Recent advances in the application of pulsed light processing for improving food safety and increasing shelf life

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Abstract

Background: New technologies of non-thermal disinfection such as pulsed light (PL) have emerged lately as an alternative to traditional (thermal and chemical) disinfection and preservation methods. PL can be used to decontaminate a wide variety of foods as well as to decontaminate contact surfaces, thus improving safety in foods and extending their shelf life. Moreover, this technology can prevent or reduce some of the detrimental effects of traditional methods on nutrients and bioactive compounds of food products.

Scope and approach: The combination of PL with other techniques such as ultraviolet light (UV), thermosonication (TS), pulsed electric fields (PEF), manothermosonication (MTS), etc., can improve the effectiveness of the decontamination process. Therefore, in this review, some of the most relevant studies evaluating the potential application of PL treatments to decontaminate food samples, and its impact of nutritional and physicochemical quality parameters will be discussed.

Key findings and conclusions: PL treatments are suitable for microbial decontamination in transparent drinks and for surface contaminated foods without complex microstructures. They also can be used for meat, fish and their by-products However, it is still necessary to evaluate the appropriate treatment conditions (number of light flashed, voltage, distance between sample and flash light, spectral range of light flashes and contamination) for each food and microorganism separately to improve the effectiveness and minimize the appearance of negative attributes reducing the quality of product as, in some cases, PL can have a negative impact on the photosensitive compounds and sensory properties of food products.
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Abstract

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Keywords: microbial inactivation; non-thermal technology; sensory properties; nutritive value; pulsed light
1. Introduction

Pulsed light (PL) is a non-thermal innovative technique used for food preservation among other relevant novel technologies such as high-pressure processing, pulsed electric fields and high electrical voltage discharges (Stoica et al., 2013). It is based on the application of short time light pulses with an intense broad spectrum (Barba et al., 2018). These pulses act inactivating the microorganisms at a surface level of food and the packaging material (Elmnasser et al., 2007). The microbial DNA absorbs UV light which leads physio-chemical changes in its structure, thus resulting in damage of genetic information, impaired replication and gene transcription as well as eventual death of the cell (McDonald et al., 2002).

PL includes the employment of inert gas flash lamps to transform short duration as well as high power electric pulses into short duration and high-power pulses of radiation having similar spectrum to that of the sun (200–1100 nm), including infrared (IR), visible light (VL) and ultraviolet (UV) (Kramer and Muranyi, 2014). Moreover, flashes can be produced for several seconds with the help of xenon flash lamp. At an industrial level, PL is a useful tool to decrease microbial counts in foods, as well as food packaging materials. It can be also used to decrease microbial contamination of food contact surface, equipment and media (e.g. air and water) implicated in the production processes (Sun, 2005).

PL treatment employs 1-20 flashes/second with an energy density ranging from 0.01 to 50 J/cm² at the surface and it has potential application in food processes requiring a rapid disinfection. During the last decades, various studies have confirmed the germicidal effect of PL in alfalfa seeds, blueberries, corn meal, carrot, honey, lettuce, milk, fish fillets, spinach, strawberries and food contact surfaces made of stainless steel (Elmnasser et al., 2007; Oms-Oliu et al., 2010). Particularly for food industrial applications, the PL technology has been successfully applied to decontaminate food
packaging materials. Some of the current applications is on caps, cups, bottles, foils and flexible food packages that can be processed in continuous flows at 7,000-90,000 caps and up to 90,000 bottle preforms per hour (CLARANOR, 2018a). Moreover, PL system can be applied to decontaminate beverages in continuous regime before filling stage (bottles and cans, for instance) at pilot scale (XENON, 2016; Pataro et al., 2011).

In this review, some recent studies, conducts over the last years, about the potential of PL and PL together with other technologies for microbial inactivation in both liquid and solid matrices published will be discussed.

2. PL system

Although the first studies about PL in physics dates back to 1960’s, its potential use as potential technology for food decontamination is related to more recent investigations when selected pathogenic and spoilage microorganisms such as Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Salmonella enteritidis, and Staphylococcus aureus in Petri dishes were exposed to PL (MacGregor et al., 1998; Rowan et al., 1999). These experiments indicate that PL effect is dependent of treatment conditions. UV source, number of pulses and selected microorganism are relevant variables explored in these studies to achieve satisfactory reduction levels. One of the first applications of PL technology to decontaminate food was carried out with bovine milk (Smith et al., 2002).

It is worth mentioning that some patents from this period explored the application of PL technology to decontaminate food. An equipment patented in 1985 is one of the oldest records of the specific use of PL treatment to destruct microorganisms (Hiramoto, 1984). In this apparatus, the application is dedicated to decontaminate surfaces. Likewise, the equipment patented by Dunn et al. (1989) achieved from 1 to 3 log10 reduction of Pseudomonas spp. on cottage cheese curds, molds in
white bread, coliform and psychrotrophic bacteria in fresh fish. Moreover, food packaging material was also successfully decontaminated by PL treatment by Dunn’s apparatus.

Further experiments evaluated the PL effect on food microorganisms has gained major attention, particularly PL decontamination in food matrix. For instance, *Serratia marcescens* in bovine milk (Smith et al., 2002), *Saccharomyces cerevisiae* in wheat flour and black pepper (Fine and Gervais, 2004) and *Apsergillus niger* spores in corn meal (Jun et al., 2003) were subjected to PL treatment.

In the period of 2000-2010 the number of studies about the use of PL technology to decontaminate food has grown. Moreover, important advances regarding the use of PL technology was dedicated to achieve industrial level, particularly for surface decontamination. The creation of companies exclusively dedicated to manufacture PL equipment and the insertion of existing companies into this new market occurred in this period facilitated the advances in food industry. Decontamination of food packaging materials is one of the main successful industrial applications of PL technology in food industry (CLARANOR, 2018a; XENON, 2016).

Moreover, PL equipment differs according to manufacturer, but a typical PL system consists of a high-voltage power supply, a storage capacitor, a pulse-forming network which establishes the spectrum properties and pulse shape, gas-discharge flash lamp and a trigger that initiates discharging the electrical energy to the flash lamp (John & Ramaswamy, 2018). Some of the most commonly used equipment are steripulse XL 3000c PL sterilization system (Xenon Corporation, MA, USA), covering a spectral range of emission from 200 nm to 1100 nm (Caminiti et al., 2011), and in some cases an automatic laboratory system (Steribeam Xe-Matic-2L-A, Kehl, Germany) equipped with standard clear 18 cm long UVC transparent quartz Xenon lamps (Maftei et al., 2014). A commercial automatic PL unit (CLARANOR S.A., Avignon,
France) equipped with 8 automatic flash xenon lamps placed all around the sample holder was also used to process whole tomatoes (Aguiló-Aguayo et al., 2013). For continuous treatment of liquid samples, a dynamic flow through pilot unit (Maria PUD system, Claranor, Monosque, France) was used (Artiguez et al., 2011). The Figures 1-2 show some of the different PL equipment models. This scenario illustrates the important advances made in only two decades of constant evolution and application of PL technology to achieve industrial level, which also include the food processing area.

3. Factors determining the effectiveness of the PL process

Several studies published over the last years displayed that the effectiveness of PL treatments on microorganism inactivation depends on several factors such as the number of flashes, the voltage applied, the distance between sample and lamps, the spectral range of light flashes, the time between contamination and treatment, the type of sample treated and the type and amount of microbial contamination (Heinrich et al., 2016).

In this regard, Koch et al. (2019) explore the effect of PL fluence (0.52-19.11 J/cm²), distance between the lamp and sample (8.3-13.4 cm), and treatment time (1-30 s). In this study pork skin was inoculated with pathogenic bacteria (Salmonella Typhimurium and Yersinia enterocolitica) and subjected to PL treatment. The authors observe that only by combining the lowest distance, highest PL fluence and the longest treatment time (8.3 cm, 19.11 J/cm², and 30 s) induced significant reduction on Salmonella Typhimurium (2.97 log CFU/cm²) and Yersinia enterocolitica (4.19 log CFU/cm²) inoculated on pork skin. Similar results were reported by Cheigh et al. (2013) who studied the inactivation of L. monocytogenes on seafood such as salmon, shrimp fillets and flatfish using intense PL at different light doses (0.11-1.75 mJ/cm² per pulse). They noticed that the application of PL at 1.75 mJ/cm² per pulse and 6900 pulses achieved reductions of 1.9, 2.1
and 2.4 log CFU/cm² of *L. monocytogenes* previously inoculated onto flatfish fillets, salmon and shrimp, respectively. Moreover, the fluence of wavelength below 300 nm seems play a central role in the inactivation effect of PL technology. This outcome was observed by Levy et al. (2012) who reported a drastic reduction in the inactivation effect on spores of *Bacillus subtilis* and *Aspergillus niger* exposed to fluences in the range 300-1100 nm. Conversely, exposing both microorganisms to fluences that included wavelengths below 300 nm produced 6 log reduction.

