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Effect of Spectral Range in Surface Inactivation of *Listeria innocua* Using Broad-Spectrum Pulsed Light

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ABSTRACT

Pulsed light (PL) treatment is an alternative to traditional thermal treatment that has the potential to achieve several log-cycle reductions in the concentration of microorganisms. One issue that is still debated is related to what specifically causes cell death after PL treatments. The main objective of this work was to elucidate which portions of the PL range are responsible for bacterial inactivation. Stainless steel coupons with controlled surface properties were inoculated with a known concentration of *Listeria innocua* in the stationary growth phase and treated with 1 to 12 pulses of light at a pulse rate of 3 pulses per s and a pulse width of 360 μs. The effects of the full spectrum (λ = 180 to 1,100 nm) were compared with the effects obtained when only certain regions of UV, visible, and near-infrared light were used. The effectiveness of the treatments was determined in parallel by the standard plate count and most-probable-number techniques. At a fluence of about 6 J/cm^2, the full-spectrum PL treatment resulted in a 4.08-log reduction of *L. innocua* on a Mill finish surface, the removal of $\lambda < 200$ nm diminished the reduction to only 1.64 log, and total elimination of UV light resulted in no lethal effects on *L. innocua.* Overwhelmingly, the portions of the PL spectrum responsible for bacterial death are the UV-B and UV-C spectral ranges ($\lambda < 300$ nm), with some death taking place during exposure to UV-A radiation ($300 < \lambda < 400$ nm) and no observable death upon exposure to visible and near-infrared light ($\lambda > 400$ nm). This work provides additional supporting evidence that cell death in PL treatment is due to exposure to UV light. Additionally, it was shown that even a minor modification of the light path or the UV light spectrum in PL treatments can have a significant negative impact on the treatment intensity and effectiveness.

Pulsed light (PL) treatment has emerged in recent years as a feasible alternative to thermal treatment for killing pathogenic and spoilage microorganisms *(1, 2, 17).* PL technology relies on a series of very short, high-power pulses of broad-spectrum light, typically emitted by a xenon lamp, to destroy bacteria (both vegetative cells and spores), yeasts, molds, and even viruses *(3, 9, 11, 13, 17).* Its use was approved by the U.S. Food and Drug Administration (FDA) in 1996 for the decontamination of food or food contact surfaces under the condition that xenon flashlamps are used as the PL source and the cumulative treatment does not exceed 12 J/cm^2 *(14).*

PL treatment results in various levels of inactivation of spoilage and pathogenic microflora on the surface of a wide variety of solid foods. Comprehensive reviews of the literature in this field have been compiled by the FDA *(15)* and by Woodling and Moraru *(17).* The variability of the results (a 2- to 8-log reduction was generally reported) is most likely due to the different challenge microorganisms used in various studies, the intensity of the treatment, and the different properties of the treated substrates *(1, 2, 5, 8, 11, 17).* Woodling and Moraru *(17)* demonstrated that the efficacy of PL is affected by substrate properties such as topography and hydrophobicity, which affect both the distribution of microbial cells on the substrate surface and the interaction between light and the substrate (i.e., reflection and absorption of light).

One source of controversy in PL treatment concerns the mechanisms of microbial inactivation. Because a significant portion of the PL spectrum includes the UV range, UV radiation damage is thought to play an important role in the inactivation of microorganisms by PL. While the effect of UV radiation and the mechanisms by which it causes cell death are clearly understood *(4, 7, 18),* the role of the visible (VIS) and near-infrared (NIR) portions of the PL spectrum on cell death is less clear. The FDA report *(15)* supported the hypothesis that the high energy and intensity of PL amplify the mechanisms of destruction of cellular components at different wavelengths, causing extensive irreversible damage to DNA, proteins, and other macromolecules. Physical destruction of *Aspergillus niger* spores as a result of structural collapse after instantaneous overheating of the cellular constituents *(16)* and of *Saccharomyces cerevisiae* yeast cells due to enlarged vacuoles *(13)* after PL treatment have been reported, and both research groups stressed the importance of the high peak power and UV wavelengths in microbial inactivation. Rowan et al. *(9)* found only minimal heating after treating a variety of food-related bacteria with both high-level and low-level UV light sources. These researchers proposed that the major reason for inactivation is structural damage of DNA and that damage of membranes, proteins, and other macromolecules play a minor role. Thus, many questions remain unanswered regarding how PL lethally affects microorganisms.

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This study was designed to further the understanding of the factors and mechanisms responsible for microbial inactivation by PL by clearly identifying the spectral range associated with the lethal effect in PL treatment of surfaces. Stainless steel, chosen as a model substrate to mimic food contact surfaces, was inoculated with a known concentration of the challenge microorganism, *Listeria innocua,* and exposed to different PL treatments in various spectral domains in the UV and NIR ranges of the electromagnetic spectrum. The effectiveness of the process was evaluated by enumerating the survivors using both the standard plate count (SPC) and most-probable-number (MPN) techniques, which were used in parallel to capture any sublethal injury and cellular recovery that might have occurred beyond the resuscitation procedures already in use.

MATERIALS AND METHODS

Culture and inoculum preparation. A culture of *L. innocua* FSL C2-008 (environmental isolate, ACME Smoked Fish Corp. processing plant, Brooklyn, N.Y.) was obtained from the culture collection maintained by the Food Microbiology and Safety Laboratory in the Food Science Department at Cornell University (Ithaca, N.Y.). This culture was maintained on tryptic soy agar slants (TSA; Becton Dickinson, Sparks, Md.). Cells were transferred to 10 ml of tryptic soy broth (TSB; Becton Dickinson) and incubated at 23°C for 24 h, resulting in a population of about 10^9 CFU/ml. One-milliliter aliquots were then transferred to 9 ml of Butterfield's phosphate buffer to produce an initial inoculum of about 10^8 CFU/ml.

