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ARTICLE

Effect of Atmospheric Pressure Plasma Jet on Inactivation of *Listeria monocytogenes*, Quality, and Genotoxicity of Cooked Egg White and Yolk

Hyun Jung Lee, Hyun Pa Song[1], Heesoo Jung[2], Wonho Choe[3], Jun Sang Ham[4], Jun Heon Lee, and Cheorun Jo*

Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea
[1]Department of Radiation Biotechnology, Advanced Radiation Technology Institute, Jeongeup 580-185, Korea
[2]Agency for Defense Development, Daejeon 305-152, Korea
[3]Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea
[4]Quality Control and Utilization Division, National Institute of Animal Science, RDA, Suwon 441-706, Korea

Abstract

The objective of this study was to evaluate the effects of an atmospheric pressure plasma (APP) jet on *L. monocytogenes* inactivation, quality characteristics, and genotoxicological safety of cooked egg white and yolk. APP treatment using He gas resulted in a 5 decimal reduction in the number of *L. monocytogenes* in cooked egg white, whereas that using He+O_2, N_2, and N_2+O_2 decreased the number further, and to undetectable levels. All treatments of cooked egg yolk resulted in undetectable levels of inoculated *L. monocytogenes*. There were no viable cells of total aerobic bacteria after APP treatment on day 0 while the control showed approximately 3-4 Log CFU/g. On day 7, the numbers of total aerobic bacteria had increased by approximately 3 log cycles in cooked egg white, but there were no viable cells in cooked egg yolk after 2 min of APP jet. APP treatment decreased the L*-values of cooked egg white and yolk significantly on day 0. No significant sensory differences were found among the cooked egg white samples, whereas significant reductions in flavor, taste, and overall acceptability were found in cooked egg yolks treated with APP jets. SOS chromotest did not reveal the presence of genotoxic products following APP treatments of cooked egg white and yolk. Therefore, it can be concluded that APP jets can be used as a non-thermal means to enhance the safety and extend the shelf-life of cooked egg white and yolk.

Key words: atmospheric pressure plasma jet, cooked egg, *L. monocytogenes*, quality, genotoxicity

Introduction

Even though product safety is the ultimate goal in the food industry, outbreaks of foodborne diseases become more frequent every year. According to the Korea Food and Drug Administration, foodborne diseases in Korea have increased 1.2 folds from 2009 to 2010 (KFDA, 2011).

To enhance food safety, studies on non-thermal treatments such as irradiation, high hydrostatic pressure, and natural antimicrobials have been encouraged due to their possible use in fresh foods (Devlieghere *et al*., 2004). These non-thermal treatments are known to be more favorable as they are characterized by minimum changes in sensorial, nutritional, and functional characteristics compared with thermal treatment (Yun *et al*., 2010). However, they also have some limitations. Irradiation may produce sensorial and nutritional losses, off-flavor, and lipid oxidation after treatment of certain dose levels or higher, and, more importantly, it must overcome the negative impression held by customers. High pressure has limitations in commercial use due to batch size and its adverse effects on lipid oxidation and texture in treated foods (Kruk *et al*., 2011).

Atmospheric pressure plasma (APP) is considered as an emerging method for enhancing food safety (Lee *et al*., 2006). APP was developed to be applied for surface modification in engineering and environmental and biomedical fields (Bogaerts *et al*., 2002). The active species generated include atoms, molecules, radicals, and UV photons, have an inactivation effect through chemical reactions, resulting in the destruction of the genetic materials of microorganisms (Moisan *et al*., 2001; Moisan *et al*., 2002). Basaran *et al*. (2008) applied sulfur hexafluoride (SF_6) and air APP to the surface of nuts and reported

*Corresponding author: Cheorun Jo, Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea. Tel: 82-42-821-5774, Fax: 82-42-825-9754, E-mail: cheorun@cnu.ac.kr

effective inactivation of *Aspergillues parasiticus* using both gases. Furthermore, sensorial acceptance of different nuts did not change upon APP treatment. Niemira *et al*. (2008) investigated the inactivation effect of APP in apples and found that exposure time and flow rate were factors affecting the inactivation effect of APP as well as the characteristics of the microorganisms. Moon *et al*. (2009) investigated the effect of APP treatment on pork and human skin. There was no electrical or thermal damage produced by the treatment. Ragni *et al*. (2010) reported effective pathogen inactivation with no specific quality changes in egg, including surface color, pH, weight loss, and yolk index, when APP was applied to egg shells. Kim *et al*. (2011) whilst treating bacon and found that the inactivation of pathogens increased as exposure time and input power were increased. Also, O_2 addition increased the efficiency of the APP, which is agreed well with Gweon *et al*. (2009). The authors also reported that no specific quality changes were found in APP-treated bacon, including pH, TBARS value, and surface color.

