FOODBORNE PATHOGENS AND DISEASE
Volume 6, Number 10, 2009
© Mary Ann Liebert, Inc.
DOI: 10.1089/fpd.2009.0328



# Pulsed Light Inactivation of *Listeria monocytogenes* Through Different Plastic Films

Manuela Fernández, Susana Manzano, Lorenzo de la Hoz, Juan A. Ordóñez, and Eva Hierro


## Abstract

The efficacy of decontamination by pulsed light technology through different plastic films has been assayed using *Listeria monocytogenes* Scott A as target microorganism. A 12-$\mu$m polyethylene film, a 48-$\mu$m polyamide/polyethylene/vinyl acetate–based copolymer, and a 60-$\mu$m polyamide/polyethylene copolymer were tested. Noble agar plates were surface inoculated and wrapped with different films. Unwrapped plates were also analyzed as control. Fluences of 0.175 and 0.35 J/cm$^2$ were applied. Pulsed light treatment achieved the same degree of inactivation (5–5.5 log cfu/cm$^2$) in either wrapped or unwrapped samples. All the polymers showed the same behavior. These results indicate that pulsed light technology could be suitable for decontamination of packaged foods.


## Introduction

PULSED LIGHT TREATMENT is a promising nonthermal technology for microbial decontamination in the food industry. The process consists of the application of short duration pulses ($10^{-3}$ to $10^2$ ms) of an intense broad spectrum (200–1000 nm) light. The UV-C region of the spectrum (200–290 nm) is the main frequency band responsible for the bactericidal effect, which is primarily attributed to a photochemical damage on DNA (Wang *et al.*, 2005). It is well known that UV light is effective for the inactivation of microorganisms (Stermer *et al.*, 1987; Bintsis *et al.*, 2000). Pulsed light is a more recent approach that allows a greater energy input than the conventional continuous systems. However, as pulsed light has a low penetration depth, it is mainly indicated for the superficial decontamination of foods, packaging materials, and food contact surfaces.

*Listeria monocytogenes* is a major safety concern that has emerged as the cause of many foodborne infections during the past decades and, in recent years, it has been associated to the consumption of ready-to-eat products, a growing sector in the food market (Meloni *et al.*, 2009). It is a ubiquitous psychrotrophic organism, which can survive in biofilms and resist diverse environmental conditions, such as low pH and high NaCl concentrations (Doyle *et al.*, 1997). Therefore, its control is of paramount importance in the food industry. Previous works have shown that this organism is among the most resistant to pulsed light (Gómez-López *et al.*, 2005).

The purpose of the present work was to study the capability of pulsed light technology to penetrate different plastic materials using *L. monocytogenes* as target organism. This would be a preliminary step to assess the efficacy of this new technology for decontamination of packaged foods.

## Materials and Methods

*L. monocytogenes* Scott A (CIP 103575, serotype 4b) was supplied by the Colección Española de Cultivos Tipo (i.e., Spanish Type Culture Collection). Freeze-dried bacteria were subcultured two times in trypticase soy broth (TSB) to the stationary growth phase. The assays were performed on 1.5% Noble agar (Difco, Sparks, MD) as holding matrix. Petri dishes containing 30 mL of agar were superficially inoculated with 0.1 mL of the appropriate dilution to reach a microbial count of $10^6$ cfu/cm$^2$. Plates were left to dry and then treated. Four batches were processed: 1, unwrapped plates; 2, 3, and 4, plates wrapped with a 12-$\mu$m polyethylene film (Cofresco Ibérica S.A., Madrid, Spain), a 48-$\mu$m polyamide/polyethylethylene/vinyl acetate–based copolymer (Cryovac, Sealed Air, Barcelona, Spain), and a 60-$\mu$m polyamide/polyethylene

Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain.

copolymer (Plastiñi, Plastinal S.L., Arrubal, Spain), respectively. Three experiences were performed in 3 different days to account for a total of 15 plates assayed per condition.

Pulsed light treatment was applied in a desktop equipment Steribeam SBS-XeMatic-2L-A (SteriBeam Systems, Kehl/am Rhein, Germany). It consists of a metal housing surrounding a treatment chamber made with polished stainless steel (20 cm wide, 14 cm deep, and 12 cm high) and equipped with two (upper and lower) xenon lamps and a quartz table located at the center. Only the upper lamp was operated, and the equipment was adjusted to apply flashes of 150 J, equivalent to a fluence (incident light energy per unit of surface area) of 0.175 J/cm$^2$. The inoculated Petri dishes were placed on the quartz table and flashed with one and two pulses. Pulse duration is 250 $\mu$s. During the treatment, the agar plate temperature was monitored using a type K thermocouple (Omega Engineering, Stamford, CT). No warming of the medium was observed.

For enumeration, the agar was aseptically removed from the dishes and mixed with 70 mL of peptone water in a sterile plastic bag. The mixture was homogenized in a Colworth Stomacher 400 for 2 min, and serial 10-fold dilutions were prepared. A spiral plate system (Eddy Jet, IUL Instruments, Barcelona, Spain) was used for plating. Survivors were enumerated on trypticase soy agar (TSA) after incubation at 32°C for 24 h. To assess the possible survival below the detection limit, the agar/peptone homogenate was simultaneously incubated under the same conditions and inoculated on TSA plates. When no colonies were detected on the initial dilutions, but growth was observed on the plates inoculated with the homogenate, results were expressed as $<$0.9 log cfu/cm$^2$ of the survivors.

A one-way analysis of variance was conducted to compare the results of the different assays, using Statgraphics Plus 5.0.

