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Effect of in–package cold plasma treatments on the quality of minimally processed apples

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Summary

Cold plasma technology is being increasingly used for food preservation and, incipiently, for minimal processing of fruit. Therefore, the objective of this work was to study the effect of in-package cold plasma (generated in atmospheric–pressure air by a low–frequency -50 Hz- dielectric barrier discharge operated at 30 kV) on the quality of minimally processed apples during refrigerated storage. Apple slices were subjected to the different treatments following a completely randomized design with 3 x 3 factorial arrangement. The independent variables were the exposure time (0, 1, 3 min) and the storage times (1, 4, 7 days). Cold plasma treatments preserved the quality of the fruit, maintaining the tissue structure. Plasma treatment applied for 1 min rendered apple slices with the highest antioxidant content but only at day 1. Even though polyphenoloxidase activity was reduced by the treatment, it was not sufficient to stabilize the antioxidant content during storage.

Keywords: cold plasma, fresh-cut, apple, browning, antioxidants, microstructure

Running title: Cold plasma for fresh-cut apple processing

Conflicts of interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

1.1 Introduction

Over the last decades, there has been a marked increase in the demand for minimally processed fruit by consumers. However, the preservation of these products represents a technological challenge, since only few strategies can be used to maintain their original quality (Bagheri & Abbaszadeh, 2020; Mao et al., 2021). An innovative though rather underexplored strategy to overcome this problem is the application of cold plasma. To generate cold plasma, energy is applied in the form of an electrical discharge to a gas, which induces ionization, excitation, and dissociation reactions, leading to the formation of different active compounds such as radicals, UV light and charged particles (Bagheri & Abbaszadeh, 2020). In air and similar mixtures, reactive oxygen, and nitrogen species such as atomic oxygen, OH radicals and nitric oxide will have an important effect on biological samples (Bruggeman et al., 2017).

The most common ways to generate atmospheric cold plasma for food applications are dielectric barrier discharge (DBD) and plasma jets. The main advantages of the DBDs are the convenience of the discharge ignition and the possibility of treating foods inside sealed packages, which eliminates the risk of post-process contamination. The dielectric properties of the package itself will help limit the charge transported, allowing the production of a stable discharge (Misra et al., 2014). Then, the gas contained in the package will generate inside a particular plasma field rendering an extended plasma exposure for the enclosed product (Misra et al., 2013). Due to its non-thermal nature, cold plasma may be a suitable technology for the treatment of heat-sensitive foods such as fruits and vegetables.

In a preliminary test, we found that cold plasma treatments provoked, in fresh cut apples, significant reductions (1-2 log CFU g^{-1}) in mesophilic aerobic counts and in yeast and molds. Similar inactivation was reported in other works. Among them, Dong and Yang (2019) found a decrease in bacteria (by 2 log CFU g^{-1}) and fungi (by 1 log CFU g^{-1}) in blueberries exposed for 10 min to plasma (DBD plasma at atmospheric pressure air). The authors attributed this effect to the increases in DNA damage and guanine oxidation. Coincidently, Hu et al. (2021) observed that blueberries treated for up to 15 min with air DBD plasma discharge (at atmospheric pressure, a peak-to-peak voltage of 4 kV, and a frequency of 8 kHz) inhibited the microbial development and natural decay of fruits during the storage without affecting the quality of the product. On the other hand, cold plasma generated by a diffuse coplanar surface barrier discharge system with

air (peak-to-peak voltage of 20 kV, frequency of 15 kHz for up to 10 min) reduced the yeasts and molds load of red currants, up to 1.28 log CFU g^{-1} (Limnaios et al., 2021). Similarly, cold plasma generated by a DBD (60 kV and a frequency of 50 Hz for 15 min), reduced 2 log CFU g^{-1} of microbial loads in strawberries (Rana et al., 2020). Zhou et al. (2020) and Perinban et al. (2022) also observed significant reductions in microbial counts on fresh-cut apples treated with cold plasma treatments. In turn, Bagheri and Abbaszadeh (2020) and Mao et al (2021) reviewed different works in which cold plasma treatments significantly inhibited microorganisms and extended the shelf life of fresh-cut fruits with minimal impacts on physicochemical and sensorial quality of the products. Recently, Zhou et al. (2022) showed that cold plasma treatments contributed to the shelf-life extension of fresh cut cantaloupes.

Regarding the biochemical and physiological effect, previous studies showed that the application of atmospheric cold plasma with 0.5% NO_2 for 10 min, was able to reduce in minimally processed potato and apple pieces the activity of browning-related enzymes (polyphenoloxidase -PPO- about 62% and 77% in apple and potato tissue, respectively, and peroxidase -POD- about 65% and 89%) (Bußler et al., 2017).

Despite the presence of phenolic compounds could lead, in their role as substrates, to browning reactions when tissues are damaged, many studies have also identified them as positive bioactive compounds involved in health promoting mechanisms, mostly those related to oxidative stress, by limiting the negative effects of reactive oxygen species (ROS) (Debelo et al., 2020). From this viewpoint, processing technologies generally used for preservation could also be potentially applied under milder conditions, which would exert a limited abiotic stress to the plant tissues, able to increase the levels of antioxidant compounds (Denoya et al., 2022). To the best of our knowledge, the application of cold plasma technology has not been widely explored for this purpose. Previous studies reported interesting results by applying a cold plasma treatment of 60 kV for 5 min to minimally processed pitaya packed in trays covered with a polypropylene film. This treatment was able to increase in 78% the content of phenolic compounds and the antioxidant capacity of the product in 48% (Li et al. 2019a). Recently, Perinban et al. (2022), applying cold plasma in an innovative and indirect way to fresh-cut apples, obtained an increase of 32 % in total phenols after the 5 min-treatment with activated water through the application of cold plasma (10 kV peak-to-peak voltage, 20 kHz frequency, gas mixture: argon/oxygen 98:2), during 10 min.