Stainless steel coupon preparation. Food grade stainless steel coupons were obtained from Pacific Sensor, Inc. (Fountain Valley, Calif.). Two surface finishes were used: mill finish, which simulates normal stainless steel surfaces encountered in food-processing environments, and an aluminum oxide–treated finish, which simulates surfaces that have undergone some degree of wear and corrosion. Total roughness of the two surfaces used in the study was 2.88 μm for the mill finish and 17.45 μm for the aluminum oxide–treated finish *(17).* All coupons were rectangular and 2 by 4 in. (50.8 by 101.6 mm).

Before the treatments, all coupons were washed for 30 min in FS30H ultrasonic cleaner (Fisher Scientific, Pittsburgh, Pa.) that contained a 30:1 dilution of Fisherbrand Versa-Clean (Fisher Scientific). Each coupon was then individually rinsed three times in distilled water, allowed to air dry under a laminar hood, and then autoclaved at 121°C for 1 h in covered glass beakers.

Coupon inoculation. The autoclaved coupons were placed under a laminar flow hood on sanitized aluminum foil and then inoculated immediately by placing 1 ml of the inoculum in the center of each coupon. The inoculated coupons were then left to dry at room temperature under the laminar flow hood for about 90 min to allow the bacterial cells to adhere to the metal surface. The time required for complete air drying of the aqueous inoculum was established experimentally. The drying step was carried out to create a worst-case scenario of bacterial contamination of a food contact surface, in which the bacterial cells are settled on the solid surface, rather than suspended in a transparent liquid, and thus are more difficult to kill.

PL treatment. The PL treatments were performed with an RS-3000C SteriPulse System (Xenon Corporation, Woburn, Mass.). The system consists of a controller unit and a treatment chamber that houses a xenon flash lamp. Each inoculated and dried coupon was centered individually on an adjustable stainless steel shelf in the PL unit at 2 in. (50.8 mm) beneath the xenon lamp and treated with 1 to 12 pulses of light, at a pulse rate of 3 pulses per s and a pulse width of 360 μs. To evaluate the effect of different portions of the PL range, three optical filters (16 by 3.5 by 0.12 in. [406.5 by 90 by 3 mm]; QSP Technologies, Santa Ana, Calif.) were used to selectively allow the transmission of certain portions of the broad spectrum of light. The filters used were (i) a UV-transmitting filter, which allowed partial passage of UV-A (λ = 300 to 400 nm) and NIR ($\lambda > 700$ nm), (ii) a UV-blocking filter, which only transmitted the light of $\lambda > 400$ nm, and (iii) a cold mirror, with partial transmission (about 50%) of UV (λ = 200 to 400 nm), total blocking of VIS, and nearly full transmission of NIR ($\lambda > 700$ nm). The transmission spectra for the UV-blocking and UV-transmitting filters and the reflection spectrum for the cold mirror are shown in Figure 1.

The filters were placed in the PL unit 5 mm from the lamp housing on a solid metallic shelf that was specially designed to minimize secondary reflections of light or the passage of light around the filters. To account for any changes in the path of light inside the PL chamber caused by the use of filters, an untreated fused quartz plate (Corning 7980 material, Technical Glass Products, Painesville, Ohio) of dimensions identical to those of the filters was used as a control. According to the manufacturer, the fused quartz plate has near 100% transmission in the spectral range of 200 to 2,000 nm (http://www.technicalglass.com/), which matches closely the spectral range emitted by the xenon lamp.

Fluence measurements. The intensity of each treatment (fluence, expressed in joules per square centimeter) was measured with a Nova II power meter equipped with a PE25-BBH-V2 pyrodetector head (Ophir Optronics, Inc., Wilmington, Mass.). The fluence measurements were performed at well-defined distances from the quartz face of the xenon lamp using an aperture cover with a circular opening of 1 cm^2 and a pulse-width meter setting of 1.0 ms. All fluence measurements were performed in triplicate.

Recovery and resuscitation procedures. Cells were recovered from the surface of the coupons using the procedure described by Woodling and Moraru *(17).* The inoculated and treated coupons were individually placed in Whirl-Pak bags that contained 100 ml of TSB. Each bag was massaged by hand for a total of 2 min, rubbing the surface of the coupon for 45 s, shaking the bag vigorously for 15 s, and then repeating the procedure. Preliminary work indicated zero survivors when the recovery broth was plated immediately after massaging the PL-treated coupons, regardless of the severity of the treatment. This finding suggested that sublethal injury occurred during PL treatment of *L. innocua,* and therefore a resuscitation step was developed *(17).* Resuscitation consisted of transferring the recovery TSB to another Whirl-pak bag and then incubating this bag at 30°C for 3 h.

