Food Research International 76 (2015) 342–347

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Inactivation of murine norovirus-1 and hepatitis A virus on fresh meats by atmospheric pressure plasma jets

San-Cheong Bae [a], Shin Young Park [a], Wonho Choe [b], Sang-Do Ha [a,*]

[a] School of Food Science and Technology, Chung-Ang University, 72-1 Nae-Ri, Daedeok-Myun, Ansung, Kyunggido 456-756, Republic of Korea
[b] Department of Physics, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 18 June 2015
Accepted 23 June 2015
Available online 10 July 2015

Keywords:
Norovirus
Hepatitis A virus
Atmospheric plasma jet
Fresh meat

ABSTRACT

In the current study, inactivation effect of atmospheric pressure plasma (APP) jets (10 s–20 min) was investigated against murine norovirus (MNV-1), as a norovirus (NoV) surrogate and hepatitis A virus (HAV) associated with three types of fresh meats (beef loin, pork shoulder and chicken breast). The quality characteristics of fresh meats, such as surface color, moisture content and thiobarbituric acid reactive substance (TBARS) were also examined. After 5–20 min of treatment with APP jets, the reduction in MNV-1 titers (initial inoculums of 10^7 plaque-forming units (PFU)) were >2 $\log_{10}$ PFU/mL in the three types of meat. After 5–20 min treatment with APP jets, the reduction in HAV titers (initial inoculums of 10^6 PFU) were >1 $\log_{10}$ PFU/mL in the three types of meat. There was no significant difference ($p > 0.05$) in the L*, a*, and b* values for APP jet treatment times below 5 min. Furthermore, there was no significant difference ($p > 0.05$) in the water content (%) value for treatment times under 5 min. Although the TBARS values gradually increased with increase in APP jet treatment times, these TBA values were below 1.0 mg MA/kg (an indicator of meat rancidity). The results of the current study indicate that 5 min of APP jet treatment showed >99% reduction (2 $\log_{10}$ PFU/mL) of MNV-1 titer and >90% reduction (1 $\log_{10}$ PFU/mL) of HAV titer without concomitant changes in meat quality; thus, this procedure can be considered in fresh meat production, processing and distribution processes to enhance fresh meat safety.

1. Introduction

The consumption of fresh meat and its products has been increasing in many countries. Accordingly, the safety of fresh meat is of great importance throughout the world. The major cause of worldwide food-borne outbreaks is norovirus (NoV) and hepatitis A virus (HAV), it also have been associated with the consumption of processed and raw meats (Malek et al., 2009; Mattison et al., 2007; Park, Kim, & Ko, 2010). During the slaughtering of infected animals and the processing of their raw meat by food handlers, bacteria and viruses are likely to be transmitted (Anang, Rusul, Bakar, & Ling, 2007; Hong et al., 2008; Malek et al., 2009; Robesyn et al., 2009). Furthermore, the contamination of meats can happen as a result of a poor sanitary environment or contaminated utensils (D'Souza et al., 2006; Richards, 2001). The Center for Science in the Public Interest (CSPI) reported 136, 72 and 176 cases of NoV infections associated with beef, pork and chicken, respectively, in the United States in the 5 years from 2007 to 2011 (CSPI, 2014). Moreover, 5 and 10 cases of HAV infection associated with meats in the United States in 1991 and 2000 year, respectively (CSPI, 2014). Human NoV belongs to the *Norovirus* genus of the Caliciviridae family; it can cause clinical illness gastroenteritis, with symptoms like vomiting and diarrhea, which appears within 1–3 days of exposure to a dose of a very few particles (10–100) (Siebenga et al., 2009). HAV are non-enveloped icosahedral viruses with a single-stranded RNA genome, which belong to the *Hepatovirus* genus and Picornaviridae family (Acheson & Fiore, 2004). HAV infections occur periodically in developing countries as well as in developed countries (Koopmans & Duizer, 2004). NoV and HAV infections are usually transmitted by person-to person contact, contaminated foods and water, fecal material and the surface of utensils during food processing (Hewitt, Rivera-Aban, & Greening, 2009; Moore et al., 2004).