Although some fruit products were treated with in-package cold plasma treatment (Misra et al., 2014; Li et al., 2019a; Li et al., 2019b; Zhou et al., 2022), the use of this way of application in fresh-cut apples has not been already reported. Besides, the microstructure of this type of products was scarcely studied.

The objective of the present work was to evaluate the effect of in-package cold plasma treatments on the overall quality, phenol content, antioxidant capacity, microstructure, and enzymatic browning prevention of fresh-cut apples.

2. Materials and methods

2.1 Plant material and processing

Apples (*Malus domestica*) var. Granny Smith were obtained from a local market in Buenos Aires, Argentina. Fruit similar in size, quality and maturity level were used for the assay. On the day of processing, the apples were washed with tap water, peeled, and cut into 0.5 cm thick slices. Nine pieces were placed in Cryovac BB2620 bags (O_2 transmission rate 6-14 $cm^3/m^2/24$ h at 22-24°C and 0% RH) prepared for this purpose and confining the volume to be filled with air by means of a frame, constituting the experimental unit. The experimental unit was the container with 9 slices of apple.

2.2 DBD plasma source

Figures 1A and 1B show a diagram and a picture of the DBD plasma source for the fruit treatments, respectively. The DBD system consisted in a needle-array power electrode and a plate ground electrode, both covered by dielectric barriers (100 μm thick Mylar film for the high–voltage electrode and 3 Thernophase films of 400 μm thickness for the ground electrode) between which six Cryovac BB2620 bags containing the food samples were placed. The Cryovac BB2620 bag also served as a dielectric barrier. The gap between the upper surface of the ground electrode barrier and the tip of the needles (tip radius about 50 μm) was fixed to 20 mm during the experiments. The power supply was a high-voltage sine AC power supply (0–30 kV) operating at 50 Hz. All treatments were conducted in ambient air. The atmospheric air conditions at the time of packaging and treatment were 48 ± 1% relative humidity and 25 ± 1°C temperature. The in–package apple samples were subjected to cold plasma treatments for 1 and 3 min. A voltage of 30 kV (peak value) was applied across the electrodes during the cold plasma application.

2.3 Cold plasma treatments, experimental design, and statistical analysis

For each treatment (unless for the controls (Ctrl) that the processing ended after the operations described in section 2.1), three replicates were done placing six experimental units with nine apple slices each in the active plasma region on the dielectric barrier. During the experiments, the packed apple slices were treated with plasma exposure times of 1 (P1) and 3 min (P3). A completely randomized design with a 3x3 factorial arrangement was used. The independent variables were the exposure time (0, 1 and 3 min) and the storage times at 4°C (1, 4 and 7 days).

The samples were transported refrigerated and stored in chambers at 4°C. The determinations detailed below were carried out at 1, 4 and 7 days and performed in triplicate. For each treatment, three replicates were done (eighteen experimental units -containers- for each treatment). Then, for each sampling date, we took one package of each replicate (three in total) to have three experimental units of different replicates and for the different sampling dates (three for each one of the three sampling dates -9-) and the different determinations (the ones that should be done the same day of sampling (9-color parameters, firmness and microstructure) and the ones that could be performed after frozen the samples at -80°C, all the other ones -9-). In the case of PPO analysis, it was performed only for day one in three pooled samples from slices of each one of three experimental units, to study the effect of the treatments on the enzyme inactivation. For the analysis of the microstructure, the micrographs were obtained after the observation of samples from different experimental units stored 4 days at 4°C to evaluate the preservation of the tissue in treated and untreated samples. The average and standard error of the experimental data for each treatment were determined. Differences were evaluated for significance by analysis of variance, which was carried out using the General Linear Model procedure from SAS (SAS Institute Inc., University Edition, Cary, NC, USA). Duncan's test (significance level of 0.05) was used for the data showed in tables and figures.

2.4 Sample Analyses

2.4.1 Chromatic parameters

Chromatic parameters of three apple slices (three points on each one) from each experimental unit were determined with a chromameter (CR-400, Konica Minolta Sensing, Inc. Osaka, Japan). Results were

expressed in CIE scale L*a*b*, where L* indicates lightness, a* indicates redness (positive values) or greenness (negative values), and b*, yellowness (positive values) or blueness (negative values). The instrument was set up for illuminant D_{65}.

2.4.2 Firmness

Firmness of the apple slices was determined by performing a puncture test with a Texture Analyzer (TA-XT plus, Stable Micro Systems LTD. Surrey, England) with a cylindrical probe of 2 mm of diameter and a cell load of 5 kg. Three slices of each experimental unit were individually measured in three different points. The conditions were: velocity of the probe during the test: 1 mm s^{-1} and the distance penetrated by the probe inside the sample was set in 3 mm. Firmness was measured in Newtons (N).

2.4.3 Soluble solid content

Soluble solid content (SSC) was determined at 20°C by measuring the refractive index of the juice of the apple slices with a refractometer (Atago Co. Ltd, Tokyo, Japan). SSC was measured in % °Brix.

2.4.4 Total phenols content

The total phenol content was determined according to Denoya et al. (2020). It was calculated based on a calibration curve with gallic acid (Carlos Erba, Milan, Italy) and was expressed as grams of gallic acid equivalents (GAE) per kilogram. Results were expressed on a fresh weight basis.

2.4.5 Antioxidant capacity

The antioxidant capacity was measured on the extracts based on three methods: ABTS (electron scavenging assay) and DPPH (radical scavenging assay) and Ferric reducing/antioxidant power (FRAP), related to the reducing/oxidizing ability of the extracts. These determinations were carried out according to Denoya et al. (2020). The antioxidant capacity was expressed in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (TEAC): µM eq. Trolox per gram in the case of ABTS method and FRAP method. The antioxidant capacity by DPPH method was expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight: mg GAE g-1.

