweish & Naidu, 2000; Tiwari et al., 2010). Ozone has also been proved effective to remove mycotoxins and pesticide residues, a positive outcome due to the health risks associated with these compounds (Cataldo, 2008; Perry & Yousef, 2011; Tiwari et al., 2010).

Early and more recent literature studies have suggested that microorganisms possess various sites or targets for ozone action that lead to inactivation (Perry & Yousef, 2011). Ozone destroys microorganisms by reactions with the enzymatic systems and the unsaturated lipids of the cell membranes as well as with bacterial spore coats (Khadre & Kim, 2001). Damage to membranes not only results in leakage of cytoplasm components but also allows ozone to penetrate in the cell, causing DNA breaks (Ishizaki, Sawadarski, Miura & Shinriqui, 1987; Komanapali & Lau, 1998). Both, molecular ozone and its decomposition products (mainly hydroxyl radical) would be responsible for the antimicrobial effects.

Effectiveness of ozone against microorganisms depends not only on the amount used, but also on the residual ozone in the medium. Several environmental factors, such as medium pH, temperature, humidity, additives (surfactants, sugars, etc.), amount of organic matter surrounding the cells (Restaino, Frampton, Hemphill & Palnikar, 1995) and solid content (Choi et al., 2012), also influence its effectiveness. Ozone is readily soluble in water, and its solubility increases with decreasing temperature. Ozone is a very reactive molecule and reacts with nearly all organic and inorganic compounds; if the ozone demand of the media increases, the amount of required ozone (for microbial inactivation or residue degradation) will increase (Kim, Yousef & Dave, 1999).

In general, the decrease of microorganisms' population during ozone exposure does not follow first-order kinetics. Cullen, Tiwari, O'Donnell and Muthukumarappan (2009) reviewed modeling approaches employed for describing the kinetics of microbial inactivation taking into account the observed non-linearity. The researchers indicated the difficulty in predicting the effect of ozone treatment on microorganisms in complex food materials. However they remarked the usefulness of kinetic models to assess the influence of ozone treatment parameters, such as ozone concentration, treatment time and gas flow rate. Recent studies by Patil et al. (2010a,b) have shown that the Weibull model and the shoulder-log linear model accurately described ozone inactivation kinetics of *E. coli* ATCC 25922 and NCTC 12900 in apple juice. Weibull model also accurately fitted *Listeria innocua* NTTC 11288 and *Listeria monocytogenes* ATCC 7644 inactivation curves in orange juice. Other researchers have reported biphasic survival curves for various microorganisms in ozonized water with or without added organic material (Restaino et al., 1995).

Fruit juices, acidic products with substantial amounts of fermentative sugars, constitute an ideal environment for spoilage by yeasts, molds and for those few acid-tolerant bacteria (Stratford, Hofman & Cole, 2000). Due to acidity, pathogenic bacteria do not grow in fruit juices but maintain viable for various days (Miller & Kaspar, 1994). A number of foodborne outbreaks that occurred in the US during the 1990s were associated with the consumption of unpasteurized fruit juices (Stratford et al., 2000). One pathogen acid tolerant of concern is *Escherichia coli* O157:H7 and several incidents had been reported from pressed, unpasteurized apple juices. The source of contamination seemed to be related to fecal material in contact with apples (McLellan & Splittstoesser, 1996). Although, to date, *L. monocytogenes* has not been implicated in any case of juice-borne illness, it has been isolated in unpasteurized apple juice (pH 3.78) and apple/raspberry juice blend (pH 3.75) (Sado, Jinneman, Busby, Sorg, & Omiecinski, 1998). Because of its high-phenotypic similarity to *L. monocytogenes*, *L. innocua* is often used as a nonpathogenic indicator for *L. monocytogenes* microorganisms (Fairchild & Foegeding, 1993).

The objectives of this study were to investigate: 1) the efficacy of ozone at two concentrations (10 and 18 ppm) at 20 °C for killing *E. coli* ATCC 11229, *L. innocua* ATCC 33090 and *Saccharomyces cerevisiae* KE 162 inoculated in peach juice; 2) the suitability of modified Weibull and Coroller models to characterize the inactivation kinetics; and 3) the growth dynamics of inoculated microorganisms in ozone decontaminated peach juice throughout 14 days in cold storage.

E. coli ATCC 11229 was selected as a representative microorganism for the enterohemorrhagic foodborne pathogen *E. coli* O157:H7 and *L. innocua* ATCC 33090 as an indicator for the common foodborne pathogen *Listeria monocytogenes*, respectively. *S. cerevisiae* KE162 was selected as a typical spoiler of fruit juices.

2. Materials and methods

2.1. Strains and preparation of inocula

Experiments were performed using *E. coli* ATCC 11229, *L. innocua* ATCC 33090 and *S. cerevisiae* KE162. Bacterial inocula were 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 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 centrifuge, New Jersey, USA), washed twice with saline and re-suspended in peptone water to give a cell density of about 10^7 or 10^9 CFU/mL for yeast and bacteria suspensions respectively. For inoculation, 4.5 mL of the microbial suspension was added to 450 mL peach juice prior to ozone treatment.

2.2. Preparation of peach juice samples

Ripe peaches (*Prunus persica*, Pavía cv, 13 ± 1 °Brix, pH = 4.1 ± 0.2) were purchased in a local market and maintained at 4–5 °C until use. Before being processed, whole fruits were rinsed with 0.03% sodium hypochlorite and sterile water to eliminate surface microbial load and gently dried with sterile cloth. Peaches were hand peeled and cut into slices. Juice was obtained under aseptic conditions in a 90 % ethanol sanitized and 10 min UV-C exposed householder juicer (Black and Decker, JE 1500, China), and centrifuged to reduce pulp amounts (2400 rpm, 10 min, 5 °C, Eppendorf, model 5804 R, Hamburg, Germany). The juice obtained was immediately frozen at −80 °C (Panasonic ultrafreezer, model MDF-U55V, Japón). Frozen juice samples were ozone processed within two months of juice preparation.

