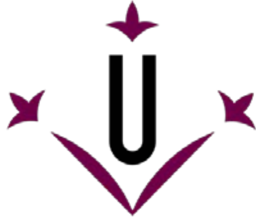

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Surface decontamination of spinach by intense pulsed light treatments: impact on quality attributes

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Short version title: **Pulsed light applied to spinach**

Abstract

Intense pulsed light (IPL) treatments constitute an emerging non-thermal technology proposed to decontaminate food surfaces. In this study, the bactericidal effect of IPL against *Listeria innocua* and *Escherichia coli* inoculated on spinach leaves was evaluated and mathematically modeled. Also, the impact of IPL treatments (20 and 40 kJ m^{-2}) on headspace gas composition, microbial quality, antioxidant properties and colour of spinach was assessed immediately after treatment and during refrigerated storage. IPL treatments were effective for reducing the naturally-occurring microbial load on the raw material by 0.4 - 2.2 log CFU g^{-1}, depending on the applied fluence. IPL treatments also reduced the growth rates of microbial populations through storage. Changes in the package headspace composition were significantly affected by IPL treatments. In-package production of CO_2 increased at a higher rate than for untreated spinach leaves, while O_2 concentrations decreased. Total polyphenolic content and antioxidant capacity of spinach exhibited significant increases in the range of 5-10% and 32-34% for the samples treated with 20 or 40 kJ m^{-2}, respectively. Despite these initial increases, treated spinach leaves presented an accelerated decrease in these quality indicators during refrigerated storage. At the end of storage, IPL-treated samples presented a slightly lower phytochemical quality but significant better microbial quality than control samples.

Keywords: Intense pulsed light; spinach; microbial stability; antioxidant capacity; polyphenolic content; colour.

23 ## 1. Introduction

24 Consumer's demand for fresh-like convenience products has promoted the
25 development of minimally processed vegetables, which have been related with an
26 increase in foodborne illnesses outbreaks (Gupta et al., 2012; Sagong et al., 2011; Ilic
27 et al., 2012; Olaimat and Holley, 2012). In particular, leafy greens have been
28 associated with multiple outbreaks with high numbers of illnesses worldwide. Taking
29 into account that fresh-cut produce is frequently eaten raw, its microbial safety has
30 become an important priority for agrifood and public health authorities (WHO, 2008). As
31 a consequence, procedures and practices during production (in field) and processing
32 (in industry) have been placed under a high degree of scrutiny (Ilic et al., 2012; Lynch
33 et al., 2009), Among these practices, disinfection procedures are commonly carried out
34 through washing with chlorinated water (Allende et al., 2008). Although sodium
35 hypochlorite is an effective an inexpensive sanitizing alternative to disinfect water to
36 avoid cross-contamination between clean and contaminated produce (Gil et al., 2009)
37 several studies have questioned its use due to the formation of products derived from
38 the oxidation of organic material with carcinogenic and mutagenic effects and toxicity
39 tested on kidney and lung (Martin-Diana et al., 2008; Nieuwenhuijsen et al., 2000; Rico
40 et al., 2007). Consequently, many countries have reduced the approved limits of
41 chlorine for use in the disinfection of vegetables. Even countries such as Germany,
42 Holland, Switzerland and Belgium have banned its use (Ölmez and Kretzschmar,
43 2009). In any case, the antimicrobial effect of chlorine when applied to fresh-cut
44 produce is rather limited. It has been demonstrated that sanitation achieves reductions
45 in microbial load only between 1 and 2 log cycles (Allende et al., 2006). This has
46 prompted research worldwide to find and develop new disinfection strategies to
47 overcome this drawback (Ölmez and Kretzschmar, 2009). Different disinfection
48 methods have been proposed as an alternative to chlorine for use in the fresh-cut
49 industry. Among these, the use of essential oils with antimicrobial activity, the addition
50 of organic acids for the reduction of pH or the use of emerging non-thermal

51 technologies stand as most prominent (Meireles et al., 2016). Among these, intense
52 pulsed light (IPL) treatments may reduce and control microbial growth while minimally
53 affecting sensorial and nutritional aspects of food products (Ramos-Villarroel et al.,
54 2012a). This technique decontaminates food surfaces by killing microorganisms using
55 short time light pulses of a broad spectrum, which have a significant UV-C component
56 (Elmnasser et al., 2007; Gómez-López et al., 2007). The lethal action of IPL has been
57 primarily attributed to a photochemical mechanism, by which UV absorption produces
58 severe damage in the DNA of microorganisms, preventing their replication. A
59 photothermal effect associated to the temporary overheating caused by the dissipation
60 of a certain amount of the incident radiation may also be involved (Gómez-López et al.,
61 2007). Another mechanism probably involved in surface decontamination by IPL has a
62 photophysical nature, through the effect of light on membranes, proteins and other cell
63 constituents (Gómez-López et al., 2007).

64 Several studies have explored the potential of this technology for the
65 inactivation of pathogenic and spoilage microorganisms in foods and its impact on
66 several quality aspects. In this way, some authors (Ramos-Villarroel et al., 2011;
67 2012a; 2012b) have studied the effect of IPL spectral distribution on the inactivation of
68 inoculated microorganisms, native microflora and quality aspects of avocado,
69 mushrooms and watermelon. Oms-Oliu et al. (2010) determined the impact of IPL
70 treatments on the microbiological, nutritional and sensorial quality of mushrooms.
71 Izquier and Gómez-López (2011) modeled the degree of inactivation of naturally
72 occurring microorganisms by IPL treatments applied on lettuce, cabbage and carrot.
73 Gómez-López et al. (2005a) found reductions of 0.56 – 2.04 log CFU g^{-1} in mesophilic
74 aerobic counts when treating spinach, celery, lettuce, white cabbage, bean sprouts and
75 green bell pepper with 7 J of IPL by side. They concluded that differences in
76 inactivation levels among substrates could be related with differences in resistance of
77 native microflora in each vegetable, the location of microorganisms as well as the
78 presence of protective substances in some of the cases under study. Thus, several

