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Effect of atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two different gas compositions

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ABSTRACT

Atmospheric pressure plasma (APP) is an emerging non-thermal pasteurization method for the enhancement of food safety. In this study, the effect of APP on the inactivation of pathogens inoculated onto bacon was observed. Sliced bacon was inoculated with *Listeria monocytogenes* (KCTC 3596), *Escherichia coli* (KCTC 1682), and *Salmonella Typhimurium* (KCTC 1925). The samples were treated with APP at 75, 100, and 125 W of input power for 60 and 90 s. Two gases, helium (10 lpm) or a mixture of helium and oxygen, (10 lpm and 10 sccm, respectively) were used for the plasma generation. Plasma with helium could only reduce the number of inoculated pathogens by about 1–2 Log cycles. On the other hand, the helium/oxygen gas mixture was able to achieve microbial reduction of about 2–3 Log cycles. The number of total aerobic bacteria showed 1.89 and 4.58 decimal reductions after plasma treatment with helium and the helium/oxygen mixture, respectively. Microscopic observation of the bacon after plasma treatment did not find any significant changes, except that the L^*-value of the bacon surface was increased. These results clearly indicate that APP treatment is effective for the inactivation of the three pathogens used in this study, although further investigation is needed for elucidating quality changes after treatment.

1. Introduction

Food safety is a large concern for government authorities, industry as well as consumers. The major food-borne pathogens found in food are *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. (Bell and Kyriakides, 1999). *L. monocytogenes* is a problematic microbe for the food industry due to its significant public health risks and economic impact with high mortality (Posfay-Barbe and Wald, 2004). *Salmonella* spp. and *E. coli* including O157:H7 also have increased worldwide over recent years (Boonmar et al., 1998; Rodrigue et al., 1990). Therefore, sterilization methods for the elimination of these food-borne pathogens have been suggested and developed.

Traditional sterilization methods typically rely on lethal heat treatment including high frequency heating, ohmic heating, steam pasteurization, autoclaving, etc. However, thermal technologies such as autoclaving cannot be applied to heat-sensitive materials. Hydrogen peroxide and ethylene oxide have been alternatively used as low-temperature sterilization techniques (Lee et al., 2006), but these chemical treatments have consumer health concerns. Alternative non-thermal pasteurization methods such as ionizing radiation, high hydrostatic pressure, pulsed electrical field, oscillating magnetic field, and high power ultrasound (Raso and Barbosa-Canovas, 2003) have been developed and significantly studied in recent years. Both irradiation and high pressure are effective non-thermal sterilization methods for food products commercially available. However, these processes has disadvantages which include high initial cost for facility, required specialized equipment, safety measures, and trained personnel, some quality changes of foods, and consumer acceptance (Yun et al., 2010; Kruk et al., 2010).

Plasma processing is an emerging non-thermal pasteurization technique for the improvement of food safety (Lee et al., 2006). Plasma treatment has been used for the sterilization of medical instruments and treatment of material surfaces. Formerly, plasma treatments were carried out under vacuum conditions, but researchers have now developed an atmospheric pressure plasma system, resulting in reduced cost, increased treatment speed, and industrial applicability (Yoon and Ryu, 2007; Yun et al., 2010). The ability to generate non-thermal plasma discharges at atmospheric

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pressure makes the decontamination process easier and more inexpensive. In addition, the gas temperature of such plasmas remains relatively low, allowing their use in heat-sensitive products (Song et al., 2009).

Laroussi et al. (2002) reported that disruption of the cell membrane, sometimes to the point of cell lysis, is the main mechanism by which atmospheric pressure plasma kills cells. Ohkawa et al. (2006) observed the antibacterial effect of atmospheric pressure glow (APG) plasma using spore-forming bacteria, mold, and yeast-like fungus. On the other hand, Gweon et al. (2010) reported that APP-treated human hepatocytes exhibit distinct zones of necrotic and live cells. The authors also found that uncharged cytoskeletal intermediate filaments are only minimally disturbed by plasma, indicating the possibility that plasma-induced electrostatic effects selectively destroy charged proteins. Recently, Song et al. (2009) and Yun et al. (2010) reported that APP inactivates *L. monocytogenes* inoculated onto sliced cheese and ham and disposable food containers, respectively.

However, the effect of APP on microbial reduction in a real food system has not been studied extensively. Moreover, there have been no reports on food quality characteristics after plasma treatment. Therefore, the objective of the present study was to investigate the effect of APP with different gas compositions on the inactivation of *E. coli*, *L. monocytogenes*, and *Salmonella* Typhimurium inoculated onto bacon and followed quality changes.

