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Application of cold plasma on fresh cut carrots for the inactivation of *Arcobacter* spp., an emerging food-borne zoonotic pathogen

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ABSTRACT

Cold plasma is a promising non-thermal technology that has the potential to inactivate microorganisms in food. In this study, the inactivation of two strains of *Arcobacter butzleri* (BZg74 and BZs206) artificially inoculated onto freshly cut carrots was investigated using two different configurations: Cold Atmospheric Plasma (CAP), Plasma Activated Water (PAW). Contamination levels of both the strains were significantly reduced. *A. butzleri* BZg74 showed a higher sensitivity, with a reduction of more than 2 log CFU/g after 20 min and values below the detection limit reached after 30 min of CAP treatment. On the other hand, a maximum reduction of 2 log CFU/g was achieved for the strain BZs206 after 30 min. As for the indigenous microbiota, CAP treatments resulted in a significant reduction of 1.5 log for mesophiles (20 min) and for Enterobacteriaceae (30 min). In contrast, washing with PAW led to an immediate (5 min) and significant effect of 1.3 log CFU/g reduction against the mesophilic population. In terms of product quality, the CAP treatment showed a stronger effect, reflected in a deterioration of colour, pH and dry matter with longer treatment times, while the PAW treatment allowed better preservation. The inactivation of peroxidase activity was strongly dependent on the type of configuration, confirming the complexity of the interaction of plasma reactive species with food matrices and the need for specific adaptation of processing parameters depending on the impact on the quality characteristics of the product.

1. Introduction

Fresh-cut fruit and vegetables are minimally processed foods that have been washed, peeled, cut into a usable form, and packaged to remain fresh and convenient for immediate consumption. Due to changing lifestyles, the consumption of fresh-cut produce has increased over the last ten years. However, because of damage occurring during handling and processing, these products spoil faster than intact produce (Perinban et al., 2022). Moreover, cutting exposes internal tissues, increasing susceptibility to microbial contamination, enzymatic degradation, and spoilage (Dar et al., 2020). Produce surfaces can also become contaminated before harvest by pathogenic microorganisms originating, for example, from manure, compost, contaminated soil, or irrigation water (Black et al., 2021).

According to the European Union One Health 2023 Zoonoses Report (published in 2024), the most common foodborne diseases are caused by *Campylobacter*, *Salmonella*, *Yersinia*, *Escherichia coli*, and *Listeria*. Ready-to-eat foods and unpasteurized fruit and vegetable juices were among the food matrices reported as positive for *Salmonella*, *E. coli*, and *Listeria monocytogenes*. Accordingly, decontamination strategies for ready-to-eat vegetables have largely focused on these pathogens, together with the endogenous microbiota.

Less attention has been paid to emerging pathogens that are increasingly recognized as transmissible to humans through contaminated fruit and vegetables, such as *Arcobacter* species (Molina-Hernandez et al., 2025). Bacteria of the genus *Arcobacter* are Gram-negative, slightly curved rods that may present a polar or bipolar flagellum/flagellum (Dieguez et al., 2017; Kietsiri et al., 2021). The pathogenic potential of *Arcobacter* spp. is associated with diarrhoeal disease and occasional systemic infections in humans, such as bacteraemia and peritonitis. Among these, *Arcobacter butzleri* has been listed as a serious human health hazard by the International Commission on

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Microbiological Specifications for Food (ICMSF), also considering that multidrug resistance has been reported in *A. butzleri* isolates from several sources (Buzzanca et al., 2024; Chiarini et al., 2024).

The most probable route of transmission to humans is the consumption of contaminated food and water; for fruits and vegetables, contamination is mainly linked to irrigation water and contaminated soil (Alegbeleye & Sant'Ana, 2020). Nevertheless, data on the occurrence of *Arcobacter* spp. in vegetables remain scarce, particularly for ready-to-eat products (González et al., 2017; Mottola et al., 2016; Mottola et al., 2021). Similarly, only a limited number of studies have evaluated the effectiveness of decontamination strategies against this emerging pathogen.

Among innovative strategies for microbial decontamination of fresh and fresh-cut produce, cold plasma has shown promising potential as a non-thermal and environmentally friendly technology. Plasma, often referred to as the fourth state of matter, is defined as a partially or fully ionised gas consisting of atoms in ground and excited states, ions, photons, and free electrons, while maintaining an overall neutral charge (Misra et al., 2011). Cold plasma can be generated using various electrical discharge methods, each exhibiting distinct characteristics suitable for specific processing requirements. The most common configurations in food research are plasma-activated water (PAW) and cold atmospheric plasma (CAP). (Liu et al., 2025). The selected configuration significantly influences the composition and flux of active species, thereby affecting treatment efficacy and its appropriateness for particular food matrices and contamination scenarios. The antimicrobial effectiveness of cold atmospheric plasma (CAP) is mainly attributed to physical damage to microbial cells and to the interaction between reactive chemical species generated during discharge and cellular components (Shishir et al., 2025). Under atmospheric pressure, cold plasma produces reactive oxygen and nitrogen species (RONS), enabling residue-free processing with relatively short treatment times (Zhang et al., 2021). When water or another liquid medium is exposed to cold atmospheric plasma, reactive species in the gas phase interact with the liquid and generate additional RONS in the aqueous phase, including OH, H_2O_2, HO_2, nitrite (NO_2^-), nitrate (NO_3^-), peroxynitrite ($OONO^-$), peroxynitrous acid (ONOOH), nitrous acid (HNO_2), and nitric acid (HNO_3) (Chuea-uan et al., 2023; Kosumsupamala et al., 2022; Poramapijitwat et al., 2020; Thana et al., 2020). The concentration of these species in the plasma activated water (PAW) can be modulated by adjusting plasma generation parameters such as voltage, carrier gas, temperature, pulse frequency, and treatment duration (Soni et al., 2021). However, depending on the operating conditions, a substantial fraction of reactive species may be converted into longer-lived aqueous compounds, which can limit PAW reactivity and, consequently, antimicrobial efficacy. Direct CAP application is not always feasible, and its microbial inactivation efficacy depends on multiple factors, including plasma source type, geometry, power delivery, gas composition, humidity, temperature, and the texture of the target material. In particular, uneven and rough surfaces may reduce inactivation efficiency due to the non-uniform distribution of reactive species (Mentheour et al., 2022). In contrast, PAW treatment offers greater versatility, as water acts as a medium that can reduce direct surface damage from charged particles, ultraviolet radiation, heat, and electricity. In addition, PAW can be more easily applied to produce of different sizes and shapes, and to matrices with variable surface roughness.

From an industrial point of view and considering scaling up issues, PAW appears more suitable to be integrated in already existing washing steps. Misra et al. (2024) described an industrial-scale continuous Plasma Activated Water (PAW) system, specifically designed for the fresh produce industry and optimized for micro to small enterprises that allows for continuous operations. On the other side, CAP showed some interesting results regarding the in-package treatment, a method that can be performed at atmospheric conditions, leads to reduced equipment costs, reduced energy consumption, improved operating convenience and it allows the reduction of the risk of post-processing contamination.

