Food Sci Biotechnol (2021) 30(4):609–618
https://doi.org/10.1007/s10068-021-00895-y

RESEARCH ARTICLE

Antimicrobial activity of 405 nm light-emitting diode (LED) in the presence of riboflavin against *Listeria monocytogenes* on the surface of smoked salmon

Min-Jeong Kim[1] · Min Da Jeong[2] · Qianwang Zheng[3] · Hyun-Gyun Yuk[4]

Received: 18 November 2020 / Revised: 28 January 2021 / Accepted: 18 February 2021 / Published online: 12 March 2021
© The Korean Society of Food Science and Technology 2021

Abstract This study investigated the antimicrobial activity of 405 nm light-emitting diode (LED) with and without riboflavin against *Listeria monocytogenes* in phosphate buffered saline (PBS) and on smoked salmon at different storage temperatures and evaluated its impact on food quality. The results show that riboflavin-mediated LED illumination in PBS 25 °C significantly inactivated *L. monocytogenes* cells by 6.2 log CFU/mL at 19.2 J/cm^2, while illumination alone reduced 1.9 log CFU/mL of *L. monocytogenes* populations at 57.6 J/cm^2. *L. monocytogenes* populations on illuminated smoked salmon decreased by 1.0–2.2 log CFU/cm^2 at 1.27–2.76 kJ/cm^2 at 4, 12, and 25 °C, regardless of the presence of riboflavin. Although illumination with and without riboflavin caused the lipid peroxidation and color change in smoked salmon, this study demonstrates the potential of a 405 nm LED to preserve the smoked salmon products, reducing the risk of listeriosis.

Keywords 405 nm LED · Photodynamic inactivation · Riboflavin · Smoked salmon · *Listeria monocytogenes*

✉ Hyun-Gyun Yuk
yukhg@ut.ac.kr

Min-Jeong Kim
mjkim0425@korea.kr

Min Da Jeong
941018@naver.com

Qianwang Zheng
zhqianw@scau.edu.cn

[1] Food Safety Risk Assessment Division, Food Safety Evaluation Department, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, 187 Osong saengmyeong 2-ro, Cheongju-si, Chungbuk 28159, Republic of Korea

[2] School of Food Science and Technology, Chung-Ang University, 4726, Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi-do 17546, Republic of Korea

[3] Institute of Food Biotechnology, College of Food Science, South China Agricultural University, Guangzhou, Guangdong 510640, People's Republic of China

[4] Department of Food Science and Technology, Korea National University of Transportation, 61, Daehak-ro, Jeungpyeong-gun, Chungbuk 27909, Republic of Korea

Introduction

A light-emitting diode (LED) is a semiconductor device which can emit very narrow visible light wavelengths (Kim et al., 2016; Mori et al., 2007). LEDs have merits, including lower energy consumption, long life, reduced heat output, reduced heat, and easy manipulation in small sizes and various shapes over the traditional visible light sources (Ghate et al., 2013; Mori et al., 2007). Inactivation of foodborne pathogens using a LED technology has recently received the interest of researchers due to its advantages and antimicrobial effect. Previous studies demonstrated that 405 and 460 nm LEDs inactivated *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus* in phosphate buffered saline (PBS) or tryptic soy broth (TSB) at refrigeration temperatures (Ghate et al., 2013; Kim et al., 2015, 2016). In addition, blue LEDs inactivated a variety of foodborne pathogens on food matrices such as cooked chicken, salmon, mango, papaya, pineapple, and orange juice without the aid of photosensitizers (Ghate et al., 2016; Ghate et al., 2017; Kim et al., 2017a; b; c; Li et al., 2018).

The antimicrobial mechanism of blue LEDs could be explained by photodynamic inactivation (PDI) requiring

photosensitizers and visible lights in the presence of oxygen (Luksienė and Zukauskas, 2009). Once bacterial cells are exposed to light energy, the photosensitizer, such as intracellular porphyrin molecules, is excited and then produced reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radicals, superoxide, and singlet oxygen. The ROS can attack cellular components, including DNA, lipids, and proteins, causing oxidative damage and eventually bacterial death (Kim et al., 2017a, c; Luksienė and Zukauskas, 2009). Recent studies have focused on LED illumination with exogenous photosensitizers such as chlorophyllin and hypericin to enhance the antimicrobial effect on foods (Aponiene et al., 2015; Luksienė and Paskeviciute, 2011). Our previous study has also demonstrated that 460 nm LED illumination with the aid of riboflavin resulted in 0.7–1.2 log CFU/cm^2 reductions of *L. monocytogenes* cells on smoked salmon at chilling temperatures (Josewin et al., 2018). However, no study has been conducted with 405 nm LED illumination with exogenous riboflavin on *L. monocytogenes* on smoked salmon.

Smoked salmon as one of ready-to-eat (RTE) foods is commonly purchased vacuum-packed and stored at refrigeration temperature for weeks to extend the shelf-life (Rørvik, 2000). *L. monocytogenes* have recently been associated with several foodborne illness outbreaks due to cold-stored RTE foods, such as meat, soft cheese, and especially cold-smoked fishery products, since they have the ability to grow 4 °C (Heir et al., 2019; Kim et al., 2015; Ye et al., 2008). In salmon processing industries, *L. monocytogenes* existing in raw salmon can be contaminated to food contact surface, such as conveyer belts, slicing machines, and knives (Pang et al., 2019). RTE fish products can be also cross-contaminated with *L. monocytogenes* during handling and further processing at food establishments, although smoked salmon may involve other processes, such as drying, salting, smoking, and packaging (Ricci et al., 2018; Rørvik, 2000). For this reason, human listeriosis outbreaks are frequently associated with smoked salmon due to eating it without the additional cooking step (Ye et al., 2008).

To control the growth of *L. monocytogenes* in RTE fisher products, several physical intervention technologies have been applied, including irradiation, pulsed UV-light treatment, high pressure processing, and microwave heating (Tocmo et al., 2014). However, these technologies have shortcomings, such as safety concerns, high cost, or changes in the food quality (Josewin et al., 2018). Thus, the implementation of suitable preservation technologies in fresh fishery products should be needed to minimize the risk of listeriosis. In this study, the efficacy of 405 nm LED illumination with and without riboflavin on *L. monocytogenes* on the surface of smoked salmon at different storage temperatures was evaluated and the quality changes of smoked salmon after long-term LED illumination were investigated.

