A Dissertation for the Degree of Master

Effects of dielectric barrier discharge plasma on physico-chemical and microbiological properties of sliced cheese and functional property of egg white protein

슬라이스 치즈의 이화학적 및 미생물학적 특성과 난백 단백질의 기능적 특성에 대한 유전체 장벽 방전 플라즈마의 영향

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Contents

Chapter III.

Influence of encapsulated dielectric barrier discharge plasma on structure and functional properties of egg white protein

List of Tables

Chapter II.

Chapter III.

List of Figures

Chapter II.

Chapter III.

List of Abbreviations

$^1O_2$	Singlet oxygen
a^*	Redness
b^*	Yellowness
CAP	Cold atmospheric plasma
DBD	Dielectric barrier discharge
EDBD	Encapsulated dielectric barrier discharge
EWS	Egg white solution
FXDBD	Flexible dielectric barrier discharge
hr	Hour
min	Minutes
NO	Nitrogen monoxide
NO_2	Nitrogen dioxide
NOx	Nitrogen oxides
O_3	Ozone
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RONS	Reactive oxygen and nitrogen species
SH	Free sulfhydryl
TBARS	2-Thiobarbituric acid reactive substances
UV	Ultraviolet

Chapter I.

General Introduction

Food safety is a major issue of public concern (Yeung & Morris, 2001). Therefore, heat treatment techniques such as autoclaving, microwave, radio frequency, and ohmic heating have been traditionally used for microbial sterilization. However, this technique causes unwanted changes in the sensory properties of the food or lowers the nutritional value of the food (Stocia et al., 2013). Thus, various non-thermal technologies such as plasma, ultrasound, irradiation, ultra high-pressure, pulsed light, and pulsed electric field have been attracted as novel technologies for microbial inactivation and minimal quality change of food (Osae et al., 2020).

Non-thermal plasma is a new discipline in food processing. Plasma is an ionized gas that occupies more than 99% of the universe as the fourth state of matter, that can be generated by the interaction between a gas and an electromagnetic field (Nehra et al., 2008). It is comprised of highly reactive species including electrons, positive and negative ions, free radicals, gas atoms, molecules in the ground or excited state, and quanta of electromagnetic radiation photons (ultraviolet) (Kim et al., 2013).

Plasma can be distinguished into high-temperature (thermal) and low-temperature (non-thermal) plasma depending on the type of supply and amount of energy transferred to the plasma including electron temperature and density (Nehra et al., 2008). In high-temperature plasma, molecules are completely dissociated and become an atomic state, and all electrons confined to the atom depart from the

electron orbit to free electrons and become a gaseous state consisting of ions and electrons. In other words, high temperature plasma implies that electrons, ions, and neutral species are in a thermal equilibrium state. The limitations of thermal plasma include the energy efficiency, the high quenching effect of chemical reactions, and the operating at temperatures much higher than ambient temperature, which can impact their applicability to food products (Moreau et al., 2008). Whereas, low-temperature (cold) plasma is produced highly reactive species (radicals) such as incompletely dissociated molecules and ionized ionic molecules, or ionic atoms (Yoo, 2015). Therefore, low-temperature plasma operates under atmospheric pressure and therefore requires less power to generate active plasma (Conrads & Schmidt, 2000). Plasma has been mainly used in the industrial field, but in recent years, the application of cold atmospheric plasma (CAP) or cold plasma attracts attention in the food industry due to cost-effectiveness and flexibility in practice including economic and technical efficiency (Oh et al., 2011; Ekezie et al., 2017). CAP can avoid nutrient destruction and quality change in food (Mandal et al., 2018). To be specific, CAP has lots of advantageous features: (i) bactericidal and virucidal effects; (ii) inexpensive facilities and operation costs; (iii) ease of use; and (ix) high concentrations of energetic particles such as reactive oxygen species (ROS), reactive nitrogen species (RNS), other reactive species, electrons, ions, and UV photons (Heuer et al., 2015; Jayasena et al., 2015).

The types of CAP are as follows: corona discharge, dielectric barrier discharge (DBD), atmospheric pressure plasma jets (APPJ), radio-frequency plasma, and microwave discharge (Fridman et al., 2005). Especially, DBD plasma is the most

preferred and widely employed CAP source (Tolouie et al., 2017), because it has no electric shock and can handle a large area at once (Yu et al., 2013, Liao et al., 2017). DBD plasma is generated by one or both electrodes covered with dielectric layers (Brandenburg, 2017). The advantages of DBD are effective in eliminating pathogens, as well as simplicity, the availability of efficient and affordable power supplies (Kim et al., 2013). DBD discharges are mainly achieved at high voltages (1–100 kV) and frequencies between 0.05 and 500 kHz, to ignite the gas discharge without the formation of sparks (Tolouie et al., 2018).

DBD plasma generated reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as O_3, $^1O_2$, ·OH, NO·, and ·NO_2, and the food industry is paying attention as an antibacterial and sterilization method (Niemira, 2012). ROS and RNS (RONS) play an important role in microbial inactivation by damaging chemical components such as proteins, lipids, and nucleic acids (Mandal et al., 2018). In fact, several studies used DBD plasma for microbial inactivation in meat products (Lee et al., 2017). Recently, various types of DBD plasma are required for wider applications according to various types of food. Therefore, a flexible DBD plasma was developed, and their microbial inactivation efficacy of flexible DBD plasma was verified in pork butt and beef loin (Jayasena et al., 2015), sliced cheese (Yong et al., 2015), chicken breast (Lee et al., 2016a), and beef jerky (Yong et al., 2017). However, further research and development are required for flexible DBD plasmas for low prices and convenient production for the industry.

The reactive species generated by CAP can affect not only microbial inactivation but also the physico-chemical properties of food due to oxidation stress

(Tolouie et al., 2018). Previous researches have been conducted on the effect of changes in the protein structure of the food (Segat et al., 2015; Ji et al., 2015; Sharifian et al., 2019).

The aims of this study were: i) to investigate the effect of the newly developed inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma in consideration of an industrial application on microbial inactivation ability and quality change on sliced cheddar cheese; ii) to determine the effect of encapsulated dielectric barrier discharge (EDBD) plasma on structure and functional properties, i.e. emulsion and foam stability, and foaming capacity of the egg white proteins.

Chapter II.

Effect of inkjet-printed flexible dielectric barrier discharge plasma on inactivation of pathogen and quality changes on sliced cheddar cheese

2.1. Abstract

The objective of this study was to investigate microbial inactivation and quality changes in sliced cheddar cheese treated with inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma. Sliced cheddar cheeses were treated with FXDBD plasma for 0, 5, 10, 15, and 20 min. After 20 min of plasma treatment, the surviving populations of *Escherichia coli* O157:H7 and *Listeria monocytogenes* were significantly reduced by 2.65 and 2.03 log CFU/g, respectively. In inkjet-printed FXDBD plasma, the reactive species that mainly act in microbial inactivation were O_3 and NOx. The pH of the plasma-treated sample was significantly lower than that in the control group, whereas there was no significant difference among treatment times. With increasing plasma treatment time, lightness (L^*) values decreased, while yellowness (b^*), total color difference (ΔE), and 2-thiobarbituric acid reactive

substances (TBARS) values increased ($P < 0.05$). Meanwhile, the redness (a^*) value remained unchanged. These results indicate that inkjet-printed FXDBD plasma treatment can effectively inactivate pathogens; however, it causes an increase in lipid oxidation on sliced cheddar cheese. In conclusion, the newly developed inkjet-printed FXDBD plasma may be applicable because it could be possible to improve microbiological safety with slight quality changes.

Keywords: Inkjet-printed flexible dielectric barrier discharge plasma, Sliced cheese, Pathogen inactivation, Quality change

Student Number: 2019-20390

2.2. Introduction

Consumer concerns about food safety continue from the past to the present, and changes in dietary patterns have led to increased consumption of ready-to-eat foods. Ready-to-eat (RTE) food products can be consumed immediately without additional cooking; therefore, more attention should be paid to pathogen contamination (Huang et al., 2020b). Cheeses have become a major consumer product in the dairy industry. *Escherichia coli* O157:H7 and *Listeria monocytogenes* are important pathogens that are addressed in the dairy industry (Park & Ha, 2019). In particular, as RTE food, sliced cheese can be contaminated by pathogens after cutting, slicing, packaging, and during transportation (Park & Ha, 2020).

Previously, traditional thermal technologies such as heating, boiling, steam, and autoclaving were used to remove pathogens (Huang et al., 2020b). Although thermal technologies are effective in sterilizing microorganisms, they can change the sensory, nutritional, and functional characteristics of food (Awuah et al., 2007). Thus, various non-thermal technologies have been studied for microbial inactivation and minimal quality change in food. Non-thermal technologies include ultrasound, irradiation, ultrahigh-pressure, pulsed light, and pulsed electric field (Osae et al., 2020). These techniques are effective in inactivating microorganisms in food. However, they also have disadvantages such as high equipment costs, residual substances, and consumer perception (Yun et al., 2010).

Recently, plasma has been attracting attention as a non-thermal technology in the food industry. Plasma is the fourth state of matter, generated by the interaction between a gas and an electromagnetic field, comprises reactive species, positive and

negative ions, and UV photons. Microorganisms can be inactivated by various free radicals that are formed by plasma (Yoo et al., 2020). Recently, cold atmospheric plasma (CAP), such as corona discharge, microwave discharge, atmospheric pressure plasma jets (APPJ), and dielectric barrier discharge (DBD), have attracted interest as non-thermal processes in the food industry. Among various discharge types, DBD plasma is the most widely used cold atmosphere plasma source (Jayasena et al., 2015). Not only can it be handled in a large area and is composed of a relatively simple structure, but it also has the availability of efficient and affordable power supplies (Kim et al., 2013).

With the increase in the necessity of plasma technology in the food industry, the demand for flexible DBD plasma is increasing rapidly because of the variety of food shapes and packaging forms (Kim et al., 2018). Additionally, to prevent secondary contamination in food, many studies on the flexible plasma in the form of in-packaging were conducted (Jayasena et al., 2015; Yong et al., 2015; Lee et al., 2016a). However, previous studied flexible plasma requires a high cost to produce a disposable plasma. Therefore, low-cost and simple production technology for flexible plasma is critical towards developing plasma applications.

Flexible dielectric barrier discharge (FXDBD) is produced by inkjet printing using conductive ink as the printing material. This technology offers the advantage of being time efficient for mass production and easy application anywhere (Kim et al., 2018). Therefore, the objective of this study was to investigate the pathogen inactivation and quality changes in sliced cheddar cheeses after inkjet-printed FXDBD treatment.

2.3. Materials and methods

2.3.1. Bacterial strain and culture condition.

The Gram-negative bacteria *Escherichia coli* O157:H7 (NCCP 15739) and Gram-positive bacteria *Listeria monocytogenes* (ATCC 19111) were provided by the National Culture Collection for Pathogen (Osong, Korea) and the Korean Culture Center of Microorganisms (Seoul, Korea), respectively. *E. coli* O157:H7 was cultivated in fresh sterile tryptic soy broth (TSB; Difco, Becton Dickinson Co., Sparks, MD, USA) and *L. monocytogenes* was cultivated in fresh sterile tryptic soy broth TSB (TSB; Difco, Becton Dickinson Co., Sparks, MD, USA) containing 0.6% yeast extract (Difco), respectively, at 37 °C for 24 h. The cultures were washed twice with sterile 0.85% saline solution by centrifugation at 2,265 ×*g* for 15 min at 4 °C using a refrigerated centrifuge (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). The pellets were then resuspended in sterile 0.85% saline solution at a final concentration of 10^8 to 10^9 colony forming units (CFU)/mL.

2.3.2. Sample preparation and inoculation

Sliced cheddar cheeses (Seoul Milk Co. Ltd., Seoul, Korea) were purchased from a local market and immediately stored at 4 °C. They were cut into 40 × 40 × 2 mm pieces and sanitized with an ultraviolet light on a laminar flow clean bench for 30 min. A 50 μL aliquot of each bacterial suspension (10^8 to 10^9 CFU/mL) was placed at nine different points onto the slice of cheddar cheese, and spread with a sterile spreader for an even distribution and attachment. The inoculated cheeses were dried

for 30 min on a clean bench.

2.3.3. Inkjet-printed flexible dielectric barrier discharge plasma

Inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma used polyethylene terephthalate (PET) films (NB-TP-3GU100, Mitsubishi Paper Mill, Tokyo, Japan) with a thickness of 100 μm as a barrier and silver nanoparticle ink (NBSIJ-MU01, Mitsubishi Paper Mill) to produce the patterned electrodes. The designed width of the electrode was 400 μm. The inkjet-printed FXDBD printed by a printer (DCP-T300, brother, Daegu, Korea), which has a piezoelectric-type print nozzle and a refillable cartridge.

2.3.4. Treatment with inkjet-printed FXDBD plasma and microbial analysis

A food-package system designed for generating inkjet-printed FXDBD plasma within the package was prepared using low-density polyethylene (LDPE) zippered bags (180 × 200 × 450 mm). The electrode-printed and electrode-attached flexible dielectric barrier was installed inside the package, and the distance between the electrodes and cheese sample was kept 450 mm using a support fixture that was applied to maintain a constant. (Figure 1A, B). In addition, the cheese sample was placed in a sterilized petri dish and placed at the bottom and the center of the package. The package was sealed immediately and the plasma was generated at a frequency of 3 kHz and voltage of 2.170 kV with atmospheric air. Sliced cheese samples inoculated with *E. coli* O157:H7 and *L. monocytogenes* were treated for 0, 5, 10, 15, and 20 min. Immediately after treatment, each cheese sample (5 g) was blended with

45 mL of sterile 0.85% saline solution using a stomacher (BagMixer 400, Interscience, St Nom, France) for 2 min and then serially diluted in sterile saline. The eosin-methylene blue agar (EMB; Difco, Becton Dickinson Co., Sparks, MD, USA), and Listeria Selective Agar (Sigma-Aldrich GmbH, Steinheim, Germany) were prepared for cultivation of *E. coli* O157:H7 and *L. monocytogenes*, respectively. Each diluent (100 μL) was plated onto the EMB agar (Difco) plates and Listeria selective agar (Sigma) and incubated at 37 ℃ for 24 h. All colonies were counted, and the population of microorganisms was expressed as log CFU/g.

