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Effect of a continuous flow-through pulsed light system combined with ultrasound on microbial survivability, color and sensory shelf life of apple juice

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ABSTRACT

The aim of this work was to investigate the effect of a continuous flow-through pulsed light system (PL_c, 0.73 J/cm^2, 155 mL/min, ***EEO***: 1.8×10^3–4.1×10^3 kW·h/m^3/order) single or combined with ultrasound (US, 30 min, ***EEO***: 4.4×10^5 − 1.1×10^6 kW·h/m^3/order) at ambient temperature on *Escherichia coli* ATCC 35218, *Salmonella* Enteritidis MA44 and *Saccharomyces cerevisiae* KE 162 and indigenous flora in commercial (CAJ) and freshly pressed (NAJ) apple juices. In addition, for the combined treatment, color evolution, sensory shelf life and consumer sensory field studies were also conducted during NAJ cold storage (4 °C). The Weibull model adequately characterized inactivation curves (R^2_{adj}: 95.0–99.1%). No differences in single or combined PL effectiveness were observed between CAJ and NAJ, resulting in 1.8–4.2 log reductions for single PL_c while US + PL_c led up to 3.7–6.3 log reductions of inoculated microorganisms. Moreover, the combined treatment delayed yeast and mold recovery and prevented from browning development during storage. Processed NAJ was well accepted by a group of consumers who highlighted its fresh natural apple taste. Sensory shelf life was determined by 6 days (25% rejection) with 95% confidence.

Industrial relevance: There is a growing consumer demand for fresh-like products as traditional thermal processing may have undesirable effects over the sensory and nutritional properties of fruit juices. From an industrial perspective, the content of this publication has the potential to be used for the development of novel products, with enhanced quality, processed by a continuous flow-through pulsed light system combined with ultrasound, both emerging technologies with good prospects for the decontamination of foods. In particular, this study showed that apple juice processed by US and PL ensured microbiological safety and was widely accepted by a group of consumers interested in sour products and its fresh natural apple taste.

1. Introduction

During the last decade considerable research on the application of non-thermal technologies for juice preservation has been developed to replace, at least partially, traditional pasteurization processes. These emerging technologies must assure the absence of pathogens like *Escherichia coli* O157:H7 and *Salmonella* Enteritidis, which may form part of the juice's microflora (Vojdani, Beuchat, & Tauxe, 2008), prevent from yeast spoilage, predominantly caused by *Saccharomyces* spp. (Fleet, 1992), and achieve improved quality (Hogan, Kelly, & Sun, 2005).

There is a wide range of modern agents that cause physical or chemical inactivation of microorganisms at ambient or sub-lethal temperatures. Some of these emerging technologies that are under research include high electric field pulses (PEF), high hydrostatic pressure (HHP), ultrasound (US), pulsed light (PL), short-wave ultraviolet light (UV-C), ozone and hydrogen peroxide, among others. These non-thermal technologies are being encouraged for fruit preservation because, without the need for severe heating, they cause minimal damage to flavor, texture and nutritional quality of some foods (Ross, Griffiths, Mittal, & Deeth, 2003).

Pulsed light (PL) is a non-thermal technology for microbial decontamination, which involves short time pulses (100–400 μs) of an intense broad spectrum between 100 and 1100 nm (Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010a). PL has gained increasing attention because of the very short treatment time required to achieve the desired microbial inactivation. The lethal action of PL has been mostly attributed to dimmer formation, which impairs DNA replication and subsequent cell division (photochemical effect). In addition, the photothermal and photophysical effects, caused by the high peak power and the visible

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to near-infrared portions of PL spectrum, respectively, seem to be involved (US FDA, 2001) and may coexist, leading to cell structure damage (Wekhof, 2000; Takeshita et al., 2003).

The main food parameters that influence PL effectiveness for microbial inactivation are the intrinsic transparency of the material allowing light penetration, the reflection coefficient and the surface condition of the item. This means that product surface should be smooth, clear and without pores or grooves which could exert a "shadow effect" to light penetration in the microbial cells, thus decreasing process effectiveness (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007; Palmieri & Cacace, 2005). Other parameters such as presence of particulate materials, treatment time, distance of sample from the light source, composition of emitting spectrum, volume of the sample, number of lamps, orientation, and design of lamps, have a direct relevance and affect the sample–light interaction (Pataro et al., 2011). These limitations have suggested PL use under a hurdle approach (Guerrero, Alzamora, & Ferrario, 2014).

Combining emerging technologies with conventional preserving ones or with other novel techniques to interfere with the homeostatic mechanisms of microorganisms has been successfully explored in the last years (Guerrero, López-Malo, & Alzamora, 2001; Guerrero, Tognon, & Alzamora, 2005; Ferrante, Guerrero, & Alzamora, 2007; Ferrario, Alzamora, & Guerrero, 2015; Lado & Yousef, 2002; Raso & Barbosa-Cánovas, 2003; Ross et al., 2003; Schenk, GarcíaLoredo, Raffellini, Alzamora, & Guerrero, 2012). If PL is combined with other preservation techniques which sensitize the organism structure to the action of light, microbial disruption, and then inactivation will be probably enhanced (Guerrero et al., 2014).

High-intensity ultrasound (US) has been proposed as an emerging method to disrupt cells by the cavitation phenomenon which produces intense localized changes in pressure and temperature, causing shear-induced breakdown of cell walls, disruption and thinning of cell membranes and DNA damage via free radical production (Guerrero et al., 2001 and 2005; López-Malo, Guerrero, & Alzamora, 1999; Ross et al., 2003). Recent studies revealed that the effects of US are multi-targeted, and at least the cell wall, the cytoplasmic membrane, the DNA, the internal cell structure and the outer membrane are affected by this emerging technology (Alzamora, Guerrero, Schenk, Raffellini, & López-Malo, 2011; Ananta, Voight, Zenker, Heinz, & Knorr, 2005). US is not considered for its use as a unique preservation factor because high levels of ultrasonic waves are needed to effectively kill all microorganisms, adversely modifying the nutritional and sensory properties of food (Ferrante et al., 2007). The use of US in combination with other hurdles (PL, ultraviolet light, natural antimicrobials, moderate temperature) has proved to enhance the observed microbial inactivation (Char, Mitilinaki, Guerrero, & Alzamora, 2010; Guerrero et al., 2001 and 2005; Ferrante et al., 2007; Ferrario, Alzamora, & Guerrero, 2013a).

Consumers' demand for a preservation technology that retains fresh-like quality has resulted in a growing interest for nonthermal processing methods (Santhirasegaram, Razali, Soloman George, & Somasundram, 2015). Despite the fact that over the last 15 years many combined non-thermal preservation processes involving PL and/or US have been proposed for a varied range of foods, quality aspects have received less attention than microbial stability and safety. In particular, Muñoz et al. (2012b) examined pH, °Brix, color, non-enzymatic browning and antioxidant activity of apple juice subjected to different combinations of PL and thermosonication. Whereas, Caminiti et al. (2011 and 2012) evaluated pH, °Brix, color, non-enzymatic browning changes and conducted sensory analysis to evaluate sweetness, acidity, odor and overall acceptability of apple juice treated by PEF and PL and orange–carrot blend subjected to combinations of manothermosonication and PL.

The objective of this study was to investigate: i) the effect of single PL_c, or combined with US (US + PL_c) on the inactivation of some microorganisms of relevance in fruit juices, ii) the suitability of the Weibull model to characterize single and combined treatment inactivation kinetics, iii) changes in color of apple juice after being processed by PL_c and US + PL_c and during cold storage (4 ± 1 °C), iv) sensory attributes and overall acceptability of NAJ after applying the combined treatment US + PL_c, and v) sensory shelf life of NAJ processed with US + PL_c during cold storage.

