Food Microbiology 28 (2011) 14–20

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Effectiveness of High Intensity Light Pulses (HILP) treatments for the control of *Escherichia coli* and *Listeria innocua* in apple juice, orange juice and milk

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ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form
9 July 2010
Accepted 30 July 2010
Available online 18 August 2010

Keywords:
High intensity light pulses
Beverages
Non-thermal preservations
Listeria innocua
Escherichia coli

ABSTRACT

High Intensity Light Pulses (HILP) represent an emerging processing technology which uses short (100–400 μs) light pulses (200–1100 nm) for product decontamination. In this study, model and real foods of differing transparencies (maximum recovery diluent (MRD), apple and orange juices and milk) were exposed to HILP in a batch system for 0, 2, 4 or 8 s at a frequency of 3 Hz. After treatment, inactivation of *Escherichia coli* or *Listeria innocua* was evaluated in pre-inoculated samples. Sensory and other quality attributes (colour, pH, Brix, titratable acidity, non-enzymatic browning, total phenols and antioxidant capacity (TEAC)) were assessed in apple juice. Microbial kill decreased with decreasing transparency of the medium. In apple juice (the most transparent beverage) *E. coli* decreased by 2.65 and 4.5 after exposure times of 2 or 4 s, respectively. No cell recovery was observed after 48 h storage at 4 °C. No significant differences were observed in quality parameters, excepting TEAC and flavour score, where 8 s exposure caused a significant decrease ($p < 0.05$). Based on these results, HILP with short exposure times could represent a potential alternative to thermal processing to eliminate undesirable microorganisms, while maintaining product quality, in transparent fruit juices.

1. Introduction

Food-borne diseases still remain a global problem with major public health implications (Rowan et al., 1999). Thermal treatment techniques such as pasteurization of beverages have been used extensively to reduce biological hazards and extent product shelf-life. More recently, changes in consumer preferences towards healthier and minimally processed beverages has led to increased research interest in non-thermal processing technologies in order to manufacture safe and fresh-like products, with enhanced retention of organoleptic quality (Leistner and Gorris, 1995). Despite the emergence of such technologies, thermal treatments still remain the most extensively used. However, the degradation of heat sensitive compounds can impair the quality of thermally processed foods (Elmnasser et al., 2008) and therefore it is critical that innovative processing technologies are developed to give equivalent levels of safety and stability (Lado and Yousef, 2002), while minimizing impact on quality.

High Intensity Light Pulses (HILP), also known as Pulsed Light (PL), is an emerging non-thermal technology which uses light pulses of short duration (100–400 μs), ranging from ultraviolet to infrared wavelengths, for microbial inactivation. The intensity of these light pulses is approximately 20,000 times greater than that of sunlight projected onto the earth's surface (Dunn, 1996). This technology has been reported to have the potential for inactivating both spoilage and pathogenic microorganism (Rowan et al., 1999; Takeshita et al., 2003; Gómez-López et al., 2007). In addition, it can limit the negative effects on product quality in terms of flavour, colour and nutritional value as there is no substantial increase in temperature during the treatment (Ross et al., 2003).

During the application of PL, energy is stored in a high power capacitor and released over a short period of time producing several high energy flashes per second, thus increasing the instantaneous energy intensity that contributes to the inactivation of microbial cells (Turtoi and Nicolau, 2007).

The lethal effect of PL on microorganisms is mostly attributed to the photochemical action of the UV part of the spectrum emitted by the flash lamp. Microbial DNA absorbs UV light that induces chemical modifications in its structure, resulting in damage of genetic information, impaired replication and gene transcription

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0740-0020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2010.07.023

and eventually death of the cell (McDonald et al., 2002). Takeshita et al. (2003) found that PL can also induce damage to membranes, proteins and other macromolecules within microorganisms, thereby increasing the efficiency of microbial inactivation. Along with a photochemical effect, a photothermal effect can be noticed. The relative contribution of the two-inactivation mechanisms depends on the energy dosage (fluence) and on the target microorganism (Gómez-López et al., 2007). Wekhof et al. (2001) reported that fluence values ranging from 10 to 30 kJ/m^2 at which the temperature increased were not sufficient to thermally inactivate *Aspergillus niger* spores, while at higher fluence values (50–60 kJ/m^2) the contribution of photothermal effect is more relevant. Pulsed UV light was found to be a more effective and rapid way of inactivating microorganisms due to the instantaneous delivery of more intense energy than continuous UV light, for the same total energy supplied. A greater inactivation of *Escherichia coli* by PL was reported by Bohrerova et al. (2008), who found that pulsed UV irradiation was approximately 2.4 times more effective than equivalent levels of UV irradiation produced by a continuous medium-pressure system.

