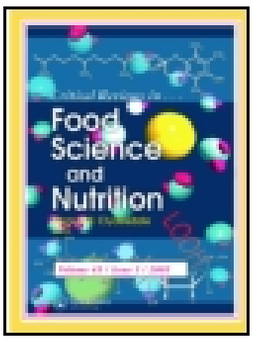

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Pulsed light treatment of different food types with a special focus on meat: a critical review

V. Heinrich[ab], M. Zunabovic[a], *T. Varzakas[c], J. Bergmair[b], and W. Kneifel[a]

[a]Department of Food Sciences and Technology, Institute of Food Science, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria.

[b]Ofi-Austrian Research Institute for Chemistry and Technology, Brehmstraße 14A, 1110 Vienna, Austria

[c]Higher Technological Educational Institute of Kalamata, School of Agricultural Technology, Dept. of Food Technology, Hellas

*Corresponding author, Theo Varzakas, theovarzakas@yahoo.gr

Abstract

Today, the increasing demand for minimally processed foods that are at the same moment nutritious, organoleptically satisfactory and free from microbial hazards challenges the research and development to establish alternative methods to reduce the level of bacterial contamination. As one of the recent emerging non-thermal methods, pulsed light (PL) constitutes a technology for the fast, mild and residue-free surface decontamination of food and food contact materials in the processing environment. Via high frequency, high intensity pulses of broad-spectrum light rich in the UV fraction, viable cells as well as spores are inactivated in a non-selective multi-target process that rapidly overwhelms cell functions and subsequently leads to cell death. This review provides specific information on the technology of pulsed light and its suitability for unpackaged and packaged meat and meat products as well as food contact materials like production surfaces, cutting tools and packaging materials. The advantages, limitations, risks and essential process criteria to work efficiently are illustrated and discussed with relation to implementation on industrial level and future aspects. Other issues addressed by this paper are the need to take care of the associated parameters such as alteration of the product and utilized packaging material to satisfy consumers and other stakeholders.

Keywords UV, pulsed light treatment, minimal processing, *Listeria monocytogenes*, ready-to-eat, packaging, meat

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1. INTRODUCTION

Societal and demographic changes as well as consumer trends, expectations and preferences have led to a steady increase in proportion and variety of meat and meat products, pre-packed meals and ready-to-eat (RTE) foods on the international market (Sofos, 2008; Havelaar et al., 2010). These products are expected to be of excellent quality, including sensory, dietary, hygienic and toxicological properties, convenience and both, economic and ecological factors (Aymerich et al., 2008). Hence, foods nowadays tend to be fat-, salt- and nitrite-reduced or less preserved, minimally processed, easy-to-handle and of fresh-like appearance and taste (Aymerich et al., 2008; Sofos, 2008; Havelaar et al., 2010; Weiss et al., 2010). Simultaneously, the persisting lack of food handling education of both, food handlers and consumers, pricing pressure, the increasing number of population at risk for severe infection (YOPIs: Young, Old, Pregnant, Immunocompromised) lay additional pressure on the food processing industry. Complex food distribution, consumer concerns and influenced purchasing patterns intensify this effect (Gerba et al., 1996, Anderson, 2000; Sofos, 2008; Havelaar et al., 2010). Also, governmental, health related and consumer advocating stakeholders and organizations are paying augmented attention to food safety as important quality attribute of food, intensified by several food scares in the (recent) past (Aymerich et al., 2008; Sofos, 2008; Havelaar et al., 2010).

To handle the risk originating from food-borne pathogens and spoilage organisms, research and development is challenged to establish alternative preservation methods (WHO, 2007; Aymerich et al., 2008; Havelaar et al., 2010). Further it is aimed to lower the evidently high per capita food loss and food waste all over the world (FAO, 2011). Additionally to the precaution that is already taken to lower the burden of food-borne disease, non-thermal – “cold” - technologies alone or in

combination with a sub-lethal multiple hurdle concept are considered as promising attempt to disinfect but not sterilize food products. Here, next to other food processing technologies – e.g. high pressure, pulsed electric filed (PEF), osmotic dehydration, radio frequency electric field, ultrasound, irradiation and chemical and biochemical hurdles (e.g. organic acids, enzymes, plant derived antimicrobials) - pulsed light (PL) application promises to reduce the hazard of food as a vector for bacterial infection and toxin production. In addition, different sources describe PL as fast and mild alternative method that retains the natural appearance of the foods while being of energy saving and of environmental interest (Butz and Tauscher, 2002; Leistner, 2002; Palmieri and Cacace, 2005; Sofos, 2005; Ortega-Rivas, 2012).

The unique interaction of intrinsic factors such as nutrient composition and extrinsic factors such as manufacturing techniques constitute meat as an optimal vehicle for spoilage and pathogenic microorganisms (Sofos, 2005; Aymerich et al., 2008; Nørrung and Buncic, 2008; Sperber, 2009; Cerveny et al., 2009; Havelaar et al., 2010).

With regard to meat and products thereof, pathogens of current concern in the European Union are infectious zoonotic agents like *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and verotoxigenic *E. coli* (EFSA, 2012; RASFF, 2012). In general, fresh meat products are closely associated to *Salmonella* spp., *Campylobacter* spp., *E. coli* and *Yersinia* spp. (EFSA, 2012). RTE meat products are at particular risk to be contaminated with *Listeria* spp. because of their ubiquity, potential to post-process recontamination and growth under refrigerated conditions following decontamination treatments (FDA/FIS, 2003; EFSA, 2012). In addition, the food production industry has to be aware of new pathogens of potential concern. These are not previously known, (re-) emerging, evolving or associated with animal health and could have

significant impact on human health problems and economic losses. *Myobacterium avium* subsp. *paratuberculosis*, *Escherichia albertii* and *Clostridium difficile* can be named as examples (Doyle and Erickson, 2006; Sofos, 2008; Havelaar et al., 2010; Newell et al., 2010).

PL is an emerging technology for the rapid and efficient surface decontamination of food and food contact materials in the production environment (Dunn et al., 1995). Synonymously PL is described as pulsed UV-light (PUV) (Sharma and Demirci, 2003), intense pulsed light (IPL) (Choi et al., 2010), high-intensity pulsed UV light (HIPL) (Ngadi et al., 2012), high intensity broad-spectrum pulsed light (BSPL) (Roberts and Hope, 2003), intense light pulses (ILP) (Gómez-Lopéz et al., 2005b) and pulsed white light (PWL) in the scientific literature (Dunn, 1996; Marquenie et al., 2003).

Today mainly applied for decontamination reasons in the food packaging industry, continuous UV light *per se* is widely known for its germicidal effect (Kowalski, 2009). Since the first use in context to food in the late 1930s, intensive studies and confirmation of the germicidal effect of (pulsed) UV light have been carried out throughout the last decades (Gates, 1928; Hiramoto, 1984; Dunn et al., 1995; Gómez-López et al., 2005b; Levy et al., 2012).

As further development of the conventionally used continuous-wave (CW) UV light (coherent (UVC) light of a defined wavelength), PL asserts itself as a good alternative or supplement to conventional physical (thermal) or chemical intervention strategies in the recent past (Ngadi et al., 2012). Including the addition of preservatives as well as heating, dehydration and freezing steps, these traditional preservation methods likely impair the composition and perceived organoleptic quality of the treated goods. Minimal temperature effects of the non-thermal technologies such as PL in consequence pave the way for demanded, nutritious, organoleptically

satisfactory and at the same moment minimally processed foods that are free from pathogens and food spoilage microorganisms (Palmieri and Cacace, 2005; Ngadi et al., 2012; Ortega-Rivas, 2012).

The objective of the present review is to provide current and practical information on the technique and suitability of PL treatments for meat and meat products, as well as the measurement of bacterial inactivation regarding the methodological approach. Further, the advantages, limitations, risks and essential process criteria to work efficiently with this technique are discussed.

2. TECHNOLOGICAL ASPECTS

With its early beginning in the late 1970s in Japan, the technology of PL was first patented by Hiramoto (1984) (US Patent 4464996). According to Palmieri and Cacace (2005), the rights were purchased by PurePulse Technologies® (subsidiary of Maxwell Technologies®) in 1988, and the technique of producing high intensity light pulsed was progressively adapted by Dunn et al. (1989). The developed broad-spectrum application was subsequently patented as PureBright® (US Patent 4871559) (Dunn et al., 1989). Further research resulted in the characterization of the germicidal effect, onward developments, devices and patents on the international market (Palmieri and Cacace, 2005).

The FDA´s (Food and Drug Administration) approval for “pulsed UV light in the production, processing and handling of food” finally gave rise to numerous scientific works in the field of food, food contact surfaces and processing environments (Palmieri and Cacace, 2005; FDA, 1996).