2. Materials and methods

2.1. Strains and preparation of inocula

Experiments were performed using *E. coli* ATCC 35218, *Salmonella* Enteritidis MA44 and *Saccharomyces cerevisiae* KE 162 (all strains were generously provided by Medica-Tec SRL, Buenos Aires, Argentina). Initial bacterial inocula were prepared by transferring a loopful of Trypticase Soy Agar plus 0.6%*w*/w Yeast Extract (TSAYE) slant stock culture to a 20 mL Erlenmeyer-flask of Trypticase Soy Broth supplemented with 0.6%*w*/w Yeast Extract. The inoculum was incubated at 37 °C under agitation for 18 h until it reached the stationary phase. A similar procedure was repeated for the yeast culture, where the initial inoculum was prepared by transferring a loopful of a fresh stock culture maintained in Potato Dextrose Agar (PDA) to an Erlenmeyer-flask containing 20 mL of Sabouraud Dextrose Broth. Incubation was performed at 27 °C for 24 h. All inocula were harvested by centrifugation (1475 *g*, 5 min) (Labnet, USA), washed twice with saline and re-suspended in peptone water to give a cell density of 10^8–10^9 CFU/mL. All microbiological procedures were done in a Class II biological safety cabinet (Nuaire Inc., Plymouth, USA). All microbiological media used in this study were from Britania (Buenos Aires, Argentina).

2.2. Preparation of produce samples

In order to evaluate the influence of suspended particles on treatment efficacy two types of matrices were used, commercial clarified apple juice without any additives (CAJ; CEPITA, Coca-Cola, Argentina; pH: 3.5 ± 0.1; 11.1 ± 0.9 °Brix; $A_{254\ nm}$: 0.031 ± 0.001; $A_{660\ nm}$: 0.063 ± 0.003; particle size: 1.37 ± 0.15 nm) and centrifuged freshly pressed apple juice (NAJ; *Pyrus malus* L., var. Granny Smith, pH: 3.5 ± 0.1; 12.6 ± 0.6 °Brix; $A_{254\ nm}$: 0.070 ± 0.007; $A_{660\ nm}$: 0.071 ± 0.005; particle size: 1068.33 ± 137.46 nm) were used in this study. NAJ was aseptically obtained from apples that were rinsed with 0.02% sodium hypochlorite and sterile water to eliminate surface microbial load, and gently dried with a sterile cloth. Juice was obtained under aseptic conditions in a 90% ethanol sanitized and 10 min UV-C exposed household apple press (Bluesky, Ningbo, China), centrifuged in order to reduce pulp amounts (2213 g, 10 min) (Eppendorf, model 5804 R, Hamburg, Germany), stored in caramel bottles at −80 ± 1 °C and defrosted at 4 ± 1 °C for its immediate use.

2.3. Measurements of physic chemical juice parameters

Juice turbidity was measured by centrifuging samples (198 *g*, 10 min, Eppendorf, model 5804 R, Hamburg, Germany), and measuring the supernatant absorbance at 660 nm (Rivas, Rodrigo, Martínez, Barbosa-Cánovas, & Rodrigo, 2006). Measurements were performed in triplicate. Particle size of apple juices ranging from 0.6 nm to 6 μm was determined in triplicate by dynamic light scattering (DLS) at 20 °C in a Zetasizer Nano-Zs (Malvern, Worcestershire, UK) provided with a He–Ne laser (633 nm) and a digital correlator (Model ZEN3600). Measurements were carried out at a fixed scattering angle of 173°, with a measuring range according to the manufacturer. The relationship between particle size and diffusion coefficient is defined by the Stokes–Einstein equation ($d(H) = (k \cdot T) / (3 \cdot \pi \cdot \eta \cdot D)$) (Malvern Instruments, 2004), where, $d(H)$: hydrodynamic diameter (m), D: translational diffusion coefficient ($m^2 \cdot s^{-1}$), k: Boltzmann's constant (1.38×10^{-23} N m K^{-1}), T: absolute temperature (K), and η: solvent viscosity (N s m^{-2}). The intensity distribution obtained was converted to volume distribution, using the Mie theory (Malvern Instruments, 2004). A refractive index (RI) of 1.35 and an absorption

parameter of 0.1 were used, according to the specifications provided by the manufacturer for colored samples (Malvern Instruments, 2004). For these studies, uninoculated juice samples were used.

2.4. Treatments

2.4.1. Ultrasonic treatment

US treatment was carried out in a 600 mL-double wall cylindrical vessel (diameter 8 cm; height: 13 cm) containing the juice (250 mL) which was serially connected to a thermostatically controlled water bath (HAAKE, Model Rotovisco RV12, Germany), to attain 25 ± 1 °C. Ultrasound (Vibracell®, net power output: 600 Watt, Sonic Materials Inc., Newtown, CT, USA) at 20 kHz and 95.2 (80%) μm of wave amplitude was applied to the medium through an immersed 13 mm diameter probe. After 3 min of sonication, the desired temperature was reached, and it was maintained constant at 25 ± 1 °C throughout the experiment. Five (5) milliliter microbial suspension was inoculated into the vessel and treated for 30 min. Due to bubbles generated by the cavitation process, the system was always highly mixed from the start of the experiment. The temperature of samples was continuously monitored by a thermocouple attached to the US device. Sonicated juice samples were immediately PL_c processed (combined treatment US + PL_c). Experiments were performed in triplicate.

2.4.2. Pulsed light treatment

PL_c treatment was performed with a RS-3000B Steripulse-XL device (Xenon Corporation, Wilmington, MA, USA), which produces polychromatic radiation in the wavelength ranging from 200 to 1100 nm according to the spectral profile provided by the manufacturer (Xenon Corporation, 2008). It generated high intensity PL at a pulse rate of 3 pulses/s and a pulse width of 360 μs. According to the specifications supplied by the manufacturer, each pulse delivered 1.27 J/cm^2 for an input of 3800 V at 1.9 cm below the quartz window surface of the lamp. Juice (250 mL) was poured into the vessel and was recirculated at 155 mL/min using a peristaltic pump (CPX-400, Cole Parmer, Illinois, USA) through two consecutive quartz tubes (1 mm i.d. 20 cm length) placed 10 cm below the light source exposed to PL_c for 10 min (maximum fluence: 0.73 J/cm^2, maximum total energy input: 0.0175 J/mL) (Fig. 1). Only for microbial challenge studies, 5 mL of inoculum was added to 245 mL of juice. PL_c treatment conditions applied to apple juice are listed in Table 1. According to previous studies, fluence was measured constant in a middle position of the tubes, and up to 10 cm forwards and backwards from this spot (Gómez, Salvatori, García Loredo, & Alzamora, 2011). Therefore, only 20 cm of the total length of each tube was exposed to PL (Fig. 1), while the non-exposed area of the tubes was covered to block light exposure. Inlet and outlet of the juice to the serially connected tubes were carried out by autoclavable flexible hoses (Cole-Parmer, Masterflex, L/S 15, Illinois, USA), which discharged into a 400 mL vessel subjected to agitation and immersed in a water–ice bath. Juice samples were taken at regular intervals from the vessel. The exposed area of the quartz tubes was countersunk in grooves in an aluminum unit containing a circulating coolant. Juice temperature was always below 25 °C. Assuming that the whole volume of juice was treated in one cycle, different PL doses were obtained by multiplying the dose of a single cycle by the number of cycles corresponding to each treatment time. The dose corresponding to a single cycle was calculated according to Pataro et al. (2011) (Table 1).

Table 1
Pulsed light (PL_c) treatment conditions applied to apple juice.

PL_c conditions	
Dose per pulse[a]	0.398 J/cm^2
Flow rate	155 mL/min
t_r[b]	0.12 s
Pulse frequency	3 Hz
Pulse width	360 μs
No. Reynolds	220
n[c]	1.8
Treatment time	10 min
PL_c fluence[d]	0.73 J/cm^2
Total specific energy input	0.0175 J/mL
Number of cycles	5
EEO[e]	1.8×10^3–4.1×10^3 kW·h/m^3/order

[a] Measured at 10 cm from the lamp.
[b] Theoretical residence time calculated as the ratio between the volume of the quartz tubes (0.314 mL) and the flow rate.
[c] Number of pulses calculated by multiplying t_r, pulse frequency (3 Hz) and the number of cycles.
[d] PL_c fluence calculated by multiplying the dose per pulse by n.
[e] Electric energy per order estimated according to Bolton et al. (2001).

