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Title: Efficacy of ultraviolet light (UV-C) and pulsed light (PL) for the microbiological decontamination of raw salmon (*Salmo salar*) and food contact surface materials.

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Abstract

The decontamination effect of two light-based technologies on salmon, polyethylene (PE) and stainless steel (SS) was evaluated. Optimization of treatment conditions for ultraviolet light (UV-C) and pulsed light (PL) was carried out on raw salmon, obtaining inactivation levels of 0.9 and 1.3 log CFU/g respectively. The effects of treatments on several microbial groups present in salmon were then evaluated. For both technologies, *Pseudomonas* spp. were found to be the most resistant group of microorganisms tested. Three different strains from within this group were isolated and speciated, including a *P. fluorescens* strain which was selected for subsequent studies. PE and SS surfaces were inoculated with a suspension of the *P. fluorescens* suspended in a 'salmon juice' solution, and treated with UV-C and PL at different doses (mJ/cm^2). PE surfaces were effectively decontaminated a low doses for both technologies, with a reduction of >4 log cycles observed. Decontamination of SS was also effective when treated with PL, although at higher doses than for PE. When SS was treated with UV-C, the maximum reduction of *P. fluorescens* achieved was 2 log cycles, even at the highest dose.

Keywords

Ultraviolet light, pulsed light, contact surfaces, decontamination, salmon, Pseudomonas

1. Introduction

Globally, salmon is one of the most important high-value fish species in the seafood sector. Within Europe, salmon is the third most commonly consumed species behind tuna and cod. The average reported consumption *per capita* is 2.17 kg/year, which is mainly consumed fresh or smoked, and represents 15% of fresh fish products (EUMOFA, 2017; FAO, 2016).

Fresh fish are highly perishable and identifying technologies to extend shelf-life is considered a priority within the fish processing industry. The application of novel processing technologies to fish has been investigated in recent years in order to try and control spoilage (Leisner & Gram, 2014).

Fish is a very complex matrix where the natural microbiota can be present in the skin, gills, eyes, muscle, internal organs, digestive tract and even in larvae and eggs (Austin, 2006). The microbial species and their evolution during post-capture storage can be very diverse and only a sub-population of this microbiota can be considered as spoilage organisms. Specific spoilage organisms (SSO) have been identified as the main groups responsible for causing deterioration in the quality of fresh fish (Gram & Huss, 1996). Fish spoilage can be defined as a series of biochemical changes, mainly induced by microbial growth which leads to undesirable sensory changes (Gram & Huss, 1996). Microorganisms present in fish may occur naturally, or can be introduced during processing through cross-contamination, poor handling and hygiene practices (Møretrø, Moen, Heir, Hansen, & Langsrud, 2016).

The processing environment can be an important source of spoilage and pathogenic bacteria. In the salmon processing industry, significant SSO species such as *Pseudomonas*, *Shewanella* and *Photobacterium* species have been widely detected

on equipment and machines even after cleaning and sanitising, highlighting the importance of controlling these contaminants in order to maximise product shelf-life (Møretrø et al., 2016).

The application of light-based technologies such as ultraviolet or pulsed light could be used as an alternative or an additional tool to compliment traditional sanitization techniques. The mode of action of UV light as a decontamination technology is based on the emission of radiation within the ultraviolet region (100-400 nm), more specifically the UV-C region (200-280 nm) which has been shown to be germicidal. This wavelength represents the peak of maximum absorption for DNA (260 nm) (Acra, Jurdi, Allem, Karahagopian, Raffoul, 1990; Kowalski, 2009). The germicidal effect is primarily due to the formation of DNA photoproducts (such as pyrimidine dimers) which inhibit transcription and replication and can lead to cell death (Gayán, Condón & Álvarez, 2013). Depending on the UV dose applied, some bacteria may initiate a repair mechanism, known as photoreactivation, which is dependent on exposure to visible light (Lasagabaster & Martínez de Marañón, 2014).

High intensity light pulses (HILP), also known as pulsed light (PL), is an emerging technology which can be used to decontaminate surfaces by generating short time high-energy light pulses (millionths or thousands of a second) of an intense broad spectrum (200-1100 nm) (Gómez-López, Devlieghere, Bonduelle & Debevere, 2005a). The antimicrobial effect has been attributed to DNA damage (from the UV-C region) although other structural damage in cell walls, membranes and internal structures may also be involved (Cheigh, Hwang & Chung, 2013).

UV-C light has been used to decontaminate air and water as well as a wide variety of contact surfaces and materials (Haughton et al., 2011a; Koutchma, 2014). PL has also proven effective in the decontamination of a large number of surfaces (Ringus &

Moraru; Woldling & Moraru, 2005), packaging materials (Haughton et al. 2011b; Turtoi & Nicolau, 2007) and even liquids (Birmpa, Vantarakis, Paparrodopoulos, Whyte & Lyng, 2014). Both technologies have also demonstrated their efficacy for food surface decontamination (Fan, Huang & Chen, 2017; Gómez-López, Ragaert, Debevere & Devlieghere, 2007; Heinrich, Zunabovic, Bergmair, Kneifel & Jäger, 2015). However, few studies have investigated the ability of these light-based technologies to decontaminate fish (Cheigh et al., 2013; Molina, Sáez, Martínez, Guil-Guerrero & Suárez, 2014; Nicorescu, Nguyen, Chevalier & Orange, 2014).

The aim of the present study was to evaluate the effect of UV-C and PL on the typical microbiota of salmon in order to identify the most resistant microorganism to both technologies and to evaluate their decontamination efficacy on related contact surfaces such as polyethylene and stainless steel.

2. Materials and methods

2.1. UV-C and PL equipment

The UV unit was custom-made and consisted of four 95 W low-pressure mercury lamps of 50 cm length (Baro Applied Technology, Manchester, UK), housed in an enclosed stainless steel cabinet with internal dimensions of 790 x 390 x 345 mm (L x W x H) (Figure 1).

