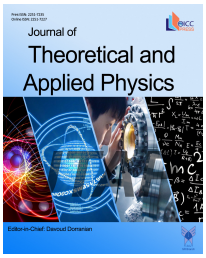

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Experimental investigation on food decontamination by low-temperature dielectric barrier discharge (LT-DBD) plasma: Application to bread-born mold

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Original Research

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Abstract:

The microbial inactivation ability of low temperature plasma treatment generated by dielectric barrier discharge (DBD) on bread-born mold (black bread mold) is investigated. It is shown that the plasma treatment can effectively decrease the mold growth rate. The longer plasma treatment time is, the more mold spores are inactivated. On the other hand, plasma treatment has not concrete effects on growth initiation time of bread-born mold. The effects of mold locations in DBD device (between the electrodes or out of electrodes gap), use of transparent barrier, electrode materials, and carrier gas on inactivation capability of DBD on bread-born molds has been analyzed. The possibility of different microbial inactivation mechanisms of plasma treatment on bread-born mold is surveyed, and a rough estimate of their shares is presented. In the best conditions, the 82% decrement in mold growth is achieved by only 5 s plasma treatment.

Keywords: DBD plasma; Plasma treatment; Microbial inactivation; Bread mold

1. Introduction

Plasma is an ionized gaseous compound composed of partially or wholly charged particles such as ions and electrons as well as atoms in ground or excited states. The net charge of plasma is neutral since the numbers of negatively charged particles in plasma are equal to numbers of positively charged ones [1]. Various sources such as radiation, laser light, heat, or electricity can be employed to induce energy to a gas and transform it to plasma state. For instance, applying electrical field between 2 electrodes having a gap filled with a gas can transform the gas to plasma state. In this method, which is named dielectric barrier discharge (DBD), a dielectric barrier is used in the gap to distribute the current flow and generate plasma [2]. Plasma has a wide range of applications in the scopes of materials synthesis and modification [3–5] to the agriculture and medical industry [6, 7].

Over the past decade, non-thermal (cold) plasma has been undergone an increasing utilization in food industry such as to decontaminate fresh or processed food products [8]. Unlike the thermal plasma, in which the heavy plasma products (such as ions, atoms, and radicals) are in thermal equilibrium ($T \cong 10^6 - 10^8$ K) with electrons (light plasma products), the heavy species in cold plasma have a much lower temperature ($T \cong 300 - 10^3$ K) and kinetic energy in comparison to the electrons. In other words, most of energy is distributed between electrons, rather than ions, radicals, and neutral species. Due to much lower weight of electrons in comparison to the weight of heavy plasma products, the average temperature of the plasma is mainly affected by heavy products temperature. As a result, by lowering the energy input of plasma and disturbing the thermodynamic equilibrium of the plasma, cold plasma can be generated [9].

In food industry, cold plasma is applied to decontaminate surfaces of inorganic or organic materials, while minimizing the adverse effects of high temperature on foods and their nutrition ingredients [2].

Many researchers have utilized cold plasma treatment to decontaminate foods such as lettuce, tomato, carrots [10], bell pepper [11], apple [12], nuts [13], meat [14], milk [15], whey protein [16], fish oil [17], wheat seeds [18], and

red pepper [19]. Some of them used atmospheric pressure DBD to inactivate microbial components in food products such as turmeric [20], cheese [21], strawberries [22], cherry tomatoes [23], wheat flour [24], almonds [25], orange juice [26], chicken [27]. Atmospheric plasma jet has also been exploited in some literatures to treat bread molds. They observed that plasma treatment for $5-20$ min can decrease the total bread mold weight [28–30].

Many microorganisms which have considerable effects on both foods production and their spoilage also prevail in food processing or preservation. Regarding bakery products, such as bread, mold growth is the main cause for microbiological spoilage. Common fungi molds deteriorating the bakery products are genera Penicillium, Monilia, Mucor, Cladosporium, Aspergillus, Endomyces, Fusarium and Rhizopus [31]. The last one, which is also known as black bread mold, is the most common spoilage element in bread.