2.4.6 PPO activity

The PPO activity was determined according to Denoya et al. (2015) and was expressed as units per gram of fresh weight of fruit. One unit of enzyme is the quantity necessary to produce an increase in absorbance of 0.01 min^{-1} at 420 nm.

2.4.7 Light microscopy

Thin slices, in at least 5 samples of each treatment, were sectioned by the conventional method for fresh plant tissues (hand sections), using a sharp razor blade (Zarlasky, 2014). Sections were temporarily mounted in distilled water on slides with coverslips. Observations and micrographs were made using a light microscope (Nikon Eclipse E200, Tokyo, Japan) with a digital camera (Moticam®, China) at the Botany Laboratory of Luján National University (Buenos Aires, Argentina). The scales were included in the micrographs as white bars representing 100 μm in the case of the 40x samples and 50 μm in the case of the 10x samples.

3. Results and discussion

3.1 Chromatic parameters

Figure 2 shows the L* and a* parameters (CIE color system) of the in-packed apple slices exposed to the different treatments. In general, L* values from control samples tend to be, along the 7 days of evaluation, lower than those from treated samples, although not significantly different ($p<0.05$). Besides, during refrigerated storage, L* decreased significantly ($p<0.05$) only for Ctrl and to P3 treatments and between day 1 and day 4. At day 7, a* parameter was higher ($p<0.05$) in control samples, following the order Ctrl > P1 > P3, evidencing the higher enzymatic browning underwent by control samples. This is in agreement with previous studies, where cold plasma treatment (Surface Discharge Plasma atmospheric plasma treatment, voltage: 5kV, plasma discharge power: 50 W, time of exposure: 3 min) prevented the browning of fresh cut apples after 6 days of refrigerated storage (Zhou et al., 2020). Tappi et al. (2014), by using a computer vision system to evaluate browning extent, also observed a higher browning in control samples in comparison to apple slices treated at 15 kV for different times of exposure (10, 20, 30 min). In a subsequent study, these authors found that the browning kinetic of plasma-treated fruit strongly depended on the apple variety (Tappi et al., 2019). The above-mentioned studies were carried out by assessing the effect of cold plasma treatment on unpacked

fresh-cut apples but in the present work, we applied the treatments after packing the product in bags (in-package treatment). Although it would be expected that this process may change the conditions of the treatment (adding an additional dielectric barrier and generating the reactive species in the headspace of the product), results on browning prevention were similar to those obtained by the other ways used to apply cold plasma but by using a shorter treatment time (1 min). Bagheri & Abbaszadeh (2020), after reviewing different studies on the application of cold plasma on fresh-cut fruit, concluded that cold plasma could stabilize the color in fruit prone to suffer browning. The proposed mechanism would be linked to the loss of the functionality of the enzymes involved in this alteration upon plasma exposure, which would be in turn provoked by the oxidation of the side-chain amino acids by ROS, therefore altering the secondary structures of these proteins.

3.2 Firmness

Table 1 shows the firmness (N) of the apple slices exposed to the different treatments. Although no significant differences were observed each day of evaluation among the different treatments, there was a reduction of firmness at day 7 of refrigerated storage in all the samples. The increase in ripening rate, commonly observed in fresh-cut fruit, is usually caused by the physiological response to wounding, leading to a loss of firmness during storage (Toivonen & Brummell, 2008). A non-significant effect of the plasma treatments on the firmness and the decrease of the firmness during refrigerated storage (4°C) of control and treated samples were also reported in fresh-cut apples treated with a direct application of atmospheric cold plasma (voltage: 5 kV, plasma discharge power: 50 W, for 1 to 3 min) (Zhou et al., 2020). As mentioned before, the present study was performed with in-package treated product, and also by this way of cold plasma application, no decrease in firmness was observed after treatment. Misra et al. (2014), working with in-package strawberries, reported a similar response after a plasma treatment with a DBD system generated by atmospheric in-package cold plasma (60 kV for 5 min).

3.3 Soluble solids

Figure 3 shows the soluble solids content (SSC) of apple slices subjected to the different treatments during the 7 days of refrigerated storage. No significant differences were found immediately after treatment (day 1) among the different treatments. However, at days 4 and 7, treated samples (P1 and P3) presented lower

($p<0.5$) SSC values than the control. In the case of cashew apple juice indirectly treated with nitrogen plasma generated by glow discharge (80 kHz, for 15 min, Rodríguez et al., 2017), the decrease evidenced in sugar content was linked to the molecular degradation brought about by oxygen radicals generated during ozonolysis, which would react with sugars, forming acid compounds. It is therefore speculated that the plasma generated from N_2 would also produce nitrogen radicals, prone to react with sugars.

3.4 Total phenols

Figure 4-A shows the total phenols content of apple slices subjected to the different treatments during the 7 days of refrigerated storage. At day 1, apples treated with P1 had the highest ($p<0.05$) total phenol content (94% increase in comparison to the control), although this difference with the other samples was not maintained during storage. The increase in total phenolic compounds had been previously observed in grape pomace treated with cold plasma (DBD, 60 kV for 5 min), in percentages that can reach around 20 % (Bao, Reddivari, & Huang, 2020a). In other products such as strawberry wedges (in-package atmospheric cold plasma, DBD, 45 kV for 1 min) this increase was verified up to day 3 of storage, thereafter followed by a decrease, in a similar way to the behavior observed in the present study (Li et al., 2019b). These authors also observed, like us, a slow but steady increase during storage in control samples. Conversely to our results, Hu et al. (2021) observed that anthocyanin concentrations were decreased at the first day of study in blueberries treated with cold plasma (air DBD plasma discharge at atmospheric pressure and a peak-to-peak voltage of 4 kV, 8 kHz). The authors attributed this effect to the anthocyanin leakage from the damaged fruit peels and to the fact that the compounds could be consumed by the reactive species generated by the cold plasma. However, the treatment times applied in this work (10 min and 20 min) were longer than those used in ours (1 min and 3 min) and the maximum reduction was observed with the longest treatment time. In turn, Dong and Yang (2019) observed an increase in anthocyanins content of blueberries with the plasma treatments coincidently with our work, although in this study the increase in the compounds was maintained during storage.

