

1 **Chicken fillets subjected to UV-C and pulsed UV light: reduction**  
2 **of pathogenic and spoilage bacteria, and changes in sensory quality**

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13 **Short title: UV light reducing bacteria on chicken fillets**

14  
15 **Keywords: chicken, continuous UV-C light, pulsed UV light, ESBL, modified atmosphere**  
16 **packaging**

## 17 **Abstract**

18 We have compared the efficacy of continuous UV-C (254 nm) and pulsed UV light in reducing  
19 the viability of *Salmonella* Enteritidis, *Listeria monocytogenes*, *Staphylococcus aureus*,  
20 enterohemorrhagic *Escherichia coli*, *Pseudomonas* spp., *Brochothrix thermospacta*,  
21 *Carnobacterium divergens* and Extended-Spectrum  $\beta$ -Lactamase (ESBL) producing *E. coli*  
22 inoculated on chicken fillet surface. Fluences from 0.05 to 3.0 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, from 5 to  
23 300 s) used for UV-C light resulted in average reductions from 1.1 to 2.8 log CFU/cm<sup>2</sup>. For  
24 pulsed UV light, fluences from 1.25 to 18.0 J/cm<sup>2</sup> gave average reductions from 0.9 to 3.0 log  
25 CFU/cm<sup>2</sup>. A small change in the odour characterized as sunburnt and increased concentration  
26 of volatile compounds associated with burnt odour posed restrictions on the upper limit of  
27 treatment, however no changes were observed after cooking the meat. Treatments under  
28 modified atmosphere conditions using a UV permeable top film gave similar or slightly lower  
29 bacterial reductions.

30

## 31 **Practical applications**

32 UV light may be used for decontaminating the surface of food products and reduce viability of  
33 pathogenic and spoilage bacteria. Exposure of raw chicken fillet surface to various doses of  
34 continuous UV-C or pulsed UV light proposed in the present work represent alternatives for  
35 microbiological improvement of this product. Chicken fillets can be treated in intact packages  
36 covered with UV permeable top film, thus avoiding recontamination of the meat. UV-C light  
37 treatment is a low cost strategy with low maintenance, whereas pulsed UV light involves more  
38 elaborate equipment, but treatment times are short and less space is required. Both methods can  
39 be helpful for producers to manage the safety and quality of chicken fillets.

## 40 1 | INTRODUCTION

41 The desired long shelf life in today's food industry has led to increasing demands in the  
42 development of methods for improving microbial safety and quality. According to the Food and  
43 Agriculture Organization of the United Nations (FAO), the average annual consumption of  
44 chicken meat pro capita worldwide increased from 10.2 kg in 1999 to 13.8 kg in 2015 (FAO,  
45 2015). The global meat consumption is projected to rise more than 4% per person over the next  
46 10 years, and for poultry it is predicted to rise more than 10% (OECD/FAO, 2016). As live  
47 poultry animals contain microorganisms on their skin, feathers, and in their digestive tract,  
48 contamination of the carcasses during slaughtering procedures can not be completely avoided  
49 when live animals are converted to meat for consumption.

50 Food contamination is a major global burden because of foodborne illnesses that can  
51 result from it. Poultry may be the vector of *Salmonella* spp., *Campylobacter* spp.,  
52 *Staphylococcus aureus*, *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* and  
53 other pathogens (Capita, Alonso-Calleja, Garcia-Fernandez, & Moreno, 2002; Hafez, 1999;  
54 Zhao, Ge, De Villena, Studler, Yeh, Zhao, White, Wagner, & Meng, 2001). The first two  
55 mentioned are the most common causes of human foodborne bacterial diseases linked to poultry  
56 (EFSA, 2015; Hafez, 2005). According to the Community Summary Reports of the European  
57 Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control  
58 (ECDC), 2008, campylobacteriosis and salmonellosis accounted for 214,779 and 82,694,  
59 respectively, confirmed human cases in the EU (EFSA, 2015). The number of confirmed  
60 listeriosis cases in humans was 1,763, where a high fatality rate of 15.6% was reported among  
61 the cases. Antibiotic-resistant bacteria, such as the Extended-Spectrum Beta-Lactamase  
62 (ESBL)-producing *E. coli*, have become a growing public health threat (Briongos-Figuero,  
63 Gomez-Traveso, Bachiller-Luque, Dominguez-Gil Gonzalez, Gomez-Nieto, Palacios-Martin,  
64 Gonzalez-Sagrado, Duenas-Laita, & Perez-Castrillon, 2012; Lu, Liu, Toh, Lee, Liu, Ho, Huang,  
65 Liu, Ko, Wang, Tang, Yu, Chen, Chuang, Xu, Ni, Chen, & Hsueh, 2012; Picozzi, Ricci, Gaeta,  
66 Macchi, Dinang, Paola, Tejada, Costa, Bozzini, Casellato, & Carmignani, 2013; Pitout, 2010).  
67 The ESBL-producing strains are feared as they produce the enzyme beta-lactamase that has the  
68 ability to break down commonly used antibiotics like penicillins and cephalosporins, and render  
69 them ineffective for treatment. In 2014, the World Health Organization (WHO) warned that the  
70 antibiotic resistance crisis is becoming dire, with diseases that have been curable for decades  
71 becoming increasingly difficult to treat (Michael, Dominey-Howes, & Labbate, 2014; WHO,

72 2014). The presence of ESBL genes has been clearly documented in *Enterobacteriaceae*  
73 isolated from food-production animals, and especially from chickens (Machado, Coque,  
74 Canton, Sousa, & Peixe, 2008; Overdeest, Willemsen, Rijnsburger, Eustace, Xu, Hawkey,  
75 Heck, Savelkoul, Vandenbroucke-Grauls, van der Zwaluw, Huijsdens, & Kluytmans, 2011;  
76 Smet, Martel, Persoons, Dewulf, Heyndrickx, Catry, Herman, Haesebrouck, & Butaye, 2008).  
77 Occurrence of cephalosporin-resistant *E. coli* on poultry in Norway ranged from 8 to 43% (Mo,  
78 Norstrom, Slettemeas, Lovland, Urdahl, & Sunde, 2014).

79 Food rendered unfit for human consumption because of product spoilage results in  
80 significant economic losses when products must be removed from the market. The  
81 accumulation of metabolic by-products or the action of extracellular enzymes produced by  
82 spoilage bacteria multiplying on these foods, leads to deterioration like discoloration, texture  
83 change, and formation of off-flavours, off-odours and slime. The meat acquires an offensive  
84 odour when the bacterial flora reaches about  $10^7$  CFU/cm<sup>2</sup> of the surface, and when reaching  
85  $10^8$  CFU/cm<sup>2</sup>, the surface becomes slimy (Borch, Kant-Muermans, & Blixt, 1996; Holck,  
86 Pettersen, Moen, & Sorheim, 2014; Molin, 2000). Common spoilage microorganisms on  
87 poultry stored aerobically at 4°C are *Pseudomonas* spp., *Brochothrix* spp. and  
88 *Enterobacteriaceae*. A widely used strategy for increasing shelf life of poultry meat is modified  
89 atmosphere packaging (MAP) (Holck, Pettersen, Moen, & Sorheim, 2014; van Velzen &  
90 Linnemann, 2008). Storage with high CO<sub>2</sub> (70% CO<sub>2</sub>, 30% N<sub>2</sub>) can lead to lactic acid bacteria  
91 like carnobacteria dominating the flora (Holck, Pettersen, Moen, & Sorheim, 2014; Vihavainen,  
92 Lundstrom, Susiluoto, Koort, Paulin, Auvinen, & Bjorkroth, 2007). Although some strains of  
93 carnobacteria show little influence on the sensory properties of a product, others can spoil the  
94 product (Laursen, Bay, Cleenwerck, Vancanneyt, Swings, Dalgaard, & Leisner, 2005; Leisner,  
95 Laursen, Prevost, Drider, & Dalgaard, 2007).

96 Various physical and chemical methods to reduce microbes on poultry products have  
97 been studied, such as water spraying, air chilling, ultrasound, irradiation, trisodium phosphate,  
98 and lactic acid (Capita, Alonso-Calleja, Garcia-Fernandez, & Moreno, 2002; Loretz, Stephan,  
99 & Zweifel, 2010). Potential disadvantages using these methods are sensory changes,  
100 deterioration of product appearance and quality, and safety concerns. In recent years, there has  
101 been a growing interest in using ultraviolet (UV) light for decontamination of poultry. UV light  
102 is widely known for its germicidal effect by damaging nucleic acids (Kowalkski, 2009). The  
103 high energy associated with short-wavelength UV energy (UV-C), primarily at 254 nm, is

104 absorbed by cellular RNA and DNA. This energy absorption initiates a reaction between  
105 adjacent pyrimidine bases to form dimer lesions, which in turn inhibit replication and  
106 transcription in cells (Harm, 1980; Weber, 2005).