All experiments were performed in triplicate. Before use, the PL_c device was thoroughly flushed with sterile distilled water. After treatment,

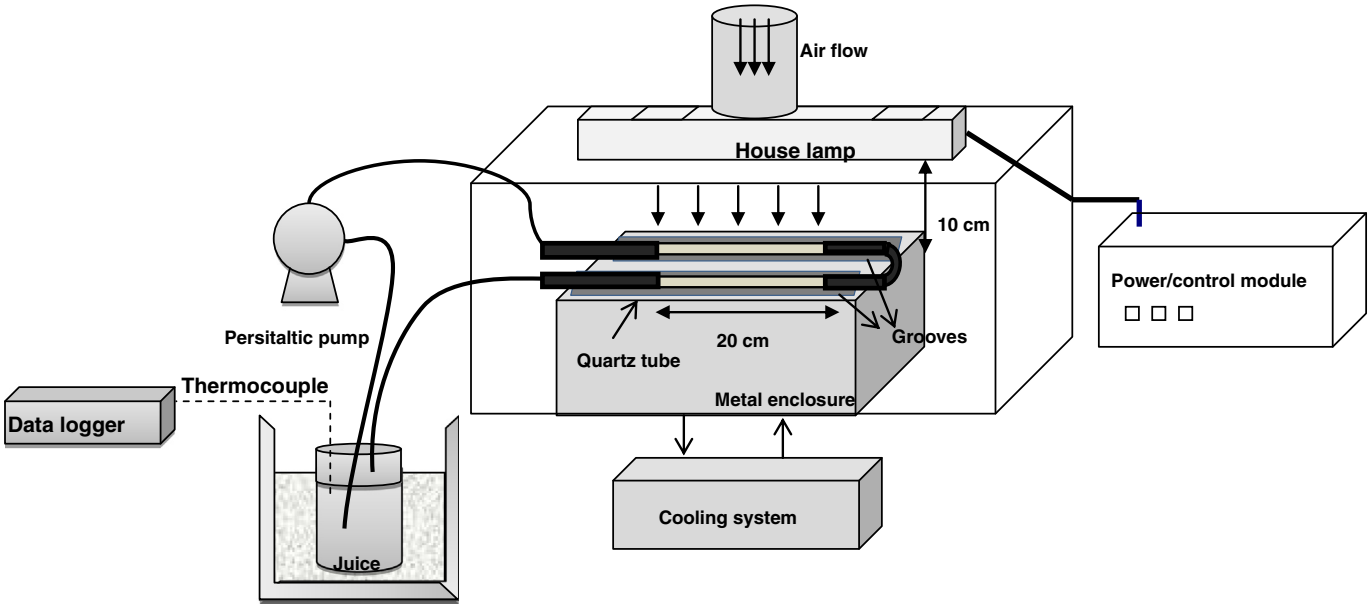

Fig. 1. Schematic diagram of the continuous flow-through pulsed light system (PL_c).The white section of the two serially connected quartz tubes represents the area exposed to PL_c.

a 10 min cleansing wash with 5%v/v sodium hypochlorite solution was performed followed by sterile distilled water.

For the combined US + PL_c treatments, 250 mL of pre-sonicated (20 kHz, 95.2 μm, 30 min, 25 ± 1 °C, juice were immediately PL_c (155 mL/min, 0–0.73 J/cm^2, 0–0.0175 J/mL, processed as described above). Inoculated untreated samples were used as controls. Temperature evolution of juices during PL_c treatment was monitored using a T-type thermocouple connected to a data logger Digi-Sense model 69202–30 (Barnant Company Division, Barrington, USA). All experiments were performed in triplicate.

2.4.3. Electric energy per order estimation

Electric energy per order (***EEO***), a figure-of-merit which was defined as the electric energy in kilowatt hours [kWh] required to reduce a given microbial load by one order of magnitude in 1 m^3 of contaminated sample, was calculated to determine the involved energy delivered to the US and PL treatments and thus, their efficiency inactivating the different microorganisms. The ***EEO*** values were estimated according to the equations proposed by Bolton, Bircher, Tumas, and Tolman (2001) for batch and flow-through operations in electric-energy-driven systems.

2.5. Microbial challenge study and predictive modeling

For microbial challenge tests, *E. coli*, *S.* Enteritidis and *S. cerevisiae* were separately inoculated in CAJ while only the former one in NAJ. After inoculation, juice samples were immediately treated by single PL_c and combined (US + PL_c) treatments according to the methodology previously described in Section 2.4. At selected time intervals, triplicate samples were taken for the analysis of survivors. Peptone water (0.1%w/v) tenfold dilution aliquots were surface plated by duplicate onto TSAYE for *E. coli* and *S.* Enteritidis, or PDA for *S. cerevisiae* using a spiral plater (Autoplate 4000, Spiral Biotech, USA). When PL treatment resulted in low counts (longer treatment times), 1-mL of fruit juice was directly pour plated into each Petri dish. Plates were incubated for 72 h at 37 ± 1 °C (bacteria) and 27 ± 1 °C (yeast), respectively. A counting grid was used for enumeration of colonies in the case of spiral plating. Survival curves were generated from experimental data by plotting log N/N_0 (where N is the number of CFU/mL at a given time and N_0 the initial number of CFU/mL) versus total specific energy input (J/mL).

Microbial inactivation data corresponding to the inoculated microorganisms in apple juice samples were fitted with the cumulative form of a Weibull type distribution of resistances (Peleg & Cole, 1998):

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

where $S(t)$ is the fraction of survivors at a given time and b and n are the scale and the shape parameters, respectively. They were derived using a nonlinear regression technique. The values of b and n were then used to generate the resistance frequency curves using the following equation:

$$\frac{d}{dt_c} = b.n.t_c^{n-1}\exp(-b.t_c^n) \tag{2}$$

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, variance, σ^2_{tc}, and coefficient of "skewness", υ_1) were calculated from the following equations (Peleg & Cole, 1998):

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

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

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

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

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

Additionally, in order to compare microorganism's sensitivity to PL_c and US + PL_c treatments, decimal reduction (D_{10}) dose values were obtained from the linear portion of the dose–response survival curves (Koutchma & Parisi, 2004).

2.6. Native flora

In order to determine the effect of US + PL_c treatment in the evolution of indigenous flora in NAJ, uninoculated and kept overnight (in order to increase native microflora level) apple juice was used for this study. Control and treated by US + PL_c samples were dispensed into 10 mL caramel flasks and cold stored (4 ± 1 °C) for 10 days. Throughout storage, three flasks were taken at preset time intervals (24–72 h) for analysis in triplicate of total mesophilic aerobic and yeast and mold survivors. Total mesophilic aerobic populations were determined on Plate Count Agar (PCA, Britania S.A., Argentina) plates incubated for 72 h at 37 ± 1 °C, whereas yeast and mold populations were cultured on Potato Dextrose Agar (PDA; Britania, Buenos Aires, Argentina; pH: 3.5 adjusted with tartaric acid) plates and incubated at 25 ± 1 °C for 5 days. Plots of log N versus treatment time were obtained. A total count not exceeding 10^7 CFU/mL for the aerobic colony were taken as a requirement according to the Health Protection Agency (2009).

2.7. Color measurement

Color of juices was measured with a handheld tristimulus reflectance spectrocolorimeter (Minolta Co. Model CM-508-d, Japan) by using a 1.4 measuring aperture with white and black background. Control, PL_C and US + PL_C samples were aseptically dispensed into 10 mL caramel flasks in quadruplicate. They were immediately stored in the dark at 4 ± 1 °C for 12 days. During storage, three flasks of each condition were taken at preset time intervals for determination. Three (3) milliliter of sample was measured using an illuminant C and 2° observer. Before the test, the instrument was calibrated with a standard provided by the manufacturer. The CIE color coordinates (X, Y, Z) and the L* (lightness), a* (green–red), and b* (blue–yellow) components of the CIELAB space were recorded. These numerical values were converted into "browning index" (BI) color function using Eqs. (7) and (8) (Buera, Lozano, & Petriella, 1986).

$$\mathrm{BI} = \frac{[100(x-0.31)]}{0.172} \tag{7}$$

where,

$$x = \frac{\mathrm{X}}{\mathrm{X}+\mathrm{Y}+\mathrm{Z}} \tag{8}$$

The relative change between the BI corresponding to a given storage time and initial BI (BI/BI_o) was analyzed at preset time intervals. Multivariate analysis of variance (MANOVA) was applied to detect differences in the L*, a*, b* and BI/BI_o values corresponding to the juices according to the factors "treatment" and "time" and the interaction

"treatment * time". Significance level was set at $p < 0.05$. Multivariate outliers were detected by Mahalanobis distance and removed from the data set. In the case of finding significant differences, post-hoc multiple comparisons among multivariate means of factors were performed by the Hotelling test based on the Bonferroni correction. Statistical analyses were carried out using InfoStat 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina).

