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Reductions of *Listeria monocytogenes* on cold-smoked and raw salmon fillets by UV-C and pulsed UV light

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ABSTRACT

Salmon is the food most frequently reported in the RASFF (Rapid Alert System for Food and Feed) database in conjunction with *Listeria monocytogenes* and consumption of cold-smoked salmon have led to severe outbreaks of listeriosis infections. UV-C and pulsed UV light were investigated for their ability to reduce *L. monocytogenes* on salmon. Cold-smoked and raw salmon were spiked with a mix of ten *L. monocytogenes* strains (10^4 CFU/sample) and subsequently exposed to UV-C light (0.0075–0.6 J/cm^2) or high intensity pulsed UV light (1.3–10.8 J/cm^2). Reductions of *L. monocytogenes* on smoked salmon were 0.7–1.3 log, depending on the fluence. Corresponding reductions for raw salmon muscle side and skin side were 0.2–0.9 log and 0.4–1.1 log, respectively. Generally, reductions using UV-C and pulsed UV light were within the same range, but with some treatments statistically different. *L. monocytogenes* surviving UV treatments on smoked and raw salmon grew at the same rate as controls during storage at 4 °C, but reached the levels of the controls 13 and 7 days later, respectively. No sensory changes were detected in UV-C treated (0.05 J/cm^2) smoked salmon.

Industrial relevance: Due to the lack of critical control points in salmon production, it is not possible to ensure products that are consistently free from *L. monocytogenes* in the absence of mitigation strategies. Taking into account the reported generally low levels of *L. monocytogenes* on contaminated salmon, UV treatments should be considered important tools for the industry to contribute to lower prevalence and levels of *Listeria*. The present work on microbial and quality effects of UV-C and pulsed UV light treatments performed under industry relevant conditions on raw and cold-smoked salmon provides important information to the salmon industry for implementation of UV-light as risk reducing mitigation tools. This has key relevance for industry and consumers and will contribute to enhanced food safety, reduction of costly recalls and longer shelf-life.

1. Introduction

Listeria monocytogenes is a ubiquitous foodborne pathogen and has been found in a range of foods including milk and dairy products, meat and egg products, seafood, vegetables, and other ready-to-eat (RTE) foods (Farber & Peterkin, 1991). It can multiply at low temperatures and under high salt conditions. *L. monocytogenes* is a facultative intracellular parasite being capable of living and reproducing either inside or outside cells.

In 2016, 2536 confirmed invasive cases of human listeriosis were reported in the European Union (European Food Safety Authority, 2017). The incidence of listeriosis was 0.47 cases annually per 100,000 population. The EU case fatality rate was 16.2% among the 1524 confirmed cases with known outcome.

Sporadic cases and outbreaks of listeriosis have generally been associated with those RTE foods that are held for extended periods at refrigeration and chill temperatures which allow growth to high numbers at the time of consumption (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017). Foods involved in sporadic cases and outbreaks have been reported to contain 10^1–10^9 *L. monocytogenes*/g (European Commission, 1999). Only few, although increasing number of cases of listeriosis have been linked to cold-smoked fish. An outbreak of listeriosis in Sweden was probably caused by *L. monocytogenes* in "gravad" or cold-smoked rainbow trout (Ericsson et al., 1997). Here, *L. monocytogenes* of the same clonal type was found in six of nine patients and also in unopened packages of fish from the suspected producer. In a Norwegian survey, fish-associated isolates, based on multiple-locus variable number tandem repeat analysis (MLVA typing), were also found to match types isolated from humans (Lunestad, Truong, & Lindstedt, 2013). One of these was associated with outbreaks from

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other foods in Norway indicating that fish could be a possible food vehicle in conjunction with listeriosis. In Denmark, at least three outbreaks have been caused by cold-smoked fish in the period 2014–2017 (Lassen et al., 2016; Schjorring et al., 2017). The cooperate use of whole genome sequencing (WGS) and epidemiological methods was key in solving these serious outbreaks which in total comprised at least nine deaths and extensive recall of smoked fish products in Danish supermarkets. These and other studies have shown the strength of WGS to determine links between isolates from food, environments and human cases and to identify low-intensity, multi-country outbreaks that otherwise could have gone unresolved. Therefore, routine WGS will increase the frequency of finding food causing outbreaks and likely reinforce the suspicion that RTE fish products are important sources of *L. monocytogenes* infection (Fagerlund, Langsrud, Schirmer, Moretro, & Heir, 2016; Lassen et al., 2016; Lüth, Kleta, & Al Dahouk, 2018).

L. monocytogenes is widely distributed in food processing environments (Buchanan et al., 2017), although the prevalence may vary considerably from < 1% up to 80% of environments tested (Jami, Ghanbari, Zunabovic, Domig, & Kneifel, 2014). Generally, presence of *L. monocytogenes* in the food processing environment is thought to be the primary source of post-processing contamination during food manufacturing (Buchanan et al., 2017; Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014; Moretro & Langsrud, 2004). The main cause of listeriosis is consumption of food contaminated from sources in the food processing environments (Ferreira et al., 2014) or at retail level (Endrikat et al., 2010; Pradhan et al., 2010). *L. monocytogenes* is often found in smoked fish production environments (Ferreira et al., 2014; Moretro et al., 2017; Moretro & Langsrud, 2004) including machines for salting, skinning and slicing and occasionally in seawater, and from fish under processing and from final products.

Listeria spp. are components of the indigenous microbiota in surface water, where fish can be contaminated with *L. monocytogenes* on the fish surface, in the stomach lining, gills, and their intestines (Jami et al., 2014). Contaminated fish can transfer *L. monocytogenes* into processing facilities and be a source to both processing environment and final product contamination.

Different studies report variations in prevalence of *L. monocytogenes* (1.3 to 80.3%) on cold-smoked salmon (Jami et al., 2014; Tocmo et al., 2014). A European-wide baseline survey in 2010 and 2011 revealed that 17.4% of 599 cold-smoked fish samples were contaminated with *L. monocytogenes* at sampling (Anon., 2013). Generally, the levels were low with 2.0% of 3053 samples exceeding 100 CFU/g at the end of shelf-life.

The legislation regarding *L. monocytogenes* in RTE food products in different countries has been summarized (Jami et al., 2014). The U.S. Food and Drug Administration (FDA) requires absence of the bacterium in 25-g samples of RTE seafood products (FDA, 2011). EU has a zero tolerance for infant foods and for RTE foods for medical purposes, while RTE foods that contain < 100 CFU/g at the end of shelf-life are accepted (Anon., 2005). Presence of *L. monocytogenes* in traded products are considered representing health risks and are notified in the EU Rapid Alert System for Food and Feed (RASFF) notification database. Smoked salmon was in 2016 the food most often notified in conjunction with *L. monocytogenes* (Anon, 2017).

