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1 **Browning response of fresh-cut apples of different *cultivars* to cold gas plasma treatment**

2

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14

15 **Abstract**

16 The present work aims to study the effects of cold gas plasma on some quality parameters of apple  
17 slices belonging to four different cultivars (Pink Lady®, Fuji, Red Delicious and Modì®), with  
18 particular attention to polyphenoloxidase (PPO) inhibition and related changes in colour and visual  
19 quality.

20 Upon plasma exposure a noticeable reduction of superficial browning was observed in all cultivars  
21 but not always proportionally to treatment time; the effect on PPO activity was very variable and not  
22 correlated to the effect on enzymatic browning. Textural parameters were affected by plasma  
23 treatments only in Red Delicious apples. Generally, the response of the tissue to the treatments was  
24 variable according to the cultivar considered. The results obtained in this study indicate the necessity

25 of further investigation about consequences of plasma treatment on specific tissue physiology in order  
26 to choose the better treatment parameters, optimizing its effect for the specific final product.

### 27 **Industrial relevance**

28 The application on cold plasma to minimally processed fruit and vegetable has shown a good potential  
29 for enzymatic browning inhibition making it an interesting alternative to traditional dipping methods.  
30 Nevertheless, the effect on the tissue to the exposure to plasma active particles is not fully known yet.  
31 For the industrial application of the treatment, the response for example of different cultivars to the  
32 treatment is of high importance, in the first place for the selection of the more appropriate raw material  
33 but also to eventually adapt the process parameters to the specificity of the matrix.

34

### 35 **Keywords**

36 Fresh-cut apples; Pink Lady®; Fuji; Modì®; Red Delicious; cold plasma; enzymatic browning.

37

### 38 **1. Introduction**

39 Processing operations for fresh-cut fruit production trigger a complex physiological process in the  
40 tissues that involves various physico-chemical and biochemical modifications that affect quality  
41 maintenance of the final product during storage. In particular, peeling and cutting operations promote  
42 tissue disruption and the contact between enzymes and their substrates, triggering a number of  
43 reactions that lead to an immediate increase in the respiration rate and endogenous metabolic activity  
44 (Soliva-Fortuny & Martin-Belloso, 2003). As a consequence, a general deterioration of the product  
45 quality characteristics occurs, among which visual quality (especially colour) and texture (tissue  
46 softening), very appreciated aspects by the consumer (Toivonen & Brummell, 2008).

47 The traditional techniques aimed at the enzymes inhibition are based on immersion (dipping) in  
48 solutions containing organic acids in combination with calcium salts, carboxylic acids, thiols-  
49 containing compounds and phenolic acids that are able to reduce pH and/or act as antioxidants (Oms-

50 Oliu et al., 2010), coupled to modified atmosphere packaging (MAP) that reduces O<sub>2</sub> availability for  
51 oxidation reactions (Rocculi et al., 2004).

52 Recently, concerning fresh-cut apples, several innovative treatments have been tested with the aim of  
53 inhibiting enzymatic browning: in particular promising results have been obtained in relation to  
54 exposure to UV-C (200-280nm) on the account of its reaction with free radicals (Manzocco et al.,  
55 2011) and short contact with nitrous oxide (NO) supposedly for its radical scavenging activity  
56 (Pristijono et al., 2006).

57 Atmospheric gas plasma is an ionised gas characterized by active particles such as electrons, ions,  
58 free radicals and atoms that are located in both fundamental and excited states; species in the excited  
59 state emit a photon (especially UV photons) when they return to the ground state (Moreau et al.,  
60 2008). Various gas mixtures can be used to generate plasma discharges, leading to different  
61 composition in radicals and active particles. Reactive oxygen species (ROS), in particular ozone (O<sub>3</sub>),  
62 atomic oxygen (O), the hydroxyl radical (OH<sup>•</sup>) and nitrogen radical species (RNS) are the main  
63 components of plasma generated by ionization of atmospheric air as feed gas. The oxidative species  
64 produced during the ionisation can cause lipid peroxidation and oxidation of proteins and DNA  
65 (Montie et al., 2000).