2.8. Sensory studies

Only the combined (US + PL_c) treatment was evaluated by this sensory method as it fulfills the 5 log reduction requirement in pathogens issued by the FDA (USFDA, 2001). Combined (US + PL_c) treated NAJ samples were sensory evaluated by untrained panelists to determine overall acceptability and some relevant sensory parameters by applying a consumer field test. In addition, sensory shelf life of combined processed NAJ samples stored under refrigeration (4 ± 1 °C) was estimated by the Survival Analysis Methodology (SAM).

2.8.1. Consumer field test

Seventy seven (77) unpaid volunteers recruited from personnel and students of the Buenos Aires University, 40 male and 37 female aged 25–55 years and consumers of apple juice, participated in the monadic test. Each subject evaluated a 15 mL sample of NAJ treated with US + PL_c. Samples were presented to the panelists at consume temperature (5–7 °C) in red plastic cups. The evaluations were carried out in individual booths under white light (ISO 8589:, 1988). The questionnaire was designed according to the general recommendations cited by Lawless and Heymann (2010) for this type of test. Firstly, the subjects were instructed to judge the general acceptability of each sample on a nine-point hedonic scale (1 dislike extremely; 9 like extremely), item which was immediately followed by open-ended questions for liking or disliking with an appropriate "skip pattern". The skip pattern dropped to reasons for liking if the respondent was positive, and then probed any dislikes, and vice versa. Additionally, more specific attributes were investigated through the use of intensity and just right 5-point scales. In particular, apple flavor was evaluated in a short intensity scale with labeled ends (1: without apple flavor; 5: too much apple flavor). Juice's sour taste was evaluated in a just right scale labeled as "not sour enough" at the left end; "extremely sour" at the right end, and "just right" in the middle. Previous to each session, each scale and terminology contained in the questionnaire was explained several times until the panelists fully understood. Data obtained from panelists were analyzed by converting assigned positions into numbers. Results were reported as an average of the individual values. Responses from open-ended questions were collected and qualitatively analyzed by grouping into categories the common attributes described by the panelists. An agglomerative hierarchical cluster analysis was carried out using weighted average linkage and Euclidean distance to find whether there was segmentation in the preference of consumers for the NAJ processed by US + PL_c (Lawless, 2013). Principal component analysis (PCA) was applied to illustrate the association between the scores obtained for the overall impression with the ones corresponding to the juice's attribute evaluation. The overall goodness of fit was measured by the cophenetic correlation coefficient (CCC) (Lawless, 2013). An adequate fit for the cluster and PCA analyses is described by a CCC value close to 1. Statistical analyses were carried out using InfoStat 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina).

2.8.2. Sensory shelf life estimation

Survival Analysis Methodology (SAM) was used to estimate the sensory shelf life of NAJ processed by US + PL_c and stored at 4 ± 1 °C for 0, 2, 3, 4, 5 or 7 days. Eighty (80) untrained volunteers who were apple juice consumers, from the Buenos Aires University staff, participated in the test. Each panelist evaluated all NAJ samples corresponding to the different storage times previously mentioned, which were randomly presented in red plastic glasses at consume temperature (15 mL; 5–8 °C). Consumers were instructed to taste each sample and answer if they would be willing to consume it by selecting a positive (yes) or negative (no) response. Water was available for rinsing between sample evaluations, which were carried out in individual booths under white light (ISO 8589:, 1988). The survival function $S(t)$ can be defined as the probability of a consumer accepting a product beyond time t, so $S(t) = P(T > t)$. Alternatively, the cumulative distribution rejection function $F(t) = 1 - S(t)$, can be defined as the probability of a consumer of rejecting the juice sample before time t, that is $F(t) = P(T \le t)$, T being the storage time at which the consumer rejects the sample (Hough, 2010). As usual, survival data is not normally distributed; models such as log-linear, log-normal and Weibull distribution for estimating T are chosen (Klein & Moeschberger, 1997). The Weibull distribution was the model that better fitted rejection data among other models in a previous trial (data not shown).

The rejection function given by the Weibull model is:

$$F(t) = 1 - \exp\left[-\exp\left(\frac{\ln(t) - \mu}{\sigma}\right)\right] \quad (9)$$

where μ and σ are the model's parameters.

The shelf life of US + PL_c processed NAJ samples was defined as the time corresponding to a predefined percentage of rejection by consumers, which was fixed at 25% (P_{25}) and 50% (P_{50}) in the present study. According to SAM, subjects who rejected the stored sample at time 0 were discarded from the analysis, and only the responses which met this criterion, were analyzed. Due to the large amount of participants and juice samples that were needed to comply with this procedure, only the juice subjected to the combined treatment US + PL_c was evaluated. Survival analysis calculations were performed using the free software *R statistical package* 3.1.1 (www.r-project.org).

3. Results and discussion

3.1. Effects of single and combined PL treatments on inoculated microorganisms

During PL_c treatments, juice temperature increased with time due to the energy absorption (Ferrario et al., 2013a). On average, temperature of treated samples after 0.73 J/cm^2 (0.0175 J/mL) of PL_c, increased by 4.4 ± 0.3 °C and 6.7 ± 0.5 °C for NAJ and CAJ, respectively. Nevertheless, the final temperature of the juices subjected to single US, PL_c and US + PL_c was always below 25 °C (data not shown).

Fig. 2 shows experimental and predicted by Weibull model *S.* Enteritidis, *E. coli* and *S. cerevisiae* inactivation curves in CAJ and *E. coli* inactivation curve in NAJ processed by single PL_c or the combined US + PL_c treatment at different doses.

In general, PL_c inactivation curves resulted in linear, reaching 4.2 and 1.8 log reductions in CAJ for *S.* Enteritidis and *S. cerevisiae*, respectively, at a maximum dose of 0.73 J/cm^2 (0.0175 J/mL, ***EEO***: 1.8×10^3–4.1×10^3 kW·h/m^3/order) (Fig. 2a, b, c), while 3.1 log reductions were obtained for *E. coli* in both juices (Fig. 2d). In a previous published work we investigated the inactivation of *E. coli*, *S.* Enteritidis and *S. cerevisiae* by PL (batch mode operation, 2.4–71.6 J/cm^2, T < 12 °C, ***EEO***: 6.2×10^6–3.8×10^7 kW·h/m^3/order) in commercial (pH: 3.5, 12.5 °Brix) and naturally squeezed (pH: 3.4, 11.8 °Brix) apple juices (Ferrario et al., 2013a). In that study, inactivation curves showed an important decay during the first 10 s of treatment (<12 J/cm^2) followed by a second period with a lower microbial decrease. In contrast with those findings, in the present work, overall PL_c and US + PL_c survival curves resulted linear. In addition, batch mode PL resulted more effective in CAJ than NAJ due to the lower absorbance, reflection and scattering of light of the former juice, whereas in the present study no significant differences in *E. coli* inactivation by single or combined continuous flow-through PL of CAJ and NAJ were observed. The difference in the