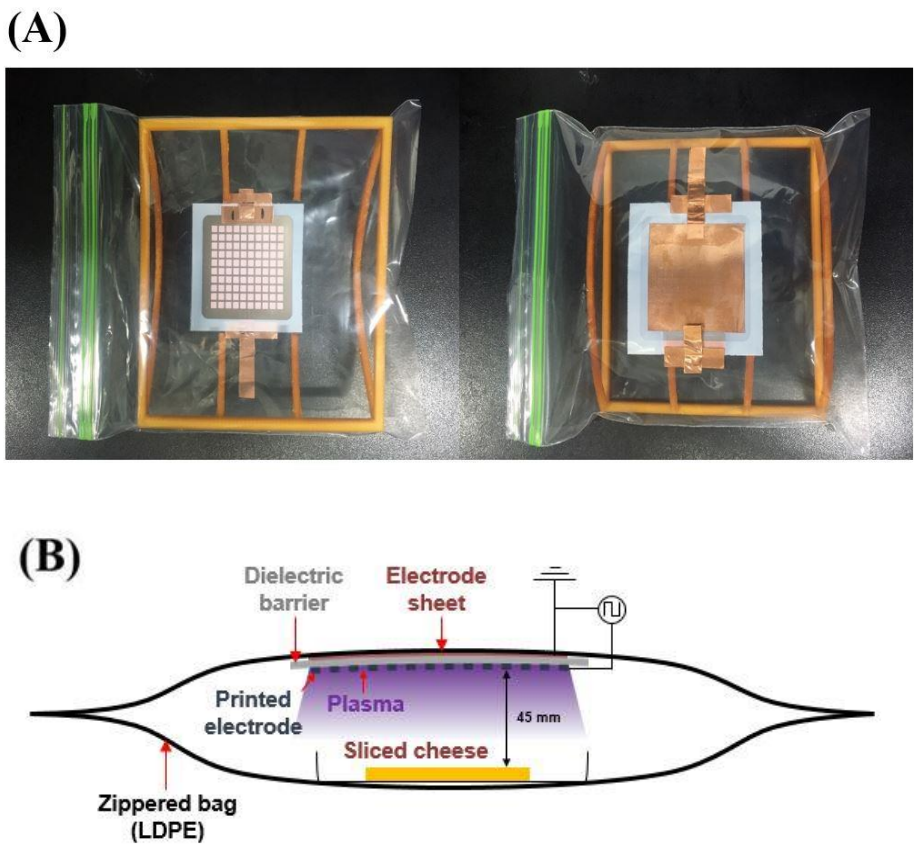

Figure 1. (A) Inkjet-printed flexible dielectric barrier discharge (FXDBD) application in low density polyethylene (LDPE) zippered bag, (B) Schematic diagram of the experimental setup for preparation of inkjet-printed (FXDBD) plasma

2.3.5. Concentration measurement of O_3, NO, and NOx

The gas-tight chamber used for the measurement was made of a stainless-steel cuboid shape with an inner width of 150 mm and a height of 100 mm. Before each measurement, the inside of the chamber was cleaned with ethyl alcohol, and the inkjet-printed FXDBD source was replaced with a new one. The O3, NO, and NOx density measurements inside the chamber were performed by dry air injection from the hole on the sides of the lid and measured by an analyzer (nCLD63, Eco physics, Duerdan, Switzerland) and ozone monitor (106-M, 2B Technology, Boulder, United States). The gas present in the chamber was replaced by dry air several times between each experiment. The gas temperature in the center of the chamber was measured using an optical thermometer (FTI-10, FISO Technologies Inc., Quebec, Canada).

2.3.6. pH

The samples (1 g) after inkjet-printed FXDBD plasma treatment were homogenized (T10 Basic, Ika Co., Staufen, Germany) in 9 mL of distilled water for 30 s (30,000 rpm). After homogenization, the suspension was centrifuged at 2,265 ×*g* for 10 min at 4 °C using a refrigerated centrifuge (UNION 32R, Hanil Science Inc., Gimpo, Korea). The pH values of the supernatant filtered with filter paper (Whatman No. 4, Whatman PLC., Middlesex, UK) were measured using a pH meter (Seven 2Go, Mettler-Toledo Inc., Schwerzenbach, Switzerland).

2.3.8. Thiobarbituric acid reactive substance (TBARS) values

Each sliced cheddar cheese sample (3 g) was homogenized (T25 Basic, Ika Co., Staufen, Germany) at 9,600 rpm for 30 s in 9 mL deionized water and 50 μL of 7.2% butylated hydroxytoluene (BHT, in ethanol). Then, the homogeneous sample (2 mL) was mixed with 4 mL of a thiobarbituric acid (20 mmol/L)/trichloroacetic acid (15%) solution in a 15 mL conical tube. The tubes were heated at 90 °C in a water bath for 30 min, cooled in water, and centrifuged (Hanil Science Industrial Co. Ltd.) at 2,265 ×*g* for 15 min. A spectrophotometer (DU 530; Beckman Instruments Inc., Brea, CA, USA) was used to measure the absorbance of the supernatant at 532 nm. The TBARS value (mg malondialdehyde/kg sample) was calculated using a standard curve.

2.3.7. Color

The color of the inkjet-printed FXDBD plasma-treated sliced cheddar cheese surface was measured using a spectrophotometer CM 3500d (Konica Minolta Censing Inc., Japan) using CIE color value, L* (lightness), a* (redness), and b* (yellowness). The instrument was calibrated using a white and black standard plate before analysis. The CIE color values were monitored with a computerized system utilizing spectra magic software (Konica Minolta Sensing, Inc.). The total color difference (ΔE) of the samples after plasma treatment was measured using the following equation:

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* + b_0^*)^2]^{\frac{1}{2}}$$

$$= [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{\frac{1}{2}}$$

2.3.9. Statistical analysis

All the experiments were conducted in triplicates. The data were analyzed using the SAS program (version 9.4, SAS Institute Inc., Cary, NC, USA). Statistical analysis was performed by one-way analysis of variance (ANOVA). Significant differences among the mean values were identified using Tukey's multiple test with a significance level of $P < 0.05$.

2.4. Results and discussion

2.4.1. Inactivation of the E. coli O157:H7 *and L. monocytogenes on the sliced cheese by the inkjet-printed FXDBD plasma treatment*

The inactivation patterns of inkjet-printed FXDBD plasma-treated *E. coli* O157:H7 *and L. monocytogenes* on the sliced cheese are depicted in Figure 2. The numbers of *E. coli* O157: H7 (Gram-negative bacteria) on the sliced cheese were initially 6.07 log CFU/g. The surviving population of *E. coli* O157:H7 reduced significantly to 1.74 log CFU/g after 5 min of plasma treatment and decreased substantially in proportion to the treatment time. After 20 min of plasma treatment, the number of *E. coli* O157:H7 reduced sharply to 2.65 log CFU/g ($P < 0.05$). However, *L. monocytogenes* (Gram-positive bacteria) showed 2.24 log CFU/g reduction after treatment for 5 min but there were no significant differences found among the samples treated with the inkjet-printed FXDBD plasma for 20 min.

UV photons and reactive species produced by plasma can inactivate microorganisms (Dobrynin et al., 2009; Guo et al., 2015; Liao et al., 2017). When a cell reacts with a UV photon, the nucleic acid of the cell forms a thymine dimer, which inhibits the replication ability of bacteria (Oguma et al., 2001). However, the ratio of N_2 and O_2, UV photons generated in CAP using air as a carrier gas, are not suitable for microbial inactivation (Guo et al., 2015). In addition, UV photons formed by CAP did not fall within the sterilization wavelength range of 220-280 nm or may have been absorbed by the surrounding atmosphere (Ehlbeck et al., 2010). Reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS)

play an important role in the inactivation of pathogens (Laroussi & Leipold, 2004; Lu et al., 2008; Yusupov et al., 2013). The reactive species trigger oxidative stress that damages cell membranes, intracellular DNA, RNA, and proteins (Joshi et al., 2011; Guo et al., 2015; Ji et al., 2018; Huang et al., 2020a). ROS generated during plasma generation cause strong oxidative stress, and cells are damaged by enzyme inactivation, lipid peroxidation, and DNA cleavage. Additionally, RNS is highly toxic and can damage DNA, leading to cell death (Han et al., 2016). Baek et al. (2020) proposed that chemical species formed by plasma-activated fine droplets (PAD) can affect the intracellular component by penetrating or attacking the cell envelope. Another study suggested that H_2O_2 and NO_3 generated from the plasma induce lipid peroxidation of bacterial cell walls and inactivate them (Ji et al., 2018). Therefore, it is suggested that the bacterial reduction in our results is due to cellular damage caused by various active species formed by inkjet-printed FXDBD plasma treatment. However, there were different trends between Gram-negative and Gram-positive bacteria. *E. coli* Ol57:H7 (Gram-negative bacteria) increasingly sterilized with increasing plasma treatment time ($P < 0.05$). On the other hand, *L. monocytogenes* (Gram-positive bacteria) showed no significant difference among the treatment times ($P > 0.05$).

In our study, inkjet-printed FXDBD plasma treatment was more effective for against Gram-negative than for Gram-positive bacteria. This result was similar to that of previous studies (Dirks et al., 2012; Shi et al., 2011; Korachi et al., 2010). According to several studies (Mai-Prochnow et al., 2016; Han et al., 2016; Baek et al., 2020; Huang et al., 2020a; Yoo et al., 2020), Gram-negative bacteria have high

plasma sensitivity due to their differences in the cellular structure with Gram-positive bacteria. The relative ineffectiveness of inkjet-printed FXDBD plasma against Gram-positive bacteria might be explained by the fact that the cells are surrounded by peptidoglycan structures that resists chemicals (Dirks et al., 2012; Laroussi, 2002). Additionally, Gram-positive bacteria have a thick peptidoglycan layer which occupies about 80-90 % of the cell wall, while the cell wall of Gram-negative bacteria is composed of a thin peptidoglycan layer and an outer membrane composed of lipopolysaccharides and phospholipids. The outer membrane of Gram-negative bacteria, which is composed of lipopolysaccharides and phospholipids, can lead to lipid peroxidation by RONS, which can damage cells (Huang et al., 2020a). In addition, the presence of a pore-forming protein (porin) facilitates the penetration of reactive species, thus increasing the sensitivity of Gram-negative bacteria to plasma (Mai-Prochnow et al., 2016). In contrast, the thick peptidoglycan layer of Gram-positive bacteria provides higher tensile strength and rigidity of the bacteria and less sensitivity to chemical oxidation (Huang et al., 2020a), as well as relatively less sensitive to plasma because it is slower than crossing the cell wall of Gram-negative bacteria (Mai-prochnow et al., 2016). Thus, in our results, the populations of *L. monocytogenes* did not decrease after 5 min of the inkjet-printed FXDBD plasma treatment.

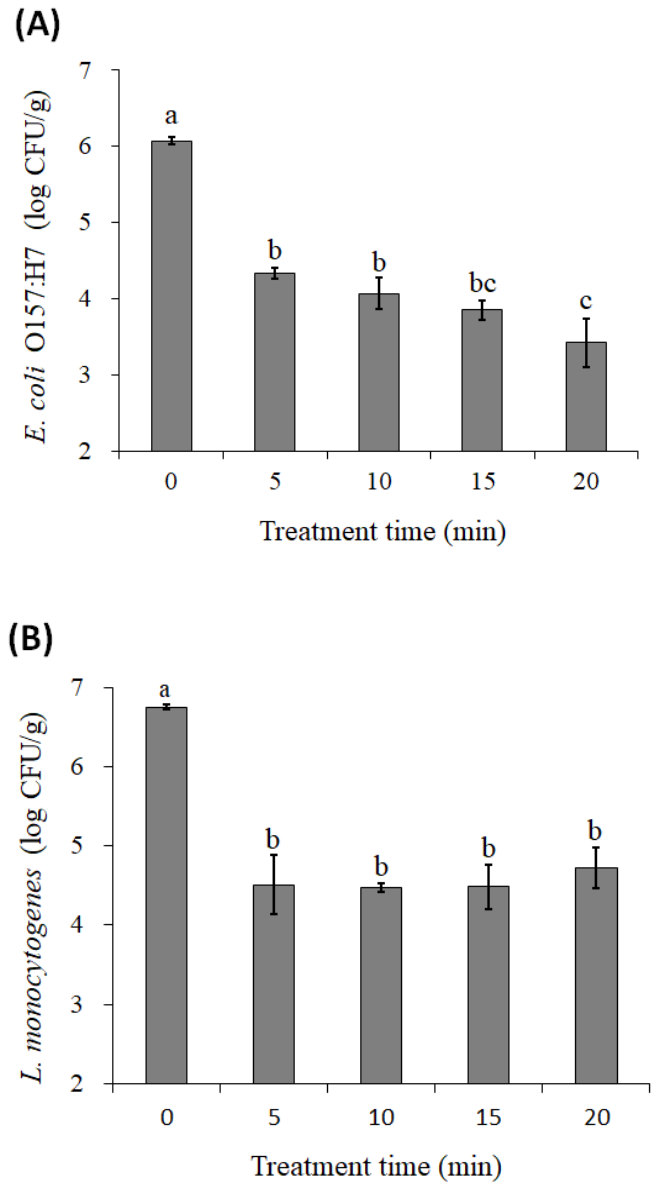

Figure. 2. The population (log CFU/g) of *E. coli* O157:H7 (A) and *L. monocytogenes* (B) on sliced cheddar cheese after inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma treatment. Error bars indicate standard deviation. [a-c]Different letters represent significant differences ($P < 0.05$).

