



Microbiological study of the effect of a dielectric barrier discharge interaction on processed orange juices exposed to the environment

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Abstract

Non-pasteurized juices may contain microorganisms that cause spoilage and public health problems. Owing to their physical properties, dielectric barrier discharge plasmas show high efficiency in the inactivation of microorganisms; in this study, coliforms, *Escherichia coli* and *Salmonella* spp. were identified in samples of orange juice collected from street stalls. Microbiological inactivation in orange juice was analyzed using the Weibull model. Using optical emission spectroscopy, the oxidant particles responsible for the inactivation of bacteria are observed. The 200 mL samples were treated for 2.5, 3.5, 5.0, and 6.0 min using a plasma with electrical power of 40 W. It was observed that for aerobic mesophilic, the colony-forming units were reduced from 4.47 to 1.00 Log; for coliforms, from 4.00 to 1.00 Log; and for yeast, from 4.75 to 3.18 Log. The evaluated physicochemical parameters indicate that there are no significant changes in the properties of the juice; therefore, the interaction process with dielectric barrier discharge is a technique that has viability in the inactivation of microorganisms and offers an alternative for the food industry in treating juices.

Keywords: bacteria; yeast; coliforms; plasma; inactivation.

Practical Application: In the current study the efficiency of the dielectric barrier discharge plasma in the inactivation of different microbial groups (e.g., aerobic mesophilic and coliforms including *E. coli*) was determined. It could offer an alternative for the food industry in treating juices.

1 Introduction

In developing countries, it is usual to observe regular food consumption sales to be carried out at the markets with few or no sanitary controls (Nawawee et al., 2019). Juice intake, especially orange juice, is a very common practice in the general population owing to its associated health benefits such as being a good source of vitamins A and C and antioxidants; it is also known that orange juice contributes to cholesterol lowering (Rampersaud & Valim, 2017). However, incorrect processing techniques can lead to yeast, and mold presence, which can produce metabolites that are toxic to human health, whereas yeast represent the main problem of fermentation and deterioration in citrus concentrates (Delage et al., 2003). The presence of mesophilic bacteria is used to estimate the level of global microbiological food sample contamination, whereas coliforms are indicators of fecal contamination that affect the microbiological safety of the product (Nawawee et al., 2019). Microbial contamination has caused food-related outbreaks from the consumption of fruit, vegetable, and cider juices. The main causal agents reported are *Salmonella*, *Cryptosporidium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter jejuni*, which put public health at risk (Tournas et al., 2017; Pankaj et al., 2018).

Currently, new methods of food preservation are being sought that do not affect food quality and safety. Thermal processing is the main technique used in the food industry; however, the use of heat causes physical, chemical and nutritional changes (Almeida et al., 2015; Pankaj et al., 2018).

The use of cold plasma has proven to be a good technique for microbial deactivation and extension of the exposed products' "shelf life" (Mir et al., 2020). It is essential to understand the effect of plasma on food quality to have acceptance for its application because food can be exposed to strong electric fields and subjected to numerous species of reactive gases that may affect physical and chemical attributes, however, several studies have mentioned that the application of plasma to treat fruits and vegetables maintains quality and minimizes effects on their nutritional properties compared to those caused by thermal processes (Mir et al., 2020; Liao et al., 2018).

Owing to their physical properties, dielectric barrier discharge (DBD) and jet plasma are recommended for environmental, biological, and biomedical applications (Bourke et al., 2017). Cold plasma such as DBD plasma emits antibacterial species,

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including electrons, cations, anions, free radicals, neutral atoms, UV photons, reactive nitrogen species (RNS), and reactive oxygen species (Kim et al., 2020; Fernandes & Rodrigues, 2021). In previous investigations using different types of low-temperature plasmas generated at atmospheric pressure, such as dielectric barrier plasmas, for reduce sugar content and improve phenolics, vitamin C, carotenoids, and the antioxidant capacity of fruit and fruit juices and the elimination of pathogenic microorganisms (Aneja et al., 2014; Fernandes & Rodrigues, 2021). The efficiency in the inactivation of several microorganisms, including viruses and bacteria, has been observed these plasmas have been applied to different fruit juices, studies have shown efficiency in this process, attributing particularly to RNS and OH oxidizing particles as responsible for the inactivation process (Montenegro et al., 2002; Shi et al., 2011; Surowsky et al., 2014; Almeida et al., 2015; Scholtz et al., 2015; Liao et al., 2017). Likewise, dielectric barrier plasmas can be generated using different physical parameters such as: power and frequency; the difference between the parameters is due to the composition of each juice studied, demonstrating that all of them are efficient.

Some plasmas used in fruit juices have achieved reduction of 2.7 Log of *E. coli* inoculated in apple and melon juices (Ferrario & Guerrero, 2016). Liao et al. (2018) used a dielectric discharge and exhibited its strong bactericidal effect on *E. coli* in apple juice. Using an input power of 30–50 W, a reduction of 3.98–4.34 Log CFU/mL of *E. coli* in apple juice was obtained.

Likewise, Preetha et al. (2020), when determining the inactivation kinetics of *E. coli* (MTCC 433) inoculated in 100 mL of fresh coconut water, orange juice, and pineapple, obtained a logarithmic reduction of 4.0, 4.5, and 5.33, respectively. The volumes used ranged from 10 to 250 mL, with interaction times of 30 s to 20 min (Kim et al., 2020).

