Influence of Surface Topography on the Effectiveness of Pulsed Light Treatment for the Inactivation of 
Listeria innocua on Stainless-steel Surfaces

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ABSTRACT: Pulsed light (PL) treatment has been proven effective for killing a wide variety of microorganisms on foods and food contact materials. However, there is concern regarding how shading may impact the effectiveness of PL when applied to imperfect surfaces. The main objective of this work was to examine how surface properties, particularly topography, influence the microbialic effect of PL. Four types of stainless-steel surfaces were inoculated with Listeria innocua and treated with up to 12 pulses of light. The highest level of inactivation achieved was about a 4-log reduction. Initially, an increase in inactivation with increasing treatment intensity was observed, but the inactivation curves tailed off above 3 light pulses. The differences in inactivation levels among the 4 finishes at specific treatment levels were rather insignificant, but some interesting trends were observed. At low treatment levels, inactivation on the smoothest finish was slightly lower than for the other surfaces, due to clustering of the cells on the highly hydrophobic smooth surface and to its reflective nature. For the roughest surface, scanning electron microscopy (SEM) imaging confirmed the preferential location of the cells inside surface features, which also promoted a relatively uniform distribution of the cells across the surface. This counterbalanced to some extent of the shading effects, and as a result inactivation on the roughest surface was comparable to inactivation on the smoother surfaces. These results demonstrate that PL can be effective on both smooth and rough surfaces, but also indicate a complex effect of various surface properties on inactivation.

Keywords: pulsed light, Listeria innocua, stainless steel, topography, shading

Introduction

Pulsed light (PL) treatment is one of the newest technologies that have been proposed as a feasible alternative to thermal treatment for killing pathogenic and spoilage microorganisms, and its use has already been approved by the USFDA for the decontamination of food or food contact surfaces (USFDA 1996). PL technology relies on a series of very short, high-power pulses of light to destroy bacteria, yeasts, molds, and even viruses. Electricity is stored in a capacitor over a period of several seconds and then released in a gas discharge lamp in only a fraction of a second. Typically, the light source is a Xenon lamp, which emits broadband radiation that ranges from ultraviolet (UV) to near infrared (NIR) (200 to 1100 nm).

The specific mechanisms by which PL causes cellular inactivation still remain unclear. Because a significant portion of the PL spectrum includes UV, it is expected that the resulting UV damage plays an important role in the inactivation of microorganisms by PL. The antimicrobial effects of UV light on bacteria are attributed to the absorption of radiation by conjugated carbon-carbon double bonds in proteins and nucleic acids and subsequent DNA structural changes (Tyrrell 1973; Rosenstein and Ducore 1983; Farkas 1997; Bintsis and others 2006; Jay 2000), as well as abnormal ion flow, increased cell membrane permeability, and depolarization of the cell membrane (Wuytack and others 2003). The physical destruction of Aspergillus niger spores as a result of structural collapse (Wekhof 2003) and of Saccharomyces cerevisiae yeast cells due to enlarged vacuoles (Takeshita and others 2003) after PL treatment have also been reported. Wekhof (2003) attributed the inactivation to the disintegration of the cells after instantaneous overheating of the cellular constituents. On the other hand, Rowan and others (2000) found only minimal heating after treating a variety of food-related microorganisms with 2 light sources with slightly different emission spectra.

