1 TITLE PAGE				
2 - Food S	cience of Animal Resources -			
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ARTICLE INFORMATION	Fill in information in each box below			
Article Type	Research article			
Article Title	Effect of UV-C irradiation on foodborne pathogen inactivation in prosciutto and changes on its physicochemical properties			
Running Title (within 10 words)	Changes in prosciutto quality by UV-C treatment			
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Special remarks – if authors have additional information to inform the editorial office				
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.			
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This research was partially supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2022R1A6A1A03055869). This paper also was supported by the KU Research Professor Program of Konkuk University.			
Author contributions (This field may be published.) Ethics approval (IRB/IACUC)	Conceptualization: Choi MJ. Data curation: Lee J, Lee S, Jung US. Methodology: Lee J, Lee S, Jung US, Lee SY Software: Lee J, Lee SY, Hong GP. Validation: Lee J, Lee S, Jung US, Hong GP. Investigation: Lee J, Lee S, Jung US. Writing - original draft: Lee J. Writing - review & editing: Lee J, Choi MJ. This article does not require IRB/IACUC approval because there are no human			
(This field may be published.)	and animal participants.			

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9	Effect of UV-C irradiation on foodborne pathogens in prosciutto and changes in
10	physicochemical properties
11	
12	Abstract
13	Ready-to-eat sliced prosciutto samples were treated with ultraviolet (UV) light (wavelength:
14	265 and 275 nm/intensity: 10 and 50 mW) after inoculation with hepatitis E virus (HEV),
15	Escherichia coli O157:H7, or Listeria monocytogenes. The parameters of prosciutto quality
16	assessed were: color, texture, pH, water content, water holding capacity, oxidative rancidity,
17	and freshness. The prosciutto sample under UV-C light was influenced on changes of color,
18	lipid and protein oxidation, and protein oxidation due to free radical generation. UV-C light
19	reduced E. coli O157:H7 and L. monocytogenes counts by up to 2.23 Log and 1.85 Log
20	colony forming units/g, respectively. The amount of HEV was also significantly reduced after
21	treatment (p<0.05). HEV was no longer detected after 10 min of treatment at an intensity of
22	50 mW. Consequently, UV-C treatment above 265 nm at 50 mW for 10 min is an effective
23	strategy for maintaining product quality and improving food safety. The results of this study
24	advance the application of non-thermal food preservation.
25	
26	Keywords: UV-C; hepatitis E virus; prosciutto; pathogens; quality
27	

28 Introduction

29 Contamination of fresh and dried meat by pathogens is a persistent challenge in the food 30 industry. Fresh meat, at the point of slaughter and in meat processing, is a transmitting 31 medium for viruses, such as hepatitis E, hepatitis A, and norovirus (Bae et al., 2015; Doyle, 32 2010; Park et al., 2010). Liu et al. (2019) reported that protein deterioration and lipid 33 oxidation during storage are potential consequences of bacterial contamination in dry-cured 34 meat products. Meat product spoiling mechanisms are associated with biochemical reactions 35 involving both external microbes and internal enzymes (Pedrós-Garrido et al., 2018). Gram-36 positive bacteria, such as Staphylococcus aureus and Brochothrix thermosphacta and gram-37 negative aerobic bacteria, such as *Pseudomonas* and *Enterobacteriaceae*, are common 38 spoilage microorganisms. Escherichia coli and Listeria monocytogenes are also major food pathogens. E. coli O157:H7 has caused numerous foodborne outbreaks in a variety of foods, 39 40 such as hamburger meat, apple cider, leafy greens, and poultry products (Ravishankar et al., 2009). In terms of the total cost of foodborne illness, L. monocytogenes is among the top five 41 42 pathogens (Ha & Kang, 2014). Thus, inactivating microorganisms and viruses is essential for 43 ensuring the safety of food (Kim et al., 2024; Kulawik et al., 2022). 44 Conventional thermal processing can negatively affect the sensory and nutritional qualities of food products; however, non-thermal processing is a preservation technique that has a 45 46 minimal negative impact on the nutritional and quality properties of foods (Hong et al., 2008; 47 Jeon & Ha, 2018; Rosario et al., 2021). The use of ultraviolet (UV) light irradiation as an alternative and less expensive method of effectively reducing the number of microbes on 48 49 food surfaces has attracted interest in food science and in the food industry (Ha et al., 2016; 50 Usaga et al., 2017). In a number of studies, UV light irradiation has been used to control

51 major food-borne pathogens, such as *Escherichia coli* O157: H7, *Salmonella enterica*, and

Listeria monocytogenes (Fan et al., 2021; Holland et al., 2020; Keklik, 2020; Yeh et al.,
2018).