2.3. Ozone equipment and treatment

Ozone was generated with a corona discharge equipment model UTK-O-4 (UNITEK, Mar del Plata, Argentina). Pure oxygen was supplied via an oxygen cylinder (Oxigena Central, Argentina) at 0.62 bar regulated by a control valve (model LRP-1/4-2.5, Festo, Argentina) and the flow rate was controlled at 5 L/min using a rotameter (UNITEK, Mar del Plata, Argentina). The setup of the ozone processing equipment was similar to that reported by Tiwari, O'Donnell, Patras, Brunton and Cullen (2009b) and Patil et al. (2010b). The ozone generator was connected to a bubble

column (0.1 m in diameter and 0.24 m in height) with an inbuilt diffuser to sparge gaseous ozone into the liquid phase. To investigate the influence of ozone concentration, the potentiometer of the generator was adjusted to obtain two levels of ozone in the gas supply, 10 ppm and 18 ppm. Ozone concentration in the gas supply was recorded using an ozone gas analyzer model UV-100 (Eco Sensors, USA). Excess ozone was neutralized by diverting the gas stream into a glass Erlenmeyer containing 2 % w/v potassium iodide solution. Dissolved ozone in the liquid phase could not be determined through the indigo colorimetric method or the iodometric method because of the large amount of organic matter in the peach juice. Ozone processing was made at ambient temperature (20 ± 1 °C). Protective cloth, gloves and masks were worn while running the experiments.

For treatments, 450 mL inoculated peach juice (20 ± 1 °C) was put inside the bubble column and exposed to both gaseous ozone concentrations (10 and 18 ppm). Microbial counts were assessed during ozone processing. For microbial analysis, treated juice samples (1 mL) were taken at different times and each sample was mixed with 0.1 mL neutralizing solution (0.005 M sodium thiosulfate, Anedra, Argentina) to stop ozone reactions (Kim & Yousef, 2000). All experiments were performed in triplicate. Juice samples not subjected to ozone treatment were designated as the control group.

2.4. Microbial analysis

To obtain inactivation curves, triplicates corresponding to a given treatment time were collected. Peptone water (0.1% w/v) tenfold dilution aliquots were surface plated by duplicate onto TSAYE for *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 or PDA for *S. cerevisiae* KE162 using a spiral plater (Autoplate 4000, Spiral Biotech, USA). When ozone treatment resulted in low counts (longer treatment times), up to 1 mL of fruit juice was directly pour plated into each Petri dish. Plates were incubated for 72 h at 37 ± 1 °C (for bacteria) or 27 ± 1 °C (for yeast). 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.

For storage studies, control (unprocessed juice) and 12 min-ozone exposed samples were put on glass jars and stored at 4 °C (±1 °C) in the darkness during two weeks. Survival of treated inoculated microorganisms along storage was analyzed at 3, 5, 7, 9, 12 and 14 days in triplicate.

2.5. Mathematical modeling

Survival curves were fitted with the cumulative form of a Weibull type distribution of resistances (Peleg & 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 is related to the rate of inactivation of microbial 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:

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

where t_c is the inactivation time (a measure of the organism's resistance or sensitivity) and $\frac{d\varnothing}{dt_c}$ is the Weibull distribution corresponding to t_c. Other statistical parameters which better explain the observed frequencies (distribution mode, t_{cm}; mean, $\overline{t_c}$; variance, ${\sigma_{tc}}^2$; and coefficient of "skewness", υ_1) were calculated from the following equations (Peleg & Cole, 1998):

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

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

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

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

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

A modified 4-parameter version of the previous model proposed by Coroller, Leguerinel, Mettler, Savy and Mafart (2006), based on mixed Weibullian distributions of resistances for characterizing two microbial subpopulations, was also applied:

$$\log\left(\frac{N}{N_0}\right) = \log\left\{\frac{1}{1+10^{\alpha}}\left[10^{-\left(\frac{t}{\delta_1}\right)^p+\alpha} + 10^{-\left(\frac{t}{\delta_2}\right)^p}\right]\right\} \tag{7}$$

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

2.6. Statistical analysis

Statistical analyses were carried out using InfoStat 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina). Model performance was evaluated using the root mean square error (RMSE) (Alzamora, Guerrero, Viollaz & Welti, 2005); the Akaike information criterion (AIC) (Akaike, 1973) and the Bayesian Schwarz criterion (BIC) (Quinn & Keough, 2002):

$$\mathrm{RMSE} = \sqrt{\frac{\sum\left(\mu_{observed} - \mu_{predicted}\right)^2}{N}} \tag{8}$$

$$\mathrm{AIC} = N\left[\ln\left(\frac{2\pi\sigma^2}{N}\right) + 1\right] + 2 \tag{9}$$

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

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 Bayesian's theories, the most accurate and parsimonious model yields the smallest AIC and BIC values (Quinn & 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 (Ferrario, Alzamora & Guerrero, 2013).

3. Results and discussion

3.1. Kinetics of microorganisms' inactivation

E. coli ATCC 11229, *L. innocua* ATCC 33090, and *S. cerevisiae* KE162 counts after 1 h of being inoculated in fresh peach juice without exposing to ozone (controls) showed in average 0.15 ± 0.05, 0.32 ± 0.03 and 0.39 ± 0.13 log reductions, respectively, revealing that these microorganisms were able to survive at the low pH of peach juice.

Survival curves for *E. coli* ATCC 11229, *L. innocua* ATCC 33090, and *S. cerevisiae* KE162 in peach juice processed by ozone at both concentrations are presented in Fig. 1. Bacteria inactivation curves were clearly nonlinear. They exhibited downward concavity, excepting in the case of *L. innocua* ATCC 33090 exposed to 18 ppm ozone, where a slight upward concavity was detected The highest ozone concentration resulted in lower counts of *E. coli* ATCC 11229 during most part of the treatment; however, after 12 min exposure, coliform counts were reduced by approximately 4.3 log-cycles in peach juice exposed to both ozone levels (Fig. 1a). The inactivation curve of *E. coli* ATCC 11229 treated with 10 ppm ozone showed a lag time of 4 min before death. The lag time was reduced by approximately 50% when the highest ozone concentration (18 ppm) was used. The effect of ozone level on lag time before inactivation had been previously reported by Steenstrup and Floros (2004), who observed lag time values of 2 min and 3 min in apple cider when *E. coli* O157:H7 was subjected to 1000 and 860 ppm of gaseous ozone, respectively. Patil et al. (2010a) reported also a 4 min lag time for *E. coli* NCTC 12900 in apple juice treated with an ozone concentration equal to 0.048 mg/min/mL.