PL has been proven effective in reducing the microbial populations, both vegetative cells and spores, on the surfaces of foods, food contact materials, and medical devices (USFDA/CFSAN 2000; Ozen and Floros 2001; McDonald and others 2002). By killing surface spoilage microflora, PL treatment was found to extend the shelf life of baked goods, fish, shrimp, eggs (Dunn and others 1995, 1996), grain (Jun and others 2003), and produce (MacGregor and others 1998; Marquenie and others 2003). PL has also been shown to possess the ability to inactivate certain pathogens in foods, which include 2-log cycles of reduction for Salmonella serovars on chicken wings and up to 8-log reduction of Salmonella enteritidis on eggshells, both reported by Dunn and others (1995). The pulsed UV treatments applied by Sharma and Demirci (2003) to alfalfa sprouts resulted in up to 8-log reduction of Escherichia coli O157:H7. Rowan and others (1999) reported that PL is efficient in reducing the population of Listeria monocytogenes on agar surfaces. One of the main limitations of PL is its limited efficacy for the in-depth treatment of opaque substrates, caused by the absorption and scattering of light, and therefore this technique is probably more appropriate as a method to control surface microflora. Because food and food contact surfaces are rarely smooth, it is expect-
ed that their surface topography, in particular the presence of cracks or crevices, may hinder to some extent the effectiveness of PL.

The main objective of this work was to examine how surface properties influence the effectiveness of PL in reducing the microbial population on surfaces. Listeria innocua was chosen as the challenge microorganism, and stainless steel with a range of factory-controlled surface topography was used as a substrate. These substrates were chosen because they mimic equipment or processing surfaces with different finishes or levels of wear and at the same time represent a good model system for investigating the influence of surface properties on PL effectiveness in general.

### Materials and Methods

#### Culture and inoculum preparation

A culture of L. innocua FSL C2-008 (environmental isolate from the ACME smoked fish corp. processing plant, Brooklyn, N.Y., U.S.A.) was obtained from the culture collection maintained by the Food Microbiology and Safety Laboratory in the Food Science Dept. at Cornell Univ. (Ithaca, N.Y., U.S.A.). This culture was used to maintain a culture on Tryptic Soy Agar (TSA; Becton Dickinson, Franklin Lakes, N. J., U.S.A.) slants. 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 10⁸ colony-forming units (CFU)/mL. One-milliliter aliquots were then transferred to 9 mL of Butterfield's Phosphate Buffer (BPB) to produce an initial inoculum population of about 10⁶ CFU/mL.

#### Stainless-steel coupon preparation

To simulate stainless-steel surfaces that may be encountered in a food-processing environment, food-grade stainless-steel coupons with 4 different types of factory-controlled surface finishes were obtained from Pacific Sensor, Inc. (Fountain Valley, Calif., U.S.A.). All coupons were rectangular (2 in x 4 in). The surface finishes were electropolished (the smoothest), mill finish, glass bead blast finish, and aluminum oxide treated (the roughest). Before the treatments, each coupon was washed in FS30H ultrasonic cleaner (Fisher Scientific; Pittsburgh, Pa., U.S.A.) that contained a 30:1 dilution of Fisher-Price detergent. All coupons were then transferred to 9 mL of Butterfield's Phosphate Buffer to produce an initial inoculum population of about 10⁶ CFU/mL.

#### Recovery and resuscitation procedures

Recovery and resuscitation procedures

To establish a reliable method for recovering the treated cells from the metallic surfaces and quantifying the survivors, a quantitative comparison between a manual recovery procedure and stomaching was performed. The manual procedure entailed placing an inoculated, untreated coupon in a sterile Whirl-pak bag that contained 100 mL of TSB. Each bag was massaged by hand for a total time of 2 min: the surface of the coupon was rubbed for 45 s, the bag was shaken vigorously for 15 s, and then the procedure was repeated once again. The stomaching procedure involved individually placing of inoculated, untreated coupons in 100 mL of TSB in sterile Whirl-pak bags and stomaching them for 2 min. Preliminary experiments indicated that not all the cells that were inoculated on the metallic surfaces could be recovered in the TSB, regardless of the recovery procedure. These losses could be due to the slight attachment of the cells onto the metallic surfaces during their limited time of contact and the inability of the recovery method to detach all the cells from the surface. These losses will be called “recovery losses.” The recovery results indicated that less than 1 log CFU cycle of loss per coupon was achieved by hand massaging for all 4 surface finishes, with standard deviations <0.1 log CFU cycles per coupon. In comparison, the loss from stomaching was in the range of 3 log CFU cycles per coupon. Therefore, cell recovery by massaging the inoculated stainless-steel coupons with TSB was adopted.