Various treatment methods for inactivating viruses have been used; these include thermal treatment, ultraviolet (UV) irradiation and food preservatives (Chun, Kim, Chung, Won, & Song, 2009; Zhou, Xu, & Liu, 2010). However, there is a limit in controlling the virus because most viruses have resistance and the methods can have a negative effect on the foods (Ko, Ma, & Song, 2005). Irradiation can produce an off-flavor and cause lipid oxidation (Lee, Jung, Ham, & Jo, 2012). Thermal treatment is especially likely to damage heat-sensitive food sensorial and nutritional effects (Awuah, Ramaswamy, & Economides, 2007). Recently, non-thermal treatment with atmospheric pressure plasma (APP) has gained considerable attention as a new method for improving food safety related to food-borne illnesses (Lee et al., 2011; Song et al., 2009). APP has been used in various fields, for example, for surface modifications, in

* Corresponding author at: Dept. of Food Science and Technology, Chung-Ang University, 72-1 Nae-ri, Daeduk-myun, Ansung, Gyunggido 456-756, Republic of Korea.
E-mail address: sangdoha@cau.ac.kr (S.-D. Ha).

http://dx.doi.org/10.1016/j.foodres.2015.06.039
0963-9969/

the environmental and biomedical fields (Bogaerts, Neyts, Gijbels, & van der Mullen, 2002). Plasma is ionized gas in a quasi-neutral condition, produced by high-energy. Plasma consists of photons, electrons, positive and negative ions, free radicals, neutral atoms, UV photons, reactive oxygen (ozone, atomic oxygen and singlet oxygen) and reactive nitrogen species (NO radicals, NO_2, NO_3, N_2O_3, N_2O_4 and $ONOO^-$) (Ahlfeld et al., 2015; Gweon et al., 2010; Wan, Coventry, Swiergon, Sanguansri, & Versteeg, 2009). Various active species and UV photons were inactivating the virus particles, bacteria, fungi and yeast by destroying the genetic material (Ahlfeld et al., 2015; Fridman et al., 2007; Moisan et al., 2001, 2002). In addition, the beginning of the exposure to the APP, these elements generate a synergistic effect (Ahlfeld et al., 2015).

Due to the absence of an animal or a cell culture system for NoV, a surrogate model is required (Straub et al., 2007). The murine norovirus (MNV-1) and the feline calicivirus (FCV) are used as surrogates in studies predicting NoV inactivation (Bae & Schwab, 2008; Jean, Morales-Rayas, Anoman, & Lamhoujeb, 2011). Recently, MNV-1 has been recognized as a more suitable surrogate model than the FCV because its genome and routes of spread are more similar to those of the human form (Jean et al., 2011; Wobus, Thackray, & Virgin, 2006).

Several studies on the inactivation of pathogenic microorganisms in food by APP jets have been carried out (Lee et al., 2012; Song et al., 2009). Furthermore, Noriega, Shama, Laca, Diaz, and Kong (2011) and Rød et al. (2012) demonstrated the impact of APP inactivation on chicken meat and ready-to-eat meat (bresaola) targeting *Listeria innocua*. Also, the influence of APP on NoV strain was described by Ahlfeld et al. (2015). However, studies of the effects of APP on virucidal contaminated with foods are not enough relatively. There is still a need to study the inactivation of viruses, especially associated on fresh meats. Therefore, we used a cold arc plasma device to investigate the effects of APP jets (0.5–20 min) on the inactivation of MNV-1, as an NoV surrogate and HAV in three kinds of fresh meat (beef loin, pork shoulder, and chicken breast) and on the quality (surface color, moisture content, 2-thiobarbituric acid reactive substance (TBARS)) of these meats.

2. Materials and methods

2.1. Viruses and cell lines

MNV-1 was provided by Dr. Skip Virgin, Washington University. The mouse leukemic monocyte macrophage cell line, RAW 264.7, was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). HAV (strain HM-175) and fetal rhesus monkey kidney (FRhK-4) cells were kindly provided by Professor M. D. Sobsey (University of North Carolina, Chapel Hill, NC, USA).

2.2. Cell culture

RAW 264.7 and FRhK-4 cells were grown in Dulbecco's Minimum Essential Medium (DMEM; SIGMA, Saint Louis, Missouri, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, New York, USA), 44 mM sodium bicarbonate (SIGMA, Saint Louis, Missouri, USA) and 1% antibiotics–antimycotics (penicillin–streptomycin; Gibco, Grand Island, New York, USA), in 75 cm^2 culture flasks, and incubated at 37 °C in a humidified 5% CO_2 incubator. Cells were sub cultured every 2–3 days.

