



## Effect of Non-Thermal Plasma Treatment Duration on Microbial Load Reduction in Selected Dried Fruits

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### ABSTRACT

The increasing demand for safe and minimally processed foods has driven research toward non-thermal sterilization technologies. This study aimed to evaluate the effect of treatment duration on the microbial load reduction of selected dried fruits figs (*Ficus carica*), apricots (*Prunus armeniaca*), and raisins (*Vitis vinifera*) using a Double Dielectric Barrier Discharge (DDBD) non-thermal plasma system. The plasma was generated at constant conditions of 1.97 Hz frequency, 1.7 A current, and an air flow rate of 2 L/min, while treatment durations were varied at 1, 2, 5, 10, and 15 minutes. Microbial analysis included determination of Total Viable Count (TVC), Total Fungal Count (TFC), and *Escherichia coli* levels using standard plating techniques. Results revealed a significant, time-dependent reduction in microbial counts across all fruit types ( $p < 0.05$ ). The highest reductions were recorded after 15 minutes of exposure, with TVC decreasing from  $3.37 \pm 0.42$  to  $1.87 \pm 0.32 \log_{10}$  CFU/g in figs,  $2.89 \pm 0.17$  to  $2.06 \pm 0.34$  in apricots, and  $2.51 \pm 0.61$  to  $2.11 \pm 0.31$  in raisins. Similarly, TFC declined by 50.5%, 12.4%, and 19.6% in figs, apricots, and raisins, respectively. These findings confirm that increasing plasma exposure duration significantly enhances microbial inactivation due to the cumulative generation of reactive oxygen and nitrogen species (ROS and RNS). The study demonstrates that DDBD non-thermal plasma offers a safe, chemical-free, and energy-efficient approach for improving the microbial safety of dried fruits without compromising their nutritional and sensory attributes.

## **Introduction**

The relationship between the foodborne pathogen, the host, the food, and the environment determines the incidence and importance of these foodborne illnesses. Abdominal discomfort, diarrhea, vomiting, nausea, fever, respiratory problems, and, in extreme situations, death are common signs of foodborne infections. Like in the case of foodborne infections, these symptoms are brought on by the microorganisms that have been consumed (Gourama, 2020). People all across the world love dried fruits because they are convenient and shelf-stable substitutes for fresh fruit. The most varied phenolic profiles can be found in certain dried fruits, including raisins, figs, dates, cranberries, apricots, and prunes (Alasalvar et al., 2023).

Apricots are rich in beta-carotene, chlorogenic acid, polyphenols, and catechins. Traditional dried apricots are great for the health of the skin, bones, intestines, and eyes. They are frequently included to dry fruit mixes. Because it contains the proper amounts of potassium, zinc, magnesium, calcium, boron, manganese, amino acids, xanthophyll and, carotenoids, it is one of the most potent antioxidants. 70% of the sugar in raisins is fructose and glucose. Even though it lacks salt and vitamin C, this dry fruit is nevertheless one of the best antioxidants. They lower the chance of cancer cell malignancies in the G.I. system and promote healthy blood pressure and digestive system functioning (Banerjee, 2020). The dried fruits are a reduced-moisture version of fresh fruits that are higher in nutrients. The traditional dried fruit list includes: apples, dates, apricots, figs, peaches, mulberries, prunes, pears, and raisins. It also includes fruits without added sugar (Alasalvar et al., 2020). Because they are less expensive and can be preserved simply, dried fruits are becoming indispensable ingredients in meals, drinks, and cooking. While these items are low in sodium, saturated fat, and total fat, they can be a valuable source of micronutrients and antioxidants. They also contain vitamins, folate, potassium, magnesium, and fiber (Jeszka-Skowron & Czarzyńska-Goślińska, 2020). The earliest food preservation technique that lowers the meal's water activity is drying. At water activity levels less than 0.87, 0.88, and 0.90, respectively, yeasts, molds, and bacteria cannot proliferate.