107 As a means for controlling surface microorganisms on food products, regulations in  
108 conjugation with using conventional continuous UV-C light (henceforth referred to as UV-C  
109 light) in the US are given by the U.S. Food and Drug Administration (FDA) (FDA, 2010). UV-  
110 C light can be employed in Europe, however in Germany the use is limited to water, fruit and  
111 vegetable products and stored hard cheeses (Anon, 2000). Decontamination of raw boneless,  
112 skinless chicken or broiler breast fillets by the use of UV-C light has been reported to reduce  
113 bacterial counts of various pathogens by 0.6 to 1.7 log depending on the conditions used (Chun,  
114 Kim, Lee, Yu, & Song, 2010; Haughton, Lyng, Morgan, Cronin, Fanning, & Whyte, 2011;  
115 Isohanni & Lyhs, 2009; Sommers, Scullen, & Sheen, 2016). High intensity pulsed UV light has  
116 been approved by the FDA up to 12 J/cm<sup>2</sup> (FDA, 2010). The UV energy spectrum of pulsed  
117 UV light consists of a continual broadband spectrum from deep UV to infrared light, especially  
118 rich in UV range below 400 nm, which is germicidal. In addition to creating dimer lesions,  
119 pulsed UV light has been proposed to cause cell damage and cell death by inducing damage of  
120 the cell membrane and to cause rupture of the bacteria by thermal stress (Krishnamurthy,  
121 Tewari, Irudayaraj, & Demirci, 2010; Takeshita, Shibato, Sameshima, Fukunaga, Isobe,  
122 Arihara, & Itoh, 2003; Wekhof, 2000). The use of this technology for food decontamination  
123 has previously been reviewed (Demirci & Panico, 2008; Gomez-Lopez, Ragaert, Debevere, &  
124 Devlieghere, 2007). Pathogen reduction on boneless skinless chicken breast has been reported  
125 to vary from 1.2 to 2.4 log depending on the conditions used (N. M. Keklik, Demirci, & Puri,  
126 2010; Paskeviciute, Buchovec, & Luksiene, 2011). Several investigations have demonstrated  
127 the effectiveness of UV light on microbial reduction *in vitro*, and a wide range of bacterial  
128 species were reduced by 5-7 log when treated on petri dishes under different conditions (Farrell,  
129 Garvey, Cormican, Laffey, & Rowan, 2010; Gomez-Lopez, Devlieghere, Bonduelle, &  
130 Debevere, 2005; Paskeviciute, Buchovec, & Luksiene, 2011; Rowan, MacGregor, Anderson,  
131 Fouracre, McIlvaney, & Farish, 1999).

132 The objective of our investigation was to study and compare the efficacy of UV-C and  
133 pulsed UV light against bacteria often found as natural contaminants on fresh chicken meat, of  
134 which several of the species have not previously been investigated for UV light treatment on  
135 food. To our knowledge, studies on UV light exposure of intact packages of MAP-chicken fillet

136 for bacterial reduction have not been reported, thus we aimed at undertaking this issue using a  
137 UV permeable top film. We also aimed at determining whether the UV light treatments had  
138 adverse effects on the sensory quality of chicken fillets.

## 139 2 | MATERIALS AND METHODS

140

### 141 2.1 | Bacterial strains, media and growth conditions

142 The bacterial strains used in this work are listed in Table 1. The strains were maintained at -  
143 80°C in their respective media supplemented with 20% glycerol (v/v). Rifampicin resistant  
144 (Rif<sup>R</sup>) derivatives were prepared for all isolates by growing strains in liquid media containing  
145 200 µg/ml rifampicin as described by Heir et al. (Heir, Holck, Omer, Alvseike, Hoy, Mage, &  
146 Axelsson, 2010), except for the ESBL-producing *E. coli* strains already resistant to several types  
147 of antibiotics. Growth experiments using a Bioscreen C instrument (Labsystems) where the  
148 Optical Density (OD) at 600 nm was monitored, showed no significant difference in growth  
149 between the original strains and their Rif<sup>R</sup> mutants in their respective media and growth  
150 conditions. The different bacterial strains of each species were cultured separately.  
151 *Carnobacterium divergens* was grown in cystein-deMan Rogosa Sharpe broth (cMRS, Oxoid)  
152 with 200 µg/ml rifampicin (Sigma-Aldrich; 48 h incubation, 30°C), ESBL-producing *E. coli* in  
153 Brain Heart Infusion broth (BHI; Oxoid) with 50 µg/ml ampicillin (Sigma-Aldrich; 16 h  
154 incubation, 37°C), and tryptic soy broth (TSB, Oxoid) with 200 µg/ml rifampicin was used for  
155 *Pseudomonas* spp. (16 h incubation, 30°C), *Brochothrix thermospacta* (48 h incubation, 30°C),  
156 *Salmonella* Enteritidis, *L. monocytogenes*, *S. aureus* and EHEC (16 h incubation, 37°C). Before  
157 decontamination experiments, bacterial cultures of each of the different strains of the same  
158 species were mixed in equal amounts, e.g. bacterial cultures of each of the four strains of *L.*  
159 *monocytogenes* were mixed 1:1:1:1. An exception was *E. coli*, for which the ESBL-producing  
160 *E. coli* strains and the EHEC strains were separated from each other.

161

### 162 2.1 | UV illumination experiments of chicken and agar surface inoculated with bacterial 163 cells

164 Fresh skinless chicken breast fillets were purchased from local Norwegian supermarkets. The  
165 meat was cut into pieces of 10 cm<sup>2</sup>, and one side of the chicken was inoculated by spreading 15  
166 µl suspension of a multi strain mix of one species (described above) to obtain bacterial levels  
167 of 10<sup>5</sup>-10<sup>7</sup> CFU/cm<sup>2</sup>. The inoculated chicken samples were left at room temperature to dry for  
168 1 h prior to UV light treatment. To assess the indigenous background flora of the chicken,

169 uninoculated samples were also analyzed. For *in vitro* illumination experiments, serial 10-fold  
170 dilutions of each multi strain mix were made and plated onto the respective agar media  
171 (described below). In the UV-C light experiments, samples were treated in a custom made  
172 aluminium chamber (1.0x0.5x0.6) m<sup>3</sup> equipped with two UV-C lamps (UV-C Kompaktleuchte,  
173 2x95 W, BÄRO GmbH, Leichlingen, Germany) in the ceiling. The UV-C light was emitted  
174 essentially at 253.7 nm, measured using a UVX Radiometer (Ultra-Violet Products Ltd.,  
175 Cambridge, UK) equipped with a UV-C sensor (model UVX-25, Ultra-Violet Products Ltd.,  
176 Cambridge, UK). Both sample distance (6 cm) from the lamps and duration of the exposures  
177 were chosen with aim to be relevant for industrial production lines. Exposures were thus at 10  
178 mW/cm<sup>2</sup>, which is close to a maximum when using commercial lamps, for 5, 10, 30, 60 or 300  
179 s, giving fluences of 0.05, 0.1, 0.3, 0.6, 3.0 J/cm<sup>2</sup>, respectively. For the pulsed UV light  
180 experiments, a semi-automated intense pulsed UV system instrument XeMaticA-SA1L  
181 (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany) was used. Samples were placed  
182 in the instrument chamber at a 6.5 cm distance from the xenon lamp (19 cm), which was water  
183 cooled, had an aluminum reflector (10 cm x 20 cm), and the spectral distribution was 200-1100  
184 nm, with up to 45% of the energy being in the UV-region (maximal emission at 260 nm). The  
185 fluences were set according to the manufacturers specifications, and were adjusted to 1.25 J/cm<sup>2</sup>  
186 (low) or 3.6 J/cm<sup>2</sup> (high). The lowest level of exposure would result in limited bacterial  
187 reductions, and fluences up to and above the limit value of 12 J/cm<sup>2</sup>, which is the maximum  
188 permitted dose by FDA (FDA, 2010), were tested. Samples were exposed either once to the low  
189 pulse, or one, three or five times to the high pulse (3.6, 10.8 or 18.0 J/cm<sup>2</sup>, respectively). Three  
190 parallels of both treated samples and untreated controls were produced for each experiment,  
191 and the experiments were repeated three times on different days.

