EFFECT OF DIELECTRIC BARRIER DISCHARGE PLASMA ON THE DISCOLORATION OF MYOGLOBIN

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Abstract – The aim of this study was to elucidate the effect of dielectric barrier discharge (DBD) plasma on the discoloration of myoglobin. When myoglobin dissolved in distilled water was exposed to DBD plasma, protein structure of myoglobin and its molecular weight were not changed but it appeared green color. With the increasing plasma treatment time, total color difference was also increased in myoglobin, hemin, protoporphyrin IX in potassium phosphate buffer (PPB), respectively. DBD plasma treatment also resulted in disruption of heme group in myoglobin solution and the decrease of absorbance intensity in hemin and protoporphyrin IX solution. It is suggested that green-colored myoglobin after plasma treatment may be within one of the pathways in disruption of the heme pigments including protoporphyrin IX.

Key Words – Color, Cold plasma, Heme protein

I. INTRODUCTION

Food safety is a large concern for government authorities, industry as well as consumers. In recent years, substantial efforts have been made to develop plasma-based sterilization methods. When a gas is given enough energy, the gas molecules are dissociated to form an ionized gas called plasma. Plasma consists of different antimicrobial substances, including reactive oxygen species (ROS), reactive nitrogen species (RNS), ions, electrons, and ultraviolet photons [1].

Previous studies applied the various plasma systems on raw meats and evaluate the bactericidal effect and the meat quality changes. Consequently, it was demonstrated that minor deterioration of meat quality, especially color, had observed after plasma treatment. For example, a^* values (redness/greenness) of each fresh and frozen pork were significantly decreased after treatment of the dielectric barrier discharge (DBD) plasma and corona discharge plasma, respectively [2, 3]. With the flexible thin-layer DBD plasma treatment, a^* value was lowered but b^* value (yellowness/ blueness) was increased in beef loin, which means that the surface color becomes greener [4]. Likewise, Fröhling et al. [5] reported the green coloring effect on fresh pork by indirect plasma treatment.

Discolored meats are considered as inferior in quality or contaminated, thereby nearly 15% of retail beef is discounted in price due to discoloration, which corresponds to annual revenue losses of one billion dollars [6]. Despite the importance of meat color, however, there were no studies that identify the discoloration mechanism of raw meat with plasma treatment.

The most responsible inherent factor for raw meat color is myoglobin, which contains a globin protein attached to a porphyrin ring containing a heme iron. Concentration of myoglobin and their chemical states can determine the meat color [6]. Therefore, the objective of the present study was to investigate the effects of encapsulated DBD plasma on myoglobin and elucidate the discoloration mechanism using myoglobin, hemin, and photoporphytin IX.

II. MATERIALS AND METHODS

Sample preparation

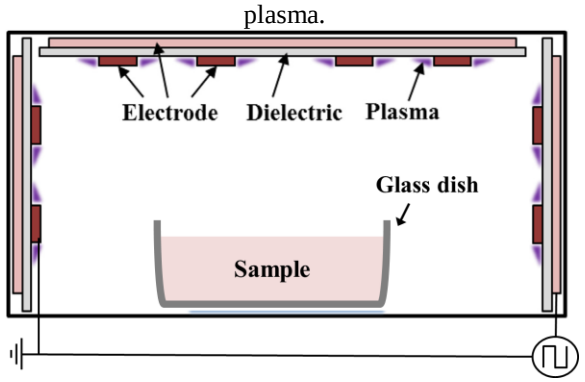
Horse skeletal muscle myoglobin, hemin, and protoporphyrin IX were purchased from the Sigma Chemical Co. (St. Louis, USA) and used without further purification. Myoglobin (60 mM) was dissolved in distilled water (DW) and potassium phosphate buffer (PPB) at pH 6.8,

respectively. Each hemin and protoporphyrin IX (0.08 mM) was dissolved in PPB.

Treatment of encapsulated DBD plasma

An encapsulated DBD plasma source was fabricated using a rectangular, parallelepiped plastic container (137 × 104 × 53 mm) (Fig. 1). The plasma was generated as a base for material treatment [1]. Ambient air was used as the carrier gas. Each sample (20 mL) was placed in a glass dish at the bottom of the container. Then, it was treated with the DBD plasma source for 0, 5, 10, and 20 min.

