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Review

# Pulsed light for food decontamination: a review

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Pulsed light (PL) is a technique to decontaminate surfaces by killing microorganisms using pulses of an intense broad spectrum, rich in UV-C light. The present review is focused on the application of PL for food decontamination. It revises the mechanism of microbial inactivation (UV-C as the most important part of the spectrum, photothermal and photochemical mechanisms, inactivation curve, peak power dependence, and photoreactivation), the factors affecting its efficacy, the advantages and problems associated with PL treatment, and results obtained *in vitro*. Examples of applications to foods are given, including microbial inactivation, and effects on food matrices.

## Introduction

Pulsed light (PL) is a technique to decontaminate surfaces by killing microorganisms using short time pulses of an intense broad spectrum, rich in UV-C light. UV-C is the portion of the electromagnetic spectrum corresponding to the band between 200 and 280 nm. PL is produced using technologies that multiply the power manyfold. Power is magnified by storing electricity in a capacitor over relatively long times (fractions of a second) and releasing it in a short time (millionths or thousandths of a second). The emitted light flash has a high peak power and consists of wavelengths from 200 to 1100 nm (Dunn, Bushnell, Ott, & Clark, 1997; Dunn, Ott, & Clark, 1995). The technique used to produce flashes originates, besides high peak power, a greater relative production of light with shorter bactericidal wavelengths (MacGregor *et al.*, 1998).

This technique has received several names in the scientific literature: pulsed UV light (Sharma & Demirci, 2003), high intensity broad-spectrum pulsed light (Roberts & Hope, 2003), pulsed light (Rowan *et al.*, 1999) and pulsed white light (Marquenie, Geeraerd, *et al.*, 2003). Barbosa-Canovas, Schaffner, Pierson, and Zhang (2000) reviewed the literature on PL some years ago, warning that most results presented in their report should be confirmed by independent researchers. Nowadays, a higher amount of independently originated data exists that justifies an updated review.

According to Wekhof (2000), the first works on disinfection with flash lamps were performed in the late 1970s in Japan, and the first patent dates from 1984 (Hiramoto, 1984). Bank, John, Schmehl, and Dracht (1990) seems to be the first work published in the scientific literature on the application of PL to inactivate microorganisms. By using a UV-C light source of 40 W at maximum peak power, a 6–7 log decrease in viable cell numbers was achieved. Additional information on this work was published also in Bank (1992).

The technique of UV-C treatment to preserve foods was discovered in the 1930s (Artés & Allende, 2005). PL is a modified and claimed improved version of delivering UV-C to bodies. The classical UV-C treatment works in a continuous mode, called continuous-wave (CW) UV light. Inactivation of microorganisms with CW UV systems is achieved by using low-pressure mercury lamps designed to produce energy at 254 nm (monochromatic light), called germicidal light (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). More recently, medium-pressure UV lamps have been used because of their much higher germicidal UV power per unit length. Medium-pressure UV lamps emit a polychromatic output, including germicidal wavelengths from 200 to 300 nm (Bolton & Linden, 2003). Another possibility for UV-C treatments is the use of excimer lasers, which can emit pulsed light at 248 nm (Crisosto, Seguel, & Michailides, 1998). PL works with

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Xenon lamps that can produce flashes several times per second.

The following units are commonly used to characterize a PL treatment.

- Fluence rate: is measured in Watt/meter$^2$ (W/m$^2$) and is the energy received from the lamp by the sample per unit area per second.
- Fluence: is measured in Joule/meter$^2$ (J/m$^2$) and is the energy received from the lamp by the sample per unit area during the treatment.
- Dose: used sometimes as a synonym of fluence.
- Exposure time: length in time (seconds) of the treatment.
- Pulse width: time interval (fractions of seconds) during which energy is delivered.
- Pulse-repetition-rate (prr): number of pulses per second (Hertz [Hz]) or commonly expressed as pps (pulses per second).
- Peak power: is measured in Watt (W) and is pulse energy divided by the pulse duration.

Formal definitions can be found in IUPAC (1996).

Proper determination of the fluence received by the treated body is the most important factor in characterizing a PL treatment; however, it is sometimes neglected or improperly reported. The same problem also exists in the literature on CW UV treatments (Bolton & Linden, 2003; Hijnen, Beerendonk, & Medema, 2006). Fluence determination can be complex, requiring a good knowledge of light properties. The assistance of experts is sometimes necessary. Researchers with scarce physics background are encouraged to look for advice before planning experiments involving light pulses. Precaution should be taken in reporting the energy received by the sample, which is substantially different from the energy delivered by the light source. Since research on PL is relatively scarce, especially in food applications, no selection was done in this review regarding the accuracy of fluence determinations. Recommendations on fluence determinations for future research can be found in Bolton and Linden (2003), Jin, Mofidi, and Linden (2006), and Ryer (1997).