The relationship between contamination time and PL treatment was also studied. In this regard, Rajkovic *et al.* (2010) evaluated the applicability of PL treatments (3 J/cm² with an input voltage of 3000 V) in the decontamination of *L. monocytogenes* and *E. coli* O157:H7 found on the surface of a meat-slicing knife at different times between the contamination and the application PL treatment. The best result (6.5 log CFU/side of knife, complete microbial inactivation) was reached when the knife surface was treated by PL treatments quickly (after knife’s contact with meat products of ≤60 s). The average reductions were 5.82, 5.01 and 2.58 log₁₀ N₀/N after 4 min, 15 min and 1 h, respectively (Rajkovic *et al.*, 2010). More recently, Rajkovic *et al.* (2017) assessed the efficacy of pulsed UV light treatments at 3 J/cm² (1 pulse) or 15 J/cm² (5 pulses) after 1 or 30 min and every pulse was manually started at a rate of a pulse/2 s to reduce *L. monocytogenes*, *E. coli* 0157:H7, *S. typhimurium* and *S. aureus* on the surface of dry fermented salami inoculated with 6.3 log CFU/g. The authors found a significant effect of PL treatment time, observing the best results after 1 min of applying PL (2.18-2.42 log CFU/g reduction), while after 30 min the reduction varied from 1.14 to 1.46 log CFU/g.

Other important factors influencing the process effectively are: i) the type of contamination, ii) the initial concentration of microorganism and iii) the matrix composition. In this line, some authors (Rajkovic *et al.*, 2010; Aguero *et al.*, 2016) found that the Gram-negative bacteria displayed higher
resistance at PL treatments than fungal spores and Gram-positive bacteria. In addition, Pataro et al. (2011) assessed the influence of PL application on apple and orange juice inoculated with the Gram-positive (L. innocua 11288) and Gram-negative (E. coli DH5-α) bacteria. Among the studied bacteria, E. coli cells presented higher susceptibility to application of PL compared to L. innocua cells in both juices. After applying PL treatment at 4 J/cm², microbial decreases were of 4.00 and 2.90 log-cycles for E. coli and 2.98 and 0.93 log-cycles for L. innocua, in apple and orange juices, respectively. In the same way, Rajkovic (2010) demonstrated that ILP treatment was more effective for the inactivation of E. coli O157:H7 on the surface of the slicing knife than for L. monocytogenes. The difference in the results achieved between both pathogens, was <0.1 log_{10} N_0/N for L. monocytogenes (Gram-positive) and 4.62 log_{10} N_0/N for E. coli O157:H7 (Gram-negative). The decontamination efficacy also decreased at high initial contamination levels; this fact could be due to light attenuation when high population densities are found, thus preventing the pulsed light incidence on microorganism placed in the lower layers (Maftei et al., 2014).

The surface characteristics and nutrient composition of sample were also studied as they could be important factors to be considered in the decontamination efficacy after applying PL treatments. In this regard, Koch et al. (2019) inoculated pork loin with Salmonella Typhimurium and Yersinia enterocolitica and applied PL treatment (Pl fluence of 0.52-19.11 J/cm², 8.3-13.4 cm between the lamp and sample, and 1-30 s of treatment time). The authors also did not obtain markable differences on pork loin (reductions in the range 0.4-1.6 and 0.4-1.7 log CFU/cm² for S. Typhimurium and Y. enterocolitica, respectively). According to authors, this effect could be explained by the surface roughness, porosity and hydrophobicity, which may have caused a shading effect and eventual protection against PL exposure.
Palgan et al. (2011a) evaluated the feasibility of using high intensity light pulses (HILP) (frequency of 3 Hz/0-8 s) to reduce *E. coli* and *L. innocua* in matrices with differing transparencies (orange juice, apple juice and milk), observing that microbial inactivation dropped with reducing transparency of the medium. For apple juice (the most transparent media), the reduction of *E. coli* and *L. innocua* after 8 s was of 4.7 and 1.93 log$_{10}$ CFU/mL, respectively, whereas for milk (the opaquest medium) was of 1.06 and 0.84 log$_{10}$ CFU/mL, respectively. A similar trend was reported by Aguirre et al. (2014) when they studied the decontamination of *Listeria innocua* in culture media with different colorations, observing higher microbial resistance for more coloured media. In general, PL process presented a higher effectiveness for inactivating bacterial cells in clear media (Pollock et al., 2017; Aguirre et al., 2014). Take into account this fact, it is of great importance to optimize the PL process prior to improve its efficacy, thus avoiding product impairment and surface heating.

### 4. Advantages/disadvantages of PL process

Some of the most important advantages of PL compared to other type of treatments are discussed below. For instance, PL is an effective tool against a great variety of pathogenic and contaminating agents due to the inactivation mechanism of PL process, which is ascribed to the UV component of the broad spectrum of the flash and impacts of the high peak power (Oms-Oliu et al., 2010; Rajkovic et al., 2010). Moreover, PL allows the decontamination of food packed and unpacked and contact surfaces and does not generate residual compounds because PL treatments use xenon flash lamps, which are nontoxic due to mercury-free properties, and do not use chemicals disinfectants and/or synthetic preservatives (Ortega-Rivas, 2012; Ferrario, and Guerrero, 2018). In addition, this technology presents low operation cost for each treatment, good consumer’s acceptance, as they prefer fresh and healthy foods and minimally processed
with high enhanced of organoleptic quality (Palgan, 2011b) as well as the possibility to operate in continuous or batch mode. Finally, PL involves the use of short processing times and high throughput since PL light is a more efficient and fast method of inactivating microorganisms (due to the instantaneous delivery of more intense energy) than continuous UV light, for the same total energy supplied (Bohrerova et al. 2008). It also has an easy integration with other processes such as temperature (Artíguez and Marañon, 2015), ultrasound, (Ferrario et al., 2015), combination with other disinfectants (Kramer et al., 2017) or other technologies such as thermosonication (Muñoz et al., 2011) and the combination of based-coatings with PL (Donsi et al., 2015).

In addition to these facts, PL technology has been associated with improved decontamination effect in comparison to other approaches commonly applied in food industry, such as the use of chlorine, organic acids and hydrogen peroxide (Table 1). According to the experiment carried out by Huang and Chen (2018), the PL treatment achieved higher levels of microbial reduction against inoculated Salmonella enterica than chlorine (1.5-2.8 vs 0.63-1.62 log$_{10}$ CFU/mL, respectively). In a similar way, organic acids are interesting alternatives to chlorine to disinfect food. In this context, Salinas-Roca et al. (2016) evaluated the impact of PL, malic acid, and its binary combination to disinfect mango slices inoculated with Listeria innocua. PL and malic acid induce reductions of 2.5 and 2.9 log$_{10}$ CFU/mL, respectively. Interestingly, the binary combination of PL with malic acid induce a 4.5 log reduction. However, combining PL, malic acid and alginate produced reductions in the range 3.0-4.0. The authors argued that combing PL with malic acid is an effective treatment to reduce Listeria innocua contamination in mango slices.

Finally, the disinfecting effect of PL technology was compared to chlorine (10 and 100 ppm), hydrogen peroxide (H$_2$O$_2$, 300 ppm), thymol (0.2 mg/mL), and citric acid (1 mg/mL) on green onion
leaves and stem inoculated with *Escherichia coli* (Xu et al., 2013). Both PL and chlorine treatments induced a reduction in the range of 0.9-0.12 log$_{10}$ CFU/mL in the leaves and stem. Differently, all the samples treated by H$_2$O$_2$, thymol, and citric acid achieve less than 1 log$_{10}$ CFU/mL. These results support the role of PL technology in disinfection of food due to its similar and even higher disinfection capacities against common pathogenic bacteria in comparison to other compounds with antimicrobial activity.

However, the PL technology also presents some important drawbacks. This technology is a surface decontamination technology, thus opacity and composition of matrix influence in a great manner the process effectiveness. Moreover, it also presents a high investment cost (Palmieri and Cacace, 2005; Heinrich *et al.*, 2016), short life time temporary lamps, changes in pH and color at high fluence and overheating of samples (Heinrich *et al.*, 2016), possible formation of ozone (Koch *et al.*, 2019). In order to reduce the heating of the samples some authors have introduced the water-assisted pulsed light (WPL) technology (Huang and Chen, 2014; Huang and Chen, 2015). It is important to highlight that, in some cases, PL treatment has a negative effect on some compounds that can lead to changes in sensory characteristics of food products, for example, promoting the degradation of some natural pigments, browning, as well as bad flavor and smell. For instance, Ignat *et al.* (2014) indicated the PL treated apple slices (157.5 kJ/m$^2$) were associated with anomalous flavor, reduced apple flavor, and more intense brown color in comparison to untreated samples.