79 factors associated with processing and those inherent to the raw material need to be
80 considered when studying the effectiveness of IPL treatments. Accordingly, a case by
81 case study is required when evaluating the suitability of IPL for different food
82 substrates.
83 Nevertheless, safety of fresh-cut products stands still as an important issue, as neither
84 IPL nor any of the before-mentioned antimicrobial treatments can be applied on fresh-
85 cut commodities with a pasteurizing intensity in order to avoid the development of
86 deleterious effects on the product fresh-like quality. The main objective of this work was
87 to explore the suitability of IPL treatments to inactivate *Listeria innocua* and *Escherichia*
88 *coli* inoculated on spinach, as well as to evaluate the effects of IPL treatments on the
89 native microflora and quality attributes of spinach evaluated immediately after
90 treatments and over storage.
91

92 **2. Materials and Methods**

93 2.1. Raw material and sample preparation

94 Spinach (*Spinacia oleracea* L.) cv. Polka bunches were purchased at a local supplier in
95 Lleida, Spain, immediately transported to laboratory and processed. To avoid the
96 natural variability of the raw material, only whole leaves uniform in size and color and
97 lacking of defects were used. Selected leaves were dipped in tap water for 5 minutes
98 and centrifuged for 30 s in a domestic centrifuge to remove the excess of water. The
99 washed produce was packed in a monolayer, avoiding overlapping of leaves, in units of
100 5 g (experimental unit) in polyethylene trays (length: 124 mm, width: 129 mm, height:
101 25 mm, headspace volume: 350 mL) under aseptic conditions. The amount of sample
102 was selected to avoid the interaction of shadow effects on microbial inactivation. After
103 IPL treatments, the trays were thermo-sealed using an ILPRA FoodPack Basic V/6
104 packaging machine (ILPRA Systems, CP, Vigevono, Italy) with a 40-μm polypropylene
105 film with an oxygen permeability of 110 cm^3 O_2 m^{-2} bar^{-1} d^{-1} at 23ºC and 0% RH (ILPRA

106 Systems Spain, S.L. Mataró, Spain). The packages were stored at 5 ºC in darkness
107 until each sample was randomly withdrawn for analysis.
108
109 2.2. Inactivation of surrogate microorganisms
110 *2.2.1. Strains and growth conditions*
111 *Listeria innocua* was used as a surrogate microorganism for the pathogenic *L.*
112 *monocytogenes* because both microorganisms are closely related from a physiological
113 point of view (Soares Pinto et al., 2009). Strains of *L. innocua* 1.17 (Laboratoire de
114 répression des Fraudes, Montpellier, France) and *Escherichia coli* 1.107 (Laboratoire
115 de répression des Fraudes, Montpellier, France) were provided from the culture
116 collections of the Department of Food Technology, University of Lleida, Spain. The
117 original strains were kept in inclined test-tubes with Tryptone Soy Agar (Biokar
118 Diagnostics, Beauvais, France) at a temperature of 5 ºC until their use.
119 *L. innocua* and *E. coli* were grown in 150 mL of Tryptic Soy Broth (Biokar
120 Diagnostics, Beauvais, France) supplemented with 0.6% yeast extract (TSYE) at 35 ºC
121 for 15 h and 180 rpm and 37 ºC for 11 h and 120 rpm, respectively, to obtain the
122 desired cells concentration (10^9 CFU mL^{-1}), determined by optical density using a CE
123 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Cells concentration
124 was then adjusted to 10^8 CFU mL^{-1} using dilutions in TSYE.
125 *2.2.2. Sample inoculation*
126 Each experimental unit (5 g) was inoculated by spreading 500 µL of the *L. innocua* or
127 *E. coli* culture over the entire upper surface of each with a sterile micropipette, to obtain
128 an initial count of approximately 10^7 CFU g^{-1} in spinach samples. After inoculation, the
129 trays were sealed as explained previously.
130 *2.2.3. Treatment*
131 IPL treatments were carried out with a XeMaticA-2L System (SteriBeam Systems
132 GmbH, Germany) equipped with Xenon flash lamps with a maximum energy emission

133 of 700 J. The emitted spectrum wavelengths (λ) ranged from 180 to 1100 nm with 17%
134 of the light in the UV region. A 65% of the emitted UV light was in the range between
135 200 and 305 nm (UV-B, UV-C), whereas the other 35% was in the upper range
136 between 305-400 nm (UV-A, UV-B). Energy calculations were carried out according to
137 the calibration of the equipment with a standard light source estimated by photodiode
138 readings and following manufacturer's directions. Duration of each pulse was 0.3 ms
139 with a fluence of 4 kJ m^{-2} from one lamp situated 8.5 cm above the sample holder.
140 Samples were exposed to doses of 0 (control), 2, 5, 10, 15, 20 and 30 pulses
141 corresponding to 0, 8, 20, 40, 60, 80 and 120 kJ m^{-2}, respectively.

142 *2.2.4. Enumeration of Listeria innocua and Escherichia coli*

143 Triplicate samples were taken from each treatment. Five g of each sample were
144 aseptically mixed with 45 mL of sterile 1 g kg^{-1} peptone water using a homogenizer
145 (BagMixer® 400, Interscience Laboratories Inc., France). Serial dilutions were carried
146 out using sterile 1 g kg^{-1} peptone water. *L. innocua* or *E. coli* counts were performed
147 using the plate count Palcam selective medium (Biokar Diagnostics, Beauvais,
148 France), added with Palcam Selective Suplement (SR0150, Biokar Diagnostics,
149 Beauvais, France), or Mac Conkey agar (Biokar Diagnostics, Beauvais, France),
150 respectively, incubated for 24-48 h at 35-37 ºC. Olive-green colonies surrounded by a
151 black halo or red colonies were counted as *L. innocua* or *E. coli*, respectively, and the
152 results were expressed as log CFU g^{-1}.