66 Although the main biological applications of cold plasma treatment are undoubtedly in the medical  
67 field aimed at the microbial decontamination of expensive equipment and heat-sensitive materials  
68 (Fridman et al., 2008), cold plasma treatments have been also applied for the decontamination of food  
69 products, as an alternative to washing procedures with chemicals. Since the temperature of the  
70 product during the treatment is very close to the ambient, this technique can be suitable for the  
71 processing of temperature sensitive products such as fresh cut fruit and vegetables. Moreover, the  
72 potential direct application on packed products seems promising (Misra et al., 2014a; Misra et al.,  
73 2014b). The cold plasma antimicrobial efficacy has been tested on various food products and on  
74 different microbial species (Shama & Kong, 2012).

75 Recently, the potential of plasma treatment for food products has been investigated also with the aim  
76 to inhibit enzymatic activity (Misra et al., 2016) provided a review of the current knowledge about  
77 the interaction between plasma species and enzyme functionality summarising the related research  
78 findings present in the literature. The accepted mechanism for the observed loss of enzymatic  
79 functionality upon plasma exposure is **an oxidation of the side-chain amino-acids that cause an**  
80 alteration of the secondary structures of protein operated by the reactive species. This could be  
81 particularly interesting for the inactivation of enzymes responsible for quality degradation in fresh  
82 vegetable products, such as of peroxidase (POD), polyphenoloxidase (PPO) and pectimethylesterase  
83 (PME). This issue has been addressed by some researches on whole apples (Gozzi et al., 2013),  
84 strawberries (Misra et al., 2014**b**) fresh-cut apples (Tappi et al., 2014), melon (Tappi et al., 2016),  
85 kiwifruit (Ramazzina et al., 2015) and fresh-cut potatoes (Bußler et al., 2016). Nevertheless, **while**  
86 **the mechanism has been clarified in model systems, further investigation is needed in real food**  
87 **systems.**

88 Different *cultivars* present diverse PPO enzymes and substrate (polyphenols) concentration and the  
89 amount is strictly bound to the physiological state of the tissue (e.g. ripening degree) (Trejogonzales  
90 et al., 1991).

91 **In a previous research (Tappi et al., 2014), we showed how plasma treatment reduces enzymatic**  
92 **browning in Pink Lady apples. Nevertheless, for the industrial applicability of this technology, the**  
93 ***cultivar* is a very important variable to consider in order to optimize cold gas plasma treatment for**  
94 **fresh-cut apple stabilization.**

95 Given this, the present work aims to study the effects of the non-thermal atmospheric plasma on some  
96 quality parameters of apple slices belonging to four different cultivars (Pink Lady®, Fuji, Red  
97 Delicious and Modì®), with particular attention to polyphenoloxidase (PPO) inhibition and related  
98 changes in colour and visual quality, being enzymatic browning the most important phenomenon  
99 limiting the shelf life of this type of product.

100

101 **2. Materials and methods**

102

103 **2.1. Raw material**

104 Fruits of four apple cultivars (Pink Lady®, Fuji, Modi® and Red Delicious) were harvested at  
105 commercial maturity in November 2012, from Apofruit Italia Soc. Coop. Agricola fields sited in  
106 Emilia Romagna region (Italy). Fruits of homogeneous size were stored at  $2 \pm 0.5$  °C and about 100%  
107 relative humidity (RH), in plastic boxes, for two weeks. After this period, fruits free from defects  
108 were moved in a refrigerated chamber at 4 °C in the dark for one week. At the time of the experiments,  
109 fruits were characterized for soluble solid content, titratable acidity, dry matter and porosity, colour,  
110 PPO activity and texture (Table 1).