Materials and methods

Bacterial strains and culture conditions

Four *L. monocytogenes* serovars were used in this study: 1/2a (SSA81) and 3a (LM8) isolated from pre-paced smoked salmon from supermarkets and 1/2b (LM4) and 4b (LM2) isolated from smoked salmon in salad bar obtained from the National Environment Agency (Chau et al., 2017). All *L. monocytogenes* serovars were preserved on beads into cryoinstant vials (DeltaLab, Barcelona, Spain) at − 70 °C. Frozen cultures were activated in 10 mL of sterile tryptone soy broth (TSB; Oxoid, Basingstoke, UK) for 18–24 h at 37 °C. After two consecutive transfers for 18–24 h at 37 °C, a cocktail culture of *L. monocytogenes* was prepared by combining equal portions (0.25 mL) of each strain to be a total of 1 mL and centrifuged at $6000\times g$ for 10 min at 4 °C, followed by washing twice with sterilized phosphate buffered saline (PBS; Vivantis Technologies Sdn Bhd, Malaysia). The resultant pellets were resuspended in 1 mL of PBS and serially diluted to ca. 10^6 CFU/mL for inoculation.

Light-emitting diode (LED) source and illumination system

LEDs (8 × 8 mm) at 405 nm wavelength were purchased from Shenzhen Getian Opto-Electronics Co., Ltd. (Shenzhen, Guangdong, China) and attached to a fan and a heat sink to reduce heat transfer to samples. The irradiance of a 405 nm LED unit was 16 ± 1 mW/cm^2 at the surface of samples, which was measured with a Compact Power and Energy Meter Console (PM100D; Thorlabs GmbH, Dachau, Germany). The dose applied to each sample was calculated with the following equation (Ghate et al., 2013; Kim et al., 2015):

$$E = Pt$$

where E = dose (energy density) in J/cm^2, P = Irradiance (power density) in W/cm^2, and t = time in s.

To adjust the height of each LED system, the system was set up in an acrylonitrile butadiene styrene (ABS) housing. To illuminate the entire surface of bacterial suspension and smoked salmon, 10 mL bacterial suspension (ca. 1 cm depth) and smoked salmon (ca. 0.2 cm depth) were deposited in a sterile Petri dish (60 mm and 90 mm diameter, respectively) and then placed directly below the LED at distance of 5.5 cm and 4.5 cm, respectively

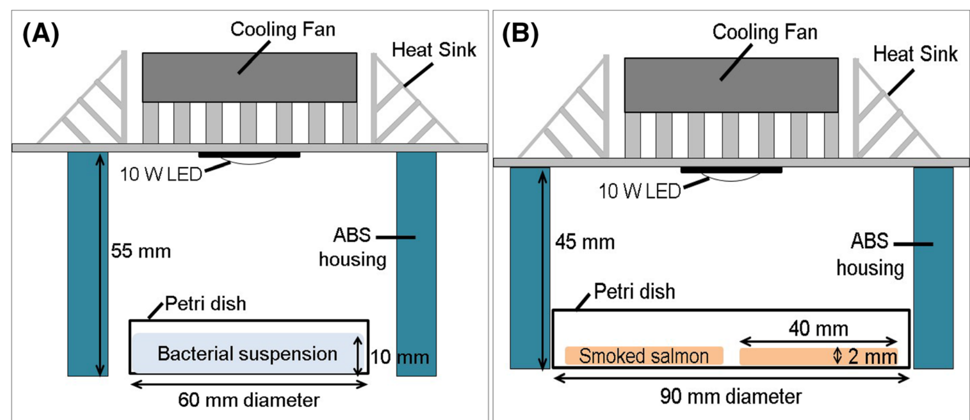

Fig. 1 Schematic diagrams of 405 nm LED illumination systems in phosphate-buffered saline (PBS) solution (**A**) and on the surface of smoked salmon samples (**B**)

(Fig. 1A and B). The surface temperatures of smoked salmon were monitored using a Fluke 5.4 thermocouple thermometer (Everett, WA, USA) during exposure to LED illumination at doses up to 288 J/cm^2 (for 300 min).

Riboflavin-mediated LED illumination in PBS

To evaluate the antibacterial efficacy of 405 nm LED illumination in the presence of riboflavin, a 10 mL bacterial suspension (ca. 10^6 CFU/mL in PBS) containing 0, 50 and 100 µM riboflavin (RBF; Sigma-Aldrich, St. Louis, MO, USA) was placed in the LED system as described above and illuminated up to 57.6 J/cm^2 (for 1 h) at 25 °C in a temperature-controlled incubator (MIR-154, Panasonic Healthcare Co., Ltd., Osaka, Japan). Non-illuminated control samples were placed in the incubator in the dark. A 0.1 mL aliquot was withdrawn at selected time intervals and serially diluted with 0.1% (w/v) sterile peptone water (PW; Oxoid), if necessary. The diluents were pour-plated in tryptone soy agar (TSA; Oxoid). After incubation at 37 °C for 48–72 h, the number of surviving cells was enumerated with a colony counter (Rocker Scientific Co. Ltd., Taipei, Taiwan) and reported as log CFU/mL.

Preparation of smoked salmon and inoculation

Norwegian smoked salmon (Norlax, Outrup, Denmark) in the absence of preservation and coloring agent was purchased from a local supermarket in Singapore and used for the experiments within two days. Smoked salmon was cut into ca. 4.0 ± 0.5 g slices (4 × 4 cm) in a biosafety cabinet. A 20 µL aliquot of the diluent (ca. 10^6 CFU/mL) as previously described was spot inoculated at 5 sites on the salmon surface to reach a final concentration of ca. 5 × 10^3 CFU/cm^2, followed by dry for 20 min in a biosafety cabinet.

To prepare non-illuminated control and illuminated salmon samples with riboflavin, riboflavin was added on the surface of inoculated salmon samples and spread evenly using a sterile spreader, followed by dry for 10 min in a biosafety cabinet. All samples were individually wrapped with a polyethylene film to keep moisture of salmon samples during storage. No significant ($p < 0.05$) difference in the intensity of LED was observed when the intensity sensor was applied with/without the film in the preliminary study (data not shown).

Riboflavin-mediated LED illumination on smoked salmon

Two inoculated samples with/without riboflavin were placed in each LED illumination system at 4, 12, and 25 °C in an incubator at doses of 1.27–2.76 kJ/cm^2 (for 22–48 h). Non-illuminated control samples with/without riboflavin were also placed in an incubator without LED illumination (dark condition). Each of two samples were withdrawn at a selected time interval, immediately placed in a sterile stomacher bag containing 36 mL of 0.1% PW, and homogenized for 2 min with a Paddle blender (Silver Masticator, IUL Instruments GmbH, Königswinter, Germany). The homogenized sample was serially diluted if necessary. A 100-µL diluent was spread on a thin agar layer (TAL) medium consisting of top thin layer of TSA (non-selective agar) and bottom layer of PALCAM Medium Base supplemented with PALCAM Antimicrobial Supplement (selective agar; Difco™, Becton, Dickinson and Co., Sparks, MD, USA) in order to recover injured cells and to selectively enumerate *L. monocytogenes* cells, respectively, followed by incubation for 48–72 h at 37 °C. The number of colonies was enumerated with a colony counter (Rocker Scientific Co. Ltd., Taipei, Taiwan) and expressed in log CFU/cm^2.

