1 **Antimicrobial effect of synergistic interaction between UV-A light and Gallic Acid against**
2 ***Escherichia coli* O157:H7 in fresh produce wash water and biofilm**

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16

17 ABSTRACT

18

19 A synergistic interaction between gallic acid (GA) and UV-A light (UV-A+GA) to inactivate *E. coli*
20 O157: H7 in spinach wash water and in biofilm was evaluated. A 30-min exposure to UV-A light in
21 presence of 10 mM GA had relevant biological effect in the inactivation of *E. coli* O157:H7 in
22 suspension in the absence ($> 5 \log(\text{CFU/mL})$) and the presence of organic content ($>3-$
23 $\log(\text{CFU/mL})$ in 2,000 mg O₂/L COD (Chemical Oxygen Demand) organic load), and resulted in
24 ~80% decrease in the metabolic activity of *E. coli* O157:H7 biofilm. GA solutions could be
25 recycled through at least 3-cycles of UV-A treatment without a significant loss in antibacterial
26 effect. Catalase reduced the extent of *E. coli* O157:H7 inactivation from the UV-A+GA treatment
27 suggesting that generation of hydrogen peroxide was predominantly responsible for the observed
28 antimicrobial effect. The UV-A+GA treatment was also found to be effective in causing more than 3
29 $\log(\text{CFU/mL})$ reductions in *E. coli* O157:H7 on the surface of spinach leaves. UV-A + GA treatment
30 can serve as an effective intervention in the fresh produce sanitation.

31

32 KEYWORDS

33 Photodynamic treatment, photosensitization, Gallic Acid, *Escherichia coli* O157:H7, wash water,
34 biofilm

35

Industrial Relevance

The results of this study show that a synergistic interaction between gallic acid (GA) and UV-A (365 nm) light is an effective treatment for sanitation of fresh produce and water used to wash fresh produce. It was also found to be effective against the *E.coli* O157:H7 biofilm. The attractive attributes of this technology include a relatively low cost; specific, light-triggered activity; non-toxic nature and scalability. Thus, this technology has potential to replace conventional chemical sanitizer-based sanitation approaches.

50 1. INTRODUCTION

51

52 Various technologies are used in the food industry for the sanitation of food processing equipment
53 and food materials, particularly fresh produce. One of the key goals in the sanitation processes is to
54 limit the cross-contamination between food materials and contaminated equipment or vice-versa.
55 **Microbial cross-contamination has been identified as one of the leading factors that can lead to**
56 **nationwide outbreaks of foodborne illnesses.** To limit cross-contamination, both physical methods
57 such as pulsed electric fields, pulsed light and sonication; (US FDA, 2015a) and chemical
58 methodologies such as **chlorine- or peroxide-based** sanitizers (Virto et al., 2005) have been
59 proposed. The chemical sanitizers are often preferred for the sanitation of process water and
60 equipment due to cost effectiveness, large scale of process equipment and relatively large quantity
61 of process water and ease of implementation and control. Despite their significant advantages, the
62 key limitations for the several chemical sanitizers include: (a) limited efficiency in the presence of
63 organic content (Van Haute et al., 2013), (b) production of harmful by-product (Nieuwenhuijsen et
64 al., 2000) and (c) the absence of a regeneration cycle to enable re-use of the added sanitizer.

65 Although photosensitizers (PS) are widely used for the sanitation in biomedical field (Luksiene and
66 Brovko, 2013), their application to sanitize process water used during food processing has been
67 only recently introduced (Cossu et al., 2016; Ercan et al., 2016). The generation of reactive oxygen
68 species (ROS) by PS upon exposure to ultraviolet (UV) or visible (VIS) light and the subsequent
69 killing of microorganisms can provide an effective alternative to address some of the key limitations
70 of chemical sanitizers. Recent studies have demonstrated the effectiveness of **food-grade**
71 photosensitizers with the synergistic effect of visible light (Cossu et al., 2016) or UV-A light (Ercan
72 et al., 2016) for the treatment of **wash water**. These prior studies focused on the use of **food-grade**
73 dyes or inorganic particles as PS agents for the inactivation of viral and bacterial targets in the
74 presence of organic content in **wash water**. The agents selected in these prior studies have a

75 restrictive use in food applications.

76 In this study, we evaluated the enhanced antimicrobial activity through a synergistic interaction
77 between gallic acid (GA) UV-A (340-400 nm) (referred to as UV-A+GA treatment henceforth) light
78 against planktonic bacteria and bacteria in a biofilm model in the presence of wash water containing
79 added organic matter from spinach. GA is a phenolic compound naturally present in food matrices
80 such as tea leaves and spices like sumac, produced after the hydrolysis of an ester bond in gallated
81 catechins (Narumi et al., 2014). GA is a generally regarded as safe (GRAS) compound (US FDA,
82 2015b). Thus, a successful application of GA as an effective sanitizer will provide a food-grade
83 antimicrobial compound to address a critical challenge of microbial cross-contamination. Although
84 GA is known to act as a mild antimicrobial agent by itself (Díaz-Gómez et al., 2014), little is known
85 about its enhanced antimicrobial activity upon exposure to UV-A radiation. A previous study
86 evaluated antimicrobial activity of 4 mM GA upon exposure to 400 nm blue light emitting diode
87 (LED) light and observed that more than 5-log reduction in *Staphylococcus aureus* could be
88 achieved after 15 min of exposure without the presence of organic content. They attributed this
89 enhanced antimicrobial activity of GA to lipid oxidation of bacterial cell membrane from ROS such
90 as hydrogen peroxide and hydroxyl radicals generated from exposure of GA to LED light
91 (Nakamura et al., 2012). Another recent study by the same group evaluated LED light enhanced
92 antibacterial activity of diverse phenolic compounds including GA on Gram-positive and Gram-
93 negative bacteria and found that although the treatment was effective on both types of bacteria,
94 Gram-positive strains were more resistant to inactivation (Nakamura et al., 2015). Nevertheless, the
95 literature lacks a systematic study involving (a) ability of UV-A light to enhance the antibacterial
96 activity of GA; (b) efficacy of the proposed process in the presence of realistic levels of organic
97 load in wash water from food processing industry, (c) ability of UV-A+GA treatment to inactivate
98 biofilms, and (d) understanding of the mechanisms responsible for the observed enhancement of
99 antimicrobial activity by synergistic interaction between UV-A and GA. This study attempts to

100 address each of these gaps in the literature.

