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In-Package Atmospheric Pressure Cold Plasma Treatment of Cherry Tomatoes

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In-package atmospheric pressure cold plasma treatment of cherry tomatoes

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Abstract

Cold plasma is increasingly under research for decontamination of foods, especially fresh fruits and vegetables. The impact of cold plasma on food quality, however, remains under researched. This study investigates the effects of cold plasma generated within a sealed package from a dielectric barrier discharge (DBD) on the physical quality parameters and respiration rates of cherry tomatoes. Respiration rates and weight loss were monitored continuously, while other parameters are reported at the end of storage period. Differences among weight loss, pH and firmness for control and treated cherry tomatoes were insignificant towards the end of storage life. Changes in respiration rates and colour of tomatoes were recorded as a function of treatment, which were not drastic. The results implicate that cold plasma could be employed as a means for decontamination of cherry tomatoes while retaining product quality.

Keywords

Cold Plasma; Spectroscopy; Tomato; Respiration Rate; Quality; Nonthermal; Barrier Discharge

Introduction

Raw agricultural produce has frequently been associated with foodborne outbreaks. Microorganisms can grow on raw and minimally processed produce at populations ranging from 10^3 to 10^9 CFU/g (1). Sanitizing with chemicals, often chlorine based, is a most common intervention aimed at providing produce safety and preservation. However, in a number of European countries, such as the Netherlands, Sweden, Germany and Belgium, the use of chlorine on minimally processed vegetable products is prohibited due to the association of chlorine with the possible formation of carcinogenic chlorinated compounds in water (2, 3). Considering this, there is a need to provide the fresh produce industry with intervention technologies to effectively eliminate pathogenic microorganisms associated with fresh produce (4).

Recently cold plasma has been added to the list of nonthermal processes offering potential for the decontamination of fresh produce. A gas energised to such degree whereby the constituent molecules of the gas split to yield free electrons, radicals, positive and negative ions, quanta of electromagnetic radiation, while some molecules may still remain neutral is known as plasma. The term "cold" plasma refers to the fact that the temperature of electrons (T_e) is much higher than that of the ions, neutrals and global gas (T_g) temperature ($T_e >> T_g$). Thus, the overall temperature of cold plasma is limited to ambient temperatures, even at atmospheric pressures. The various aspects of application of cold plasma technology for inactivation of foodborne pathogens were reviewed by Misra et al. (5).

Several research works have identified the potential of cold plasma technology in decontaminating fresh produce and other foods. Majority of studies, to this end report the use of plasma jets for treatment of foods. Recently, Baier et al. (6) reported the use of a plasma jet operated with Ar gas for treatment of corn salad leaves, while Bermúdez-Aguirre et al. (7) employed a plasma jet array operating in Ar for decontamination of lettuce, carrots and tomatoes. In order for food industries to adopt the cold plasma technology, the operating cost of the gas would play an important role. Often noble gases are employed for inducing and sustaining plasmas, which increase the cost of treatments. An ideal gas for such treatments would be the use of ambient air.

An alternative plasma source for treatment of foods is the use of a dielectric barrier discharge (DBD) set-up, which allows treatment over large volumes in air and discharge gaps, when sufficiently high potential difference is maintained across the gas gap. In particular, a DBD set-up offers further advantage in that it allows treatment of produce inside sealed packages, which eliminates the risk of post-process contamination. The use of in-package plasma technology for treatment of foods is now well-established (8-10). We have recently demonstrated the in-package cold plasma decontamination of strawberries, without compromise in their quality (2), and also inactivation of peroxidase enzymes in tomatoes (11).

Extending our investigations further, in the present work we investigated the use of a DBD set-up to generate cold plasma in ambient humid atmospheric air inside a package containing cherry tomatoes. The package itself served as the dielectric material and helped to limit the charge transported, thereby permitting the generation of a stable discharge. This also eliminated the need for additional charge barriers. The effects of cold plasma treatment on the respiration rates and quality parameters are reported here.

Materials and Methods

Produce Characteristics

Whole fresh cherry tomatoes (Class I, origin- Egypt) were used for respiration rate and storage experiments. The required amount was bought from a whole-sale agricultural produce market (Smithfield, Dublin) and chosen based on bright red colour, indicating ripeness. Tomatoes were divided into two groups- one group was used as control, while the other for in-package plasma treatment.

