

High intensity light pulses to reduce microbial load in fresh cheese

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The present study focused on the utilisation of High Intensity Light Pulses (HILP) treatment to preserve mozzarella cheese. First, the susceptibility of *Pseudomonas fluorescens* and Enterobacteriaceae to HILP (fluences from 0.39 to 28.0 J/cm²) in a transparent liquid was evaluated (*in-vitro* tests). Afterwards, the effects on inoculated mozzarella cheese were also assessed. Then untreated (Control) and HILP treated samples were packaged and stored at 10 °C for 2 weeks. Enterobacteriaceae, *Pseudomonas* spp. and pH were monitored during storage. In a transparent liquid (*in-vitro* tests) there was a significant microbial inactivation just with 2 s of treatment. On the inoculated cheese a relevant microbial reduction of about 1 log cycle was observed, according to the exposure to the treatments. For *Pseudomonas* spp. in particular, in the treated samples, the microbiological acceptability limit (10⁶ cfu/g) was never reached after 2 weeks of refrigerated storage. To sum up, the efficacy of this treatment is very interesting because a microbial reduction was observed in treated samples. HILP treatment is able to control the microbial growth and may be considered a promising way to decontaminate the surface of mozzarella cheese.

Keywords: High Intensity Light Pulses, non-thermal technology, microbial inactivation, mozzarella cheese.

Nowadays consumers are more demanding and are constantly looking for higher quality foods. Their expectations are that such quality will be maintained during the period between purchase and consumption and for this reason one of the major issues facing food manufacturers is an increasing need to extend the shelf life of food products. In terms of cheese, mozzarella is one of the most appreciated products worldwide but due to its composition it has a relatively short shelf life.

One of the most critical factors affecting the shelf life of this fresh product is the proliferation of spoilage bacteria. Characteristics of the mozzarella cheese, such as its spun soft dough nature, its high moisture content (50–60%) and the fact that its soft texture is preserved by storing in trays or bags with brine at 4 °C are all factors which provide an environment which is well suited to the growth of spoilage bacteria. In general this product is very perishable and very susceptible to degradation processes in particular to the growth of *Pseudomonas* spp. and some members of

the Enterobacteriaceae family (Cantoni et al. 2003; Haouet et al. 2008; Mastromatteo et al. 2015). The proper combination of storage time and temperature creates an ideal growth environment for these psychrophilic microorganisms with the result that they become the dominant non-lactic bacteria population in cheese (Morales et al. 2005; De Jonghe et al. 2011; Franciosi et al. 2011; Martin et al. 2011). The presence of very high loads of *Pseudomonas* spp. and Enterobacteriaceae allow us to consider those bacteria as being the main species responsible for casein hydrolysis, exfoliation of the outer surface, anomalous discoloration and in general alteration of odour and taste during storage (Cantoni et al. 2003; Martin et al. 2011; Baruzzi et al. 2012).

Several strategies have been proposed to preserve mozzarella cheese. For example the use of different modified atmosphere packaging and bio-based active coatings are both proposed as valid approaches to control microbial proliferation and minimise sensory changes during storage (Gammariello et al. 2010; Incoronato et al. 2011; Angiolillo et al. 2014; Mastromatteo et al. 2015).

Recently, non-thermal novel technologies have been introduced as physical alternative methods to control microbial proliferation and preserve food quality (Arroyo et al.

