Plasma Medicine 4(1-4): 57-65 (2014)

Treatment of Microorganisms in Vegetables and Fruits by Gliding Arc

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ABSTRACT: In this study, we constructed a gliding arc to investigate the treatment of microorganisms in vegetables and fruits at atmospheric pressure using the microorganism *Erwinia carotovora* subsp. *carotovora*. The gliding arc was driven by direct current (DC) voltage between two knife-shaped electrodes. The treatment of potatoes using this method was effective against this pathogen. The disinfection duration was a few seconds, which is rather fast, with no overheating. The use of a thermal image sensor enabled us to determine the percentage of the concentration of individual temperatures in the arc region. Using this method, we were able to identify thermal and nonthermal regions of the arc. The temperature increase of the potatoes during plasma treatment by gliding arc at atmospheric pressure was only few degrees above room temperature, assuring a mild treatment of biomaterials.

KEY WORDS: Food security, gliding arc, *Erwinia carotovora* subsp. *carotovora*, disinfection, Infrared imaging

I. INTRODUCTION

Food preservation by microorganism control is an important issue in food security worldwide.[1] Among untraditional methods, avoiding chemical treatment or radioactive exposure, nonthermal plasma is considered as a promising technique for food preservation.[2] Nonthermal plasma at atmospheric pressure has the advantage of treating biomaterials moderately without overheating or applying high pressure while retaining all nutritional value.[3] Plasma treatment is always accompanied by a pronounced antimicrobial action due to the existence of radicals, UV photons, charged particles, and reactive species, especially reactive oxygen and nitrogen species (RONS). The gliding arc has very interesting features in food preservation because it supports selective chemical processes at atmospheric pressure and controllable temperature suitable for food treatment.[4] A gliding arc is generated between two or more diverging electrodes subjected to fast gas flow. The applied alternating current (AC) or DC high voltage provides the necessary electric field for breakdown to occur at the shortest distance between the electrodes. If the gas flow is strong enough, it forces the discharge to move along the diverging electrodes and elongates.[5] The gliding arc has been used to treat vegetables and fruits to reduce microorganisms such as bacteria and fungus, preventing their attacks and enhancing plant preservation. We focus here on the treatment of one of common microorganism in potato,[6] *Erwinia carotovora* subsp. *carotovora* (*E. c. carotovora*).

1947-5764/14/$35.00

II. MATERIALS AND METHODS

The gliding arc plasma discharge used in this study consists of two knife-shaped stainless steel electrodes 96 mm long, 26 mm wide and 4 mm thick, as shown in Fig. 1a and 1b. The discharge voltage is supplied by a DC power supply delivering up to 5 kV - 2.5 Amps. The normal working condition is reached at 2 kV- 1 A, between the two electrodes in the presence of argon gas injected through a nozzle placed on the top between the two electrodes. The gap at electrodes neck is adjusted to approximately 3 mm, and the distance between electrode lower extreme tips and target position is set at 13 cm. Those values remain unchanged during all experiments.

A. Sample Preparation

First, the *E. c. carotovora* strain on both potato tubers and nutrient agar are exposed to plasma. Bacterial suspensions are prepared by growing fresh cultures of isolates overnight in tryptic soy broth (TSB) at 37°C until the logarithmic growth phase is reached. The bacteria concentration is adjusted to colony-forming units of 10^8 CFUs/ml and reaches 10^6 CFUs/ml by serial dilutions. Bacterial suspension is applied on peeled slices of potato tubers. Prior to peeling, all potato tubers are put in fresh water for 5 hours to remove any soil from their outer surfaces. Then, to inactivate inherent microorganisms on the outer skin and remove any organic residuals, potato tubers are sterilized by 70% ethanol solution followed by rinsing several times with sterile distilled water. After they dry in air, a sterilized potato peeler is used to peel 5-mm-thick slices of area 1 cm^2 from the tuber skin; the peeler is flame sterilized between samples. Potato slices are placed on sterilized glass Petri dishes covered uniformly by 100 µl *E. c. carotovora* suspension (containing approximately 10^5 CFUs). Petri dishes are then exposed to plasma for different time intervals from 15 to 60 sec in 15-sec interval steps. After treatment, the potato slices are immersed in sterile glass tubes containing

