U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
June 2, 2000

Kinetics of Microbial Inactivation for Alternative Food Processing Technologies

Pulsed Light Technology

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Scope of Deliverables

Processes designed for the bacterial inactivation of foods and packages with pulsed light are described here. Possible mechanisms of bacterial inactivation are also discussed as well as critical factors that influence the efficiency of the process. Also, the need for future research is addressed. Much of the information comes from industry sources; therefore, independently conducted research is needed to validate the effectiveness of pulsed light processes for food preservation.

1. Definition, Description and Applications

Pulsed light is a method of food preservation that involves the use of intense and short duration pulses of broad-spectrum "white light". The spectrum of light for pulsed light treatment includes wavelengths in the ultraviolet (UV) to the near infrared region. The material to be treated is exposed to a least 1 pulse of light having an energy density in the range of about 0.01 to 50 J/cm^2 at the surface. A wavelength distribution such that at least 70% of the electromagnetic energy is within the range from 170 to 2600 nm is used. The material to be sterilized is exposed to at least 1 pulse of light (typically 1 to 20 flashes per s) with a duration range from 1 μs to 0.1 s (Dunn and others 1991). For most applications, a few flashes applied in a fraction of a s provide a high level of microbial inactivation.

Pulsed light is produced using engineering technologies that multiply power many fold. Accumulating electrical energy in an energy storage capacitor over relatively long times (a fraction of a s) and releasing this storage energy to do work in a much shorter time (millionths or thousandths of a s) magnifies the power applied. The result is a very high power during the duty cycle, with the expenditure of only moderate power consumption (Dunn and others 1995).

The technology for using light pulses is applicable mainly in sterilizing or reducing the microbial population on the surface of packaging materials, transparent pharmaceutical products, or other surfaces (Dunn 1996). Usually, the packaging material used in aseptic

processing is sterilized with hydrogen peroxide, which may leave highly undesirable residues in the food or package (Barbosa-Cánovas and others 1997). Light pulses may be used to reduce or eliminate the need for chemical disinfectants and preservatives. Pulsed light may also be used to extend the shelf-life or improve the quality of produce.

MacGregor and others (1998) described the power light source suitable for inactivation of microorganisms. The test assembly used for experiments consisted of a rectangular PVC housing, a pulsed generator and associated switching and controlled circuitry as illustrated in Fig. 1. Internally, the light source was mounted 4-5 cm above the 2 sample holders, which were set 45 degrees to the horizontal. This arrangement permitted 2 petri dish samples to be located at right angles and irradiated simultaneously, with each sample receiving the same average exposure. The light source employed was a Hearaeus Noble light XAP Series that was constructed from a clear fused quartz tube filled with Xenon to pressure of 450 torr. The dimension of the tube was 3 mm dia and 7.5 cm arc length. The tube had an enriched light trigger along the length of the envelope and was capable of being operated with an average power of about 100 W. The pulsed generator was a single stage, inverting PFN Marx generator, which was charged to 30 kV dc. The generator was fired using a trigatron via a high voltage auto transformer. The source capacitance of the generator was 6.4 nF and the source impedance, when fired, was 6.25 Ohms.