192 For ESBL-producing *E. coli* and *C. divergens*, UV light treatments were also performed  
193 under modified atmosphere conditions as follows: Chicken sample with inoculated bacteria  
194 placed in a tray was packaged using a Polimoon 511VG tray sealing machine (RPC Promens  
195 AS, Kristiansand, Norway) and UV permeable top film with 65 µm thickness and an ethylene  
196 vinyl alcohol (EVOH) barrier layer (Opalen<sup>TM</sup> 65, Bemis, Oshkosh, Wisconsin, USA). A gas  
197 mixture of 60% CO<sub>2</sub> and 40% N<sub>2</sub> (AGA, Oslo, Norway) was used for the packages. The film  
198 had an oxygen transmission rate (OTR) of 5 ml/m<sup>2</sup>/24 h/atm at 23°C/50% RH, and the trays of  
199 dimension 208 x 146 x 32 mm had a barrier layer of high density polyethylene (HDPE; RPC  
200 Promens 528) with an OTR of 3.5 ml/m<sup>2</sup>/24 h/atm at 23°C/50% RH. Intact packages (MAP-



201 chicken) were exposed to UV light doses similar to the chicken samples treated in air  
202 (unpackaged chicken), allowing for comparison of bacterial reduction between the two. Three  
203 parallels of both treated samples and untreated controls were produced for each experiment.  
204 The experiments were repeated three times on different days.

205         Temperatures were measured using a Raynger MX infrared thermometer (Raytek  
206 Corporation, Santa Cruz, USA). Samples were subjected to microbial and physiochemical  
207 analyses as described below. The experiments with pathogens were performed in a Biosafety  
208 level 3 pilot plant.

209

## 210 **2.2 | Microbial analyses**

211 Chicken samples were added 90 ml of peptone water and the samples were homogenized for 1  
212 min in a stomacher (AES Smasher, AES Chemunex, Bruz, France). Serial 10-fold dilutions  
213 from each sample were prepared. Quantification of *C. divergens* (CFU/cm<sup>2</sup>) was performed  
214 using a Whitley Automatic Spiral Plater (Don Whitley Scientific Ltd., West Yorkshire, UK) on  
215 cMRS agar (Oxoid) with 200 µg/ml rifampicin (48 h incubation, 30°C), ESBL-producing *E.*  
216 *coli* on BHI (Oxoid) with 50 µg/ml ampicillin (16 h incubation, 37°C), and tryptic soy agar  
217 (TSA, Oxoid) with 200 µg/ml rifampicin was used for *Pseudomonas* spp. (16 h incubation,  
218 30°C), *B. thermospacta* (48 h incubation, 30°C), *S. Enteritidis*, *L. monocytogenes*, *S. aureus* and  
219 EHEC (16 h incubation, 37°C). The number of colonies were determined using an automatic  
220 plate reader, and the detection limit was 20 CFU/cm<sup>2</sup>. Since rifampicin resistant strains were  
221 used, the indigenous background flora on the chicken was negligible.

222

## 223 **2.3 | Packaging film analyses**

224 The UV permeable top film Opalen™ 65 was evaluated for its ability to transmit UV light by  
225 measuring UV light at 254 nm (described above). The extended O<sub>2</sub> barrier properties of the top  
226 film was evaluated by using empty packages with 100% N<sub>2</sub> that were initially exposed to four  
227 different UV-C and pulsed UV light treatments up to 10.8 J/cm<sup>2</sup> in addition to an untreated  
228 control, with five packages per treatment. The packages were analysed for concentrations of  
229 residual oxygen at packaging and after 21 days of storage with a Dansensor Checkmate 3  
230 (Dansensor, Ringsted, Denmark). The top films of the trays used for oxygen analysis were also

231 evaluated for structural damages by UV light by scanning electron microscopy, where the  
232 samples were mounted on an aluminum stub using double-sided tape coated with carbon, before  
233 being coated with gold/palladium using a SC7640 auto/manual high resolution sputter coater  
234 (Quorum Technologies, Ashford, UK). An EVO-50-EP environmental scanning electron  
235 microscope (Zeiss, Cambridge, UK) was used to study the samples at a magnification of x8000.  
236

#### 237 **2.4 | Preparation of chicken samples for sensory analyses**

238 Fresh skinless chicken breast fillets obtained from a local producer were mixed to achieve an  
239 equal number of CFU per cm<sup>2</sup> on the surface. One set of chicken samples were exposed to UV  
240 light in air (unpackaged chicken), and were thereafter packaged in modified atmosphere, while  
241 a parallel set of chicken samples were exposed to UV light under modified atmosphere (MAP-  
242 chicken), as described above. None of these chicken samples were inoculated with bacterial  
243 culture, and both sample sets were then stored at 4°C for 6 days before being used for the  
244 sensory analyses described below. The color stability of the chicken fillets were evaluated by  
245 visual inspection of the chicken before and after UV light exposure, and after storage.  
246

#### 247 **2.5 | Sensory evaluations**

248 Descriptive sensory profiling was conducted by a trained sensory panel of ten assessors at  
249 Nofima AS, Norway, according to Generic Descriptive Analysis (Lawless & Heymann, 2010).  
250 All panellists were selected and trained in accordance with ISO 8586:2012 (International  
251 Organisation for Standardisation, 2007). The following chicken samples treated in air and under  
252 modified atmosphere were prepared: untreated control, chicken exposed to UV-C at fluence 0.1  
253 J/cm<sup>2</sup> (10 s at 10 mW/cm<sup>2</sup>), chicken exposed to UV-C at fluence 0.6 J/cm<sup>2</sup> (60 s at 10 mW/cm<sup>2</sup>),  
254 chicken exposed to pulsed UV light at low intensity at fluence 1.25 J/cm<sup>2</sup> and chicken exposed  
255 to pulsed UV light three times at high intensity giving a fluence of 10.8 J/cm<sup>2</sup>. Based on a pre-  
256 trial performed by the panellists, a consensus list of attributes for the profiling was developed:  
257 Smell of raw chicken (sour odour, sunburnt odour, burnt odour, metallic odour, sulphur odour,  
258 off-odour, cloying odour and rancid odour) and odour/taste/flavour of cooked chicken  
259 (sunburnt odour, burnt odour, sour flavour, burned flavour, metallic flavour, off-flavour,  
260 cloying flavour and rancid flavour). Both raw and cooked chicken fillet samples were evaluated.  
261 For the raw samples, the panellists were given 1/6 raw chicken fillet served at room temperature

262 on white plastic cups coded by random three-digit numbers. The cooked samples were heated  
263 (100°C, 100% steam, 30 min) in an Electrolux Air-o-steam oven (Combi LW 6 GN 1/1 Gas) to  
264 a core temperature of 78°C ± 3°C. After heating, the samples rested for five minutes before  
265 each panellist were served ¼ cooked chicken fillet in a white porcelain bowl with lid marked  
266 with a random three-digit number, that had been pre-heated at 65°C. Samples were kept at 65°C  
267 for the evaluation. The panellists had unsalted crackers and lukewarm water for rinsing the  
268 palate between samples. The coded samples were evaluated in duplicate and served randomized  
269 according to sample, panellist and replicate. Each panellist recorded their results at individual  
270 speed using an unstructured line scale with labelled endpoints ranging from no intensity (1), to  
271 high intensity (9), using the EyeQuestion Software (Logic8 BV, the Netherlands) for direct  
272 recording of data.

273 Changes in the quality or sensory properties of raw chicken as a result of UV light  
274 exposure were also assessed by a smaller consumer test. Twenty randomly chosen test persons  
275 were asked if they would want to use the chicken samples for dinner. In addition, they assessed  
276 the quality of the chicken on a scale ranging from very bad (1), to very good (9).