101 We evaluated the antimicrobial effect of UV-A+GA treatment on *Escherichia coli* O157:H7 in
102 simulated wash water and as a biofilm. We also investigated the re-usability of GA for this process
103 after multiple cycles of sanitation processes, and evaluated its mechanism of action. In addition, we
104 also examined the localization of GA at the cellular level. A successful demonstration of the
105 effectiveness of UV-A+GA treatment as an antimicrobial agent will offer new opportunities for the
106 food industry to introduce sanitation systems based on GRAS compounds and address the existing
107 challenges pertaining to sanitation.

108 2. MATERIAL AND METHODS

109

110 2.1 UV-A light Device

111 A bench-top, batch type equipment was used for the UV-A light treatment. The equipment consisted
112 of four UV-A light fluorescent bulbs (320-400 nm, peak wavelength 360 nm, 18 Watt, Actinic BL,
113 Royal Philips, the Netherlands) mounted on the ceiling of a closed box. A more detailed description
114 of the system is reported in a previous study (Cossu et al., 2016). The intensity of the UV-A light
115 applied to the surface of the sample placed in the equipment was $2646 \pm 212 \mu\text{W}/\text{cm}^2$ as measured
116 with the radiometer model UV-340A (Lutron, Taipei, Taiwan).

117

118 2.2 Bacterial culture

119 *Escherichia coli* O157:H7 (ATCC 700728, Manassas, VA) was a gift from Prof. Linda Harris at the
120 University of California-Davis. The bacterium has been depleted of two shiga toxin-like genes (*stx1*
121 and *stx2*) and is rifampicin-resistant (Moyne et al., 2011). The bacterium was cultured in Lysogenic
122 broth (LB) (Casein peptone 10 g/L, Yeast extract 5 g/L, NaCl 10 g/L), added with rifampicin 50
123 $\mu\text{g}/\text{mL}$ or in LB agar with the same antibiotic (Fisher Scientific, Hampton, NH, USA). Bacteria
124 were grown at 37°C at 250 rpm until an absorbance at 600 nm of 1.5, reflecting a stationary phase,
125 was reached (approximately 7×10^8 CFU/mL).

126

127 2.3 Preparation of clarified spinach homogenate and COD measurement

128 Spinach (*Spinacia oleracea*) leaves were bought from a local retail. Ten g of spinach were added to
129 90 mL of sterile milliQ water and blended at high speed in a blender (Simple Blend™ 100 Blender,
130 Oster®, USA) with 2 pulses of 30 sec each. Homogenate was recovered and transferred to 50-mL
131 conical tubes for a following centrifugation at $13,000 \times g$ for 10 min. Supernatant was recovered
132 and centrifuged an additional time as above. The resulting supernatant was diluted and analyzed for

133 the **chemical** oxygen demand (COD) using high range COD digestion vials (Hach, Loveland, CO,
134 USA) as already described (Cossu et al., 2016). A resulting COD content of 2,800 mg/L O₂ was
135 measured. The supernatant was appropriately diluted in sterile water for the subsequent experiments
136 of microbial reduction in the presence of varying organic loads.

137

138 **2.4 Inactivation of *Escherichia coli* O157:H7 by synergistic interaction between UV-A light** 139 **and GA (UV-A+GA) treatment**

140 A culture of *E. coli* with an A₆₀₀ nm = 1.5 OD was diluted in different amounts of organic loads
141 derived from the blended spinach suspension (0, 500 and 2,000 mg/L COD) to approximately 2×10⁶
142 CFU/mL. One milliliter of the bacterial suspension was added to 1 mL of gallic acid (GA) (Sigma,
143 St. Louis, MO, USA) solutions with identical organic load to achieve 0, 1, 5 or 10 mM of GA
144 concentrations. The volume containing 1×10⁶ CFU/mL was placed within a well of a 12-well flat
145 bottom polystyrene plate (Corning, Corning, NY, USA) and exposed to UV-A light in the **photo-**
146 **reactor** at a distance of 8 cm from the light source for 0, 5, 15 and 30 min. An aliquot of the sample
147 was withdrawn for each time point, and serially diluted in **phosphate-buffered** saline (PBS) (USB
148 Corporation, Cleveland, OH, USA). Aliquots of 100 µL were plated on LB agar added with
149 **rifampicin 50 µg/mL** for the bacterial count. Plates were incubated at 37°C for 24 h and the
150 population of *E. coli* was interpreted in terms of log(CFU/mL) sample. Controls consisting in
151 incubated bacteria at room temperature for up to 1 h either in the presence of GA but without UV-A
152 light or in the absence of GA but in the presence of UV-A light were also performed. Moreover, as a
153 control to see the effect of pH on treatment, the pH of DI water was adjusted to 3.0 with HCl and
154 mixed with bacteria to reach 1×10⁶ CFU/mL population. Then, the reductions of the bacteria
155 population under UV-A and in the dark were observed.

156

157 **2.5 Mathematical modeling of inactivation of *Escherichia coli* O157:H7**

158 The Weibull model was used to describe the inactivation by UV-A and UV-A irradiated gallic acid
159 treatments. This model includes nonlinearity of semi-logarithmic survivor curves and the classical
160 first-order approach (Van Boekel, 2002).

161

$$\log_{10} \frac{N}{N_0} = \frac{1}{2.303} \left(\frac{t}{\alpha} \right)^\beta \quad (1)$$

163

164 Where, N is the number of surviving population (CFU/ml), N_0 is initial number of population
165 (CFU/mL), t is the treatment time (min), α is the characteristic time (min), β shape parameter
166 (unitless).

167 Then, α and β were used to calculate the reliable life, t_R , which correspond to the D-value for the
168 first-log reduction.

169

$$t_R = \alpha \times (2.303)^\beta \quad (2)$$

171

172 The models were constructed using averages from two experimental data sets, and a third data set
173 was used to confirm the fit. Non-linear least squares regression method was used to predict α and β .
174 The goodness-of-fit for the fitted models was evaluated by root mean square error (RMSE) and
175 mean absolute error (MAE) between the calculated data from the model and the experimental data.

176

177 **2.6 Re-usability of GA for the UV-A+GA treatment**

178 This experiment was performed to evaluate the re-usability of GA for multiple cycles of sanitation
179 treatment. Two milliliter of 10mM GA solution were added with bacteria to a final concentration of
180 1×10^6 CFU/mL in water and exposed to UV-A light for 30 min. A 100 μ L aliquot of the sample was
181 subjected to serial dilution and bacterial count. Then, the solution was transferred to a syringe and

182 filtrated through a sterile 0.22 µm filter (Millipore, Cork, Ireland) and added with fresh 100 µL of a
183 2×10^7 CFU/mL bacterial culture in water to achieve a final concentration of 1×10^6 CFU/mL
184 assessed by bacterial count. The sample was subjected to UV-A light exposure for 30 min prior to
185 perform an additional bacterial count. An additional cycle of filtration, addition of bacteria and
186 bacterial count was performed on the same GA solution to achieve a total of three UV-A light
187 activation cycles. Control samples without GA or GA not exposed to UV-A light were subjected to
188 the same cyclic treatments.