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2012; Huo et al. 2013; Sharma et al. 2013; Manzocco et al. 2015; Lacivita et al. 2016). High Intensity Light Pulses (HILP) is one such technology, its mode of action being non-thermal, and it has considerable potential in the decontamination of food surfaces (Marquenie et al. 2003; Ferrario et al. 2013). It uses short light pulses (pulse width of 100–400 μ s) with wavelength distributed in the ranges from 180 to 1100 nm, including UV (180–380 nm), visible light (380–700 nm) and infrared (700–1100 nm). The effects of HILP on microorganisms are mostly due to three mechanisms. The photophysical effect, the photochemical action of the UV-C component of the light spectrum and to a lesser extent due to photo-thermal effects of the infrared component. These mechanisms can cause an instantaneous heat generation inside the product, rupture of the cell wall and membrane, inactivation of bacteria due to DNA damage by UV photons absorption with eventual cell death (Gómez-López et al. 2007; Miller et al. 2012). Studies on HILP treatments have been reported for fruit and vegetable (Oms-Oliu et al. 2010a; Izquier, & Gómez-López, 2011; Ramos-Villaruel et al. 2011, 2012), beverages such as fruit juice and milk (Palgan et al. 2011; Caminiti et al. 2011, 2012), but the technique has received limited attention in terms of its application to dairy products, particularly cheeses.

On the base of these considerations, the aim of the present work was to evaluate the surface decontamination effect of HILP on mozzarella cheese. The criteria were to achieve significant reduction without damage to this fresh cheese. First the susceptibility of *Pseudomonas fluorescens* and *Enterobacteriaceae* to treatment in a transparent liquid (Maximum Recovery Diluent, MRD) was evaluated. Afterwards, the decontamination efficacy of HILP on inoculated mozzarella cheese was assessed. Subsequently, the effectiveness of HILP treatment on naturally contaminated mozzarella cheese, during storage at 10 °C for 2 weeks, was evaluated.

Materials and methods

Microbial groups for sample inoculation

For preliminary *in-vitro* tests the experiments were conducted using *P. fluorescens* (DSM 50090) and *Enterobacteriaceae* microorganisms in a Maximum Recovery Diluent (MRD, Oxoid). The *P. fluorescens* strain was grown overnight at 25 °C in Plate Count Broth (PCB Oxoid) and then grown on Plate Count Agar (PCA Oxoid) for 24 h at 25 °C. The *Enterobacteriaceae* were previously isolated from chicken skin, were grown overnight at 37 °C in Violet Red Bile Glucose Agar (VRBGA, Oxoid) and then grown on Plate Count Agar (PCA Oxoid) for 24 h at 25 °C. Following, the colonies were taken from the surface of the Petri dish by adding 3 ml of sterile MRD. With a sterile spatula the colonies were suspended in the liquid. Then the suspension was used as initial microbial inoculum and was subjected to serial dilutions in the MRD, depending on the microbial

concentration to be reached. 15 ml of both *P. fluorescens* and *Enterobacteriaceae* suspensions (10^8 cfu/ml) were placed in sterile petri dishes of 90 mm diameter (Sterilin Limited, UK), resulting in a liquid depth of 2 mm. These samples were then exposed to HILP for various exposure times.

In terms of treating the cheese samples, mozzarella (30 g pieces) were purchased at a Dublin local market and preserved at 4 °C. Prior to treatment, cheese was inoculated with a 150 μ l of each microbial suspension, which was directly spread onto the sample surface (final concentration of 10^4 – 10^5 cfu/g). Mozzarella cheeses were then stored under refrigerated conditions at 4 °C for 2 h before HILP treatment in order to allow microorganisms to attach to the cheese surface.

To carry out the antimicrobial test of HILP during cheese storage, untreated controls and HILP treated samples without any inoculation were packaged in commercial plastic trays (PP, thickness 69 mm) with 50 ml brine (0.6% NaCl solution) and stored at 10 °C for 2 weeks. Periodically, the microbial quality and pH were monitored, according to details reported below.

High intensity light pulses (HILP) technology

HILP treatments were performed using the Steri-Pulse XL3000 Pulsed Light Sterilization System (Xenon Corporation, MA, USA). It consists of a stainless steel sterilisation chamber (Fig. 1) 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 between 200 and 1100 nm, with a maximum emission in the UV range. All samples were placed at a distance of 2.5 cm from the light source and exposed to HILP (frequency 3 Hz; pulse width of 360 μ s; fluence 1.17 J/cm²/pulse).