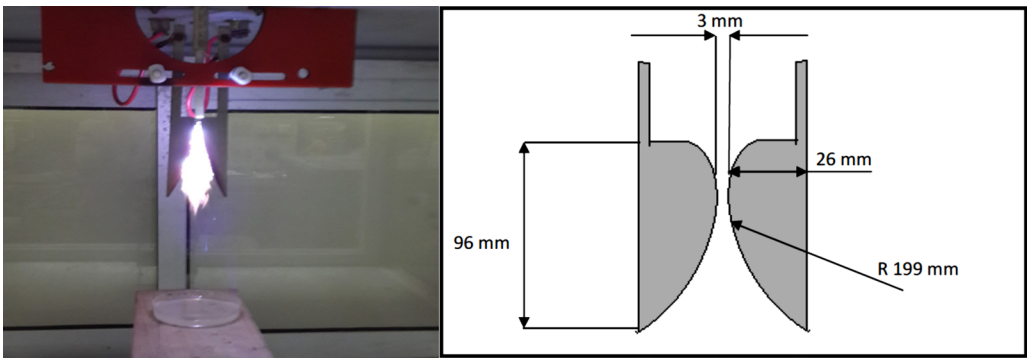

FIG. 1: (a) Photo of gliding discharge. **(b)** Schematic diagram of electrodes

2 ml phosphate buffered saline (PBS) solution (pH 7.2) and are vortexed for 2 minutes to separate the bacteria from the treated slices. To count CFUs, 100 μL of the homogenate PBS containing cell suspension are spread uniformly over nutrient agar plus 1% glucose on plates, which are then incubated at 37°C for 24 hours. Cultures of *E. c. carotovora* on nutrient agar are prepared similarly. Bacterial suspensions on potato tubers or nutrient agar are treated with the gliding arc plasma for time durations ranging from 15 to 60 seconds, as previously stated.

II. RESULTS

Decontamination actions of plasma generated by the gliding arc on *E. c. carotovora* strains spread on potato slices were assessed using a colony-counting technique described previously. Different plasma exposure times compared with the untreated control specimens are shown in Figure 2.

The decay of *E. c. carotovora* strains on potato tubers, expressed in CFUs per mL of solution, was plotted against plasma exposure times (Fig. 3). Two distinct slopes can be seen. For each one, we calculated the decimal reduction time (D-value), which is the time required to reduce the original microbial population by 90%, or 1 log cycle (factor

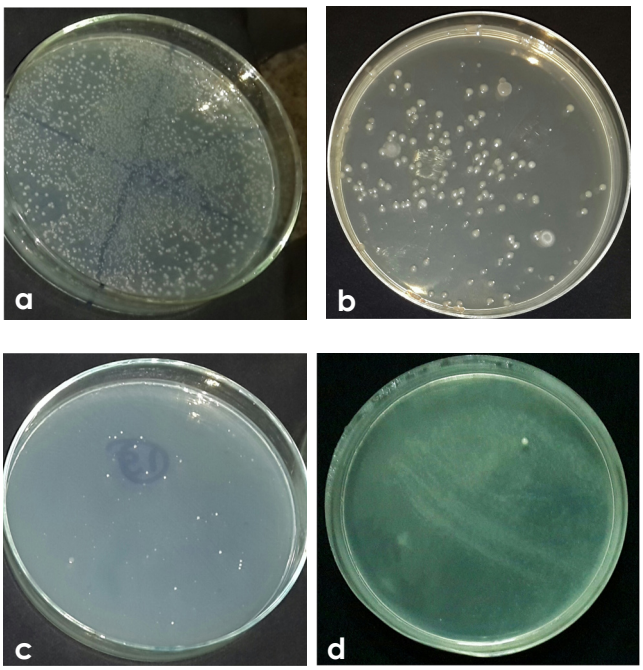

FIG. 2: Photos of Petri dishes with *E. c. carotovora* strains **(a)** control , **(b)** treated for 15 sec, **(c)** treated for 30 sec, **(d)** treated for 60 sec

of 10). As shown in Figure 3, the D-value of the first slope is D_1 = 30 sec and that of second slope is D_2 = 4.54 sec.