To estimate the volume filled by the tomatoes inside the package, their density was determined. For this, the mass of the cherry tomatoes was obtained using a precision balance (Sartorius, Germany) and the volume by the method based on Archimedes principle. The tomatoes were fixed with a thin, straight and hard copper wire and introduced into beaker filled with a known mass (kg) of water. The temperature of water was recorded using a thermometer to be 15 ± 0.2 °C. The resulting force measured as weight with the balance corresponds to buoyancy of the cherry tomatoes and equals the volume of the water displaced by the tomatoes. The apparent density was determined using the following equation-

$$d = \frac{W_{Tomato}}{W_{Water}} \qquad (1)$$

where d is the density, W_{Tomato} is the mass of the tomato and W_{Water} is the mass of volume of water displaced by the tomato (equal to mass of the tomato). The apparent density of the tomatoes was estimated to be 1.026 ± 0.003 based on water displacement measurements for 10 samples. This value is in agreement with that reported by Stertz et al. (12) for organically grown Brazilian cherry tomato variety.

Package design

Flexible packages made from high barrier Cryovac B2630 film of size 34 cm × 33 cm were used to pack 107 ± 3 g of tomatoes (10 tomatoes in each package). The average thickness of the film was 48 µm. The oxygen and carbon dioxide transmission rates for this film were 4.5 cm^3 (STP)/(m^2-24 h-atm) and 66 cm^3 (STP)/(m^2-24 h-atm) at 0% RH, 4 °C. The water vapour transmission rates of the film was 0.45 g/(100 in^2-24h) at 100% relative humidity and 37 °C. The bags were filled to approximately 3.5 litres with air using a laboratory electric pump. It may be noted that the thickness of the packaging film employed in this study is higher than those used in actual practice. However, the high barrier nature of the packaging film allows retention of the active plasma species without leakage and also serves as a stable dielectric material preventing a transition of the discharge to arc regime (13). Studies in our laboratory have revealed that nature and thickness of packaging material plays an important role in preventing sparks. The atmospheric conditions at the time of treatment were 22 ± 1 °C and 45 ±4 % relative humidity, as measured using a humidity-temperature probe connected to a data logger (Testo 176 T2, Testo Ltd., UK).

Nonthermal Plasma treatment

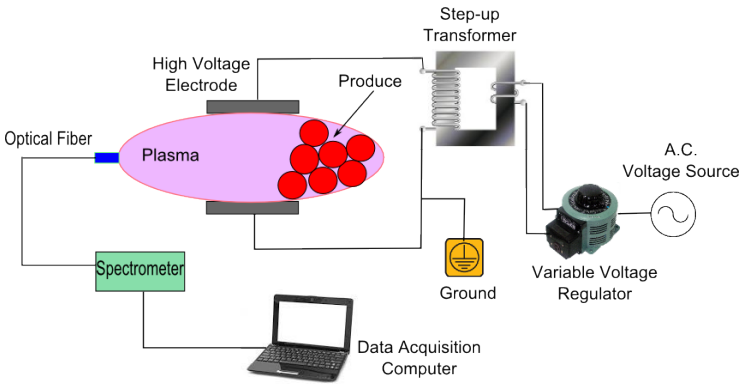

Figure 1 The experimental setup for indirect DBD plasma treatments of cherry tomatoes. The cold plasma is generated inside the bag. The tomatoes are placed outside the inter electrode space.

The cold plasma was generated in air inside the bag using a dielectric barrier setup, as shown in Figure 1. The electrode separation was fixed at 2.2 cm and powered using a high-voltage (60 kV) source, pulsed at 50 Hz from a step-up transformer operated at 60% of the input voltage (120 V) using a variac. The electrodes had a contact surface area of 249.64 cm^2. A single value of 30 kV RMS voltage and four different treatment times, 30 s, 60 s, 180 s and 300s were selected for the experiments, while each experiment was performed in duplicate. This voltage is much higher than those commonly employed in most studies, thereby allowing drastic reduction in treatment times, and use of large packages. Treatments were carried out by indirect mode, meaning that the tomatoes were placed away from the inter-electrode zone thereby ensuring homogeneous discharge. When indirectly

treated, the charged particles and short-lived transient-state species do not affect the sample under treatment, as they recombine before reaching it (10). This leaves only stable reactive species to act on the samples. The control and treated packages were stored at 20 ± 2 ^{o}C temperature and 60 ± 5% relative humidity conditions in an environmental incubator (MLR-350HT, Sanyo Electric Biomedical Co. Ltd., Japan). These storage conditions were selected to simulate room temperature storage conditions.