The US Federal Register has identified some pertinent pathogens associated with beverages (e.g. fruit juices and milk) including *E. coli* and *Listeria monocytogenes*. The latter has become increasingly important as a food-borne pathogen (Jemmi and Stephan, 2006), as it can be found in a wide range of foods and in varied and challenging environments (Gomez-Lopez et al., 2005). *Listeria* infection can cause severe disease in humans, often requiring hospitalization with high mortality rates among young, old, pregnant and immuno-compromised people (Buchrieser and Rusniok, 2003).

E. coli is an acid resistant microorganism widely distributed in nature and can survive for long periods in foods including those with a low pH such as juices or apple cider (Donahue et al., 2004), especially at low temperature conditions (Gorden and Small, 1992). Pathogenic strains, particularly enterohaemorrhagic *E. coli*, may contaminate foods and are a major public health concern because of their ability to produce toxins and cause severe diseases such as hemorrhagic colitis and haemolytic uremic syndrome (Ngadi et al., 2003).

The objectives of this study were: (a) to investigate the susceptibility of *E. coli* and *Listeria innocua* to application of PL treatment, (b) to evaluate the role of product transparency on the effectiveness of PL treatments, (c) to assess the effect of post-treatment storage time on microbial populations, (d) to examine the effect of PL on selected chemical, physical and sensory aspects of quality in microbiologically acceptable treated products.

2. Materials and methods

2.1. Product preparation

Maximum recovery diluent (MRD) (Oxoid, Basingstoke, Hampshire, UK) was prepared in Citric acid/Na_2HPO_4 buffer (McIlvaine, 1921) at pH 3.6 and autoclaved at 121 °C for 15 min.

Juices were prepared by diluting commercial apple and orange concentrates (Batchelors, Dublin, Ireland) with still water (Ballygowan, Newcastlewest, Ireland) to obtain the required dilutions of 1:7.8 and 1:7.15, respectively, which were typical of those used in commercial practice. The respective pH and °Brix values of the juices (measured as described in Section 2.4) were 3.70 and 11.2 for apple juice and 3.85 and 11.2 for orange juice. The juices used for the evaluation of microbial inactivation were prepared using deionised sterile water (15 min at 121 °C), and sterilized full fat milk (Glanbia, Kilkenny, Ireland) was obtained by autoclaving at 110 °C for 10 min.

2.2. Bacterial strains, culture conditions and enumeration

Experiments were performed using *L. innocua* NCTC 11288 and *E. coli* K12 DSM 1607 as surrogates for their pathogenic counterparts. Stock cultures were stored in glycerol at −20 °C. Strains were grown overnight in Tryptone Soya Broth (Oxoid) at 37 °C, in a shaking bath for *E. coli* and without shaking for *L. innocua*. Samples of juices or milk were inoculated at 0.1% with the overnight culture in order to achieve an initial population of approximately 6 log cfu/ml.

The following selective and non-selective media were used to enumerate organisms in pre- and post-processed samples: Tryptone Soya Agar (TSA) (Oxoid) for total bacterial counts, Eosin Methylene Blue (EMB) (Oxoid) for *E. coli* and *Listeria* Selective Agar (LSA) with selective supplement (Oxoid) for *L. innocua.* Ten-fold dilution series of samples were prepared using 1/4 strength Ringers (Oxoid) and 0.1 ml aliquots of relevant dilutions plated out on solid media. Plates were incubated at 37 °C for 48 h and survivors (cfu/ml) were enumerated. In order to establish the background microflora, fresh uninoculated juice and milk were also plated on TSA and incubated at 37 °C for 48 h to determine total bacterial populations. Treated and untreated samples were enumerated immediately and also following storage at 4 °C for 24 h and 48 h, to verify if microbial cells recovered during storage. A period of 20 min following inoculation was allowed for adaptation of the cultures to the product environment prior to the treatments.