The previous studies clearly demonstrate potential of using APP for enhancing safety and extending shelf-life of foods. However, there are some limitations, including broad variation in inactivation efficiency stemming from the different surface characteristics of the foods when using large area-type APP (Song *et al*., 2009a; Yun *et al*., 2010). For food use, previous studies revealed that APP with a pen-type device possesses high inactivation efficiency against *Listeria monocytogenes* in a model system and/or on the surface of chicken breast and ham (Lee *et al*., 2011). However, quality aspect, including sensory quality which is the most important in food use for APP and information on the genotoxicological safety of APP-treated food products remains unknown.

Therefore, the objective of this study was to evaluate the effects of APP jet on the inactivation of *L. monocytogenes* and quality characteristics of model cooked egg white and yolk product. The genotoxicological safety of APP-treated samples was also screened using SOS chromotest.

Materials and Methods

Sample preparation

Eggs were purchased from a local market in Daejeon, Korea. The samples were transferred to a laboratory, broken, and separated into egg white and yolk. Egg white and yolk were poured into petri dishes (45 mL each, Difco Laboratories, USA) and steamed in water bath for 30 min at 90°C. After cooking and cooling in air, the cooked egg white and yolk samples were taken out and cut into 10×10×1 mm (length×width×height) sections. Prior to inoculation of *L. monocytogenes*, the samples in petri dishes (50×10 mm, Difco) were sterilized using UV light for 30 min.

Microorganism and inoculation

L. monocytogenes (KCTC 3596) obtained from the Korean Collection for Type Culture (KCTC, Korea) was cultured at 37°C for 18 h in tryptic soy broth (50 mL) (Difco Laboratories, USA). The culture was transferred to a 50 mL centrifuge tube and centrifuged (2,090 *g* for 10 min at 4°C) in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial Co., Ltd., Korea). The pellet was then washed twice with sterile saline (0.85%) and suspended in saline to a final concentration of approximately 10^8 CFU/mL. The test culture suspension (10 μL) was inoculated into 5 points of the prepared cooked egg white and yolk and spread. To facilitate the attachment of microorganisms to the samples, the samples were left for 1 h at room temperature (approximately 22°C).

Treatment with APP jet

The device for APP used for the present study was a micro plasma jet (Jung *et al*., 2011; Lee *et al*., 2011). The sample was treated with the plasma produced at 2 kV peak-to-peak voltage. The cooked egg white and yolk samples were treated under plasma for 2 min. He and N_2 were used to generate plasma at a fixed flow rate of 7 L/min. In order to observe the effect of the gas mixture, O_2 (0.07 L/min) was added to each gas treatment. For plasma treatment, inoculated samples were placed on the bottom conductor in direct contact with the plasma. The distance between the powered electrode and the treatment surface was maintained at 4 cm. After the treatment, the samples were stored under commercial storage conditions (10°C) and then measured for their microbial population on days 0 and 7.

Microbiological analysis

After 2 min of APP treatment, cooked egg white and yolk (0.5 g) were vortexed with 4.5 mL of sterile saline (0.85%) for 5 min each. The samples for microbial counts were prepared in a series of decimal dilutions utilizing sterile saline. The media used for *L. monocytogenes* and total aerobic bacterial counts were tryptic soy agar (Difco Laboratories). Each diluent (100 μL) was spread in triplicate, and the plates were incubated at 37°C for 24 h. The

number of microorganisms was counted and expressed as log CFU/g.

Surface color

The samples were placed on a round-shaped quartz cell (8 mm diameter), after which the CIE color value was measured using a Color Difference Meter (Spectrophotometer CM 3500d, Minolta Co., Ltd., Japan). The instrument was calibrated to standard black and white plate before analysis. A small size aperture (3 mm diameter) was used, and three measurements at different sites of each sample were averaged and used as one replication.

pH

The cooked egg white and yolk samples (1 g) were homogenized (1,130 *g*, T25 Basic, Ika Co., Germany) with 9 mL of distilled water for 30 sec, and pH was measured using a pH meter (Model 750, iSTEC, Korea) after calibration using standard buffers from the manufacturer at pH 4, 7, and 10 at room temperature.

2-Thiobarbituric acid-reactive substances (TBARS) value

Three grams of each cooked egg white and yolk sample was homogenized (IKA) with 9 mL of distilled water for 30 sec (1,130 *g*). The homogenate (1 mL) was transferred to a 15 mL test tube and then mixed with TBA/TCA solution (2 mL). The tubes were then heated for 30 min in a water bath (90°C), cooled, and centrifuged at 2,090 *g* (UNION 32R, Hanil Science industrial, Co., Ltd., Korea). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (DU®530, Beckman Instruments Inc., USA). TBARS value (mg malondialdehyde/kg sample) was calculated using a standard curve.