54 In general, studies have shown that applying UV light to meat, poultry, and fish products 55 can preserve their nutritional value and quality (Liu et al., 2019; Mikš-Krajnik et al., 2017; Pedrós-Garrido et al., 2018). However, considering the probable modifications of 56 57 photosensitive molecules, UV-C treatment has the potential to affect the physicochemical 58 characteristics of meat products, and several undesirable changes may occur in nutritional 59 attributes. Absorption of UV energy by the food material depends on the wavelength of the 60 UV light and the structure and photosensitivity of the food molecules (Söbeli et al., 2021). 61 Bintsis et al. (2000) reported that short-wavelength ultraviolet light (UV-C range: 200-280 62 nm) is the most harmful to cells among UV because it is much easily absorbed by DNA than longer-wavelength (UV-A range: 320-400 nm) or mid-wavelength (UV-B range: 280-320 63 nm) ultraviolet light. Moreover, factors that affect the design and performance of UV 64 sterilizers include mechanical properties such as UV light intensity output, density, and 65 66 irradiation dose. Among them, pathogen resistance varies depending on the UV intensity, which is a major factor in the efficiency of UV-C treatment (Rosario et al., 2021). Hayashi et 67 al. (2021) reported that 265 nm is suitable for inactivating pathogens (E. coli, Staphylococcus 68 69 aureus, and Bacillus cereus) due to protein damage. In addition, Bowker et al. (2011) 70 reported that UV inactivation of *E. coli* was more effective at 275 nm than at 255 nm. 71 Studies on inactivation utilizing UV-C irradiation have shown its efficacy in reducing 72 bacterial counts on meat, with or without altering quality (Reichel et al., 2020; Söbeli et al., 73 2021). UV-C radiation cannot penetrate deeper tissue layers; hence, its effect is restricted to 74 surface decontamination. The effectiveness of UV-C treatment at various wavelengths is 75 influenced by the chemical composition of the food product; hence it is unclear to what extent this preservation technique reduces bacterial contamination of processed meat products 76

77	(Guerrero-Beltrán & Barbosa-Cánovas, 2004; Sastry et al., 2000). The present study
78	investigated the effect of the physicochemical properties and inactivation efficiency
79	according to UV-C wavelength and intensity on the inactivation of E. coli O157:H7, L.
80	monocytogenes, and HEV in prosciutto. Additionally determined was the impact of UV-C
81	light on the quality characteristics of meat products.
82	
83	Materials and Methods
84	Materials
85	OURHOME (Seoul, South Korea) provided the prosciutto. Chung-Ang University (Seoul,
86	Korea) provided the HEV. Before usage, the bacteria Escherichia coli O157:H7 (NCCP
87	15739) and Listeria monocytogenes KCCM 40307 were diluted in sterile saline water (Difco,
88	Detroit, MI, USA). All the chemicals used for the analysis were purchased from a local
89	supplier and were of reagent grade.
90	
91	Sample preparation
92	The steps described here were according to the instructions of the supplier (OURHOME),
93	as illustrated in Fig. S1. In total, 42 packages (14×3) of prosciutto were subjected to
94	ultraviolet C-band (UV-C) analysis (400 mm \times 300 mm \times 200 mm, W \times L \times H) (OURHOME,
95	Seoul, Korea). The UV-C treatment was performed using one pack at a time, and the
96	wavelength was 265 and 275 nm (different inactivation effect on pathogens), intensity was 10
97	and 50 mW, and the exposure time was 0–900 s. From one batch (one pack containing 10
98	slices of prosciutto), five slices were randomly chosen for texture analysis and the remaining
99	slices for chemical analysis.
100	
101	Visible appearance and color measurements

- 102 A digital camera (α 350; Sony, Tokyo, Japan) was used to obtain images of prosciutto
- 103 samples, and the characteristics were observed. The color values were measured with a color
- 104 reader (CR-10; Konica Minolta Sensing Inc., Tokyo, Japan) using a white standard plate as a
- 105 calibration: lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*) were 97.83, +0.43,
- 106 and +1.98, respectively. The device was positioned directly on the prosciutto's surface at
- 107 various points. The total color difference (ΔE) was calculated via computation as follows:

(1)

108 $\Delta \mathbf{E} = \sqrt{(\Delta \operatorname{CIE} L^*)^2 + (\Delta \operatorname{CIE} a^*)^2 + (\Delta \operatorname{CIE} b^*)^2}$

109 where $\Delta \operatorname{CIE} L^*$, $\Delta \operatorname{CIE} a^*$, and $\Delta \operatorname{CIE} b^*$ represent the change in each color after plasma

110 treatment. Two slices from each of the three batches totaled six slices for the color

- 111 measurement. (n = 6, three batches \times two replications).
- 112

113 *pH measurement*

A pH meter (S-220; Mettler Toledo Co., Zurich, Switzerland) was used to measure the pH of 5 g prosciutto samples after homogenizing for 60 s at 25 °C with 45 mL of distilled water. For every sample, three readings were obtained. The pH meter was calibrated at 25 °C using a standard buffer solution (pH 4, 7, and 10). This measurement was made with six slices in total, two slices from each 3-batch (n = 6, three batches \times two replications).