To account for variation in recovery as a result of changing environmental conditions, control samples were rerun several times over the course of the experiments, which were conducted over several months. All values obtained for recovery losses were then averaged and used to evaluate the net efficiency of the PL treatments.

Preliminary experiments, in which the TSB used for recovery was plated immediately after massaging the treated coupons, indicated zero survivors after the PL treatment, irrespective of the severity of the treatment. This suggested the possibility that sublethal injury occurred during PL treatment of L. innocua, and therefore a resuscitation step was designed, according to the procedures recommended by Jay (2000) and Ryser and Donnelly (2001). Resuscitation consisted in transferring the recovery TSB to another Whirl-pak bag and then incubating it at 30 °C. The appropriate recovery time was determined by plating samples on TSA every hour for 4 h (data not shown). Simultaneously, turbidity measurements (% transmittance) of the recovery broth were taken every 30 min using a Jenway 6300 Spectrophotometer (Jenway, Essex, England), to confirm that recovery and not cell multiplication took place during the incubation at 30 °C. There data were used to determine a resuscitation time of 3 h at 30 °C. These findings support the hypothesis that sublethal injury of L. innocua occurred after PL treatment, a phenomenon that has also been observed by Wuytack and others (2003) when treating Salmonella enterica with pulsed white light, and also highlights the importance of including a resuscitation step in the accurate evaluation of PL efficacy.

#### Evaluation of PL efficiency

After the resuscitation step, the TSB samples were serially diluted and plated on TSA. Plates were incubated at 37 °C for 24 h, at which point presumptive counts were taken. Final counts were taken after 48 h of incubation at 37 °C. The results were expressed in CFU/50 cm² (or CFU/coupon, 50 cm² representing the surface area of a coupon). For each PL treatment, 2 types of losses were reported: the “raw loss,” obtained by subtracting the survivor counts from the initial inoculum, and the “adjusted loss,” which also accounted for the recovery loss:

\[
\text{Adjusted loss} = \text{Initial inoculum} - \text{Survivors} - \text{Recovery loss, CFU/50 cm}^2
\]

The use of adjusted loss led to a slight underestimation of the effectiveness of the PL treatment because it assumed that all the unrecoverable microorganisms were alive. Realistically, it is very likely that a fraction of the cells lost during the recovery procedure were dead, meaning that the real number of microorganisms killed by PL lied
between the calculated raw loss and adjusted loss. Yet, this conservative approach was preferred to an overestimation of PL effectiveness.

Most probable number technique
For the treatments that yielded counts of less than 25 CFU/mL for the lowest dilution, the survivors were enumerated using the most probable number (MPN) technique. Following the procedure reported by Tay and others (2003), aliquots of the inoculum in the recovery broth were transferred into 100 mL of TSB in the following distribution: 3 with 10 mL, 3 with 1 mL, and 3 with 0.1 mL. The TSB was then incubated at 37 °C for 48 h. Turbidity was used to presumptively identify positive samples, and the positives were further confirmed by streaking on TSA and incubating at 37 °C for 48 h and identifying typical L. innocua colonies by their bluish gray color (Ryser and Donnelly 2001). The MPN estimates were based on the procedure of Ryser and Donnelly (2001). The final results were also expressed in CFU/50 cm².