Polyphenols in general, and especially chlorogenic acid, were increased to 39.85 % in cold plasma treated strawberry (air DBD plasma discharge at atmospheric pressure for 10 min, Rana et al. 2020). Similarly to our study, the polyphenol content was significantly decreased with an extended storage of 2 days. The authors

considered that this could be linked to the oxidation of polyphenols, in presence of elevated number of reactive species. The differences between our results and those reported by other authors could be due to different variables such as the type of fruit treated, the different way of sample processing and the conditions of the cold plasma treatment applied. In one related treatment, Perinban et al. (2022), also working with fresh-cut apples but applying plasma activated water for 10 min instead of direct cold plasma application, observed an increase of 32 % in total phenols at day one.

In some fruit, phenolic compounds are bounded to cell membranes. Then, a certain level of energy should be necessary to release them and increase the content of these compounds in the samples. Among the alternatives to exert this effect and break down even covalent bonds with cell membranes, it can be mentioned the generation of reactive species, charged particles and photons generated from cold plasma (Herceg et al., 2016). Plasma ions could also provoke the rupture of plant cells and therefore increase the penetration of the UV radiation that, together with heat, are generated during the plasma treatment. Interestingly, irradiation treatments were shown to induce stress responses in plants foods, eventually leading to an increase in the antioxidant synthesis (Elez Garofulic et al., 2015).

3.4 Antioxidant capacity

According to nowadays standards, different analytical methods should be used to evaluate the antioxidant capacity, since only their combined assessment would provide a more complete picture of the antioxidant capacity of foods. In this regard, the antioxidant capacity could be measured by using metal reduction capacity (FRAP) and organic radical-scavenging capacity (DPPH and ABTS) (Rodriguez et al., 2017).

Figure 4 shows the antioxidant capacity of apple slices subjected to the different treatments during the 7 days of refrigerated storage, as measured by three different methods (ABTS, DPPH and FRAP). At day 1, apples of the P1 group presented a significantly ($p<0.05$) higher antioxidant capacity in comparison to the control, as measured by FRAP (increase in 58% figure 4-D) and DPPH (increase in 35% figure 4-C) methods. In the case of ABTS method (Figure 4-B), both P1 and P3 groups presented higher ($p<0.05$) antioxidant capacity than control samples, although at a lesser extent (increase in 22% and 10%, respectively). However, in all the cases, this difference was not maintained along the storage. A recent research (Li et al., 2019b) with fresh-cut strawberries also showed an increase in DPPH antioxidant capacity after a cold plasma treatment (in-package

DBD cold atmospheric plasma treatment; 45kV for 1 min), but it was only around 10%. As in the present study, the increased antioxidant capacity of the product was not maintained during storage (1 week at 4°C).

In the above-mentioned study of Rana et al. (2020), cold plasma treated strawberries for 10 min presented an increase of 14.5 % of antioxidant capacity by DPPH method, but similarly to our study, the increase was not maintained during storage. In the above-mentioned study with plasma activated water, Perinban et al. (2022) also observed in fresh cut apples an increase of antioxidant capacity by DPPH and FRAP method on the products but during storage. They attributed this effect to the induced oxidative stress in the treated samples. Li et al. (2019a) observed that the cutting process itself induced in fresh-cut pitaya an increase in antioxidant capacity of the product, while samples treated with cold plasma (60 kV for 5 min) evidenced a further enhancement of 21 % in the antioxidant capacity of the product.

As expected, the behavior of the antioxidant capacity of the samples paralleled that of the total phenols, since most of the antioxidants of this fruit belong to the group of polyphenols in general, and flavonoids in particular, which especially influence the FRAP measurements (Rodriguez et al., 2017). Some authors consider that the increase in antioxidant capacity produced by cold plasma treatments is due not only to an increase in polyphenols content, but also to the higher extractability of the compounds brought about by the treatment (Bao et al., 2020b).

3.5 PPO activity

PPO is the principal enzyme that catalyzes cut edge browning in white flesh fruit such as apples and pears (Toivonen & Brummell, 2008). Table 1 shows the PPO activity of apple slices subjected to the different treatments. Enzyme activity of apple slices treated with P1 was significantly lower ($p<0.05$) than that measured in samples subjected to P3 and control ones, with a percentage of reduction of 20% in relation to the control.

Bubler et al. (2017) informed in fresh cut apples a reduction of 52% achieved with a direct treatment of cold atmospheric plasma (generated from air by a microwave plasma torch at a frequency of 2.45 GHz, power 1.2 kW and a gas flow of 20 L min^{-1} for 2.5 min). The extension of the exposure time from 2.5 min to 10 min

only provoked a slight increase in the percentage of inactivation (58%), although the difference was not statistically significant.

In minimally processed apples, Tappi et al. (2014) reported a significant reduction in PPO activity after the application of direct cold atmospheric plasma treatment (potential difference of 15 kV, input voltage 19 V and frequency of 12.7 kHz for 30 min), attaining an enzyme activity about 45% lower than that found in untreated samples (control). In the same type of product but applying plasma activated water, Perinban et al. (2022) reported a maximum reduction of 32.3 % in PPO activity.