Treatments with ozone (10 or 18 ppm) for 12 min decreased *L. innocua* ATCC 33090 counts by 3.9 and 4.9 log-cycles, respectively (Fig. 1b). This microorganism presented a 2 min lag time for 10 ppm ozone concentration; this time was lower than that observed for *E. coli* ATCC 11229 treated with the same ozone concentration, indicating that *L. innocua* ATCC 33090 would be more sensitive to ozone. The presence of the lag time in *L. innocua* ATCC 33090 response is in agreement with the results reported by other authors, who observed a 2 min lag time for *L. innocua* NCTC 11288 in orange juice treated with 0.098 mg/min/mL ozone (Patil et al., 2010b). However, *L. innocua* ATCC 33090 survival curve during treatment with 18 ppm ozone showed a sigmoid inactivation pattern with lack of lag time.

The occurrence of a shoulder on the microbial kinetics had been discussed by Cullen et al. (2009) and Steenstrup and Floros (2004). These researchers concluded that a minimum ozonation time and a minimum ozone concentration were required to observe inactivation, which results in a lag phase in the survival curves. They also reported that lag time depended on the type of treated microorganism and it would also occur due to the presence of ozone demanding organic material in the liquid phase. Peach juice contains various organic compounds, such as carotenoids, ascorbic acid and amino acids, that may be oxidized due to direct reaction with ozone or indirect reaction of secondary oxidizing agents; thus the presence of lag times was expected. As ozone level in the peach juice increased, depending on microorganism sensitivity to ozone, lag phase decreased or was not detected.

For *S. cerevisiae* KE162, ozone treatment was less effective, since only 1 log-cycle of reduction was achieved regardless of ozone concentration (Fig. 1c). These curves were not modeled since inactivation was scarce.

Relative resistances of assayed microorganisms to ozone were in agreement with literature results (Cullen et al., 2010): *E. coli* ATCC 11229, a Gram-negative bacteria, appeared to be more resistant to ozone than *L. innocua* ATCC 33090, a Gram-positive bacteria, and both bacteria were more sensitive than the yeast, *S. cerevisiae* KE162.

Fig. 1 also shows the fitting of experimental inactivation data using the cumulative Weibull distribution function (Eq. (1)) and the modified version of the model proposed by Coroller et al. (2006) (Eq. (7)). Table 1 showed the estimated parameters from fitting the models to experimental data. Table 2 enumerated the specific statistics related to the Weibullian distribution calculated according Eqs. (2)–(6). In order to compare the goodness of fit of the models, Table 3 displays RMSE, AIC and BIC values associated to the prediction of survival curves.

Weibull type model was appropriate for representing survival data of both bacteria. High adjusted coefficient of determination (R^2_{adj}) values were obtained, showing that between 94.1% and 98.7% of the variation in the experimental data could be explained by the selected model (Table 1). Inactivation curves exhibited n values > 1 (except *L. innocua* curve for 18 ppm ozone, that showed $n < 1$), as expected according to the downward concavity, more notorious for *E. coli* ATCC 11229 inactivation at the lowest ozone level. When $n > 1$, it is generally assumed that remaining cells become increasingly susceptible to treatment, in other words, there is a cumulative damage making it increasingly difficult for the cells to survive (van-Boekel, 2002). However, it must be highlighted that the presence of organic matter is also responsible of the shoulder or lag time before ozone killing. In the case of *L. innocua* ATCC 33090 exposed to 18 ppm ozone, an n parameter lower than 1 would indicate that the weak members of the population were destroyed at fast rate, leaving behind a little fraction of higher resistance survivors. In spite of ozone organic demand, killing of this bacteria would be detected from the beginning of the treatment because of its high ozone sensitivity.

For *E. coli* ATCC 11229, b parameter varied between 0.003 $\text{min}^{-2.96}$ (10 ppm ozone) and 0.23 $\text{min}^{-1.18}$ (18 ppm ozone); and for *L. innocua* ATCC 33090, it varied between 0.21 $\text{min}^{-1.19}$ (10 ppm ozone) and 0.68 $\text{min}^{-0.8}$ (18 ppm ozone), indicating that the inactivation rate of the microorganisms was strongly influenced by ozone concentration.

The Weibull distribution considers that the individual microorganisms in a population do not have identical resistances and that microbial sensitivity to lethal agents is distributed (Peleg & Cole, 1998). A single microorganism is either alive or it dies because of lethal agents such as heat, disinfectants, pressure, and radiation. It is unlikely that all cells behave in the same way and the inactivation time varies to some extent for each microorganism in a population, even if the population is pure (van-Boekel, 2002). Consequently, the survival curve could be assumed as the cumulative form of the underlying distribution of the individual inactivation times (Peleg & Cole, 1998).

The b and n parameters were used to generate the frequency distribution of resistances (Fig. 2) and to calculate the associated statistics: mode, mean, variance and coefficient of skewness (Table 2). Frequency distribution profiles markedly changed with ozone level and the microorganism assayed. Frequency distribution of resistances for *E. coli* ATCC 11229 treated with 10 ppm ozone was rather symmetric (coefficient of skewness near to 1) and characterized by similar mode (6.2 min) and mean (6.4 min) values (Fig. 2a). On the contrary, frequency distributions corresponding to *E. coli* ATCC 11229 treated with 18 ppm ozone (Fig. 2a) and *L. innocua* ATCC 33090 treated with 10 ppm ozone (Fig. 2b) were asymmetric (coefficient of skewness: 1.8), skewed to the right and characterized by a mode lower than the mean (Table 2). Distribution of resistances for *L. innocua* ATCC 33090 treated with 18 ppm ozone lacked of mode and was strongly skewed to the right (coefficient of skewness: 2.7), showing that the majority of the microorganisms in the population were inactivated by ozone at low exposure times (Fig. 2b). Clearly, *L. innocua* ATCC 33090 was more sensible that *E. coli* ATCC 11229 cells to ozone. It is noteworthy that the greater the ozone level, the lower the mean for both bacteria, which populations were more sensitive on average. For *L. innocua* ATCC 33090, the distribution of resistances was narrower (lower variance value) when ozone concentration increased, meaning a more uniform sensitivity of the population to ozone treatment. However, for *E. coli* ATCC 11229, the heterogeneity of the response continued being