153 *2.2.5. Mathematical modelling*

154 In order to mathematically describe the changes in microbial counts as a function of
155 treatment fluence, a model based on the Weibull distribution function was applied. This
156 model has been extensively applied to model lifetime data in medical, biological and
157 engineering sciences (Soliman et al., 2006). Several authors have used this model to
158 predict inactivation of different microbial populations inoculated on vegetables after
159 non-thermal processing (Alexopoulos et al., 2013; Huang et al., 2014; Izquier and

160 Gómez-López, 2011; Kim et al., 2014; Martínez-Hernández et al., 2015), highlighting
161 its simplicity and high versatility to provide a good description of complex and highly
162 variable processes.

163 In the present study, *L. innocua* or *E. coli* counts are the dependent variables in
164 the Weibull model which can be mathematically expressed following equation 1:

165 $$\log(N) = \log(N_0) - \left(\frac{F}{\delta}\right)^{\rho} \qquad \text{Eq. 1}$$

166 where δ is the scale parameter (kJ m^{-2}) representing the fluence required for the first
167 decimal reduction, ρ is the shape parameter (dimensionless) representing the
168 concavity or convexity of the curve, and F is the fluence (kJ m^{-2}) applied in the
169 treatment (Izquier and Gómez-López, 2011).

170

171 2.3. Impact of IPL treatments on quality parameters of spinach

172 In a second assay, samples prepared following the same previously described
173 protocol, but without inoculation, were treated with 20 and 40 kJ m^{-2} (5 and 10 light
174 pulses, respectively) to determine the impact of these treatments on headspace gas
175 composition, native microflora, nutritional and sensorial aspects of spinach. These
176 doses were selected considering the results of the previous inoculation studies. One lot
177 of spinach samples, prepared with the same protocol but without IPL treatment, was
178 considered as control. Samples were stored under refrigerated storage (4 ± 2°C) and
179 analytical determinations were carried out periodically after treatment and over
180 refrigerated storage on the product obtained from three independently treated
181 packages.

182

183 *2.3.1. Headspace gas analysis*

184 The gaseous composition of the package headspace was determined using a Micro-
185 GC CP 2002 gas analyzer (Chrompack International, Middelburg, Netherlands)
186 equipped with a thermal conductivity detector. A sample of 1.7 mL was automatically

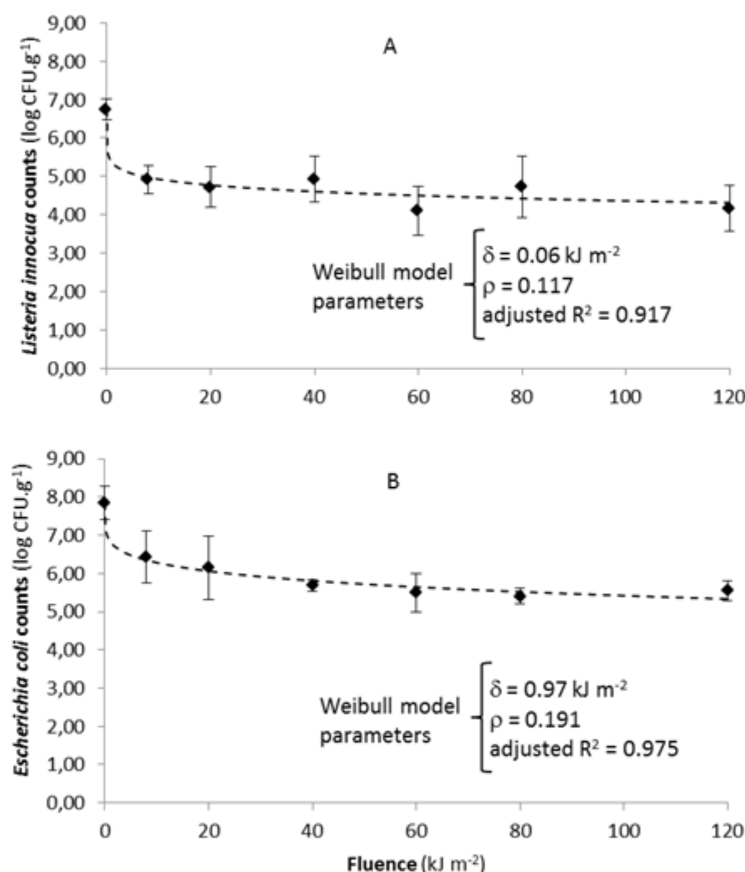
187 withdrawn from the headspace atmosphere with a syringe using a rubber septum
188 sticker. Portions of 0.25 and 0.33 mL were injected for O_2 and CO_2 determination,
189 respectively. The O_2 content was analyzed with a CP-Molsieve 5 A packed column
190 (Chrompack International, Middelburg, Netherlands (4 m x 0.32 mm, df = 10 mm) at 60
191 ºC and 100 kPa. On the other hand, a Pora-PLOT Q column (Chrompack International,
192 Middelburg, Netherlands) (10 m x 0.32 mm, df = 10 mm) was held at 70 ºC and 200
193 kPa for CO_2 quantification. Four trays were withdrawn at each storage time for every
194 treatment.
195
196 *2.3.2. Native microflora counts*
197 Mesophilic bacteria (MB), psychrotrophic bacteria (PB), coliforms (C) and yeasts and
198 molds (Y&M) counts were evaluated with the methodology suggested by Ponce et al.
199 (2008). Additionally, *Listeria* spp. was counted. Ten grams of spinach leaves were
200 macerated for 2 min with 90 mL of sterile 1 g kg^{-1} peptone water using a homogenizer
201 (Stomacher Lab Blender 400, Seward medical, London, England). Serial dilutions were
202 carried out using sterile 1 g kg^{-1} peptone water. Enumeration of MB and PB was
203 performed using plate count agar (PCA) incubated at 32-35 ºC for 48-72 h and 5-7 ºC
204 for 5 days, respectively. For C counts, Mac Conkey agar was incubated at 32-35 ºC for
205 48-72 h; enumeration of Y&M was done using yeast glucose chloramphenicol agar
206 (YGC) incubated at 28 ºC for 72 h. *Listeria* spp. counts were determined using Palcam
207 agar incubated at 32-35 ºC for 24-48 h. All culture mediums were purchased from
208 Biokar Diagnostics (Beauvais, France). Results were expressed as log CFU g^{-1}.
209
210 *2.3.3. Color measurement*
211 The color of spinach was determined with a tristimulus Minolta CR-400 colorimeter
212 (Konica Minolta Sensing, INC. Osaka, Japan) using a D75 illuminant and an
213 observation angle of 10º. A standard white tile (Y = 94.00, x = 0.3158, y = 0.3322) was