2. Materials and methods

2.1. Sample preparation and inoculation

Bacon (Lotte ham, Co., Ltd., Gimcheon, Korea) was purchased from a local market in Daejeon, Korea. Prior to inoculation of pathogens, bacon samples were sterilized by irradiation (35 kGy) in a cobalt-60 gamma irradiator at the Advanced Radiation Technology Institute, Jeongeup, Korea.

L. monocytogenes (KCTC 3596), *E. coli* (KCTC 1682), and *S.* Typhimurium (KCTC 1925) were obtained from the Korean Collection for Type Culture (KCTC, Daejeon, Korea). The strains were cultivated at 37 °C for 18 h in tryptic soy broth (50 mL) (Difco Laboratories, Detroit, MI, USA), after which cultures of each strain were transferred aseptically to a 50 mL centrifuge tube. *L. monocytogenes*, *E. coli*, and *S.* Typhimurium were centrifuged (209 × *g* for 10 min at 4 °C) in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). The resulting pellet was washed twice with sterile saline (0.85%) and then suspended in saline at a final concentration of approximately 10^9 CFU/mL. Radiation-sterilized bacon samples were aseptically cut into 60 × 6 mm (1 g) rectangles in order to fit the plasma generator. The samples were then inoculated at 10 different areas with 10 μL of the stock inoculum. The samples were sealed in a plastic petri dish and incubated at 10 °C for 1 h to facilitate bacterial attachment to the bacon samples.

To measure the number of total aerobic bacteria, samples were stored in abused temperature (approximately 20 °C) for 2 days to accelerate growth.

2.2. Treatment of atmospheric pressure plasma (APP)

The plasma generator used in the experiment was a large area (dimensions: 110 × 15 mm) system consisting of a powered rod electrode covered with a dielectric material and a bottom ground electrode placed under the powered electrode that was used as a base for material treatment (Gweon et al., 2009). Gases were supplied between the powered electrode and the rectangular electrically grounded conducting case, flowing downward toward the sample. The electrode was powered by a 13.56 MHz radio-frequency (rf) supply through an impedance matching network. The input rf powers in this study were 75, 100, and 125 W, and the exposure times were 60 and 90 s. Helium gas with at fixed flow rate of 10 lpm (liter per minute) was introduced for stable plasma generation. To observe the effect of the gas mixture on plasma effectiveness, a mixed gas with helium and O_2 (10 sccm) was also used.

For plasma treatment, inoculated samples were placed on the bottom conductor in direct contact with the plasma at room temperature (approximately 20 °C). The gap distance between the powered electrode and the treatment surface was maintained at 3 mm. After plasma treatment, the samples were immediately stored under commercial storage conditions (10 °C) and then analyzed.

2.3. Microbial analysis

After plasma treatment, samples were blended with sterile saline for 2 min using a stomacher (BagMixer® 400, Interscience Ind., St. Nom, France). A series of decimal dilutions was prepared with sterile saline. Each diluent (0.1 mL) was spread in triplicate on each medium. Media used for the enumeration of *E. coli*, *L. monocytogenes*, and *S. Typhimurium* as well as for total aerobic bacteria was tryptic soy agar (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37 °C for 24 h, and the microbial counts were expressed as Log CFU/g.

2.4. Physicochemical properties

2.4.1. pH

The bacon samples (3 g) were homogenized (T25 Basic, Ika Co., Staufen, Dermany) with 27 mL of distilled water for 1 min (1130 × *g*), after which the pH levels of the homogenates were measured using a pH meter (Model 750, iSTEC, Seoul, Korea).

2.4.2. 2-Thiobarbituric acid reactive substances (TBARS) measurement

A 5 g bacon sample was placed in a 50 mL centrifuge tube containing 50 μL of BHA (7.2% in ethanol) and 15 mL of distilled water and then subjected to homogenization (T25 basic, Ika Co., Staufen, Germany). Then, the homogenate (1 mL) was mixed with 2 mL of a thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA in 15% TCA), heated in boiling water, and centrifuged for 15 min at 2090 × *g* (Hanil Science Industrial Co., Seoul, Korea). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (DU®530, Beckman Instruments Inc., Fullerton, CA USA). The TBARS value (mg malondialdehyde/kg sample on the basis of wet weight) was calculated using a standard curve.