2.3. PL processing

For the purpose of PL processing, 2 ml of the products were pipetted onto a petri dish (50 mm diameter) to ensure that the entire dish surface was covered with sample to a depth of 1 mm. The PL system employed in this study was the Steri-Pulse XL 3000 Pulsed Light Sterilization System (Xenon Corporation, MA, USA), which consisted of a stainless steel sterilization chamber with a lamp housing mounted on top and a control module (Model No. RC 747, Xenon). The xenon lamp emits high intensity light in the wavelength range between 200 nm and 1100 nm, with a maximum emission in the UV range. Light pulses are delivered at a frequency of 3 Hz and a pulse width of 360 μs, delivering 1.17 J/cm^2/pulse at the distance of 2.5 cm from light source. As the lamp emission spectrum included a segment of IR light heat was generated within the chamber, requiring air to be continuously pumped through the system to provide cooling. Petri dishes containing the selected medium were placed at a distance of 2.5 cm from the lamp in a box filled with ice to minimise temperature increases of samples (Fig. 1). Inoculated samples were exposed to light for 2, 4 or 8 s, in order to receive total energy doses of 7, 14 or 28 J/cm^2, respectively.

Product temperature increases under the processing conditions used were determined by T-type thermocouples (Industrial

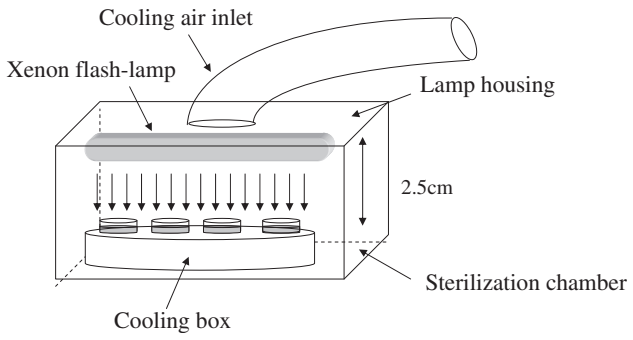

Fig. 1. Schematic diagram of the PL chamber.

Temperature Sensors, Naas, Ireland) and recorded at 1 s intervals using a data logger (Model No. SQ 2020, Grant Instruments Ltd., Cambridge, UK).

2.4. Chemical and physical analysis

The pH of selected products was measured by 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) was used to evaluate the °Brix before and after the treatments. Colour attributes were measured in the Hunter Lab colour space with a tristimulus colorimeter (Model No. CR 300 Chroma Meter, Minolta, Osaka, Japan). L (lightness), *a* (redness) and *b* (yellowness) values were recorded for each sample and the total colour difference (ΔE) was calculated using equation (1):

$$\Delta E = \sqrt{\left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)} \quad (1)$$

The non-enzymatic browning index (NEBI) was determined using the procedure described by Meydav et al. (1977). The total phenolic content was assessed by the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965) and the results were expressed as gallic acid equivalents (GAE, mg/L). The total antioxidant activity was determined according to the method of Kim et al. (2002) but using an absorbance reading of 0.820 ± 0.020 at 734 nm. Standard Trolox solutions were also evaluated and the results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

2.5. UV transparency

In order to establish the transparency of the different products to UV light, absorbance was measured in 1 cm-path quartz cuvettes at 254 nm using an UV–VIS spectrophotometer (UV-Mini – 1240 Shimadzu, Columbia, MD, USA). 'Absorbance coefficients' (ε) were calculated by measuring the absorbance of different dilutions of the samples and determining the slope of absorbance vs. concentration.

2.6. Sensory analysis

Pulsed light processed apple juice was used for sensory evaluation. A total of 31 untrained panelists (15 male and 16 female) participated in the consumer acceptance test. A 20-ml volume of each treated sample and untreated control were served in a randomized order in clear plastic cups and accompanied by unsalted crackers and still water (Ballygowan, Ireland) to rinse the palates between samples. Panelists evaluated the colour, odour, flavour, sweetness, acidity and overall acceptability in a 9-point hedonic scale. On this scale, 1 and 9 were the lowest and highest scores respectively for all the attributes with the exception of sweetness and acidity which were optimum graded at the midpoint of the scale (5). Untreated reconstituted apple juice was used as a control.