Despite the effort made, the potential of PL on food is still under investigation and the gap between the basic and applied science seems to be remaining (Marquenie et al., 2003; Gómez-López et al., 2005b; Ozer and Demirci, 2006). In food processing the technique of PL is not yet applied at industrial scale (precompetitive stage), although systems for other applications are already patented or launched on the market (Ortega-Rivas, 2012). Examples include the pharmaceutical and medical sector as well as sanitation of surfaces and atmospheres (aseptic technology) in various processes to reduce or eliminate the need for chemical disinfection/preservation agents (Dunn et al., 1989; Wekhof, 2003). In the context of PL, the expanded field of foods dealt with in scientific papers is comprised in Table 1.

The PL technology basically comprises the emittance of short-duration, high-power pulses of electromagnetic radiation (light) from an inert-gas flash lamp, which causes microbial cell deterioration. Regarding the peak power dependence it is generally stated that the shorter the pulse duration the higher the energy deliverance and the higher the bactericidal effect is (Dunn et al., 1989).

Basic essentials of the equipment (Figure 1) being utilized to generate these pulses are: (i) a power unit to generate high-power electrical pulses and (ii) the treatment chamber where a light source transforms the generated pulses into high-power light pulses. First the (low power, low voltage) alternating current coming from the primary energy source is transformed to direct current via a converter. Then a capacitor is charged to a certain voltage (within fractions of a second), and the current coming from the capacitor is discharged by a high voltage switch (nanoseconds to milliseconds). The so obtained (high-power, high-voltage) high direct pulsed

electric current is then used to excite the lamp(s) within the treatment chamber (Dunn et al., 1995; Palmieri and Cacace, 2005; Elmnasser et al., 2007; Ortega-Rivas, 2012). The driving circuit further includes a timing control and a trigger generator (Anderson et al., 2000; Barbosa-Canovas et al., 2000; Choi et al., 2010).

The lamp types used comprise tubes of various materials, such as quartz and shapes (e.g. spherical, spiral) and are almost exclusively filled with xenon gas or alternatively krypton or a mixture of noble gases (Dunn et al., 1989; Palmieri and Cacace, 2005; Gómez-López et al., 2007). The main focus of this review is laid on broad-spectrum light xenon flash lamps, since the FDA approval for the treatment of food is solely for these lamps (FDA, 1996).

Varying in terms of wavelength, frequency and energy, the emitted broad spectrum radiation of xenon lamps ranges from approximately 180 to 1100 nm and encompasses next to the infrared (IR 700 - 1100 nm) and visible light (VIS 400 – 700 nm) the fraction of ultra violet light (UV 180 – 400 nm), each contributing 30, 45 and 25 % to the whole spectrum, respectively (Dunn, 1996).

Light pulses approximately have 20.000 times the intensity of the sunlight at the surface of the earth and the emitted spectrum is to some extent similar to sunlight - except for some UV fractions (UVC) filtered out by the atmosphere of the earth (Dunn et al., 1995). This is why the commonly used “working rule” that a flash lamp mimics the sun spectrum is criticized (Wekhof, 2000). In addition, the spectral components of a single light pulse are time-dependent, starting with the shortest wavelengths (UV) and ending with the longest wavelengths (IR) (Ortega-Rivas, 2012).

Basic information on light technology can also be taken from Ryer (1998), Bolton and Linden (2003) and Jin *et al.* (2006).

To better understand and to characterize the effects of PL, some basic measures should be taken into consideration and consistently used (Palmieri and Cacace, 2005; BIPM, 2006; Lagunas-Solar and Gómez-López, 2006; Gómez-López et al., 2007; IUPAC (International Union of Pure and Applied Chemistry), 2007):

- The fluence rate (F) [W m^{-2}]: describes the total radiant power incident from all directions that is received by a small sphere divided by the cross-sectional area of that sphere.
- The fluence (F) [J m^{-2}]: describes the total radiant energy that is received from the light source by the matrix per unit area during the treatment time (in seconds).
- Number of pulses (n) applied during the whole treatment.
- Pulse width (t) [s]: the duration of one pulse or time interval in which the energy is delivered by the light source.
- Exposure time (t_{tot} = n*t) [s]: is the total length of the treatment.
- Frequency (f = 1/t) [Hz]: also called pulse-repetition rate (prr) or reported as pulses per second (pps) depicts the number of pulses generated per second.
- Peak Power [W]: describes the proportion of pulse energy to pulse duration.

Lagunas-Solar and Gómez-López (2006) critically highlighted the fact that most of the available publications on UV light disinfection for CW UV as well as PL treatments lack the basic

information on the influencing factors light generating source and target matrix (e.g. geometry and condition), negatively affecting the credibility of the outcomes (inactivation rate). However, descriptions of materials and methods used are often limited to the type of apparatus being used (including lamp manufacturer and specifications but lacking information on light source, geometry of irradiation and installed reflectors) and the geometric set up, such as the space between the light source and target. This is further complicated by the fact of simplifying the physical basis of these processes. Commonly, energy (light) output by the lamp and incident on the target are assimilated without taking care of the possible discrepancies arising from differences in physical units, the conversion formula, geometry and experimental set up used (Lagunas-Solar and Gómez-López, 2006).

3. FACTORS AFFECTING PL TREATMENTS

Based on the available scientific literature, three major variables that influence the efficacy of a PL treatment can be identified: (i) the type of matrices treated, such as food, food contact surfaces or culture media (solid/liquid), (ii) the quantity and quality of microbial contamination, (iii) the process parameters, such as the geometry of the treatment chamber and the intensity of light.

Thus, it appears that PL treatments and resulting outcomes vary significantly due to difficulties in the standardization of the parametrical configuration allowing interpretable, comparable and reproducible decontamination outcomes. In addition, not only the decontamination rate but also the avoidance of damages to the matrix should be of upmost interest to the users of this technology (Gómez-López et al., 2007).

The variables in this review (Table 2) are discussed from a general point of view in relation to different kinds of foods and food contact materials.

3.1. TREATED MATRIX

PL is a technique that is only effective on surfaces. This superficial character can be explained by transparency/opacity means and diameter of the body (or fluid) under consideration and should not be considered as disqualifier since washing solutions act similar (Dunn et al., 1989; Palmieri and Cacace, 2005; Gómez-López et al., 2007). Regarding fresh meat it should be also taken into account that inner layers of intact muscle tissue are free from bacteria, which is particularly advantageous in this context (Gill and Penney, 1977).

The optimization towards a low reflection-, high absorption- and transmission coefficient increases the penetration depth and concurrently allows for increased decontamination even in layers beyond the surface of the matrix (Palmieri and Cacace, 2005; Gómez-López et al., 2007). Yet, penetration depth and inactivation of enzymes remain restricted to the upmost layers (Dunn et al., 1989). For in-depth studies both, the light incidence at a defined layer beneath the surface and the temperature rise can be calculated. The former can be calculated using the Lambert-Beer law and the latter on the basis of heat transfer law (Dunn et al., 1989).

The surface characteristics and nutrient composition are important factors to better understand which meat and meat products are best suited for surface decontamination with PL.

The surface topology (tree-dimensional structure) for example does not allow vast irregularities and light absorbing (organic/inorganic) matter (hurdle) between the light source and the treated surface, shielding the target (microbial contamination) from the photon (light) incidence (Dunn

et al., 1995; Gómez-López et al., 2005a; Palmieri and Cacace, 2005; Gómez-López et al., 2007; Sommers et al., 2009). This also applies to dust that may have accumulated on the xenon lamps (Lagunas-Solar and Gómez-López, 2006). Additionally, thin items, slices or pieces positively affect the inactivation because of a lower total fluence needed for penetration (Krishnamurthy et al., 2004). Despite the complex surface having microscopic unevenness and cracks, smooth surface meat cuts and meat products are described as well suited for decontamination, achieving up to 3 Log reductions in microbial counts as reported in the literature (Dunn et al., 1995; Green et al., 2003). Table 3 comprises the data published in scientific literature about the inactivation of microorganisms on meat and meat products using PL. It is apparent that the research activities for red meat focus on fresh meat products, sausages and cold cuts. White meats are represented by (skinless) chicken meat, parts of (or whole) chicken carcasses and unpacked and packed sausages. In both cases, decontamination of unpacked and packed products is addressed.

The other prerequisite for successful PL decontamination is the suitable composition of the matrix, which is mainly dependent on the proportion of protein, fat, carbohydrates and water able to competitively absorb light (Gómez-López et al., 2005a; Rajkovic et al., 2010a). A matrix or surface rich in fat and protein will impair the decontamination process (Gómez-López et al., 2005a; Rajkovic et al., 2010a). This is either caused by the absorption of UV light on the molecular basis of isolated as well as conjugated double bonds or by increased absorption at 280 nm in the UVB range (Hollósy, 2002). A lack in this particular fraction of the applied broad-spectrum light affects the efficacy to a certain extent but does not totally offset the destructive power. For carbohydrates these pronounced detrimental characteristics are missing (Gómez-López et al., 2005a). Contrary to these findings, some photon-sensitive substances are described

in the literature, which are capable of selectively enhancing the absorption of specific wavelengths. This characteristic is used for the selective inactivation of microorganisms. An area of application is for example the surface decontamination in packaging processes. Some colorants suitable for foods would be carotene, lime green, black cherry and cooking oils (Dunn et al., 1989).

