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Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin

Hyun-Joo Kim [a], Hae In Yong [a], Sanghoo Park [b], Wonho Choe [b], Cheorun Jo [a,*]

[a] Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

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ABSTRACT

This study aimed to evaluate the use of a dielectric barrier discharge (DBD) plasma system to improve the safety of pork loins. When pork loin was exposed to DBD plasma with the input gases He and He + O_2, the population of *Escherichia coli* was reduced by 0.26 and 0.50 log cycles following a 5-min treatment and by 0.34 and 0.55 log units following a 10-min treatment, respectively. That of *Listeria monocytogenes* was also reduced from 0.17 to 0.35 and 0.43 to 0.59 log cycles when the samples were exposed to DBD for 5 and 10 min using He and He + O_2, respectively. The pH and L^*-values (lightness) of the samples decreased significantly with DBD plasma treatment, but a^*- (redness) and b^*-values (yellowness) exhibited no obvious changes. Lipid oxidation, measured by TBARS values, was greater in samples with He + O_2 than in other samples. Significant reductions in sensory quality parameters (appearance, color, odor, acceptability, etc.) were observed in DBD-treated samples. These results indicate that the DBD plasma system has potential for use in sanitizing pork loins by inactivation of foodborne pathogens, although the effect was limited. In order to meet market requirements, however, a method to overcome sensory deterioration of pork loins should be developed and applied.

1. Introduction

The most serious meat safety issues resulting in immediate consumer health problems and recalls of potentially contaminated products from the market place are associated with microorganisms, especially bacterial pathogens. Recently, some highly publicized outbreaks of foodborne disease in the US, caused by pathogenic bacteria, such as *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes*, have brought meat safety and associated issues to the forefront of societal concerns [1]. The World Health Organization (WHO) [2] reported that foodborne pathogens result in 325,000 hospitalizations and 5000 deaths every year. According to data from the Korea Food and Drug Administration, the number of foodborne diseases in 2010 increased 2-fold compared to the number in 2003. Several technologies have been developed to reduce the occurrence of pathogens on meat products [3,4]. Thermal treatment can effectively inactivate pathogens, but induces side effects in the sensory, nutritional, and functional properties of foods, especially for fresh products [5]. To overcome these disadvantages, non-thermal methods, such as chemical treatment, UV, irradiation, and high-pressure processing were developed. However, these technologies also have some drawbacks, including the high cost of application, requirements for specialized equipment, generation of undesirable residues, extended processing times, and lower efficiencies [6].

Recently, atmospheric pressure plasma (APP) has been investigated as a non-thermal inactivation technique in food processing. Plasma, electrically energized matter in the gaseous state, can be generated by electrical discharge [7] and is often called the fourth state of matter. Inside the atmospheric pressure plasma, there are abundant chemically active free radicals, such as reactive oxygen species and reactive nitrogen species, metastables, excited atoms and molecules, UV photons, and charged particles, such as electrons and ions [8]. Highly reactive species can overcome natural defense mechanisms, resulting in damage to DNA, proteins, lipids, and membranes [9]. Due to these species, plasma has demonstrated bactericidal, fungicidal, and virucidal effects [10–12]. Therefore, the generation and utilization of plasma at atmospheric pressure may be competitive due to its simplicity and reduced cost compared to other microbial inactivation methods [13]. In addition, Isbary et al. [14,15] demonstrated the safety of the APP devices that did not lead to changes in the tissue/meat after multiple treatments.

Methods of plasma excitation for sterilization can be divided into several categories, i.e., APP jet, microwave discharge,

* Corresponding author. Tel.: +82 42 821 5774; fax: +82 42 825 9754.
E-mail address: cheorun@cnu.ac.kr (C. Jo).

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dielectric barrier discharge (DBD), and DBD-based atmospheric pressure glow discharge (APGD), etc. Compared with APP jet and microwave discharge methods, DBD and APGD can achieve a more stable discharge at a higher power level, and they are simpler and more effective, without heating [16].

The DBD plasma is generated by one or two electrodes covered with dielectric layers such as glass, polymer layers, and ceramic materials, thereby stopping the electric current and preventing streamer formation [10]. The advantages of DBD are its simplicity and the availability of efficient and affordable power supplies. Compared to pulsed corona discharge, DBD does not require sophisticated pulsing circuits [17]. Fridman et al. [18] found that DBD plasma minimizes the negative effects on living targets when it is used under appropriate conditions.