Specifically, the study was organised in two phases. In the first one, the susceptibility of microorganisms (10^8 cfu/ml) to the treatment in a transparent liquid (MRD) was evaluated (*in-vitro* tests). Petri dishes with microbial inoculums (15 ml) were exposed to increasing treatment times from less than 1 to 8 s, in order to receive total energy doses ranging from 1.17 to 28 J/cm², respectively. Subsequently, the surface decontamination efficacy of HILP on inoculated mozzarella samples (i.e. 10^4 – 10^5 cfu/g) was investigated. In this case treatment times from 1 to 8 s were chosen for treatments. On the second phase, the effect of HILP on normal samples (samples without inoculated microorganisms) was evaluated. Treatment times of 2 and 4 s were chosen for treatments with corresponding fluence of 7.02 and 14.04 J/cm², respectively. After the treatment, untreated and HILP treated samples were packaged and stored. Microbial quality and pH were monitored.

Microbiological analyses

For *P. fluorescens* detection, 0.1 ml of dilution was spread plated in duplicate onto *Pseudomonas* Agar Based (PAB,

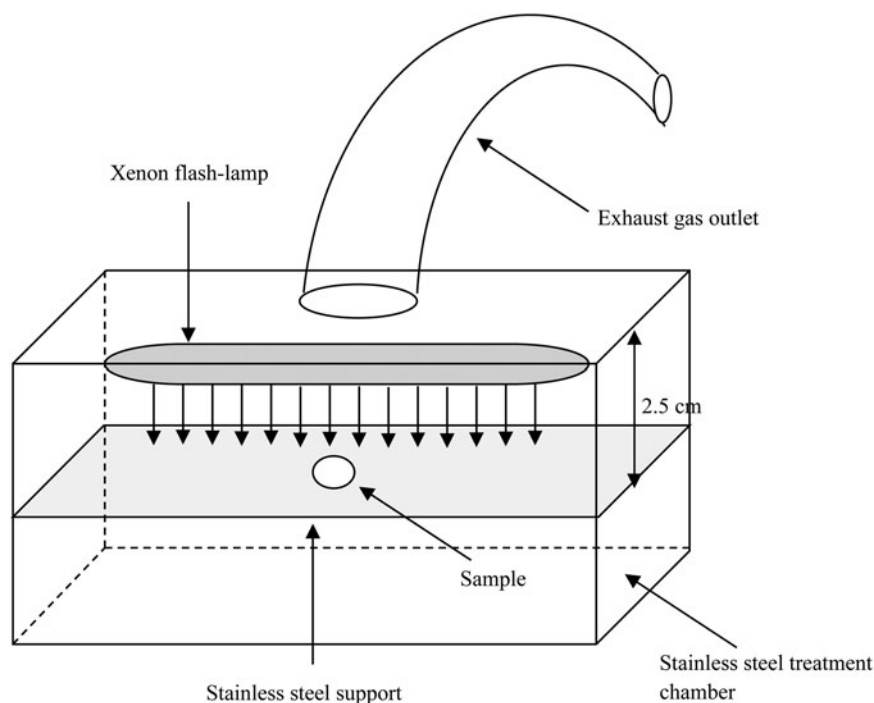


Fig. 1. Schematic representation of High Intensity Light Pulses (HILP) treatment of mozzarella cheese.

Oxoid) properly modified by adding *Pseudomonas* C-F-C SR103 (Oxoid) selective supplement. For enumeration of *Enterobacteriaceae* 1 ml of dilution was plated in duplicate onto Violet Red Bile Glucose Agar (VRBGA, Oxoid). The plates were incubated at 25 °C for 48 h and 37 °C for 24 h, respectively. For the mozzarella cheese samples 10 g were aseptically removed and immediately transferred to sterile stomacher bags, diluted with 90 ml of MRD (Oxoid) and homogenised with a Stomacher LAB Blender 400. Subsequently, decimal dilutions of homogenates were made using the same diluent (MRD) and the dilutions (0.1 or 1 ml) were plated on appropriate media as described above, for enumeration of *Pseudomonas* spp. and *Enterobacteriaceae*. Colony forming units were counted and expressed as log cfu/g (Isohanni & Lyhs, 2009). Microbial thresholds of 10^6 and 10^5 cfu/g were set for *Pseudomonas* spp. and *Enterobacteriaceae*, respectively (Mastromatteo et al. 2015).