119

120 Water content measurement

- 121 The heat-drying method (AOAC, 2012) was used to determine the water content of the
- 122 samples. A dry oven (OF-105; Daihan Scientific Co., Ltd., Gangwon-do, Korea) was used to
- 123 weigh and dry a sample of prosciutto weighing about 2 g for about 6 h, or until a constant
- 124 weight was achieved. Water content has been determined as follows:

125 Water content (%) = $[(W_1 - W_2)/W_1] \times 100$ (2)

where W_1 and W_2 are the initial and final weights of the sample, respectively. This measurement was made with six slices in total, two slices from each 3-batch (n = 6, three batches × two replications).

129

130 Water holding capacity (WHC)

With minor adjustments, the technique outlined by Choi et al. (2018) was used to evaluate the prosciutto samples' capability to hold onto moisture. A centrifuge tube containing about 1 g of prosciutto sample was used, and the tube was centrifuged at 3,000 g for 10 min at 4°C.

134 The WHC was calculated as:

135 WHC (%) = $(W_2/W_1) \times 100$ (3)

136 where W_1 is the initial sample weight and W_2 is the sample weight after centrifugation. This

137 measurement was made twice per batch (n = 6; three batches × two replicates).

138

139 Hardness

140 The texture of the samples was determined using a texture analyzer (CT3; Brookfield Co.,

141 USA). A circular plate probe (TA4/1000; 38.1 mm in diameter) was used to compress

samples that were 1 cm by 1 cm twice, to 50% of their initial height. The compression was

143 performed at a speed of 1 mm/s with a trigger load of 100 g. Using new batches of prosciutto

144 $(n = 15, three batches \times five replications)$, every step was repeated five times.

145

146 Scanning electron microscopy (SEM)

147 Prosciuttos were cut into thin slices and the morphology was observed using a scanning

148 electron microscope (TM4000Plus; Hitachi, Tokyo, Japan) with backscattered electron

149 detection at 15 kV and $500 \times$ magnification.

151 2-Thiobarbituric acid reactive substances (TBARS)

152 A minor modification to the procedure provided by Lee et al. (2021) allowed for the 153 determination of secondary lipid oxidation from the TBARS values. After homogenizing the 154 samples (4 g) in 16 mL of distilled water, the samples eluted for 30 min. After the homogenates (20 mL) were filtered, 0.5 mL of the sample was combined with 4.5 mL of 155 156 TBA solution—a mixture of 0.375% TBA reagent, 15% trichloroacetic acid, and 0.25 N 157 hydrochloric acid—to measure the amount of TBARS present. The sample was then heated in 158 a shaking water bath (MaXturdy 45, DAIHAN[®]) for 15 min at 95°C. After the heated 159 mixture was cooled for thirty minutes at 25°C, it was centrifuged for ten minutes at 25°C at 160 3,000 g. A spectrophotometer (MultiskanTM GO UV/VIS; Thermo Fisher, Waltham, MA, 161 USA) was used to measure the absorbance of the supernatant at 532 nm. This measurement was made with six slices in total, two slices from each 3-batch (n = 6, three batches \times two 162 163 replications).

164

165 Volatile basic nitrogen (VBN) content

166 With minor adjustments, the Conway microdiffusion method (Lee et al., 2021) was used to determine the VBN content. A total of six slices were used, two from each of the three 167 168 batches (n = 6, two batches \times three replications). After homogenizing the sample (4 g) in 16 169 mL of distilled water, the sample was left to elute for 30 min. Whatman No. 1 filter paper 170 (GE Healthcare Life Science, Sheffield, UK) was used to filter the homogenate (20 mL). The 171 1 mL of filtered sample was added to the outer section of the Conway dish along with 1 mL of 0.01 N H₃BO₃ and 100 µL of Conway solution. The inner portion of the Conway dish was 172 173 filled with a mixture of 0.066% methyl red and 0.066% bromocresol green in aqueous ethanol. In addition, the outer portion of the dish received 1 mL of 50% K₂CO₃. Following a 174 175 2 h incubation period at 37 °C, the Conway dish was titrated with $0.02 \text{ N H}_2\text{SO}_4$ until the

176 Conway reagent turned red. The VBN values were determined using the following factors:

177 the weight (g) of the sample (S), the dilution amount (C), the factor of $H_2SO_4(f)$, the titration

178 volume of $0.02 \text{ N H}_2\text{SO}_4$ (mL) (A), and the titration volume of the blank (mL) (B).

179 VBN (mg/100 g) = $[14.007 \times (A-B) \times f \times 100 \times C]/S$ (4)

180

181 Preparation and inoculation of prosciutto

Samples were prepared with minor modifications to the Lee et al. (2023) method in order to verify the effects of UV-C irradiation. The following were added to a 20-g sample taken from each of the 15 packages: 1 mL of a 50-fold diluted HEV stock (106 plaque-forming units (PFU)/mL), 100 μ L of a colony-forming unit (CFU)/mL of *E. coli* O157:H7 stock, and 100 μ L of a CFU/mL of *L. monocytogenes* stock. The mixture was then packed into sterile polyethylene pouches. The packages were vacuum-packed and exposed to UV-C radiation after drying.