Surface roughness profile determination
For each type of stainless-steel finish, surface roughness was quantitatively evaluated with a contact surface profilometer (Mitutoyo Surface Roughness Tester SJ-301; Aurora, Ill., U.S.A.). The following amplitude parameters have been determined: Ra (arithmetic mean roughness), Rq (mean root squared roughness), Rz (10-point height), and Rt (total roughness). Ra and Rq are frequently used to express surface roughness but cannot differentiate between the peaks and valleys of a surface and therefore are considered somewhat deceptive. Because Rz and Rt are able to assess the amplitude of the surface defects, they are considered a more accurate way to express roughness and reliable predictors of surface cleanability (Frank and Chmielewski 2001). The assessment length for the roughness measurements was 0.15 in (3.81 mm) for all surfaces.

Surface reflectance
The surface reflectance of the 4 types of stainless-steel surfaces in the UV-VIS range was determined using a BYK Gardner TCS II spectrophotometer (BYK-Gardner, Geretsried, Germany).

Scanning electron microscopy
To determine the distribution of cells on the surfaces of the coupons, samples were analyzed using scanning electron microscopy (SEM) at the Cornell Center for Materials Research; 1-in × 2-in coupons of each surface finish were inoculated with 0.25 mL of the inoculum, allowed to dry, and then treated with 6 pulses. The coupons were then sputter-coated with a 60:40 mixture of Au:Pd, using a Denton Vacuum Desk II Cold Sputter Etch Unit. An exposure time of 30 s was used, which yielded about 10 nm of coating. The sputter-coated samples were then viewed with a Leica Stereoscan 440 SEM (Leica Cambridge Ltd.; Cambridge, England).

Contact angle measurement
Dynamic underwater contact angle measurements were taken using a Rame-Hart 100 goniometer (Rame-Hart, Inc., Landing, N.J., U.S.A.) and an immersion chamber, as described by Andrade and others (1979); 1-in × 2-in coupons of the 4 different surfaces were held under water using a magnet and double-sided tape. The samples were held in double distilled water and air bubbles of 1- to 2-μL volume (radii of 0.6 to 0.8 mm) were injected under water using an air-filled microsyringe and then allowed to settle on the underwater surface of the coupons. The bubbles were viewed with the eyepiece of the goniometer and their contact angles (θ) determined. The contact angles determined with this technique were intermediate between advancing and receding angles (Drellich and Miller 1994). All measurements were performed in quadruplicate and average values of θ were reported and used as a measure of surface hydrophobicity.

Surface temperature measurements
To quantify any substrate heating that might have taken place as a result of the PL treatment, surface temperatures of stainless-steel coupons were taken before and after the PL treatment. Temperature measurements were performed in triplicate using a non-contact, type K infrared thermometer (Fisher Scientific, Pittsburgh, Pa., U.S.A.). To take all measurements at the same distance from the metallic surface, the sensing head of the thermometer was placed directly on the surface of the coupons when taking the readings.

Statistical analysis
A 1-way analysis of variance (ANOVA) test was run on the data to determine any significant differences between the survivors counts obtained for the different surface finishes and number of pulses. The ANOVA test was also run on the values obtained for contact angle measurements. All statistical analysis was performed using Minitab versions 5 and 6 (Minitab Inc., Minneapolis, Minn., U.S.A.).

Results and Discussion
Effectiveness of PL treatment as influenced by surface characteristics
The 4 different surface finishes were chosen to represent a range of stainless-steel surfaces found in food processing plants. The electropolished finish was a highly polished sample with a near mirror finish. Electropolished surfaces are sometimes used in the food industry on the premise that surface polishing provides a more cleanable surface. The mill finish stainless steel most closely represented the stainless-steel surfaces typically used in food-processing facilities, the glass bead finish was used to simulate stainless steel that had experienced mild to moderate wear, whereas the aluminum oxide finish simulated a surface that exhibited severe levels of wear. The topographical differences between the 4 types of surfaces can be clearly observed in the SEM micrographs in Figure 1. The electropolished surface (a) was microscopically featureless. The mill finish (b) was also smooth, but showed the granularity of the metal. Neither of these 2 surfaces presented crevices where bacteria could be harbored. By contrast, the bead blasted finish (c) and particularly the aluminum oxide treated finish (d) had very rough and irregular surfaces.