214 used as a reference. Five readings of L* (lightness), a* (green-red chromaticity) and b*
215 (blue-yellow chromaticity) coordinates were recorded from each spinach sample.
216
217 *2.3.4. Antioxidant potential*
218 *2.3.4.1. Antioxidants extraction*
219 Spinach leaves were ground and two samples of 1 g were macerated for 2 min with 10
220 mL methanol (80%) using an high-speed homogenizer (Ultra-Turrax® T 25 basic, IKA®
221 WERKE, Staufen, Germany). The homogenates were centrifuged at 5400 x g for 20
222 min at 4 ºC (AVANTI^{TM} J-25 centrifuge, Beckman Instruments Inc., Fullerton, CA, USA)
223 and then filtered through a Whatman no. 1 filter. The supernatant was separated and
224 solids were reextracted with 10 mL of methanol (80%) under ultrasonication (Hielscher
225 sonifier, model UP400S, Hielscher Ultrasound Technology, Teltow, Germany) at a
226 frequency of 24 KHz and 400 W of nominal power for 5 min. A second centrifugation
227 and filtration was carried out and both supernatants were considered as the source of
228 phenolic compounds and antioxidants.
229
230 *2.3.4.2. Total phenolic compounds*
231 The concentration of total phenolic compounds was determined according to the Folin-
232 Ciocalteu procedure (Singleton et al., 1999) with some modifications. An aliquot of 0.4
233 mL of the supernatant was added to 0.2 mL of Folin-Ciocalteu solution. After 3 min, 0.6
234 mL of saturated sodium carbonate solution were added and brought up to 4 mL with
235 distilled water. The absorbance at 765nm was measured after incubation at 20 ºC for 1
236 h in darkness conditions using a spectrophotometer. Total phenolics concentration was
237 calculated and expressed as gallic acid (GA) equivalents on a fresh weight basis (mg
238 kg^{-1}).
239
240 *2.3.4.2. Antioxidant capacity*

241 The antioxidant capacity was analyzed through the determination of free radical-
242 scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical
243 according to the procedure described by (Viacava et al., 2015). Aliquots of 0.4 mL of
244 the supernatant were mixed with 3.6 mL of methanolic DPPH solution. The
245 homogenate was shaken vigorously and kept in darkness for 30 min. Thereafter,
246 absorption at 515 nm was measured with a spectrophotometer. Blank solutions
247 (without DPPH) were prepared to correct any influence due to spinach extract color. A
248 calibration curve of the DPPH solution and a standard curve for ascorbic acid were
249 used to express the antioxidant capacity of spinach extracts as ascorbic acid
250 equivalents on a fresh weight basis (mg kg^{-1}).
251
252 2.4. Statistical analysis
253 Data were analyzed using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). For
254 all experiments, General Linear Model procedure was used for analysis of variance
255 (ANOVA) with different variation sources depending on the experiment. For
256 determination of *L. innocua* and *E. coli* inactivation, the effect of fluence was evaluated
257 through an ANOVA test. Additionally, non-linear regressions for Weibull model fittings
258 were calculated using SYSTAT 5.03 software (SYSTAT Inc., Evanston, IL, USA). The
259 impact of IPL treatment fluence (0, 20 or 40 kJ m^{-2}) and storage time on quality indices
260 of spinach leaves were also evaluated with an ANOVA. In all cases, differences
261 between levels of factors under analysis were assessed by multiple comparison Tukey-
262 Kramer tests with a confidence level of 95%.
263
264 **3. Results and Discussion**
265 3.1. Survival of *Listeria innocua* and *Escherichia coli*
266 Figure 1 presents of the counts of *L. innocua* and *E. coli* achieved on spinach leaves
267 after IPL treatments of increasing fluence. In addition, microbial inactivation predicted
268 by the Weibull model is displayed. IPL treatments presented a significant lethal effect