This study describes the use of DBD plasma to observe its effect on randomly collected orange juice produced at street stalls and public markets; the result of the interaction is determined based on microbial activity.

2 Materials and methods

2.1 Obtaining the samples

This study monitored 20 orange juice samples from 4 different street stalls to analyze whether the contamination was recurrent in the preparation of the product. The sample collection was performed by avoiding all external contamination (both environmental and human) to ensure its integrity; 2 L of juice was collected in sterile polyethylene bags that were labeled and transported to the laboratory in a cooler (NOM-109-SSA1-1994) (México, 1994). In the laboratory, the samples were filtered and placed in sterilized bottles for the corresponding analyses.

2.2 Physicochemical properties

The total soluble solids content (SST) of the samples was determined by the Brix degrees evaluation (°Bx) using a digital pocket refractometer (Atago3810 PAL-1). Hydrogen ion concentration (pH), temperature, and conductivity were measured

using a potentiometer (HANNA HI 83141). The determination of titratable acidity was performed by placing 100 mL of each samples of treated and untreated juice; the mixture was titrated with a 0.1 M NaOH solution, and the results were expressed as (g citric acid/100) (ISO 750:1998) (International Organization for Standardization, 1998) (Equation 1):

$$\%(\text{Ac}) = \frac{Vb \times Cb \times f \times 100}{m}, \text{ Eq. (A.1)} \quad (1)$$

Where Vb corresponds to NaOH volume consumption in mL; Cb , NaOH concentration; m , juice volume in mL; and f , acid factor (ISO 750:1998) (International Organization for Standardization, 1998).

2.3 Microbiological analysis

During the first stage, sampling was performed in orange juice outlets to detect the contamination of aerobic mesophilic, coliforms, molds, and yeast. The samples were processed by preparing serial dilutions (10^{-2}) using the plate pouring technique; the determination of aerobic mesophilic was performed using the standard method of agar incubation at 35 °C for 48 h. For coliforms, MacConkey agar was used; the plates were incubated at 35 °C for 24 h (ISO 6887-1:2017; NOM-092-SSA1-1994; NOM-110-SSA1-1994) (International Organization for Standardization, 2017; México, 1995a, 1995b).

Molds and yeast were quantified with Potato Dextrose Agar (BIOXON) acidified with 10% tartaric acid, and the plates were incubated at 25 °C for 5 days (and another series was incubated at 35 °C for 48 h) for the determination of yeast. The quantification of yeast was supplemented with Yeast Extract Agar (BIOXON) acidified with tartaric acid at the temperature of 35 °C for 48 h (Tournas et al., 2017).

Colonies were seeded on selective media for the detection of *E. coli* and *Salmonella* spp. The agars used were Mac Conkey (BIOXON), Chromogenic O157:H7 (DIBICO), Methylene Blue Eosin (BIOXON), Brilliant Green (BIOXON), *Salmonella Shigella* (BIOXON), and Xylose Lysine Desoxycholate (DIBICO). The plates were incubated at 35 °C for 24 h.

All physicochemical and microbiological analyses were performed in triplicate before and after interaction with DBD plasma. A completely randomized design was followed to evaluate the response of the physicochemical and microbiological parameters to the interaction of DBD plasma as a microbial inactivation method. The obtained results were evaluated using ANOVA ($p < 0.05$); when significant differences were observed, a 5% Tukey test was applied using the statistical program STATGRAPHICS Centurion 18.1 (Statgraphics Technologies, Inc., 2021).

2.4 Mathematical quantifying of inactivation kinetics

The microbiological inactivation in orange juice was analyzed using the Weibull model described by (Equation 2) (Alenyorege et al., 2019; Kim et al., 2020; Liao et al., 2018; van Boekel, 2002). The data were fitted and analyzed using the nonlinear least-squares regression method in the Origin v.9.0 software (OriginLab, Northampton, MA, USA):

$$\text{Log}_{10} \frac{N_t}{N_0} = -\frac{1}{2.303} \left(\frac{t}{\alpha} \right)^\beta, \quad \text{Eq. (A2)} \quad (2)$$

where N_t corresponds to the survival population number at time t [colony-forming units (CFU)/mL], N_0 is the initial bacterial population number (CFU/mL), t is the exposure time (min), α is the scale factor (s), and β is the dimensionless shape factor of the inactivation curve. If $\beta < 1$, the concavity of the survival curve would show upward concavity, and if $\beta > 1$, a downward survival curve would be obtained.