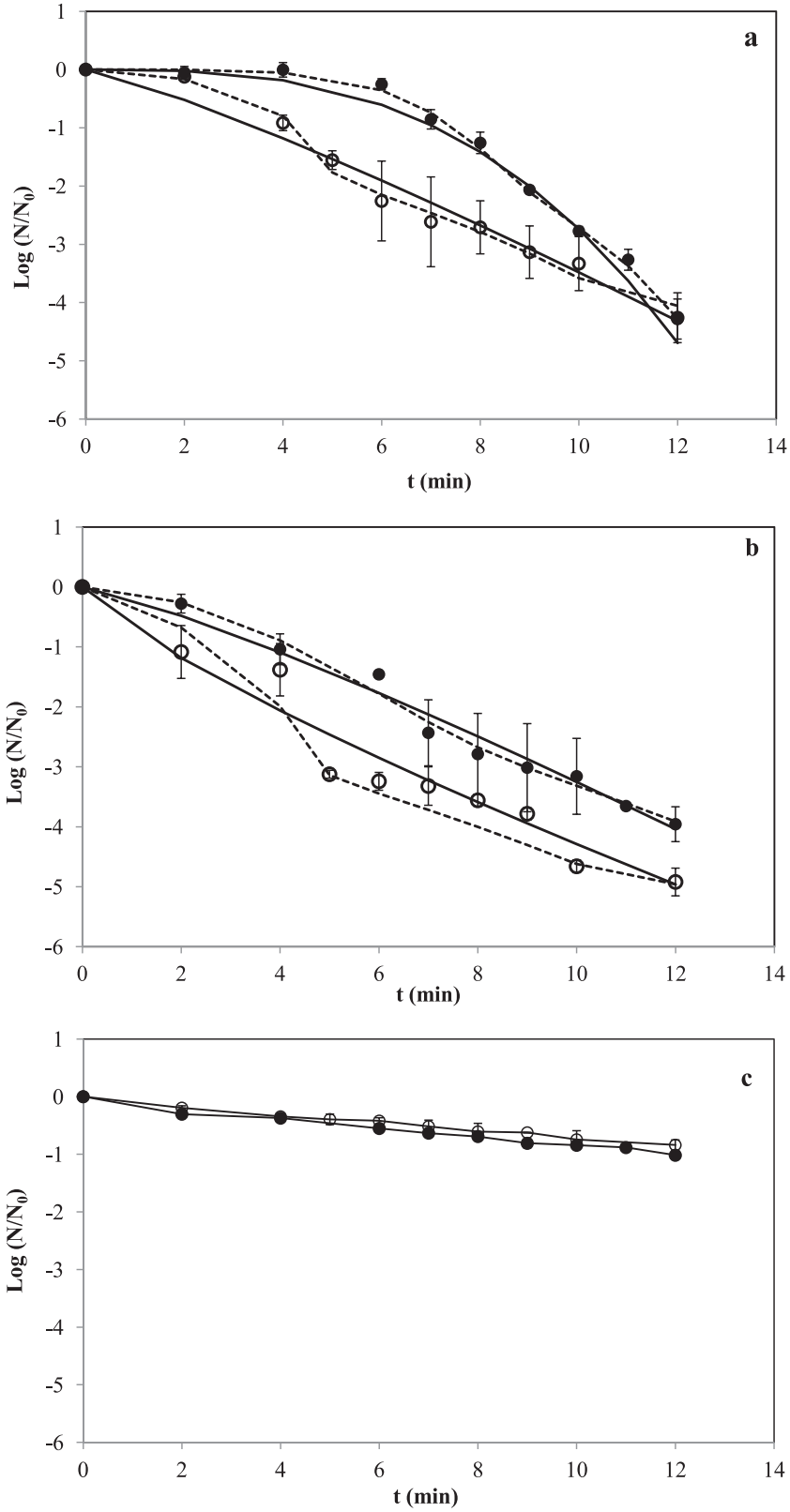

Fig. 1. Experimental survival curves (points) and fitted values derived from Weibull (solid line) and Coroller (dashed line) models for (a) *E. coli* (b) *L. innocua* and (c) *S. cerevisiae* treated with 10 (●) and 18 ppm (○) ozone at 20 °C during 12 minutes; standard deviation (|).

important as it was reflected by the tails of the distributions and great variance values.

The Coroller model was also appropriate for representing survival data as shown by the high R^2_{adj} values obtained, ranging between 93.5% and 99.8% (Table 2), and the low RMSE values (Table 3). The α parameter, representing the log relationship between the most sensible population proportion (f) and the most resistant population proportion ($1-f$), was greater than 1 for both microorganism, indicating that the

Table 1
Estimated parameters of Weibull and modified Coroller models corresponding to *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 survival in natural peach juice treated with ozone at different concentrations.

Microorganism	O_3 concentration (ppm)	b (min^{-n})	n (−)	R^2_{adj}	α (−)	δ_1 (min)	p (−)	δ_2 (min)	R^2_{adj}
E. coli	10	0.003 (0.001)	2.96 (0.19)	0.987	1.62 (0.19)	7.45 (0.12)	4.83 (0.32)	9.83 (0.51)	0.996
	18	0.23 (0.06)	1.18 (0.11)	0.967	2.11 (0.08)	4.17 (0.08)	2.82 (0.19)	9.16 (0.27)	0.998
L. innocua	10	0.21 (0.05)	1.19 (0.11)	0.977	1.84 (0.56)	4.22 (0.36)	1.8 (0.4)	8.02 (1.5)	0.982
	18	0.68 (0.16)	0.8 (0.1)	0.941	2.35 (0.72)	2.54 (0.59)	1.59 (0.63)	6.57 (2.54)	0.935

(Value): standard error for each parameter.

majority of population was the sensitive one, which died in the first minutes during ozone treatment. When the population was exposed to 18 ppm ozone, the proportion of sensitive members in the population increased.