There are no critical control points during the cold-smoking process that will guarantee the elimination of *L. monocytogenes* on the final product. Given the ubiquitous nature of *L. monocytogenes*, the lack of listericidal steps in the cold-smoking procedure, and the ability of the organism to become established in the processing environment and contaminate products, it is not possible to produce cold-smoked fish consistently free of *L. monocytogenes*. By adhering strictly to Good Manufacturing Practices (GMPs) and Good Hygienic Practices (GHPs) to prevent recontamination, by obtaining the raw materials from known sources (for example, from producers with a history of non-contaminated fish), by freezing or limiting shelf-life of the product or by using preservatives that can inhibit growth at refrigerated temperatures, it is possible to produce cold-smoked fish with low levels of *L. monocytogenes*, preferably at < 1 cell/g at the time of production (Anon, 2001). Novel emerging decontamination technologies may also contribute to reducing the level of contamination.

Depending on the processing and storage conditions, *L. monocytogenes* can grow to high numbers on salmon fillets and cold-smoked salmon. This leads to enhanced interest in additional decontamination strategies of which several have been tested including the use of organic acids or their salts, phages (Soni & Nannapaneni, 2010), nisin (Soni, Shen, & Nannapaneni, 2014), protective cultures (Matamoros et al., 2009), and lauryl arginate (Soni et al., 2014) and a number of other compounds (Tocmo et al., 2014). Several of these are not approved for use in the EU on cold-smoked salmon.

In recent years, the use of UV light as a surface decontamination method has been met with increasing interest (Holck, Liland, Drømtorp, Carlehøg, & McLeod, 2017; McLeod et al., 2018). UV-C light is emitted primarily at 254 nm, while the UV energy spectrum of pulsed UV light is caused by bremsstrahlung (braking radiation) and covers the whole spectrum from UV (200 nm) into the infrared region (1100 nm). UV-C light provides effective inactivation of microorganisms by damaging nucleic acids through creating nucleotide dimers, and thus leaving the microorganisms unable to perform vital cellular functions. In addition to creating nucleotide dimers, pulsed UV light has been suggested to cause cell death by induction of cell membrane damage (Takeshita et al., 2003) and rupture of the bacteria by overheating caused by absorption of all UV light from the flash lamp (Wekhof, Tropeter, & Franken, 2001). Also, disturbances caused by high-energy pulses have been suggested to contribute to cell damage (Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2010).

Only few studies on the use of UV light and pulsed UV light in conjunction with salmon are reported. Generally, reductions have been obtained in the range 0–1.9 log depending on the type of UV treatment, the fluence, and the product tested (Cheigh, Hwang, & Chung, 2013; Miks-Krajnik, Feng, Bang, & Yuk, 2017; Ozer & Demirci, 2006; Shaw, 2008).

Regulations in conjunction with using conventional continuous UV-C light and pulsed UV light in the USA are given by the FDA (U.S. Food and Drug Administration, 2017). Pulsed UV light has been approved by the FDA up to 12 J/cm^2 as a means for controlling surface microorganisms on food products. UV-C light can be employed in the EU; however, in Germany the use is limited to water, fruit and vegetable products and stored hard cheeses (Anon, 2000).

The effectiveness of UV-C and pulsed UV light for decontamination depends on the time a microorganism is exposed, the intensity and wavelength of the illumination, the microorganism's ability to withstand the UV exposure, properties of the food surface, the penetration of the UV light and the presence of particles shielding the microorganisms. To the end of enhancing food safety, the efficiency of UV-C and pulsed UV light against a mix of fish-associated *L. monocytogenes* strains on cold-smoked salmon, raw salmon muscle and skin under conditions relevant for practical implementation was evaluated. The influence of UV treatments on sensory properties of the fish products was also investigated.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The ten *L. monocytogenes* strains used in the experiments are shown in Table 1. The 10 strains used included six strains isolated from salmon and salmon processing facilities (Moretro et al., 2017), three strains associated with human listeriosis and one strain was isolated from cattle. The strains represented three serotypes commonly associated with human listeriosis and different multilocus sequence typing (MLST) and MLVA types. The strains were maintained at −80 °C in Brain Heart Infusion (BHI) broth with 15% (v/v) glycerol. For each experiment,

Table 1
Strains used in the present work.

Strain no.	Serotype	MLVA/ST[a]	Source[b]	Other designations; Reference
MF3860	1/2a	6-10-5-16-6/20	Salmon processing, Plant S4	(Moretro et al., 2017)
MF3939	1/2a	5-8-15-10-6/14	Salmon processing, Plant S3	(Moretro et al., 2017)
MF4001	1/2a	5-8-15-10-6/14	Salmon processing, Plant S2	(Moretro et al., 2017)
MF4077	1/2a	6-9-18-16-6/8	Salmon processing, Plant S1	(Moretro et al., 2017)
MF4588	1/2a	7-7-10-10-6/7	Salmon processing, Plant S1	(Moretro et al., 2017)
MF4804	1/2a	6-7-14-10-6/121	Salmon processing, Plant S2	(Moretro et al., 2017)
MF2184	1/2b	7-8-0-16-0/3	Meat processing, outbreak	2583/92; (Rudi, Zimonja, Hannevik, & Dromtorp, 2006)
MF3009	1/2b	n.d./5	Cattle	FSL J2-064; (Fugett, Fortes, Nnoka, & Wiedmann, 2006; National Institutes of Health, 2018)
MF3039	4b	n.d./6	Human, cerebrospinal fluid, outbreak	FSL N1-227; (Fugett et al., 2006)
MF3710	4b	7-7-20-6-10/n.d.	Human, cerebrospinal fluid	CCUG3998; Culture Collection University of Gothenburg

[a] MLVA designation according to (Moretro et al., 2017). ST numbers refer to Institute Pasteur MLST database (Moura et al., 2017), n.d., not determined.
[b] Plant designation according to (Moretro et al., 2017).

strains were cultured separately on BHI agar at 37 °C, 24 h and single colonies were picked to inoculate 2-ml BHI broth before incubation at 37 °C for 24 h. These pre-cultured strains were thereafter again inoculated (1%) separately in 2-ml BHI broth. After incubation at 37 °C for 24 h, the bacterial cultures were mixed to contain approximately equal cell numbers of each of the strains. The ten-strains cell-culture mix was stored at 4 °C for 20–24 h for cold adaptation. Dilutions to working solutions were performed in 0.9% (w/v) NaCl.