Figure 1. Schematic diagram of the experimental system for the generation of encapsulated DBD plasma.



Color value

L^*, a^*, and b^* values were determined with a spectrophotometer (CM-5, Minolta Censing Inc., Osaka, Japan). Then, the total color difference ($\Delta E=\sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$) was calculated.

pH

The pH value were measured using a pH meter (SevenGo, Mettler-Toledo International Inc., Schwerzenbach, Switzerland)

UV absorbance spectrum

UV-visible spectra of samples were obtained using a UV-visible spectrophotometer (X-ma 3100, Human Co. Ltd., Seoul, Korea).

Statistical analysis

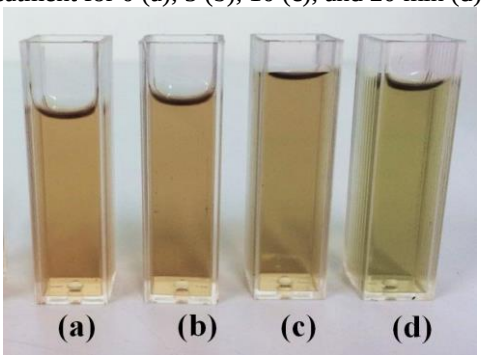
Statistical analysis was performed by one-way analysis of variance (ANOVA), and significant differences between mean values were identified using the Tukey's multiple comparison test in SAS software (SAS Institute Inc., Cary, NC, USA) at a significance level of $P<0.05$.

III. RESULTS AND DISCUSSION

Color and pH

When myoglobin in DW was exposed to DBD plasma, secondary and tertiary structure contents, as well as the molecular weight size of myoglobin were not changed (Data not shown). Despite of these molecular properties, however, L^*, a^*, and b^* values were significantly decreased and it appears green color in the same myoglobin solution (Fig. 2, Table 1).

Figure 2. Myoglobin in DW with DBD plasma treatment for 0 (a), 5 (b), 10 (c), and 20 min (d).



Compared to myoglobin in DW, myoglobin in PPB shows a less change in total color difference and pH with plasma treatment. These results suggest that pH might be affected myoglobin color in a certain degree. According to Renerre [7], myoglobin undergoes denaturation at < pH 5, and a low pH also reduces the stability constant for the heme–globin linkage, thus, porphyrin being released from globin at < pH 5.

Green coloring of meat can also occur with irradiation if sulfmyoglobin is formed in the presence of hydrogen sulfide and oxygen [5]. Motohashi et al. [8] suggested that aqueous electrons produced by radiolysis of water may promote sulfur release from sulfer-containing amino acid in globin protein. However, Table 1 presents that hemin (protoporphyrin IX and Fe III complex) also discolored after DBD plasma treatment without any globin protein or amino acids.

Protoporphyrin IX, a part of myoglobin, was also exposed to DBD plasma in odor to investigate the myoglobin discoloration more closely. As a

results, L^* value was increased but a^* and b^* values were decreased with increasing plasma exposure time (Table 1).

Table 1. Effect of DBD plasma on color and pH value of myoglobin, hemin, and protoporphyrin IX.