277

## 278 **2.6 | Dynamic headspace gas chromatography mass spectrometry**

279 The same set of raw chicken samples used in the pre-trial sensory evaluation was subjected to  
280 dynamic headspace gas chromatography mass spectrometry (GC/MS) analysis. Based on  
281 variation found both in the sensory results and the GC/MS results of the pre-trial, chicken  
282 samples that showed the greatest variation were further selected for analysis of volatile organic  
283 compounds. These included: untreated control, chicken exposed to UV-C light at fluence 0.60  
284 J/cm<sup>2</sup> (60 s at 10 mW/cm<sup>2</sup>) and pulsed UV light three times at high intensity giving a fluence  
285 of 10.8 J/cm<sup>2</sup> treated in air, and pulsed UV light at low intensity at fluence 1.25 J/cm<sup>2</sup> treated  
286 under modified atmosphere. A gas chromatography analysis was carried out on chicken samples  
287 as previously described (Olsen, Vogt, Veberg, Ekeberg, & Nilsson, 2005). Fifteen gram aliquots  
288 of homogenized sample (the samples were analyzed in duplicate) were distributed evenly in  
289 250 ml Erlenmeyer flasks. The samples were heated to 70°C in a water bath and purged with  
290 100 ml/min nitrogen through a Drechsel-head for 30 min. Volatile compounds were adsorbed  
291 on Tenax GR (mesh size 60/80). Water was removed from the tubes by nitrogen flushing (50  
292 ml/min) for 5 min in the opposite direction of sampling. Trapped compounds were desorbed at  
293 250°C for 5 min in a Perkin Elmer Automatic Thermal Desorption System ATD400 and

294 transferred to an Agilent 6890 GC System with an Agilent 5973 Mass selective detector, which  
295 is a quadrupole, operated in electron impact (EI) mode at 70 eV. The scan range was from 33  
296 to 300 amu. The compounds were separated on a DB-WAXetr column from J&W  
297 Scientific/Agilent (0.25 mm i.d., 0.5  $\mu$ m film, 30 m). Helium (99.9999%) was used as carrier  
298 gas. The temperature program started at 30°C for 10 min, increased 1°C/min to 40°C, 3°C/min  
299 to 70°C, 6.5°C/min to 160°C, 20°C/min to 230°C with a final hold time of 4 min. Integration  
300 of peaks and tentative identification of compounds were performed with HP Chemstation  
301 (G1701CA version C.00.00, Agilent Technologies), Wiley 130 KMass Spectral and NIST98  
302 Mass Spectral. Comparison of retention times and mass spectra of the sample peaks with those  
303 of pure standards confirmed identities of several of the components. Heptanoic acid ethyl ester  
304 was used as internal standard. System performance was checked with blanks and standard  
305 samples before, during and after the sample series, and the selected major compounds (80–  
306 100%) on a peak area basis were included in the data analysis.

307

## 308 **2.7 | Statistical analysis**

309 Bacterial reductions  $\log$  CFU/cm<sup>2</sup> between control and UV light treated samples were  
310 calculated. Analysis of variance (ANOVA) and Tukey's multiple comparison test were used to  
311 determine statistically significant effects on the reduction by the treatments (R 3.3.2; R Core  
312 Team (2017)) using a significance level of 0.05. For sensory evaluation, the same analyses were  
313 performed on the descriptive sensory data from the trained panel in order to identify sensory  
314 attributes that discriminated between samples.

315

## 316 **2.8 | Weibull models**

317 For each species, a two-parameter Weibull distribution was fitted to the observed log reductions  
318 to produce predictive models of the effects of UV exposure. The chosen Weibull model is  
319 defined as:

$$320 \quad \log_{10} \left( \frac{N}{N_0} \right) = \frac{-1}{\log_e(10)} \left( \frac{f}{\alpha} \right)^{\beta},$$

321 where  $N_0$  and  $N$  denote the number of bacteria per square cm before and after UV exposure,  
322 respectively,  $f$  is the UV dose (fluence),  $\alpha$  is the scale parameter (describes how sharply the

- 323 curve drops in the beginning), and  $\beta$  is the shape parameter (describes the shape of the curve).
- 324 Common models were produced based on log reduction data for all the bacterial species.

## 325 3 | RESULTS

326

### 327 3.1 | Bacterial reductions on skinless chicken fillets

328 We investigated the effect of UV-C and pulsed UV light against microbial flora associated with  
329 fresh, skinless chicken fillets. Resulting bacterial log reductions CFU/cm<sup>2</sup> of the food pathogens  
330 *S. Enteritidis*, *L. monocytogenes*, *S. aureus* and EHEC, and chicken spoilage bacteria  
331 *Pseudomonas* spp., *B. thermospacta*, *C. divergens*, and ESBL-producing *E. coli* applied to  
332 chicken meat surface are shown in Figure 1, Figure 2 and Table S1.

333 UV-C light exposure with fluences from 0.05 to 3.0 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, from 5 to 300  
334 s), gave the largest reduction of 2.8 log for *C. divergens* after the highest fluence treatment,  
335 while only 1.7 log reduction was obtained for EHEC. The lowest fluence level gave up to 2.2  
336 log reduction for *S. aureus*, and EHEC was reduced the least with 1.1 log. By comparing UV-  
337 C light results using ANOVA within each species, some of the shorter treatments were  
338 considered statistically different from the treatments of longer duration for *S. Enteritidis* (Figure  
339 1a, range 1.6-2.4 log), *Pseudomonas* spp. (1e, 2.0-2.7 log), *C. divergens* (1g, 1.9-2.8 log), and  
340 ESBL-producing *E. coli* (1h, 1.7-2.8 log), while none of the treatments were statistically  
341 different from each other for *L. monocytogenes* (1b, 1.5-1.8 log), *S. aureus* (1c, 2.2-2.6 log),  
342 EHEC (1d, 1.1-1.7 log) and *B. thermospacta* (1f, 1.7-2.7 log).

343 Sensitivities against pulsed UV light, where fluences from 1.25 to 18.0 J/cm<sup>2</sup> were used,  
344 seemed to be more similar between the different species than for UV-C light. Reductions after  
345 pulsed UV light exposure at the highest fluences (10.8 and 18.0 J/cm<sup>2</sup>) ranged from 1.6 log for  
346 *L. monocytogenes* and *C. divergens* to 3.0 log for *S. aureus*, *Pseudomonas* spp. and *B.*  
347 *thermospacta*. For the low fluence exposure of 1.25 J/cm<sup>2</sup>, reductions ranged from 0.9 log for  
348 *S. Enteritidis* to 1.7 log for *Pseudomonas* spp. ANOVA on the pulsed UV light results within  
349 each species defined the treatment at low fluence statistically different from some or all of the  
350 higher intensity treatments, thus increased reduction was obtained by increasing the UV dose.  
351 The range of reduction was 0.9-2.4 log for *S. Enteritidis* (Figure 1a), 1.1-2.0 log for *L.*  
352 *monocytogenes* (1b), 1.3-3.0 log for *S. aureus* (1c), 1.1-2.9 log for EHEC (1d), 1.7-3.0 log for  
353 *Pseudomonas* spp. (1e), 1.3-3.0 log for *B. thermospacta* (1f) and 1.3-2.8 log for ESBL-  
354 producing *E. coli* (1h). *C. divergens* deviated from this pattern, for which none of the treatments

355 were considered statistically different from each other and reductions ranged from 1.5 and 1.8  
356 log (Figure 1g).

357 In the *in vitro* illumination experiments of petri dishes, the UV light treatments  
358 inactivated all the bacterial species by 5-7 log, except from *L. monocytogenes* that was able to  
359 withstand the low fluence 1.25 J/cm<sup>2</sup> treatment with pulsed UV light better than the other  
360 species, showing approximately 4 log reduction (not shown).

361 Bacterial reductions after exposure with UV-C and pulsed UV light against *C. divergens*  
362 and ESBL-producing *E. coli* on MAP-chicken, are shown in Figure 2 and Table S1. Samples  
363 were stored under an anaerobic atmosphere with 60% CO<sub>2</sub> and 40% N<sub>2</sub>, and the UV permeable  
364 top film allowed for UV light exposure of intact packages. *C. divergens* reduction after UV-C  
365 light treatments ranged from 1.3 to 1.8 log, and after pulsed UV light treatments from 0.5 to 1.5  
366 log. The UV-C light treatments at the lowest fluences (0.05, 0.1, 0.3 J/cm<sup>2</sup>) resulted in  
367 approximately 0.7 log lower reduction on MAP-chicken compared with unpackaged chicken,  
368 and 1.4 log lower reduction was seen for the highest fluence treatment (3.0 J/cm<sup>2</sup>). ANOVA on  
369 the UV-C light results confirmed the observed differences statistically (results not shown).  
370 After pulsed UV light exposure, reductions were similar for MAP-chicken and unpackaged  
371 chicken samples for the highest fluences (10.8 and 18.0 J/cm<sup>2</sup>), while for fluences of 1.25 and  
372 3.6 J/cm<sup>2</sup>, 0.9 and 0.7 log lower reductions, respectively, were seen on MAP-chicken, which  
373 were confirmed statistically by ANOVA (not shown). Reduction of ESBL-producing *E. coli*  
374 after UV-C light treatments ranged from 1.5 to 2.5 log, and after pulsed UV light treatments  
375 from 0.6 to 1.7 log. ANOVA on the UV-C light results confirmed statistically that reductions  
376 on MAP-chicken and unpackaged chicken samples were similar (not shown). For pulsed UV  
377 light, lower reductions were seen for the MAP-chicken samples regardless of UV dose, 0.7, 1.1,  
378 0.9 and 1.3 log lower reductions for fluences of 1.25, 3.6, 10.8 and 18.0 J/cm<sup>2</sup>, respectively,  
379 confirmed statistically by ANOVA (not shown). The applied UV light up to 10.8 J/cm<sup>2</sup> did not  
380 impair the oxygen barrier properties and structural integrity of the UV permeable top film, and  
381 the O<sub>2</sub> concentrations of the trays increased from approximately 0.12±0.03% at packaging to  
382 0.69±0.02% after 21 days, and were similar for the different UV light treatments and the  
383 untreated control. Scanning electron microscopy analysis showed no structural damages to the  
384 UV treated films (not shown). The ability of the film to transmit UV light was measured as  
385 80.5% at 254 nm, which was compensated for by increasing the UV doses accordingly in the  
386 illumination experiments.