## Susceptibility of microorganisms

Anderson, Rowan, MacGregor, Fouracre, and Farish (2000) and Rowan *et al*. (1999) reported the following trend of susceptibility in decreasing order: Gram-negative bacteria, Gram-positive bacteria and fungal spores. The colour of the spores can play a significant role in fungal spore susceptibility. *Aspergillus niger* spores are more resistant than *Fusarium culmorum* spores, which could be because the pigment of the *A. niger* spores absorbs more in the UV-C region than that of *F. culmorum* spores, protecting the spore against UV (Anderson *et al*., 2000). In contrast, Gómez-López, Devlieghere, Bonduelle, and Debevere (2005a) did not observe any sensitivity pattern among different groups of microorganisms, after studying 27 bacterial, yeast and mould species.

## Inactivation mechanism

### UV-C as the most important part of the spectrum

Xenon flash lamps have an emission spectrum ranging from ultraviolet to infrared light. The UV-C part of the spectrum is the most important for microbial inactivation. Rowan *et al*. (1999) reported that the inactivation of food related microorganisms (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Enteriditis, *Pseudomonas aureginosa*, and *Saccharomyces cerevisiae*) was 5–6 log CFU/plate using a high UV flash, whereas with low UV light only 1–2 log CFU/plate was achieved. Using a monochromator, Wang, MacGregor, Anderson, and Woolsey (2005) determined the germicidal efficiency against *E. coli* as a function of wavelength over the range 230–360 nm using about 6 mJ/cm$^2$. The results showed a maximum inactivation around 270 nm, and no measurable inactivation was observed to occur above 300 nm. Furthermore the authors concluded that the rich UV content from 220 to 290 nm in the UV spectrum provides the major contribution to inactivation, whichever type of UV source is used.

### Photothermal and/or photochemical mechanism

Considerable research has been performed on the mechanism of microbial inactivation by light pulses. The lethal action of PL can be due to a photothermal and/or a photochemical mechanism. It is possible that both mechanisms coexist, and the relative importance of each one would depend on the fluence and target microorganism. Most of the authors explain their results based on the photochemical effect. For example, since Rowan *et al*. (1999) achieved inactivation with less than 1 °C rise in temperature, they concluded that the lethality can be attributed to the photochemical action of the shorter UV wavelengths.

The mechanism of microbial inactivation by PL is frequently explained based on studies using CW UV, in which the inactivation is photochemical. Although the mechanism of inactivation by PL can have similarities with that of CW UV, some differences might exist. The germicidal effect of UV light on bacteria is primarily due to the formation of pyrimidine dimers, mainly thymine dimers (Giese & Darby, 2000; Mitchell, Jen, & Cleaver, 1992). The dimer inhibits the formation of new DNA chains in the process of cell replication, thus resulting in the inactivation (inability to replicate, called clonogenic death) of affected microorganisms by UV (Bolton & Linden, 2003). On bacterial spores, UV-C treatment results mainly in the formation of the ‘‘spore photoproduct’’ 5-thyminyl-5,6-dihydrothymine, and in single-strand breaks, double-strand breaks and cyclobutane pyrimidine dimers (Slieman & Nicholson, 2000).

Comparing the wavelength sensitivity for *E. coli* inactivation with previously reported absorption spectra of the

purine and pyrimidine bases of DNA, Wang *et al.* (2005) supported the hypothesis that the photochemical effect produced as a consequence of the UV absorption by DNA is the major cause of microorganism inactivation by PL.

There is however evidence that the photothermal effect can also occur. Hiramoto (1984) proposed that the rays absorbed into *A. niger* are expected to heat the mould instantaneously, providing a sort of thermal sterilization. Dunn *et al.* (1989) explained the inactivation of microorganisms with PL by both mechanisms, emphasizing that light pulses heat a superficial layer of food in such a way that the heat that is produced on the surface will eventually be conducted into the interior of the product. However, the total quantity of heat that is produced may be small relative to the amount of heat that would be needed to substantially raise the temperature of the whole product. Wekhof (2000) proposed that with a fluence exceeding 0.5 J/cm$^2$, the disinfection is achieved through a rupture of bacteria during their momentous overheating caused by absorption of all UV light from a flash lamp. Later on, Wekhof, Trompeter, and Franken (2001) provided evidence of this hypothesis by showing electron-microscope photographs of flashed *A. niger* spores presenting severe deformation and rupture. A ruptured top of a spore was presented as evidence of an escape of an overheated content of the spore, which became empty after such an internal ''explosion'' and ''evacuation'' of its content took place during the light pulse.

Different authors have interpreted their results on PL application based on the same photochemical mechanism occurring during inactivation by CW UV. However, they have also proposed or identified additional effects on microbial cells. It is not clear if those effects have a photochemical or a photothermal origin. Based on the relative inactivation of *Salmonella* cells plated on selective and non-selective agars, Wuytack *et al.* (2003) concluded that pulsed white light inactivation should be regarded as a multitarget process. In this, structural changes to DNA would be a major reason, and damage to membranes, proteins and other macromolecules plays a minor role. The hypothesis is in line with the results published by Takeshita *et al.* (2003), who compared the inactivation of *S. cerevisiae* by CW UV and broad-spectrum flash lights. Those authors observed that the DNA damage induced in yeast cells was essentially the same for both methods. It consisted in formation of single strand breaks and pyrimidine dimmers. However, an increased concentration of eluted protein and structural change in the flashed cells were observed only in the case of pulsed light. This suggested cell membrane damage induced by pulsed light. Flashed yeast cells showed raised and expanded vacuoles and cell membrane distortion/damage, and their shape changed to circular. On the other hand, after treatment with CW UV light, the yeast cell structure was almost the same as that of non-treated cells.