In the present work, we obtained applying in-package cold plasma treatments a lower reduction of the PPO activity compared to that obtained in the above-mentioned works. However, as previously mentioned, the package added an additional barrier to the browning development, which could explain why the results for color parameters were similar to those obtained in the cited studies.

Regarding the biochemical basis underlying the PPO inhibition, the mentioned authors consider different phenomena, such as the losses of α-helical secondary structures together with an increase in the β-sheet region, the degradation of protein integrity, chemical changes of reactive sidechain of the amino acids induced by radicals, and the decomposition of C-H, C-N and N-H bonds. They also mention that the inactivation is only partially due to the low penetrability of the plasma into the tissues.

It is important to highlight that the effect (inactivation or activation of enzymes) strongly depends on the treatment conditions and on the type of product treated (Zhou et al., 2020). In this sense, Tappi et al. (2019) studied the application of different cold plasma treatments on fresh-cut apples of different varieties and concluded that the effect of the treatment on PPO activity is not always proportional to treatment time and surely not similar among the different varieties evaluated. These authors also observed an increase of PPO activity in fresh-cut Red Delicious apples after the longest treatment times.

3.6 Microstructure

To the best of our knowledge, information about the effects of cold plasma treatments on the microstructure of vegetable tissues is rather scarce. The few studies found in the literature were mainly focused on the surface of the products. To our standpoint, the study of the microstructure of the treated tissues will be highly

relevant to better understand some of the underlying effects of the cold plasma treatments on products such as minimally processed apples. Figure 5 shows the micrographs of the apple tissues exposed to the different treatments after four days of refrigerated storage. No major changes are observed in treated apple tissues (P1 and P3) (Figure 5. D-F, G-I) compared to the control (C1) (Figure 5 A-C). The general structure was well maintained in all cases, as well as the characteristics of the intercellular spaces and the cell walls. The only change observed in treated samples was a lower content of cellular content (Figure 5 E; 5 H, I), which could be associated to a slight dehydration of the material. On the other hand, the control samples, when cut, were more hydrated than the treated samples (Figure 5, A-C). In the treated samples, the amyloplasts were more evident, especially in the P1 group (Figure 5 F), probably because of the slight dehydration, as previously mentioned. In conclusion, no substantial structural changes can be observed in the treated material with respect to the control (Figure 5), with the cells remaining well preserved in the treated samples. The only difference observed is a more evident presence of amyloplasts in the treated samples, probably related to the reduction in soluble solids or to the partial dehydration. A more desiccated appearance and visible signals of stressed was previously reported in lamb´s lettuce treated with direct cold plasma generated from pure oxygen by RF-glow discharge at 150 W for 60 s (Grzegorzewski et al., 2010). Other observation in previous studies were the loss of the cell integrity and rupture of cell walls in tomato pomace when treated with the plasma generated by various gases (air, argon, helium, and nitrogen) under 60 kV for 15 min (Bao et al., 2020b). In this last research the structure presented more damaged when using pure gases for generating the plasma, in comparison to the cold plasma generated from air, probably because of the type and the number of species generated.

4. Conclusions

In–package cold plasma treatments evaluated in the present study proved suitable to preserve the chromatic characteristics of minimally processed apples, adequately maintaining the tissue structure, with only a minor decrease in soluble solids evidenced during storage and with no negative impact on textural parameters. Among the alternatives tested, results show that the in-package DBD atmospheric cold plasma treatment (30 kV-50 Hz) applied for 1 min showed the best performance, rendering apple slices with the highest antioxidant capacity and total phenols content but only for day 1 because the increase in antioxidant content was not

maintained during refrigerated storage. It is important to mention that although PPO activity was reduced by the treatment, it was not sufficient to stabilize the antioxidant content of the treated samples during storage, for which the combination with other preservation strategies remains to be evaluated in future studies.

5. Acknowledgments

This work was supported by Instituto Nacional de Tecnología Agropecuaria, INTA (INTA PD-E7-I153), PIP CONICET (ID#11220210100718CO) and Universidad Tecnológica Nacional, UTN (PID UTN 5418 and PID UTN 5447).