2.2. UV treatment of salmon spiked with L. monocytogenes

Fresh and cold-smoked salmon fillets were obtained from a salmon processor and local producer, respectively. Pieces of approximately 3.1 × 3.1 × 0.5 cm^3 were cut, maintaining the original surface of the salmon fillets, and 20 µl of the *L. monocytogenes* cocktail (5 × 10^5 CFU/ml) were spread on the surface of the salmon piece by a sterile plastic spreader unless otherwise stated. The surface was subjected to UV treatment after approx. 5–10 min unless otherwise stated. In some experiments, the *L. monocytogenes* was added to the salmon in small droplets (4 × 5 µl). Also, in some experiments, fish samples were illuminated twice with the samples lying on a flat surface for the first exposure and being bent over a scaffold for the second exposure. Fish samples were held at 4 °C. In the continuous UV-C light experiments, samples were treated in a custom made aluminum chamber (1.0 × 0.5 × 0.6 m^3) equipped with two UV-C lamps (UV-C Kompaktleuchte, 2 × 95 W, BÄRO GmbH, Leichlingen, Germany) in the ceiling. The UV-C light was emitted essentially at 253.7 nm, and measured using a UVX Radiometer (Ultra-Violet Products Ltd., Cambridge, UK) equipped with a UV-C sensor (model UVX-25, Ultra-Violet Products Ltd., Cambridge, UK). Samples of salmon were placed in empty petri dishes for illumination. Parameters of intensity and exposure times were chosen with aim to be relevant in industrial production lines. Samples were exposed at a power intensity of 2 mW/cm^2 for 3.75 and 7.5 s giving fluences of 0.0075 and 0.015 J/cm^2, respectively, or 10 mW/cm^2 for 5, 10 or 60 s, giving fluences of 0.05, 0.1, 0.6, J/cm^2, respectively. For pulsed UV light treatments, the instrument XeMaticA-SA1L (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany) was employed. The instrument was equipped with a xenon flash lamp (19 cm), which was water cooled, with an aluminum reflector (with opening 10 cm × 20 cm), and emitted light of 200–1100 nm with up to 45% of the energy being in the UV-light region with maximal emission at 260 nm for high energy pulses (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany). Samples were illuminated at 6.5 cm distance barely beneath the opening of the reflector. At this distance, the fluence could be calculated according to the manufacturer's specifications as the total discharge energy of the lamp divided by the opening area of the reflector. The fluence of each pulse was adjusted to 1.3 J/cm^2 (low) or 3.6 J/cm^2 (high) by adjusting the discharge voltage. The samples were exposed with single pulses either once to the low pulse (1.3 J/cm^2), or one or three times to the high pulse (3.6 or 10.8 J/cm^2), respectively. Three parallels for each UV treatment and three or six untreated controls were used in each experiment. The UV experiments were repeated three times on different days. In storage experiments, UV treated samples were stored in vacuum bags at 4 °C for 28 days (cold smoked salmon) and 14 days (raw salmon). Storage experiments were carried out once with five (raw salmon) or six (cold-smoked salmon) parallels for each sampling point. All reductions are given as $\log_{10}$ CFU/cm^2 reductions (abbreviated log in manuscript). Temperatures were measured using a Raynger MX infrared thermometer (Raytek Corporation, Santa Cruz, USA). The experiments were performed in a Biosafety level 3 pilot plant.

2.3. Microbial analyses

Illuminated samples were transferred to stomacher bags and 40-ml peptone water (0.1% (w/v) bacteriological peptone, Oxoid Ltd., England, 0.85% (w/v) NaCl) was added. The samples were stomached for 1 min and appropriate 10-fold dilutions in peptone water were plated on RAPID'L.mono agar (Bio-Rad, CA, USA) and incubated at 37 °C for 24 h. Total counts were determined by plating on blood agar petri dishes (Oxoid blood agar base supplemented with 50 ml horse blood/l, Oxoid,UK) and incubating aerobically at 15 °C for 5 days.

2.4. Consumer test

Odor and appearance of cold-smoked salmon after UV light exposure were assessed in a consumer test. Five different treatments of cold-smoked salmon pieces were evaluated: controls without UV exposure, pieces subjected to UV-C fluences 0.0075 J/cm^2 or 0.05 J/cm^2, and pieces exposed to pulsed UV light at 1.3 or 3.6 J/cm^2. Both the fish muscle side and the skin side of the pieces were evaluated 19 days after illumination. Samples were held at room temperature in plastic dishes covered with a lid. Samples were evaluated in a randomized manner by 40 untrained panelists (consumers) in two sessions, muscle and skin side separately. The consumers were asked what they thought about the overall quality of the sample on a hedonic category scale from 1 (very bad) to 9 (very good). They were also asked one question: “Would you use this sample in a meal?”

2.5. Sensory analysis by a trained sensory panel

A trained panel of nine assessors at Nofima performed a sensory descriptive analysis according to “Generic Descriptive Analysis” as described by Lawless and Heymann (Lawless & Hildegard, 2010) and ISO 13229 Sensory analysis – Methodology - General guidance for establishing a sensory profile (2016). The assessors were tested, selected and trained according to ISO standards (ISO 8586:2012), and the sensory laboratory used followed the ISO standards (ISO, 8589:2007). Commercial cold-smoked salmon was obtained vacuum-packed and refrigerated from a local processor one day after production. Smoked

salmon pieces were subjected to UV-C light at different fluences: 0 (control), 0.0075 J/cm^2, 0.05 J/cm^2 and 0.1 J/cm^2. Samples were randomized so that pieces from the front, middle and rear sections of the fillets received all UV treatments. The pieces were vacuum-packed and stored at 4 °C for 19 days, before being cut into 0.4 cm thick slices and served to panelists for examination. The samples were served at room temperature on white dishes identified by random three-digit numbers. Each panelist recorded their results at individual speed on a 15 cm non-structured continuous scale with the left side of the scale corresponding to the lowest intensity, and the right side of the scale corresponding to the highest intensity. The computer transformed the responses into numbers between 1 = low intensity, and 9 = high intensity. Samples were served in two replicates in randomized order following a balanced block experimental design. Twenty two sensory attributes were evaluated in the descriptive sensory analysis of the smoked salmon: sourness odor, marine odor, fish odor, smoke odor, sunburnt odor, cloying odor, rancid odor, color hue, color intensity, whiteness, sourness flavor, salty taste, bitter taste, marine flavor, fish flavor, smoke flavor, metallic flavor, cloying flavor, rancid flavor, hardness, juiciness, toughness.

2.6. Physical analyses

pH was determined using a sensION + pH 31 pH meter, (Hach Company, Loveland, CO, USA). Water activity (a_w) determinations were carried out employing an Aqualab dew point water activity meter 4TE (Decagon devices, Inc., Pullman WA, USA).

2.7. Statistical analyses

Analysis of variance (ANOVA) was used to determine statistically significant effects on the bacterial reduction by the treatments. All analyses were performed in R (R_Core_Team, 2016). A significance level of $\alpha = 0.05$ was used, meaning that samples were considered statistically different for P-values < 0.05. For both consumer test and sensory analysis with trained panel, analysis of variance (ANOVA) was used. A two-way model, with interactions and with the consumer/assessor and interaction effects considered random, was performed on the sensory data in order to identify the parameters that discriminated between samples. The statistical software used in consumer and sensory analysis was EyeOpenR® (Logic8 BV, Utrecht, the Netherlands).

