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Optimization of Short-Pulsed Dielectric Barrier Discharge for In-Package Disinfection

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ABSTRACT: Plasma treatment for bacterial inactivation has been studied via the direct application of dielectric barrier discharges or the indirect afterglow of atmospheric pressure plasma jets. Industrial application of this technology is limited to open contact between plasma and bacteria, which limits the potential use of atmospheric plasma for food safety. In this paper, we show that uniform plasma treatment using microsecond-pulsed and nanosecond-pulsed dielectric barrier discharge can be applied directly to a bread surface through two layers of plastic bread packaging material. We show inactivation of *Escherichia coli* inoculated on the bread surface inside of packaging material without significantly modifying the plastic. This discharge will be used for the prevention of mold growth on bread in future studies.

KEY WORDS: atmospheric-pressure plasmas, food products, food packaging, plasma applications, pulsed power supplies

I. INTRODUCTION

Food safety is a growing concern with increasing food production and the rise of antibody resistant pathogenic organisms that cause foodborne outbreaks of infectious disease.[1–3] Current methods to mitigate the contamination of fresh produce postharvest are to wash with water, chlorine-based sanitizers, ozone, or other disinfecting agents.[4,5] These chemical-based approaches put both consumer and produce quality at risk at higher concentrations required for microbial inactivation. There is a need for newer technologies that reduce microbial load while maintaining produce quality. Studies show that cold atmospheric plasma is one such technology that addresses these challenges because it is nonchemical and nonthermal.[6–9]

A gas phase phenomenon, plasma is typically generated on the surface of the food, and it is delivered to the surface either directly by dielectric barrier discharge (DBD) or indirectly as a plasma afterglow or plasma-treated water.[10,11] Treatment of produce with these methods was shown to inactivate microorganisms on lettuce, strawberries, apples, and other produce.[12–15] The discharges typically are generated through high voltages (> 20 kV). Additionally, the mechanism of antimicrobial activity is through reactive oxygen species known to produce ozone and hydroxyl radicals that are toxic to microorganisms.[15] Recently, in-package disinfection has garnered interest for commercial

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implementation at ambient conditions, and it may also reduce the possibility of contamination after packaging.[16–18]

In the present work, we use microsecond- and nanosecond-pulsed DBD plasma to treat the surface of packaged bread inoculated with pathogenic organisms. The plastic material of the package is used as a secondary dielectric, similar to other recent DBD studies for in-package disinfection.[19] As an example of a packaged food, we use standard bread loaves baked without antimicrobial additives. The purpose is to demonstrate the feasibility of in-package disinfection with pulsed DBD discharge for future work investigating inhibition of mold growth on bread. Since the packaging material is not permeable to microorganisms (inoculated on the bread), once the bread is treated, it will be protected from additional airborne pathogens until it is removed from the package.

II. EXPERIMENTAL SECTION

A. Microsecond- and Nanosecond-Pulsed DBD Plasma Parameters

Microsecond-pulsed DBD (mspDBD) and nanosecond-pulsed DBD (nspDBD) were produced by applying high voltage pulses to an electrode placed 1 mm above the target for treatment. The microsecond power supply was used with the round electrode, 25.4 mm in diameter, as described previously.[20] Plasma was applied at 2500 Hz and 26 kV unless stated otherwise. The nanosecond power supply (FPB-20-05NM, FID GmbH, Germany) generated plasma at 17 kV and 500 Hz with a rise time of 2 ns and a pulse width of 20 ns, as previously described by the authors.[21]

B. Detection of Oxidation Due to DBD Plasma through Layers of Plastic

Indigo carmine is an established indicator of oxidation, frequently used to evaluate plasma treatment of water; thus, we used it to signify the presence of plasma.[22] Indigo carmine powder (Fisher Scientific, USA) was dissolved in distilled water at a concentration of 2 g-L^{-1}. Standard office paper was soaked in the solution for 2 minutes before treatment. mspDBD was applied to the soaked paper directly or through layers of plastic for up to 90 seconds. As an example of packaged food, we mimicked the double layered packaging of bread loaves by wrapping the bread in an inner bag and an outer bag. The top layer that directly interacts with plasma was a 31.75 μm monolayer of low density polyethylene (LDPE),and the layer underneath was 29.97 μm of biaxially oriented polypropylene (BOPP). The electrode was placed 1 mm above the LDPE, with a 1 mm gap between LDPE and BOPP and another 1 mm between BOPP and the soaked paper.