As any other method, PL has its positive and negative effects. Most controversially discussed, but studied to a minor extent are possible damages of meat and meat products due to visual changes (color, graying processes), oxidation reactions (mainly fatty acids), nutrient alterations and textural changes including moisture loss (Palmieri and Cacace, 2005; Oms-Oliu et al., 2010; Wambura and Verghese, 2011). Observations vary among the published literature (Table 3). It is remarkable that the outcome strongly depends on the product, packaging and treatment parameters (Dunn et al., 1995; Keklik, 2009; Keklik et al., 2009; Uesugi and Moraru, 2009; Keklik et al., 2010; Paskeviciute et al., 2011; Haughton et al., 2011; Wambura and Verghese, 2011; Hierro et al., 2011; Hierro et al., 2012; Ganan et al., 2013). This leads to the conclusion, that treatment optimization in terms of decontamination intensity and impact on the treated matrix is unavoidable to guarantee consumer acceptance and elongated shelf life (Hierro et al., 2011; Wambura and Verghese, 2011).

PL treatment could be carried out at several steps in the production chain. A way to keep the microflora of fresh meat (carcasses) as low as possible from the start could be the integration of PL in the refrigeration step or before continuation of the processing (Wong et al., 1998; Lyon et al., 2007). Hence, the microbial burden in the production and thus the chance of (re-)contaminating already processed products is reduced (Lyon et al., 2007). PL can also be used as

an intervention strategy in processing lines. Here, the aim is the avoidance of cross-contamination between the equipment and the final product (Rajkovic et al., 2010a). Another or a complementary strategy to combat microbial contamination of (minimally) processed foods can be seen at the very end of the production chain. Here both, the (primary) decontamination of the unpacked products and the (terminal) treatment through the packaging material are possible (Uesugi and Moraru, 2009). The former, for example, can be easily integrated in the processing line (e.g. after slicing) to reduce the possible post-heat treatment contamination prior to packaging (Fernández et al., 2009; Rajkovic et al., 2010b). Generally, PL treatment can be used in batch (single pulse, burst of pulses, random sequences) or continuous mode (array of pulses) to match the prevailing process conditions (Ortega-Rivas, 2012).

Related to food contact materials like packaging films and trays, conveyor belts, diverse surfaces in production, cutting tools such as meat slicing knives and slicing equipment can be highlighted along with the following treatment prerequisites.

The material for post-packaging application of PL should be transparent for the used wavelengths (mainly UV) while constituting a sufficient barrier (OTR: oxygen transfer rate; WVP: water vapor permeability) and mechanic (stability) properties to protect the perishable meat products (Dunn et al., 1989; Elmnasser et al., 2007; Han, 2007; Oms-Oliu et al., 2010; Ščetar et al., 2010). Suitable materials for PL treatments for example are polyethylene (PE), polypropylene (PP), polyolefin (PO) and polyvinyl chloride (PVC) (Keklik, 2009; Keklik et al., 2009; Keklik et al., 2010; Haughton et al., 2011).

Moreover, matrices that may interfere with light absorption, such as ink printed labels or drawings, should be avoided (Palmieri and Cacace, 2005). The optimal package for PL treatment

should be open for UV light and allow light incidence from any direction to guarantee uniformity of decontamination. Experiments on decontamination through packaging made in the recent past report the same inactivation efficacy for packed and unpacked products (Table 3) (Keklik, 2009; Keklik et al., 2010; Haughton et al., 2011).

The surface characteristics of food contact materials in general can significantly interfere with spatial distribution and decontamination characteristics of microorganisms (Levy et al., 2012). A rough surface finish for example favors uniform 2-dimensional distribution (hiding) of cells and an even decontamination success. A too smooth surface whereas leads to cell clustering due to hydrophobicity and a decreased decontamination success (Woodling and Moraru, 2005).

As for the organoleptic properties of the various products, food contact surfaces like packaging films are affected by the treatment according to the prevalent conditions. Parameters analyzed in the studies (Table 4) are: elastic modulus, yield strength and percent elongation at yield point, maximum tensile strength, percent elongation at break and hydrophobicity (Keklik, 2009; Keklik et al., 2009; Ringus and Moraru, 2012).

As a preliminary conclusion from this, the research field of packaging characterization and targeted development for PL applications needs to be more intensively addressed. In this context, Han (2007) describes the balanced research and development as an important tool to encourage the commercial use of non-thermal decontamination technologies.

In-vitro experiments on solid surfaces (agar) and fluids (broth, buffer) do not bear tremendous differences to the above described facts. Here also light attenuation, shadowing (particulate

material), composition and propagation of light play a significant role (Gómez-López et al., 2007).

3.2 CONTAMINATION

MICROBIAL AGENTS

Contaminating microbial agents show different sensitivities to PL treatment according to the physiological constitution, population density and growth parameters (growth rate and lag time), but are thought to be all inactivated in the same manner –a non-selective multi-target process that overwhelms cell functions and leads to cell death (Dunn, 1989; Augustin et al., 2011). The application of surrogate strains for foodborne pathogens in challenge tests is inevitable and should consider key characteristics such as lack of pathogenicity, inactivation kinetics, being genetically stable etc. (Komitopoulou, 2011). Although some general rules can be drawn from the research efforts made so far on this scientific topic, there are still several uncertainties that have to be addressed in future works.

VARYING SUSCEPTIBILITY

Lack of clarity, for example, exists when the sensitivity of microorganisms to PL is addressed by means of cell morphology. Some authors report a significantly higher resistance of Gram-positive in comparison to Gram-negative bacteria in their experiments (Rowan et al., 1999; Anderson et al., 2000; Farrell et al., 2010). The reason could be due to an evolutionary adaptation. Bacteria more frequently exposed to sunlight (e.g. *Bacillus cereus*) are thought to be less susceptible to light emitting treatment, due to induced protective and repair mechanisms (Anderson et al., 2000). However, Gómez-López et al. (2005b) and Rajkovic et al. (2010a) could

not prove this thesis. One way of explanation could be found in the different experimental set-up and equipment used (Rajkovic et al., 2010a,b).

Also, mucoid as well as pigment forming bacteria exhibit higher resistance to PL at increased cell populations. This has been attested for *Pseudomonas aeruginosa*, which showed strain dependant susceptibility to PL (Farrell *et al*., 2010). Furthermore, fungi are more resistant to PL than bacteria and spores are more resistant than viable cells (Rowan et al., 1999; Anderson et al., 2000). A difference in susceptibility is also evident between fungal and bacterial spores. This is due to the role of spore color (pigment) in cell protection (absorbance of light) (Dunn et al., 1991; Anderson et al., 2000; Levy et al., 2012; Esbelin et al., 2013).

Due to a higher surface to volume ratio, which eases the dissipation of heat from the cell (depending on the surrounding matrix), smaller bacteria are described to be more resistant to PL than larger ones (Wekhof, 2000).

High population densities (> 6.9 Log cm^{-2}) in general presuppose a shading effect due to overcrowding and light attenuation. Nevertheless, these so called “point concentrations” are reported to be successfully decontaminated with PL. This phenomenon also applies for spore producing stalks, where numerous spores are entrapped in conglomerates. In most cases, this overcrowding leads to a protection of the underlying cells by the (inactivated) upmost cell layer to a certain extent and thereby declined inactivation rate (Hiramoto, 1984; Anderson et al., 2000; Gómez-López et al., 2005b; Farrell et al., 2010).

TIME-DEPENDENCY OF TREATMENT

The time between contamination and treatment additionally has to be taken into consideration as an influencing factor. The more extended the time span between contamination and PL treatment is, the less likely even successive flashes of light can guarantee a sufficient decontamination success (attested for *Listeria monocytogenes* and *Escherichia coli O157:H7* on stainless steel) (Rajkovic et al., 2010a). Since stationary state growth phase causes a constant but reduced decontamination effect, it is therefore recommended to start the treatment promptly after a possible contamination (e.g. cutting of meat), before an increase in the endogenous microflora starts (Gómez-López et al., 2005b).