### Inactivation curve

The shape of the inactivation curve for microbial inactivation by CW UV light is sigmoid. The initial plateau is due to an injury phase. Once the maximum amount of injury has been surpassed, minimal additional UV exposure would be lethal for microorganisms and survivor numbers would rapidly decline (Barbosa-Canovas *et al.*, 2000). The end of the curve has a tailing phase that has received several explanations, which have been summarized by Yaun, Summer, Eifert, and Marcy (2003): lack of homogeneous population, multi-hit phenomena, presence of suspended solids, the use of multiple strains that may vary in their susceptibility to UV-C, varying abilities of cells to repair DNA mutations, and the shading effect that may have been produced by the edge of the Petri dishes used in some experiments. Another possible explanation was given by McDonald, Curry, Clevenger, Brazos, *et al.* (2000) when explaining the tailing of the inactivation curve of *Bacillus subtilis* treated with PL: the probability of exposing a biological element to the requisite conditions for lethality is reduced with decreasing population density.

For PL treatments, the shape of the inactivation curve of conidia of *Botrytis cinerea* and *Monilia fructigena* was also found to be sigmoid (Marquenie, Geeraerd, *et al.*, 2003). Anderson *et al.* (2000) and MacGregor *et al.* (1998) reported that the higher the number of pulses the higher the lethal effect. It can be observed in their results that the microbial population as a function of the number of pulses keeps constant until a certain point beyond which the inactivation starts. The same observation was reported by Fine and Gervais (2004) on the viability of *S. cerevisiae* cells dried on a quartz plate, which suggested a threshold level of energy for total destruction. These findings are in line with the sigmoid pattern discussed before for CW UV lamps. However, complete inactivation of microorganisms and absence of tailing have also been reported (Krishnamurthy, Demirci, & Irudayaraj, 2004; Otaki *et al.*, 2003; Wang *et al.*, 2005), although the effect of the detection limit of the enumeration method should be better assessed.

### Peak power dependence

Some research has been conducted to investigate if PL sources really yield improved microbial inactivation rates in comparison with CW UV light sources, as claimed by early literature coming from private sources. A peak power effect would not be in line with the Bunsen–Roscoe law (Sommer, Haider, Cabaj, Heidenreich, & Kundi, 1996). As a general rule in photochemical processes, the principle of equi-effectivity of the product of fluence rate and exposure time is valid. This principle is known as the Bunsen–Roscoe reciprocity law. It asserts that for the effectiveness of radiation it does not matter whether the fluence is reached with high fluence rate and short exposure time or with low fluence rate and long exposure time. An exception to this principle has been found for CW UV light treatment (Sommer *et al.*, 1996). The diversity of findings does not

allow setting a definitive conclusion on the validity of this principle for microbial inactivation by PL, although results point towards possible peak power dependence. According to McDonald, Curry, and Hancock (2002) several theories predict a more rapid kill of vegetative cells with PL. The most probable theory postulates that the high photon flux emanating from a pulsed source simply overwhelms the cellular repair mechanisms before repair can be completed.

Rice and Ewell (2001) examined the peak power dependence in the UV inactivation of bacterial spores by comparing the output of a high-peak-power UV source at 248 nm from an excimer laser to a low-power CW UV source (254 nm) used to inactivate *B. subtilis* spores. The two UV sources differed by eight orders of magnitude in peak power. Results showed no discernible peak power effect. Therefore, it appears that the total number of photons delivered is the important parameter and not the number of photons delivered per unit time (peak power). The results agree with the principle of Bunsen–Roscoe. A similar conclusion can be derived from the work of Otaki *et al.* (2003), who compared the inactivation of three strains of *E. coli* and two coliphage types by a low-pressure UV lamp emitting at 254 nm and a broad-spectrum flash lamp. Working at the same germicidal dose, no differences were found between both lamp sources. Moreover, Wang *et al.* (2005) concluded that the germicidal efficiency obtained with a Xenon flash lamp used to inactivate *E. coli* shows no obvious difference to the published data on inactivation using CW UV low-pressure mercury lamps at the same wavelength.

However, a single research group working with *B. subtilis* spores has reported conflicting results and conclusions. Two articles concluded that given the same fluence, no differences in the inactivation efficacy were observed when comparing the results obtained with a CW UV lamp producing 3.9 mW/cm$^2$ on the target to those obtained with two kinds of flash lamps producing in excess of 60 W/cm$^2$ on the target, in the fluence range 0–200 mJ/cm$^2$ (Hancock, Curry, McDonald, & Altgilbers, 2004; McDonald *et al.*, 2002). On the other hand, other articles concluded that pulsed UV light exhibits a minor improvement over that of the CW UV source over the fluence range 0–80 mJ/cm$^2$ (McDonald, Curry, Clevenger, Brazos, *et al.*, 2000) or that PL significantly outperforms CW UV light in aqueous suspensions and on surfaces (McDonald, Curry, Clevenger, Unklesbay, *et al.*, 2000).