The times for the first decimal reduction (δ_1) decreased about 44% and 40% for *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 respectively, as ozone concentration increased. Furthermore, *L. innocua* ATCC 33090 presented minor δ_1 than *E. coli* ATCC 11229 when exposing to both ozone concentrations, indicating that the first microorganism was more sensible to ozone. The times to first log reduction values corresponding to the second subpopulations (δ_2) also decreased as ozone level increased, although in a lesser degree than δ_1 did (about 7% and 18% for *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 respectively).

The models considered represent different types of assumptions that can be used regarding differences in population resistance to ozone treatment: (1) a population with a distribution of sensitivities represented by a unique nonlinear behavior (Weibullian model), and (2) two subpopulations associated to a double Weibull distribution of resistances (modified Coroller model). Estimated model parameters explained, from a different point of view, the influence of ozone on microbial response. Weibull parameters allow knowing the frequency distribution of resistances to ozone in the microbial population, while Coroller parameters give information about the relationship between sensitive and resistant subpopulations.

Statistic criteria to evaluate the fitting capacity of Weibull and Coroller demonstrated that both models constituted good alternatives to quantify the microbial response to ozone (R^2_{adj} in the range 0.935–0.996) (Table 3). However, based on the RMSE, AIC and BIC values, the modified Coroller model showed the best performance in the most cases, except for the inactivation kinetics of *L. innocua* treated with 18 ppm ozone, where there was a difference in favor of the Weibull model in RMSE value. Coroller model had good predictive ability according to AIC and BIC criteria, which take both, fit and parsimony, into account (Coroller et al., 2006)

3.2. Growth dynamics of microorganisms in decontaminated peach juice

Most previous studies in the literature had focused in the inactivation of microorganisms by ozone but they did not evaluate the behavior of microorganisms following oxidative stress. However, different patterns of microbial response in decontaminated minimally processed vegetables and in fruit juices have been identified by Gómez-López, Ragaert, Debevere and Devlieghere (2008) and Ferrario, Alzamora and Guerrero (2014) respectively, evidencing difficulties to control microbial loads of these products during storage at low temperatures: 1) no decontamination occurred but growth rate of microorganisms in treated samples was slower than that in untreated samples or exhibited a longer lag phase than that in untreated samples; 2) decontamination occurred and growth rate of microorganisms in treated samples was slower (or counts decreased) or was equal than that in untreated samples; and 3) decontamination occurred and microorganisms in treated samples did not grow or exhibit lag phase; or growth rate was faster than that in untreated samples.

Table 2
Weibull model related statistics[a], corresponding to *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 survival in natural peach juice treated with ozone at different concentrations.

Microorganism	O_3 concentration (ppm)	t_{cm} (min)	$\bar{t}_c$ (min)	σ_{tc}^2 (min^2)	v_1 (−)
E. coli	10	6.2	6.4	5.5	1.1
	18	0.7	3.3	7.8	1.8
L. innocua	10	0.8	3.5	8.7	1.8
	18	–	1.8	5.3	2.7

[a] Statistics of the Weibullian model: t_{cm}, mode; $\bar{t}_c$, distribution's mean; σ_{tc}^2, variance; v_1, coefficient of skewness.

In this work, growth of inoculated microorganisms during refrigerated storage was monitored in peach juice processed with 10 and 18 ppm ozone during 12 min (Fig. 3). Microbial behavior during storage was dependent not only on the type of microorganism but also on the level of ozone applied. *E. coli* ATCC 11229 populations did not significantly grow in untreated and treated peach juices until 7 day storage, as it would be expected due to the low temperature condition (Fig. 3a). At 12 and 14 day storage, growth achieved in peach juices treated with both ozone levels was lower than 1 log cycle.

Counts of *L. innocua* ATCC 33090 slightly increased (≈0.6 log cycles) after a 2 day lag phase in the untreated juice, while in decontaminated samples, growth (≈0.3 and 1 log cycles) occurred after a greater lag phase (5 days). Then *L. innocua* ATCC 33090 populations remained approximately constant until the end of storage in ozone processed juices as well as in control juice (Fig. 3b).

S. cerevisiae KE162 was able to grow in peach juice stored at 5 °C, showing a similar growth rate in 10 ppm ozone processed juice and in nondecontaminated juice. Yeast counts increased approximately by 1 and 2 log cycles at 6 and 8 day storage, respectively. Then they remained approximately constant by the end of storage (Fig. 3c). However, following exposure to 18 ppm ozone stress, no spoilage occurred (Fig. 3e). After this lag phase, yeast population gradually increased, with a faster growth rate when compared with the control juice, although yeast counts at the end of storage remained lower than those in the untreated juice (Fig. 3e). *S. cerevisiae* is one of the most important yeasts causing spoilage of fruit juices and can be considered as shelf-life indicator (Patil et al., 2011). In spite of the low effectiveness of ozone for reducing the initial load of the yeast, ozone treatment at the highest concentration delayed its growth during refrigerated storage.

According to the response of *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 (indicators for the enterohemorrhagic foodborne pathogen *E. coli* O157:H7 and *L. monocytogenes*) during storage, 12 min ozone exposure at the greatest concentration (18 ppm) would be effective in increasing safety of refrigerated peach juice, and, considering *S. cerevisiae* KE162 response, it would extend the shelf-life of the juice.

Regarding the impact on peach juice quality, we found that ozone processing using similar doses/conditions than in this work slightly increased browning and decreased pseudoplasticity of peach juice

Table 3
Minimum RSME, AIC and BIC values for the survival curves of the assayed microorganisms in ozone treated peach juice.

Microorganism	O_3 concentration (ppm)	RMSE		AIC		BIC	
		Weibull	Coroller	Weibull	Coroller	Weibull	Coroller
E. coli	10	0.2	0.083	0.96	−15.67	1.86	−14.15
	18	0.26	0.061	4.92	−22.58	5.83	−21.07
L. innocua	10	0.21	0.18	0.96	−0.62	1.86	0.89
	18	0.38	0.4	13.08	15.06	13.99	16.57

as well as significantly reduced PPO and POD enzyme activities (Jaramillo, 2014). These studies also support the suitability of the ozone treatment for peach juice.