COMBINATION OF PL WITH OTHER TREATMENTS

For the reason that sub-lethal injury can occur, one should be aware of possible over-estimation of the lethal effect (Woodling and Moraru, 2005). At the same time, the sub-lethal injury renders the integration into the hurdle concept proposed by Leistner (2002) perfectly possible (Green et al., 2003; Woodling and Moraru, 2005). Following that, hurdles hamper recovery of the cells, which enhances the effect of the PL treatment (Woodling and Moraru, 2005). PL can be, for example, applied in combination with several thermal and non-thermal decontamination steps in the food production chain. Compatible treatments, mentioned in the literature with possible synergistic effects, are sub-lethal stress conditions (e.g. acid, salt (NaCl)) or thermal treatment (as final step). Also MAP (Modified Atmosphere Packaging), HPP (High Hydrostatic Pressure), antimicrobial chemical additives (e.g. nitrates), “natural” antimicrobial essential oils, extracts or bacteriocins (e.g. nisin) as well as hydrogen peroxide can be applied as a supplement (McDonald, 2002; Marquenie et al., 2003; Green et al., 2003; Uesugi and Moraru, 2009; Bradley

et al., 2012). Furthermore, a very promising attempt to increase the sensitivity of microorganisms towards light treatment in general is the photodynamic approach (Kreitner et al., 2001). In this combination PL treatment is used together with food-grade compounds, so called photosensitizers. These substances are, when exposed to light, capable of producing free radicals inside the cell which leads to cell disintegration (Kreitner et al., 2001; Lukšienė and Zukauskas, 2009). Some photosensitizing agents used in experiments are for example haematoporphyrin (HP) and sodium chlorophyllin (CHL). Therefore, photosensitization seems to be a rapidly expanding research field for food and non-food applications (Kreitner et al., 2001).

INACTIVATION MECHANISMS

Today the multi-target process overwhelming the microbial cell is seen as an interaction of at least two mechanisms: (i) the photochemical (cell death due to DNA lesions), and (ii) the photothermal (cell death due to disruption and structural changes) (Dunn et al., 1989; Cheigh et al., 2012). Acting in parallel or in sequence these interactions make a treatment with PL more effective than the conventionally used CW UV systems (Dunn, 1989). In the context of cell membrane damage, vacuole expansion, elution of proteins as well as structural deformation of cells and spores a third effect – the photophysical effect (structural cell damage) - is occasionally described in the literature (Wekhof et al., 2000; Wekhof and Trompeter, 2001; Takeshita et al., 2003).

The heat potentially generated during non-thermal methods such as PL is not a primary (physical) cause of decontamination. It is more seen as a “secondary effect” which can positively affect the decontamination effect (Koutchma, 2009).

The photochemical effect causes similar to slightly less DNA damage (clonogenic death: double-/single- strand DNA breaks, cyclobutane dimer formation) than with CW UV at almost the same inactivation rate (Takeshita et al., 2003; Cheigh et al., 2012).

The UV fraction of the light, being only responsible for this chemical effect, is also mostly responsible for the photothermal effect mentioned above. Exceeding a certain fluence of 0.5 J cm^{-2} causes temporary overheating and subsequent rupture of cells without significantly increasing the surface temperature of the matrix treated (Wekhof, 2000; Farrell et al., 2010). This destruction of cells is so far reported for PL but not for CW UV treatments, where only alteration of the cell wall is detected (Cheigh et al., 2012). Cheigh et al. (2012) illustrate this effect via transmission electron microscopy for *L. monocytogenes* KCCM40307 and *E. coli* O157:H7 comparing CW UV and PL treatment.

The cause of cell rupture is seen in the considerable absorption differences between the cell and the surrounding matrix. Since the total rate of cooling is less than the total energy disposition into the cell, overheating is inevitable (Wekhof, 2000). The spontaneous evaporation of water (formation of micro-bubbles) in the cell causes rupture of functional membranes and leads to emission of cell contents and to possible melting of the organisms into the matrix when a certain surface temperature of the organism is reached (Wekhof and Trompeter, 2001; Takeshita et al., 2003; Cheigh et al., 2012). Wekhof and Trompeter (2001) illustrate the melting phenomenon of cells into the matrix through electron-microscope photographs displaying craters around overheated spores on packaging material (PET with a melting temperature of about 120 °C) and describe the cell rupture effect as pulsed UV disintegration (PUVD). This effect seems to occur if the pulse energy is high enough, the pulse duration short enough and the distance from the

flash lamp limited. Otherwise, the inactivation is comparable with that of CW UV light (SteriBeam, 2013b).

The relative importance of the photothermal and/or photochemical mechanism depends on both, the fluence and the target organism (Gómez-López et al., 2007). In absence of UV light the photothermal effect is regarded as the primary cause of destruction (Mertens and Knorr, 1992). In order to understand the mechanisms underlying microbial destruction in detail, further research is needed (Elmnasser et al., 2007).

INACTIVATION KINETICS AND REPAIR MECHANISMS

Repetitively authors highlight the inactivation curve of PL treatments to be non-linear with a sigmoid shape (Luksiene et al., 2007; Farrell et al., 2010). Three major phases of inactivation have been identified. The initial plateau is registered through a cell injury phase (characteristic shoulder effect) (Gómez-López et al., 2007; Farrell et al., 2010). The second phase describes the rapid decline of surviving cells after a maximum amount of injury and a minimum of additional energy required to cause tremendous cell death rates (MacGregor et al., 1998; Fine and Gervais, 2004; Gómez-López et al., 2007). Finally, a tailing phase is described in the literature. Here, the lack of homogeneous population, multi-hit phenomena, varying susceptibility of different bacterial strains, cell repair activity, shading by suspended solids or objects (e.g. petri dish wall in *in-vitro* experiments) and the declined probability of exposure to the conditions are named as the underlying reasons (McDonald et al., 2000; Yaun et al., 2003). If the fluence rate of the PL treatment is high enough from the beginning on, the shoulder effect is absent (Farrell et al., 2010). Absence of the tailing phase and/or complete inactivation due to low initial cell population is also reported (Otaki et al., 2003; Wang et al., 2005; Farrell et al., 2010).

Cell repair mechanisms like photoreactivation (PHR), the spore repair system or the dark repair mechanism are widely known to be active under certain conditions after CW UV treatments, allowing revising some or all of the damage caused to the cell (Setlow, 1992; Cleaver, 2003). The most frequently described mechanism is the PHR. Under the influence of visible light the enzyme photolyase catalyzes the cleavage of UV-induced cyclobutane dimers in the DNA (Weber, 2005; Kao et al., 2005). It seems that this effect (in a mitigated manner) is also common for PL treatments under certain preconditions, e.g. when cells are not irreversibly damaged (Otaki et al., 2003; Gómez-López et al., 2005b; Farrell et al., 2010).

Since this effect is not shown with reasonable certainty for PL, (Paskeviciute *et al.* (2011) were not able to show this effect) and the link to food and food-contact materials is missing, it is recommended to precautionary protect the treated matrix from light after the treatment for a few hours (Gómez-López, 2012). This avoids increased numbers of surviving cells, underestimation of the lethality effect as well as a high variability of results and eases shelf-life estimation of the products (Kelner et al., 1949; Gómez-López et al., 2005b). Practically, this can be realized by wrapping the matrix in, for example, aluminum foil (in-vitro experiments and small goods) or immediate storage in a dark room in industrial production (MacGregor et al., 1998; Rowan et al., 1999; Anderson et al., 2000; Gómez-López et al., 2005b; Paskeviciute et al., 2011; Farrell et al., 2010).

RESISTANCE FORMATION

Lack of information is evident on resistance formation of certain bacterial species through long-term application of PL, which is so far described for *Listeria monocytogenes*, *E. coli* O157:H7 and *Pseudomonas aeruginosa* (Barbosa-Canovas et al., 2000; Marquenie et al., 2003; Gómez-

López et al., 2005b; Elmnasser et al., 2007; Rajkovic et al., 2009; Massier et al., 2012). Next to this potential selection for more resistant organisms, the NACMCF (National Advisory Committee on Microbiological Criteria for Foods) points out that eliminating the competitive microflora necessitates to protect the food products from recontamination with pathogens (NACMCF, 2006). Also it is recommended to take preventive actions against resistant sub-populations in production environments (Gómez-López et al., 2005b; Rajkovic et al., 2009).

In the broader sense of resistance formation, it can be stated that until now no evidence is found that multiple resistances to antibiotics interfere with the susceptibility to PL treatments (Farrell et al., 2010).

MICROBIOLOGICAL ANALYSIS

The determination of the effectivity of PL is significantly affected by the method applied. Traditional culture-dependent methods require 1-5 days for the detection and enumeration of specific bacteria obtaining results on culturable bacteria and therefore might not include sub-lethally injured and stressed cells. Also the presence of low bacterial counts can lead to false negative results (Cocolin et al., 2011). Alternatively, molecular techniques can be applied (e.g. PCR) obtaining faster results but are still hampered by challenging validation tasks. Conventional PCR based methods are not able to discriminate between dead and viable cells and also cannot give quantitative information regarding initial concentration of target molecules. However, quantitative molecular methods such as qPCR (quantitative PCR) are available. Certain approaches like RT (reverse transcriptase)-qPCR, EMA (ethidium monoazide)-qPCR, PMA (propidium monoazide)-qPCR) enable the discrimination between live and dead cells, although the discriminatory power strongly depends on the protocol applied (Cocolin et al.,

2011). Additionally, for the determination of the sublethal injury of bacteria the role of stress-response factors (e.g. RpoS and SigB) related to a certain treatment condition should be evaluated (Ouazzu et al., 2012).

