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Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation

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Abstract. A direct-current, atmospheric-pressure air cold plasma microjet (PMJ) was applied to disinfect *Salmonella* directly deposited on fresh fruit and vegetable slices. Effective inactivation was achieved on sliced fruit and vegetables after 1 s plasma treatment. The physiochemical properties of the slices, such as water content, color parameters, and nutritional content were monitored before and after plasma treatment. It was found that the physiochemical properties changes caused by the plasma were within an acceptable range. Reactive oxygen species, which are believed to be the major bactericidal agents in the plasma, were detected by electron spin resonance spectroscopy and optical emission spectroscopy.

1 Introduction

Fresh fruits and vegetables are indispensable components in our daily diet. Food safety incidents have increased rapidly due to the widespread use of pesticides and due to the contamination that occurs during food processing [1,2]. Bacterial contamination accounts for 34% of all global food safety issues every year [3]. The recent *Escherichia coli* O157:H7 contaminations of cucumbers in Europe and Listeria-tainted cantaloupe in the US have raised renewed awareness to food safety across the world [4]. Thousands of people were infected and dozens ultimately died. Therefore, effective and easy-to-apply inactivation approaches for fruits and vegetables have taken on a high priority.

Conventional thermal methods of food sterilization, while effective, are unsuitable for fruits and vegetables, since heating causes inevitable changes of color, smell, flavor, and a loss of nutritional value [5]. Many efforts to develop alternative sterilization techniques have been proposed and used, such as the use of ozone, UV radiation, ultrasound, and other physical and chemical methods [6–8]. However, these approaches have drawbacks in terms of cost, potential hazards, and maintaining controllable and reproducible conditions of application, which limits their large-scale practical utilization.

Recently, an emerging technology, the use of cold plasmas operating at ambient pressure in air (or other operating gases and gas mixtures), gained prominence for use in biological and biomedical applications. These plasmas have been reported to efficiently inactivate microorganisms including bacteria, bacterial spores, fungi, and biofilms [9–11] as well as degrade bio-macromolecules such as proteins and saccharides [12,13]. It has been established that reactive radicals and other active species in the cold plasma play a key role in inactivation processes. Since the lifetimes of these species are very short, they will not leave any residual trace contaminants behind [14]. These attributes render atmospheric-pressure cold plasmas a viable alternative for the inactivation of foods. Ragni et al. studied the decontamination power of a cold plasma device on samples of shell eggs experimentally inoculated with *Salmonella Enteritidis* and *Salmonella Typhimurium* (5.5–6.5 Log CFU/egg-shell) [15]. After 90 min of treatment, a reduction about 2.5 Log CFU/eggshell and 4.5 Log CFU/eggshell were observed for *S. enteritidis* using air with a low and high moisture content, respectively. No significant negative effects of the gas plasma were observed on the egg quality traits. Song et al. evaluated the efficacy of atmospheric pressure plasma in sliced cheese and ham inoculated with 3-strain cocktail of *Listeria monocytogenes* [16]. Their results indicated that the inactivation of *L. monocytogenes* by a cold plasma is strongly dependent on the type of food. There also have been several reports on the applications of cold plasmas to sanitize fresh fruits and vegetables. Vleugels et al. used

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the biofilm-forming bacterium *Pantoea agglomerans* and bell peppers as a typical example of plant tissue, to show that atmospheric He-O_2 plasmas can be effective inactivation agents without causing unacceptable levels of discoloration to the peppers, and that they are superior to low-pressure ultraviolet sources [17]. Niemira and Sites applied a gliding arc cold plasma to inactivate *Escherichia coli* and *Salmonella stanley* on the surfaces of apples [18]. They showed that both pathogens were inactivated, and the rate of inactivation increased with increasing gas flow. Perni et al. investigated the effects of a cold plasma on mangoes and melons pericarps with four kinds of pathogens [5]. While the plasma-induced inactivation rate for the four pathogens varied, all four pathogens were inactivated in a matter of minutes. These authors also examined the decontamination efficacy on inoculated membrane filters and fruit surfaces and found a markedly reduced efficacy on the cut surfaces compared to membrane filters [19]. However, the physiochemical properties of the fruit, such as the color, flavor, and nutritional value, which are important for fruits, were not measured or studied in those experiments.