2.3. Virus preparation

MNV-1 and HAV preparation was performed as previously described with slight modifications (D'Souza & Su, 2010). When monolayers of RAW 264.7 and FRhK-4 in 150 cm^2 culture flasks were 90% confluent, the growth medium was removed by aspiration. The monolayers were washed with phosphate-buffered saline (PBS, pH 7.4). A 1 mL aliquot of virus inoculums was added to the flasks, and the flasks were incubated at 37 °C in a 5% CO_2 atmosphere for 90 min to allow virus adsorption. Then, 25 mL of maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotic–antimycotic) was added to the flasks, and they were incubated at 37 °C in a 5% CO_2 atmosphere for 3 days in the case of MNV-1 and 7 days for HAV. If cytopathic effects (CPE) above 90% were observed, the virus-infected flasks were frozen and thawed three times. Viruses were released by cell lysis during this step. The contents were centrifuged at 1500 *g* for 10 min to remove cell debris and the supernatants harvested. Viruses were stored at −70 °C until use.

2.4. Sample preparation and inoculation

Samples were purchased from a local market in Anseong, Korea. We prepared samples type of beef loins, chicken breasts and pork shoulder. The meats were uniformly cut into pieces of 3.0 × 2.5 × 0.5 cm (3 g ± 0.05) using a sterile stainless steel knife and immediately used for the experiments. The samples were transferred to sterile petri-dishes (50 mm in diameter and 10 mm in depth) and evenly inoculated with 200 μL of virus suspension (6–7 $\log_{10}$ PFU/mL) on the surface. To allow the virus to attach to the fresh meats, the samples were placed on a clean bench for 1 h at room temperature.

2.5. Treatment with APP jets

Samples were treated by APP jets based on cold arc plasma. The cold arc plasma source was made of a cylindrical powered electrode with a sharpened tip and an emission hole diameter of 1.5 mm. This electrode was covered by a grounded metal, which also used a cathode nozzle with a cooling system (Fig. 1). The plasma was produced at a condition of peak voltage of 3.5 kV, a frequency of 28.5 kHz and a N_2 (99.9%) flow rate at 6 standard liters per minute. The distance of between the jet nozzle and the surface of the sample was 4 cm. During the APP jet treatment, samples were covered with a glass container with a hole in the top. Fresh meats were treated with APP in triplicates at 0.5, 1, 3, 5, 10, and 20 min.

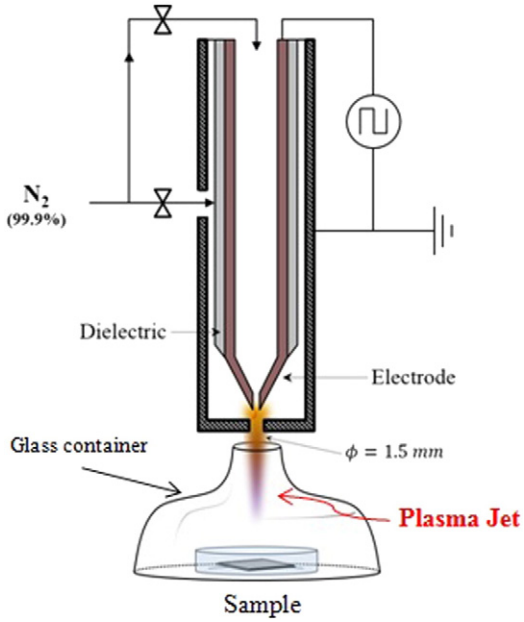

Fig. 1. Schematic diagram of the experimental system of atmospheric pressure plasma jets (APP jets).

2.6. Sample processing for virus recovery

Virus recovery was performed as previously described with slight modifications (Son et al., 2014). Following the treatment, each virus-contaminated meat fillet (3 g) was soaked in 27 mL of PBS in a 50-mL conical tube. The samples were vortexed for 2 min and shaken in a shaking incubator (Vision Scientific Co., Seoul, Korea) at 300 rpm for 1 h to elute the virus. After centrifuging at 10,000 *g* for 1 h at 4 °C, the supernatants were filtered using 5, 1.2, 0.8, and 0.45 μm filters. Each eluted viral suspension was 10-fold serially diluted in DMEM. The virus infection titers were analyzed with a plaque assay.