6. Data Availability Statement: Data available on request from the authors

7. Ethical Guidelines: Ethics approval was not required for this research.

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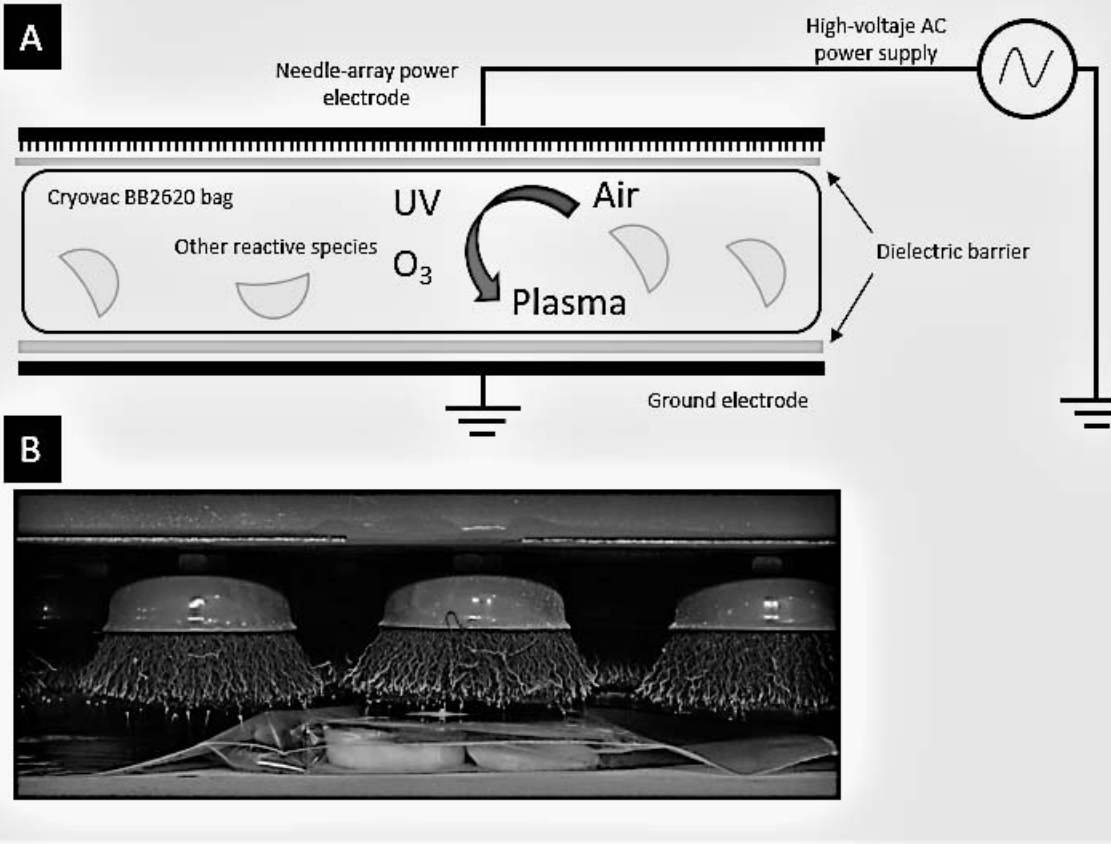
Table 1: Firmness (N) and polyphenol oxidase (PPO) activity of minimally processed apples treated at different cold plasma treatments.

Firmness (N)	day 1	day 4	day 7	PPO activity (UE/g FWF) day 1
Control	8.1 ± 0.4 aB	9.8 ± 0.5 aA	6.9 ± 0.3 aB	53.87±4 a
P1	8.7 ± 0.8 aA	8.1 ± 0.6 aAB	6.3 ± 0.4 aB	42.88±4 b
P3	8.7 ± 0.4 aA	8.0 ± 0.8 aA	5.4 ± 0.8 aB	56.48±5 a