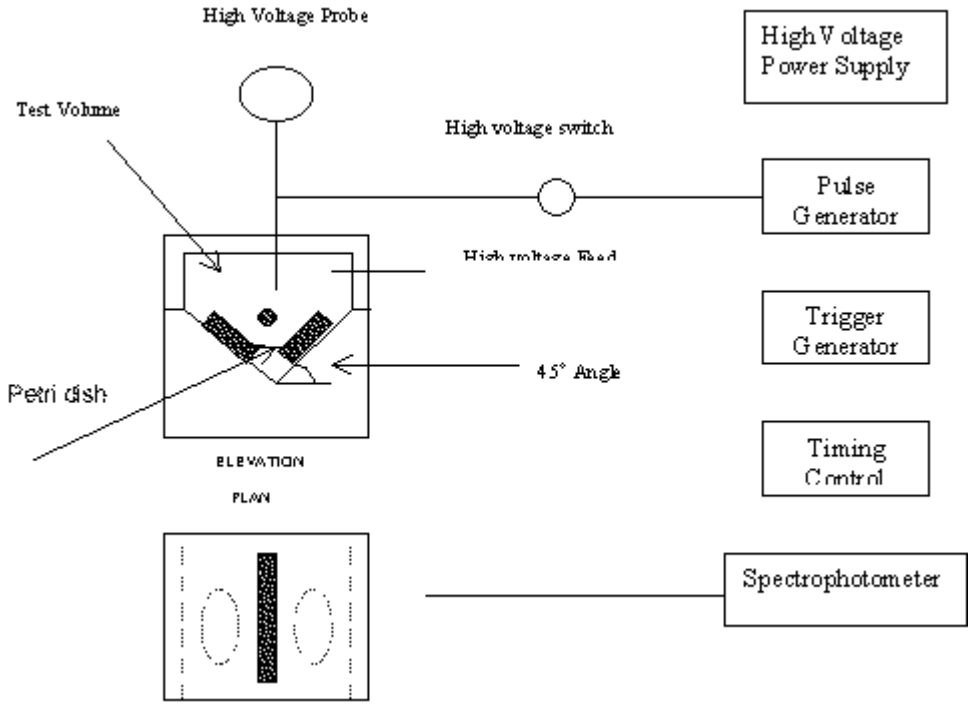


Figure 1. Experimental facility for bacterial inactivation using a pulsed light generator.

The pulsed light process developed by PurePulse Technologies Inc. utilizes flashes of intense broad-spectrum pulsed light (BSPL*)* to sterilize pharmaceuticals, medical devices, packaging, and water. The spectrum closely mimics the spectrum of sunlight at sea level with an important difference: The system delivers a spectrum 20,000 times more intense

than sunlight at the earth□s surface during the pulse. The intense flashes of light are less than 1 millisecond in duration. Several pulses can be delivered per s, creating sterilized material at a greater speed than conventional processes. The efficacy of the process has been tested against a broad range of microorganisms, including bacteria (vegetative cells and spores), fungi, viruses, and protozoa (PurePulse Technologies Inc. 1999).

Figure 2 illustrates the treatment system. The basic electrical components are shown schematically in the upper portion of the Fig. In the lower portion of the Fig., energy per unit time diagrams are shown below the system components to emphasize the power magnification available through pulsed energy processing.

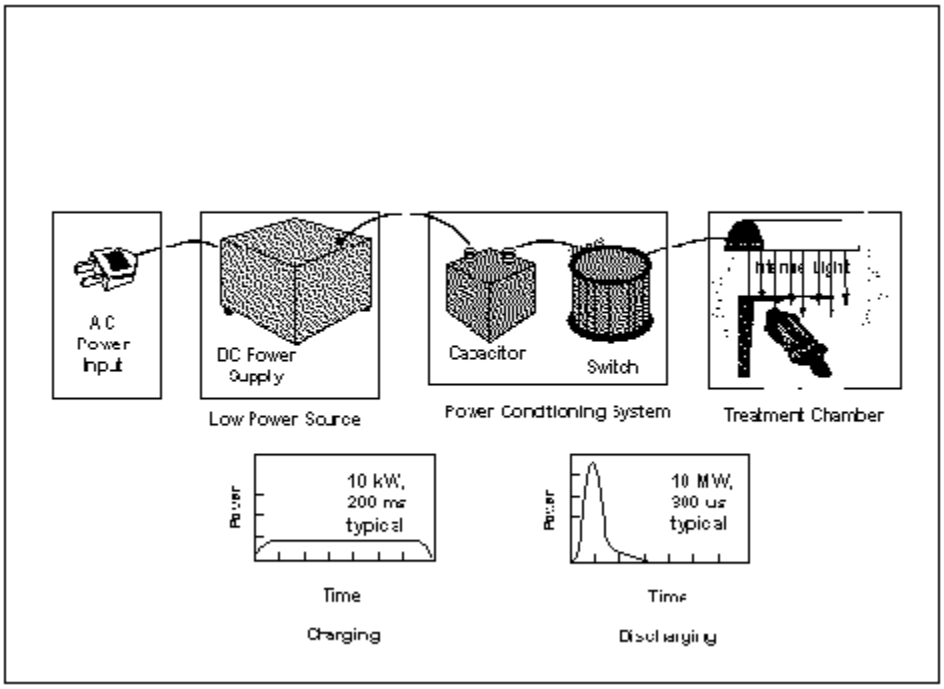


Figure 2. Schematic diagram of a pulsed light treatment system.