2.7. Plaque assay

The plaque assay was performed as previously described with slight modifications (Bidawid, Malik, Adegbunrin, Sattar, & Farber, 2003). RAW 264.7 and FRhK-4 cells were seeded in 12-well plates (2 mL for each well containing 4×10^5 cells) and incubated at 37 °C in 5% CO_2 conditions for 24 h until they reached 90% cell confluency. Virus suspensions eluted from the samples were serially diluted in maintenance medium from 10^1 to 10^4(DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotic–antimycotic). All dilutions virus suspensions (100 μL) were added into the wells containing cells. In the case of 10^4 dilution levels suspensions added 200 μL. After shaking the plates for 10 min using a shaker (FMS2, FINEPCR, Korea), they were incubated at 37 °C in 5% CO_2 conditions. After 1 h of incubation, 2 × type II agarose (SIGMA, Saint Louis, Missouri, USA) supplemented with 2 × DMEM was added to the inoculated cells; each well received from 1 mL to 2 mL of this mixture. The plates were then left at room temperature for 20 min and then incubated for 2–3 days for MNV-1 and 7–8 days for HAV at 37 °C in 5% CO_2 conditions. The cells were fixed with 1 mL of 3.7% formaldehyde for 4 h. The formaldehyde was discarded and the 2 × Type II agarose and 2 × DMEM mixture overlays were removed carefully using tap water. The fixed cells were dyed with a 0.1% (w/v) crystal violet solution for 20 min to visualize the plaques (Fig. 2). The plaques were counted and the virus infectivity titer has been described as PFU/mL.

2.8. Surface color

APP jets treated and untreated control samples (each 3 different samples) were prepared. The surfaces of samples were measured using Color Difference Meter (UltraScan PRO, Hunterlab, USA). Prior analysis, the color difference meter was calibrated with standard black and white tiles. Values L* (lightness), a* (redness +, greenness–) and b* (yellowness +, blueness–) were recorded and the mean of 3 measurements was recorded for each sample (Hunter & Harold, 1987).

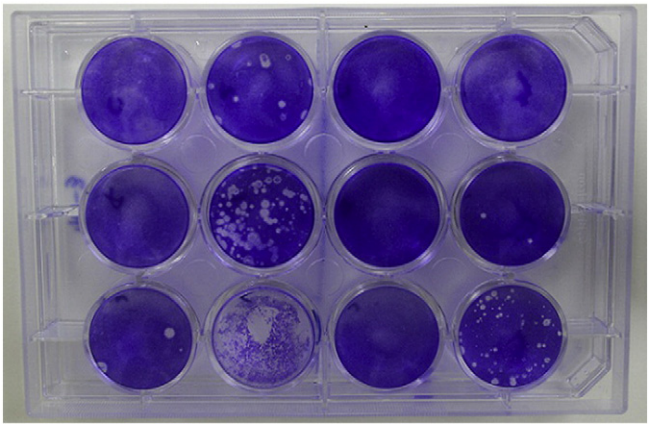

Fig. 2. The form of plaques in the 12-well plate after staining with crystal violet.

2.9. Moisture content (%)

Control and treated meats were placed into a 105 °C infrared moisture content tester (Precisa Gravimetrics AG, Switzerland) and dried to a constant weight. The moisture content tester utilizes the heat/dry weight change measurement principle. This allows for an accurate (as low as 0.01%) determination of the total moisture content.

2.10. 2-Thiobarbituric acid-reactive substances (TBARS) value

The TBARS assay was performed as described by Turner et al. (1954). Following the treatment, the samples were added into a 50 mL conical tube with 5 ml of 20% trichloroacetic acid (TCA; WAKO Pure Chemical Industries, Osaka, Japan) in 2 M phosphoric acid (WAKO pure chemical industries, Osaka, Japan) and 10 ml of 0.01 M thiobarbituric acid (TBA; SIGMA, Spruce Street, Louis, USA), and the solution was vortexed. The mixture was then heated in a water bath for 30 min and cooled quickly with ice water for 10 min. A 15 ml mixture of isoamyl alcohol (JUNSEI Chemical, Tokyo, Japan): pyridine (SIGMA, Spruce Street, Louis, USA) (2:1, v/v) was added to the conical tube, and the solution was vortexed for 2 min. The mixture was centrifuged at a speed of 3000 *g* for 15 min. The absorbance of the supernatant was measured at 538 nm (Spectra Max 190, Molecular Devices, USA).