5. Food processing applications

Several studies reported the application of PL to reduce microbial counts from different commodities of vegetable and animal origin. Some of the most important findings are discussed below and in Tables 2-5.
5.1. Liquid foods

The use of PL as a non-thermal technology for conservation purposes is a new tendency in liquid food processing research. Some of the most recent studies about the use of this technique in beverages are shown in Table 2. Although the application of PL in beverages has been reported to significantly reduce the initial number of microorganisms, this reduction is variable and depends on the transparency of the beverages. For instance, as can be seen in Table 2, transparent beverages such as water (2.96 to 5.0 log$_{10}$ N$_{0}$/N) and apple juice (1.0 and 4.9 log$_{10}$ N$_{0}$/N) presented lower microbial counts after PL treatment than other opaque drinks such as grape, strawberry, and orange juices or milk, independently of the targeted microorganism. Artíguez and Marañón (2015) evaluated the impact of a PL system with continuous flow on the inactivation of *L. innocua* in whey, skimmed whey, diluted whey and distilled water. The authors observed a higher reduction in *L. innocua* when the number of pulses and total fluence were increased. For a similar total fluence, treatments consisting of a greater pulse number but with lower voltage were more effective. In water, for total fluencies of 11 J/cm$^2$, a decrease of 5 log CFU/mL in the number of initial pathogens was reached. Moreover, the self-life was increased by 7 days at 4 ºC compared with untreated group. The microbial inactivation by PL treatment differed according to the amount of light transmitted through the fluid, which explains the highest inactivation of *L. innocua* cells in diluted whey samples.

The efficiency of PL treatment on the inactivation of different microorganism has been studied by several authors. *Salmonella enteritidis*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Listeria innocua* are the most commonly evaluated microorganism. In general, Gram-negative bacteria (e.g. *E. coli* and *Salmonella enteritidis*) presented higher susceptibility to PL treatment than Gram-positive bacteria (Table 2). For instance, Pataro *et al.* (2011) investigated the impact of PL treatment, in the range of 1.8 to 5.5 J/cm$^2$, on apple and orange juice inoculated with the Gram-negative (*E. coli* DH5-
α) and Gram-positive (L. innocua 11288) bacteria. A laboratory scale continuous flow PL system with xenon flash-lamp emitting high intensity in the range of 100-1100 nm was used (frequency of 3Hz/360 µs). The most important inactivation effect was observed when the highest amount of energy was applied to the juice stream. E. coli presented higher susceptibility compared to L. innocua cells in both juices (reductions in apple and orange juices were of 4.00 and 2.90 log-cycles for E. coli and 2.98 and 0.93 log-cycles for L. innocua, respectively) when subjected to PL treatment at 4 J/cm².

Maftei et al. (2014) assessed the effectiveness of PL applications on the inactivation of Penicillium expansum inoculated in apple juice. Several critical processing attributes including number of pulses (5, 10, 15, 20, 30 and 40 flashes), depth of the juice layer (6, 8 and 10 mm), fluence (0.2 and 0.4 J/cm² per pulse) and inoculation level (2.3 x 10⁴ CFU/mL and 3x 10⁵ CFU/mL) were assessed. The lethality caused by PL treatment on P. expansum was reported to be dependent on the depth of the juice layer, fluence and mold contamination level. The inactivation of P. expansum improved using high intense PL applications, thinner juice layers and lower contamination levels. Microbial inactivation only slightly increased, with log reductions varying between 3.76 and 1.27 log CFU/mL for 32 J/cm² (40 flashes per side) and energy fluences of 4 J/cm² (5 flashes per side), respectively. The study also indicated a reduced protective effect against enzymatic browning in apple juice using 0.2 and 0.4 J/cm². Moreover, the study also indicated that increasing PL treatment intensity amplified color changes.

5.2. Meat, fish and derived products

Table 3 shows the most recent works evaluating PL decontamination of fish, meat and derived products. In general, around 2.0 log CFU/mL reduction of the initial count of microorganisms was achieved after PL treatments, independently of the target microorganism and the matrix. Listeria monocytogenes and Salmonella enterica were the main studied microorganisms in fish after applying
PL treatments. Overall, similar values of *Listeria monocytogenes* decontamination were found in both meat (0.9-2.24 log CFU/mL) and fish/seafood (0.7-2.4 log CFU/mL), whereas for *Salmonella enterica* the values were ≈ 2.0 log CFU/mL (Table 3). However, PL promoted some changes in the sensory characteristics of these products. The study carried out by Koch et al. (2019) with pork skin and loin indicated that the lowest level of PL fluence (0.52 log CFU/cm²) was the only sensory accepted samples in comparison to more intense treatments (4.96 and 12.81 log CFU/cm²). Moreover, both pork skin and loin treated with 0.52 log CFU/cm² received the same scores of untreated samples for rancid odor. Interestingly, the authors did not associate this effect to lipid peroxidation due to low oxidation level induced by PL treatments on pork skin (<0.12 µg/g). Moreover, Hierro et al. (2012) assessed the feasibility of PL treatments (0.7, 2.1, 4.2, 8.4 and 11.9 J/cm²) to enhance the safety of beef and tuna carpaccio. The results indicated a significant reduction in the initial microbial count (≈1 log CFU/cm²) of the samples inoculated with *Vibrio parahaemolyticus*, *E. coli*, *L. monocytogenes* and *S. typhimurium* after applying PL treatments (8.4 and 11.9 J/cm²), and obtained a significant improvement in the food safety of these products. However, PL treatments at high doses (8.4 and 11.9 J/cm²) resulted in color variation (lower redness and yellowness than untreated sample) and a negative impact on sensorial quality (lower score for color and odor in comparison to untreated samples).

The effectiveness of PL treatments in surfaces in contact with food was also studied. Rajkovic et al. (2010) assessed the applicability of PL treatment (3 J/cm² with an input voltage of 3000 V) in the inactivation of *L. monocytogenes* and *E. coli* O157:H7 present on the surface of meat slicing knife (meat extract, pork meat and fermented sausage). The inactivation effectiveness differed according to the type of meat product in touch with the treated surface and on the remaining time between the contamination and the application of PL treatment. The best result (6.5 log CFU/side of...
knife, complete microbial inactivation) was found when the knife surface was treated with PL treatments quickly (after the knife contacted with meat products ≤60 s) and when the knife surface was in touch with products with lower protein and fat amount (Rajkovic et al., 2010).

Regarding the impact on sensory properties, the study carried out by Koch et al. (2019) indicate associated the most intense PL treatments (4.96 and 12.81 J/cm²) with unpleasant, ozoneous, pungent, ammoniacal, and off-odor perception in pork skin and loin. Conversely, the samples subjected to 0.52 J/cm² were perceived as “less porky” and “slightly chemical”, which support the indication that excessive PL treatment reduced the quality of food. Additionally, significant changes on color caused by PL treatment were also indicated particularly for redness.

5.3. Fruits and Vegetables

Over the last years, many studies have been published dealing to the microbial decontamination after applying PL treatments to fruits (blueberries, melon apple, raspberry and strawberry) and vegetables (tomatoes, beans, salad, onions and avocado) (Table 4). In most of these studies, the decontamination was assessed on samples of fresh products artificially inoculated or in native microflora. Although complete microbial inactivation was not achieved, reductions of ≈1-6 log CFU/mL were achieved in vegetables and fruits without compromising of the nutritional value of products.