Evaluation of PL effectiveness. After the resuscitation step, the TSB samples were serially diluted and plated on TSA. Plates were incubated at 37°C for 48 h, and the survivors (N) were enumerated and expressed as log CFU. Inactivation of *L. innocua* by the various treatments was expressed as the difference between N and the initial inoculum concentration (N_0) and was expressed as $\log(N/N_0)$. Experimental data collected over several months indicated that not all the cells that were inoculated onto the metallic surfaces could be recovered in the TSB, presumably because of both slight attachment of the cells to the metallic surface and/or cell death during the drying step. These losses were quantified at <1 log CFU, with standard deviations of <0.1 log CFU as previously reported *(17).* In the present study, the microbial reduction

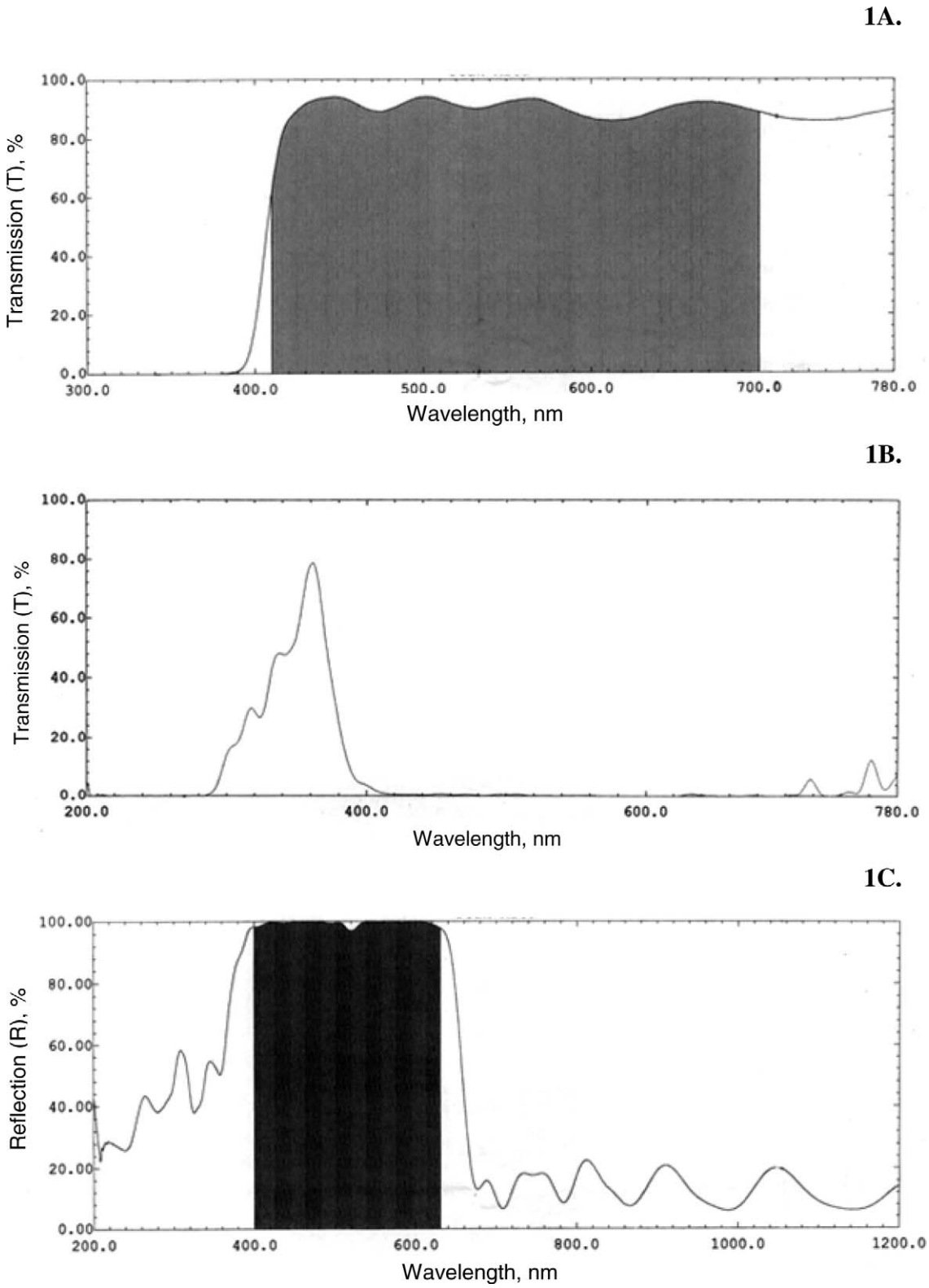

FIGURE 1. *Specific spectra for the three filters used in the study. (A) Transmission spectrum for the UV-blocking filter. (B) Transmission spectrum for the UV-A–transmitting filter. (C) Reflection spectrum for the cold mirror.*

by PL treatment was calculated by subtracting the survivors from the initial inoculum, which leads to a slight overestimation of the effectiveness of the PL treatment. Each data point represents the average of a minimum of six replicates (three PL treatments and two microbiological replicates per treatment). For certain treatments, up to 28 replicate measurements were made (19 PL treatments and two microbiological replicates per treatment) (Tables 1, 3, and 4).

MPN technique. In addition to the SPC procedures, the survivors were also enumerated using the MPN technique. Following the procedure reported by Swanson et al. *(12),* aliquots of the

TABLE 1. *Reduction of* L. innocua *on coupons with a mill finish and an aluminum oxide (AO) finish after exposure to PL light treatments*