Results reported by Takeshita *et al.* (2003), also supported a violation of the principle of Bunsen–Roscoe. The authors compared the effect of peak power on *S. cerevisiae* cells, using 4655 and 2473 kW. Their results revealed that under high-peak-power conditions, the killing effect and concentration of eluted protein were higher than under low-peak-power conditions.

Furthermore, the photothermal effect is not in agreement with the Bunsen–Roscoe law. It seems that under certain extreme conditions, PL causes different kinds of damage than CW UV.

### Photoreactivation

Photoreactivation means the reversal of ultraviolet damage in bacteria by illumination with visible light (Cleaver, 2003). It is a well known phenomenon in the CW UV treatment field. It is catalysed by the enzyme photolyase, which uses light energy to split UV-induced cyclobutane dimers in damaged DNA through a radical mechanism. "Photolyase is a flavoprotein and contains two noncovalently bound chromophores. One chromophore is the fully reduced flavin-adenine dinucleotide ($FADH^-$), the catalytic cofactor that carries out the repair function upon excitation by either direct photon absorption or resonance energy transfer from the second chromophore (methenyltetrahydrofolate or deazaflavin) that harvests sunlight and enhances the repair efficiency. The excited flavin cofactor transfers an electron to the cyclobutane pyrimidine dimer to generate a charge separated radical pair. The anionic ring of the dimer is split, and the excess electron returns to the flavin radical to restore the catalytically competent $FADH^-$ form and close the catalytic photocycle" (Kao, Saxena, Wang, Sancar, & Zhong, 2005).

In the PL research field, Rowan *et al.* (1999) wrapped their samples with aluminium foil after PL treatment as a precaution to avoid photoreactivation. Otaki *et al.* (2003) found photoreactivation after a PL treatment, being the photoreactivation rate slower than after a CW UV treatment. The photoreactivation suppression was assumed to have been due to the difference in wavelength. The wider wavelength light of the pulsed Xenon lamp was considered to have some effects on photoreactivation; for example, shorter wavelengths damage some kinds of enzyme related to this process. Evidence of photoreactivation in flashed cells has also been given by Gómez-López *et al.* (2005a). However, future research is needed to better quantify this phenomenon.

There are two other repair mechanisms for UV damage that might reactivate PL treated cells. One is the dark repair mechanism, which does not require light as photoreactivation does. The other is specifically related to spores. Spores can repair themselves from the spore photoproduct by the common excision repair system, or the spore photoproduct specific repair system (Setlow, 1992).

## Advantages and disadvantages

The short pulse width and high doses of the pulsed UV source may provide some practical advantages over CW UV sources in those situations where rapid disinfection is required (Wang *et al.*, 2005). For example, Rice and Ewell (2001), in the aforementioned experiment, needed 3 h to deliver $10^4$ J/m$^2$ using a CW UV lamp and 40 s to deliver the same total fluence using a laser with a repetition rate of 10 Hz. Other advantages of PL treatment are the lack of residual compounds, and the absence of applying

chemicals that can cause ecological problems and/or are potentially harmful to humans. Xenon flash lamps are also more environment friendly than CW UV lamps because they do not use mercury.

Sample heating is perhaps the most important limiting factor of PL for practical applications. Heat can originate from the absorption of light by the food or by lamp heating. When studying the inactivation of *A. niger* spores on corn meal, Jun, Irudayaraj, Demirci, and Geiser (2003) found that some experimental factor settings resulted in sample temperatures of 120 °C, which might have been due to the large amount of heat generated by the lamps, even though their device had a cooling system. Heating also limited the treatment of alfalfa seeds (Sharma & Demirci, 2003), grated carrots (Gómez-López, Devlieghere, Bonduelle, & Debevere, 2005b), and raw salmon fillets (Ozer & Demirci, 2006). It was also demonstrated to occur in honey (Hillegas & Demirci, 2003) and agar (Gómez-López *et al*., 2005a).

Another disadvantage of PL treatments is the possibility of shadowing occurring when microorganisms readily absorb the rays, as in the case of *A. niger*, and are present one upon another. This makes the organisms in the lower layers very hard to destroy in contrast to those in the upper layer (Hiramoto, 1984), although the use of relatively high peak powers can overcome the shadowing effect.

In order for a PL treatment to inactivate microorganisms, contact between photons and microorganisms should occur. Therefore, any body between the light source and the microorganism that absorbs light will impair the disinfection process. This restriction is different when flashing solid foods, and when flashing fluid foods or microorganism suspensions.

For the decontamination of solid foods, the situation can be divided into three cases. The first and most important case is that food components absorb light. Therefore, opaque solid foods can only be decontaminated superficially. The most important implication of this fact is a food safety concern. It has been demonstrated that pathogenic microorganisms can be internalised in produce tissues (Beuchat, 2006). PL cannot inactivate those microorganisms because the light will be absorbed at the surface, and the more opaque and thicker the food item, the lower the inactivation below the surface. This drawback should not be overestimated since the superficial character of PL treatment is also common with washing solutions such as chlorinated water and its substitutes, applied to decontaminate raw fruits and vegetables.