pH measurements

pH was evaluated on homogenates of cheese as prepared for microbiological analyses and on brine in duplicate with a pH meter (Crison, Barcelona, Spain) after appropriate calibration.

Statistical analysis

Results are reported as mean value \pm SD. The statistical analysis was performed by one-way ANOVA test. A Duncan's

multiple range test, with the option of homogeneous groups ($P < 0.05$), was used to determine significance among differences. To this aim, STATISTICA v. 7.1 for Windows (StatSoft Inc., Tulsa, OK, USA) was used.

Results and discussion

Inactivation of P. fluorescens and Enterobacteriaceae in transparent liquid

The preliminary experiments with HILP were performed on inoculated MRD with a microbial concentration of 10^8 cfu/ml. The log cycles reduction was evaluated. Table 1 shows the behaviour of *P. fluorescens* and *Enterobacteriaceae* populations in MRD. These results showed that the microbial count for both microorganisms decreased with the increase in light fluence (Palgan et al. 2011; Miller et al. 2012). In particular, with a treatment time of 0.4 s corresponding to a HILP treatment with 1 pulse and a fluence of 1.17 J/cm², microbial reduction of *P. fluorescens* and *Enterobacteriaceae* of 2.54 and 2.12 log cycles, respectively, were observed. In contrast, a treatment time of 1 s corresponding to a fluence of 3.51 J/cm² reduced the microbial loads by 5.27 and 5.21 log cycles for *P. fluorescens* and *Enterobacteriaceae*, respectively. The microbial loads for both microorganisms were inactivated below the detection limit (<10 cfu/ml for *Pseudomonas fluorescens* and <1 cfu/ml for *Enterobacteriaceae*) after 2 s exposure to HILP treatment with a maximum fluence of 7.02 J/cm². These results showed that in a transparent liquid, there was a

Table 1. Inactivation of *Pseudomonas fluorescens* (DSM 50090) and *Enterobacteriaceae* in Maximum Recovery Diluent (MRD) by High Intensity Light Pulses (HILP)

Treatment times (s)	Pulses	Fluence (J/cm ²)	<i>P. fluorescens</i> (Log cfu/ml)	<i>Enterobacteriaceae</i> (Log cfu/ml)
0	0	0	8.74 ± 0.45 ^a	8.63 ± 0.15 ^a
0.4	1	1.17	6.20 ± 0.04 ^b	6.51 ± 0.09 ^b
0.7	2	2.34	5.28 ± 0.11 ^c	5.76 ± 0.04 ^c
1	3	3.51	3.47 ± 0.21 ^d	3.42 ± 0.32 ^d
2	6	7.02	<DL	<DL
4	12	14.04	<DL	<DL
8	24	28.08	<DL	<DL

DL, Detection limit for *Pseudomonas fluorescens*: 10 cfu/ml and *Enterobacteriaceae*: 1 cfu/ml in MRD, placed to 2.5 cm from the high intensity light source
^{a-d}Data with different superscript letters within a column are significantly different ($P < 0.05$)

significant microbial inactivation using this novel non-thermal technology, with just a few seconds of treatment (2 s). This finding is in agreement with what was observed in the study carried out on different liquid foods inoculated with different microbial strains (Palgan et al. 2011). Documented studies suggest that the ultraviolet (UV) portion of the spectrum is the most important for microbial inactivation (Gómez-López et al. 2007; Innocente et al. 2014). In fact the lethal effect of pulsed light on microorganisms is mostly attributed to the photochemical action of the UV portion (180–380 nm) of the spectrum emitted by the xenon flash lamp (Bintsis, et al. 2000; Oms-Oliu et al. 2010b; Innocente et al. 2014; Ignat et al. 2014). This leads to a microbial DNA absorption of UV light that induces chemical modifications, with impairment of cell replication and ultimately cell death (Gómez-López et al. 2007; Palgan et al. 2011; Miller et al. 2012; Ignat et al. 2014; Innocente et al. 2014).