3. Results

3.1. Reduction of L. monocytogenes on cold-smoked salmon fillets

The bacterial reductions after continuous UV-C and pulsed UV light of the fillet surface (muscle side) of cold-smoked salmon were between 0.7 log and 1.3 log, depending on the UV dose (Fig. 1 and Supplemental material Table S1). Some additional reduction could in most cases be obtained by increasing the UV dose. However, this effect appeared variable. For example, additional reduction was obtained by increasing the UV-C dose from 0.0075 J/cm^2 to 0.05 J/cm^2, and by increasing the pulsed UV fluence from 1.3 J/cm^2 to 3.6 J/cm^2. However, a further increase in the fluence did not give enhanced reduction. By comparing UV-C and pulsed UV treatments, it was apparent that the reductions were in the same range. When comparing these treatments using ANOVA, the *L. monocytogenes* reduction obtained using the 0.050 J/cm^2 UV-C treatment was statistically different ($P = 0.002$) from the 0.0075 J/cm^2 UV-C, 0.015 J/cm^2 UV-C and the 1.3 J/cm^2 pulsed UV treatments.

In experiments where *L. monocytogenes* was spread on agar plates with a smooth surface and subjected to the similar treatments as above, 5- and 6-log reductions were obtained even with mild UV treatments (Holck et al., 2017). Some bacteria may be shielded from the UV light due to the uneven surface of the smoked salmon (Gomez-Lopez, Ragaert, Debevere, & Devlieghere, 2007). Therefore, the effect of

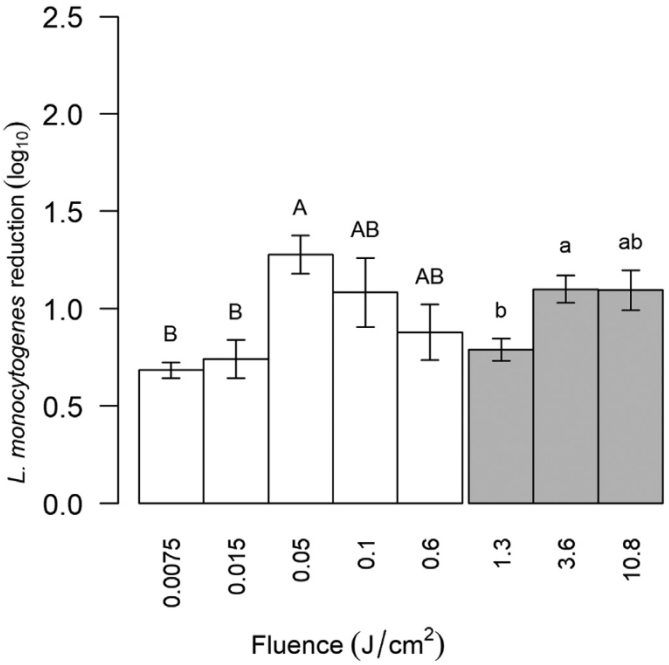

Fig. 1. Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light treatments on cold-smoked salmon fillet muscle surface lying flat. Samples with upper and lower case letters were analysed separately by ANOVA. Samples containing the same letter were not considered different.

illuminating the smoked salmon with two exposures of UV light with either the fish lying on a flat surface for both exposures or with the fish lying flat on the first exposure and being bent over a scaffold for the second illumination was compared (Fig. 2). In these sets of experiments, with reductions ranging from 0.7 log to 1.6 log, the increase in total fluence lead to enhanced reduction of *L. monocytogenes*. No statistically enhanced (all P-values > 0.2) reduction was obtained when exposing the samples for an additional dose of UV light when the samples were lying flat or in a combination of flat and bent position.

Fish may be contaminated in different ways, by direct contact or by bacteria in aerosols or suspended in liquid. It may also be of importance for efficiency of UV illumination how long the *Listeria* have been attached to the meat surface prior to UV-treatment. Therefore, the smoked salmon muscle surface was contaminated by spreading *L. monocytogenes* with a sterile plastic rod and by adding the contamination in small droplets. The contaminated salmon was treated with UV light and analysed immediately after contamination or treated with UV light 24 h after contamination (Fig. 3). Depending on the conditions, reductions ranged from 0.4 to 2 log. For *L. monocytogenes* spread on the surface, there was no difference in reduction if the bacteria were treated with UV light and analysed directly after contamination or after 24 h. However, when the *Listeria* contamination was added in droplets, the reduction was 1 log higher when UV-C treatment was performed immediately after contamination compared with treatments after 24 h. For the pulsed UV treatment the corresponding difference was 0.5 log.

3.2. Reduction of L. monocytogenes on raw salmon fillets

L. monocytogenes were also applied to the fillet muscle surface and skin side of raw salmon fillets. The pieces were subsequently subjected to different fluences of continuous UV-C and pulsed UV light, resulting in bacterial reductions between 0.2 log and 1.1 log, depending on the UV treatment (Fig. 4 and Supplemental material, Table S2). For UV-C treatments an additional reduction of *L. monocytogenes* was obtained when increasing the UV fluence. The reduction was, however, low. For *L. monocytogenes* contaminating raw salmon meat and skin, increasing

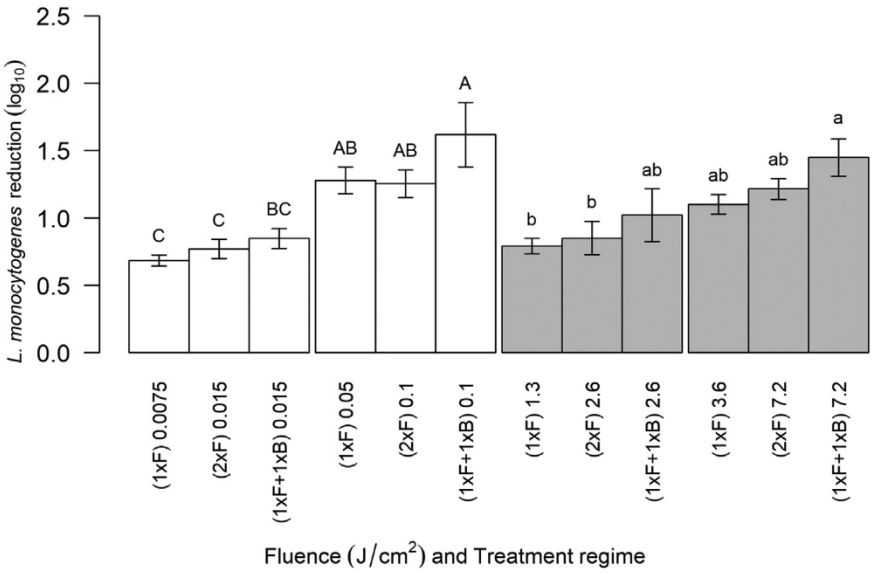

Fig. 2. Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light treatments on cold-smoked salmon fillet muscle surface lying flat and bent. Illuminations were either given as a single dose while the fish was lying flat (1 × F) or as two separate doses while the fish was lying flat (2 × F) or the first dose while lying flat and the other dose when bent (1 × F + 1 × B). Samples with upper and lower case letters were analysed separately by ANOVA. Samples containing the same letter were not considered different.