The superficial absorption of light should be regarded in view of how deep the light can penetrate into the food, i.e. the superficial character of the PL decontamination process should not be considered limited to an infinitesimal superficial layer of the food because some degree of inactivation can occur below it. The inactivation of microorganisms below the surface was demonstrated and modelled by Gardner and Shama (2000) for CW UV. Although it has been claimed that PL has a big penetration power, independent experiments have not been reported.

The second case is that the entire surface of the food piece should be flashed in order to achieve the decontamination of its whole surface, where irregularities of the food surface complicate the achievement of the goal. The other case is that food pieces can shadow each other when treated together. Both cases require engineering solutions that need sometimes equipment with radically new designs (Gardner & Shama, 2000).

Regarding fluid foods and microorganism suspensions, the liquid will absorb light depending on its absorption coefficient and depth. The challenge consists in promoting the flow of the fluid in an adequate way to drive microorganisms close to the light source in order to achieve a uniform exposure. Coping with this problem also requires engineering solutions, and a possibility has been offered by Forney, Pierson, and Giorges (2005) for CW UV applications.

PL is safe to apply but some precautions have to be taken to avoid exposure of workers to light and to evacuate the ozone generated by the shorter UV wavelengths.

## Factors determining the efficacy of a PL treatment

The most important factor determining the effect of PL is the fluence incident on the sample. The energy emitted by the flash lamp is different from the energy incident on the sample. Factors such as distance from light source to target, and propagation vehicle (air, water, fruit juice) affect the level of energy than ultimately reaches the target.

The inactivation efficacy of PL is higher when treated samples are closer to the lamp (Hillegas & Demirci, 2003; Ozer & Demirci, 2006). An equation to describe the effect of distance taking into account both the photochemical and the photothermal effects was described by Gómez-López *et al*. (2005a). The effect of distance was modelled by Sharma and Demirci (2003) for inactivation of *E. coli* O157:H7 on inoculated alfalfa seeds, and by Jun *et al*. (2003) for *A. niger* spores in corn meal.

Furthermore, tests on microbial inactivation are generally performed by placing the sample directly below the lamp. The effect of the placement of samples at other positions inside the treatment chamber was studied by Gómez-López *et al*. (2005a). The authors demonstrated that when a group of samples is placed at a short vertical distance from the lamp, those located directly below the lamp will be decontaminated while the rest will undergo almost no decontamination. When the vertical distance is increased, the decontamination will be less intense in those samples located directly below the lamp but the rest of the samples will be also decontaminated.

Sample thickness is another limiting factor for microbial inactivation with PL. Due to the restricted penetrability of the UV light, overlapping opaque samples shield surfaces from decontamination and also light is attenuated during

the treatment of fluid samples. That was observed by Sharma and Demirci (2003) for alfalfa seeds and by Hillegas and Demirci (2003) for honey.

The decontamination efficacy decreases at high contamination levels, which is also related to light attenuation. At high population densities, microorganisms overlap each other. Therefore, microorganisms placed in the upper layers will become inactivated, but will shadow the rest from the light (Gómez-López *et al*., 2005a).

Food composition also affects the efficacy of the decontamination by PL. Gómez-López *et al*. (2005b) treated *Photobacterium phosphoreum*, *L. monocytogenes* and *Candida lambica* inoculated onto surfaces of agars supplemented with several food components. The results demonstrated that proteins and oil decreased the decontaminant efficacy of PL, whereas when water or starch was added to the agar, no particular trends were observed. Roberts and Hope (2003) also found that the addition of protein to a buffered saline solution decreased the efficacy of virus inactivation. Therefore, high protein and fat containing food products have little potential to be efficiently treated by PL. Vegetables, on the other hand, could therefore be suitable for PL treatment.

With regard to the long term applicability of PL, the possible development of resistant strains should be taken into account. However, Marquenie, Geeraerd, *et al*. (2003) observed no development of resistance in fungi. This was also found by Gómez-López *et al*. (2005a) in the case of *L. monocytogenes*.

## Pulsed light devices

The pioneer company producing PL equipment for disinfection was Purepulse Technologies Inc. (San Diego, California), a subsidiary of Xenon Corp., which commercialized the PureBright™ system. Applications included water purification systems and virus inactivation systems for biopharmaceutical manufacturers. References by Dunn *et al*. (1995, 1997) correspond to the early efforts of this company, which is no longer active, to promote this novel technology. A brief history of the evolution of the pioneer companies related to PL can be found in Wekhof (2000).

As far as we know, there are nowadays two commercial companies producing disinfection systems based on PL. One is SteriBeam Systems from Germany, the other is Xenon Corporation from USA. References by Kaack and Lyager (2007), Wekhof (2000), and Wekhof *et al*. (2001) are associated with SteriBeam, while results reported by Demirci (Hillegas & Demirci, 2003; Jun *et al*., 2003; Krishnamurthy *et al*., 2004; Ozer & Demirci, 2006; Sharma & Demirci, 2003) were obtained with a Xenon Corp. device, mainly the model SteriPulse™-XL 3000. Information regarding devices for industrial applications can be found at the websites of these companies.

