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ATMOSPHERIC COLD PLASMA PROCESS FOR VEGETABLE LEAF DECONTAMINATION: A FEASIBILITY STUDY ON RADICCHIO (RED CHICORY, CICHORIUM INTYBUS L.)

Authors

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Abstract

Cold plasma is a novel non-thermal technology that could be used for large scale leaf decontamination in food manufacturing as an alternative to chlorine washing. The effect of atmospheric cold plasma apparatus on the safety, quality and antioxidant activity of radicchio (red chicory, *Cichorium intybus* L.) was investigated after treatment and during storage. Cold plasma treatment caused significant changes to the external appearance of the radicchio leaves during storage as assessed by a sensory and image analysis. Chroma values of radicchio leaves were also affected by the cold plasma treatments. The antioxidant activity of radicchio leaves, assessed by the ABTS and ORAC assays, was not affected by cold plasma treatments (15 and 30 min). *E. coli* inoculated on radicchio leaves was significantly reduced after 15 min cold plasma treatment. However, a 30 min plasma treatment was necessary to achieve a significant reduction of *L. monocytogenes* counts. Atmospheric cold plasma appears to be a promising technology for the decontamination of leafy vegetables when applied under the optimised conditions.

**Key words:** cold plasma, decontamination, antioxidant activity, colour, *Listeria monocytogenes*, *Escherichia coli*
1. INTRODUCTION

In recent years vegetables are consumed more frequently due to their nutritional benefits. This has led to the development of a wide variety of minimally processed vegetable based products (Ramos et al., 2013). Commercially, fresh vegetables need to be decontaminated prior to packaging. Several chemical and physical technologies have been found to be efficient in reducing bacterial contamination in fresh vegetables (Parish et al., 2003). The majority of minimally processed fresh produce manufacturers use chlorine washing (50–200 mg/L). However due to the increasing safety concerns regarding the formation of potentially carcinogenic chlorinated compounds in water, and its demonstrated limited efficiency in reducing foodborne pathogens on fresh produce (Oliveira et al. 2012), alternative methods have been sought out by the food industry that can ensure safety and at the same time are environmentally friendly (Baur et al. 2004; Siroli et al. 2014).

Other chemical technologies including washing with organic acids (e.g. citric and ascorbic), hydrogen peroxide and application of ozone are also available. However, restrictions associated to low direct antibacterial activity, pH dependence, and influence on sensory parameters, have been reported (Ramos et al., 2013). On the other hand, physical non thermal technologies such as irradiation, ultraviolet light, pulsed light, high pressure processing, and ultrasound are considered more promising alternatives. Among these, cold plasma technology has drawn a lot of attention as a minimal processing technology (Olaimat & Holley 2012; Srey et al. 2014; Ziuzina et al. 2014). Cold plasma is produced by excitation of gas molecules through the use of electrical discharges. Molecules become ionised or dissociate by collisions with the background resulting in the production of a plasma (Gadri et al., 2000). The antimicrobial effect of cold plasma is the result of charged particles and reactive species present in the plasma that can cause DNA damage, breaking of chemical bonds, damages to the cell membrane which can lead to further penetration of reactive species into the cell (Fernández & Thompson 2012). Plasma ions could catalyse processes such as oxidation and peroxidation that take place inside the cell as well as in the external environment, and
result in inactivation (Dobrynin et al. 2009). Moreover, cold plasma efficiency also depends on biological parameters such as the type of substrate and microorganism characteristics (type, load, physiological state) (Moreau et al., 2008; Misra et al., 2011; Stratakos & Koidis, 2015). The decontamination efficiency of non-thermal gas plasma treatments has been evaluated against Gram-negative bacteria, Gram-positive bacteria, spores, yeasts, moulds and viruses (Montie et al., 2000). The first applications on agricultural products were conducted targeting foodborne pathogens such as *Escherichia coli*, Salmonella, and *Listeria monocytogenes* and spoilage organisms inoculated on the surface of fruits and vegetables; with results showing significant reductions depending on the treatment time and the technology used to produce the gas plasma (Critzer et al. 2007; Perni et al. 2008).