2.7. Statistical analysis

Statistical analysis of the data was performed using the SAS software (2005). A one-way ANOVA was used to compare the experimental treatments and a Student's *t*-test was used to determine significant differences between the means. All microbiological and physical/chemical/quality experiments were performed in duplicate and triplicate, respectively.

3. Results

3.1. Effect of PL on apple juice, orange juice and milk temperatures

Fig. 2 shows the temperature change in apple juice, orange juice and milk samples following PL treatments of 2, 4 and 8 s. It shows strong linear relationships between temperature attained and PL treatment time ($r^2 = 0.97$, 0.99 and 0.97 respectively for apple juice, orange juice and milk) with a maximum average temperatures recorded of 36.2, 40.6 and 40.1 °C respectively for apple juice, orange juice and milk following the longest a PL treatment time of 8 s.

3.2. Effect of PL on the inactivation of *E. coli* and *L. innocua* in liquid media of varying transparency to light

3.2.1. MRD – model solution

The inactivation effect of PL on *E. coli* and *L .innocua* was investigated using both non-selective (TSA) and selective agars (EMB and LSA for *E. coli* and *L. innocua*, respectively). In general, greater recovery rates of both organisms were observed from samples using non-selective medium. Consequently, results described below relate to recovery rates observed for non-selective media, while results obtained using selective media are commented upon subsequently.

Exposure of MRD inoculated with *E. coli* or *L. innocua* to PL led to a reduction of the bacterial population below the detection level ($\leq$1 $\log_{10}$ cfu/ml) for all treatment times (Fig. 3a and b). A reduction of 4.67 $\log_{10}$ for *E. coli* and 5.13 $\log_{10}$ for *L. innocua* was observed when compared to corresponding untreated control ($p < 0.001$). As the reductions in populations of both organisms to below detection levels were observed even for the shortest treatment time, it was not possible to determine differences in inactivation in response to different residence times within this medium.

Levels of *E. coli* and *L. innocua* were not significantly different following 24 and 48 h storage compared to samples analysed immediately post-treatment.

3.2.2. Apple juice

While similar initial cell concentrations (approximately 6 log cfu/ml) of *E. coli* and *L. innocua* were used, it was observed that counts following a 20 min adaptation period in apple juice were reduced to 5.66 and 3.88 log cfu/ml, respectively. All of the treatment times had a significant impact on *E. coli* populations ($p < 0.001$). The reduction in *E. coli* counts in apple juice was 2.65,

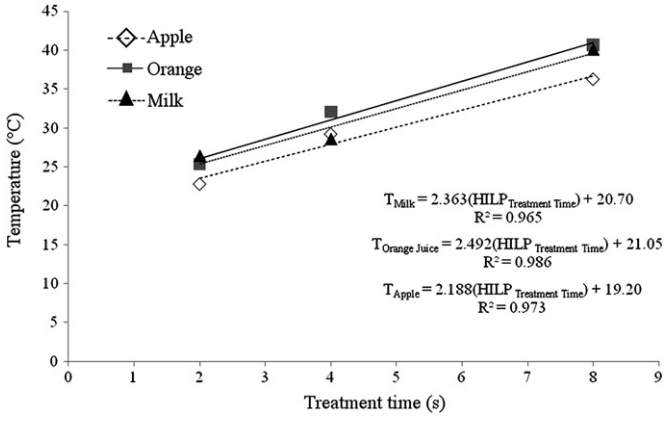

Fig. 2. Maximum temperatures recorded in apple juice, orange juice and milk during PL treatments of 2–8 s duration.

