EFFECT OF DIELECTRIC BARRIER DISCHARGE PLASMA ON BACTERIA AND SURFACE COLOR OF PORK LION

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Abstract: The aim of this study was to investigate the effect of cold atmospheric pressure plasma on decontamination and surface color of pork lion. Meat samples were treated at 80 kV for 60-180 s inside sealed polypropylene trays containing air, 40% O_2 / 60% N_2 and 60% O_2 / 40% N_2, respectively. Results showed that time and oxygen concentration had significant effects on total aerobic bacteria of pork, which was reduced by 53% for 180 s with 60% O_2. The L^* values of the samples exhibited no obvious changes following with dielectric barrier discharge（DBD） plasma treatment, but a^* values and b^* values changed significantly ($P < 0.05$). These results indicated that plasma could be applicable in surface decontamination of pork lion.

Keywords: Cold Sterilization, Gas Composition, Quality.

I. INTRODUCTION

Meat and meat products are contaminated by bacterial pathogens, which resulted in highly publicized outbreaks of foodborne disease in the world. The World Health Organization (WHO) reported that foodborne pathogens lead to 325,000 hospitalizations and 5000 deaths every year [1]. Various decontamination technologies have been proposed including the use of various chemical agents and physical methods [2]. These processes have disadvantages including high initial cost for facility, required specialized equipment, safety measures, some quality changes of foods, and consumer acceptance [3].

Recently, a new cold sterilization technology was developed for fresh meat by use of plasmas, which are ionized gas. Plasma is a quasineutral state comprised by mixtures of electrons, ions, atomic species, reactive oxygen species and UV, all of which recognized to exert microbial-inactivation effects [4]. The DBD plasma system is regard as the most widely used cold atmospheric pressure plasma (APP) system, which generate plasma by one or two electrodes covered with dielectric layers such as quartz, ceramics, and polymer layers [5]. DBD plasma has been tested in a number of microorganisms, such as bacteria, fungus, virus, which successfully results in the degree of inactivation. Several factors might affect the degree of inactivation, such as the type of microorganisms, number of cells, and operating gas mixture [6]. Dobrynin *et al.* [7] suggested that the lower water content of mediums increased the inactivation of effect of plasma. Kelly-Wintenberg *et al.* [8] concluded that plasma decontamination of a packed product was at least as effect as open exposure, but treatment objects in a sealed container can avoid cross contamination and can be used on a moving conveyer belt, so it's fit for industrial scale online decontamination. The objective of the present study was to investigate the decontamination effect of DBD plasma on pork lion in sealed food package. Moreover, change in color of pork lion was also studied.

II. MATERIALS AND METHODS

Sample preparation

Fresh, raw, pork lion was purchased at a local supermarket in Nanjing province and transported immediately to the laboratory, where they were kept in a refrigerator overnight at 4 °C before use. Before packaging, muscles were cut into 2 cm thickness and associated external subcutaneous fat was trimmed off and mixed, and then randomly packaged in polypropylene trays type TQBC-0775 (oxygen transmission rate: 10 $cm^3/m^2/24$ h at 23 °C, water vapor transmission rate: 15 $g/m^2/24h$ at 38 °C). Trays were flushed with the desired gas mixture using a DT-6D packaging machine (Dajiang Machinery Equipment Co., Ltd., Wenzhou, China) and sealed with oxygen-barrier film (oxygen transmission rate: 25 $cm^3/m^2/24h$ at 23 °C, water vapor transmission rate: 10 $g/m^2/24h$ at 4 °C; Lid 1050; Sealed Air Corp., Danbury, USA). Packaging types: 60 % O_2-MAP (60% O_2 + 20% N_2), 40% O_2-MAP (40% O_2 + 60% N_2), or air. The modified atmospheres were validated by testing sample packages at the beginning of each treatment with a gas analyzer (PBI-Dansensor A/S; CheckPoint O_2/CO_2, Ringsted, Denmark).