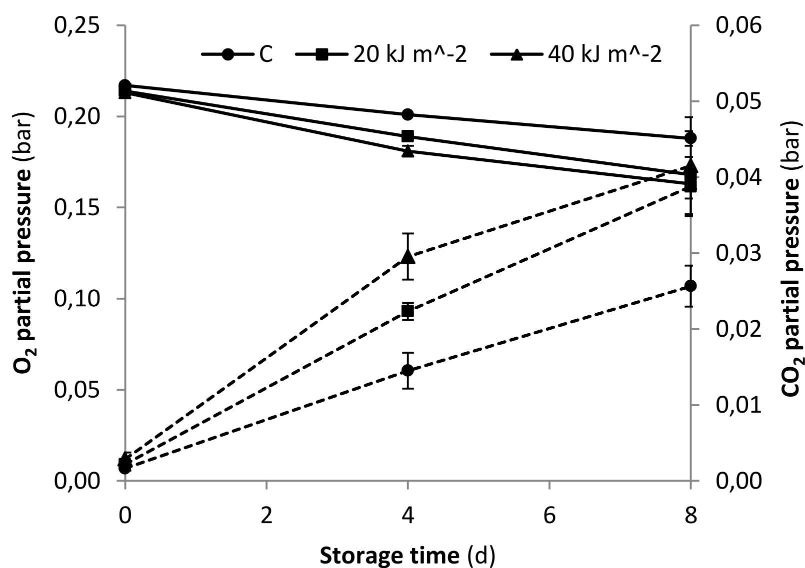
269 against the two tested microorganisms even when the lowest doses were applied. In
270 this way, reductions of 1.85 and 1.72 log CFU g^{-1} were obtained for *L. innocua* and *E.*
271 *coli*, respectively, after the application of 2 light pulses. For both microorganisms,
272 fluencies much lower than 10 kJ m^{-2} were enough to get a log-reduction in microbial
273 counts higher than 1. Increases in the fluence applied did not produce proportional
274 increases in the effectiveness of the treatment. In fact, using 15 times more intense
275 treatment did not improve the inactivation of microorganisms in the same magnitude
276 and decreases of 2.6 and 2.3 log CFU g^{-1} of *L. innocua* and *E. coli*, respectively, were
277 obtained when the highest dose (120 kJ m^{-2}) was applied. This trend could also be
278 observed through the analysis of ρ parameter obtained when fitting the Weibull model
279 to results. In both cases, this value was significantly lower than 1 (0.117 and 0.191 for
280 *L. innocua* and *E. coli*, respectively), indicating that the rate of inactivation decreased
281 as fluence is greater (van Boekel, 2002).

282



283

284 ***Figure 1.*** *Survival curve of Listeria innocua (A) and Escherichia coli (B) of spinach*
285 *leaves treated with intense pulsed light. Experimental data are represented as bullet*
286 *points whereas dotted lines display the values predicted by the adjusted Weibull model.*
287 *Vertical bars stand for standard deviation. Results are the mean obtained from three*
288 *replicate measurements.*
289
290 Other authors have reported decreases in *Listeria* and *E. coli* counts inoculated on
291 vegetable surfaces after applying IPL treatments of a similar fluence than those used in
292 the present work. Among them, Ramos-Villarroel et al. (2012b) found decreases in the
293 range of 2.66-3.03 log CFU g^{-1} for these microorganisms inoculated on fresh-cut
294 mushrooms. However, it is worth noticing that, they found higher sensibility to IPL in *E.*
295 *coli* than *L. innocua* and attributed this result to the cell wall structure of each
296 microorganism. In the present work, the behavior was opposite; however differences
297 could be attributed to the initial counts of these microorganisms. A higher initial count
298 could be associated with lower efficiency in the treatment (Gómez-López et al., 2005b).
299 Taking into account these results, two treatments of 20 kJ m^{-2} and 40 kJ m^{-2} (5 and 10
300 pulses of 4 kJ m^{-2}, respectively) were selected and applied over spinach leaves to
301 study the impact of this technology on quality indices immediately after treatment and
302 over refrigerated storage.
303
304 *3.2. Package headspace gases*
305 A continuous increase in CO_2 concentration accompanied by a decrease in O_2
306 concentration was found in all packages during refrigerated storage (Figure 2).
307



308

309 ***Figure 2.*** *Headspace oxygen (continuous lines) and carbon dioxide (discontinuous*
310 *lines) partial pressures in spinach trays treated with IPL and stored at 5ºC. Vertical*
311 *bars stand for standard deviation. Results are the mean obtained from three replicate*
312 *measurements.*

313

314 Oxygen consumption and carbon dioxide production is associated with spinach
315 respiration as it is a living tissue even after harvest. Also, respiration of native
316 microflora could contribute to the gases balance (Ramos-Villarroel et al., 2012b). IPL
317 treatments significantly affected the changes in the headspace gases composition,
318 increasing the production of CO_2 at a higher rate than untreated spinach and
319 decreasing the O_2 concentration. These effects were influenced by the intensity of the
320 treatment, being highest when the most intense treatment (40 kJ m^{-2}) was applied.
321 These changes could be associated with a physiological stress or even physiological
322 damage caused by IPL treatments, which in turn could affect the metabolic activity of
323 the vegetable tissue. Levels of oxygen at the end of storage were high enough to
324 prevent anaerobic respiration which could lead to fermentation in spinach tissues. The
325 increase in O_2 consumption and CO2 production in irradiated samples is in accordance
326 with the results previously reported by other researchers for fresh-cut produce after IPL