Treatment	Coupon finish	Avg inactivation of *L. innocua* ($\log(N/N_0)$)[a]					
		1 pulse	2 pulses	3 pulses	6 pulses	9 pulses	12 pulses
No filter	Mill	−3.19 ± 0.07 A	−4.47 ± 0.12 B	−3.66 ± 0.16 AC	−4.08 ± 0.10 BC	−4.02 ± 0.10 BC	−5.32 ± 0.14 D
	AO	−3.98 ± 0.14 A	−3.35 ± 0.05 ABD	−4.05 ± 0.07 AB	−4.98 ± 0.25 C	−3.88 ± 0.23 ABD	−3.95 ± 0.16 D
Quartz filter (control)	Mill	−1.64 ± 0.23 A	−1.24 ± 0.07 A	−1.82 ± 0.22 A	−1.64 ± 0.14 A	−2.89 ± 0.22 B	−3.26 ± 0.40 B
	AO	0.11 ± 0.16 A	−0.12 ± 0.10 A	−1.97 ± 0.18 BC[b]	−1.75 ± 0.09 B[b]	−2.70 ± 0.06 C[b]	−2.12 ± 0.03 B
UV blocking filter (UV-B)	Mill	0.10 ± 0.03 AC	0.23 ± 0.05 AB	0.19 ± 0.06 A	0.48 ± 0.08 B	−0.05 ± 0.05 C	0.07 ± 0.06 AC
	AO	1.00 ± 0.04 A	1.29 ± 0.24 A	1.01 ± 0.03 A	1.01 ± 0.06 A	0.12 ± 0.19 B	0.32 ± 0.05 B
UV-A transmitting filter (UVT)	Mill	0.46 ± 0.07 A	0.41 ± 0.06 A	−0.41 ± 0.06 B	−0.45 ± 0.07 B	−0.47 ± 0.09 B	−0.21 ± 0.08 B
	AO	0.54 ± 0.03 A	0.56 ± 0.03 B	0.87 ± 0.03 ABC	0.96 ± 0.10 BC	−0.16 ± 0.09 D	−0.31 ± 0.12 D
Cold mirror	Mill	−0.08 ± 0.04 A	−0.20 ± 0.08 AB	−0.53 ± 0.22 AB	−0.74 ± 0.20 B	−0.47 ± 0.14 AB	−0.41 ± 0.10 AB
	AO	−0.28 ± 0.04 A	0.31 ± 0.08 A	−0.20 ± 0.03 B	−0.38 ± 0.05 B	−0.36 ± 0.09 B	−0.62 ± 0.04 C

[a] Values are mean ± SE. Each value for the mill finish treatments represents the mean of 12 to 28 replicates, and each value for the AO finish treatments represents the mean of 6 to 16 replicates. Different letters indicate significantly different levels of inactivation between treatments for each surface and type of filter: $P < 0.01$ for most situations, and $P < 0.05$ for a small number of situations.

[b] Less than six replicates were used to calculate the mean because of plate contamination.

recovery TSB were diluted from 10^{-2} to 10^{-7}, inoculated into three replicate tubes for each dilution level, and incubated at 37°C for 48 h. Turbidity was used as an indicator of positive samples, and any atypical positive samples were further confirmed by streaking onto TSA and incubating at 37°C for 48 h and identifying typical *L. innocua* colonies by their bluish gray color *(10).*

Statistical analysis. For each treatment, the mean, standard deviation (SD), and standard error (SE) of inactivation were calculated. The significance of differences between various treatments was evaluated with the Tukey test, which consists of a pairwise comparison of the means of multiple data sets. Statistical analysis was performed with Minitab version 6 (Minitab Inc., Minneapolis, Minn.) and Microsoft Excel (Microsoft Corp., Redmond, Wash.).

RESULTS AND DISCUSSION

Inactivation of *L. innocua* under different PL treatment conditions. Although the filter support was designed such that the path of the light in the PL chamber was only minimally altered, one of the primary concerns that had to be addressed was whether the use of the filter setup would significantly change the output of the PL treatment. A comparison between PL treatments performed without the filter setup and with the filter support and a fused quartz (control) filter was carried out. The substrates were coupons with two different types of stainless steel surfaces: mill finish and aluminum oxide–treated finish. Each coupon was inoculated with *L. innocua* at 10^7 to 10^8 CFU. The inactivation on the aluminum oxide finish was consistently lower than that on the mill finish under similar treatment conditions. This result is consistent with earlier findings *(17)* and can be attributed to the reduced treatment effect on cells lodged in the surface imperfections of the rougher surface.

The use of the filter setup and the control filter resulted in inactivation levels that were lower by 1 to 2 log CFU than the inactivation levels obtained without the filter setup (Table 1). This difference probably was due to both a slight modification of the light path and the absorption by the quartz plate of UV light of $\lambda < 200$ nm.

Experimental data clearly revealed that when filters altered the spectral range of the PL treatment, particularly the UV component, the inactivation of *L. innocua* was extremely small (Table 1). Although for the PL treatments performed with minimal or no alterations of the PL spectrum the level of inactivation increased significantly with fluence, for the UV-absorbing filters the inactivation levels were almost negligible and relatively constant throughout the PL range used (1 to 12 pulses). Significant differences are indicated by different letters in Table 1. The low levels of *L. innocua* inactivation obtained when using the filters could be attributed to significantly lower fluence in the presence of filters, removal of regions of the UV spectrum that are critical for bacterial inactivation, or both.

To correctly compare microbial inactivation, fluence measurements were performed for all PL treatments in this study. The use of filters had a significant effect on the treatment intensity at the substrate level. Although the quartz (control) filter reduced the PL fluence by only about 10%, for all other filters the fluence reduction exceeded 50% be-

TABLE 2. *Estimated fluence values for all PL treatments*

PL treatment	Transmitted wavelengths (λ)	PL treatment intensity (fluence)		
		Fluence (F) vs distance from the xenon lamp (x)	Fluence/pulse (J/cm^2)[a]	% fluence transmitted
No filter	$180 < \lambda < 1{,}100$ nm (light emitted by xenon lamp)	$F = 2.20e^{-0.015x}$ ($r^2 = 0.97$)	1.06	100
With filter				
Fused quartz filter (control)	Total transmission for $200 < \lambda < 2{,}000$ nm	$F = 1.86e^{-0.013x}$ ($r^2 = 0.98$)	0.96	91
Cold mirror	Partial transmission for $200 < \lambda < 400$ nm; almost total transmission for $\lambda > 700$ nm	$F = 1.07e^{-0.014x}$ ($r^2 = 0.98$)	0.54	51
UV-A transmitting	Partial transmission for $300 < \lambda < 400$ nm	$F = 0.81e^{-0.014x}$ ($r^2 = 0.98$)	0.41	38
UV blocking	$\lambda > 400$ nm	$F = 1.06e^{-0.014x}$ ($r^2 = 0.98$)	0.54	50