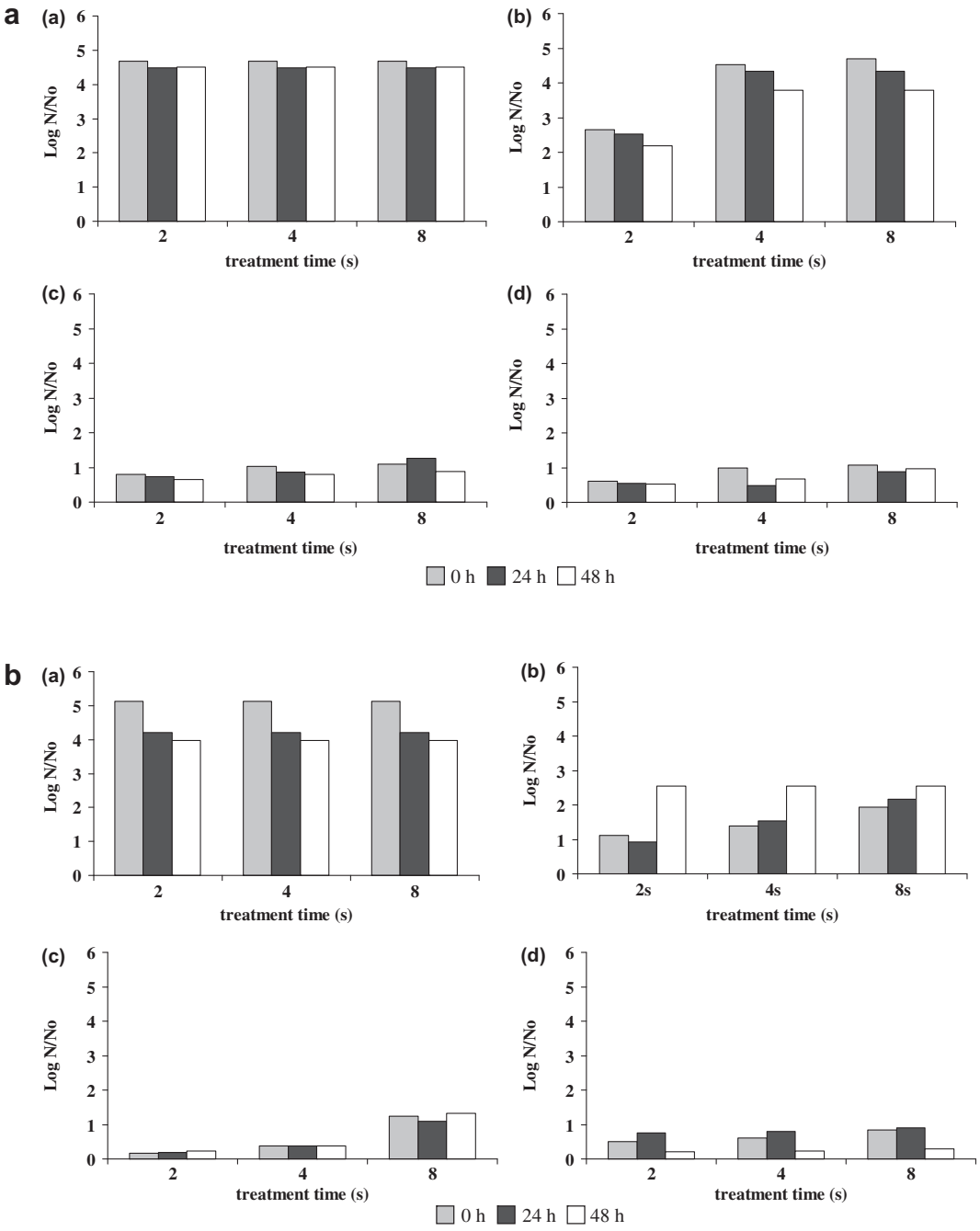

Fig. 3. a. Effect of PL treatment duration and storage times (grey 0 h, black 24 h, white 48 h) on inactivation of *Escherichia coli* in (a) maximum recovery diluent (MRD), (b) apple juice, (c) orange juice, (d) milk. Results expressed as log reduction cfu/ml (Log N/No). b. Effect of PL treatment duration and storage times (grey 0 h, black 24 h, white 48 h) on inactivation of *Listeria innocua* in (a) maximum recovery diluent (MRD), (b) apple juice, (c) orange juice, (d) milk. Results expressed as log reduction cfu/ml (Log N/No).

4.5 and ≥ 4.7 $\log_{10}$ cfu/ml (counts for the latter were below the detection limit) for 2, 4 and 8 s exposure times, respectively. Significantly greater reductions in *E. coli* populations were observed in samples exposed to PL for either 4 or 8 s when compared to 2 s treatments ($p < 0.001$).

