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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