Surface decontamination of inoculated mozzarella cheese by HILP treatment

To investigate the effectiveness of HILP treatment as a surface decontamination system, the inoculated mozzarella cheeses were exposed to HILP treatment at increasing times of up to 8 s. Table 2 shows the microbial counts of *P. fluorescens* and *Enterobacteriaceae* inoculated on the product surface. There was a greater reduction for both microbial strains until 4 s exposures but then, as the dose increased, the

population maintained approximately constant, without significant differences in general. In particular, significant reductions of 1.07 and 1.06 log cycles were achieved for *P. fluorescens* and *Enterobacteriaceae* with a fluence of 14.04 and 7.02 J/cm², respectively. Similar results were obtained by Dunn et al. (1995) in a study on curds inoculated with *Pseudomonas* spp. and treated with pulsed light (fluence of 16 J/cm²), resulting in a 1.5 log reduction of microbial population after the treatment. These observations are also in agreement with another study where reductions up to 1.69 log cycles for different microbial strains on chicken exposed to HILP have been achieved (Haughton et al. 2011). Other treatment times did not result in a significant reduction compared to untreated control, the reduction was less than 1 log cycle, which could be attributed to a non effective access of the radiation due to the irregular cheese surface. Of the two organisms studied *Enterobacteriaceae* appear slightly more sensitive to HILP treatment. At the same treatment conditions (fluence of 3.5 J/cm²) there was a 0.99 and a 0.55 log cycle reduction in *Enterobacteriaceae* and *P. fluorescens* samples respectively. The microbial reduction observed on mozzarella was not as high as that detected in the preliminary study conducted on transparent liquid (MRD). This is probably due to the protective effect of the cheese composition vs. a liquid buffer and to the fact that the cheese surface is rough and has pores or crevices where microorganisms will hide from light (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Palgan et al. 2011; Choudhary & Bandla, 2012; Innocente et al. 2014).

Table 2. Microbial counts of *Pseudomonas fluorescens* (DSM 50090) and *Enterobacteriaceae* inoculated on mozzarella cheese exposed to increasing fluence of High Intensity Light Pulses

Samples	Treatment times (s)	Fluence (J/cm ²)	<i>P. fluorescens</i> (Log cfu/g)	<i>Enterobacteriaceae</i> (Log cfu/g)
Control	0	0	4.82 ± 0.03 ^a	4.65 ± 0.14 ^a
A	1	3.51	4.27 ± 0.05 ^b	3.66 ± 0.06 ^c
B	2	7.02	3.91 ± 0.29 ^{b,c}	3.59 ± 0.02 ^c
C	3	10.53	3.83 ± 0.08 ^c	3.76 ± 0.02 ^{b,c}
D	4	14.04	3.75 ± 0.02 ^c	3.88 ± 0.04 ^b
E	5	17.55	3.97 ± 0.02 ^{b,c}	3.77 ± 0.10 ^{b,c}
F	6	21.06	3.99 ± 0.17 ^{b,c}	3.75 ± 0.11 ^{b,c}
G	7	24.57	3.92 ± 0.44 ^{b,c}	3.71 ± 0.01 ^{b,c}
H	8	28.08	3.99 ± 0.13 ^{b,c}	3.74 ± 0.14 ^{b,c}

^{a-c}Data with different superscript letters within a column are significantly different ($P < 0.05$)