C. Fourier Transform Infrared Spectroscopy Analysis of Plasma Treated BOPP

BOPP was cut into 10 × 10 cm squares prior to nspDBD plasma treatment. Samples were treated with nspDBD plasma for 15 seconds and immediately analyzed. Fourier

transform infrared (FTIR) spectroscopy of untreated and treated BOPP was done using a Nicolet 8700 spectrometer with a Smart iTR sampling accessory and analyzed using OMNIC Specta software. Each spectrum was averaged over 32 repeated measurements and acquired at a resolution of 4 nm. The experiment was run in triplicate.

D. Measurement of the Puncture Force

BOPP was cut into squares as previously mentioned and taped down taut on all four corners around a hollow cylinder 5 cm in diameter. mspDBD and nspDBD were applied for 15 seconds at the parameters described earlier. Treated plastic was subjected to the puncture test immediately after treatment. A dynameter with a sharp attachment was used to calculate the force until the plastic punctured. The data was recorded and repeated three times for both untreated and treated BOPP.

E. Inoculation of *Escherichia coli* on Bread Samples

Bread samples were cut out from loaves and inoculated with a rifampicin-resistant strain of *Escherichia coli (E. coli* O157:H7; gift from Dr. Nitin Nitin of the University of California at Davis, ATCC#700728) prior to treatment. Fresh cultures were prepared as described previously.[23,24] Briefly, 5 mL of Bacto Tryptic Soy Broth (Becton, Dickinson and Company, USA) containing 10 mg-mL^{-1} rifampicin (Sigma Aldrich, USA) and 1 mL of *E. coli* suspension overnight at 37°C. *E. coli* suspensions for experiments were diluted in Hank's Buffered Salt Solution (HBSS, Corning Life Sciences, USA) to 10^8 CFU-mL^{-1} by comparison to the turbidity-based McFarland No. 2 standard (Hardy Diagnostics, USA), then serially diluting to 10^5 CFU-mL^{-1} in 1 mL of HBSS. Samples of bread baked without antimicrobial additives (gift from Dr. Reha Azizoglu, Campbell's Soup in Camden, NJ) were prepared by excising 12.5 × 12.5 × 2.5 cm pieces from the side crust. Bread was inoculated by dripping 10 µL of 10^5 CFU-mL^{-1} *E. coli* suspension on the surface of the bread and allowing it to dry.

F. mspDBD Treatment of Inoculated Bread

Inoculated bread samples were treated with mspDBD plasma applied for 15 seconds up to 10 minutes. Following treatment, samples were placed in 1 mL of HBSS. The bread and HBSS were vortexed and the HBSS was collected, spread over tryptic soy agar (TSA) plates (Hardy Diagnostics, USA), and incubated at 37ºC overnight. CFUs were counted the following morning.

G. Statistical Analysis

Each treatment was performed in duplicate. Data was analyzed using through one-way analysis of variance (ANOVA) parametric tests to assess the significance between the

treated samples and the control samples (0 mJ). Samples were plotted with the mean and standard error of the mean.