Volatile basic nitrogen (VBN)

VBN measurement in cooked egg white and yolk was carried out following the method of Kruk *et al*. (2011). Three grams from both samples were homogenized (IKA) with 3 mL of distilled water and 6 mL of 10% TCA for 30 sec (1,130 *g*), followed by centrifugation at 2,090 *g* (Hanil) for 15 min. The supernatant was filtered using a filter paper (Whatman No 4), after which the filtrate was placed in a test tube and then made up to a final volume of 30 mL with 5% TCA. A volume of 0.01 N boric acid as a VBN absorber was placed in the inner section of a Conway micro-diffusion cell (Sibata Ltd., Japan). Then, 1 mL of sample solution and 1 mL of saturated K_2CO_3 were also placed into the outer section of the same cell, and the lid was immediately closed. TCA solution (5%) was used as a blank. The cell was incubated at 37°C for 120 min and then titrated against 0.02 N sulfuric acid. VBN value was calculated as ammonia equivalent using the following equation:

$$\text{VBN value (mg\%)} = [0.28\times(\text{titration volume of sample} - \text{titration volume of blank})\times 10]\times 100$$

Sensory evaluation

Ten panelists were used for the sensory evaluation. The panelists had at least 1 year of experience in sensory evaluation, but were not trained specifically for this experiment. The samples, APP-treated cooked egg white and yolk, were cut into 30×30×1 mm (length×width×height) sections before serving to the panelists and then evaluated using a 9-point hedonic scale (1, dislike extremely; 5, neither dislike nor like; 9, like extremely). The parameters for sensory evaluation tested were color, flavor, taste, texture, and overall acceptance. A white-colored plastic tray with a random three-digit number was used to provide the samples to each panelist, and water was provided to rinse the oral cavity during the sensory session.

SOS chromotest

SOS chromotest was carried out following the method of Quillardet and Hofnung (1985) and Song *et al*. (2009b). *E. coli* PQ37 suspension was diluted to 1:50 (v/v) using L-medium (bacto tryptone, bacto yeast extract, NaCl, and ampicillin), then cultured for 2 h (5×10^6 CFU/mL). The solution was prepared in a series of decimal dilutions utilizing L-medium for direct test and S9 mix for indirect test. S9 mix was made using S9 fraction and cofactor (Wako Co., Japan). APP-treated samples and control group (20 μL) were added to the prepared solution (0.6 mL) and then cultured at 37°C for 2 h to induce SOS response. To prevent the influence from the absorbed spectrum, culture solution was centrifuged in a refrigerated centrifuge, the upper layer was discarded, and then L-medium (0.6 mL) was suspended. β-Galactosidase and phosphatase assays were performed with the suspension (0.2 mL). β-Galactosidase assay was performed with 0.2 mL of suspension and 1.8 mL of B buffer (Na_2HPO_4, NaH_2PO_4, H_2O, KCl, $MgSO_4$, $7H_2O$, sodium dodecyl sulfate, and β-mercaptoethanol) at 37°C for 10 min, after which 0.4 mL of o-nitrophenyl-β-D-galactoopyranoside (ONPG) was added for color reaction. Na_2CO_3 (1 M, 1.4 mL) was added to stop the response, and then the absorbance of the supernatant was measured at 415 nm using a spectrophotome-

ter. For alkaline phosphatase assay, P buffer (SDS, Tris), P-nitrophenyl phosphate disodium (PNP, 4 mg/mL), and 3.75 M HCl (0.7 mL) were used instead of B buffer, ONPG, and 1 M Na_2CO_3, respectively. Tris solution (2 M, 0.7 mL) was added for color reaction after 10 min, and then the absorbance of the supernatant was measured at 400 nm using a spectrophotometer.

Statistical analysis

Three different trials were individually carried out, and two observation numbers were obtained per trial. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and significant differences between mean values were identified by Student-Newman-Keul's multiple range test using SAS software with a confidence level at $p<0.05$ (SAS, Release 9.2, SAS Institute Inc., USA). Mean values and standard errors of the mean (SEM) are reported.

Results and Discussion

Inactivation of *L. monocytogenes*

The inoculated level for *L. monocytogenes* was 6.68 and 7.07 Log CFU/g in cooked egg white and yolk, respectively, at the initial stage. APP treatment for 2 min using He resulted in a 5.68 log reduction in the number of *L. monocytogenes*, whereas that using He combined with O_2 decreased the number further to an undetectable level ($<10^1$ CFU/g) in cooked egg white (Fig. 1). APP treatment using N_2 and N_2+O_2 also showed significant inactivation (approximately 7 decimal reductions) of *L. monocytogenes*. All treatments in cooked egg yolk, regardless of the gas treatment used, resulted in undetectable levels of inoculated *L. monocytogenes*. Our previous study in inactivation of *L. monocytogenes* reported 1.37-4.73 log reductions in chicken breast and 1.94-6.52 log reductions in ham using a similar APP jet. APP jet for 2 min reduced the number of *L. monocytogenes* by 0.87, 4.19, 4.26, and 7.59 log cycles when He, He+O_2, N_2, and N_2+O_2 were used, respectively, on an agar model system (Lee *et al*., 2011). This result clearly indicates that APP jet was effective in inactivating *L. monocytogenes* in various food products. When Song *et al*. (2009a) used a large area-type APP at 100 and 150 W of input power, the number of three-strain cocktail *L. monocytogenes* on cheese decreased approximately 2 and 8 log cycles, respectively, but only 1 log cycle on the surface of ham. Kim *et al*. (2011) reported only three decimal reduction upon treating bacon with the same type of APP for 1.5 min at 125 W of input power. The authors also indicated that 150 W of input power produced a significant amount of heat during APP production. Thus, the maximum power for treatment is possibly 125 W when large area-type APP is used.