The basic benchtop equipment for laboratory studies is composed of a treatment chamber and a control module (Fig. 1). The treatment chamber is built of stainless steel. It has a shelf to hold the samples (microorganisms on agar in Petri dishes, or food samples), which can be displaced vertically, allowing to regulate the distance between the target and light source. The light source is a linear Xenon flash lamp located at the top centre of the chamber, inside the lamp housing. A basic benchtop equipment was used in the works by Gómez-López *et al*. (2005a, 2005b), Hillegas and Demirci (2003), Krishnamurthy *et al*. (2004), Lammertyn, De Ketelaere, Marquenie, Molenberghs, and Nicolaï (2003), Marquenie, Geeraerd, *et al*. (2003), Marquenie, Michiels, Van Impe, Schrevens, and Nicolaï (2003), Ozer and Demirci (2006), Sharma and Demirci (2003), Takeshita *et al*. (2003) and Wuytack *et al*. (2003) Experimental units used by Jun *et al*. (2003) and Kaack and Lyager (2007) were equipped with two lamps, and that used by Jun *et al*. (2003) had also a blower. By using a blower, a filtered air stream can flow around the lamp serving two functions: dissipating the heat generated by the lamp, and avoiding the accumulation of high levels of toxic ozone produced by the shortest wavelengths. Anderson *et al*. (2000), MacGregor *et al*. (1998), and Rowan *et al*. (1999) used a benchtop experimental facility where two inoculated Petri dishes inclined 45° received equivalent doses.

Experimental units with more complicated configurations have also been used. Fine and Gervais (2004) used a fluidized bed to mix powders and to increase particle exposure, and Huffman, Slifko, Salisbury, and Rose (2000) plumbed three PureBright™ units to treat water continuously. Most of the cited references provide schematic representations of the experimental units.

A control cable connects the light source with the control module, in which the electric current is modulated to produce a specific prr, pulse width and peak power. The control module has a switch to start the flashing period and a timer to control the exposure time. High peak power is produced by pulse power energization techniques. Information related to this kind of technique can be found in Anderson *et al*. (2000), Ghasemi, Macgregor, Anderson, and Lamont (2003) and Hancock *et al*. (2004).

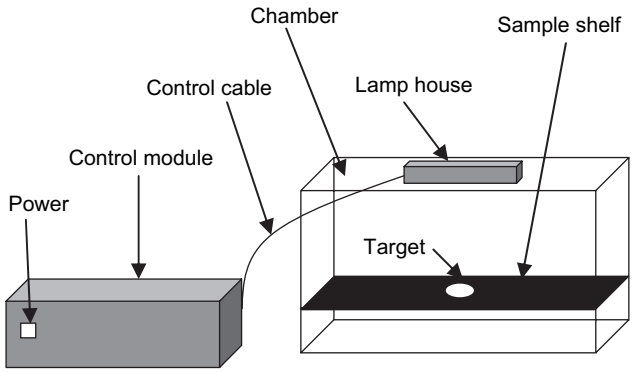


**Fig. 1**. Schematic representation of a bench top experimental unit of pulsed light.

## Inactivation of microorganisms *in vitro*

Several *in vitro* studies have reported high levels of inactivation of human pathogens by PL. MacGregor *et al.* (1998) found that 512 μs of light (512 pulses with a duration of 1 μs, a prr of 1 Hz, and a power density in the light source of 380 kW/cm$^2$) resulted in 6 and 7 log CFU/g reduction of *E. coli* O157:H7 and *L. monocytogenes*, respectively, when microorganisms were treated on an agar surface. Moreover, Krishnamurthy *et al.* (2004) reported a 7–8 log CFU/ml reduction in buffer suspended or agar seeded *S. aureus* cells treated for 5 s, and Fine and Gervais (2004) achieved a 7 log reduction of *S. cerevisiae* dried in glass beads and quartz plates. However, regarding results of experiments with microorganisms spread on agar surfaces, Gómez-López *et al.* (2005a) warned about a potential overestimation of the lethality due to the possibility that two or more survivor microorganisms situated very close to each other can form just one colony, and therefore be counted as only one.

Regarding liquid samples, Huffman *et al.* (2000) reported >7 log inactivation of *Klebsiella*, and >4 log inactivation of poliovirus, rotavirus and *Cryptosporidium parvum* in water. Moreover, Ghasemi *et al.* (2003) reported a 9 log order reduction in suspended *E. coli* and *Salmonella* after treatment with 100 pulses with each pulse providing 9 J.

Photosensitizers can enhance the microbicidal action of light. Photosensitized UV disinfection uses a combination of direct interaction between UV light and biologically important molecules and an indirect mechanism that involves the photochemical generation of reactive molecules and radicals to inactivate microorganisms. McDonald, Curry, Clevenger, Brazos, *et al.* (2000) reported a synergism between pulsed UV light and hydrogen peroxide, which yielded a gain of nearly 2 logs in the inactivation of *B. subtilis* spores in comparison with treatment with pulsed UV only. The authors did not propose the application of photosensitized UV treatment to food surfaces, but the possibility could be assayed since hydrogen peroxide *per se* has also been tested as a disinfectant of MPV.