Optical Emission Spectroscopy

The energy transferred to the plasma produces various gaseous species in excited states. Some of these species can be identified based on the characteristic optical emission spectra (OES) of the plasma. Therefore, a computer controlled Stellarnet EPP 2000C-25 spectrometer was employed for optical emission spectroscopy (OES). The light emission from the plasma generated inside an empty package was captured via an optical fibre, directed towards the centre of the inter-electrode space where the species density is likely to be highest. Empty packages were employed since the presence of produce posed difficulties in maintaining a consistent alignment of the optical fibre. The diffraction grating of the spectrometer had a radius of curvature of 40 mm, 590 grooves per mm and an entrance slit width of 25 µm. The spectrometer had a resolution of 1.5 nm and operated in the spectrum of 190 to 850 nm. The fibre had a numerical aperture of 0.22 and was suitable for use in the ultraviolet and visible portion of the spectrum. The integration time was 5000 ms and 5 samples were averaged for the collection of spectra. The spectra were noise cancelled, averaged and analysed using National Institute of Standards and Technology (NIST) atomic spectra database and published works (14, 15) for identification of active chemical species.

Gas concentration analysis

The change in gas composition (O_2 and CO_2) inside each package was monitored over time using gas analyser (Systech Instruments, UK). Gas extractions were performed with a hypodermic needle inserted through an adhesive septum previously fixed to the bags, at a flow rate of 150 mL/min for 10s. The instrument is based on electrochemical sensor to record O_2 concentration, and uses a mini-IR spectrophotometer to record CO_2 concentrations (accuracy: 0.1% v/v O_2; 2% v/v CO_2). Initial experiments showed that sampling had no significant influence on gas concentration in the bag, as the bag volume was much greater than the total volume sampled by the instrument during the experiment.

A two parameter, non-exponential equation was fitted to average O_2 and CO_2 concentrations of control and treated packages at different storage periods (16, 17) as shown in equations (2) and (3) to determine the values of the coefficients:

$$G_{O_2} = 0.209 - \left[\frac{t}{K_1 t + K_2}\right] \quad (2)$$

$$G_{CO_2} = \frac{t}{K_1 t + K_2} \quad (3)$$

where K_1 and K_2 (h) are the regression coefficients, *t* is the time of storage in hour, G_{O_2} is the oxygen concentration in decimal and G_{CO_2} is the carbon dioxide concentration in decimal. The rate of change of gas concentration was determined from the first derivative of the regression functions. Thus, at each sampling time, the respiration rates in terms of CO_2 evolution and O_2 consumption were calculated using equations (4) and (5):

$$R_{CO_2} = \frac{d[G_{CO_2}]}{dt}\frac{V}{W} = \left(\frac{1}{(K_1 t + K_2)} - \frac{K_1 t}{(K_1 t + K_2)^2}\right)\left(\frac{V}{W}\right) \quad (4)$$

$$R_{O_2} = -\frac{d[G_{O_2}]}{dt}\frac{V}{W} = -\left(\frac{K_1 t}{(K_1 t + K_2)^2} - \frac{1}{(K_1 t + K_2)}\right)\left(\frac{V}{W}\right) \quad (5)$$

Weight loss, visual fungal and pH measurement

Quality parameters of weight loss and visual fungal were monitored for up to two weeks of storage. Weight loss was expressed as percentage of initial weight of sample. For visual fungal, the quality of cherry tomatoes was

visually assessed on a daily basis by examining for signs of growth of filamentous hyphae or black spots of botrytis. The pH of the cherry tomatoes was determined by using a handheld pH-meter with spear electrode (Eutech Instruments, Thermo Fisher Scientific Inc., Netherlands). Tomato pH was measured for fresh tomatoes before packaging, and of control and treated groups at the end of storage period in triplicate for five tomatoes.