Consequently, germs are prevented from growing due to a decrease in water activity caused by drying. Drying preserves the nutritional value of food and some other qualitative features of the original item or product by preventing the growth of microbes as well as other moisture-driven reactions that lead to deterioration (Shah et al., 2022). The cold plasma technique uses ionized gases created by microwaves and electrical discharges. A variety of reactive species, including free radicals such as reactive nitrogen species, or RNS, reactive oxygen species, or ROS negative and positive ions, and ultraviolet radiation, are created in the cold plasma. The topography of the food surface can alter as a result of a variety of physical and chemical processes brought on by the diffusion of reactive species on the material surface. These changes can be either positive or negative (Loureiro et al., 2021).

Plasma is a gas that is partially or completely ionized and contains free electrons, neutral atoms, negative and positive ions, and charged molecules that can be neutral or charged. Non-thermal plasma generates UV radiation, reactive nitrogen species (RNS) and reactive oxygen species (ROS) with the help of a gas discharge method. The gas used can be oxygen, argon, or air. Non-thermal plasma technique is efficient in the inactivation of microorganisms in diverse foods in a laboratory-scale setup. Various techniques are used to generate plasma including dielectric barrier discharge, surface dielectric barrier discharge, and double dielectric barrier discharge technique. Non-thermal plasma is also known as cold plasma technique (Anuntagool et al., 2023). In the medical world, the focus on non-thermal plasma technique is increasing because of its bacterial effect. The reactive species that are generated by non-thermal plasma have an antimicrobial effect (Kasa et al., 2022). Double dielectric barrier discharge is widely used in fields such as energy,

environment, and surface treatment. DDBD uses two discharge sources to generate the non-thermal plasma (Li et al., 2022). In this study, three dried fruits were selected including apricots, raisins, and figs which were exposed to the non-thermal plasma generated from a double dielectric barrier discharge, and the results were calculated and compared.

Plasma makes up the majority of the universe that is visible and is thought to be the fourth state of matter. Plasma has a natural energy since it uses constant energy to remove electrons. Unlike conventional matter, they can exist across a wide range of temperatures without changing their state. It does not originate from human innovation; rather, it can be found in nature as "here" in the Sun, stars, sparks, and lightning bolts (Sera & Sery, 2018). Nonthermal plasma (NTP) uses an electric field to produce an ionized gas in a reactor with a comparatively low temperature. When ions' free electrons interact with substances like odorants, they change into oxidized forms. For instance, NTP transforms molecular oxygen (O<sub>2</sub>) into ozone (O<sub>3</sub>), a potent oxidant that degrades the majority of fragrances and volatile organic compounds (VOCs). The NTP reactor's catalyst passes the air that has to be treated through it. The amount of air that needs to be treated is enormous, thus even though the treatment duration is brief, the reactor(s) for composting scenarios need to be able to handle a high air flow rate. While there may be some niche uses for NTP in odor management, its main potential seems to be in food and medical application disinfection (O'Neill et al., 2022). A partially or completely ionized gas with a neutral net charge is called plasma. It is composed of free radicals, ions, electrons, UV radiation, photons, reactive nitrogen and oxygen species, and molecules in their excited or ground states. Plasma comprises 99 percent of all visible matter in the cosmos and can be either manmade or naturally generated. The sun, the fire of Saint Elmo, Australis, the Aurora Borealis, and lightning are a few examples of natural plasmas (Lotfy et al., 2022).

For many food sectors, non-thermal cold plasma technology (NTCP) is an environmentally friendly method of eliminating pollutants, breaking down pesticides, and inactivating microbes and enzymes. However the use of CP technology in food production is still relatively new, and much research needs to be done before its full potential is realized for a variety of food products. Moreover, microbial inactivation takes place on the food's surface due to the restricted ability of plasma species to penetrate it. This represents a constraint associated with plasma technology (Asl et al., 2022). The use of NTP to sterilize a variety of surfaces in dentistry and medical equipment as well as food sector packaging. These days, at atmospheric pressure, the sterilization of both living and non-living items is more commonly accomplished with NTP. Its low ionization level, tissue-tolerable temperature, and air pressure set it apart. Numerous microorganisms, including viruses, fungi, and bacteria, were discovered to be rendered inactive following NTP therapy. About ten years ago, the concept of using plasma to sterilize fruit or seed surfaces was first proposed (ŠerÁ & ŠerÝ, 2018).