In the food sector, cold plasma has proven to be effective in inactivating indigenous bacteria, yeasts, and moulds on fresh and ready-to-eat fruit and vegetables (Ansari et al., 2022; Laurita et al., 2021; Sharma et al., 2025; Tappi et al., 2016). It has also been investigated for limiting biofilm formation and for inactivating spores (Molina Hernandez et al., 2022a, 2022b, 2023). Nevertheless, effectiveness can vary widely depending on several parameters, including the type and level of contamination, the physiological state of the microbial population, feed gas and plasma generation conditions, product surface properties, and treatment time, among other factors (Mao et al., 2021).

While extensive literature testing cold plasma technology to improve food safety is available, being *E. coli*, *Salmonella* and *Listeria monocytogenes* the mostly widely addressed foodborne pathogens, to our knowledge, the effect of cold plasma on *Arcobacter* spp. has never been investigated. Therefore, this study aimed to apply cold plasma both as a gas (cold atmospheric plasma, CAP) and as plasma activated water (PAW) to fresh-cut carrots deliberately inoculated with two *Arcobacter butzleri* strains. The inactivation efficacy was compared with that observed for a reference pathogen (i.e., *Escherichia coli*), and the impact on product quality was also assessed.

2. Material and methods

2.1. Raw material preparation

Whole fresh carrots (*Daucus carota* L.) were purchased from a local supermarket in Cesena (Italy), stored at 5 °C, and used within 2 days. Carrots had an average mass of 478.55 ± 11.12 g per carrot and a total soluble solids (TSS) content of approximately 13.0°Brix. All chemicals used in this study were of analytical grade.

To evaluate the inactivation of inoculated microorganisms, whole carrots were washed with sodium hypochlorite (30 ppm for 2 min) and air-dried for 10 min in a chamber before processing. Each carrot was manually peeled and cut into julienne strips using a vegetable cutter (GAM International, model TVEC UJMN230p, Igea Marina, Italy). Samples were prepared fresh each morning and then inoculated with the target pathogens.

To assess the effect of plasma treatments on the indigenous microbiota and product quality, unwashed carrots were processed under the same conditions.

2.2. Bacterial strains and inoculum preparation

Two strains of *Arcobacter butzleri* (BZg74 and BZs206), provided by the Department of Agricultural, Forest and Food Sciences (University of Turin, Italy), and one strain of *Escherichia coli* (NCFB 555), from the Department of Agricultural and Food Sciences (University of Bologna, Italy), were used in this study. The *A. butzleri* strains had been previously isolated from chicken skin and intestines, whereas the *E. coli* strain had been isolated from raw milk.

Both *A. butzleri* and *E. coli* strains were grown overnight in Brain Heart Infusion broth (BHI; Oxoid) at 37 °C, reaching a final concentration of approximately 10^9 colony-forming units (CFU)/mL. Cultures were then diluted and resuspended in sterile saline solution (0.9% NaCl) to obtain an inoculum of about 5 log CFU/mL for each strain.

The concentration of each suspension used for sample inoculation was confirmed by plating appropriate serial dilutions on BHI agar and incubating at 37 °C for 24 h.

2.3. Carrot inoculation

For each strain, batches of approximately 500 g of shredded carrots, prepared as described in Section 2.1, were inoculated by immersion into 1.5 L of the cell suspension (~10^5 CFU/mL) for 2 min. After inoculation, carrots were drained and air-dried under a laminar flow hood at

25 °C for 1 h, turning the product every 15 min to ensure uniform drying, before plasma treatments. The inoculated carrots had an initial microbial load of approximately 5 log CFU/g.

2.4. Gaseous Cold Atmospheric Plasma (CAP) treatment

A detailed description of the plasma system has been provided elsewhere (Molina-Hernandez et al., 2023; Molina-Hernandez, Capelli, et al., 2022); however, the key features are summarised here for completeness. Treatments were carried out using a surface dielectric barrier discharge (SDBD) plasma source operated in ambient air and powered by a high-voltage generator (Alma Pulse, Alma Plasma s.r.l.) (Fig. 1).

The SDBD unit consisted of four rectangular high-voltage electrodes (115 cm^2 each). A 2 mm thick mica sheet served as the dielectric barrier, while a mesh electrode in contact with the dielectric acted as the grounded electrode. This mesh also functioned as the lid of a closed box, creating a confined treatment atmosphere. The box had an internal volume of 18.5 L and a maximum height of 20 cm.

The high-voltage generator delivered a sinusoidal waveform with a peak voltage of 6 kV and a repetition frequency of 23 kHz. The power absorbed by the plasma source was 425.35 ± 25.79 W, corresponding to a surface power density of 2.6 W/cm^2 and to a transitional NO_x regime.

Treatments were carried out at room temperature (26 ± 1 °C). A 55 g portion of fresh-cut (julienne) carrots (maximum chamber capacity: 200 g) was uniformly distributed on a metallic mesh to obtain a homogeneous treatment surface. The sample holder was positioned 4 cm below the SDBD electrodes (perpendicular arrangement) and exposed to CAP for 5, 10, 20, or 30 min.

The electrode-to-sample distance and sample mass were selected based on preliminary experiments aimed at maximising microbial inactivation. During CAP treatments, the temperature inside the chamber did not exceed 36 °C in the NO_x regime at a 4 cm distance from the plasma source (Laika et al., 2024). This temperature is well below the reported lethal temperature for *Arcobacter* spp. (50 °C) (D'sa & Harrison, 2005). After treatments, samples were placed into polyethylene bags and stored at 5 °C until analysis.

Each treatment was performed in duplicate in two independent experiments. In addition to inoculated samples, uninoculated julienne carrots were treated under the same operating conditions.

2.5. Plasma activated water (PAW) treatments

Plasma activated water (PAW) was prepared by treating 500 mL of sterile distilled water (SDW) with a pulsed corona discharge for 1 min, generated by a high-voltage power supply (AlmaPulse, AlmaPlasma s.r.l., Bologna, Italy) operating at a maximum voltage of 18 kV and a pulse repetition frequency of 5 kHz (Laurita et al., 2021). Plasma was generated at the water surface within the 5 mm air gap between the tip of the stainless-steel working electrode and the liquid surface. The reaction chamber was not hermetically sealed; therefore, the discharge was produced in ambient air under static conditions (Fig. 2).

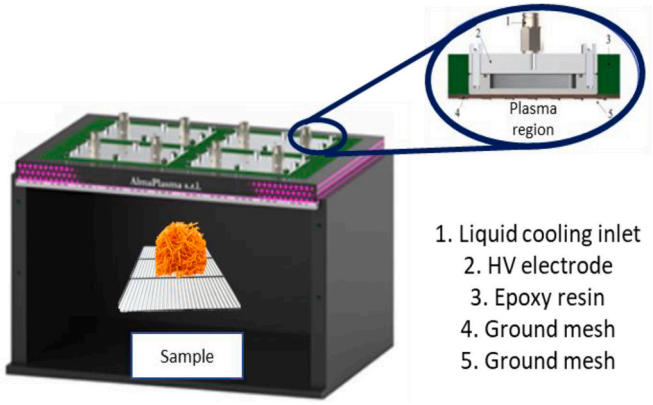

Fig. 1. Surface dielectric barrier discharge (SDBD) used in this study.

