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Study of the inactivation of spoilage microorganisms in apple juice by pulsed light and ultrasound

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ABSTRACT

The aim of this study was to evaluate the effect of ultrasound (US) (600 W, 20 kHz and 95.2 μm wave amplitude; 10 or 30 min at 20, 30 or 44 ± 1 °C) and pulsed light (PL) (Xenon lamp; 3 pulses/s; 0.1 m distance; 2.4 J/cm^2–71.6 J/cm^2; initial temperature 2, 30, 44 ± 1 °C) on the inactivation of *Alicyclobacillus acidoterrestris* ATCC 49025 spores and *Saccharomyces cerevisiae* KE162 inoculated in commercial (pH: 3.5; 12.5 °Brix) and natural squeezed (pH: 3.4; 11.8 °Brix) apple juices. Inactivation depended on treatment time, temperature, microorganism and matrix. Combination of these technologies led up to 3.0 log cycles of spore reduction in commercial apple juice and 2.0 log cycles in natural juice; while for *S. cerevisiae*, 6.4 and 5.8 log cycles of reduction were achieved in commercial and natural apple juices, respectively. In natural apple juice, the combination of US + 60 s PL at the highest temperature build-up (56 ± 1 °C) was the most effective treatment for both strains. In commercial apple juice, US did not contribute to further inactivation of spores, but significantly reduced yeast population. Certain combinations of US + PL kept on good microbial stability under refrigerated conditions for 15 days.

1. Introduction

Consumers are increasingly aware of health benefits and risks associated with food consumption and tend to opt for healthy foods that have been subjected to less extreme treatments (less heat and chill damage), with lower levels of salts, fats, acids, and sugars and/or the complete or the partial removal of chemically synthesized additives. Thermal processing ensures the safety and shelf life of fruit juices, but can result in the loss of this claimed sensory and nutritional quality. In the last decade, emerging preservation procedures have been included as hurdles in combined preservation systems to ensure food safety and to retain or improve food quality (Alzamora et al., 2011). This type of processing techniques for food preservation reduce microbial load and at the same time, allow better retention of product flavour, texture, colour and nutrient content than comparable conventional treatments. There is a wide range of novel alternative physical agents, intensely investigated in the last 25 years, which can cause inactivation of microorganisms at ambient or sublethal temperatures (*i.e.*, high hydrostatic pressure, pulsed electric fields (PEF), ultrasound (US), pulsed light (PL), and ultraviolet light (UV), among others). The choice of non-thermal hurdles involved in the combined processes depends on the target within the microbial cells (e.g. cell membrane, DNA or enzymes system) or the extrinsic environment surrounding them (e.g. pH, temperature or water activity).

Because of its acidity, fruit juices were thought to be spoiled by yeasts, predominantly *Saccharomyces* spp. (Pontius et al., 1997; Martinez et al., 2000); moulds like *Aspergillus ochraceus*, and lactic acid bacteria like *Lactobacillus* and *Leuconostoc* spp. (Stratford et al., 2000) However, *Alicyclobacillus acidoterrestris* is a thermoacidophilic, heat-resistant bacteria that is capable of surviving the pasteurization procedures normally applied to fruit juices (Bahçeci et al., 2005). Therefore, the endospores can germinate and increase in products to cell concentrations high enough to produce taint compounds (Smit et al., 2010). Few scientific studies focus on the effects of emerging preservation technologies to inactivate *A. acidoterrestris*. Baysal et al. (2013) examined the efficiency of UV-C (0.5 J/cm^2) at constant depth (0.15 cm), as an alternative to thermal treatment, on the inactivation of *A. acidoterrestris* spores in fruit juices. They reported significant population reductions in white grape (5.5 log-cycles) and apple (2.1 log-cycles) juices.

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Among physical hurdles, US (*i.e.* intensities higher than 1 W/cm^2; frequencies between 18 and 100 kHz) has attracted considerable interest in food preservation applications (Knorr et al., 2004; Alzamora et al., 2011). The lethal effect of US has been attributed to the cavitation phenomenon, in which microbubbles of gas and/or vapour formed within a liquid, undergo violent collapse during the compression cycle of the wave (Guerrero et al., 2001, 2005). The mechanism of microbial inactivation is mainly due to the thinning of cell membranes, localized heating and production of free radicals (Butz and Tauscher, 2002; Alzamora et al., 2011). The use of ultrasonic waves as a unique preservation factor is unable to effectively kill all the microorganisms since the high levels needed of US could adversely modify nutritional and sensory properties of the food. This limitation has suggested that US could be more effective when used in combination with other techniques such as heat (Guerrero et al., 2001; Bermúdez-Aguirre and Barbosa-Cánovas, 2008), pressure (Raso et al., 1998), natural antimicrobials (Guerrero et al., 2005; Ferrante et al., 2007), UV light (Lopez-Malo et al., 2005a) for inactivation of some pathogenic and spoilage microorganisms.