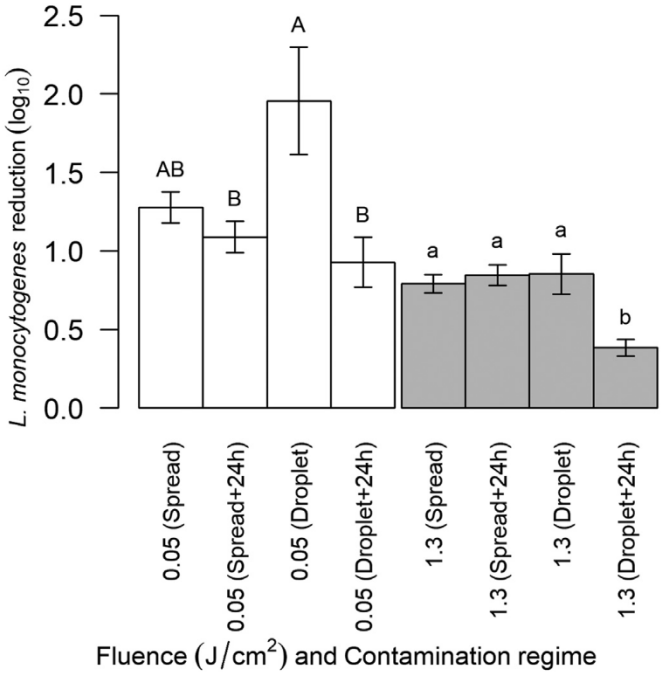

Fig. 3. Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light treatments on cold-smoked salmon fillet muscle surface contaminated by spreading or by application in small droplets. The contamination was either spread by a sterile plastic rod and UV treated and analysed immediately (Spread) or UV treated after 24 h (Spread + 24 h), or added as droplets and UV treated and analysed immediately (Droplet) or UV treated after 24 h (Droplet + 24 h). Samples with upper and lower case letters were analysed separately by ANOVA. Samples containing the same letter were not considered different.

the UV-C dose 80-fold only gave 0.6 and 0.7 log increase in reduction up to 0.9 and 1.1 log reduction for the meat and skin surface, respectively. For pulsed UV, the increase in fluence did not lead to an increase in *L. monocytogenes* reduction, which remained in the ranges 0.4–0.5 and 0.7–0.9 for muscle and skin side, respectively. When comparing UV-C and pulsed UV treatments, the pulsed UV treatments were not statistically different from the UV-C treatments in the range 0.015 to 0.1 J/cm^2.

The reduction was somewhat higher on the skin side compared with the raw salmon meat side, as was confirmed by ANOVA when comparing over all fluences, both for UV-C and pulsed UV light (not shown). Also, when comparing *Listeria* reductions for raw salmon muscle side and raw salmon skin side with those of smoked salmon, reductions for unsliced smoked salmon were higher or similar to those of raw skin and consistently higher than those for raw salmon muscle (not shown).

Fresh salmon muscle were subjected to two exposures of UV light with either the fish lying on a flat on a flat surface for both exposures or with the fish lying flat on the first exposure and being bent over a scaffold for the second illumination (Fig. 5). Also, in these sets of experiments the increase in total fluence lead to enhanced reduction in *L. monocytogenes* ranging from 0.2 to 0.9 log for UV-C treatments. No such dose-response effect was achieved for the pulsed UV treatments. No statistically enhanced reduction was obtained when exposing the samples of raw fillet muscle to an additional dose of UV light when the samples were lying flat or in a combination of flat and bent treatments, neither for UV-C nor pulsed UV treatments.

The influence of applying the *Listeria* contamination on fresh salmon meat and skin, as a direct contact contamination or as droplets, and how time (24 h) between contamination and UV treatment affected *L. monocytogenes* reductions were examined. For UV-C treatments the reductions were 0.4 log and 0.7–0.8 log for raw salmon muscle and skin side, respectively, regardless of application mode and whether samples were analysed immediately after contamination and UV treatment or exposed to UV light 24 h after contamination (not shown). Similarly, the corresponding results for pulsed UV treatments were 0.6 log and 0.9 log reductions for raw salmon muscle and skin side, respectively, regardless of application mode and whether samples were analysed directly after contamination and UV treatment or UV treated 24 h after contamination (not shown).

Weibull models were constructed from the reduction data for UV-C and pulsed UV treatments for cold-smoked salmon, raw salmon fillets and raw salmon skin (Fig. 6, Supplemental material Table S3). The models confirmed the general impression that *Listeria* directly exposed to UV light is killed at low doses, and that the doses must be increased many-fold to achieve some additional reduction. Also, the models indicated that reduction is lower when treating fresh salmon fillet compared with cold-smoked salmon.

3.3. Growth of *L. monocytogenes* during storage

To determine whether the *L. monocytogenes* surviving UV treatment behaved similarly to untreated cells, contaminated cold-smoked and

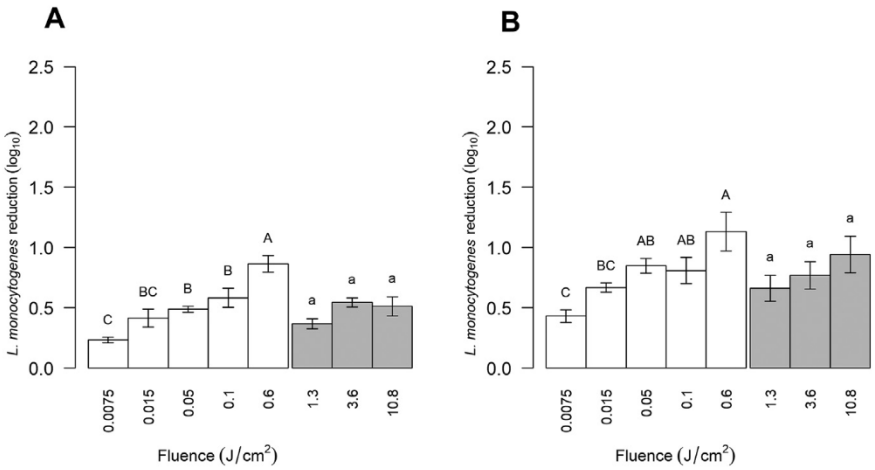

Fig. 4. Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light treatments on (A) raw salmon fillet muscle surface and (B) raw salmon skin side. Samples with upper and lower case letters were analysed separately for Fig. A and B by ANOVA. Samples containing the same letter were not considered different.

