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1 **Effect of plasma activated water (PAW) on rocket leaves decontamination and** 2 **nutritional value**

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18

19

20 **Abstract**

21 Plasma Activated Water (PAW) obtained by exposing water to cold atmospheric pressure plasma,
22 has recently emerged as a promising alternative for food decontamination, compared to the use of
23 traditional chemical sanitizers. The aim of the study was to evaluate the efficacy of PAW treatments
24 for rocket salad decontamination. Washing with PAW for 2, 5, 10 and 20 min was assessed against
25 different endogenous spoilage microorganisms and compared to **untreated water and** hypochlorite
26 solution. The chemical composition of PAW as a function of treatment and delay time was
27 characterized and the effect on product quality and nutritional parameters was evaluated.

28 Results showed that PAW allowed an average reduction of **1.7-3 Log** CFU/g for total mesophilic and
29 psychrotrophic bacteria and *Enterobacteriaceae* following 2-5 min washing with minimal variation
30 of qualitative and nutritional parameters. Overall, experimental results highlighted the potentiality of
31 PAW treatments as a promising alternative to chlorine having the advantage of a minor adverse
32 impact on environment and consumers' health.

33

34 **Industrial relevance**

35 To meet consumers demand, the minimally processed fruit and vegetable industry needs to find
36 sustainable solutions as alternative to the use of traditional chemical sanitizers that allow to increase
37 product shelf-life and preserve safety, qualitative and nutritional characteristics.

38 Plasma activated water represents a promising strategy for food decontamination, but its effects on
39 foods have been only limitedly investigated. The present research is the first study on the use of
40 plasma activated water on fresh rocket leaves, providing new and important information on microbial
41 inactivation and quality of the fresh cut product.

42

43 **Keywords:** emerging technologies; plasma activated water; microbial decontamination; food quality;
44 food spoilage

45

46 **1. Introduction**

47 The market of fresh and minimally processed vegetables has rapidly grown in the last decades as
48 these products are very attractive to consumers looking for healthy and convenient meals. However,
49 they are characterized by a very short shelf-life that limit their market opportunity and that leads to
50 high amount of wasted product. The main limiting factor for these products shelf-life is surely
51 microbial spoilage. Mesophilic aerobic bacteria, coliforms, *Pseudomonas* spp., *Bacillus* spp., lactic
52 acid bacteria, as well as yeast and moulds are considered to be the main microorganisms found in
53 fresh and ready-to-eat salads with loads up to 10^7 Log CFU/g. Decontamination of fresh products
54 from pathogenic and spoilage microorganisms is usually performed through washing with chemical
55 sanitizers, being sodium hypochlorite the most commonly used one. However, there are several
56 concerns related to human health and environmental pollution that have led to the search for
57 alternative methods. Moreover, wide variability in disinfection efficacy has been reported in
58 numerous studies due to several factors, e.g. microbial protection by internalization within the
59 vegetable tissue or biofilm formation and depletion of free-chlorine during washing (Yoon & Lee,
60 2018).

61 On one side, microorganisms are becoming more resistant to existent traditional technologies, and
62 they are able to survive under various stress conditions, resulting in potential risk for human health
63 (Zhao, Patange, Sun, & Tiwari, 2020). On the other side, the necessity to preserve the fresh-like
64 characteristics and the content of bioactive compounds requires a mild technology in the
65 decontamination process. Hence, the combination of effective sanitizing measures, while preserving
66 quality and nutritional features, is the main issues for producers to improve the fresh products' shelf-
67 life. Moreover, the environmental sustainability of the overall process has to be taken into account.

68 The use of cold atmospheric pressure plasma (CAP) represents a novel approach for sanitation of
69 food products that involves the application of an ionised gas mixture composed by charged particles,
70 electric fields, ultraviolet (UV) photons, and reactive species exploiting its strong oxidative power.
71 Previous research has demonstrated that CAP can efficiently inactivate a wide range of
72 microorganisms, including bacteria, fungi, viruses, bacterial spores and biofilms (Ikawa, Tani,
73 Nakashima, & Kitano, 2016; Kolb et al., 2008; Machala et al., 2013; Pan et al., 2013). Reactive
74 oxygen and nitrogen species (RONS) are the major bactericidal agents, which cause damage to DNA
75 and proteins in microbial cells (Joshi et al., 2011; Laurita, Barbieri, Gherardi, Colombo, & Lukes,
76 2015).