Data are expressed as means ± standard error (n=3). For each storage time, means at different treatment followed by the same lowercase letter were not significantly different according to Duncan's test (p=0.05). For each plasma treatment, means at different storage time followed by the same uppercase letter were not significantly different according to Duncan's test (p=0.05). P1=Cold Atmospheric Plasma treatment by Dielectric Barrier discharge for 1 min. P3=Cold Atmospheric Plasma treatment by Dielectric Barrier discharge for 3 min. Control = without Cold plasma treatment. UE: Units of enzyme FWF: Fresh weight of fruit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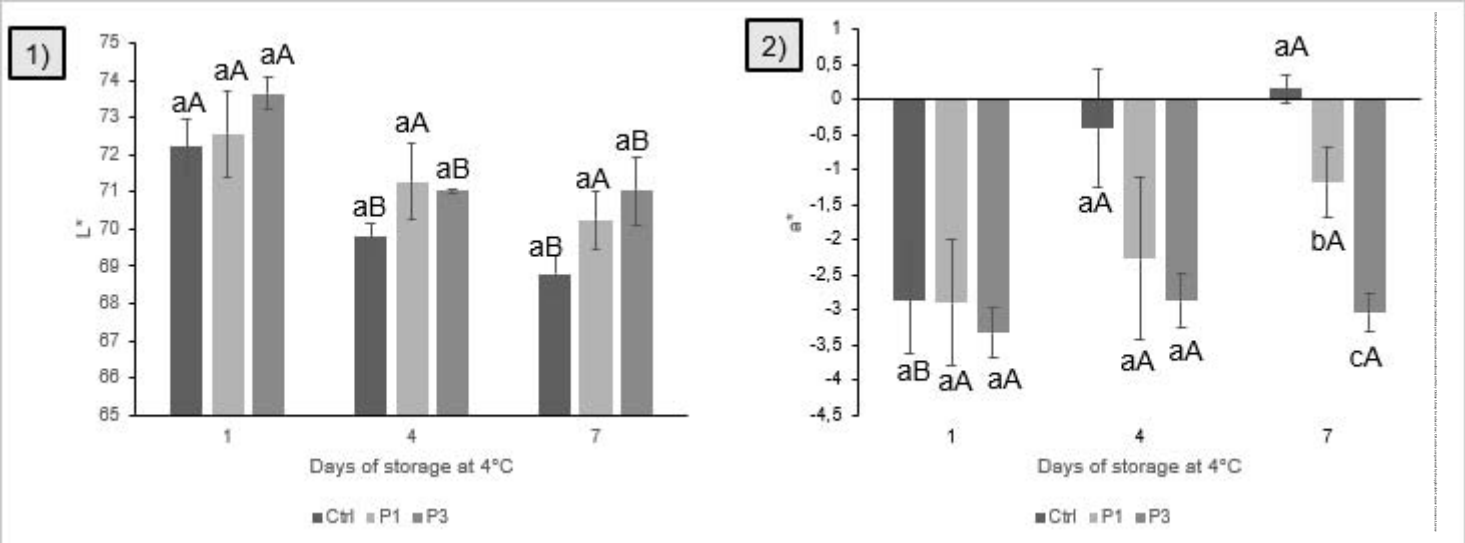
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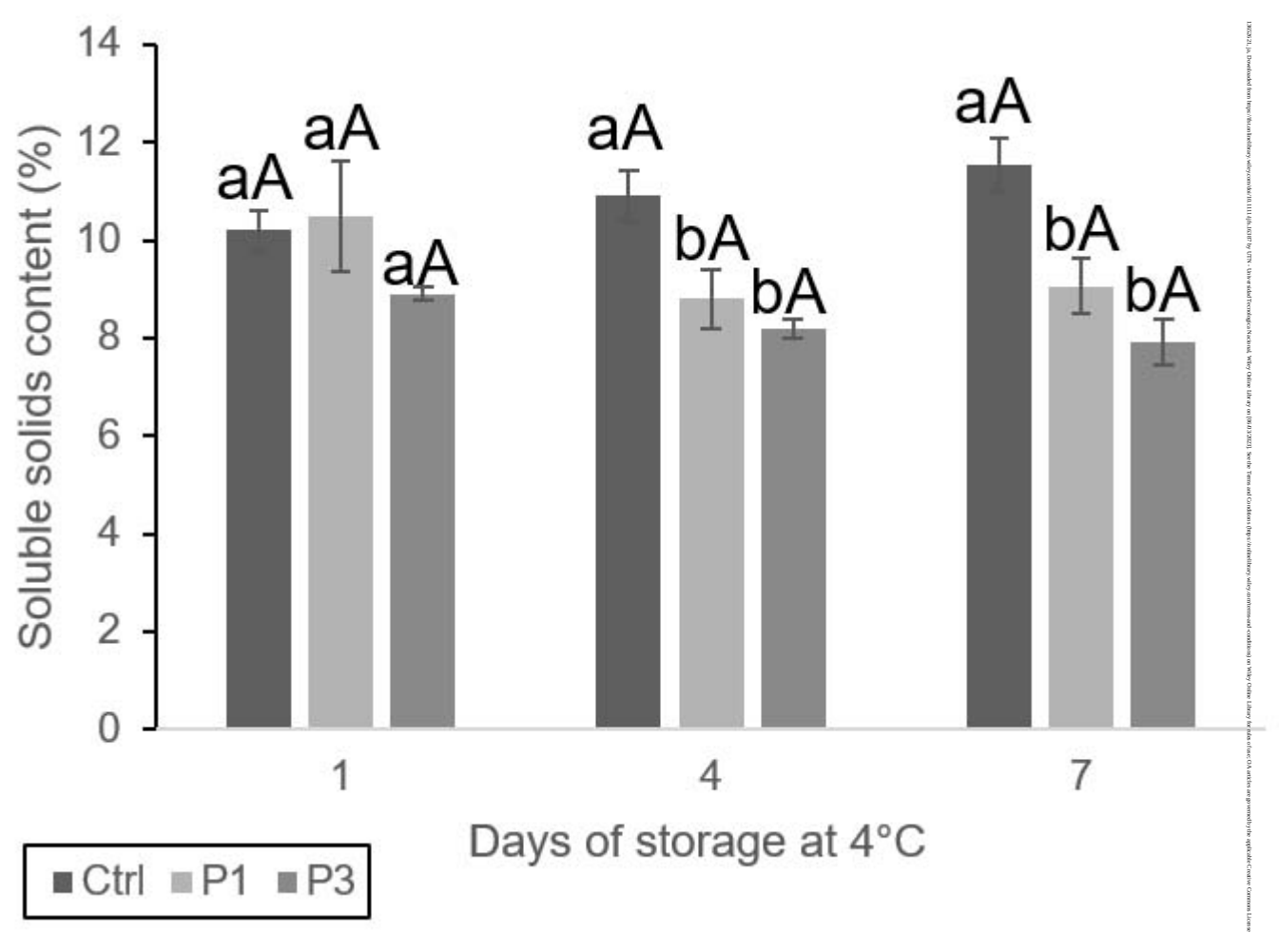