2.4.2. Concentration of O_3, NO, and NOx

Concentrations of O_3, NO, and NOx after inkjet-printed FXDBD plasma treatment were measured to confirm the major reactive species for inactivating bacteria. The concentrations of O_3, NO, and NOx according to the FXDBD plasma treatment time is presented in Figure 3. The O_3 concentration rapidly increased within 2.5 min after plasma generation and remained stable. On the other hand, NOx increased in proportion to the plasma treatment time, but NO was negligible. NOx includes NO, NO_2, NO_3, N_2O_4, and N_2O_5. The chemical reactions are as follows:

$$O_2 + e \rightarrow 2O + e$$

$$O + O_2 + e \rightarrow O_3 + e$$

$$O_2 + N_2 + e \rightarrow 2NO + e$$

$$O_3 + NO \rightarrow NO_2 + O_2$$

$$O_3 + NO_2 \rightarrow NO_3 + O_2$$

$$NO + NO_2 \rightarrow N_2O_3$$

$$2NO_2 \rightarrow N_2O_4$$

The concentrations of O_3, NO, and NOx in the 5 min of inkjet-printed FXDBD plasma treatment were approximately 302.71, 0.086, and 8.361 ppm, and approximately 298.193, 0.174, and 28.083 ppm after 20 min of treatment, respectively. As shown in the equation, NO was negligible because it reacts with O_3 to form NO_2 (Park et al., 2018). In contrast, O_3 and NOx are considered the main active species in inkjet-printed FXDBD. The microbial reduction (Figure 2)

observed in the early stages (5 min) of inkjet-printed FXDBD treatment is caused by O_3, and in the second half (20 min) is caused by O_3 accumulation and NOx production. Thus, the bacterial reduction by inkjet-printed FXDBD plasma treatment could be attributed to the accumulation of O_3 and the production of NOx.

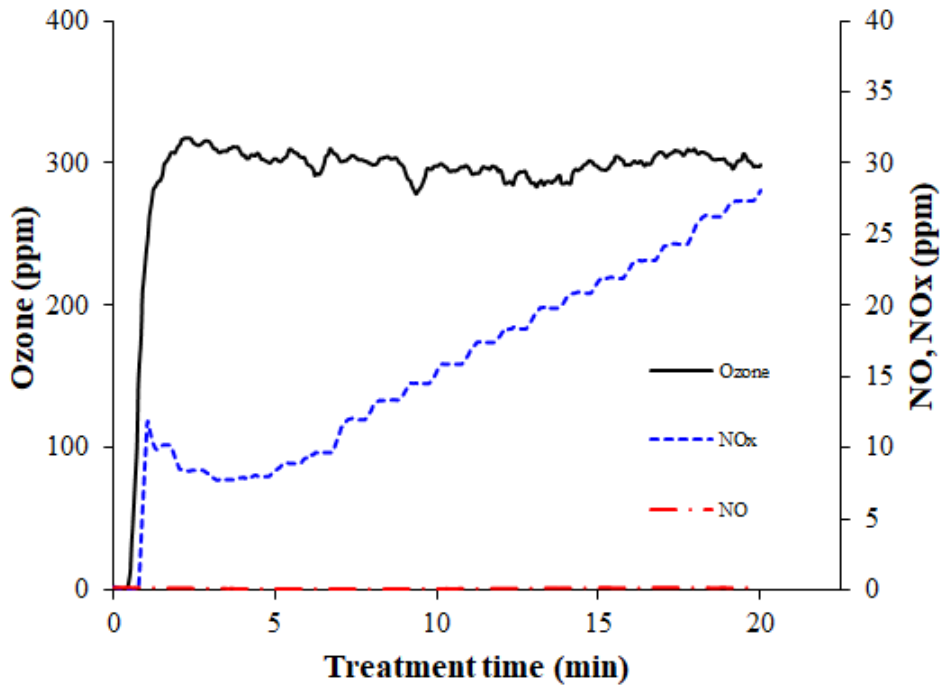

Figure 3. Concentration of O_3, NO, NOx in accordance with the inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma treatment

2.4.3. pH

pH is one of the factors affecting microbial survival and plays a vital role in pathogen inactivation (Aronsson & Ronner, 2001). Table 1 shows the pH value in sliced cheddar cheese after inkjet-printed FXDBD plasma treatment. The pH value of the samples was 6.44 before plasma treatment. The pH values of samples after plasma treatment for 5, 10, 15, and 20 min was 6.39, 6.37, 6.36, and 6.35, respectively. When the sliced cheddar cheese was treated with inkjet-printed FXDBD plasma for more than 5 min, the pH value decreased. Compared with the untreated group, the pH value was significantly decreased in the plasma treatment group ($P < 0.05$). However, no difference among the plasma treatment times was observed ($P > 0.05$). Several studies have also confirmed the decrease in pH due to plasma treatment in various food samples such as milk, cheeses, brown rice, chicken breast, and fruit juice (Kim et al., 2015; Yong et al., 2015; Lee et al., 2016b; Wan et al., 2019; Moutiq et al., 2020; Ozen & Singh, 2020). The decrease in pH by plasma treatment may be due to generation of higher levels of acidogenic molecules, including NOx, by plasma treatment (Stoffels et al., 2008; Fröhling et al., 2012). The decrease in pH after treatment with cold plasma was due to increase in the H^+ separated from bacterial molecules or H_2O (Korachi & Aslan, 2011). RONS, including O, O_3, and NOx, form low concentrations of nitrate and nitrite, which are acidogenic molecules due to the interaction of the moisture content of the sample (Wan et al., 2019; Mahnot et al., 2020). The chemical reaction equations are as follows:

$N_2 + O_2 + e \rightarrow 2NO + e$

$2NO + O_2 \rightarrow 2NO_2$

$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$

In fact, according to our results (Figure 3), NO_2 increased in proportion to inkjet-printed FXDBD plasma treatment time. However, the concentration of NO is maintained at a low level in the control group and treatment groups, which is presumed to be due to conversion to NO_2.

Table 1. pH changes in sliced cheddar cheese treated by inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma

Treatment time (min)	pH
0	6.44±0.028[1,a]
5	6.39±0.024[b]
10	6.37±0.012[b]
15	6.36±0.008[b]
20	6.35±0.004[b]

[1]Data represent the mean±standard deviation
[a,b]Different letters within the same column differ significantly ($P < 0.05$)

2.4.4. TBARS

TBARS values were obtained to measure lipid oxidation in sliced cheddar cheese by inkjet-printed FXDBD plasma treatment (Table 2). As shown in Table 2, in response to 5 min treatment, the TBARS values of the samples showed no difference compared to those of the non-treated samples ($P > 0.05$), whereas in response to 10, 15, and 20 min treatments, the TBARS values of the samples increased significantly compared to those of the control group. However, TBARS values of 10, 15, and 20 min inkjet-printed FXDBD treatment samples (0.212-0.244 mg MDA/kg) were low compared with other studies (Shan et al., 2011; Mahajan et al., 2015). Therefore, it can be inferred that the quality change of lipid oxidation by plasma treatment is insignificant. However, further investigation is needed. From our results, inkjet-printed FXDBD plasma generates RONS such as O_3 and NOx, which lead to lipid oxidation due to oxidative stress. Previous research shows that plasma can generate radicals and compromise the functions of fatty acids (especially unsaturated fatty acids), thereby inducing lipid oxidation (Laroussi, 1996; Montie et al., 2000; Kim et al., 2013). In addition, ozone generated by flexible thin-layer DBD plasma can also react with fatty acids, inducing the production of malonaldehyde (Roehm, et al., 1971).

As with our microbial inactivation results, it is presumed that the TBARS value increased at 10,15, and 20 min treatment compared to the 5 min treatment due to the accumulation of O_3 and the production of NOx as the treatment time increased. Similar results were obtained when using flexible thin-layer DBD (FTDBD) in the following studies (Jayasena et al., 2015; Yong et al., 2015). FTDBD plasma

treatment for 5 and 10 min increased the TBARS value in the same sliced cheese (Yong et al., 2015). In addition, Jayasena et al., (2015) also reported that the TBARS values of FTDBD plasma treated pork butt and beef loin were higher than those of the untreated samples. However, in plasma-treated bacon, chicken breasts, and cooked eggs, there was no significant difference when compared with the untreated group (Kim et al., 2011; Lee et al., 2016a). Thus, the changes in TBARS values could be influenced by properties such as plasma type and the fat content or fatty acid composition of the plasma-treated sample (Jo et al., 1999; Kim et al., 2011).

Table 2. TBARS values in sliced cheddar cheese treated by inkjet-printed flexible dielectric barrier discharge FXDBD plasma

Treatment time (min)	TBARS value (mg malondialdehyde/kg)
0	0.012±0.010[1,b]
5	0.023±0.020[b]
10	0.212±0.024[a]
15	0.244±0.047[a]
20	0.227±0.050[a]

[1]Data represent the mean±standard deviation
[a,b]Different letters within the same column differ significantly ($P < 0.05$)

2.4.5. Color

The color of cheese is a quality factor that has an important influence on consumer preferences (Wadhwani & McMahon, 2012). Table 3 shows the surface L^*, a^*, b^* values, and ΔE of sliced cheddar cheeses before and after plasma treatment. The L^* and b^* values of cheese samples were affected by the inkjet-printed FXDBD plasma treatment. L^* value slightly decreased significantly when the exposure time was increased, while b^* and ΔE values values increased ($P < 0.05$). The high b^* values of the samples were prominent when the samples were treated with plasma for 15 and 20 min. These findings are in agreement with those reported by Huang et al. (2020b) and Yong et al. (2015). On the other hand, there was no difference in a^* value and ΔE ($P > 0.05$). The sliced cheddar cheese used in our research contained oleoresin paprika for coloring. Paprika is commonly used in processed cheeses for color or taste advantage, which gives it a yellow/orange color (Sharma et al., 2020). A slight decrease in surface L^* of sliced cheese by inkjet-printed FXDBD plasma treatment was thought to be due to the reduction of moisture in the sample by plasma treatment (Sanabria et al., 2004). Huang et al. (2020b) also observed L^* reduction when the sliced cheese sample was treated with non-thermal atmospheric gas plasma. Oxidation by plasma treatment could increase b^* in cheese, which was caused by a higher oxidative damage effect proportional to plasma treatment time (Lee et al., 2012). Lipid oxidation can cause browning in food, especially brown-colored oxypolymers from milk proteins in sliced cheese (Yong et al., 2015). Therefore, in this experiment, it can be said that the increase in b^* by inkjet-printed FXDBD plasma treatment was might be caused by browning due to lipid oxidation. However,

there is no significant difference in the ΔE value, which is an indicator of the overall color change. In fact, a distinct difference was not observed in the surface color of sliced cheddar cheeses after inkjet-printed FXDBD plasma treatment (Figure 4). Therefore, the effect of inkjet-printed plasma on the color quality of sliced cheese can be said to be insignificant.

Table 3. Surface color of sliced cheddar cheese treated by inkjet-printed flexible dielectric barrier discharge FXDBD plasma

Treatment time (min)	L^*	a^*	b^*	Total color difference (ΔE)
0	76.53±0.668[1,a]	19.34±0.726	37.56±0.168[c]	-
5	75.57±0.638[ab]	19.63±0.766	40.50±0.373[b]	3.37±0.29[b]
10	75.07±0.634[b]	19.87±0.349	41.97±0.761[ab]	4.69±0.61[ab]
15	75.01±0.196[b]	20.40±0.747	42.78±0.846[a]	5.64±0.66[ab]
20	75.11±0.155[b]	20.16±0.243	42.75±0.757[a]	5.45±0.77[a]

[1]Data represent the mean±standard deviation
[a-c]Different letters within the same column differ significantly ($P < 0.05$)

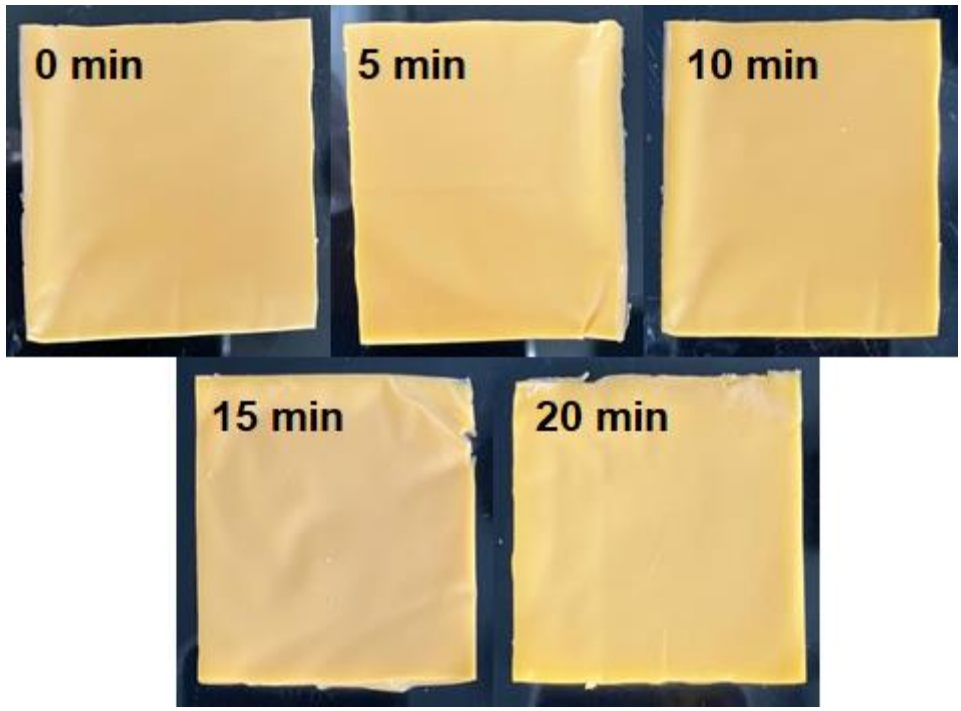

Figure 4. Pictures of the surface of sliced cheddar chesses after inkjet-printed flexible dielectric barrier discharge (FXDBD) plasma treatment.