Thiobarbituric acid reactive substance (TBARS) assay

A thiobarbituric acid reaction substance (TBARS) assay was carried out in order to analyze lipid peroxidation of smoked salmon by 405 nm LED illumination with or without riboflavin. Malondialdehyde (MDA), an indicator of lipid peroxidation, is produced naturally when ROS attack lipids containing a carbon–carbon double bond, in particular polyunsaturated fatty acids (PUFAs) (Ayala et al., 2014; Kim et al., 2017a).

Smoked salmon samples (ca. 2 g) containing 0–50 μM riboflavin were illuminated to 405 nm LED at 4 °C at a total dose of 2.76 kJ/cm^2 in an incubator. Non-illuminated control samples were stored in the dark. To quantify lipid peroxidation, each of two non-illuminated and illuminated samples with or without riboflavin was withdrawn at a selective time interval. Each sample was mixed with 4 mL of deionized water, 40 μL of 100 × butylated hydroxytoluene (BHT; Sigma-Aldrich, St. Louis, MO, USA), and 4 mL of 10% trichloroacetic acid (TCA; Sigma-Aldrich) in a 50 mL Falcon tube, followed by vigorous vortexing. The mixture was centrifuged at 3,000 × g for 2 min. One milliliter of the supernatant was transferred to a new 15 mL Falcon tube and subsequently added to 1 mL of thiobarbituric acid (TBA; Sigma-Aldrich) reagent freshly prepared (5.2 mg TBA per 1 mL of 5% TCA). The mixture was incubated at 95 °C for 30 min and cooled to room temperature in an ice bath for 10 min. Afterwards, 200 μL of the mixture was transferred to a 96-well plate in duplicate and measured at 532 nm with a Synergy HT multidetection Microplate reader (Bio-Tec Instruments Inc., Winooski, VT, USA). The degree of lipid peroxidation was calculated using a standard curve between 0 and 100 μM MDA (Merck, Darmstadt, Germany) and expressed as μg MDA per g fresh weight (FW).

Color analysis

The color of non-illuminated and illuminated salmon samples with or without riboflavin (0–100 μM) was measured with a reflectance spectrometer (CM-3500d; Konica Minolta Sensing Inc., Osaka, Japan) by set to a D65 illuminant and an observation angle of 10°. The color parameters of L^* (lightness), a^* (red to green), and b^* (yellow to blue) were measured via reflectance values. The values were used to calculate the white index (WI) between non-illuminated control and illuminated samples with or without riboflavin using the following equations (Bermúdez-Aguirre and Barbosa-Cánovas, 2013):

$$WI = 100 - \sqrt{(100 - L^*) + a^{*2} + b^{*2}}$$

Statistical analysis

All experiments were carried out in independent triplicate with duplicate sampling (n = 6). Data were expressed by mean ± standard deviation. Significant differences in the mean values were calculated at the 95% confidence interval ($p < 0.05$) using one-way analysis of variance (ANOVA) and means were separated by least significant difference (LSD) with the IBM SPSS statistical software (version 17.0; SPSS Inc., IBM Co., Armonk, NY, USA).

Results and discussion

Change in temperature during 405 nm LED illumination

The temperature fluctuation on the surface of smoked salmon was monitored for 300 min with 1-min interval during 405 nm LED illumination at different storage temperatures to determine the heat transfer from the LED to salmon samples. To simulate the ideal refrigeration condition, temperature abuse at food establishments, and the room temperature condition, the different storage temperatures at 4, 12, and 25 °C, respectively, were chosen in this study. Results show that the surface temperature of smoked salmon increased by approximately 4.5 °C within 30 min during LED illumination, irrespective of set temperatures, and the elevated temperature was maintained until the end of measurement when the set temperatures of the incubator were 0, 7.5, and 20.5 °C (Fig. 2). Hence, non-illuminated control salmon samples were performed at the incubator temperatures of 4, 12, and 25 °C, respectively, to eliminate the effect of temperature difference under non-illuminated and illuminated conditions during long-term storage. On the other hand, the bacterial suspension in PBS was held at the incubator temperature of 25 °C for both non-illuminated control and illuminated suspension with/without riboflavin due to short-time exposure.

Antibacterial effect of riboflavin mediated 405 nm LED illumination in PBS

Riboflavin, known as a water-soluble vitamin B_2, is essential for the metabolism of nutrients and antioxidant protection for human health (Ashoori and Saedisomeolia, 2014). However, riboflavin is sensitive to lights in aqueous solution and its absorbance spectrum are a sharp peak at 270 nm and shorter peaks at 380 and 445 nm (Ashoori and Saedisomeolia, 2014; Josewin et al., 2018; Liang et al., 2013). Riboflavin could be photodegraded by several pathways including the excited states upon light exposure such as ultra-violet (UV) and visible light at blue

