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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.

Key words: *Listeria monocytogenes*, Salmon, UV light, microbial decontamination

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 were associated with outbreaks from 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

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72 outbreaks which in total comprised at least nine deaths and extensive recall of smoked fish products

73 in Danish supermarkets. These and other studies have shown the strength of WGS to determine links

74 between isolates from food, environments and human cases and to identify low-intensity, multi-

75 country outbreaks that otherwise could have gone unresolved. Therefore, routine WGS will increase

76 the frequency of finding food causing outbreaks and likely reinforce the suspicion that RTE fish

77 products are important sources of *L. monocytogenes* infection (Fagerlund, Langsrud, Schirmer,

78 Moretro, & Heir, 2016; Lassen, et al., 2016; Lüth, Kleta, & Al Dahouk, 2018).

79   *L. monocytogenes* is widely distributed in food processing environments (Buchanan, et al., 2017),

80 although the prevalence may vary considerably from less than 1% up to 80% of environments tested

81 (Jami, Ghanbari, Zunabovic, Domig, & Kneifel, 2014). Generally, presence of *L. monocytogenes* in the

82 food processing environment is thought to be the primary source of post-processing contamination

83 during food manufacturing (Buchanan, et al., 2017; Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014;

84 Moretro & Langsrud, 2004). The main cause of listeriosis is consumption of food contaminated from

85 sources in the food processing environments (Ferreira, et al., 2014) or at retail level (Endrikat, et al.,

86 2010; Pradhan, et al., 2010). *L. monocytogenes* is often found in smoked fish production

87 environments (Ferreira, et al., 2014; Moretro, et al., 2004; Moretro, Schirmer, Heir, Fagerlund,

88 Hjemli, & Langsrud, 2017) including machines for salting, skinning and slicing and occasionally in

89 seawater, and from fish under processing and from final products.

90   *Listeria* spp. are components of the indigenous microbiota in surface water, where fish can be

91 contaminated with *L. monocytogenes* on the fish surface, in the stomach lining, gills, and their

92 intestines (Jami, et al., 2014). Contaminated fish can transfer *L. monocytogenes* into processing

93 facilities and be a source to both processing environment and final product contamination.

94   Different studies report variations in prevalence of *L. monocytogenes* (1.3 to 80.3 %) on cold-

95 smoked salmon (Jami, et al., 2014; Tocmo, Krizman, Khoo, Phua, Kim, & Yuk, 2014). A European-wide

96 baseline survey in 2010 and 2011 revealed that 17.4% of 599 cold-smoked fish samples were

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97 contaminated with *L. monocytogenes* at sampling (Anon., 2013). Generally, the levels were low with

98 2.0% of 3053 samples exceeding 100 CFU/g at the end of shelf-life.

99 The legislation regarding *L. monocytogenes* in RTE food products in different countries has been

100 summarized (Jami, et al., 2014). The U.S. Food and Drug Administration (FDA) requires absence of

101 the bacterium in 25-g samples of RTE seafood products (FDA, 2011). EU has a zero tolerance for

102 infant foods and for RTE foods for medical purposes, while RTE foods that contain less than 100

103 CFU/g at the end of shelf-life are accepted (Anon, 2005). Presence of *L. monocytogenes* in traded

104 products are considered representing health risks and are notified in the EU Rapid Alert System for

105 Food and Feed (RASFF) notification database. Smoked salmon was in 2016 the food most often

106 notified in conjunction with *L. monocytogenes* (Anon, 2017).

107 There are no critical control points during the cold-smoking process that will guarantee the

108 elimination of *L. monocytogenes* on the final product. Given the ubiquitous nature of *L.*

109 *monocytogenes*, the lack of listericidal steps in the cold-smoking procedure, and the ability of the

110 organism to become established in the processing environment and contaminate products, it is not

111 possible to produce cold-smoked fish consistently free of *L. monocytogenes*. By adhering strictly to

112 Good Manufacturing Practices (GMPs) and Good Hygienic Practices (GHPs) to prevent

113 recontamination, by obtaining the raw materials from known sources (for example, from producers

114 with a history of non-contaminated fish), by freezing or limiting shelf-life of the product or by using

115 preservatives that can inhibit growth at refrigerated temperatures, it is possible to produce cold-

116 smoked fish with low levels of *L. monocytogenes*, preferably at < 1 cell/g at the time of production

117 (Anon, 2001). Novel emerging decontamination technologies may also contribute to reducing the

118 level of contamination.

119 Depending on the processing and storage conditions, *L. monocytogenes* can grow to high

120 numbers on salmon fillets and cold-smoked salmon. This leads to enhanced interest in additional

121 decontamination strategies of which several have been tested including the use of organic acids or

122   their salts, phages (Soni & Nannapaneni, 2010), nisin (Soni, Shen, & Nannapaneni, 2014), protective

123   cultures (Matamoros, et al., 2009), and lauryl arginate (Soni, et al., 2014) and a number of other

124   compounds (Tocmo, et al., 2014). Several of these are not approved for use in the EU on cold-

125   smoked salmon.

126   In recent years, the use of UV light as a surface decontamination method has been met with

127   increasing interest (Holck, Liland, Drømtorp, Carlehøg, & McLeod, 2017; McLeod, Liland, Haugen,

128   Sorheim, Myhrer, & Holck, 2018). UV-C light is emitted primarily at 254 nm, while the UV energy

129   spectrum of pulsed UV light is caused by bremsstrahlung (braking radiation) and covers the whole

130   spectrum from UV (200 nm) into the infrared region (1100 nm). UV-C light provides effective

131   inactivation of microorganisms by damaging nucleic acids through creating nucleotide dimers, and

132   thus leaving the microorganisms unable to perform vital cellular functions. In addition to creating

133   nucleotide dimers, pulsed UV light has been suggested to cause cell death by induction of cell

134   membrane damage (Takeshita, et al., 2003) and rupture of the bacteria by overheating caused by

135   absorption of all UV light from the flash lamp (Wekhof, Tropeter, & Franken, 2001). Also,

136   disturbances caused by high-energy pulses have been suggested to contribute to cell damage

137   (Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2010).