The initial numbers of total aerobic bacteria were 2.89 and 2.32 Log CFU/g in cooked egg white and yolk, respectively. There were no viable cells counted in any of the samples on day 0 after 2 min of APP jet (Fig. 2). On day 7, the numbers of total aerobic bacteria had increased by approximately 3 log cycles in cooked egg white but no viable cells in cooked egg yolk after 2 min of APP jet. There were no differences found among the samples treated with APP jet using He, He+O_2, and N_2 gas; however, the gas mixture N_2+O_2 showed a significantly lower number of total aerobic bacteria compared to those of the other treatments. Lee *et al*. (2011) also reported APP jet supplied with N_2+O_2 was the most effective in inactivating *L. monocytogenes* on processed meat surfaces.

The results indicate that the inactivation rate was dependent on the nature of the gas, and inactivation was more effective when the gas was combined with O_2 due to the

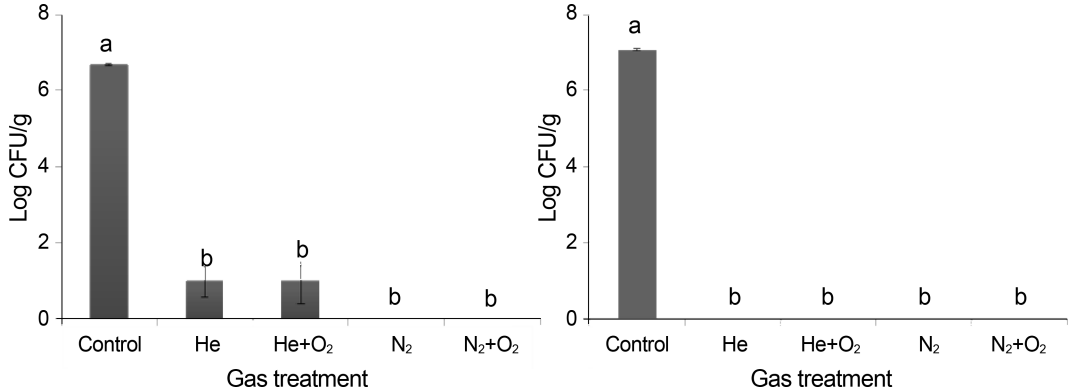

Fig. 1. Effect of atmospheric pressure plasma with different gas compositions on inactivation of *Listeria monocytogenes* inoculated onto cooked egg white (left) and yolk (right). [a,b]Different letters within the same storage day differ significantly ($p<0.05$). [x,y]Different letters within the same gas treatment differ significantly ($p<0.05$).

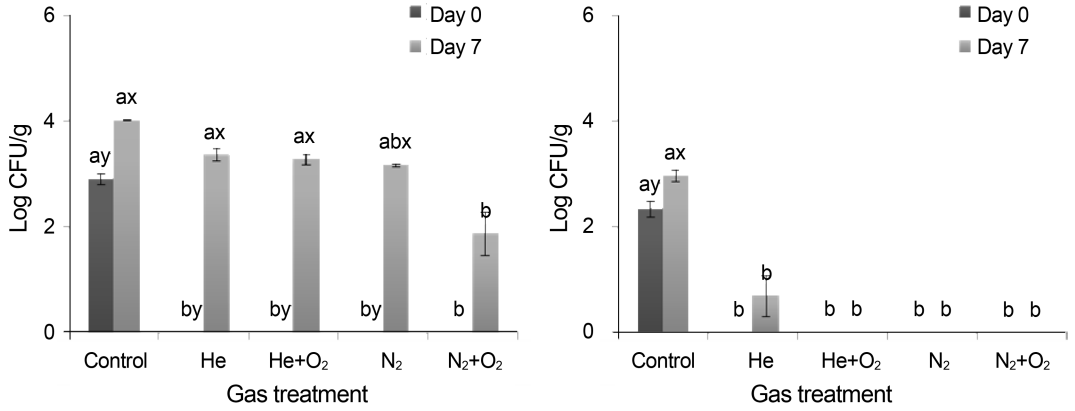

Fig. 2. Effect of atmospheric pressure plasma with different gas compositions on the growth of aerobic bacteria in cooked egg white (left) and yolk (right) during storage for 7 d at 10°C. [a,b]Different letters within the same storage day differ significantly ($p<0.05$). [x,y]Different letters within the same gas treatment differ significantly ($p<0.05$).

greater production of radicals based on O_2 and ozone by APP treatment (Gweon *et al*., 2009; Marsili *et al*., 2002). Yu *et al*. (2006) and Kim *et al*. (2011) also previously found that O_2 addition resulted in more effective inactivation of inoculated pathogens. Montie *et al*. (2000) reported that the inactivation rate of different microorganisms is dependent on cell wall structure. Gram-negative cells tend to be more effectively inactivated since their cell wall is thinner compared to Gram-positive cells. In another research, a 7 decimal reduction in apple juice was obtained by treatment with APP, making the authors concluded that the inactivation rate was more effective as input power was increased (Montenegro *et al*., 2002). However, pathogen inactivation by different APP systems used in previous studies cannot be compared directly due to the different discharge conditions.