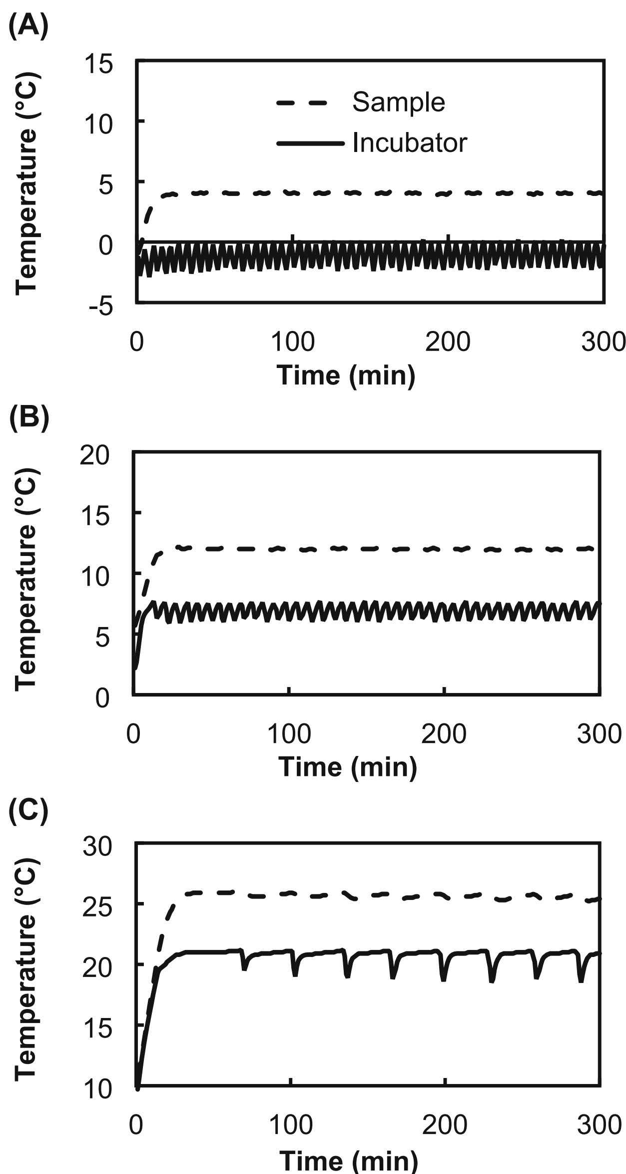

Fig. 2 Temperature profile on the surface of smoked salmon at 4 °C (**A**), 12 °C (**B**) and 25 °C (**C**) during 405 nm LED illumination

wavelengths (Ashoori and Saedisomeolia, 2014; Liang et al., 2013), generating ROS as previously described. Thus, in this study, the antimicrobial activity of 405 nm LED illumination with the aid of exogenous riboflavin against *L. monocytogenes* in PBS at doses as high as 57.6 J/cm^2 at 25 °C was evaluated. Two concentrations of riboflavin (50 and 100 µM) were selected in this study since the maximum water solubility of riboflavin is about 120 µM (Liang et al., 2015) and our previous study also showed that 460 nm LED illumination with 25 µM of riboflavin was less effective than 50 and 100 µM in inactivating *L. monocytogenes* on smoked salmon (Josewin et al., 2018). The present results show that LED illumination alone inactivated 1.9 log CFU/mL of *L. monocytogenes* cells at 57.6 J/cm^2, while riboflavin-mediated LED illumination reduced the populations by 6.2 log CFU/mL (below the detection limit) at 19.2 J/cm^2, revealing that the antibacterial effect of 405 nm LED illumination could be significantly ($p < 0.05$) enhanced in the presence of riboflavin (Fig. 3). There was no significant ($p \geq 0.05$) difference in the inactivation of *L. monocytogenes* between 50 and 10 µM of riboflavin.

Similarly, a previous study demonstrated that 400 nm LED irradiation (total irradiance of 260 mW/cm^2) in pure water for 10 min significantly ($p < 0.05$) reduced 0.9 to 3.0 log CFU/mL of *Streptococcus mutants, Escherichia coli*, and *Pseudomonas aeruginosa,* whereas PDI in the presence of polyphenols, such as caffeic acid and chlorogenic acid, was enhanced by ≥ 5 log reduction (Nakamura et al., 2015). Another study conducted by Luksienė et al. (2013) showed that chlorophyllin-based 405 nm LED illumination in PBS for 5 min (at 3.6 J/cm^2) inactivated by 7 log CFU/mL of *L. monocytogenes* cells, while aminolevulinic acid-based photosensitization caused less than 4 log reduction of the populations for 20 min (14.4 J/cm^2). Wong et al. (2016) reported that 405 and 465 nm LED irradiation at 9 ± 1 °C enhanced the inactivation rate of *S. aureus* in the presence of riboflavin-5′-phosphate (FMN), but *S. aureus* populations were susceptible to 405 nm LED illumination than 465 nm LED, regardless of FMN. Unlike the present study, 465 nm LED irradiation with 60 and 120 µM FMN for 2 h (total doses of 14.4 J/cm^2) inactivated by 41.1% and 94.9% of *S. aureus* populations, respectively, exhibiting that the higher concentration of FMN could cause the better inactivation rate of *S. aureus* under 465 nm LED exposure.

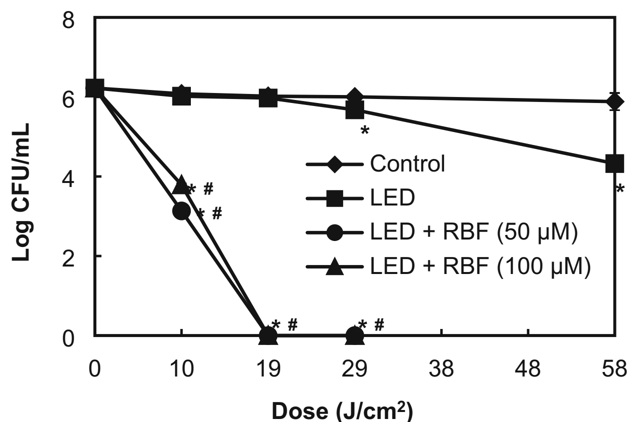

Fig. 3 Antimicrobial activity of 405 nm LED illumination with different concentrations of riboflavin (RBF) on *L. monocytogenes* in phosphate-buffered saline (PBS) at 25 °C. Asterisk (*) indicates significant ($p < 0.05$) difference in cell counts between non-illuminated control and LED illumination with/without riboflavin. Hash (#) indicates significant ($p < 0.05$) difference in cell counts between LED illumination alone and riboflavin-mediated illumination

Behavior of *L. monocytogenes* on smoked salmon surface during LED illumination with/without riboflavin

For food application, the efficacy of 405 nm LED illumination with and without riboflavin (0–100 μM) on *L. monocytogenes* on the surface of smoked salmon was investigated at 4, 12, and 25 °C, respectively, until total doses of 1.27–2.76 kJ/cm^2. As shown in Fig. 4, non-illuminated control cells on smoked salmon remained unchanged during storage at 4 and 12 °C, whereas riboflavin-mediated LED illumination inactivated *L. monocytogenes* populations on smoked salmon by 1.7 to 2.0 log CFU/cm^2 until total doses of 2.76 kJ/cm^2. But no further inactivation was observed at doses greater than 1.27 kJ/cm^2, regardless of the riboflavin concentration. Interestingly, *L. monocytogenes* cells on smoked salmon were found to be the most ($p < 0.05$) susceptible to LED illumination alone after 1.96 kJ/cm^2 at chilling temperatures, compared to riboflavin-mediated LED illumination. At 25 °C, non-illuminated control cells on smoked salmon grew to 6.1 log CFU/cm^2 at the end of storage, while LED illumination reduced the populations of *L. monocytogenes* to 2.2–2.8 log CFU/cm^2 at 1.27 kJ/cm^2, regardless of the presence of exogenous riboflavin (Fig. 4c).