In the case of culture-based methods, it is necessary to ensure that cell-clusters that could form a single colony are separated and that the exposure to light is kept as low as possible to avoid photoreactivation in the laboratory procedure (Gómez-López et al., 2005b). In their scientific work, Gómez-López et al. (2005b) thoroughly discuss the advantages and disadvantages of the different *in-vitro* methods and the comparability to the results obtained from food matrices. For *in-vitro* tests on agar surfaces the most widely used method in scientific papers is the incubation method (MacGregor et al., 1998; Rowan et al., 1999; Anderson et al., 2000). Here, a defined amount of bacteria – ideally a monolayer (to avoid overlapping of cells), which complies to less or equally 3×10^7 colony forming units (cfu) cm^{-2} (calculated from the average size of a bacterium, which is 1 μm x 3 μm) – is spread on to a Petri dish.

Plates are treated with PL and afterwards incubated, which allows possible occurring cell conjugates to form a single colony, which in turn makes overestimation of the kill effect likely (about 1 Log). So, the findings are hardly comparable to those from food matrices, where the plating method is most frequently applied. Here, by comparison, the sample is weigh in, diluted, homogenized, serially diluted, transferred to the Petri dish and covered with medium. Of course it is possible to use the plating method in *in-vitro* tests, but the unavoidable photoreactivation (about 0.3 Log) during processing of the sample is a major drawback. Since photoreactivation is little and incomplete separation is limited, Gómez-López et al. (2005b) recommend the strike

method as the most reliable *in-vitro* testing procedure. Here, the microorganisms are once more spread after the PL treatment to assure cell separation and further incubated.

Based on these findings, reports on *in-vitro* reductions have to be analyzed more critically (Gómez-López et al., 2005b).

OTHER USES OF PL

Scientific literature is further expanded to the field of viruses and protozoa like *Cryptosporidium parvum*, which is of great concern in the water industry and in communal water supply systems (Huffman et al., 2000; Roberts and Hope, 2003; Lamont et al., 2007; Bohrerova et al., 2008; Lee et al., 2008; Jean et al., 2011). Next to the significant impairment of virus inactivation in the presence of protein (demonstrated in fluid media), Roberts and Hope (2003) highlight the size of the genome encoding essential genes, genome packaging, capsid structure as well as the presence of an envelope as factors that may influence the susceptibility of viruses to PL. Due to the fact that PL treatment is limited to the treatment of surfaces, helminths like *Trichinae* are not affected by the PL treatment (especially important for pork meat) (Green et al., 2003).

3.3 PROCESS SETUP

The process set-up has to be fully reported in terms of materials used and experimental conditions chosen to make *in-vitro* as well as *in-vivo* experiments comparable to each other (Elmnasser et al., 2007; Gómez-López, 2012). Specific information is often missing when needed to interpret a certain effect in literature (Elmnasser et al., 2007).

This section therefore aims to give information on how the process may be influenced and critical interactions.

The whole span of wavelengths in the broad-spectrum (pulsed) light (BSPL) can contribute to a lethal effect. Wavelength adjustment by filters (e.g. glass, pyrex or makrolon or water) as well as alteration in density of current for lamp excitation can be used to limit the application to a selected range of wavelengths in a specific treatment (Dunn et al., 1989; Wekhof, 2000; Wekhof and Trompeter, 2001). This makes it possible to change the light spectrum without changing the lamp and to concurrently adapt the treatment to the target matrix and population density as well as to limit the treatment temperature (mainly due to the IR content of the light) (Panico, 2005; Ortega-Rivas, 2012).

Shorter wavelengths bear higher energy levels; hence the UV (C) content of the light is of pronounced importance for the microbiocidal action (Dunn et al., 1991; Wekhof, 2000; Takeshita et al., 2003; Levy et al., 2012). The UV C fraction, for instance, alone is sufficient for inactivation, having a maximum interference at 270 nm (Wekhof, 2000; Wang et al., 2005). Since light with a higher UV content has higher effectiveness, a decrease in UV and especially omission of wavelengths below approximately 300 nm offsets the lethal effect to a certain extent (Rowan et al., 1999; Wekhof, 2000; Wang et al., 2005; Woodling and Moraru, 2007; Levy et al., 2012). Therefore, variation of UVA, UVB and UVC allows for a great flexibility in applications in accordance to the biological substrate or product treated (Dunn et al., 1989; Wekhof, 2000). Overall system performance monitoring in consequence is possible by either (constantly) measuring the UV output (silicone photodiode) or the lamp current as indication for the intensity and spectrum of the light emitted (Barbosa-Canovas et al., 2000; Clark et al., 2006). Allowing for corrective actions, an additional critical control point could be easily introduced in the processing chain to assure safe products (Rajkovic et al., 2010b; Ortega-Rivas, 2012).

Continuing with the process characterizing measures, the fluence incident on the treated matrix can be described as one of the most important factors to be determined (Gómez-López et al., 2007; Levy et al., 2012). Variations within the energy delivered by the light source and the actually received energy by the sample make proper measurement and reporting unavoidable (Gómez-López et al., 2007). From the technical point of view, the treatment intensity needed to accomplish the intended decontamination in most cases does not exceed the cumulative limit of 12.0 J m^{-2} suggested by the FDA (FDA, 1996).

A pulse number of 1 to 20 is commonly reported to be sufficient for inactivation (Dunn et al., 1989). Although there is proportionality between the number of pulses applied and the magnitude of lethal effect, it is possible to achieve a significant Log reduction to an even entire decontamination against a wide range of microorganisms and matrix with only a single pulse of sufficient fluence (MacGregor et al., 1998; Anderson et al., 2000; Luksiene et al., 2007; Farrell et al., 2010; Levy et al., 2012). In general, a single pulse of higher fluence is regarded to be more effective than a higher number of lower fluence. Additionally, a threshold value of pulses or energy stands in line with the sigmoid pattern of the inactivation curve described above (Anderson et al., 2000).

Short pulse duration in turn is regarded as a key factor that makes PL advantageous in comparison to CW UV treatments, when rapid disinfection is required (Hiramoto, 1984; Wang et al., 2005). Complementary, short-duration pulses (1 μs to 0.1 sec) do not only prevent the cell surface to be cooled down by the surrounding but also minimize the treatment temperature notably (Wekhof, 2000; Panico, 2005). Additionally, the lipid oxidation is limited due to the short half-life of arising π-bonds and concurrent prevention of efficient coupling with dissolved

or free oxygen present. Apart from this, the low pulse number required supplementary decreases the potential for oxidation reactions (Fine and Gervais, 2004).

The frequency of pulses is found to be independent from the inactivation in a range of 1 to 5 Hz by Luksiene et al. (2007), meaning that the total energy accepted and not the pulse frequency is determent in this case. In terms of process design, the features and the efficacy of the lamp cooling system limit the maximum pulse frequency of a single lamp (or simultaneously flashed lamps) (Dunn, 1989). Commonly, frequencies of 0.5 to10 Hz are used (Ortega-Rivas, 2012). A notable increase in effective pulse rate in consequence can be achieved by using sequentially flashed lamps or by introducing relative movement between the light source(s) and the matrix (Dunn et al., 1989).

The negative and therefore limiting effect of superficially matrix (over-)heating can be additionally diminished when the cooling period between the pulses is sufficient to allow the heat to dissipate from the surface (Panico, 2005). This period can be 0.1 to 5 seconds long, but should be preferably set below 2 seconds to retain the cumulative effect of the pulsed application (Dunn et al., 1989).

For the practical implementation of PL, the geometry of the laboratory or industrial device has to be taken into consideration as a major efficiency-determining factor (Gómez-López et al., 2005b; Demirci and Keklik, 2012). Hence, the position and orientation of the light source(s) should allow for a multidirectional and uniform exposure of all surfaces of the matrix (Dunn, 1989; Sommers et al., 2009; Hsu and Moraru, 2001). This for example can be realized by: (random) movement of the matrix (e.g. rotation on a moving belt), a conveyor having transparent sections or the usage of reflectors (e.g. aluminum with a rough, patterned raster) (Dunn et al., 1989;

Sommers et al., 2009; Luksiene et al., 2007). Complementary, the absolute and relative distance between the sample and the lamp is important for decontamination capability. The shorter the absolute vertical distance, the higher the effect is (Gómez-López et al., 2005b; Ozer and Demirci, 2006; Farrell et al., 2010). However it must be noted that a shorter distance causes a narrower frame of high efficiency treatment and elevated matrix heating (Gómez-López et al 2005b).

As a consequence, the general trend is to minimize the distance for the purpose of high-energy incidence on the matrix and minimal treatment times. Conversely, globular bodies having curvatures are likely to exhibit intrinsic variations in decontamination even when rotated about one-axis. Practically, this can be overcome by increasing both, the distance and the treatment time. The relative position of the matrix to the light source however plays a significant role since the intensity of a xenon flash lamp decreases from the geometric center towards the ends (Laguans-Solar and Gómez-López, 2006).