189

190 Microbiological analyses

191 To verify the effects of UV-C, the samples were processed following Lee et al. (2023) procedures. After being aseptically moved to a filtered stomacher bag (3M Science, MN, 192 193 USA), each sample was homogenized for 5 min and diluted with an equal amount of saline 194 water. The mixture and the supernatant were centrifuged at 4°C for 30 min at 10,000 g and 15 195 min at 8,000 g, respectively. Using the QIA amp Viral RNA Mini Kit (QIAGEN, Hilden, 196 Germany) and the manufacturer's instructions, RNA was extracted from the supernatant. 197 The 1.8 mL of sterile saline was used to suspend 200 mg of the sample, which was then 198 serially diluted with sterile saline to assess the inactivation of E. coli and L. monocytogenes. 199 The samples were then blended with a stomacher blender (BKST-04C; BioKonvision, Gwacheon, Korea) for 2 min while enclosed in bags. Following homogenization, the samples 200

- 201 were divided into 20 mL of Oxford agar (Oxford Listeria agar, Oxoid Ltd., Basingstoke, UK)
- 202 for L. monocytogenes strain KCCM 40307 and EC 3M (Petrifilm) (3M Microbiology

203 Products, St. Paul, MN, USA) for E. coli O157:H7 at 36 °C for 24 h. For every experiment (n

- 204 = 3; 1 batch \times 3 replicates), microbiological parameters were estimated in triplicate.
- 205

206 Statistical analysis

207 To test for the main effects (different UV-C wavelengths, intensities, and time conditions), 208 the packages were randomized. All data obtained in this study were analyzed using a linear 209 mixed model. To evaluate the physicochemical and microbiological parameters, the main 210 effects and their interactions were included as fixed effects and random terms of replications 211 (batch). Analysis of variance (ANOVA) was conducted to determine the significance of the 212 model using SPSS software (ver. 24.0, IBM, Chicago, IL, USA). When the main effects 213 (wavelength, intensity, and irradiation time) were significant (p<0.05), Duncan's multiple 214 range test was performed as a post-hoc procedure.

215

216 Results and Discussion

217 Appearance, SEM and color

218 Changes in the appearance, SEM, and color of the prosciutto under various conditions are 219 shown in Fig. 1 and Table 1. For the appearance of samples, the structural and colorimetric 220 modification by irradiation condition were not obvious (Fig. 1). Accordingly, the 221 microstructure of samples after irradiation seemed not to be markedly changed (Fig. 2). CIE L^* and CIE a^* values of the control and treated samples were not significantly different 222 223 (p>0.05). The CIE L^* value of the control was 60.53, which varied with the treatments, 224 ranging from 55.27 and 63.16. Generally, UV-C irradiation did not have a significant impact on the CIE L* values of prosciutto (p>0.05), except for 265/10 for 1 min. In CIE a*, the 225

226 values decreased after rise with irradiation time. Short-term irradiation at 275 nm 227 significantly increased the CIE a^* values of the samples (p<0.05). At 275/10, the CIE a^* 228 value of the samples raised from 15.54 to 18.60 for the 1 min treatment, likewise those of the 229 samples were over 19 with irradiation at 275/50 for 1 and 5 min. However, CIE a^* of the 275 nm-treated samples were reduced with longer irradiation treatments, presenting no marked 230 231 difference from the control (p>0.05). The CIE b^* value of the control was the lowest, and 232 irradiation increased the CIE b^* of prosciutto (p<0.05), except at 265/10 for 1 min. However, 233 it was hard to find the effect on CIE b^* by wavelength, intensity, or irradiation time. 234 Color is one of the most crucial factors in defining the appearance of food products and 235 influencing consumer preferences is color (Hong et al., 2012). UV-C irradiation can induce 236 meat discoloration by forming metmyoglobin (Renerre, 1990). Park and Ha (2015) stated that chicken frankfurters with increasing stepwise UV-C (60, 3,600 mWs/cm²) showed a decrease 237 in CIE L* value, indicating that higher UV-C doses resulted in lower L* values and higher 238 CIE a^* and CIE b^* values. Similarly, lower CIE L^* values of chicken legs and breasts were 239 reported after UV-C irradiation (82.56 mWs/cm²) for 1 min (Wallner-Pendleton et al., 1994), 240 241 consistent with the results of the 1 min treatment in this study. Isohanni and Lyhs (2009) showed that activated oxygen lowers CIE L^* value and increases the CIE a^* and CIE b^* 242 243 values in meat after UV irradiation. The light-induced degradation of proteins and lipids (i.e., photooxidation) can change the color from red to brown (Söbeli et al., 2021). However, the 244 245 CIE L* value rebounded and CIE a* value decreased with long-term irradiation. This was somewhat unclear, and thus, the total color differences among the samples were not 246 247 significantly different (p>0.05), except for some samples, which remained below 10-unit in 248 all samples. Jung et al. (2003) found that meat with a 10-unit increase in ΔE exhibits 249 significant changes in appearance. Thus, the color change of prosciutto induced by UV-C 250 irradiation was not perceptible to human eye in this study (Fig. 1). No significant differences

251 were observed among the samples. This is consistent with the results of Park and Ha (2015),

252 who reported no visual changes in meat color after UV-C irradiation.