189

190 **2.7 Inactivation of *Escherichia coli* O157:H7 biofilm by UV-A+GA treatment**

191 A stationary phase culture of *E. coli* O157:H7 was diluted 1:100 in LB low salt (LB-LS) (Tryptone,
192 10 g/L, yeast extract, 5 g/L NaCl, 5 g/L) and 1 mL aliquots of the bacterial suspension were
193 deposited in the wells of a multiwell 24-well transparent polystyrene plate (Corning, Corning, NY,
194 USA) and incubated at room temperature for 72 h in the dark. After 3 days, the medium was
195 removed and the wells were filled with 1 mL of fresh LB-LS. Plates were incubated for additional 3
196 days. After a total of 6 days of incubation, the medium was removed and the biofilm on the bottom
197 of the wells was washed twice with PBS. PBS was removed by inverting the plate and shaking it.
198 One milliliter of 5 to 10 mM GA was added to each well containing the biofilm and placed under
199 UV-A light for 60 min exposure. Appropriate controls of with the same GA solution in the presence
200 of biofilm at T0 min or T60 min in dark condition were also performed as well as in the absence of
201 biofilm.

202 After UV-A exposure, the volume was removed and remaning biofilm was washed twice with PBS.
203 Washed biofilm was then added with 1 mL of LB-LS with 50 µM of Resazurin (Sigma, St. Louis,
204 MO, USA) and incubated for 18 h in the dark at room temperature. The day after an aliquot of 100
205 µL of the supernatant was recovered for each well and analyzed by fluorescence detection with a
206 fluorescence plate reader (Spectrafluor Plus, Tecan, Durham, NC, USA) with filters of excitation of

207 530 nm and emission at 580 nm. Biofilm metabolic activity was measured as a percentage of
208 fluorescence intensity calculated from the metabolic activity of the biofilm treated with the GA
209 solution at T0 min without UV-A light exposure. Triplicate biological readings were done for each
210 biofilm reduction experimental condition.

211

212 **2.8 Fate of UV-A irradiated GA**

213 To evaluate the fate of UV-A irradiated GA, we measured the degradation of 10 mM GA after 30
214 min of UV-A exposure. Samples of both UV-A+GA and GA stored in dark were collected, diluted
215 20 fold in DI water and analyzed for the concentration of GA using high-pressure liquid
216 chromatography (HPLC). HPLC analysis was conducted on a Shimadzu® model LC-2010A
217 (Kyoto, Japan) HPLC controlled by LabSolution V 5.81 Software, and with a LC-2010 pump, a LC-
218 2010 autosampler and a LC-2010 UV-VIS detector. The column used was C18 (250 mm × 4.6 mm,
219 10 µm) from Phenomenex® (Torrance, CA, USA). The chromatographic separation was carried out
220 in isocratic elution using a mobile phase composed of 0.1 N phosphoric acid (84%) and acetonitrile
221 (16%), at a flow rate of 0.6 mL/min. The wavelength of detection was 215 nm. The column
222 temperature was 33°C and the injection volume was 10 µL. A standard curve was prepared using
223 50-775 µM GA solution.

224 To complement the HPLC measurements we also performed mass spectroscopic analysis on a 5
225 mM GA solution exposed to UV-A for 30 min. The GA solution before and after UV-A treatment
226 was analyzed by mass spectrophotometry to detect formation of decomposition products of GA
227 from exposure to **UV-A light. A time-of-flight mass** spectrometer (AccuTOF, JEOL, USA, Inc.)
228 with a resolving power of 6000 (FWHM) was equipped with an ESI ion source and coupled with an
229 Agilent 1100 HPLC system set in electronegative mode. The AccuTOF MS settings were as
230 follows: orifice 1 temperature = 100 °C, orifice 1 V= 30 V, orifice 2 = 5 V, ring V= 10 V. The
231 desolvating chamber temperature was set at 250°C, and the flow rates of the nebulizing and

desolvating gases were 0.6 and 3.0 L/min, respectively. The needle voltage was 2300 V. The flow rate of the mobile phase was set at 0.25 mL/min. The mobile phases were 0.1% formic acid in water and methanol. The injected sample volume was 10 μ L.

2.9 Effect of catalase on the antimicrobial activity of UV-A+GA treatment

To evaluate the role of ROS such as hydrogen peroxide generated from the UV-A+GA treatment, we investigated its antimicrobial activity in presence of catalase, an enzyme previously known to degrade hydrogen peroxide into water and oxygen. *E. coli* suspensions were prepared as discussed earlier with no organic content. One milliliter volume containing 2×10^6 CFU/mL was mixed with GA to a final concentration of 10 mM GA and 3000 U of native catalase from bovine liver (Sigma, St. Louis, MO, USA) to a final volume of 2 mL with 1×10^6 CFU/mL. To validate that the effect of catalase on the antimicrobial activity of UV-A+GA was due to the ability of the enzyme to quench hydrogen peroxide and not because of its contribution to the organic load in the solution, an experiment was also performed where bacterial sample containing GA was mixed with 3000 U of heat denatured (70°C for 30 min) catalase.

2.10 Association of GA with in *Escherichia coli* O157:H7

Diphenylboric acid 2-aminoethyl ester (DPBA) (Sigma, St. Louis, MO, USA) was used to detect GA associated with *E. coli*. A culture of 1×10^8 CFU/mL containing 10 mM GA was incubated in the dark for 30 min. The cells were washed twice with DI water to remove the extracellular GA and centrifuged for 2 min at $10,000 \times g$ at 4°C. Then, the pellet was re-suspended in 100 μ L of DI water. To this, 5 mM DPBA was added and the association GA with bacterial cell was measured using fluorescence spectroscopy. A 150 μ L of mixture was placed within a well of a 96-well plate and the fluorescence intensity of the solution was measured using Spectramax M5e plate reader (Molecular Devices LLC, Sunnyvale CA) with the excitation and emission wavelengths at 405 and 465 nm,

257 respectively.