2.5 Determination of the electronic excitation temperature

To characterize the DBD plasma used in this study, the electronic excitation temperature is obtained. Assuming that plasma is in a stable state, the distribution of the number of particles in the energy level of the excited state obeys the Boltzmann distribution. The intensity ratio of two spectral lines of the same element with different magnitudes of excitation energy is as follows (Yuan et al., 2020) (Equation 3):

$$\frac{I_1}{I_2} = \frac{v_1 g_1 A_1}{v_2 g_2 A_2} \cdot \exp\left(\frac{E_2 - E_1}{kT}\right), \quad \text{Eq. (A3)} \quad (3)$$

Solving for temperature, (Equation 4)

$$T = \frac{5040(E_1 - E_2)}{\ln \frac{A_1 g_1}{A_2 g_2} - \ln \frac{\lambda_1}{\lambda_2} - \ln \frac{I_1}{I_2}}, \quad \text{Eq. (A4)} \quad (4)$$

Where the values of excitation energy E , statistical weight g , and transition probability A can be obtained from the database of the National Institute of Standards and Technology. The spectral intensity I is obtained from the measured spectrum. T is the electron excitation temperature in the plasma.

2.6 Experimental system (plasma generation)

The developed system consists of a cylindrical quartz chamber (Figure 1) with a diameter of 5 cm and a total volume of 255 cm³; inside the chamber, there is a concentrically placed cylindrical electrode (Figure 1d) that is machined from stainless steel (A304) of 2.5 cm in diameter. The separation distance between the internal surface of the quartz chamber and the electrode is 1.25 cm. The chamber is sealed by acrylic caps on both sides. In the upper part, an inlet is placed to admit the juice (Figure 1a) in such a manner that it falls on the electrode and runs evenly along the walls of the metal electrode. At the bottom, the inner electrode goes through the cap, and the joint is sealed with Teflon. The lower lid has an outlet for the juice (Figure 1e) toward the container for its recirculation. A copper winding is placed around the quartz tube (Figure 1c), which acts as a second electrode; this winding completely covers the length of the central electrode to obtain a homogeneous discharge on the metal surface. The fastening of acrylic caps is done using four posts made from Nylamid to avoid electric shock.

The potential difference, or voltage, is supplied employing a pulsed power supply (GBS ELECTRONIK); the center electrode is connected to earth ground, and the winding is connected to

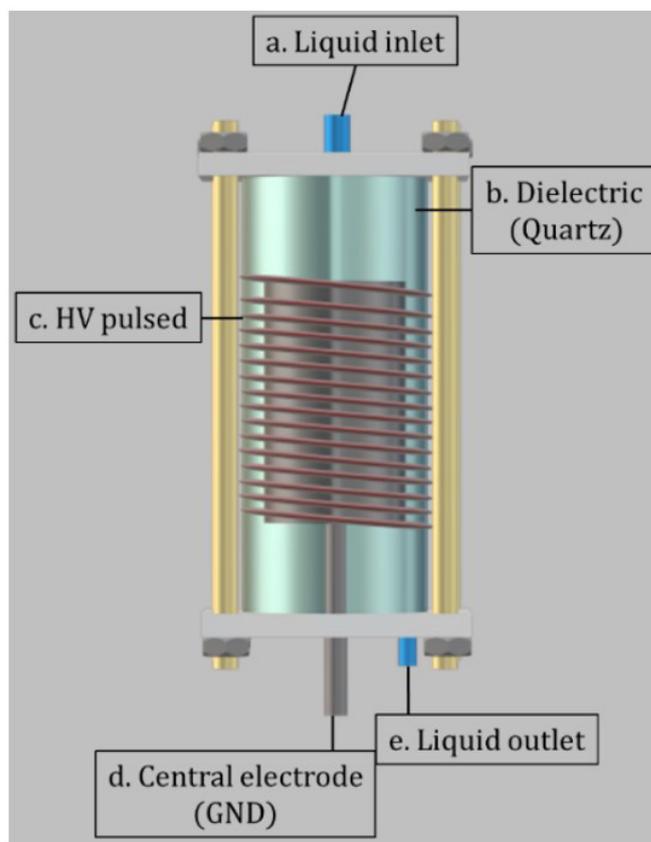


Figure 1. Main DBD reaction chamber components.

the high-voltage output. Once the juice is recirculating through the system with a flow of 100 mL/min, a voltage of 12 kV and a frequency of 14.6 kHz are applied (at the power of 40 W) to the volume of 200 mL of juice for different interaction times (2.5, 3.5, 5, and 6 min and the control group). An optical fiber is placed on one side of the fixed chamber with a support and connected to an Ocean Optics spectrometer (FLAME-T-UV-Vis). Optical emission spectra of the plasma are observed to identify oxidants that interact in the system.

3 Results

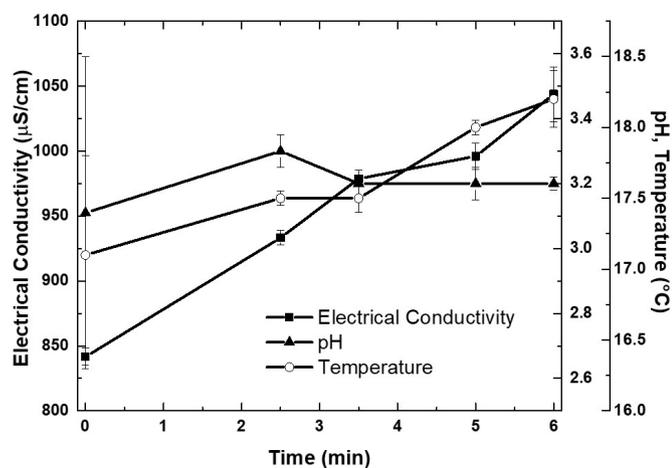
The mean values of aerobic mesophilic, coliforms and yeast were 3.7, 4.4, and 2.2 Log CFU/mL, respectively. This result highlights the constant microbial contamination to which consumers are exposed when juices that have not undergone any pasteurization process are purchased. The presence coliforms, aerobic mesophilic and yeast > 2 Log CFU/mL, as it happened in all samplings, indicates poor sanitary quality of the product, hygienic conditions of the raw material, and the way it is handled during the production (Maturin & Peeler, 2021; Red Nacional de Laboratorios Oficiales de Análisis de Alimentos, 2014).