For instance, Aguiló-Aguayo et al. (2013) assessed the impact of PL, at fluencies of 2.68 and 5.36 J/cm², on the decontamination (natural and inoculated microorganisms) of red-ripe tomatoes. The application of PL at fluence of 4 J/cm² decreased tomato total microflora by 1 log₁₀ CFU/mL. On the other hand, treatment at fluencies of 2.2 J/cm² and 4 J/cm² caused a decrease of 2.3 log₁₀ CFU/mL of S. cerevisiae inoculated. In addition, Aguiló-Aguayo et al. (2014) assessed the influence of PL (3.6, 6.0 and 14 J/cm²) on avocado microbial content,
observing reductions in aerobic mesophilic microorganisms (1.20 log CFU/g) after PL treatment of 14 J/cm². The growth of molds and yeasts was also delayed during 3 days and the shelf-life was increased to 15 days. In addition, Ignat et al. (2014) assessed the impact of different PL fluencies (17.5, 52.5, 105.0 and 157.5 kJ/m²) against the growth of L. brevis and L. monocytogenes inoculated on fresh sliced apples during storage at 6 ºC. Independently of the fluence used, the application of PL treatments significantly reduced the total viable counts (1 log CFU/g) and inoculated bacteria (3 log CFU/g) of the samples. Differently, Xu et al. (2016) studied the impact of PL application on the inactivation of Salmonella spp. and E. coli O157:H7 on fresh raspberries stored for 10 days at 4 ºC. In comparison with untreated samples, the fresh raspberries treated with PL during 5-30 s had lower pathogen survival. However, the most efficient treatment was obtained by using PL for 30 s (fluence of 28.2 J/cm²), which resulted in reductions of 4.5 and 3.9 log₁₀ CFU/g in Salmonella spp. and E. coli O157:H7 populations, respectively.

In addition to safety related analysis, the physicochemical properties (weight, texture and color, for instance) and nutritional properties of the samples after applying PL treatments were also studied. For example, Aguiló-Aguayo et al. (2013) assessed the influence of PL in color, texture, weight and ascorbic acid of tomato stored at 20 ºC for 15 days. Regarding nutritional quality, ascorbic acid amounts did not change during storage, while total lycopene, α-carotene and β-carotene amounts and percentage of lycopene isomerization in both tomato tissues and chloroplast were higher in tomatoes treated with high PL dose (30 J/cm²). In another study, Aguiló-Aguayo et al. (2014) evaluated the effect of PL application (3.6, 6.0 and 14 J/cm²) on lipid oxidation chlorophyll stability and color of avocado after 15 d at 4 ºC. These authors observed that after 15 days, the color was maintained, and the chlorophyll content was increased. The authors argued that enzymatic browning could explain this effect, particularly due to cell
disruption that facilitated the enzyme-substrate contact while cutting the fruits but the PL treatment did not prevent enzymatic browning. Moreover, the lipid fraction exhibited a minimal peroxide formation and the rancidity processes were not increased.

In addition to the positive effect of PL processing in reducing the microbial counts present on fruits and vegetables, some studies reported the negative effect of this technology at high and long pulses on the food appearance and nutritional quality of the foods. In this line, Aguiló-Aguayo et al. (2013) noticed that although the microbial counts present in tomatoes after 3 days of storage were reduced, and the appearance (wrinkled skin and softening) and weight loss were also detected in PL treated samples. In addition, Ignat et al. (2014) investigated the influence of PL on the appearance of sliced fresh apples during storage at 6 °C. The use of PL treatment at 17.5 kJ/m² did not induce any protective effect against enzymatic browning in comparison to untreated samples. Moreover, the use of 157.5 kJ/m² favored more intense color changes than untreated and PL treated samples at 17.5 kJ/m². The main reason for this particular effect was related to disruption of apple cell membranes. The authors also observed a high microbial reduction at high fluencies verifying that the high fluencies negatively affected to color and sensorial attributes of apple slices. In a similar way, a study with fresh-cut mangoes indicated that PL treatment (4 pulses at 2.80 J/cm²) indicated non-significant changes on luminosity but yellow color (b* value) was better preserved during 7 days of storage at 6 °C for up to 7 days (Lopes et al., 2017).

The study carried out by Charles et al. (2013) evaluated the impact of PL (four pulse with a total of 8 J/cm²) on selected endogenous enzymes (polyphenoloxidase and phenylalanine ammonia lyase) fresh cut fresh-cut mangoes. The results of PL treatment indicated that both Polyphenoloxidase and Phenylalanine ammonia lyase activity were improved during storage of mangoes cuts but a non-significant effect on color was observed between untreated and PL-treated
samples. Likewise, PL treatment (4, 6, and 8 J/cm$^2$) did not induce important changes in pectin methylesterase and polygalacturonase activity of fresh-cut tomatoes. This outcome was obtained during storage at 5 °C for 20 days (Valdivia-Nájar et al., 2018). Finally, Xu et al. (2016) assessed the influence of PL applications on fresh raspberries stored for 10 days at 4 °C, observing that the PL treatment negatively affected to the color and texture of raspberries. However, no significant changes were observed in total phenolic compounds (TPC) and the total antioxidant capacity (TAC) levels of the treated samples compared to the untreated ones. The initial population of total bacteria, total yeast and mold in raspberries decreased with all PL treatments.

5.4. Salad

Recently, several studies have evaluated the application of washing cycles with PL-treated water for microbial disinfection of salad samples (Table 4). Manzocco et al. (2015) studied the effectiveness of using recycled water from lamb’s lettuce washing after applying PL treatments (pulse light dose ranges from 0 to 17.5 KJ/m$^2$). The authors observed a complete inactivation of most of the autochthonous microflora and a significant reduction (5-6 log CFU/g) in inoculated microorganisms (E. coli, L. monocytogenes and S. enterica) using PL doses of 11 KJ/m$^2$.

Moreover, the influence of increasing the washing cycles in the decontamination process and hygienic quality of washed lamb’s lettuce was also investigated. Increasing wash cycles up to 5 did not decrease the effectiveness of decontamination (reduction of ≈4 log CFU/g in native microflora). Moreover, a decrease of 1 log CFU/g in native microflora was reached in the washed salad. Kramer et al. (2017) compared the application of PL alone or in combination with the traditional disinfectants (chlorine and chlorine dioxide) on the reduction of microbial counts (L. innocua) from bean sprouts and endive salad during a simulated wash process for up to 60 s with PL dose of 320 mJ/cm$^2$. The best results were obtained using PL wash, where the microbial
counts were reduced by up to 2.5 log in endive salad and 0.45 log CFU/g in beans sprouts, demonstrating the efficiency of PL treatments in comparison to other traditional disinfectants (Table 4). Therefore, the application of PL is a good alternative to inactivate suspended microorganisms in the washed water. In addition, PL is an economical alternative method, which can be used to reduce the generation of wastewater without losing the efficiency of the disinfection process.

6. PL together with other technologies

In order to enhance the efficiency of the PL applications, some authors proposed the combination of PL with subsequent storage at low temperatures. For instance, Ferrario et al. (2013) assessed the inactivation kinetics of E. coli ATCC 35218, Saccharomyces cerevisiae KE162, Salmonella enteritidis MA44 and L. innocua ATCC 33090 inoculated in apple, strawberry juices, melon and orange using HIPL. The samples were subjected to irradiation for 2-60 s as pre-treatment, corresponding to fluencies between 2.4 and 71.6 J/cm². After 60 s at 71.6 J/cm², microbial reductions in the range 0.3-6.9 log-cycle were observed. These authors showed the potential of using PL processing, for inactivation of some microorganism in different types of fruit juices at low temperature (<20 °C).

In a further study, Ferrario et al. (2015b) evaluated the impact of PL treatments on the inactivation of native flora and inoculated microorganisms (S. cerevisiae E. coli, S. enteritidis, L. innocua) in strawberry juices, orange and apple inoculated with these microorganisms. The application of PL treatments at 71.6 J/cm² resulted in 0.3–2.6 and 0.1–0.7 log reductions in inoculated microorganisms and in native flora, respectively. The turbidity of the samples and the size of the particles had a negative effect on PL treatment efficiency. These authors also reported
that the application of PL was ineffective in reducing the native microorganisms of strawberry
juices, orange and apple stored for 10 days under refrigerated conditions.

Table 5 summarizes several studies recently published that display the effect of PL
treatments together with other technologies. Moreover, in these studies, apart from obtaining a
significant reduction in microbial contamination, minimally processed foods with improved
nutritional and sensorial profiles. Ultrasound (Ferrario et al., 2015, 2016 and 2017),
thermosonication (Muñoz et al., 2011 and 2012), disinfectant products (Xu et al., 2013),
advanced oxidation processes using hydrogen peroxide (Huang et al., 2015; Huang y Chen
2015), combination with water (Xu et al., 2013; Huang y Chen 2014 and 2015) and edible
coating (Donsi et al., 2015) are the most common and reliable strategies to be used combined
with PL treatments.