258

259 **2.11 Antibacterial activity of UV-A+GA on *E.coli* O157:H7 inoculated on spinach leaves**

260 Spinach leaves were bought in a local retail market, soaked in 2% (w/v) bleach for 10 mins
261 followed by washing in sterilized water and drying before use. *E. coli* was cultured in LB added
262 with rifampicin 50 µg/mL and incubated as already described. The culture was then diluted 100 fold
263 in water to achieve 1×10^6 CFU/mL. One hundred µL of the diluted bacterial suspension were
264 spotted on the surface of a spinach leaf and 900 µL of a sterile 11.1 mM gallic acid solution were
265 added to the bacteria droplet on the leaf. The leaf was then exposed to UV-A light for 30 min.
266 Controls consisting in contaminated leaves incubated in the dark with gallic acid or in the dark with
267 water were also carried out. After treatment, leaves were carefully transferred into plastic bags
268 (whirl-pak, Fisher Scientific Inc., Pittsburgh PA, USA) where 9 mL of water were added to
269 resuspend bacteria from the leaves surface. The bags were then rubbed by hand to mix the sample
270 and an aliquot of 100 µL of the liquid was serially diluted in PBS, and plated on LB agar added
271 with rifampicin 50 µg/mL. Plates were incubated at 37 °C for 24 hours and the population of *E.coli*
272 was interpreted as log(CFU/mL) by plate counting. Each treatment was performed in 4 replicates.

273

274 **2.12 Statistical analysis**

275 Each treatment was performed in triplicate. The significant difference between microbial reductions
276 in different treatments were analyzed with the analysis of variance Tukey's method at the 95%
277 confidence interval by using the MINITAB Statistical Software package (Version 17; State College,
278 PA, USA).

279 3. RESULTS

280

281 3.1 Bacterial inactivation by a synergistic UV-A+GA treatment

282 Figure 1 (Fig. 1) illustrates a reduction in the bacterial count upon exposure to a combination of
283 UV-A light and GA in the absence (Figure 1A) and the presence of varying levels of organic content
284 (Figure 1B and 1C). The results demonstrate that UV-A light itself only caused 0.56 ± 0.46
285 $\log(\text{CFU/mL})$ reduction in *E. coli* population after 30 min of treatment, while 10 mM of GA caused
286 a maximum reduction of $0.91 \pm 0.23 \log(\text{CFU/mL})$ after 30 min of incubation in the dark. When 10
287 mM GA and UV-A light were simultaneously present, a $4.95 \pm 0.19 \log(\text{CFU/mL})$ reduction in the
288 microbial count was achieved within 15 min of light exposure. To demonstrate the observed
289 increase in the antimicrobial activity was not due to the acidity of GA, the bacterial suspension was
290 also incubated with acidified water (pH 3) for 15 min to mimic the acidic conditions for the 10 mM
291 GA solution (pH 3.14). A mere 0.36 ± 0.08 and $0.37 \pm 0.19 \log(\text{CFU/mL})$ reduction was observed
292 in 10 mM GA solution and acidified water respectively after 15 min of incubation and the reduction
293 was not significantly different from each other ($p > 0.05$), indicating that the effect was not
294 associated with the pH of the medium. When the GA concentration was lowered to 5 and 1 mM,
295 only $1.28 \pm 0.37 \log(\text{CFU/mL})$ ($p = 0.6665$), and $0.74 \pm 0.49 \log(\text{CFU/mL})$ ($p = 0.1035$)
296 reductions were observed during 30 min of UV-A light exposure, respectively indicating that a GA
297 concentration greater than 5 mM was required to achieve more than 2 $\log(\text{CFU/mL})$ reduction in
298 bacterial count.

299 Figures 1B and 1C (Fig. 1) show a reduction in bacterial count by 1, 5 or 10 mM GA upon exposure
300 to UV-A light at varying concentration of organic load. The organic load was designed to simulate
301 wash water conditions in fresh produce environment as described in the materials and methods
302 section. The results demonstrate that GA at concentration levels of 5 mM or below did not achieve
303 greater than 1 $\log(\text{CFU/mL})$ reduction in bacterial count in the presence of organic content (COD

values of 500 or 2,000 mg/L). In case of 10 mM GA, 5.09 ± 0.10 log(CFU/mL) and 3.74 ± 0.10 log(CFU/mL) reduction in bacterial count was achieved for the COD values of 500 and 2,000 mg/L respectively after 30 min of UV-A light exposure. Thus, UV-A+GA treatment at 10 mM GA concentration was effective in achieving greater than 3 log(CFU/mL) reduction in 30 min even in the presence of high organic content (COD of 500 or 2,000 mg/L).

To further characterize the kinetics of bacterial inactivation, the experimental data was fitted to non-linear kinetic equations. The inactivation by UV-A light in the presence of various concentrations of gallic acid at various organic contents was described with the Weibull model (Tab. 1). The reliable life (t_R) value, that represents the amount of time required for the first log reduction was calculated from the Weibull model constants, α (characteristic time), and β (shape parameter). Compared to the absence GA, the presence of 1, 5, 10 mM GA decreased the t_R values by 1.27, 3.64, 11.32 fold respectively when organic matter was absent in the solution. Although an increase in organic content to 2000 COD, dramatically increased the t_R values for each condition compared to that in the absence of organic content, 10 mM GA was still effective in lowering the time required for the first log reduction at 2000 COD.

3.2 Retention of activity of GA after multiple UV-A light exposure cycles

The ability of GA recycled from the previous treatment to effectively inactivate *E. coli* O157:H7 in a subsequent cycle of UV-A exposure is shown in Figure 2 (Fig. 2). The 10 mM GA solution achieved 4.99 ± 0.05 log(CFU/mL) reduction in the first cycle of 30 min UV-A exposure, 4.99 ± 0.10 log(CFU/mL) reduction in the second cycle and 4.98 ± 0.04 log(CFU/mL) reduction in the third cycle, indicating a near complete retention of antimicrobial activity of UV-A irradiated GA through multiple cycles. The bacterial cultures incubated with the same recycled GA solution in the dark achieved less than 1 log(CFU/mL) reduction in each cycle except in the last cycle where a slightly higher inactivation of 1.36 ± 0.35 log(CFU/mL) reduction was achieved.

329

330 **3.3 Effect of UV-A+GA treatment on the metabolic activity of *E. coli* O157:H7 biofilm**

331 The metabolic activity of *E. coli* O157:H7 biofilm was assessed upon UV-A light exposure in
332 presence of 0-10 mM GA in suspension (Fig. 3). Results showed that at 5 mM concentration, GA
333 alone had a marginal impact on reduction of metabolic activity of biofilm and the presence of UV-A
334 light did not enhance this effect. At 10 mM concentration, GA alone again had a marginal impact on
335 reducing the metabolic activity of biofilm; however, in the presence of UV-A light and 10 mM GA,
336 the metabolic activity of biofilm reduced to 26.2 ± 4.7 % of the original activity indicating a lethal
337 effect on the biofilm at this concentration.