111

112 **2.2. Gas plasma generator**

113 The gas plasma generator was a double barrier discharge type consisting of three parallel pairs of  
114 brass electrodes (supplied by a DC power supply consuming 150W powered by high voltage  
115 transformers) placed at the top of a hermetic chamber of 29 dm<sup>3</sup> internal volume. The dielectric  
116 material used was a 5 mm thick glass. Over the electrodes three fans were directing the discharge  
117 towards the samples, placed at a distance of 9 cm. The measured air speed was 1.5 m/s at the  
118 electrodes and 0.8 m/s at the apple surface. The system has been electrically and chemically  
119 characterized in previous studies (Ragni et al., 2010). A schematic representation of the electrodes  
120 configuration is reported in Fig. 1. In the present experiment, the gas plasma generation has been  
121 obtained by atmospheric air. Its chemical characterization has been carried out in a previous work  
122 (Ragni et al., 2010).

123

124 **2.3. Gas plasma treatments and samples storage**

125 Apple samples (rectangular slices of 40 x 10 x 10 mm) of Pink Lady® (PL), Fuji (F), Modi® (M)  
126 and Red Delicious (RD) were prepared from the central part of the fruit mesocarp. From each sample,

127 prepared using about eight fruits, two sub-samples **were** taken, one used as control, and the other one  
128 subjected to the plasma treatment.

129 The treatment times, selected on the basis of preliminary experiments and of previous researches  
130 (Tappi et al., 2014), were 30 and 60 min (respectively 15+15 and 30+30 min for each major slice  
131 side).

132 Considering this factor, gas plasma treatments were conducted at a RH of 60 % (22 °C). This  
133 condition was selected on the basis of preliminary experiments, considering that the emission of OH  
134 radicals can be increased by increasing the air humidity (Ragni et al., 2010) but an excess of water  
135 vapour (> 80 %) can decrease the gas plasma effectiveness (Muranyi et al., 2008). During the  
136 treatments, each control sample was stored for the same time and at the same temperature (22 °C)  
137 and RH (60 %) of the tested treatment. Each treatment **was** performed in triplicate.

138 After treatment, control and treated samples were stored in a climatic chamber in controlled  
139 atmospheric conditions (4 °C and 95 % RH). For browning assessment, samples were analysed every  
140 15 min up to **4 h**. For texture analysis samples were analysed after 0, 24 and 48 h.

141 Sampling intervals were chosen after preliminary experiments aimed at evaluating the kinetics of  
142 variation of each considered parameter.

143

## 144 **2.4. Qualitative assessment of fresh-cut apples**

145

### 146 **2.4.1. Chemical parameters**

147 Soluble solid content (SSC) was determined by assessing the refractive index of the juice obtained  
148 from apple slices after filtering through Whatman #1 filter paper with a digital refractometer mod.  
149 PR1 (Atago Co.Ltd, Tokyo, Japan) at 20 °C calibrated with distilled water.

150 Titratable acidity (TA) was measured by titration with NaOH 0.1 N until pH 8.1 was reached (AOAC  
151 Official Method 942.15, 2000), and expressed as mg of malic **acid/kg on a fresh weight basis**.

152 For each sample, SSC and TA were determined in triplicate on the juice obtained by nine apple slices,

153 taken from the three replicated treatments.

154 Dry matter content of apple samples was determined gravimetrically by difference in weight on about  
155 5 g of finely chopped apples exactly weighted before and after drying at 70 °C, until a constant weight  
156 was achieved (AOAC International, 2002).