- 253
- 254 pH

Changes in the pH values of UV-C-treated and untreated prosciutto are shown in Fig. 3A. 255 256 The pH values of all samples ranged from to 5.75-5.94. There was a somewhat increasing tendency, although there was no distinct tendency caused by treatment because of the narrow 257 258 distribution of values despite the statistical differences among samples. The pH of prosciutto 259 depends on the rate of water loss, the increase in free amino acid content by proteolysis, and 260 the drying environment (Draghici et al., 2013; Petrovic et al., 2016). The pH of dry-cured 261 ham was reported as approximately 5.8 (Alba et al., 2012; Bover-Cid et al., 2011), and UV-C 262 irradiation did not significantly affect the pH of meat (Chun et al., 2010; Park & Ha, 2015; Söbeli et al., 2021), which were consistent with this result. In addition, Bintsis et al. (2000) 263 264 reported no differences between foods treated and untreated with UV-C light. Monteiro et al. (2019) described that UV-C causes the production of reactive oxygen species, which 265 subsequently cause the oxidation of water molecules. This process increases the ionic 266 strength of the protein and exposes additional water-binding sites, thereby increasing its 267 268 charge state. Though, it was regarded that the pH of samples was in the range of 5.75-5.94 269 regardless of UV-C doses and intensity, which might be due to the low moisture content of 270 prosciutto.

271

272 Water content and WHC

The water content and WHC of the prosciutto samples depending on the UV-C
wavelength, intensity, and irradiation time, are presented in Fig. 3B and 3C. The water
content of control was approximately 40%. After UV-C irradiation, except 265/10 for at 5

276 min, the water content of all samples did not change significantly (p>0.05). The WHC of the 277 control was approximately 97%. After the irradiation, Except for 265/10 for 1 and 10 min and 278 265/50 for 10 min, the WHC of all samples significantly increased to above 98% (p<0.05). 279 Though, WHC of prosciutto was ranged into 97-99% regardless of UV irradiation conditions. 280 The water content of prosciutto decreases from 60% to about 30% in one month during 281 dry aging (Draghici et al., 2013). Pleadin et al. (2017) reported that the water content of a household-manufactured prosciutto was 35%. Short-term treatment with no more than 15 min 282 283 of irradiation had no effect on the water content of the prosciuttos. The WHC of meat is the 284 capacity of muscle to retain moisture from external forces (Huff-Lonergan and Sosnicki, 285 2002). The increase in the WHC of prosciutto might be somewhat correlated with the pH 286 rather than with irradiation. Arnau et al. (1998) reported that high-pH hams had higher WHC 287 than low-pH hams. The pH trend was partially similar to that of the WHC results, except at 275/50. This can be induced by tissue aggregation, which contributes to the blocking of 288 289 moisture loss. Wu et al. (2015) reported that the gel strength of gelatin exposed to UV 290 irradiation increased owing to the UV-induced formation of new hydrogen bonds and the 291 consequent aggregation of gelatin helices. As reported by Monteiro et al. (2019), during the 292 entire storage period at 4°C for 15 days, the tilapia fillets treated with UV-C (103 mJ/cm²) 293 displayed significantly higher WHC compared to untreated control groups, which may be 294 because more water-binding sites were exposed after UV-C treatment. However, in this 295 study, it was thought that UV-C irradiation did not markedly effect to water content and 296 WHC of prosciutto.

297

298 Hardness

Table 1 shows the hardness of the prosciutto after UV-C irradiation. The hardness of the control was the lowest (34.88 N) and UV-C irradiation significantly increased the hardness of

the prosciutto (p<0.05). Excluding some samples, such as 265/10 for 1 min, 265/50 for 5 min, and 275/50 for 15 min, which were approximately 49 N, the UV-C treatment significantly increased the hardness of the prosciutto to over 50 N (p<0.05). However, the individual effect of wavelength and intensity on hardness of samples appeared to hard to find obviously. There was no significant difference in the hardness among the samples within the same irradiation time (p>0.05).

307 Textural parameters are crucial for determining quality, which affects consumer 308 satisfaction (Hong et al., 2005). Esua et al. (2021) reported that tissue hardening was 309 associated with myofibrillar protein accumulation and denaturation. However, numerous 310 studies have reported that UV-C treatment does not affect the textural properties of chicken 311 breast, poultry, or pork (Chun et al., 2010; Monteiro et al., 2021). Degala et al. (2018) noted 312 an increase in the hardness of UV-C-treated goat meat, although the difference was not statistically significant (p>0.05) ascribing to tissue aggregation and structural changes, which 313 314 leaded to the increase in WHC and hardness (Wu et al., 2015). In this study, any 315 distinguishable modification in the morphology of prosciutto was not detected after 316 irradiation (Fig. 2).