PAW parameters (pH and electrical conductivity) were measured according to Laurita et al. (2021). The resulting 500 mL of PAW was used to wash julienne carrots on an orbital shaker at 250 rpm for 5, 10, 20, or 30 min. The carrot-to-liquid ratio was kept constant at 1:20 (*w/v*).

The sample:PAW ratio was selected based on preliminary experiments to maximise microbial inactivation and to approximate ratios commonly used in industrial washing processes for fresh-cut products. Each treatment was performed in duplicate. In addition to inoculated samples, uninoculated carrots were treated under the same operating conditions.

2.6. Analytical determination

2.6.1. Characterization of reactive species in CAP and PAW

The quantitative determination of species concentrations was performed by optical absorption spectroscopy (OAS), following the Lambert–Beer law, as described in our previous studies (Molina-Hernandez et al., 2023; Molina-Hernandez, Capelli, et al., 2022):

$$\frac{I}{I_0} = e^{(-L\sigma n)} \quad (1)$$

where the absorber concentration (n) is related to the light attenuation over an optical path length (L) and is expressed through the ratio between the incident light intensity (I_0) and the transmitted light intensity after absorption (I). The absorption cross-section, σ, depends on the wavelength (σ = σ(λ)). In the present experiments, the optical path length was 25 cm.

The wavelengths selected for the analysis and the corresponding absorption cross-sections for O_3 and NO_2 are reported in Table 1. Wavelengths were chosen according to Moiseev et al. (2014) to maximise absorption by the target species while minimising interference from other absorbing molecules.

For all experiments, the optical path length (L) was 25 cm. Contributions from background radiation and spontaneous plasma emission were accounted for during data processing and subtracted from the acquired I and I_0 signals. Each experiment was performed three times, and results are reported as mean ± standard deviation.

At increasing power densities, nitrogen oxides formation is favoured, which promotes ozone depletion, leading to a nitrogen oxides (NO_x) regime.

For PAW characterization, hydrogen peroxide and nitrite/nitrate concentrations were measured using the Amplex® Red Hydrogen Peroxide Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and a nitrate/nitrite colorimetric assay (Roche, Basel, Switzerland), respectively, with a microplate reader (Rayto, P.R. China). In addition, pH and electrical conductivity were measured using an inoLab® pH 7110 m and an Oakton Con 6+ meter. All measurements were performed at least in triplicate immediately after treatment.

2.6.2. Microbiological analysis

Untreated (control), CAP- or PAW-treated carrot samples, collected immediately after the treatments, were serially diluted in sterile saline solution (0.9% NaCl) and homogenized in a Stomacher 400 apparatus (Seward Ltd., Worth ing, West Sussex, UK) (2 min). The determination of cell counts of both *Arcobacter* spp. and *E. coli* was made using Brain-Heart- Infusion media (BHI; Oxoid, Milan, Italy). Plates were incubated 24 h at 37 °C. For the enumeration of the indigenous microbiota, both total mesophilic count and Enterobacteriaceae were detected following the method reported by Laurita et al. (2021). Two independent replicates were conducted for each treatment, and results of microbial counts at each time point were expressed as mean log CFU/g ± SD.

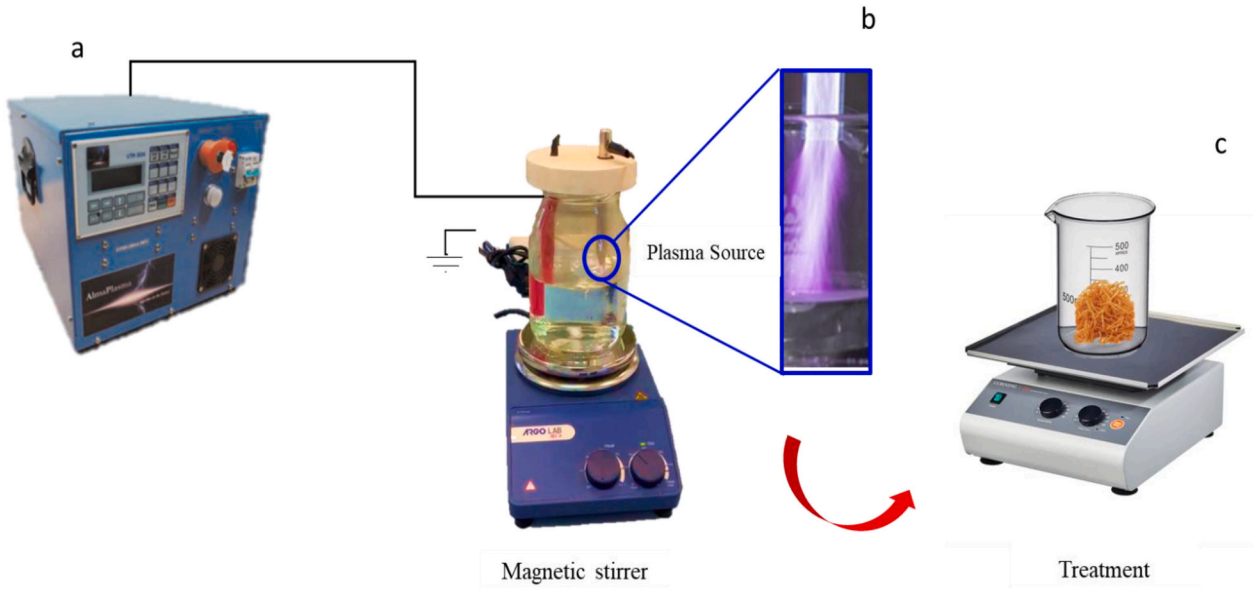

Fig. 2. (a) Schematic of the experimental setup and pictures of the corona source during the production of plasma activated water with (b) light on. (c) Treatment of julienne carrots with plasma activated water.

Table 1
Absorption cross-sections in cm^2 of the species of interest at each selected wavelength.

Selected wavelength	O_3 cross-section	NO_2 cross-section
253 ± 1.2 nm	(1.12 ± 0.02) E-17	(1.1 ± 0.3) E-20
400 ± 1.2 nm	(1.12 ± 0.08) E-23	(6.4 ± 0.2) E-19

2.6.3. Physico-chemical parameters

Water Activity and pH of the treated carrots were measured according to the AOAC methods 981.12 and 925.1, and dry matter according to the method 920.15. Water activity (aw) was determined using a hygrometer AquaLab CX 4-TE (Decagon DevicesInc., Pullman, WA, USA). To measure the pH, 10 g of carrot were processed and mixed with 30 mL of distilled water, and the pH values were then determined using a laboratory pH meter (MP220, Mettler Toledo International, Polaris Parkway, OH, USA). Dry matter content was determined gravimetrically by difference in weight before and after drying at 70 °C, until a constant weight was achieved. All measurements were performed in triplicate.