2.11. Statistical analysis

All experiments were carried out in triplicates. For statistical analysis, we used the Statistical Analysis System software version 9.2 (SAS Institute, Cary, NC, USA). Differences among means were examined with Duncan's multiple range test and differences at the 5% level were considered significant. Model and parameter adequacy were considered significant at the $p < 0.05$ level unless otherwise noted. Each mean value represents the average of measurements from three samples and means were considered significantly different at $p < 0.05$. Least squares mean separation slope differences were analyzed by SAS as described above and considered significant at $p < 0.05$.

3. Results and discussion

3.1. Inactivation effects of APP jets on MNV-1 and HAV on the fresh meats

To examine the effects of APP jets against MNV-1 and HAV on the surface of fresh meats, we treated the samples with APP jets for a range of times between 0.5 and 20 min. MNV-1 titers were determined after APP jets at different exposure times as shown Fig. 3(a). The MNV-1 titer from beef loins, pork shoulders and chicken breasts decreased with increase in APP jet treatment time. For the beef, pork and chicken, there was an initial steep drop of 2.05, 2.11 and 2.01 $\log_{10}$ PFU/mL, respectively, in the MNV-1 titer up to 5 min of treatment time. The total MNV-1 titer of 2.09 $\log_{10}$ PFU/mL in beef, 2.15 $\log_{10}$ PFU/mL in pork and 2.07 $\log_{10}$ PFU/mL in chicken decreased after the maximum treatment of 20 min. The total MNV-1 titer significantly decreased ($p < 0.05$) at longer exposure times to APP jets (0.5–20 min). However, there were no significant differences ($p > 0.05$) in the MNV-1 titers after APP jet treatment between 5 and 20 min. Besides, there were no significant differences ($p > 0.05$) in the MNV-1 titers in the three kinds of meat after APP jet treatment of 0.5–20 min.

The overall average HAV titers with different exposure times to APP jets were analyzed (Fig. 3(b)). Same as the trends shown in MNV-1 titer, the HAV titer from beef loins, pork shoulders and chicken breasts decreased as the APP jet treatment time increased. There was an initial steep drop of 1.45 $\log_{10}$ PFU/mL in beef, 1.49 $\log_{10}$ PFU/mL in pork, and 1.47 $\log_{10}$ PFU/mL in chicken in the HAV titer up to 5 min of APP jet treatment time. The total HAV titer of 1.47 $\log_{10}$ PFU/mL in beef, 1.54 $\log_{10}$ PFU/mL in pork and 1.51 $\log_{10}$ PFU/mL in chicken decreased after 20 min as the maximum treatment time. There were no significant

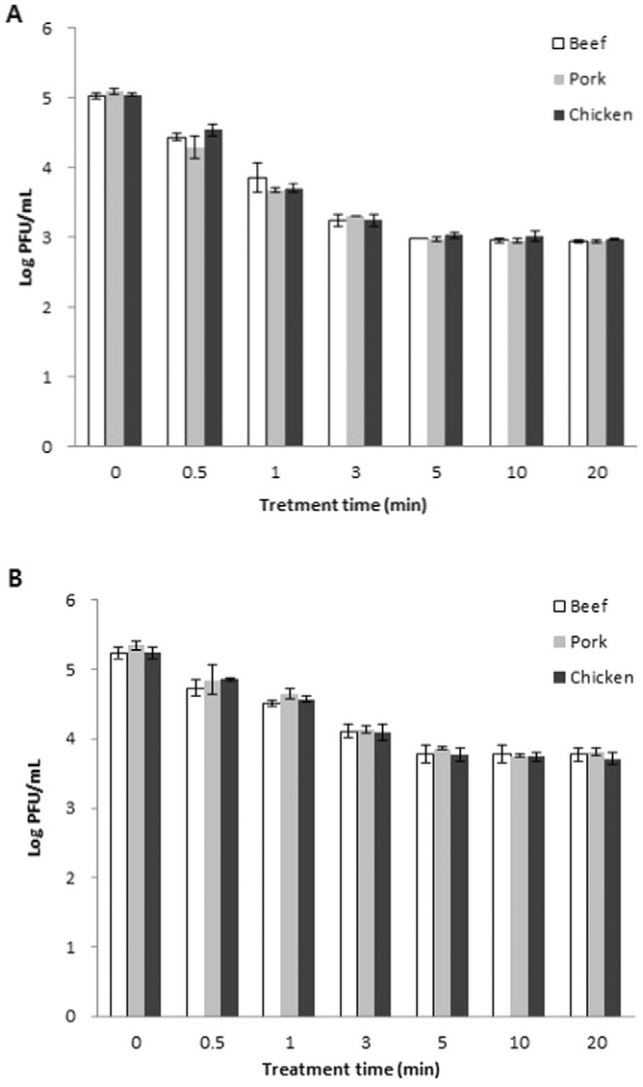

Fig. 3. Inactivation of MNV-1 and HAV on fresh meats by APP jets. (A) MNV-1, (B) HAV.