III. RESULTS AND DISCUSSION

A. Oxidation of Indigo Carmine–Soaked Paper by mspDBD Plasma

A protocol for use of indigo carmine–soaked paper as an indicator of plasma treatment was first established, following the standard protocols found in literature.[25,26] mspDBD plasma was applied to indigo carmine–soaked paper for up to 90 seconds to determine whether treatment damaged the paper and how much time it took for the change in color to saturate. Images were taken every 5 seconds and analyzed for whiteness index. The whiteness index *(WI)* was calculated using Eq. (1), where *L* is the lightness of the color, *a* is the position between red and green, and *b* is the position between yellow and blue:[27,28]

$$WI = L + 3a - 3b \tag{1}$$

As shown in Fig. 1, treatment applied for 50 seconds saturated the change in color from blue to white on the Stensby scale. Additionally, the paper did not burn for the duration of the plasma treatment. To evaluate whether mspDBD plasma could penetrate through packaging for sterilization of packaged goods, plasma was applied as shown in Fig. 2. Indigo carmine–soaked paper was layered with BOPP and LDPE with a 1 mm gap set by microscope slides placed between each layer. mspDBD plasma was applied to the top of the LDPE with a 1 mm gap distance for 30 seconds (to observe the ink's

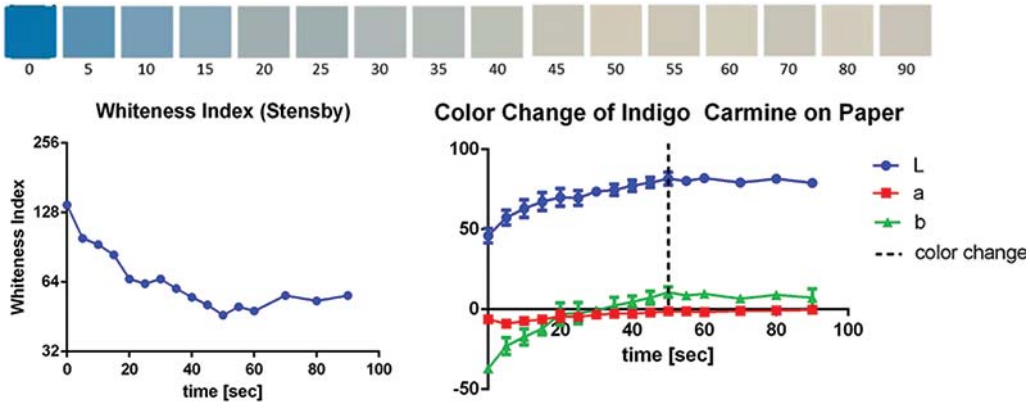

FIG. 1: Plasma treatment saturates the color change from indigo carmine–soaked paper after 50 seconds of treatment. (Top) mspDBD plasma treatment oxidizes the indigo carmine, changing the color from blue to white. (Bottom) The Stensby Whiteness Index was at a minimum after 50 seconds.