Colour measurement

The colour was quantified using L^*-a^*-b^* colorimeter (using Colour Quest XE Hunter Lab, Northants, U.K.) after 14 days of storage. The colour measurement was performed on (along four symmetrical sections) each tomato and average values reported. The instrument was calibrated using white (L^* = 93.97, a^* = 0.88 and b^* = 1.21) and green (L^* = 56.23, a^* = 21.85, b^* = 8.31) standard tiles. The hue angle was calculated as $h^* = \tan^{-1}(b^*/a^*)$, chroma as $C^* = (b^{*2}+a^{*2})^{1/2}$ and tomato colour index as $TI = (a^*/b^*)$. The CIE L^*, a^*, b^* parameters were used to report the total colour difference as $(L^{*2} + a^{*2} + b^{*2})^{1/2}$.

Firmness

The firmness of control and treated samples was analysed using an Instron texture analyser (Instron 4302 Universal Testing Machine, Canton MA, USA). The texturometer was mounted with a 500 N load cell and equipped with a 2 mm flat head stainless steel cylindrical probe which punctures the sample at a download speed of 200 mm/min and a distance of 10 mm (18). A single whole tomato was placed on the stage for each measurement. The maximum force (N) required to puncture the sample was used as an indication of firmness. Data were analysed by using Bluehill software. The firmness of 3 tomatoes from each package was measured individually and an average firmness value was reported.

Statistical Analysis

Multiple comparison analysis by Tukey method was used to analyse the significance of differences between different treatments. Statistical analysis was carried out in Statgraphics software (centurium XV; Statistical Graphics Co., Rockville, USA). Means were considered significant at $p \leq 0.05$. Nonlinear least-squares regression was carried out for model fitting and parameter estimation using the Levenberg–Marquardt algorithm available in Statgraphics software.

Results and Discussion

Optical Emission Spectroscopy (OES)

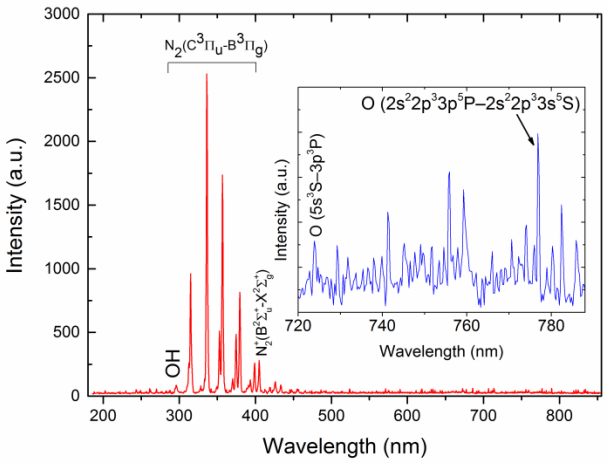

Figure 2 Optical emission spectrum of the in-package plasma discharge.

The emission spectra of the cold plasma generated inside the package is presented in Figure 2. Most of the intense peaks in the spectra correspond to the emissions in the near UV region by the excited species of nitrogen, namely

nitrogen second positive system $N_2(C-B)$ and first negative system $N_2^+(B-X)$ (19). The OH peak around 300 nm was also identified. The low intensity, in spite of ~22% relative humidity at the time of treatment can be explained by the loss of the $OH^\bullet$ $(A^2\Sigma^+)$ state. This in turn has been primarily attributed to the process of radiative de-excitation and the quenching by collisions with various molecules, for example, the quenching time by H_2O is reported to be 100 ns (20). Such observation for discharges operating in filamentary mode has also been observed by Chiper et al. (21). Peaks associated with optical transitions of O atom are observed at low intensities: 725.4 from O ($5s\ ^3S \rightarrow 3p\ ^3P$), 533.0 nm from O ($5d\ ^5D\text{-}3p\ ^5P$), and 777.4 from O ($2s^22p^33p^5P \rightarrow 2s^22p^33s^5S$) (inset in Figure 2). Air plasma chemistry is believed to comprise of more than 75 species and almost 500 reactions at different time and length scales, which reflects the underlying complexity (22). Thus, currently there exists a limitation over identification of the short time scale species existing in the plasma discharge. Besides those observed through OES, the dielectric barrier discharge system employed here is also a source of ozone, as reported in our previous studies (9, 10). The ozone forms by the dissociation of oxygen when highly energetic electrons bombard the molecules, followed by recombination of the singlet oxygen with oxygen molecules. Summing up, the plasma source in its current set-up is a source of reactive oxygen species (ROS) and excited nitrogen species, which have proven anti-microbial effects.