In this study, we investigated the inactivation efficacy as well as the physiochemical property changes of fresh fruits and vegetables caused by cold plasma treatment. A direct-current atmospheric-pressure cold plasma was applied to decontaminate slices of cucumbers, carrots and pears with *Salmonella*. *Salmonella* causes the most common infections that lead to nausea, vomit, fever, diarrhea or even death. The inactivation efficacy was evaluated by counting the colony forming units (CFU) before and after plasma treatment. The colors of the slices were measured by a colorimeter to obtain precise values for the color change. Moisture content and vitamin C content of the fresh slices were also quantitatively monitored before and after plasma treatment. Electron spin resonance (ESR) spectroscopy and optical emission spectroscopy (OES) were applied to identify some of the reactive species in the plasma.

2 Methods and materials

2.1 Plasma microjet device

A direct-current negative-polarity high-voltage power supply (Matsusada AU5R120) is connected to the plasma microjet (PMJ) device through a 5 kΩ ballast resistor. The PMJ device consists of two metal tubes as electrodes separated by a ceramic tube. The openings in the two electrodes were about 0.8 mm in diameter, and the depth of the exit opening is ∼1 mm. The outer electrode is grounded for safety reasons. Compressed air was used as the working gas at a flow rate of 5 slm. The operating output of the power supply is 30 mA at 500 V. A schematic diagram and further details of this plasma system can be found in references [10,20]. The plasma plume typically extended between 8–10 mm beyond the exit nozzle of the device. Figure 1 shows the treatment of fruit and vegetable slices using the PMJ.

2.2 Microorganisms cultivation and preparation of fruits and vegetables slices

The isolates of *Salmonella sp.* CGMCC 1.1552 (referred to as *Salmonella* from here on) utilized in this research were acquired from China General Microbiological Culture Collection Center (CGMCC). Fresh cultures of the isolates were grown overnight in tryptic soy broth (TSB) at 37 °C until it reached the logarithmic growth phase. The bacteria concentration is approximately 10^9 CFU/mL, as determined by serial dilution and CFU count on tryptic soy agar (TSA). Subsequently, the concentration was diluted to 10^6 CFU/mL.

Carrots, cucumbers, and pears were purchased from a local supermarket and stored at 4 °C for no more than 2 days before use. The carrots, cucumbers and pears were cleaned with sterilized water and 95% (v/v) ethanol, and then placed under UV light (peak intensity at 254 nm) for half an hour to inactivate the inherent microorganism on the outer skins and to remove other residual organics (e.g. fertilizer, organic pesticides, etc.) prior to peeling with fully sterilized peeling tools.

Slices of fruit and vegetables (pears: 3 cm × 3 cm square, 0.5 cm thick; cucumbers and carrots: round slices, 3 cm in diameter, 0.5 cm thick) were cut with a knife cleaned with 70% (v/v) ethanol. All other tools used in this process were also sterilized with 70% (v/v) ethanol. 100 μL *Salmonella* suspension (containing roughly 10^5 CFUs) was deposited onto the slices uniformly without flowing over the edges of the samples. Subsequently, these slices were allowed to dry for half an hour at room temperature to ensure that all the cells in the suspension adhered to the surface of the slice.

2.3 Procedure and inactivation analysis

Slices of fruit and vegetables were put in a petri dish and exposed to the plasma at a distance of 1 cm from the exit nozzle of the device (Fig. 1). To ensure that the entire surface was treated by the plasma, the petri dish was moved under the spatially fixed PMJ with a constant speed of 4 mm/s along the gridlines as shown in Figure 2. Each path from the top left corner to the bottom right corner of the 3 cm × 3 cm square area corresponds to a treatment time of 60 s. Increase of plasma dosage is achieved by repeating the treatment cycle up to 8 times. It has to be noted that, if we assume an active plasma area of ∼2 mm in diameter, each part of the surface of the slice was exposed to the plasma for only about 0.5 s during each plasma treatment cycle. The total treatment time of 1 to 8 min corresponds to an actual plasma exposure time ranging from 0.5 to 4 s. We shall refer to the actual plasma exposure time from here on.