Physicochemical properties

APP treatment decreased the L*-values of cooked egg white and yolk significantly on day 0 (Table 1). Kim *et al*. (2011) also reported a decrease in L*-value with an increase in a*-value of bacon after the treatment of large area-type APP. Moisture content is known to correlate with lightness, such that moisture evaporation from the sample during APP treatment can be a possible explanation for the decrease in L*-value (Kim *et al*., 2011). However, there were no notable changes in a*- and b*-value of cooked egg white and yolk as well by sensory evaluation (Table 3). Other studies also reported no visible changes in color when plasma was treated onto nuts (Basaran *et al*., 2008), apples (Niemira *et al*., 2008), pork (Moon *et al*., 2009), or shell egg (Ragni *et al*., 2010).

Longer treatment resulted in a lower pH because H^+ concentration dissociated from bacterial molecules and H_2O increased during APP treatment (Korachi *et al*., 2010). Korachi *et al*. (2010) observed a pH decrease from 7.5 to 1.2 after 20 min of APP treatment in liquid. However, specific pH changes were not shown in the present study (Table 2). Exposure time may not be long enough to cause pH decrease. Kim *et al*. (2011) also did not observe pH changes between APP-treated and non-treated bacon after 60 or 90 s of APP.

It is known that the radicals generated using APP either with or without O_2, can accelerate the production of peroxides, which are intermediate products of lipid oxidation, so that the TBARS value during storage after APP treatment may increase. Kim *et al*. (2011) reported that the TBARS value of APP-treated bacon was lower than that of non-treated bacon on day 0 but then increased after 7 d of storage resulting in higher TBARS value of APP-treated bacon. However, TBARS value was not specifically affected in both APP-treated samples on days 0 and 7 in the present study (Table 2). The changes in TBARS value possibly depends on fat content and fatty acid composition of APP-treated foods (Kim *et al*., 2011). High pressure treatment, another emerging non-thermal technology, treated to chicken breast fillet at pressures up to 600 MPa increased the TBARS value with pressure on day 0 (Kruk *et al*., 2011). Gamma or electron beam irradiation also results in accelerated lipid oxidation (Kim *et al*., 2010). Based on these results, the changes in TBARS value were much smaller in the sample treated with APP compared to other non-thermal processes, indicating APP can be a good candidate of non-thermal technology for food products with high fat content.

VBN content is a method used to measure nitrogen compounds produced during protein catabolism by enzymes from microorganisms (Egan *et al*., 1981). As the micro-

Table 1. Effect of atmospheric pressure plasma with different gas compositions on the surface color of cooked egg white and yolk during storage for 7 d at 10°C

Color value	Input gas[1)]	Cooked egg white		SEM[3)]	Cooked egg yolk		SEM[3)]
		Day 0	Day 7		Day 0	Day 7	
L*	None	76.83^{ax}	75.11^{cy}	0.178	72.82^{ax}	71.77^{ay}	0.118
	He	76.81^{ax}	76.06^{by}	0.110	69.26^{b}	69.13^{b}	0.469
	He+O_2	76.46^{ab}	75.79^{b}	0.176	60.25^{dx}	55.10^{cy}	0.770
	N_2	76.03^{bx}	75.16^{cy}	0.073	61.52^{dy}	69.52^{bx}	0.386
	N_2+O_2	75.04^{cy}	77.43^{ax}	0.299	66.37^{cy}	68.34^{bx}	0.291
	SEM[2)]	0.212	0.152		0.525	0.386	
a*	None	-4.21^{b}	-4.30^{c}	0.077	11.01^{b}	11.19^{b}	0.202
	He	-3.63^{a}	-3.83^{a}	0.055	12.46^{ax}	11.54^{aby}	0.169
	He+O_2	-3.60^{ax}	-4.05^{aby}	0.071	11.24^{b}	11.57^{ab}	0.191
	N_2	-4.11^{b}	-4.08^{b}	0.078	11.34^{by}	12.35^{ax}	0.099
	N_2+O_2	-4.15^{by}	-3.93^{abx}	0.053	10.83^{b}	11.23^{b}	0.421
	SEM[2)]	0.068	0.067		0.201	0.277	
b*	None	5.66^{ax}	2.55^{dy}	0.123	47.36^{by}	51.18^{ax}	0.412
	He	3.77^{b}	3.65^{b}	0.150	53.06^{ax}	46.99^{by}	0.509
	He+O_2	3.89^{bx}	3.27^{cy}	0.056	45.52^{b}	47.38^{b}	1.192
	N_2	3.92^{b}	3.47^{bc}	0.177	46.23^{by}	51.49^{ax}	0.193
	N_2+O_2	3.22^{by}	5.54^{ax}	0.268	46.97^{b}	46.28^{b}	0.361
	SEM[2)]	0.210	0.116		0.765	0.471	

[1)]Gas flow rate, 7 lpm for He and N_2 and 70 sccm for O_2
[2)]Standard error of means (n=6)
[3)](n=15)
[a-d]Different letters within the same column differ significantly (p<0.05).
[x,y]Different letters within the same row differ significantly (p<0.05).