### 387 **3.2 | Weibull models describing bacterial reduction**

388 Weibull models created to predict the log reduction patterns for the different bacterial species  
389 are shown in Figure 3 and parameters for the models are listed in Table 2. RMSE values  
390 indicating the goodness of fit, were the lowest for *S. aureus* exposed to UV-C light (0.20) and  
391 the highest for *Pseudomonas* spp. exposed to pulsed UV light (0.55). Determination coefficient  
392 ( $R^2$ ) values ranged from 0.41 to 0.80 for UV-C light and from 0.47 to 0.89 for pulsed UV light.  
393 Since  $R^2$  indicates the proportion of variation in log reduction explained by the fitted Weibull  
394 model, a value approaching 1 would signify perfect predictability. Since all of the  $\beta$  (shape  
395 parameter) values were less than 1, the Weibull fits of the reduction data were concave upward.  
396 The highest  $\beta$  values were obtained for EHEC and *S. Enteritidis* (0.32 and 0.31, respectively)  
397 for pulsed UV light. The  $\alpha$  (scale parameter) values were very small, implying concentrated  
398 distribution, as seen by how sharp the curve drops in the beginning. There was a noticeable  
399 difference between the two UV methods, where higher  $\alpha$  values were obtained for UV-C light  
400 than for pulsed UV light, with *C. divergens* as an exception. Common models based on log  
401 reduction values for all the species gave a good fit for the majority of the species, but for *L.*  
402 *monocytogenes* exposed to both UV-C and pulsed UV light, reduction was overestimated. The  
403 same was seen for EHEC exposed to UV-C light and *C. divergens* exposed to pulsed UV light.

404

### 405 **3.3 | Sensory evaluation of UV light treated chicken**

406 Changes in quality or sensory properties of chicken fillets as a result of UV light treatments  
407 were assessed by ten trained assessors. Their evaluation results are shown in Figure 4, where  
408 raw chicken samples were evaluated for odour and cooked chicken samples for  
409 odour/taste/flavour. A statistically significant difference between the samples was only  
410 registered for the odour characterized as sunburnt ( $p < 0.001$ ), which is associated with that of  
411 sunburnt human skin. Most notably, treatment with the highest dose of pulsed UV light (10.8  
412  $\text{J}/\text{cm}^2$ ) in air gave the highest intensity of the sunburnt odour (sensory intensity value score of  
413 3.4). After cooking, this effect of the UV light treatment could not be detected. From the  
414 consumer test, UV light exposed raw chicken fillet samples assessed by 20 random consumers  
415 could not be differentiated from untreated control samples (data not shown). By visual  
416 inspection, the color stability was not affected by the treatments at the doses used (data not  
417 shown).



418 Denaturation of proteins in chicken has been considered to be initiated at temperatures  
419 higher than 56°C (Murphy, Marks, & Marcy, 1998). Only minor elevation of the temperature  
420 was observed, 2.5-4.0°C and 4.0-6.5°C for UV-C light treatments at fluences 0.6 J/cm<sup>2</sup> and 3.0  
421 J/cm<sup>2</sup>, respectively, and 0.5-2.5°C and 2.5-3.5°C for pulsed UV light treatments at fluences  
422 10.8 J/cm<sup>2</sup> and 18.0 J/cm<sup>2</sup>, respectively. The rise in surface temperature was only temporary  
423 since the surface was rapidly cooled by the low temperature of the interior of the chicken fillet.

424

### 425 **3.4 | Volatile organic compounds**

426 Nearly 100 different volatile organic compounds were detected by dynamic headspace/GC-MS  
427 in the raw chicken samples that were analyzed, of which approximately 70 compounds could  
428 be identified. The major compounds were ketones (C2-C5, C7), alcohols (C2-C8), acids (C2-  
429 C7), fatty and non-fatty aldehydes (C2-C9), hydrocarbons (C5-C7) and sulfides. Only a few  
430 compounds were observed to increase in concentration as a result of exposure to UV light. This  
431 included dimethyltrisulfide, pentane, heptane, propanoic acid, 2-pentanone, 1-pentanol and  
432 hexanal (Figure 5). Linear correlation with the odour scores were calculated, and gave  
433 correlations with the sunburnt odour scores as follows: dimethyltrisulfide  $r=0.70$  ( $p<0.01$ ), 2-  
434 pentanone  $r=0.95$  ( $p<0.0025$ ), 1-pentanol  $r=0.91$  ( $p<0.005$ ), pentane ( $r=0.92$ ,  $p<0.005$ ), heptane  
435 ( $r=0.81$ ,  $p<0.01$ ), propanoic acid ( $r=0.98$ ,  $p<0.001$ ), and hexanal ( $r=0.81$ ,  $p<0.01$ ). The sample  
436 in which all the compounds increased the most, was chicken exposed to pulsed UV light at  
437 fluence 10.8 J/cm<sup>2</sup> treated in air.

## 438 **4 | DISCUSSION**

439

### 440 **4.1 | Effect of UV treatment on inoculated bacteria**

441 There are large differences between the conventional continuous UV-C light and pulsed UV  
442 light with respect to wavelengths, intensities and exposure times. In this work, we have  
443 compared the efficacy of continuous UV-C light and pulsed UV light in reducing bacteria on  
444 chicken fillet. We used multi strain mixtures of the same species and bacterial cells that were  
445 in the same state during the different treatments. In earlier studies, single strains were often  
446 used which may not show reductions representative for the species. Differences in reduction  
447 within species have been reported, and state of the cells can influence the sensitivity to UV light  
448 (Farrell, Garvey, Cormican, Laffey, & Rowan, 2010; Haughton, Lyng, Morgan, Cronin,  
449 Fanning, & Whyte, 2011). To avoid possible changes in sensory perception, it is desirable to  
450 maximize bacterial reduction without treating the surface of a product more than necessary.  
451 Treatment levels employed for both UV methods were practical and relevant within industrial  
452 production, from weak exposures resulting in limited bacterial reduction, up to levels exceeding  
453 the maximum permitted dose by the FDA for pulsed UV light (FDA, 2010). The fluences are  
454 not directly comparable between the two methods, since the different wavelengths in the UV  
455 spectrum have different germicidal effectiveness (Bintsis, Litopoulou-Tzanetaki, & Robinson,  
456 2000). For UV-C exposure at  $0.05 \text{ J/cm}^2$ , the germicidal effect was comparable to a fluence of  
457  $1.25 \text{ J/cm}^2$  for the pulsed UV light. UV-C light showed a higher germicidal effect when the  
458 same fluence was employed for the two methods, which can be explained by most of the energy  
459 being emitted at 254 nm, where the germicidal effect is close to the maximum (Bintsis,  
460 Litopoulou-Tzanetaki, & Robinson, 2000).