Heating of the matrix and also of lamp(s) is further determined and limited by the treatment duration, which should be as short as possible (Dunn et al., 1989; Gómez-López et al., 2005b). The needed exposure time to PL is 4 to 6 magnitudes lower than with CW UV and there is no heating throughout the first few seconds or hyperthermic effect when PL is correctly applied (MacGregor et al., 1998; Rowan et al., 1999; Elmnasser et al., 2007; Luksiene et al., 2007). The (facultative) cooling system further provides a filtered air stream around the light source and matrix to avoid (over-) heating, impairment of product quality and accumulation of high ozone levels (Gómez-López et al., 2005b; Elmnasser et al., 2007; Gómez-López et al., 2007). Other systems, for example, include lamps jacketed for water-cooling. Additionally, the lamp lifetime can be extended herewith (Dunn et al., 1989).

It has to be highlighted that, despite the fact that PL is seen as a safe treatment technology, the operator security in terms of light and ozone (preferably generated by the shortest wavelengths between 170 and 200 nm exposure) has to be assured at all times (Gómez-López et al., 2007; Kowalski, 2009; Farrell et al., 2010). Automatic door locks, which prevent from opening the treatment chamber or room when operating, venting or lamps that filter out the ozone forming wavelengths of the light respectively can realize this (Gómez-López, 2012).

4. IMPLEMENTATION OF PL, ADVANTAGES AND DISADVANTAGES

The companies listed in Table 5 offer information material regarding laboratory-scale and/or industrial size (static conditions) applications on their websites or provide customized information on demand. For instance, SteriBeam (2013c) offers recommendations to avoid common pitfalls when implementing PL at the production site.

With regard to meat and meat products, some useful systems exist. Exemplarily, one of these is a UV-tumbling process (USA, C&S Equipment Co.) where the goods (e.g. fresh or frozen meats) are agitated by the rotating drum or screw conveyor to allow illumination from all sides (equipment originally dimensioned for CW UV) (Koutchma, 2008; Rajkovic et al., 2010b). In scientific work by Isohanni and Lyhs (2009) two commercially available systems (both equipment with CW UV lamps) (EU, Finland; www.biocid.com) are used: an irradiator for experiments in a laminar flow hood and an ultraviolet chamber with an active oxygen generator for the decontamination of poultry are used. Patents by Dunn et al. (1991) and Clark et al. (2006) describe machines (PL) for fabricating, filling, sealing and sterilizing packages for various products.

Some of the frequently applied xenon lamps are mentioned in Table 6.

Despite the decontamination capabilities, alternative non-thermal technologies such as PL may not be implemented in a food processing company when there is a major discrepancy in one of the following criteria: (i) improvement of food safety and shelf-life, (ii) maintenance of the organoleptic and nutritional qualities, (iii) no residues, (iv) convenience, (v) economic efficiency and (vi) no objections from both, consumers and legislative (Raso et al., 2005). Therefore, the present review attempts to describe some of the advantages and disadvantages that are associated with the use of PL (Table 7).

PL is particularly safe to apply for the operator if some precautions are taken and barely affects the environment (Palmieri and Cacace, 2005; Gómez-López et al., 2007; Farrell et al., 2010). In addition one can relinquish, under normal conditions, required chemicals, disinfectants and preservatives, which enable to produce high quality foods with no residual compounds from the decontamination process (Dunn et al., 1989). Available literature on this topic also highlights the xenon lamps as good alternative to the conventionally used CW UV lamps, which contain environmentally hazardous mercury (Ortega-Rivas, 2012). The low energy input finally rounds off the ecological advantages (Dunn et al., 1989).

The possible formation of ozone is disadvantageous but it could be controlled (Gómez-López, 2012).

When talking about convenience of the technique, the main benefit is seen in the short processing times, the possibility to operate in batch or continuous mode, the ability to fit PL treatment into existing processes and minimum space requirements (Dunn et al., 1989; Rajkovic

et al., 2010b; Ortega-Rivas, 2012). All applications that currently use CW UV lamps can be adapted to the PL technology. Instant action of the light source without the need of a warming phase (in comparison to CW UV), natural cooling of the lamps and matrix between the pulses as well as the adjustable light spectrum (by the pulse forming network or applied filters) with high UV output permit to set-up customized high efficiency plants with high throughput (Dunn et al., 1989; Wekhof, 2000; Wekhof and Trompeter, 2001; Gómez-López, 2012). Disadvantageous, however, are the occasionally difficult to standardize geometric configurations and process parameters (Gómez-López et al., 2007). To date, also the market situation with few global players and some minor market competitors may impair the desired flexibility of food companies.

The high investment costs of industrial PL equipment may discourage from the buying intention and make the technology predominantly interesting for high added value foods or particular market situations (Palmieri and Cacace, 2005). Arguments for the costs are the highly sophisticated and costly driving circuits (10 to 100 times more expensive than for CW UV) that are necessary to guarantee preferably high UV outputs and long lifetime of the lamps. Average lifetime depends on the operating parameters and can be defined from 6 to 12 months. However, the costs are a few times higher than for CW UV lamps. This compares to low running expenses and therefore elevated cost efficiency of the described emerging technology (SteriBeam, 2013a).

The pronounced inquiry for minimally processed foods by the consumer makes the PL technique attractive for both present and future markets (Palmieri and Cacace, 2005). Serious marketing problems however may arise due to misunderstanding by the consumer, when the term “irradiation” instead of “illumination” is chosen, even though PL treatment is a non-ionizing

technique that does not promote the formation of radioactive by-products (Dunn et al., 1995; Lagunas-Solar and Gómez-López, 2006). The NACMCF (2006) also concludes that further consumer research is required to elaborate label statements that are understood by the final consumer.

While the FDA received the petition for approval of PL for food applications in 1994 (Dunn et al., 1995) and approved it in 1996 from a technology-orientated approach for the United States of America (Code 21CFR179.41) (FDA, 1996), PL in the European Union is judged from a different approach. This food-ingredient oriented point of view is covered by the regulation 258/97 (1997) “novel foods and novel food ingredients” (article 1, item f). Here amongst other criteria, foods as well as food ingredients need to be evaluated regarding possible changes in nutritional value, metabolism and level of undesirable substances when treated with a production process that is not currently used. Based on the statements above, specific foods but not the treatment intrinsically can be approved in the EU at the moment (EU, 1997).

5. DISCUSSION

The information derived from *in-vitro* tests performed throughout the last decades reveals that the PL technology is of high potential for the decontamination of both, vegetative cells and spores, from surfaces and liquids (Bank et al., 1990; MacGregor et al., 1998; Rowan et al., 1999; Anderson et al., 2000; Takeshita et al., 2003; Wuytak et al., 2003; Gómez-López et al., 2005b; Luksiene et al., 2007; Van Houteghem et al., 2008; Rajkovic et al., 2009; Ben Saïd, 2010; Choi et al., 2010; Farell et al., 2010; Bradley et al., 2012; Levy et al., 2012; Massier et al., 2012).

For meat and meat products where specific information is generated gradually, industrial application is hampered. Further, a clear need for more focused research on a comparable basis is evident to close the gap between laboratory scale interventions and the stepwise upscale to high-throughput (continuous) processing lines in the meat processing industry.

While *in vitro* tests spotlight microbial reductions of up to 6 Log on solid media, it has been shown that the more complex the supporting medium in terms of structure and composition is, the less inactivation can be expected. Hence, even the decontamination of relatively simple appearing food-contact surfaces like plastic packaging materials (Table 4) show approximately halving of the decontamination efficiency depending on the material characteristics (Ringus and Moraru, 2012).

Regardless the hardly comparable treatment parameters of the published data (Table 3) for meat and meat products, the actual achieved microbial reduction ranges from 1 Log for whole chicken carcasses (pilot scale plant) to 2.4 Log for skinless, boneless chicken breast meat (bench-top equipment) (Paskeviciute et al., 2001; Keklik, 2009). For unpackaged and vacuum packaged products almost the same reduction can be reported, depending on the polymeric material and the treatment conditions chosen (Keklik et al., 2009; Keklik et al., 2010; Haughton et al., 2011). Although the packaging material can necessitate slightly extended treatment times to achieve the same reduction, Keklik et al. (2010) report the positive side effect of slowing down the visual color change of boneless chicken breast meat caused by the PL treatment. Hand in hand with the post-packaging application of PL to prevent unwanted recontamination in the production chain, the need for more research on the packaging material *per se* has become evident. Since non-thermally processed foods exhibit unique quality parameters, packaging designs and materials

should be changed accordingly. This research includes interactions between the material and the product as well as chemical and physical requirements of the material (Han, 2007).

This highlights that not only the degree of decontamination but also the associated parameters are of upmost interest to provide consumers and other stakeholders with the demanded nutritious, organoleptically satisfactory, minimally processed and at the same time safe foods. Particularly, the sensory product alterations will constitute a field of attention in the future research.