The curves of these two slopes has been explained by Kelly-Wintenberg et al.[7] as being due to the effect of plasma reactive species on the cells outer membranes, which induces their alteration. This is manifested in the first part of the curve with longer D-value, D_1, followed by a quick penetration of the reactive species in the cells causing more rapid cells death, which is apparent during the second part of the curve with shorter D-value, D_2. Complete disinfection is reached after 60 sec of plasma treatment. The efficacy of plasma disinfection of fresh-cut legumes is competitive with other traditional food disinfection methods. The D-values concerning plasma disinfection of common microorganisms on potatoes are measured in seconds; other methods, such as as chemicals, pulsed electric field or radioactive irradiation, generally have D-values rated in minutes.[8] To identify the morphology of *E. c. carotovora* cells after plasma treatment, we exposed *E. c. carotovora* culture on nutrient agar to a gliding arc for 60 seconds, which we subsequently analyzed with scanning electron microscope (SEM) and transmission electron microscope (TEM).

The samples were examined by scanning electron microscope (JEOL-JSM-5500 LV) using high-vacuum mode. Figure 4 shows scanning electron microscope photomicrographs illustrating the effect of gliding arc plasma on the morphology of *E. c. carotovora*. The SEM results clarify that the bacterial membranes show surface cracks and irregular shapes after 60 seconds of plasma treatment. The dissimilarities of magnifications and scales are notable between Figs. 4a and 4b; however, cell alterations between control and treated samples are obvious. Samples were also examined with a JEOL 1010 transmission electron microscope (Fig. 5). On the TEM images, we could see that leakages of cell contents occurred through holes in cell membranes after 60

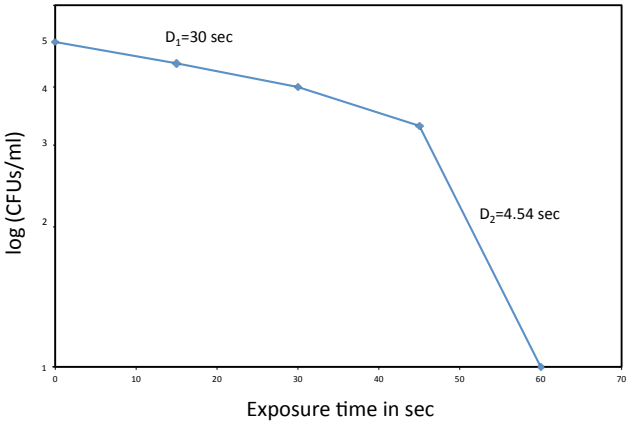

FIG. 3: Inactivation of *E. c. carotovora* on potato tubers showing decay of colony-forming units per ml against plasma exposure times showing different D-values

seconds of plasma treatment.

A. Temperature Distribution Along the Gliding Arc

The gliding arc infrared image was obtained using a FLIR A5sc thermal imager sensor with spatial resolution of 2.78 mrad and spectral resolution of 7.5–13 μm. Figure 6 shows the surface temperature image in the arc region during treatment of biomaterials determined from the infrared emission. The infrared sensor was placed 80 cm in front of the electrodes. The mesurements were taken under the following parameters: emissivity of polished stainless steel

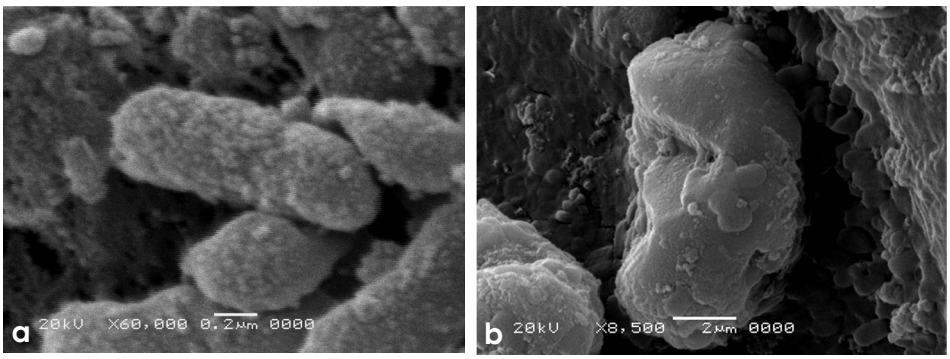

FIG. 4: Scanning electron microscope images of (a) control sample of *E. c. carotovora*. (b) treated sample of *E. c. carotovora*