Mean reductions in *L. innocua* of 1.1, 1.4 and 1.93 $\log_{10}$ cfu/ml were obtained in samples treated with PL for 2, 4 and 8 s, respectively. In contrast to *E. coli* following the 8 s treatment, *L. innocua* survivors could still be detected (1.95 $\log_{10}$ cfu/ml). No significant differences in counts were observed when samples exposed 2 or 4 s treatments were compared ($p \geq 0.05$) while significant differences were noticed between the 2 and 8 s treatments ($p < 0.05$). Sample storage for 24 or 48 h following treatment did not significantly alter levels of surviving cells, thus indicating no subsequent recovery of cells occurred.

Although differences in inactivation levels for each of the microorganisms was observed, similar linear pattern occurred with, increasing treatment times resulting in linear reductions in the number of survivors ($p < 0.001$).

For selective media, EMB and LSA, recovery levels were generally lower when compared to TSA, although statistical analysis showed no significant differences ($p \geq 0.05$).

3.2.3. Orange juice

To study how changes in the transparency of medium would affect the microbial inactivation as a result of differences in the ε of the juices (orange juice $\varepsilon = 79.7$ vs. 5.81 for apple juice), orange juice was subjected to PL using the same treatment times as described previously for apple juice. While reductions in microbial population were observed with increasing treatment time, a maximum decrease of approximately 1 log cfu/ml was achieved after 8 s for both *E. coli* and *L. innocua*. No significant differences were found between any of the treated samples inoculated with *E. coli*, irrespective of exposure times used, however, the samples were significantly different from the untreated control ($p < 0.01$). In contrast, significant reductions in *L. innocua* counts were found in orange juice treated for 8 s compared to the juice treated for 0, 2 or 4 s ($p < 0.001$). The data in relation to storage for up to 48 h at 4 °C did not confirm any sign of post-treatment microbial recovery.

3.2.4. Milk

The results of the application of PL to milk, an opaque medium ($\varepsilon = 1275.2$), are shown in Fig. 3a and b. After treatment times of 2, 4 and 8 s, the microbial inactivation was 0.61, 0.99 and 1.06 $\log_{10}$ cfu/ml respectively and for *E. coli* and 0.51, 0.62, 0.84 $\log_{10}$ cfu/ml for *L. innocua*. No significant differences were observed in *E. coli* counts when samples were treated for 2 s ($p \geq 0.05$), while significant reductions ($p < 0.05$) were found after exposure for 4 s and 8 s compared to untreated control. In contrast, all selected treatment times significantly affect *L. innocua* counts compared to control ($p < 0.001$), while no significant differences were observed in samples exposed to different treatment times. Although the applied treatments were not sufficient to successfully inactivate the selected microorganisms in milk, there was a linear increase in inactivation of *E. coli* as the exposure time increased ($p < 0.001$). Similar to the others products, storage time or plating media showed no significant effect on recovery rates.

3.3. Physical and chemical properties of reconstituted apple juice

Physical and chemical analyses were also performed on apple juice to assess the impact of PL treatments on key quality attributes. No significant differences ($p \geq 0.05$) were observed in the pH, °Brix, NEBI and total phenol content of apple juice treated by PL compared to the control. The overall mean values for these attributes were 3.66, 11.8, 0.149 and 135 mg GAE/l, respectively. The colour analysis also did not reveal any significant differences between processed apple juice samples and the control (Table 1). A significant decrease ($p < 0.05$) was noted after 8 s exposure time in the TEAC value, while shorter treatment times (2 s and 4 s) did not cause any significant decrease compared to the control (Table 1).

3.4. Sensory analysis of reconstituted apple juice

The results of the sensory study conducted on reconstituted apple juice exposed to PL are shown in Table 2. No significant change was perceived in terms of colour by the panellists, which was in agreement with instrumental measurements. In addition, none of the PL treatments significantly changed the sweetness, acidity or odour of apple juice. In contrast, samples exposed for 8 s scored the lowest in terms of flavour (4.5), which was significantly different ($p < 0.001$) from either the control or samples PL-treated for shorter times. The overall acceptability decreased linearly ($p < 0.01$) with the exposure time from a value of 6.0 in the control to 4.7 after 8 s of PL treatment. A positive correlation ($R^2 = 0.77$) was found between overall acceptability and flavour of apple juice.