138   Only few studies on the use of UV light and pulsed UV light in conjunction with salmon are

139   reported. Generally, reductions have been obtained in the range 0 - 1.9 log depending on the type of

140   UV treatment, the fluence, and the product tested (Cheigh, Hwang, & Chung, 2013; Miks-Krajnik,

141   Feng, Bang, & Yuk, 2017; Ozer & Demirci, 2006; Shaw, 2008).

142   Regulations in conjunction with using conventional continuous UV-C light and pulsed UV light in

143   the USA are given by the FDA (U.S. Food and Drug Administration, 2017). Pulsed UV light has been

144   approved by the FDA up to 12 J/cm^2 as a means for controlling surface microorganisms on food

145   products. UV-C light can be employed in the EU; however, in Germany the use is limited to water,

146   fruit and vegetable products and stored hard cheeses (Anon, 2000).

147   The effectiveness of UV-C and pulsed UV light for decontamination depends on the time a

148   microorganism is exposed, the intensity and wavelength of the illumination, the microorganism's

149   ability to withstand the UV exposure, properties of the food surface, the penetration of the UV light

150   and the presence of particles shielding the microorganisms. To the end of enhancing food safety, the

151   efficiency of UV-C and pulsed UV light against a mix of fish-associated *L. monocytogenes* strains on

152   cold-smoked salmon, raw salmon muscle and skin under conditions relevant for practical

153   implementation was evaluated. The influence of UV treatments on sensory properties of the fish

154   products was also investigated.

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156   **2. Materials and Methods**

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158   *2.1. Bacterial strains and culture conditions*

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160   The ten *L. monocytogenes* strains used in the experiments are shown in Table 1. The 10 strains

161   used included six strains isolated from salmon and salmon processing facilities (Moretro, et al.,

162   2017), three strains associated with human listeriosis and one strain was isolated from cattle. The

163   strains represented three serotypes commonly associated with human listeriosis and different

164   multilocus sequence typing (MLST) and MLVA types. The strains were maintained at -80 °C in Brain

165   Heart Infusion (BHI) broth with 15% (v/v) glycerol. For each experiment, strains were cultured

166   separately on BHI agar at 37°C, 24 h and single colonies were picked to inoculate 2-ml BHI broth

167   before incubation at 37 °C for 24 h. These pre-cultured strains were thereafter again inoculated (1%)

168   separately in 2-ml BHI broth. After incubation at 37°C for 24 h, the bacterial cultures were mixed to

169   contain approximately equal cell numbers of each of the strains. The ten-strains cell-culture mix was

170   stored at 4 °C for 20-24 h for cold adaptation. Dilutions to working solutions were performed in 0.9%

171   (w/v) NaCl.

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173 *2.2. UV treatment of salmon spiked with L. monocytogenes*

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175   Fresh and cold-smoked salmon fillets were obtained from a salmon processor and local producer,

176 respectively. Pieces of approximately 3.1 x 3.1 x 0.5 cm^3 were cut, maintaining the original surface of

177 the salmon fillets, and 20 µl of the *L. monocytogenes* cocktail (5x10^5 CFU/mL) were spread on the

178 surface of the salmon piece by a sterile plastic spreader unless otherwise stated. The surface was

179 subjected to UV treatment after approx. 5 - 10 min unless otherwise stated. In some experiments,

180 the *L. monocytogenes* was added to the salmon in small droplets (4 x 5 µl). Also, in some

181 experiments, fish samples were illuminated twice with the samples laying on a flat surface for the

182 first exposure and being bent over a scaffold for the second exposure. Fish samples were held at 4°C.

183 In the continuous UV-C light experiments, samples were treated in a custom made aluminium

184 chamber (1.0 x 0.5 x 0.6 m^3) equipped with two UV-C lamps (UV-C Kompaktleuchte, 2x95 W, BÄRO

185 GmbH, Leichlingen, Germany) in the ceiling. The UV-C light was emitted essentially at 253.7 nm, and

186 measured using a UVX Radiometer (Ultra-Violet Products Ltd., Cambridge, UK) equipped with a UV-C

187 sensor (model UVX-25, Ultra-Violet Products Ltd., Cambridge, UK). Samples of salmon were placed in

188 empty petri dishes for illumination. Parameters of intensity and exposure times were chosen with

189 aim to be relevant in industrial production lines. Samples were exposed at a power intensity of 2

190 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

191 5, 10 or 60 s, giving fluences of 0.05, 0.1, 0.6, J/cm^2, respectively. For pulsed UV light treatments, the

192 instrument XeMaticA-SA1L (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany) was

193 employed. The instrument was equipped with a xenon flash lamp (19 cm), which was water cooled,

194 with an aluminum reflector (with opening 10 cm x 20 cm), and emitted light of 200-1100 nm with up

195 to 45% of the energy being in the UV-light region with maximal emission at 260 nm for high energy

196 pulses (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany). Samples were illuminated at 6.5

197 cm distance barely beneath the opening of the reflector. At this distance, the fluence could be

198   calculated according to the manufacturer's specifications as the total discharge energy of the lamp

199   divided by the opening area of the reflector. The fluence of each pulse was adjusted to 1.3 J/cm^2

200   (low) or 3.6 J/cm^2 (high) by adjusting the discharge voltage. The samples were exposed with single

201   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

202   J/cm^2), respectively. Three parallels for each UV treatment and three or six untreated controls were

203   used in each experiment. The UV experiments were repeated three times on different days. In

204   storage experiments, UV treated samples were stored in vacuum bags at 4 °C for 28 days (cold

205   smoked salmon) and 14 days (raw salmon). Storage experiments were carried out once with five (raw

206   salmon) or six (cold-smoked salmon) parallels for each sampling point. All reductions are given as

207    $\log_{10}$ CFU/cm^2 reductions (abbreviated log in manuscript). Temperatures were measured using a

208   Raynger MX infrared thermometer (Raytek Corporation, Santa Cruz, USA). The experiments were

209   performed in a Biosafety level 3 pilot plant.