However, the application of DBD plasma to food safety improvement is still very limited. Our previous studies have proposed recommendations to develop a specific APP jet system for food application [19,20]. It is also necessary to evaluate a wide range of nutritional and quality attributes of APP-treated foods for obtaining evidence and general acceptance of this method as a food decontamination process. Compared with most of other types of discharge, DBD can be scaled up without additional difficulties [17]. Recently, Lee et al. [21] confirmed that the DBD plasma system may be an effective method for the reduction of microorganisms in cheese slices. Therefore, the objective of this study is to evaluate the quality characteristics of pork loins treated with DBD plasma.

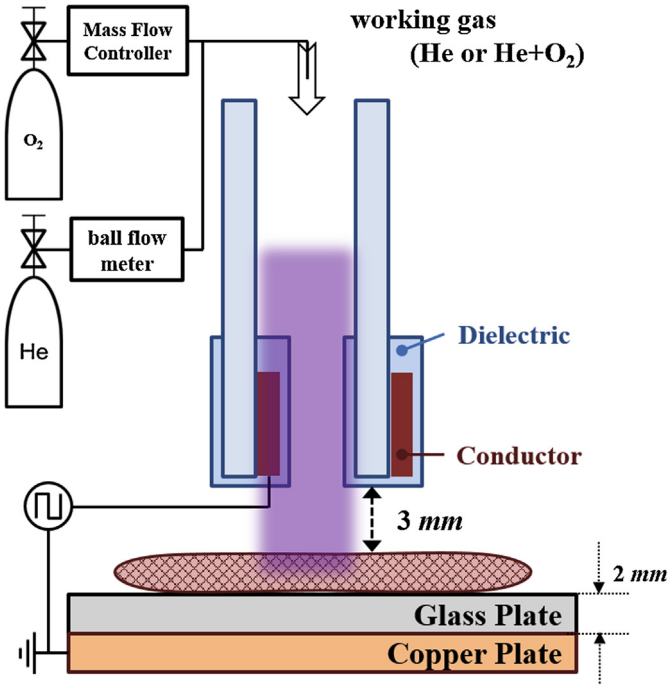

Fig. 1. Schematic diagram of the experimental setup for preparation of dielectric barrier discharge plasma.

2. Materials and methods

2.1. Sample preparation and inoculation

Oval-shaped slices of pork loin measuring approximately 85 × 60 × 3 mm were purchased from a local market in Daejeon, Korea. Two hours prior to inoculation of pathogens, the samples were sterilized by irradiation (35 kGy) in a linear electron beam RF accelerator (2.5 MeV, 40 kW; EB Tech, Daejeon, Korea).

E. coli (KCTC 1682) and *L. monocytogenes* (KCTC 3569) were obtained from the Korean Collection for Type Culture (KCTC, Daejeon, Korea). *E. coli* and *L. monocytogenes* were cultivated in tryptic soy broth and tryptic soy broth containing 0.6% yeast extract, respectively. Sterilized broth was inoculated from agar slant culture, and after 24 h incubation at 37 °C, 0.1 mL of culture was transferred to new broth and cultivated for 18 h. The cultures were centrifuged at 3000 rpm for 15 min at 4 °C in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). Cultures were washed twice with sterile saline water. The pellet was finally suspended in sterile saline water at a cell density of approximately 10^7–10^8 CFU/mL. The test culture suspension (100 μL) was inoculated and spread on the prepared pork loins. To facilitate attachment of the microorganisms to the samples, the samples were incubated for 20 min at 10 °C [20].

2.2. Treatment with DBD plasma and visible emission spectrum

A DBD plasma was generated by a 3 kV, 30 kHz bipolar square wave. In order to produce a linear DBD between samples and the actuator by obtaining a high aspect ratio from the gas outlet, we used acrylic plates (100 × 100 mm) facing with each other with a 0.5 mm gap distance. As indicated in Fig. 1, electrically conducting tapes attached to the near end of the acrylic plates were used as the powered and the floating electrodes. Pure helium gas (99.999%) was introduced between the acrylic plates at 10 slpm (standard liter per minute) and exited the plates uniformly. In addition to helium, 0.3% oxygen was introduced in order to confirm the effects of reactive oxygen species (ROS).