As mentioned before, low temperature plasma methods, such as DBD, are vastly used for decontamination of various foods. However, to the best of our knowledge, this method is not yet employed to inactivate bread contaminations such as their molds. In this article, we develop two simple DBD devices to treat bread and mold with plasma in order to investigate the ability of atmospheric low temperature (LT) plasma in decontamination of bread. The effects of the position of bread-born mold in the DBD device, the stream gas, and the electrode materials were studied in this article.

2. Materials and method

We have procured the toast bread from a local market and prepared it for plasma treatment by slicing it with a size of approximately $1 \times 1 \times 1$ cm. Moreover, intact toast bread was incubated (T: 30 °C) to grow substantial black bread mold for plasma treatment. The DBD devices used for plasma treatment of bread and bread-born black mold (Rhizopus stolonifer) are described as below.

***DBD number* #1**: This device is composed of a circular solid electrode (aluminum, copper, or steel) and a circular mesh-like steel electrode with 20 cm diameter. The thickness of electrodes (both the solid and mesh-like electrodes) is 1 mm, and mesh-like electrode has 1 mm^2 square holes. A circular dielectric barrier composed of mica with 20 cm diameter and 1 mm thickness is placed between the electrodes. Mesh-like electrode is surrounded by a wood ring, acting as a holder. Four cylindrical Teflon spacers with 5 mm and 1 mm in diameter and height, respectively, are placed between the wood ring and dielectric barrier, above which circular solid electrode is placed. The whole device is placed above the 4 cylindrical Teflon spacers with 1 cm and 3 cm in diameter and height, respectively. The samples are placed below the mesh-like electrode, permitting the both UV-light and plasma species to pass and reach the surface of the sample. Fig. 1 shows the schematic diagram of the device. Power applied for plasma generation was 10 kV at 12 kHz.

***DBD number* #2**: This device is composed of a solid copper electrode with 20.3, 5.1, and 0.2 cm in length, width and thickness, respectively, and mesh-like steel electrode with 1 mm thickness and 1 mm^2 square holes. The mesh-like electrode is placed between 2 glass plate with 17.5, 13.5, and 0.5 cm in length, width, and thickness, respectively, acting as both electrode holder and dielectric barrier. Moreover, the glass plate passes the UV-light through itself, but inhibits the plasma species to pass. The upper (mesh-like) electrode and its holders (2 glass plates) are placed above the 2 plastic holders, which form 3 mm space between the copper (bottom) electrode and dielectric barrier.

Figure 2 shows a schematic diagram of the device layers.

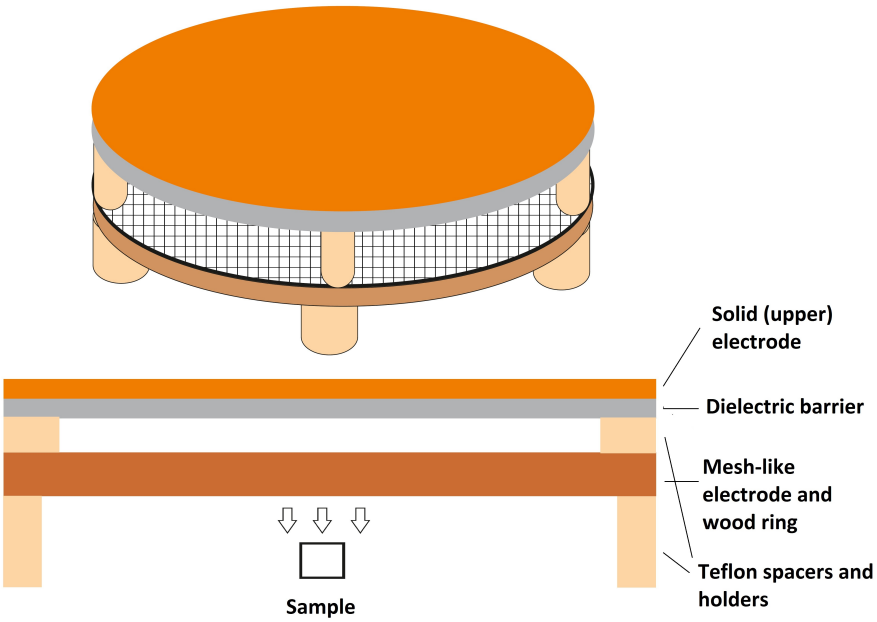

Figure 1. Schematic diagram of DBD device #1.