Respiration rate of cherry tomatoes

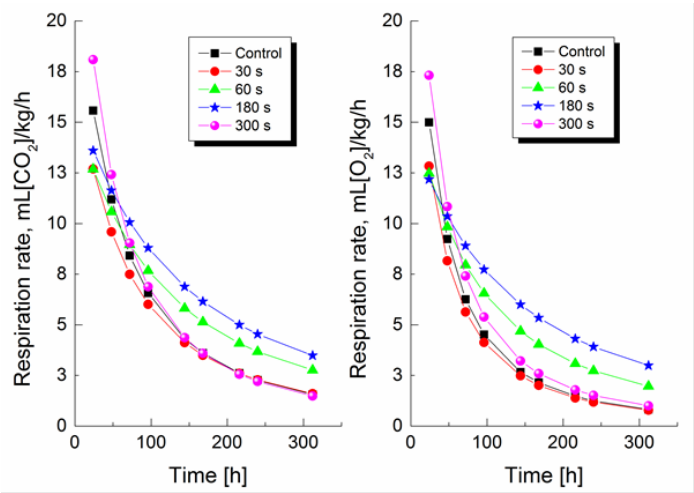

Figure 3 Respiration rates of control and plasma treated cherry tomatoes.

Based on the density and weight of tomatoes, the free volume of air in the packages was determined to be approximately 3.4 litres. The coefficients of regression, R^2 *(adj)* were found to be 0.85 or higher for all cases. The respiration rates in terms of CO_2 evolution and O_2 consumption are presented in Figure 3. The respiration rate of fresh produce is a very important factor in quality preservation. Respiration involves the oxidation of energy-rich organic molecules of the cells (such as starch, sugar and organic acids) to simpler molecules (CO_2 and H_2O), with the simultaneous production of energy (in the form of ATP and heat) and other molecules. This energy is used by the cell for synthetic reactions. The rate of respiration is often a good index of the storage life of horticultural products- the higher the rate, the shorter the life; the lower the rate, the longer the life (23). In this study, the respiration rate was found to decrease with time for the control as well as treated tomatoes due to decreasing O_2 concentration and increasing CO_2 concentration in the gaseous environment. At the end of storage period, the respiration rates appeared to converge to similar values. Such observations have also been reported by Tappi et al. (24), who hypothesise the alteration of the cellular respiratory pathway. In general, a drastic change in the respiration rate was not observable. The free volumes of the package in the order of 3.3 l for different treatments are suspected to result in respiration rates which are insignificantly different from each other. It may be noted that diffusion across the packaging film also occurs, which in our study has been ignored. This approximation is justifiable to some extent, considering the high barrier nature of the packaging material. However, as mentioned earlier the packaging requirements for this novel technology needs further studies to attain a balance

between the plasma kinetics, respiratory dynamics, and diffusion across the film.

Weight loss and visual fungal

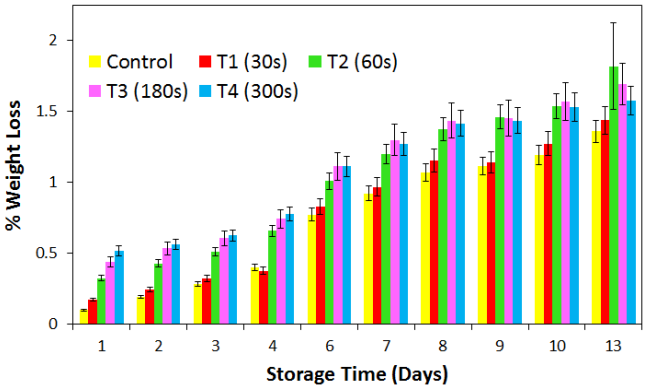

Figure 4 Weight loss (expressed as % of initial weight) of control and in-package treated cherry tomatoes over the storage period.