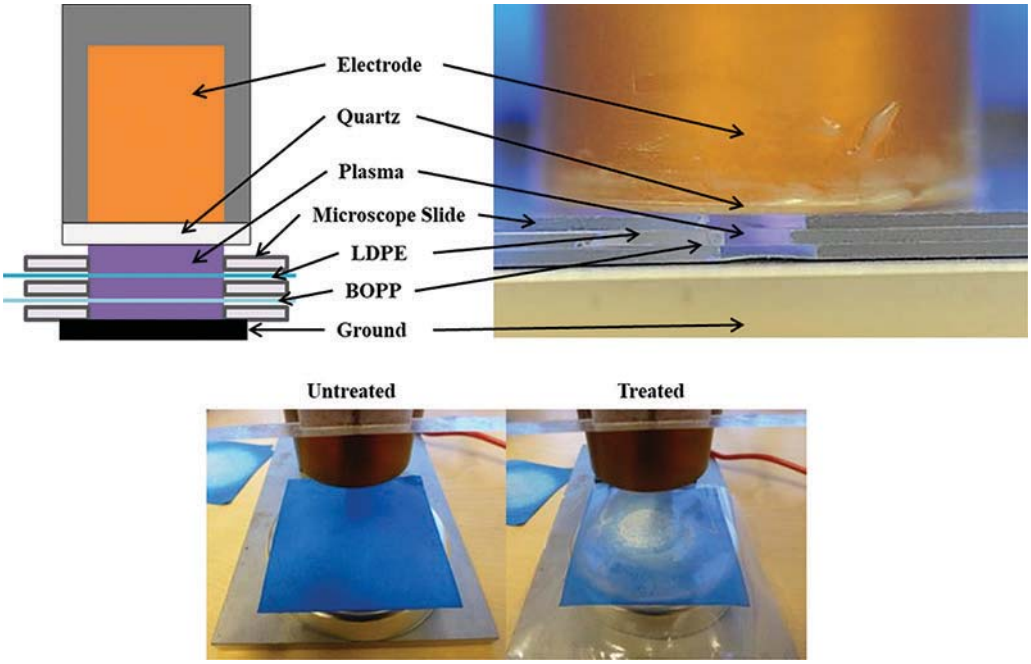

FIG. 2: mspDBD plasma oxidizes indigo carmine–soaked paper through two layers of plastic. (Top Left) A schematic representation of plasma discharge occurring above LDPE, between LDPE and BOPP, and below BOPP and the ground plate.

behavior at an intermediate plasma dose). The change in color of the indigo carmine–soaked paper suggests that plasma is discharged onto the paper and partially oxidized the indigo carmine within 30 seconds. There were no observable changes to LDPE after plasma treatment with the microsecond or nanosecond power supply; however, the BOPP underneath had a faint discoloration on the surface. To further evaluate the effect of DBD treatment on BOPP, FTIR spectroscopy was performed and the results were analyzed. These results are indicative of a "safe zone" for plasma disinfection of bread samples where bacteria on the bread, inside of a package, is inactivated whereas the packaging materials remain intact.

B. Plasma Treatment Does Not Significantly Alter the Puncture Force or Chemical Composition of BOPP

To assess if nspDBD or mspDBD plasma treatment altered the puncture force of BOPP, a dynameter was used to measure the force required to puncture both treated and untreated BOPP. This allows us to evaluate the structural integrity of the packaging layer. Plasma was applied for 15 seconds as described earlier. After treatment, BOPP retained the same discoloration observed for the nspDBD plasma treatment with no visible changes when

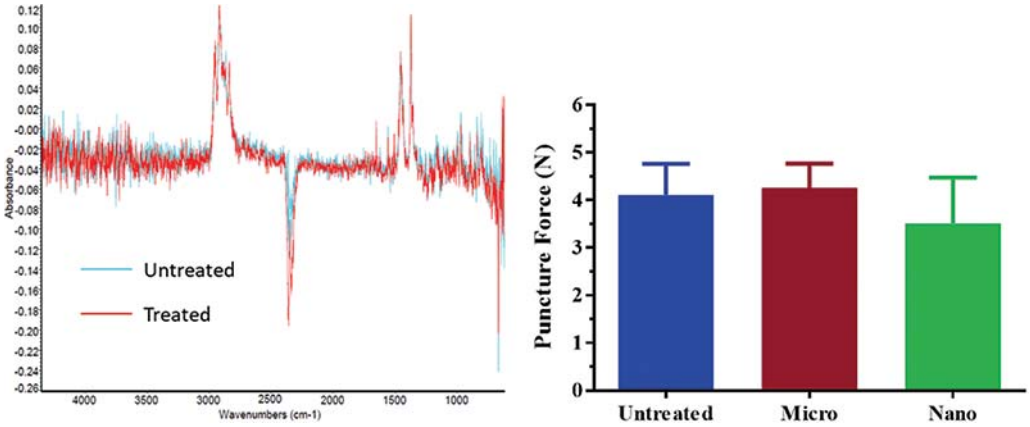

FIG. 3: Plasma treatment does not affect the physical or chemical properties of BOPP. (Left) FTIR spectra of untreated and treated BOPP overlap, showing minor differences after nspDBD plasma treatment. (Right) Puncture force measured directly after plasma treatment with either mspDBD or nspDBD. The force required to puncture did not change between untreated and treated BOPP.

exposed to mspDBD treatment. As shown in Fig. 3, both microsecond and nanosecond plasma treatments did not significantly change the force required to puncture the plastic. Thus, plasma treatment does not affect the fracture strength of the material.