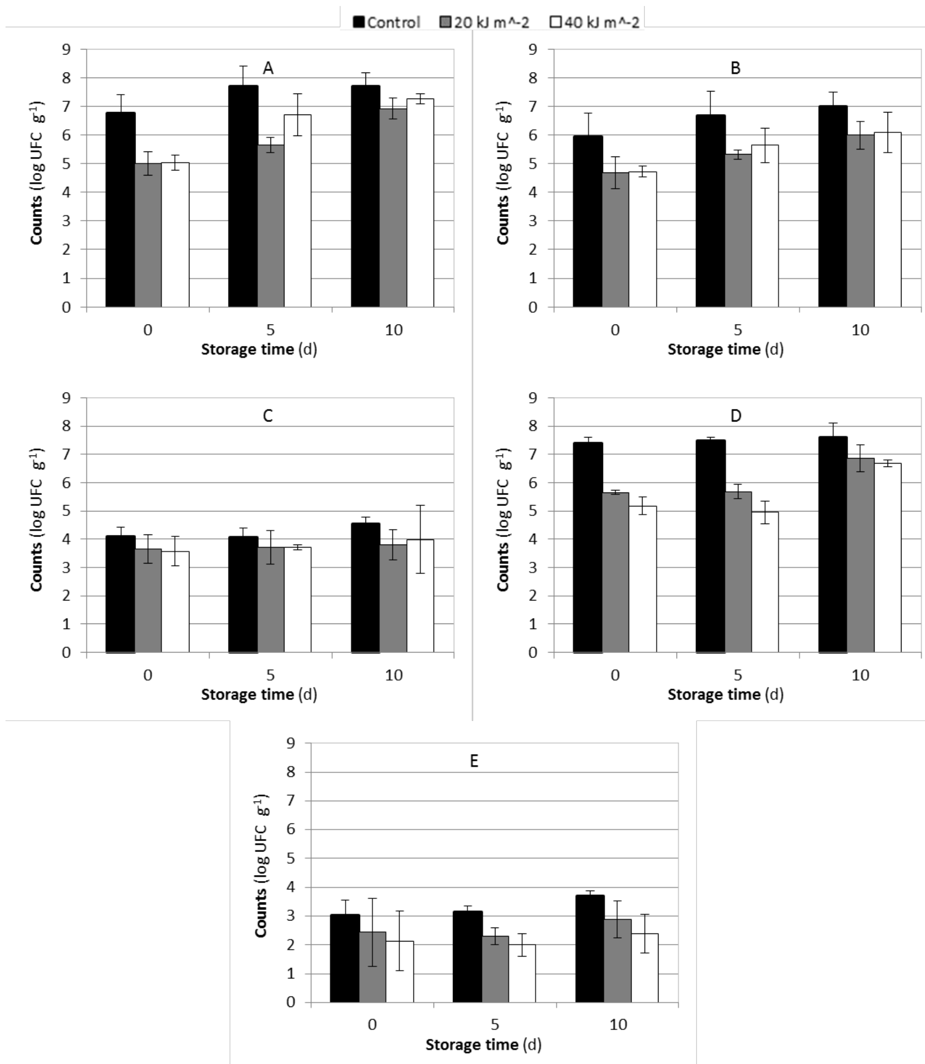
327 or UV-C treatments (Artés-Hernández et al., 2009; Escalona et al., 2010; Oms-Oliu et
328 al., 2010). Nevertheless, it must be stated that the modification of the in-package
329 atmosphere was little compared to that occurring in commercial packages. This needs
330 to be considered for estimating the storage shelf-life as affected by microbial growth
331 and physicochemical changes, although it provides useful information regarding the
332 changes in respiration as affected by IPL treatments from a qualitative point of view.
333

334 *3.3. Microbiological quality*

335 Figure 3 shows the effect of IPL treatments on microbial population of spinach as well
336 as the changes in microbial counts over refrigerated storage for both untreated and
337 IPL-treated samples. Spinach leaves presented higher initial counts, especially in
338 mesophilic aerobic bacteria and coliforms, than usually reported for leafy vegetables at
339 harvest (Escalona et al., 2010; Izquier and Gómez-López, 2011; Moreira et al., 2003).
340 Many factors may account for this difference, such as the type and variety of spinach,
341 pre-harvest specific conditions such as soil, climate and crop management conditions,
342 and post-harvest factors such as temperature and humidity during harvesting and
343 distribution of raw materials, among others.
344 IPL light treatments were effective for reducing the initial microbial load of raw material.
345 In fact, reductions from 0.4 to 1.8 log CFU g^{-1}, depending on the microbial population
346 under consideration, were observed in spinach leaves treated with 20 kJ m^{-2} (5 pulses)
347 and in the range of 0.5 to 2.2 for samples treated with 40 kJ m^{-2} (10 pulses). Gómez-
348 López et al. (2005a) reported differences in the degree of inactivation, depending on
349 the food substrate, when applying IPL treatments to different minimally processed
350 vegetables. Particularly, for shredded spinach, although the treatment intensities (160
351 and 640 kJ m^{-2}) were significantly higher than those used in our work, they reported a
352 smaller reduction in mesophilic aerobic counts (0.34 and 0.90 log CFU g^{-1},
353 respectively). Differences in treatment effectiveness might be related to different
354 resistances of natural microbial populations, the location of microorganisms on and into

355 the product, among others. Oms-Oliu et al. (2010) working with fresh-cut mushrooms,
356 also found an initial reduction in microbial counts in the same order of magnitude than
357 those found in the present study.