Around 3-5% of the post-harvest weight loss of produce is accounted for by the escape of CO_2 from the cells. Diffusion of gases across the fruit boundary and loss of water vapours from the fruit occur either through aqueous/waxy layers of the epidermis or through gaseous pores (25). For the present study, the relative loss in weights of cherry tomatoes at the end of thirteen day storage is presented in Figure 4. It can be seen that the total loss in weight did not exceed 2 % for all the samples including controls. This is less than that reported by Javanmardi and Kubota (26), who observed up to 5% weight loss after 7 days storage at room temperature (25-27 ^{o}C) for cluster tomatoes (cv. *Clermon*). This weight loss can be accounted partly based on the CO_2 that escapes from the tissue and possibly, the higher rates of transpiration in room temperature stored tomatoes (26). Venta et al. (27) have reported that ozonated cultivar tomatoes (25 and 45 mg m^{-3} ozone for 2 h/day over a 16 day period) tended to have a smaller weight loss than the control tomatoes. However, in the present study such an effect was not observed, probably due to the differences in treatment conditions and the tomato cultivar used. It is worth noting that the weight losses in the present study (up to a maximum of ~1.7%) are much less than that of Venta et al. (27) (up to a maximum of 5.7%). It has been shown that storage duration, storage temperature, and treatment have significant effects on weight loss (28). It is generally considered that fruits and vegetables are deprived of their characteristic freshness when they lose more than 3-5% of their weight (23).

The control and treated cherry tomatoes were constantly monitored for any sign of fungal growth over the entire storage period. On day thirteen, fungal growth (tiny white mass of filamentous hyphae near the stem scar and evolution of black spot) was observed on one tomato in the control (untreated) package only (which means a 10% visual fungal (29), while no visual fungal was detected in any of the treated tomatoes. Following this, the packages were opened to measure the instrumental colour and firmness. It is worth mentioning that our recent studies have revealed the inactivation of a range of bacteria on cherry tomatoes, following in-package cold plasma treatments (manuscript submitted).

Changes in colour

Colour is probably the first quality factor judged by tomato product consumers. There was no significant difference ($p \geq 0.05$) between the mean L*, a* and b* values of fresh, control and treated group of tomatoes at 95% confidence level (Table 1). The overall change in hue and chroma value (indicating colour saturation) of the control and treated samples was also insignificant (except T3). The difference in C* and h* was however, significant between fresh and control samples, as would be expected. Differences in perceivable colour can be analytically classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3) and small difference (TCD < 1.5) (30). The TCD between fresh (Day 0) and control, T1, T2, T3, T4 tomatoes were 2.88, 2.81, 3.14, 3.30, and 2.45 respectively. The control (2.88) and 30s treated tomatoes had a distinct colour from that of fresh tomatoes.

However, tomatoes treated for extended durations had a very distinct perceivable colour than fresh tomatoes. These changes were attributed to the inherent variability in the colour of produce, considering that the L^*, a^* and b^* values were not significantly different from each other. Moreover, any changes in colour of the tomatoes were visually unperceivable. These results are in agreement with those of cold plasma treated strawberries, where changes in colour among control and treated groups were found to be insignificant (2). Bermúdez-Aguirre et al. (7) have also reported insignificant changes in the colour of tomatoes following cold plasma treatments using a plasma jet array with Argon gas. The relatively lower tomato colour index (TI), which was significant ($p \leq 0.05$), indicated the possibility of degradation of carotenoid pigments, which needs further investigation.

Table 1. The CIE L^*-a^*-b^* values of fresh, control and treated group of tomatoes. Values represent mean (±S.D.) of measurements made on three tomatoes in quadruplicates along four different sections‡.

Sample	***L****	***a****	***b****	***C****	***h****	***TI***
Fresh	49.12 ± 1.19^a	31.57 ± 2.59^b	35.45 ± 1.77^c	47.98 ± 3.19^a	0.79 ± 0.03^a	1.00 ± 0.06^a
Control	48.15 ± 1.22^a	33.86 ± 2.61^b	33.98 ± 1.75^c	46.66 ± 2.27^b	0.86 ± 0.05^b	0.87 ± 0.08^b
T1 (30s)	48.27 ± 1.03^a	33.82 ± 1.95^b	33.99 ± 1.39^c	45.60 ± 1.38^b	0.87 ± 0.04^b	0.84 ± 0.07^b
T2 (60s)	48.04 ± 1.28^a	34.08 ± 2.11^b	33.88 ± 2.35^c	46.37 ± 1.62^b	0.89 ± 0.06^b	0.81 ± 0.10^b
T3 (180s)	48.11 ± 1.19^a	34.34 ± 2.06^b	33.96 ± 1.73^c	47.96 ± 1.56^c	0.81 ± 0.05^a	0.95 ± 0.09^c
T4 (300s)	48.87 ± 0.88^a	34.00 ± 2.22^b	35.23 ± 1.04^c	46.65 ± 1.10^b	0.89 ± 0.05^b	0.81 ± 0.08^b