However, the application of this technology as a food decontamination method might have limitations in terms of the irreversible changes that might occur due to the interaction between the oxidative species and the product. Alterations of the nutritional and quality/sensory characteristics could potentially take place depending on the product characteristics and residues of the oxidation processes. Only recently, studies have started to investigate the effects of cold plasma on quality associated characteristics. Depending on time and exposure conditions, pigments can be affected by the treatment; changes in colour parameters of tomatoes and carrots as well as in the photosynthetic activity in cucumber and fresh corn salad leaves have been shown (Baier et al. 2013; 2014; 2015). Moreover, due to the nature of the cold plasma technology and the operating conditions, the antioxidant potential of the tested food sample has to be monitored. Reductions of antioxidant compounds (e.g. vitamin C) were observed on cold plasma treated cucumber (Wang et al., 2012), on the surface of *Abate Fetel* pear in terms of ABTS antioxidant capacity (Berardinelli et al., 2012), and on peel and pulp of *Fuji* apples as determined by the DPPH antioxidant assay (Gozzi et al., 2013).
Based on the above, further studies should be conducted in order to understand the role of the reactive species in the decontamination efficacy and chemical modifications on different foods. In addition, both microbiological and quality parameters during realistic storage conditions are essential in order to apply this technology on highly perishable products such as leafy vegetables. The present study explores the suitability of atmospheric cold plasma generated by means of a dielectric barrier discharge (DBD) device on the inactivation of *Listeria monocytogenes* and *Escherichia coli* experimentally inoculated on radicchio leaves (red chicory, *Cichorium intybus* L.).

Radicchio has been selected for a number of reasons. Firstly, it is part of many Ready-to-Eat meals so it is normally chlorine washed. Secondly, it has a delicate texture and a characteristic red colour which is challenging to maintain after any processing. This makes radicchio a benchmark and perhaps the best candidate for applications of alternative technologies such as cold plasma. Therefore, to complement this study, the effect of cold plasma on radicchio was also assessed in terms of visual and compositional parameters. Differently from previous efforts, the effect of storage was also taken into consideration.

2. MATERIALS AND METHODS

2.1 Gas plasma generator and vegetable treatments

Treatments were conducted at atmospheric conditions (at approximately 22 °C and 60 % of Relative Humidity, RH) by placing radicchio leaves samples at about 70 mm beneath the plasma emission generated between three couples of parallel plates electrodes made of brass (Figure 1). One electrode of each couple was covered by a 5 mm thick glass sheet according to a dielectric barrier discharge (DBD) configuration. The discharge was directed on the vegetable surface by three fans mounted over the electrodes powered by a DC power supply (input voltage of 19 V). The electrodes and the treated samples were confined inside a cabinet as described by Ragni et al. (2010). The chemical characterisation of the emission in the 200-450 nm wavelength range was
carried out by means of an optic fibre probe (Avantes, FC-UV400-2) placed at about 20 mm from the discharge and connected to a spectrometer (Avantes, AvaSpec-2048).

Radicchio also known as red chicory *(Cichorium intybus L.)* was purchased in bulk from local wholesalers (Cesena, Italy) and was used unwashed. Treatment times of 15 and 30 min were chosen after preliminary tests aimed at avoiding evident surface damages immediately after the treatment. Control samples were conditioned at the same atmospheric (temperature and relative humidity) and ventilation (about 0.5 m/sec on the vegetable surface) settings defined for the plasma tests.

2.2 Qualitative assessments

The layout of qualitative assessments conducted on radicchio leaves before and after the treatments (15 and 30 min) and during storage at 4 °C and 90 % of Relative Humidity is illustrated in Figure 2. Six radicchio leaves for each storage time (before and immediately after the treatment, and after further 2 h, 1 and 3 d) and each treatment time were evaluated.