The differences in surface roughness were quantified by means of 4 roughness parameters: Ra, Rq, Rz, and Rt (Figure 2a). Both the electropolished and the mill finish surfaces had very low values of the roughness parameters, similar to those reported by Frank and Chmielewski (2001) for several electropolished stainless-steel surfaces used in commercial applications. The values of the roughness parameters for the bead blasted and the aluminum oxide treated surfaces suggested that these could allow, in average, the “hiding” of about 1 (the bead blasted surface) to up to 3 (the aluminum oxide–treated surface) layers of bacterial cells. The Rq (mean root squared roughness) values for these surfaces (Figure 2b) were 1.01 μm and 2.88 μm, respectively, whereas the cell size of L. innocua was in the 1- to 2-μm range. However, one must take into account that Rq is an average value, which does not accurately characterize the presence of surface defects. Judging by the Rt values, which were 6.21 μm for the bead blasted finish and 17.45 μm for the aluminum oxide finish (Figure 2b), in certain locations these 2 finishes could harbor multiple layers of bacteria cells inside the surface imperfections, and thus shield them from the effect of PL.

A visual confirmation of bacterial cell deposition on the various surface finishes was obtained by electron microscopy. SEM micro-
Influence of topography on pulsed light...

Graphs of the inoculated aluminum oxide–treated surface revealed the agglomeration of _L. innocua_ cells in the “valleys” of the surface (Figure 3). This spatial distribution of the cells was confirmed by stereoimaging, which involved tilting of the analyzed coupon under a 7° angle, followed by differential coloring and superimposition of tilted and non-tilted images of the same analyzed field. This allowed a 3-dimensional visualization of the surface using red/blue anaglyphic glasses, which confirmed that the darker areas in Figure 3 represent deep valleys in the surface, whereas the brighter areas represent protuberances above the mean line. Cell agglomeration could also be observed in the imperfections of the bead blasted surface, but was much less pronounced. The “hiding” of bacteria cells in surface imperfections is extremely important because it could potentially shield the cells from the full effect of the PL treatment, this being one of the major concerns related to the use of PL technology for surface decontamination in general and of foods in particular (USFDA/CFSAN 2000).

The 4 different types of surfaces were inoculated with approximately 10^8 CFU/coupon and treated with varying numbers of pulses of light to observe any trends in the inactivation levels as a function of surface finish. At lower treatment levels (1 and 3 pulses), the adjusted loss of _L. innocua_ on the stainless-steel surfaces increased from the electropolished surface (1.31-log and 1.93-log reduction for the 1 and 3 pulses, respectively) to the bead blasted surface (2.68-log reduction and 2.77-log reduction for the 1 and 3 pulses, respectively) (Table 1). Due to the variability of the data, the reduction levels for the 2 smoothest surfaces (electropolished and mill) were not statistically different from each other but were significantly lower than for the 2 roughest surfaces (bead blasted and aluminum oxide–treated). Based on the surface topography and the potential hiding effects discussed previously, it would have been expected that the rough surfaces should yield a lower level of reduction than the smooth surfaces. The surprising reversed trend found in this study pointed to the fact that topography was not the only surface property to affect microbial inactivation by PL. However, it was also determined that the initial inoculum level may have affected the reduction level, as initial findings indicated that as the inoculum level increases, the level of reduction also increases, which would explain some of the variability. Research is currently in progress to further clarify this phenomenon.