Treatment of dielectric barrier discharge plasma

The DBD system used in this study consisted of a high voltage transformer, a voltage variac (0 ~ 100%, output voltage controlled within 0~120 kV). This system was operated at 80 kV of potential between the high voltage circular aluminium electrodes (12 cm diam, 4.5 cm gap) with polypropylene insulation layers: top (2.0 mm) and bottom (3.0 mm). The distance between two polypropylene insulation layers was 40 mm which was equal to the height of the polypropylene trays (310 × 230 × 40 mm) utilized as a sample holder. Treatment of all samples occurred at room temperature. Plasma treatment times (60, 120, & 180 s) were applied for pork lion at 80 kV. After DBD treatment, samples were subsequently stored at room temperature for 24 h. All experiments were performed in triplicate to ensure reproducibility of the experimental data.

Microbiological Analysis

After plasma treatment, samples were blended with sterile saline for 2 min using a stomacher (BagMixer 400, Interscience Ind., St. Nom, France). A series of decimal dilutions was prepared with sterile saline. Each diluent (0.1 mL) was spread in triplicate on each medium. Media used for the enumeration total aerobic bacteria (TAB) was tryptic soy agar (LandBridge Co., Ltd., Beijing, China). The plates were incubated at 37℃ for 48 h, and then the numbers of colonies on the plates were counted.

Surface color

Color of the pork loin sample surface was evaluated using a Color Difference Meter (Spectrophotometer CR-400, Konica Minolta Sensing, Inc., Osaka, Japan), and L^* values, a^* values, and b^* values, were determined. The instrument was calibrated to a standard black and white plate before analysis, and the measurements were performed in triplicate.

Statistical analysis

Statistical analysis was carried out with the statistical analysis system (Version 9.0, SAS Institute, Cary, NC, USA, 2006). Experiments adopted a split-plot design. Color parameters was analyzed by one-way (general linear model procedure) analysis of variance, and the differences between means were detected using student newman keuls (SNK) test at 5% significance ($P < 0.05$) level. The MIXED procedure was used with packaging treatments, treatment time and their interaction as fixed factors and animal as random factor. Mean values and standard error of the means are reported.

III. RESULTS AND DISCUSSION

Packaged pork lion was treated directly with DBD at 80 kV for 60, 120 and 180 s, respectively. Survival curves for TAB was illustrated in Fig. 1. When pork loin was exposed to DBD plasma with the gases air, 40% O_2-MAP and 60% O_2-MAP, the survival rate of TAB was reduced by 15.2%, 26.7% and 32.4% at 60 s and by 26.2%, 39.4% and 45.1% at 120 s, respectively. When treatment time was increased to 180 s, survival rate of total aerobic bacteria showed 42.5%, 49% and 53.2% reduction with gases air, 40% O_2-MAP and 60% O_2-MAP, respectively. Kim *et al.*[9] reported that after inoculation, the number of *L. monocytogenes* found on pork lion ranged from 0.26 to 0.5 log cycles in DBD-treated samples with He and He + O_2, but only 0.08 and 0.05 log cycles were reduced when treatment time changed from 5 min to 10 min with He and He + O_2. Korachi *et al.* [10] reported that the reduction effect of atmospheric corona discharge plasma on *E. coli* in water was increased with longer treatment time. Several studies have suggested that sample parameters can influence the inactivation effects of plasma. The interactions between plasma and samples can be influenced by various factors including surface roughness, adsorption of diffusing plasma, and moisture [11], and some or all of these factors may affect the survival of pathogens.

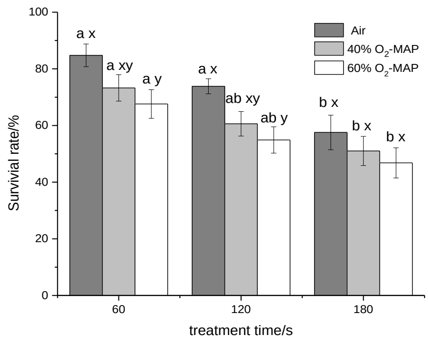

Fig. 1. Survival rate of TAB on pork lion. The black bars showed the standard deviation. Columns with different letters (a, b) differ significantly ($P < 0.05$) as a result of different treatment time using the same power. Columns with different letters (x, y) differ significantly ($P < 0.05$) as a result of different gas.