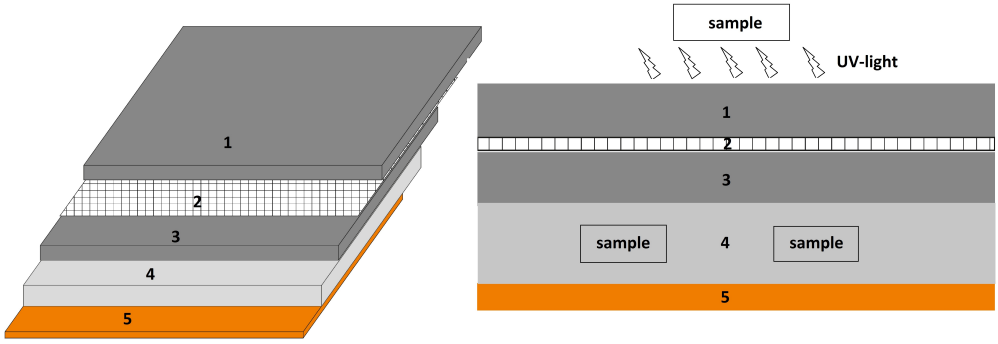

Figure 2. Schematic diagram of DBD device #2 layers: 3D view (left) and cross-sectional view (right). The layers from top to down are: (1) glass plate – (2) mesh-like electrode – (3) glass plate – (4) hollow space (plastic holder) – (5) copper (bottom) electrode.

The space in which the plasma is generated has 14 cm (the length of mesh-like electrode), 5.1 cm (the width of solid copper electrode), and 0.3 cm (the space between the copper electrode and dielectric barrier) in length, width, and thickness, respectively. Power applied for plasma generation was 10 kV at 12 kHz.

2.1 Analyzing the effect of plasma on mold growth initiation time

For this purpose, sliced bread pieces were subjected to plasma treatment for $0-30$ min by DBD device number#1 with copper, aluminum, or steel electrodes. The plasma treated samples were maintained in incubator (31 °C temperature and 31% humidity) until the first spores of bread mold were formed. During the incubation time, the microscopic examination with optical microscope (Zeiss Co.) was conducted every 24 hrs to find the appearance time of first spores.

2.2 Analyzing the effect of plasma on inactivation of bread-born molds

In order to investigate this effect, bread-born molds were exposed to plasma treatment for various times by DBD number #1 and 2. After that, the molds were placed directly on Potato Dextrose Agar (PDA, as a culture medium) surface, or diluted several times (for reaching the best dilution to attain countable colonies) and then placed on PDA surface. To manufacture PDA, 16.8 g of agar powder (purchased from Liofilchem, Italy) poured in 400 mL distilled water, then was heated to temperature below the boiling point. Meanwhile, the solution was stirred up. By reaching the boiling point, the solution were picked up from the heater and autoclaved for 2 hrs (at approximately 130 °C) to be sterilized. Then, sufficient amount of solution was poured in Petri dishes under cleaned oven and placed in incubator (31 °C temperature and 31% humidity) for 24 hrs. After that, if there was no contamination in the synthesized PDA, it was placed in refrigerator for further utilization.
The transfer of untreated or plasma treated molds to PDA surface was conducted under cleaned oven. The mold-containing PDAs were placed in incubator (31 °C temperature and 31% humidity) until the mold colonies were appeared on it. The incubation times were varied upon each examination, but were a constant time for the samples in each experiment. However, it is worth mentioning that the incubation time has not any effects on the total number of colonies, but only affects the size of colonies. As a result, the incubation time was set in order to attain reasonable colony sizes for counting purpose.