For the electropolished finish both the direct, visual observation of the inoculated surface and its SEM analysis (Figure 4) revealed a very distinct spatial distribution of the inoculum and bacterial cells on this surface as compared with the other 3 surfaces. The liquid inoculum was particularly difficult to spread on the electropolished finish because it tended to bead up in the center of the coupon. Visual assessment indicated that both the bead blasted and the mill finish allowed for imperfect, but near complete spreading of the aqueous inoculum, whereas the aluminum oxide–treated surface allowed for complete coverage and uniform spreading, which suggested a difference in hydrophobicity among the 4 surfaces. The spreadability of the liquid inoculum on the 4 surfaces correlated with the extent of recession that occurred during drying. The inoculum that was deposited on the aluminum oxide surface dried evenly and did not recede at all while it dried, whereas the inoculum on the electropolished surface did recede significantly. As a result, a ring of thicker inoculum was observed on the center of the inoculum area of the electropolished surface after the drying had occurred.

The surface hydrophobicity of the 4 surfaces was quantitatively characterized by their underwater contact angles. The more hydrophobic a surface is, the larger its underwater contact angle: a con-

**Table 1—Inactivation levels of _Listeria innocua_ for stainless-steel samples after pulsed light (PL) treatment with 1 and 3 pulses**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surface type</th>
<th>Electropolished finish</th>
<th>Mill finish</th>
<th>Bead finish</th>
<th>Aluminum oxide finish</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pulse</td>
<td>Raw loss</td>
<td>2.97 ± 0.34 A</td>
<td>3.19 ± 0.25 A</td>
<td>4.07 ± 0.57 B</td>
<td>3.98 ± 0.49 B</td>
</tr>
<tr>
<td></td>
<td>Adjusted loss</td>
<td>1.31</td>
<td>1.81</td>
<td>2.68</td>
<td>2.51</td>
</tr>
<tr>
<td>3 pulses</td>
<td>Raw loss</td>
<td>3.59 ± 0.04 C</td>
<td>3.66 ± 0.86 C, D</td>
<td>4.16 ± 0.34 D</td>
<td>4.05 ± 0.17 D</td>
</tr>
<tr>
<td></td>
<td>Adjusted loss</td>
<td>1.93</td>
<td>2.28</td>
<td>2.77</td>
<td>2.59</td>
</tr>
</tbody>
</table>

*aDifferent letters in the raw loss rows indicate a significant difference among the means of at least 3 replicates plated in duplicate.
Influence of topography on pulsed light . . .

Contact angle (θ) of 0° results in wetting, θ of 0° to 90° results in spreading of the liquid on the surface, whereas θ > 90° indicates that the liquid will bead on the solid surface. The contact angles showed in Table 2 indicate a significant increase in surface hydrophobicity as surface roughness decreased, the electropolished surface being the most hydrophobic of all, with an average θ of 91.2°. The mill finish and the bead blasted finish were insignificantly different from each other in terms of hydrophobicity, with the bead blasted finish averaging θ = 73.9° and the mill finish averaging θ = 69.8°, whereas the aluminum oxide–treated surface was the least hydrophobic of all, with an average contact angle of only 45.0°.

Another factor that could have affected the distribution of bacteria on the metal surfaces was the hydrophobicity of the bacterial cells themselves. The effect of bacteria surface properties is particularly important in biofilm formation (Samuelsson and Kirchman 1990), which, however, is not very relevant for this work because the contact time between bacteria and the stainless-steel surface was rather short. In the present study, the difference in hydrophobicity between the metal surfaces, the bacterial cells, and the watery media can be used to explain the spatial distribution of the cells on the stainless-steel coupons.

Listeria cells exhibit rather hydrophilic properties and low values of the water contact angles. Meylheuc and others (2002) reported for L. innocua cells water contact angles ranging from 27° to 36.5° (at 20°C), depending on their growth stage. This means that in a stainless steel/L. innocua cells/watery media system the bacteria would be preferentially located between the metal and the watery media because the latter has a more pronounced tendency to “run away” from the hydrophobic metal surface and a higher affinity for the bacteria. This is particularly true of the highly hydrophobic electropolished finish, and it explains why on that surface the watery inoculum beaded up during inoculation and drying, creating a protective, relatively thick layer of media that covered the cells, shielding them from the direct effect of PL. A visual confirmation of this layer and the Listeria cells underneath is shown in the SEM micrograph in Figure 4.