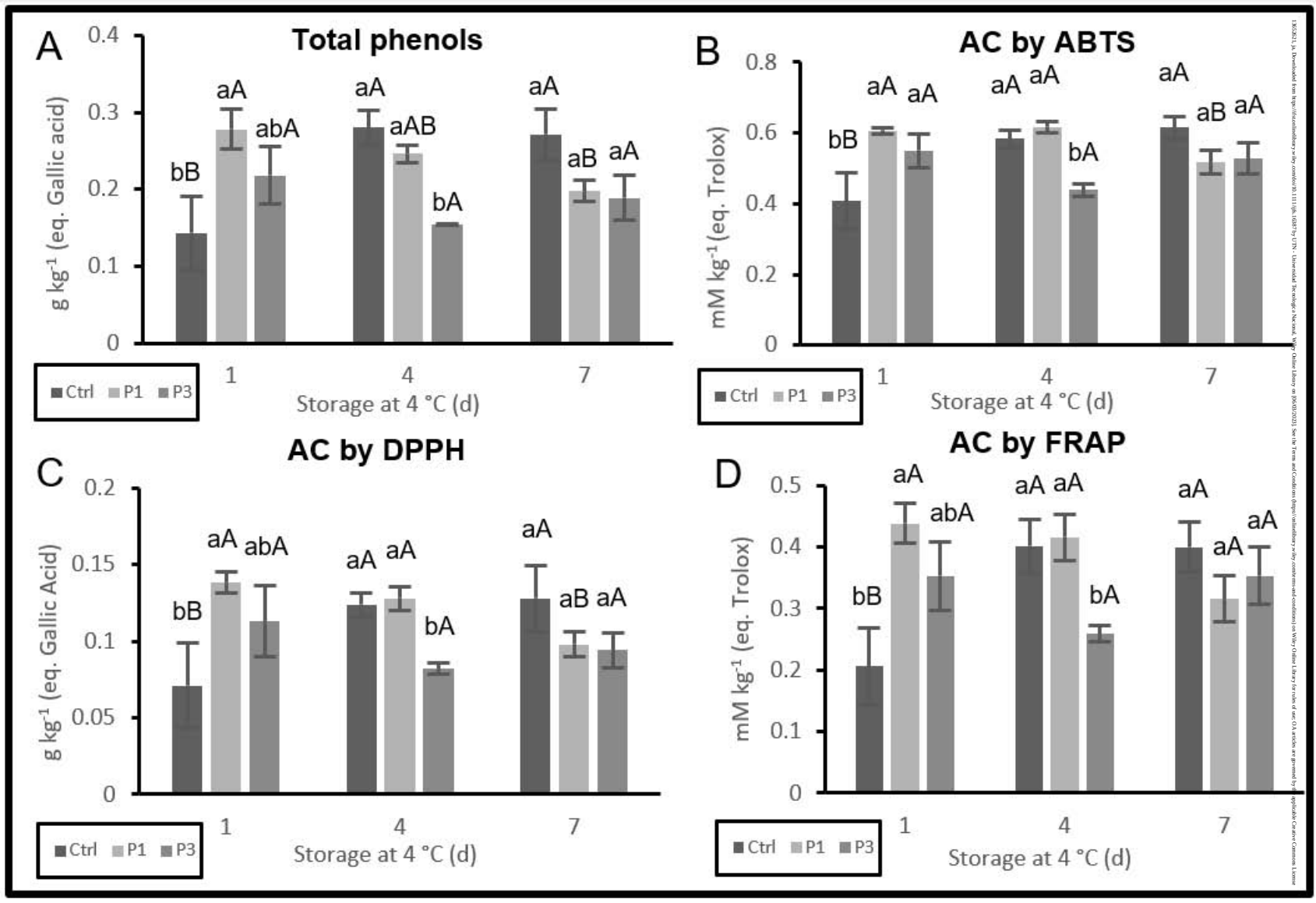

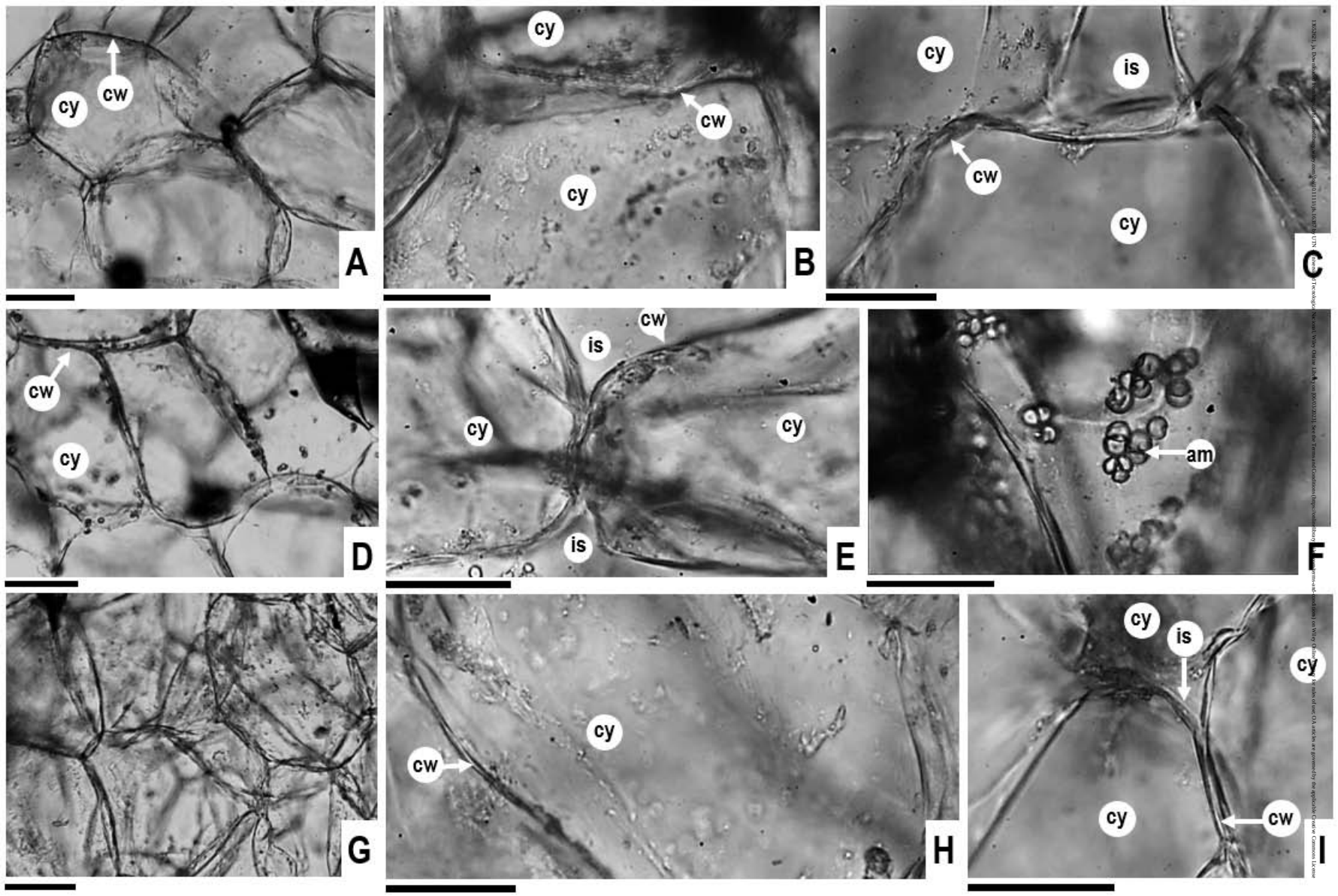
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