338

339 **3.4 Fate of GA upon UV-A exposure**

340 Degradation of 10 mM GA during exposure to UV-A light was monitored using HPLC with a
341 sensitivity to detect a 50µM/L change in the concentration of GA. We observed that the
342 concentration of GA before UV-A exposure and after 30 min of UV-A exposure was 9.9 ± 0.4 mM
343 and 10.0 ± 0.1 mM respectively indicating no significant change in the absorbance. GA stored in
344 dark showed a marginal change in the concentration from 10.0 ± 0.1 mM at the beginning of
345 storage, to 9.3 ± 0.1 mM after 30 min. Based on these results, it is apparent that GA did not degrade
346 into new chemical compounds during UV-A exposure. To further validate the results from the
347 HPLC, we compared the mass spectroscopy spectrum of un-treated GA and GA exposed to UV-A
348 light for 30 min (Fig. 4). Both MS spectra showed prominent peaks at m/z of 167.9, 168.9, 169.9
349 and 339. The peak at m/z 169.9 represents a small fraction of GA that was un-dissociated. A peak at
350 m/z 168.9 represents GA with a deprotonated carboxylic acid group, as one would expect from the
351 negative ion electron spray. A peak at 167.9 likely represents one electron oxidized GA, where one
352 of the hydroxyl groups were oxidized resulting in the formation of a quinone. Since, this peak was
353 observed in both control (relative intensity 2116) of and UV-A light treated samples (relative

intensity 2354), it is likely that this was a result of oxidation of some of the GA during the high-voltage electron spray. This phenomenon is known in the literature (Chen and Cook, 2007). Nevertheless, a small increase (~11%) in the intensity of the peak at m/z 167.9 in UV-A treated sample may be a result of the oxidation of GA by UV-A light. A peak at m/z of 339 is possibly a result of a dimer formed between two GA molecules due to hydrogen bonding. Such dimerization has been observed previously in the case of caffeic acid, a molecule similar to GA (Arakawa et al., 2004). However, no new peaks were detected in the mass spectrum of UV-A treated GA and consistent with HPLC measurements, the signal intensity from GA (m/z 168.9) was virtually identical in the two spectra (~2600) indicating that the two solutions had a similar concentration of GA.

3.5 Effect of catalase on the antibacterial activity of UV-A+GA treatment

To illustrate the potential antimicrobial mechanism of UV-A+GA treatment based on its ability to generate ROS such as hydrogen peroxide, we evaluated the antibacterial activity of UV-A+GA in the presence of catalase. Catalase is an enzyme that is a known inhibitor of hydrogen peroxide activity (Abucanyon et al., 2014).

Figure 5 (Fig. 5) shows the comparison between the inactivation of *E. coli* O157:H7 upon simultaneous exposure to 10 mM GA and UV-A in the absence and presence of active catalase or denatured catalase. In the control samples (UV-A+GA), 30 min of light exposure resulted in a $5.10 \pm 0.12 \log(\text{CFU/mL})$ reduction in the bacterial count, while in the presence of catalase enzyme, only $1.52 \pm 0.19 \log(\text{CFU/mL})$ reduction in bacterial count was achieved. Samples treated with catalase with or without UV-A light exposure showed a reduction of less than 1 $\log(\text{CFU/mL})$ as well as the samples with GA and catalase not exposed to UV-A light. In the presence of denatured catalase, a $3.44 \pm 0.39 \log(\text{CFU/mL})$ reduction was observed. This inactivation was significantly ($p < 0.05$) lower than the control (no catalase) but significantly higher than the treatment in presence

of catalase ($p < 0.05$). Denatured catalase lowered the extent of bacterial inactivation because denaturation of catalase resulted in slight increase in turbidity induced by enzyme aggregation and it also contributed to the organic content in the solution. A previous study indicated that flavonoid compounds such as epigallocatechin gallate can bind with catalase and inhibit its activity (Krych and Gebricka, 2013). To investigate whether catalase retained its activity in the presence of GA, we measured the ability of catalase to decompose hydrogen peroxide in the absence and presence of GA and found that catalase was able to decompose as high as 800 μM hydrogen peroxide into water and oxygen within 5 min indicating that its activity was not affected by GA. Overall, the results of the study clearly highlight that catalase enzyme was able to inhibit the antimicrobial activity of UV-A+GA. To further delineate the role of hydrogen peroxide in the observed antibacterial activity, we also performed experiments where gallic acid was replaced by hydrogen peroxide (supplementary figure 1).

391

3.6 Detection of GA associated with *E. coli* O157:H7

DPBA is a flavonoid specific dye that becomes fluorescent upon conjugation with flavonoid compounds and has been used previously to localize these compounds within cells (Peer et al., 2001; Chen et al., 2015). We used this approach to detect association of GA with the bacterial cell using fluorescence spectroscopy. The fluorescence intensity of bacterial suspension mixed with GA and DPBA was 272.7 ± 7 unit, compared to 41.6 ± 0.5 unit for the bacterial suspension mixed with DPBA alone, indicating that a fraction of GA was associated with the bacteria.

399

3.7 Inactivation of *Escherichia coli* O157:H7 on spinach leaves by UV-A+GA treatment

Figure 6 shows the remaining bacteria on spinach samples after the treatments. Although GA in absence of UV-A light did not cause a significant bacterial inactivation, a simultaneous treatment of UV-A+GA resulted in at least 3.50 ± 1.04 log(CFU/mL) reduction without any visible color or

404 texture change in leaves. This result demonstrates that the synergistic interaction of UV-A light and
405 GA treatment was effective in inactivating *E.coli* inoculated on the surface of the spinach leaves.
406 The magnitude of inactivation was lower than that observed in the model wash water. This can be
407 attributed to a higher variability in the extent of inactivation on spinach surface compared to that in
408 the solution. Nevertheless, the leaf surface bacterial inactivation assay offers a proof-of-concept of
409 the synergistic effect in inactivating the bacteria on the surface of spinach.

410

411 4. DISCUSSION

412

413 Sanitation of fresh produce and wash water with common, low-cost sanitizers such as peroxides and
414 chlorine-based compounds is still a common practice within the industry. However, there is
415 emerging and growing need for new sanitation systems that are not only low-cost, scalable, and
416 effective but also are environmental friendly and safe for the workers at the processing plants. UV-A
417 activated phenolic acids such as GA could represent an emerging class of light activated sanitizers
418 for the fresh produce and food processing industry.