The PL unit was a benchtop Steri-Pulse-XL Pulsed Light Sterilization system (Xenon Corporation, MA, US) with internal dimensions of 406 x 203 x 127 mm (L x W x H). The treatment chamber, separated by a quartz glass window, is equipped with a high-energy pulsed ultraviolet/visible flash lamp (Type C, 190nm spectral cut-off point) which generates broad spectrum light pulses (200 to 1000 nm), with a power

of 1516 W. The pulse width was 360 μs with a fixed pulsed rate of 3 pulses per second (Hz).

In both systems the energy received by the sample varied depending on treatment time and distance from the light source. Dose or fluence (mJ/cm^2) received by samples was measured with a radiometer-ILT1700 (International Light Technologies, MA, US) coupled with a solar blind vacuum photodiode detector (SED240/NS254/W) for the UV-C lamp measurements, at 254 nm. For the PL lamp, a broadband silicone detector (SED033/QNDS2) was used, working in the range 200-1100 nm. The energy of several positions was measured within each chamber and the location that consistently delivered the highest irradiance was selected for further study (Haughton et al., 2011a). The doses (mJ/cm^2) received by each sample side are presented in Table 1, and were calculated by using the following formula:

$$D = I \times t$$

where D is the dose (mJ/cm^2), I is the dosage rate (Dr, in W/m^2) measured at 254nm or from 200-1000nm for UV-C and PL respectively and t is the retention time (s).

The electrical consumption (kWh) was calculated for each technology, as well as an estimation of the cost according to the EU-28 average electricity price for non-household consumers (EUR 0.114 per kWh) (Eurostat, 2017). For UV-C consumption was 22.8 kWh at 2.6 € and for PL was of 90.9 kWh at 10.36 €.

2.2. Treatment conditions

2.2.1. Raw salmon

Skin-packed raw salmon (*Salmo salar*) fillets were purchased in a local supermarket and were stored at 4°C in a refrigerator until the end of their indicated shelf-life. This

was performed in order to standardize total bacterial counts (10^6-10^7 CFU/g). Fillets were cut aseptically in smaller pieces (3 cm x 5 cm) with skin on and those with a similar thickness (2.5 - 3 cm) were selected and placed on sterile petri dishes and then refrigerated until treated. Salmon pieces were treated within the petri dishes (without lid) on both sides using either UV-C or PL. All samples were turned aseptically with sterile forceps after the corresponding treatment by side and were immediately wrapped in foil and refrigerated at 4°C for 2-4h in order to avoid photoreactivation (Lasagabaster & Martinez de Marañon, 2014).

To optimize the treatment conditions for UV-C and HILP on salmon, a number of inactivation kinetics of mesophilic viable counts (MVC) were performed. For UV-C, three different distances from the irradiation source (26, 16 and 6 cm) were used along with treatment times ranging from 0 to 90 s which resulted in the delivery of a range of energy doses (Table 1).

Once treatment conditions were optimised, salmon pieces, as described above, were treated using one set of conditions (t/distance) with either UV-C or PL. Counts of various groups of bacteria were then carried out and compared to untreated controls. All treatments were carried out at least in triplicate.

2.2.2. Fish contact surfaces

Polyethylene cutting board (PE) and stainless steel (SS) sections of 25cm^2 (5 x 5 cm) were kept in 70% ethanol overnight and then transferred aseptically to sterile 90mm diameter petri dishes until required.

Salmon juice was prepared using a method previously described (Jørgensen & Huss, 1989) for cod with slight modifications. Briefly, minced salmon fillets without skin were mixed with maximum recovery diluent (MRD) at a ratio of 1:2 and

homogenised in a Stomacher (Lab-blender 400, Seward) for 15 min. The mixture was heated to 70°C to coagulate and precipitate the majority of proteins, and was filtered before centrifuging at 4°C for 15 min at 4000 rpm. The supernatant was transferred to glass bottles and autoclaved at 121°C for 15 min and stored at 4°C until required.

Before treatment with the light technologies, salmon juice inoculated with the test organism (*P. fluorescens*) to a concentration of 1-4 x 10^6 CFU/mL was transferred to the upper surfaces of the SS and PE pieces by applying an aerosolized inoculum with a spray bottle (previously cleaned with 70% ethanol and MRD to remove possible residues) (Haughton et al., 2011a). Each surface section received a single spray (~0.8 mL) inside a laminar flow cabinet to give a final concentration ~4 log CFU/cm^2.

UV-C and PL treatments of the contact surfaces were carried out at different distances from the light source and energy doses applied were calculated (Table 1). For PL, treatments were given in 2 s intervals (6 pulses) to avoid excessive increases in temperature which could cause thermal inactivation of bacteria present. After treatments, the plates containing the pieces of SS and PE were also wrapped in foil to avoid photoreactivation. All treatments were repeated at least three times.

2.3. Microbiological analysis

2.3.1. Raw salmon

Following treatment of salmon pieces, 10 g were transferred aseptically to sterile stomacher bags (Stomacher ® 400 classic, Seward, UK) containing 90 mL of MRD (Maximum recovery diluent, Oxoid) and homogenized in a mechanical homogenizer (Stomacher Lab-blender 400, Seward, UK). Ten-fold dilution series of homogenates

were prepared and 0.1 or 1 ml aliquots (depending on the agar media) were added to a range of solid culture media in triplicate.

For experiments carried out to optimise treatment conditions, total MVC were enumerated on Plate Count Agar (PCA, Oxoid) containing 1% NaCl, which were incubated for 48 h at 30°C.