Double dielectric barrier discharge (DDBD) is an extensively studied and used method for the production of non-thermal plasma (NTP). It is used in various sectors including energy, environment, and surface treatment. A large number of free radicals, atoms, and excited particles are generated in a double dielectric barrier discharge (DDBD) reactor at room temperature and atmospheric pressure in controlled conditions (Li et al., 2022). It is one of the most efficient sources of the cold plasma technique and has drawn attention for surface inactivation and thin functional coating deposition (Ribeiro et al., 2017). As compared to alternative methods like fast pulsed high-pressure discharges, low-pressure discharges, and or electron beam injection, the best feature of a double dielectric barrier discharge (DDBD) reactor is that it easily maintains the non-thermal equilibrium of the plasma state. Other important benefits include its adaptability when it comes to operational parameters, geometrical arrangements, and operating mediums. At high pressures, a double dielectric barrier discharge (DDBD) reactor segregates the discharge in

multiple equally spaced micro discharges that take place in the discharge gap or over the discharge zone.(Farag & Kadhemi, 2022).

A low temperature plasma device, which covers one or both of the metal electrodes to limit electric current, and two metal electrodes make up an atmospheric dielectric barrier discharge (DBD) device. The idea is to apply a voltage between the two electrodes in order to release atmospheric pressure. One proposed next-generation non-thermal technique for food sterilization is atmospheric DBD plasma. The DBD plasma device operates straightforwardly and continuously by producing plasma in an air environment (Kim et al., 2020).

### **Objectives of Study**

1. To evaluate the effect of different non-thermal plasma treatment durations on the total viable bacterial count (TVC) in selected dried fruits.
2. To determine the impact of plasma exposure time on total fungal count (TFC) and assess the time-dependent antifungal efficiency of the treatment.
3. To compare microbial reduction efficiency among different dried fruits subjected to varied plasma treatment durations.
4. To examine the relationship between treatment duration and reactive species generation (ROS and RNS) responsible for microbial inactivation.
5. To establish the optimal plasma exposure duration that achieves maximum microbial decontamination while preserving product quality and nutritional integrity.

### **Methodology**

#### **Sample Selection and Preparation**

Three varieties of commercially available dried fruits figs (*Ficus carica*), apricots (*Prunus armeniaca*), and raisins (*Vitis vinifera*) were selected for this study due to their high nutritional value and susceptibility to microbial contamination. Samples were procured from local markets in clean, sealed packaging to minimize pre-experimental contamination. Each fruit type was visually inspected to remove damaged or visibly spoiled units. Approximately 100 grams of each fruit sample were weighed using a digital balance and divided into experimental groups for plasma treatment at different time intervals. The data collection and experimental work were conducted between March and June 2024. Before treatment, the fruits were placed on sterile glass plates to ensure uniform exposure to plasma discharge. All samples were analyzed within 24 hours of procurement to maintain microbial viability and minimize environmental contamination.

#### **Study Type**

This study follows a laboratory-based experimental design, where controlled conditions were set up to investigate the effects of the non-thermal plasma treatment on the selected dried fruit surfaces.

#### **Study Locale**

The research experiment was conducted in Nutrition Sciences Laboratory and Plasma Laboratory, Allama Iqbal Open University, Islamabad.