2.2.1 Digital Image analysis

A digital camera model D7000 (Nikon, Shinjuku, Japan) equipped with a 60 mm lens mod. AF-S micro, Nikkor (Nikon, Shinjuku, Japan) was used to acquire digitalized images of radicchio leaves (exposition time ½ sec; F-stop f/16) placed inside a black box under controlled lighting condition. The digitalized images were analysed with Image Pro-Plus v. 6.2 (Media Cybernetics, USA). On the basis of the chromatic characteristics, two different pixel ranges were defined corresponding to “light red area” and “dark red area” for the samples evaluated until 2 h of storagae. For the samples stored for 1 day, a different data analysis was conducted because the leaves were very chromatically different from the other samples; two different pixel ranges were redefined corresponding to “dark red area” and “brown area”.

All pixels were then assessed by the model in terms of percentage of each area on the total.
2.2.2 Instrumental colour analysis

Instrumental colour measurements were conducted by means of a Minolta ChromaMeter CR-400 reflectance colourimeter (Minolta, Milan, Italy). For each acquisition, an average value of three measurements for each leaf taken at different spots was calculated. The CIELab system L*, a* and b* was considered (CIE, 1976) and the Chroma values were calculated as \( C^* = \sqrt{a^* + b^*} \). The acquisitions were performed on both white and red area of the radicchio leaves.

2.2.3 Sensory test

A hedonic test was conducted with 10 untrained assessors who scored the acceptability of 4 attributes (freshness, colour, odour and texture) using the following 1-5 point scale: 1) unacceptable, very poor, strong defects; 2) poor, major defects; 3) fair, acceptable defects; 4) good, acceptable defects; 5) typical attribute, very good without defects. In addition, ‘overall acceptability’ was assessed using a 1-9 point scale ranged from 1 (dislike extremely) to 9 (like extremely). A total of 6 different samples were presented to assessors (four cold plasma treated samples for both treatment times and two respective controls) at 0, 1 and 3 days of storage. All test samples were appropriately randomised to avoid bias.

2.2.4 Antioxidant activity assays

Treated and not treated radicchio samples were freeze-dried just after the treatments and then analysed for ABTS radical-scavenging activity and oxygen radical absorbance capacity (ORAC). The ABTS assay is based on the discolouration of the radical cation 3-ethyl-benzothiazoline-6-sulfonic acid (ABTS•+; Sigma, UK.). The procedure was performed according to Miller et al. (1993) and as improved by Re et al. (1999). The ABTS•+ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand overnight in the dark at room temperature. The radical remained stable for 48 h when stored in the dark at room
temperature. A working solution of the ABTS$^{•+}$ was prepared by diluting the radical stock solution in 80% methanol to an absorbance of 0.700 ± 0.020 nm at 734 nm. Radicchio extract was obtained by vortexing 0.5 g freeze dried radicchio in 10 mL 80% methanol at 2500 rpm for 20 min and centrifuged for 10 min at 3800 rpm. Radicchio extract (20 μL) was added to 980 mL of ABTS$^{•+}$ solution and incubated under dark at room temperature. Absorbance was measured at 734 nm after 10 min reaction. A calibration curve was constructed using Trolox (6-hydroxy-2,5,7,8-tetramethylochroman-2-carboxylic acid). All measurements were carried out three times, and in duplicate. The results are expressed as μmol Trolox equivalents per g of dried weight.

The ORAC assay was performed according to Huang et al. (2005) with some modifications. 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH; Sigma, UK.) was completely dissolved in 75 mM phosphate buffer (pH 7.4) to a final concentration of 369 mM. Fluorescein stock solution (4.19 μM) was made in 75 mM phosphate buffer (pH 7.4). A 0.586 μM fluorescein working solution was made fresh before analysis by further diluting the stock solution in 75 mM phosphate buffer. Trolox dissolved in 75 mM phosphate buffer (pH 7.4) was used to build the calibration curve. The same radicchio extracts were used as the ABTS assay. The procedure was as follows: 25 μL of extracts/blank/standard were added to a 96 well plate, subsequently 100 μl of fluorescein working solution was added to all wells. The plate was then heated to 37°C for 30 min. After the incubation, 75 μl of AAPH were added and the fluorescence of the samples was recorded for 100 min at 2 min intervals using a plate reader (Teca, Safire 2190, UK). Excitation wavelength was set at 485 nm and emission wavelength at 530 nm. ORAC values were calculated using the areas under the fluorescein decay curves (AUC), between the blank and the sample, using the following equation. Results were expressed as μM Trolox equivalents (TE) per g of dried weight.

\[
AUC = 0.5 + f_1/f_0 + \ldots f_i/f_0 + \ldots + f_{99}/f_0 + 0.5(f_{100}/f_0)
\]

where: \(f_0\) = initial fluorescence reading at 0 min and \(f_i\) = fluorescence reading at time \(i\).