Once treatment conditions were optimized for each technology, a range of bacterial groups were investigated using selective and non-selective media. MVC and psychrotrophic viable counts (PVC) were cultured in PCA+1% NaCl, and incubated 48h at 30°C and 7 days at 6°C respectively. Enterobacteriaceae (ENT) were cultured in double layer VRBGA (violet red bile glucose agar, Oxoid) incubated for 48 h at 30°C. Lactic acid bacteria (LAB) counts were carried out under anaerobic conditions in MRS agar (de Man, Rogosa & Sharpe, 1960; Oxoid) for 5 days at 30°C. *Pseudomonas* spp. counts (PSE) were performed in Pseudomonas CFC (Cetrimide, Fucidin, Cephalotin) selective agar (Oxoid) incubated for 48 h at 25°C. Levels of *Brochothrix thermosphacta* (BRT) were performed in STA medium (streptomycin sulphate and thallous acetate, Oxoid) (NMKL, 1991). The presumptive count of *Listeria* spp. (LIS) was carried out in chromogenic *Listeria* agar with Chromogenic *Listeria* Selective Supplement (ISO) and Brilliance *Listeria* Differential Supplement (Oxoid), and incubated for 24h at 37°C. Counts of *Photobacterium phosphoreum* (PHP) were performed by counting the luminous colonies in darkness after 7 days at 6°C using Long and Hammer's medium (Broekaert, Heyndrickx, Herman, Devlieghere & Vlaemynck, 2011). The detection limit for MVC, PVC, ENT, LAB, and *Listeria* spp., was 10 CFU/g, and for PSE, BRT and PHP was 100 CFU/g. All microbial analyses were performed in triplicate.

2.3.2. Isolation and identification of the most resistant microorganism

After UV-C and PL treatments on raw salmon, the most resistant microbial group to both technologies was determined. Then, three different colonies from the selective agar used for this group (CFC), were isolated and streaked onto new plates to ensure the purity of cultures and incubated for 48 h at 25°C. A single colony from each plate was transferred into 5 mL of Tryptic Soy Broth (TSB) and incubated again until turbidity was detected (~24h). Then, 1 mL was centrifuged at ~8000 *g* (10,000 rpm) for 5 min in an Eppendorf centrifuge (model 5417 R, Eppendorf AG 22331, Hamburg, Germany), the supernatant was discarded and the pellet was re-suspended in sterile MRD. This process was repeated twice and was done inside a laminar flow cabinet. Bacterial pellets were re-suspended in 500 µL of lysis buffer (Fisher Scientific, New Hampshire, US) and sent overnight to an external laboratory (Eurofins Medigenomix GmbH, Ebersberg, Germany) for sequencing by partial 16S rRNA gene analysis. The received sequences (forward and reverse) were introduced in the Basic Local Alignment Search tool (BLAST) from the US National Centre for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov) to be identified.

One isolate within the most resistant group was then selected for the surface decontamination study. This strain was stored at -80°C on Protect ^{TM} beads until required (Technical Services Consultants Ltd, Lancashire, UK). The suspensions were prepared by transferring one bead to 5 mL of TSB and incubating until turbidity was detected; a loopful was then streaked onto the corresponding selective agar to ensure homogeneity of the culture. After incubation for 48h at 25°C, a single colony was transferred to 10 mL of TSB, which was incubated overnight at 25°C in a shaker incubator (160 rpm, Orbital shaker MaxQ 4000, ThermoFisher Scientific). Following

this step, 50 mL of TSB containing sterile glass beads were inoculated with 1 mL of overnight culture and incubated, as previously described, until the stationary phase was reached (3-5 x 10^9 CFU/mL) (Gayán, García-Gonzalo, Álvarez & Condón, 2014).

2.3.3. Fish contact surfaces

The selected microorganism, used to inoculate the food contact surfaces, was recovered following treatments by using pre-moistened swabs and sampling the surface of the PE and SS pieces in 4 directions. The swab was then placed in 5 mL of MRD and shaken in a vortex for 30 s. Subsequently, decimal dilutions were performed and were plated in heart infusion agar (HIA) supplemented with 0.5% NaCl. Previous analysis confirmed that the target microorganism in pure culture was able to grow to equivalent levels in both HIA and its selective agar (data not shown). All analyses were performed in triplicate.

2.4. Physicochemical analysis

The temperature of each side of the treated salmon samples were measured with an infrared thermometer (RS 1327K, RS Components, Corby, Northamptonshire, UK) to ensure excessive temperatures were not reached (>30°C). SS and PE surfaces were also measured with the infrared thermometer immediately after treatment to ensure that any observed inactivation was due to light-based technologies rather than a thermal effect.

Instrumental colour analysis was carried out on salmon surfaces with a Chroma Meter (CR-400 Konica Minolta sensing, Japan) measuring the CIE parameters L*

(lightness), a* (redness) and b* (yellowness) (CIE, 1976) from three random locations per piece.

Lipid oxidation of salmon fillets was also measured before and after treatment using the 2-thiobarbituric acid (TBA) previously described by Pfalzgraf, Frigg and Steinhart (1995). Absorbance was measured at 532 nm in a spectrophotometer (Thermo Spectronic, BioMate 5, UK). The standard curve with TMP (1,1,3,3-tetramethoxypropane, Sigma Aldrich) was performed to calculate the TBARS (TBA reactive substances) for each analysis. Results were expressed as mg of malonaldehyde (MA)/kg of sample. All analysis were performed in triplicate.

2.5. Statistical analysis

Statistical analyses were carried out using two-way ANOVA with GraphPad PRISM 5.0 software (GraphPad software, San Diego, CA, US). Statistical significance was assigned to p values <0.05.

3. Results

3.1. Optimization of salmon treatment conditions

A series of treatments with UV-C and PL were given to salmon pieces in order to obtain a number of inactivation curves for MVC. The corresponding doses (mJ/cm^2) for each treatment, in terms of treatment time and distance from the light source are presented in Table 1. The log reductions of MVC after UV-treatments in salmon are shown in Figure 2 (A). A high variability was observed between samples, however, with the exception of the 5 s treatment, reductions of MVC in treated samples were significantly lower ($p<0.05$) compared to corresponding controls for all distances

studied. In general, the inactivation profile for each distance consisted of an initial reduction within ~15-30 s with no additional significant reductions observed when treatment times were extended.

For PL treatments, similar inactivation levels in MVC were observed when compared to those for UV-C treatments (Figure 2-B). All treatments were significantly lower than controls, and no differences were found between 11 and 7.5 cm. For both technologies, the maximum inactivation levels were achieved at the closest distance from the light source, -1.0±0.1 log CFU/g at 6 cm after 60 s in UV-C and -1.3±0.1 log CFU/g at 3.5 cm for 12 s in PL.