Table 1 summarizes the data obtained in different physicochemical analyses performed before and after the application of DBD plasma; in addition, Figure 2 illustrates the electrical conductivity behavior, pH, and temperature during the exposure of orange juice to DBD plasma without indicating a significant difference.

Table 1. Physicochemical variables of orange juice.

Time treatment (min)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Temperature ($^{\circ}\text{C}$)	SST ($^{\circ}\text{Bx}$)	AT (%)	IM
0 (Control)	841.6 \pm 6.6a	3.11 \pm 0.48a	17.1 \pm 0.70a	12.4a \pm 0.29a	1.3 \pm 0.07a	9.74a \pm 0.75a
2.5	933.2 \pm 5.5b	3.30 \pm 0.05a	17.5 \pm 0.05bc	12.2 \pm 0.20a	1.4 \pm 0.05a	8.90 \pm 0.37a
3.5	978.8 \pm 6.5bc	3.20 \pm 0.01a	17.5 \pm 0.10bc	12.1 \pm 0.20a	1.4 \pm 0.04a	9.42 \pm 0.40a
5	996.0 \pm 10cd	3.20 \pm 0.05a	18.0 \pm 0.05cd	12.0 \pm 0.10a	1.4 \pm 0.03a	9.62 \pm 0.45a
6	1,043.8 \pm 21d	3.20 \pm 0.02a	18.2 \pm 0.20d	11.6 \pm 0.30b	1.2 \pm 0.01b	9.67 \pm 0.50a

Means followed by the same letters indicate the absence of statistically significant difference by Tukey's test ($p < 0.05$). pH = hydrogen ion concentration; SST = total soluble solids; AT = titratable acidity; IM = maturity index.

**Figure 2.** Behavior of pH, electrical conductivity, and temperature of orange juice during the time of interaction with DBD plasma.

The temperature and conductivity parameters are related and show minimal significant effects ($p < 0.05$) with the formation of four groups; however, they do not affect a decrease in the microbial load. Although the temperature increased by 1.1 $^{\circ}\text{C}$ for the maximum interaction time of 6 min, this increase does not have any effect on the physicochemical and microbiological variables. The changes in the increase in conductivity are attributed to the increase in the formation of oxidant particles generated by the plasma as the exposure time increases. Theoretically it is possible to verify that when the plasma interacts with the juice the electrical conductivity are affected because of the system is supplied with high energy electrons, causing ionization, dissociation, and recombination processes with water molecules, generating OH^{\bullet} and H^{\bullet} radicals, as described in reaction R1 [A] (Equation 5):



Plasma promotes an increase in the H^{\bullet} concentration, owing to the process of dissociation of water molecules, resulting in an increase in the electrical conductivity in the system.

SST ($^{\circ}\text{Bx}$) has high range because the fruit used to make the juice is more mature. The obtained results indicate three different groups, noting a decrease after 5 min of exposure to DBD plasma. The high range of $^{\circ}\text{Bx}$ indicates high sugar content (80%–85%), and sucrose, glucose, and fructose are most abundant. The remaining composition is made up of acids,

minerals, amino acids, and water-soluble compounds (Keng et al., 2015). The use of dielectric barrier discharge plasma in natural juices does not produce physicochemical changes that affect the quality of the product.

3.1 Microbial load assessment

A decrease in CFU for aerobic mesophilic, coliforms and yeast is determined by the time of exposure to DBD plasma in such a manner that all treatments presented a statistically significant difference by Tukey's test ($p < 0.05$). The initial value of coliforms of 4.0 ± 0.20 Log (CFU/mL) gradually decreased to 3.37 ± 0.17 , 3.27 ± 0.16 , 2.69 ± 0.13 and 0.00 ± 0.05 Log (CFU/mL) in 2.5, 3.5, 5.0 and 6.0 min of exposure with DBD plasma, respectively. Similarly, the initial value of the aerobic mesophilic was 4.47 ± 0.22 Log (CFU/mL) and decreases as a function of the interaction time with the DBD Plasma to 4.18 ± 0.21 , 4.13 ± 0.20 , 3.89 ± 0.19 and 0.00 ± 0.05 Log (CFU/mL) in 2.5, 3.5, 5.0 and 6.0, respectively. This behavior in the decrease is also presented by the value of the yeasts, its initial value was 4.75 ± 0.24 Log (CFU/mL), in 2.5 min 4.37 ± 0.22 , later in 3.5 min 4.12 ± 0.21 , after in 5.0 min 3.89 ± 0.19 and finally in 6 min the value was 3.18 ± 0.16 , this last value is different from that found for aerobic mesophilic and coliforms at the same time of interaction with plasma.

