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/cm² (H) or 4.0 J/cm² (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.

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**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, 2011).
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; Walking-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, Jensen, & 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; Welkof, 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, Newry, Northern Ireland) following the method 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.

2.2. 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 380 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/cm²/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.

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>
<thead>
<tr>
<th>Table 1</th>
<th>Pulsed electric field (PEF) ‘high’ and ‘low’ energy treatment conditions applied to apple juice.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PEF parameters</strong></td>
<td><strong>Low</strong></td>
</tr>
<tr>
<td>Electric field</td>
<td>24 kV/cm</td>
</tr>
<tr>
<td>Voltage</td>
<td>4.8 kV</td>
</tr>
<tr>
<td>Flow rate</td>
<td>17 ml/min</td>
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<tr>
<td>Residence time</td>
<td>5.95 s</td>
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<tr>
<td>Pulse frequency</td>
<td>15 Hz</td>
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<tr>
<td>Pulse width</td>
<td>1 μs</td>
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<tr>
<td>N pulses</td>
<td>89</td>
</tr>
<tr>
<td>Treatment time</td>
<td>89 μs</td>
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<td>Total specific energy input</td>
<td>130.3 J/ml</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>High intensity light pulses (HILP) ‘high’ and ‘low’ energy treatment conditions applied to apple juice.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HILP parameters</strong></td>
<td><strong>Low</strong></td>
</tr>
<tr>
<td>Flow rate</td>
<td>17 ml/min</td>
</tr>
<tr>
<td>Residence time</td>
<td>1.11 s</td>
</tr>
<tr>
<td>Pulse frequency</td>
<td>3 Hz</td>
</tr>
<tr>
<td>Pulse width</td>
<td>360 μs</td>
</tr>
<tr>
<td>N pulses</td>
<td>33</td>
</tr>
<tr>
<td>Treatment time</td>
<td>1.20 μs</td>
</tr>
<tr>
<td>HILP fluence</td>
<td>4 J/cm²</td>
</tr>
<tr>
<td>Total specific energy input</td>
<td>51.5 J/ml</td>
</tr>
</tbody>
</table>
2.6. Microbial preparation and analysis

Experiments were conducted on *E. coli* K12 DSM 1607 to assess the impact of combined PEF-HILP treatments. For long term storage, cultures were maintained in glycerol at −20 °C. *E. coli* used for inoculation of apple juice was grown overnight in Tryptone Soya Broth (Oxoid, Basingstoke, Hampshire, UK), at 37 °C, in a shaking bath to reach a stationary phase of growth. The overnight culture was centrifuged (Sigma, Model No. 4K15, DJB Labcare Ltd, Buckinghamshire, England) at 4 °C for 10 min at a relative centrifugal force of 10,375 × g.

The apple juice was inoculated with *E. coli* to achieve an initial population of approximately 7 log cfu/ml. Tryptone Soya Agar (TSA) (Oxoid) was used as non-selective medium to enumerate inoculated strains for total bacterial counts in pre- and post-processing samples. Ten-fold serial dilutions were prepared using 1/4 strength Ringers solution (Oxoid) and 0.1 ml aliquots of the appropriate dilution plated out. Plates were incubated at 37 °C for 48 h and survivors (cfu/ml) were enumerated. In order to establish background microbiota, fresh uninoculated juice was also placed on TSA and incubated at 37 °C for 48 h to determine total bacterial populations. A period of 20 min after inoculation was allowed for adaptation to the media before treatments were applied. All the microbiological experiments were performed in duplicate.

2.7. Chemical and physical analysis

The pH and the Brix of the juice pre- and post-processing were measured with a pH meter (Model No. 9450, Unicam Ltd., Cambridge, UK) and a hand held refractometer (0–50% Sugar Refractometer, Bellingham & Stanley Ltd., Tunbridge Wells, UK), respectively. A tristimulus colorimeter (Model No. CR 300 Chroma Meter, Minolta, Osaka, Japan) was used to evaluate the colour of apple juice. The Hunter Lab colour parameters \( L \), \( a \) and \( b \) were recorded for each set of samples and the total colour difference (ΔE) was calculated.
The spectrophotometric assay described by Meydav, Saguy, and Kopelman (1977) was used to determine the non-enzymatic browning index (NEBI) of the apple juice samples. Total polyphenols were assessed using the Folin–Ciocalteu colorimetric method of Singleton and Rossi (1965), while the total antioxidant activity was determined by the method of Kim, Lee, Lee, and Lee (2002), but using (1 = dislike extremely and 9 = like extremely) (Peryam & Pilgrim, 1957) for all the parameters. In addition, intensity levels of colour, sweetness and acidity were assessed with 1 = low intensity and 9 = high intensity.