Next, we used FTIR spectroscopy to determine the influence of nspDBD plasma on the chemical composition of BOPP after treatment. Discoloration of BOPP was not observed after direct treatment with mspDBD. Thus, FTIR spectroscopy was not performed on mspDBD treated samples. The plasma parameters used for treatment were the same as described previously. The spectroscopic profile of BOPP untreated is not significantly altered post nspDBD treatment, as shown in Fig. 3. Therefore, nspDBD treatment applied to the plastic does not affect the chemical structure of the packaging material.

C. Sterilization of *E. coli* on Bread Samples

We have demonstrated that plasma can penetrate plastic barriers without damaging the plastic. Since it has been shown that plasma inactivation of *E. coli* with direct or indirect treatment on TSA plates or in solution, we hypothesized that plasma could directly inactivate *E. coli* placed on the surface of a loaf of bread.[10,29] To assess if mspDBD plasma treatment could inactivate bacteria on bread, we inoculated bread samples *E.coli* just prior to plasma treatment. Bread samples were cut from fresh loaves baked without antimicrobials. As shown in Fig. 4, mspDBD plasma treatment results in a 2-log reduction after 10 minutes. These observations show that plasma treatment inactivates *E. coli* on the bread surface in a time dependent manner. nspDBD treatment of inoculated bread was not conducted because of the poor conductivity of the bread samples. This analysis must be optimized for future experiments.

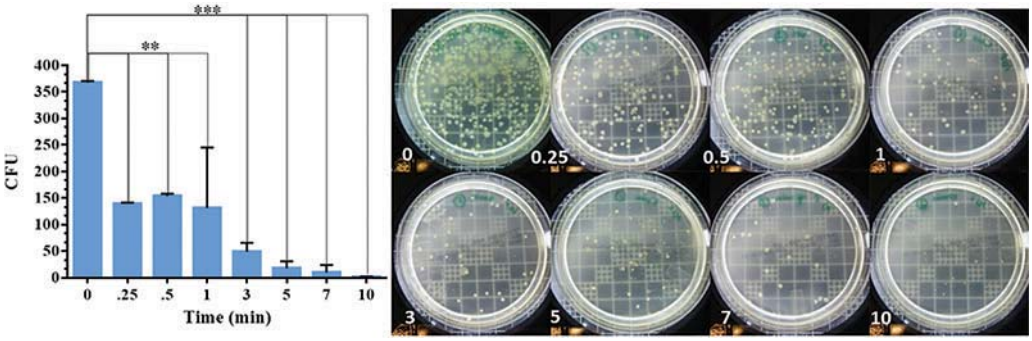

FIG. 4: mspDBD plasma inactivates *E. coli* inoculated on bread in a dose-dependent manner. (Left) The number of CFUs decreases with longer plasma treatment time. (Right) Pictures of TSA plates with *E. coli* 1 day after plasma treatment show inactivation after 10 minutes of treatment. The significance of the data was determined with the one-way analysis of variance (ANOVA) test, where ** and *** signify $p < 0.01$ and $p < 0.001$, respectively.

IV. CONCLUSION

In this work, we demonstrate that we were able to generate uniform dielectric barrier discharge (DBD) on the surface of bread samples and through two layers of packaging materials. Key observations include: (1) mspDBD plasma treatment effectively inactivates pathogenic organisms on the surface of the bread through two layers of plastic; (2) uniform plasma discharge is generated inside of the package, as demonstrated by experiments to determine the level of oxidation of indigo carmine; and (3) the chemical and physical properties of packaging material are unaffected by the plasma treatment. A challenge to this technology is the scalability for an industrial process and the ability to provide sufficient grounding for short-pulsed discharges. We hope to address these issues by using nspDBD to maintain volumetric plasma uniformity and effective sterilization.[30,31] Future studies will focus on disinfection of mold inoculated on packaged bread using nspDBD.

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