The survival level of aerobic mesophilic detected in the orange juice studied. The initial value of aerobic mesophilic was 4.47 Log (CFU/mL), and it gradually decreased to total inactivation of 1.0 Log (CFU/mL) in 6 min of exposure to the DBD plasma and had a reduction of 3.0 Log (CFU/mL) during the interaction time with the DBD plasma. Similar for coliforms in orange juice, Figure 3 indicates that in the interval from 0 to 5 min, there is only a reduction of 12.97%, whereas from 5 to 6 min of interaction with plasma, the greater reduction in the value of total aerobic mesophilic is observed, i.e., the remaining 87.03%. The magnitudes of coliforms and yeasts do not reach 1.0 Log (CFU/mL) in 6 min; however, indicates that the shape of the curve is convex and predicts that the magnitude of yeasts will continue to decrease as a function of time of exposure to plasma (Figure 3).

The survival level of coliforms decreases as a function of treatment time at the constant electrical power of 40 W and illustrates gradual reduction as a function of the interaction time with plasma. In the first 2.5 min, there is a decrease of 15.75%; in 3.5 min, 18.25%; in 5 min, 32.75%; and in the interval of 5–6 min, the remaining 67.25% (Figure 3).

The colonies obtained were positive for *E. coli* y *Salmonella* spp. This represents a risk to the health of consumers. By subjecting the samples to the DBD plasma for different exposure times, the inactivation efficiency of 100% for these microorganisms is observed.

The inactivation efficiency of the plasma is due to the presence of reactive N_2 species (RNS) generated by the DBD plasma, which were observed by optical emission spectroscopy. The observed reactive species were formed since the gas used was air at atmospheric pressure containing 78% nitrogen. The spectroscopic analysis performed indicates the presence of mainly N_2 species (second positive system or 2PS system), which are found at wavelengths of 316.58, 337.7, 354.15, 358.11 and 380.25 nm. The reactive species are formed as follows; the 2PS system corresponds to the transition between the $C^3\Pi_u$ and $B^3\Pi_g$ electronic states; this feature dominates the spectral region at 300-490 nm; this 2PS band is typically found in nitrogen discharges or atmospheric discharge plasmas, and the latter can be obtained in a laboratory.

The electron excitation temperature was calculated by selecting the primary ionization transition line [N_2^+ (0, 0)] of nitrogen NII (391.4 nm) and the secondary ionization transition line [N_{2n} (2, 5)] of nitrogen NIII (394.3 nm) (Yuan et al., 2020); the optical emission spectrum allows obtaining the intensity values (I) to determine the plasma electron excitation temperature using

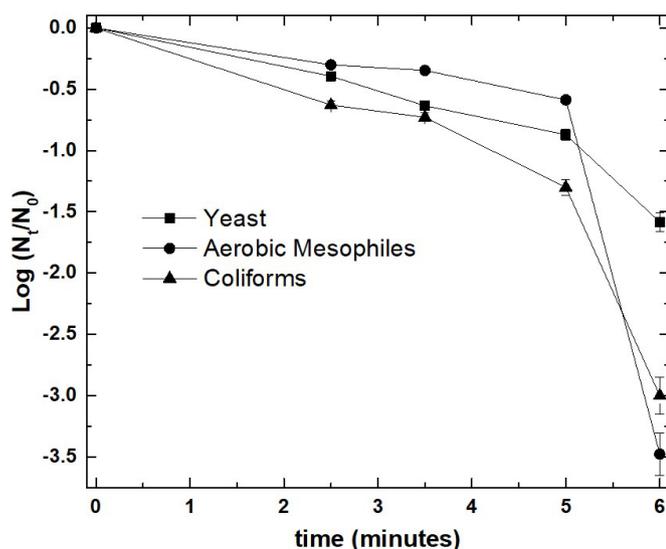


Figure 3. Survival curve of coliforms, aerobic mesophilic and yeast in orange juice treated with the DBD atmospheric plasma.

Table 2. Parameters of the fitted Weibull model.

Input power (W)	N_0 (Log CFU/mL)	α scale factor (s)	β dimensionless shape factor	Reports
40	4.00 ± 0.20	3.20	3.38	This work: coliforms
40	4.47 ± 0.22	3.92	3.43	This work: aerobic mesophilic
40	4.75 ± 0.24	9.42	4.28	This work: yeast
40	8.30 ± 0.22	13.45	2.09	DBD plasma Apple juice (Liao et al., 2018)

CFU=Colony Forming Units

Equation 4 and considering the excited nitrogen species, NII and NIII at 391.4 and 394.3 nm, respectively. In this study, the electron excitation temperature determined for the DBD plasma used in the disinfection of orange juice is of the order of 740 K.

Mathematical modeling of the inactivation kinetics was performed using Equation 1, as described earlier, and the values of α and β were obtained (Table 2). The β form factor for the conditions described in this study is greater than 1, which indicates that the remaining bacterial population became increasingly damaged and more susceptible to lethal stressors (Liao et al., 2018).

The results obtained indicate that for the three reported parameters aerobic mesophilic, coliforms and yeast, the treatment of orange juice reduces magnitudes as a function of time that the juice is subjected to plasma. All three parameters present a convex curvature with the β form factor > 1 , which indicates that the disinfection treatment by this process is effective, and the contaminants after 6 min will not be able to regenerate.