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211   *2.3. Microbial analyses*

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213   Illuminated samples were transferred to stomacher bags and 40-ml peptone water (0.1 % (w/v)

214   bacteriological peptone, Oxoid Ltd, England, 0.85 % (w/v) NaCl) was added. The samples were

215   stomached for 1 min and appropriate 10-fold dilutions in peptone water were plated on

216   RAPID'L.mono agar (Bio-Rad, Ca., USA) and incubated at 37 °C for 24h. Total counts were determined

217   by plating on blood agar petri dishes (Oxoid blood agar base supplemented with 50 ml horse blood/l,

218   Oxoid,UK) and incubating aerobically at 15 °C for 5 days.

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220   *2.4. Consumer test*
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223   Odor and appearance of cold-smoked salmon after UV light exposure were assessed in a

224   consumer test. Five different treatments of cold-smoked salmon pieces were evaluated: controls

225   without UV exposure, pieces subjected to UV-C fluences 0.0075 J/cm^2 or 0.05 J/cm^2, and pieces

226   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

227   pieces were evaluated 19 days after illumination. Samples were held at room temperature in plastic

228   dishes covered with a lid. Samples were evaluated in a randomised manner by 40 untrained

229   panellists (consumers) in two sessions, muscle and skin side separately. The consumers were asked

230   what they thought about the overall quality of the sample on a hedonic category scale from 1 (very

231   bad) to 9 (very good). They were also asked one question: "Would you use this sample in a meal?"

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233   *2.5. Sensory analysis by a trained sensory panel*

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235   A trained panel of nine assessors at Nofima performed a sensory descriptive analysis according to

236   "Generic Descriptive Analysis" as described by Lawless and Heymann (Lawless & Hildegard, 2010)

237   and ISO 13229 Sensory analysis – Methodology - General guidance for establishing a sensory profile

238   (2016). The assessors were tested, selected and trained according to ISO standards (ISO 8586:2012),

239   and the sensory laboratory used followed the ISO standards (ISO, 8589:2007). Commercial cold-

240   smoked salmon was obtained vacuum-packed and refrigerated from a local processor one day after

241   production. Smoked salmon pieces were subjected to UV-C light at different fluences: 0 (control),

242   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,

243   middle and rear sections of the fillets received all UV treatments. The pieces were vacuum-packed

244   and stored at 4 °C for 19 days, before being cut into 0.4 cm thick slices and served to panelists for

245   examination. The samples were served at room temperature on white dishes identified by random

246   three-digit numbers. Each panelist recorded their results at individual speed on a 15 cm non-

247   structured continuous scale with the left side of the scale corresponding to the lowest intensity, and

248   the right side of the scale corresponding to the highest intensity. The computer transformed the

249   responses into numbers between 1 = low intensity, and 9 = high intensity. Samples were served in

250   two replicates in randomized order following a balanced block experimental design. Twenty two

251   sensory attributes were evaluated in the descriptive sensory analysis of the smoked salmon: sourness

252   odor, marine odor, fish odor, smoke odor, sunburnt odor, cloying odor, rancid odor, color hue, color

253   intensity, whiteness, sourness flavor, salty taste, bitter taste, marine flavor, fish flavor, smoke flavor,

254   metallic flavor, cloying flavor, rancid flavor, hardness, juiciness, toughness.

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256   *2.6. Physical analyses*

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258   pH was determined using a sensION+pH31 pH meter, (Hach Company, Loveland, CO, USA). Water

259   activity (a_w) determinations were carried out employing an Aqualab dew point water activity meter

260   4TE (Decagon devices, Inc, Pullman WA, USA).

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262   *2.7. Statistical analyses*

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264   Analysis of variance (ANOVA) was used to determine statistically significant effects on the

265   bacterial reduction by the treatments. All analyses were performed in R (R_Core_Team, 2016). A

266   significance level of $\alpha = 0.05$ was used, meaning that samples were considered statistically different

267   for P-values < 0.05. For both consumer test and sensory analysis with trained panel, analysis of

268   variance (ANOVA) was used. A two-way model, with interactions and with the consumer/assessor

269   and interaction effects considered random, was performed on the sensory data in order to identify

270   the parameters that discriminated between samples. The statistical software used in consumer and

271   sensory analysis was EyeOpenR® (Logic8 BV, Utrecht, the Netherlands).

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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 illuminating the smoked salmon with two exposures of UV light with either the fish laying on a flat surface for both exposures or with the fish laying 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 laying flat or in a combination of flat and bent position.

298   Fish may be contaminated in different ways, by direct contact or by bacteria in aerosols or

299   suspended in liquid. It may also be of importance for efficiency of UV illumination how long the

300   *Listeria* have been attached to the meat surface prior to UV-treatment. Therefore, the smoked

301   salmon muscle surface was contaminated by spreading *L. monocytogenes* with a sterile plastic rod

302   and by adding the contamination in small droplets. The contaminated salmon was treated with UV

303   light and analysed immediately after contamination or treated with UV light 24 h after contamination

304   (Fig. 3). Depending on the conditions, reductions ranged from 0.4 to 2 log. For *L. monocytogenes*

305   spread on the surface, there was no difference in reduction if the bacteria were treated with UV light

306   and analysed directly after contamination or after 24 h. However, when the *Listeria* contamination

307   was added in droplets, the reduction was 1 log higher when UV-C treatment was performed

308   immediately after contamination compared with treatments after 24 h. For the pulsed UV treatment

309   the corresponding difference was 0.5 log.

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311   *3.2.*   Reduction of L. monocytogenes on raw salmon fillets*.*
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313   *L. monocytogenes* were also applied to the fillet muscle surface and skin side of raw salmon fillets.

314   The pieces were subsequently subjected to different fluences of continuous UV-C and pulsed UV

315   light, resulting in bacterial reductions between 0.2 log and 1.1 log, depending on the UV treatment

316   (Fig. 4 and Supplemental material, Table S2). For UV-C treatments an additional reduction of *L.*

317   *monocytogenes* was obtained when increasing the UV fluence. The reduction was, however, low. For

318   *L. monocytogenes* contaminating raw salmon meat and skin, increasing the UV-C dose 80-fold only

319   gave 0.6 and 0.7 log increase in reduction up to 0.9 and 1.1 log reduction for the meat and skin

320   surface, respectively. For pulsed UV, the increase in fluence did not lead to an increase in *L.*

321   *monocytogenes* reduction, which remained in the ranges 0.4-0.5 and 0.7-0.9 for muscle and skin side,

322   respectively. When comparing UV-C and pulsed UV treatments, the pulsed UV treatments were not

323   statistically different from the UV-C treatments in the range 0.015 -to 0.1 J/cm^2.