461 In the range tested, a limited dose-response effect was observed, likely caused by  
462 shading effects of the irregular surface structure of the chicken fillet. The increase in reduction  
463 with increasing dose was though more apparent for the pulsed UV light. Any substance between  
464 the light source and the bacterium that absorbs light will impair the decontamination process  
465 (Gomez-Lopez, Ragaert, Debevere, & Devlieghere, 2007). Even when a surface appears  
466 smooth to the naked eye, it may harbour crevices and cracks where bacteria are shielded against  
467 direct exposure, and bacteria may also be covered by protein or other organic matrices.  
468 Moreover, the average size of a bacterium is approximately  $1 \mu\text{m} \times 2 \mu\text{m}$ , and although its

469 spreading was carried out carefully, it is practically impossible to avoid some overlapping. A  
470 shielding effect for colonies of *L. monocytogenes* growing on petri dishes where the upper cells  
471 of a colony appeared to protect the lower cells has previously been described (Gomez-Lopez,  
472 Devlieghere, Bonduelle, & Debevere, 2005). At high fluence rates, the light should be able to  
473 penetrate deeper, but still, the efficiency of using UV light for decontamination of foods is  
474 lower than when tested on smooth surfaces. Reductions of 5-7 log achieved on agar in petri  
475 dishes was in accordance with previous reports (Farrell, Garvey, Cormican, Laffey, & Rowan,  
476 2010; Gomez-Lopez, Devlieghere, Bonduelle, & Debevere, 2005; Paskeviciute, Buchovec, &  
477 Luksiene, 2011; Rowan, MacGregor, Anderson, Fouracre, McIlvaney, & Farish, 1999), and the  
478 observed higher resistance of *L. monocytogenes* to pulsed UV light, reduced only 4 log after  
479 treatment at low fluence of 1.25 J/cm<sup>2</sup>, has also been reported previously (Gomez-Lopez,  
480 Devlieghere, Bonduelle, & Debevere, 2005; Lasagabaster & de Maranon, 2012). In general, the  
481 reductions of inoculated bacteria on chicken fillet surface observed in this study correlated well  
482 with previous findings, both for UV-C (Chun, Kim, Lee, Yu, & Song, 2010; Haughton, Lyng,  
483 Cronin, Morgan, Fanning, & Whyte, 2011; Isohanni & Lyhs, 2009; Sommers, Scullen, &  
484 Sheen, 2016) and for pulsed UV light (N. M. Keklik, Demirci, & Puri, 2010; Paskeviciute,  
485 Buchovec, & Luksiene, 2011), including for *C. divergens*, *Pseudomonas* spp. and *B.*  
486 *thermospacta*, for which previous reports on UV light inactivation on food surfaces does not  
487 exist or are scarce. EHEC seemed to resist the UV-C light treatments better than ESBL-  
488 producing *E. coli*, and better than the other species tested as well.

489         The Weibull distribution is suitable for the analysis of bacterial reduction (Chen, 2007;  
490 N. M. Keklik, Demirci, Puri, & Heinemann, 2012; Martin, Sepulveda, Altunakar, Gongora-  
491 Nieto, Swanson, & Barbosa-Canovas, 2007; Ugarte-Romero, Feng, Martin, Cadwallader, &  
492 Robinson, 2006; van Boekel, 2002), and was previously demonstrated to be more successful  
493 than models such as the log-linear model and first-order kinetic model (Chen, 2007; N. M.  
494 Keklik, Demirci, Puri, & Heinemann, 2012; Martin, Sepulveda, Altunakar, Gongora-Nieto,  
495 Swanson, & Barbosa-Canovas, 2007). The model seemed to be a useful tool to describe the  
496 reduction patterns and give clues to how pathogens and spoilage bacteria on chicken fillet  
497 surfaces are likely to respond to UV light treatments. The Weibull fits of the reduction data  
498 were concave upward, indicating that exposed cells were destroyed and that the more resistant  
499 cells or those shaded from exposure were left undamaged.

500 To our knowledge, studies on UV light treatment of intact packages of MAP-chicken  
501 fillet for reducing bacteria on the chicken surface have previously not been reported. UV light  
502 reduction of bacteria on various packaging materials have, however, been studied (Haughton,  
503 Lyng, Morgan, Cronin, Fanning, & Whyte, 2011), and vacuum-packaged chicken breast  
504 inoculated with *Salmonella* Typhimurium treated with pulsed UV light were shown to give  
505 about 2 log reduction, but with double the exposure time (30 s) in comparison with unpackaged  
506 samples (15 s) (N. M. Keklik, Demirci, & Puri, 2010). The additional bacterial reduction  
507 obtained on ready packaged chicken fillet product would increase shelf life and safety.  
508 Treatment after packaging should be simple to implement at industrial packaging lines without  
509 reductions in production efficiency.

510

#### 511 **4.2 | Sensory quality of the chicken fillets**

512 Meat exposed to UV light can develop off-flavours caused by the absorption of ozone and  
513 oxides of nitrogen, or because of photochemical effects on the lipid fractions of the meat  
514 (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Lipid oxidative rancidity is regarded as the  
515 most important non-microbial factor responsible for meat deterioration, resulting in adverse  
516 changes in appearance, texture, odour and flavour (Frankel, 1998). An increase in fatty  
517 aldehydes due to lipid oxidation during irradiation of poultry meat has been documented (Du,  
518 Ahn, Nam, & Sell, 2000, 2001; Du, Hur, Nam, Ismail, & Ahn, 2001; Kim, Nam, & Ahn, 2002).  
519 The major fatty aldehyde hexanal is a typical volatile secondary lipid oxidation product  
520 (Beltran, Pla, Yuste, & Mor-Mur, 2003; Jayasena, Ahn, Nam, & Jo, 2013; Shi & Ho, 1994).  
521 Although we observed an increase in the concentration of hexanal, particularly for unpackaged  
522 chicken exposed to UV light, no significant effect was found on the corresponding rancid  
523 related sensory attributes in the professional sensory evaluation. This suggests that lipid  
524 oxidation does not have a negative impact on the perceived odour and flavour of the chicken  
525 meat at the applied UV doses. The higher intensity of the sunburnt odour for chicken exposed  
526 to the most intense dose of pulsed UV light, does however seem to pose restrictions on the  
527 upper limit of treatment of unpackaged chicken. The sensory intensity value was though only  
528 3.4, which is considered relatively low, and for lower doses relevant in industrial application,  
529 the odor should not be a problem. Detected changes in concentrations of volatile compounds  
530 correlated well with the sensory observations. Increased levels were seen in unpackaged  
531 chicken after UV light exposure. Hydrocarbons may be generated during irradiation of poultry

532 meat (Du, Ahn, Nam, & Sell, 2000, 2001; Du, Hur, Nam, Ismail, & Ahn, 2001; Kim, Nam, &  
533 Ahn, 2002), where increased concentrations of propanol and butanol have been documented  
534 (Du, Ahn, Nam, & Sell, 2000, 2001; Du, Hur, Nam, Ismail, & Ahn, 2001). In accordance, we  
535 detected increased levels of pentane, heptane and 1-pentanol. Sulfur compounds with low odour  
536 thresholds are important to odour associated with irradiation (Angelini, Merritt, Mendelsohn,  
537 & King, 1975; Batzer & Doty, 1955; Patterson & Stevenson, 1995). Dimethyltrisulfide,  
538 although only detected in small amounts in unpackaged chicken after UV light exposure, was  
539 reported by Patterson and Stevenson (Patterson & Stevenson, 1995) to be the most potent off-  
540 odour compound in irradiated raw chicken. Other compounds that showed an increase and  
541 which character could be associated with sunburnt/irradiated odour and flavour, were 2-  
542 pentanone (roasted sweet) and 1-pentanol (roasted meat) (Brewer, 2009). Together these three  
543 compounds likely contribute to the sensory perceived sunburnt odour. Irradiation of poultry  
544 meat is though based on irradiation by electrons using an accelerator, representing far higher  
545 dose in terms of energy exposure per area compared to our applied UV doses, thus the results  
546 may not be directly comparable. Paskeviciute et al. (Paskeviciute, Buchovec, & Luksiene,  
547 2011) investigated chemical changes in pulsed UV light treated chicken breasts, and reported  
548 that the intensity of lipid peroxidation in control and treated chicken samples differed in 0.16  
549 mg malondialdehyde per kilogram of chicken meat. However, taste panellists did not observe  
550 any changes in organoleptic properties of treated raw chicken, chicken broth or cooked chicken  
551 meat in comparison with control. Although treated raw chicken samples could not be  
552 differentiated from an untreated control sample by the 20 random chosen consumers in the  
553 present study, more extensive consumer studies could aid in determining whether such UV light  
554 treatments are acceptable.

555         The color of raw or cooked poultry meat is by origin pale with a low content of the  
556 muscle pigment myoglobin. Furthermore, the color of raw meat is dependent on the oxidation  
557 state of myoglobin (Mugler & Cunningham, 1972; USDA, 2013). Chicken breasts exposed to  
558 high doses of UV light was previously reported to turn darker, show more redness and a slight  
559 increasing amount of yellow coloration (Park & Ha, 2015). The color of the chicken fillets was  
560 not affected by the treatments at the doses used in our experiments, as in agreement with other  
561 reports (Chun, Kim, Lee, Yu, & Song, 2010; Haughton, Lyng, Cronin, Morgan, Fanning, &  
562 Whyte, 2011). Together these results indicate that sensory and quality changes are small or  
563 negligible both after UV-C and pulsed UV light treatments.