A sample was placed on the ground electrode covered by the glass plate. The gap between the DBD actuator and the top of the sample was maintained at approximately 3 mm during plasma treatments. To facilitate treatment time for large areas, we used an xyz stage with a DBD actuator and scanned the entire sample area. The speed, acceleration, and distance of the scan were 1 mm/s, 5 mm/s^2, and 100 mm, respectively. The pork loin samples inoculated with *E. coli* and *L. monocytogenes* were treated for 5 or 10 min. After DBD treatment, the samples were immediately stored under commercial storage conditions at 4 °C.

The visible emission spectrum of the discharge was obtained through spectrometers (MAYA2000 Pro) with the relevant optical setups.

2.3. Microbial analysis

After DBD plasma treatment, samples were blended with sterile saline using a stomacher (BagMixer 400, Interscience Ind., St. Nom, France). Serial dilutions were prepared with sterile saline solution. Media for the enumeration of *E. coli* and *L. monocytogenes* were tryptic soy agar (Difco Laboratories, Detroit, Michigan, USA) and tryptic soy agar containing 0.6% yeast extract (Difco), respectively. The plates were incubated at 37 °C for 48 h, and the reduction in pathogens was observed.

2.4. pH

After treatment with DBD plasma, the pork loin samples (1 g) were homogenized (1130× *g*, T25 Basic, Ika Co., Staufen, Germany) with 9 mL of distilled water for 30 s, and the pH was measured using a pH meter (Model 750, iSTEC, Seoul, Korea) after calibration using standard buffers from the manufacturer at pH 4, 7, and 10 at room temperature.

2.5. Color measurement

Color of the pork loin sample surface was evaluated using a Color Difference Meter (Spectrophotometer CM-3500d, Konica Minolta Sensing, Inc., Osaka, Japan), and Hunter color values, L^* (lightness), a^* (redness), and b^* (yellowness), were determined. The instrument was calibrated to a standard black and white plate before analysis. The Hunter values were monitored by a computerized system using spectra magic software (Konica Minolta Sensing, Inc.), and the measurements were performed in triplicate.

2.6. Lipid oxidation

Lipid oxidation was determined by calculating 2-thiobarbituric acid reactive substances (TBARS) values. Briefly, each sample (3 g) and 9 mL of distilled water was homogenized (Ika) with 50 μL BHT (7.2%) for 30 s (1130× *g*). The homogenate (1 mL) was transferred to a 15-mL test tube and then mixed with 2 mL thiobarbituric acid (20 mM)/trichloroacetic acid (15%) solution was added. The tubes were then heated for 30 min in a water bath (90 °C), cooled, and centrifuged at 2090× *g* (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer, and lipid oxidation was reported as mg malondialdehyde per kg sample.

2.7. Sensory evaluation

Each panelist that participated in sensory evaluation had at least 1 year of experience in the analysis of meat quality. Pork loins treated with DBD plasma for 10 min in the raw state were cut into 20 × 20 × 3 mm sections, and sensory evaluation was conducted with both raw and cooked loins. The raw loins were provided to the panelists and were tested for appearance, color, odor, and acceptability. In the case of cooked loins, plasma-treated loins were packaged in oxygen permeable polyethylene, followed by cooking in an 80 °C water bath. The cooked loins were served to the panelists with drinking water, which provided to clean the mouth cavity, and panelists tested 2 more sensory parameters, i.e., flavor and texture, in addition to the sensory evaluation of raw loin. In both sensory evaluations, samples were served to each panelist on a white-colored plastic tray with a random 3-digit number. Samples were scored on a 9-point hedonic scale by sensory panelists to assess various meat quality attributes (extremely dislike = 1 to extremely like = 9).

2.8. Statistical analyses

The data were analyzed using SAS software (Release 9.2, SAS Institute, Inc., Cary, NC, USA). Statistical analysis was performed by one-way analysis of variance (ANOVA). When significant differences were detected, the differences among the mean values were determined by Duncan's multiple comparison test at a confidence level of $p < 0.05$. Mean values and standard errors of the mean are reported.