Some authors have proposed several alternatives to PL in order to avoid sample heating
and browning effect when this technology is used for sanitization of fresh vegetables and fruits.
For instance, water-assisted pulse light (WPL) treatment has been suggested as a useful tool to
overcome these limitations. In this regard, Huang and Chen (2014) compared the inactivation of
Salmonella spp. And E. coli O157:H7 in blueberries using dry PL and WPL at different times (5-60
s). These authors observed an effective inactivation of both pathogens after all treatments. However,
the aspect of blueberries was negatively influenced after applying dry PL treatments. Moreover, in
another study, the application of WPL treatments was found to be more effective on inactivating
both pathogens than chlorine washing. After WPL treatment for 60 s, the populations of E. coli
O157:H7 inoculated on skin and calyx of blueberries decreased by >5.8 and 3.0 log CFU/g,
respectively. A similar trend was found for Salmonella spp., with >5.9 log and 3.6 CFU/g reduction
on blueberry skin and calyx, respectively.
On the other hand, the effect of WPL application alone (5-60s) or together with 1% H\textsubscript{2}O\textsubscript{2} or 100 ppm sodium dodecyl sulfate (SDS) on the decrease of murine norovirus (MNV-1), *Salmonella* and *E. coli* O157:H7 in raspberries and strawberries was investigated (Huang and Chen, 2015). *E. coli* reduction in strawberries and raspberries differed according to WPL time, observing reduction values of 1.4-2.4 log units and 1.6-4.5 log units, respectively. The inactivation of *Salmonella* spp. and *E. coli* O157:H7 was higher after using the WPL+H\textsubscript{2}O\textsubscript{2} combination for 60s in comparison to other treatments (e.g. chlorine, SDS, H\textsubscript{2}O\textsubscript{2}, WPL and the combination of WPL+H\textsubscript{2}O\textsubscript{2} and WPL+SDS). *E. coli* O157:H7 cell number reduction on raspberries and strawberries was of 5.3 and 3.3 log CFU/g, respectively, whereas for *Salmonella* was 4.9 and 2.8 log CFU/g, respectively. Regarding MNV-1, the application of WPL and H\textsubscript{2}O\textsubscript{2} did not improve the treatment effectiveness for raspberries, while it was slightly improved in strawberries (WPL: 1.8 log CFU/g and WPL+H\textsubscript{2}O\textsubscript{2}: 2.2 log CFU/g). The potential of using WPL alone or in combination with H\textsubscript{2}O\textsubscript{2} was demonstrated as a good alternative for decontamination of fresh and frozen berries.

More recently, Avalos et al. (2016) assessed the influence of PL application with a quality-stabilizing dip (0.5% w/v CaCl\textsubscript{2} and 1% w/v N-acetylcysteine) on the microbial count reduction, the quality and antioxidant features of fresh-cut apples during 15 days storage at 5 °C. A significant reduction of the naturally-occurring microbiota without compromising the quality of apples was achieved. The application of quality-stabilizing pre-treatments inhibited the browning phenomena and oxidation on the cut-tissue surface. Oxidation and browning in PL-treated samples were not promoted. Compared to untreated samples, vitamin C and phenolic compounds were higher in PL-treated fresh-cut apples. Moreover, the quality and antioxidant properties of the samples were better maintained at the doses of 8 and 16 J/cm\textsuperscript{2}.
The combination of PL processing with edible coatings (e.g. chitosan, pectin, alginate and
gellan) is another interesting strategy that has been taken into account and could be employed for
improving the safety of vegetables and fruits. Recently, Chen et al. (2017) evaluated the use of
this technique to increase the shelf-life of fresh-cut cantaloupes. These authors combined
alginate and PL (fluence of 0.9 J/cm²) and observed an increase on shelf-life of fresh-cut
cantaloupes up to 28 days. After 28 days, the total viable counts and yeast mold counts were
reduced ≈4 log CFU/g.

Donsi et al. (2015) investigated the combination of high pressure processing (HPP) and PL
with a modified chitosan based-coating containing a nanoemulsion of mandarin essential oil on
green beans preservation. Coated and uncoated green bean samples (2.0 × 10⁻² to 2.2 × 10⁻² kg,
respectively) inoculated with *L. innocua*, were exposed to PL treatments at 3 × 10⁴ and 1.2 × 10⁵
J/m² energy dose, respectively. No synergistic or additive antimicrobial influence was observed
against *L. innocua* in PL-treated and bioactive coated samples during storage. Moreover, no
effect was observed in the firmness or coating integrity of the samples treated with the
combination of PL processing and bioactive coating during storage.

The use of PL together with other technologies increased the microbial inactivation and
shelf-life of food products. For example, Muñoz et al. (2011) observed that PL together with
thermosonication was more effective to reduce the initial concentration of *E. coli* in orange juice
than PL alone. In this regard, reductions of 3.93 log CFU/g were obtained after the application of
PL+thermosonication, whereas reductions of 2.92 log CFU/g were found using PL alone. After
the application of US+PL, Ferrario et al. (2015) observed an increased reduction of
*Alicyclobacillus terrestris* and *Sacharomyces cerevisiae* counts in apple juice. The application of
PL alone showed a reduction of 4.4 log and 3.0 and CFU/g for *Sacharomyces cerevisiae* and

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Alicyclobacillus terrestris, respectively, whereas the application of US+PL presented a reduction of 5.9 and 6.4 log CFU/g for Alicyclobacillus terrestris and Sacharomyces cerevisiae, respectively.

7. Industrial relevance of PL technology in food sector

The application of PL technology in the food industry has been gaining more attention and potential applications, especially in the last 10 years. The use of PL technology to decontaminate food packaging material is the most concrete application, as indicated in previous sections. The safety levels achieved in this sector of food industry are relevant, support the continuous research in the area and also expand to new applications. The small size and design, in order to be implemented on existing processing lines, are also important characteristics available for packaging materials.

However, the use of PL technology to decontaminate food still require more efforts to achieve industrial scale of direct food decontamination. At the current level, few pilot scale studies have been carried out and revealed important considerations. The decontamination of sesame seeds carried out by Hwang et al. (2017) achieved 1.46 log reduction operating at 44.46 J/cm² for 120 s in a pilot scale semi-continuous system (up to 3 kg recirculating in the system). The shadow effect, which can prevent the exposure of microorganisms to PL irradiation and reduce treatment efficiency, remains as the main concern. A possible alternative to increase energy exposure during PL treatment is enhancing the area exposed to PL. A promising outcome was reported by Krishnamurthy et al. (2007) who achieved total inactivation of Staphylococcus aureus in milk with a continuous system operating at 20 ml/min. This particular apparatus was equipped with a V-groove reflector, which reflected the energy back to the quartz tube where milk was treated.
An important consideration is the development of efficient PL systems for heat liable food and packaging materials. Increasing temperature may cause changes and potential quality decrease. For instance, temperature increase around 20 °C may occur in most intense PL treatments and achieve higher decontamination levels, by combining short distance between lamp and sample and high PL fluence, as indicated by Koch et al. (2019). Either a cooling system or a more efficiency PL fluence approach are necessary to reduce temperature increase when considering scaling up.

The current information disclosed by companies in the area of food package decontamination indicate that operational cost is reduced, considering the same amount of material. For instance, in order to decontaminate 10,000 cups of 28 mm, the total operational cost is estimated in 42 €, whereas the estimated cost to carry out the same decontamination with peracetic acid is around 266 €. Reduced water consumption, particularly for decontamination step, can also be achieved (CLARANOR, 2018b). However, further studies and thorough evaluation of costs associated with starting, operating and maintaining the processing line, which take into account the type of food, microorganism, reduction level, amount of processed food and other variables presented in this review are still necessary.

8. Conclusion

PL treatment is a successful, fast and environmentally friendly decontamination technology with many potential applications in the food industry, especially as a promising non-thermal technology to be used for food safety purposes. It has been found that PL treatments are suitable for microbial decontamination in transparent drinks and for surface contaminated foods that not presenting complex microstructures.
High-power PL increased the shelf life of meat, fish and derivate products as well as on food contact surfaces. However, for other types of food it is still necessary to evaluate the appropriate treatment conditions (number of flashes, voltage, distance between sample and flash light, spectral range of light flashes and contamination) for each food and microorganism separately to improve the effectiveness and minimize the appearance of negative attributes that reduce the quality of product. In this sense, more studies about the effect of PL on the microbial inactivation and food quality characteristics are necessary at laboratory and pilot scale. The process economic evaluation also deserves more attention, few information is available for both researchers and professionals working on this promising food industry area.

To avoid these problems, it is necessary to optimize the treatment conditions and take into account that the effectiveness of the treatments depends on the time among contamination, PL treatment parameters and food matrix. Currently, to avoid these existent limitations some authors have complemented the PL treatments with other techniques, which can keep the food conservation with minimal impact on the food quality. Finally, it is necessary to carry out studies on a large scale in order to introduce this disinfection technique at industrial level.