4 Discussion

The properties of orange juice are determined by microbiological, enzymatic, chemical, and physical factors that affect its sensory and nutritional characteristics. Most studies performed with plasmas have focused on microbial inactivation without highlighting the impact that this has on the physicochemical qualities related to quality attributes (Pankaj et al., 2018).

The obtained results on the concentration of hydrogen ions complied with the specifications indicated in NMX-F118-1984 without indicating a significant difference compared to the juice that was not treated with DBD plasma; these results differ from those in the studies in which there has been a decrease in the values of pH in samples treated with plasmas DBD in addition to the fact that this treatment does not affect the percentage of microbial inactivation (Parish, 1997; Preetha et al., 2021). The pH measures free H^+ ions in the solution, which interact with the taste buds of the tongue and are related to the taste of citrus juices (Segurondo et al., 2013). Because there are no significant changes, juice preserves sensory characteristics for which the consumer is looking.

The achieved range complies with the international standard (CODEX STAN 247-2005) for orange juices. High titratable acidity indicates a high organic acid content ratio. The Brix-acidity ratio expressed as anhydrous citric acid complies with international regulations that establish a limit of 15 (CODEX STAN 247-2005) (Codex Alimentarius, 2005).

The source of entry of microorganisms in fresh orange juices from environment exposure and may contain spoilage and pathogenic microorganisms (Jesús et al., 2022). The results of previous sampling in commercial outlets indicated the absence of molds because its detectability depends on the degree of invasion compared to that obtained by Aneja et al. (2014), who reported the presence of molds such as *Rhodotorula mucilaginosa* and *Aspergillus flavus* in juice samples. The high incidence of yeast causes the deterioration and decomposition of orange juices, with the production of an unpleasant smell and taste (Tournas et al., 2017). The presence of coliforms and *Escherichia coli* can indicate safety in the food processing environment and recent fecal contamination and unhealthy processing (Feng et al., 2020).

In the present study spoilage and pathogenic microorganisms such as *E. coli* were identified and the decrease in microorganisms by the DBD plasma is analogous to that reported in sugarcane juice by Manzoor in 2020, who obtained 3.6 Log for aerobic mesophilic and 0.5 Log for yeasts in contrast to Ahmadnia in 2021, decreased 1.46 and 2.75 for aerobic mesophilic and yeast for strawberry at a time of 20 min (Manzoor et al., 2020).

In previous studies on apple juice treatment with DBD plasma at lower volumes of juice, such as the one performed by Liao et al. (2018), the behavior depended on the electrical power with which the DBD plasma was generated in the inactivation process of *E. coli* in apple juice (Liao et al., 2018). The effect on the reduction of total microorganisms in food using plasma has been previously studied by Pankaj et al. (2018) for strawberry (DBD plasma), radish sprouts (microwave plasma), melon (DBD plasma), and cherry tomatoes (DBD plasma); there was always an effective decrease depending on the time of exposure to plasma.

The inactivation efficiency is attributed to the interaction of reactive N₂ species (RNS) with the contaminants, this presence of RNS observed in the optical emission spectra of plasma. These reactive species tend to react when in contact with living cells and normal metabolism may be disrupted. These components reach the cell wall and cytoplasmic membrane through the periplasmic space and internalize reactive species that cause damage to the cell (Fernandes & Rodrigues, 2021). The reactive species with antimicrobial potential emitted by the DBD plasma cross the outer membrane of Gram-negative bacteria (Bourke et al., 2017; Sholtz et al., 2015) that is composed of phospholipids, lipopolysaccharides, and lipoproteins. The chemical reactions caused by DBD plasma treatment can provide benefits such as improving quality microbiology of fruit juices.

5 Conclusions

Microbial contamination of natural orange juices affects sanitary quality, which can cause health risks owing to the presence of *Escherichia coli* and *Salmonella spp.* The presence of aerobic mesophilic and yeast is responsible for product spoilage and the presence of coliforms and *E. coli* is related to the sanitary quality of juice and is responsible for diarrheal diseases; coliforms and *E. coli* inactivation can be reduced by applying the DBD plasma with a power of 40 W to samples of juice. The obtained results indicate that after 6 min of treatment with the DBD plasma, the microbial load in orange juices completely decreases,

which affects the sanitary quality and shelf life of the product without detrimental changes in physicochemical parameters and product quality.