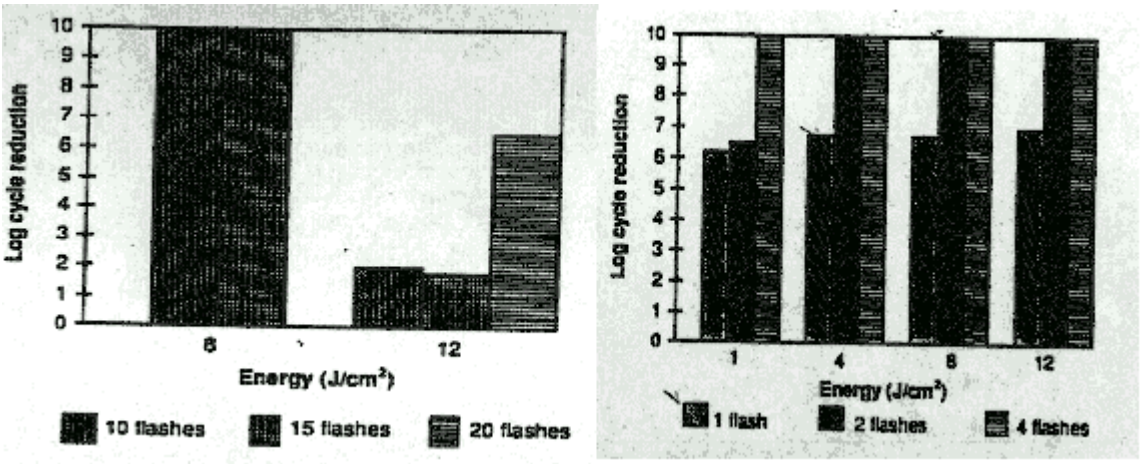
The lamp unit consists of 1 or more inert gas lamps arranged to illuminate the desired treatment area. A high voltage cable connects the lamps to the power unit. To flash the lamp, a high-voltage, high-current pulse of electricity is applied. The high current passing through the gas in the lamp causes it to emit an intense pulse of light that lasts a few hundred microseconds. The frequency of flashing is adjustable, allowing optimization of the process in conjunction with any particular processing speed. Systems are normally designed for each application to provide flexibility in the number of lamps, the flashing configuration, and the flash rate. Lamps can be flashed simultaneously or sequentially.

A different system using pulsed UV light for microbial control has been patented in the United States (Lagunas-Solar and Pyne 1994), with patent pending in Chile. This technology uses monochromatic excimer lamps at a wavelength of 247 nm rather than the xenon flash tubes described above. This technology is in commercial production in Chile and is being used on grapes exported to the United States.

2. Inactivation of Microorganisms

Pulsed light provides shelf-life extension and preservation when used with a variety of foods. Because pulsed light applications are limited to the surfaces of products, most studies have tested the effectiveness of pulsed light on food or packaging surfaces. For instance, the process was reported to be effective to inactivate molds in a variety of baked goods and to extend their shelf-lives (Dunn and others 1995). Similarly, shrimp treated with pulsed light and stored under refrigeration for 7 d remained edible, while untreated shrimp showed extensive microbial degradation and were discolored, foul smelling, and not edible (Dunn and others 1995). More than 7-log cycles of *Aspergillus niger* spore inactivation resulted with a minimal number of pulsed light flashes with 1 J/cm^2 (Dunn and others 1991). A variety of microorganisms including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* have been inactivated by using 1 to 35 pulses of light with an intensity ranging from 1-2 J/cm^2 as illustrated in Fig. 3, 4, and 5. *Salmonella* serovars were reduced by 2-log cycles on chicken wings in samples inoculated with either 5 or 2 log/cm^2. *Listeria innocua* was reported to be reduced by 2-log cycles on hot dogs (inoculated with 3 or 5 log/wiener) after pulsed light treatment (Dunn and others 1995).

Curds of commercially dry cottage cheese inoculated with *Pseudomonas* spp. and treated with pulsed light with an energy density of 16 J/cm^2 and pulse duration of 0.5 ms reduced the microbial population by 1.5-log cycles after 2 flashes. The temperature at the surface of the curd increased by 5 ^{o}C (Dunn and others 1991). Sensory evaluation with trained panelists showed no effects of the treatment on the taste of the cheese. A combination of high-pressure wash and exposure to pulsed light was reported to reduce the psychrotroph and coliform population on the surface of fish tissue by 3-log cycles. The sensory attributes of the fish remained acceptable after 15 d of refrigerated storage (Dunn and others 1988).