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Figure captions:

Fig. 1. Schematic diagram of a pulsed light chamber

Fig. 2. Schematic diagram of a continuous flow pulsed light system
| Food product                        | Decontamination method | Microorganism treated | Reduction (log$_{10}$ CFU/mL) | Reference                  |
|------------------------------------|------------------------|-----------------------|------------------------------|---------------------------|
| Lettuce shreds                     | Wet PL                 | *Salmonella enterica* | 1.5-2.8                      | Huang and Chen (2018)     |
|                                    | Chlorine               |                       | 0.63-1.62                    |                           |
| Mango slices                       | PL                     | *Listeria innocua*    | 2.5                          | Salinas-Roca et al. (2016)|
|                                    | Malic acid (2%)        |                       | 2.9                          |                           |
|                                    | Malic acid (2%) + PL   |                       | 4.5                          |                           |
| Green onion leaves (L) and stem (S)| PL (30 and 60 s)       | *Escherichia coli*    | 1.2 (L) and 0.9 (S)          | Xu et al. (2013)          |
|                                    | Chlorine (10 and 100 ppm) |                       | 0.9-1.2 (L and S)           |                           |
|                                    | H$_2$O$_2$ (300 ppm)   |                       | 0.4 (L) and 0.6 (S)         |                           |
|                                    | Thymol (0.2 mg/mL)     |                       | <0.5 (L and S)              |                           |
|                                    | Citric acid (1 mg/mL)  |                       | <0.5 (L and S)              |                           |
Table 2. Microbial reduction levels for liquid foods after innovative non-thermal processing

| Food product                  | Microorganism treated | Operation conditions                                                                 | Reduction ($\log_{10}$ CFU/mL) | Reference                    |
|-------------------------------|-----------------------|--------------------------------------------------------------------------------------|--------------------------------|-------------------------------|
| Orange juice                  | *Escherichia coli*    | Frequency (Hz): 3; Total fluence (J/cm²): 5.10; Peak power (J/cm²/pulse): 1.213; Pulse width (µs): 360; Exposure time (s): 2.81; Distance from the lamp (cm): 1.9 | 2.42                           | Muñoz et al. (2011)          |
| Apple and orange juices, Milk | *Escherichia coli*    | Frequency (Hz): 3; Total fluence (J/cm²): 28; Peak power (J/cm²/pulse): 1.17; Pulse width (µs): 360; Exposure time (s): 8; Distance from the lamp (cm): 2.5 | Apple juice: 4.7 Milk: 1.06 Orange juice: 1 | Palgan et al. (2011a)        |
|                               | *Listeria innocua*    | Frequency (Hz): 3; Total fluence (J/cm²): 4; Peak power (J/cm²/pulse): 1.21; Pulse-repetition-rate (pulses/s): 3; Discharge voltage (V): 3800; Pulse width (µs): 360; Distance from the lamp (cm): 1.9 | Apple juice: 2.98 Orange juice: 0.93 | Pataro et al. (2011)        |
|                               | *Escherichia coli*    | Number of pulses: 40; Total fluence (J/cm²): 32; Peak power (J/cm²/pulse): 0.4; Pulse width (µs): 300; Distance from the lamp (cm): 0.60 | Apple juice: 4.0 Orange juice: 2.90 | Pataro et al. (2011)        |
|                               | *Penicillium expansum*| Number of pulse: 10; Total fluence (J/cm²): 11; Peak power (J/cm²/pulse): 1.1; Discharge voltage (V): 3000; Pulse width (µs): 300 |                         | Maftei et al. (2014)        |
|                               | *Listeria innocua*    | Number of pulse: 10; Total fluence (J/cm²): 11; Peak power (J/cm²/pulse): 1.1; Discharge voltage (V): 3000; Pulse width (µs): 300 | Whey and skimmed whey: <0.5 Diluted whey (3/4, 1/2, 1/4 and 1/8): 0.5, 1.4, 2.3 and 5.4 Distilled water: 5.0 | Artíguez and Martínez Marañón (2015) |
|                               | *Escherichia coli*    | Frequency (Hz): 3; Total fluence (J/cm²): 71.6; Peak power (J/cm²/pulse): 1.213; Pulse-repetition-rate (pulses/s): 3; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10 | Apple juice: 2.1 Orange and strawberry juice: 0.3-0.8 | Ferrario et al. (2015b)      |
|                               | *Listeria innocua*    | Frequency (Hz): 3; Total fluence (J/cm²): 15.3; Peak power (J/cm²/pulse): 1.17; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10 | Apple juice: 1.6 Orange and strawberry juice: 0.3-0.8 | Ferrario et al. (2015b)      |
|                               | *Salmonella enteritidis* | Frequency (Hz): 3; Total fluence (J/cm²): 1.7; Peak power (J/cm²/pulse): 1.17; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10 | Apple juice: 2.4 Orange and strawberry juice: 0.3-0.8 | Ferrario et al. (2015b)      |
|                                                 | Saccharomyces cerevisiae                                                                 | Apple juice: 1.0 Orange and strawberry juice: 0.3-0.8 |
|------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------|
| Native flora                                     | Frequency (Hz): 3; Total fluence (J/cm^2): 2.4-71.6; Peak power (J/cm^2/pulse): 1.27; Pulse-repetition-rate (pulses/s): 3; Discharge voltage (V): 3800; Pulse width (μs): 360; Exposure time (s): 2-60; Distance from the lamp (cm): 10 | Commercial juice: 3.0 Natural juice: 1.5 |
| Aliceyclobacillus acidoterrestris                |                                                                                         | Commercial juice: 4.4 Natural juice: 2.0 |
| Apple juice (commercial and natural)             |                                                                                         | Ferrario et al. (2015a) |
|                                                 | Pseudomonas aeruginosa Total fluence (J/cm^2):0.97 for Mineral water and isotonic beverage; 12.7-24.35 for apple juice, carbonated beverage and plum juice; 29.21 for milk, coffee without milk, orange juice and grape juice. | Mineral water, isotonic beverage, apple juice, plum juice and carbonated beverage: 7.0 Orange juice, grape juice, milk, coffee without milk: 0.5-2.0 |
| Mineral water, isotonic beverage, apple juice, grape juice, carbonated beverage, plum juice, milk, coffee without milk |                                                                                         | Hwang et al. (2015) |
|                                                 | Goat milk Escherichia coli Total fluence (J/cm^2): 10; Peak power (J/cm2/pulse): 0.187; Exposure time (s): 8 | 6 Kasahara et al. (2015) |
| Apple juice (commercial and natural)             | Escherichia coli Total fluence (J/cm^2): 0.73; Peak power (J/cm^2/pulse): 0.398; Discharge voltage (V): 3800; Pulse width (μs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10 | 3.1 Ferrario et al. (2016) |
| Salmonella Enteritidis                           |                                                                                         | 4.2 |
| Saccharomyces cerevisiae                         |                                                                                         | 1.8 |
| Water                                           | Escherichia coli Frequency (Hz): 5; Total fluence (J/cm^2): Distance from the lamp (cm): 9 | 3.35 |
| Murine norovirus                                 |                                                                                         | 4.79 Yi et al. (2016) |
| Aerobic and facultative anaerobic                |                                                                                         | 2.91 |
| Apple juice (commercial and natural)             | Saccharomyces cerevisiae Frequency (Hz): 3; Total fluence (J/cm^2): 71.6; Peak power (J/cm^2/pulse): 1.27; Discharge voltage (V): 3800; Pulse width (μs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10 | Commercial: 3.9 Natural: 1.0-2.0 |
| Turnip juice                                    | Candida inconspicua Total fluence (J/cm^2): 19.71; Discharge voltage (V): 3800; Pulse width (μs): 360; Exposure time (s): 60; Distance from the lamp (cm): 5 | 2.80 Karaoglan et al. (2017) |
| Apple juice                                     | Alicyclobacillus acidoterrestris Frequency (Hz): 3; Total fluence (J/cm^2): 71.6; Peak power (J/cm^2/pulse): 1.27; Discharge voltage (V): 3800 | 3.0--3.5 Ferrario et al. (2018) |
Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10
Table 3: Microbial reduction levels for meat, fish, derived products and cheese after pulsed light processing