2.9. Statistical analysis

The dataset was analysed as a factorial design using the general linear model (GLM) procedure (SAS, 2005). The effect of the sequence and energy levels applied was compared. Multiple pairwise comparisons of means (Student’s t test) were performed to identify significant differences (p<0.05) between the treatments.

3. Results and discussion

3.1. Microbiological results

To our knowledge, no studies have reported on the preservation effect of combined PEF and HILP treatments in apple juice. The main focus of the present study was to investigate the effect of the sequence of the selected hurdles (PEF/HILP and HILP/PEF) and the impact of different energies applied to apple juice as presented in Table 3.

The inactivation effect of the combinations of these technologies on E. coli in apple juice is shown in Fig. 3. The hurdle sequence had a significant impact on the bacterial counts (p<0.0001). When PEF was followed by HILP, the viable counts were below the detection level (<1 log cfu/ml). In contrast, the number of survivors was 1.85 log cfu/ml when the reverse sequence was applied. The enhanced synergistic effect observed with the PEF/HILP sequence may be due to the greater damage to the cell membrane induced by PEF as a first hurdle, possibly causing increased susceptibility to the UV component of the subsequent HILP treatment. In general, as the energy applied through the two hurdles increased, E. coli inactivation significantly increased (p<0.001). An interaction between the sequence and the energy was also observed with energy level only having a significant effect in the HILP/PEF sequence (p<0.001).

When treatments within the PEF/HILP sequence were tested, counts were below the detection limit at all energy levels, confirming a microbial reduction of ≥6.42 log cycles (p<0.001). When PEF and HILP hurdles were applied individually, the log reductions observed were 1.8 (L) and 3.45 (H) for PEF and 3.30 (L) and 3.90 (H) for HILP. The results obtained for PEF (L) followed by HILP (either L or H) suggest a synergistic effect on microbial inactivation (Fig. 4). When the PEF (H) treatment was applied in combination with HILP (L or H), the remaining population of E. coli after the first hurdle of each sample were presented in a randomized order in clear plastic cups and accompanied by unsalted crackers and still water (Ballygowan, Ireland) for cleansing the palate between samples. The panellists were asked to evaluate the colour, odour, sweetness, acidity and overall acceptability of the products. A 9-point hedonic scale was used (1 = dislike extremely and 9 = like extremely) (Peryam & Pilgrim, 1957) for all the parameters. In addition, intensity levels of colour, sweetness and acidity were assessed with 1 = low intensity and 9 = high intensity.

![Fig. 3. Inactivation of E. coli K12 in apple juice treated by a combination of pulsed electric field (PEF) and high intensity light pulse (HILP) at different energy levels (E1, E2, E3, and E4).](image)

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PEF energy input (J/ml)</th>
<th>HILP energy input (J/ml)</th>
<th>Energy level</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF (L)/HILP (L)</td>
<td>130.5</td>
<td>51.5</td>
<td>E1</td>
</tr>
<tr>
<td>PEF (L)/HILP (H)</td>
<td>130.5</td>
<td>65.4</td>
<td>E2</td>
</tr>
<tr>
<td>PEF (H)/HILP (L)</td>
<td>261.9</td>
<td>51.5</td>
<td>E3</td>
</tr>
<tr>
<td>PEF (H)/HILP (H)</td>
<td>261.9</td>
<td>65.4</td>
<td>E4</td>
</tr>
</tbody>
</table>

* Hurdles in the reverse sequence were applied using identical processing conditions.

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was not sufficient to evaluate the presence or absence of additive or synergistic effects of these combinations.

The results of reverse combinations (HILP followed by PEF) showed that different energy levels of PEF significantly affected microbial counts (p < 0.001), while no significant differences were found when the low and high energy applied by HILP was applied. The HILP(H)/PEF(H) treatment was found to be the most effective in inactivating E. coli, achieving total elimination (p < 0.001). Lower energy combinations also led to significant microbial reductions of 6.22, 4.47, and 4.95 log cycles for HILP(L)/PEF(H), HILP(H)/PEF(L) HILP(L)/PEF(L) respectively, when compared to untreated controls (p < 0.001). Comparison of the combination of HILP(L)/PEF(L) to the summed effect of the individual hurdles (4.95 vs. 5.1 log cycles), suggested an additive effect.