References

- Ahmadnia, M., Sadeghi, M., Abbaszadeh, R., & Ghomi Marzdashti, H. R. (2021). Decontamination of whole strawberry via dielectric barrier discharge cold plasma and effects on quality attributes. *Journal of Food Process and Preservation*, 45, e15019. <https://doi.org/10.1111/jfpp.15019>.
- Alenyorege, E. A., Ma, H., & Ayim, I. (2019). Inactivation kinetics of inoculated *Escherichia coli* and *Listeria innocua* in fresh-cut Chinese cabbage using sweeping frequency ultrasound. *Journal of Food Safety*, 39(6), e12696. <http://dx.doi.org/10.1111/jfs.12696>.
- Almeida, F. D. L., Cavalcante, R. S., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N., & Rodrigues, S. (2015). Effects of atmospheric cold plasma and ozone on prebiotic orange juice. *Innovative Food Science & Emerging Technologies*, 32, 127-135. <http://dx.doi.org/10.1016/j.ifset.2015.09.001>.
- Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. (2014). Microbes associated with freshly prepared juices of citrus and carrots. *International Journal of Food Science*, 2014, 408085. <http://dx.doi.org/10.1155/2014/408085>. PMID:26904628.
- Bourke, P., Ziuzina, D., Han, L., Cullen, P., & Gilmore, B. F. (2017). Microbiological interactions with cold plasma. *Journal of Applied Microbiology*, 123(2), 308-324. <http://dx.doi.org/10.1111/jam.13429>. PMID:28245092.
- Codex Alimentarius. (2005). *General standard for fruit juices and nectars (CXC 247-2005)*. Retrieved from <https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/>
- Delage, N., d'Harlingue, A., Ceccaldi, B. C., & Bompeix, G. (2003). Occurrence of mycotoxins in fruit juices and wine. *Food Control*, 14(4), 225-227. [http://dx.doi.org/10.1016/S0956-7135\(03\)00010-0](http://dx.doi.org/10.1016/S0956-7135(03)00010-0).
- Feng, P., Weagant, S. D., Grant, M. A., & Burkhardt, W. (2020). *BAM chapter 4: enumeration of Escherichia coli and the Coliform Bacteria*. Retrieved from <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria#citrus>
- Fernandes, F. A. N., & Rodrigues, S. (2021). Cold plasma processing on fruits and fruit juices: a review on the effects of plasma on nutritional quality. *Processes*, 9(12), 2098. <http://dx.doi.org/10.3390/pr9122098>.
- Ferrario, M., & Guerrero, S. (2016). Study of the inactivation of spoilage microorganisms in apple and melon juices by pulsed light and ultrasound. *INNOTEC*, 11, 9-17.
- International Organization for Standardization – ISO. (1998). *ISO 750:1998. Fruit and vegetable products — determination of titratable acidity* (2nd ed.). Geneva: ISO.
- International Organization for Standardization – ISO. (2017). *ISO 6887-1:2017. Microbiology of the food chain — preparation of test samples, initial suspension and decimal dilutions for microbiological examination — part 1: general rules for the preparation of the initial suspension and decimal dilutions* (2nd ed.). Geneva: ISO.
- Jesús, R. L. O., Ibáñez, A. T. G., Puebla, I. R., García, N. P., Siclán, M. L. S., & Romero, L. D. C. (2022). Microbiological quality and presence of enteropathogenic bacteria in orange juice sold in popular markets. *Food Science and Technology*, 42, e09621. <http://dx.doi.org/10.1590/fst.09621>.
- Keng, S. E., Al-Karkhi, A. F. M., Talib, M. K. M., Easa, A. M., & Hoong, C. L. (2015). An investigation of potential fraud in commercial