4. Conclusions

Ozone treatment in a bubble column was effective for inactivation of *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 inoculated in peach juice. Reductions ranged from at least 3.9 up to 4.9 log units according to ozone level (10 ppm or 18 ppm) and microorganism, being *L. innocua* ATCC 33090 more sensitive than *E. coli* ATCC 11229 at both ozone concentrations. For *S. cerevisiae* KE162, the treatment was less effective (only 1 log-cycle of reduction). Non-linear inactivation curves were successfully fitted with Weibull type and modified Coroller models, although, based on the RMSE, AIC and BIC values, the modified Coroller model showed the best performance in most cases. Model parameters and statistics showed that, when ozone level increased, the treatment time at which the majority of population dies and the inactivation time on average decreased, while the proportion of ozone sensitive members in the population increased. Growth dynamics in decontaminated juice during cold storage was dependant on inoculated microorganism and on the ozone level applied, but surviving microorganisms face more difficulties to grow than in unprocessed juice, especially at

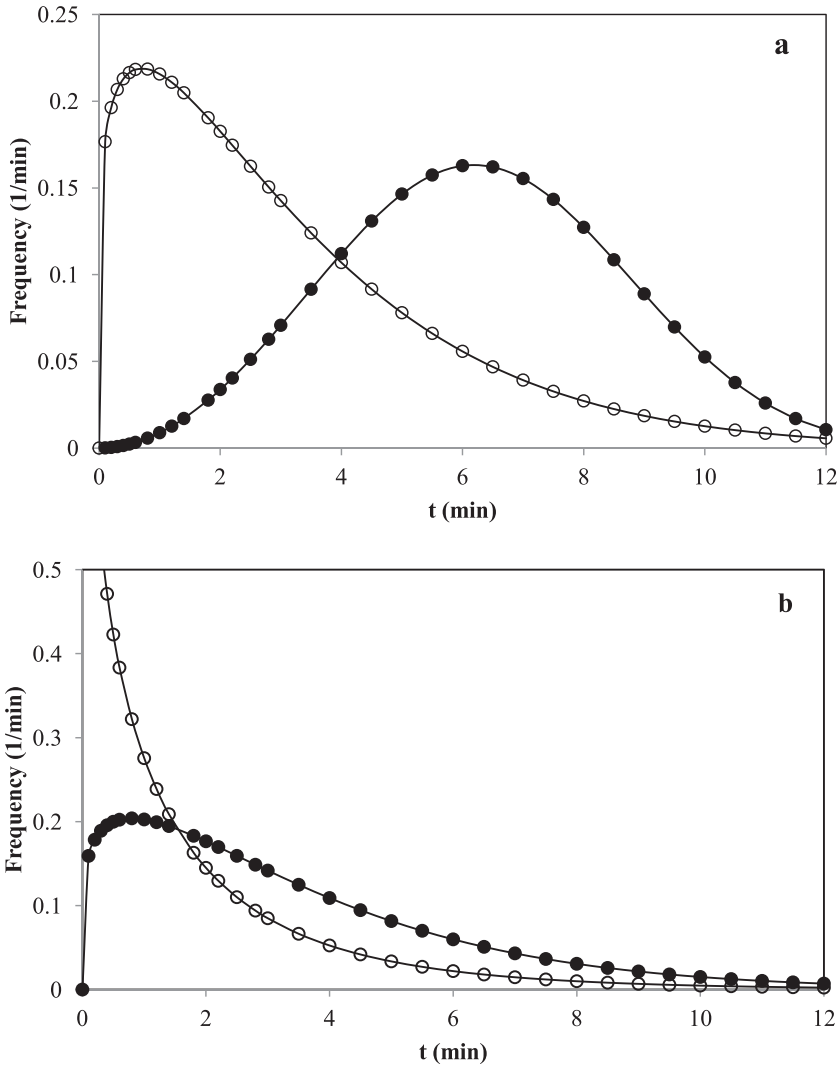

Fig. 2. Weibull frequency distributions of resistances corresponding to survival curves of (a) *E. coli* and (b) *L. innocua* treated with 10 (●) and 18 ppm (○) at 20 °C during 12 minutes.