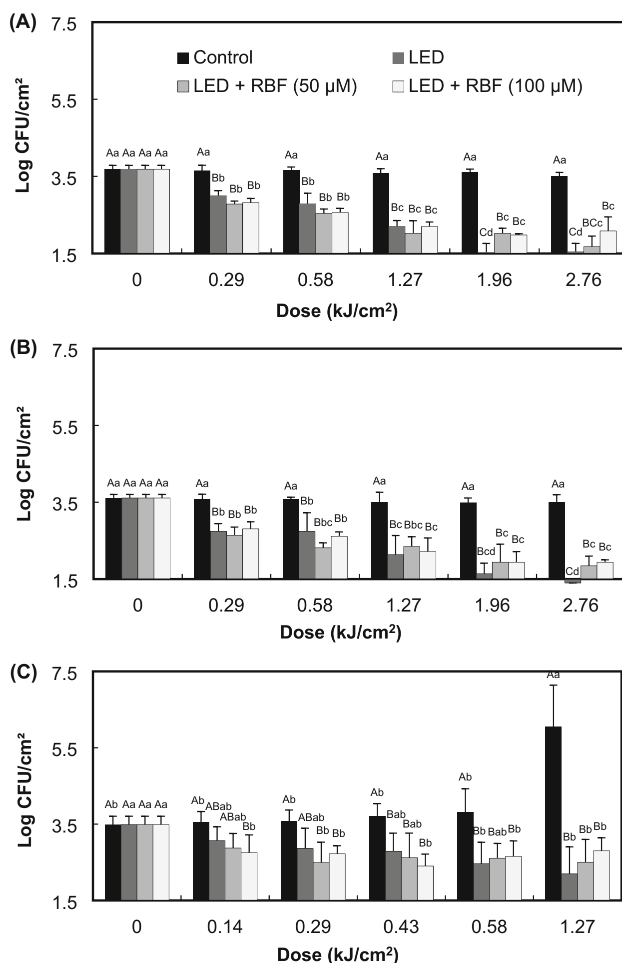

Fig. 4 Effects of 405 nm LED illumination with different concentrations of riboflavin (RBF) on survival of *L. monocytogenes* on the surface of smoked salmon at 4 °C (**a**), 12 °C (**b**), and 25 °C (**c**). Uppercase letters (A–B) for the same exposure dose and lowercase letters (a–d) for the same bar indicate significant ($p < 0.05$) difference

The antibacterial effect of 405 nm LED with and without the additional riboflavin on smoked salmon was considerably decreased when compared with that in PBS. Similarly, our previous study has shown that *S.* Enteritidis in PBS were more susceptible to 405 nm LED illumination than that on cooked chicken (Kim et al., 2017b). Another study conducted by Ghate et al. (2016) reported that 460 nm LED illumination inactivated by 5 log CFU/mL of *Salmonella* populations in orange juice. In contrast, 460 nm LED illumination alone on smoked salmon did not inactivate *L. monocytogenes* populations (Josewin et al., 2018). This might be because liquid samples can be evenly illuminated by LEDs, while the efficacy of LED illumination can be limited on the surface of solid food matrices due to uneven and opaque surface such as shadow effect.

Furthermore, unlike PBS, no synergistic effect was observed under 405 nm LED illumination combined with riboflavin on smoked salmon. Food components could also affect bacterial behaviors on smoked salmon by LED illumination. Salmon has high levels of fat contents (5.7 to 17.6%), especially polyunsaturated lipids ($\sim$ 38% of total fat contents) (Nicorescu et al., 2014; Wold et al., 1996). The double bonds in polyunsaturated lipids are susceptible to deterioration by oxidation, resulting in lipid peroxidation (Ma et al., 2018). Lipid peroxidation products, such as reactive aldehydes (MDA and 4-hydroxynonenal), can also react with DNA directly (Catalá, 2009; Ma et al., 2018). On the other hand, as described above, riboflavin is sensitive to light exposure, but also act as antioxidants under oxidative stress, particularly lipid peroxidation and oxidative injury (Ashoori and Saedisomeolia, 2014; Nicorescu et al., 2014), potentially playing a crucial role in preventing lipid peroxidation (Ma et al., 2018). Thus, it is postulated that some of unexcited riboflavin due to shadow effect on smoked salmon might act as antioxidant protection by quenching ROS generated by LED illumination.

In our previous study, 460 nm LED illumination with riboflavin on smoked salmon effectively inactivated *L. monocytogenes* by 0.5–1.6 log CFU/cm^2 at 2.4 kJ/cm^2 (Josewin et al., 2018), while 405 nm LED alone on smoked salmon nearly reached below the detection limit (< 1.4 log CFU/cm^2) at chilling temperatures in this study. These findings indicate that 405 nm LED could be much more effective in inactivating *L. monocytogenes* than 460 nm LED. Amit et al. (2016) also reported that the greater antibacterial effect was observed in 405 nm LED than

460 nm LED. Bacterial inactivation by visible light can differ depending on the absorption spectrum of photosensitizers. For instance, porphyrin compounds naturally produced by some bacteria have a strong absorption band at around 400 nm (Kim and Yuk, 2017; Redi and Jori, 1988), resulting in the production of ROS by 405 nm LED illumination in the presence of oxygen and then indirectly damages to bacterial components, such as DNA, lipids, proteins, and membrane (Amit et al., 2016; Kim and Yuk, 2017). Another possible explanation could be because some portions of 405 nm LED spectrum were ranged in the UV which could directly cause DNA damage (Amit et al., 2016). For these reasons, 405 nm LED itself might enhance the bactericidal effect by generating much more ROS than 460 nm LED.

The present results show that storage temperatures did not affect the bactericidal efficacy of 405 nm LED illumination on smoked salmon, irrespective of the presence of exogenous riboflavin. Unlike this, our previous studies using 405 nm LED on *Salmonella* on fresh-cut papaya and cooked chicken proved the greater antibacterial effect at 4 °C than those of 10 and 20 °C (Kim et al., 2017a, 2017b). Kim et al. (2017c) also showed the greater antibacterial effect against *L. monocytogenes* on fresh-cut mango at lower temperatures of 4 and 10 °C than that of 20 °C, whereas *Salmonella* on mango were susceptible to LED illumination at all temperatures tested. On the contrary, the antibacterial effect of 460 nm LED illumination against *Salmonella* in orange juice was enhanced at room temperature rather than chilling temperatures (Ghate et al., 2016). These findings indicate that the different bacterial susceptibility to LED illumination might be dependent on species as well as be affected by experimental design, LED wavelengths, illumination temperatures, and food components such as antioxidants, lipids, and proteins (Kim et al., 2017a, b, c).