419 The results of this study demonstrated that a combined UV-A+GA treatment exerts a significant
420 antimicrobial effect on *E. coli* O157:H7 both in the presence of high and medium levels of organic
421 content. Light activated GA treatment achieved approximately 6 log(CFU/mL) reduction in the
422 presence of medium level (500 mg/L O₂ COD) and approximately 4 log(CFU/mL) reduction in the
423 presence of high level (2000 mg/L O₂ COD) of organic content. In the presence of high organic
424 content, common sanitizers based on chlorine can only achieve reductions between 2 and 3
425 log(CFU/mL) when used with standard treatments parameters in the wash water (Lee et al., 2014).
426 Although peroxyacetic acid (PAA) can achieve a reduction up to 5 log(CFU/mL) against non-
427 pathogenic bacteria such as *E. coli* K-12, *Listeria innocua* and *Lactobacillus plantarum* (Grace Ho
428 et al., 2011), its concentration needs to be constantly monitored and replenished as PAA is

429 consumed by non-specific reactions with organic matter. In contrast to this, our experiment
430 investigating the antimicrobial activity of the recycled GA solution showed that GA maintained the
431 biological activity against *E. coli* after multiple cycles of UV-A exposure, indicating an attractive
432 recycling potential of GA (Fig. 2). These results are also consistent with the LC-MS measurements
433 which demonstrated that GA was not degraded by UV-A light.

434 In addition to planktonic bacteria in wash water, biofilms are a significant risk factor for cross-
435 contamination. Standard sanitizers have limited efficacy against biofilms (Percival et al., 2014). The
436 formation of *E. coli* O157:H7 biofilm on food contact surfaces can increase the risk of cross-
437 contamination (Dourou et al., 2011). New tools for the eradication of biofilm from food contact
438 surfaces are needed and the use of GRAS compounds activated by the light could be a potential
439 environmentally friendly tool for the treatment of surfaces susceptible to biofilm formation. Our
440 results showed that a 10 mM GA was able to achieve more than 70% reduction in metabolic activity
441 of the biofilm, highlighting the promise of this approach for the inactivation of biofilm. Previous
442 studies that used GA alone against the biofilms of Gram positive and Gram negative bacteria
443 concluded that the MIC concentration of GA for biofilm control was in the order of 5 mg/mL (29
444 mM) (Borges et al., 2012) or 8 mg/mL (47 mM) (Shao et al., 2015) for the majority of the biofilms
445 tested. The GA concentration used in those previous works was approximately 3 to 5 fold higher
446 than the concentration needed in our work to inhibit the metabolic activity of the *E. coli* O157:H7
447 biofilm indicating a significant improvement in the antimicrobial activity of GA upon its interaction
448 with UV-A light.

449 Catalase inhibited the antimicrobial activity of UV-A+GA, indicating that one of the possible
450 mechanisms responsible for the antibacterial effect was generation of hydrogen peroxide. This is
451 consistent with the previous studies where exposure of GA to 400 nm LED light resulted in
452 generation of hydrogen peroxide as detected by electron spin resonance spectroscopy (Nakamura et
453 al., 2012; Nakamura et al., 2015). Another study showed generation hydrogen peroxide by

454 compounds such as epigallocatechin gallate and gallic acid at physiological pH (Sakagami et al.,
455 2001). Similarly, UV-C exposure of chemical compounds **structurally similar** to GA such as 1,2-,
456 1,3- and 1,4-benzene diols resulted in formation of hydrogen peroxide (Clapp et al., 1990).
457 However, it is interesting to observe that despite using excess concentration of catalase (about 1
458 mg/mL) in this study, the antimicrobial activity of UV-A+GA was not completely inhibited and a
459 1.52 ± 0.19 **log(CFU/mL)** reduction was still observed. Catalase is a large molecular weight protein
460 (250 kDa) (Schroeder et al., 1964) that is not permeable through the cell membrane (Schimmel and
461 Bauer, 2002). Thus the residual antibacterial activity in presence of catalase can be attributed to: (a)
462 GA uptaken and internalized within the bacterial cell, where it exhibited antimicrobial effect,
463 possibly through generation of hydrogen peroxide upon exposure to UV-A light; (b) involvement of
464 other ROS species such as singlet oxygen or superoxide anion or (c) other potential non-ROS based
465 mechanisms such as changes in the membrane permeability. The results pertaining to antibacterial
466 activity of UV-A exposed hydrogen peroxide (supplementary figure 1) highlight that when
467 hydrogen peroxide is internalized within the bacteria, it can be more effective against the bacteria.
468 However, hydrogen peroxide is a reactive and thus unstable molecule that can be rapidly dissociated
469 to other short lived but reactive species by trace metal ions in the solution or may be decomposed
470 by reaction with organic matter. However, GA is a more stable molecule, and based on the
471 fluorescence spectroscopic observations, it is evident that a fraction of GA was associated with and
472 likely internalized within the bacterial cells, where it may exert antibacterial effect upon exposure to
473 UV-A light either by generation of hydrogen peroxide or other mechanisms. This is also consistent
474 with a previous study that discussed the metabolism of GA within the bacterial cells (Nogales et al.,
475 2011). Thus, rather than adding a relatively large amount of hydrogen peroxide to the **wash water** to
476 inactivate bacteria, it may be more attractive to utilize the ability of GA to generate of hydrogen
477 peroxide *in-situ* upon exposure to UV-A light. Nevertheless, further research is needed to evaluate
478 the generation and role of other ROS on the antibacterial activity of UV-A+GA treatment. Research

479 is also needed to investigate whether non-ROS based mechanisms such as changes in membrane
480 permeability are also responsible for the antibacterial effect.

481

482 **5. CONCLUSIONS**

483

484 We evaluated the synergistic interaction of UV-A and GA against *E. coli* O157:H7. Simultaneous
485 treatment with UV-A and GA resulted in a significant (>5 log) inactivation of *E. coli* O157:H7 in
486 suspension. While the presence of organic matter lowered the efficacy of the treatment, at least 3-
487 log reductions were observed at the highest organic load (2000 mg O₂/L COD) in 30 min of
488 treatment. The combined treatment was also effective on the biofilm. Since GA retained its effect
489 during multiple cycles of UV-A treatment, it can be recycled and reused, making it a cost-effective
490 treatment. Generation of hydrogen peroxide from GA in the presence of UV-A was one of the
491 mechanisms responsible for the enhanced antimicrobial activity of the combined treatment;
492 however, it is plausible that other ROS and non-ROS based mechanisms are also involved. The
493 simultaneous treatment was also effective in achieving more than 3-log reductions in bacterial count
494 on the surface of spinach leaves. Based on the relatively low cost of GA, its efficacy and its
495 regulatory status as GRAS, the technology explored in this study holds a significant promise in the
496 sanitation industry. Research is needed to fully understand the mechanism of inactivation to further
497 enhance the efficacy of this treatment.

498

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506 REFERENCES

507

508 Abucayon, E., Ke, N., Cornut, R., Patelunas, A., Miller, D., Nishiguchi, M. K., & Zoski, C. G.
509 (2014). Investigating catalase activity through hydrogen peroxide decomposition by bacteria
510 biofilms in real time using scanning electrochemical microscopy. *Analytical Chemistry*, 86(1), 498–
511 505.