77 Plasma-activated water (PAW) is generated by exposing water to a plasma discharge above or
78 underneath water surface. The plasma-generated reactive species interact with water molecules and
79 trigger several chemical reactions, which produce a unique mixture of biochemical reactive
80 chemistries. In recent years, PAW has also been confirmed to possess outstanding biological activity
81 in biomedical and agricultural sectors (Kaushik et al., 2018; Sajib et al., 2020).

82 PAW can represent a promising alternative to washing in chlorinated water for fresh fruit and
83 vegetables, since it does not leave any residual chemicals in the products and it is considered more
84 environmentally friendly compared with some traditional chemical sanitizers (Zhao et al., 2020).

85 **There are few studies (Baier et al., 2013; Giannoglou et al., 2020; Hertrich, Boyd, Sites, & Niemira,**
86 **2017; Silvetti et al., 2021) that report the efficacy of cold plasma on the microbial inactivation of**
87 **fresh cut salads, assessing endogenous microbiota or inoculated pathogens load reduction. However,**
88 **in all of these studies plasma in the gaseous form was employed, while, to our knowledge, no reports**
89 **are available on the use of PAW.**

90 **On the contrary,** several authors have reported on the efficiency of PAW in inactivating natural
91 microbiota and pathogens deliberately contaminated on **other types of** fresh fruits and vegetables,
92 such as strawberries, button mushrooms, Chinese bayberries, and fresh-cut apples (Liu et al., 2020;
93 Ma et al., 2016; Ma et al., 2015; Xu, Tian, Ma, Liu, & Zhang, 2016), but no study on the effect of
94 PAW on background spoilage microbiota of rocket leaves is available in literature. Moreover, the
95 effect on microbial load has been investigated by many authors, while the effect on products quality
96 and nutritional value is often scarcely considered.

97 Fresh fruit and vegetables are rich in bioactive compounds that exert a pronounced effect on human
98 health, due to their antioxidant properties. Hence, the preservation of these compounds is fundamental
99 for product quality. A previous research showed that exposure to RONS produced by CAP treatment
100 reactive species could lead to the oxidation of some phenolic compounds in leafy vegetables such as
101 lams lettuce, but mainly on model systems, while the matrix protected these compounds

102 (Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011). Nevertheless, no differences in the
103 antioxidant activity of fresh cut apples (Liu et al., 2020), pears (Chen et al., 2019) and button
104 mushrooms (Xu et al., 2016) were observed after washing with PAW.

105 The aim of the present research was to investigate the decontamination of endogenous spoilage
106 microbiota of rocket leaves induced by PAW washing and compare it to the effect of hypochlorite
107 solution washing, used as traditional reference method. The effect on product quality was evaluated
108 by measuring pH, color parameters and some bioactive compounds content.

109

110 **2. Materials and Methods**

111

112 **2.1 Raw materials**

113 Fresh rocket (*Rucola sativa*) leaves were purchased at the local market in Cesena, Italy and stored in
114 a refrigerated cell at 2 ± 1 °C for maximum 24 h before processing.

115

116 **2.2 Corona discharge for the production of PAW: electrical analysis and chemical** 117 **characterization of RONS in PAW.**