## Collection & Preparation of Sample

100 g of dry fruit samples including apricots, raisins and figs were purchased from a local market in Islamabad. Once the samples were collected, they were kept in an air-tight bag to avoid any contamination. Initially, all the equipment was sterilized and laboratory isolation units were disinfected before sample preparation. 20 grams of dry fruit sample was mixed with 180 ml of peptone (Oxoid, Basingstoke, UK) water and homogenized for 2 minutes ( $10^{-1}$  dilution). After homogenization in a stomacher for two minutes, the sample was serially diluted (decimal) into the peptone solution. Once the solution was ready, pre-prepared agar plates were used for the spread method where 1 ml sample of the appropriate dilutions was poured on relevant agar plates. Using the spreader, the poured sample was spread throughout the prepared agar plates. This process was repeated for all three samples for the pour plate method on relevant agar plates.

## Experimental Design for Plasma Treatment

The variables used in the experiments were time and voltage. The constants used in this experiment were current, frequency, and air flow rate. Six different time durations (1, 2, 5, and 10 minutes) and five different power inputs (25, 35, 45, and 50 volts) were used. Air was used as a carrier. 4 g of fruit sample in sliced form was placed inside a double dielectric barrier discharge non-thermal plasma discharge tube for different time durations and voltages. Table 3.1 shows the time durations and voltages that were used to experiment.

## Results

**Table 1: Effect of Plasma Treatment Duration on Total Viable Count (TVC) in Dried Fruits**

| Fruit Type | Control (0 min) | 1 min           | 2 min           | 5 min           | 10 min          | 15 min          | Mean $\pm$ SD (Final) |
|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------------|
| Figs       | 3.37 $\pm$ 0.42 | 3.10 $\pm$ 0.38 | 2.75 $\pm$ 0.33 | 2.22 $\pm$ 0.27 | 2.01 $\pm$ 0.29 | 1.87 $\pm$ 0.32 | ↓44.5%                |
| Apricots   | 2.89 $\pm$ 0.17 | 2.74 $\pm$ 0.18 | 2.49 $\pm$ 0.22 | 2.28 $\pm$ 0.28 | 2.15 $\pm$ 0.33 | 2.06 $\pm$ 0.34 | ↓28.7%                |
| Raisins    | 2.51 $\pm$ 0.61 | 2.43 $\pm$ 0.55 | 2.32 $\pm$ 0.49 | 2.22 $\pm$ 0.43 | 2.16 $\pm$ 0.38 | 2.11 $\pm$ 0.31 | ↓15.9%                |

Table 1 indicated that the total viable count (TVC) of all tested dried fruits decreased progressively with longer plasma exposure times, confirming the time-dependent bactericidal action of non-thermal plasma. Figs showed the greatest bacterial reduction (44.5%) after 15 minutes, indicating higher surface responsiveness to plasma treatment. Apricots and raisins demonstrated moderate declines in viable bacterial counts of 28.7% and 15.9%, respectively. Statistical analysis ( $p < 0.05$ ) confirmed that exposure duration had a significant influence on microbial reduction across all fruits. The reduction trend was nearly linear, suggesting that the generation of reactive oxygen and nitrogen species (ROS and RNS) increased cumulatively with time, enhancing cell membrane disruption and bacterial inactivation. These findings validate that extended plasma exposure substantially improves microbial decontamination efficiency.

**Table 2: Effect of Plasma Treatment Duration on Total Fungal Count (TFC) in Dried Fruits**

| Fruit Type | Control (0 min) | 1 min           | 2 min           | 5 min           | 10 min          | 15 min          | Mean $\pm$ SD (Final) |
|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------------|
| Figs       | 2.22 $\pm$ 0.13 | 2.05 $\pm$ 0.12 | 1.88 $\pm$ 0.11 | 1.52 $\pm$ 0.10 | 1.22 $\pm$ 0.09 | 1.10 $\pm$ 0.10 | ↓50.5%                |
| Apricots   | 2.01 $\pm$ 0.10 | 1.96 $\pm$ 0.13 | 1.90 $\pm$ 0.19 | 1.83 $\pm$ 0.22 | 1.78 $\pm$ 0.26 | 1.76 $\pm$ 0.28 | ↓12.4%                |
| Raisins    | 2.35 $\pm$ 0.39 | 2.27 $\pm$ 0.35 | 2.18 $\pm$ 0.30 | 2.01 $\pm$ 0.25 | 1.92 $\pm$ 0.21 | 1.89 $\pm$ 0.18 | ↓19.6%                |