After plasma treatment, the fruit and vegetable slices were transferred into a sterilized bag with 10 mL PBS, and treated for 10 min at the highest speed in a stomacher. 100 μL of the homogenate (cell suspension) of the PBS were spread uniformly over TSA agar plates, which were

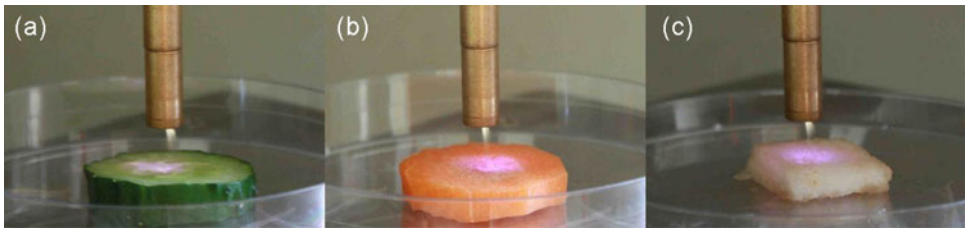

Fig. 1. (Color online) Plasma inactivation of (a) cucumber slice, (b) carrot slice, (c) pear slice.

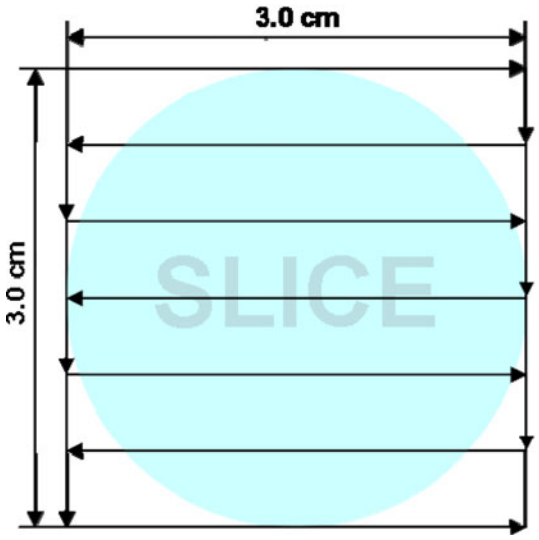

Fig. 2. (Color online) Schematic diagram of the petri dish movement in plasma treatment.

then incubated at 37 °C for 24 h for CFU counting. The inactivation rate is defined as $(1-\mathrm{CFU}_{\mathrm{treated}}/\mathrm{CFU}_{\mathrm{control}})\times 100\%$. All experiments were repeated three times for statistical evaluations.

2.4 Measurement of physical and chemical properties

To test the moisture content of the vegetable and fruit slices after plasma treatment, a direct drying method was applied [21]. Fruit and vegetables slices were placed in dried weighing bottles and kept at 105 °C for at least 3 h in the drying oven. They were then allowed to cool down to room temperature in desiccators for half an hour and weighed again. The difference in this final weight and the initial weight before drying is the weight of moisture. In an effort to eliminate as much moisture as possible, this cycle was repeated until the final weight obtained in two consecutive cycles differed by less than 2 mg. The total moisture content was then defined as the difference between the initial weight prior to the first drying cycle and the final weight after the last drying cycle.