## Effects of PL on foods of vegetable origin

The inactivation of microorganisms naturally present on vegetable surfaces by PL has been demonstrated by Hoornstra, de Jong, and Notermans (2002). The authors treated five vegetables, namely white cabbage, leek, paprika, carrots and kale with two pulses of wide spectrum PL that amounted to a fluence of 0.30 J/cm$^2$. The reduction in aerobic count at the surface of the vegetables varied from 1.6 log CFU/cm$^2$ for carrots to >2.6 log CFU/cm$^2$ for paprika. No significant increase in the inactivation was observed after using a third pulse. Moreover, no adverse effects on sensorial quality were observed after treating the vegetables with three pulses and storage at 7 and 20 °C for up to 7 days with one exception, iceberg lettuce showed some discoloration after 48 h at a storage temperature of 20 °C. Their own calculations demonstrated that a reduction of 2 log CFU/cm$^2$ increases the shelf-life at 7 °C of cut vegetables by about 4 days, which is remarkable given a treatment of only 0.4 ms effective duration. The efficacy of PL to inactivate vegetable spoilage microflora was again proved afterwards. Mesophilic aerobic counts were reduced between 0.56 and 2.04 log CFU/g after treating with more than 2000 pulses several minimally processed vegetables (spinach, radicchio, lettuce, cabbage, carrot, green bell pepper) and soybean sprouts (Gómez-López *et al.*, 2005b). Counts of *S. cerevisiae* cells inoculated onto carrot slices were reduced by more than 4 log CFU/g after 24 pulses.

The inactivation of microorganisms present on food surfaces does not necessarily result in an extension of shelf-life. PL treatment failed to prolong the shelf-life of shredded Iceberg lettuce and shredded white cabbage, stored under modified atmosphere packaging at 7 °C. For Iceberg lettuce, a 0.46 log reduction was achieved in psychrotroph counts. However, no-flashed and flashed samples were rejected at the third day of storage due to excessively high psychrotroph counts and bad sensory quality. For white cabbage, a 0.54 log reduction was achieved in psychrotroph counts. But after 2 days of storage, no-flashed and flashed samples had the same psychrotroph counts; therefore, the benefit of the decontamination was readily lost (Gómez-López *et al.*, 2005b). It should be mentioned that the failure in shelf-life prolongation does not necessarily imply the inefficacy of PL, but can also be a consequence of the storage conditions.

Regarding fruits, Marquenie, Geeraerd, *et al.* (2003) reported a maximal inactivation of 3 and 4 log units for conidia of *B. cinerea* and *M. fructigena in vitro*, which are fungi responsible for important economical losses during postharvest storage and transport of strawberries and sweet cherries. PL however had no effect on strawberries or on the development of *B. cinerea* inoculated on strawberries. The treatment did not induce resistance against fungal infection (Marquenie, Michiels, *et al.*, 2003). This could be due to insufficient dose or inadequate exposure.

PL has also been tested to decontaminate food powders and seeds. Jun *et al.* (2003) reported a 4.96 log reduction of *A. niger* spores inoculated in corn meal, but Fine and Gervais (2004) achieved less than 1 log reduction in *S. cerevisiae* cells inoculated onto wheat flour and black peppers. For seeds, Sharma and Demirci (2003) showed >4 log reduction in *E. coli* O157:H7 inoculated on alfalfa seeds.

The colour of foods can be modified by PL. Fine and Gervais (2004) compared the effects of light pulses on the inactivation of *S. cerevisiae* on black pepper and wheat flour. Colorimetric results indicated a rapid modification of product colour well before the decontamination threshold was reached and this was clearly more rapid for black pepper than for wheat flour. This colour modification was attributed to overheating combined with oxidation. The

difference in colour modification between wheat flour and black peppers was explained by the difference in initial colour, dark products absorb more light energy than pale products. On the other hand, Lammertyn *et al.* (2003) found that PL treatment did not significantly reduce strawberry sepal quality decay rate during storage for 10 days at 12 °C.

PL can affect the enzymatic activity of flashed vegetables as well as cause physiological changes also observed due to application of CW UV. Since proteins have a strong absorption at about 280 nm (Hollósy, 2002), broad-spectrum pulsed light could be useful to inactivate enzymes and as such inhibit adverse effects due to enzymatic activity. Dunn *et al.* (1989) claimed that by using two to five flashes of light at a fluence of 3 J/cm$^2$, it is possible to inhibit potato slice browning. The polyphenol oxidase extract recovered from the treated slices exhibited less activity than that from the untreated slices. PL can also increase the respiration rate of vegetables. Gómez-López *et al.* (2005b) reported an 80% increase in the respiration rate of shredded Iceberg lettuce, but no significant effects on the respiration rate of shredded white cabbage treated under the same conditions.