- orange juice products in Malaysian market by cluster analysis and principal component analysis. *The Malaysian Journal of Analytical Sciences*, 19(2), 377-387.
- Kim, J. Y., Jeon, E. B., Choi, M.-S., Choi, E. H., Lim, J. S., Choi, J., & Park, S. Y. (2020). The efficiency of atmospheric dielectric barrier discharge plasma against *Escherichia coli* and *Bacillus cereus* on dried laver (*Porphyra tenera*). *Foods*, 9(8), 1013. <http://dx.doi.org/10.3390/foods9081013>. PMID:32731394.
- Liao, X., Li, J., Muhammad, A. I., Suo, Y., Chen, S., Ye, X., Liu, D., & Ding, T. (2018). Application of a dielectric barrier discharge atmospheric cold plasma (Dbd-Acp) for *Escherichia coli* inactivation in apple juice. *Journal of Food Science*, 83(2), 401-408. <http://dx.doi.org/10.1111/1750-3841.14045>. PMID:29355961.
- Liao, X., Xiang, Q., Liu, D., Chen, S., Ye, X., & Ding, T. (2017). Lethal and sublethal effect of a dielectric barrier discharge atmospheric cold plasma on *Staphylococcus aureus*. *Journal of Food Protection*, 80(6), 928-932. <http://dx.doi.org/10.4315/0362-028X.JFP-16-499>. PMID:28459615.
- Manzoor, M. F., Ahmad, N., Ahmed, Z., Siddique, R., Mehmood, A., Usman, M., & Zeng, X.-A. (2020). Effect of dielectric barrier discharge plasma, ultra-sonication, and thermal processing on the rheological and functional properties of sugarcane juice. *Journal of Food Science*, 85(11), 3823-3832. <http://dx.doi.org/10.1111/1750-3841.15498>. PMID:33073398.
- Maturin, L., & Peeler, J. T. (2021). *BAM chapter 3: aerobic plate count*. Retrieved from <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count>
- México. (1994, November 4). Proyecto de norma oficial mexicana NOM-109-SSA1-1994, bienes y servicios. Procedimientos para la toma, manejo y transporte de muestras de alimentos para su análisis microbiológico. *Diario Oficial de la Federación*.
- México. (1995a, December 12). Norma oficial mexicana NOM-092-SSA1-1994, bienes y servicios. Metodo para la cuenta de bacterias aerobias en placa. *Diario Oficial de la Federación*.
- México. (1995b, October 16). Norma oficial mexicana NOM-110-SSA1-1994, bienes y servicios. Preparación y dilución de muestras de alimentos para su análisis microbiológico. *Diario Oficial de la Federación*.
- Mir, S. A., Siddiqui, M. W., Dar, B. N., Shah, M. A., Wani, M. H., Roohinejad, S., Annor, G. A., Mallikarjunan, K., Chin, C. F., & Ali, A. (2020). Promising applications of cold plasma for microbial safety, chemical decontamination and quality enhancement in fruits. *Journal of Applied Microbiology*, 129(3), 474-485. <http://dx.doi.org/10.1111/jam.14541>. PMID:31800143.
- Montenegro, J., Ruan, R., Ma, H., & Chen, P. (2002). Inactivation of *E. coli* O157: H7 using a pulsed nonthermal plasma system. *Journal of Food Science*, 67(2), 646-648. <http://dx.doi.org/10.1111/j.1365-2621.2002.tb10653.x>.
- Nawawee, N. S. M., Bakar, N. F. A., & Zulfakar, S. S. (2019). Microbiological safety of street-vended beverages in Chow Kit, Kuala Lumpur. *International Journal of Environmental Research and Public Health*, 16(22), 4463. <http://dx.doi.org/10.3390/ijerph16224463>. PMID:31766289.
- Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of cold plasma on food quality: a review. *Foods*, 7(1), 4. <http://dx.doi.org/10.3390/foods7010004>. PMID:29301243.
- Parish, M. E. (1997). Public health and nonpasteurized fruit juices. *Critical Reviews in Microbiology*, 23(2), 109-119. <http://dx.doi.org/10.3109/10408419709115132>. PMID:9226110.
- Preetha, P., Pandiselvam, R., Varadharaju, N., Kennedy, Z. J., Balakrishnan, M., & Kothakota, A. (2021). Effect of pulsed light treatment on inactivation kinetics of *Escherichia coli* (MTCC 433) in fruit juices. *Food Control*, 121(5), 107547. <http://dx.doi.org/10.1016/j.foodcont.2020.107547>.
- Rampersaud, G. C., & Valim, M. F. (2017). 100% citrus juice: nutritional contribution, dietary benefits, and association with anthropometric measures. *Critical Reviews in Food Science and Nutrition*, 57(1), 129-140. <http://dx.doi.org/10.1080/10408398.2013.862611>. PMID:25831042.
- Red Nacional de Laboratorios Oficiales de Análisis de Alimentos – RENALOA. Administración Nacional de Medicamentos, Alimentos y Tecnología Médica – ANMAT. (2014). *Análisis microbiológico de los alimentos: metodología analítica oficial*. Buenos Aires: ANMAT. Recuento de aerobios mesófilos en muestras de alimentos: técnica de recuento en placa (Microorganismos Indicadores, Vol. 3, pp. 43-52).
- Scholtz, V., Pazlarova, J., Souskova, H., Khun, J., & Julak, J. (2015). Nonthermal plasma—a tool for decontamination and disinfection. *Biotechnology Advances*, 33(6 Pt 2), 1108-1119. <http://dx.doi.org/10.1016/j.biotechadv.2015.01.002>. PMID:25595663.
- Segurondo, L. R., Pantoja, M. R., & Rocha, E. (2013). Determinación de la genuinidad en jugos de naranja comercializados en los supermercados de la Ciudad de La Paz. *Revista CON-CIENCIA*, 1(1), 105-112.
- Shi, X.-M., Zhang, G.-J., Wu, X.-L., Li, Y.-X., Ma, Y., & Shao, X.-J. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Transactions on Plasma Science*, 39(7), 1591-1597. <http://dx.doi.org/10.1109/TPS.2011.2142012>.
- Statgraphics Technologies, Inc.. (2021). *Statgraphics Centurion 18*. The Plains: Statgraphics Technologies, Inc.
- Surowsky, B., Fröhling, A., Gottschalk, N., Schlüter, O., & Knorr, D. (2014). Impact of cold plasma on *Citrobacter freundii* in apple juice: inactivation kinetics and mechanisms. *International Journal of Food Microbiology*, 174, 63-71. <http://dx.doi.org/10.1016/j.ijfoodmicro.2013.12.031>. PMID:24462703.
- Tournas, V., Stack, M. E., Mislivec, P. B., Koch, H. A., & Bandler, R. (2017). *BAM chapter 18: yeasts, molds and mycotoxins*. Retrieved from <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins>.
- van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, 74(1-2), 139-159. [http://dx.doi.org/10.1016/S0168-1605\(01\)00742-5](http://dx.doi.org/10.1016/S0168-1605(01)00742-5). PMID:11930951.
- Yuan, Q., Ren, P., Liu, S., Wang, J., & Yin, G. (2020). The optical emission spectroscopy of nitrogen plasma driven by the 94.92 MHz/13.56 MHz dual-frequency. *Physics Letters A*, 384(12), 126367. <http://dx.doi.org/10.1016/j.physleta.2020.126367>.