#### 564 **4.3 | Advantages and disadvantages of continuous UV-C and pulsed UV treatments**

565 Both UV-C and pulsed UV light treatments provide effective tools for reduction of  
566 microorganisms. They are rapid and efficient non-chemical, non-ionizing, and non-thermal  
567 surface decontamination treatments and can be used in continuous processing. The methods  
568 have been shown as effective technologies for decontamination of stainless steel conveyors and  
569 surfaces in the production environment (Haughton, Lyng, Morgan, Cronin, Fanning, & Whyte,  
570 2011; Sommers, Sites, & Musgrove, 2010). They can be used on foods and synergistically with  
571 other treatments (Mukhopadhyay & Ramaswamy, 2012). The methods require little energy use,  
572 are easy to implement and require no increase in work load. UV light is safe to apply, but some  
573 precautions have to be taken to avoid exposure of workers to light and to evacuate any ozone  
574 generated by the shorter UV wavelengths (Gomez-Lopez, Ragaert, Debevere, & Devlieghere,  
575 2007). The effect of both UV-C and pulsed UV light is impaired in opaque matter, where  
576 bacteria are shielded from direct exposure such as by food surface topography, organic matter  
577 or by other bacteria. The UV light treatments of this study did not alter the properties of the  
578 EVOH film used, as was also the case with polyethylene, polypropylene and  
579 polyvinylidichloride films (Tarek, Rasco, & Sablani, 2015). The top film used transmitted  
580 approximately 80% of the UV light, while in previous studies, films with polypropylene and  
581 polyethylene barrier layers transmitted 75% (N. M. Keklik, Demirci, & Puri, 2009) and 72%  
582 (N. M. Keklik, Demirci, & Puri, 2010), respectively, of pulsed UV light at 1.27 J/cm<sup>2</sup>. By using  
583 a packaging film with a high UV transmission, the chicken fillets could be packaged before the  
584 UV light treatment, therefore avoiding the risk of recontamination. Both methods would be  
585 beneficial for large scale industrial UV decontamination operations. UV-C light treatment is a  
586 low cost strategy with low maintenance (N.M. Keklik, Krishnamurthy, & Demirci, 2012). The  
587 treatment time is somewhat longer in comparison with pulsed UV light treatment, and therefore  
588 the equipment may require more space if installed over for example a conveyor belt. Pulsed UV  
589 light provides rapid decontamination, but involves equipment that is more elaborate. The xenon  
590 flash lamps used for pulsed UV light are also more environment friendly than the mercury-  
591 vapor lamps typically used in UV-C light treatment (Gomez-Lopez, Ragaert, Debevere, &  
592 Devlieghere, 2007).

593 **5 | CONCLUSION**

594 Despite good hygiene practices during production of fresh meat, contamination of carcasses  
595 with pathogens and spoilage bacteria cannot be completely prevented. There is pressure on the  
596 food industry for nutritious, fresh and healthy food products, to maximize the shelf life of the  
597 products, and for reducing costs and waste. Antimicrobial interventions that effectively reduce  
598 the bacterial load are feasible in slaughter and product processing. They should be safe,  
599 economic, and easy to handle. Also, interventions should not change the organoleptic quality  
600 of the food and should be widely accepted by consumers. The exposure of raw chicken fillet  
601 surface to various doses of UV-C or pulsed UV light proposed in this work represents useful  
602 alternatives for reducing the viability of pathogenic and spoilage bacteria on this product.

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609 respectively.

610 **COMPETING INTERESTS**

611 The authors declare that there is no conflict of interest regarding publication of this paper.



612 **FIGURE LEGENDS**

613

614 **FIGURE 1** Reductions of (a) *S. Enteritidis*, (b) *L. monocytogenes*, (c) *S. aureus*, (d)  
615 enterohemorrhagic *E. coli* (EHEC), (e) *Pseudomonas* spp., (f) *B. thermospacta*, (g) *C. divergens*  
616 and (h) ESBL-producing *E. coli* on chicken fillet meat after continuous UV-C (white bars) and  
617 pulsed UV light (grey bars) exposures at different fluences ( $J/cm^2$ ). Three separate ANOVA  
618 were performed for each species, represented by upper case letters (comparing UV-C and  
619 pulsed UV light treatments), numbers (comparing UV-C light treatments) and lower case letters  
620 (comparing pulsed UV light treatments). Samples containing the same letter/number were not  
621 considered different.

622

623 **FIGURE 2** Reductions of (a) *C. divergens* and (b) ESBL-producing *E. coli* on MAP-chicken  
624 exposed to continuous UV-C (white bars) and pulsed UV light (grey bars) at different fluences  
625 ( $J/cm^2$ ). A gas mixture of 60% CO<sub>2</sub> and 40% N<sub>2</sub> and a UV permeable top film was used for the  
626 packages. Three separate ANOVA were performed for each species, represented by upper case  
627 letters (comparing UV-C and pulsed UV light treatments), numbers (comparing UV-C light  
628 treatments) and lower case letters (comparing pulsed UV light treatments). Samples containing  
629 the same letter/number were not considered different.

630

631 **FIGURE 3** Weibull models for bacterial log reduction as a function of UV exposure. Models  
632 for each species (black continuous line) and common models (red dotted line) are shown for  
633 bacterial reduction on chicken fillet meat after (a) continuous UV-C and (b) pulsed UV light  
634 exposures at different fluences ( $J/cm^2$ ).

635 **FIGURE 4** Sensory analysis of (a) raw chicken fillet samples and (b) cooked chicken fillet  
636 samples. Chicken samples were exposed to continuous UV-C light at 10 mW/cm<sup>2</sup> for 10 s  
637 (UVC-10) and 60 s (UVC-60), giving fluences of 0.1 J/cm<sup>2</sup> and 0.60 J/cm<sup>2</sup>, respectively, and  
638 pulsed UV light to a low pulse with fluence of 1.25 J/cm<sup>2</sup> (PUV-L) and three times to a high  
639 pulse giving a fluence of 10.8 J/cm<sup>2</sup> (PUV-Hx3), both in air (O<sub>2</sub>) and anaerobic (CO<sub>2</sub>:N<sub>2</sub>)  
640 atmospheres, representing unpackaged chicken and MAP-chicken, respectively. The intensities  
641 of different odours of raw samples and odour/taste/flavour of cooked samples were registered,  
642 1 = no intensity and 9 = high intensity. The letters above the columns indicate grouping  
643 according to ANOVA and Tukey multiple comparison test. Samples with the same letter are  
644 considered being equal for the specific property.

645

646 **FIGURE 5** Volatile organic compounds from chicken which showed an increase in  
647 concentration (pg/g) as a result of exposure to UV light. The samples included were chicken  
648 exposed to pulsed UV light at low intensity at fluence 1.25 J/cm<sup>2</sup> (PUV-L) treated under  
649 anaerobic (CO<sub>2</sub>:N<sub>2</sub>) atmosphere (MAP-chicken), an untreated control (Untreated), chicken  
650 exposed to UV-C light at 10 mW/cm<sup>2</sup> for 60 s (UVC-60) giving a fluence of 0.60 J/cm<sup>2</sup> and  
651 pulsed UV light three times at high intensity (PUV-Hx3) giving a fluence of 10.8 J/cm<sup>2</sup> treated  
652 in air (O<sub>2</sub>). The precision of replicate measurements were within 15%.