2.4.3. Microscopic observation

The samples were cut to 10 mm diameters for plasma treatment and were prepared from independently the microbial test samples. The surfaces of the bacon samples were observed (×60) using a microscope (Stereoscopic zoom microscope SMZ 800, Nikon Co., Ltd., Tokyo, Japan) before and after plasma treatment.

2.4.4. Surface color

Plasma-treated bacon was placed on a round-type quartz cell (8 mm diameter), after which the CIE color value was measured using a Color Difference Meter (Spectrophotometer CM-3500d, Minolta Co., Ltd., Osaka, Japan). The instrument was calibrated to standard black and white plate before analysis. A small size

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aperture was used, and three measurements at different sites were averaged for combined treatment.

2.5. Statistical analysis

Three different trials were individually carried out and considered as replicates with each 2 observation numbers. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and significant differences between mean values were identified by the Student–Newman–Keul's multiple range test using SAS software with a confidence level at $P < 0.05$ (SAS, Release 8.01, SAS Institute Inc., Cary, NC). Mean values and standard error of the means are reported.

3. Results and discussion

3.1. Microbiological quality

3.1.1. Inoculation test

Table 1 shows the inactivation of *E. coli* (KCTC 1682), *L. monocytogenes* (KCTC 3569), and *S.* Typhimurium (KCTC 1925) by APP treatment with helium or the helium/oxygen mixture. The populations of *E. coli*, *L. monocytogenes* and *S.* Typhimurium were more reduced as the input power and plasma exposure time were increased, regardless of gas composition. When the input rf power was increased to 125 W, *E. coli*, *L. monocytogenes*, and *S. Typhimurium* showed 1.6, 2.0, and 1.5 decimal reductions after 90 s. Feng et al. (2009) reported that high voltage plasma (400–600 V and 20–35 mA) using air and nitrogen gas can rapidly reduce the number of *E. coli* and *Micrococcus luteus* cells on agar by 90–95% in the first 30 s, resulting in full elimination after 90 s. Likewise, Korachi et al. (2009) reported that the reduction effect of atmospheric corona discharge plasma on *E. coli* in water is increased with longer treatment time. Song et al. (2009) and Yun et al. (2010) also observed the same trend. Therefore, these previous reports along with the present study indicate that plasma treatment at high voltage or high power is effective for eliminating pathogens over short time periods. Niemira and Sites (2008) reported that high voltage cold plasma (15–20 kV, 60 Hz AC power) significantly reduces the viable populations of *Salmonella* Stanley and *E. coli* O157 cells inoculated onto apple. The authors also reported that a higher rate of gas flow (10 vs 40 lpm) better promotes the effects of inactivation. However, there was no difference found in gas flow rate (6 vs 10 lpm) for helium gas (data not shown). Moon et al. (2009) inoculated *E. coli* (KCTC 1682) onto pork and then compared APP with UV sterilization. They found that UV irradiation for 1 min has a similar effect to that of APP treatment for 30 s. However, the authors suggested that APP treatment is more effective and safer than UV sterilization due to the harmful effects of UV rays on human skin.

Effective microbial reduction was achieved using the helium/oxygen mixture. The initial counts (7–8 Log CFU/g) of *E. coli*, *L. monocytogenes*, and *S. Typhimurium* were significantly reduced to 4.80, 5.79, and 6.46 Log CFU/g after plasma treatment at 125 W for 90 s (Table 1).

APP is a partially ionized gas consisting of ions, electrons, UV photons, and reactive species such as radicals, excited atoms, and molecules with sufficient energy to break covalent bonds and initiate various chemical reactions (Moisan et al., 2001). These highly reactive species can overcome natural defense mechanisms, resulting in damage to DNA proteins, lipids, and membranes (Montie et al., 2000). Therefore, the plasma produced by the helium/oxygen mixture generated a larger amount of free radicals than that produced by helium only. Hury et al. (1998) reported that oxygen, H_2O_2, and CO_2-based plasmas were more effective than argon plasma. For example, oxygen-based plasma destroys microorganisms via slow combustion with the oxygen atoms and oxygen-containing radicals present in the plasma.

The calculated *D*-values (decimal reduction time; the exposure time required to inactivate 90% of a population) of *E. coli* were 142, 76.9, and 55.6 s and 90.9, 66.7, and 28.6 s upon plasma treatment with helium gas and helium/oxygen, respectively (Fig. 1). The *D*-values of *L. monocytogenes* and *S. Typhimurium* also showed a similar trend. Moisan et al. (2002) reported that the survival curve after plasma treatment with pure argon indicates inactivation by UV irradiation, since no radicals from the gaseous phase were available. On the other hand, the addition of oxygen, which will partially dissociate to individual O atoms, will contribute along with UV to the inactivation of the spores. Nelson and Berger (1989) reported that the inactivation effect of plasma is very efficient against *Bacillus subtilis* and *Clostridium sporogenes* when combined with oxygen.