3. Results and discussion

3.1. Visible emission spectrum

Optical emission spectroscopy was conducted by a spectrometer (MAYA2000Pro, Oceanoptics) with a relevant optical setup (Fig. 2). Excited He atomic lines (667 and 706 nm) were observed due to the He feeding gas. Due to the ambient air, the following related nitrogen and oxygen lines were also presented in the emission spectrum: NOγ ($A^2\sum^+ - X^2\prod$), N_2 ($C^3\prod_u - B^3\prod_g$, second positive system), N_2^+ ($B^2\Sigma_u^+ - X^2\Sigma_g^+$, first negative system), OH ($A^2\sum^+ - X^2\prod$), and O I (777, 844, and 926 nm). With the addition of oxygen, the nitrogen emission intensities were reduced. However, the oxygen emission intensity was increased by more than 2-fold. ROS, such as OH, NOγ, and O I, were considered as the effective factor of sterilization. In addition to the radicals mentioned above, we suggest that UV radiation at a wavelength ranging from 200 to 300 nm may also affect sterilization.

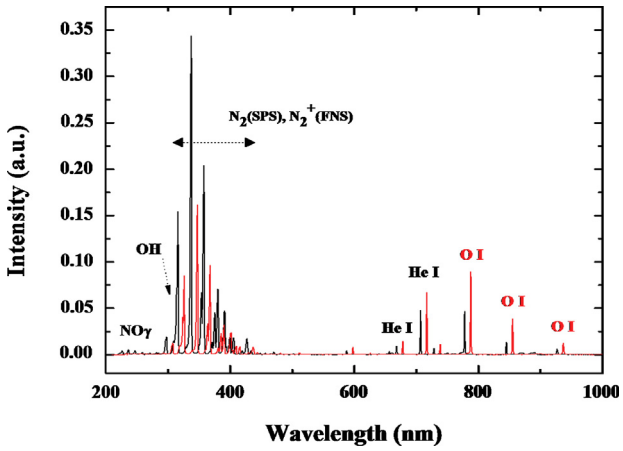

Fig. 2. A typical emission spectrum of the plasma. Helium atomic lines due to helium feeding gas, NO, OH, N_2, N_2+ molecular spectra, and excited oxygen atomic emission lines due to the ambient air are observed. *Black line: He, red line: He + O_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Inactivation of pathogens

E. coli and *L. monocytogenes* were initially loaded at 8.54 and 8.10 log CFU/g when inoculated, respectively (Fig. 3). When pork loin was exposed to DBD plasma with the input gases He and He + O_2, the population of *E. coli* was reduced by 0.26 and 0.50 log cycles at 5 min and by 0.34 and 0.55 log units at 10 min, respectively. That of *L. monocytogenes* was also reduced from 0.17 to 0.35 and 0.43 to 0.59 log cycles when the samples were exposed to DBD for 5 and 10 min using He and He + O_2, respectively. Lee et al. [21] reported that after inoculation, the number of *S. aureus* found on cheese slices ranged from 0.05 to 0.45 log cycles in DBD-treated samples with He and from 0.08 to 0.91 log cycles in DBD-treated samples with He + O_2.

The DBD can create specific types of ROS, such as oxygen atoms, ozone, metastable oxygen molecules, peroxide, superoxide, and hydroxyl radicals, and all of these are bactericidal. These ROS have strong oxidizability and are prone to act with the bacteria cells [6,22]. Ma et al. [23] reported that ROS play a main role in the inactivation of microorganisms treated with DBD plasma.

The results indicate that addition of oxygen improved the efficiency of microbial reduction when compared to He alone. Marsili et al. [24] showed that addition of oxygen yields more radicals based on oxygen and ozone during APP plasma treatment and acts as an inactivation agent. Moreover, Hury et al. [25] reported that oxygen, H_2O_2, and CO_2-based plasmas were more effective than argon plasma. Namely, oxygen-based plasma destroys microorganisms via slow combustion with the oxygen atoms and oxygen-containing radicals present in the plasma. This result suggests that adding oxygen could increase the inactivation rate due to increased production of active radicals [26].