differences ($p > 0.05$) in the HAV titers after APP jet treatment between 5 and 20 min although the total HAV titer significantly decreased ($p < 0.05$) at longer exposure times to APP jets. Furthermore, no significant differences ($p > 0.05$) in the HAV titers were observed in the three kinds of meat after APP jet treatment of 0.5–20 min.

Kayes et al. (2007) reported that, after the initial treatment of foodborne pathogens (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella enteritidis*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, and *Shigella flexneri*) with atmosphere uniform glow discharge plasma, inactivation curves sharply decreased with a shouldering pattern. However, there was a decrease in the inactivation rate after long exposure times (Kayes et al., 2007). These results are somewhat similar to our current observations with MNV-1 and HAV treated with APP jets on fresh meats. It has been reported that several factors can influence the inhibitory effects, such as microbial species, exposure type, injected gas type, and the number of cell layers in the sample (Song et al., 2009). The plasma emits antiviral and antimicrobial materials, such as photons, electrons, positive and negative ions, free radicals, neutral atoms, UV photons, as well as reactive oxygen and reactive nitrogen species, with enough energy to affect the foodborne pathogens (Wan et al., 2009; Ahlfeld et al., 2015). During the exposure to the plasma, these particles have a sterilization effect by directly being in contact with the surface. Active oxygen and active nitrogen species are likely to affect the cells by reacting with the diverse

Table 1
Color measurement of the three kinds of fresh meats treated by APP jets.

Meat types	Treatment time (min)	Color		
		L* value	a* value	b* value
Beef loin	0	44.36 ± 0.18^{a}	17.64 ± 0.45^{ab}	14.22 ± 0.49^{a}
	0.5	44.12 ± 0.23^{a}	17.49 ± 0.46^{ab}	15.38 ± 0.99^{bc}
	1	44.04 ± 0.05^{a}	17.36 ± 0.27^{a}	15.23 ± 0.64^{bc}
	3	43.90 ± 0.34^{ab}	17.26 ± 0.23^{a}	14.84 ± 0.61^{ab}
	5	43.90 ± 0.40^{ab}	17.45 ± 0.07^{ab}	15.25 ± 0.19^{ab}
	10	43.16 ± 0.89^{bc}	18.33 ± 0.45^{bc}	16.24 ± 0.47^{d}
	20	42.83 ± 0.41^{c}	18.01 ± 0.20^{c}	16.50 ± 0.36^{d}
Pork shoulder	0	49.27 ± 0.26^{a}	16.84 ± 0.53^{a}	16.84 ± 0.53^{a}
	0.5	49.05 ± 0.12^{a}	16.62 ± 0.28^{a}	17.07 ± 0.01^{ab}
	1	48.92 ± 0.23^{a}	16.83 ± 0.45^{a}	16.68 ± 0.28^{a}
	3	48.88 ± 0.03^{a}	17.30 ± 0.08^{ab}	16.79 ± 0.51^{a}
	5	48.83 ± 0.19^{a}	17.32 ± 0.17^{ab}	16.65 ± 0.57^{a}
	10	48.04 ± 0.73^{b}	18.03 ± 0.53^{bc}	18.04 ± 0.09^{c}
	20	47.77 ± 0.59^{b}	18.33 ± 0.52^{c}	17.89 ± 0.89^{c}
Chicken breast	0	63.10 ± 0.51^{a}	6.24 ± 0.13^{a}	18.98 ± 0.21^{a}
	0.5	62.43 ± 0.16^{ab}	6.26 ± 0.06^{a}	19.34 ± 0.43^{ab}
	1	63.18 ± 0.17^{a}	6.76 ± 0.08^{ab}	19.82 ± 0.49^{a}
	3	62.13 ± 0.56^{a}	6.95 ± 0.60^{b}	18.98 ± 0.17^{a}
	5	63.05 ± 0.69^{ab}	7.04 ± 0.50^{b}	19.69 ± 0.44^{a}
	10	61.68 ± 0.66^{c}	7.93 ± 0.11^{c}	20.19 ± 0.58^{c}
	20	61.67 ± 0.14^{c}	8.26 ± 0.45^{c}	18.66 ± 0.06^{c}