In this study, changes in color parameters due to plasma exposure were another property of concern for the fruit and vegetable slices. In order to quantify the color differences of fruit and vegetable slices, the slices were examined with a colorimeter (Quest XE, Hunter Lab UltraScan PRO) using CIE ($L^*a^*b^*$) system in it. The CIE ($L^*a^*b^*$) system is a standard system for evaluating the color changes which allows for a color specification in 3-D space. The L^*-axis represents the degree of brightness within a sample, ranging from 0 (black) to 100 (white). The a^*-plane denotes the degree of green/red while the b^*-plane represents the degree of blue/yellow in the sample. Δ denotes the value difference before and after the treatment in each group. The total color difference (ΔE^*) of the specimen can be calculated from the following formula [22]:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}.$$

A slice was placed in front of the camera of the colorimeter and the L^*, a^*, b^* values were determined, from which, ΔE^*, was calculated.

Vitamins are important ingredients in fresh fruit and vegetables, and among them, vitamin C is the most common ingredient. We assessed the changes in vitamin C content caused by cold plasma treatment with High Performance Liquid Chromatography (HPLC). The method used in this study is similar to the method described in reference [23]. As a pretreatment step, fruit (vegetable) slices were placed in an ultrasonic bath (SB-5200DTD, Ningbo Scietz Biotechnology Co., Ltd.) in metaphosphoric acid (4.5%) (m:m=1:1) for 10 min (40 Hz, 20 °C). The treated slices were blended into a homogenate, which was then filtered using normal filter paper and hydrophilic membranes (0.45 μm). The vitamin C concentrations of the extractions were measured by HPLC.

2.5 Electron spin resonance (ESR) spin-trapping spectroscopy

Electron spin resonance (ESR) spin-trapping spectroscopy was applied to detect free radicals in the plasma treated liquid system. 5, 5-dimethyl -1-pyrroline-N-oxide (DMPO, Sigma Aldrich Co., Ltd.) was used to trap hydroxyl radicals (•OH). A solution composed of 1 mL oxygen-free ultrapure water and 20 μL DMPO (0.8 mol/L) was exposed to the PMJ at a distance of 1 cm for 1 min. We also used 2, 2, 6, 6-tetramethylpiperidine (TEMP, Sigma Aldrich Co., Ltd.) to trap singlet molecular oxygen ($^1O_2$). 20 μL TEMP (99.9%) was added to 1 mL H_2O, and then treated with the PMJ for 20 s. These treatment times were chosen to maximize the signal-to-noise ratio. Measurements of spin-trapped adduct (DMPO-OH and TEMPO) signals

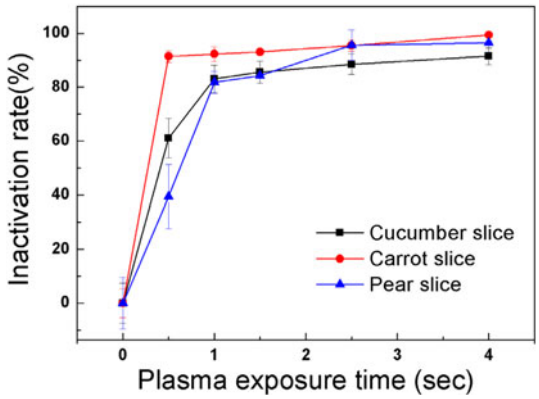

Fig. 3. (Color online) Cold plasma inactivation of *Salmonella sp* CGMCC 1.1552 on the surface of cucumber, carrot, and pear slices. Time presented here is based on the assumption of a 2 mm diameter plasma spot moving at a speed of 4 mm/s.

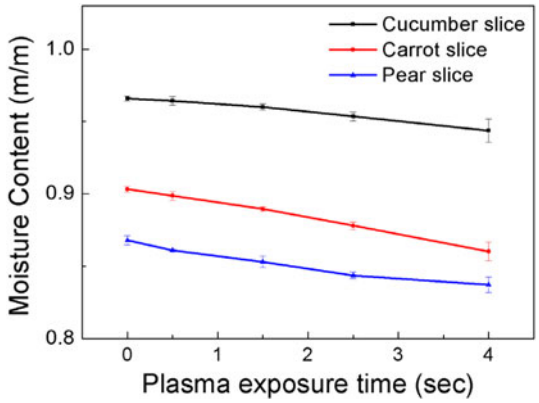

Fig. 4. (Color online) Cold plasma effect on the moisture content of cucumber, carrot, and pear slices.