157

#### 158 **2.4.2. Porosity**

159 The apparent density ( $\rho_a$ ) of apple was determined by volume displacement in a pycnometer using  
160 appropriate aqueous isotonic sucrose solutions as reference liquid (Gras et al., 2003). The real solid-  
161 liquid density ( $\rho_r$ ) was also obtained by volume displacement using sample purees obtained by  
162 manually grinding the samples using a mortar and pestle. The purees were placed in a Büchner flask  
163 and degasified for 10 min by creating vacuum in the flask. The total porosity of the sample ( $\varepsilon$ ) is the  
164 dimensionless ratio of air volume to total volume, and varies between 0 and 1 (Eq. 1) (Lozano et al.,  
165 1980):

166

$$167 \quad \varepsilon = 1 - (\rho_a / \rho_r) \quad (1)$$

168

#### 169 **2.4.3. Colour**

170 A spectrophotometer (Colorflex, Hunterlab) was used to measure surface colour of apple slices  
171 (D65 illuminant and 10° standard observer). For each piece, measurements were performed on each  
172 side. The L\*, a\* and b\* parameters of the CIELAB scale were measured, Hue angle ( $h^\circ =$   
173  $\arctan[b^*/a^*]$ ) values were also calculated (CIE, 1987). Results were expressed as average of 10  
174 measurements for sample.

175

#### 176 **2.4.4. Visual quality by computer vision system (CVS)**

177 Digitalized images of apple pieces were acquired by positioning the samples inside a black box under  
178 controlled lighting condition. A digital camera mod. D7000 (Nikon, Shinjuku, Japan) equipped with

179 a 60 mm lens mod. AF-S micro, Nikkor (Nikon, Shinjuku, Japan) was used to acquire the images.  
180 The CVS **was** calibrated with standard colour, according to Romani et al. (2009).  
181 For each treatment time, acquisitions (exposition time 1/2 s; F-stop f/16) were conducted on samples  
182 of 20 apple slices each (10 for the treatment and 10 for the control) immediately after the treatment  
183 and every 15 min up to 1 h, and then every 30 min up to 4 h of storage in controlled conditions (4 °C,  
184 95% RH), in order to understand the treatment effect on the browning kinetic.  
185 **The length of the assessment was based on preliminary tests aimed at evaluating the browning kinetics**  
186 **of the slices and on previous studies (Quevedo et al., 2016). The intervals were chosen as often as**  
187 **possible in order to properly follow the kinetic.**  
188 Digitalized images were evaluated with an advanced Image Analysis Software (Image Pro-Plus v.  
189 6.2, Media Cybernetics, USA) using RGB scale. Total and browned areas were selected and a colour  
190 model was set up according to Rocculi et al. (2004). Two different pixel ranges were identified on  
191 the basis of different chromatic characteristics, considered as ‘not browned’ and ‘browned’ area (**BA**).  
192 The model was then applied to each digitalized image, and by evaluating all pixels; the percentage of  
193 each chromatic area was calculated by the software.

194

#### 195 **2.4.5. Texture**

196 A Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, UK) equipped with a 50 N  
197 load cell and a 6-mm diameter stainless steel cylinder was used for conducting penetration tests using  
198 a compression test speed of 0.5 mm s<sup>-1</sup> and a maximum deformation of 90 %. For each treatment  
199 time 30 apple slices (15 controls and 15 treated) were analysed after 0, 24 and 48 h of storage in  
200 controlled conditions (4 °C and 95 % RH). From the analysis of the acquired curves, the following  
201 parameters were evaluated: Firmness F (N) as the first peak force value representing the limit of the  
202 flesh elasticity and the linear distance (LD) between F and the first 20 s (time required to attain a flesh  
203 deformation of about 85 %), calculated as follow (Stable Micro Systems, 2000):

204

205 
$$L_d = \sum_{x=1}^{x=n} \sqrt{[F(x+1) - F(x)]^2 + [D(x+1) - DS(x)]^2}$$
 (2)

206

207 where  $f$  is the force (N) and  $d$  is the distance (mm).

208

#### 209 **2.4.6. Polyphenol oxidase activity (PPO)**

210 Enzyme extraction was carried out according to Baritoux et al. (1991) with slight modifications.

211 Briefly, 50 g of sample were homogenised in 100 mL of cold McIlvaine's buffer solution at pH 7.5

212 containing 0.5 % Triton X100, 25 mM ascorbic acid and 0.5% PVPP, using an Ultra-Turrax blender

213 for 30 s. The homogenate was kept under agitation and in the dark at 0 °C for 15 min and then

214 centrifuged for 30 min at 4 °C and 25000 g. The supernatant was filtered and used as extract.