512

513 Borges, A., Saavedra, M. J., & Simões, M. (2012). The activity of ferulic and gallic acids in biofilm
514 prevention and control of pathogenic bacteria. *Biofouling*, 28(7), 755–767.

515

516 Arakawa, R., Yamaguchi, M., Hotta, H., Osakai, T., Kimoto, T. (2004). Product analysis of caffeic
517 acid oxidation by on-line electrochemistry/electrospray ionization mass spectrometry. *Journal of*
518 *American Society for Mass Spectroscopy*, 15(8), 1228-1236.

519

520 Chen, J., Song, M., Wu, X., Zheng, J., He, L., McClements, D. J., Decker, E., & Xiao, H. (2015).
521 Direct Fluorescent Detection of a Polymethoxyflavone in Cell Culture and Mouse Tissue. *Journal*
522 *of Agricultural and Food Chemistry*, 63(49), 10620–10627.

523

524 Chen, M., Cook, K. D. (2007). Oxidation artifacts in the electrospray mass spectroscopy of A β
525 peptide. *Analytical Chemistry*, 79(5), 2031-2036.

526

527 Clapp, P. A., Du, N., & Evans, D. F. (1990). Thermal and photochemical production of hydrogen
528 peroxide from dioxygen and tannic acid, gallic acid and other related compounds in aqueous
529 solution. *Journal of the Chemical Society, Faraday Transactions*, 86(14), 2587–2592.

530

531 Cossu, A., Ercan, D., Tikekar, R. V., & Nitin, N. (2016). Antimicrobial Effect of Photosensitized
 532 Rose Bengal on Bacteria and Viruses in Model Wash Water. *Food and Bioprocess Technology*, 9(3),
 533 441–451.

534

535 Díaz-Gómez, R., Toledo-Araya, H., López-Solís, R., & Obreque-Slier, E. (2014). Combined effect
 536 of gallic acid and catechin against *Escherichia coli*. *LWT - Food Science and Technology*, 59(2),
 537 896–900.

538

539 Dourou, D., Beauchamp, C. S., Yoon, Y., Geornaras, I., Belk, K. E., Smith, G. C., Nychas G. J. E., &
 540 Sofos, J. N. (2011). Attachment and biofilm formation by *Escherichia coli* O157:H7 at different
 541 temperatures, on various food-contact surfaces encountered in beef processing. *International*
 542 *Journal of Food Microbiology*, 149(3), 262–268.

543

544 Ercan, D., Cossu, A., Nitin, N., & Tikekar, R. V. (2016). Synergistic interaction of ultraviolet light
 545 and zinc oxide photosensitizer for enhanced microbial inactivation in simulated wash water.
 546 *Innovative Food Science & Emerging Technologies*, 33, 240–250.

547

548 US FDA (2015a). Safe Practices for Food Processes - Kinetics of Microbial Inactivation for
 549 Alternative Food Processing Technologies.
 550 <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm100158.htm>.
 551 Accessed 11.25.2015

552

553 US FDA (2015b) GRAS Substances (SCOGS) Database - Select Committee on GRAS Substances
 554 (SCOGS) Opinion: Tannic acid (hydrolyzable gallotannins). WebContent.
 555 <http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm261485.htm>. Accessed

556 11.21. 2015

557

558 Grace Ho, K.-L., Luzuriaga, D. A., Rodde, K. M., Tang, S., & Phan, C. (2011). Efficacy of a novel
559 sanitizer composed of lactic acid and peroxyacetic acid against single strains of nonpathogenic
560 *Escherichia coli* K-12, *Listeria innocua*, and *Lactobacillus plantarum* in aqueous solution and on
561 surfaces of romaine lettuce and spinach. *Journal of Food Protection*, 74(9), 1468–1474.

562

563 Krych, J., & Gebicka, L. (2013). Catalase is inhibited by flavonoids. *International Journal of*
564 *Biological Macromolecules*, 58, 148–153.

565

566 Lee, H.-H., Hong, S.-I., & Kim, D. (2014). Microbial reduction efficacy of various disinfection
567 treatments on fresh-cut cabbage. *Food Science & Nutrition*, 2(5), 585–590.

568

569 Luksiene, Z., & Brovko, L. (2013). Antibacterial Photosensitization-Based Treatment for Food
570 Safety. *Food Engineering Reviews*, 5(4), 185–199.

571

572 Moyne, A.-L., Sudarshana, M. R., Blessington, T., Koike, S. T., Cahn, M. D., & Harris, L. J. (2011).
573 Fate of *Escherichia coli* O157:H7 in field-inoculated lettuce. *Food Microbiology*, 28(8), 1417–1425.

574

575 Nakamura, K., Ishiyama, K., Sheng, H., Ikai, H., Kanno, T., & Niwano, Y. (2015). Bactericidal
576 Activity and Mechanism of Photoirradiated Polyphenols against Gram-Positive and -Negative
577 Bacteria. *Journal of Agricultural and Food Chemistry*, 63(35), 7707–7713.

578

579 Nakamura, K., Yamada, Y., Ikai, H., Kanno, T., Sasaki, K., & Niwano, Y. (2012). Bactericidal
580 Action of Photoirradiated Gallic Acid via Reactive Oxygen Species Formation. *Journal of*

581 *Agricultural and Food Chemistry*, 60(40), 10048–10054.

582

583 Narumi, K., Sonoda, J.-I., Shiotani, K., Shigeru, M., Shibata, M., Kawachi, A., Tomishige, E., Sato,

584 K., & Motoya, T. (2014). Simultaneous detection of green tea catechins and gallic acid in human

585 serum after ingestion of green tea tablets using ion-pair high-performance liquid chromatography

586 with electrochemical detection. *Journal of Chromatography B*, 945–946, 147–153.

587

588 Nieuwenhuijsen, M. J., Toledano, M. B., & Elliott, P. (2000). Uptake of chlorination disinfection

589 by-products; a review and a discussion of its implications for exposure assessment in

590 epidemiological studies. *Journal of Exposure Analysis and Environmental Epidemiology*, 10(6),

591 586–599.

592

593 Nogales, J., Canales, A., Jiménez-Barbero, J., Serra, B., Pingarrón, J. M., García, J. L., & Díaz, E.

594 (2011). Unravelling the gallic acid degradation pathway in bacteria: the gal cluster from

595 *Pseudomonas putida*. *Molecular Microbiology*, 79(2), 359–374.