2.3 Microbiological assessments
Radicchio samples were experimentally contaminated with a cocktail of five *Listeria monocytogenes* strains (LR 102, 0227-359, VI 51028, 0113-131 and VI51010) or *Escherichia coli* (O157:H7 VTx, Thermo Fisher NTCT12900). Cultures were grown at 37°C using brain heart infusion (BHI, Thermo Fisher, Milan, Italy) and tryptic soy broth (TSB, Thermo Fisher) for *L. monocytogenes* and *E. coli*, respectively.

2.3.1 *Listeria monocytogenes*

One hundred microliters of a cellular suspension of an OD 0.08-0.1 at 625 nm of the *L. monocytogenes* cocktail in physiological saline (NaCl 0.9%) were spotted on the surface of the radicchio samples (4 x 4 cm). After inoculation, the leaves were stored under laminar flow in a biohazard cabinet for 30 min in order to let the inoculum dry. After each treatment (15 and 30 min) and after 3 days of storage at 4°C and 90% RH, each radicchio leaf was transferred into 160 mL of Buffer Peptone Water (BPW; Thermo Fisher, Milan, Italy) and homogenised by a Stomacher® (Seward, UK) for 2 min at normal speed. After one hour of storage at room temperature, serial ten-fold dilutions were performed and plated onto Thin Agar Layer (TAL) plates for colony counting. The TAL method involves overlaying 14 mL of nonselective medium (Tryptic Soy Agar, TSA, Thermo Fisher) onto a prepoured, pathogen-specific, selective medium in order to allow the recovery of sub-lethally injured cells (Wu and Fung, 2001). In the present study *L. monocytogenes* enumeration was performed on Agar Listeria according to Ottaviani and Agosti (ALOA, Biolife, Milan, Italy) overlayed with 14 mL of TSA. TAL plates and BPW were incubated for 24 h at 37°C. Upon observation of no colonies, the ISO 11290 was performed from the enriched BPW for qualitative assessment of the presence/absence of *L. monocytogenes* in the sample.
2.3.2 Escherichia coli

The bactericidal effect of gas plasma on *E. coli* was assessed in triplicate after 15 min of treatment using the most probable number counting methods to enumerate the surviving bacteria on the surface of radicchio leave samples. This experiment was set up to control the presence of interfering background flora that can also include other *E. coli* strains. The use of selective supplements such as antibiotics, tellurite and bile salts could inhibit bacterial cells exposed to the gas plasma treatment. Three series of eight ten-fold dilutions of a microbial suspension with OD 0.08-0.1 at 625 nm of *E. coli* colonies in physiological saline (NaCl, 0.9%) containing approximately 8 log CFU/mL were used for the inoculum of radicchio leave samples (1 cm x 1 cm). After treatment, all samples were transferred in tubes containing 10 mL of BPW and homogenized for 1 min, then the tubes were incubated at 37 °C for 24 h. These cultures were seeded on the surface of Sorbitol MacConkey agar supplemented with cefixime (0.05 mg/L) and tellurite (2.5 mg/L) (CT-SMAC, Thermo Fisher) and the agar plates were incubated overnight at 37 °C. Sorbitol non-fermenting colonies were assessed with latex agglutination test (*E. coli* O157 Latex Test Kit, Thermo Fisher). The BPW tubes containing viable *E. coli* O157 were considered positive and on this basis, the most probable number (MPN) of *E. coli* was assessed using MPN tables (USDA-FSIS, 2013).