215 A solution containing 4-methylcatechol 50 mM prepared in McIlvaine's buffer solution at pH 7.5 was

216 used as a substrate for the assay. 200 µL of extract were added to 3.2 mL of substrate solution. The

217 determination of PPO activity was carried out immediately after plasma exposure by measuring the

218 variation of the absorbance of the mixture at 420 nm and 25 °C during 5 min compared to the initial

219 value with a spectrophotometer (UV-1601, Shimadzu) and calculated on the basis of the slope of the

220 linear portion of the curve ( $\Delta A/\text{min}$ ). One unit (U) is defined as the quantity of enzyme necessary to

221 obtain an increase in absorbance of 1U in 1 min under the assay conditions.

222 PPO residual activity (%) was calculated for each sample in relation to its control considered as 100

223 %.

224

#### 225 **2.5. Data analysis**

226 Significant differences ( $p < 0.05$ ) were evaluated by using the Analysis of Variance (ANOVA)

227 according to LSD post-hoc test. Differences were investigated among values relative to the raw

228 material of different cultivars, between control and treated samples for PPO activity and among values

229 of texture relative to control and treated samples at different storage times.

230 Image analysis data were modelled according to the power law or Weibull model that according as  
231 suggested by Quevedo et al (2016):

232

$$233 \quad \frac{BA_t}{BA_0} = e^{\alpha t^\beta} \quad (3)$$

234 Where  $BA_t$  is the browned area at time  $t$ ,  $BA_0$  is the browned area at time 0,  $t$  represents time (min),  
235  $\alpha$  is a rate parameter that defines an exponential growth or decline depending whether it's positive or  
236 negative,  $\beta$  is a shape factor related to the concavity. When  $\beta$  is 0 or 1, the equation describes a zero  
237 or first empirical order kinetic.

238 The coefficients of the equation with their relative standard errors were reported with the  
239 determination coefficient ( $R^2$ ) and the RSME of the model.

240 All statistical analyses were carried out using the software STATISTICA 8.0 (Statsoft Inc., Tulsa,  
241 UK).

242

### 243 3. Results

244

#### 245 3.1. Characterization of the raw materials

246 **Table 1** presents the initial physico-chemical characteristics of the four apple cultivars considered.  
247 Soluble solid content was in the range between 12.7 (Modì®) and 13.9 % (Red Delicious). TA was  
248 similar for all cultivars, except than Red Delicious that showed the lowest value (2.21 g malic acid  
249  $\text{kg}^{-1}$ ).

250 Significant variations between samples were observed in the porosity of the tissues. In particular,  
251 Pink Lady® showed the highest value (24.58 %), while Fuji showed the lowest one (17.35 %). These  
252 values roughly fall in the range reported in literature for apples (between 19 to 27 % depending on  
253 cultivar and ripening degree) (Del Valle et al., 1998; Muujica-Paz et al., 2003; Salvatori et al., 1998).

254 Colour parameters are indicators of freshness and quality of fruit and vegetable (Altisent et al., 2014).  
255 L\* and h° values were similar for the four cultivars considered that were all characterized by a  
256 yellowish flesh.

257 The highest PPO activity data were observed in the Fuji apple variety, the lowest in the Modì® one;  
258 Pink Lady® and Red Delicious apples had significantly similar PPO activity values.

259 Textural parameters were similar for Pink Lady® and Fuji that showed the highest values for both  
260 firmness and crunchiness, while Red Delicious apples were characterized noticeably by lower values.

261

### 262 **3.2. Effect of plasma treatment on browning kinetics**

263 The Weibull equation was used as an empirical model to fit browning data measured by CVS.  
264 Constants of Equation 3 ( $\alpha$  and  $\beta$ ) are reported in **Table 2**.