[a] Fluence was calculated at the substrate surface (49.8 mm from the face of the xenon lamp housing).

cause of absorption of light in various spectral ranges (Table 2). Regardless of the filter used, fluence decayed exponentially with distance from the lamp (Fig. 2). The quantitative fluence versus distance relationships were determined for all PL treatments (Table 2) and were then used to calculate the exact fluence per pulse at the substrate level.

Effect of spectral range on the PL inactivation of *L. innocua*. Even at the same fluence, inactivation was lower for the quartz (control) filter treatment than for the no-filter treatments (Figs. 3 and 4). The PL treatment performed through the quartz filter resulted in maximum losses of *L. innocua* that were about 2 log CFU less than those for the no-filter treatments at comparable fluences (approximately 12 J/cm^2). This reduced inactivation probably is due to the removal of a small portion of the UV spectrum by the quartz filter ($\lambda < 200$ nm), which clearly indicates that the UV range is important in inactivation, as expected, and that even a slight modification of the UV profile in the PL treatment can impact treatment effectiveness.

The most striking results regarding the effect of portions of the broad light spectrum were obtained when using the UV-blocking filter, which resulted in practically no reduction of *L. innocua* over the entire range of treatment intensities (Table 1). In some instances microbial growth rather than microbial inactivation was observed, as indicated by the positive $\log(N/N_0)$ values. Positive $\log(N/N_0)$ values were recorded for most treatment intensities and both surface types and were attributed to cell growth that possibly took place during the resuscitation procedure. Thus, when the wavelengths of <400 nm were removed from the treatment and the *L. innocua* cells were exposed only to the VIS and NIR portions of the spectrum (>400 nm) the cells did not receive even sublethal injury.

The lethal effects of the individual subranges of UV light could not be investigated in this study because no commercially available filters enabled a clear isolation of the UV-A, UV-B, and UV-C wavelengths. However, some useful conclusions can be drawn when comparing the effect of the UV-A–transmitting filter and the cold mirror. The cold mirror partially transmitted the UV light in the range of 200 to 400 nm, reflected all the visible light (400 to 700 nm), and only slightly transmitted the NIR wavelengths (>700 nm) (Fig. 5). For the mill finish, the use of the cold mirror resulted in a very modest degree of inactivation. Because the results from the UV-blocking filter indicated that the VIS and NIR ranges do not have microbicidal effects, any microbial reduction obtained when using the cold mirror probably was due to the UV light that escaped reflection by this filter (200 to 400 nm).

TABLE 3. L. innocua *inactivation for the mill finish coupons subjected to similar PL fluences but different spectral ranges*[a]

Treatment	No filter	Quartz filter	Cold mirror	UVT	UV-B
3 J/cm^2 (treatment between groups: $F = 90.44$, $P < 0.0001$)					
Treatment intensity	3 pulses, $F = 3.2$ J/cm^2	3 pulses, $F = 2.9$ J/cm^2	6 pulses, $F = 3.2$ J/cm^2	6 pulses, $F = 2.4$ J/cm^2	6 pulses, $F = 3.2$ J/cm^2
Inactivation ($\log(N/N_0)$)	−3.66 ± 0.16 A	−1.82 ± 0.22 B	−0.74 ± 0.20 C	−0.45 ± 0.07 C	0.48 ± 0.08 D
6 J/cm^2 (treatment between groups: $F = 127.61$, $P < 0.0001$)					
Treatment intensity	6 pulses, $F = 6.4$ J/cm^2	6 pulses, $F = 5.8$ J/cm^2	12 pulses, $F = 6.5$ J/cm^2	12 pulses, $F = 4.9$ J/cm^2	12 pulses, $F = 6.4$ J/cm^2
Inactivation ($\log(N/N_0)$)	−4.08 ± 0.10 A	−1.64 ± 0.14 B	−0.41 ± 0.10 C	−0.21 ± 0.08 CD	0.07 ± 0.06 D

[a] Values are mean ± SE. Values followed by different letters are significantly different ($P < 0.01$).

TABLE 4. L. innocua *inactivation for the aluminum oxide-finish coupons subjected to similar PL fluences but different spectral ranges*[a]

Treatment	No filter	Quartz filter	Cold mirror	UVT	UV-B
3 J/cm^2 (treatment between groups: $F = 251.40$, $P < 0.0001$)					
Treatment intensity	3 pulses, $F = 3.2$ J/cm^2	3 pulses, $F = 2.9$ J/cm^2	6 pulses, $F = 3.2$ J/cm^2	6 pulses, $F = 2.4$ J/cm^2	6 pulses, $F = 3.2$ J/cm^2
Inactivation ($\log(N/N_0)$)	-4.05 ± 0.07 A	-1.97 ± 0.18 B[b]	-0.38 ± 0.05 C	0.96 ± 0.10 D	1.01 ± 0.06 D[b]
6 J/cm^2 (treatment between groups: $F = 743.50$, $P < 0.0001$)					
Treatment intensity	6 pulses, $F = 6.4$ J/cm^2	6 pulses, $F = 5.8$ J/cm^2	12 pulses, $F = 6.5$ J/cm^2	12 pulses, $F = 4.9$ J/cm^2	12 pulses, $F = 6.4$ J/cm^2
Inactivation ($\log(N/N_0)$)	-4.98 ± 0.25 A	-1.75 ± 0.09 B	-0.62 ± 0.04 C	-0.31 ± 0.12 CD	0.32 ± 0.05 D

[a] Values are mean ± SE. Values followed by different letters are significantly different ($P < 0.01$).
[b] Values for these two treatments are significantly different at $P < 0.05$.