118 PAW was produced treating distilled water with a corona discharge, as reported in Laurita et al.
119 (2021). Briefly, the plasma source consists of a stainless-steel pin-electrode connected to a **high**
120 **voltage** microsecond pulsed generator (AlmaPulse, AlmaPlasma s.r.l.) at a distance of 5 mm from the
121 liquid surface. A volume of 450 ml of distilled water, in a borosilicate Erlenmeyer flask on a stirrer
122 (IKA Magnetic Stirrers RCT basic), was grounded. **The CAP is generated in the air gap between the**
123 **tip of the high voltage electrode and the liquid surface.** The generator was operated at a peak voltage
124 of 9 kV, with a fixed frequency of 5 kHz. The voltage (V) and the current (i) were measured using a
125 high voltage probe (Tektronix P6015A) and a current probe (Pearson 6585) connected to a digital
126 oscilloscope (Tektronix DPO4034, 350 MHz, 2.5 GSa s⁻¹). The average power (P) dissipated in the
127 discharge was determined applying the following formula:

$$128 \quad P = \frac{1}{T} \int_0^T i(t) V(t) dt, \quad (1)$$

129 where T is the applied voltage period.

130

131 The concentrations of H₂O₂, NO₂⁻ and dissolved O₃ produced in PAW by the CAP treatment were
132 measured. The Amplex® Red Hydrogen Peroxide Assay Kit (Thermo Fisher Scientific, Waltham,
133 MA, USA) and the Nitrate/Nitrite colorimetric assay (ROCHE, Baesel, Switzerland) were used for
134 the measurement of H₂O₂ and NO₂⁻, respectively. The analysis of their concentrations was performed
135 photometrically according to the manufacturer's protocols using a microplate reader (Rayto, P.R.

136 China). The O₃ concentration produced in the liquid phase was evaluated according to the
137 manufacturer's protocols using Chematest 20 (SWAN Analytics). Moreover, the pH and the
138 conductivity were evaluated by the means of inoLab® pH 7110 and Oakton Instrument: Con 6+
139 Meter, respectively. The RONS concentrations were assessed for four different treatment times (1, 2,
140 3 and 4 min) and four different delay times (2, 5, 10 and 20 min). All measurements were performed
141 at least 3 times.

142

143 **2.3 PAW treatment**

144 PAW was produced exposing distilled water **the aforementioned corona discharge for 4 min.**
145 Immediately after the treatment, rocket samples were immersed in PAW for 2, 5, 10 and 20 min in
146 the ratio product:liquid of 1:20 (w:v) at room temperature. **20 g of rocket leaves were treated each**
147 **time.** During immersion, samples were constantly agitated in an orbital agitator.

148 After dipping, rocket leaves were removed from PAW and blotted with adsorbent paper to remove
149 excess liquid. For each treatment time, two independent treatments were carried out.

150 Untreated rocket leaves were considered as control. Moreover, in order to evaluate the effect of PAW
151 treatment, **washing in untreated water was tested and** a dipping in sodium hypochlorite (NaClO) 100
152 ppm for 2, 5, 10 and 20 min was used as common reference washing procedure.

153 For analysis of bioactive compounds, after each treatment time, rocket samples from both washing
154 replicates were immediately freeze dried after treatment, the obtained samples were stored at -20°C
155 until analysis.

156

157 **2.4 Analytical determinations**

158

159 **2.4.1 Microbiological analysis**

160 Immediately after each washing treatment, rocket samples were aseptically transferred to sterile saline
161 solution (0.9 %, w/v, 1:10 w/v) and homogenized in a Stomacher 400 apparatus (Seward Ltd.,
162 Worthing, West Sussex, UK) for 2 min. Ten-fold serial dilutions of the homogenates were then
163 performed and inoculated on suitable media for counting viable cells of various microbial groups.
164 Total aerobic mesophilic and psychrotrophic microorganisms were evaluated onto Standard Plate
165 Count Agar (Oxoid, Basingstoke, UK) plates incubated at 30 °C for 48 h and at 4 °C for 10 d,
166 respectively. *Enterobacteriaceae* were counted on Violet Red Bile Glucose agar (Oxoid, Basingstoke,
167 UK) incubated at 37 °C for 24 h. For each washing replicate (n=2), microbiological analyses were
168 carried out at least in duplicate and the data were expressed as the mean of log colony forming units
169 (CFU) per gram ±standard deviation.

170

171 **2.4.2 Physico-chemical parameters**

172 pH was measured in triplicate with a pH-meter (Crison, Barcellona, Spain) on the juice obtained by
173 10 g of treated or untreated sample. Water content was determined in triplicate by gravimetric method,
174 drying samples in an oven at 70°C until constant weight was achieved. Results were expressed as
175 g/100g.