Values are mean ± standard deviation.
[a–d]Means in the same column are significantly ($p < 0.05$) different by Duncan's multiple range test.
L* values = lightness (0 = dark, 100 = bright).
a* values = redness/greenness (+ = red, − = green).
b* values = yellowness/blueness (+ = yellow, − = blue).

intracellular macromolecules (Fridovich, 1995). Several studies have been performed on the antiviral and antimicrobial effects of plasma on foods and food-related environments. Basaran, Basaran-Akgul, and Oksuz (2008) observed that low-pressure cold plasma is effective at reducing *Aspergillus parasiticus* on the surfaces of different kinds of nuts. Lee et al. (2012) reported that APP jets are effective against *L. monocytogenes* on the surface of agar and processed meat surfaces. Song et al. (2009) showed that *L. monocytogenes* on sliced cheese and ham was effectively controlled by APP jets, and that various factors influenced the effectiveness of inactivation. Yong et al. (2015) reported that *L. monocytogenes* on sliced cheese was effectively reduced by encapsulated atmospheric pressure dielectric barrier discharge plasma. Ahlfeld et al. (2015) demonstrated that APP treatment impacts the inactivation of NoVII.4 strains on NoV positives stool sample; 1.23 log and 1.69 log genomic equivalents/mL reduction after exposure 10 and 15 min, respectively (initial quantity of 2.36×10^4 genomic equivalents/mL). Alshraiedeh, Alkawareek, Gorman, Graham, and Gilmore (2013) reported that APP as non-thermal treatment is useful disinfection process of rapid antiviral activity against NoV surrogate as MS2 bacteriophage. Park and Ha (2015) observed that, MNV-1 and HAV on fresh chicken breasts were reduced up to 1 log by UV-C light with 3600 mWs/cm^2 (irradiation dose of UV-C; 1000 mW/cm^2,

Table 2
Moisture content (%) measurement of the three kinds of fresh meats treated by APP jets.

Treatment time (min)	Moisture content (%)		
	Beef loin	Pork shoulder	Chicken breast
Control	52.48 ± 0.49^{a}	53.33 ± 0.47^{a}	59.27 ± 1.80^{a}
0.5	51.72 ± 0.27^{ab}	53.00 ± 0.12^{a}	58.27 ± 1.01^{ab}
1	50.52 ± 0.92^{b}	52.21 ± 0.70^{ab}	57.72 ± 0.41^{ab}
3	50.31 ± 2.05^{b}	52.12 ± 0.24^{ab}	56.92 ± 0.38^{b}
5	49.99 ± 0.62^{b}	51.52 ± 1.29^{b}	57.02 ± 0.30^{b}
10	43.32 ± 0.10^{c}	46.50 ± 0.99^{c}	50.16 ± 1.61^{c}
20	40.88 ± 0.56^{d}	43.80 ± 0.33^{d}	50.00 ± 0.80^{c}

Values are mean ± standard deviation.
[a–d]Means in the same column are significantly ($p < 0.05$) different by Duncan's multiple range test.

Table 3
2-Thiobarbituric acid-reactive substances (TBARS) value measurement of the three kinds of fresh meats treated by APP jets.

Treatment time (min)	TBARS value (OD at 538 nm)						
	Control	0.5	1	3	5	10	20
Beef loin	0.059 ± 0.01[a]	0.062 ± 0.01[a]	0.063 ± 0.01[a]	0.074 ± 0.00[b]	0.075 ± 0.01[b]	0.080 ± 0.01[c]	0.082 ± 0.01[c]
Pork shoulder	0.054 ± 0.00[a]	0.063 ± 0.01[b]	0.068 ± 0.01[b]	0.073 ± 0.01[c]	0.075 ± 0.01[c]	0.081 ± 0.01[d]	0.094 ± 0.00[e]
Chicken breast	0.058 ± 0.01[a]	0.077 ± 0.00[b]	0.079 ± 0.01[b]	0.091 ± 0.01[c]	0.126 ± 0.00[d]	0.147 ± 0.01[e]	0.151 ± 0.01[e]

Values are mean ± standard deviation.
[a–e]Means in the same column are significantly ($p < 0.05$) different by Duncan's multiple range test.
TBARS expressed as mg MA (Malonaldehyde)/kg sample.

exposure time; 60 min). There is a limit to reduction of viruses. Also, at increased UV-C exposure time, meat is difficult to maintain the freshness. The current study showed that, APP treatment effectively reduces viruses up to 2 log after exposure 5 min, without change quality characteristic of three kinds of meats. Moreover, APP treatment does not leave chemical residues (Ahlfeld et al., 2015).