4. Discussion

4.1. Effect of PL on apple juice, orange juice and milk temperatures

The temperatures recorded during PL treatments would have been insufficient to induce thermal inactivation of either *E. coli* or *L. innocua* populations, as the maximum temperature attained during treatment was actually close to the optimum growth temperature for both microorganisms (Adams and Moss, 2000).

4.2. Microbial analyses

Previous studies on the effectiveness of PL have shown that this technology may result in significant reductions in levels of microbial pathogens, moulds and yeasts (Hillegas and Demirci, 2003; Takeshita et al., 2003; Gómez-López et al., 2007; Turtoi and Nicolau, 2007). However differences between the product formulations used and experimental conditions applied make comparisons difficult.

Several critical parameters should be considered when designing experiments to assess the suitability of PL, such as the number of pulses, transparency of the medium and the depth of the samples.

The penetration of UV radiation through liquids is very limited, with the exception of clear water (Guerrero-Beltrán and Barbosa-Cánovas, 2004). In juice products 90% of the light absorption occurs within a depth of 1 mm (Keyser et al., 2008). In a previous study carried out using honey, a direct correlation was found between levels of microbial reduction and depth of sample (Hillegas and Demirci, 2003). As a result the depth of the samples was maintained at 1 mm to maximise the effect of the PL treatment in the current studies.

Table 1
Chemical and physical analysis of reconstituted apple juice processed by PL.

Exposue times (s)	*L*	*a*	*b*	ΔE[a]	TEAC[b] (mM)
0	25.64	1.74	6.62	–	1.45[a]
2	25.42	1.65	6.46	1.45	1.44[a]
4	25.20	1.64	6.24	1.37	1.41[ab]
8	25.27	1.43	6.22	1.07	1.37[b]
SED	*0.505*	*0.121*	*0.489*	*0.346*	*0.017*
p-Value	*NS*	*NS*	*NS*	*NS*	*

Within columns, means not followed by the same superscript are significantly different ($p < 0.05$).
SED: Standard Error of Difference between treatment means.
*$p < 0.05$; NS: not significant.
[a] ΔE values calculated from the *L*, *a* and *b* replicate values.
[b] TEAC: Trolox Equivalent Antioxidant Activity.

Table 2
Sensory analysis of reconstituted apple juice exposed to PL.

Exposure times (s)	Colour (x)	Odour (x)	Sweetness (y)	Acidity (y)	Flavour (x)	Acceptability (x)
0	5.8	5.7	6.3	4.0	6.1[a]	6.0[a]
2	5.9	5.6	6.1	4.3	5.5[a]	5.6[a]
4	5.7	5.6	5.9	4.4	5.6[a]	5.5[ab]
8	5.6	4.8	5.8	4.7	4.5[b]	4.7[b]
SED	*0.33*	*0.40*	*0.41*	*0.45*	*0.39*	*0.42*
p-Value	*NS*	*NS*	*NS*	*NS*	***	*

Scale (x): 1 = represent maximum dislike. 9 = represent maximum like.
Scale (y): 1 = represent too low; 9 = represent too high.
Within columns, means not followed by the same superscript are significantly different ($p < 0.05$).
SED: Standard Error of Difference between treatment means.
***$p < 0.001$; *$p < 0.05$; NS: not significant.

Initial experiments were performed on MRD to investigate the ability of different energy dosages to inactivate microorganisms in medium with good transparency ($\varepsilon = 0.74$). The energy doses selected were sufficient to achieve a level of microbial inactivation of approximately 5 $\log_{10}$ cfu/ml, which is in line with the minimum inactivation recommended by the Food and Drugs Administration (FDA, 2001) in fruit juices. In a study carried out by Huffman et al. (2000) water treated with pulsed light at 0.25 J/cm^2 (flow rate 15.41 l/min) gave a reduction of >7.4 $\log_{10}$ cfu/ml for *Klebsiella terrigena* after two pulses. These results show that transparency of the media allowed successful microbial inactivation using this technology.