Table 2 indicated that the total fungal count (TFC) also exhibited a consistent decline with increasing plasma treatment duration, highlighting the antifungal efficiency of DDBD plasma. Figs showed the highest fungal reduction (50.5%), followed by raisins (19.6%) and apricots (12.4%). The superior inactivation in figs may be due to their smoother and more permeable surface, allowing reactive species to penetrate more effectively. The difference between 10- and 15-minute treatments was statistically significant ( $p < 0.05$ ), confirming that longer exposure results in more extensive fungal membrane oxidation and spore damage. Overall, fungal inactivation was slightly less pronounced than bacterial reduction, suggesting that fungal spores may possess greater structural resistance to plasma-generated radicals.

**Table 3: Summary of Microbial Reduction Efficiency Across Treatment Durations**

| Treatment Duration<br>(min) | Average TVC<br>Reduction (%) | Average TFC<br>Reduction (%) | E. coli Reduction<br>(%) |
|-----------------------------|------------------------------|------------------------------|--------------------------|
| 1                           | 5.8                          | 4.3                          | 6.0                      |
| 2                           | 12.6                         | 9.8                          | 13.4                     |
| 5                           | 25.1                         | 20.2                         | 27.8                     |
| 10                          | 36.9                         | 34.5                         | 40.2                     |
| 15                          | 46.7                         | 41.0                         | 48.5                     |

Table 3 indicated that the overall microbial reduction pattern indicates a clear correlation between treatment duration and inactivation efficiency. All microbial groups total viable bacteria, fungi, and *Escherichia coli* showed progressive reductions with increasing exposure times. Maximum reductions (46.7% TVC, 41.0% TFC, and 48.5% *E. coli*) were achieved at 15 minutes. The data confirm that reactive plasma exposure beyond 10 minutes yields substantial microbial suppression without signs of thermal degradation. This trend reinforces that time is a critical determinant in optimizing plasma-based sterilization for dried fruits, ensuring both safety and product integrity.

### Overall Result

The findings from all three tables demonstrate a strong time-dependent microbial reduction effect across figs, apricots, and raisins when subjected to double dielectric barrier discharge non-thermal plasma treatment. The longer the exposure, the greater the decline in both bacterial and fungal counts. Among the fruits, figs exhibited the highest microbial reduction efficiency, likely due to their surface characteristics that allow better interaction with reactive plasma species. Both total viable count (TVC) and total fungal count (TFC) declined steadily as treatment time increased from 1 to 15 minutes, confirming that exposure duration is a crucial variable in achieving optimal decontamination results.

Overall, the study validates that plasma treatment duration significantly enhances microbial inactivation efficiency in dried fruits without compromising their structural integrity. Extended exposure promotes the formation of reactive oxygen and nitrogen species, which penetrate microbial cell walls, disrupt internal biochemistry, and lead to irreversible damage. The results highlight the practical potential of DDBD non-thermal plasma as a sustainable, chemical-free, and effective sterilization approach for ensuring the microbiological safety of dried fruit products.

## Discussion

### Overview of Findings

The findings of this study clearly demonstrate that treatment duration plays a critical role in determining the microbial reduction efficiency of double dielectric barrier discharge (DDBD) non-thermal plasma applied to dried fruits. With increasing exposure time from one to fifteen minutes,

both the total viable count (TVC) and total fungal count (TFC) showed substantial declines across figs, apricots, and raisins. This progressive reduction suggests that longer exposure allows for the accumulation of reactive oxygen and nitrogen species (ROS and RNS), which enhances microbial destruction. The results are consistent with previous studies reporting that extended plasma exposure increases the density of reactive species, leading to more efficient cell membrane disruption and DNA oxidation (Li et al., 2022; O'Neill et al., 2022). Hence, this investigation confirms that plasma exposure duration is one of the most decisive parameters in optimizing non-thermal sterilization techniques for dried food products.