were carried out using an ESR spectrometer (Bruker ER-200D-SRC). Detailed information about the ESR detection can be found in our previous publications [24,25]. In an effort to verify the detected spin adduct signals, we added 0.75 mol D-Mannitol (D-Man, a quencher of •OH) or 6 mg L-Histidine (L-His, a scavenger of •OH and $^1O_2$) into the liquid system before plasma treatment and measured the change of the DMPO-OH and TEMPO signal intensities. Both D-Man and L-His were obtained from the Shanghai Secondary Military Science Academy.

2.6 Optical emission spectroscopy (OES)

OES was applied to identify some of the active species in plasma. End-on light emissions were collected via a fiber optics cable and imaged to the entrance slit of a 0.75-m spectrometer (Princeton Instrument/Acton Spectra Pro 2750) equipped with an 1800-groove/mm blazed holographic grating. To obtain a reasonable signal-to-noise ratio with sufficient spectral resolution to isolate the major emission lines, the entrance slit was set to 100 μm. An intensified CCD camera (Princeton Instrument I-Max-512) was used to record the dispersed emission spectra.

3 Results and discussion

3.1 Inactivation on fruit and vegetable slices

As described above, about 10^5 CFUs *Salmonella* were suspended on the slice surfaces. The inactivation results are shown in Figure 3. While the inactivation curves show a similar general trend for cucumbers, carrots, and pears, there are notable differences in the actual shapes of the survival rates of *Salmonella* for short periods of plasma treatment. In half a second, 90% of the bacteria on the carrot slices were inactivated. By contrast, in the case of the cucumber slices, only about 60% of the *Salmonella* were inactivated. As for the pear slices, the inactivation was even less effective with only about 40% being inactivated. After 1 s treatment, the inactivation rates of bacteria on cucumber and pear slices reached above 80%. Further plasma treatment caused a further reduction in the surviving bacteria, but change of inactivation rates decreased considerably. A 4 s plasma treatment inactivated all the pathogens on the carrot slices. However, about 10% of the *Salmonella* on both the pear and cucumber slices still survived.

The differences in the inactivation efficiency may be attributed to differences in the surface liquid water, or more likely to juice emanating from the internal tissue. Even though the slices were dried for half an hour to let the surface liquid water to evaporate, internal juices in the tissue continue to permeate out. According to reference [5], the more residual moisture remains on the slice, the easier it is for the microbial cells to move and penetrate deep into the slice, which enhances the chance of their survival. Since there was less liquid on the surface of the carrot slices than on the pear and cucumber slices, we attribute the incomplete inactivation of *Salmonella* cells on the pear and cucumber slices to the moist environment on the surfaces of these slices. Perni et al. [5] computed the migration velocity of pathogens into the fruit tissues and arrived at a similar conclusion about the different inactivation rates. Other factors which may contribute to different inactivation rates are the texture and hardness of the slices, which can enhance or hinder the penetration of the microbial cells into the tissue [26].

3.2 Evaluation of moisture content of fruit and vegetable slices

The moisture content was slightly affected by the plasma treatment as can be seen from Figure 4. We found a moisture loss of less than 5% for all three species after 8 min cold plasma treatment. The moisture loss may be attributed (1) to the air flow from the plasma device, which is 5 liters per minute and (2) to the slightly elevated temperature of air plasma, which is about 30–45 °C. These two factors will accelerate the evaporation of water on the surface of the fruit and vegetable slices. However,

Table 1. The L^*, a^*, b^*, and ΔE^* values for slices of cucumber, carrot, and pear after 4 s plasma treatment (C refers to control group, PT refers to plasma treatment).