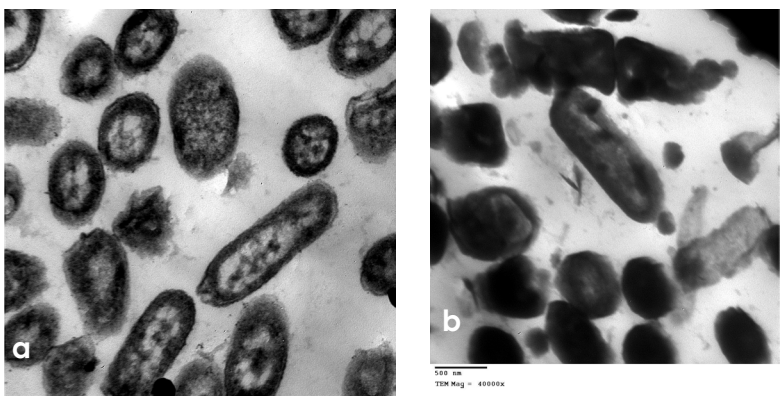

FIG. 5: Transmission electron microscope images of (a) control sample of *E. c. carotovora*. (b) treated sample of *E. c. carotovora*

electrodes was taken to be 0.14, room temperature 24.5°C, and relative humidity 78%.

For further investigations, we recorded temperatures distribution in the arc region along two axis,[9] one vertical Li1 and the other horizontal Li2 (Fig. 6). The use of a thermal imager sensor enabled us to determine the percentage concentrations of individual temperatures in the arc region (Figs. 7 and 8). The maximum, minimum, and average temperatures are indicated in Figures 7 and 8 for the two axes. The maximum temperature recorded at the central region of the gliding arc plasma column was assumed to occur in a thermal zone. The minimum temperature recorded at the outer contour of the gliding arc plasma column was assumed to occur in nonthermal zone. The temperature at the Petri dish location (13 cm from electrode tips) was monitored during treatment time by a separate infrared thermometer and was found to increase slightly depending on treatment time, reaching values 6–8°C above room temperature, which is quite acceptable for the treatment of biomaterials.

III. DISCUSSION

When applied to biomaterials, plasma has two main effects. The first is the inactivation of microorganisms and the second is biomaterial cells alteration. The inherent advantage of using nonthermal plasma in decontamination of food from common pathogens is now recognized. However, the action of plasma on nutritive food constituents as protein, lipid, and sugar has yet to be elucidated. To picture the effect of plasma on cell materials, the extensive work that has been done in the investigation of effects of pulsed electric fields (PEF) on biomaterials must be considered.[10] PEF, due to elec-

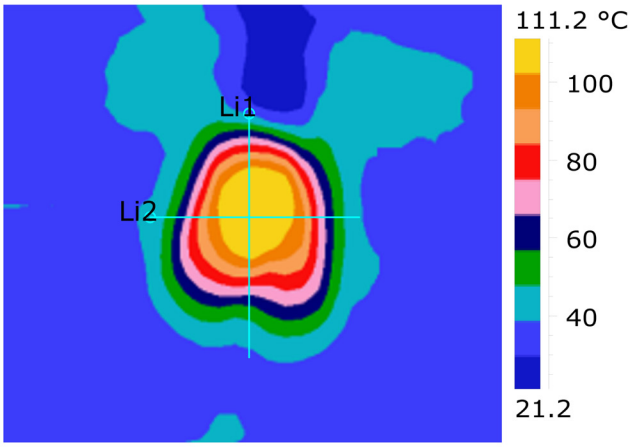

FIG. 6: Thermal image of the gliding arc showing temperatures at different contours in the arc region