176 Image analysis was used to determine samples color. Digital images were acquired placing the
177 samples inside a black box and illuminated using four parallel lamps (mod. TL-D deluxe, natural
178 daylight, 18W/965, Philips, NY, USA) with a colour temperature of 6500 K. The Colour Digital
179 Camera (mod. D7000, Nikon, Shinjuku, Japan) used was equipped with a 60 mm lens (mod. AF-S
180 micro, Nikkor) and located vertically over the sample at a fixed distance of 23 cm and at an angle
181 with the lightning source of approximately 45°. The pre-processing of RGB images, segmentation
182 and colour quantification were performed with ImageJ analysis software (NIH, USA). The average
183 value of the segmented pixels was registered as L, R, G and B values. For each sample, images of 10
184 leaves were acquired and analysed.

185

186 **2.4.3 Nutritional quality**

187 Total Phenolic Content (TPC) was determined spectrophotometrically according to Mustafa et al.
188 (2016) method with some modifications. Briefly, 0.5 mL of extracts solution (100 mg freeze dried
189 sample extracted with 10 mL ethanol 70% with Formic acid (1.5%, v/v) was introduced into test
190 tubes, then 2.5 mL of Folin-Ciocalteu reagent solution and 7 mL of Na₂CO₃ (7.5% w/w in water)
191 solution were added. The reaction mixture allowed to stand at room temperature in the dark for 2 h
192 and absorption was measured at 765 nm. The TPC was expressed as mg of gallic acid equivalents
193 (GAE) per 100 g of dry weight of the rocket samples using gallic acid calibration curve.

194 Total Flavonoid Content (TFC) was determined following a method described by (Chen, Chen, Xiao,
195 & Fu, 2018) with slight variations. Briefly, 0.5 mL of extract solution (10 mg/mL), 0.15 mL of NaNO₂
196 (0.5 M), 3.2 mL of methanol (30% v/v) and 0.15 mL of AlCl₃·6H₂O (0.3 M) were mixed. After 5
197 min, 1 mL of NaOH (1 M) was added. The solution was mixed well, and the absorbance was measured
198 against the blank reagent at 506 nm. The standard calibration curve for TFC was made using quercetin
199 standard solution (0 to 100 mg/L) under the same procedure as described above. TFC was expressed
200 as mg of quercetin equivalents (QE) per 100g of dried extract.

201 Analytical determinations of TPC and TFC were carried out in triplicate for each freeze-dried sample.

202

203 **2.5 Statistical analysis**

204 To investigate statistical significance of observed differences in microbial, qualitative and nutritional
205 data, a one-way analysis of variance (ANOVA) followed by the Tukey's HSD test (multiple range
206 test) was used ($p < 0.05$). Analysis was conducted using Microsoft Excel 365 and Minitab ver. 19.0.

207

208 **3. Results and Discussion**

209

210 **3.1 Electrical analysis and chemical characterization of RONS in PAW**

211

212 **Figure 1** shows current and voltage waveforms for the corona discharge operated at a peak voltage
213 of 9 kV and a frequency of 5 kHz, referring to two applied voltage periods representative of the
214 discharge electrical behavior during the treatment of the distilled water. Each applied voltage period
215 (200 μ s) exhibited four voltage peaks, whose intensities reached maximum values up to 9.24 kV. The
216 average discharge dissipated power, which can be calculated directly from the measured current and
217 voltage reported in **Figure 1**, is 480.95 ± 33.42 W.

218 pH and conductivity of PAW as a function of treatment time are shown in **Figure 2**. As previously
219 reported, the plasma treatment induced a reduction of pH (Laurita et al., 2015) up to 3.3 (dotted black
220 line) after 4 min of plasma treatment, and an increase of conductivity (dotted purple line) up to 200
221 mS/cm.