In a recent study, Gachovska, Kumar, Thippareddi, Subbiah, and Williams (2008) reported an additive effect on E. coli in apple juice when treated by a combination of PEF (60 kV/cm; 11.3 pulses) followed by UV processing (exposure for 1.8 s in a 30 cm length chamber), and for the reverse sequence. Noci et al. (2008) also reported an additive effect on the inactivation of native microbiota in freshly squeezed apple juice, when a batch UV pre-treatment was followed by PEF at 40 kV/cm.

3.2. Chemical and physical analysis

In contrast to the microbial data, results of the chemical and physical analysis showed that the sequence of the hurdles had no major impact on the quality attributes measured. This could be due to the fact that concentrated apple juice had been exposed during preparation to a thermal treatment, resulting in possible modification of certain quality attributes which would be important in a fresh juice. However, some PEF/HILP combinations affected the lightness L and yellowness b colour attributes (p < 0.05) in comparison to the untreated control (L = 34.10; b = 13.65). In particular, a decrease of 3.0 and 2.9% respectively in the latter parameters was observed in the samples with the PEF(H)/HILP(L) combination, regardless of the sequence. The latter combinations also produced the greatest ΔE (1.17), though all combinations showed “slightly noticeable” colour changes (0.5 < ΔE < 1.5) (Cserhalmi et al., 2006). None of the PEF/HILP combinations examined had any effect on pH, Brix, NEBI, total phenolics or TEAC of apple juice. Mean values for these parameters were 3.56, 12, 0.142, 341 mg GAE/L and 1.87, respectively. Similarly, thermal pasteurisation (94 °C for 26 s) of apple juice did not affect any of the parameters with the exception of total phenolics where a slight though significant decrease of 2.5% (p < 0.05) was observed.

To our knowledge, no study has described the effect on product quality of combined HILP and PEF treatments. However, Walking-Ribeiro et al. (2008) found no significant difference (p ≥ 0.05) in colour, pH and °Brix between an untreated reconstituted apple juice and the product processed by UV/PEF combined treatments, while thermal processing (94 °C for 26 s) caused a significant change in all colour attributes. In a recent study on fresh apple juice Noci et al. (2008) found that batch UV and PEF (40 kV/cm) combinations which produced satisfactory microbial inactivation, also had less adverse effects on juice colour and the level of phenolic compounds than pasteurisation by heat. However, total phenolics were significantly (p < 0.05) reduced compared to levels present in the unprocessed juice.

Overall, the physical and chemical data obtained in the present study suggest that HILP, an alternative light based technology to UV, which is compatible with PEF in a continuous hurdle combination, could represent a satisfactory alternative technology for processing apple juice, in terms of maintaining the measured quality attributes.

3.3. Sensory analysis

A number of studies have reported on the impact of PEF or UV-light based technologies as stand-alone processing methods on the sensory quality of fruit-based beverages, but the effects of HILP and PEF applied as combined treatments have not been evaluated. Evrendilek et al. (2000) showed that the acceptability of fresh apple cider exposed to PEF (35 kV/cm for 94 s) was not significantly different to that of an untreated control, whereas Caminiti et al. (2010) found that reconstituted apple juice exposed to continuous UV light at energy dosages lower than 10.6 J/cm² was comparable to an unprocessed sample in all the sensory attributes evaluated. The results of the present sensory study are shown in Fig. 5. Significant differences between the treatments were found for odour (p < 0.05) and for flavour (p < 0.001). Pasteurised samples received a significantly higher score for odour (6.3) than those treated by the selected combinations, with the exception of juice processed by the HILP(H)/PEF(H) sequence (5.7). The score for flavour of the heat-processed product was significantly higher than any hurdle treated samples. Panellists did not perceive any difference in colour, sweetness and acidity attributes of samples processed by the selected treatments compared to the heat treated juice. The HILP(L)/PEF(L) processed sample proved to be the most acceptable among the non-thermally treated samples with a score of 6.1, which was not significantly different from the pasteurized control (6.7). Statistical analysis did not reveal any significant effect between the samples receiving “low” and “high” energy inputs. Furthermore, the treatment sequence did not impact on any of the sensory attributes examined.

4. Conclusions

The present study has shown that a PEF/HILP hurdle combination could represent an alternative preservation technology to thermal pasteurisation for bacterial control in reconstituted apple juice. The reverse sequence (HILP/PEF) resulted in a slightly lower microbial inactivation. Further studies are required to optimise the processing parameters with regard to improving product sensory quality.

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