The colour of the fresh-cut carrots was assessed on the surface before and immediately after the CAP and PAW treatments. The analysis was performed using a Konica Minolta Chroma Metre CR-5 spectrophotocolorimeter (Konica Minolta, Osaka, Japan) equipped with a D65 light source. Measurements were performed directly on a target mask with a measuring area of 8 mm using standard 10° observers. For each sample, 10 different measurements were taken and the CIELab colorimetric coordinates were recorded in two different areas. The coordinates L* (luminosity), b* (yellowness) and a* (greenness) were analysed and the colour difference was calculated according to Molina-Hernandez et al. (2023).

2.6.4. Enzymatic activity

The activity of polyphenol oxidase (POD) was determined using the spectrophotometric method described by Tappi et al. (2016) with some modifications. 3 g of the sample were mixed for 3 min with 50 mL of 0.1 M potassium phosphate buffer (pH 6.5) using a T25 digital Ultraturrax (IKA®-Werke GmbH&Co. KG, Staufen, Germany). After filtration, the solution was centrifuged at 4 °C for 15 min at 10,000 g and the supernatant was collected and considered as an enzymatic extract. A solution containing 99.8 ml of 0.1 M potassium phosphate buffer (pH 6.5), 0.1 mL of 99.5% guaiacol and 0.1 mL of 30% hydrogen peroxide was considered as POD substrate. The enzymatic activity was determined by mixing 150 μL of the enzyme extract with 3 mL of the substrate solution in glass cuvettes with 10 mm path length and then observing the increase in absorbance at 470 nm at 25 °C for 3 min. The results were expressed as residual enzymatic activity (%), which was considered as the ratio between the CAP-treated or the PAW-treated sample and the untreated sample and measured on three independent extracts.

2.6.5. Carotenoids content analysis

The total carotenoids content was determined using the spectrophotometric method described by Molina-Hernandez et al. (2019) with some modifications. 0.1 g of julienne carrots, was placed in a tube that had previously been covered with aluminium foil. Then 7 mL of ethanol-hexane mixture (4:3 *v*/v) were added and placed in a heat bath (16 °C) for 1 h while stirring with a shaker (100 rpm). Finally, 1 ml distilled water was added and shaken again. The absorbance at a wavelength of 450 nm was measured with a spectrophotometer, which was previously calibrated with hexane. The concentration of total carotenoids (β-carotene) was then calculated by applying the Lambert-Beer equation.

2.7. Statistical analysis

All analyses were performed in triplicate and expressed as mean ± SD. All data were subjected to one-way ANOVA for mean comparisons, standard deviation (SD) and significant differences means at a significance level of $p \leq 0.05$ were calculated according to Tukey HSD post-hoc test. Data were processed using XLSTAT (XLSTAT 2022; Addinsoft, New York, NY, USA) software.

3. Results and discussion

3.1. Concentration of RONS by CAP and PAW

Nitrogen dioxide concentrations measured after different CAP treatment times are shown in Fig. 3. Under the high-power condition, the system operated in the nitrogen oxides (NO_x) regime; accordingly, ozone concentrations were below the detection limit (<0.3 ppm). The temporal evolution of NO_2 was monitored by optical absorption

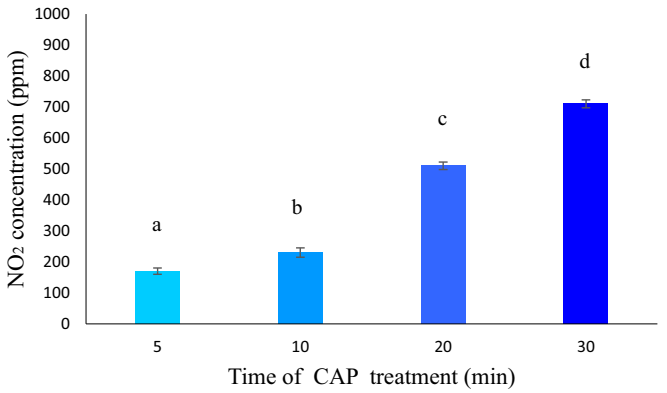

Fig. 3. Values of NO_{2-} after different treatment times. The values are the mean of three repetitions. In each panel, data are mean ± SD and statistical significance is specified with letters (*p ≤ 0.05 as determined by paired Student *t*-test).

spectroscopy (OAS). Consistent with the high surface power density applied during treatment, no O_3 was detected, whereas NO_2 was measurable and increased with exposure time (Fig. 3).

With regard to PAW composition, the concentrations of the main long-lived species are reported in Table 2. The results indicate the formation of hydrogen peroxide and nitrite(s), together with a decrease in pH during PAW generation, consistent with previous reports (Gebremical et al., 2023).

3.2. Microbial counts

Shredded carrots were deliberately and separately inoculated with two *Arcobacter butzleri* strains (BZs206 and BZg74) and one *Escherichia coli* strain (NCFB 555) to achieve initial loads between 4.5 and 5.5 log CFU/g. Immediately after inoculation, samples were subjected to CAP or PAW treatments for 5, 10, 20, or 30 min. Results are shown in **Figs. 4** and **5A,B** and evidenced strain-dependent responses, which also differed between plasma configurations.

CAP treatments significantly reduced viable counts of *A. butzleri* ($p < 0.05$), with marked differences between strains. Strain BZg74 was more sensitive, showing a reduction of >2 log CFU/g after 20 min and falling below the detection limit (<10 CFU/g) after 30 min. In contrast, strain BZs206 showed a maximum reduction of 2 log CFU/g after 30 min.

By comparison, *E. coli* exhibited only an ~1 log CFU/g decrease after 30 min of CAP exposure, with no statistically significant differences versus untreated controls ($p > 0.05$). Overall, short CAP treatments (5 and 10 min) did not result in significant reductions ($p > 0.05$) relative to controls, regardless of bacterial species or strain.

In the case of PAW treatment, significant reductions ($p < 0.05$) were already observed after 5 min, irrespective of bacterial species or strain. Notably, these reductions (1.4–1.7 log CFU/g for *E. coli* and *A. butzleri* BZs206, and ~ 2 log CFU/g for *A. butzleri* BZg74) remained unchanged when treatment time was extended up to 30 min.

Previous studies have shown that CAP treatment can exert a bactericidal effect against *E. coli* on foods, with reductions of up to 5 log reported, strongly depending on plasma set-up, operating conditions and product-related factors such as the feed gas, exposure time, distance from the plasma source, and sample load (Ahmed et al., 2025).

Table 2
Concentrations of NO_2^-, H_2O_2 and pH measured in PAW during 30 min after generation.