3. Result and discussions

3.1 The effect of plasma treatment on growth initiation time of bread-born-mold

Bread slices were treated by DBD device number #1 with different electrode materials (aluminum, stainless steel, and copper) at various treatment times ($0-30$ min). The samples treated more than 5 min were dried, indicating the high temperature after such treatment times. Our calculation revealed that plasma treatment more than 5 min increases the temperature more than 40 °C resulting in drying. As a result, the treatment time during the study was set as far as the temperature remains low to inhibit drying of the samples in order to minimize the thermal inactivation.
The microscopic investigation of untreated and plasma treated bread slices showed that the first mold spores appear approximately at the same time (after 72 hrs) in the untreated and treated bread slices. Utilizing different electrode materials also had not any substantial effect on mold spore appearance time.
To the best of our knowledge, there were not any authentic article investigations on the effect of LT-plasma on growth initiation time of not only mold spore, but also other food decontaminations and microbial systems. The ineffectiveness of plasma treatment on growth initiation time of bread-born mold spores can be realized by the fact that surface and within the bread were not evenly treated by plasma, and plasma treatment can not decontaminate 100% of the foods. As a result, after plasma treatment even for 5 min, there is still unaffected contamination in foods, which can grow during incubation time (after treatment).

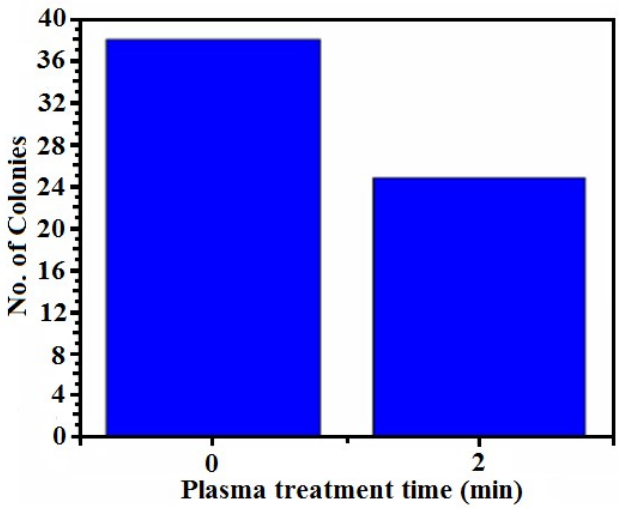

Figure 3. Colony numbers of untreated and 2 min plasma treated samples after transferring to PDA without dilution and incubation time.

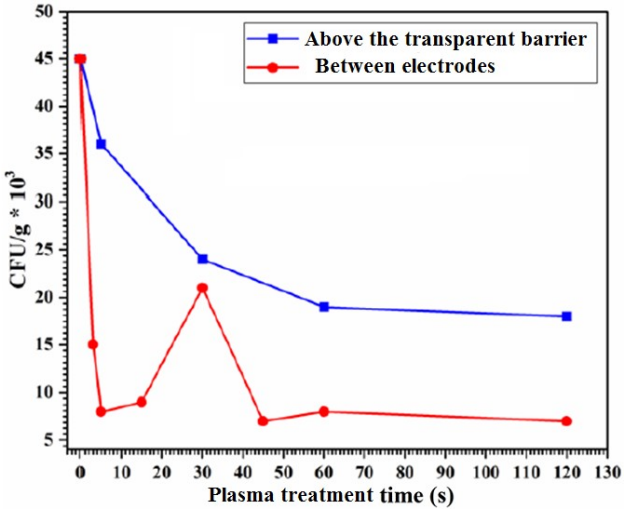

Figure 4. Colony numbers versus plasma treatment time for mold samples located between the electrodes and above the transparent barrier of DBD device number #2.

3.2 The effect of plasma on inactivation of bread-born mold spores

Figure 3 shows the calculated colonies in reference mold and plasma treated mold by DBD device number #2 for 2 min which transferred on PDA without dilution. In this case, the mold samples were placed directly on the surface of PDA without spreading on the surface. As a result, mold spores should move on the PDA surface to feed from PDA, grow, and form colonies. Consequently, the colonies appeared on the surface of PDA after incubation time, are formed by movable (alive) mold spores, which survive in plasma treatment. Accordingly, this method (transferring mold samples without dilution on the PDA surface and counting colonies after incubation time) can effectively represent the intact mold spores during plasma treatment. As seen in Figs. 3, 2 min plasma treatment on mold could decrease the grown colonies from 38 to 25 (on 23.75 cm^2 of plate surface).