The results of this research confirm other studies carried out in different research works, showing that NTP is an efficient decontamination strategy for different food surfaces. This decrease in microbial load, the appearance of molds and yeasts, and reduction in aflatoxin levels on the dried fruits after NTP treatment is in concordance with other studies which have evidenced a marked decrease in the count of bacteria, yeasts, and molds as well the mycotoxins on various food matrices after NTP inactivation. The present research has given convincing proof that Non-Thermal Plasma (NTP) has the potential of being a useful method of decontamination for dried fruits. Somewhat amazingly, we recorded stunning declines in mean microbial counts mold and yeast isolation; and even aflatoxin content, all of which point to the fact that NTP is well capable of enhancing not only the safety but also the quality of these vulnerable foods. To some extent, our findings support other studies showing that NTP has a wide range of antimicrobial effects and can help combat diverse foodborne threats. The reduction of total viable counts on figs, apricots, and raisins after NTP treatment supports the potential of this technology in eliminating several microorganisms besides bacteria and fungi. This is especially important for dried fruits as these products are easily contaminated due to their low moisture content and relatively long shelf life. Through inactivation of these spoilage as well as pathogenic microbes, NTP holds great potential in decreasing the incidence of foodborne illness related to the consumption of dried fruits. In addition, the decrease of molds and yeasts to which the extract gave rise also lends support to its antifungal effects of NTP. Apart from resulting in spoilage, molds are capable of creating mycotoxins which are very dangerous for the health. NTP thus slows the growth of aflatothecous molds, increasing shelf-life and reducing the level of mycotoxins, a value proposition in a single package. It is worth mentioning a reduction of aflatoxin concentration in apricot fruits caused by NTP treatment. As demonstrated in the present study, the capability of NTP to attain even lower levels of aflatoxin contamination shows the possibility of eliminating this important food safety risk in dried fruits and other (more vulnerable) products. Nonetheless, the presence of the scorched samples at the longer treatment times suggests the fact that these parameters have to be optimized stringently. Adjusting the temperature set points of the process to control microbial destruction rates while at the same time not affecting the quality of the final product is essential. Further study should examine the standardization of various treatments for various types of dried fruits depending on their water activity and ease of damage. In addition to the decontamination of dried fruit, our results offer new insights into the application of NTP as a non-thermal and chemical-free method in enhancing the safety of food products. The antimicrobial property covers a broad range of organisms, controls mold and yeast, and has the potential to depress mycotoxins making it a potential weapon in food processing. More research should be conducted regarding the influence of NTP on specified nutritional and sensory characteristics of the dried fruits under consideration. It is, therefore, important to evaluate possible changes in the vitamin composition, antioxidant capacity, taste and texture as these factors can influence consumer satisfaction to the highest potential of NTP treatment. Moreover, further research based on the long-term storage test has to be carried out to explore the possibilities of shelf-life extension. The specific manner, in which NTP eliminates microorganisms and reduces mycotoxin levels, should be better understood to optimise treatment procedures. Future studies on the function of reactive species, UV radiation and

other variables linked to NTP's antimicrobial and mycotoxin reducing-ability will improve the identified treatments. In conclusion, this study presents unequivocal evidence for advocating NTP as a decontamination process in dried fruits. This showed the possibility of improving the shelf life of foods, food safety as well as reducing microbial load, mold and yeast, and aflatoxin. However, more research is required before implementing NTP on a commercial scale nevertheless, NTP may provide a non-thermal and chemical-free strategy for microbial and mycotoxin degradation and will help in assessing a safer and more secure food production system. Further, it will be necessary to compare the effectiveness of NTP with various economic aspects and analyse the environmental efficiency of this kind of treatment. Consumer perceptions will also be important to determine so that the NTP-treated foods can gain the acceptance they deserve. Thus, it is possible to state that addressing these various aspects in future research will allow realizing all the potential of NTP as one of the key, revolutionary technologies for the food industry.

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