The UV-transmitting filter, which allowed partial transmission of the UV-A range (300 to 400 nm) (Fig. 5), resulted in non-zero reduction of *L. innocua* at higher treatment intensities. The reduction obtained with this filter was at consistently lower levels than the reduction obtained with the cold mirror at equivalent treatment intensities for both types of substrate (Figs. 3 and 4). This result indicates a more significant role of the lower wavelength UV light in the inactivation of *L. innocua* compared with the higher wavelength UV light. Therefore, although the entire UV range seemed to contribute to the inactivation of *L. innocua,* the effect of the UV-B and UV-C ranges ($\lambda < 315$ nm) was stronger than the effect of the UV-A range (315 to 400 nm). Although it is generally believed that most of the microbicidal effect of the UV range lies in the lower wavelength, higher energy domain (UV-B and UV-C), some microbicidal effects of UV-A light have been reported. Fargues et al. *(3)* observed that the viability of *Paecilomyces fumosoroseus* spores was diminished during prolonged exposure to continuous UV-A wavelengths at energy levels of 10 to 60 J/cm^2, which is severalfold higher than the energy levels used in this study. Below 10 J/cm^2, less than 1-log reduction by UV-A radiation was reported *(3),* which is consistent with the findings of the present study. Rowan et al. *(9)* reported similar findings for several microorganisms, including *L. monocytogenes,* that were exposed to high-level and low-level UV PL. *L. monocytogenes* was the most resistant of the tested organisms to the lower wavelength UV treatments *(9),* and this resistance was attributed to the gram-positive physiology of this pathogen *(7).*

To determine whether the findings obtained in the present study were significant, a pairwise comparison of the inactivation means obtained with the different filters was performed using the Tukey test. Because a direct comparison between treatments is only relevant at similar treatment intensities, two treatment levels were used for the statistical analysis: 3 and 6 J/cm^2. Table 3 shows the results of the statistical analysis for the mill finish, and Table 4 shows the results for the aluminum oxide finish. As indicated by the different letters, the inactivation means for most of the treatments were significantly different from each other (with a probability of error $P < 0.01$), particularly when comparing the treatments with different UV profiles, i.e., no-filter versus the quartz filter, partial transmission UV filters (UV transmitting and cold mirror), and the UV-blocking filter.

Although the fluences achieved with the cold mirror, UV-transmitting, and UV-blocking filters were lower than

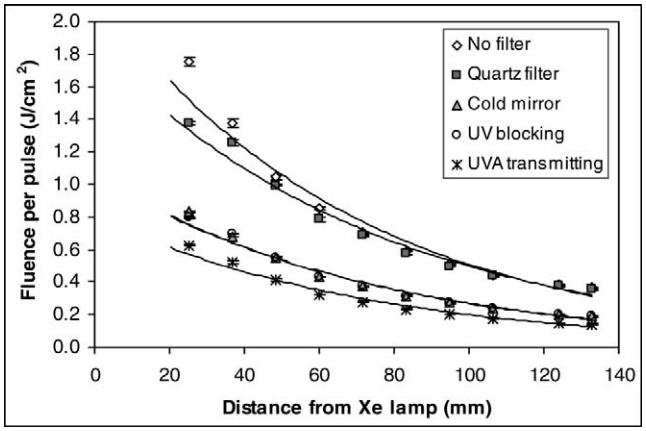

FIGURE 2. *Fluence as a function of distance for all PL-treatment setups (with and without filters).*

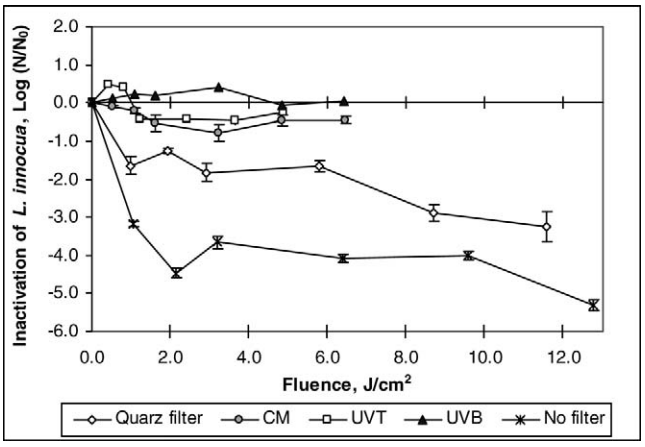

FIGURE 3. *PL inactivation of* L. innocua *on the mill finish surface with and without filters (SPC data). Error bars represent standard errors calculated for each individual treatment.*