Salmon surface temperatures did not exceed 30°C after all UV-C treatments, but treatments over 45 s at 6 cm (190.8 mJ/cm^2) caused a detectable colour change. Similarly, for PL treatments, doses higher than 200 mJ/cm^2 (Table 1) caused a cooked appearance on the salmon surface resulting from an increase in temperature to over 35°C. These colour changes were due to an increase in the L* value and a reduction in the a* index (data not shown).

Lipid oxidation in salmon samples exposed to both technologies was also evaluated for each distance and treatment time (Table 2). For UV-C, significantly higher values with respect to controls (t = 0) were observed for 60 s at 26 cm, 45 s at 16 cm and 5 s at 6 cm treatments. Generally, no significant differences were found between treatment distances, except for 26 and 16 cm at 45 s, and between 16 and 6 cm at 5s.

In general, PL treated salmon showed higher levels of lipid oxidation than UV-C treated samples; however no significant differences were found between distances and modest differences among treatment times for each distance (Table 2).

In addition, although no sensory analysis was performed as such, changes in colour and lipid oxidation were also detected organoleptically by the laboratory personnel (2-3 people) after treatments. The highest energy treatments for both technologies resulted in the development of detectable sensory changes in salmon (samples were perceived as paler and with a strong rancid odour).

When excessive surface temperatures, undesirable colour changes, increases in lipid oxidation or other sensory changes together with microbial inactivation levels were taken into account the following conditions were selected for further investigation: 30 s at 6 cm (127.2 mJ/cm^2) for UV-C, and 9 s at 3.5 cm (152.6 mJ/cm^2) for PL treatments on salmon.

3.2. Characterisation and identification of the most resistant microbial group

The aim of this characterisation study was to identify the microbial group which showed the lowest inactivation rates with respect to untreated samples for both technologies.

Mean log reductions of *Listeria* spp. (LIS), lactic acid bacteria (LAB), *Brochotrix thermosphacta* (BRT), *Photobacterium phosphoreum* (PHP), psychrophilic and mesophilic viable counts (PVC and MVC), Enterobacteriaceae (ENT) and *Pseudomonas* spp. after treatment with UV-C and PL technologies are shown in Figure 3.

Generally, no significant differences in levels of inactivation were observed when PL and UV-C treatments were compared ($p>0.05$) except *Listeria* spp., where inactivation levels with PL were significantly higher ($p<0.05$). Reductions after UV-C treatment ranged from 0.42 ± 0.1 log CFU/g for PSE up to 1.33 ± 0.1 log CFU/g for

PHP. Inactivation levels after PL treatment ranged from 0.6 ± 0.1 log CFU/g for PSE to 1.6 ± 0.3 log CFU/g in the LIS group. Hence, the microbial group that showed the highest resistance to both light-based technologies was *Pseudomonas* spp.

As mentioned in section 2.4.2., three visually different colonies from the treated salmon (2 from PL and 1 from UV-C treatments) were isolated from the CFC selective media and sequenced by partial 16S rRNA gene analysis (Mardis, 2008). Following BLAST analysis, two of the isolates were confirmed as *Pseudomonas* sp. for forward and reverse sequences, one was 99% identical to *P. lurida* and the other had 99% identity to *P. fluorescens*. The third isolate was defined as an "uncultured bacterium clone". Therefore, the wild type *P. fluorescens* strain was selected for the following studies as it was considered to be most relevant to fish processing operations.

3.3. Fish contact surfaces decontamination

Salmon juice was inoculated with the *P. fluorescens* isolate at levels of ~10^4 CFU/cm^2 on sections of PE and SS. These sections were treated immediately with UV-C and PL at different distances from the irradiation source and times.

For UV-C, mean log CFU/cm^2 counts following exposure to various treatment doses (mJ/cm^2) (Table 1), are represented in Figure 4 (A). The greatest reductions were observed for PE surfaces even at lower doses, and treatments greater than 19 mJ/cm^2 inactivated the organism to below the limit of detection. In contrast, for SS surfaces, the maximum inactivation levels were on average 2 log CFU/cm^2, even when the highest doses were applied.

The effect of different treatments using PL on counts of *P.fluorescens* inoculated on PE and SS surfaces is presented in Figure 4 (B). Unlike UV-C, PL treatments were more effective for both contact surface materials. For PE, the inoculated test bacteria were under the detection limits even when lower doses were applied. For SS, although higher doses were necessary, inactivation below the detection limits was achieved (>80 mJ/cm^2).

4. Discussion

One of the first objectives of this study was to assess the effect of light-based technologies (UV-C and PL) to decontaminate the typical microbiota present on salmon. In order to obtain maximum inactivation rates and to avoid any potentially undesirable effects caused by these technologies, several treatment conditions were investigated. For UV-C, the optimal conditions to maximise bacterial reductions while limiting deterioration in some quality attributes were found to be 30 s at 6 cm from the UV lamp, which corresponds to a dose of 127.2 mJ/cm^2. Maximum inactivation levels were reached at 6 cm for 60 s, but no further significant differences were found between 30 and 60 s. Moreover, doses higher than 190mJ/cm^2 (45 s – 6 cm) produced slight colour changes in the fillet surface, thereby enabling conditions to be identified which would maximise bacterial inactivation while preserving organoleptic properties of the fish.