596

597 Peer, W. A., Brown, D. E., Tague, B. W., Muday, G. K., Taiz, L., & Murphy, A. S. (2001). Flavonoid

598 accumulation patterns of transparent testa mutants of arabidopsis. *Plant Physiology*, 126(2), 536–

599 548.

600

601 Percival, S. L., Williams, D., Cooper, T., & Randle, J. (2014). *Biofilms in Infection Prevention and*

602 *Control: A Healthcare Handbook*. (1st ed.). New York: Elsevier.

603

604 Sakagami, H., Arakawa, H., Maeda, M., Satoh, K., Kadofuku, T., Fukuchi, K., & Gomi, K. (2001).

605 Production of hydrogen peroxide and methionine sulfoxide by epigallocatechin gallate and

606 antioxidants. *Anticancer Research*, 21(4A), 2633–2641.

607

608 Schimmel, M., & Bauer, G. (2002). Proapoptotic and redox state-related signaling of reactive
 609 oxygen species generated by transformed fibroblasts. *Oncogene*, 21(38), 5886–5896.

610

611 Schroeder, W. A., Shelton, J. R., Shelton, J. B., & Olson, B. M. (1964). Some Amino acid sequences
 612 in bovine-liver catalase. *Biochimica Et Biophysica Acta*, 89, 47–65.

613

614 Shao, D., Li, J., Li, J., Tang, R., Liu, L., Shi, J., Huang, Q., & Yang, H. (2015). Inhibition of Gallic
 615 Acid on the Growth and Biofilm Formation of *Escherichia coli* and *Streptococcus mutans*. *Journal*
 616 *of Food Science*, 80(6), M1299–1305.

617

618 Van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of
 619 microbial vegetative cells. *International Journal of Food Microbiology*, 74(1-2), 139–159.

620

621 Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical Quality and
 622 Chemical Safety of Chlorine as a Reconditioning Agent and Wash Water Disinfectant for Fresh-Cut
 623 Lettuce Washing. *Applied and Environmental Microbiology*, 79(9), 2850–2861.

624

625 Virto, R., Mañas, P., Álvarez, I., Condon, S., & Raso, J. (2005). Membrane Damage and Microbial
 626 Inactivation by Chlorine in the Absence and Presence of a Chlorine-Demanding Substrate. *Applied*
 627 *and Environmental Microbiology*, 71(9), 5022–5028.

628 **Table 1. Parameters for the Weibull models that describe the inactivation of *E. coli* at**
629 **stationary phase by UV-A treatment in presence of 0, 1, 5 and 10 mM gallic acid.**

630

Sample	alpha	beta	t _R	MAE	RMSE
0 COD 0 mM Gallic acid	31.16	0.81	61.24	0.04	0.06
0 COD 1 mM Gallic acid	12.27	1.64	48.20	0.14	0.22
0 COD 5 mM Gallic acid	4.96	1.46	16.80	0.02	0.24
0 COD 10 mM Gallic acid	2.29	1.03	5.41	0.11	0.48
500 COD 0 mM Gallic acid	17.91	1.05	43.00	0.01	0.08
500 COD 1 mM Gallic acid	13.84	1.24	38.93	0.08	0.16
500 COD 5 mM Gallic acid	3.00	0.60	4.94	0.22	0.36
500 COD 10 mM Gallic acid	0.16	0.47	0.23	0.08	0.10
2000 COD 0 mM Gallic acid	562.71	0.29	716.72	0.02	0.03
2000 COD 1 mM Gallic acid	566.71	0.27	709.87	0.07	0.08
2000 COD 5 mM Gallic acid	151.07	0.40	210.91	0.05	0.06
2000 COD 10 mM Gallic acid	9.31	1.80	41.79	0.04	0.1

631

632 **FIGURE CAPTIONS**

633

634 **Figure 1. Logarithmic reduction of *E. coli* O157:H7 during UV-A+GA treatment.**

635 Concentrations in a range of 0 to 10 mM of GA were tested against 1×10^6 CFU/mL bacteria with
636 UV-A light in spinach wash water at 0 (A), 500 (B) or 2000 COD (C) of organic content. CFU
637 counts are reported in a logarithmic scale. Controls composed of no UV-A light exposure but
638 incubation with 10mM GA (GA 10mM L-) or no GA but UV-A exposure (L+) are also shown.

639

640 **Figure 2. Logarithmic reduction of *E. coli* O157:H7 after multiple exposures of GA to UV-A**

641 **light.** A solution of 10 mM of GA was subjected to 3 cycles of 30 min UV-A light exposure with
642 1×10^6 CFU/mL bacteria in water. The same solution of GA was filtrated and recycled for the
643 additional two photosensitization cycles. CFU counts are reported in a logarithmic scale. Controls
644 composed of no UV-A light exposure but incubation with 10mM GA (GA 10mM L-) or no GA but
645 UV-A exposure (L+) are also shown.

646

647 **Figure 3. Metabolic inactivation of *E. coli* O157:H7 biofilm UV-A+GA treatment.** A range of 0

648 - 10 mM concentration of GA was tested against a 6 days biofilm with 0 COD water. Metabolic
649 activity of the biofilm after 1 h of GA UV-A photosensitization (L+) was assessed by incubation of
650 the biofilm with 50 μ M of Resazurin in LB medium for 18 h at room temperature in the dark.
651 Fluorescence of the reduced resazurin was detected with ex530nm/em580nm filters. Biofilm
652 metabolic activity was measured as percentage of fluorescence intensity calculated from the
653 metabolic activity of the biofilm treated with the GA solution at 0 min without UV-A treatment.
654 Controls with the same GA concentration but without exposure to UV-A light (L-) are also shown.

655

656 **Figure 4. Mass spectrum of 5 mM gallic acid solution (A) before UV-A exposure and (B) after**

657 **30 mins of UV-A exposure.**

658

659 **Figure 5. Antimicrobial activity of UV-A+GA treatment against *E.coli* O157:H7 in the presence**
660 **of catalase enzyme.** A 10 mM concentration of GA was tested against bacteria at a titer of 1×10^6
661 CFU/mL and incubated for 10 min (GA+). Bacteria were then added with native catalase enzyme 1
662 mg/mL (3000 U) (CAT NAT+) or denatured catalase enzyme 1 mg/mL (3000 U) (CAT DEN+)
663 immediately before a 30 min UV-A treatment (L+) in 0 COD. CFU counts are reported in a
664 logarithmic scale. Bacterial counts without exposure to UV-A light (L-) are also shown.

665

666 **Figure 6: Antimicrobial activity of UV-A+GA treatment on *E.coli* O157:H7 inoculated on the**
667 **surface of spinach (GA+L+).** Controls consisted of incubation with GA in dark (GA+L-) and
668 incubation of bacteria without UV-A and GA (GA-L-).

669

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6