The optical properties of the 4 surfaces are also expected to have played a role in the microbial inactivation by PL. As shown by Figure 5, the electropolished sample was the most reflective of all, followed by the mill finish surface and the bead blasted finish. A high degree of reflectivity could potentially result in less light being absorbed by the L. innocua cells, which could have also contributed to the lower-than-expected inactivation levels found for the smooth, electropolished surface. The reflectivity of the aluminum oxide finish was very low and the measurements resulted in scattered values (data not shown). Conversely, the aluminum oxide finish absorbed a part of the incident light, which resulted in a slight increase in surface temperature (Figure 6).

The increase in temperature was most pronounced for the aluminum oxide–treated surface, and it increased systematically from the smoothest to the roughest surface. The most significant increase in temperature was recorded for the smooth, electropolished surface. The reported temperature increase was evaluated on the substrate, after cessation of the treatment and after considerable cooling of the coupons might have taken place in the PL chamber in the few seconds that passed until the measurement was completed. It is perfectly possible that the heating effects at the cell level, during the very short duration of the treatment, might have been significant and therefore contributed to their death. This hypothesis has yet to be verified and warrants further investigation.

Table 2—Surface properties of the different stainless-steel surfaces

<table>
<thead>
<tr>
<th>Surface finish</th>
<th>Ra</th>
<th>Rq</th>
<th>Rz</th>
<th>Rt</th>
<th>Contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electropolish</td>
<td>0.12</td>
<td>0.19</td>
<td>1.05</td>
<td>1.95</td>
<td>91.2 ± 2.9 A</td>
</tr>
<tr>
<td>Mill finish</td>
<td>0.24</td>
<td>0.30</td>
<td>2.07</td>
<td>2.88</td>
<td>69.8 ± 3.9 B</td>
</tr>
<tr>
<td>Bead blasted</td>
<td>0.78</td>
<td>1.01</td>
<td>5.15</td>
<td>6.21</td>
<td>73.9 ± 1.8 B</td>
</tr>
<tr>
<td>Aluminum oxide–treated</td>
<td>2.30</td>
<td>2.88</td>
<td>12.93</td>
<td>17.45</td>
<td>45.0 ± 1.3 C</td>
</tr>
</tbody>
</table>

*aDifferent letters in the same column indicate a significant difference among the means of 3 replicates. 
*bDrop radius = 0.6 to 0.8 mm.

Figure 3—Scanning electron microscopy (SEM) image of inoculated aluminum oxide–treated surface at 2260x magnification

Figure 4—Scanning electron microscopy (SEM) image of inoculated electropolished surface at 2260x magnification
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From another perspective, if such a low level of heating were encountered when treating a food surface with PL, this is not expected to result in any significant changes of the sensory and chemical attributes of that particular food, which is beneficial from a food quality standpoint. Yet, light absorption and subsequent heating can vary considerably from 1 substrate to another, depending on their composition, color, and surface roughness, and therefore this particular phenomenon has to be verified for each type of food substrate.

The differences in inactivation levels among the 4 finishes were less evident at higher treatment levels, particularly due to the large standard deviations, but still indicated an overall lower reduction on the electropolished surface as compared with the other 3 (Figure 7).

Inactivation kinetics for the reduction of *L. innocua* by PL

The effect of treatment levels ranging from 1 to 12 pulses of light on the stainless-steel surfaces inoculated with *L. innocua* is shown in Figure 7. Figure 7a represents the raw losses, and Figure 7b shows the adjusted losses. The difference between Figure 7a and Figure 7b is represented by the average loss caused by sample preparation (mostly drying) for each coupon type over the range of environmental conditions encountered over the duration of the experiments. These differences are represented by only slight changes in temperature and humidity from summer to winter, as all the experiments were performed under environmental conditions in a air-conditioned laboratory. In Figure 7b, the accurate calculation of standard deviation and plotting of the error bars was not possible, due to the combined variability in both the PL treatments and the recovery process.