324   The reduction was somewhat higher on the skin side compared with the raw salmon meat side, as

325   was confirmed by ANOVA when comparing over all fluences, both for UV-C and pulsed UV light (not

326   shown). Also, when comparing *Listeria* reductions for raw salmon muscle side and raw salmon skin

327   side with those of smoked salmon, reductions for unsliced smoked salmon were higher or similar to

328   those of raw skin and consistently higher than those for raw salmon muscle (not shown).

329   Fresh salmon muscle were subjected to two exposures of UV light with either the fish laying on a

330   flat surface for both exposures or with the fish laying flat on the first exposure and being bent over a

331   scaffold for the second illumination (Fig. 5). Also, in these sets of experiments the increase in total

332   fluence lead to enhanced reduction in *L. monocytogenes* ranging from 0.2 to 0.9 log for UV-C

333   treatments. No such dose-response effect was achieved for the pulsed UV treatments. No

334   statistically enhanced reduction was obtained when exposing the samples of raw fillet muscle to an

335   additional dose of UV light when the samples were laying flat or in a combination of flat and bent

336   treatments, neither for UV-C nor pulsed UV treatments.

337   The influence of applying the *Listeria* contamination on fresh salmon meat and skin, as a direct

338   contact contamination or as droplets, and how time (24 h) between contamination and UV

339   treatment affected *L. monocytogenes* reductions were examined. For UV-C treatments the

340   reductions were 0.4 log and 0.7-0.8 log for raw salmon muscle and skin side, respectively, regardless

341   of application mode and whether samples were analysed immediately after contamination and UV

342   treatment or exposed to UV light 24 h after contamination (not shown). Similarly, the corresponding

343   results for pulsed UV treatments were 0.6 log and 0.9 log reductions for raw salmon muscle and skin

344   side, respectively, regardless of application mode and whether samples were analysed directly after

345   contamination and UV treatment or UV treated 24 h after contamination (not shown).

346   Weibull models were constructed from the reduction data for UV-C and pulsed UV treatments for

347   cold-smoked salmon, raw salmon fillets and raw salmon skin (Fig. 6, Supplemental material Table S3).

348   The models confirmed the general impression that *Listeria* directly exposed to UV light are killed at

349 low doses, and that the doses must be increased many-fold to achieve some additional reduction.

350 Also, the models indicated that reduction is lower when treating fresh salmon fillet compared with

351 cold-smoked salmon.

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353 *3.3 Growth of L. monocytogenes during storage*
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355   To determine whether the *L. monocytogenes* surviving UV treatment behaved similarly to

356 untreated cells, contaminated cold-smoked and raw salmon were subjected to 0.050 J/cm^2 UV-C

357 treatments and stored under vacuum at 4 °C for 28 and 14 days, respectively (Fig. 7). For smoked

358 salmon an immediate *L. monocytogenes* reduction of 0.85 log was obtained by the UV-C treatment.

359 During storage, the growth curves indicated a similar growth rate of the UV treated and the control

360 samples up to 21 days of storage, with the UV treated samples being 0.9 log lower. The reduction of

361 *L. monocytogenes* implied that levels reached at day 15 for the untreated samples were reached at

362 day 28 for the UV treated samples. The curves show that the surviving *L. monocytogenes* had a

363 similar lag phase and grew equally well as untreated cells. It also indicated that any reduction of the

364 background flora by UV light did not influence the proliferation of the *Listeria*. Similar results were

365 obtained when exposing spiked raw salmon to 0.050 J/cm^2 of UV-C light. After the UV treatment, an

366 immediate reduction of *L. monocytogenes* of 0.7 log was observed. On the average, this difference

367 remained essentially unchanged during the 10 first days of storage. The level of *L. monocytogenes*

368 reached at day 7 for the untreated samples was not reached until day 14 for the UV-treated samples.

369 The results again indicated that the *Listeria* surviving UV treatment would grow at the same rate as

370 untreated cells and that any reduction of the background flora would not influence the growth. In a

371 similar set of experiments, cold-smoked and raw salmon were subjected to 0.050 J/cm^2 UV-C light

372 treatment, thereafter spiked with *L. monocytogenes* and then stored under vacuum at 4 °C for 28

373 and 14 days, respectively. The *Listeria* grew equally well on UV-C treated samples and corresponding

374 untreated samples, again indicating that any reduction in the natural background flora by the UV

15

375 light would not influence the growth of *L. monocytogenes* (results not shown). The total background

376 flora of untreated smoked salmon was 3 log CFU/cm^2 at the start of the storage experiments at 4 °C,

377 increasing to approximately 4 log CFU/cm^2 after 14 days with a further increase up to 6.3 log

378 CFU/cm^2 at day 28. For raw salmon, the endogenous background flora grew from 6.11 +/- 0.54

379 CFU/sample on day 0 to 9.18 +/- 0.13 log CFU/sample after 14 days of storage.

380

381 3.4 Sensory analyses of cold-smoked salmon.
382

383   Quality of odor and appearance of cold-smoked salmon after UV light exposure were assessed in a

384 consumer test with 40 respondents. Samples subjected to UV-C fluences 0.0075 J/cm^2 or 0.05 J/cm^2,

385 and samples exposed to pulsed UV light at 1.3 or 3.6 J/cm^2 were evaluated. The respondents were

386 asked "What do you think about the quality of this piece of cold-smoked salmon?" Averaged answers

387 for the fillet side ranged from 5.83 to 6.22 on a scale from 1 to 9, of which none were statistically

388 different from the untreated control (score 6.05). Similarly, corresponding results for the skin side of

389 the samples ranged from 5.88 to 6.20, which were not statistically different from the control (score

390 5.95). The respondents were also asked if they would use the sample in a meal. For the fillet and skin

391 side, the answers were 87% and 77.5% yes, respectively, with no statistical differences between the

392 treated and the control samples. In conclusion, no consistent changes in the sensory properties were

393 detected after the UV treatments of cold-smoked salmon by the consumers.