Properties	Plasma treatment time (min)				SEM[1)]
	0	5	10	20	
Myoglobin in DW					
L^*	62.87[a]	60.32[b]	58.41[c]	57.93[d]	0.015
a^*	18.31[a]	15.83[b]	9.69[c]	0.21[d]	0.015
b^*	42.40[a]	38.86[c]	36.33[d]	39.44[b]	0.016
ΔE	0.00[d]	5.03[c]	11.46[b]	19.00[a]	0.019
pH	7.43[a]	5.50[b]	4.21[c]	3.39[d]	0.074
Myoglobin in PPB					
L^*	79.92[a]	79.60[a]	79.10[b]	78.48[c]	0.091
a^*	9.50[a]	7.87[b]	6.58[b]	4.86[c]	0.343
b^*	30.41[a]	29.83[b]	29.77[b]	29.58[c]	0.034
ΔE	0.00[c]	1.76[b]	3.10[b]	4.94[a]	0.332
pH	6.86[a]	6.85[a]	6.82[ab]	6.79[b]	0.010
Hemin in PPB					
L^*	68.62[d]	69.60[c]	69.96[b]	70.85[a]	0.014
a^*	9.66[a]	8.89[b]	8.55[c]	7.64[d]	0.016
b^*	48.06[a]	47.13[b]	46.68[c]	45.63[d]	0.012
ΔE	0.00[d]	1.55[c]	2.22[b]	3.87[a]	0.019
pH	6.86[a]	6.85[a]	6.83[ab]	6.80[b]	0.009
Protoporphyrin IX in PPB					
L^*	92.32[d]	93.47[c]	93.78[b]	93.92[a]	0.006
a^*	0.80[a]	0.04[b]	-0.03[c]	-0.10[d]	0.003
b^*	22.58[a]	19.45[b]	18.77[c]	18.55[c]	0.072
ΔE	0.00c	3.41b	4.16a	4.42a	0.066
pH	6.86[a]	6.85[a]	6.83[ab]	6.80[b]	0.009

[1)]Standard error of the mean (n=12).
[a-d]Values with different letters within the same row differ significantly (P <0.05).

UV-absorbance spectrum

UV-absorption spectra of the myoglobin showed a typical spectrum of the protein containing heme group which has a maximum absorbance around 409 nm. However, DBD plasma treatment resulted in a decrease of the absorbance around 409 nm, which means that the heme group is disrupted (Fig. 3).

Formation of green pigments has been also noted in irradiated meat and in myoglobin solutions. Sulfmyoglobin exhibits strong absorption in the red region of the spectrum (616 nm) and is visually green [9]. When H_2O_2 is present, the porphyrin ring can oxidize producing cholemyoglobin which has an absorption maximum at 628 nm and is also visually green [10]. However, Figure 3 showed that absorption at around 505 nm (blue region of spectrum) was decreased and that at around 585 nm were increased in myoglobin solutions with increasing plasma treatment time.

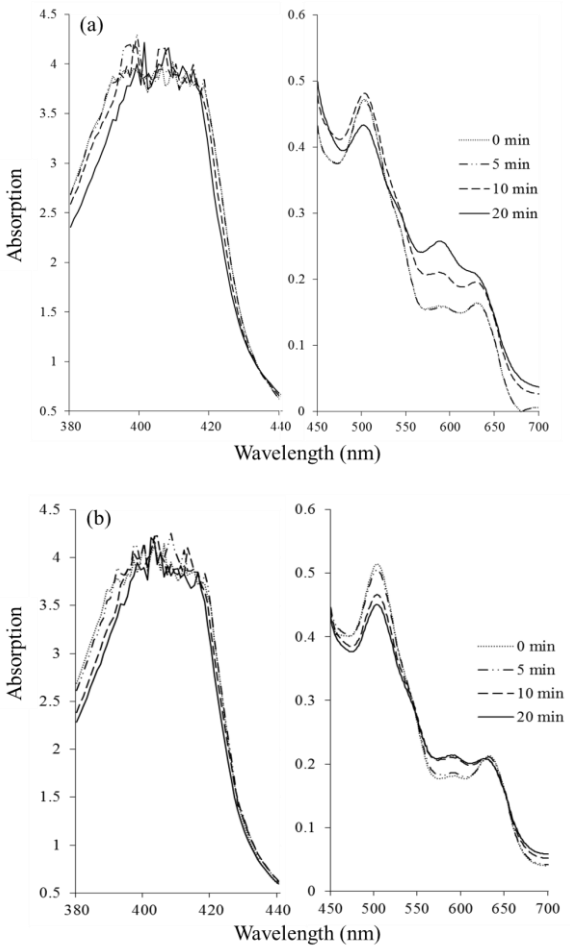


Figure 3. UV-absorption spectra of myoglobin in DW (a), PPB (b) with DBD plasma treatment for 0, 5, 10, and 20 min