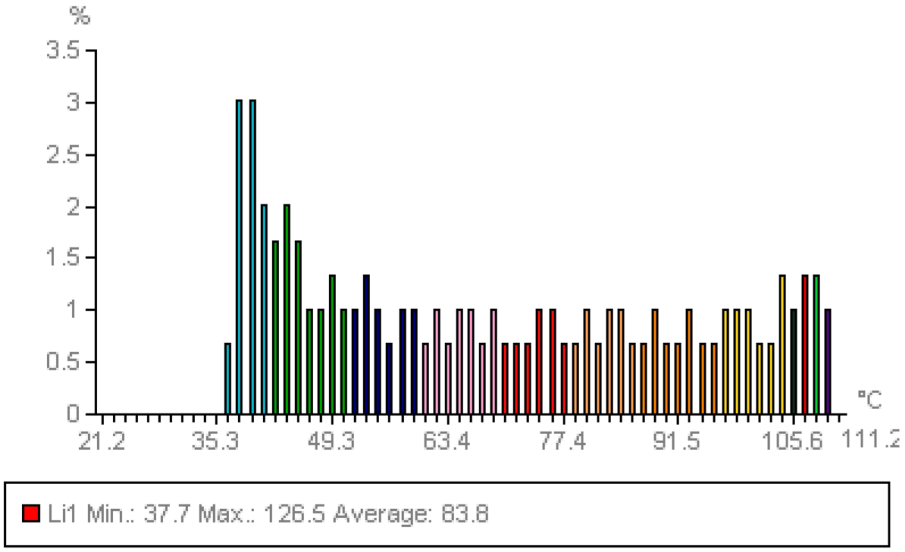

FIG. 7: Temperatures distribution in the arc region along the vertical axe Li1. The minimum, maximum, and average temperatures along this axis are indicated

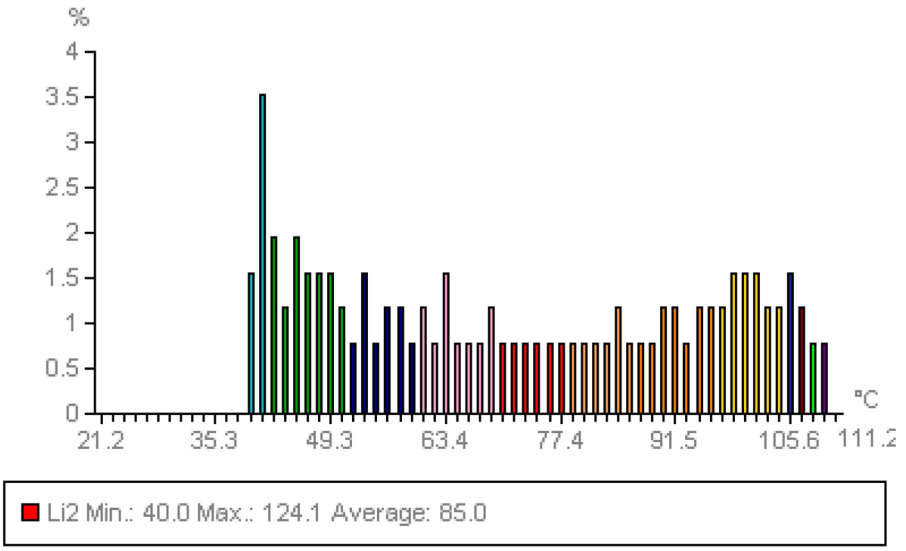

FIG. 8: Temperatures distribution in the arc region along the horizontal axe Li2. The minimum, maximum, and average temperatures along this axis are indicated

trical charge action, produces electroporation of the cell membrane, leading to membrane permeabilization and the extraction of food plant nutritive materials. The action of plasma on biomaterials seems to be more complicated due to the presence of highly energetic reactive plasma species, which allows plasma to act at low temperature, gently maintaining all nutritive materials. This action should be analyzed extensively to localize any nutritive materials changes after plasma treatment.

IV. CONCLUSION

The nonthermal plasma treatment of biomaterials is a promising method for pathogen elimination. The disinfection of potato tubers from E. c. carotovora by gliding arc at atmospheric pressure is efficient and quick with only moderate temperature increase of the tuber surface. The temperature distribution in the gliding arc region is an important factor considering plasma chemistry issues. The further study of temperature distribution in the arc region can clarify the formation of reactive species and their diffusion in biomaterial cells. In addition to treating plant tubers with plasma, which is important for the food industry, this method can be used to treat seeds to prevent the spread of common pathogens.

ACKNOWLEDGMENTS

E. c. carotovora strains are supplied by the faculty of Agriculture, Ain-Shams University, Cairo, Egypt. Electron microscopy measurements are done at the *Regional Center for Mycology and Biotechnology*, *Al-Azhar University*, Cairo, Egypt. Sample preparation and bacteria counting are done at the Faculty of Medicine, Zagazig University, Zagazig, Egypt.

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