Treatment	$NO2^-$(mg/L)	H_2O_2 (mg/L)	pH
0	13.62 ± 1.202	1.49 ± 0.021	3.6 ± 1.202
5	13.3 ± 2.601	1.15 ± 0.051	3.54 ± 1.601
10	12.97 ± 2.403	0.87 ± 0.051	3.2 ± 1.312
20	12.66 ± 0.602	0.66 ± 0.091	3.2 ± 1.212
30	12.39 ± 0.502	0.42 ± 0.021	3.15 ± 1.302

The disparity observed in the present study between CAP and PAW efficacy may be attributed to differences in the generation, delivery, and lifetime of reactive species in the two configurations. Direct comparisons with other studies are challenging because surface power density is rarely reported, limiting the possibility of matching plasma "dose" across experimental setups. Nevertheless, the literature consistently indicates that CAP treatment involves continuous plasma generation inside the chamber, leading to a progressive increase in the concentration and activity of reactive species over time (Fig. 3; Table 1). This behaviour is consistent with the time-dependent inactivation observed for *A. butzleri*, which increased as processing time increased. In contrast, PAW reaches its maximum reactivity immediately after generation, when the concentrations of short-lived reactive oxygen and nitrogen species (ROS/RNS)—including $^1O_2$, $^{\bullet}OH$, $O_2^{\bullet-}$, $^{\bullet}NO_2$, $^{\bullet}NO$, and $ONOO^-$—are highest. These species, with half-lives ranging from nanoseconds to seconds, rapidly react to form more stable compounds. On the other hand, PAW also contains long-lived species such as NO_2^-, NO_3^-, and H_2O_2, whose half-lives range from minutes to days (Han et al., 2022; Wong et al., 2023). According to the literature, long-lived species are largely responsible for microbial inactivation, through mechanisms involving disruption of bacterial membrane integrity as well as DNA damage and denaturation (Han et al., 2022; Hu et al., 2024).

A similar pattern was observed for uninoculated shredded carrots, analysed for total mesophilic count and Enterobacteriaceae. CAP treatments resulted in significant reductions ($p < 0.05$) of 1.5 log for mesophiles (after 20 min) and for Enterobacteriaceae (after 30 min) (Fig. 5A). By contrast, PAW washing produced an immediate (5 min) and significant effect ($p < 0.05$), with a 1.2–1.5 log reduction in the mesophilic population (Fig. 5B). Conversely, Enterobacteriaceae showed a lower, non-significant reduction ($p > 0.05$).

Similar trends have been reported in the literature, where PAW has shown to provide rapid initial reductions of microbial loads on fresh produce surfaces, whereas CAP to yield gradual but progressive inactivation with longer exposure times (Misra et al., 2018; Ziuzina et al., 2014). Overall, these findings are consistent with the reactive-species dynamics of the two configurations, supporting the potential of PAW for rapid decontamination in high-throughput settings, while CAP may provide continuous microbial suppression over time.

In addition to the observed inactivation results, several factors may further influence the effectiveness and practical implementation of CAP and PAW treatments on fresh-cut carrots, particularly produce matrix properties and species-specific resistance mechanisms.

Surface characteristics of shredded carrots, including microstructural irregularities and the presence of organic residues, may affect CAP and PAW efficacy. Irregular surface features can promote the formation of micro-niches that physically shield bacterial cells from oxidative agents, while organic load may scavenge reactive species, thereby reducing their availability for microbial inactivation (Niemira, 2012).

Moreover, the different sensitivities observed between *A. butzleri* and *E. coli* may reflect inherent, species-specific resistance traits, including differences in cell envelope structure, stress-response pathways, and antioxidant defence systems. *E. coli* possesses well-characterized oxidative stress response mechanisms, involving various specialized detoxifying enzymes such as glutathione (GSH), catalase, and superoxide dismutase (SOD), which can mitigate damage induced by reactive species during CAP and PAW treatments (Imlay, 2013).

Although these activities were not directly assessed in the present study, previous work suggests that *E. coli* can activate intracellular antioxidant defences in response to PAW-based treatments. For example, Wen et al. (2025) reported that exposure of *E. coli* to PAW combined with ultrasound triggered activation of the intracellular antioxidant enzyme system, with a significant increase in superoxide dismutase (SOD) activity ($p < 0.05$) after treatment. The authors further suggest that the enhancement of SOD activity indicated that cells

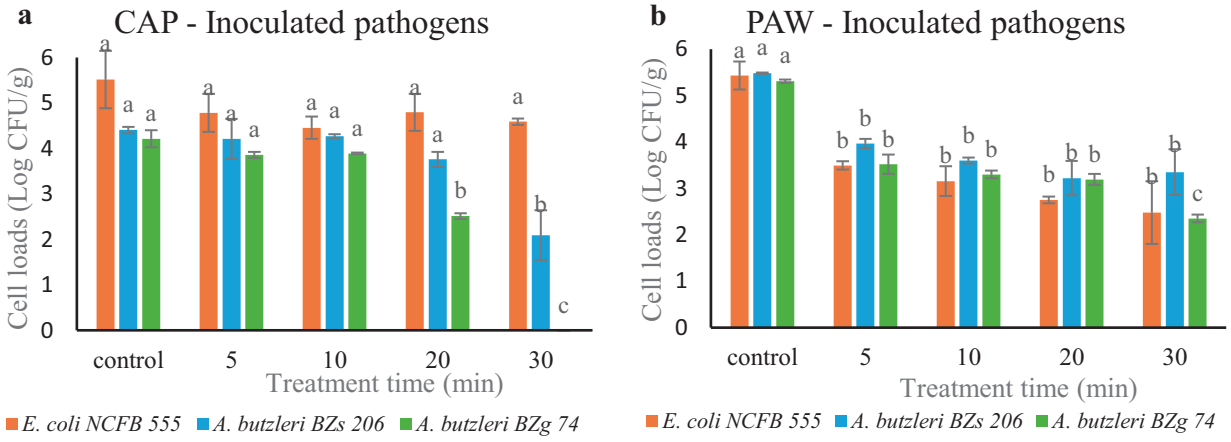

Fig. 4. Cell loads of *Escherichia coli* NCFB 555, *Arcobacter butzleri* BZs206 and BZg74 inoculated on fresh cut carrots untreated or treated with CAP (**A**) and PAW (**B**) for 5, 10, 20 and 30 min. Different letters above the bars indicate significant differences between different treatment times on the same microorganism (Tukey HSD, $p < 0.05$).