Sun et al. [17] reported that DBD can achieve a more stable discharge at a higher power level and is simpler and more effective

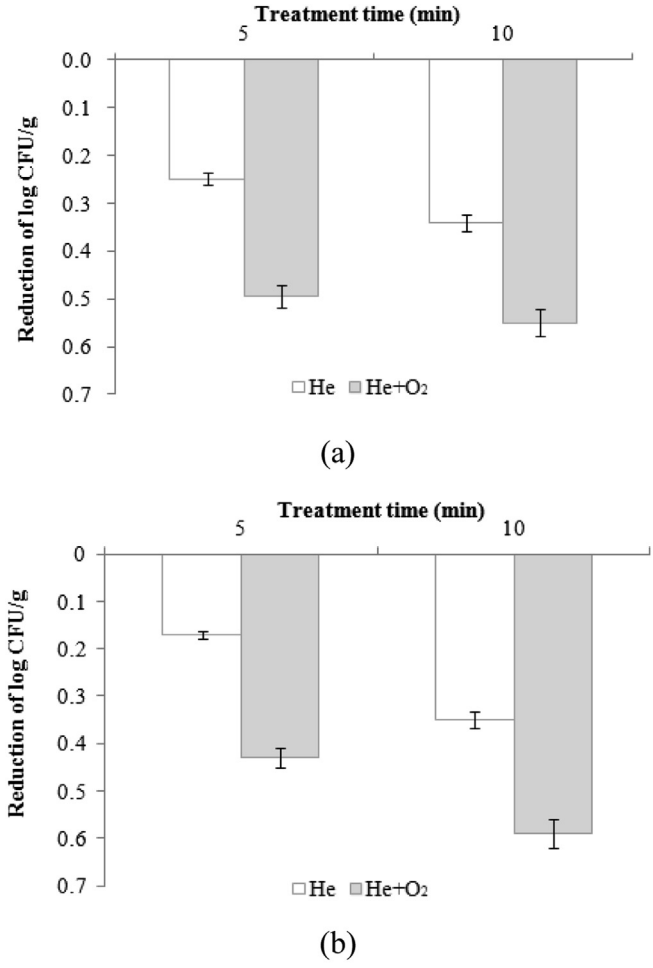

Fig. 3. Inactivation of *Escherichia coli* (a) and *Listeria monocytogenes* (b) inoculated on pork loins after exposure to dielectric barrier discharge plasma using He or He + O_2.

at eliminating pathogens than APP jet and microwave discharge methods. However, Maisch et al. [27] showed that the reduction rates (*E. coli*, *S. aureus*, and MRSA) of a surface micro discharge device on ex-vivo porcine skin were much higher than this DBD device. Several studies have suggested that numerous experimental parameters can influence the inactivation effects of plasma. There are physical parameters (gas composition, flow rate, input power, type of discharge, etc.), microbiological parameters (Gram negative or positive, bacteria or fungi, cell concentration of microorganism, etc.), and sample parameters (type of sample, humidity, etc.). Because of complicated parameters and sample differences, results can be different [28,29].

Therefore, DBD plasma has good potential for inactivation of pathogens on meat products such as pork loins. However, further studies are required to develop an efficient DBD plasma system that can be applied effectively in the food industry.

3.3. pH

pH changes in pork loins treated with DBD plasma are shown in Fig. 4. The pH decreased significantly following treatment with DBD plasma. Cured meats, such as bacon or sausage, have pH values ranging from 4.6 to 5.3 [30], and the pH of pork loins treated with DBD plasma was about 5.3. Fröhling et al. [31] reported that the pH of indirect air plasma-treated pork loin muscle was significantly lower than that of untreated control meat. The significantly larger

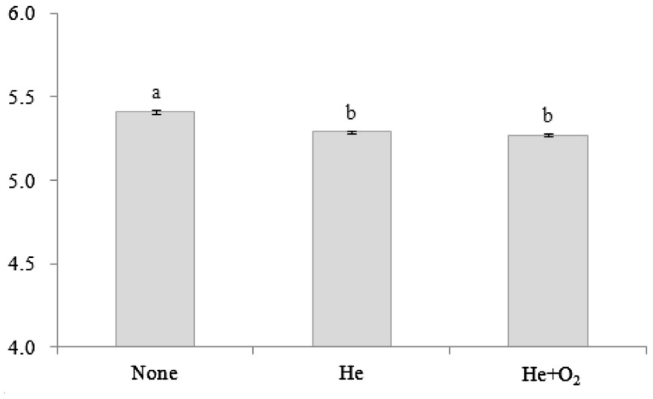

Fig. 4. pH changes in pork loins treated with dielectric barrier discharge plasma.