| Food product                          | Microorganism treated                  | Operation conditions                                                                 | Reduction (log_{10} CFU/g) | Reference                  |
|---------------------------------------|----------------------------------------|---------------------------------------------------------------------------------------|---------------------------|---------------------------|
| Stainless steel in contact with meat  | *Listeria monocytogenes* <br> *Escherichia coli* | Total fluence (J/cm²): 3; Pulse width (µs): 300; Discharge voltage (V): 3000; Distance from the lamp (cm): 10 | 6.5                       | Rajkovic et al. (2010)    |
| Tuna carpaccio Beef carpaccio         | *Listeria monocytogenes* <br> *Escherichia coli* <br> *Salmonella Typhimurium* <br> *Vibrio parahaemolyticus* | Total fluence (J/cm²): 11.9; Peak power (J/cm²/pulse): 0.175; Pulse width (µs): 250 | Beef carpaccio: 0.9 <br> Tuna carpaccio: 0.7 <br> Beef carpaccio: 1.2 <br> Beef carpaccio: 1.0 | Hierro et al. (2012) |
| Shrimp, salmon, flatfish              | *Listeria monocytogenes*               | Frequency (Hz): 5; Total fluence (J/cm²): 6900; Peak power (J/cm²/pulse): 0.00175; Exposure time (s): 1380 | Shrimp: 2.4 <br> Salmon: 2.1 <br> Flatfish: 1.9 | Cheigh et al. (2013) |
| Dry cured meat products (salchichón and loins) | *Listeria monocytogenes* <br> *Salmonella enterica serovar Typhimurium* | Total fluence (J/cm²): 11.9; Peak power (J/cm²/pulse): 0.7; Pulse width (µs): 250 | Salchichón: 1.81 <br> Loins: 1.61 <br> Salchichón: 1.48 <br> Loins: 1.73 | Ganan et al. (2013) |
| Raw pork roast, roast pork and raw salmon | *Aerobic flora* <br> *Pseudomonas fluorescens* | Frequency (Hz): 1; Total fluence (J/cm²): 30; Distance from the lamp (cm): 3 | Raw pork roast: 0.96 <br> Roast pork: 0.99 <br> Raw Salmon: 0.7 | Nicorescu et al. (2014) |
| White cheddar cheese                  | *Listeria innocua* <br> *Pseudomonas fluorescens* <br> *Escherichia coli* | Total fluence (J/cm²): 3.1; Pulse width (µs): 360; Pulse-repetition-rate (pulses/s): 3 | 3.0 <br> 3.0 <br> 5.0 | Proulx et al. (2015) |
| Seafood Isolates                      | *Listeria monocytogenes* <br> *Listeria innocua* | Pulse energy (J/cm²): 0.316; Peak power (J/cm²/pulse): 0.053 Pulse width (µs): 325; Distance from the lamp (cm): 11 | 2.4 <br> 5.4 | Lasagabaster et al. (2017) |
| Sliced fermented salami               | *Listeria monocytogenes* <br> *Escherichia coli* <br> *Salmonella Typhimurium* <br> *Staphylococcus aureus* | Total fluence (J/cm²): 3; Number of pulses: 1; Pulse width (µs): 300; Discharge voltage (V): 3000; Distance from the lamp (cm): 10 | 2.24 <br> 2.29 <br> 2.25 <br> 2.12 | Rajkovic et al. (2017) |
| Pork skin                             | *Salmonella typhimurium* <br> *Yersinia enterocolitica* | Total fluence (J/cm²): 19.11; Distance from the lamp (cm): 8.3; Pulse width (µs): 300; Treatment time: 30 s | 2.97 <br> 4.2 | Koch et al. (2019) |
| Pork loin                             | *Salmonella typhimurium*               | Total fluence (J/cm²): 0.52-19.11; Distance from the lamp (cm): 3; Pulse width (µs): 300; Treatment time: 30 s | 0.4-1.6 | |
| Species          | Lamp (cm): | Pulse width (µs): | Treatment time: | 
|------------------|------------|------------------|----------------| 
| *Yersinia enterocolitica* | 8.3-13.4   | 300              | 1-30 s         | 0.4-1.7         |
Table 4: Microbial reduction levels for fruits and vegetables after pulsed light processing

| Food product                               | Microorganism treated                              | Operation conditions                                                                 | Reduction (log$_{10}$ CFU/g) | Reference                      |
|--------------------------------------------|----------------------------------------------------|--------------------------------------------------------------------------------------|------------------------------|-------------------------------|
| Tomato fruit                               | Microflora in skin and peduncle                    | Total fluence (J/cm$^2$): 4 (Microflora) and 2.2 (S. cerevisiae); Discharge voltage (V): 2500; Pulse width (µs): 250 | 1.0                          | Aguiló-Aguayo et al. (2013)  |
|                                            | Saccharomyces cerevisiae                            |                                                       | 2.3                          |                               |
| Avocado                                    | Aerobic mesophilic microorganisms                  | Total fluence (J/cm$^2$): 14; Pulse width (µs): 360; Distance from the lamp (cm): 5 | 1.20                         | Aguiló-Aguayo et al. (2014)  |
|                                            |                                                    |                                                       | Spot inoculation: 4.8 for stems and 4.1 leaves Dip inoculation: 0.2 for stems and 0.6 leaves |                               |
| Green Onions                               | Escherichia coli                                   | Total fluence (J/cm$^2$): 5; Peak power (J/cm$^2$/pulse): 1.27; Exposure time (s): 5 | 4.3 calyx and >6.7 skin      | Xu et al. (2013)              |
| Blueberries                                | Escherichia coli                                   | Total fluence (J/cm$^2$): 5; Peak power (J/cm$^2$/pulse): 1.27; Discharge voltage (V): 3800; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 15 | 4.1 calyx and 5.7 skin       | Huang and Chen (2014)         |
|                                            | Salmonella                                         |                                                       |                              |                               |
|                                            |                                                    |                                                       |                              |                               |
| Fresh-cut apples                           | Total viable counts                                | Frequency (Hz): 0.5; Pulse energy (J/cm2): 17.5; Pulse width (µs): 50; Distance from the lamp (cm): 10 | 1.0                          | Ignat et al. (2014)           |
|                                            | Lactobacillus brevis                               |                                                       | 3.0                          |                               |
|                                            | Listeria monocytogenes                             |                                                       | 2.7                          |                               |
|                                            |                                                    |                                                       |                              |                               |
|                                            | Escherichia coli                                   | Frequency (Hz): 1; Total fluence (J/cm$^2$): 3; Distance from the lamp (cm): 10 | Endive salad: 2.34           | Kramer et al. (2015)          |
|                                            | Listeria innocua                                   |                                                       | Mung bean sprouts: 1.91      |                               |
|                                            |                                                    |                                                       | Endive salad: 2.54           |                               |
|                                            |                                                    |                                                       | Mung bean sprouts: 1.55      |                               |
|                                            |                                                    |                                                       |                              |                               |
|                                            | Native microflora                                  | Total fluence (J/cm$^2$): 11.0; Discharge voltage (V): 3000; Pulse width (µs): 50; Distance from the lamp (cm): 10 | ~ 4.7                        | Manzocco et al. (2015)        |
|                                            | Salmonella enterica                                |                                                       | ~ 5.5                        |                               |
|                                            | Listeria monocytogenes                             |                                                       | ~ 6                          |                               |
|                                            | Escherichia coli                                   |                                                       | 5.3                          |                               |
|                                            |                                                    |                                                       |                              |                               |
|                                            | Mesophilic aerobic bacteria                         | Total fluence (J/cm$^2$): 20 and 40; Pulse width (ms): 0.3 | 0.5-2.2                      | Agüero et al. 2016            |
|                                            | Psychrotrophic bacteria                            |                                                       |                              |                               |
|                                            | Coliforms                                          |                                                       |                              |                               |
|                                            | Listeria spp.                                      |                                                       |                              |                               |
|                                            | Yeasts and molds                                   |                                                       |                              |                               |
|                                            |                                                    |                                                       |                              |                               |
|                                            | Fresh-cut apples Mesophilic and psychrophilic      | Number of pulse: 40; Total fluence: (J/cm$^2$): 16; Peak | >1.55                        | Avalos et al. (2016)          |
| Sample Type                  | Microbial Target       | Conditions                                                                 | Fluence (J/cm²) | Power (J/cm²/pulse) | Pulse Width (µs) | Distance (cm) | Ref.          |
|-----------------------------|------------------------|----------------------------------------------------------------------------|-----------------|---------------------|-----------------|---------------|---------------|
| Cantaloupe melon slice      | Moulds and yeast       | Total viable count, Total fluence (J/cm²): 15.6 followed by storage at 4 ± 1 °C for 28 days. Peak power (J/cm²/pulse): 0.3 | 1.39            | 0.4                 | 300             | 10            | Koh et al. (2016b) |
| Cantaloupe melon slice      | Yeast and moulds       | Total viable count, Fluence (J/cm²): 0.9 every 48 h up to 28 days of storage at 4 ± 1 °C | 1.45            | 0.3                 | 10              | 6             | Koh et al. (2016a)  |
| Raspberries                 | Salmonella             | Frequency (Hz): 1; Total fluence (J/cm²): 28.2; Peak power (J/cm²/pulse): 1.27; Discharge voltage (V): 3000; Exposure time (s): 15; Storage temperature (°C): -20 | 4.5             | 1.39               |                 |               | Xu & Wu (2016) |
| Endive salad and Mung bean sprouts | Listeria innocua    | Frequency (Hz): 1; Peak power (J/cm²/pulse): 580; Discharge voltage (V): 2000; Exposure time (s): 60; Distance from the lamp (cm): 5 | 3.9             | 1.5                 |                 |               | Kramer et al. (2017) |
| Strawberries and Blueberries | Murine norovirus (MNV-1) | Total fluence (J/cm²): 22.5; Exposure time (s): 24; Distance from the lamp (cm): 16 | 4.5             | 1.5                 |                 |               | Huang et al. (2017) |
| Blueberries                 | Salmonella             | Total fluence (J/cm²): 6; Peak power (J/cm²/pulse): 0.066; Pulse-repetition-rate (pulses/s): 3; Pulse width (µs): 360; Exposure time (s): 30 | 0.9             | 0.9                 | 360             | 30            | Cao et al. (2017) |
| Fresh-Cut tomatoes          | Listeria innocua      | Total fluence (J/cm²): 8; and kept cold at 4 °C for 20 days | 1.4             | 0.9                 |                 |               | Valdivia-Nájar et al. (2017) |
| Fresh-Cut tomatoes          | Escherichia coli       | Total fluence (J/cm²): 4, 6, and 8; and kept cold at 5 °C for 20 days | 1.4             | 1.4                 |                 |               | Valdivia-Nájar et al. (2018) |
| Sesame seeds                | Total viable count     | Total fluence (J/cm²): 44.46; Discharge voltage (V): 2400; Exposure time (s): 120; Pulse width (µs): 3.0 | 1.46            | 1.46                |                 |               | Hwang et al. (2017) |
| Malting barley | *Aspergillus carbonarius* | *Aspergillus flavus* | Total fluence (J/cm²): 18; Pulse width (µs): 360; Exposure time (s): 15; Distance from the lamp (cm): 10 | 1.2 | 1.7 | Zenklusen *et al.* (2018) |
### Table 5: Microbial reduction levels in foods using PL in combination with other technologies