(a) (b)

Figure 3. Inactivation of (a) *Escherichia coli* vegetative cells using filtered spectrum-light and (b) *Bacillus subtilis* spores using full-spectrum light. (Dunn and others 1988).

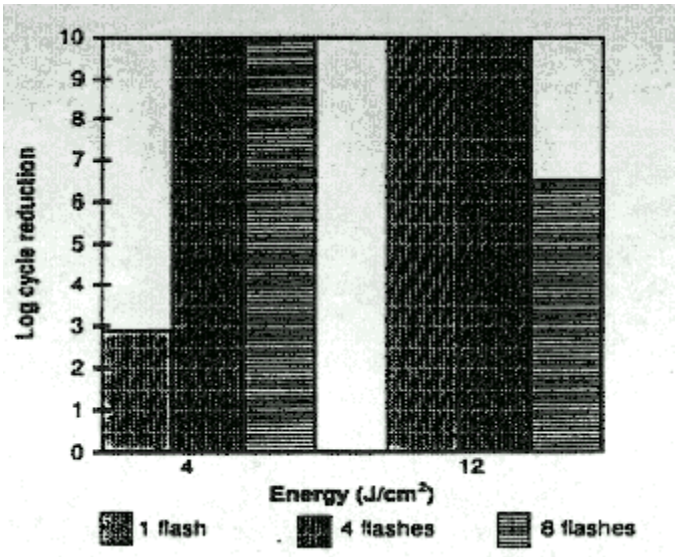


Figure 4. Inactivation of *Aspergillus niger* spores using full-spectrum light. (Dunn 1988).

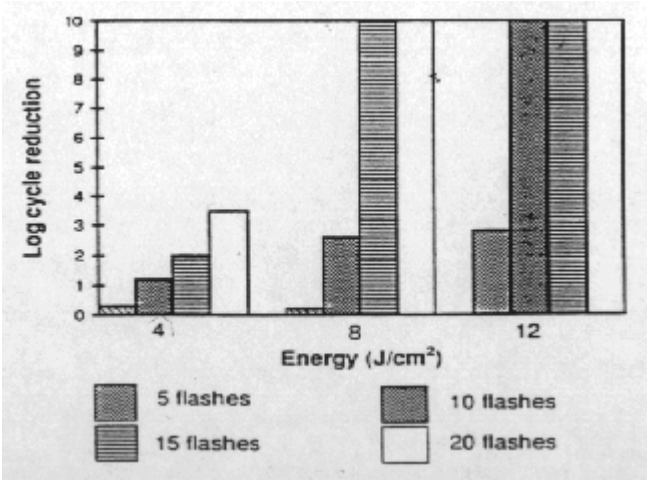


Figure 5. Inactivation of *Aspergillus niger* spores using filtered-spectrum light

(Dunn and others 1988).

Pulsed light was reported to be effective in eliminating microbial contamination from the surface of eggshells. Up to an 8-log reduction of *Salmonella* Enteriditis was achieved for commercial or raw eggs, which were treated with 0.5 J/cm^2 for 8 flashes (Dunn 1995). On the surface of different packaging materials inoculated with 10-1000 cfu/cm^2, a single light pulse inactivated *S. aureus* with an intensity as small as 1.25 J/cm^2, while *B. cereus* and *Aspergillus* spp. spores were inactivated with intensities greater than 2 J/cm^2 (Dunn and others 1991).

The inactivation of microorganisms on dry surfaces by pulsed light has been demonstrated using *Salmonella* Enteriditis ATCC 13076, *E. coli* O157:H7, *Salmonella* Typhimirium ATCC 13312, *Listeria monocytogenes* ATCC 15314, and *S. aureus* ATCC 6539, among other microorganisms. Information of the initial inoculation levels, though,

is not available. The challenge studies are initiated by first inoculating the surface to be treated with a uniform layer of the test organism. This is accomplished by a spray inoculation method developed for this application by PurePulse Technologies Inc. (PurePulse Technologies Inc. 1999). Once the microbial layer is allowed to dry, it is exposed to treatment. Since the exposure is less than 1 s, treatment is quantified by the lamp output expressed as "fluence", the time integrated light energy per unit surface.