Groups	C			PT			
	L^*	a^*	b^*	L^*	a^*	b^*	ΔE^*
	63.57	–6.25	20.53	63.15	–6.17	19.76	
Cucumber	62.44	–6.21	19.68	63.01	–6.16	19.84	0.666 ± 0.118
	64.54	–6.48	20.58	62.95	–6.19	19.87	
	55.49	20.87	24.60	54.81	20.01	23.30	
Carrot	55.38	20.90	24.82	54.30	20.42	23.91	1.510 ± 0.628
	55.31	20.99	24.88	54.13	20.57	24.13	
	68.51	–0.55	6.69	69.14	–0.25	7.83	
Pear	68.40	–0.48	6.68	69.05	–0.24	7.83	1.339 ± 0.091
	68.32	–0.48	6.70	68.96	–0.24	7.85	

moisture lost is quite common in fresh-cut fruits and vegetables. When fruits and vegetables are cut in the commercial processing operations, it is inevitable that some of the extracellular fluid bleeds out and the cell sap runs off. After a short time, only the cells of cut surface sections lose moisture, which has little negative effect on the rest of the slice. In general, a moisture loss of less than 5% will not significantly influence the tissue hardness and is considered acceptable.

3.3 Evaluation of surface color parameters of fruit and vegetable slices

In the evaluation of the surface color parameters of the slices with method described in Section 2.4, we found that the plasma treatment affected the lightness (L^*), the red/green contrast (a^*), and the blue/yellow contrast (b^*), as shown in detail in Table 1. A plasma treatment of 8 min (4 s exposure of each part of the surface area) resulted in ΔE^* values of 0.666 ± 0.118, 1.510 ± 0.628 and 1.339 ± 0.091 for the cucumber, carrot and pear slices, respectively. According to reference [27], total color differences (ΔE^*) are considered as very distinct ($\Delta E^* > 3$), distinct ($1.5 < \Delta E^* < 3$), and small differences ($\Delta E^* < 1.5$). In our experiments, the ΔE^* values of all three slices are in the small differences range. The ΔE^* value of the carrot slices changed somewhat more noticeably compared to the cucumber and pear slices, perhaps because of a higher degree of oxidation of the surface carotene. Another reason may be the loss of liquid on the slice surface, which can significantly influence the lightness of each slice. Nevertheless, no unappealing differences in the surface colors after plasma treatment can be ascertained by visual inspection of the slices, as shown in the pictures of the slices with no plasma treatment, treated with plasma for 0.5 s and 4 s, respective (Fig. 5).

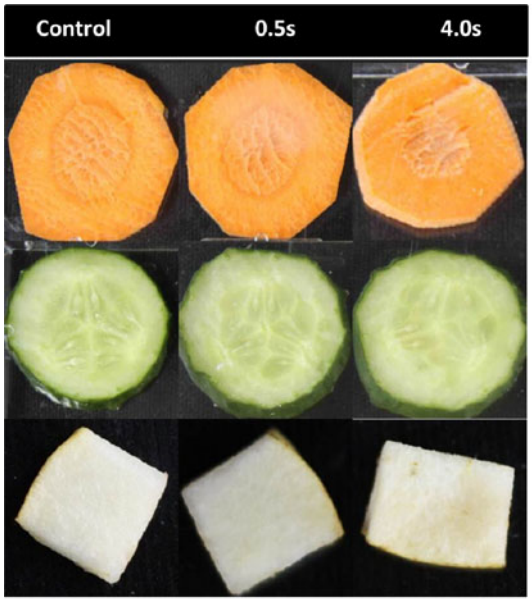

Fig. 5. (Color online) Images of sample carrot, cucumber and pear slices from the control group (left column), the 0.5 s treatment group (middle column) and the 4 s treatment group (right column).