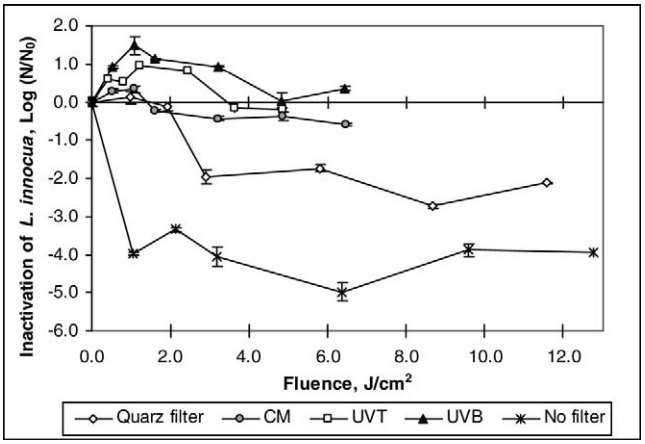

FIGURE 4. *PL inactivation of* L. innocua *on the aluminum oxide–treated surface with and without filters (SPC data). Error bars represent standard errors calculated for each individual treatment.*

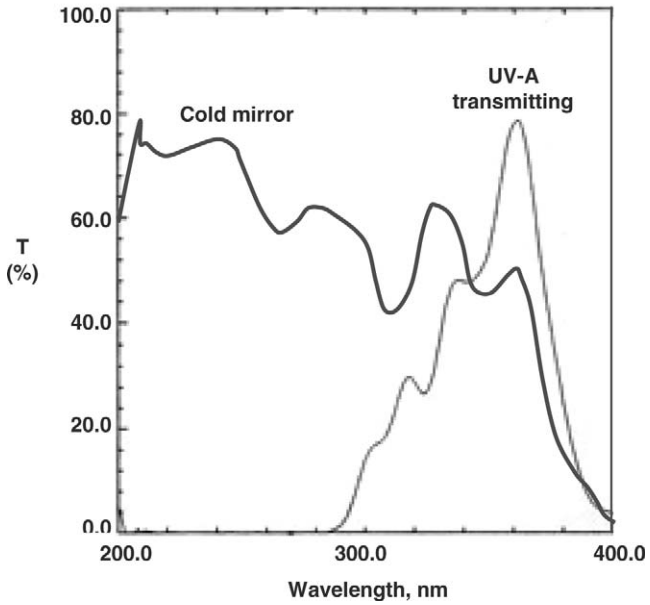

FIGURE 5. *Comparison between the transmission properties (T) of the two filters with partial transmission of UV light.*

those for the no-filter or control-filter setups (Figs. 3 and 4), the clear trends in inactivation observed for treatment intensities up to about 6 J/cm^2 were considered conclusive enough for the purpose of this study, and it was not considered necessary to extend the range for the filter treatments to 12 J/cm^2.

Confirmation of findings using the MPN procedure. Furukawa et al. *(6)* reported that numerous organisms treated with PL were incapable of light or dark repair even after 28 days. In our earlier experiments, plating samples on TSA immediately after treatment yielded no survivors. However, after a resuscitation step was performed (incubating the PL-treated cells in TSB at 30°C for 3 h), significant numbers of survivors were obtained. This finding raised the question of whether additional recovery of sublethally injured cells occurs beyond the time allowed by the resuscitation step, which could have affected the conclusions of the present study. To determine whether further cell repair occurred beyond the resuscitation step, survivors from all treated samples were enumerated using the MPN technique, to allow identification of additional repaired cells.

For all treatments, the SPCs were comparable to their MPN counterparts, but small differences between the SPCs and the MPN estimates were observed. Therefore, each treatment was individually checked for differences between SPCs and MPN estimates by determining whether the SPC for survivors fell within the 95% confidence interval (CI) of the MPN estimate, as determined with the three-tube sampling plan of Swanson et al. *(12).* The SPCs for survivors differed significantly from the MPN estimates, determined as the survivor SPCs that fell below the 95% CI for the MPN survivor estimates for the same treatment (Table 5). Using a one-sample test of proportions, all treatments except those performed with the UV-blocking filter had a significant number of samples (>5%) with MPN survivor estimates that were significantly higher than the concurrent survivor SPCs. At the same time, each treatment set, with the exception of the UV-blocking treatment, also had a significant number of samples (>5%) for which the survivor SPCs were significantly higher than the concurrent survivor MPN estimates. Thus, in some instances cells were able to repair beyond the resuscitation step, whereas in other instances this additional repair did not occur. No trends in terms of what specific treatment might have been conducive for additional cell repair were identified; the significantly different SPC-MPN pairs were randomly distributed across the PL treatments performed with the quartz filter, cold mirror, and UV-A–transmitting filter. When comparing the mean survivor MPN estimates and the mean survivor SPCs for the quartz filter, which had the highest proportion of significantly different MPN-SPC pairs, no significant differences between the two data sets were found (Fig. 6). The absolute superimposition of the MPN estimates and the SPCs for the UV-blocking filter supported the hypothesis that the microbicidal effects of the PL treatment are associated only with the UV range of the electromagnetic spec-

TABLE 5. *On mill finish coupons, some SPCs differed significantly from MPN estimates*[a]

	Control (quartz) filter		UV-B filter		UVT filter		Cold mirror	
No. of pulses	SPC < MPN	SPC > MPN	SPC < MPN	SPC > MPN	SPC < MPN	SPC > MPN	SPC < MPN	SPC > MPN
1	3/8	0/8	0/9	0/9	3/9	3/9	1/9	2/9
2	2/7	0/7	0/9	0/9	0/9	1/9	0/9	2/9
3	1/7	0/7	0/9	0/9	0/6	0/6	1/9	0/9
6	0/8	3/8	0/8	0/8	0/8	0/8	3/9	0/9
9	0/5	1/5	0/9	0/9	0/8	0/8	0/9	3/9
12	2/6	0/6	0/9	0/9	0/9	0/9	2/9	0/9
Total	8/41	4/41	0/53	0/53	3/49	4/49	7/54	4/54
Total %	20	10	0	0	6	8	13	7

[a] Values are number of samples that differed/total number of samples.