The ability of pulsed light technology to sterilize liquids after filling in containers has been tested against a broad range of microorganisms (Table 1). At a Japanese Congress (PurePulse Technologies Inc. 1999), Nissin Pharmaceutical presented a case study on the effectiveness of pulsed light technology for achieving sterility in liquids. Low-density polyethylene (LDPE) containers were filled with 20% (w/v) glucose, physiological saline, or water for injection using blow/fill/seal technology (Table 1). Subsequent to filling, samples were aseptically inoculated with the challenge microorganisms and treated with pulsed light under sterilizing conditions. Each set of 20 containers was tested for sterility by the membrane filter technique described in the Japanese Pharmacopoeia. Pulsed light was applied in 2 modes. In 1 mode, containers were treated using a lamp reflector that illuminated each container from above (single lamp/reflector or SLR mode). For SRL mode, 20 flashes at 1 J/cm^2/flash were used (Dunn and others 1991). In the second treatment mode, the containers were treated in a reflective cavity containing a single lamp, which was flashed 10 times (cavity treatment or CT mode). For the sterility test, 0.5, 5, and 15 ml samples were assayed directly for the presence of survivors using 20, 40, and 60 ml pour plates, respectively. In addition, 120 ml samples were assayed by filtration through a 0.45 µm filter.

Table 1 Treatment of Blow-Fill Seal Packaging using pulsed light

Organism	**Inoculation (in 20 mL)**
Bacillus subtilis var. niger spores	4.8 x 10^6
Bacillus pomilus spores	3.0 x 10^6
Bacillus stearothermophilus spores	4.4 x 10^6
Clostridium sporogenes spores	2.4 x 10^6
Aspergillus niger spores	1.4 x 10^6
Candida albicans	3.0 x 10^6
Deinococcus radiodurans	2.2 x 10^6
Staphylococcus aureus	5.6 x 10^6
Enterococcus faecalis	2.0 x 10^6

Escherichia coli	3.4×10^6
Salmonella choleraesuis	1.8×10^6
Pseudomonas aeruginosa	2.8×10^6

Dunn and others (1991) reported that in the SLR mode, 36 out of 40 *A. niger* samples were sterile, and all 40 *Bacillus pumilus*, *B. subtilis*, and *Bacillus stearothermophilus* spore inoculated samples were sterile. No viable organisms were recovered from any of the 160 samples treated using the CT mode.

3. Mechanisms of Microbial Inactivation

The lethality of the light pulses is different at different wavelengths. Therefore, the full spectrum or selected wavelength may be used to treat the foods. Wavelengths known to produce undesirable products in foods are eliminated by filtering through glass or liquid filters. Light pulses induce photochemical or photothermal reactions in foods. The UV-rich light causes photochemical changes, while visual and infrared lights cause photothermal changes. UV light has been shown to inactivate pathogens and indicator organisms (Chang and others 1985). The antimicrobial effects of these wavelengths are primarily mediated through absorption by highly conjugated carbon-to-carbon double-bond systems in proteins and nucleic acids (Jay 1992).

The mode of action of the pulsed light process is attributed to unique effects of the high peak power and the broad-spectrum of the flash. A primary cellular target is nucleic acids. Inactivation occurs by several mechanisms, including chemical modifications and cleavage of the DNA. The impact of pulsed light on proteins, membranes, and other cellular material probably occurs concurrently with the nucleic acid destruction. For example, the motility of *E. coli* ceases immediately after exposure to pulsed light. In additional studies, loss of motility of protozoan sporozoites was observed after pulsed light treatment of oocysts. As with any lethal physical agent, it is difficult to determine the actual sequence of events due to the possible "domino effect" (PurePulse Technologies Inc. 1999).