The effect of UV-C light has been previously studied for surface decontamination of fruits and vegetables (Fino & Kniel, 2008), egg shells (Kuo, Carey & Ricke, 1997), seeds (Sharma & Dermici, 2003) and meat or meat products (Haughton et al., 2011a; Sommers, Geveke, Pulsfus & Lemmenes, 2009). However, relatively few

studies have evaluated the effects of UV on fish. Huang and Toledo (1982) obtained levels of decontamination of 2-3 log cycles in vacuum packed mackerel stored at low temperatures, with similar treatment doses to those used in the current study (120-300 mJ/cm^2). Molina et al. (2014) assessed the effect of UV-C on the natural microbiota of sea bass at doses of 7.9 and 15.8 kJ/cm^2, and observed a decrease in microbial counts of 2.4-2.6 log CFU/g 4 days after treatment. However, the authors also reported decreased collagen content and increased lipid oxidation. Mikš-Krajnik, Feng, Bang and Yuk (2017) evaluated the effect of high doses of UV-C (3.08 kJ/cm^2) on the natural microbiota of salmon, obtaining reductions from 0.1 to 0.4 log CFU/g, and also observed visual colour changes when compared to controls. Cheigh et al. (2013) inoculated flatfish, salmon and shrimp with *Listeria monocytogenes* which were treated with UV-C at doses from 0-0.16 J/cm^2, and recorded no significant reductions. Generally, in the present study, higher inactivation levels for MVC were obtained by using UV-C at lower doses than other studies reported to date.

For PL treatments, the optimal condition was 9 s at 3.5 cm (152.6 mJ/cm^2). Similarly to UV-C treatments, no significant differences were found between treatment times of 9 s and 12 s, where the maximum inactivation level was reached and no detectable colour changes or excessive temperature increases were observed. PL technology has also proven its effectiveness in several other food matrices such as fruits and vegetables (Ferrario, Alzamora & Guerrero, 2013; Gómez-López et al., 2005b), eggs (Lasagabaster, Arboleya & Rodríguez de Marañón, 2011) and poultry (Haughton et al., 2011b). Some studies have been carried out on fish. Hierro, Ganan, Barroso and Fernández (2012) studied the application of PL at doses ranging from 0.7 to 11.9 J/cm^2 for the decontamination of tuna carpaccio inoculated with *Vibrio parahaemolyticus* and *L. monocytogenes*, with maximum inactivation

rates of 1.0 and 0.7 log CFU/cm^2 reported, respectively. However, they reported that the most severe treatments applied resulted in colour changes and negatively affected the sensory quality. Nicorescu et al. (2014) reported microbial reductions ranging from 0.7 to 1 log CFU/g for aerobic counts and *P. fluorescens* in salmon when treatment doses of 3.0 to 30 J/cm^2 were applied. However, undesirable changes in sensory and quality parameters were reported at the higher doses.

In the present study inactivation levels of MVC on salmon were generally achieved at lower doses than those reported in several other studies published to date for both UV-C and PL technologies. There could be several reasons to explain these observations, for example, the equipment and lamps used or the type of food matrix and their characteristics. Another possible explanation could be due to the fact that samples in our study were covered with foil immediately after treatment to avoid photoreactivation. Lasagabaster and Martinez de Marañon (2014) reported that conditions following light treatments, such as temperature and presence of daylight (visible sprectrum) could affect microbial counts. They suggested that the photorepair mechanism can be activated immediately after treatment and can peak after 30 min exposure to light. Thus, maintaining the samples at refrigeration temperatures and in darkness could prevent this mechanism becoming activated. A further possible reason, related to the lower doses applied, could be due to the fact that microbial inactivation with UV light can occur during the first seconds of treatment (Condón-Abanto, Condón, Raso, Lyng, & Álvarez, 2016), generating inactivation kinetics similar to those observed (Figures 2 and 4). These results showed a significant decrease during the first seconds followed by a tail as longer treatment times did not produced significantly greater levels of inactivation. Therefore, in the present study it was decided to optimize treatment conditions in

order to avoid excessive treatments to raw salmon which could adversely affect organoleptic characteristics.

A number of microorganisms considered capable of causing spoilage and microbial groups naturally present in skin-packaged salmon were exposed to UV-C and PL light in order to assess their decontamination potential. Inactivation levels after either UV-C or PL were similar for all groups assessed with the exception of *Pseudomonas* spp. which were more resistant.

Many factors can affect the efficacy of light-related technologies in food decontamination, including food composition, product thickness, initial contamination levels and the dose applied (Gómez-López et al., 2007). A number of factors could explain why *Pseudomonas* spp. appeared to be the least susceptible group investigated in this study. Initial counts of all groups were similar (10^6-10^7 CFU/g) with the exception of Enterobacteriaceae and *Listeria* spp. (10^3-10^4), so differences in levels of inactivation observed were unlikely to be due to differences in initial concentrations of each bacterial group.

Moreover, *Pseudomonas* spp. have the ability of create biofilms (Danielsson, Norkrans & Bjornsson, 1977). Biofilms are aggregated populations of microorganisms embedded in a matrix of extracellular polymeric substances which can adhere to surfaces and create a symbiotic environment where cells are protected from external factors (Pozo, Olmos, Orgaz, Božanić & González-Benito, 2014). In these biofilms, bacteria can be present in multiple layers, which can cause a shadowing effect, with those in the lower layers shielded from the light (Hiramoto, 1984).

Among the *Pseudomonas* spp. isolated after treatments, *P. fluorescens* was selected to be tested against light based technologies in fish contact surfaces. *P. fluorescens* is an important fish spoilage bacterium which has an ability of form thick biofilms which may also serve as an anchoring layer for pathogenic microorganisms (Pozo et al., 2014). As a result, *P. fluorescens* has been widely isolated in salmon processing plants, equipment and machines even after sanitization which could be an important source of contamination between products and batches (Møretrø et al., 2016). Therefore, it was considered as an interesting target microorganism for investigating the efficacy of these technologies to decontaminate fish contact surfaces.