2.5. Conclusion

In sliced cheese, inkjet-printed FXDBD plasma showed effective inactivation against *E. coli* O157:H7 and *L. monocytogenes* showed the most significant reduction at a treatment time of 20 min. O_3 and NOx generated by FXDBD plasma were determined to play a major role in inactivating microorganisms. Considering the change in the quality of sliced cheese, FXDBD plasma treatment for 5 min could be the optimum. Based on the results, inkjet-printed FXDBD plasma can be considered a potential as a non-thermal sterilization technology. However, quality changes should be studied through additional research for industrial applications.

Chapter III.

Influence of encapsulated dielectric barrier discharge plasma on structure and functional properties of egg white protein

3.1. Abstract

The objective of this study was to investigate the effect of encapsulated dielectric barrier discharge (EDBD) plasma on structure and functional properties of egg white protein. Egg white was diluted with 0.1 M phosphate buffer (pH 7.4) to protein concentration 10.36±0.191 mg/mL (EWS). Twelve mL of diluted EWS was treated by EDBD plasma for 0, 10, 20, 30, and 40 min, respectively. As the plasma treatment time increased, the temperature of the EWS increased and the pH decreased. However, the changes in temperature and pH did not affect EWS. At 30 min of EDBD plasma treatment, the protein structure in EWS was unfolded which confirmed through the results of free sulfhydryl groups and surface hydrophobicity. On the other hand, the aggregates were formed when EDBD plasma was treated for 40 min. To evaluate the functional properties of EWS, the emulsion properties and foaming properties were measured. The results indicated that emulsion stability and

foaming capacity gradually increased as the plasma treatment time increased up to 30 min and decreased after 40 min, with the highest at 30 min. The droplet size of the emulsion decreased the most at 30 min and increased at 40 min after EDBD plasma treatment ($P < 0.05$). The decrease in droplet size means increasing emulsion stability, which could be supported by the emulsion stability results. In conclusion, EDBD plasma treatment had a significant influence on emulsion properties and foaming capacity of egg white protein solution and 30 min would be the most effective treatment time to improve the functional properties. The mechanism of this phenomenon should be investigated through further studies and an application study for plasma-treated egg white protein should be needed.

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Keywords: Encapsulated dielectric barrier discharge plasma, Egg white protein, Functional properties

Student Number: 2019-20390

3.2. Introduction

Protein is one of the essential nutrients and determines the texture, sensory and nutritional properties. The functional properties of food proteins are affected by physical, chemical, and structural changes, which can be induced by high pressure treatment, irradiation, plasma, pulse electric field, heating, microfluidization, oxidation, hydrolysis, and so on (Alavi et al., 2019). Important functional properties of proteins in the food industries are protein solubility, water holding capacity, emulsification properties, foaming properties, gelling ability, and fat binding properties (Zayas, 2012). In food processing, proteins are often exposed to oxidative conditions and subjected to oxidative stress (Wu et al., 2009). Proteins are especially easily affected by oxidation due to their structure and the presence of particular amino acid residues such as tyrosine, tryptophan, cysteine, methionine, lysine, proline, and histidine (Sharifian et al., 2019). Oxidative stress can lead to several structural changes in protein, including unfolding, degradation, and aggregation (Duan et al., 2018).

Egg white protein (EWP) is widely used as desirable ingredients in the food industry such as bakery products, meringues, meat products (Stefanovic et al., 2017). It can be used as an emulsifier and foaming agents to stabilize food emulsions and foams due to its ability to lower the surface tension by adsorbing at the interface (Alavi et al., 2019). Egg white contains different proteins that are important for functionality, such as ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%) and lysozyme (3.5%) (Abeyrathne et al., 2013). In particular, ovalbumin, the main protein responsible for egg white functionality, is a monomeric phosphoglycoprotein

with coagulation and gelation properties and has excellent emulsifying ability and stability in acidic conditions (Mine et al., 1991; Machado et al., 2007). However, emulsions prepared with egg white protein at neutral and alkaline pH are very unstable, limiting the application of egg white protein in non-acid emulsion food systems as emulsifiers and stabilizers (Chang et al., 2017). Therefore, various technologies are being applied to overcome instability.

In recent years, non-thermal technologies have attracted considerable interest in the food industry to improve the specific functions of food ingredients (Ashokkumar et al., 2008). Non-thermal technologies prevent the thermal decomposition of food components and can preserve the sensory and nutritional quality of fresh food, unlike thermal technologies (Pereira & Vicente, 2010). Irradiation, ultrasound, pulsed electric fields, high-pressure are included (Liu et al., 2009; Arzeni et al., 2012; Wu et al., 2014; Chen et al., 2019). However, these various non-thermal techniques have disadvantages: unpleasant odor occurs during irradiation (Liu et al., 2009) and instability of EWP foam caused by ultrasonic treatment (Sheng et al., 2018).

Plasma, a non-thermal technology that is receiving new attention in the food industry, is the fourth state of a substance and consists of reactive species, UV photons, and electromagnetic field, and free electrons (Yadav et al., 2019). Cold atmospheric plasma (CAP) generated at atmospheric pressure has already been proven effective in microbial inactivation in dairy and meat products such as milk, cheese, pork, beef, chicken by previous studies (Kim et al., 2015; Jayasena et al., 2015; Yong et al., 2015; Lee et al., 2016a; Yong et al., 2017). This is known to be

due to the damage from the oxidative stress of cell membranes, intracellular DNA, RNA, and protein by reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by the plasma (Ji et al., 2018; Huang et al., 2020a). In recent, there have been studies that induce structural changes in protein molecules by RONS generated by CAP and have a beneficial effect on the interface-related functional properties of proteins using whey protein, peanut protein, and myofibrillar protein (Segat et al., 2015; ji et al., 2018; Sharifian et al., 2019). However, there is little research on applying DBD plasma to egg white protein. Therefore, the aim of this study is to analyze protein structural changes and the improved functional properties of egg white protein using encapsulated dielectric barrier discharge (DBD) plasma, one of the CAP.

3.3. Materials and methods

3.3.1. Materials

Egg white solution with 10 % protein content (wet base) was obtained from a local market and immediately stored at 4 ℃. The protein contents of egg white solution were quantified using bicinchoninic acid (BCA) assay (BCA Protein Assay Kit; Pierce, Rockford, IL, USA) according to the manufacturer's instruction. Bovine serum albumin was used to draw a standard curve. Egg white solution (EWS) used in the experiment was diluted 10 times with phosphate buffer (100 mM, pH 7.4) to a final concentration of 1%. Soybean oil used in the emulsion property test was purchased from a local market and used immediately without further purification.

3.3.2. Encapsulated dielectric barrier discharge (EDBD) plasma

Encapsulated dielectric barrier discharge (EDBD) plasma was generated from plastic container (137 × 104 × 53 mm) described by Yong et al. (2015). To briefly explicate, power electrode and ground electrode were connected with copper tape attached in a container and a polytetrafluoroethylene (PTFE) sheet was attached between copper tape as a dielectric barrier. Plasma was generated atmospheric air condition and discharge conditions were 2.2 kHz and 8.4 kVpp. Twelve mL of EWS was placed in 60 × 15 mm sterilized petri dishes and placed in the center of the container for EDBD plasma treatment. All experiments were performed at a discrete voltage of 4.2 kV applied for 0, 10, 20, 30, and 40 min using ambient air at atmospheric pressure conditions. Treatments were carried out in duplicate.

3.3.3. Temperature and pH measurement

The temperature and pH of EWS were measured immediately after treatment. The temperature of the EWS was measured using a digital thermometer (TM-747DU, Tenmars Electronics Co., Ltd., Taipei, Taiwan) and pH measured using a pH meter (Seven 2Go, Mettler-Toledo Inc., Schwerzenbach, Switzerland) immediately after EDBD plasma treatment. Control samples without EDBD plasma treatment were measured immediately after refrigeration.

3.3.4. Free sulfhydryl (SH) group content

The concentration of free SH group of the samples were performed according to Ellman's procedure (1959) with the modifications of Ji et al. (2018), Sharifian et al. (2019). To determine, the exposed SH group 0.5 mL of EWS was added in pH 8.0 Tris-Glycine buffer 2.5 mL. The Tris-Glycine buffer composed of 86 mM Tris (Bio-rad Laboratories Inc., Hercules, USA), 90 mM glycine (Bio-rad), and 4 mM EDTA (Sigma Chemical Co., St. Louis, USA). The pH was adjusted with Hydrochloric acid (Duksan Pure Chemical Co., Ansan-si, Korea). Then, 0.02 mL Ellman's reagent (4 mg/mL 5, 5′- dithiobis (2-nitrobenzoic acid), (DTNB) in Tris-Glycine buffer) was added to develop color. After 1 hr at room temperature for stabilizing, absorbance was measured at 412 nm using UV/Vis spectrophotometer (X-ma 3100, Human Co. Ltd, Seoul, Korea). The total SH group contents were also measured in the same protocol but using a denaturing buffer containing 8 M Urea (Samchun Chemical Co., Seoul, Korea) and 0.5% sodium dodecyl sulfate (SDS, Bio-rad) in Tris-glycine buffer. The free SH groups were calculated as follows:

$\mu M\ SH/g = (75.53 \times A_{412} \times D)/C$

Where, A_{412} is the absorbance at 412 nm, C is the EWS concentration (mg/mL), and D is the dilution factor.

3.3.5. Surface hydrophobicity (H_0)

Surface hydrophobicity (H_0) of EWS to confirm the protein structure was determined using the method with a slight modification described by Segat et al., (2015) and Alavi et al., (2019). EWS were dilutions with 100 mM phosphate buffer (pH 7.4) to obtain a range of sample concentration (0.05-0.4 mg/mL). The hydrophobic fluorescence probe Magnesium (II) 8-Anilino-1-naphthalenesulfonate (ANS-Mg) solution of 20 µL (8 mM in 100 mM phosphate buffer, pH 7.4) was added to each EWS of 2 mL. Spectrofluorophotometer (SpectraMax M2e; Molecular Devices, Sunnyvale, CA, USA) was used to measure the fluorescence intensity at excitation and emission wavelengths of 390 nm and 470 nm, respectively. Surface hydrophobicity was calculated from the initial slope of the plot of fluorescence intensity as a function of protein concentration.

3.3.6. Foaming properties

Foaming properties were measured by referring to the method described in Kuan et al., (2011). To determine foaming properties, 12 mL EWS were homogenized (T10 Basic, Ika Co., Staufen, Germany) at 30,000 rpm for 1 min in a glass tube for foam formation. Immediately and 1 hr after foam formation, the volume of foam (overrun) and volume of liquid drainage was measured and

calculated into the following equation.

$$Foaming\ capacity\ (\mathrm{FC})\ \% = \frac{\text{volume of foam} - \text{volume of liquid drainage}}{\text{original volume of liquid}} \times 100$$

$$Foam\ stability\ (\mathrm{FS})\ \% = \frac{\mathrm{FC}\ (0\ \mathrm{min})}{\mathrm{FC}\ (60\ \mathrm{min})} \times 100$$

After foam formation, standing for 1 hr at room temperature.

3.3.7. Emulsion properties

The emulsion properties of EWS were measured using the turbidimetric method (Pearce & Kinsella, 1978; Oh & Lee, 2004; Kuan et al., 2011). The EWS samples and soybean oil in the ratio of 3:1 (v/v) were homogenized (T25 Basic, Ika Co., Staufen, Germany) at 12,000 rpm for emulsion formation. Immediately and 2 hr after 1 min of homogenization, 100 μL emulsion was added in 5 mL of 0.1 % SDS and measured absorbance at 500 nm using UV/Vis spectrophotometer (X-ma 3100, Human Co. Ltd, Seoul, Korea) to determine the turbidity and calculated using the following equation.

$$T = \frac{2.303 \times \mathrm{A}}{l}$$

$$Emulsion\ stability\ (\%) = \frac{T\ of\ emulsion\ after\ 2\ hr}{T\ of\ initial\ emulsion} \times 100$$

Where T is turbidity, A is the absorbance at 500 nm and l is the path length of the cuvette. The 2 hr stand for emulsion stability was carried out at room temperature.

3.3.8. Droplet size

The EWS emulsions for droplet size were prepared as the above method. The plasma-treated and untreated EWS samples were measured using Laser Diffraction Particle Size Analyzer (Mastersizer 3000, Malvern Instruments Ltd., Worcestershire, UK). The dispersant was deionized water and the emulsion was diluted in a dispersion circulator tank with stirring at 2,500 rpm. The calculation of final size distribution was performed by Malvern software (Version 3.00, Malvern Instruments Ltd) using the Mie scattering with a particle absorption index of 1.0, particle refractive index of 1.520, and a dispersive refractive index of 1.33 (water). Results were expressed as area average diameter (D[3,2]).

3.3.9. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA). Significant differences among the mean values were identified using the Tukey's multiple test using SAS program (version 9.4, SAS Institute Inc., Cary, NC, USA) with a significance level of $P < 0.05$. All experiments conducted in this study were performed in triplicate.