265 Browning kinetic data and fitting capability of the model can be graphically observed in the **Fig. 2**,  
266 where Eq. 3 was used to model browning parameters for Pink Lady® (a), Fuji (b), Modì® (c) and  
267 Red Delicious (d) apples.

268 In general, the model showed a good fit to experimental data, as high R<sup>2</sup> values were found (**Table**  
269 **2**), confirming its suitability for describing enzymatic browning phenomena in apples. RSME was  
270 found high in the control sample for Pink Lady apples, probably because of the natural high variability  
271 of the browning values.

272 The shape factor ( $\beta$ ) was for all samples  $< 0$  except for Pink Lady® after 30+30 min, indicating that  
273 the kinetic do not follow an empirical first order kinetic model a downward concavity, as observed  
274 by Quevedo et al. (2009) This shape reflects a development of browned areas characterized by a  
275 higher initial rate followed by a slower one.

276 In all samples, the rate parameter ( $\alpha$ ) was strongly reduced upon plasma treatment, with the exception  
277 of Red Delicious after the 15+15 min treatment that showed a value very close to the control one.

278 Nevertheless, the variations observed in the browning kinetic parameters and the final degree of  
279 browning measured after 240 min were not always proportional to treatment time (Fig. 2). Indeed,

280 after 15+15 min, the reduction of the browned area at the end of the observed period was around 50  
281 % for Pink Lady®, Fuji and Modi®, while only 17 % for Red Delicious. After the longest treatment  
282 (30+30). The reduction ranged from 86 % for Pink Lady® to 58 % for Modi®.

283

### 284 **3.3. Effect of plasma treatment on textural parameters**

285 **Table 3** reports mean values of firmness (N) and crunchiness (LD) textural parameters of the four  
286 different apple cultivars as affected by plasma treatment time and storage.

287 Generally, during 48 h of storage, control samples of all cultivars showed an increase of firmness and  
288 a decrease of crunchiness although differences were not always significant.

289 After plasma treatment, samples belonging to Pink Lady®, Fuji and Modi® cultivars showed an  
290 increase of firmness compared to the untreated sample, but this difference did not appear to be related  
291 to treatment time and was not always maintained over storage. Differences found among samples in  
292 crunchiness values did not indicate any clear trend but seem related more to natural variability.  
293 Conversely, while control samples of Red Delicious variety did not show variation for both textural  
294 parameters during storage, after treatment apple samples were characterized by a lower firmness and  
295 a lower crunchiness index. Furthermore, upon the longer treatment (30+30), these values decreased  
296 significantly over storage.

297

### 298 **3.4. Effect of plasma treatment on PPO activity**

299 **Fig. 3** shows the PPO residual activity of apple samples as a function of plasma treatment time  
300 expressed in percentage considering the activity of the untreated samples of the same variety as 100.

301 As it can be observed, the effect was very variable between the different cultivars.

302 In Pink Lady® apple, the effect seemed proportional to treatment time as already observed in a  
303 previous research (Tappi et al., 2014), but, in absolute terms, PPO inhibition was definitely lower  
304 compared to the abovementioned study. Previously, after 15+15 min treatment a 46 % of reduction

305 was obtained, while in the present study, PPO residual activity was not significantly different  
306 compared to untreated sample, and after 30+30 min was about 79 %.

307 The highest inhibition level was observed in Fuji apple, showing a residual activity of 50 and 10 %  
308 after respectively the 15+15 and the 30+30 min treatment. On the contrary, in Modi®, after 15+15  
309 min, PPO activity was not significantly affected by the treatment, while with a longer exposure, it  
310 was reduced of about 50%. Generally, Red Delicious did not show a significant reduction of PPO as  
311 a consequence of plasma treatment.