decrease in pH after indirect plasma treatment may be attributed to acidogenic molecules such as NO_x that are normally generated in air plasma [32]. Korachi et al. [33] indicated that longer treatment resulted in a lower pH because H^+ dissociated from bacterial molecules and H_2O increased during APP treatment. However, the procedure used to treat the sample, the plasma type, and the process gas was different, making these methods and their effects difficult to compare. Thus, additional investigations are required to determine which components are mainly responsible for these effects.

3.4. Color measurement

Meat color is an important factor in meat quality because the coloring of meat is used as an indicator of freshness and quality by the consumer; therefore, the consumer's purchase decision is highly influenced by meat color [34]. Hunter color value changes in pork loins treated with DBD plasma are shown in Table 1. The L^*-values decreased significantly with DBD plasma treatment. Cheng et al. [35] treated various fibrous materials with APP and reported a 6.67%–14.81% increase in the yellow index. Similarly, the L^*-values of bacon were decreased by plasma treatment [19,35] and were consistent with the present results. Moisture content is known to correlate with lightness, such that moisture may explain the

Table 1
Surface color values of pork loins treated with dielectric barrier discharge plasma.

Hunter color	Gas treatment[a]	Storage (day)			SEM[b]
		0	3	7	
L^*	None	46.33ay	48.74ax	48.54ax	0.485
	He	41.95b	44.58b	43.65b	0.804
	He + O_2	44.76a	45.03b	44.55b	0.700
	SEM[c]	0.497	0.601	0.874	
a^*	None	−1.26	−2.16	−2.84	0.450
	He	−1.92x	−2.64xy	−3.23y	0.241
	He + O_2	−1.06	−1.46	−1.68	0.753
	SEM[c]	0.609	0.476	0.481	
b^*	None	9.07	8.42	7.71	1.042
	He	8.00	8.82	7.36	0.423
	He + O_2	9.41	9.47	8.38	0.826
	SEM[c]	0.761	0.886	0.763	

DBD plasma condition: 3 kV, 30 kHz Low Frequency (LF).
[a, b] Different letters within the same column for different times differ significantly ($p < 0.05$).
[x, y] Different letters within the same row for different gas treatments differ significantly ($p < 0.05$).
[a] Gas 10 lpm, O_2 30 sccm.
[b] Standard error of means ($n = 9$).
[c] ($n = 9$).

decrease in L^*-value [19]. During storage, the a^*-value slightly decreased for pork loins treated with DBD plasma, especially that prepared with He. Green coloring of meat can occur if myoglobin reacts with hydrogen peroxide to form choleglobin or if sulfmyoglobin is formed in the presence of hydrogen sulfide and oxygen [31]. Thus, generated hydrogen peroxide can react with myoglobin, causing the plasma-treated meat to appear greener. There was no notable changes in the b^*-value of pork loins treated with DBD plasma. Other studies have also reported no visible changes in color when pork [36] and egg shells [37] were treated with plasma.

3.5. Lipid oxidation

The TBARS value was used to investigate the inhibition of lipid oxidation in non-thermal treated samples. TBARS values of He + O_2-treated samples were greater than those of other samples (Table 2). Gamma or electron beam irradiation, another emerging non-thermal technology, results in accelerated lipid oxidation [38]. This is an indication of oxidation of lipids by the irradiation process through the production of OH radicals formed mainly by water radiolysis [39]. Joshi et al. [40] suggested that ROS were the products of plasma-induced oxidative stress, demonstrating that intact *E. coli* cells and isolated membrane-rich fractions undergo lipid peroxidation in a manner that is proportional to the amount of plasma energy. Oxygen radicals produce lipid oxidation byproducts, such as hexanal and malondialdehyde [41]. Kim et al. [19] reported that the TBARS value of APP-treated bacon was lower than that of untreated bacon at day 0, but then increased after 7 days of storage, resulting in higher TBARS values in APP-treated bacon. Moreover, radicals generated using plasma can accelerate the production of peroxides, which are formed as intermediate products of lipid oxidation, such that the TBARS value in DBD plasma-treated samples may increase during storage.