During the present study, we found that the quantity and stability of generated plasma was dependent upon the environment. For instance, the quantity and stability of plasma were higher at a higher humidity (data not shown). This can be explained by the fact that higher humidity increased the efficiency of free radical production compared to dry or normal conditions. Moreover, the temperature of the powered electrode, the closest apparatus to the sample, increased gradually as treatment time was elongated at higher input power (data not shown). Therefore, the electrode of the APP apparatus used in this study was cooled periodically, especially for treating 125 W, in order to avoid any effect of temperature on pathogen inactivation. Due to this reason, APP was not conducted at a higher power (>125 W).

3.1.2. Total aerobic bacteria

The initial number of total aerobic bacteria (TAB) on bacon was 7.08 Log CFU/g (Fig. 1). Using the helium/oxygen mixture, APP significantly reduced the initial number of microbes on bacon to 4.65, 4.02, and 2.55 Log CFU/g after being performed at 75, 100, and

Table 1
Inactivation of pathogens inoculated onto bacon by atmospheric pressure plasma (Log CFU/g).

Time (s)	Input power (W) and oxygen addition[a]							SEM[b]
	0	75	100	125	$75+O_2$	$100+O_2$	$125+O_2$	
	Escherichia coli							
0	7.80	7.80^{x}	7.80^{x}	7.80^{x}	7.80^{x}	7.80^{x}	7.80^{x}	
60	7.80^{a}	7.33^{yb}	7.01^{yc}	6.28^{ye}	7.09^{ybc}	6.72^{yd}	5.12^{yf}	0.094
90	7.80^{a}	7.13^{yb}	6.58^{zcd}	6.23^{ye}	6.80^{yc}	6.44^{yde}	4.80^{yf}	0.110
SEM[c]		0.131	0.127	0.130	0.131	0.124	0.161	
	Listeria monocytogenes							
0	8.39	8.39^{x}	8.39^{x}	8.39^{x}	8.39^{x}	8.39^{x}	8.39^{x}	
60	8.39^{a}	7.77^{yb}	6.96^{yde}	7.08^{yd}	7.54^{ybc}	7.42^{yc}	6.69^{ye}	0.104
90	8.39^{a}	7.46^{zb}	7.09^{yc}	6.33^{zd}	7.45^{yb}	7.16^{ybc}	5.79^{ze}	0.112
SEM[c]		0.044	0.125	0.063	0.067	0.092	0.150	
	Salmonella Typhimurium							
0	0	75	100	125	$75+O_2$	$100+O_2$	$125+O_2$	
60	8.19	8.19^{x}	8.19^{x}	8.19^{x}	8.19^{x}	8.19^{x}	8.19^{x}	
90	8.19^{c}	7.66^{yd}	7.43^{ye}	7.30^{ye}	7.68^{yd}	7.27^{ye}	7.27^{ye}	0.070
SEM[c]	8.19^{a}	7.56^{yb}	7.43^{ybc}	6.87^{zd}	7.39^{zbc}	7.24^{yd}	6.46^{ze}	0.095
		0.035	0.032	0.063	0.067	0.059	0.139	

Values with different letters (x–z) within the same column differ significantly ($P < 0.05$).
Values with different letters (a–f) within the same row differ significantly ($P < 0.05$).
[a] Number indicates input power with helium gas only and numbers + O_2 indicate helium/oxygen mixture.
[b] Standard error of the means ($n = 21$).
[c] ($n = 9$).