Lipid peroxidation and color change of smoked salmon by LED illumination with/without riboflavin

Lipids are known as one of chemically unstable food components and easily undergo oxidation and autoxidation processes (German, 1999; Nicorescu et al., 2014). Rancidity and off-flavor in salmon are caused by lipid peroxidation, being able to impact the sensory quality (Refsgaard et al., 1998). Therefore, chemical quality of illuminated smoked salmon with and without riboflavin should be evaluated since polyunsaturated lipids and color are known to be susceptible to light. In this work, no significant ($p \geq 0.05$) changes in TBARS values were observed in non-illuminated smoked salmon with and without riboflavin during storage at 4 °C (Fig. 5). On the other hand, TBARS values for illuminated smoked salmon significantly

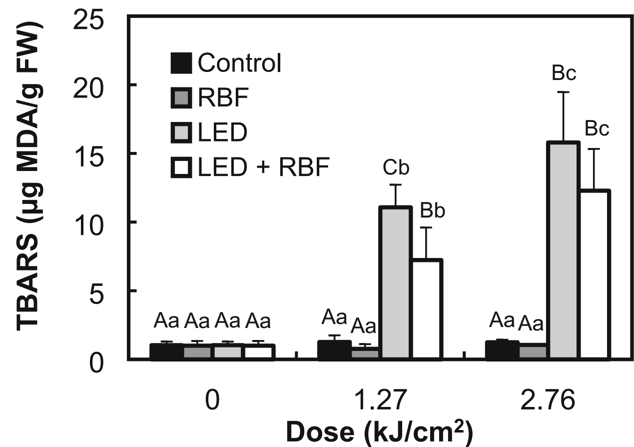

Fig. 5 Lipid peroxidation of smoked salmon by 405 nm LED illumination with different concentrations of riboflavin (RBF) at 4 °C. Uppercase letters (A–C) for the same exposure dose and lowercase letters $^{(a–c)}$ for the same bar indicate significant ($p < 0.05$) difference

($p < 0.05$) increased with the increase in exposure doses, regardless of the presence of riboflavin, but the mean values of illuminated smoked salmon without riboflavin were higher than riboflavin-mediated illuminated smoked salmon. A previous study reported that pulsed light (PL) treatment at 30 J/cm^2 induced lipid peroxidation in raw salmon samples (Nicorescu et al., 2014). Another study conducted by Pedrós-Garrido et al. (2018) revealed that PL and UV-C treatments for 30–90 s also caused the lipid peroxidation in salmon samples. In contrast, Colejo et al. (2018) demonstrated that lipid peroxidation in UV-C treated smoked salmon samples remained unchanged until 900 mJ/cm^2, while TBARS values for smoked salmon treated with non-thermal atmospheric plasma (NTAP) after 4 min were higher than those for control untreated samples. However, lipid peroxidation observed in these previous studies did not exceed the consumption limit of 7 to 8 mg MDA/kg (Coleio et al., 2018; Oğuzhan, 2013), whereas our results were over the consumption limit probably due to the long-term exposure. Furthermore, the lipid peroxidation may induce color changes due to the co-oxidation of water-soluble and fat-soluble pigments such as carotenoids and myoglobin in food products (Nicorescu et al., 2014). Thus, color modification on illuminated smoked salmon samples was also investigated for further experiment.

Whiteness index (WI) is useful to quantify the degree of discoloration during processing, which is generally used for consumer's preference of white colors (Anyasi et al., 2015). In this study, changes in WI of smoked salmon illuminated by 405 nm LED illumination with and without riboflavin were measured to determine if ROS generated by LED illumination causes discoloration. As shown in Fig. 6, the WI values of non-illuminated smoked salmon with and without riboflavin remained unchanged ($p \geq 0.05$) during

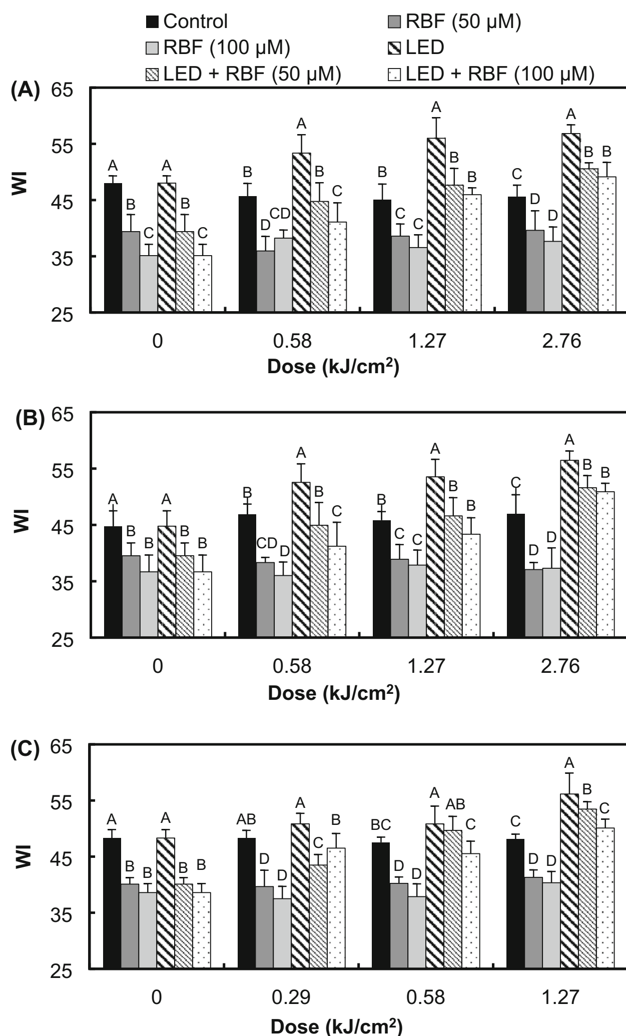

Fig. 6 Comparison of white index (WI) between 405 nm LED illuminated and non-illuminated smoked salmon with/without RBF at different temperatures of 4 °C (**a**), 12 °C (**b**), and 25 °C (**c**). Uppercase letters (A–D) for the same exposure dose indicate significant ($p < 0.05$) difference