Implementation of PL to inactivate microorganisms has gained interest due to the short-time treatments involved (Krishnamurthy et al., 2010). This technology uses short time pulses (100–400 μs) of an intense broad spectrum between 100 and 1100 nm with 54% of emitted energy in the ultraviolet range (Gómez-López et al., 2007; Oms-Oliu et al., 2010). Exposure to PL causes the formation of pyrimidine dimmers which impairs the process of cell replication (photochemical mechanism) (Gómez-López et al., 2007). Moreover, membrane disruption was also reported (Wekhof, 2000; Ferrario et al., 2013a) as a result of a momentous overheating. This phenomenon is attributed to a difference in UV light absorption between the microorganism and its surrounding environment (photothermal effect). Besides, structural damages in microbial cells like cytoplasmic membrane shrinkage were also reported (photophysical effect) by Krishnamurthy et al. (2010). It is possible for these mechanisms to coexist and, the relative importance of each one would depend on the fluence and target microorganism (Gómez-López et al., 2007). PL application in fruit juices is very promising as some studies reflect its effectiveness as a decontamination technique. Among them, Sauer and Moraru (2009) studied the inactivation of *Escherichia coli* in apple juice (pH: 4.0; 11.3 °Brix) and reported 2.7 log reductions (12.6 J/cm^2, 1.3 mm layer liquid thickness) under static conditions. In addition, they observed up to 7.3 log reductions (8.8 J/cm^2) when generated turbulence in the liquid substrate during the PL treatment (3000 rpm, orbital shaker).

Certain combinations of PL with other technologies like PEF, manothermosonication, UV-C and heat have already been reported for microbial inactivation (Palgan et al., 2011; Muñoz et al., 2011, 2012a, 2012b; Marquenie et al., 2003). In particular, Muñoz et al. (2011, 2012a) assayed combinations of PL and thermosonication (TS) in a continuous flow system to inactivate *E. coli* in orange and apple juices. They observed that all the different combinations tested showed an additive effect. Combinations of PL with mild temperatures were also reported. Marquenie et al. (2003) found that the combination of PL with thermal treatment at 40 °C in culture media had a significant higher fungal inactivation than single treatments. (In addition, Krishnamurthy et al. (2007) suggested that the heat produced during the PL process itself could have been responsible for higher *Staphylococcus aureus* inactivation in milk treated with PL in a continuous flow.

The combination of US and PL may improve food microbial stability due to their different inactivation mechanisms. The purpose of this study was to analyse the response of *Saccharomyces cerevisiae* cells and *A. acidoterrestris* spores in apple juice as affected by combinations of US and PL with different temperature build-up generated from the PL process itself. Storage studies were also carried out for some US-PL combinations to assess the evolution of treated microorganisms under refrigerated conditions.

2. Materials and methods

2.1. Strains and preparation of inocula

Experiments were performed using *S. cerevisiae* KE162 and *A. acidoterrestris* ATCC 49025 spores. Initial yeast inoculum was prepared by transferring a loopful of Potato Dextrose Agar (PDA; Britania, Buenos Aires, Argentina) slant stock culture to a 20 mL Erlenmeyer-flask of Sabouraud Dextrose Broth (Britania, Buenos Aires, Argentina). It was incubated at 27 ± 1 °C under agitation for 24 h until it reached stationary phase. Yeast inocula was harvested by centrifugation (5000 rpm, 5 min) (Labnet International Inc., Edison, NJ, USA), washed twice with saline and re-suspended in peptone water to give a cell density of 10^7–10^8 CFU/mL. For bacterial culture, the initial inoculum was prepared by transferring a loopful of a fresh stock culture maintained in *Bacillus acidoterrestris* medium (BAM) to an Erlenmeyer-flask containing 20 mL of BAM Broth and subsequently incubated at 43 ± 1 °C for 24 h. The production of spores was performed by sowing the inoculum onto bottles containing *A. acidoterrestris* medium (AAM) and incubating 1 week at 43 ± 1 °C. Spores were removed by the procedure explained by Silva and Gibbs (2001), and maintained at −18 ± 1 °C until use. The BAM used in this study was prepared according to Silva and Gibbs (2001).

2.2. Apple juice samples

Commercial (CEPITA, Coca- Cola, Argentina) (pH: 3.5 ± 0.1; 12.5 ± 0.1 °Brix; $A_{660\ nm}$: 0.063 ± 0.003) and natural squeezed apple juice (*Pyrus malus* L, var Granny Smith, pH: 3.4 ± 0.1; 11.8 ± 0.6 °Brix; $A_{660\ nm}$: 0.071 ± 0.005) were used in this study. Commercial apple juice characterized by a high penetration of light was used in order to compare it with a matrix with dissolved particles. Natural juice was aseptically obtained from fruits that were rinsed with 0.02% sodium hypochlorite and sterile water to eliminate surface microbial load and gently dried with a sterile cloth. Juice was obtained under aseptic conditions in a 90% ethanol sanitized and 10 min UV-C exposed household juicer (Bluesky, Ningbo, China), centrifuged in order to reduce pulp amounts (5000 rpm, 10 min) (Eppendorf, model 5804 R, Hamburg, Germany) and collected for subsequent processing. Juice turbidity was measured by centrifuging samples (1500 rpm, 10 min), and measuring the supernant absorbance at 660 nm (Rivas et al., 2006). Measurements were performed in triplicate. For these studies, uninoculated juice samples were used.

2.3. Ultrasonic treatment

Treatments were carried out in a 150 mL-double wall cylindrical vessel (diameter 6.3 cm; height: 7.6 cm) connected to a thermostatically controlled water bath (HAAKE, Model Rotovisco RV12, Germany), to attain 20, 30 or 44 ± 1 °C in the vessel. Ninety-five (95 mL) of juice were poured into the vessel. Ultrasound (Vibracell®, net power output: 600 W, Sonic Materials Inc., Newtown, CT, USA) at 20 kHz and 95.2 (80%) μm of wave amplitude was applied to the medium with an immersed 13 mm diameter probe. The equipment has automatic amplitude compensation to ensure uniform probe amplitude regardless of the varying loading conditions and line voltage fluctuations. The probe was previously calibrated following the steps of the manufacturer. After three minutes of sonication, the desired temperature was reached, and it was maintained constant at the predetermined temperature value

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