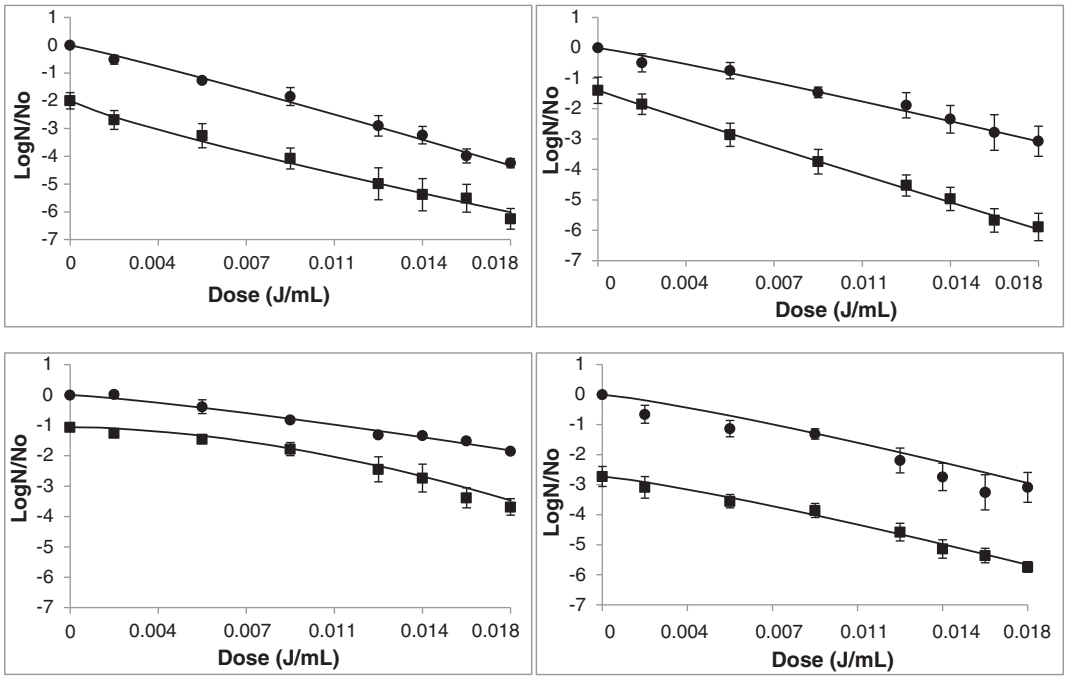

Fig. 2. Experimental survival curves (points) and fitted values derived from the Weibull model (line) for *S.* Enteritidis (a), *E. coli* (b) and *S. cerevisiae* (c) treated in CAJ, and for *E. coli* in NAJ (d). PL_c (●) or US + PL_c (■). (I) Standard deviation.

survival curve shape may be attributed to the lower fluence applied in the present work. Notwithstanding, it is important to highlight that PL_c was more effective in inactivating these microorganisms than batch mode PL in apple juice, as 1.8–4.2 log cycles were achieved after applying 0.73 J/cm^2 of PL_c, whereas 0.4–1.7 were obtained after exposure to batch mode PL at the lowest dose examined (2.4 J/cm^2). Moreover, the continuous PL operation used in this study seemed to be more efficient since its ***E*EO** value was approximately 3 orders of magnitude lower than the ***E*EO** estimated for batch mode PL (Ferrario et al., 2013a). In concordance with this study, Pataro et al. (2011) reported similar inactivation of *E. coli* (~3 log-cycles) in apple juice (pH: 3.5, 10.9 °Brix) after PL exposure (continuous device without recirculation, 0.0–5.5 J/cm^2). Despite the fact that they observed a change in the inactivation curve slope after PL exposure at a dose of 2.5 J/cm^2, they did not study microbial inactivation at low doses as in the present work. Moreover, Muñoz et al. (2012a) studied the inactivation of *E. coli* and *L. innocua* by PL in a continuous device (without recirculation, 3.3 J/cm^2, 42.5 J/mL) in citric acid–disodium phosphate buffer reporting 3.6 and 2.7 log reduction cycles, respectively. In addition, Muñoz et al. (2012b) obtained 3.1 and 4.9 log reductions of *E. coli* after 4.0 J/cm^2 (51.5 J/mL) and 5.1 J/cm^2 (65.4 J/mL) PL exposure (continuous flow) in CAJ (pH: 3.6, 12 °Brix). In the present study, a higher distance from the lamp and a lower residence time were assayed, thus obtaining a lower PL dose compared to the reported literature, but recirculation of juice ensured similar microbial inactivation values.

When US (***E*EO**: 4.4×10^5–1.1×10^6 kW·h/m^3/order) was applied prior to PL_c treatment, slight differences in the shape of the PL_c survival curves were observed comparing to single PL_c treated samples. The use of single US resulted in scarce inactivation as 2.0, 1.4 and 1.1 log reductions of *S.* Enteritidis, *E. coli* and *S. cerevisiae* were achieved in CAJ after 30 min of US treatment, respectively (Fig. 2a, b, c). Subsequent PL_c treatment invoked additional reductions of 4.2, 3.1 and 1.8 log cycles for these strains (Fig. 2a, b, c). Consequently, the combined treatment US + PL_c led up to 6.3, 5.9 and 3.7 log reductions for *S.* Enteritidis, *E. coli* and *S. cerevisiae*, respectively, in CAJ (Fig. 2a, b, c). Conversely, 2.7 and 3.1 log reductions of *E. coli* in NAJ were obtained after single US and PL_c, respectively, while application of US + PL_c, provoked 5.7 *E. coli* log reductions (Fig. 2d). Therefore, US + PL_c treatment exhibited an additive effect as the inactivation obtained after combination of the mentioned hurdles was equal to the sum of both effects taken separately. The ***E*EO** values (***E*EO:** 441,800–1,104,100 kW·h/m^3/order) estimated for the combined treatment US + PL_c, as the sum of the individual ***E*EO**s, were closer to the ***E*EO** corresponding to the single US treatment since this value was significantly higher in proportion (2–3 orders of magnitude) than the ***E*EO** value corresponding to PL_c. Literature focused on ***E*EO** estimation is available mainly for measurements of the cost effectiveness of UV based AOP (Advanced Oxidation Process) systems (Behnajady, Vahid, Modirshahla, & Shokri, 2009; Li, Hokanson, Crittenden, Trussell, & Minakata, 2008). Unfortunately, there is a lack of information regarding ***E*EO** estimation for reducing microbial load in juices or other food matrixes processed by PL or UV-C light for comparison purposes.

Muñoz et al. (2012a) studied the combination of PL (42.5 J/mL, 0.019 m from the lamp) with thermosonication (TS, 500 W, 20 KHz, 48–50 °C, 100 J/mL) in a continuous flow system, for inactivating *E. coli* and *L. innocua* inoculated in buffer solutions (pH 4.0, for *E. coli* and pH 7.0, for *L. innocua*). They observed that the combined treatment contributed to an additive increase in the inactivation by PL_s for *Listeria innocua*, but not for *E. coli*. Moreover, Palgan et al. (2011a) reported that combinations of PL (continuous device, 0.019 m from the lamp, 3.3 J/cm^2) and manothermosonication (20 kHz, 43 °C, 750 W, 400 kPa) led to 6 log reductions of *E. coli* and *Pichia fermentans* in a fresh blend of apple cranberry juice. These authors were not able to detect the presence or absence of synergistic or additive effects as very low counts which were obtained after the application of combined treatments. Besides, Muñoz et al. (2011 and 2012b) studied the combination of PL (35 °C, 4.03–5.10 J/cm^2) and TS (24 kHz, 400 W, 14 mL/min, 40 °C or 8 mL/min, 53 °C) in the inactivation of *E. coli* in orange and apple juices in a continuous device. In agreement with our results, they found that the combined treatments showed an additive effect, reaching ~5.0 log reductions in apple juice and ~3.2 log in orange juice. In a previous research, we also observed an additive effect when applying US (600 W, 20 kHz and 95.2 μm wave amplitude; 10 or 30 min at 20, 30 or 44 ± 1 °C) combined with PL (batch mode, 23.9 or 71.6 J/cm^2, T_{final}: 12–56 °C) in *S. cerevisiae* cells in CAJ and NAJ

Table 2
Decimal reduction dose (D_{10}), Weibull model parameters (*b* and *n*) and related statistics[a] corresponding to *E. coli*, *S. cerevisiae* and *S.* Enteritidis survival in CAJ and NAJ treated with PL_c or $US + PL_c$.