2.4 Data analysis

Significant differences (*p*-level < 0.05) between subgroups (control and treated samples and during storage) were determined by analysis of variance (ANOVA), Tukey test as used for post hoc comparisons. All analysis was conducted with SPSS 22.0 (IBM, Somers, New York).

3. RESULTS AND DISCUSSION

3.1 Emission characterisation
Irradiance values of the atmospheric dielectric barrier discharge emission show typical peaks of the second N\textsubscript{2} positive system (\(\lambda = 290-440\) nm, transition between \(C^3\Pi_u\) and \(B^3\Pi_g\) electronic states) and of the positive ion N\textsubscript{2}\textsuperscript{+} (\(\lambda = 391.4\) nm transition between \(B^3\Sigma_u^+\) and \(X^3\Sigma_g^+\), as expected for air non-equilibrium discharges. The generation of NO (\(\gamma\) systems, transition between \(A^3\Sigma^+\) and \(X^3\Pi\)) and OH radicals was also respectively detected at \(\lambda = 226-248\) and \(\lambda = 305-309\) nm. As previously described the presence of NO and OH radicals play an important role in microbial decontamination (Laroussi and Leipold, 2004).

3.2 Qualitative assessments

3.2.1 Image and colour analyses

Results of digital image analysis, in terms of mean values of the calculated dark red area, are reported in Table 1. For control samples, no significant differences emerged during storage for both 15 and 30 min. For treated samples, significant increments in terms of dark red area were observed after a further 1 day of storage (with respect to “before treatment samples”: about 20.8% and 35.5% for 15 and 30 min, respectively). Immediately and after the first 2 h from the treatments, no significant changes on the radicchio leaves could be observed. These results can be seen in Figure 4.

Results of the colour measurements, in terms of Chroma (C\textsuperscript{*}), are summarised in the Table 2. C\textsuperscript{*} was selected because is considered the quantitative expression of colourfulness perceived by consumers (Pathare et al., 2013). In relation to the white area, no significant differences were observed for all samples. On the other hand, in relation to the red area of the treated samples (15 and 30 min) a significant decrease of the C\textsuperscript{*} values was observed during storage. For the control samples, this parameter showed a slight but not significant decrease (\(p>0.05\)) during storage. Changes in colour characteristics have also been found to take place in carrot and cucumber slices after a microjet cold plasma treatment (Wang et al. 2012).
The results obtained by digital image and instrumental colour analyses are in agreement with previous studies carried out on lettuce leaves. Although different methods to generate the ionized gas and different storage times were used, results suggested that the treatment can induce an irreversible damage to the cellular structure of lettuce leaves (Grzegorzewski et al., 2011; Bermúdez-Aguirre et al., 2013). Accordingly in the present study, a surface erosion of radicchio leaves caused by oxidation of cell components can be hypothesized. This hypothesis is in line with the visual observation of treated leaves after 1 day of storage (Figure 4).

3.2.3 Sensory evaluation

The mean scores of the organoleptic analysis are reported in Table 3. The results show that, after one day of storage, the treated samples were significantly different (P<0.05) from the control samples. The mean scores for both control and plasma treated samples for freshness, colour, odour, texture and overall acceptability decreased significantly during storage. Although, the scores were significantly reduced for control samples during storage illustrating the very perishable nature of the radicchio leaves, treated samples showed even lower score. The results from the sensory evaluation are consistent with the decreased of C* values observed during storage.