## Decontamination of other foods

Besides its application on foods of vegetable origin, PL has been tested for efficacy to inactivate microorganisms in a very limited number of other foods. As much as 0.97 log reduction was obtained for spores of *Clostridium sporogenes* inoculated in honey. Also in this case, the longer the treatment time and the shorter the distance between sample and lamp, the higher the inactivation, but also the higher the temperature. When the depth of the honey was decreased the spore kill increased. It appeared that the UV light has a limited penetration in the honey. The heat generated does not appear to have a synergistic effect on the inactivation of *C. sporogenes* in honey (Hillegas & Demirci, 2003).

The inactivation of *E. coli* O157:H7 and *L. monocytogenes* inoculated on raw salmon fillets by pulsed UV light was studied by Ozer and Demirci (2006), using a broad-spectrum lamp that generated 5.6 J/cm$^2$ per pulse at the lamp surface and three pulses per second. Two different surfaces were treated, the skin side and the muscle side. For *E. coli* O157:H7, the maximum log reduction was 1.09 on the muscle side and 0.86 on the skin side, while for *L. monocytogenes*, the maximum log reduction was 0.74 on the muscle side and 1.02 on the skin side. Some fish fillets were overheated at shorter distances and longer treatment times. The higher inactivation on the skin side was attributed by the authors to a higher temperature in comparison with the muscle side.

CW UV light has been used for meat treatments, but oxidation reactions (notably of lipids) resulted in significant changes. PL systems should effectively limit oxidation reactions because of the short pulse duration and the half-life of π-bonds, which prevent efficient coupling with dissolved or free oxygen (Fine & Gervais, 2004).

Other applications are described in the US patent of Dunn *et al.* (1989), such as disinfection of curds of dry cottage cheese, hard crusted white bread rolls, cake, packaging materials, and shrimps.

## Nutritional and toxicological aspects

Neither the effect of PL on nutritional components of vegetables nor the potential formation of toxic by-products has been studied yet. Since the wavelengths used for PL are too long to cause ionisation of small molecules and are in the nonionising portion of the electromagnetic spectrum (Dunn *et al.*, 1995), the formation of radioactive by-products is not expected.

PL treatment of foods has been approved by the FDA (1996) under the code 21CFR179.41. According to Dunn *et al.* (1997), in assessing the safety of foods treated with all forms of radiation, the agency considers changes in chemical composition of the food that may be induced by the proposed treatment, including any potential changes in nutrient levels. The legal status of PL in the European Union has a different approach, since the legislation is not technology oriented but food and food ingredient oriented. This technology would fall in the scope of regulation 258/97 on novel foods and novel food ingredients, article 1, item f (European Union, 1997). Among other categories, this legislation applies to foods and food ingredients to which a production process not currently used has been applied, and evaluates possible changes in nutritional value, metabolism and level of undesirable substances. The EU therefore would not approve PL technology as such, but specific foods and food ingredients treated with PL.

It has been proved that CW UV treatment can increase the concentration of phytochemicals in fruits. Cantos, Espín, and Tomás-Barberán (2001) found that UV treatment can increase by more than 10-fold the levels of resveratrol in grapes. Given the similarities between both techniques, PL might also have the same effect.

## Photosensitization: another application of light to inactivate microorganisms

Photosensitization is another technique that might be useful to decontaminate food surfaces by using light. It has been proposed as a milder alternative to the emerging non-thermal technologies for food preservation. Photosensitization has been defined by Lukšiene (2005) as a treatment involving a photoactive compound that accumulates in microorganisms followed by illumination with visible light. The combination of photosensitizer and light, in the presence of oxygen results in the destruction of microorganisms. After the work by McDonald, Curry, Clevenger, Brazos, *et al.* (2000) described before, it is foreseeable that this definition can be expanded to include the UV part of the spectrum. The primary field of application of

photosensitization has been photodynamic cancer treatment. Haematoporphyrin, sodium chlorophyllin, riboflavin and psoralen are examples of photodynamic active plant food constituents that could be used as photosensitizers for foods (Kreitner *et al.*, 2001).

Lukšiene (2005) published a comprehensive review on photosensitization encompassing history, photosensitizers, light sources, and mechanism of microbial inactivation. As an example of photosensitization, Kreitner *et al.* (2001) inactivated as much as 3.9 log CFU/ml of *S. aureus* cells after incubation for 1 h with haematoporphyrin, followed by illumination for 1 h. To date, its potential application in food preservation has only been tested for the inactivation of bacteria, yeasts (Kreitner *et al.*, 2001), and fungal food contaminants *in vitro* (Lukšiene, Pečiulyte, Jurkoniene, & Puras, 2005; Lukšiene, Pečiulyte, & Lugauskas, 2004). Therefore, more research is necessary to evaluate its future in food preservation.

## Conclusions

Light pulses are a high potential novel technology for food decontamination. This technology is fast and environment friendly. Proper reporting of treatment conditions, including fluence, will enable researchers to better understand and compare results from different experiments. Knowledge of the mechanism of inactivation by light pulses and the number of reports on food applications have increased considerably during the last 15 years. Its most important technological problems are to find ways to control food heating, and to treat homogeneously foods. More research is however needed on the nutritional consequences and possible formation of toxic by-products, and on the applicability of photosensitization to foods.

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