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

Color	PM [a]	Treatment Time (second) [b]	*P* value

value		60	120	180	PM	TT [c]	MAP×TT
L^*	air	50.26±0.37	52.22±0.24 [x]	50.64±1.33			
	40%	50.81±0.24	50.80±0.41 [xy]	50.30±0.38	0.389	0.676	0.497
	60%	51.20±1.30	50.23±0.59 [y]	50.70±0.17			
a^*	air	7.80±0.21 [dz]	5.35±0.34 [ey]	3.66±0.12 [fy]			
	40%	8.38±0.17 [dy]	8.16±0.12 [ey]	6.73±0.03 [fy]	$P < 0.05$	$P < 0.05$	$P < 0.05$
	60%	9.63±0.08 [dx]	9.27±0.28 [ex]	8.55±0.08 [fx]			
b^*	air	8.04±0.49 [ey]	8.23±0.12 [ez]	9.35±0.15 [dz]			
	40%	8.37±0.21 [fy]	10.19±0.05 [ey]	13.43±0.04 [dy]	$P < 0.05$	$P < 0.05$	$P < 0.05$
	60%	10.87±0.14 [fx]	13.51±0.10 [ex]	15.38±0.53 [dx]			

[a] PM= packaging methods:40%: 40% O_2 + 60% N_2; 60%: 60% O_2 + 40% N_2.
[b] Results (n=3) are expressed as the mean ± standard error.
[c] TT=treatment time.
[d, e, f] Different letters within the same row for different times differ significantly ($P < 0.05$).
[x, y, z] Different letters within the same column for different gas differ significantly ($P < 0.05$).

Treatment time of 10 s gave > 3 log reductions of *L. innocua* on membrane filters, an 8 min treatment gave 1 log reduction on chicken skin, and a 4 min treatment gave > 3 log reduction on chicken muscle [12]. These results showed that the efficacy of gas plasma treatment was greatly affected by surface topography of mediums, effect of bacteria migrate could be responsible for this. Bacteria which attached on irregular surface migrated depths up to about 140 μm through capillary action [9]. Microorganisms at these distances from the surface have been reported to be largely unaffected by sterilization treatment. And also fissures result of radial shrinkage of muscle fibres could provide a route for bacterial penetration [12]. All those factors would affect sterilization efficiency of DBD.

Meat color is an important quality parameter of meat products because it is used as an indicator of freshness and quality by the consumer [5]. The L^* values, a^* values, and b^* values of the pork lion treated with plasma were observed in Table 1. No significant differences were found on L^* values between any of the plasma treated samples according to treatment time. L^* values of pork lion were decreased to 50.23 at higher oxygen concentration with the treatment time of 120 s. No significant difference was detected in L^* values on bresaola surface after DBD treatment [13]. Kim found that L^* values of the bacon surface treated with APP were decreased at a higher input power and exposure time [14]. Kim *et al.* [15] reported the L^* values of pork lion decreased significantly with DBD plasma treatment under various gas. However, this reduction was only detected when the plasma was produced using helium gas instead of a mixture of helium and oxygen. The L^* values of pork and beef were not significantly different from those of the untreated samples [16]. Sanabria *et al.* [17] found a high correlation coefficient between L^* values and moisture content, such that moisture may explain the decrease in L^* values. In this article, we found a^* values is susceptible to oxygen concentration and treatment time. More oxygen and less treatment time will lead to positive effects. Under high oxygen concentration, production of oxymyoglobin and deeper oxygen penetration can explain stabilized the a^* values. Zakrys et al. [18] reported that beef with O250 % maintained high a* values for up to 3 days of retail display. Previous findings by Jayasingh et al. [19] reported that ground beef, packed in high oxygen MAP maintained a bright red colour for 10 days. Jakobsen *et al.* [20] reported the stability of a^* values stored in HiOx (80% O_2/20% CO_2) in beef steaks. Moreover, Attri *et al.* [21] demonstrated that hemoglobin structural changed in presence of RS generated using DBD plasma in the presence of various gases. We proposed that DBD can also induce change of structure of myohemoglobin which can influence a^* values of meat. For b^* values, it's significantly increased with oxygen concentration and treatment time. Similarly, the b^* values of bacon did not change when helium was used, but it's increased at a higher input power using helium/oxygen [16].

IV. CONCLUSION

In the study, DBD effectively enhance microbial safety of raw pork lion. However, the present DBD plasma may be developed further with higher pathogen inactivation efficiency and safety of foods for consumption.

ACKNOWLEDGEMENTS

This project was supported by the Agricultural Science and Technology Independent Innovation of Suzhou, China (Grant No. CX(15)1049)

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