Figure 4 shows the colony numbers in untreated mold and mold samples placed between the electrodes and above the transparent barrier of DBD device number #2 and plasma treated at various times. As seen, increment in plasma treatment time reduces the colony numbers of bread-born mold. When the mold sample is placed above the transparent barrier, applying LT-DBD plasma for 2 min decreases the spore colonies in 1 g of mold from 45×10^3 to 18×10^3, indicating 60% decrement in number of colonies.

It is worth mentioning that the slope of colony decrement decreases by increment in exposure time. When the exposure time exceeds 60 s, the slope substantially decreases. Plasma treatment for only 60 s results in 58% decrement in mold colonies, while continuing the plasma treatment for extra 1 min, just decreases 2% of colonies. It shows that exposure times longer than 1 min in the DBD device number #2, and when the mold is located above the transparent barrier is redundant and do not have any considerable effect on inactivation of molds and decontamination of breads.

On the other hand, when the mold is located between the electrodes, the plasma treatment substantially inactivates the bread-born molds in a short exposure time. The related graph can be divided into two sections, i.e., (1) exposure time for 5 s and less and (2) exposure time for longer than 5 s. As seen, increment in plasma exposure time in the first region considerably decreases the mold colonies. The slope of the graph in this region is very high. plasma treatment of 5 s reduces the colonies from 45×10^3 to 8×10^3 CFU/g, indicating of 82% effectiveness of such method to mold decontamination in just 5 s. On the contrary, increment in plasma treatment time more than 5 s did not have any significant effect on mold inactivation. The higher colonies in 30 s plasma treated sample is probably related to experimental errors or addition of other contamination to PDA during handling. Such trends have been also reported by Feichtinger et al. [32] for Bacillus Subtilis and Saccharomyces Cerevisiae. However, they did not mention the probable cause of this observation.

The different capability of the DBD plasma in mold inactivation is relied on the sample location, resulting in various contributions of inactivation mechanisms. Generally, three food decontamination mechanisms can be triggered during LT plasma treatment, namely, (1) reactions between cell membranes with reactive, charged, and radical species, (2) the damage caused by UV radiation induced to cell components and membrane, and (3) breaking and damaging of DNAs caused by UV radiation [2]. Reaction with plasma species with target can be physical or chemical. Charged particles can accelerate in the electrical field of plasma and hit the target with high velocity, inducing damage to it. Moreover, reactive species can chemically react (such as oxidation) with cell membrane and harm it [1, 8].

When the mold is located between the electrodes, all plasma species, including ions, electrons, unstable species, and reactive particles, are able to reach the mold surface and induce damage to it by either physical collisions or chemical reactions. Moreover, recombination of plasma species can generate UV light, damaging the mold as above mentioned

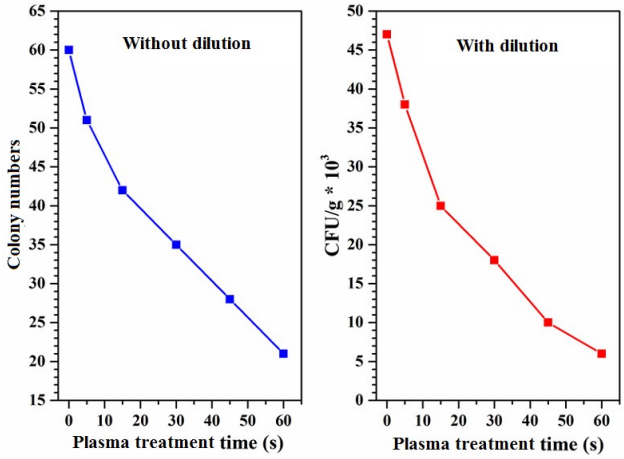

Figure 5. Colony numbers versus plasma treatment time for mold samples located beneath the DBD device #1: with (right) and without (left) dilution.