The current study on apple juice showed that a reduction of 4.7 $\log_{10}$ cfu/ml for *E. coli* was obtained after 8 s treatment. When the same conditions were applied to this microorganism inoculated into orange juice, the maximum extent of microbial inactivation after 8 s was only of the order of 1 $\log_{10}$ cfu/ml. This confirms the profound effect which decreased transparency of the liquid medium can have on light penetration and consequently on levels of microbial inactivation, even when using only a 1 mm sample thickness.

In the case of milk, the overall level of inactivation was unsatisfactory, which is most likely due to the lack of light penetration in this opaque medium ($\varepsilon = 1275.2$). Even increasing the exposure time beyond 8 s (data not shown) was not sufficient to significantly inactivate microbial populations.

In relation to the susceptibility of Gram-positive *L. innocua* vs. Gram-negative *E. coli* to pulsed light treatment, the results indicated significant differences ($p < 0.01$) in viable counts, most clearly seen in the data for apple and orange juice. This variation in light sensitivity may be due to structural/compositional differences in the cell walls and membranes, in particular the thicker peptidoglycan cell wall (20–80 nm) of Gram-positive compared to Gram-negative organisms (1–2 nm) (Alcamo, 1997).

4.3. Chemical and physical analyses of apple juice

Quality changes in liquid products processed by UV or broader spectrum PL have not been investigated in great depth (Oms-Oliu et al., 2008). UV light is known to induce a range of adverse effects in food products due to the generation of free radicals through a wide variety of photochemical reactions, which can damage vitamins, antioxidants, while also inducing lipid oxidation and colour changes (Koutchma, 2009). In the present study short PL exposure times (2 or 4 s) caused no changes in the measured quality attributes of apple juice. In a study performed by Kasahara et al. (2004) on clarified apple juice, exposure to pulsed UV light at different energy dosages (from 1850 to 3354 mJ/cm^2) did not cause significant differences from the control sample in terms of soluble solids, turbidity, acidity, viscosity and colour. However instrumental colour measurement also performed in the present study, revealed a slightly noticeable ($0.5 < \Delta E < 1.5$ (Cserhalmi et al., 2006)) total colour difference was found in the apple juice processed by PL, regardless of the exposure time.

The NEBI of the processed juice was not significantly different from the untreated control at any exposure time, suggesting that the PL treatment did not cause any browning of the juice, as confirmed by the instrumental measurements of lightness. The total phenol content of apple juice was retained after all PL processing times. In addition, the antioxidant activity of the reconstituted apple juice PL processed for 2 and 4 s was not significantly different from the control, though the TEAC of the juice showed a 5.5% decrease ($p < 0.05$) after 8 s exposure time. Overall, these results suggest that bioactive compounds are unlikely to be damaged under short PL exposure conditions.

4.4. Consumer acceptability of PL processed apple juice

Few investigations have evaluated the effects of light-based technologies on sensory properties of food. Donahue et al. (2004) reported that apple cider exposed to UV light (energy dosage of 17.54 mJ/cm^2) showed no significant difference to an untreated control in terms of flavour. Similarly, Tandon et al. (2003) reported that UV treated apple cider tested by sensory panel immediately after processing did not differ from thermally pasteurized samples in preference scores or average preference ranking. Matak et al. (2007) found that UV treatment (energy dosage 15.8 mJ/cm^2) affected the odour of goat milk, while in the study of Dunn et al. (1989), no visible discolouration nor changes in taste were found in dry cottage cheese exposed to energy dosage of 2000 mJ/cm^2. In the present work a consumer acceptance study was carried out on apple juice, because PL showed the greatest potential for microbial inactivation in this product. The results showed that shorter exposure times (2 or 4 s) did not affect any of the measured sensory attributes of apple juice, while a longer exposure time (i.e. 8 s) negatively impacted on product flavour.

In conclusion, this study has shown the possibility of successfully inactivating *E. coli* in reconstituted apple juice, by PL technology used as non-thermal preservation technique. Short exposure time achieved acceptable inactivation levels without compromising the quality parameters selected, showing a clear potential in terms of product safety and quality.

Acknowledgements

The authors would like to acknowledge the financial support of the Non-Commissioned Food Institutional Research Measure, administered by the Department of Agriculture, Fisheries and Food, Ireland and the technical support provided by Batchelor's Ltd., Ireland.

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