394   UV-C treatment of cold-smoked salmon was thereafter chosen for analysis by a trained sensory

395 panel. The salmon had pH 5.95 +/- 0.01 and a_w = 0.961 +/- 0.006. Cold-smoked salmon fillets were

396 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

397 stored for 19 days before analysis. Of the 22 evaluated sensory attributes, the only statistically

398 different attributes were rancid flavor and salty taste. For the rancid flavor, the samples exposed to

399 0.0075 J/cm^2 scored higher (score 2.17 on the scale from 1 to 9) than the samples exposed to 0.1

400   J/cm^2 (score 1.37). However, none of them were statistically different from the untreated control

401   (score 1.39). For salty taste, samples exposed to 0.05 J/cm^2 UV-C scored higher (score 6.06) than the

402   control (score 5.14). However, the samples exposed to 0.0075 J/cm^2 and 0.1 J/cm^2, were not

403   different from the control.

404

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

431   importance when fish is contaminated at a slaughter house and then transported to another facility

432   for smoking. Generally, relatively small changes in reductions were observed when varying the

433   fluences, the mode of application of the contamination and the time the contamination was allowed

434   to reside on the food prior to treatment. One exception was the enhanced reduction observed when

435   *L. monocytogenes* was added to cold-smoked salmon in droplets and analysed immediately after

436   contamination and UV treatment. In this case the pathogen appeared less shielded from the UV light

437   and thus a more pronounced reduction occurred. The observed tendency of lower *L. monocytogenes*

438   reductions for samples contaminated 24 h prior to UV treatments could be due to occasional

439   diffusion of *L. monocytogenes* to niches in the humid fillets not reached by UV light during the

440   subsequent treatment. In most cases there was also a tendency to an average additional reduction

441   when the salmon was bent on a scaffold to "open" the surface structure to expose more *Listeria* to

442   the UV light. UV exposure during this bending of the salmon fillets was applied to mimic possible UV

443   exposure strategies along the processing line in the salmon industry. However, the tendency of

444   additional reduction by bending was not statistically significant. Generally, the reductions were

445   higher on the surface of cold-smoked salmon and the skin side of raw salmon compared with the

446   muscle side of raw salmon. This difference is probably due to the smoother surface of the two

447   former.

448   Reductions of microorganisms using UV light are often described mathematically using Weibull

449   models, which have previously been demonstrated to be more successful than other models such as

450   the log-linear model and first order kinetic model (Chen, 2007; Keklik, Demirci, Puri, & Heinemann,

451   2012; Martin, Sepulveda, Altunakar, Gongora-Nieto, Swanson, & Barbosa-Canovas, 2007). The

452   strongly concave models confirm the general impression that *Listeria* directly exposed to UV light are

453   killed at low doses, and that other *Listeria* are shielded from the UV light. The doses must therefore

454   be increased many-fold to achieve some additional reduction.

455   Little information is available in the literature on the reduction using UV-C light of *L.*

456   *monocytogenes* on cold-smoked salmon surfaces. Ceiling mounted UV-C light has been used to

457   disinfect food processing surfaces in a fish smoke house (Bernbom, Vogel, & Gram, 2011). After 48 h

458   of UV-C exposure, the number of *L. monocytogenes* positive samples was reduced from 30 to 8 (of

459   68), showing the efficiency of the UV light. In the present report, reductions in the range 0.7 -1.3 log

460   were obtained depending on the fluence used. Likewise, information is scarce on the use of pulsed

461   UV light on cold-smoked salmon. A reduction of 1.8 log of a mix of three stains of *Listeria innocua*

462   was reported for cold-smoked salmon when subjected to pulsed light at a fluence estimated at 1.6 -

463   2.9 J/cm^2 (Shaw, 2008). For fresh salmon fillets muscle side we obtained reductions in the range 0.2

464   to 1.1 log depending on the fluence employed. When a mix of three *L. monocytogenes* strains spiked

465   onto raw salmon fillets were subjected to 10 mW/cm^2 for 5 to 10 min (3 to 6 J/cm^2), approximately

466   0.5 log reduction was obtained (Miks-Krajnik, et al., 2017). In contrast, Cheigh et al. did not obtain

467   any reduction of a strain of *L. monocytogenes* on raw salmon fillets when using UV-C light for up to

468   1960 s (Cheigh, et al., 2013). However, when the same group subjected raw salmon fillets to pulsed

469   UV light a 1.9 log reduction was achieved after 3600 pulses for 720 s using a total fluence of 6.3

470   J/cm^2. When *L. monocytogenes* Scott A was exposed pulsed UV light treatments for 60 s, reductions

471   were 0.74 log and 1.02 log for the muscle and skin side, respectively (Ozer, et al., 2006). However,

472   the fillets' surface temperature rose in these cases up to 100 °C.

473   The growth patterns *of L. monocytogenes* on cold-smoked salmon during storage after UV

474   treatment differed somewhat from growth curves obtained using the food spoilage and safety

475   predictor (FSSP) modelling program (Technical University of Denmark, 2010). The phenol

476   concentration due to smoking of the product in the present report is not known, therefore a direct

477   comparison is difficult. However, the model predicted a lag phase of 10 to 15 days with phenol conc.

478   of 5 and 15 ppm, respectively, before growth, followed by a 2.5 log increase in *L. monocytogenes*

479   during a subsequent storage period at 4 °C of 18 days under vacuum (with phenol conc. 5 ppm). Our

480   results indicated a lag phase of only 5 days and an approx. 2.5 log growth during the following 23

481   days. The pathogen modelling program (United States Department of Agriculture Agricultural

482   Research Service, 2018) for aerobic storage of smoked salmon indicated a 5 to 8 days lag phase

483   followed by a growth period with 1 log increase per 5 to 7 days depending on the phenol

484   concentration.

485   For fresh salmon the FSSP model suggested a lag phase of approx. 6 days with a subsequent

486   growth of 1.7 log during following 8 days. Our results gave approx. 1.5 log increase during the 14

487   days of storage with no significant lag period.

488   The observation that growth of *L. monocytogenes* resumed after UV treatment indicated that the

489   treatment could be combined with other methods that do not necessarily kill *Listeria*, but may inhibit

490   growth. Several such strategies exist, including increasing the degree of smoking, super-chilling,

491   treatment with salts of organic acids (Singh, Lee, Park, Shin, & Lee, 2016), protective cultures

492   (Matamoros, et al., 2009) or storage in modified atmosphere (Masniyom, Benjakul, & Visessanguan,

493   2006).