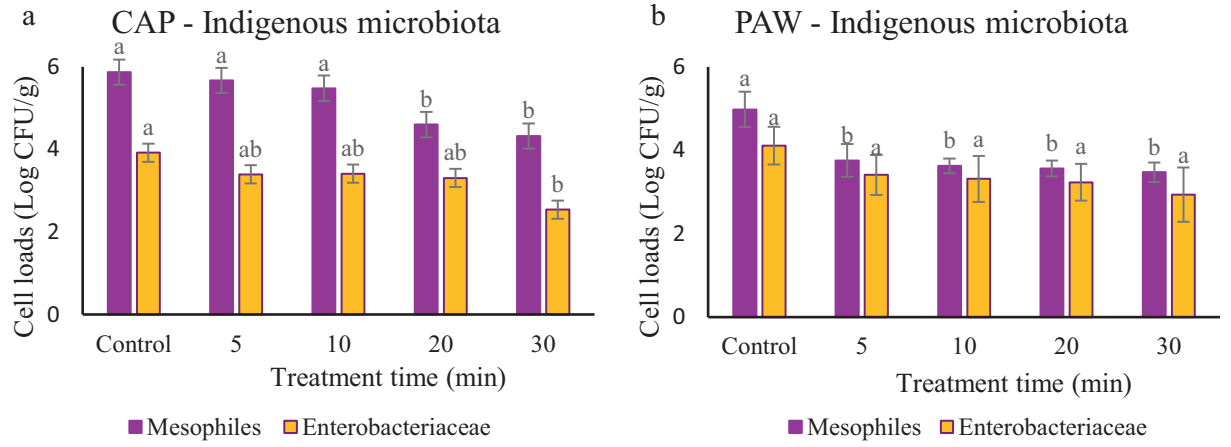

Fig. 5. Cell loads of the total mesophilic bacteria and Enterobacteriaceae of fresh cut carrots untreated and treated with CAP (**A**) and PAW (**B**) for 5, 10, 20- and 30-min. Different letters above the bars indicate significant differences between different treatment times on the same microbial group (Tukey HSD, $p < 0.05$).

entered a defensive state, potentially supported by increased energy metabolism.

Consistently, Hu et al. (2025) observed that PAW-treated *E. coli* exhibited a SOD activity of 30.37 U/mL, corresponding to a 52% increase compared with the untreated control ($p < 0.05$). An increase in catalase (CAT) activity was also reported, further supporting the induction of antioxidant responses associated with cellular defence against oxidative stress following PAW exposure.

Additionally, the presence of efflux pumps and DNA repair systems may further reduce *E. coli* susceptibility under oxidative stress, potentially requiring longer treatments or combined hurdles to achieve effective inactivation. By contrast, although *A. butzleri* exhibits aerotolerance compared to *Campylobacter*— due to catalase and SOD activity—it appears to display lower oxidative stress resistance than *E. coli*. Moreover, *E. coli* possesses genetic systems that enable survival under extremely low pH conditions (De Biase & Lund, 2015), whereas *A. butzleri* has been reported to be sensitive to acidic ecosystems (Cervenka, 2007; D'Sa & Harrison, 2005). This difference may have contributed to the greater viability losses observed in the present study, also considering the mildly acidic conditions resulting from plasma treatments.The limited effectiveness of CAP against *E. coli* observed here (~1 log reduction after 30 min) may also be related to its tolerance to the chemico-physical stresses associated with CAP exposure. Adaptation to one stressor can result in cross-protection of the surviving cells against single or multiple subsequent stresses. Direct comparisons with the literature remain difficult due to differences in experimental set-ups and the well-known variability among strains within the same species in response to chemical-physical stresses. Nevertheless, Little et al. (2024) reported that *E. coli* cells pre-adapted to acidic conditions (pH 4.6–4.8) showed higher survival after high-voltage atmospheric cold plasma (HVACP) treatment in pineapple juice compared with non-acid-adapted cells (pH 7.0–7.2). The higher number of survivors detected 24 h post-treatment in the acid-adapted samples suggested cross-protection against the bactericidal effects of HVACP induced by prior growth under acidic conditions. Similarly, studies evaluating the response of the same *E. coli* strain used in the present work (NCFB 555) to sub-lethal concentrations of natural antimicrobial compounds (hexanal and 2-(*E*)-hexenal) reported its ability to modulate membrane fatty acid composition (Patrignani et al., 2008), thereby maintaining membrane integrity and functionality in response to environmental stress.

In Gram-negative bacteria, membrane integrity is in fact of paramount importance in protecting microbial cells from hostile environments such as that generated during CP treatments. The strain-dependent susceptibility to plasma treatments observed for *A. butzleri* could be linked to variations in the bacterial cell envelope, differentially protecting cells from RONS and acidic conditions, or genetic differences in stress response. On the other hand, phenotypic and genotypic differences have been reported among *A. butzleri* strains isolated from

different sources which can be related to the ability to survive in both animal samples and on environmental surfaces. Comparative analysis of 56 *A. butzleri* isolates, characterized by different antibiotic resistance profiles and deriving from different sources (slaughterhouse environment after cleaning procedures and broiler carcasses), indicated 31 different strains from 13 strains groups and 18 unique strains (Buzzanca et al., 2024; Chiarini et al., 2024). Moreover, the observed correlation between genes related to antibiotic resistance and environmental stressing conditions characterizing the isolation sourceis consistent with the presence of genomic traits enabling resistance and survival under specific pressures which may be associated with the food ecosystems.

3.3. Physico-chemical parameters

Table 3 reports mean values (± SD) of pH, water activity (aw), and dry matter (%) for untreated and treated julienne carrots subjected to CAP or PAW. For CAP-treated samples, pH decreased from 6.28 ± 0.02 (control) to 5.15 ± 0.05 after treatment, while for PAW-treated samples it decreased from 6.41 ± 0.02 (control) to 5.51 ± 0.05. Both treatments caused a significant pH reduction ($p < 0.05$), already evident after 5 min and reaching approximately 1 pH unit after the longest treatments (5.15 ± 0.05 for CAP and 5.51 ± 0.05 for PAW).

A comparable pH decrease (up to 1.13 and 0.9 units, respectively) has also been reported during carrot sanitation using chlorine washing, which is a standard industrial procedure (Amanatidou et al., 2000). Changes in pH during cold plasma treatment are mainly attributed to interactions between reactive gas species and the intrinsic moisture of the food matrix, promoting the formation of nitrate and nitrite species even at low concentrations (Judée et al., 2018; Pankaj et al., 2018). In addition, plasma-generated NO_x can react with water to form longer-lived reactive nitrogen species (e.g., nitrite and nitrate), which contribute to pH reduction, particularly in PAW treatments.

In our plasma system, NO_2 concentrations reached 1200 ppm under high-power conditions, whereas ozone remained below the detection limit (<0.3 ppm) (Molina-Hernandez et al., 2022). Consistently, previous studies have shown that PAW generation leads to significant acidification as RONS produced during discharge dissolve in water due to their solubility (Thirumdas et al., 2018).

In terms of dry matter, a significant increase ($p < 0.05$) was observed after CAP treatments longer than 10 min compared with the control. This result is consistent with Ramazzina et al. (2015), who reported that DBD cold plasma significantly increased dry matter in minimally processed kiwifruit. The observed changes may be attributed to surface moisture loss associated with tissue damage, as well as to physiological activity in living tissues (Misra et al., 2016). By contrast, no significant differences in aw were detected in any of the samples.

Table 3
Effect of CAP and PAW treatments on physico-chemical parameters of fresh cut carrots.