Prior reports have suggested that microbial inactivation by PL follows 1st-order kinetics (PPT 1999). However, the results of this work showed a slight increase in the level of inactivation between up to 3 pulses, but then the inactivation curve quickly leveled off to a plateau as treatment intensity increased. The adjusted data showed a more progressive increase in microbial reduction than the raw data for the lower treatment levels, but confirmed the flattening of the inactivation curve above 3 pulses. Because for the 9 and 12 pulses treatments the plating procedure resulted in less than 25 CFU/mL for the lowest dilution, the MPN technique was used to determine the number of survivors accurately. The plating data that were below the accurate identification threshold indicated survivor counts of about 10^3 CFU/coupon, but this was not included in the calculated averages from Figure 7. The MPN data for the same experimental conditions showed estimated counts higher than those observed by plating, suggesting that additional resuscitation took place during the MPN procedure (Table 3). One explanation for this finding could be the fact that the sublethally injured cells repaired themselves further in the nonselective TSB used for the MPN procedure, resulting in higher survivor levels compared with those obtained by plating on TSA. This hypothesis has yet to be verified and is the subject of an ongoing study. The averaging between serial dilution data and MPN data for the 9 and 12 pulses could explain the apparent decrease in inactivation levels at 9 pulses (Figure 7).
Influence of topography on pulsed light .

Table 3—Comparison of standard plate count SPC data versus most probable number (MPN) data obtained at 9 and 12 pulse treatment levels*

<table>
<thead>
<tr>
<th>Surface type</th>
<th>SPC count after 9 pulses (CFU/coupon)</th>
<th>MPN count after 9 pulses (CFU/coupon)</th>
<th>SPC count after 12 pulses (CFU/coupon)</th>
<th>MPN count after 12 pulses (CFU/coupon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill</td>
<td>&lt;2.5e3</td>
<td>1.50 × 10^4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mill</td>
<td>&lt;2.5e3</td>
<td>2.10 × 10^4</td>
<td></td>
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<tr>
<td>Electro</td>
<td>&lt;2.5e3</td>
<td>1.50 × 10^4</td>
<td></td>
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<tr>
<td>Bead</td>
<td>&lt;2.5e3</td>
<td>&gt;1.10 × 10^5</td>
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<tr>
<td>Al oxide</td>
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*aCFU = colony-forming units; SPC = standard plate count.

Conclusions

This study demonstrates that surface topography has a complex influence on the efficacy of PL treatment for surface microbial reduction. A high degree of surface roughness allows some hiding of microbial cells inside surface details, yet it can also allow for a relatively uniform 2-dimensional distribution of the cells on the treated surface, which favors treatment uniformity. On the other hand, a smooth surface does not necessarily warrant a high level of microbial inactivation by PL treatment if that surface is highly hydrophobic and thus leads to cell clustering. The interaction between light and substrate, such as light reflection and absorption, also plays a role in the inactivation process.

The experimental data also suggest that inactivation does not appear to follow 1st-order kinetics, at least for the system studied in this work, and that sublethal injury and subsequent recovery of the treated cells can occur. The ability of PL to result in a high level of sublethal injury holds promise for its use in a hurdle approach, where another type of treatment (such as a sanitizer in case of food contact surfaces) could be applied to the sublethally injured cells immediately after the PL treatment, which could impede their recovery and thus lead to a much higher level of reduction than would result from the individual application of each microbicidal technique. Yet, more research is required to clearly understand the extent of sublethal injury caused by PL.

Overall, the fact that a significant level of microbial reduction can be achieved after a very short treatment time indicates much promise for the use of PL as a quick and relatively inexpensive method of reducing the microbial load on a range of different surfaces in food-processing environments.

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