494

495   *4.2. Sensory analyses*
496

497   Meat exposed to UV light can develop off-flavours caused by the absorption of ozone and oxides

498   of nitrogen, or because of photochemical effects on the lipid fractions of the meat (Bintsis, et al.,

499   2000). Lipid oxidative rancidity is regarded as the most important non-microbial factor responsible

500   for meat deterioration, resulting in adverse changes in appearance, texture, odor and flavor (Frankel,

501   1998). Neither the trained sensory panelists nor the consumer panelists did observe any consistent

502   changes in organoleptic properties of UV-C treated cold-smoked salmon in comparison with the

503   untreated control. When smoked salmon was subjected to pulsed light up to 10 pulses with a total

504   fluence of 10 J/cm^2, little changes in lipid oxidation, color and sensory description were detected

505   (Nicorescu, Nguyen, Chevalier, & Orange, 2014). Rainbow trout fillets were subjected to UV-C light

506   for 60 s using a total fluence of 0.1 J/cm^2, and thereafter vacuum packed or stored using modified

507   atmosphere packaging (Rodrigues, et al., 2016). Generally, only small changes were observed in

508   treated products regarding thiobarbituric acid reactive substances (TBARS), ammonia, and biogenic

509   amine values. This indicated that UV-C treatment of raw salmon could also be feasible from an

510   organoleptic viewpoint. Any changes in organoleptic properties of raw salmon due to UV light must

511   also take into consideration the large sensory changes occurring by cooking or frying during

512   preparation of a meal.

513

5. Conclusions

514

515

516   Due to the lack of critical control points in salmon production, it is not possible to ensure products

517   that are consistently free from *L. monocytogenes*. In this situation both UV-C and pulsed UV light

518   should be considered important tools to contribute to lower prevalence of *Listeria* positive samples,

519   with higher efficiency on cold-smoked than on raw salmon. UV light treatments will contribute to

520   reducing the contamination levels of *L. monocytogenes* and thereby reducing the frequency of

521   products reaching 100 CFU/g at the end of shelf-life. UV treatments may thus contribute to reduced

522   human illness and costly recalls. The sensory changes appear small or negligible both after UV-C and

523   pulsed UV light treatments provided employing reasonable fluences and storage times and

524   conditions. UV methods are surface decontamination treatments that can be used in many stages in

525   continuous processing on raw materials, processed fish and final products. They can be used on

526   foods and synergistically with other treatments. The methods require little energy use, are easy to

527   implement, require no increase in work load and are safe to apply.

539

540   **Supplementary data**

541

542   Supplementary data related to this article can be found at http://…..

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711   *Journal of Food Science, 70*(7), M345-M351.

712

713

Legend to figures

715

716 **Fig. 1.** Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light

717 treatments on cold-smoked salmon fillet muscle surface laying flat. Samples with upper and lower

718 case letters were analyzed separately by ANOVA. Samples containing the same letter were not

719 considered different.

720

721 **Fig. 2.** Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light

722 treatments on cold-smoked salmon fillet muscle surface laying flat and bent. Illuminations were

723 either given as a single dose while the fish was laying flat (1xF) or as two separate doses while the

724 fish was laying flat (2xF) or the first dose while laying flat and the other dose when bent (1xF+1xB).

725 Samples with upper and lower case letters were analyzed separately by ANOVA. Samples containing

726 the same letter were not considered different.

727

728 **Fig. 3.** Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light

729 treatments on cold-smoked salmon fillet muscle surface contaminated by spreading or by application

730 in small droplets. The contamination was either spread by a sterile plastic rod and UV treated and

731 analysed immediately (Spread) or UV treated after 24 h (Spread +24 h), or added as droplets and UV

732 treated and analysed immediately (Droplet) or UV treated after 24 h (Droplet + 24 h). Samples with

733 upper and lower case letters were analyzed separately by ANOVA. Samples containing the same

734 letter were not considered different.

735

736 **Fig. 4.** Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light

737 treatments on (A) raw salmon fillet muscle surface and (B) raw salmon skin side. Samples with upper

738   and lower case letters were analyzed separately for Fig. A and B by ANOVA. Samples containing the

739   same letter were not considered different.

740

741   **Fig. 5.** Reduction of *L. monocytogenes* by UV-C (white bars) and pulsed UV (gray bars) light

742   treatments on raw salmon fillet muscle surface laying flat and bent. Illuminations were either given

743   as a single dose while the fish was laying flat (1xF) or as two separate doses while the fish was laying

744   flat (2xF) or the first dose while laying flat and the other dose when bent (1xF+1xB). Samples with

745   upper and lower case letters were analyzed separately by ANOVA. Samples containing the same

746   letter were not considered different.

747

748   **Fig. 6.** Weibull models for *L. monocytogenes* log reduction as a function of UV exposure. Models for

749   each surface (continuous lines) and common models (dotted line) are shown for bacterial reduction

750   on salmon after (A) continuous UV-C and (B) pulsed UV light exposures at different fluences (J/cm^2).

751

752   **Fig. 7.** Growth of *L. monocytogenes* on (A) cold-smoked salmon and (B) raw salmon fillet muscle after

753   UV-C treatment. The samples were subjected 0.050 J/cm^2 UV-C and stored under vacuum at 4 °C for

754   the days indicated, (o) samples subjected to UV-C treatment, (•) untreated control samples.