222 **Figures 3** and **4** report the behaviors of the concentration of the investigated RONS as a function of
223 the treatment time and of the delay time. H_2O_2 , NO_2^- and O_3 concentrations increased with the
224 increasing of the treatment times up to 4.5 ± 0.1 mg/L, 30.4 ± 0.9 mg/L and 0.3 ± 0.1 mg/L
225 respectively. Focusing on the post-discharge kinetic of reactive species reported in **Figure 3b**, the
226 decrease in concentration of both H_2O_2 and NO_2^- is related to the post-discharge processes in acid
227 environment mediated by peroxyxynitrite (ONOOH) (Laurita et al., 2015; Lukes, Dolezalova, Sisrova,
228 & Clupek, 2014). Finally, it is worth to note that the O_3 decay reported in **Figure 4b** is in accordance
229 with the half-life values reported by Gardoni, Vailati, & Canziani (2012).

230

231 **3.2 Effect of PAW on microbial load**

232

233 The effect of PAW on the microbial loads of the target microbial groups of the rocket leaves was
234 evaluated after washing for 2, 5, 10, and 20 min and compared to **untreated water and** hypochlorite
235 solution (**Figure 5**).

236 Initial contamination levels of the untreated samples were about 5.74 ± 0.49 , 6.17 ± 0.70 and $3.67 \pm$
237 0.49 Log CFU/g for total mesophilic and psychrotrophic bacteria and *Enterobacteriaceae*,

238 respectively. These data are consistent with literature reporting rather high contamination levels for
239 rocket leaves with counts for the aerobic mesophilic and psychrotrophic microorganisms up to 7.5
240 Log CFU/g, yeasts and moulds about 5.8 Log CFU/g, and the range for *Enterobacteriaceae* 4.3–5.9
241 Log CFU/g (Abadias, M., Usall, Anguera, Solsona, & Viñas, 2008).

242 The results presented in **Figure 5** clearly show that untreated water cannot be used to effectively
243 decrease surface contamination of fresh rocket leaves since mean reductions for the target spoilage
244 bacteria did not exceed 0.6 Log units regardless washing time. By contrast, all the considered
245 microbial populations were significantly ($p < 0.05$) affected by PAW treatments depending on
246 washing time. Psychrotrophic bacterial population was the most sensible one and it was reduced by
247 2 Log CFU/g after a 2-min washing. By extending treatments up to 5 min, the decontaminating effect
248 of PAW was enhanced resulting in a 3 Log CFU/g reduction. However, no further significant
249 improvement was observed following the longest treatments. On the other hand, a 5-min washing
250 was effective in decreasing counts of mesophilic bacteria and *Enterobacteriaceae* by 1.7-2.5 Log
251 CFU/g. Similarly to the psychrotrophic population, PAW decontamination activity against
252 mesophiles and enterobacteria did not significantly increase by prolonging the treatment time.

253 Overall, PAW had a stronger effect on the spoilage-related microorganisms with respect to the
254 reference method (NaClO, 100 ppm) indicating that it can be an alternative to conventional washing
255 with a chlorine-based sanitiser. More precisely, no significant influence on the presence of total
256 mesophilic bacteria was detected over 20 min treatment with NaClO. Moreover, longer treatments
257 were necessary with NaClO than PAW to have significant differences compared to untreated samples
258 for enterobacteria and total psychrophilic population. In particular, this was observed only after 10
259 min with maximum reductions of about 1.4-1.7 Log CFU/g. However, it was less effective than PAW
260 since resulted in lower population decreases for both microbial groups which did not further improve
261 by lengthening the treatment to 20 min.

262 It is not surprising that the chlorine treatments achieved rather limited reductions and its use for
263 sanitizing fresh produce is controversial due to several issues which limit its effectiveness (Yoon &
264 Lee, 2018). Besides the possibility for indigenous microbiota to internalize within tissues of fresh
265 produce and to produce biofilm, depletion of free chlorine due to the presence of organic matters is
266 well known to affect the disinfection capacity of the treatment, thus making processing time little
267 relevant. On the other hand, the bactericidal action of PAW relies on the RONS content and its low
268 pH. An important feature of PAW used in the present study is that only limited changes occurred in
269 the content of nitrite anion by increasing washing time being its content about 25 ppm even after 20
270 min (**Figure 3**).