3.2.4 Antioxidant activity

The study of the interactions between plasma and food bioactive compounds is still at early stages. Radicchio is rich in phenolic compounds, caffeic acid derivatives, chlorogenic acid, and some flavonoids (Di Venere et al., 2005; Koukounaras & Siomos, 2010). The results from both ABTS and ORAC antioxidant assays showed that, cold plasma at either treatment times (15 or 30 min) did not cause any significant decrease in the antioxidant activity of polar fraction of the radicchio leaves (Table 4). The differences observed between the two assays can be attributed to the different principle on which they work (Zulueta et al., 2009). While ABTS is an electron transfer method, ORAC is based on hydrogen atom transfer in which antioxidant and substrate compete for peroxyl
radicals (Cilla et al., 2011). Although the polar profile of the radicchio extracts was not chromatographically analysed in this study since no significant changes were observed, literature suggests that individual polar components can vary and results are quite treatment dependent. In lettuce, for example, low pressure O₂ plasma treatment resulted in two-fold increase in the protocatechuic acid, luteolin, and disometin (Grzegorzewski et al., 2010) as determined by HPLC where Ar plasma treatment resulted in decrease in phenolic acids such as protocatechuic acid and chlorogenic acid. However, HPLC analysis of flavonoids such as luteolin and diosmetin remained in the same levels or significantly increased after treatment (Grzegorzewski et al., 2011). Different mechanisms have been proposed to explain the changes in the content of individual antioxidant compounds of the polar fraction (enhanced extractability due to penetration or favoured biosynthesis due to UV-B radiation) and the matter is under investigation (Grzegorzewski et al., 2010; 2011). In essence, the presence of multiple reactive species in cold plasma render the investigation of its effect on total antioxidant activity difficult as synergistic actions and several different reaction pathways may take place. Although in this study plasma treatment did not appear to negatively affect the antioxidant activity of the radicchio leaves, further mechanistic studies need to be conducted in order to understand the interactions between plasma and the antioxidant components.

3.3 Microbiological assessments

In several countries Listeria monocytogenes and Escherichia coli O157:H7 have been implicated in several food poisoning incidents resulting in serious illnesses and even deaths (Rangel et al. 2005; Olaimat and Holley, 2012). The results on L. monocytogenes survival after atmospheric cold plasma treatment and after 3 days of storage, are reported in Table 5. The 15 min treatment was not effective in significantly reducing the initial L. monocytogenes counts on radicchio leaves (P >0.05). However, a reduction of approximately 2.20 log CFU/cm² of L. monocytogenes counts was
observed immediately after the 30 min treatment in comparison to controls. Storage results confirmed the decontamination effect of cold plasma on *L. monocytogenes* experimentally contaminating radicchio leaves after 30 minutes of treatment. In particular, the log reduction was maintained all over the storage period with no occurrence of re-growth.

Ziuzina et al. (2014) found that treatment of strawberries with cold plasma generated by a dielectric barrier discharge system treatment time for 5 min resulted in a reduction of *L. monocytogenes* counts by 4.2 log CFU/ sample. Furthermore, a significant reduction in the number of *E. coli* surviving cells was observed (-1.35 log MPN /cm² passing from 6.32 (CI95% 5.35-4.64) to 4.97 (CI95% 4.25-5.62) log MPN /cm²), for the 15 min treatment. Similar results were found by Bermúdez-Aguirre et al. (2013) who reported reductions in *E. coli* counts of 1.5 and 1.7 log CFU in lettuce and tomato, respectively after a 10 min cold plasma treatment. The results presented here illustrate the decontamination efficiency of the cold plasma on radicchio immediately after treatment and confirms previously reported results on other fruits and vegetables as cucumber, carrot and pear slices experimentally contaminated by *Salmonella* (Wang et al., 2012). Reductions of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* counts have also been reported for apples and lettuce (Misra et al. 2012). For *E. coli*, the presence of sub-lethally injured cells (not culturable) due to the cold plasma treatment should be excluded, since the long enrichment in a non-selective culture medium (i.e. BPW for 24 h) can allow their recovery. Consequently, these cells could not recover or grow during storage on radicchio leaves. The fact that a 30 min treatment was needed to obtain a significant reduction in *L. monocytogenes* counts whereas a 15 min treatment was enough for *E. coli* could imply that Gram positive bacteria, such *L. monocytogenes*, are less susceptible to cold plasma treatment compared to Gram negative ones. This is consistent with the study of Fröhling et al. (2012) who found, using membrane integrity measurements, that different modes of plasma action exist against Gram-positive bacteria and Gram-negative bacteria.