755

756 **Table 1**

757 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

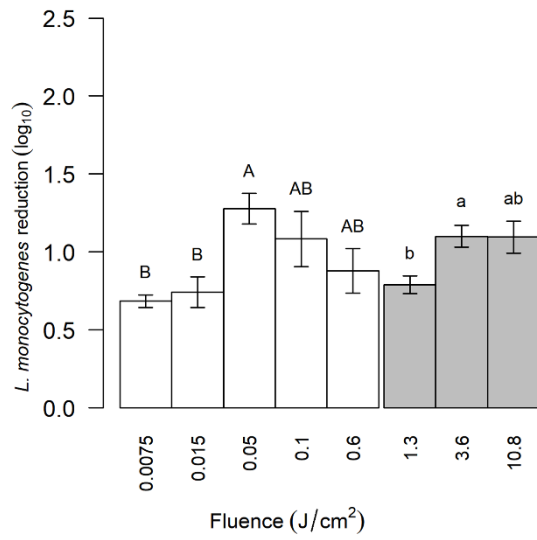758 [a] MLVA designation according to (Moretro, et al., 2017). ST numbers refer to Institute Pasteur MLST

759 database (Moura, et al., 2017), n.d., not determined
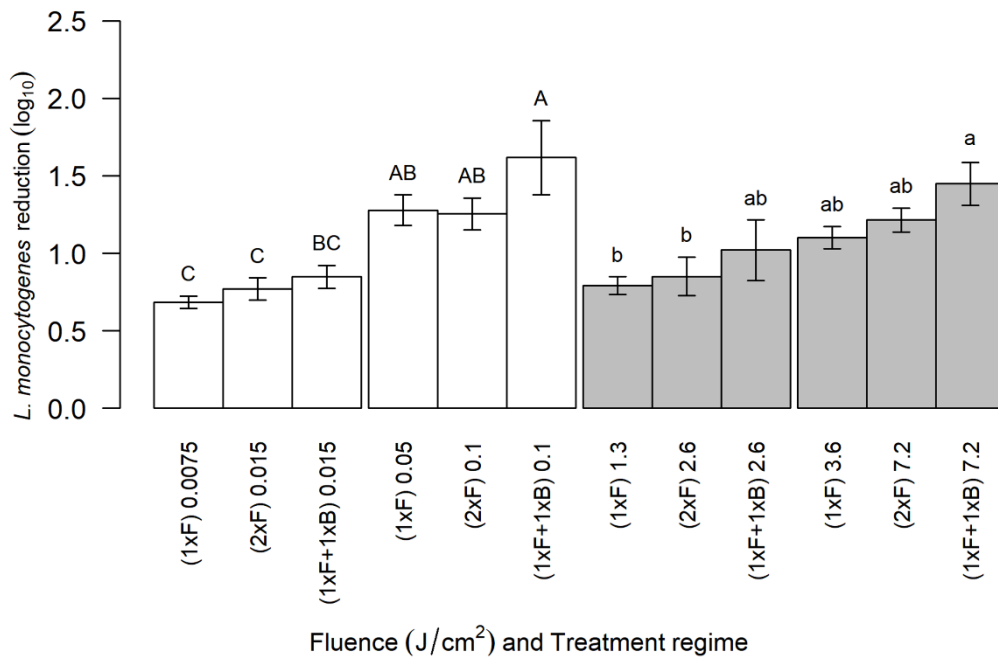
760 [b] Plant designation according to (Moretro, et al., 2017)

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766   Figure 1.

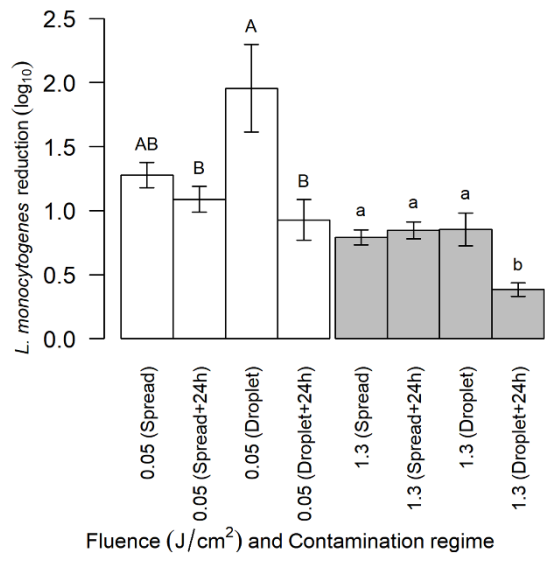767

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771   Figure 2

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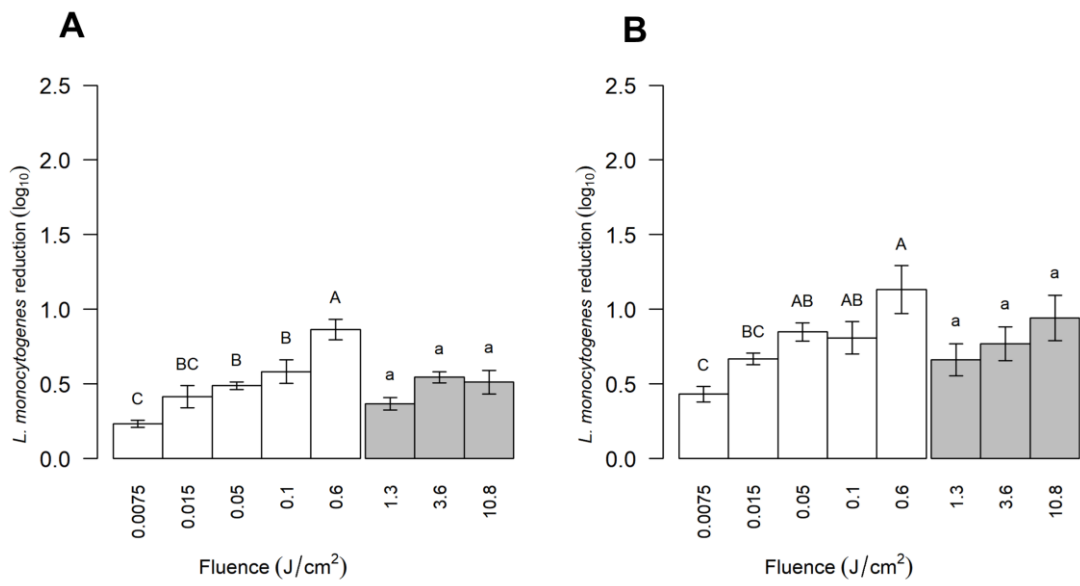

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776   Figure 3.