312

#### 313 **4. Discussion**

314 PPO is a group of enzymes mainly responsible for superficial browning in cut fruit such as apples  
315 and one of the main factor limiting their shelf-life. Browning reactions have generally been  
316 considered as the consequence of the reaction of PPO with polyphenols, made possible by the  
317 breakdown of membranes that normally keeps them separated. Comparing PPO activity found in the  
318 different cultivars, the higher value was found in Fuji, while Pink Lady® and Red Delicious were  
319 characterized by similar values and Modi® showed the lower activity. This result is in agreement  
320 with Altisent et al. (2014) that found Modi® apples to be characterized by a low browning index  
321 although showing the highest polyphenol content among various cultivars considered.

322 However, according to the browning evaluated by CVS, the untreated Modi apple seems to be  
323 subjected to a more remarkable browning phenomenon with respect to the other cultivars. This  
324 difference may be due to different reasons: the different method used for evaluating browning  
325 (colorimetric parameters instead of CVS), the different interval of time for browning development,  
326 the different phenolic profile both in quantitative than qualitative terms and the different physiological  
327 state of the fruit evidenced by the SSC and TA values.

328 As showed by the kinetic parameters, calculated applying the Peleg model, plasma exposure  
329 promoted a decrease of the rate of browning phenomena and a general lower level of browning in all

330 apple cultivars. The observed variations were not strictly proportional to treatment time but generally  
331 a strong inhibition of browning was obtained.

332 In the present study, also the response of PPO activity to plasma treatment was not always  
333 proportional to treatment time and surely not **similar** among the different cultivars considered.  
334 Moreover, an increase of activity was observed in Red Delicious apples after the longest treatment  
335 times.

336 According to various studies carried out on model systems, the inhibition of enzymatic activity after  
337 plasma exposure is due to a change in the secondary protein structure and the modification of some  
338 amino acids side chains of the enzyme (Deng et al., 2007; Takai et al., 2012). In particular, Surowsky  
339 et al. (2013) found a variation in the relative amounts of alfa-helix structures and  $\beta$ -sheet content upon  
340 plasma exposure, that was strongly correlated to the loss of enzymatic activity.

341 In a study on fresh-cut melon, Tappi et al. (2016) found that different enzymes respond in different  
342 way to the same plasma treatment probably for a different resistance to denaturation by plasma agents  
343 due to the different enzyme structure and possibly by the presence of isoenzymes.

344 Bußler et al. (2016) observed a strong reduction of PPO and POD activity in fresh-cut apples and  
345 potatoes after 10 min of exposure to microwave generated plasma.

346 Nevertheless, there are studies reporting an increase of the enzymatic activity following plasma  
347 treatment in model systems (Li et al., 2011) and in brown rice (Chen et al., 2016; Lee et al., 2016).

348 By directly treating carrot cells with a radio frequency plasma needle, Puač et al. (2014) hypothesized  
349 that the effect of increasing or decreasing enzymatic activity depends on the density of reactive  
350 species and on the ability of the tissue to cope with the highly oxidative atmosphere. The authors  
351 reported that the activation of some enzymatic systems is a mean of counteracting the oxidative stress  
352 caused by plasma active species. On the other hand, when the concentration of reactive species  
353 increases and the cell is not able to respond sufficiently, the enzymes are inactivated.

354 To this date, very few experimental researches have been conducted to evaluate the effect of cold  
355 plasma treatments on the enzymatic activity in real systems and in particular in fruit tissues, hence it

356 is difficult to evaluate the influence of the matrix. Moreover, there are no available data on the  
357 penetration power of different plasma reactive species, even if plasma treatment is roughly considered  
358 ‘superficial’. As observed previously, the four cultivars considered in this study were characterized  
359 by different porosity levels, factor that could have influenced the penetration of plasma within the  
360 tissue empty spaces. **Nevertheless, Fuji apple, which is characterized by the lowest porosity value**  
361 **(17.35 %), seems to be majorly affected, in terms of PPO reduction. Hence, the relationship between**  
362 **porosity with PPO inhibition is not clear and needs further investigation.**

363 Obtained results on enzymatic activity do not seem to be correlated to the results obtained by image  
364 analysis showing that, although PPO is the main responsible for superficial browning in cut apples,  
365 such phenomenon is more complex and depends also on other variables; the effect on the different  
366 phenolic compounds that are the substrate for the reaction should be further investigated.