317

318 TBARS

The effect of UV-C radiation on the TBARS value of prosciutto is shown in Fig. 4A. The TBARS level in the control group was 0.614 mg-MDA/kg. Generally, the TBARS of samples increased with the wavelength and intensity. Especially in 275 nm, higher intensity induced higher TBARS level of samples (p<0.05). For wavelength, the tendency was roughly found that the TBARS of 275 nm-treated samples were higher than those of 265 nm-treated samples in same irradiation time. Thus, the 275/50 treatment resulted in significantly high TBARS within 5 min irradiated samples (p<0.05). After 10 min of irradiation, the samples treated

326 with 50 mW showed significantly higher TBARS values than those treated with 10 mW (p 327 <0.05). After 15 min of irradiation, the TBARS values were reduced and were not 328 significantly different from the corresponding values in the control (p>0.05). Among the 329 main effects (wavelength, intensity, and irradiation time), the intensity of UV-C had a marked 330 effect on the TBARS of the prosciutto, although all values were less than 1.0 mg MDA/kg. 331 Lipid oxidation is a complex process and an important indicator of meat deterioration (Fan 332 et al., 2021; Lee et al., 2021). UV irradiation can result in the production of reactive chemical 333 species such as hydrogen peroxide, hydroxyl radicals, and superoxide anions (Fan et al., 334 2021). The higher intensity and longer irradiation time of UV-C could be associated with a 335 pro-oxidant effect that produces peroxide radicals and accelerates lipid oxidation (Koutchma, 336 2019). The composition of fatty acids and the amount of fat in foods exposed to UV-C 337 determine changes in TBARS values (Kim et al., 2011). Namiki (1990) reported that various 338 factors of lipid oxidation initiation, such as peroxides, the presence of oxygen, heat, light 339 irradiation, and enzymes, affect TBARS. Chun et al. (2010) reported that the TBARS value 340 of chicken breasts gradually increased during storage, even at the same UV-C dose. Fan et al. (2021) reported an increase in TBARS in tuna fillets after UV irradiation. The TBARS 341 increase was further observed in the UV-C treated goat meat, chicken breast, and tilapia fillet 342 343 (Degala et al., 2018; Lázaro et al., 2020; Park and Ha, 2015), consistent with this study. Although, the level of TBARS in all samples was below 1.0 mg MDA/kg, the threshold of 344 345 rancid odor of meat product (Kolsarıcı et al., 2010). 346

347 VBN

VBN of the prosciutto depending on UV-C irradiation is illustrated in Fig. 4B. The initial
VBN was 0.65 mg% and UV-C irradiation significantly raised the VBN contents of
prosciutto, with some exceptions (265/10 for 1 and 5 min, and 275/50 for 15 min) (p<0.05).

351 The VBN values of 275 nm-treated samples were higher than those of samples irradiated at 352 265 nm, except 50 mW for 10 and 15 min (p<0.05). However, there was no clear trend based 353 on intensity and duration of irradiation. Further, all values were below 1 mg%. 354 VBN compounds consist of ammonia and amines produced by disintegrated proteins; thus, 355 VBN is considered a freshness index for meat products (Lee et al., 2022). Monteiro et al. 356 (2019) reported that UV-C radiation acts as a pro-oxidant. Fatty acid molecules reach 357 electronically excited levels, absorb photons, and trigger dissociation reactions that generate 358 free radicals. Thus, higher doses result in higher levels of excitation, and consequently, 359 higher numbers of free radicals that enhance the oxidation of lipids and proteins (Canto et al., 360 2016; Koutchma, 2019). Oxidized lipids and proteins produce free radicals, which cause 361 changes in the myofibrillar protein structure and expose hydrophobic amino acids, making 362 them more susceptible to proteolytic enzyme action (Monteiro et al., 2019). According to Kim et al. (2019), the standard VBN value of fresh meat is below 20 mg%, and all samples 363 were susceptible to UV-C irradiation, despite a significant increase in VBN. It was thought 364 that UV-C could cause oxidative stress in the proteins and lipids of the prosciutto, although 365 its impact on VBN was minor. 366

367

368 Inactivation of microorganisms

The total numbers of aerobic bacteria are shown in Table 2. In all samples, the total number of colonies was below the detection limit (1 Log CFU/g) (Jo et al., 2020). Total aerobic bacteria ranged between 6.03 and 7.25 Log CFU/g, and there were no significant differences between the control and treated samples. Among the treated samples, the UV condition at 265/10 for 600 s showed the lowest value (p>0.05). The reduction rate was highest at irradiation times of 5 and 10 s at 10 and 50 W, respectively, regardless of the wavelength. Soro et al. (2021) reported that the application of UV light of various

wavelengths to chickens significantly reduced the mean bacterial concentrations. A lower
reduction compared to that of the control was also observed at specific treatment times;
however, the difference was not significant. A similar trend was observed in the present
study.