raw salmon were subjected to 0.050 J/cm^2 UV-C treatments and stored under vacuum at 4 °C for 28 and 14 days, respectively (Fig. 7). For smoked salmon an immediate *L. monocytogenes* reduction of 0.85 log was obtained by the UV-C treatment. During storage, the growth curves indicated a similar growth rate of the UV treated and the control samples up to 21 days of storage, with the UV treated samples being 0.9 log lower. The reduction of *L. monocytogenes* implied that levels reached at day 15 for the untreated samples were reached at day 28 for the UV treated samples. The curves show that the surviving *L. monocytogenes* had a similar lag phase and grew equally well as untreated cells. It also indicated that any reduction of the background flora by UV light did not influence the proliferation of the *Listeria*. Similar results were obtained when exposing spiked raw salmon to 0.050 J/cm^2 of UV-C light. After the UV treatment, an immediate reduction of *L. monocytogenes* of 0.7 log was observed. On the average, this difference remained essentially unchanged during the 10 first days of storage. The level of *L. monocytogenes* reached at day 7 for the untreated samples was not reached until day 14 for the UV-treated samples. The results again indicated that the *Listeria* surviving UV treatment would grow at the same rate as untreated cells and that any reduction of the background flora would not influence the growth. In a similar set of experiments, cold-smoked and raw salmon were subjected to 0.050 J/cm^2 UV-C light treatment, thereafter spiked with *L. monocytogenes* and then stored under vacuum at 4 °C for 28 and 14 days, respectively. The *Listeria* grew equally well on UV-C treated samples and corresponding untreated samples, again indicating that any reduction in the natural background flora by the UV light would not influence the growth of *L. monocytogenes* (results not shown). The total background flora of untreated smoked salmon was 3 log CFU/cm^2 at the start of the storage experiments at 4 °C, increasing to approximately 4 log CFU/cm^2 after 14 days with a further increase up to 6.3 log CFU/cm^2 at day 28. For raw salmon, the endogenous background flora grew from 6.11 ± 0.54 CFU/sample on day 0 to 9.18 ± 0.13 log CFU/sample after 14 days of storage.

3.4. Sensory analyses of cold-smoked salmon

Quality of odor and appearance of cold-smoked salmon after UV light exposure were assessed in a consumer test with 40 respondents. Samples subjected to UV-C fluences 0.0075 J/cm^2 or 0.05 J/cm^2, and samples exposed to pulsed UV light at 1.3 or 3.6 J/cm^2 were evaluated. The respondents were asked “What do you think about the quality of this piece of cold-smoked salmon?” Averaged answers for the fillet side ranged from 5.83 to 6.22 on a scale from 1 to 9, of which none were statistically different from the untreated control (score 6.05). Similarly, corresponding results for the skin side of the samples ranged from 5.88 to 6.20, which were not statistically different from the control (score 5.95). The respondents were also asked if they would use the sample in a meal. For the fillet and skin side, the answers were 87% and 77.5% yes, respectively, with no statistical differences between the treated and

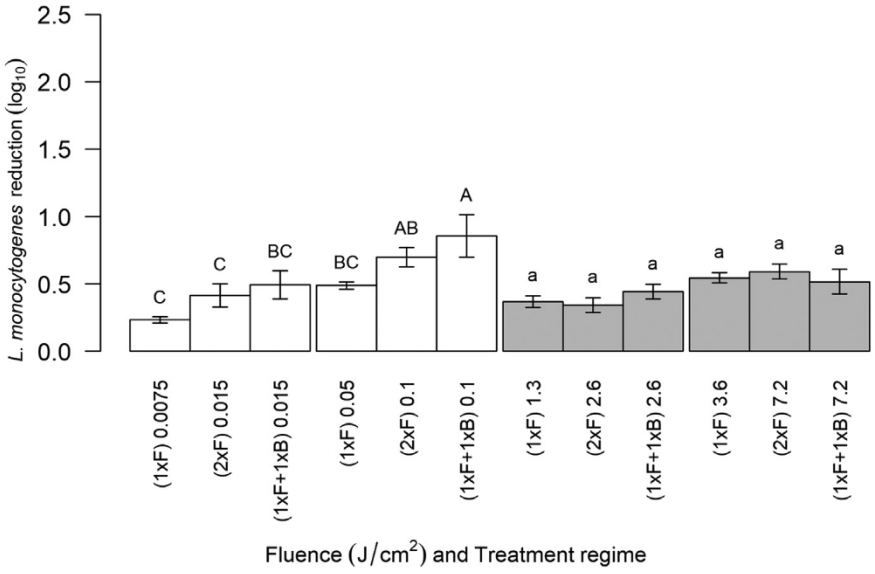

Fig. 5. Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light treatments on raw salmon fillet muscle surface lying flat and bent. Illuminations were either given as a single dose while the fish was lying flat (1 × F) or as two separate doses while the fish was lying flat (2 × F) or the first dose while lying flat and the other dose when bent (1 × F + 1 × B). Samples with upper and lower case letters were analysed separately by ANOVA. Samples containing the same letter were not considered different.

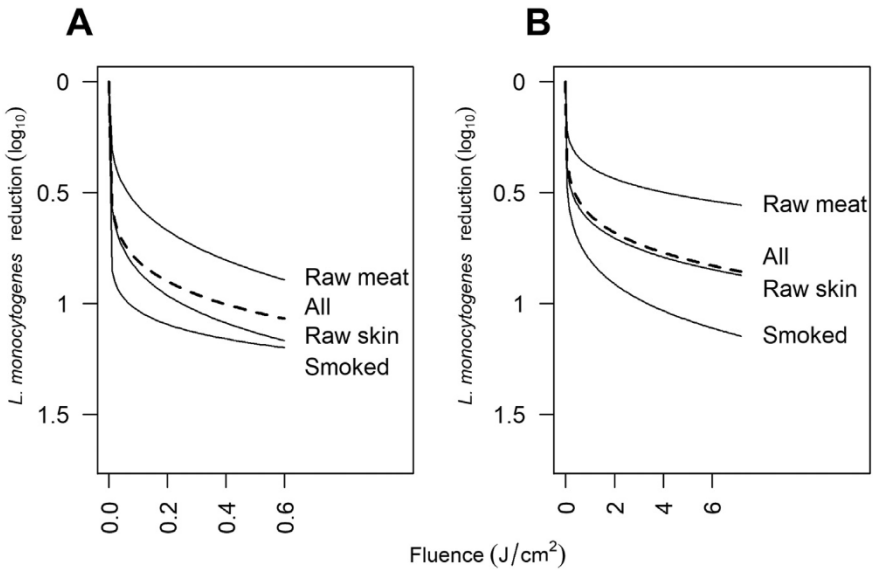

Fig. 6. Weibull models for *L. monocytogenes* log reduction as a function of UV exposure. Models for each surface (continuous lines) and common models (dotted line) are shown for bacterial reduction on salmon after (A) continuous UV-C and (B) pulsed UV light exposures at different fluences (J/cm^2).

the control samples. In conclusion, no consistent changes in the sensory properties were detected after the UV treatments of cold-smoked salmon by the consumers.