System	Strain	First-order model		Weibull model						
		D_{10} (J/cm^2)	R^2_{adj}	b (min^{-n})	n (−)	R^2_{adj}	t_{cm} (min)	$\overline{t_c}$ (min)	$\sigma^2 t_c$ (min^2)	v_1 (−)
PL_c/CAJ	*S.* Enteritidis	5.97*** (0.23)	0.99	0.36*** (0.05)	1.08*** (0.07)	0.99	0.23	2.50	5.37	1.97
	E. coli	4.18*** (0.23)	0.98	0.27*** (0.07)	1.05*** (0.12)	0.98	0.19	3.41	10.57	2.02
	S. cerevisiae	2.84*** (0.14)	0.99	0.11** (0.03)	1.22*** (0.12)	0.98	1.50	5.72	22.20	1.78
PL_c/NAJ	*E. coli*	4.43*** (0.37)	0.95	0.49** (0.11)	0.81*** (0.11)	0.95	–	2.70	11.35	2.69
$US + PL_c$/CAJ	*S.* Enteritidis	5.67*** (0.22)	0.99	0.58*** (0.09)	0.84*** (0.07)	0.99	–	2.10	6.29	2.58
	E. coli	6.40*** (0.19)	0.99	0.49*** (0.04)	0.97*** (0.04)	0.99	–	2.11	4.75	2.19
	S. cerevisiae	3.63*** (0.37)	0.93	0.04** (0.01)	1.78*** (0.14)	0.98	3.84	5.42	9.93	1.40
$US + PL_c$/NAJ	*E. coli*	4.17*** (0.22)	0.99	0.19*** (0.04)	1.19*** (0.10)	0.99	0.86	3.80	10.30	1.81

(value), standard error of each parameter. The Weibull model: t_{cm}, distribution mode, $\overline{t_c}$ distribution mean, $\sigma^2 t_c$ variance, and $\boldsymbol{v_1}$ coefficient of skewness.
*** Significant at the 0.1% level.
** Significant at the 1% level.
[a] R^2_{adj}, adjusted determination coefficient for both models.

(Ferrario et al., 2015). It is worthy to note that the majority of binary or ternary non-thermal treatment combinations reported in the literature described additive more than synergy inactivation responses (Guerrero et al., 2014).

Nonlinear semilogarithmic survival curves were characterized by the Weibull distribution of resistance model. Table 2 displays the estimated parameters obtained from fitting single PL_c and $US + PL_c$ experimental data to this model, and it also enumerates the specific statistics related to the weibullian distribution calculated according to Eqs. (3) to (6). The weibullian model was appropriate for representing survival data, as the estimated parameters were significant ($p < 0.01$). High R^2_{adj} values were obtained which showed that between 95.0% and 99.1% of the variation in the experimental data could be explained by the selected model (Table 2).

Overall, *S.* Enteritidis and *E. coli* PL_c survival curves showed n values around 1, as expected for a linear response. In contrast, *S. cerevisiae* survival curves exhibited downward concavity ($n > 1$, Table 2). No differences in the weibullian related statistics corresponding to *E. coli* were observed for each treatment between CAJ and NAJ indicating that applied treatments showed similar effectiveness in both matrixes (Table 2). *S.* Enteritidis showed the highest sensitivity to both treatments as it exhibited the lowest mean, followed by *E. coli* and *S. cerevisiae*. Consistently with the mean values obtained, decimal reduction doses (D_{10}) also reflected that *S.* Enteritidis proved to be the most sensitive strain to PL_c, followed by *E. coli* and *S. cerevisiae* in apple juice (Table 2). In addition, *E. coli* and *S. cerevisiae* showed higher D_{10} values when $US + PL_c$ was applied compared to those corresponding to PL_c processed CAJ

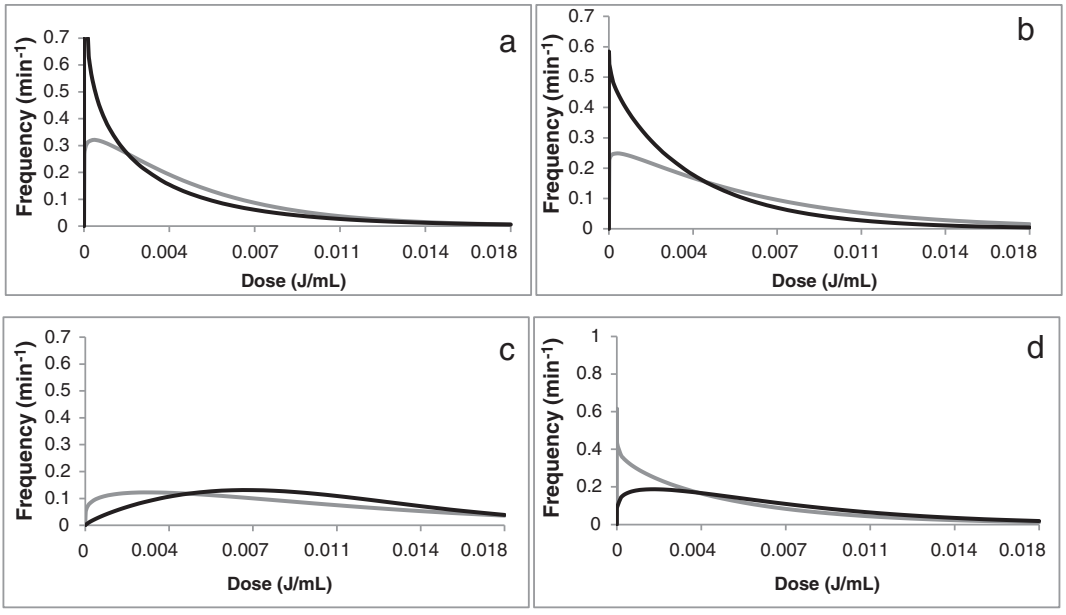

Fig. 3. Frequency distribution of resistances corresponding to *S.* Enteritidis (a), *E. coli* (b), and *S. cerevisiae* (c) in CAJ, and *E. coli* (d) in NAJ survival curves treated with PL_c (▬) or $US + PL_c$ (▬).

(Table 2), suggesting the higher sensitivity of these strains to the combined treatment.

In agreement with our findings, several studies reported that the Weibull model could quantitatively describe microbial inactivation by PL in liquid (Ferrario et al., 2013a; Sauer & Moraru, 2009; Uesugui, Woodling, & Moraru, 2007), and in solid substrates (Bialka, Demirci, & Puri, 2008; Izquier & Gómez-López, 2011).

The frequency distributions, generated according to Eq. (3) corresponding to microbial survival curves in apple juice treated by PL_c and US + PL_c are shown in Fig. 3. Frequency distributions corresponding to *S.* Enteritidis and *E. coli* in CAJ treated by PL_c exhibited mode, while US + PL_c ones did not; therefore, the combined treatment improved the observed inactivation for these strains (Fig. 3a–b). *S. cerevisiae* in CAJ treated with PL_c and US + PL_c, exhibited a large mode and a notorious tail, and high variance compared to the frequency shapes corresponding to the other strains (Fig. 3c), according to its high resistance to death by PL. No differences were observed for frequency distributions of resistances corresponding to *E. coli* subjected to single PL_c or US + PL_c (Fig. 3d).

It is worthy to remark that previous studies have shown the existence of viable non-culturable cells (VBNC) after single PL exposure (Ferrario, Guerrero, & Alzamora, 2013b; Rowan , Valdramidis, & Gómez-López 2015), which are incapable of undergoing cellular division, but are metabolic active cells, which could seriously affect shelf life in PL treated foods (Zhao et al., 2011). Nevertheless, recent studies showed that apple juice subjected to the combination of US (10, 30 min, T: 20 or 44 °C) and PL (batch mode, 71.6 J/cm^2, T_{final}: 12 or 56 °C) treatments ensured the absence of these VBNC (Ferrario, Alzamora, & Guerrero, 2014).

3.2. Native flora

The evolution of native flora (mesophilic aerobes and yeasts and molds) existent in NAJ without any treatment (control) and after US + PL_c treatment during the 10 day-storage (4 ± 1 °C) is shown in Fig. 4. The aerobic mesophilic and mold and yeast counts were 2.2 and 2.5 log CFU/mL in control NAJ, respectively, while 2.2 and 2.0 log CFU/mL were obtained after the combined treatment. Despite the fact that US + PL_c was not able to reduce the indigenous flora counts immediately after treatment, it prevented its recovery for 6 days. While by this day, untreated yeast and mold counts increased by more than 2 log cycles. Besides, the recommended limit for aerobic mesophilic counts (Health Protection Agency, 2009) was not exceeded by 10 days of refrigerated storage. Previous studies demonstrated (Ferrario et al., 2015) a scarce decrease of aerobic mesophilic and mold and yeast counts after exposure to single PL (batch mode, 71.6 J/cm^2) in apple, orange and strawberry juices, and no significant differences in the evolution of treated and control samples during storage at 5 °C. Conversely, the delay in the mold and yeast recovery observed in the present study could be attributed to an additive inhibitory effect between US and PL_c. In agreement, Palgan et al. (2011b) studied the shelf life of a blend of apple and cranberry juice treated with a combination of PL (continuous flow, 360 μs, 3 Hz, 1.213 J/cm^2/pulse) and PEF (34 kV/cm, 18 Hz, 93 μs) during cold storage. They reported that although no counts of mesophilic aerobes and molds and yeasts were observed after PL treatment, an increase of 2 log-cycles was obtained for both after 14 days of storage at 4 °C.