3.6. Sensory evaluation

Significant reductions in all parameters (appearance, color, odor, and acceptability) of DBD-treated raw pork loins were observed (Table 3). However, no significant sensory differences were found in cooked pork loins treated with DBD plasma. Previous reports have demonstrated that plasma treatment impacts flavor, odor, and overall acceptability of cheese slices [21]. Due to the high fat content in pork loin, increased production of lipid oxidation byproducts may induce off-odors in DBD-treated samples [21]. During plasma treatment, free radicals are produced, and these free radicals consequently trigger lipid and/or protein oxidation. Free radicals, the precursors of lipid hydroperoxides, are regarded as primary oxidation products that further promote the production of secondary oxidation products (alkanes, alkenes, aldehydes, alcohols, ketones, and acids) [42]; these molecules have been sensorially described as “fishy,” “metallic,” “rancid,” and “oxidized” [43]. Basaran et al. [44] demonstrated that sensory evaluations of APP-treated peanuts, hazelnuts, and pistachios were in the range of “like moderately” to “like very much,” although there was no significant difference compared with the control.

Sensorial deterioration of DBD-treated foods is possibly associated with food type. However, to be developed as an effective non-thermal food sterilization method, the sensory deterioration of DBD-treated foods should be considered and prevented. Recently, several studies have demonstrated that the original flavors of selected food flavorings, such as mint, citrus, and barbecue, were not influenced by irradiation treatment, but successfully masked the off-flavor produced from irradiation [45,46]. Furthermore, this method was successfully applied to the production of ice cream and pork jerky without harmful pathogens or spoilage bacteria and off-flavor [45,46]. Application of natural antioxidants in food products, such as α-tocopherol and polyphenolic compounds, can be another possible strategy to reduce lipid and/or protein oxidation.

Table 2
TBARS values (mg malondialdehyde per kg sample) in pork loins treated with dielectric barrier discharge plasma.

Gas treatment[a]	Storage (day)			SEM[b]
	0	3	7	
None	0.31cy	0.33cy	0.39cx	0.005
He	0.35by	0.37by	0.46bx	0.012
He + O_2	0.51ay	0.52ay	0.57ax	0.005
SEM[c]	0.004	0.006	0.012	

DBD plasma condition: 3 kV, 30 kHz LF.
[a, b] Different letters within the same column for different times differ significantly ($p < 0.05$).
[x, y] Different letters within the same row for different gas treatments differ significantly ($p < 0.05$).
[a] Gas 10 lpm, O_2 30 sccm.
[b] Standard error of means ($n = 9$).
[c] ($n = 9$).

Table 3
Sensory evaluation of pork loins treated with dielectric barrier discharge plasma.

Gas treatment[a]	Sensory parameters					
	Appearance	Color	Flavor	Odor	Texture	Acceptability
Raw						
None	6.75a	6.58a	–[b]	6.00a	–[b]	6.42a
He	4.67c	4.50b	–	4.58b	–	4.50b
He + O_2	5.38b	4.88b	–	4.88b	–	4.79b
SEM[c]	0.183	0.212	–	0.209	–	0.173
Cooked						
None	5.58	5.50	5.13	5.38	5.25	5.46
He	5.13	5.21	5.13	5.00	5.63	5.46
He + O_2	5.25	5.13	4.79	4.83	5.38	4.92
SEM[c]	0.168	0.149	0.219	0.218	0.202	0.228

DBD plasma condition: 3 kV, 30 kHz LF.
[a, b] Different letters within the same column for different times differ significantly ($p < 0.05$).
[a] Gas 10 lpm, O_2 30 sccm.
[b] Flavor and texture were not measured for raw pork loins.
[c] Standard error of means ($n = 24$).

4. Conclusion

Our findings suggest that plasma, such as DBD, may be used to improve the microbiological safety of pork loins. However, the present DBD plasma must be developed further with higher pathogen inactivation efficiency and minimum adverse effects on physicochemical and sensorial qualities.

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