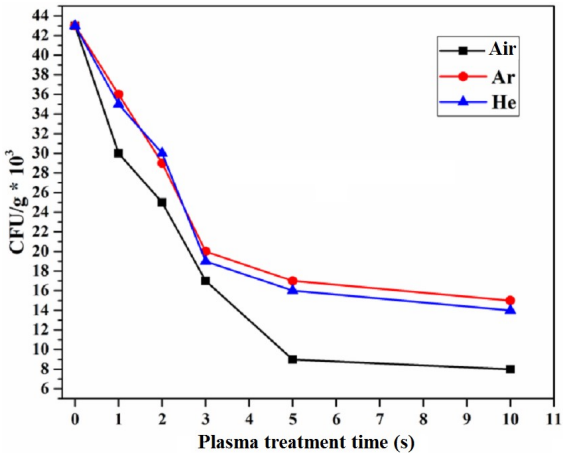

Figure 6. Colony numbers versus plasma treatment time for mold samples located between the DBD device number #2 electrodes with different carrier gases.

UV-based mechanisms (mechanisms 2 and 3).

Contribution of reactive species along with scattered UV-light brings about maximum capability of DBD generated LT-plasma for inactivation of molds in short treatment time. On the other hand, when the mold is located above the transparent barrier, it inhibits the plasma species to reach the surface of the mold. The only plasma product, which can pass through the barrier, is UV-light. As a result, the capability of DBD plasma for mold decontamination is reduced to only inactivation effect of UV-light. Regarding the shares of mold decontamination of different plasma products (reactive, radical, and charged species versus UV light), it can be inferred that:

(1) Since there is an explicit difference between the numbers of inactivated spores in two conditions, it can be deduced that both plasma products (species and UV-light) have their own substantial shares in mold inactivation.

(2) When the mold is located above the transparent barrier, plasma treatment more than a specific time of 1 min does not have any considerable effect on mold inactivation, and plasma treatment reaches its highest ability in mold decontamination. In this condition, the maximum inactivation capability is less than the maximum capability of plasma treatment in mold decontamination when the mold is located between the electrodes, indicating that long-time UV exposure can not replace the role of plasma species, especially reactive ones.

(3) Inactivation efficacies of 5 s plasma treatment in two states (%82 and 20% in the case of locating between the electrodes and above the transparent barrier, respectively) reveal that the contribution of plasma species in mold inactivation (mechanism 1) is approximately three folds of UV-light in mold inactivation. However, if the plasma generated UV-light exposure continues, approximately 2/3 mold inactivation share of plasma species can be compensated by UV-light.

Figure 5 shows the effect of plasma treatment generated by DBD device number #1 on mold inactivation. Similar to DBD device number #2, this device can effectively reduce the spore colonies in the bread-born mold. Increasing plasma treatment time also decreases the colony numbers. Plasma treatment of 60 s duration by DBD device number #1 resulted in 65% and 87% decrement in colony numbers in the cases of with and without dilution measurement, respectively. By comparing Figs. 4 and 5 (right), it seems that DBD device number #2 has the higher maximum capability in inactivation of bread-born molds. However, its maximum capability has been activated at 1 min plasma treatment, while this value for DBD device number #2 can be attained at just 5 s. The more time needed to attain the maximum inactivation molds in DBD device number #1 is related to the location of mold sample. The mold sample is located beneath the mesh electrode, which may result in sacrificial of some unsteady plasma reactive species. On the other hand, its high capability in mold inactivation may be arisen from its higher area and consequently more generated plasma species and UV-light. At last, it is worth mentioning that as proved by DBD device number #2, both plasma products, i.e., plasma species (electron, ions, reactive species, and etc.) and UV-lights are responsible for mold inactivation in DBD device number #1.

Thonglor and Amnuaycheewa [30] treated bread–born black mold with atmospheric argon plasma jet and obtained 70% reduction in total weight of mold. In our research, we have obtained 82% decrement in mold colonies with 5 s plasma treatment by DBD device number #2 and 87% decrement in mold colonies with 1 min plasma treatment by DBD device number #1, indicating the better performance of DBD air plasma in inactivation of bread-born black mold.

3.3 The effect of stream gas on mold inactivation

To examine the effects of stream gas on microbial inactivation ability of LT plasma on bread mold, the mold samples were placed between the electrodes of DBD device number #2, and various stream gases including air, argon, and helium were blown with a flow of 2 L/min. The mold samples were treated with various plasma times, diluted and transferred to PDA. The maximum treatment time was chosen 10 s, because as mentioned above, the 5 s plasma treatment can result in maximum ability of DBD device number #2 to in-

activate bread mold. Figure 6 shows the colony numbers of plasma treated mold versus plasma treatment time. It is observed that the ability of plasma treatment of DBD plasma by utilizing helium and argon as stream gases in inactivation of bread-born mold are almost the same. However, by exploiting air as stream gas for plasma treatment, better performance in food decontamination can be obtained.