3.4. Results and discussion

3.4.1. Temperature and pH

The protein structure and functional properties in foods are affected by temperature and pH (Croguennec et al., 2002; Pelegrine & Gasparetto, 2005). Table 4 shows the temperature and pH values according to EDBD plasma treatment time. As the EDBD plasma treatment time increased, the temperature increased significantly. The temperature of the EWS was increased from 7.9°C to 43°C from 0 to 40 min. In comparison to the untreated EWS, temperature of treated EWS increased with an increase in the treatment time. The temperature increase with increasing plasma treatment time could be also confirmed in other studies (Ji et al., 2018, Judée et al., 2018). However, it is believed that the temperature increase by plasma treatment did not affect protein denaturation in this study, since it is lower than the egg white protein denaturation temperature of 60°C (Mine et al., 1990).

It can be seen that the pH of EWS decreased from 7.49 to 7.19 after EDBD plasma treatment, and significantly decreased at 30 and 40 min. Previous studies found that plasma induced a decline in pH especially for liquid conditions (Oehmigen et al., 2010, Segat et al., 2015). This is because reactive species generated by plasma could form low concentrations of nitrate and nitrite which acidogenic molecules due to the interaction of the moisture content of the sample (Wan et al., 2019; Mahnot et al., 2020). Monahan et al., (1995) found that the whey proteins showed that unfolding of the protein had occurred at pH 9 and 11. Whereas, the pH of EWS after EDBD plasma treatment in this study was neutral conditions. Therefore, the increase in

temperature and decrease in pH ($P < 0.05$) after EDBD plasma treatment would not have an effect on the protein structure change.

Table 4. Temperature and pH changes in egg white solution (EWS) treated by encapsulated dielectric barrier barrier discharge (EDBD) plasma

Treatment time (min)	Temperature (°C)	pH
0	7.9±0.493[1,d]	7.49±0.012[a]
10	23.4±0.666[c]	7.45±0.026[a]
20	31.3±2.082[b]	7.33±0.067[a]
30	39.3±2.427[a]	7.23±0.067[b]
40	43.0±1.229[a]	7.19±0.030[b]

[1]Data represent the mean±standard deviation
[a-d]Different letters within the same column differ significantly ($P < 0.05$)

3.4.2. Free sulfhydryl (SH) group content

Sulfhydryl group plays an important role in biochemistry because disulfide bonds link together the amino acids needed for functional purposes in secondary, tertiary, or quaternary protein structures (Karimi et al., 2016). Also, the degree of oxidation of cysteine residues in egg white proteins can be determined by free SH groups (Alavi et al., 2019). Figure 5(A) shows the total free sulfhydryl group contents in EWS by EDBD plasma treatment time. The total free SH group contents in untreated EWS was 65.26 μM SH/g protein. Our studies found that the total free SH contents of EDBD plasma-treated EWS gradually decreased until 20 min of treatment ($P < 0.05$). This is because the SH group of EWS is oxidized by ROS and RNS generated during EDBD plasma treatment, forming a new disulfide bond (Eaton, 2006; Alavi et al., 2019). In other words, the decrease in the total free SH content was related to the oxidation of free SH groups to form disulfide bonds (Eaton 2006). In addition, according to previous studies (Asada & Kanematsu, 1976; Wu et al., 2009), radical and SH reactions can reduce the contents of the SH group.

The results of the free SH group exposed to the surface are shown in Figure 5(B). For the untreated EWS, the exposed free SH content was 1.89 μM SH/g protein. This result had shown significantly the highest at 20 and 30 min of plasma treatment and decreased again at 40 min ($P < 0.05$). A small increase in the exposed free SH content was observed after the 20 min exposure to EDBD plasma. The small increase in exposed free SH content can be attributed to the formation of unfolded molecular structures, which may be associated with the disruption of disulfide bonds during the attack with high energetic ions (Ji et al., 2018). However, a slight decrease seen at

40 min ($P < 0.05$) of EDBD plasma treatment was observed. This is because the s-s bonds of SH exposed by peroxidation increased and aggregates were formed (Zhang et al., 2015). In addition, this may be due to the gathering of protein micelles (Ji et al., 2018). Overall, our results suggest that protein oxidation by EDBD plasma treatment can cause sulfhydryl-disulfide exchange reaction between disulfide groups to unfolding the protein structure.

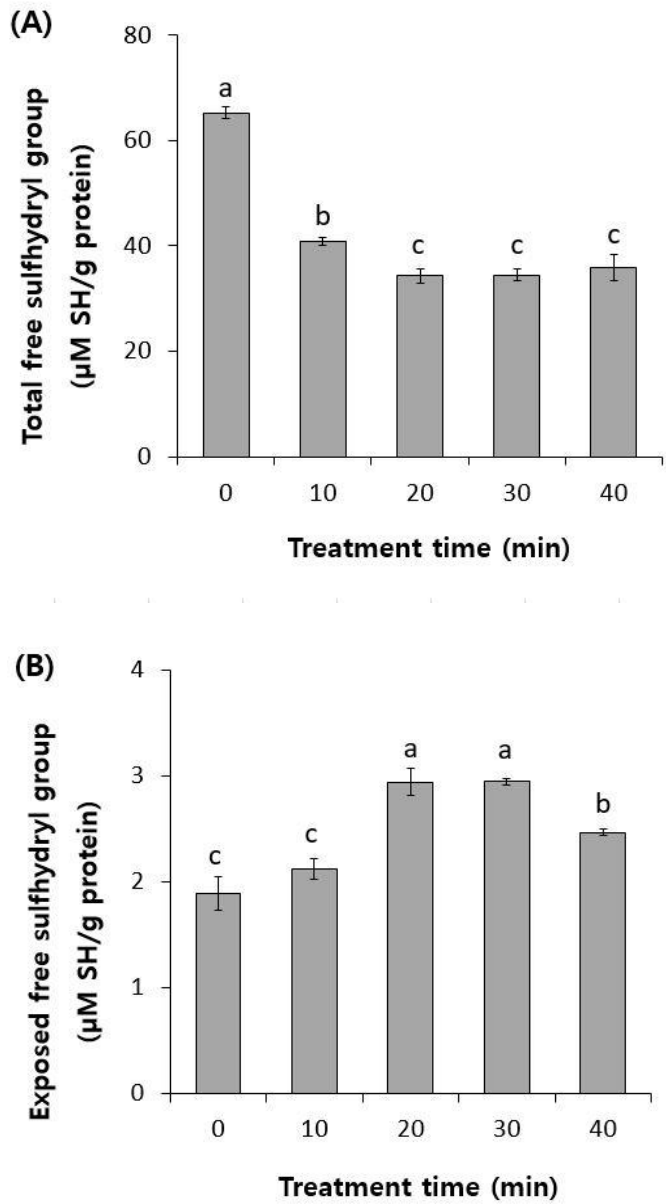

Figure 5. Total free sulfhydryl group (A) and exposed free sulfhydryl group (B) contents in egg white solution (EWS) by encapsulated dielectric barrier discharge (EDBD) plasma treatment time. Error bars indicate standard deviation. $^{a\text{-}c}$Different letters represent significant differences ($P < 0.05$).

3.4.3. Surface hydrophobicity (H_0)

H_0 is a common method for assessing structural changes in proteins which is closely related to functional properties (Wu et al., 2014). When the protein is unfolded, the hydrophobic groups are exposed and the exposed hydrophobic groups combine with the hydrophobic fluorescence probe, (8-anilino-1-naphthalene sulfonic acid-ANS) that is known to bind hydrophobic areas of proteins accessible to the aqueous solvent (Gaucheron et al., 1997; Ali et al., 1999) to increase a fluorescent. Figure 6 shows the results of surface hydrophobicity of EWS by EDBD plasma treatment time. For the plasma-treated EWS, H_0 values increased significantly until 30 min of treatment. As mentioned above, when the protein structure is unfolding, the hydrophobic group buried inside is exposed. Therefore, the increase in the H_0 value indicates that the protein structure has been unfolding by EDBD plasma treatment. Similar results have been obtained in other studies (Segat et al., 2015; Duan et al., 2018; Xiong et al., 2018). The increase in H_0 can have a positive effect on functionality by increasing the degree of adsorption at the air-water interface (Delahaije et al., 2014). However, there was a tendency to significantly decrease in the 40 min treatment ($P < 0.05$). This is because of the formation of hydrophobic bonds between the increased surface hydrophobic groups (Subirade et al., 1998). This was because the unfolding protein structure was aggregated due to the effect of peroxidation by plasma treatment for 40 min. In addition, it can be seen that the protein structure was aggregated due to the effect of peroxidation by plasma treatment for 40 min. This can support the results of the exposed free SH group content in the previous results.

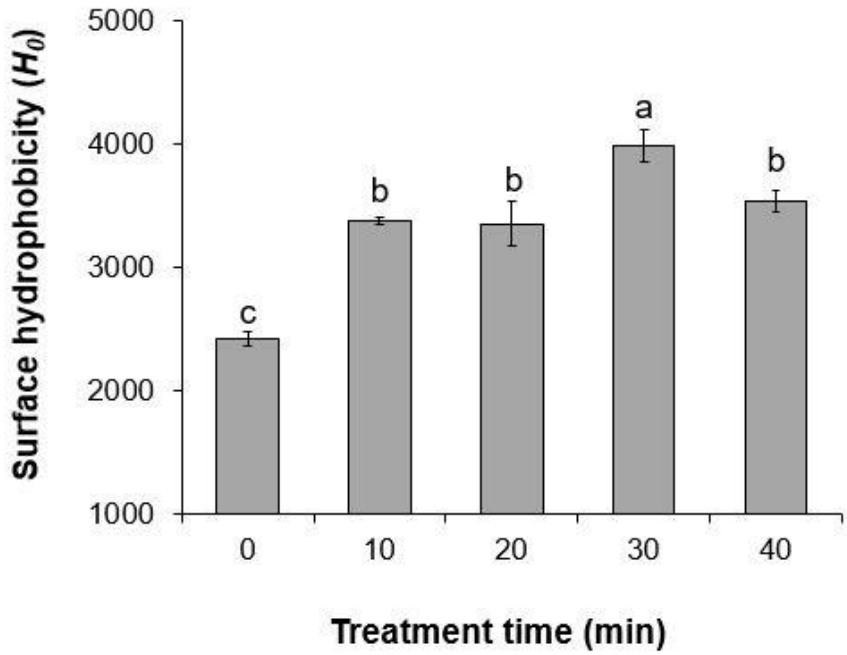

Figure 6. Surface hydrophobicity in egg white solution (EWS) by encapsulated dielectric barrier discharge (EDBD) plasma treatment time. Error bars indicate standard deviation. [a-c]Different letters represent significant differences ($P < 0.05$).

3.4.4. Foaming properties

Foaming properties were measured to confirm the functional properties of the EDBD plasma-treated EWS. The foaming capacity (FC) and foam stability (FS) of the EDBD plasma-treated EWS were shown in Figure 7. The FC of the EDBD plasma-treated EWS was significantly higher than that of untreated EWS (Figure 7A). From this result, it can be said that EDBD plasma treatment induces partial unfolding of the protein structure, adsorbs rapidly at the air-water interface, and forms a viscoelastic film to improve foam formation (Duan et al., 2018; Xiong et al., 2018). As shown in Figure 7(B), it can be confirmed that the FS of the EDBD plasma-treated EWS was significantly lower than that of untreated EWS. FS was influenced by the formation of protein structure and networks due to strong intermolecular interactions. In other words, when aggregates are formed, the resistance of the interfacial film increases, and it forms a barrier against bubble collapse and coalescence, thereby improving bubble stability (Segat et al., 2015). However, in this study, since the structure was unfolding by protein oxidation due to EDBD plasma treatment, it would have the opposite effect from the result of aggregate formation. As shown in the results, there was a significant decrease in FS compared to untreated EWS. As the protein unfolds, the formation of the protein network weakens, and the resistance of the interfacial membrane decreases, which is believed to have caused bubble collapse. Our findings indicated that EWS could have an outstanding FC after EDBD plasma treatment.

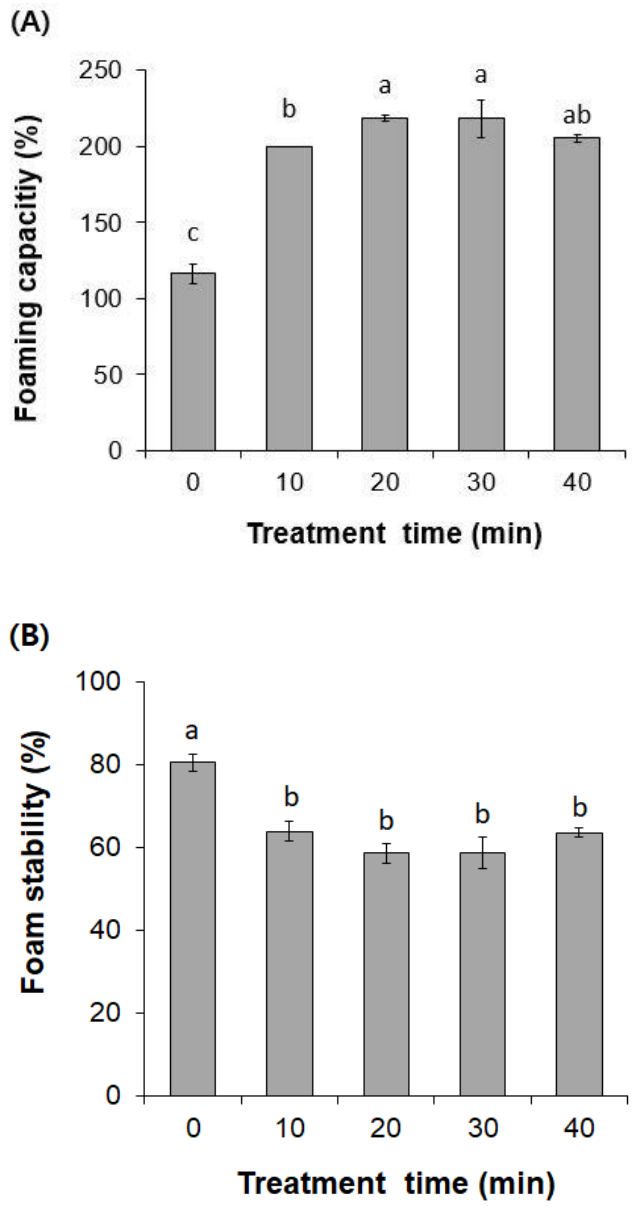

Figure 7. Foaming capacity (A) and Foam stability (B) in egg white solution (EWS) by encapsulated dielectric barrier discharge (EDBD) plasma treatment time. Error bars indicate standard deviation. [a,b]Different letters represent significant differences ($P < 0.05$).