The treatment applied was able to significantly reduce but not eliminate the bacterial pathogens inoculated on the surface of radicchio leaves. However, in this study a worse case scenario was
adopted (initial load of approx. $10^4$-$10^5$ CFU/cm$^2$) whereas usually a load of maximum $10^2$ CFU/cm$^2$ (Crépet et al., 2007) is present on the surface of leafy vegetables. Therefore, the treatment could be effective in eliminating the pathogenic microorganisms although further experiments need to be performed to confirm this.

4. CONCLUSIONS

The present work presents the results of a critical study conducted on the efficiency of atmospheric cold plasma technology in the decontamination of radicchio leaves. Results indicate maximum significant reductions of 1.35 log MPN/cm$^2$ for *E. coli* (15 min of treatment) and approx. 2 log CFU/cm$^2$ for *L. monocytogenes* (30 min treatment). These reductions can be considered promising in terms of safety considering that this kind of product can be characterised by a maximum contamination load of $10^2$ CFU/cm$^2$. In relation to the possible effects caused by the interaction of reactive species with the product, the treatments appeared to negatively affect the quality of the leaves during storage. Although immediately after the treatment and after 2 h of storage, no quality defects could be observed, a significant impact in terms of visual quality was observed after 1 day of storage with respect to the control. The nutritional quality of the radicchio leaf, if conventionally expressed here as the antioxidant capacity of its polar fraction, remained relatively intact after the cold plasma treatments. Further evaluation of nutritional compounds need to be considered also in relation to the storage. Since the cold plasma system described in this study operates in open air and does not require water, it could be easily incorporated in existing food production lines. In conclusion, based on our results, atmospheric cold plasma treatment is a promising technology for the decontamination of radicchio leaves and further optimisation needs to be undertaken to reduce or remove the negative effect on quality.
Acknowledgements

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Figure 1. Atmospheric gas plasma treatments of radicchio leaves.
Figure 2. Layout of the qualitative assessments.
Figure 3. Irradiance values of the emission acquired at about 20 mm from the discharge (input voltage: 19 V). Values in brackets refer to vibrational transition ($\nu' \rightarrow \nu''$).
Figure 4. Images of radicchio leaves treated for 30 min and relative control during 1 day of storage at 4°C and 90% of R.H.
Table 1. Mean values of the dark red area of the radicchio.

<table>
<thead>
<tr>
<th>Treatment time (minutes)</th>
<th>Sample</th>
<th>STORAGE TIME</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>2 h</th>
<th>1 day</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>C</td>
<td>72.7 (5.5)^a</td>
<td>72.4 (5.5)^a</td>
<td>75.3 (3.1)^a</td>
<td>76.5 (8.2)^a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>79.7 (3.8)^a</td>
<td>78.5 (4.9)^a</td>
<td>79.6 (2.7)^a</td>
<td>19.7 (9.2)^b</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>C</td>
<td>73.0 (3.7)^a</td>
<td>72.7 (3.7)^a</td>
<td>75.2 (2.5)^a</td>
<td>74.9 (6.1)^a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>72.7 (6.2)^a</td>
<td>78.1 (5.8)^a</td>
<td>76.1 (7.3)^a</td>
<td>16.6 (7.9)^b</td>
<td></td>
</tr>
</tbody>
</table>

Note: C: control, T: treated (standard deviation in brackets). The same lowercase letters denote no significant differences during storage, within the same sample, control or treated and the same treatment time (Tukey HSD test, p < 0.05).
### Table 2. Instrumental colour (C*) values of cold plasma treated radicchio leaves.