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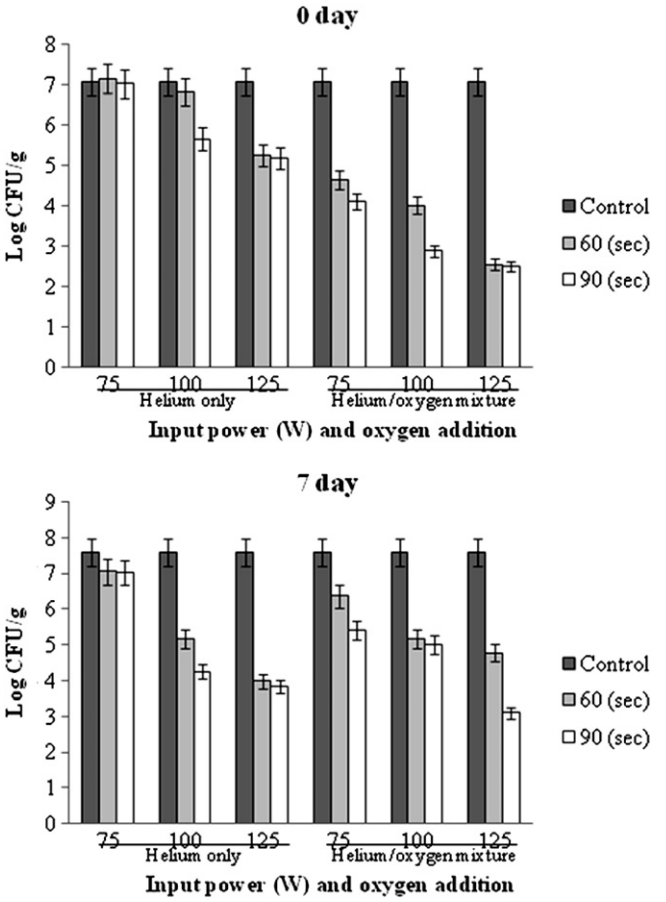

Fig. 1. Total aerobic bacterial (TAB) count on bacon stored for 7 days at 4 °C after treatment with APP.

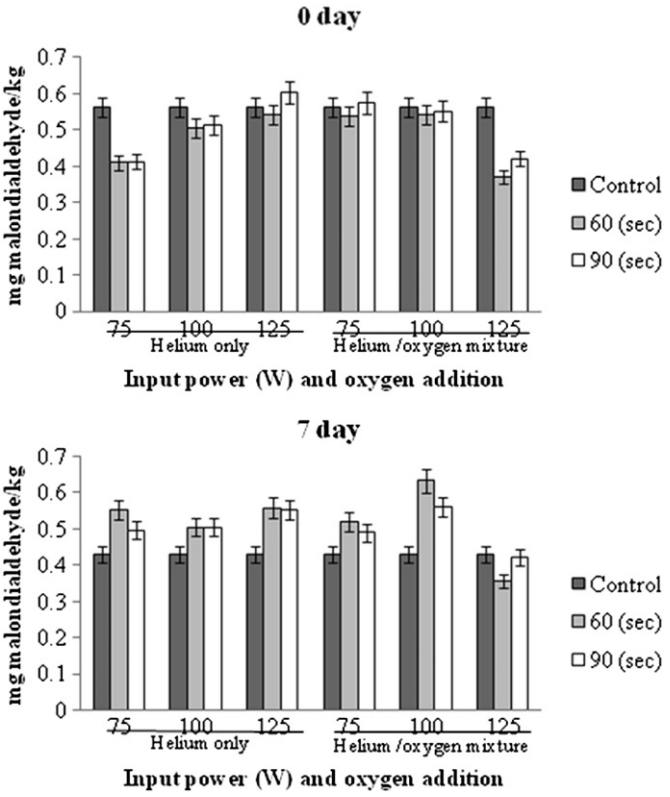

Fig. 2. 2-Thiobarbituric acid reactive substances (TBARS) values of bacon stored for 7 days at 4 °C after treatment with APP.

125 W for 60 s, respectively. As observed in the inoculation test, the number of TAB was reduced to 4.10, 2.89, and 2.50 Log CFU/g when the plasma exposure time was increased to 90 s. After storage for 7 days at 4 °C, the microbial population was still low. Therefore, the damaged cells were incapable of repair during storage after APP treatment.

3.2. Physicochemical properties

3.2.1. pH and TBARS value

There were no differences on the pH levels of bacon after APP treatment with different gas compositions, at different input power levels, and for various exposure times, indicating that APP treatment did not significantly change the pH values of bacon. Cured meats such as bacon or fermented sausage showed pH in the range of 4.6–5.3 (NIAS RDA, 2007) and the pHs of the samples ranged 4.8–5.0.

The TBARS values of plasma-treated bacon fluctuated (Fig. 2). At day 0, treatment at 75 W with helium only and at 125 W with the helium/oxygen mixture showed lower TBARS values compared to non-treated control. However, after 7 days of storage, plasma treatment for 60 or 90 s produced higher TBARS values than non-treated control, except for treatment at 125 W with mixed gas. Additionally, there were no significant differences in TBARS values upon oxygen addition. These fluctuating results may be due to variations in the fat content and fatty acid composition of the market samples, a result of limited sample size. The range of TBARS values was therefore 0.37–0.63 mg of malondialdehyde/kg sample. However, further studies should be conducted to clarify these results.