storage in the dark, regardless of storage temperatures, although the WI values of non-illuminated smoked salmon with exogenous riboflavin were lower than those of non-illuminated control smoked salmon. On the other hand, LED illumination significantly ($p < 0.05$) increased by 8–14 of the WI values until 1.27–2.76 kJ/cm^2, irrespective to storage temperatures and the presence of riboflavin, but the mean values of WI in illuminated smoked salmon were statistically ($p < 0.05$) higher than those of riboflavin-mediated illuminated samples. In contrast to the present results, our previous study has reported that no color change in illuminated smoked salmon with 460 nm LED alone was observed, while LED illumination with 100 mM riboflavin significantly ($p < 0.05$) increased the WI (Josewin et al., 2018). The color of salmon ranges from pink to orange-red and might be correlated to the content of astaxanthin that is one of the predominant carotenoids (Yagiz et al., 2010). Astaxanthin is sensitive to oxidation and irradiation due to its highly unsaturated structures (Takeungwongtrakul and Benjakul, 2016; Yagiz et al., 2010). For example, the amount of astaxanthin was decreased with the increase in irradiation dose, resulting in a loss of redness of smoked salmon (Yagiz et al. 2010). Unlike these findings, our previous studies have demonstrated that 405 nm LED illumination on fresh-cut mango and papaya did not adversely influence the color as well as contents of β-carotene, flavonoids, antioxidant capacity, and ascorbic acid compared to non-illuminated control fruits during long-term exposure, regardless of storage temperatures (Kim et al., 2017a; 2017c). The discrepancy in our previous and present studies may be attributed to types and contents of nutrition and bioactive compounds in food matrices.

In conclusion, the results in this study demonstrate that 405 nm LED illumination in the presence of riboflavin could accelerate the inactivation rate of *L. monocytogenes* in aqueous solution, while the effect of riboflavin-mediated LED illumination was highly diminished on the surface of smoked salmon that in PBS. Interestingly, LED illumination alone could more effectively reduce *L. monocytogenes* populations on smoked salmon at chilling temperatures than riboflavin-mediated LED illumination. Although illumination with and without riboflavin caused lipid peroxidation and discoloration of smoked salmon, this study proved the potential of 405 nm LED to play a role in a food preservation technology for RTE fishery products. Such technology can be applied to preserve smoked fish susceptible to post-processing contamination and can be also applicable to food establishments where smoked salmon should be sold within 2–3 days. However, the optimization of LED illumination conditions is crucial to minimize the quality changes of smoked salmon during storage. Furthermore, a scale-up study is required to be applied to commercial food systems under actual operating conditions.

Acknowledgements This work was supported by Guangdong Province Science and Technology Plan Project (International Science and Technology Cooperation Field) (Grant No. 2020A0505100049).

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Anyasi TA, Jideani AI, Mchau GR. Effect of organic acid pretreatment on some physical, functional and antioxidant properties of flour obtained from three unripe banana cultivars. Food Chemistry. 172: 515-522 (2015)

Ashoori M, Saedisomeolia A. Riboflavin (vitamin B_2) and oxidative stress: a review. British Journal of Nutrition. 111: 1985-1991 (2014)

Aponiene K, Paskeviciute E, Reklaitis I, Luksiene Z. Reduction of microbial contamination of fruits and vegetables by hypericin-based photosensitization: Comparison with other emerging antimicrobial treatments. Journal of Food Engineering. 144: 29-35 (2015)

Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. 2014: 360438 (2014)

Bermúdez-Aguirre D, Barbosa-Cánovas GV. Disinfection of selected vegetables under nonthermal treatment: Chlorine, acid citric, ultraviolet light and ozone. Food Control. 29: 82-90 (2013)

Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chemistry and Physics of Lipids. 157: 1-11 (2009)

Chau ML, Aung KT, Hapuarachchi HC, Lee PSV, Lim PY, Kang JSL, Ng Y, Yap HM, Yuk H G, Gutiérrez RA, Ng LC. Microbial survey of ready-to-eat salad ingredients sold at retial reveals the occurrence and the presistance of *Listeria monocytogenes* sequence type 2 and 87 in pre-packed smoked salmon. BMC Microbiology. 17: 46 (2017)

Colejo S, Alvarez-Ordóñez A, Prieto M, González-Raurich M, López M. Evaluation of ultraviolet light (UV), non-thermal atmospheric plasma (NTAP) and their combination for the control of foodborne pathogens in smoked salmon and their effect on quality attributes. Innovative Food Science and Emerging Technologies. 50: 84-93 (2018)

Ghate V, Ng KS, Zhou W, Yang H, Khoo GH, Yoon WB, Yuk HG. Antibacterial effect of light emitting diodes of visible wavelengths on selected foodborne pathogens at different illumination temperatures. International Journal of Food Microbiology. 166: 399-406 (2013)

Ghate V, Kumar A, Zhou W, Yuk HG. Irradiance and temperature influence the bactericidal effect of 460-nanometer light-emitting diodes on *Salmonella* in orange juice. Journal of Food Protection. 79: 553-560 (2016)

Ghate V, Kumar A, Kim MJ, Bang WS, Zhou W, Yuk HG. Effect of 460 nm light emitting diode illumination on survival of *Salmonella* spp. on fresh-cut pineapples at different irradiances and temperatures. Journal of Food Engineering. 196: 130–138 (2017)

German JB. Food processing and lipid oxidation. Advances in Experimental Medicine and Biology. 459: 23-50 (1999)

Heir E, Liland KH, Carlehög M, Holck AL. Reduction and inhibition of *Listeria monocytogenes* in cold-smoked salmon by Verdad N6, a buffered vinegar fermentate, and UV-C treatments. International Journal of Food Microbiology. 291: 48-58 (2019)

Josewin SW, Ghate V, Kim MJ, Yuk HG. Antibacterial effect of 460 nm light-emitting diode in combination with riboflavin against *Listeria monocytogenes* on smoked salmon. Food Control. 84: 354-361 (2018)

Kim MJ, Mikš-Krajnik M, Kumar A, Ghate V, Yuk HG. Antibacterial effect and mechanism of high-intensity 405±5 nm light emitting diode on *Bacillus cereus, Listeria monocytogenes*, and *Staphylococcus aureus* under refrigerated condition. Journal of Photochemistry and Photobiology B: Biology. 153: 33-39 (2015)

Kim MJ, Mikš-Krajnik M, Kumar A, Yuk HG. Inactivation by 405±5 nm light emitting diode on *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Shigella sonnei* under refrigerated condition might be due to the loss of membrane integrity. Food Control. 59: 99-107 (2016)

Kim MJ, Bang WS, Yuk HG. 405±5 nm light emitting diode illumination causes photodynamic inactivation of *Salmonella* spp. on fresh-cut papaya without deterioration. Food Microbiology. 62: 124–132 (2017a)

Kim MJ, Ng BXA, Zwe YH, Yuk HG. Photodynamic inactivation of *Salmonella* enterica Enteritidis by 405±5-nm light-emitting diode and its application to control salmonellosis on cooked chicken. Food Control. 82: 305-315 (2017b)