After 10 s plasma treatment, the reduction in mold colonies were 33×10^3 (79% efficacy), 27×10^3 (65% efficacy), and 28×10^3 (67% efficacy) CFU/g in the cases of using air, argon and helium, respectively. In fact, in each plasma treatment time, air plasma had better performance in bread decontamination, in comparison to plasmas generated by other stream gases. Such enhanced performance of LT air-plasma is arisen form the more reactive plasma species, which formed in plasma treatment, while they are absent in noble gas-based plasmas such as argon and helium-based ones. In fact, complex chemistry of air results in formation of various reactive plasma species such as O radicals, O_3 and its radicals, NO^-, NO_2^-, and OH^- [1, 33], which can damage the mold with more quantity and efficacy than noble gas plasma-generated species. As mentioned previously, these species can chemically damage the mold cells such as by oxidation. On the other hand, it seems that noble-gases plasma generated species damage the mold cells more physically rather than chemically, (due to low content of species which are able to react with microbial cells). Moreover, air has some trace elements such as noble gases which can produce noble gases plasma-generated species. As a result, if there is any particular damage-inducing mechanism in noble gas plasma generated species, there are traces of noble gases-based species in air plasma which can trigger their specific decontamination mechanism.

3.4 The effect of electrode material

Figure 7 shows the colony numbers in 1 g of black bread mold plasma treated by DBD device number #1 with various solid electrode materials at different treatment times. It is observed that all the three electrodes are able to form stable plasma and inactivate the bread-born mold. Among the electrodes, the plasma generated by aluminum one had the best performance in inactivation of bread-born mold, while the plasmas generated by steel and copper electrodes has a similar inactivation performance with a slight better performance for copper electrode. The differences between the inactivation ability of various plasmas generated by different electrodes decrease by increment in plasma treatment time.

Figure 8 shows the reduction in mold spores treated by plasmas generated by these electrodes at various treatment times. We note that the plasmas generated by aluminum, copper, and steel had the best inactivation performances, respectively. While increment in plasma time increases the inactivation percentage, the differences between inactivation percentages of plasma generated by different electrodes decrease by increment in plasma treatment time. Such behavior is also observed by Talebizadeh et al. [34] by removing NO_x gas by DBD with different electrodes. In fact, higher secondary electron emission coefficient for aluminum in comparison to other electrodes is responsible for better performance of the LT plasma generated by aluminum electrode in inactivation of bread-born mold spores. During the plasma treatment, ions and electrons strike the electrode, which may result in detachment of other electrons from electrode atoms, known as secondary electrons. The material with higher secondary electron emission coefficient (γ) generated more electrons in this situation [34, 35]. The secondary electron emission coefficients for steel, copper, and aluminum are 1.24, 1.29, and 1.5, respectively [34]. As a result, the plasma generated by aluminum electrode shows a better inactivation performance. Moreover, the higher difference between γ_{Al} and γ_{Cu} in comparison with the difference between γ_{Cu} and γ_{steel} resulted in better inactivation performance of the plasma generated by aluminum electrode in comparison with other electrodes. The better inactivation performance may be attributed to straight

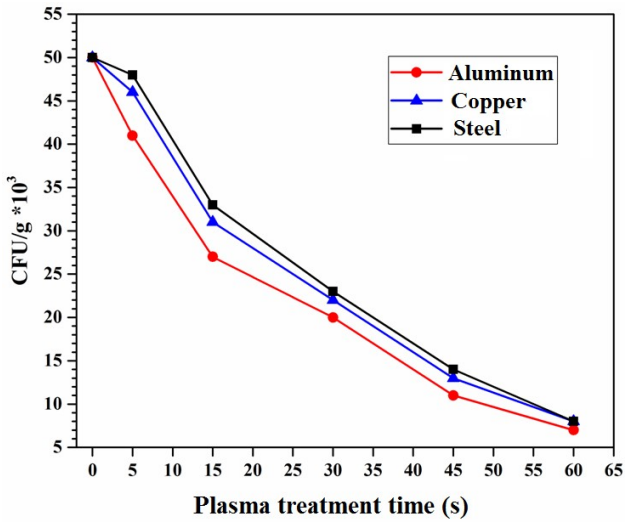

Figure 7. Colony numbers in black bread mold samples treated with DBD device number #1 with various solid electrodes for 0-60s plasma treatment.