3.2.2. Microscopic observation of plasma-treated bacon

Microscopic observation (×60) of bacon treated with APP was carried out. The surface tissues of treated samples did not show any significant changes or damage (data not shown). However, moisture evaporation from the surface of the treated samples was observed. Moon et al. (2009) reported that plasma treatment can be adapted to sterilize human skin since there no electric or thermal damage is inflicted. Therefore, plasma treatment has no effect on the surface morphology of bacon.

3.2.3. Surface color

The lightness (L^*-value), redness (a^*-value), and yellowness (b^*-value) of the bacon samples treated with plasma and helium or helium/oxygen were observed (Table 2). The L^*-value of the bacon surface treated with APP was decreased at a higher input power and exposure time. This result was more obvious when the helium/oxygen mixture was used. In contrast, the a^*-value was increased at a higher input power and exposure time only for helium gas and for the helium/oxygen mixture. Sanabria et al. (2004) found a high correlation coefficient ($r = +0.80$, $P < 0.01$) between color lightness (L^*) and moisture content. Additionally, PSE meat was characterized by high reflectance due to the exudation of water at low pH (Swatland, 2008). Therefore, evaporation of a small amount of moisture from the bacon surface can be a reason for the decrease of L^*-value after APP treatment. Lee et al. (1998) reported that cold low-pressure plasma generated by argon gas reduces the weight of cotton fabric based on the removal of moisture and contaminants from the surface. Even if differences in color values were found using instruments, there still was no difference found by visual inspection (data not shown). The b^*-value did not change when helium was used, but it did increase at a higher input power using helium/oxygen. Nevertheless, there was no difference found in

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Table 2
Color changes of the bacon surface after treatment of atmospheric pressure plasma.

Gas composition	Color value	Time	Input power (W)				SEM[a]
			0	75	100	125	
Helium	L^*	60	61.31^{a}	59.97^{xb}	60.24^{xb}	58.57^{xc}	0.240
		90	61.31^{xa}	58.32^{yc}	58.20^{yc}	59.85^{yb}	0.246
		SEM[b]	0.123	0.261	0.215	0.327	
	a^*	60	4.00^{b}	4.87^{ya}	5.05^{a}	5.06^{xa}	0.067
		90	4.00^{d}	5.59^{xa}	5.03^{b}	4.59^{yc}	0.079
		SEM[b]	0.019	0.057	0.083	0.105	
	b^*	60	10.92^{b}	10.40^{d}	10.67^{yc}	11.68^{xa}	0.077
		90	10.92^{a}	10.54^{c}	10.81^{xab}	10.65^{yab}	0.056
		SEM[b]	0.057	0.105	0.035	0.050	
Helium + oxygen	L^*	60	63.94^{a}	62.19^{xb}	58.29^{c}	56.46^{xd}	0.400
		90	63.94^{a}	59.14^{yb}	57.06^{c}	52.28^{yd}	0.448
		SEM[b]	0.535	0.382	0.038	0.378	
	a^*	60	6.84^{b}	6.67^{b}	7.64^{xa}	7.23^{ab}	0.178
		90	6.84	7.05	7.02^{y}	7.56	0.194
		SEM[b]	0.230	0.206	0.155	0.141	
	b^*	60	12.52^{d}	13.56^{c}	13.94^{b}	14.42^{a}	0.075
		90	12.52^{c}	13.58^{b}	13.88^{b}	14.43^{a}	0.106
		SEM[b]	0.120	0.068	0.118	0.025	

Values with different letters (x–y) within the same column differ significantly ($P < 0.05$).
Values with different letters (a–d) within the same row differ significantly ($P < 0.05$).
[a] Standard error of the means ($n = 12$).
[b] ($n = 6$).

redness upon visual inspection. Moon et al. (2009) also reported that the color of the pork surface was not different after APP for 1 min at an input power of 150 W.

4. Conclusion

In our study, APP effectively inactivated pathogens inoculated onto bacon. In general, APP is a promising non-thermal cold pasteurization method for improving the safety of processed meat products. However, before applying this technology to the wider food industry, further studies should be conducted, especially focusing on the enhancement of inactivation efficiency, development of a plasma system, and evaluation of quality and safety of foods for consumption.

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