271 Several studies showed that PAW can efficiently inactivate a wide variety of microorganisms from
272 different fresh fruit and vegetables, but no study on rocket has been performed yet. It should however
273 be considered that effectiveness of washing with PAW depends on several aspects related to the mode
274 and conditions of PAW generation, which affect its chemico-physical properties, as well as on the
275 different sizes, shapes, surface-to-volume ratio, and initial microbial contamination level of various
276 fruits and vegetables. Despite differences in these factors, our data are in agreement with previous
277 studies. Choi et al. (2019) reported reductions of 2.0, 2.2, 1.8, and 0.9 Log CFU/g in mesophilic
278 aerobic bacteria, lactic acid bacteria, yeast and moulds, and coliforms, respectively of shredded salted
279 Chinese cabbages. While such values are consistent with those obtained in this study, it is important
280 to point out that the PAW they used was generated following a 120 min discharge resulting in NO_2^-
281 and NO_3^- concentrations up to 88 and 394 ppm, respectively, while H_2O_2 was negligible. Xiang et al.
282 (2019) showed significant reductions in populations of total aerobic bacteria and yeasts and moulds
283 (2.32- and 2.84-Log CFU/g, respectively) of mung bean sprouts after washing for 30 min with PAW
284 produced by 30 sec (APPJ; 5 kV, 40 kHz) and characterized for a content in H_2O_2 , NO_3^- and NO_2^-
285 of about 17, 571 and 531 $\mu\text{mol/L}$, respectively. Reductions up to 1.7 Log CFU/ g for mung bean seeds
286 inoculated with *Escherichia coli* O154 were achieved by Machado-Moreira, Tiwari, Richards,
287 Abram, & Burgess (2021) but only after 3h of treatment with PAW. A shorter washing time of 5 min,
288 which is consistent with our study, was demonstrated to have a significant effect in decreasing initial
289 counts of aerobic bacteria and mould and yeast of fresh-cut 'Fuji' apples (Liu et al., 2020). Not only
290 PAW treatment reduced by approximately 1-2 Log CFU/g the indigenous spoilage microbiota, but
291 also limited their proliferation over refrigerated storage while reducing superficial browning and
292 maintaining quality features thus confirming its efficacy in prolonging the product shelf-life.
293 Compared to Liu et al. (2020), our results indicate that PAW can be a suitable treatment to reduce
294 bacterial population in rocket salad being active even after the shortest treatment. To successfully
295 extend the produce shelf-life it would be however important to test also the fate of the surviving cells
296 during storage to check whether bacteria were lethally or sub-lethally damaged by PAW and hence
297 could recover or develop stress resistance responses.

298

299 **3.3. Effect on quality and nutritional parameters**

300

301 Water content and pH of rocket leaves did not show any significant variation during washing in PAW
302 compared to the untreated sample (data not reported). Although the pH of PAW is significantly lower
303 compared to rocket leaves one (6.17 ± 0.05), washing up to 20 min did not promote any change, in
304 agreement with Xu et al. (2016).

305 **Table 1** reports the colorimetric parameters measured by the image analysis. A significant decrease
306 was observed for the parameters of L (luminosity), R (red components) and G (green parameter) in
307 all samples, but variations do not appear to be proportional to treatment time.

308 According to literature, the effect of plasma exposure on colour of fresh produce is generally quite
309 minimal but depends on the specific matrix (Baier, Ehlbeck, Knorr, Herppich, & Schlüter, 2015). In
310 a previous study, Ramazzina et al. (2015) observed an immediate reduction of chlorophyll content in
311 kiwifruit subjected to gaseous plasma treatment that was attributed to the Type II chlorophylls
312 degradation induced by reactive species. However, the author also observed a better retaining of
313 colour during the following storage, that was probably due to degradative enzymes inactivation.

