**Effect Of Gliding Arc Discharge On Reducing The Microbial Load Of Black Table Olives**

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**Abstract**

In this study, it was aimed to investigate the potential of Gliding Arc Discharge (GAD) plasma, one of the non-thermal cold plasma types, for reducing the natural microbial load in Gemlik type black olives obtained from İzmir province. The GAD plasma conditions were optimized using the Box-Behnken experimental design with black olives, harvested in 2020 and then stored at +4 °C for natural microorganism growth. Olives were treated with the GAD plasma at optimum plasma condition of 0.7 mL/min gas flow rate, 0.5 cm distance between electrodes, 5 min time with dry air (99.9%). Changes in microbiological (mold-yeast and lactic acid bacteria count) of olives with and without plasma treatment were determined. After plasma treatment, 5.4% reduction in mold-yeast count and 10.7% reduction in lactic acid bacteria count were detected. The results showed that air-GAD plasma is a promising method for the pre-decontamination of fruits that are sensitive to heat and have high moisture content.

*Keywords: Gliding arc discharge plasma, Black table olives, Mold-yeast, Lactic acid bacteria, Decontamination*

**1. Introduction**

Olive fruit (Olea europaea L.), cultivated in Mediterranean countries and widely consumed worldwide, stands as a valuable product. Turkey is one of the key countries with intense olive production. Olives have an important value not only due to their nutritional value but also in terms of their contribution to the economy, culturally and environmentally [4].

In addition to its nutritional and health-benefiting properties, olive fruit is one of the foods where microbiological spoilage is frequently encountered. Proper hygiene conditions during harvesting and storage are essential, and hygiene and quality conditions must also be maintained during olive processing. Otherwise, mold formation, a common type of spoilage, could occur in olives. Mold floras in olives are primarily composed of molds belonging to the Penicillium and Aspergillus genera [3].

Certain pre-processing steps such as salting and/or pickling process and caustic application are applied to olives to preserve their nutritional value, reduce microbiological load, minimize microbial growth during storage, and enhance stability. Non-thermal (cold) plasma technology is one of these pre-processing steps. Plasma is generated by applying electrical or electromagnetic fields to or within a gas with a high electrical potential difference between two electrodes. The reactive compounds in plasma typically include reactive oxygen species (O, O2, ozone (O3), and OH), reactive nitrogen species (NO, NO2, and NOx), ultraviolet radiation (UV), free radicals, and charged particles [6]. These compounds possess the necessary energy for decontamination. Parameters such as the type of gas used, electrodes, and production system in plasma generation lead to diversity in reactive species and variations in decontamination effectiveness [10]. Additionally, parameters like the distance to the target, application time, and direct vs. indirect application might influence decontamination efficacy.

In recent years, plasma application for sterilization and disinfection purposes has become widespread in the food industry. Plasma treatment does not elevate the temperature of the food to levels that would adversely affect on its nutritional value and not cause significant changes in the composition and physical properties of the food while providing effective antimicrobial protection [1]. Among plasma treatments, GAD is preferred due to its low equipment and operating costs, as well as its high efficiency in the inactivation of various microorganisms and its operation at atmospheric pressure with low gas flow rates [2-8]. In a study using dry air GAD plasma, reductions of 3.4 and 3.7 log CFU/ml were observed in *E. coli* O157:H7 and *Salmonella* Stanley strains on agar medium and Golden Delicious apples, respectively [7]. Khalili et al. (2018) achieved complete decontamination of *E. coli* strains in almonds with air GAD-plasma treatment for 5 min [5].

There are limited studies on GAD plasma but not on olives. The aims of this study is to explore the efficacy of cold air-GAD plasma treatment in reducing the microbial load of Gemlik-type black olives. The objectives of this study involve applying GAD plasma to Gemlik-type black olives that naturally develop mold after one year of storage at +4°C. Future research will focus on evaluating the impact of optimized plasma conditions, determined through statistical analysis, on the physicochemical, microbiological quality, and storage stability of black olives.

**2. Materials and Methods**

**2.1 Samples and Chemicals**

Gemlik type black olives, harvested in 2020 with hand picking, were suppplied from the Aliağa district of İzmir province and stored at +4°C until plasma treatment. Sodium chloride (NaCl), methanol, and other chemicals were obtained from Sigma-Aldrich (Munich, Germany). Potato Dextrose Agar (PDA), Man Rogosa Sharp (MRS) and other chemicals of analytical quality were purchased from Merck (Darmstadt, Germany). The high-purity (99.9%) dry air used in plasma generation was purchased from Ankara Gaz (Ankara, Turkey).