Treatment	pH		a_w		Dry matter (%)	
	CAP	PAW	CAP	PAW	CAP	PAW
Control	6.28 ± 0.021^a	6.41 ± 0.021^a	0.98 ± 0.005^a	0.98 ± 0.005^a	15.21 ± 0.052^a	14.31 ± 0.611^a
5	6.14 ± 0.042^b	5.62 ± 0.041^b	0.97 ± 0.004^a	0.97 ± 0.004^a	15.41 ± 0.051^a	13.39 ± 0.081^a
10	6 ± 0.011^b	5.4 ± 0.015^b	0.98 ± 0.002^a	0.98 ± 0.002^a	16.72 ± 0.021^b	13.51 ± 0.191^a
20	5.42 ± 0.042^b	5.49 ± 0.042^b	0.98 ± 0.004^a	0.98 ± 0.004^a	18.21 ± 0.120^c	14.96 ± 0.122^a
30	5.15 ± 0.051^b	5.51 ± 0.051^b	0.98 ± 0,.001^a	0.98 ± 0.001^a	18.91 ± 0.211^c	14.72 ± 0.231^a

*Different letters in the same column represent significant differences at p < 0.05 by Tukey multiple range test.

The freshness of fresh-cut products is often judged by consumers primarily on the basis of appearance and colour (Rico et al., 2007). As shown in Fig. 6a, a visible colour change was observed only after the longest CAP treatment (30 min) compared with the control, whereas no apparent modification was detected in PAW-treated samples.

Fig. 6b reports the overall colour difference (ΔE) as a function of treatment time. According to Tiwari et al. (2008), colour differences are considered very distinct when $\Delta E > 3$, distinct when $1.5 < \Delta E < 3$, and small when $\Delta E < 1.5$. Overall, CAP induced clearly visible colour changes that increased with exposure time. While ΔE remained below 3 after 5 min, it increased to approximately 6 after 10 and 20 min and reached 23.27 ± 5.40 after 30 min. In contrast, PAW-treated samples showed ΔE values below 3 at all tested times.

Similar observations have been reported for carrots treated with plasma systems. For example, Wang et al. (2012) found that long exposures using a plasma microjet increased total colour difference (ΔE), possibly due to surface oxidation of carotenes. The authors also suggested that surface water loss may influence lightness and thus contribute to colour changes. Likewise, Mahnot et al. (2020) reported that DBD-CAP treatment increased L* (lightness) and decreased a* and b* values in fresh-cut carrots—consistent with our findings—and attributed these changes to bleaching effects and pigment oxidation associated with plasma-generated oxidants. Notably, they also observed an overall good retention of carotenoids.

To clarify whether the observed colour changes were associated with carotenoid degradation, total carotenoids (mg/g) in untreated and treated samples are shown in Fig. 6c. CAP did not significantly affect total carotenoid content across the tested treatment times ($p \geq 0.05$). Conversely, PAW led to a significant increase in total carotenoids ($p < 0.05$) already after 5 and 10 min of treatment, followed by a slight decrease at longer exposure times.

Exposure of carotenoids to plasma-derived reactive species may induce structural modifications such as chain shortening or extension, hydrogenation/dehydrogenation, cyclization, double-bond migration, and isomerization (Ramezan et al., 2023). However, carotenoids are stored in plastids and are generally stable unless cellular structures are disrupted. In addition, plasma-induced acidification may promote denaturation of pigment–protein complexes, potentially facilitating carotenoid release. In the present study, no evidence of carotenoid loss was detected despite the colour changes observed after CAP treatment. The increase in total carotenoids measured after PAW treatment may instead be related to partial disruption of cellular structures and enhanced extractability, possibly due to release of carotenoids into the aqueous phase. Some authors have also hypothesised that oxidative stress may induce the biosynthesis of carotenoids and other secondary metabolites (Jìa et al., 2022), which could contribute to the observed increase.

Peroxidase (POD) is a ubiquitous oxidative enzyme in plant tissues and can contribute to quality deterioration by oxidising a range of substrates, including phenolic compounds and lipids (Morales-Blanca et al., 2002). POD has also been implicated in carrot browning, negatively affecting visual quality (Sharma et al., 2024). As shown in Fig. 7, both treatments affected POD activity.

CAP treatment led to a significant reduction in POD activity compared with the untreated control ($p < 0.05$) (Fig. 7a). After 5 min, POD activity decreased by approximately 30%; treatment time was prolonged, inactivation increased, reaching around 60%. Similar reductions in POD activity following plasma exposure have been reported previously. Bußler et al. (2017) showed that plasma-processed air generated by a microwave-driven plasma torch reduced POD activity in apples and potatoes by 62% and 77%, respectively, after 10 min of exposure. Using a gliding arc plasma, Khani et al. (2017) reported a residual POD activity of 7.32% after 7 min for tomato peroxidase. Han et al. (2019) also observed a 17% reduction in POD activity using a