3.4.5. Emulsion properties

Protein acts as an emulsifier to improve the texture of food by mixing two substances that do not mix with each other, such as water and oil (Hoffmann & Refar, 2014). Figure 8 indicates the values for emulsion properties of EWS before and after EDBD plasma treatment. In Figure 8(A), turbidity of EWS increased significantly with the increase of plasma treatment time. The turbidity is higher as the size of the fat globules is small and well dispersed, so it can be seen that the emulsion capacity increases as the turbidity increases. (Viljanen et al., 2005; Kim et al., 2019). The increase in emulsifying capacity of ESW treated with EDBD plasma was closely related to the rise in surface hydrophobicity induced by the exposure of hydrophobic groups of protein molecules (Sharifian et al., 2019). Proteins had both hydrophilic and hydrophobic amino acids and hydrophobic amino acids are usually buried inside. During plasma treatment, moisture in EWS generates OH radicals cause EWS protein oxidation (Shugang et al., 2019). Oxidized proteins can denature their structure and expose hydrophobic amino acids to the surface (Kim et al., 2013). Therefore, the protein is unfolded by oxidation by plasma treatment. This increases the hydrophobic amino acids exposed to the surface and further enables better binding with oil.

The emulsion stability increased significantly up to 30 min of EDBD plasma treatment but declined after 40 min of plasma treatment ($P < 0.05$) (Figure 8B). It reached a maximum percentage after 30 min of plasma treatment. Basically, the structural changes in proteins and their unfolding increases emulsion stability (Delahaije et al., 2014). However, the decreased emulsion stability at 40 min may be

due to the formation of aggregates by peroxidation, which reduces emulsion stability (Zhang et al., 2015). From these results, it could be confirmed that the EDBD plasma treatment can improve the emulsion properties of EWS.

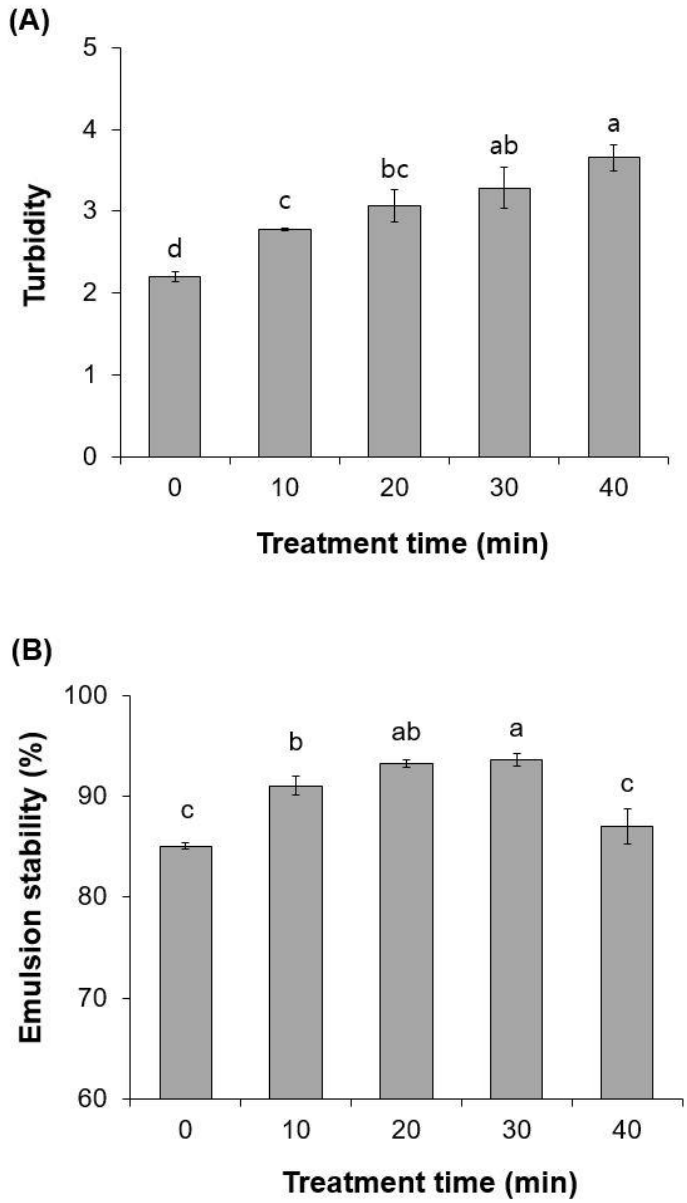

Figure 8. Turbidity (A) and emulsion stability (B) in egg white solution (EWS) by encapsulated dielectric barrier discharge (EDBD) plasma treatment time. Error bars indicate standard deviation. [a-d]Different letters represent significant differences ($P < 0.05$).

3.4.6. Droplet size

The droplet size is an important parameter that affects the capacity and stability of the emulsion (Goodarzi & Zendehboudi, 2018). The droplet size of the EWS emulsion before and after EDBD plasma treatment is indicated in Figure 9. It can be seen that the droplet size of EWS emulsion decreased significantly up to 30 min of EDBD plasma treatment but increased after 40 min of plasma treatment ($P < 0.05$). The untreated EWS has the largest D[3,2] of 18.07 μm, while the treated EWS showed the lowest size at 30 min (10.50 μm) (Table 2), which was consistent with the results of emulsion stability (Figure 8B). The increased D[3,2] size after 40 min of plasma treatment indicates that there were aggregation and coalescence, which leads to emulsion destabilization (Kuan et al., 2011). Protein denaturation and changes in structure affect surface hydrophobicity and consequently lead to improve adsorption of oil-in-water emulsion systems (Jambrak et al., 2009) Therefore, these results suggest that structural changes caused by EDBD plasma treatment resulted in improved emulsification properties.

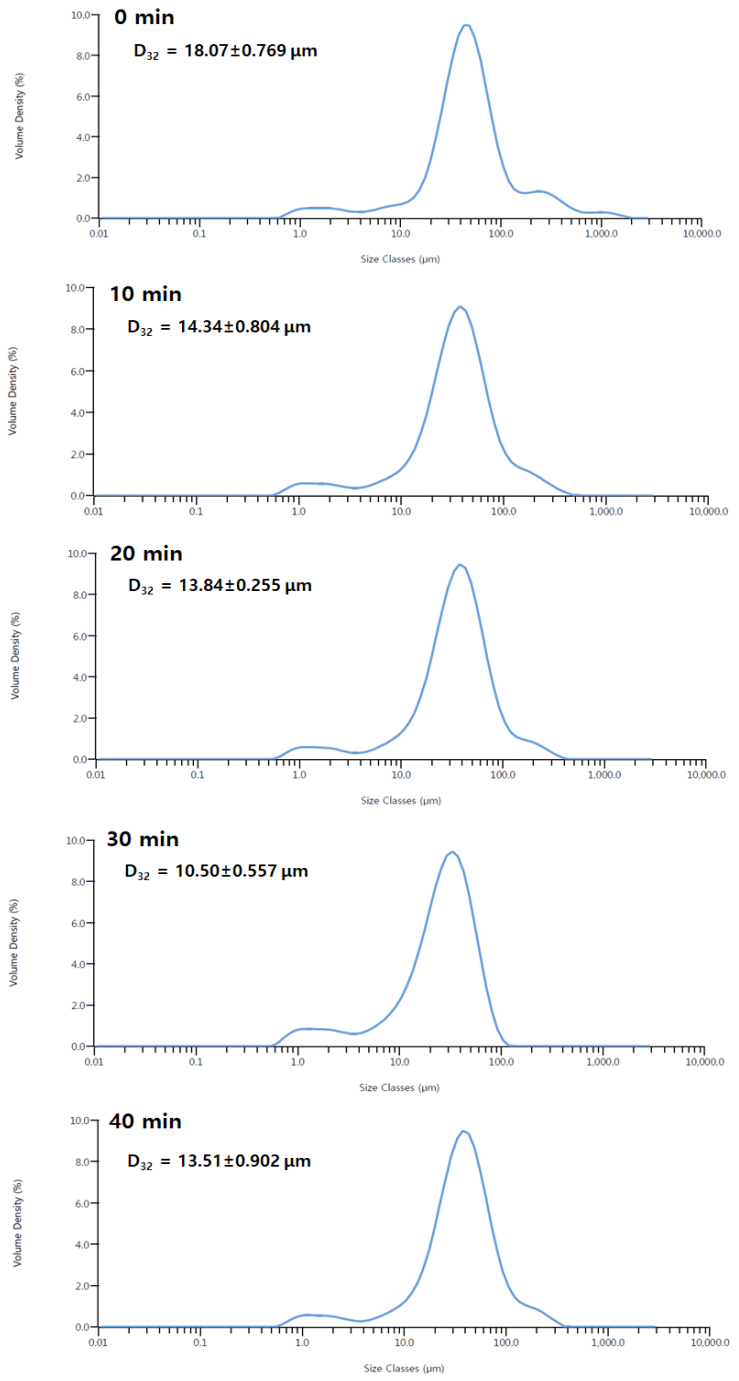

Figure 9. Egg white solution (EWS) emulsion droplet size by encapsulated dielectric barrier discharge (EDBD) plasma treatment time.

Table 4. Egg white solution (EWS) emulsion droplet size by encapsulated dielectric barrier discharge (EDBD) plasma treatment time.

Treatment time (min)	D [3,2] (μm)
0	18.07±0.769[1,a]
10	14.34±0.804[b]
20	13.48±0.255[b]
30	10.50±0.557[c]
40	13.51±0.902[b]

[1]Data represent the mean±standard deviation

[a,c]Different letters within the same column differ significantly ($P < 0.05$)

2.5. Conclusion

This paper constitutes a research on structural changes and functional properties of egg white protein solution treated with EDBD plasma. It was confirmed through the free SH group and surface hydrophobicity that 30 min of exposure to EDBD plasma led to unfolding of protein, leading to enhanced protein flexibility and improved functional properties. This experiment showed that extended treatment time (40 min) led not only to reduced emulsion capacity and stability but also to declining foaming capacity. These changes were attributed to the aggregation of proteins, as also confirmed by the observed reduction in exposed free sulfhydryl content and surface hydrophobicity. Therefore, EDBD plasma treatment may be one of the useful ways to improve the functionality of egg white protein. A further study for the detail stability mechanisms is still needed.

References

Abeyrathne, E. D. N. S., Lee, H. Y., & Ahn, D. U. (2013). Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. *Poultry Sscience*, *92*(12), 3292-3299.

Alavi, F., Emam-Djomeh, Z., Momen, S., Mohammadian, M., Salami, M., & Moosavi-Movahedi, A. A. (2019). Effect of free radical-induced aggregation on physicochemical and interface-related functionality of egg white protein. *Food Hydrocolloids*, *87*, 734-746.c

Ali, V., Prakash, K., Kulkarni, S., Ahmad, A., Madhusudan, K. P., & Bhakuni, V. (1999). 8-anilino-1-naphthalene sulfonic acid (ANS) induces folding of acid unfolded cytochrome c to molten globule state as a result of electrostatic interactions. *Biochemistry*, *38*(41), 13635-13642.

Aronsson, K., & Rönner, U. (2001). Influence of pH, water activity and temperature on the inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* by pulsed electric fields. *Innovative Food Science & Emerging Technologies*, *2*(2), 105-112.

Arzeni, C., Pérez, O. E., & Pilosof, A. M. (2012). Functionality of egg white proteins as affected by high intensity ultrasound. *Food Hydrocolloids*, *29*(2), 308-316.

Asada, K., & Kanematsu, S. (1976). Reactivity of thiols with superoxide radicals. *Agricultural and Biological Chemistry*, *40*(9), 1891-1892.

Ashokkumar, M., Sunartio, D., Kentish, S., Mawson, R., Simons, L., Vilkhu, K., & Versteeg, C. K. (2008). Modification of food ingredients by ultrasound to improve

functionality: a preliminary study on a model system. *Innovative Food Science & Emerging Technologies*, *9*(2), 155-160.

Awuah, G. B., Ramaswamy, H. S., & Economides, A. (2007). Thermal processing and quality: Principles and overview. *Chemical Engineering and Processing: Process Intensification*, *46*(6), 584-602.

Baek, K. H., Yong, H. I., Yoo, J. H., Kim, J. W., Byeon, Y. S., Lim, J., ... & Jo, C. (2020). Antimicrobial effects and mechanism of plasma activated fine droplets produced from arc discharge plasma on planktonic *Listeria monocytogenes* and *Escherichia coli* O157:H7. *Journal of Physics D: Applied Physics*, *53*(12), 124002.

Bárdos, L., & Baránková, H. (2010). Cold atmospheric plasma: Sources, processes, and applications. *Thin Solid Films*, *518*(23), 6705-6713.

Bermúdez-Aguirre, D., Wemlinger, E., Pedrow, P., Barbosa-Cánovas, G., & Garcia-Perez, M. (2013). Effect of atmospheric pressure cold plasma (APCP) on the inactivation of *Escherichia coli* in fresh produce. *Food Control*, *34*(1), 149-157.

Brandenburg, R. (2017). Dielectric barrier discharges: progress on plasma sources and on the understanding of regimes and single filaments. *Plasma Sources Science and Technology*, *26*(5), 053001.