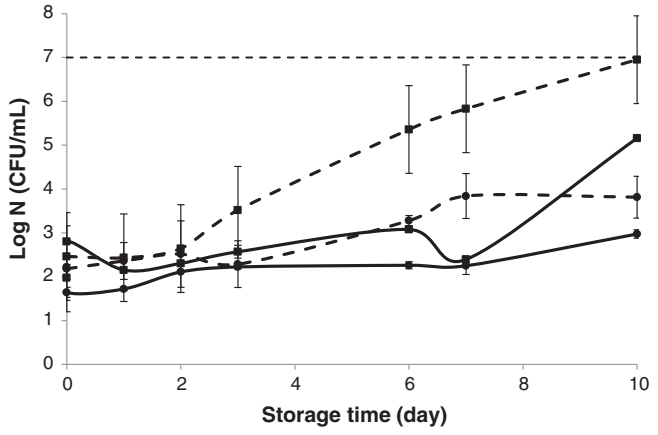

Fig. 4. Evolution of indigenous flora in NAJ stored at 4 ± 1 °C. Control (dotted line) and US + PL_c treated (solid line) of mesophilic aerobic (●) and yeast and mold counts (■). (I) Standard deviation.

3.3. Color measurement

The evolution of average L*, a*, b* and BI/BI_o values corresponding to US + PL_c treated CAJ and NAJ during storage at 4 ± 1 °C are presented in Table 3. The two-way MANOVA revealed the significance of the cross-product term *time* * *treatment* ($p < 0.001$), indicating that the evolution of juice color depended on the applied treatment.

Immediately after treatment, CAJ samples processed by PL_c and US + PL_c exhibited a decrease in a* and an increase in b* values with respect to the control samples, while US + PL_c treated samples exhibited the lowest luminosity. During refrigerated storage of CAJ samples, L* increased and a* decreased in control single PL_c samples turning them less green and with higher luminosity. On the other hand, US + PL_c treated CAJ samples exhibited a decrease in b* value (more bluish samples) during cold storage (Table 3a).

Regarding NAJ samples, they exhibited a decrease in L* and an increase in b* values immediately after PL_c and US + PL_c treatment with respect to control samples. The highest b* values were recorded for

Table 3
Mean values and standard deviations (±) of color parameters and BI/BI_o function corresponding to commercial apple juice, CAJ (a) and natural apple juice, NAJ (b) with different treatments and cold stored for 12 days.

Treatment	Time (day)	L*	a*	b*	BI/BI_o	sd[a]
a)						
Control	0	42.8 ± 0.6	−2.4 ± 0.2	8.9 ± 0.6	1.0 ± 0.1	b
	2	49.4 ± 0.7	−2.7 ± 0.1	10.9 ± 0.4	1.1 ± 0.0	ce
	5	48.3 ± 0.2	−2.8 ± 0.1	10.9 ± 0.5	1.1 ± 0.0	cd
	7	49.2 ± 0.4	−2.8 ± 0.0	11.2 ± 0.7	1.3 ± 0.1	e
	12	47.7 ± 0.3	−2.7 ± 0.2	9.8 ± 0.7	1.0 ± 0.4	d
PL_c	0	41.7 ± 0.4	−3.4 ± 0.1	12.1 ± 0.2	1.0 ± 0.0	a
	2	49.0 ± 0.4	−3.8 ± 0.1	14.1 ± 1.0	1.0 ± 0.1	h
	5	48.9 ± 0.7	−3.8 ± 0.1	13.7 ± 0.5	1.0 ± 0.1	fh
	7	48.5 ± 0.3	−3.8 ± 0.1	13.7 ± 0.5	1.0 ± 0.0	h
	12	48.6 ± 0.8	−3.6 ± 0.1	11.8 ± 0.4	0.9 ± 0.0	f
US + PL_c	0	41.3 ± 0.2	−3.3 ± 0.1	14.0 ± 0.9	1.0 ± 0.1	g
	2	42.5 ± 0.8	−3.3 ± 0.1	11.8 ± 0.9	0.8 ± 0.1	i
	5	43.0 ± 0.8	−3.2 ± 0.1	11.2 ± 0.7	0.7 ± 0.1	i
	7	43.0 ± 0.2	−3.3 ± 0.1	12.4 ± 0.3	0.8 ± 0.0	i
	12	42.3 ± 0.8	−3.4 ± 0.1	12.7 ± 0.4	0.9 ± 0.0	gi
b)						
Control	0	49.3 ± 0.4	−1.3 ± 0.2	5.19 ± 0.7	1.00 ± 0.2	fg
	2	49.6 ± 0.2	−1.2 ± 0.0	5.23 ± 0.6	1.03 ± 0.1	fg
	5	49.0 ± 0.3	−1.2 ± 0.1	4.88 ± 0.5	0.95 ± 0.1	fg
	7	49.1 ± 0.6	−1.2 ± 0.1	5.39 ± 0.4	1.08 ± 0.1	fg
	12	49.1 ± 0.3	−1.1 ± 0.1	5.81 ± 0.5	1.19 ± 0.1	f
PL_c	0	48.0 ± 0.1	−1.5 ± 0.1	10.43 ± 1.2	1.00 ± 0.1	de
	2	47.7 ± 0.2	−1.4 ± 0.1	11.40 ± 0.7	1.12 ± 0.1	d
	5	47.5 ± 0.3	−1.5 ± 0.2	10.06 ± 0.3	0.97 ± 0.1	e
	7	47.6 ± 0.3	−1.3 ± 0.1	10.21 ± 0.5	1.00 ± 0.1	e
	12	47.6 ± 0.3	−1.2 ± 0.1	10.72 ± 0.3	1.07 ± 0.0	de
US + PL_c	0	36.9 ± 0.6	−0.8 ± 0.1	23.33 ± 0.9	1.00 ± 0.0	bc
	2	37.1 ± 1.5	−1.0 ± 0.1	23.00 ± 1.7	0.98 ± 0.1	bc
	5	38.0 ± 0.7	−1.0 ± 0.1	22.90 ± 1.0	0.96 ± 0.1	c
	7	37.8 ± 0.8	−1.0 ± 0.1	23.15 ± 1.0	0.99 ± 0.1	c
	12	36.7 ± 0.5	−1.0 ± 0.1	22.73 ± 0.6	1.01 ± 0.2	b

[a] Different letters indicate significant differences (sd) among mean values performed by the Hotelling test based on the Bonferroni correction.

the combined treatment (less bluish samples), while PL_c samples were less green (lowest a*) followed by control and US + PL_c treated samples (Table 3b). During cold storage of all treated NAJ samples, no differences in color parameters were observed.

Interestingly, BI/BI_o values corresponding to US + PL_c treated CAJ and NAJ samples were the lowest ($p < 0.05$), indicating that the combined treatment prevented them from turning darker and brownish. It is well known that enzymatic browning is a consequence of polyphenol oxidase (PPO) catalyzed oxidation of phenolic substrates into quinones, which undergo further reactions which produce dark pigments called melanins. Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, and Soliva-Fortuny (2010b) reported that mushrooms flashed at doses below 12 J/cm^2 prevented samples from turning darker compared to untreated ones, suggesting that PPO could be inhibited by PL. Accordingly, Manzocco, Panozzo, and Nicoli (2013) studied the inactivation of PPO solutions (potassium phosphate buffer) by PL (batch mode, 0–1.75 J/cm^2) observing that enzyme activity progressively decreased as fluence increased, obtaining up to 7.6–23.4% activity, depending on the initial enzyme concentration. Moreover, US treatment (75–373 W/cm^2, 2–10 min) has shown to inactivate PPO in melon juice (Vidal Fonteles et al., 2012). These authors observed that a higher decrease in residual PPO activity was observed with US power intensity increase, and they reported that the highest US treatment conditions (373 W/cm^2—10 min) were the most effective in reducing PPO activity. A power intensity of 452 W/cm^2 and 30 min of sonication were applied in the present work. Therefore, the lower increase in the browning index observed for apple juice samples subjected to US + PL_c might be attributed to a possible PPO inactivation achieved by these technologies.