3.4 Evaluation of vitamin C content of fruit and vegetable slices

Figure 6 shows only slight reduction of the vitamin C content of the three types of slices after plasma treatment, with the cucumber slices showing the largest reduction (3.6%). The vitamin C loss for the carrot and pear slices was 3.2% and 2.8%, respectively. The slight reduction in vitamin C content is most likely due to its oxidation by the cold plasma. In addition, vitamin C is light sensitive [28], so UV generated by plasma may also play an important role for the vitamin C degradation. Ajibola et al. have studied vitamin C loss of tropical fruit juice (orange, lemon, lime, pineapple, paw paw and carrot) during storage in refrigerator (4 ± 1 °C). All of the juices lost more than 10% vitamin C after one week [29]. Therefore, our result of less than 4% vitamin C reduction is considered in the acceptable range.

3.5 Evaluation of reactive species

Signals of DMPO-OH (spin adduct of •OH) and TEMPO (spin adduct of $^1O_2$) show a 1:2:2:1 quartet pattern and a 1:1:1 triplet pattern in ESR spectra, respectively (Fig. 7).

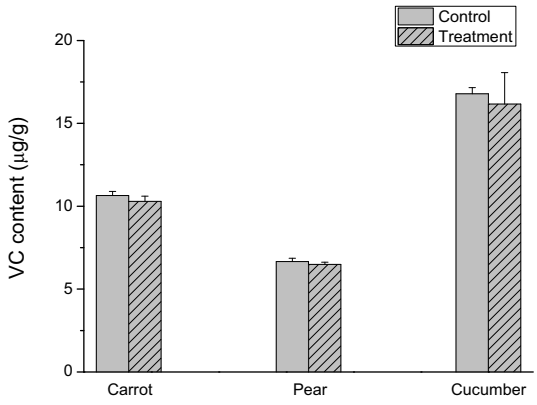

Fig. 6. Vitamin C content of carrot, cucumber and pear slices of the control group and the 4 s treatment group.

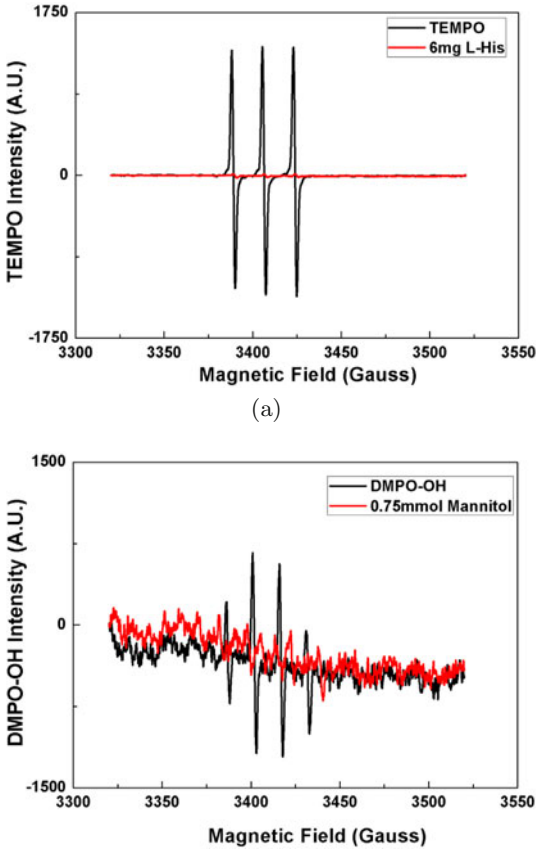

Fig. 7. (Color online) ESR signals of (a) TEMPO (spin adduct of $^1O_2$) and the effect of L-Histidine, (b) DMPO-OH (spin adduct of •OH) and the effect of D-Mannitol.

With an addition of 0.75 mol D-Man or 6 mg L-His with the spin trap reagents before the plasma treatment, and following the same ESR experiment procedure, no DMPO-OH or TEMPO were measurable within the detection limit of the apparatus, confirming the existence of •OH and $^1O_2$ in the system. Details about the generation of •OH and $^1O_2$ in the liquid can be found in our prior work [24,25].