**2.2 GAD Plasma System Setup and Optimization of Treatment Parameters**

GAD plasma system[8] with constant the electrical power is generated between copper electrodes (10.4 cm length, 1.5 mm thickness) at atmospheric pressure using high-purity air, with a discharge frequency of 20 kHz and an applied voltage of 15 kV. For the optimization of treatment conditions, a flow control mechanism (Bronkhorst, Netherlands) was used to adjust the gas flow at 0.3, 0.5 and 0.7 mL/min. As shown in Table 1, while the gas type used in this study (high-purity dry air) was kept constant, the electrode gap was set to 0.5, 0.7 and 0.9 cm. Plasma characteristics were examined by applying plasma for 1, 3, and 5 min to enhance the efficiency of plasma. The gas flow, adjusted by the flow controller, was applied to the top parts of olives stalk where visible mold formation was concentrated, from a fixed distance of 0.5 cm. The images of the plasma application are shown in Figure 1.

To determine the optimum process parameters, the Box-Behnken method under the Design of Expert menu in Minitab 17 statistical software was used.

**Table 1**. Air-GAD Plasma Experimental Design

|  |  |  |
| --- | --- | --- |
| Gas Flow Rate (mL/min) | Electrode Gap (cm) | Treatment time (min) |
| 0.3 | 0.5 | 1 |
| 0.5 | 0.7 | 3 |
| 0.7 | 0.9 | 5 |

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**Figure 1**. Laboratory stand with mini GAD plasma reactor (1: discharge chamber, 2: case, 3: connectors for working electrodes, 4: electrodes, 5: power cord connectors, 6: nozzle, 7: power supply, 8: pressure gauge, 9: gas, 10: flow rate controller, 11: sample)

**2.3 Microbiological Analyses**

Microbiological analyses were conducted both before and after plasma treatment. Olive samples, separated from 5 g of the core within a sterile cabinet, were diluted at a ratio of 1:10 (10-1) with 45 mL of sterile physiological saline solution (0.85%). Subsequently, microbial counts of total mesophilic aerobic bacteria, yeast-mold, lactic acid bacteria, and coliform were performed by inoculating different dilutions (10-2, 10-3, 10-4, and 10-5) of samples onto various agar media.

The results of mold-yeast and lactic acid bacteria counts were considered in determining the optimum treatment conditions of plasma because total mesophilic aerobic bacteria and coliform bacteria were not detected in our numerous attempts to modify inoculation procedure.

For counting total yeast and mold, PDA was prepared, sterilized, and adjusted its pH to ~3.4 by adding a 10% sterile lactic acid solution. Inoculation was performed using the spread plate method and the petri dishes were incubated at 30°C for 72 hours. The microbiological results were expressed as log CFU/g [9]. For the enumeration of lactic acid bacteria (LAB), dilutions were inoculated on MRS, using the spread plate method. After 48 hours of incubation at 30°C, the petri dishes were taken for colony counting. Throughout incubation, the petri dishes were wrapped in a refrigerator bag to create an anaerobic environment. The results were expressed as log CFU/g [9].

**3. Results and Discussion**

**3.1 GAD plasma treatment optimization**

The initial mold-yeast count was found to be 6.25 log CFU/g, while lactic acid bacteria were found to be 6.33 log CFU/g. Plasma was applied to olive samples under 15 different conditions according to the Box-Behnken experimental design. The maximum reduction in mold-yeast count, 8%, was observed at 0.7 mL/min gas flow rate, 0.7 cm electrode distance, and 5 min duration and this condition resulted in an 8% reduction in count of lactic acid bacteria. The highest reduction of 11% in lactic acid bacteria was determined after plasma treatment at 0.7 mL/min gas flow rate, 0.5 cm electrode distance, and 3 min. The post-plasma treatment temperatures of the samples were ranged from 24.46 to 30.68°C and indicated that air-GAD plasma as a cold process in food production.

Microbiological analysis results were analyzed using the Minitab 17.0 program and the "Select optimal design" menu to determine the conditions where the mold-yeast quantity was minimized while lactic acid bacteria and temperature were not optimized. Accordingly, the optimum plasma conditions were determined as 0.7 mL/min gas flow rate, 0.5 cm electrode distance, and 5 min treatment time (Table 2).

**Table 2.** The data for optimum plasma treatment conditions generated with "Select Optimum Design" menu

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gas Flow Rate (mL/min) | Electrode Gap (cm) | Treatment Time(min) | Log CFU/g | Logarithmic Reduction (%) |
| Mold-yeast | Lactic acid bacteria | Mold-yeast | Lactic acid bacteria |
| 0.7 | 0.5 | 5 | 5.44 | 5.65 | 13 | 11 |

Note: log CFU/g denotes logarithm of Colony Forming Units per gram. The percentages are calculated based on the reduction in microbial counts compared to the initial counts.

The impact of plasma application on microorganisms is complex and influenced by various factors. The high-energy ions, electrons, and radicals generated as a result of plasma induce damage to the cell membrane, DNA, and RNA structures of microorganisms. This can reduce or eliminate the reproductive capabilities of microorganisms. Simultaneously, free radicals can create oxidative stress within cells, potentially leading to cellular death.

**4. Conclusion**

The results showed that decontamination of Gemlik type black olives using air GAD plasma could be achieved without rising of temperature up 50 oC which would adversely affect food quality. Cold air GAD plasma treatment is a new food processing technology for pre-decontamination of foods either alone or in combination with other preservation methods.

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