367 In the present study, the effect on textural parameters was also evaluated. The initial differences found  
368 in the considered cultivars did not seem to be related to the porosity of the flesh, although according  
369 to Del Valle et al. (1998), varieties characterized by a similar texture have intercellular spaces of the  
370 same size and should hence have a similar porosity. On the other hand, textural parameters do not  
371 depend only on intercellular spaces size but also on the strength and properties of cell wall, middle  
372 lamellae adhesion and cell turgor (Toivonen & Brummell, 2008).

373 **Generally, the increased rate of ripening related phenomena induced by cutting (wounding response)**  
374 **promote a loss of firmness in fruit slices (Toivonen & Brummel, 2008). The variations in textural**  
375 **parameters observed in this study, namely an increase of firmness and a decrease of crunchiness,**  
376 **follow an opposite trend that may be explained by the short storage period and by a possible decrease**  
377 **of water content of samples during storage, but these aspects should need further clarification.**

378 **Nevertheless,** in the present study, the exposure to plasma seemed to affect the evolution of textural  
379 parameters of only one of the considered cultivars, Red Delicious.

380 Nevertheless, it is difficult to formulate hypothesis on the basis of the present knowledge considering  
381 that the effect of plasma exposure on structural and textural parameters has not been deeply studied.

382 In a previous research Tappi et al. (2014), using the same plasma generator, a slight decrease in the  
383 crunchiness of fresh-cut apples subjected to plasma treatment was observed and attributed to the  
384 destruction of the superficial cell layers; while other authors (Schnabel et al., 2014) did not detect  
385 significant differences in textural characteristics of apple flesh treated with a microwave-generated  
386 plasma.

387 Considering that texture, together with colour, is one of the fundamental **characteristics** determining  
388 the acceptability of fresh-cut fruit and vegetables, future in-depth investigations about the effect on  
389 micro and macrostructure are needed in order to better understand the effect of plasma treatment on  
390 fresh cut tissue physiology, **such as respiration rate, gross metabolism and other enzymatic activities,**  
391 **cell viability and structure.**

392

## 393 **5. Conclusions**

394 The effects of cold plasma treatments were evaluated on the browning kinetics and texture properties  
395 of different apple cultivars characterized by different structural characteristics and PPO activity  
396 levels. Upon plasma exposure, a noticeable reduction of superficial browning was observed in all  
397 cultivars but not always proportional to treatment time. The effect on PPO activity was very variable  
398 in the different cultivars and not correlated to the effect on enzymatic browning.

399 Textural parameters were affected by plasma treatments only in Red Delicious apples in which a loss  
400 of firmness and crunchiness was observed.

401 Cold plasma technology seems to be promising in terms of enzymatic browning inhibition in all  
402 considered apple cultivars. Nevertheless, the response of the tissue to the treatments was variable  
403 according to the cultivar considered. The results obtained in this study indicate the necessity of further  
404 **investigating** the consequences of plasma exposure on specific tissue physiology in order to choose  
405 the better treatment parameters, optimizing its effect for specific final product.

406

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410

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511

512

513 **Figure captions**

514

515 Figure 1. Schematic representation of the electrodes configuration.

516

517 Figure 2. Comparison between observed (dots) and calculated (lines) browned area in Pink Lady®,  
518 Fuji, Modi® and Red Delicious apple slices according to the power law model for control and treated  
519 samples (T15, T30).

520

521 Figure 3. PPO residual activity expressed as % compared to untreated sample of the four different  
522 apple cultivars according to treatment time. The symbol ‘\*’ indicates results that were not  
523 significantly different compared to control sample ( $p < 0.05$ ).