Muñoz et al. (2012b) examined the changes in L*, a*, and b* parameters corresponding to apple juice (pH: 3.6; 12 °Brix) treated with thermosonication (4–14 mL/min; 24 kHz, 40–50 °C) combined with PL in continuous flow arrangement (13.4–17 mL/min; 4.0–5.1 J/cm^2). In this work, authors reported a decrease in the L*, a* and b* values of combined treated samples compared to untreated ones. Otherwise, Palgan et al. (2011b) reported no differences in non-enzymatic browning index, L*, a*, and b* between untreated (pH: 3.7; 11.8 °Brix) and PL (batch mode, 0–28 J/cm^2) treated apple juice samples.

3.4. Sensory evaluation of US + PL_c treated apple juice

NAJ samples processed by US + PL_c were evaluated by using a consumer field test to determine the overall sensory impression and their opinion about some relevant sensory juice characteristics. The overall acceptability of samples averaged 6.1 in the 9-point hedonic scale, which corresponded to the category “like slightly” in the used scale. However, by applying cluster analysis for segmentation of consumer group preferences, two clusters emerged: cluster 1 (C1), with 22 consumers, encompassing categories 2 to 5 in the 9-point hedonic scale, and cluster 2 (C2), with 55 consumers, including categories 6 to 9 (data not shown). The CCC value obtained was 0.84, indicating that a good fit was achieved by this analysis. C2 had a marked interest in the product, exhibiting a liking overall mean of 7.0 in the 9-point hedonic scale (corresponding to the category “like it moderately”). They also significantly perceived the sour taste (3.6 ± 0.6), closer to the just right value (2.5) than C1 (4.5 ± 0.5) ($p < 0.0001$). Therefore, NAJ processed by US + PL_c exhibited a maximal appeal to a group of consumers, in agreement with the segmentation approach which manufacturers have largely embraced in recent years. This approach considers that constructing different products to groups of consumers who display different tastes will exhibit more acceptability than developing a unique product to the entire pool of users (Lawless, 2013). Open-ended questions revealed that a fresh natural apple taste was the attribute mostly highlighted by the consumers. A principal component analysis (PCA) was performed in order to assess the relationship between the scores corresponding to the hedonic scale and the % of responses in the apple flavor intensity and optimal sour taste of the juices. The two-dimensional representations (PCA bi-plot) are presented in Fig. 5. The CCC value was 1.0, indicating that an accurate dimension reduction was achieved with the analysis. The first two principal components (PC_1 and PC_2) explained 100% of the total variance. The first two PC (Fig. 6) explained 79.5% and 20.5% of the variance, respectively. PC_1 separated the apple flavor intensity, which was associated positively, from optimal sour taste (negatively associated). On the other hand, PC_2 was associated positively with both juice's attributes. This analysis showed that C1 (scores from 2 to 5) was associated with extreme sour taste and low apple flavor, while C2 (scores from 6 to 9) was associated with high apple flavor intensity and optimal sour taste.

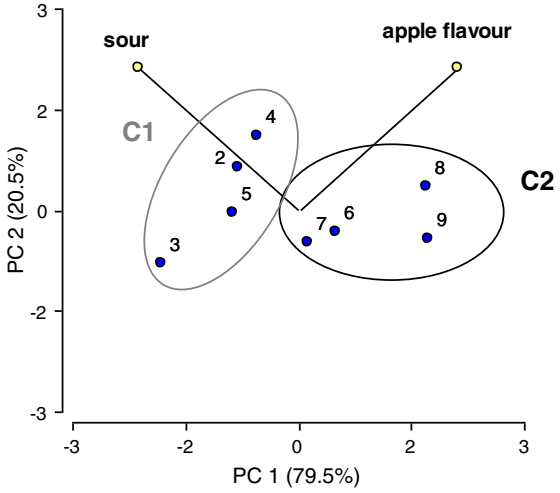

Fig. 5. Principal component analysis (PCA) bi-plot of the scores assigned by the panelists in the overall impression test, and the scores assigned during the evaluation of apple flavor intensity and optimal sour taste of NAJ processed by US + PL_c. Circles encompass scores corresponding to C1 and C2.

In agreement with our results, Bleibaum et al. (2002) evaluated the overall acceptance of commercial apple juice and commercial apple juice supplemented with 3 g/L citric acid. The former juice exhibited 6.6 points in a 9-point hedonic scale, while the latter received 5.0 points, based on responses from 97 consumers.

3.5. Shelf life estimation

Due to the fact that the combined US + PL_c treated apple juice exhibited adequate acceptability, and some positive characteristics, as its fresh-like appearance and natural apple flavor, were highlighted by most of the consumers, further sensory shelf life studies were conducted. Sensory shelf life of NAJ processed by US + PL_c was estimated by survival analysis focusing on the consumer rejecting the product, within the microbiological shelf life already estimated. The maximum likelihood estimates of the parameters by using the Weibull distribution function were $\mu = 2.00 \pm 0.05$ and $\sigma = 0.17 \pm 0.15$. Fig. 6 shows the estimated cumulative distribution function $F(t) = P(T \leq t)$ obtained by replacing these values in Eq. (9), which represents the percentage of rejection versus US + PL_c treated juice storage time. According to this plot, the shelf life for a 50% probability of consumer rejection was estimated with 95% of confidence in 7 ± 1 days. For a 25% probability of consumer rejection the shelf life would be estimated in 6 ± 1 days.

4. Conclusions

This study contributed to give an alternative to the use of novel technologies when applied to real food systems and to show how they influence microbial stability, some food quality parameters and sensory shelf life. In particular, this work provided quantitative information about the inactivation of some targeted microorganisms, and overall quality of apple juice treated with single continuous flow-through pulsed light

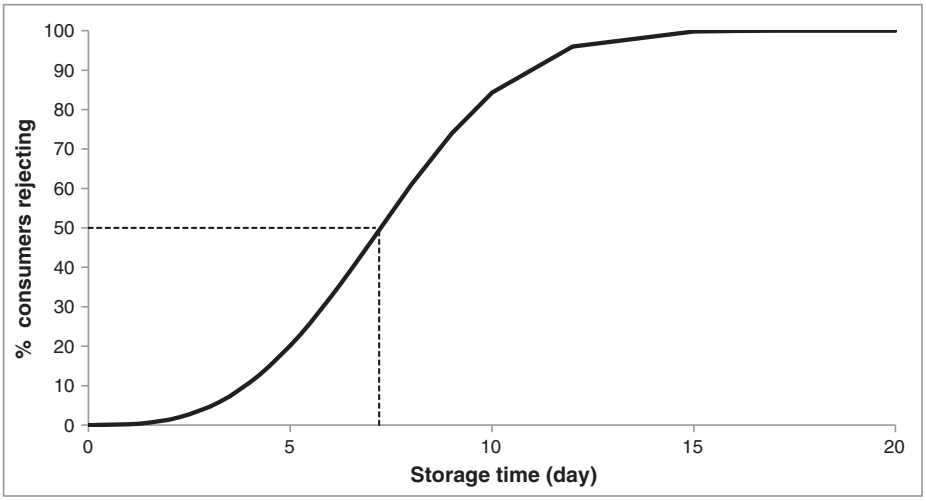

Fig. 6. Percentage of consumers rejecting NAJ processed by US + PL_c along storage time.

(PL_c), or combined with ultrasound. The combination of US and PL_c turned out to be a promising alternative in inactivating several relevant microorganisms. The Weibull model puts on evidence that, though the combined treatment reduced microorganism levels on juices, there were always residual survivors. In addition, the combined treatment US + PL_c delayed mold and yeast recovery for seven days along cold storage, showing an additive inhibitory effect between US and PL_c. Moreover, the proposed combined treatment exhibited the lowest BI/BI_o increase through storage when compared to untreated and PL_c treated samples. Segmentation approach revealed that a group of consumers showed a very strong interest in the sour taste of the product and its strong apple flavor. Nevertheless, further studies focused on extending juice shelf life need to be carried out, involving the use of both US and PL in a continuous flow-through system. Moreover, further research on PPO activity of treated juices will be conducted in order to characterize browning development.

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