UV-C treatment of cold-smoked salmon was thereafter chosen for analysis by a trained sensory panel. The salmon had pH 5.95 ± 0.01 and a_w = 0.961 ± 0.006. Cold-smoked salmon fillets were subjected to UV-C light treatments at 0.0075 J/cm^2, 0.050 J/cm^2 and 0.1 J/cm^2, vacuum packed and stored for 19 days before analysis. Of the 22 evaluated sensory attributes, the only statistically different attributes were rancid flavor and salty taste. For the rancid flavor, the samples exposed to 0.0075 J/cm^2 scored higher (score 2.17 on the scale from 1 to 9) than the samples exposed to 0.1 J/cm^2 (score 1.37). However, none of them were statistically different from the untreated control (score 1.39). For salty taste, samples exposed to 0.05 J/cm^2 UV-C scored higher (score 6.06) than the control (score 5.14). However, the samples exposed to 0.0075 J/cm^2 and 0.1 J/cm^2, were not different from the control.

4. Discussion

4.1. Reduction of L. monocytogenes by UV light

To avoid possible changes in sensory perception, it is desirable to maximize the reduction of bacteria without treating the fish more than necessary. The fluence treatment levels for UV-C light were selected within time spans suitable for practical use in commercial production. Pulsed UV light was tested at fluences from 1.3 J/cm^2 up to levels approaching the limit value of 12 J/cm^2 determined by FDA. The fluences of the two methods are not directly comparable since the different wavelengths in the UV spectrum have different germicidal effectiveness (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). The higher germicidal effect at lower fluence for the UV-C light is likely explained by most of the energy being emitted at 254 nm, where relative germicidal effect is close to the maximum (Bintsis et al., 2000).

Both continuous UV-C and pulsed UV treatments generally gave *L. monocytogenes* reductions in similar ranges for the same products. The efficacy of using UV light for decontamination of foods is often lower than when tested on smooth surfaces (Gomez-Lopez et al., 2007). The lower reductions compared with those of smooth surfaces, like those of bacteria present on nutrient agar surfaces in petri dishes under laboratory conditions, and limited dose-response effects in the ranges tested, are likely caused by shading effects of the irregular surface structure of the fish (Woodling & Moraru, 2005). UV light does not penetrate well through organic matter, such as protein and other organic matrices, which therefore also may contribute to protect the bacteria.

Contamination of salmon with *L. monocytogenes* in the processing industry can occur via many different routes, by direct contact and from water spills and aerosols formed e. g. under production or cleaning. The fish can also be contaminated from the environment outside of the processing facility. The fish was therefore contaminated in different ways, and also time from contamination till decontamination treatment as a factor for reduction was investigated. The time factor may be of importance when fish is contaminated at a slaughter house and then transported to another facility for smoking. Generally, relatively small changes in reductions were observed when varying the fluences, the mode of application of the contamination and the time the contamination was allowed to reside on the food prior to treatment. One exception was the enhanced reduction observed when *L. monocytogenes* was added to cold-smoked salmon in droplets and analysed immediately after contamination and UV treatment. In this case the pathogen appeared less shielded from the UV light and thus a more pronounced reduction occurred. The observed tendency of lower *L. monocytogenes* reductions for samples contaminated 24 h prior to UV treatments could be due to occasional diffusion of *L. monocytogenes* to niches in the humid fillets not reached by UV light during the subsequent treatment. In most cases there was also a tendency to an average additional reduction when the salmon was bent on a scaffold to "open" the surface structure to expose more *Listeria* to the UV light. UV exposure during this bending of the salmon fillets was applied to mimic possible UV exposure strategies along the processing line in the salmon industry. However, the tendency of additional reduction by bending was not statistically significant. Generally, the reductions were higher on the surface of cold-smoked salmon and the skin side of raw salmon compared with the muscle side of raw salmon. This difference is probably due to the smoother surface of the two former.

Reductions of microorganisms using UV light are often described mathematically using Weibull models, which have previously been demonstrated to be more successful than other models such as the log-linear model and first order kinetic model (Chen, 2007; Keklik, Demirci, Puri, & Heinemann, 2012; Martin et al., 2007). The strongly concave models confirm the general impression that *Listeria* directly exposed to UV light are killed at low doses, and that other *Listeria* are shielded from the UV light. The doses must therefore be increased many-fold to