358



359
360

361 ***Figure 3.*** *Mesophilic aerobic bacteria (A), psychrotrophic bacteria (B), yeasts and*
362 *molds (C), coliforms (D) and Listeria spp. (E) counts of spinach leaves treated with IPL*
363 *and stored at 5ºC. Data represent the mean of three replicate measurements. Vertical*
364 *bars stand for standard deviation.*

365

366

367 It is worth noting the effectiveness regarding the decontamination of coliform species.
368 These are one of the most important microbial populations present in leafy vegetables
369 and they are considered as quality indicators as they are usually related with
370 agricultural and manufacturing practices employed during growth, harvest and
371 postharvest handling of this produce (Olaimat and Holley, 2012). Additionally, the Gram
372 negative character of these microorganisms makes them more difficult to remove with
373 other non-thermal technologies such as natural antimicrobials, which usually show a
374 minor efficiency against coliforms (Helander and Mattila-Sandholm, 2000). In our study,
375 IPL led to higher reductions in coliform populations compared to those achieved for
376 yeasts and molds or *Listeria* spp. In contrast, in the case of yeasts and molds the effect
377 of treatments was significantly lower. However, as yeasts and molds counts are usually
378 low and do not present a significant growth in this type of product during refrigerated
379 storage, this lower efficiency of IPL to reduce their counts is not a limiting case for the
380 application of this technology. Taking into account these results, reductions detected in
381 the initial microbial counts are particularly important considering that other disinfection
382 methods of leafy vegetables, many of them currently being questioned, achieved
383 microbial reductions in the same order of magnitude as those found in this work.
384 The effect of the application of IPL technology was not only observed in the initial
385 reductions achieved but also during refrigerated storage, because the growth of
386 microbial populations was significantly affected, slowing their development. This result
387 may be attributed to the occurrence of sublethal damage in populations and a
388 consequent decrease in the adaptability to low storage temperatures. Thus, at the end
389 of storage, treated spinach leaves exhibited lower counts than control samples. These
390 results were also reported by other authors who worked with IPL treatments in other
391 food substrates (Izquier and Gómez-López, 2011; Oms-Oliu et al., 2010). In contrast
392 with our results, some studies show that the inhibitory effect of radiation on initial
393 microbial counts is progressively lost during storage, reaching similar or higher counts
394 on IPL-treated produce compared to the untreated (Escalona et al., 2010; Gómez-

395 López et al., 2005a). These differences could be related with the occurrence of
396 sublethal effects as a consequence of the applied treatments, as well as by the
397 different interactions given, in each case, by the type of microorganism and the
398 characteristics of the food matrix.

399 Finally, it is important to note that reductions in the native microflora growing on
400 spinach leaves were lower than those achieved in the *L. innocua* and *E. coli* counts on
401 inoculated samples. These results could be associated with a possible internalization of
402 endogenous microorganisms (native microflora). This phenomenon implies a serious
403 problem in superficial disinfection treatments such as IPL. In fact, it is well accepted
404 that decontamination achieved by IPL treatments is only superficial. If microorganisms
405 are internalized in tissue, IPL treatment may not be able to inactivate them because
406 light is absorbed by the surface layers (Gómez-López et al., 2007). Beyond these
407 observations, it was proven that IPL treatments at 20 and 40 kJ m^{-2} lead to a significant
408 reduction in the initial load of microbial population present in spinach and limit its
409 development during refrigerated storage.

410

411 *3.4. Colour*

412 Table 1 presents results obtained for color parameters *L*, a** and *b** as a function of
413 both the dose of IPL treatment and the storage time. No significant change was noticed
414 in the initial value of lightness (*L**) among untreated and treated spinach leaves.
415 However, a slight but significant increment of *a** values was detected in treated
416 spinach, together with a decrease in *b** values. On the other hand, only *L** values were
417 reported to change throughout refrigerated storage, with an increase in values over
418 time, whereas *a** and *b** remained unchanged over the same period. Different patterns
419 related to these color parameters have been reported for different vegetable substrates
420 exposed to IPL treatments. Particularly for spinach, Artés-Hernández et al. (2009)
421 reported a slight loss of lightness over storage of this leafy vegetable exposed to UV-C
422 doses. Chroma and hue angle values, which are closely related with *a** and *b**

423 parameters, did not change over storage, which is in contrast with the results reported
424 by Costa et al. (2006), who found a decrease in hue angle and an increase in lightness
425 during storage of broccoli treated with UV-C light (10 kJ m^{-2}).
426 Although consumer tests were not carried out during the study, it is worthwhile
427 mentioning that, in line with the instrumental results, the overall visual quality of the
428 treated spinach leaves, evaluated by an informal test panel, did not differ from that of
429 the untreated product. To sum up, the color of spinach was not significantly affected by
430 IPL treatments applied, indicating that this technology, applied at the low doses of our
431 work, is adequate from the point of view of visual appearance to treat spinach without
432 affecting its color.
433
434 **Table 1.** Colour parameters of spinach samples treated with intense pulsed light and
435 stored under refrigerated conditions. Results as expressed as mean ± standard
436 deviation. Different lowercase letters denote a significant difference between mean
437 values within a column ($p< 0.05$). Different capital letters denote a significant difference
438 between mean values within a row ($p< 0.05$).
439

Colour parameter	Time (d)	Treatment		
		Control	20 J/cm^2	40 J/cm^2
*L**	0	36.0 ± 3.5 aA	34.7 ± 2.6 aA	34.2 ± 1.9 aA
	4	36.2 ± 2.5 bA	35.9 ± 2.4 bA	35.6 ± 1.6 bA
	8	37.1 ± 2.7 cA	36.7 ± 1.2 cA	36.5 ± 2.8 cA
*a**	0	-10.3 ± 2.9 aA	-9.3 ± 1.5 aB	-7.9 ± 1.5 aC
	4	-10.2 ± 2.2 aA	-9.7 ± 1.8 aB	-8.9 ± 1.2 aC
	8	-10.2 ± 2.1 aA	-9.8 ± 1.2 aB	-9.5 ± 1.9 aC
*b**	0	13.9 ± 3.7 aA	13.0 ± 2.2 aB	11.4 ± 2.1 aC
	4	13.7 ± 2.7 aA	13.7 ± 2.5 aB	12.2 ± 1.8 aC
	8	14.6 ± 4.1 aA	13.9 ± 1.6 aB	12.9 ± 4.0 aC
Hue	0	126.4 ± 1.9 aA	125.5 ± 1.7 aA	124.7 ± 1.0 aA
	4	126.4 ± 2.0 aA	125.2 ± 1.2 aA	126.4 ± 2.0 aA
	8	125.4 ± 3.1 aA	125.1 ± 1.6 aA	127.2 ± 14 aA