Chang, C., Li, X., Li, J., Niu, F., Zhang, M., Zhou, B., ... & Yang, Y. (2017). Effect of enzymatic hydrolysis on characteristics and synergistic efficiency of pectin on emulsifying properties of egg white protein. *Food Hydrocolloids*, *65*, 87-95.

Chen, Y., Sheng, L., Gouda, M., & Ma, M. (2019). Impact of ultrasound treatment on the foaming and physicochemical properties of egg white during cold storage. *LWT*, *113*, 108303.

Conrads, H., & Schmidt, M. (2000). Plasma generation and plasma sources. *Plasma Sources Science and Technology*, *9*(4), 441.

Croguennec, T., Nau, F., & Brule, G. (2002). Influence of pH and salts on egg white gelation. *Journal of Food Science*, *67*(2), 608-614.

Cutler, T. D., & Zimmerman, J. J. (2011). Ultraviolet irradiation and the mechanisms underlying its inactivation of infectious agents. *Animal Health Research Reviews*, *12*(1), 15.

Delahaije, R. J., Gruppen, H., Giuseppin, M. L., & Wierenga, P. A. (2014). Quantitative description of the parameters affecting the adsorption behaviour of globular proteins. *Colloids and surfaces B: Biointerfaces*, *123*, 199-206.

Delahaije, R. J., Wierenga, P. A., Giuseppin, M. L., & Gruppen, H. (2014). Improved emulsion stability by succinylation of patatin is caused by partial unfolding rather than charge effects. *Journal of Colloid and Interface Science*, *430*, 69-77.

Dirks, B. P., Dobrynin, D., Fridman, G., Mukhin, Y., Fridman, A., & Quinlan, J. J. (2012). Treatment of raw poultry with nonthermal dielectric barrier discharge plasma to reduce Campylobacter jejuni and *Salmonella* enterica. *Journal of Food Protection*, *75*(1), 22-28.

Dobrynin, D., Fridman, G., Friedman, G., & Fridman, A. (2009). Physical and biological mechanisms of direct plasma interaction with living tissue. *New Journal of Physics*, *11*(11), 115020.

Duan, X., Li, M., Shao, J., Chen, H., Xu, X., Jin, Z., & Liu, X. (2018). Effect of oxidative modification on structural and foaming properties of egg white protein. *Food Hydrocolloids*, *75*, 223-228.

Eaton, P. (2006). Protein thiol oxidation in health and disease: techniques for measuring disulfides and related modifications in complex protein mixtures. *Free Radical Biology and Medicine*, *40*(11), 1889-1899.

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Von Woedtke, T., Brandenburg, R., ... & Weltmann, K. D. (2010). Low temperature atmospheric pressure plasma sources for microbial decontamination. *Journal of Physics D: Applied Physics*, *44*(1), 013002.

Ekezie, F. G. C., Sun, D. W., & Cheng, J. H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science & Technology*, *69*, 46-58.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*(1), 70-77.

Fedele, E., & Bergamo, P. (2001). Protein and lipid oxidative stresses during cheese manufacture. *Journal of Food Science*, *66*(7), 932-935.

Fernández, A., & Thompson, A. (2012). The inactivation of *Salmonella* by cold atmospheric plasma treatment. *Food Research International*, *45*(2), 678-684.

Fridman, A., Chirokov, A., & Gutsol, A. (2005). Non-thermal atmospheric pressure discharges. *Journal of Physics D: Applied Physics*, *38*(2), R1.

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.

Gaucheron, F., Famelart, M. H., Mariette, F., Raulot, K., Michela, F., & Le Graeta, Y. (1997). Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. *Food Chemistry*, *59*(3), 439-447.

Goodarzi, F., & Zendehboudi, S. (2019). A comprehensive review on emulsions and emulsion stability in chemical and energy industries. *The Canadian Journal of Chemical Engineering*, *97*(1), 281-309.

Guo, J., Huang, K., & Wang, J. (2015). Bactericidal effect of various non-thermal plasma agents and the influence of experimental conditions in microbial inactivation: A review. *Food Control*, *50*, 482-490.

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P. J., & Bourke, P. (2016). Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. *Applied and Environmental Microbiology*, *82*(2), 450-458.

Heuer, K., Hoffmanns, M. A., Demir, E., Baldus, S., Volkmar, C. M., Röhle, M., ... & Opländer, C. (2015). The topical use of non-thermal dielectric barrier discharge (DBD): Nitric oxide related effects on human skin. *Nitric Oxide*, *44*, 52-60.

Hoffmann, H., & Reger, M. (2014). Emulsions with unique properties from proteins as emulsifiers. *Advances in Colloid and Interface Science*, *205*, 94-104.

Huang, M., Zhuang, H., Zhao, J., Wang, J., Yan, W., & Zhang, J. (2020a). Differences in cellular damage induced by dielectric barrier discharge plasma between *Salmonella* Typhimurium and *Staphylococcus aureus*. *Bioelectrochemistry*, *132*, 107445.

Huang, Y. M., Chen, C. K., & Hsu, C. L. (2020b). Non-thermal atmospheric gas plasma for decontamination of sliced cheese and changes in quality. *Food Science and Technology International*, 1082013220925931.

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins. *Journal of Food Engineering*, *93*(4), 386-393.

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.

Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., & Chen, Y. (2018). Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, *11*(2), 344-354.

Ji, S. H., Ki, S. H., Ahn, J. H., Shin, J. H., Hong, E. J., Kim, Y. J., & Choi, E. H. (2018). Inactivation of *Escherichia coli* and *Staphylococcus aureus* on contaminated perilla leaves by dielectric barrier discharge (DBD) plasma treatment. *Archives of Biochemistry and Biophysics*, *643*, 32-41.

Jo, C., Lee, J. I., & Ahn, D. U. (1999). Lipid oxidation, color changes and volatiles production in irradiated pork sausage with different fat content and packaging during storage. *Meat Science*, *51*(4), 355-361.

Joshi, S. G., Cooper, M., Yost, A., Paff, M., Ercan, U. K., Fridman, G., & Brooks, A. D. (2011). Nonthermal dielectric-barrier discharge plasma-induced inactivation

involves oxidative DNA damage and membrane lipid peroxidation in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, *55*(3), 1053-1062.

Judée, F., Simon, S., Bailly, C., & Dufour, T. (2018). Plasma-activation of tap water using DBD for agronomy applications: Identification and quantification of long lifetime chemical species and production/consumption mechanisms. *Water Research*, *133*, 47-59.

Karimi, M., Ignasiak, M. T., Chan, B., Croft, A. K., Radom, L., Schiesser, C. H., & Davies, M. J. (2016). Reactivity of disulfide bonds is markedly affected by structure and environment: implications for protein modification and stability. *Scientific Reports*, *6*, 38572.

Kim, B., Yun, H., Jung, S., Jung, Y., Jung, H., Choe, W., & Jo, C. (2011). Effect of atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two different gas compositions. *Food Microbiology*, *28*(1), 9-13.

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*(7), 1420-1425.

Kim, H. J., Yong, H. I., Park, S., Kim, K., Choe, H., & Jo, C. (2015), Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. *Food Control*, 47, 451-456.

Kim, J., Park, S., Park, J., & Choe, W. (2018, July). Characteristics of Inkjet-Printed Dielectric Barrier Discharge Source. In *The 7th International Conference on Microelectronics and Plasma Technology*. Songdo Convensia.

Kim, Y., Imm, J. Y., Kim, I. H., & Kim, S. J. (2019). Functional and Emulsifying Properties of Balloonflower Seeds Oil Extracted by Supercritical Carbon Dioxide. *Journal of the Korean Applied Science and Technology*, *36*(1), 226-236.

Korachi, M., Gurol, C., & Aslan, N. (2010). Atmospheric plasma discharge sterilization effects on whole cell fatty acid profiles of *Escherichia coli* and *Staphylococcus aureus*. *Journal of Electrostatics*, *68*(6), 508-512.

Korachi, M., & Aslan, N. (2011). The effect of atmospheric pressure plasma corona discharge on pH, lipid content and DNA of bacterial cells. *Plasma Science and Technology*, *13*(1), 99.

Kuan, Y. H., Bhat, R., & Karim, A. A. (2011). Emulsifying and foaming properties of ultraviolet-irradiated egg white protein and sodium caseinate. *Journal of Agricultural and Food Chemistry*, *59*(8), 4111-4118.

Laroussi, M. (1996). Sterilization of contaminated matter with an atmospheric pressure plasma. *IEEE Transactions on Plasma Science*, *24*(3), 1188-1191.

Laroussi, M. (2002). Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. *IEEE Transactions on Plasma Science*, *30*(4), 1409-1415.

Laroussi, M., & Leipold, F. (2004). Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *International Journal of Mass Spectrometry*, *233*(1-3), 81-86.

Lee, H. J., Jung, S., Jung, H., Park, S., Choe, W., Ham, J. S., & Jo, C. (2012). Evaluation of a dielectric barrier discharge plasma system for inactivating

pathogens on cheese slices. *Journal of Animal Science and Technology*, *54*, 191-198.

Lee, H., Yong, H. I., Kim, H. J., Choe, W., Yoo, S. J., Jang, E. J., & Jo, C. (2016a). Evaluation of the microbiological safety, quality changes, and genotoxicity of chicken breast treated with flexible thin-layer dielectric barrier discharge plasma. *Food Science and Biotechnology*, *25*(4), 1189-1195.

Lee, K. H., Kim, H. J., Woo, K. S., Jo, C., Kim, J. K., Kim, S. H., & Kim, W. H. (2016b). Evaluation of cold plasma treatments for improved microbial and physicochemical qualities of brown rice. *LWT*, *73*, 442-447.

Lee, J., Lee, C. W., Yong, H. I., Lee, H. J., Jo, C., & Jung, S. (2017). Use of atmospheric pressure cold plasma for meat industry. *Korean Journal for Food Science of Animal Resources*, *37*(4), 477.

Li, S., Huang, Y., An, F., Huang, Q., Geng, F., & Ma, M. (2019). Hydroxyl radical-induced early stage oxidation improves the foaming and emulsifying properties of ovalbumin. *Poultry Science*, *98*(2), 1047-1054.

Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., & Ding, T. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control*, *75*, 83-91.

Liu, X. D., Han, R. X., Yun, H., Jung, K. C., Jin, D. I., Lee, B. D., & Jo, C. (2009). Effect of irradiation on foaming properties of egg white proteins. *Poultry Science*, *88*(11), 2435-2441.

Lu, X., Ye, T., Cao, Y., Sun, Z., Xiong, Q., Tang, Z., & Pan, Y. (2008). The roles of the various plasma agents in the inactivation of bacteria. *Journal of Applied Physics*, *104*(5), 053309.

Machado, F. F., Coimbra, J. S., Rojas, E. E. G., Minim, L. A., Oliveira, F. C., & Rita de Cássia, S. S. (2007). Solubility and density of egg white proteins: Effect of pH and saline concentration. *LWT*, *40*(7), 1304-1307.

Mahajan, D., Bhat, Z. F., & Kumar, S. (2015). Pomegranate (Punica granatum) rind extract as a novel preservative in cheese. *Food Bioscience*, *12*, 47-53.

Mahnot, N. K., Siyu, L. P., Wan, Z., Keener, K. M., & Misra, N. N. (2020). In-package cold plasma decontamination of fresh-cut carrots: microbial and quality aspects. *Journal of Physics D: Applied Physics*, *53*(15), 154002.

Mai-Prochnow, A., Clauson, M., Hong, J., & Murphy, A. B. (2016). Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. *Scientific Reports*, *6*, 38610.

Mandal, R., Singh, A., & Singh, A. P. (2018). Recent developments in cold plasma decontamination technology in the food industry. *Trends in Food Science & Technology*, *80*, 93-103.

Mine, Y., Noutomi, T., & Haga, N. (1990). Thermally induced changes in egg white proteins. *Journal of Agricultural and Food Chemistry*, *38*(12), 2122-2125.

Mine, Y., Noutomi, T., & Haga, N. (1991). Emulsifying and structural properties of ovalbumin. *Journal of Agricultural and Food Chemistry*, *39*(3), 443-446.

Monahan, F. J., German, J. B., & Kinsella, J. E. (1995). Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced

gelation of whey proteins. *Journal of Agricultural and Food Chemistry*, *43*(1), 46-52.

Montie, T. C., Kelly-Wintenberg, K., & Roth, J. R. (2000). An overview of research using the one atmosphere uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials. *IEEE Transactions on Plasma Science*, *28*(1), 41-50.

Moreau, M., Orange, N., & Feuilloley, M. G. J. (2008). Non-thermal plasma technologies: new tools for bio-decontamination. *Biotechnology Advances*, *26*(6), 610-617.

Moutiq, R., Misra, N. N., Mendonca, A., & Keener, K. (2020). In-package decontamination of chicken breast using cold plasma technology: Microbial, quality and storage studies. *Meat Science*, *159*, 107942.

Nakai, S. (1983). Structure-function relationships of food proteins: with an emphasis on the importance of protein hydrophobicity. *Journal of Agricultural and Food Chemistry*, *31*(4), 676-683.

Nehra, V., Kumar, A., & Dwivedi, H. K. (2008). Atmospheric non-thermal plasma sources. *International Journal of Engineering*, *2*(1), 53-68.

Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology*, *3*, 125-142.

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K. D., & Von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers*, *7*(3-4), 250-257.

Oguma, K., Katayama, H., Mitani, H., Morita, S., Hirata, T., & Ohgaki, S. (2001). Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. *Applied and Environmental Microbiology*, *67*(10), 4630-4637.

Oh, K. N., & Lee, S. Y. (2004). Effects of gamma irradiation on the functional and structural characteristics of soy protein isolates. *Korean Journal of Food and Cookery Science*, *20*, 256-264.

Oh, J. S., Aranda-Gonzalvo, Y., & Bradley, J. W. (2011). Time-resolved mass spectroscopic studies of an atmospheric-pressure helium microplasma jet. *Journal of Physics D: Applied Physics*, *44*(36), 365202.