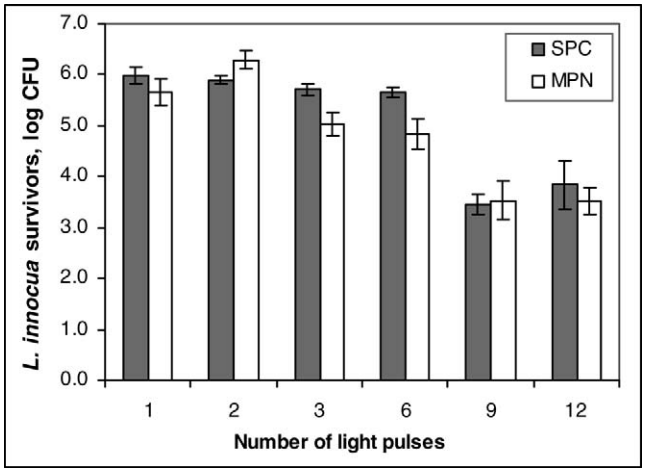

FIGURE 6. *Comparison of number of* L. innocua *survivors after PL treatment with the control (quartz) filter as determined by SPC and MPN procedures. Error bars represent standard errors calculated for each individual treatment.*

trum, denying any contribution of the VIS and NIR wavelengths, at least for the *L. innocua* used in this study.

Thus, it can be concluded that the inactivation of *L. innocua* by PL is caused by exposure to UV light, mostly the UV-B and UV-C portions of the light spectrum, and the NIR or VIS wavelengths did not have a quantifiable effect on the overall microbial inactivation. Even a slight alteration of the UV portion of the light spectrum in PL treatments can have a significant negative impact on the treatment intensity and effectiveness. The specific mechanisms of inactivation require further investigation to clearly determine whether cell death is the result of DNA damage, physical damage, or a combination of both.

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REFERENCES

1. Dunn, J., R. Clark, and T. Ott. 1995. Pulsed-light treatment of food and packaging. *Food Technol.* 49(9):95–98.
2. Dunn, J. E., T. M. Ott, and R. W. Clark. 6 February 1996. Prolongation of shelf life in perishable pod products. U.S. patent 5,489,442.
3. Fargues, J., M. Rougier, R. Goujet, N. Smits, C. Coustere, and B. Itier. 1997. Inactivation of conidia of *Paecilomyces fumosoroseus* by near-ultraviolet (UVB and UVA) and visible radiation. *J. Invertebr. Pathol.* 69:70–78.
4. Farkas, J. 1997. Physical methods of food preservation, p. 497–519. *In* M. P. Doyle, L. R. Beauchat, and T. J. Montville (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, D.C.
5. Fine, F., and P. Gervais. 2004. Efficiency of pulsed UV light for microbial decontamination of food powders. *J. Food Prot.* 67:787–792.
6. Furukawa, M., N. Enta, and T. Kawamata 1999. Brand new pulsed light sterilization technology can sterilize both injectable solution and its 20mL polyethylene container. PDA International Congress: Bridging the Centuries through Innovation and Technology, Tokyo, Japan.
7. Jay, J. M. 2000. Modern food microbiology. Aspen Publishers, Gaithersburg, Md.
8. MacGregor, S. J., N. J. Rowan, L. McIlvaney, J. G. Anderson, R. A. Fouracre, and O. Farish. 1998. Light inactivation of food-related pathogenic bacteria using a pulsed power source. *Lett. Appl. Microbiol.* 27:67–70.
9. Rowan, N. J., S. J. MacGregor, J. G. Anderson, R. A. Fouracre, L. McIlvaney, and O. Farish. 1999. Pulsed-light inactivation of food-related microorganisms. *Appl. Environ. Microbiol.* 65:1312–1315.
10. Ryser, E. T., and C. W. Donnelly. 2001. *Listeria,* p. 343–356. *In* F. P. Downes and K. Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
11. Sharma, R. R., and A. Demirci. 2003. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling. *J. Food Sci.* 68:1448–1453.
12. Swanson, K. M. J., R. L. Petran, and J. H. Hanlin. 2001. Culture methods for enumeration of microorganisms, p. 53–62. *In* F. P. Downes and K. Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
13. Takeshita, K., J. Shibato, T. Sameshima, S. Fukunaga, S. Isobe, K. Arihara, and M. Itoh. 2003. Damage of yeast cells induced by pulsed light irradiation. *Int. J. Food Microbiol.* 85:151–158.
14. U.S. Food and Drug Administration. 1996. Code of Federal Regulations. 21 CFR §179.41, p. 443.
15. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2000. Kinetics of microbial inactivation for alternative food processing technologies: pulsed light technology. Available at: http://www.cfsan.fda.gov. Accessed 1 September 2002.
16. Wekhof, A. 2000. Disinfection with flash lamps. *PDA J. Pharm. Sci. Technol.* 54:264–276.
17. Woodling, S. E., and C. I. Moraru. 2005. Influence of surface topography on the effectiveness of pulsed light treatment for the reduction of *Listeria innocua* on stainless steel surfaces. *J. Food Sci.* 70:245–351.
18. Wuytack, E. Y., L. D. Phoung, A. Aertsen, K. M. Reyns, D. Marquenie, B. D. Ketelaere, B. Masschalck, I. V. Opstal, A. M. Diels, and C. W. Michiels. 2003. Comparison of sublethal injury induced in *Salmonella enterica* serovar Typhimurium by heat and by different nonthermal treatments. *J. Food Prot.* 66:31–37.