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929 **TABLE 1** Strains used in this study

Bacterial species	Strain name <sup>a</sup>	Reference/source/strain/other
<i>Pseudomonas spp.</i>	MF6041	Chicken fillet
	MF6042	Chicken fillet
	MF6043	Chicken fillet
	MF6044	Chicken fillet
<i>B. thermospacta</i>	MF6045	Chicken
	MF6047	Chicken
	MF6049	ATCC11509 <sup>b</sup>
<i>C. divergens</i>	MF3036	DSM20623 <sup>c</sup>
	MF6031	Chicken fillet
	MF6032	Chicken fillet
	MF6034	Chicken fillet
	MF6038	Chicken fillet
<b>ESBL-producing <i>E. coli</i></b>	MF5658	Chicken <sup>d</sup>
	MF5660	Chicken <sup>d</sup>
	MF5664	Chicken <sup>d</sup>
	MF5670	Broiler <sup>d</sup>
	MF5674	Broiler <sup>d</sup>
<i>S. Enteritidis</i>	MF3817	1049-1-99 <sup>d</sup>
	MF3818	Poultry, 61-358-1 <sup>e</sup>
	MF3824	ATCC13076 <sup>b</sup>
<i>L. monocytogenes</i>	MF3508	2230/92 (Nesbakken, 1995)
	MF3509	167 (Blom, Nerbrink, Dainty, Hagtvedt, Borch, Nissen, & Nesbakken, 1997)
	MF3510	187 (Blom, Nerbrink, Dainty, Hagtvedt, Borch, Nissen, & Nesbakken, 1997)
	MF3571	EGD-e (Glaser, Frangeul, Buchrieser, Rusniok, Amend, Baquero, Berche, Bloecker, Brandt, Chakraborty, Charbit, Chetouani, Couve, de Daruvar, Dehoux, Domann, Dominguez-Bernal, Duchaud, Durant, Dussurget, Entian, Fsihi, Garcia-del Portillo, Garrido, Gautier, Goebel, Gomez-Lopez, Hain, Hauf, Jackson, Jones, Kaerst, Kreft, Kuhn, Kunst, Kurapkat, Madueno, Maitournam, Vicente, Ng, Nedjari, Nordsiek, Novella, de Pablos, Perez-Diaz, Purcell, Rimmel, Rose, Schlueter, Simoes, Tierrez, Vazquez-Boland, Voss, Wehland, & Cossart, 2001)
<i>S. aureus</i>	MF2123	ATCC25923 <sup>b</sup>
	MF2124	ATCC12600 <sup>b</sup>
	MF2125	ATCC6538 <sup>b</sup>
<b>Enterohemorrhagic <i>E. coli</i> (EHEC)</b>	MF3572	O103, fermented sausage, linked to outbreak in Norway 2006 (Schimmer, Nygard, Eriksen, Lassen, Lindstedt, Brandal, Kapperud, & Aavitsland, 2008) <sup>f</sup>
	MF3574	ATCC43895 <sup>b</sup> , O157:H7
	MF3576	O111:H, semi-dry fermented sausage, outbreak Australia 1995 (Paton, Ratcliff, Doyle, Seymour-Murray, Davos, Lanser, & Paton, 1996) <sup>g</sup>
	MF5554	O145 (McLeod, Mage, Heir, Axelsson, & Holck, 2016)

<sup>a</sup>Antibiotic resistant strains. All strains were grown in their respective medium with 200 µg/ml rifampicin, except ESBL-producing *E. coli* grown in medium with 50 µg/ml ampicillin.

<sup>b</sup>ATCC, American Type Culture Collection, Manassas, VA, USA.

<sup>c</sup>DSM, Deutsche Sammlung von Microorganismen und Zellkulturen, Braunschweig, Germany.

<sup>d</sup>Kindly received from the Norwegian Veterinary Institute, Oslo, Norway.

<sup>e</sup>Kindly received from the Technical University of Denmark, the National Veterinary Institute, Denmark.

<sup>f</sup>Kindly received from the Norwegian School of Veterinary Science, Oslo, Norway.

<sup>g</sup>Kindly received from Statens Serum Institut, Copenhagen, Denmark.

932 **TABLE 2** Parameters for Weibull models predicting bacterial  
 933 reduction on chicken fillet meat after continuous UV-C and  
 934 pulsed UV light exposures, and goodness-of-fit parameters of  
 935 the models

Continuous UV-C light	Bacterial species	$\alpha$	$\beta$	RMSE	$R^2$
	<i>E. coli</i> EHEC	2.03E-06	0.09	0.31	0.75
	<i>L. monocytogenes</i>	2.02E-09	0.07	0.47	0.41
	<i>S. Enteritidis</i>	2.35E-05	0.14	0.41	0.64
	<i>S. aureus</i>	2.22E-15	0.05	0.20	0.76
	<i>Pseudomonas</i> spp.	2.86E-09	0.09	0.39	0.68
	<i>C. divergens</i>	1.45E-08	0.10	0.37	0.74
	<i>B. thermospacta</i>	1.66E-07	0.11	0.31	0.80
	<i>E. coli</i> ESBL	1.65E-08	0.10	0.38	0.74
	All	9.89E-09	0.09	0.53	0.25

Pulsed UV light	Bacterial species	$\alpha$	$\beta$	RMSE	$R^2$
	<i>C. divergens</i>	3.79E-10	0.06	0.29	0.86
	<i>L. monocytogenes</i>	2.27E-04	0.13	0.37	0.63
	<i>S. Enteritidis</i>	6.32E-02	0.31	0.42	0.79
	<i>E. coli</i> EHEC	5.29E-02	0.32	0.41	0.79
	<i>E. coli</i> ESBL	7.58E-03	0.24	0.28	0.89
	<i>Pseudomonas</i> spp.	1.31E-03	0.20	0.55	0.71
	<i>S. aureus</i>	6.61E-03	0.24	0.47	0.47
	<i>B. thermospacta</i>	9.21E-03	0.26	0.37	0.82
	All	6.23E-03	0.22	0.54	0.46

936

937 **SUPPORTING INFORMATION**

938

939 **TABLE S1** Bacterial reductions log CFU/cm<sup>2</sup> on chicken fillet meat after continuous UV-C  
 940 and pulsed UV light treatments at different fluences (J/cm<sup>2</sup>)

941

Bacterial species	Continuous UV-C light treatment <sup>a</sup> , J/cm <sup>2</sup>					Pulsed UV light treatment <sup>a</sup> , J/cm <sup>2</sup>			
	0.05	0.1	0.3	0.6	3.0	1.25	3.6	10.8	18.0
<i>S. Enteritidis</i>	1.56 (0.15)	1.21 (0.13)	1.81 (0.16)	1.53 (0.23)	2.44 (0.17)	0.90 (0.05)	1.58 (0.09)	2.44 (0.20)	2.23 (0.19)
<i>L. monocytogenes</i>	1.49 (0.13)	1.13 (0.14)	1.82 (0.22)	1.36 (0.16)	1.81 (0.27)	1.08 (0.12)	1.83 (0.13)	2.01 (0.25)	1.63 (0.21)
<i>S. aureus</i>	2.22 (0.18)	2.23 (0.22)	2.56 (0.20)	2.05 (0.26)	2.41 (0.16)	1.25 (0.09)	2.21 (0.16)	2.95 (0.33)	2.61 (0.25)
<i>E. coli</i> EHEC	1.08 (0.18)	1.34 (0.19)	1.10 (0.12)	1.30 (0.18)	1.65 (0.17)	1.06 (0.11)	1.96 (0.22)	1.98 (0.29)	2.93 (0.21)
<i>Pseudomonas</i> spp.	2.01 (0.10)	1.85 (0.20)	1.95 (0.15)	2.23 (0.21)	2.66 (0.24)	1.67 (0.06)	2.25 (0.18)	2.45 (0.18)	3.01 (0.14)
<i>B. thermospecta</i>	1.70 (0.17)	1.74 (0.24)	2.41 (0.22)	2.33 (0.32)	2.68 (0.37)	1.30 (0.13)	2.28 (0.20)	3.00 (0.37)	2.88 (0.30)
<i>C. divergens</i>	1.90 (0.16)	1.95 (0.25)	2.32 (0.14)	2.04 (0.14)	2.82 (0.14)	1.46 (0.17)	1.59 (0.17)	1.84 (0.21)	1.61 (0.35)
<i>E. coli</i> ESBL	1.65 (0.11)	1.91 (0.11)	2.43 (0.20)	2.79 (0.15)	2.56 (0.22)	1.34 (0.09)	2.17 (0.17)	2.58 (0.17)	2.83 (0.25)
<i>C. divergens</i> MAP <sup>b</sup>	1.26 (0.15)	1.30 (0.11)	1.52 (0.21)	1.80 (0.24)	1.38 (0.14)	0.54 (0.21)	0.90 (0.22)	1.35 (0.29)	1.53 (0.08)
<i>E. coli</i> ESBL MAP <sup>b</sup>	1.46 (0.11)	1.69 (0.22)	2.12 (0.27)	2.49 (0.25)	2.33 (0.18)	0.61 (0.14)	1.09 (0.12)	1.70 (0.18)	1.56 (0.16)

<sup>a</sup>Standard error values are shown in brackets

<sup>b</sup>MAP refers to modified atmosphere packaging