Critically observed, scientific literature almost exclusively has focused on inoculated meat and meat products. Naturally contaminated commodities have been more or less neglected. In the context of RTE cooked meat products inoculated with *L. monocytogenes*, Uyttendaele et al. (2004) describe several factors that are essential to reflect a natural contamination in challenge tests. These, for example, are the choice of the microbial strain (food isolate and/or reference strain), the respective growth potential on the RTE products, the inoculum level and the effect of pre-incubation temperature.

Principally utilized pathogens are *Campylobacter* spp., *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. While the latter with certainty constitutes one of the most tenacious microorganisms concerning meat and especially RTE foods, the question concerning the most resistant microorganism of public health significance for PL decontamination remains open (FDA/FIS, 2003; NACMCF, 2006; EFSA, 2012).

In addition to the increased application of pulsed light in multiple hurdle concepts like for example in combination with nisin, the decomposition of allergens and toxins are about to become a major topic in research and development (Uesugi and Moraru, 2009).

6. CONCLUSIONS

Pulsed light as a non-thermal intervention strategy against pathogenic and spoilage microorganisms opens up new possibilities in food and particular meat and meat product decontamination.

Despite the fact that the efficiency of pulsed light is limited to the surface, the reduction of vegetative cells and spores prior to or post-packaging permits increased product safety without resorting to the conventional used thermal or chemical decontamination methods.

While the characterization of effects is mainly based on the knowledge derived from *in vitro* tests so far, the link to practical implication is often missing. Also fragmentary reporting and the lack of possibility to compare results can be regarded as a major drawback. The future research focus can be therefore seen in the closure of the gap between basic and applied research. Further, the alterations of meat and meat products as well as of food contact materials have to be more detailed specified in order to further develop and commercialize this promising emerging technology.

Conflict of interest

The authors declare to have no conflict of interest.

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Table 1 Food groups treated with pulsed light technology.

Food	Reference
Liquids (water, apple juice, apple cider, sugar syrup)	Dunn et al., 1989; Huffman et al., 2000; Sauer and Moraru, 2009; Chaine et al., 2012
Powders (corn meal, food powders, infant food, ground red and black pepper)	Jun et al., 2003; Fine and Gervais, 2004; Choi et al., 2010; Nicorescu et al., 2013
Seeds (alfalfa seeds, soybean seeds)	Sharma and Demirci, 2003; Gómez-López et al., 2005a
Fresh produce (spinach, celeriac, green bell pepper, radicchio, iceberg lettuce, soybean sprouts, white cabbage, carrot, mushrooms)	Gómez-López et al., 2005a; Ramos-Villarroel et al., 2012
Fruits (blueberries, strawberries, sweet cherries, apples)	Marquenie et al., 2003; Bialka and Demirci, 2007; Gómez et al., 2012
Fish and seafood (raw summer flounder, raw shrimp, raw salmon fillets)	Dunn et al., 1989; Ozer and Demirci, 2006
Dairy products (cottage cheese, milk)	Dunn et al., 1989; Krishnamurthy et al., 2007
Shell eggs	Keklik, 2009
Honey (clover honey)	Hillegas and Demirci, 2003
Baked products (hard crusted white bread rolls, cake)	Dunn et al., 1989

Table 2 Factors affecting the efficacy of a pulsed light treatment.

Matrix	**Microbial contamination**	**Process**
Transparency/opacity	Microorganism	Spectrum
Surface characteristics	Intrinsic conditions	Experimental parameters
Composition	Growth parameters	Geometry and set up

Table 3 Summary of published scientific data for the microbial decontamination and alteration of meat and meat products using pulsed light technology.

Food (non-sterile)	Artificially inoculated bacterial strain [inoculation range]	Parameter[a]	Light dose (at sample) (F)[b]	Re-duction (log_{10})	Product alteration at the given parameters[c]			Reference
					Positive	Negative	Neutral	
White meats								
Chicken wings	*Salmonella* [2-5 log_{10} cm^{-2}]	n.d.	n.d.	~2	n.d.	n.d.	n.d.	Dunn et al., 1995
Whole chicken carcass	*E. coli* K12 [6 log_{10} ml^{-1}]	t_{tot}=45 s; t=360 µs; f=3 Hz; 5.6 $J.cm^2$/pulse Distance to quartz window=5 cm	n.d.	~1	-	-	Color	Keklik, 2009
Chicken frankfurters	*L. monocytogenes* Scott A [5-6 log_{10} cm^{-2}]	t_{tot}=60 s; t=360 µs; f=3 Hz; 1.27 $J.cm^2$/pulse (1.5 cm from lamp surface)	n.d.	1.6	-	Color (L*, a* and b* values)	Visual appearance, Lipid peroxidation, rancid odor	Keklik et al., 2009

Vacuum packaged (poly-propylene) chicken frankfurters		Distance to quartz window=8 cm	n.d.	1.5	-	Color (b* values)	Visual appearance, lipid peroxidation, rancid odor	
Boneless chicken breast	*S. typhimurium* [5-6 $\log_{10}$ 16 cm^{-2}]	t_{tot}=15 s; t=360 µs; f=3 Hz; 1.27 $J.cm^2$/pulse (1.5 cm from lamp surface) Distance to quartz window=5 cm	n.d.	~2	-	-	Color, lipid peroxidation	Keklik et al., 2010
Vacuum packaged (poly-propylene) boneless chicken breast		t_{tot}=30 s; t=360 µs; f=3 Hz; 1.27 $J.cm^2$/pulse (1.5 cm from lamp surface) Distance to quartz window=5 cm	n.d.	~2	-	-	Color, lipid peroxidation	
Chicken breast meat	*S. typhimurium* *L. monocytogenes* [6 $\log_{10}$ ml^{-1}]	t=112 µs	5.4	2 2	n.d.	n.d.	n.d.	Gudelis and Lukšienė, 2010

Skinless, boneless chicken breast	*S. thyphimurium* DS88 (SL5676 SmR [pLM32]), *L. monocytogenes* ATCCL3 C7644 [7 $\log_{10}$ ml^{-1}]	t_{tot}=n.d. s; t=112 µs; f=5 Hz Distance to quartz window=n.d.	5.4	2 2.4	-	Lipid oxidation	Sensory (smell, odor, flavor, taste, color)	Paskeviciute et al., 2011
	Total aerobic mesophiles[d]			2.0				
Chicken skin	*C. jejuni* (Chicken and human isolates) [Dip solution of 8 $\log_{10}$ cm^{-2}]	t_{tot}=30 s; t=360 µs; f=3 Hz; Distance to quartz window= 2.5cm	n.d.	0.91	n.d.	n.d.	n.d.	Haughton et al., 2011
	E.coli (ATCC 25922) [Dip solution of 8 $\log_{10}$ cm^{-2}]		n.d.	1.51				
	S. enteritidis (ATCC 13076) [Dip solution of 8 $\log_{10}$ cm^{-2}]		n.d.	1.5				
Packaged chicken skin (polyolefine)	*C. jejuni* (Chicken and human isolates)		n.d.	1.22	n.d.	n.d.	n.d.	

	E. coli (ATCC 25922)	n.d.	1.69			
Packaged chicken skin (polyvinyl-chloride)	*S. enteritidis* (ATCC 13076)	n.d.	1.27	n.d.	n.d.	n.d.
Skinless chicken breast meat	*C. jejuni* (Chicken and human isolates)	n.d.	0.89	-	-	Color (Hunter a* value)
	E.coli (ATCC 25922)	n.d.	1.48	-	-	-
	S. enteritidis (ATCC 13076)	n.d.	1.2	-	-	-
Packaged skinless chicken breast meat (polyolefine)	*C. jejuni* (Chicken and human isolates)	n.d.	0.96	-	Color (decreased Hunter L* and b* value)	Color (Hunter a* value)
	E.coli (ATCC 25922)	n.d.	1.13	-	Color (decreased Hunter L* and b* value)	-

Packaged skinless chicken breast meat (polyvinyl chloride)	*S. enteritidis* (ATCC 13076)		n.d.	1.35	-	Color (decreased Hunter b* value)	-	
Red meats								
Retail beef	n.d.	n.d.	n.d.	n.d.	Microbial and sensorial shelf-life	n.d.	n.d.	Dunn et al., 1995
Frankfurters	*L. innocua* [3-5 $\log_{10}$/Frankfurter]	n.d.	n.d.	~2	-	-	Alteration of protein, riboflavin, nitrosamine, benzpyrene and vitamin E content	
(Skinless) Canned Vienna sausages	*L. innocua* (FSL C2-008) (isolate from fish plant)	t=360 μs; f=3 Hz; 9 pulses Distance to sample=5.8 cm	9.4	1.37	-	-	Noticeable changes in color or appearance	Uesugi and Moraru, 2009