As shown in Fig. 5A and B, both the total and active HEV RNA were significantly 380 381 reduced after UV-C treatment compared to the control, and the amount of RNA decreased 382 with increasing exposure time in all samples. UV radiation produces photoproducts that cause 383 structural DNA deformation and cell death (Mikš-Krajnik et al., 2017). Gómez-López et al. 384 (2021) reported that UV radiation affects viral inactivation by inducing protein damage. 385 However, another report stated that meat products are slightly less effective in virus 386 inactivation owing to UV ray shielding by irregular surfaces (Gómez-López et al., 2007). 387 This ccurrent study found that both E. coli and L. monocytogenes were significantly reduced 388 after UV-C treatment compared with the control (Fig. 5C and D). Based on these results, UV-389 C light effectively and dose-dependently inactivated microorganisms in protoplasts. 390 Similarly, Sommers et al. (2010) reported that sausages irradiated with UV-C light showed a reduction in L. monocytogenes, Staphylococcus aureus, and Salmonella. Other factors, such 391 392 as the species, strain, and composition of food, can also affect the effectiveness of UV-C 393 radiation in reducing bacterial counts (Reichel et al., 2020; Sommers et al., 2010). 394 Furthermore, Reichel et al. (2020) found that the surface texture was the most influential 395 factor in decreasing bacterial counts when using UV-C light. The current study found that 396 UV-C treatment effectively reduced viruses and pathogens by up to 4 Log; therefore, as 397 further evidence, the number of microorganisms in meat products can be reduced by UV-C 398 radiation. It should be emphasized that this decreasing effect may depend on the species or 399 strain of the microbe.

400

401 Conclusion

402 UV-C irradiation successfully reduced in the load of microorganisms on the prosciutto 403 without perceptible modification in appearance and microstructure. Further, the irradiation 404 did not markedly affect to water content and WHC of prosciutto. Although, the TBARS and 405 VBN of prosciutto was raised after irradiation, it was not exceeded the threshold for rancid 406 deterioration. Specifically, a significant reduction in both E. coli and L. monocytogenes was 407 observed in all UV-C-treated samples compared with the unirradiated sample. In particular, 408 the reduction of HEV by UV-C irradiation was significant, and no active RNA was detected 409 at 265/50 and 275/50 for more than 10 min. Photoproducts affect microbial inactivation by 410 inducing protein damage, which leads to DNA modification and cell death. Briefly, these 411 results indicate that UV-C above 265 nm at 50 mW for 10 min inactivated microorganisms without quality modification of the meat product. These findings support further application 412 413 of UV-C irradiation in meat processing.

414

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578 Table 1

	Irradiatio	CIE L*	CIE a*	CIE b^*	⊿E	Hardness (N)
	n time (min)					
Control	0	60.53 ± 9.78^{ab}	15.54 ± 5.32^{bc}	21.13±3.16 ^e		34.88 ± 6.34^{e}
265/10 ¹	1	55.27±5.76°	$\underset{c}{17.31{\pm}3.42^{ab}}$	22.46±3.49 ^{de}	8.56±3.33 ^a	49.59±14.10 ^{de}
	5	57.74 ± 1.11^{bc}	$19.09 {\pm} 0.68^{a}$	24.18 ± 0.24^{bc}	$5.53{\pm}0.77^{bc}$	$66.17{\pm}9.02^{abcd}$
	10	63.16±1.91 ^a	14.91±2.05 ^c	23.82 ± 1.32^{bc}	1.65±1.25 ^e	$_{d}^{64.07\pm30.37^{abc}}$
265/50	1	60.83 ± 1.22^{ab}	14.94±0.75°	24.21 ± 0.67^{bc}	3.45 ± 0.49^{d}	58.59 ± 16.74^{bcd}
	5	$58.31{\pm}2.63^{ab}$	17.8±1.37 ^{abc}	$24.97{\pm}0.76^{ab}$	5.42 ± 2.05^{bc}	49.59±15.94 ^{de}
	10	$59.17{\pm}3.08^{ab}$	16.65 ± 2.18^{ab}	25.50±0.27 ^{ab}	5.75±1.36 ^b	$_{d}^{64.06\pm10.19^{abc}}$
	15	$60.90 {\pm} 1.31^{ab}$	$14.88 {\pm} 1.41^{\rm bc}$	$26.58{\pm}0.56^{a}$	$5.79 {\pm} 0.51^{b}$	50.95 ± 11.50^{d}
275/10	1	$58.29{\pm}1.40^{ab}$	18.60±1.26ª	23.89 ± 0.34^{bc}	4.85 ± 1.40^{bc}	57.97±12.53 ^{cd}
	5	60.95±2.53 ^{ab}	15.37±2.23°	25.24 ± 0.56^{ab}	5.15 ± 0.61^{bc}	76.77 ± 5.83^{a}
	10	59.37±2.61 ^{ab} c	15.86±1.52 ^{bc}	22.83±1.24 ^{cd}	3.50±1.32 ^{cd}	75.14±19.05 ^{ab}
275/50	1	56.42 ± 1.79^{bc}	19.51 ± 1.66^{a}	24.78±0.44 ^b	$6.96{\pm}1.81^{ab}$	$60.45{\pm}6.77^{abcd}$
	5	57.54 ± 0.62^{bc}	19.42 ± 0.58^{a}	24.54 ± 0.90^{bc}	$6.01 {\pm} 0.94^{b}$	$68.89{\pm}5.26^{abc}$
	10	57.90±5.70 ^{bc}	17.64±4.19 ^{ab} c	24.93±0.50 ^{ab}	8.10±2.33 ^a	$63.14{\pm}14.88^{abc}$
	15	58.18±3.95 ^{ab}	17.08±2.31 ^{ab}	24.98±0.24 ^{ab}	6.03 ± 2.27^{b}	49.33±9.76 ^{de}