Table 2. Effect of atmospheric pressure plasma with different gas compositions on the quality characteristics of egg white and yolk during storage for 7 d at 10°C

	Input gas[1)]	Cooked egg white		SEM[3)]	Cooked egg yolk		SEM[3)]
		Day 0	Day 7		Day 0	Day 7	
pH	None	9.50^{b}	9.53^{a}	0.022	6.33^{a}	6.35	0.011
	He	9.56^{a}	9.55^{a}	0.016	6.32^{aby}	6.34^{x}	0.005
	He+O_2	9.53^{ab}	9.51^{ab}	0.009	6.30^{bcy}	6.35^{x}	0.009
	N_2	9.50^{b}	9.49^{b}	0.006	6.28^{cy}	6.35^{x}	0.006
	N_2+O_2	9.48^{b}	9.44^{c}	0.009	6.28^{cy}	6.33^{x}	0.010
	SEM[2)]	0.016	0.012		0.008	0.010	
TBARS*	None	0.16^{ay}	0.25^{abx}	0.005	2.29^{a}	2.28	0.062
	He	0.15^{aby}	0.25^{abx}	0.009	2.15^{ay}	2.49^{x}	0.033
	He+O_2	0.15^{aby}	0.25^{abx}	0.008	1.85^{by}	2.46^{x}	0.077
	N_2	0.13^{by}	0.29^{ax}	0.014	2.25^{a}	2.45	0.102
	N_2+O_2	0.16^{ay}	0.24^{bx}	0.015	2.22^{a}	2.23	0.064
	SEM[2)]	0.006	0.014		0.056	0.083	
VBN**	None	9.80^{ey}	29.87^{ax}	0.330	5.60^{b}	5.83	0.165
	He	12.37^{dy}	31.03^{ax}	0.617	6.07^{ab}	6.30	0.165
	He+O_2	17.50^{cy}	25.67^{bx}	0.165	6.07^{ab}	6.30	0.330
	N_2	28.00^{ax}	23.80^{cy}	-	6.30^{a}	6.30	-
	N_2+O_2	24.73^{bx}	11.20^{dy}	0.165	5.83^{ab}	6.30	0.165
	SEM[2)]	0.148	0.443		0.181	0.209	

[1)]Gas flow rate: 7 slpm for He and N_2 and 70 sccm for O_2
[2)]Standard error of means (n=15)
[3)](n=6)
[a-e]Different letters within the same column differ significantly (p<0.05).
[x,y]Different letters within the same row differ significantly (p<0.05).
*TBARS, 2-thiobarbituric acid reactive substance (mg malondialdehyde/kg sample)
**VBN, Volatile basic nitrogen (mg/100 g sample)

bial results showed effective inactivation with APP treatment (Figs. 3 and 4), VBN value in the APP-treated group was expected to be lower than that of the non-treated group. However, VBN value was significantly increased in APP-treated samples, especially following N_2 or N_2+O_2 treatment (Table 2). After 7 d of storage, however, VBN contents were significantly lower in the cooked egg white samples treated with N_2 and N_2+O_2 compared with the results of day 0. No differences were found in cooked egg yolk. The gases including N_2 used for plasma production may be the reason for the higher VBN content on day 0. Optical emission spectra from the present APP system indicated higher production of O_2 and N_2-related species, including N_2^+, O, and OH, as He flow was increased (Jung *et al*., 2011). Further, N_2 is able to produce more N_2^+ and N^+ groups compared with He (Naveed *et al*., 2006). After 7 days of storage, however, the sample treated with APP jet using N_2 or N_2+O_2 had the lowest VBN content prob-

Table 3. Effect of atmospheric pressure plasma with different gas compositions for 2 min on sensory qualities of cooked egg white and yolk

		Gas treatment[1)]					SEM[2)]
		None	He	He+O_2	N_2	N_2+O_2	
Cooked egg white	Color	5.6	5.8	5.6	5.2	5.4	0.21
	Flavor	4.9	4.7	4.7	4.4	4.7	0.21
	Taste	5.1	5.1	5.1	4.7	4.9	0.21
	Texture	5.0	5.1	5.3	5.0	5.0	0.31
	Acceptability	5.2	5.0	5.0	4.9	4.9	0.22
Cooked egg yolk	Color	5.6^{ab}	6.0^{a}	5.3^{ab}	5.1^{b}	5.0^{b}	0.27
	Flavor	5.5^{a}	4.8^{a}	3.9^{b}	3.4^{b}	3.3^{b}	0.29
	Taste	5.5^{a}	4.4^{b}	4.0^{bc}	3.2^{cd}	3.0^{d}	0.28
	Texture	5.5	5.4	5.5	5.6	5.5	0.29
	Acceptability	5.4^{a}	5.2^{ab}	4.2^{bc}	3.2^{cd}	2.9^{d}	0.36

The score was evaluated with 10 semi-trained panelists (1, extremely dislike; 5, neither dislike nor like; 9, extremely like).
[1)]Gas flow rate: 7 lpm for He and N_2 and 70 sccm for O_2
[2)]Standard error of means (n=50).
[a-d]Different letters within the same row in different gas treatment differ significantly (p<0.05).

