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The effect of pulsed electric fields (PEF) in combination with high intensity light pulses (HILP) on *Escherichia coli* inactivation and quality attributes in apple juice

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ABSTRACT

Treatments involving pulsed electric fields (PEF) in combination with high intensity light pulses (HILP) were applied to reconstituted apple juice in a continuous system using a 2 × 4 factorial design, with sequence and energy levels as main factors. Two PEF field strengths (24 kV/cm or 34 kV/cm) were selected (treatment time 89 μs each) corresponding to "high" (H) and a "low" (L) energy inputs (261.9 and 130.5 J/ml, respectively). Juice was also pumped through a HILP system (pulse length 360 μs, frequency 3 Hz) and exposed to energy dosages of 5.1 J/cm^2 (H) or 4.0 J/cm^2 (L) corresponding to 65.4 and 51.5 J/ml, respectively. Microbiological analysis was performed by inoculating juice with *Escherichia coli* K12 and counting microbial populations pre- and post-processing. Selected physical and chemical quality attributes were compared with those of unprocessed controls. A sensory evaluation was conducted using 31 untrained panellists and the products compared to thermally processed juice (94 °C for 26 s). With the exception of HILP (H) and PEF (L), all combinations achieved the minimum microbial reduction of 5 log units required by the FDA. The results obtained for PEF (L) followed by either HILP (L or H) suggest a synergistic effect on microbial inactivation. In general, the quality attributes were not affected by the chosen treatments and sensory evaluation revealed that the HILP(L)/PEF(L) combination was the most acceptable of the selected non-thermal treatments. *Industrial Relevance:* Heat remains the dominant microbial/enzyme inactivation technique though its impact on food quality is often at odds with increased consumer demand for minimally processed (MP) products. The reduction in intrinsic preservation in MP products raises new safety and stability risks and a major trend is the combination of inhibitory techniques to effectively preserve without the extreme use of a single technique (i.e. hurdle technology). PEF and HILP are emerging nonthermal/mild-heat technologies which have antimicrobial capabilities when applied alone or in combination with other physicochemical hurdles. Only a limited amount of work has focused on combinations of emerging technologies. As consumers have less reservations about physical (vs. chemical) preservation treatments, the objective of this paper is to assess if novel combinations of these emerging physical hurdles achieves the twin goals of food safety and quality in apple juice. This will involve assessing whether these combinations are effective vs. selected microorganisms un-/mildly heated products. In addition the nutritional/sensory quality of these MP products will be compared to untreated products.

1. Introduction

Due to growing consumer demand for more natural and nutritionally healthier food, the combination of novel, non-thermal technologies for preservation purposes is a recent trend in food processing research. Thermal processing, the most common method of preservation, can adversely affect the quality characteristics of food. As an alternative, the application of combinations of different non-thermal hurdles, used at sub-lethal levels, could maintain the organoleptic and nutritional quality while still ensuring the safety and stability of the food product (Leistner & Gorris, 1995). The choice of non-thermal hurdles 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, redox potential or water activity). When hurdles are selected from different target classes (Leistner, 1995), the combined treatment is more likely to achieve a more gentle and effective preservation measure, because of their potential to act synergistically on microbial stability (Leistner, 1978).

Pulsed electric fields (PEF) and high intensity light pulses (HILP) are examples of non-thermal technologies whose antimicrobial effects are largely believed to act upon different classes of intrinsic targets with the cell membrane and DNA affected by PEF and HILP, respectively.

PEF has been widely investigated as an alternative processing technique for decontamination of beverages (Charles-Rodríguez,

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Nevárez-Moorillón, Zhang, & Ortega-Rivas, 2007; Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006; Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003). The application of a high voltage electric field (5–80 kV/cm) in short electric pulses (1–100 μs) has been shown to disrupt the cell membrane, by formation of pores (electroporation), which increases permeability and leads subsequently to cell death (Sale & Hamilton, 1967). PEF has also been successfully combined with other non-thermal technologies such as UV irradiation to achieve bacterial inactivation in juices (Noci et al., 2008; Walkling-Ribeiro et al., 2008).

HILP is a more recent technology to emerge capable of killing pathogenic and spoilage microorganisms. It generally involves the use of a xenon flashlamp, which converts electric pulses into short-duration (1 μs–0.1 s) and high power pulses of radiation of a broad emission spectrum, ranging from ultraviolet (200 nm) to infrared light (1100 nm) (Dunn, Bushnell, Ott, & Clark, 1997; Palmieri & Cacace, 2005). The lethal effect on microorganisms is mostly attributed to the photochemical action of the UV part of the spectrum. Microbial DNA absorbs UV light inducing chemical modifications in its structure (Mitchell, Jen, & Cleaver, 1992) that results in damage of genetic information, impairing replication, gene transcription and, eventually results in the death of the cell. A photothermal mechanism of inactivation is believed to coexist at the microscopic level when the highest fluence values are applied. The consequence of such effects is the production of a localised temperature rise that can cause structural damage to membranes, proteins and other macromolecules (Takeshita et al., 2003; Wekhof, 2000).