Kim, MJ, Tang CH, Bang WS, Yuk HG. Antibacterial effect of 405±5 nm light emitting diode illumination against *Escherichia coli* O157: H7, *Listeria monocytogenes*, and *Salmonella* on the surface of fresh-cut mango and its influence on fruit quality. International Journal of Food Microbiology. 244: 82-89 (2017c)

Kim MJ, Yuk HG. (2017) Antibacterial mechanism of 405-nanometer light-emitting diode against *Salmonella* at refrigeration temperature. Applied and Environmental Microbiology. 83: e02582-16 (2017)

Kumar A, Ghate V, Kim M., Zhou W, Khoo GH, Yuk HG. Antibacterial efficacy of 405, 460 and 520 nm light emitting diodes on *Lactobacillus plantarum, Staphylococcus aureus* and *Vibrio parahaemolyticus*. Journal of Applied Microbiology. 120: 49-56 (2016)

Liang JY, Yuann JMP, Cheng CW, Jian HL, Lin CC, Chen LY. Blue light induced free radicals from riboflavin on *E. coli* DNA damage. Journal of Photochemistry and Photobiology B: Biology. 119: 60–64 (2013)

Liang JY, Cheng CW, Yu CH, Chen LY. Investigations of blue light-induced reactive oxygen species from flavin mononucleotide on inactivation of *E. coli*. Journal of Photochemistry and Photobiology B: Biology. 143: 82–88 (2015)

Li X, Kim MJ, Bang WS, Yuk HG. Anti-biofilm effect of 405-nm LEDs against *Listeria monocytogenes* in simulated ready-to-eat fresh salmon storage conditions. Food Control. 84: 513-521 (2018)

Luksiene Z, Zukauskas A. Prospects of photosensitization in control of pathogenic and harmful micro-organisms. Journal of Applied Microbiology. 107: 1415e1424 (2009)

Luksiene Z, Paskeviciute E. Novel approach to the microbial decontamination of strawberries: chlorophyllin-based photosensitization. Journal of Applied Microbiology. 110: 1274–1283 (2011)

Luksienė Z, Kokstaite R, Katauskis P, Skakauskas V. Novel approach to effective and uniform inactivation of Gram-positive *Listeria* monocytogenes and Gram-negative *Salmonella enterica* by photosensitization. Food Technology and Biotechnology. 51: 338-344 (2013)

Ma Y, Hou J, Hao W, Liu J, Meng L, Lu Z. Influence of riboflavin on the oxidation kinetics of unsaturated fatty acids at the air/aqueous interface revealed by sum frequency generation vibrational spectroscopy. Physical Chemistry Chemical Physics. 20: 17199-17207 (2018)

Mori M, Hamamoto A, Takahashi A, Nakano M, Wakikawa N, Tachibana S, Kinouchi Y. Development of a new water sterilization device with a 365 nm UV-LED. Medical and Biological Engineering and Computing. 45: 1237-1241 (2007)

Nakamura K, Ishiyama K, Sheng H, Ikai H, Kanno T, Niwano Y. Bactericidal activity and mechanism of photoirradiated polyphenols against Gram-positive and-negative bacteria. Journal of Agricultural and Food Chemistry. 63: 7707-7713 (2015)

Nicorescu I, Nguyen B, Chevalier S, Orange N. Effects of pulsed light on the organoleptic properties and shelf-life extension of pork and salmon. Food Control. 44: 138-145 (2014)

Oğuzhan P. Effect of salting and packaging on liquid-smoked rainbow trout fillets during refrigerated storage. African Journal of Microbiology Research. 50: 5719-5725 (2013)

Pang X, Wong C, Chung HJ, Yuk HG. Biofilm formation of *Listeria monocytogenes* and its resistance to quaternary ammonium compounds in a simulated salmon processing environment. Food Control. 98: 200-208 (2019)

Pedrós-Garrido S, Condón-Abanto S, Clemente I, Beltrán JA, Lyng JG, Bolton D, Brunton N, Whyte P. Efficacy of ultraviolet light (UV-C) and pulsed light (PL) for the microbiological decontamination of raw salmon (*Salmo salar*) and food contact surface materials. Innovative Food Science and Emerging Technologies. 50: 124-31 (2018)

Reddi E, Jori G. Steady-state and time-resolved spectroscopic studies of photodynamic sensitizers: porphyrins and phthalocyanines. Research on Chemical Intermediates. 10: 241-268 (1988)

Refsgaard HH, Brockhoff PB, Jensen B. Sensory and chemical changes in farmed Atlantic salmon (*Salmo salar*) during frozen storage. Journal of Agricultural and Food Chemistry. 46: 3473-3479 (1998)

Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Fernandez Escamez PS, Girones R, Herman L, Koutsoumanis K, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlstrom H, Takkinen J, Wagner M, Arcella D, Da Silva Felicio MT, Georgiadis M, Messens W. Lindqvist R. EFSA panel on biological hazards. Scientific opinion on the *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA Journal. 16: 5134 (2018)

Rørvik LM. *Listeria monocytogenes* in the smoked salmon industry. International Journal of Food Microbiology. 62: 183-190 (2000)

Takeungwongtrakul S., Benjakul, S. Astaxanthin degradation and lipid oxidation of Pacific white shrimp oil: kinetics study and stability as affected by storage conditions. International Aquatic Research. 8: 15-27 (2016)

Tocmo R, Krizman K, Khoo WJ, Phua LK, Kim M, Yuk HG. *Listeria monocytogenes* in vacuum-packed smoked fish products: sccurrence, routes of contamination, and potential intervention measures. Comprehensive Reviews in Food Science and Food Safety. 13: 172-189 (2014)

Yagiz Y, Kristinsson HG, Balaban MO, Welt BA, Raghavan S, Marshall MR. Correlation between astaxanthin amount and $a*$ value in fresh Atlantic salmon (*Salmo salar*) muscle during different irradiation doses. Food Chemistry. 120: 121-127 (2010)

Ye M, Neetoo H, Chen H. Effectiveness of chitosan-coated plastic films incorporating antimicrobials in inhibition of *Listeria monocytogenes* on cold-smoked salmon. International Journal of Food Microbiology. 127: 235-240 (2008)

Wold JP, Jakobsen T, Krane L. Atlantic salmon average fat content estimated by near-infrared transmittance spectroscopy. Journal of Food Science. 61: 74-77 (1996)

Wong TW, Cheng CW, Hsieh ZJ, Liang JY. Effects of blue or violet light on the inactivation of *Staphylococcus aureus* by riboflavin-5′-phosphate photolysis. Journal of Photochemistry and Photobiology B: Biology. 173: 672-680 (2017)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.