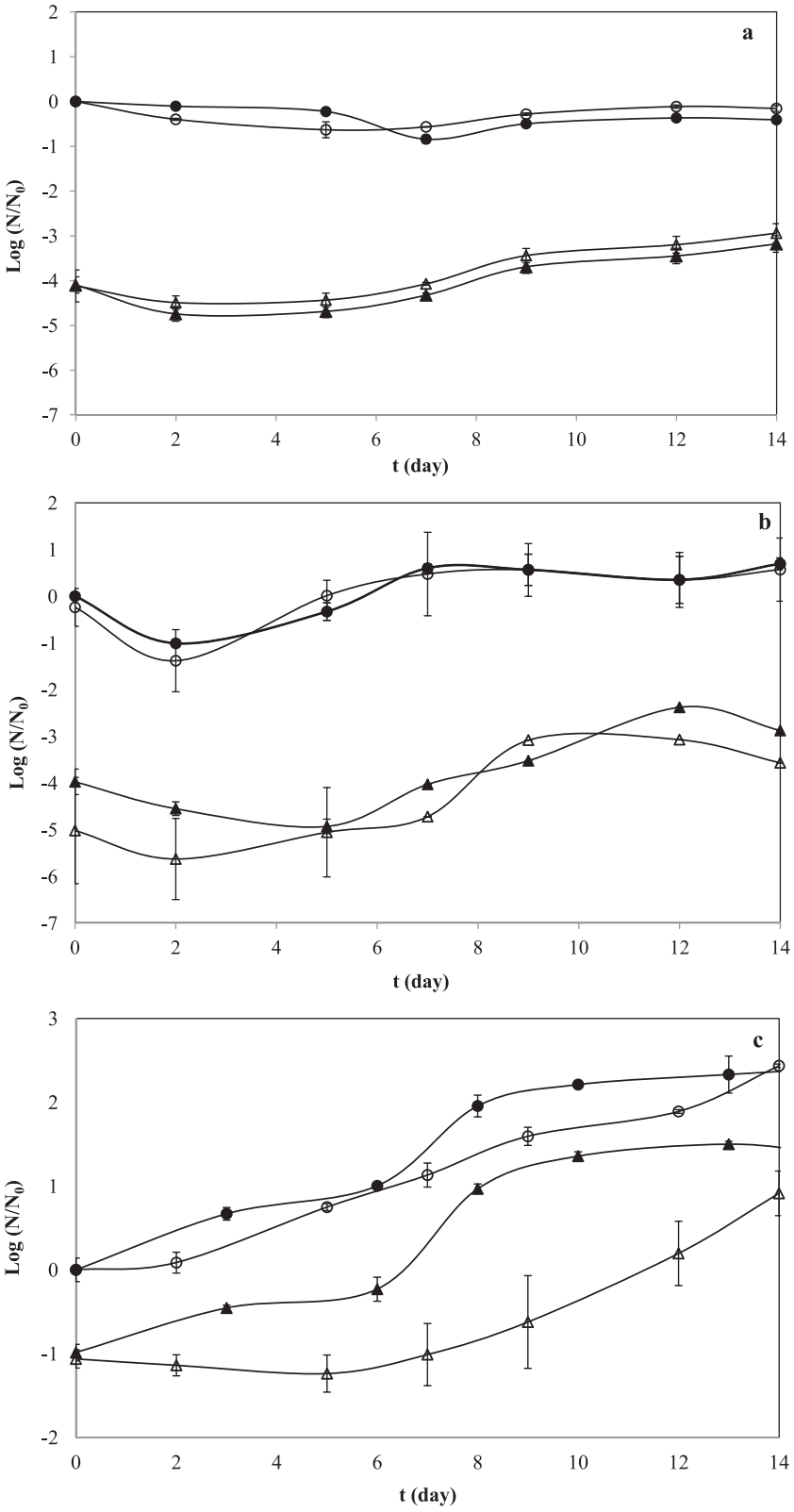

Fig. 3. Evolution of ozone treated (a) *E. coli* (b) *L. innocua* and (c) S. cerevisiae in peach juice stored at 5 ± 1 °C during 14 days. Control (●, ○); treated with 10 ppm (▲) and 18 ppm (empty triangle) ozone; standard deviation (|).

the highest ozone concentration. Ozone exposure (18 ppm) coupled with low temperature storage conditions seemed to be a good option for preserving peach juice, while it may also offer a promising alternative to thermal pasteurization.

More studies, however, are needed to determine how ozone affects organoleptic and nutritional properties as well as bioactive compounds of peach juice and the response of the indigenous microbiota of the juice.

Acknowledgments

The authors would like to acknowledge the financial support from the Universidad de Buenos Aires (20020130100388BA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, 11220120100395) and the Agencia Nacional de Promoción Científica y Técnica (ANPCyT) of Argentina and from the Banco Interamericano de Desarrollo (BID, PICT 2011-0288).

References

Abeysinghe, D.C., Li, X., Sun, C.D., Zhang, W.S., Zhou, C.H., & Chen, K.S. (2007). Bioactive components and antioxidants capacities in different edible tissues of citrus fruit of four species. *Food Chemistry*, *104*, 1338–1344.

Akaike, H. (1973). Information theory and extension of the maximum likelihood principle. In B.N. Petrov, & F. Czaki (Eds.), *Proceedings of the 2nd International Symposium of Information* (pp. 267–281). Budapest: Akademiai Kiado.

Alzamora, S.M., Guerrero, S., Viollaz, P., & Welti, J. (2005). Experimental protocols for modeling the response of microbial populations exposed to emerging technologies: Some points of concern. In G. Barbosa-Cánovas (Ed.), *Novel food processing* (pp. 591–607). New York: Marcel Dekker, Inc.

Cataldo, F. (2008). Ozone decomposition of patulin — A mycotoxin and food contaminant. *Ozone: Science & Engineering*, *30*, 197–201.

Choi, M., Liu, Q., Lee, S., Jin, J., Ryu, S., & Kang, D. (2012). Inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* in apple juice with gaseous ozone. *Food Microbiology*, *32*, 191–195.

Coroller, L., Leguerinel, I., Mettler, E., Savy, N., & Mafart, P. (2006). General model, based on a two mixed Weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. *Applied and Environmental Microbiology*, *72*, 6493–6502.

Cullen, P.J., Tiwari, B.K., O'Donnell, C.P., & Muthukumarappan, K. (2009). Modelling approaches to ozone processing of liquid foods. *Trends in Food Science and Technology*, *20*, 125–136.

Cullen, P.J., Valdramidis, V.P., Tiwari, Patil, S., Bourke, P., & O'Donnell, C.P. (2010). Ozone processing for food preservation: An overview on fruit juice treatments. *Ozone: Science & Engineering*, *32*, 166–179.

Dubois, M., Coste, G., Despres, A.G., Efstathiou, T., Nio, C., Dumont, E., et al. (2006). Safety of Oxygreen, an ozone treatment on wheat grains. Part 2. Is there a substantial equivalence between Oxygreen-treated wheat grains and untreated wheat grains? *Food Additives and Contaminants*, *23*, 1–15.

Esteve, M.J., & Frigola, A. (2007). Refrigerated fruit juices. Quality and safety issues. *Advances in Food and Nutrition Research*, *52*, 103–139.

Fairchild, T.M., & Foegeding, P.M. (1993). A proposed non-pathogenic biological indicator for thermal inactivation of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, *59*, 1247–1250.

Ferrario, M., Alzamora, S.M., & Guerrero, S.N. (2013). Inactivation kinetics of some microorganisms in apple, melon, orange and strawberry juices by high intensity light pulses. *Journal of Food Engineering*, *118*, 302–311.

Ferrario, M., Alzamora, S.M., & Guerrero, S.N. (2014). *Apple, orange and strawberry juices treated with pulsed Light. Effect on some inoculated microorganisms and native flora during refrigerated storage.* IAFP 2014. Indianapolis, Indiana, USA, August 3–6, 2014.

Gómez-López, V.M., Ragaert, P., Debevere, J., & Devlieghere, F. (2008). Decontamination methods to prolong the shelf-life of minimally processed vegetables, state-of-the-art. *Critical Reviews in Food Science and Nutrition*, *48*, 487–495.