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Sample</th>
<th>Radicchio area</th>
<th>STORAGE TIME</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>2 h</td>
<td>1 day</td>
</tr>
<tr>
<td>15</td>
<td>White</td>
<td>5.5 (0.5)a</td>
<td>5.4 (0.6)a</td>
<td>5.8 (0.4)a</td>
<td>6.1 (0.6)a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>28.3 (1.9)a</td>
<td>26.5 (2.4)b</td>
<td>25.8 (1.9)b</td>
<td>25.3 (1.6)b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>4.3 (0.6)a</td>
<td>4.3 (0.4)a</td>
<td>4.2 (0.6)a</td>
<td>4.2 (0.7)a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>29.1 (1.5)a</td>
<td>20.6 (4.2)b</td>
<td>17.6 (2.7)c</td>
<td>14.9 (0.7)d</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>White</td>
<td>5.3 (0.9)a</td>
<td>5.5 (1.2)a</td>
<td>5.0 (0.7)a</td>
<td>5.2 (0.5)a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>25.5 (4.5)a</td>
<td>23.4 (3.6)ab</td>
<td>22.4 (3.3)b</td>
<td>21.7 (3.3)b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White</td>
<td>4.2 (0.7)a</td>
<td>4.0 (0.6)a</td>
<td>4.4 (0.4)a</td>
<td>4.9 (1.3)a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>25.8 (1.5)a</td>
<td>19.5 (4.3)b</td>
<td>17.7 (2.4)bc</td>
<td>15.6 (2.1)c</td>
<td></td>
</tr>
</tbody>
</table>

Note: C: control, T: treated (standard deviation in brackets). The same lowercase letters, in the same row, denote no significant differences during storage, within the same sample, control or treated and the same treatment time (Tukey HSD test, p < 0.05).
Table 3. Sensory analysis of cold plasma treated radicchio leaves stored for 3 days.

<table>
<thead>
<tr>
<th>Treatment time (minutes)</th>
<th>Storage time (days)</th>
<th>Freshness</th>
<th>Colour</th>
<th>Odour</th>
<th>Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5(0)a</td>
<td>5(0)a</td>
<td>5(0)a</td>
<td>5(0)a</td>
<td>5(0)a</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>4(0)b</td>
<td>2(0)b</td>
<td>3.6(0.5)b</td>
<td>2.6(0.2)b</td>
<td>3.8(0.6)b</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4(0)b</td>
<td>2(0)b</td>
<td>3.7(0.6)b</td>
<td>2.1(0.3)c</td>
<td>3.7(0.5)b</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5(0)a</td>
<td>5(0)a</td>
<td>5(0)a</td>
<td>5(0)a</td>
<td>5(0)a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4(0)b</td>
<td>1.1(0.3)b</td>
<td>3.6(0.5)b</td>
<td>2.1(0.3)b</td>
<td>3.8(0.6)b</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4(0)b</td>
<td>1.1(0.3)b</td>
<td>3.7(0.6)b</td>
<td>1.1(0.3)c</td>
<td>3.7(0.5)b</td>
</tr>
</tbody>
</table>

Note: C: control, T: treated (standard deviation in brackets). The same lowercase letters denote no significant differences during storage, within the same sample, control or treated and the same treatment time (p < 0.05).
Table 4. Effect of cold plasma on the antioxidant activity of radicchio leaves assessed by ABTS and ORAC values (μM TE/g dried weight) (n=6).

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Sample</th>
<th>ABTS</th>
<th>ORAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>193(22)\textsuperscript{a}</td>
<td>98(1)\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>T</td>
<td>219(8)\textsuperscript{a}</td>
<td>117(5)\textsuperscript{a}</td>
</tr>
<tr>
<td>30</td>
<td>T</td>
<td>213(18)\textsuperscript{a}</td>
<td>97(18)\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Note: C: control, T: treated (standard deviation in brackets). The same lowercase letters denote no significant differences between control and treated samples at the same treatment time (p < 0.05).

Table 5. *L. monocytogenes* survival on the cold plasma treated radicchio leaves.

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Sample</th>
<th>Log CFU/cm\textsuperscript{2}</th>
<th>After the treatment</th>
<th>After 3 days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>C</td>
<td>5.92(0.16)\textsuperscript{a}</td>
<td>5.85(0.14)\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>5.59(0.30)\textsuperscript{b}</td>
<td>5.87(0.16)\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>C</td>
<td>4.17(0.21)\textsuperscript{a}</td>
<td>3.49(0.66)\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>1.96(0.16)\textsuperscript{b}</td>
<td>1.21(0.56)\textsuperscript{b}</td>
<td></td>
</tr>
</tbody>
</table>

Note: C: control, T: treated (standard deviation in brackets). The same lowercase letters denote no significant differences between control and treated samples at the same treatment time (p < 0.05).