In order to evaluate the decontamination effect of UV-C and PL on food contact surfaces, two commonly used contact materials (polyethylene and stainless steel) were inoculated with a wild strain of *P. fluorescens*. In order to simulate commercial processing conditions, the cells were inoculated in a spray containing salmon juice. Something similar was used by Sommers & Sheen (2015) who inoculated an avirulent strain of *Yersinia pestis* in a sterile fish exudate to contaminate several contact surfaces to be treated by UV-C. With treatment doses of 0.5 J/cm^2 they achieved a 4 log reduction, and with 1.0 J/cm^2 reported inactivation below the limit of detection. Haughton et al. (2011a) also evaluated the decontamination effect of UV-C over several contact surfaces and packaging materials. With initial contamination levels of 10^3-10^4, inactivation below the detection levels was achieved for *C. jejuni* and *Salmonella* Enteritidis at doses >0.012 J/cm^2, and for *E. coli* at doses >0.024 J/cm^2. Similarly, Haughton et al. (2011b) evaluated the inactivation of these microorganisms using PL and reported levels of inactivation below detection limits for *C. jejuni* at the lowest dose tested (0.9 J/cm^2) when an initial inoculum of 10^4-10^5

CFU was used, however for *Salmonella* Enteritidis and *E. coli* maximum inactivation levels of 4 log cycles were only reached when a higher energy dose was applied (6.0 J/cm^2). Woodling and Moraru (2005) observed 2.97-3.59 log reductions for *Listeria innocua* inoculated on to SS when PL at a dose of 0.89 J/cm^2 was applied.

The method of inoculation and the concentration of cells added can affect inactivation efficiency when light treatments are used. McDonald et al. (2000) reported that concentrations higher than 10^5 CFU of spores in an inoculum could reduce the efficiency of pulsed light treatments, probably due to the formation of agglomerates or layers of spores after drying. Levy, Bornard and Carlin (2011) confirmed that high spore concentrations produced overlapping and cluster formation which can affect treatment efficacy. A monolayer of bacteria is recommended and can be achieved by spraying test surfaces (Levy et al. 2011). In contrast, excessive cell densities can cause a shadowing effect, impeding light penetration and bacterial inactivation (Chen, Lung, Yang & Wang, 2015). This fact was also observed in our previous experiments, when higher cell concentrations resulted in significant variations between sample replicates, generating inconsistent data (data not shown). Similarly, when inoculated surfaces were left for more than one hour to dry before treatments, results were not consistent. This could be due to the hydrophobicity of the surfaces which could affect treatment efficiency. When the water contact angle and the inoculum reach a certain point (normally at high cell concentrations), clustering of bacteria can occur resulting in inconsistent results being observed (Woodling & Moraru, 2005).

Generally, PL treatments of inoculated PE and SS surfaces produced very satisfactory decontamination results with practically all the inoculated microorganisms inactivated or below the limit of detection. However, when UV-C was

applied to SS surfaces, only a 2 log reduction was observed. These differences between technologies also occurred at similar doses and could be attributed to characteristics of the SS, the light technologies, or a combination of both. SS surfaces can have some defect areas, with different degrees of roughness, and these areas can allow cells to congregate in single or multiple layers which could reduce the efficacy of the light treatments (Ringus & Moraru, 2013; Woodling & Moraru, 2005). However, this may not explain the higher inactivation levels achieved with PL on SS. Thus it may be that several mechanisms of inactivation including UV-C are produced by PL. Some authors have demonstrated that the main mechanism of inactivation by pulsed light is the same as for UV light, namely the photochemical effect which can cause damage to DNA, inhibiting cell replication and ultimately leading to death (Bolton & Linden, 2003; Giese & Darby, 2000; Mitchell, Jen & Cleaver, 1992). However, other researchers have suggested that PL also produces a photothermal effect, causing absorption of excessive doses of UV or pulsed light causing cell disruption followed by inactivation by PL (Takeshita et al., 2003; Wuytack et al., 2003). In reality, both mechanisms could co-exist during PL treatments with the significance of each dependent on the dose applied and the target microorganisms used (Cheigh et al., 2013).

Overall PL was found to be more effective than UV-C for the decontamination of salmon and contact surfaces. Even though, hourly rates of electrical consumption (90.9 kWh) were higher for the PL system compared to the UV-C equipment (22.8 kWh), it has been calculated that when equivalent treatment doses (mJ/cm^2) are applied, the energy consumption is similar for both technologies. For instance, an applied dose of 102 mJ/cm^2 with UV-C (24 s at 6cm) or with HILP (6 s at 3.5 cm) is equivalent to 0.152 kW per treatment.

5. Conclusions

The aim of the present study was to evaluate the decontamination efficacy of UV-C and PL technologies on raw salmon and food-contact surfaces. Both technologies proved to be effective in reducing bacterial populations in raw salmon. The optimal treatments for salmon decontamination were 30 s at 6 cm (127.2 mJ/cm^2) for UV-C and 9 s at 3.5 cm (152.6 mJ/cm^2) for PL. Higher doses did not result in significantly greater bacterial reductions and could produce undesirable organoleptic changes.

Of the groups investigated, *Pseudomonas* spp. were found to be the most resistant to both technologies, with lower levels of inactivation observed when compared to the other bacterial groups.

PL technology was more effective than UV-C light for food-contact surface decontamination when assessed using a wild strain of *Pseudomonas fluorescens* isolated from raw salmon. *P. fluorescens* showed higher resistance on inoculated stainless steel surfaces (2 log inactivation) compared to polyethylene surfaces (4 log inactivation).

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Table 1

Treatment doses (mJ/cm^2) for UV-C and PL applied to salmon (for each side) and contact surfaces (stainless-steel (SS) and polyethylene (PE)).

				Treatment dose (mJ/cm²)										
Technology	**Surface treated**	**Distance (cm)**	**Treatment time (s)**	**2**	**5**	**10**	**15**	**20**	**30**	**45**	**60**	**90**		
UV-C	*salmon*	**26**		_	15.9	_	47.8	_	95.6	143.4	191.3	286.9		
		16		_	19.1	_	57.3	_	114.5	171.8	229.1	343.6		
		6		_	21.2	_	63.6	_	127.2	190.8	254.4	381.6		
UV-C	*SS/PE*	**26**		_	15.9	31.9	47.8	_	95.6	_	191.3	_		
		16		_	19.1	38.2	57.3	76.4	114.5	_	_	_		
		6		8.5	21.2	42.4	63.6	_	_	_	_	_		
			Treatment time (s)	**1**	**2**	**3**	**4**	**6**	**8**	**9**	**10**	**12**	**15**	**30**
PL	*salmon*	**11**		_	_	40.1	_	80.3	_	120.4	_	160.6	200.7	401.4
		7.5		_	_	43.2	_	86.4	_	129.6	_	172.8	216.0	432.0
		3.5		_	_	50.9	_	101.7	_	152.6	_	203.4	254.3	508.5
PL	*SS/PE*	**11**		_	_		53.5	80.3	107.0	_	_	160.6	200.7	_
		7.5		14.4	_	43.2	_	86.4	_	129.6	_	172.8	_	_
		3.5		17.0	33.9	_	67.8	_	_	_	169.5	_	_	_

Table 2

Lipid oxidation values of salmon after treatments with ultraviolet light (UV-C) and pulsed light (PL) at different distances from the light source and treatment times.