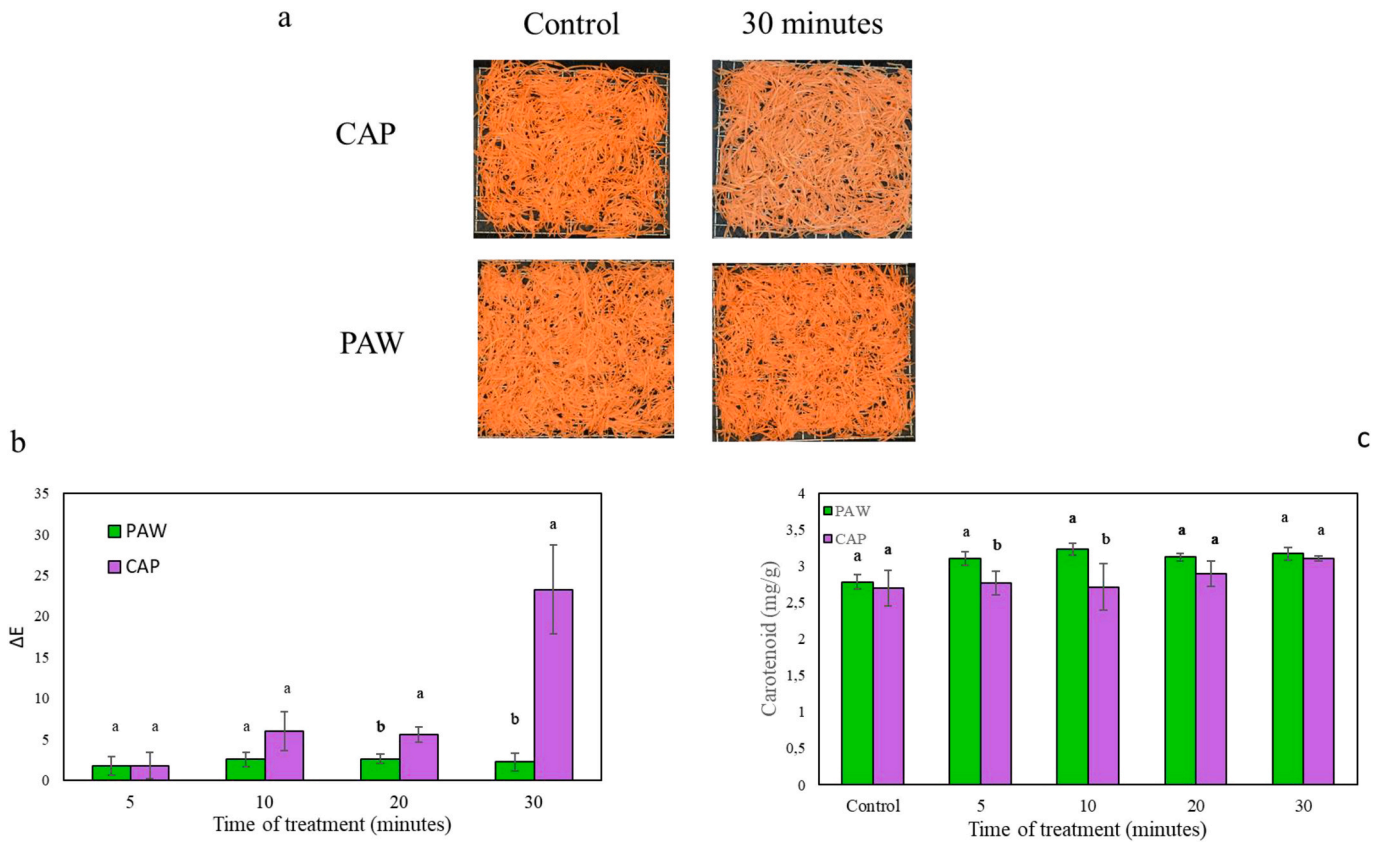

Fig. 6. Visual appearance (a), ΔE values (b) and total carotenoids (c) in control and CAP and PAW treated fresh cut carrots. Different letters above the bars indicate that the data were significantly different ($p < 0.05$) among treatment times for the same treatment (CAP or PAW respectively).

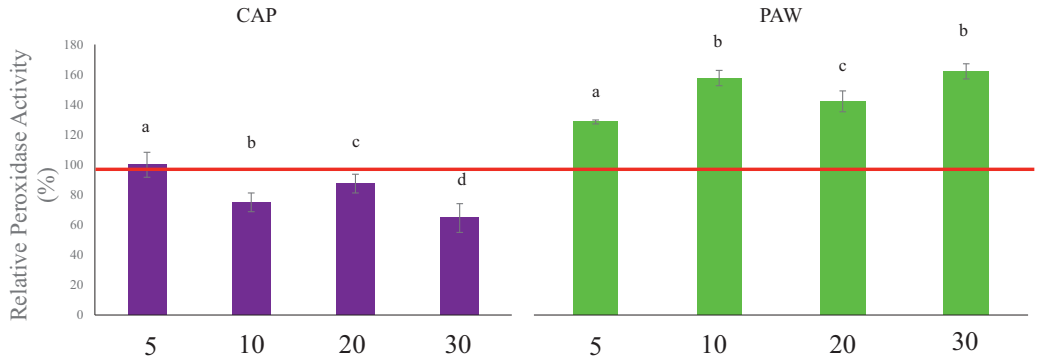

Fig. 7. Relative peroxidase activity (%) in fresh-cut carrots compared with CAP treated (purple colour) and PAW treated samples (green colour). Red line represents the control sample as 100%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microscale atmospheric-pressure plasma jet (μAPPJ).

Despite differences in plasma sources and inactivation levels, it is generally accepted that exposure to plasma-derived reactive species can oxidise amino acids, disrupt disulfide bonds, and alter secondary and tertiary protein structures, ultimately leading to loss of enzymatic activity.

In contrast, PAW induced a significant increase in POD activity (Fig. 7b) already after 5 min, reaching a maximum increase of approximately 25%. A similar response was reported by Zhao et al. (2019) in fresh-cut kiwifruit treated with PAW. In addition to a ~ 20% increase in POD activity, those authors observed an overall upregulation of several enzymes involved in endogenous antioxidant defence. Likewise, Perinban et al. (2022) reported a 40% increase in POD activity in fresh-cut apple after PAW treatment. They attributed this behaviour to differences in POD isoenzyme structure, which may affect sensitivity to oxidative stress, as well as to plant regulatory mechanisms that counteract ROS-induced lipid peroxidation.

Accordingly, we hypothesise that the POD activation observed in the present study may be linked to oxidative-stress signalling and the regulation of functional genes triggered by highly reactive species. The increase in carotenoids observed in PAW-treated samples could be related to the same response; however, additional analytical investigations are needed to clarify the underlying mechanisms.

More generally, as described by Han et al. (2019), the impact of CAP on food enzymes can vary widely—resulting in either inactivation or activation—depending on several factors, including enzyme type, plasma generation method and electrical parameters, reactive species composition, exposure mode and duration, and matrix characteristics.

4. Conclusions

For the first time, this study reports the effect of cold plasma (CP) treatments—cold atmospheric plasma (CAP) and plasma activated water (PAW)—on the inactivation of *Arcobacter butzleri*, an emerging foodborne pathogen, inoculated onto julienne carrots. Moreover, a comparison with a reference pathogen – *E. coli* - was also carried out considering that it is frequently addressed in several studies testing the efficacy of cold plasma treatments in food safety. Overall, CP reduced the viability of both *A. butzleri* strains, although differences were observed both between strains and across species. Given the growing relevance of *Arcobacter butzleri* as a food-safety concern, these findings provide useful evidence to support improved control measures for fresh and ready-to-eat (RTE) vegetables.

Both CAP and PAW showed potential for reducing *A. butzleri* and *E. coli* on shredded carrots, with distinct inactivation kinetics: PAW produced rapid reductions, whereas CAP resulted in progressive, time-dependent inactivation. In addition, both treatments significantly reduced indigenous total mesophilic populations and Enterobacteriaceae, contributing to improved microbial quality of the product.

Regarding product quality, CAP had a stronger impact, causing colour deterioration and changes in pH and dry matter, while PAW better preserved quality attributes. Enzymatic responses were strongly treatment-dependent, further highlighting the complexity of plasma–matrix interactions and the need to tailor processing parameters to balance microbial safety with quality preservation. Moreover, CAP was more effective at inactivating peroxidase (POD), the enzymes responsible for surface browning in solid foods; therefore, more effective in preventing surface browning during extended storage periods, despite the risk of minor colour degradation. Generally,

Overall, CP—particularly when treatment conditions are optimized—represents a promising approach to enhance the safety and quality of fresh-cut carrots. Further studies are warranted to elucidate the molecular mechanisms underlying *Arcobacter butzleri* responses to plasma-based treatments. and the investigation of shelf-life and sensory studies to validate the commercial potential of the proposed solutions.

CRediT authorship contribution statement

Beatrice Cellini: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Fatemeh Shanbeh Zadeh:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Junior Bernardo Molina-Hernandez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Massimo Mozzon:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Pietro Rocculi:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization. **Lucia Vannini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Silvia Tappi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data that has been used is confidential.

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