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

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

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Abstract

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

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

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

Industrial relevance

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

Keywords

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

1. Introduction

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

The traditional techniques aimed at the enzymes inhibition are based on immersion (dipping) in solutions containing organic acids in combination with calcium salts, carboxylic acids, thiols-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_2 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_3),
62 atomic oxygen (O), the hydroxyl radical ($OH^{\bullet}$) 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).

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

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

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

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

2. Materials and methods

2.1. Raw material

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

2.2. Gas plasma generator

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

2.3. Gas plasma treatments and samples storage

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

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

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

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

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

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

2.4. Qualitative assessment of fresh-cut apples

2.4.1. Chemical parameters

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

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

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

taken from the three replicated treatments.

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

2.4.2. Porosity

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

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

2.4.3. Colour

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

2.4.4. Visual quality by computer vision system (CVS)

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

a 60 mm lens mod. AF-S micro, Nikkor (Nikon, Shinjuku, Japan) was used to acquire the images. The CVS was calibrated with standard colour, according to Romani et al. (2009).

For each treatment time, acquisitions (exposition time 1/2 s; F-stop f/16) were conducted on samples of 20 apple slices each (10 for the treatment and 10 for the control) immediately after the treatment and every 15 min up to 1 h, and then every 30 min up to 4 h of storage in controlled conditions (4 °C, 95% RH), in order to understand the treatment effect on the browning kinetic.

The length of the assessment was based on preliminary tests aimed at evaluating the browning kinetics of the slices and on previous studies (Quevedo et al., 2016). The intervals were chosen as often as possible in order to properly follow the kinetic.

Digitalized images were evaluated with an advanced Image Analysis Software (Image Pro-Plus v. 6.2, Media Cybernetics, USA) using RGB scale. Total and browned areas were selected and a colour model was set up according to Rocculi et al. (2004). Two different pixel ranges were identified on the basis of different chromatic characteristics, considered as 'not browned' and 'browned' area (BA). The model was then applied to each digitalized image, and by evaluating all pixels; the percentage of each chromatic area was calculated by the software.

2.4.5. Texture

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

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

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

2.4.6. Polyphenol oxidase activity (PPO)

Enzyme extraction was carried out according to Baritaux et al. (1991) with slight modifications. Briefly, 50 g of sample were homogenised in 100 mL of cold McIlvaine's buffer solution at pH 7.5 containing 0.5 % Triton X100, 25 mM ascorbic acid and 0.5% PVPP, using an Ultra-Turrax blender for 30 s. The homogenate was kept under agitation and in the dark at 0 °C for 15 min and then centrifuged for 30 min at 4 °C and 25000 *g*. The supernatant was filtered and used as extract.

A solution containing 4-methylcatechol 50 mM prepared in McIlvaine's buffer solution at pH 7.5 was used as a substrate for the assay. 200 µL of extract were added to 3.2 mL of substrate solution. The determination of PPO activity was carried out immediately after plasma exposure by measuring the variation of the absorbance of the mixture at 420 nm and 25 °C during 5 min compared to the initial value with a spectrophotometer (UV-1601, Shimadzu) and calculated on the basis of the slope of the linear portion of the curve (ΔA/min). One unit (U) is defined as the quantity of enzyme necessary to obtain an increase in absorbance of 1U in 1 min under the assay conditions.

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

2.5. Data analysis

Significant differences ($p<0.05$) were evaluated by using the Analysis of Variance (ANOVA) according to LSD post-hoc test. Differences were investigated among values relative to the raw material of different cultivars, between control and treated samples for PPO activity and among values of texture relative to control and treated samples at different storage times.

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

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

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

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

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

3. Results

3.1. Characterization of the raw materials

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

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

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

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

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

3.2. Effect of plasma treatment on browning kinetics

The Weibull equation was used as an empirical model to fit browning data measured by CVS. Constants of Equation 3 (α and β) are reported in **Table 2**.

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

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

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

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

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

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

3.3. Effect of plasma treatment on textural parameters

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

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

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

3.4. Effect of plasma treatment on PPO activity

Fig. 3 shows the PPO residual activity of apple samples as a function of plasma treatment time expressed in percentage considering the activity of the untreated samples of the same variety as 100. As it can be observed, the effect was very variable between the different cultivars.

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

To this date, very few experimental researches have been conducted to evaluate the effect of cold 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.

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

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

5. Conclusions

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

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

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

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513 **Figure captions**

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515 Figure 1. Schematic representation of the electrodes configuration.

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517 Figure 2. Comparison between observed (dots) and calculated (lines) browned area in Pink Lady®,

518 Fuji, Modì® 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 ($p<0.05$).