579 Color and hardness of prosciutto depending on the various UV-C irradiation conditions.

580

>

¹⁾ UV-C condition: wavelength (nm)/watt (mW). ^{a-e} Means with different letters in a column are significantly different (p < 0.05). 581

	Irradiation time	Total aerobic	Reduction rate ²⁾
	(sec)	bacteria	(%)
Control	0	$\frac{(\text{Log10 copies/}\mu\text{L})}{(\text{Log10 copies/}\mu\text{L})}$	
	0	6.92 ± 0.21^{abc}	-
265/10-7	5	6.30 ± 0.00^{abc}	75.90
	10	6.80 ± 0.0 / abc	24.10
	20	6.99±0.38 ^{abc}	-16.87
	40	6.60 ± 0.22^{abc}	52.41
	120	6.92 ± 0.31^{abc}	-1.20
	300	7.00 ± 1.20^{abc}	-19.32
	600	6.03±0.73 ^c	87.21
265/50	5	6.91 ± 0.54^{abc}	1.20
	10	6.17 ± 0.70^{abc}	82.05
	20	6.13±0.47 ^{abc}	83.86
	40	6.62 ± 0.94^{abc}	49.58
	120	6.71 ± 1.07^{abc}	37.59
	300	7.16 ± 0.40^{ab}	-72.69
	600	$6.88 \pm 0.06^{\mathrm{abc}}$	8.03
275/10	5	6.23 ± 0.49^{abc}	79.76
	10	6.81 ± 0.21^{abc}	22.29
	20	6.94±0.43 ^{abc}	-4.82
	40	6.78 ± 0.18^{abc}	27.71
	120	7.25 ± 0.22^{a}	-112.85
	300	$6.97{\pm}1.08^{abc}$	-13.21
	600	6.78 ± 0.28^{abc}	26.91
275/50	5	$6.96{\pm}0.36^{abc}$	-10.24
	10	6.12 ± 0.23^{ab}	84.04
	20	6.50 ± 0.17^{abc}	62.05
	40	6.54 ± 0.37^{abc}	57.83
	120	6.71 ± 1.07^{abc}	37.59
	300	7.16 ± 0.40^{ab}	-72.69
	600	$6.88{\pm}0.06^{abc}$	8.03

 Table 2

 Effect of UV-C treatment on total aerobic bacteria of the prosciutto.

¹⁾ Wavelength (nm)/watt (mW).

²⁾ Reduction rate: 1-(N/N₀) %, N₀: Copy number of initial hepatitis E virus (control), N: Copy number of UV-irradiated hepatitis E virus.

^{a-c} Means with different letters are significantly different (p < 0.05).

Figure captions

Fig. 1. Appearance images of prosciutto. ¹⁾ UV-C condition: wavelength (nm)/watt (mW).

Fig. 2. Scanning electron microscopy images of prosciutto treated with UV-C. ¹⁾ UV-C condition: wavelength (nm)/watt (mW).

Fig. 3. pH (A), water content (B), and water holding capacity (C) of prosciutto treated with UV-C. ¹⁾ UV-C condition: wavelength (nm)/watt (mW). ^{a-h} Means with different letters are significantly different (p<0.05).

Fig. 4. TBARS (A) and VBN (B) of prosciutto treated with UV-C. ¹⁾ UV-C condition: wavelength (nm)/watt (mW). ^{a-k} Means with different letters are significantly different (p<0.05).

Fig. 5. Total RNA (A) and active RNA (B) of HEV, and inactivation of *E. coli* (C) and *L. monocytogenes* (D) of prosciutto treated with UV-C. ¹⁾ UV-C condition: wavelength (nm)/watt (mW). ^{a-c} Means with different letters in same UV-C treated time are significantly different (p<0.05). ^{A-B} Means with different letters in same UV-C wavelength and watt are significantly different (p<0.05).

Figure	1
	_

Samples	oles Treatment time (min)				
	0	1	5	10	15
Control					
265/10 ¹⁾			111	111	
265/50	100	111	111	}))	900
275/10		991	>>1	())	
275/50			171	111)))

Figure	2



Figure 3







Figure 4





Figure 5









Supplementary data 1. Diagram of the technological process for the manufacturing of prosciutto.