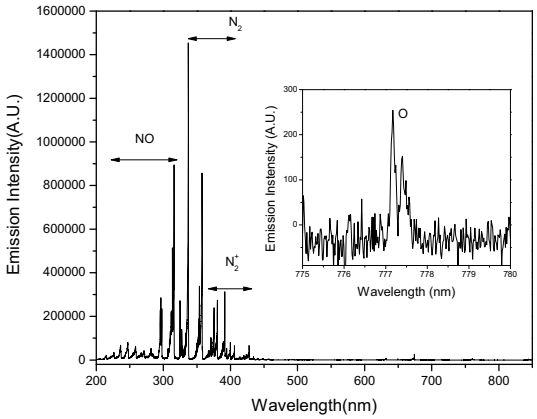

Fig. 8. (Color online) Optical emission spectrum of the cold air plasma.

•OH, whose oxidation potential is 2.8 eV, can oxidize almost any biomaterial; $^1O_2$, also with a high oxidation potential, can effectively destroy unsaturated fatty acids and channel proteins on cell membranes. Both of these species cause damage to the polysaccharides on the bacteria cell wall and initiate the breakdown of the macromolecules in the lipid bilayer, which ultimately destroys the cell.

Figure 8 shows the plasma emission spectrum from 200 to 860 nm. When operated in air, the spectrum shows very strong N_2 second positive system ($C^3\Pi_\mu$-$B^3\Pi_g$) emissions at 337.1, 353.7, and 357.7 nm. In the near UV region, NO γ-system ($A^2\Sigma^+$-$X^2\Pi$) emission bands from 211 to 300 nm and a strong emission of N_2^+ first negative system ($B^2\Sigma_u^+$-$X^2\Sigma_g^+$) at 391 nm were also observed. A weak atomic oxygen emission at 777 nm was observed (as shown in the inset of Fig. 8).

NO is an important cellular messenger molecule involved in many physiological and pathological processes in mammals. Direct cellular damage results from increased levels of NO. NO interacts with ROS in a variety of ways, either as a crucial partner in regulating the redox status of cells, determining cell fate, or in signaling the response to a number of physiological and stress conditions [30]. A few possible pathways for the generation of excited NO species are listed below [31]:

$$\begin{aligned}
&N_2 + O \rightarrow NO + N\\
&N + O_2 \rightarrow NO + O\\
&N + O + N_2 \rightarrow NO + N_2\\
&NO + O_3 \leftrightarrow NO_2 + O_2\\
&NO_2 + O_2 + h\nu \rightarrow O_3 + NO.
\end{aligned}$$

Atomic oxygen (and subsequently O_3) are also highly reactive. Aside directly contributing to the inactivation process, they also lead to the production of •OH upon interacting with the liquid water on the surface of fruit/vagetable slices [32]. The recombination of two polar •OH molecules on charge centers produces a hydrogen peroxide molecule, which may pass cell membrane relatively easily and later cause DNA damage [33].

4 Conclusion

We studied the use of atmospheric-pressure cold air plasma for the inactivation of fresh fruits and vegetables. A plasma treatment of 2 min (1 s direct plasma exposure of each part on the surface) inactivated nearly 90% of the *Salmonella* (concentration of 10^6 CFU/mL) on the carrot slices and 80% on the cucumber and pear slices. The water content, color parameters and vitamin C content of the fruits and vegetables were found to be only minimally affected by the plasma treatment, with all changes within the range of changes considered acceptable. ESR spectroscopy showed signals attributed to spin-trapped •OH and singlet oxygen in the presence of the PMJ. Our study is one of the first attempts to provide a detailed quantitative analysis of the effect of cold plasma treatment on food sensory and nutritional quality attributes beyond the frequently reported qualitative visual observation and subjective human judgment. The results presented here can be viewed as a proof-of-concept of the successful use of an atmospheric-pressure plasma microjet to combat bacterial surface contamination of fresh fruits and vegetables. However, any large-scale industrial application of cold atmospheric-plasma technology in food decontamination, after appropriate regulatory approval, would require a suitable 2D plasma source much larger than the microjet used here, with a diameter of preferably a few centimeters.

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