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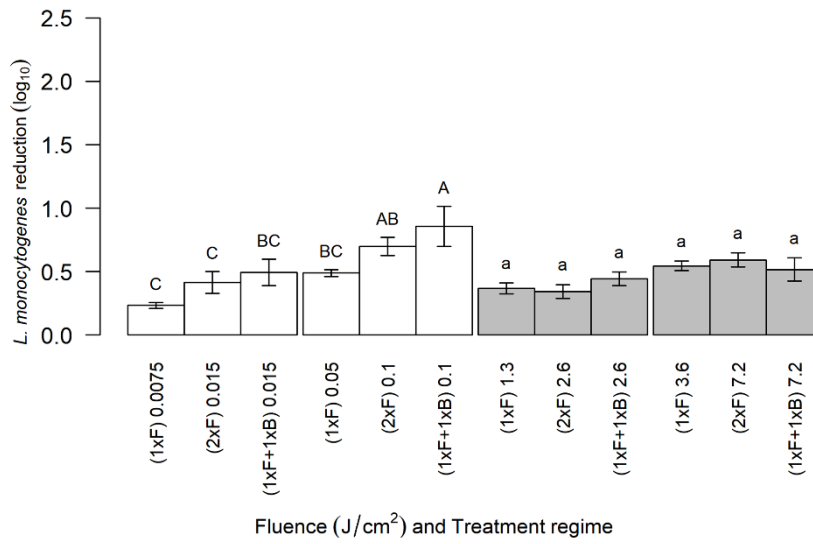
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783   Figure 4 A and B

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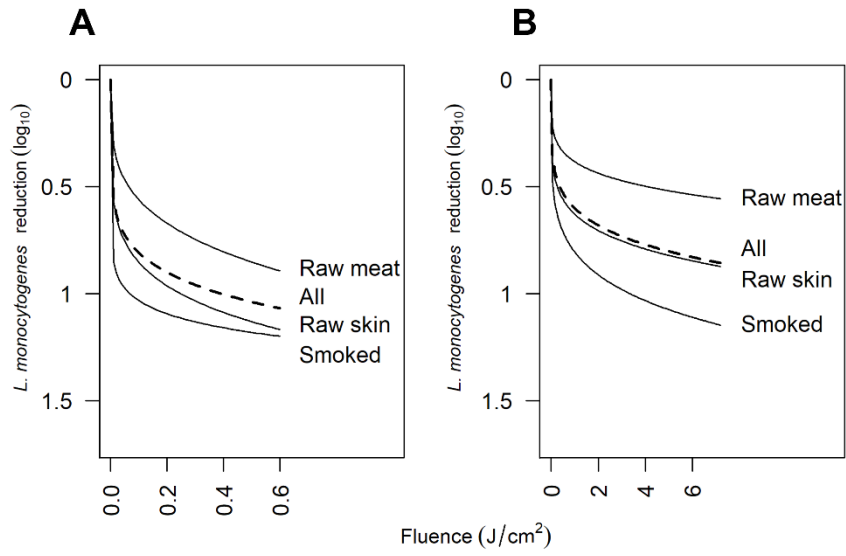
790   Figure 5

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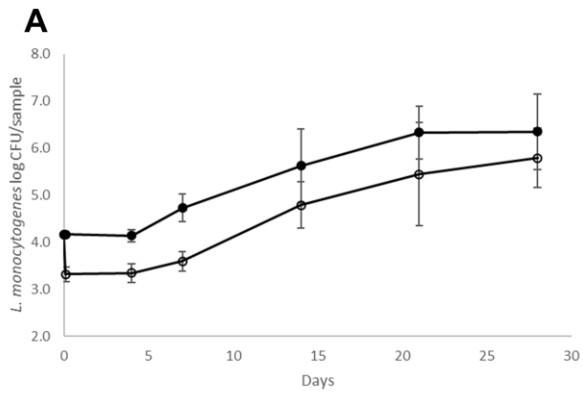796

797   Figure 6 A and B

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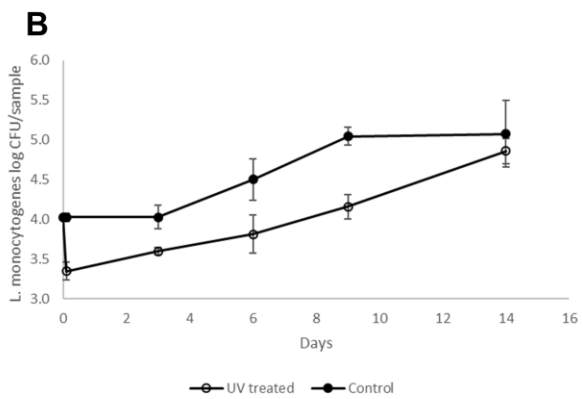
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814   Figure 7 A and B

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819 **Supplemental material**

820

821 **Table S1**. Log reduction of *L. monocytogenes* for selected fluences used in UV-C (white background)
822 and pulsed UV (grey background) treatments of cold-smoked salmon*.

J/cm²	0.0075	0.015	0.05	0.1	0.6	1.3	3.6	10.8
lower	0.645	0.643	1.180	0.905	0.736	0.733	1.030	0.994
mean	0.685	0.742	1.278	1.083	0.879	0.791	1.101	1.096
upper	0.725	0.841	1.376	1.262	1.021	0.848	1.172	1.199

823 * Mean values with lower and upper error intervals. Values correspond to Figure 1.

824

825 **Table S2**. Log reduction of *L. monocytogenes* on raw salmon for selected fluences*.

826 A:

J/cm²	0.0075	0.015	0.05	0.1	0.6	1.3	3.6	10.8
lower	0.210	0.340	0.463	0.504	0.795	0.326	0.508	0.434
mean	0.234	0.414	0.489	0.583	0.865	0.368	0.545	0.513
upper	0.257	0.489	0.515	0.663	0.934	0.411	0.582	0.592

827

828 B:

J/cm²	0.0075	0.015	0.05	0.1	0.6	1.3	3.6	10.8
lower	0.380	0.628	0.787	0.701	0.971	0.555	0.657	0.789
mean	0.432	0.667	0.848	0.809	1.132	0.662	0.770	0.941
upper	0.483	0.706	0.910	0.918	1.292	0.769	0.883	1.093

829 * Mean values with lower and upper error intervals. Values correspond to Figure 4a and b.

830

831

832 **Table S3**. Weibull parameters for Figure 6.

Material	Smoked	Raw muscle	Raw skin	Smoked	Raw muscle	Raw skin

α	3.053e-06	0.0370	0.002	0.0315	1.916	0.108
β	8.339e-02	0.259	0.175	0.179	0.188	0.166

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