Technology		**UV-C**				**PL**		
Distance (cm)		**26 cm**	**16 cm**	**6 cm**		**11 cm**	**7.5 cm**	**3.5 cm**
Treatment time (s)	**0**	0.22 ± 0.08^{a}	0.22 ± 0.08^{ab}	0.22 ± 0.08^{a}	**0**	0.27 ± 0.1^{a}	0.27 ± 0.1^{a}	0.27 ± 0.1^{a}
	5	0.22 ± 0.07^{a}	0.18 ± 0.02^{a1}	0.32 ± 0.04^{b2}	**3**	0.32 ± 0.06^{ab}	0.32 ± 0.06^{ab}	0.30 ± 0.01^{a}
	15	0.25 ± 0.04^{ab}	0.24 ± 0.08^{ab}	0.33 ± 0.01^{b}	**6**	0.39 ± 0.01^{b}	0.30 ± 0.02^{ab}	0.29 ± 0.07^{a}
	30	0.25 ± 0.03^{ab}	0.28 ± 0.03^{bcd}	0.31 ± 0.01^{b}	**9**	0.33 ± 0.04^{ab}	0.30 ± 0.01^{ab}	0.30 ± 0.08^{a}
	45	0.22 ± 0.02^{a1}	0.36 ± 0.01^{c2}	0.31 ± 0.04^{b}	**12**	0.34 ± 0.06^{ab}	0.30 ± 0.01^{ab}	0.35 ± 0.01^{a}
	60	0.32 ± 0.05^{b}	0.35 ± 0.01^{d}	0.35 ± 0.02^{b}	**15**	0.31 ± 0.03^{ab}	0.30 ± 0.02^{ab}	0.34 ± 0.03^{a}
	90	0.32 ± 0.02^{b}	0.35 ± 0.04^{d}	0.35 ± 0.01^{b}	**30**	0.34 ± 0.02^{ab}	0.39 ± 0.07^{b}	0.32 ± 0.01^{a}

Mean values (three replicates) ± Standard deviation (*SD*).

UV-C: ultraviolet light C; PL: pulsed light.

^{a,b,c} Different superscript letters in the same column denotes statistical differences between treatment times.

^{1,2,3} Different superscript numbers in the same row denotes statistical differences between treatment distances.

Figure 1

Diagram of ultraviolet (UV-C) treatment unit. 1. housing for UV lamps; 2. UV lamps; 3. safety interlock; 4. treatment chamber of 790 by 390 by 345 mm (L x W x H); 5. sample placement.

Figure 2

Mean log reductions of mesophilic viable counts in salmon after UV-C treatments (**A**) at 26 cm (●), 16 cm (■) and 6 cm (▲) over time, and after PL treatments (**B**) at 11 cm (●), 7.5 cm (■) and 3.5 cm (▲) over time. Each point represents mean of three replicates ± *SEM*.

Figure 3

Mean log reductions (CFU/g) for *Listeria* spp. (LIS), lactic acid bacteria (LAB), *Brochothrix thermosphacta* (BRT), *Photobacterium phosphoreum* (PHP), psychrophilic and mesophilic viable counts (PVC and MVC), Enterobacteriaceae (ENT) and *Pseudomonas* spp. (PSE) after UV-C treatments for 30 s at 6 cm (black bars) or PL treatments for 9 s at 3.5 cm (white bars) on raw salmon fillets. Each bar represents the mean of three replicates ± *SEM*. Different letters denote significant differences ($p<0.05$) between treatments for each microbial group.

Figure 4

Mean log CFU/cm^2 of *P. fluorescens* inoculated on polyethylene (white bars) and stainless steel (grey bars) contact surfaces after UV-C treatments (**A**) and after PL treatments (**B**) at different doses (mJ/cm$^{2)}$. Each bar represents the mean of three replicates ± *SEM*. Black dashed line represents the detection limit (0.25 log CFU/cm^2).