| Food product                  | Method of generation | Microorganism treated | Operation conditions                                                                 | Reduction (log_{10} CFU/mL) | Reference                  |
|-------------------------------|----------------------|-----------------------|---------------------------------------------------------------------------------------|-----------------------------|-----------------------------|
| Orange juice                  | HILP + TS            | *Escherichia coli*    | Frequency: 3 Hz; Total fluence (J/cm²): 5.10; Peak power (J/cm²/pulse): 1.213; Pulse width (µs): 360; Exposure time (s): 2.81; Distance from the lamp (cm): 1.9 | 3.93                        | Muñoz et al. (2011)         |
| Apple juice                   | PL + TS              | *Escherichia coli*    | Frequency (Hz): 3; Total fluence (J/cm²): 5.1; Pulse width (µs): 360; Exposure time (s): 1.52; TS: (24 kHz, 100 µm) at 40 °C for 2.9 min or 50 °C for 5 min | 4.9 at 40 °C 5.9 at 50 °C   | Muñoz et al. (2012)         |
| Green Onions (stems and leaves) | WPL                 | *Escherichia coli*    | Total fluence (J/cm²): 56.1; Peak power (J/cm²/pulse): 1.27; Exposure time (s): 60 | Sopt inoculation: Stems: 4.1 Leaves: 4.6 Dip inoculation: Stems: 0.9 Leaves: 1.2 | Xu et al. (2013)             |
|                               | PL + 100 ppm chlorine|                      |                                                                                      | Stems: 0.9 Leaves: 2.4      |                             |
|                               | PL + 1000 ppm SDS    |                       |                                                                                      | Stems: 1.4 Leaves: 3.1      |                             |
| Apple juice (natural and commercial) | PL + US          | *Alicyclobacillus acidoterrestris* | Total fluence (J/cm²): 71.6; Peak power (J/cm²/pulse): 1.27; Discharge voltage (V): 3800; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10 US: 30 min, 44 °C±1 | Commercial juice: 5.8 Natural juice: 2.0 | Ferrario et al. (2015a)     |
| Blueberries                   | WPL                 | *Escherichia coli*    | Peak power (J/cm²/pulse): 1.27; Total fluence (J/cm²): 56.1; Time (s): 60; Pulse width (µs): 360; Discharge voltage (V): 3800; Distance Calyx: 3.0 Skin: 5.8 Calyx: 3.6 Skin: 5.9 | Calyx: 3.0 Skin: 5.8        | Huang and Chen (2014)        |
| Sample                        | Method                        | Microorganism/Pathogen                     | Parameters                                                                 | Results                                                                 |
|-------------------------------|-------------------------------|---------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Green Beans                   | HHP or PL + coating           | *Listeria innocua*                         | Discharge voltage (V): 3800; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 20; 400 MPA and 5 min for HHP; 1.2 x 10^7 J/m^2 per bean side for PL | HHP + coating: 4 PL + coating: 2 Donsi *et al.* (2015)                     |
| Raspberries and blueberries   | WPL                           | *Salmonella*                               | Peak power (J/cm^2/pulse): 0.298; Time (s): 60; Pulse width (µs): 360; Distance from the lamp (cm): 16 | Raspberries: 3.0 for WPL Blueberries: 5.6 for WPL and WPL+H2O2 Huang *et al.*, (2015) |
|                               | WPL + 1% H2O2                 |                                             |                                                                             |                                                                          |
| Raspberries (R) and strawberries (S) | WPL                           | *Escherichia coli*                         | Total fluence (J/cm^2): 53.9 for raspberries and 63.2 for strawberries; Pulse width (µs): 360; Exposure time (s): 60 | S: 2.2 and 3.3 with H2O2 R: 4.4 and 5.3 with H2O2 Huang and Chen (2015) |
|                               | WPL + 1% H2O2                 | *Salmonella*                               |                                                                             |                                                                          |
|                               |                               | *Murine norovirus (MNV-1)*                 |                                                                             |                                                                          |
| Apple juice (natural and commercial) | PL + US                       | *Escherichia coli*                         | Frequency (Hz): 3; Pulse energy (J/cm^2): 0.73; Peak power (J/cm^2/pulse): 0.398; Pulse-repetition-rate (pulses/s): 3; Discharge voltage (V): 3800; Pulse width (µs): 360; Exposure time (s): 60; Distance from the lamp (cm): 10; US: 30 min at room temperature | 5.9 Ferrario *et al.* (2016)|
|                               |                               | *Salmonella*                               |                                                                             |                                                                          |
|                               |                               | *Enteritidis*                              |                                                                             |                                                                          |
|                               |                               | *Saccharomyces cerevisiae*                 |                                                                             |                                                                          |
| Blueberries                   | WPL                           | *Salmonella*                               | Total fluence (J/cm^2): 9; Peak power (J/cm^2/pulse): 0.066; Pulse-repetition-rate (pulses/s): 3; Pulse width (µs): 360 Exposure time (s): 30 | 4.4 spot inoculation and 0.8 dip inoculation Cao *et al.* (2017) |
| Apple juice (natural and commercial) | PL + US                       | *Saccharomyces cerevisiae*                 | Total fluence (J/cm^2): 71.6; Peak power (J/cm^2/pulse): 1.27; Discharge voltage (V): 3800; Pulse width (µs): 360; Distance from the lamp (cm): 10 US: 30 min at 20 °C or 44 °C | At 20 °C: Commercial: 4.9 Natural: 3.9 At 44 °C: Commercial: 6.4 Natural: 5.8 Ferrario *et al.* (2017) |
| Iceberg lettuce               | PL + Chlorine washing         | *Salmonella enterica*                      | Peak power (J/cm^2/pulse): 0.14; Pulse-repetition-rate (pulses/s): 3; Pulse width (µs): 360 | 1.5-2.7 Huang and Chen (2018) |
PL: Pulsed Light; HILP: High Intensity Light Pulses; TS: Thermosonication; US: Ultrasonics; WPL: Water-assisted pulsed light; SDS: Dodecilsulfato sódico; \( \text{H}_2\text{O}_2 \): HHP: High hydrostatic pressure.
Highlights

- Pulsed light (PL) have emerged lately as an alternative to traditional disinfection and preservation methods
- The combination of PL with other techniques can improve the effectiveness of the decontamination process
- PL can have a negative impact on the sensory properties of food products