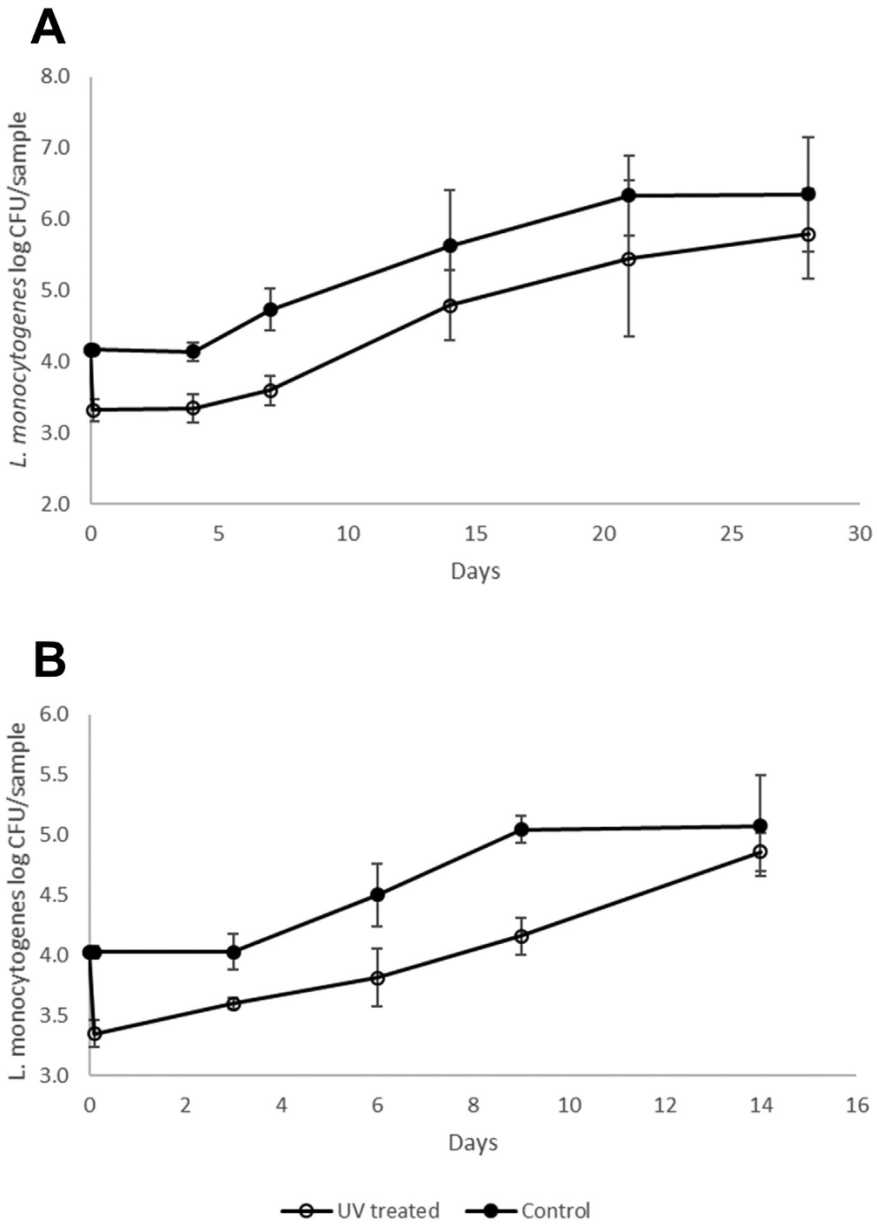

Fig. 7. Growth of *L. monocytogenes* on (A) cold-smoked salmon and (B) raw salmon fillet muscle after UV-C treatment. The samples were subjected 0.050 J/cm^2 UV-C and stored under vacuum at 4 °C for the days indicated, (o) samples subjected to UV-C treatment, (•) untreated control samples.

achieve some additional reduction.

Little information is available in the literature on the reduction using UV-C light of *L. monocytogenes* on cold-smoked salmon surfaces. Ceiling mounted UV-C light has been used to disinfect food processing surfaces in a fish smoke house (Bernbom, Vogel, & Gram, 2011). After 48 h of UV-C exposure, the number of *L. monocytogenes* positive samples was reduced from 30 to 8 (of 68), showing the efficiency of the UV light. In the present report, reductions in the range 0.7–1.3 log were obtained depending on the fluence used. Likewise, information is scarce on the use of pulsed UV light on cold-smoked salmon. A reduction of 1.8 log of a mix of three stains of *Listeria innocua* was reported for cold-smoked salmon when subjected to pulsed light at a fluence estimated at 1.6–2.9 J/cm^2 (Shaw, 2008). For fresh salmon fillets muscle side we obtained reductions in the range 0.2 to 1.1 log depending on the fluence employed. When a mix of three L. *monocytogenes* strains spiked onto raw salmon fillets were subjected to 10 mW/cm^2 for 5 to 10 min (3 to 6 J/cm^2), approximately 0.5 log reduction was obtained (Miks-Krajnik et al., 2017). In contrast, Cheigh et al. did not obtain any reduction of a strain of *L. monocytogenes* on raw salmon fillets when using UV-C light for up to 1960 s (Cheigh et al., 2013). However, when the same group subjected raw salmon fillets to pulsed UV light a 1.9 log reduction was achieved after 3600 pulses for 720 s using a total fluence of 6.3 J/cm^2. When *L. monocytogenes* Scott A was exposed pulsed UV light treatments for 60 s, reductions were 0.74 log and 1.02 log for the muscle and skin side, respectively (Ozer & Demirci, 2006). However, the fillets' surface temperature rose in these cases up to 100 °C.

The growth patterns of *L. monocytogenes* on cold-smoked salmon during storage after UV treatment differed somewhat from growth curves obtained using the food spoilage and safety predictor (FSSP) modelling program (Technical University of Denmark, 2010). The phenol concentration due to smoking of the product in the present report is not known, therefore a direct comparison is difficult. However, the model predicted a lag phase of 10 to 15 days with phenol conc. of 5 and 15 ppm, respectively, before growth, followed by a 2.5 log increase in *L. monocytogenes* during a subsequent storage period at 4 °C of 18 days under vacuum (with phenol conc. 5 ppm). Our results indicated

a lag phase of only 5 days and an approx. 2.5 log growth during the following 23 days. The pathogen modelling program (United States Department of Agriculture Agricultural Research Service, 2018) for aerobic storage of smoked salmon indicated a 5 to 8 days lag phase followed by a growth period with 1 log increase per 5 to 7 days depending on the phenol concentration.

For fresh salmon the FSSP model suggested a lag phase of approx. 6 days with a subsequent growth of 1.7 log during following 8 days. Our results gave approx. 1.5 log increase during the 14 days of storage with no significant lag period.

The observation that growth of *L. monocytogenes* resumed after UV treatment indicated that the treatment could be combined with other methods that do not necessarily kill *Listeria*, but may inhibit growth. Several such strategies exist, including increasing the degree of smoking, super-chilling, treatment with salts of organic acids (Singh, Lee, Park, Shin, & Lee, 2016), protective cultures (Matamoros et al., 2009) or storage in modified atmosphere (Masniyom, Benjakul, & Visessanguan, 2006).

4.2. Sensory analyses

Meat exposed to UV light can develop off-flavours caused by the absorption of ozone and oxides of nitrogen, or because of photochemical effects on the lipid fractions of the meat (Bintsis et al., 2000). Lipid oxidative rancidity is regarded as the most important non-microbial factor responsible for meat deterioration, resulting in adverse changes in appearance, texture, odor and flavor (Frankel, 1998). Neither the trained sensory panelists nor the consumer panelists did observe any consistent changes in organoleptic properties of UV-C treated cold-smoked salmon in comparison with the untreated control. When smoked salmon was subjected to pulsed light up to 10 pulses with a total fluence of 10 J/cm^2, little changes in lipid oxidation, color and sensory description were detected (Nicorescu, Nguyen, Chevalier, & Orange, 2014). Rainbow trout fillets were subjected to UV-C light for 60 s using a total fluence of 0.1 J/cm^2, and thereafter vacuum packed or stored using modified atmosphere packaging (Rodrigues et al., 2016). Generally, only small changes were observed in treated products regarding thiobarbituric acid reactive substances (TBARS), ammonia, and biogenic amine values. This indicated that UV-C treatment of raw salmon could also be feasible from an organoleptic viewpoint. Any changes in organoleptic properties of raw salmon due to UV light must also take into consideration the large sensory changes occurring by cooking or frying during preparation of a meal.

5. Conclusions

Due to the lack of critical control points in salmon production, it is not possible to ensure products that are consistently free from *L. monocytogenes*. In this situation both UV-C and pulsed UV light should be considered important tools to contribute to lower prevalence of *Listeria* positive samples, with higher efficiency on cold-smoked than on raw salmon. UV light treatments will contribute to reducing the contamination levels of *L. monocytogenes* and thereby reducing the frequency of products reaching 100 CFU/g at the end of shelf-life. UV treatments may thus contribute to reduced human illness and costly recalls. The sensory changes appear small or negligible both after UV-C and pulsed UV light treatments provided employing reasonable fluences and storage times and conditions. UV methods are surface decontamination treatments that can be used in many stages in continuous processing on raw materials, processed fish and final products. They can be used on foods and synergistically with other treatments. The methods require little energy use, are easy to implement, require no increase in work load and are safe to apply.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2018.10.007.

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