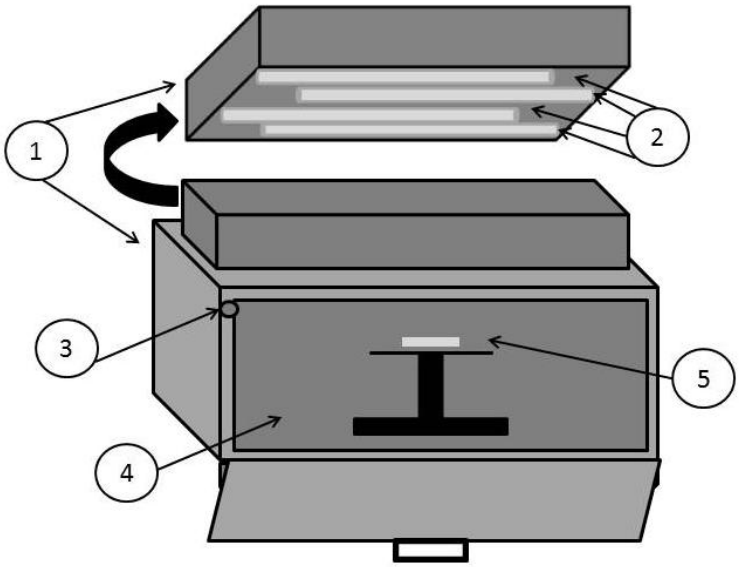
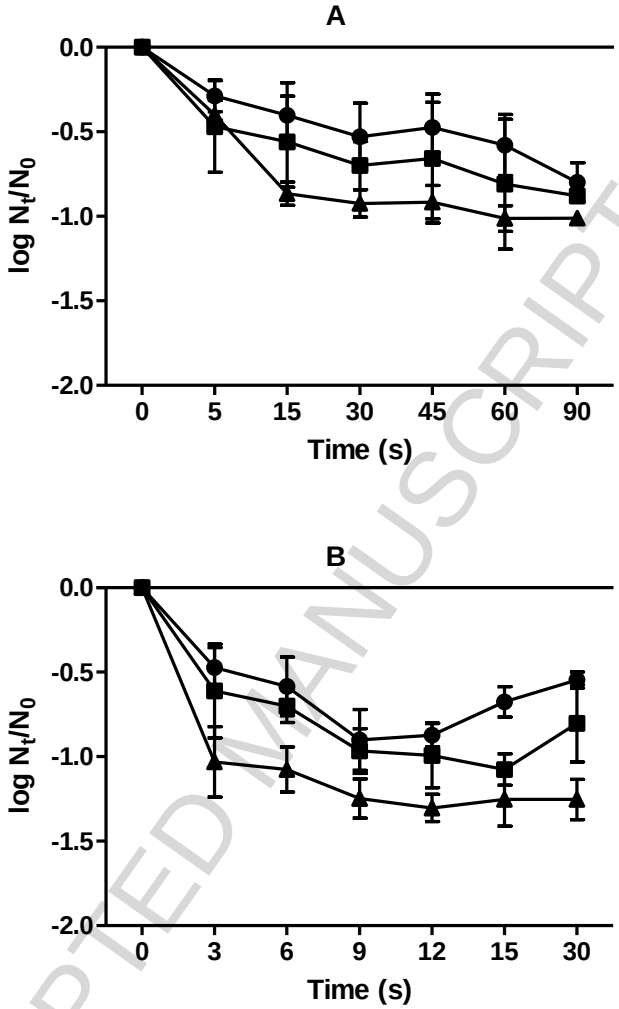


Figure 1

Figure 2



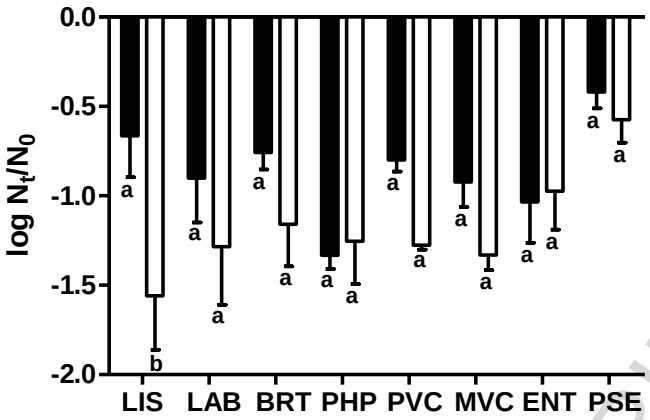


Figure 3

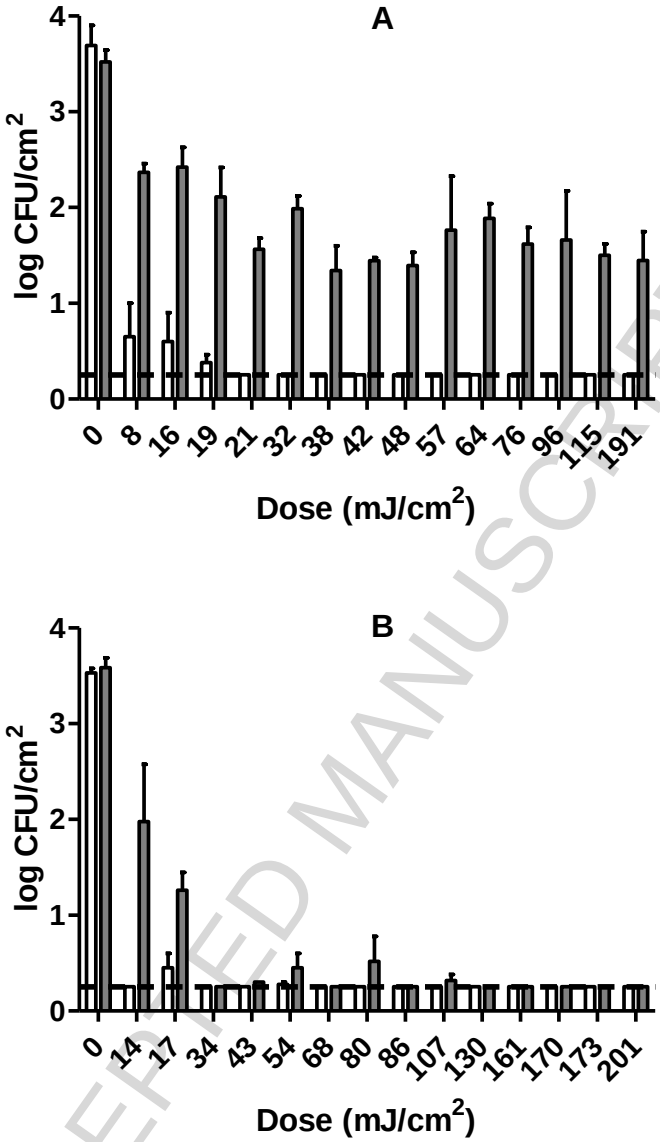


Figure 4

Highlights

- UV-C and PL treatment doses were dependant on the treatment time and the distance from the light source.
- Optimal treatment for salmon decontamination was between ~130-150 mJ/cm^2 for both technologies.
- *Pseudomonas* spp. were the most resistant bacterial group to UV-C and PL treatments on salmon
- *Pseudomonas fluorescens* inoculated on polyethylene surfaces was effectively decontaminated with low does of UV-C and PL.
- 2 log cycles of inactivation were achieved for stainless steel surfaces inoculated with *P. fluorescens*, while PL treaments resulted in reductions below the detection limit (<0.25 CFU/cm^2).