The objective of the current study was to evaluate the effect of PEF/HILP hurdle combinations, including the impact of sequence and different energy levels, on the inactivation of *E. coli* in apple juice. The effect of the selected processing technologies on quality (chemical, physical and sensory) attributes was also investigated. Clear apple juice was selected as the effectiveness of light-based hurdles depends on the transparency of the medium, while *E. coli* was chosen as the test microorganism since certain strains represent a major concern to public health.

2. Materials and methods

2.1. Juice preparation

Concentrated apple juice (Batchelors, Cabra, Dublin, Ireland) was reconstituted in water using a 1:7.8 dilution (v/v). Juices for evaluation of quality parameters were prepared by reconstituting the concentrated juice in commercial non-carbonated mineral water (Ballygowan, Newcastle West, Co. Limerick, Ireland), while samples destined for microbial evaluation were re-diluted with sterile deionised water (15 min at 121 °C) and inoculated as described in Section 2.6. The pH and the Brix of the reconstituted juice were 3.56 and 12, respectively.

2.2. PEF processing

Apple juice was processed using the lab-scale PEF system equipment described by Noci et al. (2008) and employing mono-polar pulses, with a pulse width of 1 μs. The volume of the treatment chamber was 1.68 ml with an electrode gap of 2 mm. Two different treatment conditions for a constant treatment time of 89 μs were applied in order to provide a “low” and a “high” energy input. An overview of the relevant PEF processing parameters is given in Table 1.

2.3. HILP processing

Pulsed light was generated using a Steri-Pulse XL 3000 Pulsed Light Sterilization System (Xenon Corporation, MA, USA). The length of the light pulse was 360 μs with a fixed frequency of 3 Hz. The treatment system consisted of a stainless steel sterilization chamber containing a xenon flashlamp which delivered a radiant energy of 1.213 J/cm^2/pulse. Two different treatment conditions were applied to provide a “high” and a “low” energy input (see Table 2). The cell for the continuous processing of liquid products was developed in-house. The liquid was pumped (peristaltic pump Model No. L/S 77200-60, Masterflex, Cole-Parmer Instruments, Illinois, USA) through two quartz tubes (length 30 cm, i.d. 1 mm) located at a distance of 1.9 cm from the xenon flashlamp. The total length of tube irradiated was 40 cm. The two tubes were located in grooves (30 × 3 mm and 1.5 mm deep) cut in an aluminium unit (see Fig. 1) incorporating a recirculating coolant (ethylene glycol) system kept at −10 °C to prevent overheating of the juice. The product was also cooled immediately before and after HILP exposure by means of cooling coils submerged in iced water, to minimise temperature rise. The thermocouples were located at the inlet and outlet points of the HILP sterilization chamber and temperatures were monitored using a data logger (Squirrel SQ 2020, Grant Instruments Ltd., Cambridge, UK). Before and after use, both processing units (PEF and HILP) were thoroughly flushed with water for 15 min, disinfected with a 5%, (v/v) hypochlorite-based solution for 20 min and finally rinsed again with water.

Table 1
Pulsed electric field (PEF) ‘high’ and ‘low’ energy treatment conditions applied to apple juice.

PEF parameters	Low	High
Electric field	24 kV/cm	34 kV/cm
Voltage	4.8 kV	6.8 kV
Flow rate	17 ml/min	13.4 ml/min
Residence time	5.95 s	7.54 s
Pulse frequency	15 Hz	12 Hz
Pulse width	1 μs	1 μs
N pulses	89	89
Treatment time	89 μs	89 μs
Total specific energy input	130.5 J/ml	261.9 J/ml

2.4. Thermal processing

For the thermal treatment used in this experiment, reconstituted apple juice was passed through a tubular heat exchanger (Model No. FT74T, Armfield, Ringwood, UK) at a flow rate of 94 ml/min. The temperature of the holding tube was set at 94 °C with a residence time of 26 s. Relevant processing parameters were monitored using the logging system supplied with the unit.

2.5. Experimental treatment and design

In the present study the treatments combining PEF and HILP were applied to reconstituted apple juice in a continuous system using 2 energy levels for PEF and HILP, as described in Sections 2.2 and 2.3 respectively. After being exposed to the PEF, the juice was subsequently cooled to approximately 10 °C and pumped through the HILP system (Fig. 2). The reverse sequence using identical treatment conditions was also evaluated. Overall, four treatments were applied to apple juice within each sequence (Table 3).

Table 2
High intensity light pulses (HILP) ‘high’ and ‘low’ energy treatment conditions applied to apple juice.

HILP parameters	Low	High
Flow rate	17 ml/min	13.4 ml/min
Residence time	1.11 s	1.41 s
Pulse frequency	3 Hz	3 Hz
Pulse width	360 μs	360 μs
N pulses	3.3	4.2
Treatment time	1.20 μs	1.52 μs
HILP fluence	4 J/cm^2	5.1 J/cm^2
Total specific energy input	51.5 J/ml	65.4 J/ml