Table 4. SOS chromotest (*E. coli* PQ37) of egg white treated with atmospheric pressure plasma with different gas compositions

Gas treatment[1)]	Dose (μg/assay)	S-9 mix	β-gal[b] (unit)	ap[b] (unit)	Ratio	IF[b]	S-9 mix	β-gal[b] (unit)	ap[b] (unit)	Ratio	IF[b]
D.W.		-	2.00	10.10	0.20	1.00	+	3.64	15.60	0.23	1.00
None	5000	-	2.88	12.80	0.23	1.13	+	4.99	19.88	0.25	1.09
	1250	-	2.97	12.56	0.24	1.18	+	5.21	22.01	0.24	1.03
	625	-	2.9	12.74	0.23	1.14	+	5.07	20.99	0.24	1.05
	313	-	2.66	11.15	0.24	1.19	+	4.66	19.87	0.23	1.02
	156	-	2.61	12.87	0.20	1.01	+	4.78	20.10	0.24	1.03
He	5000	-	2.98	12.90	0.23	1.16	+	4.85	20.30	0.24	1.04
	1250	-	3.02	13.45	0.22	1.12	+	5.01	19.70	0.25	1.11
	625	-	3.01	13.80	0.22	1.09	+	4.99	21.00	0.24	1.03
	313	-	2.85	13.33	0.21	1.07	+	4.78	19.60	0.24	1.06
	156	-	2.90	13.10	0.22	1.11	+	4.86	20.60	0.24	1.03
He + O_2	5000	-	3.18	13.60	0.23	1.17	+	5.11	19.70	0.26	1.13
	1250	-	3.11	13.55	0.23	1.15	+	5.23	20.60	0.25	1.10
	625	-	2.85	13.04	0.22	1.09	+	4.76	19.80	0.24	1.05
	313	-	3.01	13.65	0.22	1.10	+	4.82	19.66	0.25	1.07
	156	-	3.10	13.11	0.24	1.18	+	5.01	20.46	0.24	1.06
N_2	5000	-	3.07	13.96	0.22	1.10	+	5.28	20.66	0.26	1.11
	1250	-	2.98	12.33	0.23	1.13	+	5.40	20.05	0.27	1.17
	625	-	2.56	11.97	0.21	1.07	+	4.98	19.88	0.25	1.09
	313	-	2.56	11.83	0.22	1.08	+	4.90	19.65	0.25	1.08
	156	-	2.62	12.19	0.21	1.07	+	4.55	18.01	0.25	1.10
N_2 + O_2	5000	-	3.01	12.89	0.23	1.17	+	5.11	21.10	0.24	1.05
	1250	-	3.12	14.40	0.22	1.08	+	4.91	19.55	0.25	1.09
	625	-	2.98	13.12	0.23	1.14	+	4.91	19.21	0.26	1.11
	313	-	3.01	13.87	0.22	1.09	+	4.49	17.86	0.25	1.09
	156	-	2.66	12.65	0.21	1.05	+	4.5	18.8	0.24	1.04
4-NQO[b]	0.03	-	16.25	12.55	1.29	6.45[a]					
B(α)P[b]	2.5	+	13.66	16.54	0.83	4.13[a]					

[1)]Gas flow rate: 7 lpm for He and N_2 and 70 sccm for O_2
[a]Significantly different from the control at p<0.05.
[b]Abbreviations: β-gal, β-galactosidase; ap, alkaline phosphatase; IF, induction factor; 4-NQO, 4-nitroquinoline-N-oxide; B(α)P, benzo (α)pyrene

ably due to the inactivation of microorganisms in samples by APP treatment.

Table 3 shows the sensory evaluation of cooked egg white and yolk treated with APP jet for 2 min with different gas compositions. No significant sensory differences were found among the cooked egg white samples whereas significant reductions in flavor, taste, and overall acceptability were found in cooked egg yolk treated with APP jet. APP produced by N_2 resulted in lower sensory scores for color, flavor, taste, and acceptability compared to those of He. O_2 added to both gas compositions resulted in lower scores for taste and acceptability of cooked egg yolk. Recent study on APP-treated cheese reported that APP using He and He+O_2 led to significant reductions in flavor, odor, and acceptability after 1, 5, and 10 min of APP and when He was combined with O_2, the result showed the lowest sensory scores (data not shown). Therefore, sensorial deterioration of APP-treated foods is possibly associated with food type. In contrast to our results, Basaran *et al*. (2008) demonstrated that sensory evaluations of APP-treated peanut, hazelnut, and pistachio were in the range of "liked moderately" to "liked very much", although there was no significant difference compared with the non-treated group. However, to be developed as a good non-thermal food sterilization method, the sensory deterioration of APP-treated foods should be considered and prevented.