(Skinless) Canned Vienna sausages (+nisin after 48h, 4 °C)	plant) [7 $\log_{10}$ ml^{-1}]	sample=5.8 cm	9.4	4.03	Suppressed long-term survival of *Listeria*	-		
Sliced ham	-	t=360 µs; f=3 Hz; 0.14 $J.cm^2$/pulse on surface of lamp, various settings	n.d.	n.d.	-	Color, oxidative stability, texture, moisture loss	-	Wambura and Verghese, 2011
Vacuum packaged ham slices	*L. monocytogenes*	n.d.	8.4	1.8	Shelf-life extension	-	Sensory, lipid oxidation	Hierro et al., 2011
Vacuum packaged bologna slices	*L. monocytogenes*			1.1	-	Sensory	Lipid peroxidation, shelf-life	
Beef carpaccio (vacuum packaged, polyamide/ polyethylen)	*L. monocytogenes* Scott A (CIP 103575 Serotype 4b) E.coli O157:H7 (CECT 4972)	t_{tot}= n.d.; t=250 µs; f= Hz; 0.175 $J.cm^2$/pulse (at level of quartz table)	8.4	0.8 1.2	-	Color, odor, changes during storage	Shelf-life	Hierro et al., 2012

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	S. enterica serovar Typhimurium [3 $\log_{10}$ cm^{-2}] (CECT 443)			1.0				
Ready-to-eat cured meat product (salchichón)	*L. monocytogenes*	t=250 μs; f= Hz; 0.7 $J.cm^2$/pulse (at level of quartz table)	11.9	1.81	-	-	Sensory	Ganan et al., 2013
	S. typhimurium			1.48				
Ready-to-eat cured meat product (loin)	*L. monocytogenes*			1.61				
	S. typhimurium [4-5 $\log_{10}$ cm^{-2}]			1.73				

[a] t_{tot}, exposure time; t, pulse width; f, frequency.

[b] F, fluence.

[c] L*, lightness; a*, redness; b*, yellowness. Color space values.

[d] Natural contamination.

n.d., not determined.

Table 4 Summary of published data for the microbial decontamination and alteration of food contact surfaces using pulsed light technology.

Food contact material	Artificially inoculated bacterial strain [inoculation range]	Parameter[a]	Light dose (at sample) (F)[b]	Re-duction (log_{10})	Product alteration at the given Parameters			Reference
					Positive	Negative	Neutral	
Stainless steel bead blasted surface)	*L. innocua* FSL C2-008 (isolate from fish palnt) [8 log_{10} ml^{-1}]	1pulse; 1.27 $J.cm^2$/pulse (1.93 cm from lamp surface) Distance= 5.08 cm from lamp	n.d.	2.68	n.d.	n.d.	n.d.	Woodling and Moraru, 2005
Stainless steel (mill finish surface)	*L. innocua*	t=360 µs; F=3 Hz	6	4.08	n.d.	n.d.	n.d.	Woodling and Moraru, 2007
Poly-propylene (PP) film	-	t_{tot}=30 s; t=360 µs; f=3 Hz; 1.27 $J.cm^2$/pulse (1.5 cm below lamp surface) Distance to quartz window=8 cm	-	-	-	-	Mechanical properties	Keklik et al., 2009

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Poly-propylene (PP) film	-	t_{tot}=30 s; t=360 µs; f=3 Hz; 1.27 J.cm^2/pulse (1.5 cm below lamp surface) Distance to quartz window=8 cm	-	-	-	-	Mechanical properties	Keklik et al., 2010
Meat slicing knife (stainless steel, polished)	*L. monocytogenes* LMG 23905 *E. coli* O157:H7 LFMFP 463 [6 $\log_{10}$ cm^{-2}]	t=300 µs; 3 J.cm^2/pulse, 4 lamps, Horizontal distance =6 cm, Vertical distance =10 cm		6.5 6.5				Rajkovic et al., 2010a
Packaging materials/ contact surfaces	*Campylobacter* spp. [3 $\log_{10}$ cm^{-2}] *E. coli* *S. enteritidis* [4 $\log_{10}$ cm^{-2}]	t_{tot}= 5 s; t= 360 µs; f= 3 Hz; 1.27 J.cm^2/pulse Distance to quartz window= 2.5 cm		3.56 4.69 4.6				Haughton et al., 2011
Low density polyethylene (LDPE)	*L. innocua* (FSL C2-008)	t=360 µs; f=3 Hz; 0.67 J.cm^2/pulse	8	7.1	-	-	-	Ringus and Moraru, 2012

High density polyethylene (HPDE)	[9 log_{10}/coupon]	Distance to quartz window=10.16 cm	7.2	-	-	-
Polyethylene terephthalate (PET)/ LDPE/ ultra metalized PET/ LDPE/ Linear low density polyethylene (LLDPE) metallocene (MET)			3.5	-	-	-
LDPE/ paperboard/ LDPE			4.4	-	-	-
Polyethylene (PE)/ paperboard/ PE/ A1 foil/ TIE/ PE (EE)			4.5	-	Hydro-phobicity	-

[a] t_{tot}, exposure time; t, pulse width; f, frequency.

[b] F, fluence.

n.d., not determined.

Table 5 Producers of commercially available pulsed light equipment.

Company name	Publications related to meat, meat products and food contact surfaces
Xenon Corporation (USA, Massachusetts, www.xenoncorp.com)	Woodling and Moraru, 2006; Uesugi and Moraru, 2009; Keklik et al., 2009; Keklik et al., 2010; Wambura and Verghese, 2011; Ringus and Moraru, 2012
SteriBeam Systems GmbH (EU, Germany, www.steribeam.com)	Rajkovic et al., 2010b
Claranor (EU, France, www.claranor.com)	Hierro et al., 2012; Massier et al., 2012
Montena Technology (Europe, Switzerland, www.montena.com)	
Samtech (EU, United Kingdom, www.samtech.co.uk)	

Table 6 Producers of commercially available PL xenon lamps.

Company
Flashlamps Verre & Quartz (EU, France, www.verre-et-quartz.fr)
Hamamatsu (Japan, www.hamamatsu.com)
Heraeus Noblelight (EU, Germany, www.heraeus-noblelight.com)

Table 7 Advantages and limitations of implementing pulsed light in the food industry.

Feature	Advantages	Limitations
Quality improvement	Synergistic effect with other technologies	Surface decontamination technology
	Decontamination of food (packed/unpacked) and contact surfaces	Product (surface condition, opacity, composition) and contamination characteristics influence effectiveness
	Effective against pathogenic and spoilage microorganisms as well as other contaminating agents (photochemical and photophysical)	Packaging has to be compatible
	4 to 6 times more effective than CW UV	Control of post-process parameters critical for shelf-life
	Applicable in HACCP systems	Photoreactivation possible (precautions have to be taken)
	No resistance formation of bacteria expected so far	-
Impact on the treated matrix	Non-thermal surface treatment	Adjustment of processes necessary to avoid product impairment and surface heating
	Minimally processing technique	-
Ecological factor	Safe to apply (industrial safety)	Ozone formation (precautions have to be taken)
	Environmentally friendly (lack of residual compounds, absence of applied chemicals, disinfectants and preservatives, little solid and liquid wastes and no ionizing radiation)	-
	Xenon lamps (broad spectrum): mercury free alternative to CW UV	-
	Low energy input (energy manifold magnified)	-

Convenience	Short processing times (4 to 6 times lower than with CW UV) and therefore high throughput	Geometric configuration and process parameters difficult to standardize
	Easy to integrate into existing processes	Only few suppliers on the market
	Minimum space requirement	-
	Operable in batch or continuous mode	-
	Instant action adjustable to product flow (no warm up of lamps necessary)	-
	Natural cooling of lamps between pulses	-
	Light spectrum adjustable by pulse forming network or filters to the respective situation	-
	High UV intensities during a pulse (up to 30 % UV)	-
Economic factor	Low operation costs make the technology efficient	High investment costs (€ 300 000 – 800 000)
	High efficiency (40-50% conversion of electrical energy into optical)	Technology for high added value products or particular market situations
	Emerging technology	Lamps: life time depends on operating parameters (average life time: 6 to 12 months; costs (few times higher than for CW UV): about 700 € each due to sophisticated and costly design)
	-	Sophisticated and costly driving circuits necessary for long life time and high UV output of lamps (10 to 100 times more expensive than for CW UV)
Acceptance	Good consumer acceptance	-
	FDA approved technology	Technology per se not approved in the EU (novel food regulation)

Figure 1 Schematic layout of a pulsed light device (modified from: Dunn et al., 1995; MacGregor et al., 1998; Anderson et al., 2000; Gómez-López et al., 2007; Keklik et al., 2009; Ortega-Rivas, 2012).

0: Low voltage input (low power, low voltage, and low AC continuous current)

1: Low voltage is converted into high voltage (low power, high voltage, and low DC continuous current)

2: Capacitor is charged (switch 1: on, switch 2: off)

3: Release (switch1: off, switch 2: on) of stored energy (high power, high voltage, high DC continuous current)

4: Light source emits high power pulsed light

5: High power pulsed light hits the target matrix