Ishizaki, K., Sawadarski, K., Miura, K., & Shinriqui, N. (1987). Effect of ozone on plasmid DNA of *Escherichia coli* in situ. *Water Research*, *21*, 823–827.

Jaramillo, G.M. (2014). *Preservación de jugo de durazno mediante la aplicación de ozono. Efecto en la calidad y la actividad enzimática. Magister thesis* in Food Science and Industrial Technology. University of Buenos Aires.

Khadre, M.A., & Kim, J.G. (2001). Sporicidal action of ozone and hydrogen peroxide: A comparative study. *International Journal of Food Microbiology*, *71*, 131–138.

Khadre, M.A., Yousef, A.E., & Kim, J.G. (2001). Microbiological aspects of ozone applications in food: A review. *Journal of Food Science*, *66*, 1242–1252.

Kim, J.G., & Yousef, A.E. (2000). Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. *Journal of Food Science*, *65*, 521–527.

Kim, J.G., Yousef, A.E., & Dave, S. (1999). Application of ozone for enhancing the microbiological safety and quality of foods. A review. *Journal of Food Protection*, *62*, 1071–1087.

Kim, J.G., Yousef, A.E., & Khadre, M.A. (2003). Ozone and its current and future application in the food industry. *Advances in Food and Nutrition Research*, *45*, 168–275.

Komanapali, I.R., & Lau, B.H.S. (1998). Ozone-induced damage of *Escherichia coli* K-12. *Applied Environmental & Biotechnology*, *46*, 610–614.

Korycka-Dahl, M., & Richardson, T. (1978). Activated oxygen species oxidation of food constituents. *Critical Reviews in Food Science and Nutrition*, *10*, 209–241.

McLellan, M.R., & Splittstoesser, D.F. (1996). Reducing the risk of *E. coli* in apple cider. *Food Technology*, *50*, 174.

Miller, L.G., & Kaspar, C.W. (1994). *Escherichia coli* O157:H7 acid-tolerance and survival in apple cider. *Journal of Food Protection*, *57*, 460–464.

Muthukumarappan, K., Halaweish, F., & Naidu, A.S. (2000). Ozone. In A.S. Naidu (Ed.), *Natural food anti-microbial systems* (pp. 783–800). Boca Raton, FL: CRC Press.

Patil, S., Bourke, P., Frías, J.M., Tiwari, B.K., & Cullen, P.J. (2009). Inactivation of *Escherichia coli* in orange juice using ozone. *Innovative Food Science & Emerging Technologies*, *10*, 551–557.

Patil, S., Valdramidis, V.P., Cullen, P.J., Frias, J., & Bourke, P. (2010a). Inactivation of *Escherichia coli* by ozone treatment of apple juice at different pH levels. *Food Microbiology*, *27*, 835–840.

Patil, S., Valdramidis, V.P., Cullen, P.J., Frias, J.M., & Bourke, P. (2010b). Ozone inactivation of acid stressed *Listeria monocytogenes* and *Listeria innocua* in orange juice using a bubble column. *Food Control*, *21*, 1723–1730.

Patil, S., Valdramidis, V.P., Tiwari, B.K., Cullen, P.J., & Bourke, P. (2011). Quantitative assessment of the shelf life of ozonated apple juice. *European Food Research and Technology*, *232*, 469–477.

Peleg, M., & Cole, M.B. (1998). Reinterpretation of microbial survival curves. *Critical Reviews in Food Science*, *38*, 353–380.

Perry, J.J., & Yousef, A.E. (2011). Decontamination of raw foods using ozone-based sanitization techniques. *Annual Review of Food Science and Technology*, *2*, 281–298.

Quinn, G., & Keough, M. (2002). *Experimental design and data analysis for biologists.* Cambridge: Cambridge University Press (Chapter 13).

Restaino, L., Frampton, E.W., Hemphill, J.B., & Palnikar, P. (1995). Efficacy of ozonated water against food-related microorganisms. *Environmental Microbiology*, *61*, 3471–3475.

Sado, P.N., Jinneman, K.C., Busby, G.J., Sorg, S.M., & Omiecinski, C.J. (1998). Identification of *Listeria monocytogenes* from unpasteurized apple juice using rapid test kits. *Journal of Food Protection*, *61*, 1199–1202.

Steenstrup, L.D., & Floros, J.D. (2004). Inactivation of *E. coli* O157:H7 in apple cider by ozone at various temperatures and concentrations. *Journal of Food Processing and Preservation*, *28*, 103–116.

Stratford, M., Hofman, P.D., & Cole, M.B. (2000). Fruit juices, fruit drinks and soft drinks. In B.M. Lund, T.C. Baird-Parker, & G.W. Gould (Eds.), *Microbiological safety and quality of food* (pp. 836–869). Maryland: Aspen Publisher Inc.

Tiwari, B.K., Brennan, C.S., Curran, T., Gallaher, E., Cullen, P.J., & O'Donnell, C.P. (2010). Application of ozone in grain processing. *Journal of Cereal Science*, *51*, 248–255.

Tiwari, B.K., Muthukumarappan, K., O'Donnell, C.P., & Cullen, P.J. (2008a). Modelling color degradation of orange juice by ozone treatment using response surface methodology. *Journal of Food Engineering*, *88*, 553–560.

Tiwari, B.K., Muthukumarappan, K., O'Donnell, C.P., & Cullen, P.J. (2008b). Kinetics of freshly squeezed orange juice quality changes during ozone processing. *Journal of Agricultural and Food Chemistry*, *56*, 6416–6422.

Tiwari, B.K., O'Donnell, C.P., Brunton, N.P., & Cullen, P.J. (2009a). Degradation kinetics of tomato juice quality parameters by ozonation. *International Journal of Food Science and Technology*, *44*, 1199–1205.

Tiwari, B.K., O'Donnell, C.P., Patras, A., Brunton, N.P., & Cullen, P.J. (2009b). Anthocyanins and color degradation in ozonated grape juices. *Food and Chemical Toxicology*, *4*, 2824–2829.

United States Food, Administration, Drug, & USFDA. (2004). *Juice HACCP hazards and controls guidance. Guidance for industry* (1st ed.).

van-Boekel, M.A.J.S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, *74*, 139–159.