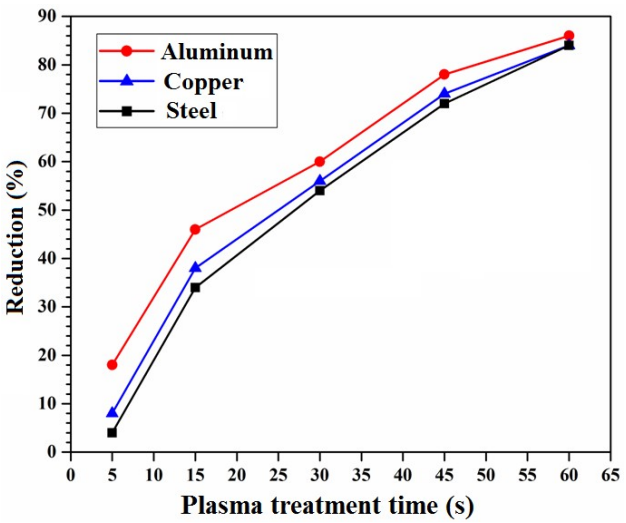

Figure 8. Reduction percentage in colony numbers of black bread mold treated via DBD device number #1 with different solid electrode materials for 5-60s plasma treatment.

role of additional electrons, or extra reactive species generated by reactions between additional electrons and plasma compounds. However, increasing in plasma treatment time reduces the effect of extra electrons, may be attributed to plasma species or inactivation saturation.

4. Conclusion

In this work, DBD-generated cold plasma treatment has been utilized to inactivate black bread mold (Rhizopus stolonifer). It was showed that plasma treatment can effectively reduce the bread-born mold growth. The following results are obtained:
(1) Plasma treatment has not significant effect on growth initiation time of bread-born mold spores. Changing the location of bread in DBD plasma, electrode materials, and bread sample do not alter the growth initiation time of bread-born mold spores.
(2) Increment in plasma treatment time enhances the inactivation of mold spores.
(3) Plasma treatment for more than a specific time results in significant temperature rise and consequently bread drying.
(4) Both the UV-light and plasma species have their own shares in microbial inactivation of bread-born mold.
(5) Locating mold sample between the electrodes results that more reactive plasma species reach the mold surface, increment in inactivation capacity of plasma treatment. Moreover, they reduce the required time to achieve maximum inactivation capacity.
(6) Utilizing transparent glass barrier hinders plasma species to reach the mold surface and allow the UV-light to pass through, resulting in decrement in microbial inactivation capacity in any plasma treatment time and achieving maximum capability, as well.
(7) Among three applied carrier gases, He and Ar plasmas had similar microbial inactivation behaviors, while air plasma had a significantly enhanced microbial inactivation behavior. After 10 s plasma treatment, microbial inactivation of air, argon, and helium plasmas were 79%, 65%, and 67%, respectively.
(8) The plasma generated by solid aluminum electrode had better inactivation performance in comparison with plasmas generated by copper and steel electrodes. The higher inactivation ability of aluminum electrode-generated plasma is attributed to high secondary electron emission coefficient of aluminum in comparison with copper and steel.
(9) Both utilized DBD devices had significant effects on inactivation of bread-born mold. One minute plasma treatment generated by DBD device number #1 resulted in 87% mold inactivation, while 5 s plasma treatment generated by DBD device number #2 brought about in 82% mold inactivation.

Authors Contributions
Setup arrangement, data acquisition, analyzing the results, and preparing the draft were carried out by MES, while DD supervised the work. Conception, designing the setup, analyzing the results, and finalizing the paper were done by DD.

Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interests
The author 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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