## Results and Discussion

The same degree of inactivation was observed in either wrapped or unwrapped plates after the pulsed light treatment (Table 1). The application of 0.175 J/cm$^2$ gave approximately 5 log cfu/cm$^2$ reductions in all samples, and no significant differences were observed. The use of a higher fluence (0.35 J/cm$^2$) provided the maximum inactivation rate (5.5 log cfu/cm$^2$). In this case, no survivors were detected on TSA, although growth was observed after the incubation of the initial homogenates.

Table 1. Inactivation (log cfu/cm$^2$) of *Listeria monocytogenes* Scott A Through Different Plastics

| | Fluence | |
|---|---|---|
| | *0.175 J/cm$^2$* | *0.35 J/cm$^2$* |
| Unwrapped plates | 5.1 ± 0.1 | 5.5[a] |
| Cofresco[b] film | 4.9 ± 0.2 | 5.5[a] |
| Cryovac[c] film | 5.1 ± 0.3 | 5.5[a] |
| Plastiñi[d] film | 5.3 ± 0.1 | 5.5[a] |

Initial contamination (mean ± SD) was 6.4 ± 0.1 log cfu/cm$^2$.
[a]No survivors were observed after incubating the plates, but growth was obtained after the incubation of the initial homogenate, which was expressed as 0.9 log cfu/cm$^2$.
[b]Polyethylene 12 $\mu$m.
[c]Polyamide/polyethylene/vinyl acetate 48 $\mu$m.
[d]Polyamide/polyethylene 60 $\mu$m.

The level of inactivation achieved in the present work is similar to the observations reported by MacGregor *et al.* (1998) and Rowan *et al.* (1999) in unwrapped trypticase soy yeast extract (TSYE) agar plates superficially inoculated with *L. monocytogenes*. On the other hand, Gómez-López *et al.* (2005) obtained a lower inactivation, 2.8 log cfu/cm$^2$, using a similar procedure. However, it is not easy to compare the observations made by different authors since pulsed light devices have different configurations and, in most cases, authors provide information on the number of pulses, the energy delivered per pulse, and/or the distance to the lamp, but no data on the fluence (J/cm$^2$) are reported.

Pulsed light efficacy is related with the surface topography (Woodling and Moraru, 2005). Therefore, a high level of inactivation could be expected when inoculated agar is treated at low fluences. When the treatment is applied to foods, the surface features (i.e, roughness and porosity) may allow shadowing of microbial cells, reducing its efficacy and requiring the application of higher fluences. For instance, Ozer and Demirci (2006) obtained 1 log cfu/g reduction of *L. monocytogenes* on raw salmon fillets applying 180 pulses of 5.6 J each, with the sample placed at 8 cm from the UV strobe.

Regardless of the fluence required to achieve the desired level of inactivation, the interest of the present work is that all the plastic films assayed were easily penetrated by light and, therefore, they would be suitable for pulsed light treatment of packaged foods. This will be the next step of the current research.

## Acknowledgments

This work has been supported by the projects AGL2007-65235-C02-02 and CONSOLIDER-INGENIO 2010-CARNISENUSA of the Ministerio de Educación y Ciencia (Government of Spain).

## Disclosure Statement

No competing financial interests exist.

## References

Bintsis T, Litopoulou-Tzanetaki E, Robinson RK, *et al.* Existing and potential application of ultraviolet light in the food industry—a critical review. J Sci Food Agric 2000;90:637–645.

Doyle MP, Beuchat LR, Montville TJ, *et al. Food Microbiol. Fundamentals and Frontiers*. Washington DC: ASM Press, 1997.

Gómez-López VM, Devlieghere F, Bonduelle V, Debevere J, *et al.* Factors affecting the inactivation of micro-organisms by intense light pulses. J Appl Microbiol 2005;99:460–470.

MacGregor SJ, Rowan NJ, McIlvaney L, Anderson JG, Fouracre RA, Farish O, *et al.* Light inactivation of food-related pathogenic bacteria using a pulsed power source. Lett Appl Microbiol 1998;27:67–70.

Meloni D, Galluzzo P, Mureddu A, Piras F, Griffiths M, Mazzette R, *et al. Listeria monocytogenes* in RTE foods marketed in Italy: prevalence and automated *Eco*RI ribotyping of the isolates. Int J Food Microbiol 2009;129:166–173.

Ozer NP and Demirci A. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. Int J Food Sci Technol 2006;41:354–360.

Rowan NJ, MacGregor SJ, Anderson JG, Fouracre RA, McIlvaney L, Farish O, *et al.* Pulsed-light inactivation of food-related microorganisms. Appl Environ Microbiol 1999;65:1312–1315.

Stermer RA, Lasater-Smith M, Brasington CF, *et al.* Ultraviolet radiation—an effective bactericide for fresh meat. J Food Prot 1987;50:108–111.

Wang T, MacGregor SJ, Anderson JG, Woolsey GA, *et al.* Pulsed ultra-violet inactivation spectrum of *Escherichia coli*. Water Res 2005;39:2921–2925.

Woodling S and Moraru C. Influence of surface topography on the effectiveness of pulsed light treatment for the inactivation of *Listeria innocua* on stainless-steel surface. J Food Sci 2005;70:345–351.

Address correspondence to:
*Manuela Fernández, Ph.D.*
*Departamento de Nutrición*
*Bromatología y Tecnología de los Alimentos*
*Facultad de Veterinaria*
*Universidad Complutense de Madrid*
*Avda Puerta de Hierro s/n*
*Madrid 28040*
*Spain*

*E-mail:* manuela@vet.ucm.es