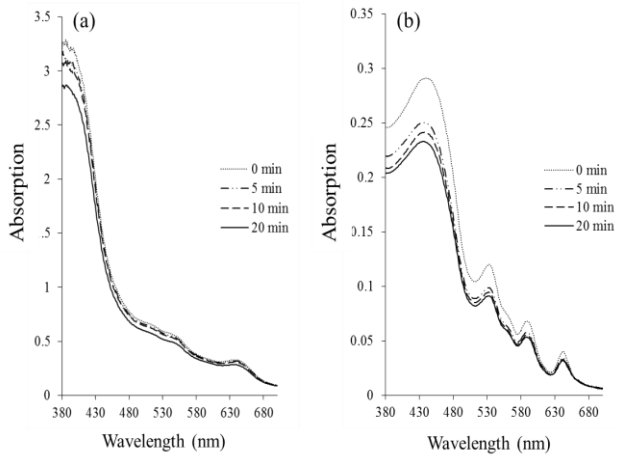


Figure 4. UV-absorption spectra of hemin in PPB (a), protoporphyrin IX in PPB (b) with DBD plasma treatment for 0, 5, 10, and 20 min

When plasma was exposed to hemin and protoporphyrin IX, the intensity of absorbance decreased without extensive modification of the shape of spectra (Fig. 4). The absorption spectrum changes with plasma treatment are more distinct in protoporphyrin IX compared to hemin. Brewer [11] reported that generation of green pigments appears to be due to breakdown of the porphyrin integrity.

IV. CONCLUSION

This study indicates that the color and chemical properties of myoglobin could be altered by DBD plasma treatment. The experiments with myoglobin, hemin, and protoporphyrin IX solutions individually may help to discriminate various discoloration mechanisms of myoglobin in meat. Further studies are needed to find a solution for meat color stabilization by plasma treatment,

ACKNOWLEDGEMENTS

This work was supported by National Research Foundation Grant funded by the Korean Government (NRF-2015H1A2A1034708).

REFERENCES

1. Yong, H. I., Kim, H. J., Park, S., Alahakoon, A. U., Kim, K., Choe, W., & Jo, C. (2015). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma. Food Microbiology 46: 46-50.
2. Kim, H. J., Yong, H. I., Park, S., Choe, W., & Jo, C. (2013). Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin. Current Applied Physics 13:1420-1425.
3. Choi, S., Puligundla, P., & Mok, C. (2015) Corona discharge plasma jet for inactivation of *Escherichia coli* O157: H7 and *Listeria monocytogenes* on inoculated pork and its impact on meat quality attributes. Annals of Microbiology 1-10.
4. Jayasena, D. D., Kim, H. J., Yong, H. I., Park, S., Kim, K., Choe, W., & Jo, C. (2015). Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. Food Microbiology 46:51-57.
5. Fröhling, A., Durek, J., Schnabel, U., Ehlbeck, J., Bolling, J., & Schlüter, O. (2012). Indirect plasma treatment of fresh pork: Decontamination efficiency and effects on quality attributes. Innovative Food Science & Emerging Technologies 16: 381-390.
6. Nam, K. C., & Ahn, D. U. (2003). Effects of irradiation on meat color. Food Science and Biotechnology 12: 198-205.
7. Renerre, M. (1990). Review: Factors involved in the discoloration of beef meat. International Journal of Food Science and Technology 25:613-630.
8. Motohashi, N., Mori, I., Sugiura, Y., & Tanaka, H. (1981). Modification of γ-irradiation-induced change in myoglobin by α-mercapto-propionylglycine and its related compounds and the formation of sulfmyoglobin. Radiation Research 86:479-487.
9. Fox, J. B., Strehler, T., Bernofsky, C., & Schweigert, B. S. L. (1958). Production and identification of a green pigment formed during irradiation of meat extracts. Journal of Agricultural and Food Chemistry 6:693-696.
10. DeMan, J. M. (1999). Color. In Principles of food chemistry (pp. 239-242). Gaithersburg, MD: Aspen Publishers, Inc.
11. Brewer, S. (2004). Irradiation effects on meat color–a review. Meat Science 68: 1-17.