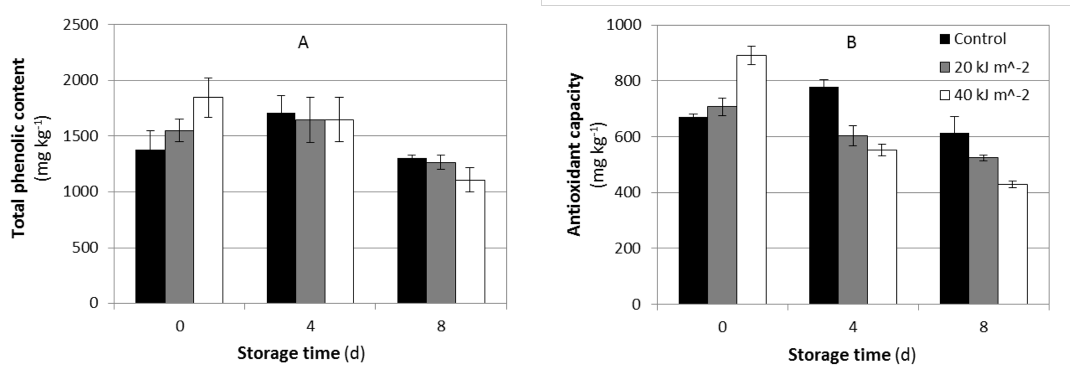
Chroma	0	17.3 ± 4.7 aA	16.0 ± 2.6 aA	13.8 ± 2.6 aB
	4	17.1 ± 3.4 aA	16.7 ± 3.0 aA	15.1 ± 2.1 aA
	8	17.8 ± 4.5 aA	17.0 ± 2.0 aA	16.4 ± 2.9 aA

440

441

442 *3.5. Antioxidant potential*

443 Figure 4 shows the effect of IPL treatments on total phenolics and antioxidant capacity
444 of spinach leaves. Both indicators underwent a significant increase after the application
445 of treatments in the range of 5-10% for the samples treated with 20 kJ m^{-2} and between
446 32 and 34% for the samples treated with 40 kJ m^{-2}. This initial increase could be
447 associated with an increase in free radicals as a consequence of the stress response
448 induced by IPL radiation applied, leading to an increase in the synthesis of antioxidants
449 (Oms-Oliu et al., 2012). This increase in the antioxidant capacity of spinach leaves by
450 the application of non-ionizing radiation was also reported by other authors who worked
451 applying these technologies in other plant substrates. Among them, Costa et al. (2006)
452 reported increases of 20% in both phenols and antioxidant capacity of broccoli florets
453 after applying UV-C treatments at doses of 10 kJ m^{-2}.



454

455 ***Figure 4.** Total phenolics content (A) and antioxidant capacity (B) of spinach leaves*
456 *treated with IPL and stored at 5ºC. Vertical bars stand for standard deviation.*

457

458 Despite the initial increase detected in polyphenols content and antioxidant capacity of
459 spinach due to IPL treatment, treated samples presented an accelerated decrease in
460 these quality indicators during refrigerated storage. However, the total polyphenols

461 content was similar in all samples at the end of storage, while the antioxidant capacity
462 was only lower in spinach leaves treated with 40 kJ m^{-2} compared to untreated spinach
463 (Figure 4). The decrease in the overall antioxidant capacity of the treated spinach
464 leaves can be attributed to a deleterious effect of this treatment on tissue integrity
465 causing membrane damage and altering the composition and content of antioxidant
466 compounds. A similar behavior was observed previously by (Artés-Hernández et al.,
467 2009), who found decreases in antioxidant capacity and polyphenolic increase
468 throughout the refrigerated storage of spinach leaves treated with 10 kJ m^{-2} of UV-C
469 radiation. Also, Oms-Oliu et al. (2010) found that samples treated with the highest IPL
470 doses accelerated the oxidative decay of fresh-cut mushrooms and associated this
471 phenomenon to deleterious effects of this treatment on tissue integrity.
472

473 **4. Conclusion**

474 Treatments with IPL allowed a significant reduction in contaminating microflora
475 on spinach leaves, even when low doses were applied, being effective for both
476 innoculated Gram positive (*Listeria innocua*) and Gram negative (*Escherichia coli*).
477 Additionally, treatments with 20 and 40 kJ m^{-2} were effective to reduce initial microbial
478 counts of this vegetable showing high efficiency for coliforms. IPL applied on spinach
479 lead to an increase in the total phenolics concentration and antioxidant capacity,
480 thereby improving the initial health-related characteristics of the product, possibly as a
481 consequence of a stress response generated in the spinach tissue by abiotic means,
482 also reflected in the increase of respiratory activity. However, IPL-treated samples
483 showed higher degradation rate of phytochemical compounds during refrigerated
484 storage. These changes did not translate into the color of the samples, which remained
485 without changes amongst samples and during storage. Finally, samples treated with
486 IPL presented lower microbial growth rate than control, maintaining better
487 microbiological quality until the end of storage. This work is a contribution to knowledge
488 of IPL treatments performance related with its efficiency to inactivate microorganisms

489 (native or contaminants) and its impact on quality attributes of a leafy vegetable.
490 Further studies will be aimed at addressing issues limiting the efficiency of IPL
491 treatments, including the need for reducing shadow effects during the treatment, which
492 are on the most important practical limitations of this technology. As well, possible
493 synergies between IPL and other techniques applied for the decontamination of fresh-
494 cut produce need to be explored.

495

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503

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