‡Values within a column followed by the same letter do not differ significantly ($p > 0.05$)

Change in pH

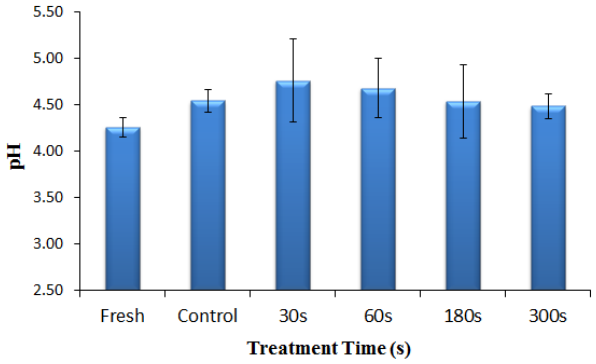

Figure 5 pH of control and treated tomatoes at the end of storage period. Values represent mean (±S.D.) of measurements made on three tomatoes in duplicate.

Among the parameters commonly analysed for the assessment of tomato quality, pH is very important because it influences the processing conditions required for producing safe products. The pH of the cherry tomatoes was found to increase at the end of storage period for the control as well as treated tomatoes (Figure 5). The relative increase in pH was slightly higher for plasma treated tomatoes, with this increment being inversely related to the treatment time. However, there was no significant difference ($p > 0.05$) between the pH of control and treated tomatoes. The pH values are comparable to those reported in literature (31, 32). An increase in pH of cherry tomatoes in storage under natural conditions has also been reported by Rodriguez-Lafuente et al. (32). The change in pH could be attributed to the metabolic changes and water loss in the tomatoes (33).

Fruit firmness

The mean values of peak force (N) required for puncturing (break) the fruits is presented in Figure 6. The firmness of the control and treated group of produce was less than that of fresh tomatoes and this difference was significant ($p \leq 0.05$). An insignificant difference ($p > 0.05$) between the firmness values of control and treated tomatoes was

recorded at the end of storage period, meaning that the tissue structure of the produce remained intact. Tappi et al. (24) recently reported that gas plasma treatments cause loss of firmness in fresh-cut apples with treatment times of the order of 10 to 30 min. This was most likely an outcome of the exposed tissues surface in their studies, which cause cell leakage during prolonged exposure, making the process more challenging. However, in the present study whole tomatoes with intact skin were treated which allowed retention of the firmness.

To summarise the findings, the plasma treatment of cherry tomatoes does not adversely affect critical quality parameters of colour, firmness, pH and weight loss. The respiration rate of cherry tomatoes does not exhibit a rise following cold plasma treatments. However, the optimisation of packaging conditions for meeting the requirements of high barrier nature for in-package plasma, while relatively high permeability desired for respiring produce needs to be addressed in future studies. In addition, the evaluation of the physical quality parameters offers only a good starting point, and changes in the produce chemistry need further investigation.

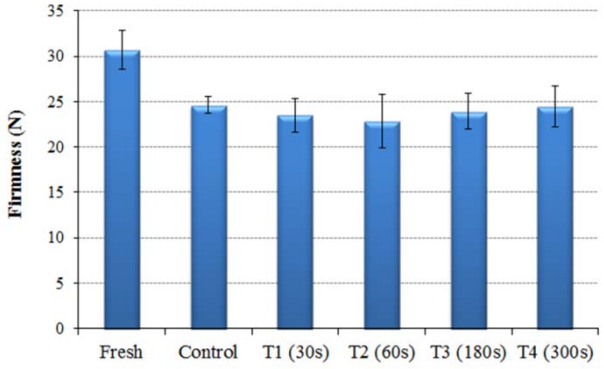

Figure 6 Firmness (force at break, N) of control and treated tomatoes at the end of storage period. Values represent mean (±S.D.) of measurements made on three tomatoes from each package.

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