Experience suggests that shorter wavelengths in the UV range of 200-320 nm are more efficient inactivation agents than the longer wavelengths due to their higher energy levels. Because DNA is a target molecule for these UV wavelengths, it is thought that 1 primary cause of killing microorganisms is through DNA structural changes (Farkas 1997). Conventional UV treatment primarily affects DNA by mechanisms that are reversible under certain experimental conditions. Cell repair systems are classified as either "dark enzymatic repair" or "light enzymatic repair" (PurePulse Technologies Inc. 1999). Experiments designed to test enzymatic repair of DNA using pulsed light have shown that this repair does not occur after pulsed light treatment. The magnitude of the damage caused by pulsed light may also be too massive for the repair mechanisms to be effective. It is conceivable the DNA repair system itself is inactivated as well as other

enzymatic functions. In summary, the high energy and intensity of pulsed light are thought to amplify the known mechanisms of destruction of cellular components caused by individual wavelengths of light. The sum of the damage caused by the broad-spectrum light is thought to produce extensive irreversible damage to DNA, proteins, and other macromolecules.

4. Validation/Critical Process Factors

4.1. Critical Process Factors

Due to failure of light to penetrate opaque and irregular surfaces, there is generally less microbial inactivation with pulsed light, compared to other technologies. Light characteristics (wavelength, intensity, duration and number of the pulses), packaging and food attributes (type, transparency and color) are considered to be critical process factors. In the case of a fluid food, transparency and depth of the fluid column become critical factors.

Despite its minimal effectiveness with opaque foods, pulsed light has been reported to have limited ability to reduce microbial counts (about 1- to 4-log cycles) on eggs, including organisms inoculated onto the surface of eggs and then drawn into egg air pores by a temperature differential (Dunn and others 1995).

The lethality of the pulsed light increases with increasing light intensity or fluence (PurePulse Technologies Inc. 1999), although formulation of a model for dose-response is not currently possible. Data presented in Table 2 summarizes experiments examining the effect of pulsed light on spores from 3 different strains of *Bacillus* spp. and *A. niger*. The spores were exposed to 3 flashes from a single lamp with flashes of 0.5 J/cm^2, 0.75 J/cm^2, and 1.5 J/cm^2 per flash (PurePulse Technologies Inc. 1999). Microbial reduction increased with increasing light intensities.

Recovery cfu/ml				
	Bacillus stearothermophilus	***Bacillus subtilis***	***Bacillus pumilus***	***Aspergillus niger***
0 J/cm^2	1.4 x 10^6	2.6 x 10^6	3.1 x 10^6	ND
0 J/cm^2	177	436	570	ND
0 J/cm^2	63	43	90	ND
0 J/cm^2	ND	ND	ND	ND

ND, not detectable

Table 2 The effects of fluence on different microorganisms subjected to pulsed light

treatment.

5. Process Deviations

In this application, each pulsed light system is designed with monitoring devices that capture lamp output (fluence) and the lamp current. The silicon photo-diode detectors measure fluence in the UV range wavelengths. The UV range is monitored because it is the most sensitive to variations in both the lamp drive and the optical characteristics of the lamp units. Due to the nature of the lamps and the lamp unit optics, a change in the UV output of the system will always be detected before any measurable changes occur in the full spectrum output. Therefore, monitoring the UV output is the best measure of overall system performance, even though all the wavelengths contribute to the microbiocidal effect. Should a flash fall below the validated minimum threshold, the pulsed light system can be programmed to automatically shut down to avoid underprocessing. Monitors are available to measure the full spectrum of light or specific wavelengths of interest. The lamp current determines the light intensity and spectrum. Monitors record the lamp current for each flash and have the capability of halting operations if an abnormal signal is detected.

6. Research Needs

A great deal of research remains to be done before pulsed light technology will be suitable for commercial use. Most results presented in this report should be confirmed by independent researchers. The following is a list of areas where relevant information is lacking:

- Identification of critical process factors and their effect on microbial inactivation.
- Suitability of the technology for solid foods and non-clear liquids where penetration depth is critical.
- Potential formation of unpalatable and toxic by-products.
- Resistance of common pathogens or surrogate organisms to pulsed light treatments.
- Differences between this technology and that of the more conventional UV (254 nm) light treatment.
- Mechanisms of microbial inactivation to determine whether they are significantly different from those proposed for UV light.
- Understanding of the mechanism and quantification of the benefit attributed to the pulse effect.

GLOSSARY

A complete list of definitions regarding all the technologies is located at the end of this document.

Broad-spectrum light. For pulsed light technology, the ultraviolet, visible and infrared light wavelengths.

Energy density. See light fluence.

Light fluence. Energy delivered from a light source per unit area (Joules/ cm^2).

High voltage switch. Device used to trigger the delivery of high intensity light pulses to foods or packaging materials.

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