314 Changes in colour of fresh products could be due to pigment degradation but also to changes in the
315 tissue microstructure. However, to our knowledge there are no reports on the use of PAW on rocket
316 leaves, so the observed effect on colour should be further clarified. Moreover, it is important to
317 determine if these changes are perceptible by consumers and how they evolve during the storage of
318 the product.

319 Consumption of bioactive compounds, especially polyphenols, plays a central role in the protection
320 of human organs against inflammatory and neurodegenerative disorders and oxidative stress.
321 However, the preservation of natural bioactive compounds in food products is a major challenge for
322 the food industry. In this sense, cold plasma has been proposed as one of the most promising solutions
323 to maximize the retention of polyphenol in food products.

324 **Table 2** shows the time dependent changes in the total phenolic content (TPC) and total flavonoid
325 content (TFC) of PAW treated rocket samples. According to the obtained results, all PAW treated
326 samples showed non-significant differences in the TPC ($p > 0.05$) compared to the control samples,
327 except for the 5 min treatment which induced a slight reduction. These results are in good accordance
328 with the previous findings reported by Xiang et al. (2019) in which the PAW treatment did not induce
329 any significant change in the total phenolic content of mung bean sprouts.

330 A significant variation in total flavonoid content was observed for PAW treated rocket samples with
331 higher values for 10 and 20 min (3098.85 ± 53.20 and 3046.88 ± 38.58 mg gallic acid/ 100g,
332 respectively) compared to the control samples (2524.88 ± 39.53 mg quercetin/ 100g).

333 The increase in the content of some specific phenolic compounds was observed after a short
334 exposition to plasma in apples (Tappi et al., 2018) and blueberries (Sarangapani, O'Toole, Cullen, &
335 Bourke, 2017) and after PAW treatment on mung bean sprouts (Fan, Liu, Ma, & Xiang, 2020). This
336 effect was attributed to a physiological response of the tissue to the stress promoted by the reactive
337 species. However, the increase of exposure time generally promoted oxidative reactions and the
338 progressive decrease in the phenolic content and antioxidant activity. In the present research, TPC

339 was not affected even at the longer treatment time, while flavonoids accumulation occurred. A deeper
340 analysis of the single phenolic compounds could help to clarify the response of this specific matrix
341 to the exposure to plasma reactive species.

342

343 **4. Conclusions**

344

345 In summary, this study demonstrates PAW has the potential to decontaminate rocket salad with
346 limited changes in quality parameters. Compared to hypochlorite, which was chosen as a reference
347 sanitizer due to its widespread use in the food industry, PAW treatments resulted to be more effective
348 against the target background microbiota. In fact, shorter treatments were necessary to significantly
349 reduce *Enterobacteriaceae* and **psychrotrophic** bacterial populations, and greater inactivations were
350 achieved for **all the spoilage microbial groups** after 2 min.

351 However, the observed slight changes in color and bioactive compounds content should be further
352 clarified. Moreover, the behavior of the surviving microbiota and quality parameters should be
353 evaluated during storage of the packed product in order to determine the effect of PAW on the shelf-
354 life in real distribution and storage conditions.

355

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361

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472

473 **Figure captions**

474

475 **Figure 1.** Voltage and current waveforms during distilled water treatment

476 **Figure 2.** pH (black) and conductivity (purple) values of not treated solution (NT) and 1, 2, 3 and 4
477 min of plasma treatment.

478 **Figure 3.** (a) Chemical analysis of the concentrations of H_2O_2 and NO_2^- after 1, 2, 3 and 4 min of
479 plasma treatment and (b) their decay kinetic at room temperature.

480 **Figure 4.** (a) Chemical analysis of the concentrations of O_3 after 1, 2, 3 and 4 min of plasma treatment
481 and (b) its decay kinetic at room temperature.

482 **Figure 5.** Mean counts of (a) total aerobic mesophilic and (b) psychrotrophic bacteria, and (c)
483 *Enterobacteriaceae* on rocket leaves collected before and after the washing with PAW, **untreated**
484 **water** or **sodium hypochlorite (NaClO, 100 ppm)** at different times. Mean values with different letters
485 significantly differ among treatment times and washing solutions ($p < 0.05$).

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