Osae, R., Essilfie, G., Alolga, R. N., Akaba, S., Song, X., Owusu-Ansah, P., & Zhou, C. (2020). Application of non-thermal pretreatment techniques on agricultural products prior to drying: a review. *Journal of the Science of Food and Agriculture*, *100*(6), 2585-2599.

Ozen, E., & Singh, R. K. (2020). Atmospheric cold plasma treatment of fruit juices: A review. *Trends in Food Science & Technology.*

Park, S., Choe, W., & Jo, C. (2018). Interplay among ozone and nitrogen oxides in air plasmas: Rapid change in plasma chemistry. *Chemical Engineering Journal*, *352*, 1014-1021.

Park, J. S., & Ha, J. W. (2019). X-ray irradiation inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* on sliced cheese and its bactericidal mechanisms. *International Journal of Food Microbiology*, *289*, 127-133.

Park, J. S., & Ha, J. W. (2020). Synergistic antimicrobial effect of X-ray and curcumin against *Listeria monocytogenes* on sliced cheese. *Food Control*, *110*, 106986.

Pastorino, A. J., Hansen, C. L., & McMahon, D. J. (2003). Effect of pH on the chemical composition and structure-function relationships of Cheddar cheese. *Journal of Dairy Science*, *86*(9), 2751-2760.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry*, *26*(3), 716-723.

Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey proteins solubility as function of temperature and pH. *LWT*, *38*(1), 77-80.

Pereira, R. N., & Vicente, A. A. (2010). Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International*, *43*(7), 1936-1943.

Rød, S. K., Hansen, F., Leipold, F., & Knøchel, S. (2012). Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Food Microbiology*, *30*(1), 233-238.

Roehm, J. N., Hadley, J. G., & Menzel, D. B. (1971). Oxidation of unsaturated fatty acids by ozone and nitrogen dioxide. *Archives of Environmental Health: An International Journal*, *23*(2), 142-148.

Ryu, Y. H., Uhm, H. S., Park, G. S., & Choi, E. H. (2013). Sterilization of Neurospora crassa by noncontacted low temperature atmospheric pressure surface discharged

plasma with dielectric barrier structure. *Journal of the Korean Vacuum Society*, *22*(2), 55-65.

Sadler, G. D., & Murphy, P. A. (2010). pH and titratable acidity. In *Food analysis* (pp. 219-238). Springer, Boston, MA.

Sanabria, C., Martin-Alvarez, P. J., & Carrascosa, A. V. (2004). Colour and moisture changes during the manufacture of Iberian dry-cured ham caused by some biotic and abiotic factors. *Food Science and Technology International*, *10*(4), 269-275.

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2015). Atmospheric pressure cold plasma (ACP) treatment of whey protein isolate model solution. *Innovative Food Science & Emerging Technologies*, *29*, 247-254.

Shan, B., Cai, Y. Z., Brooks, J. D., & Corke, H. (2011). Potential application of spice and herb extracts as natural preservatives in cheese. *Journal of Medicinal Food*, *14*(3), 284-290.

Sharifian, A., Soltanizadeh, N., & Abbaszadeh, R. (2019). Effects of dielectric barrier discharge plasma on the physicochemical and functional properties of myofibrillar proteins. *Innovative Food Science & Emerging Technologies*, *54*, 1-8.

Sharma, P., Segat, A., Kelly, A. L., & Sheehan, J. J. (2020). Colorants in cheese manufacture: Production, chemistry, interactions, and regulation. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 1220-1242.

Sheng, L., Wang, Y., Chen, J., Zou, J., Wang, Q., & Ma, M. (2018). Influence of high-intensity ultrasound on foaming and structural properties of egg white. *Food Research International*, *108*, 604-610.

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.

Smet, C., Noriega, E., Rosier, F., Walsh, J. L., Valdramidis, V. P., & Van Impe, J. F. (2017). Impact of food model (micro) structure on the microbial inactivation efficacy of cold atmospheric plasma. *International Journal of Food Microbiology*, *240*, 47-56.

Stefanović, A. B., Jovanović, J. R., Dojčinović, M. B., Lević, S. M., Nedović, V. A., Bugarski, B. M., & Knežević-Jugović, Z. D. (2017). Effect of the controlled high-intensity ultrasound on improving functionality and structural changes of egg white proteins. *Food and Bioprocess Technology*, *10*(7), 1224-1239.

Stoffels, E., Sakiyama, Y., & Graves, D. B. (2008). Cold atmospheric plasma: charged species and their interactions with cells and tissues. *IEEE Transactions on Plasma Science*, *36*(4), 1441-1457.

Stoica, M., Mihalcea, L., Borda, D., & Alexe, P. (2013). Non-thermal novel food processing technologies. An overview. *Journal of Agroalimentary Processes and Technologies*, *19*(2), 212-217.

Subirade, M., Kelly, I., Guéguen, J., & Pézolet, M. (1998). Molecular basis of film formation from a soybean protein: comparison between the conformation of glycinin in aqueous solution and in films. *International Journal of Biological Macromolecules*, *23*(4), 241-249.

Tolouie, H., Mohammadifar, M. A., Ghomi, H., & Hashemi, M. (2018). Cold atmospheric plasma manipulation of proteins in food systems. *Critical Reviews in Food Science and Nutrition*, *58*(15), 2583-2597.

Viljanen, K., Halmos, A. L., Sinclair, A., & Heinonen, M. (2005). Effect of blackberry and raspberry juice on whey protein emulsion stability. *European Food Research and Technology*, *221*(5), 602.

Wadhwani, R., & McMahon, D. J. (2012). Color of low-fat cheese influences flavor perception and consumer liking. *Journal of Dairy Science*, *95*(5), 2336-2346.

Wan, Z., Pankaj, S. K., Mosher, C., & Keener, K. M. (2019). Effect of high voltage atmospheric cold plasma on inactivation of *Listeria innocua* on Queso Fresco cheese, cheese model and tryptic soy agar. *LWT*, *102*, 268-275.

Wu, W., Hou, L., Zhang, C., Kong, X., & Hua, Y. (2009). Structural modification of soy protein by 13-hydroperoxyoctadecadienoic acid. *European Food Research and Technology*, *229*(5), 771-778.

Wu, W., Zhang, C., Kong, X., & Hua, Y. (2009). Oxidative modification of soy protein by peroxyl radicals. *Food Chemistry*, *116*(1), 295-301.

Wu, L., Zhao, W., Yang, R., & Chen, X. (2014). Effects of pulsed electric fields processing on stability of egg white proteins. *Journal of Food Engineering*, *139*, 13-18.

Wu, W., Hua, Y., & Lin, Q. (2014). Effects of oxidative modification on thermal aggregation and gel properties of soy protein by malondialdehyde. *Journal of Food Science and Technology*, *51*(3), 485-493.

Xiong, T., Xiong, W., Ge, M., Xia, J., Li, B., & Chen, Y. (2018). Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. *Food Research International*, *109*, 260-267.

Yadav, B., Spinelli, A. C., Govindan, B. N., Tsui, Y. Y., McMullen, L. M., & Roopesh, M. S. (2019). Cold plasma treatment of ready-to-eat ham: Influence of process conditions and storage on inactivation of *Listeria innocua*. *Food Research International*, *123*, 276-285.

Yeung, R. M., & Morris, J. (2001). Food safety risk. *British Food Journal*.

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.

Yong, H. I., Kim, H. J., Park, S., Kim, K., Choe, W., Yoo, S. J., & Jo, C. (2015). Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin-layer dielectric barrier discharge plasma. *Food Research International*, *69*, 57-63.

Yong, H. I., Lee, H., Park, S., Park, J., Choe, W., Jung, S., & Jo, C. (2017). Flexible thin-layer plasma inactivation of bacteria and mold survival in beef jerky packaging and its effects on the meat's physicochemical properties. *Meat Science*, *123*, 151-156.

Yoo, S. J. (2015). Status of plasma technology applied to agriculture and foods. *Vacuum Magazine*, *2*(4), 4-8.

Yoo, J. H., Baek, K. H., Heo, Y. S., Yong, H. I., & Jo, C. (2020). Synergistic bactericidal effect of clove oil and encapsulated atmospheric pressure plasma against *Escherichia coli* O157:H7 and *Staphylococcus aureus* and its mechanism of action. *Food Microbiology*, *93*, 103611.

Yu, Y. H., Kim, S. H., Park, G. S., & Choe, E. H. (2013). Sterilization of neurospora crassa by noncontacted low temperature atmospheric pressure surface discharged plasma with dielectric barrier structure. In *Proceedings of the Korean Vacuum Society Conference* (pp. 519-519). The Korean Vacuum Society.

Yun, H., Kim, B., Jung, S., Kruk, Z. A., Kim, D. B., Choe, W., & Jo, C. (2010). Inactivation of *Listeria monocytogenes* inoculated on disposable plastic tray, aluminum foil, and paper cup by atmospheric pressure plasma. *Food Control*, *21*(8), 1182-1186.

Yusupov, M., Bogaerts, A., Huygh, S., Snoeckx, R., van Duin, A. C., & Neyts, E. C. (2013). Plasma-induced destruction of bacterial cell wall components: a reactive molecular dynamics simulation. *The Journal of Physical Chemistry C*, *117*(11), 5993-5998.

Zayas, J. F. (2012). *Functionality of proteins in food*. Springer science & business media.

Zhang, T., Xue, Y., Li, Z., Wang, Y., Yang, W., & Xue, C. (2015). Effects of ozone-induced oxidation on the physicochemical properties of myofibrillar proteins recovered from bighead carp (Hypophthalmichthys nobilis). *Food and Bioprocess Technology*, *8*(1), 181-190.

Summary in Korean

슬라이스 치즈의 이화학적 및 미생물학적 특성과 난백 단백질의 기능적 특성에 대한 유전체 장벽 방전 플라즈마의 영향

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본 연구에서는 유전체 장벽 방전 플라즈마를 이용하여 슬라이스 체다 치즈의 이화학적, 미생물학적 특성 및 난백 단백질의 기능적 특성에 대한 영향을 알아보았다. 본문은 총 2개의 실험으로 구성되어 있으며 실험 1은 식품 산업에서의 효율적인 적용을 위해 개발된 새로운 기술인 inkjet-printed FXDBD를 사용하여 슬라이스 체다 치즈에서의 미생물 불활성화 및 품질 변화를 조사하였다. 슬라이스 체다 치즈에 0, 5, 10, 15 및 20분 inkjet-printed FXDBD 플라즈마 처리 하였을 때, 대장균 O157:H7(*Escherichia coli* O157:H7) 및 리스테리아 모노사이토제니스(*Listeria monocytogenes*)에서 효과적인 미생물 살균 능력을 보였으며, 플라즈마 처리 20분 뒤에는 *E. coli* O157:H7과 *Listeria monocytogenes*는 각각 2.65 log CFU/g 및 2.03 log CFU/g 살균 되었

다. 활성종 분석 결과 inkjet-printed FXDBD 플라즈마에서 주된 살균 역할을 한 활성종은 오존과 질소 산화물로 확인 할 수 있었다. 플라즈마 처리 후 슬라이스 치즈의 pH는 대조군에 비해 유의미하게 감소하였지만 처리 시간에 따른 유의한 차이는 없었다. 플라즈마 처리 시간이 길어짐에 따라 밝기 (L^*)는 감소하였고 황색도 (b^*) 및 지방산패도를 나타내는 TBARS 값은 증가한 반면 적색도 (a^*) 및 총 색 차이 (ΔE)는 변하지 않았다. 본 연구 결과를 통해 새로 개발된 inkjet-printed FXDBD 플라즈마 처리가 병원균을 효과적으로 불활성화 시킬 수 있었다. 하지만 inkjet-printed FXDBD 플라즈마는 슬라이스 체다 치즈의 일부 항목에서 미미한 품질 변화를 야기하였으며, 이를 방지하기 위한 추가 연구가 필요하다고 본다.

실험 2에서는 유전체 장벽 방전 플라즈마(EDBD)가 난백 단백질의 구조 및 기능적 특성에 미치는 영향에 대해 연구하였다. 난백 용액은 0.1 M 인산염 완충용액(pH 7.4)으로 1%의 단백질 농도로 희석하여 사용하였다. 난백 용액을 0, 10, 20, 30 및 40분 동안 플라즈마 처리하였을 때, 플라즈마 처리시간이 증가할수록 난백 용액의 온도는 유의미하게 증가하였고 pH는 유의미하게 감소하였다. 하지만 이와 같은 온도나 pH의 변화는 단백질 변성 조건인 60 ° C 및 알칼리 pH 조건에 속하지 않아 난백 단백질 구조 변화에 영향을 미치지 않았을 것으로 사료된다. Free SH기의 농도 측정 결과와 표면 소수성 결과를 통해 플라즈마 30분 처리 후 난백 단백질 구조가

펼쳐진 것을 확인 하였다. 또한, 플라즈마 처리된 난백 용액의 기능적 특성을 확인하기 위해 거품형성 특성과 유화 특성을 측정하였다. 플라즈마 30분 처리시 거품 형성능 및 유화 안정성에서 가장 좋은 결과를 보였으며, 플라즈마 40분 처리 후에는 과도한 산화에 의한 단백질 응집체가 형성되어 거품 형성능 및 유화 안정성이 다시 감소하는 경향을 보였다. 따라서 EDBD 플라즈마 30분 처리가 난백 단백질의 기능적 특성을 향상시키는 효과적인 적정 처리 시간으로 생각된다.

본 연구들의 결과에 의하면 유전체 장벽 방전 플라즈마 처리는 슬라이스 체다 치즈에서의 병원균 살균 및 난백 단백질의 기능적 특성을 향상시키는데 효과적이었으며, 식품 산업에서 유용하게 활용될 수 있을 것으로 사료된다.