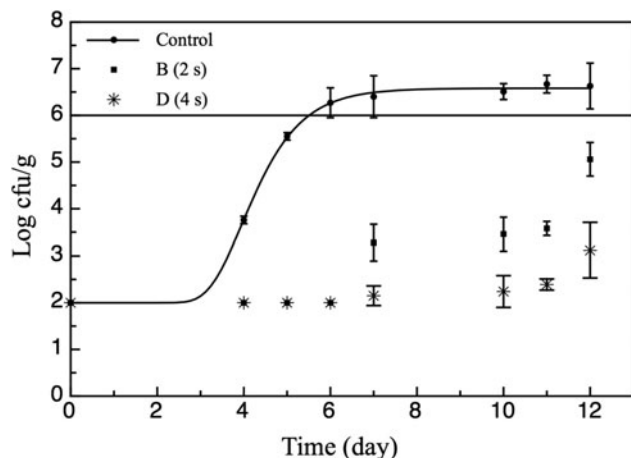


Fig. 2. Evolution of *Pseudomonas* spp. population during the refrigerated storage of mozzarella cheese exposed to two different High Intensity Light Pulses (HILP) treatments. Symbols: experimental data. Lines: best fits estimates. Control = untreated Mozzarella cheese; B = Mozzarella cheese treated for 2 s; D = Mozzarella cheese treated for 4 s.

HILP effect on normal mozzarella cheese

On the basis of the work on liquid and inoculated cheese samples, in the second phase of this study the effectiveness of HILP treatments on the native flora of mozzarella cheese was investigated without any preliminary contamination. Treatment parameters chosen were 2 s (sample B, Fig. 2) and 4 s (sample D, Fig. 2), corresponding to fluences 7.02 and 14.04 J/cm², respectively. For *Enterobacteriaceae*, no proliferation has been found in both control and treated samples. The absence of this microbial group allows us to hypothesise that good hygienic conditions were adopted during cheese making process (Gammariello et al. 2011). Fig. 2 shows the evolution of *Pseudomonas* spp. during refrigerated storage in mozzarella exposed to two different HILP treatments compared to untreated control. The horizontal line at 10⁶ cfu/g indicates the microbial limit above which changes to the quality parameters generally begin to appear (Cantoni et al. 2003; Angiolillo et al. 2014; Mastromatteo et al. 2015). The initial microbial load for all samples was below the detection limit (<10² cfu/g). After a few days of storage (4 d), an increase of *Pseudomonas* spp. population was observed in the control samples, and the microbiological limit was exceeded after 5.5 d. By contrast, for the HILP treated samples the microbial load remained below the detection limit until the 6th storage day. After this storage period a different trend was observed. In sample B (Fig. 2) a gradual increase of microbial load was noted, although the load did not reach the microbiological acceptability limit during up to 12 d storage. A slight increase of microbial count was also observed in the sample D (Fig. 2), reaching a maximum value of 3.12 log cfu/g¹ at the end of the 12-day observation period.

The pH of all samples (both cheese and brine) remained almost constant (around 6.0–6.5) during the microbial

testing, except for the control brine that reached values around 5.9–5.5, thus confirming the degradation process beginning to occur.

Conclusions

The current study investigated for the first time the effect of HILP on mozzarella cheese. The efficacy of this treatment is very interesting because a microbial reduction was observed in treated samples. From the preliminary test it was observed that in a transparent liquid (Maximum recovery diluent, MRD) there was a significant microbial inactivation with just 2 s of treatment (fluence 7.02 J/cm²). These results show that transparency of the media allowed successful microbial inactivation using this technology. In the case of inoculated mozzarella, a considerable microbial reduction was observed until 4 s of exposures, even if a lower microbial inactivation was noticed compared to that obtained in the transparent liquid. HILP treatment is able to control the microbial growth and may be considered a promising way to decontaminate the surface of mozzarella cheese. The short treatment time lends itself to application in high volume production lines. Further research is still necessary to optimise the application of HILP and also to investigate the effect of the technique on sensory parameters.

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