SOS chromotest was carried out to screen the genotoxicological safety of APP-treated samples (Tables 4 and 5). This test is a quantitative bacterial colorimeteric assay for genotoxins (Quillardet *et al*., 1985). Generally, the genotoxicity test composed of 3 different tests including bacterial reverse mutation test, *in vitro* chromosome aberration assay, and in vivo micronucleus assay. Ames test is usually used method as bacterial reverse mutation test for screening. SOS chromotest compensates for the short-

Table 5. SOS chromotest (*E. coli* PQ37) of egg yolk treated with atmospheric pressure plasma with different gas compositions

Gas treatment[1)]	Dose (μg/assay)	S-9 mix	β-gal[b] (unit)	ap[b] (unit)	Ratio	IF[b]	S-9 mix	β-gal[b] (unit)	ap[b] (unit)	Ratio	IF[b]
D.W.		-	2.39	7.20	0.33	1.00	+	3.01	15.09	0.20	1.00
None	5000	-	2.88	8.20	0.35	1.06	+	3.88	18.70	0.21	1.04
	1250	-	2.87	8.54	0.34	1.02	+	3.76	17.60	0.21	1.07
	625	-	2.79	8.44	0.33	1.00	+	3.79	16.88	0.22	1.08
	313	-	2.39	7.23	0.33	1.00	+	3.66	15.76	0.22	1.08
	156	-	2.46	7.21	0.34	1.03	+	3.43	15.99	0.22	1.09
He	5000	-	2.86	7.66	0.37	1.13	+	3.94	17.66	0.22	1.12
	1250	-	2.76	7.87	0.35	1.06	+	3.84	17.21	0.22	1.12
	625	-	2.99	8.02	0.37	1.13	+	3.77	16.88	0.22	1.12
	313	-	2.64	7.84	0.34	1.02	+	3.65	17.50	0.21	1.04
	156	-	2.51	7.50	0.33	1.01	+	3.43	17.10	0.20	1.00
He + O_2	5000	-	2.98	7.60	0.39	1.19	+	3.82	16.99	0.22	1.12
	1250	-	3.01	7.73	0.39	1.18	+	3.66	16.22	0.23	1.13
	625	-	2.76	7.90	0.35	1.06	+	3.75	17.01	0.22	1.10
	313	-	2.77	7.30	0.38	1.15	+	3.50	16.85	0.21	1.04
	156	-	2.90	8.00	0.36	1.10	+	3.62	16.43	0.22	1.10
N_2	5000	-	2.90	8.26	0.35	1.06	+	3.71	17.13	0.22	1.08
	1250	-	2.65	7.65	0.35	1.05	+	3.64	16.44	0.22	1.11
	625	-	2.70	7.9	0.34	1.04	+	3.65	16.01	0.23	1.14
	313	-	2.57	7.66	0.34	1.02	+	3.43	16.91	0.20	1.01
	156	-	2.59	7.56	0.34	1.04	+	3.68	16.80	0.22	1.10
N_2 + O_2	5000	-	3.22	7.95	0.41	1.23	+	4.22	17.62	0.24	1.20
	1250	-	3.01	7.88	0.38	1.16	+	3.98	17.73	0.22	1.12
	625	-	2.88	8.44	0.34	1.03	+	3.75	16.89	0.22	1.11
	313	-	3.11	8.32	0.37	1.13	+	3.53	16.01	0.22	1.10
	156	-	2.98	7.98	0.37	1.13	+	3.45	16.8	0.21	1.03
4-NQO[b]	0.03	-	16.25	12.55	1.29	6.45[a]					
B(α)P[b]	2.5	+	13.66	16.54	0.83	4.13[a]					

[1]Gas flow rate: 7 lpm for He and N_2 and 70 sccm for O_2

[a]Significantly different from the control at $p<0.05$.

[b]Abbreviations: β-gal, β-galactosidase; ap, alkaline phosphatase; IF, induction factor; 4-NQO, 4-nitroquinoline-N-oxide; B(α)P, benzo (α)pyrene.

comings of the Ames test, which include personal deviation on counting colonies and amino acids, especially in high protein foods such as egg. It also maintains high correlation (>90%) with the results from the Ames test (Quillardet *et al*., 1985). Results from this study showed that APP-treatment of cooked egg yolk and white did not induce any mutation response. Thus, 2 min treatment with APP jet in the present system may be considered as not leading to genotoxicity. There was no prvious report on genotoxicity test of APP-treated foods so far. Song *et al*. (2009b) reported that gamma irradiated egg yolk and white had no gentoxicity by SOS chromotest. However, further studies including mouse lymphoma test and chromosome aberration test should be conducted to confirm the genotoxicological safety of APP-treated the cooked egg product.

From the results, it can be concluded that there is potential for using APP jet as a non-thermal sterilization process to enhance safety and extend shelf-life of egg products. However, more appropriate APP system should be developed in minimizing quality changes and maximizing efficiency for food application.

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