3.2. Effects of APP jet treatment on the physicochemical properties

To assess the affect of APP jets on fresh meats, the changes in the surface color of the meat samples used in this study was analyzed. The results of the surface color evaluation are shown in Table 1. Significant differences ($p < 0.05$) of "L", "a", and "b" were observed between 0.5 and 20 min treated samples exposed to APP jets. APP treatment for 0.5–5 min did not significantly affect color characteristics of meats, regardless of the type of the meat studied. Treatment of the fresh meats, decreased the L*-values and increased the a*- and b*-values. Kim et al. (2011) observed a decrease in L*-value and an increase in a*-value in bacon after APP treatment.

To assess the effects of exposure to APP jets on the moisture content (%) of fresh meats, the changes in these values were analyzed. The moisture content (%) values were significantly decreased ($p < 0.05$) in fresh meats during exposure to APP jets (0.5–20 min). However, there was no significant difference ($p > 0.05$) in the moisture content (%) of meats exposed to APP jets until after 5 min of treatment. The results of the current study indicate that, from 0.5 min to 5 min of APP treatment, there was no change in the moisture content (%) of the three kinds of fresh meat. The moisture content of meats was known to be related to its lightness in color (Kim et al., 2011). The authors explained that the L*-value can be reduced by water evaporation. This study showed that the moisture content and the L value decreased after treatment (Table 2). Several studies showed that there was no change in the color of apples, pork and egg shells after treatment with plasma (low-pressure cold plasma, cold plasma, APP and non-thermal atmospheric gas plasma) (Basaran et al., 2008; Moon et al., 2009; Niemira & Sites, 2008; Ragni et al., 2010). The results of the current study indicate that APP jet treatment times below 5 min do not affect the surface color of fresh meats.

To assess the effects of the exposure to APP jets on the TBARS of fresh meats, the changes in these values were analyzed. The results of the TBARS evaluation were shown in Table 3. The TBARS values significantly increased ($p < 0.05$) after longer exposure to APP jets (0.5–20 min). Mattison et al. (1986) reported that the acid patch can sense when the TBA values of fresh meat were more than 1.0 mg MA/kg. Although the TBARS values gradually increased with longer exposure to APP jets, the TBARS values in these studies were below 1.0 mg MA/kg. These results showed that the treatment of the three kinds of fresh meat with APP jets did not affect the TBARS values. Changes in TBARS values depend on the fatty acid composition and fat content of foods treated with APP jets (Kim et al., 2011). Kim et al. (2011) reported that lipid oxidation rapidly increased after gamma or electron beam irradiation. The changes in TBARS values were much smaller in samples treated with APP jets than for other non-heat treatments. Thus, the APP jet treatment may be a good alternative to non-thermal processes for high-fat foods.

4. Conclusions

This study showed that all APP jet treatments decreased the levels of MNV-1 and HAV viruses in three types of fresh meat, compared to the levels in untreated control samples. The viral titers of MNV-1 and HAV from the fresh meats decreased as the APP jet exposure time increased, without affecting meat quality when treatment time was under 5 min. In the samples treated with APP jets, the change in the meat quality was much smaller for other non-heat treatments. Thus, the APP jet treatment may be a good alternative to non-thermal processes for high-fat foods. Therefore, APP jet treatment can be used effectively to control the transmission of food-borne viruses during food preparation, and can thus be expected to reduce the outbreaks related to these viruses.

References

Acheson, D., & Fiore, A. E. (2004). Hepatitis A transmitted by food. *Clinical Infectious Diseases*, *38*, 705–715.

Ahlfeld, B., Li, Y., Boulaaba, A., Binder, A., Schotte, U., Zimmermann, J. L., et al. (2015). Inactivation of a foodborne norovirus outbreak strain with nonthermal atmospheric pressure plasma. *mBio*, *6*, e02300–e02314.

Alshraiedeh, N. H., Alkawareek, M. Y., Gorman, S. P., Graham, W. G., & Gilmore, B. F. (2013). Atmospheric pressure, nonthermal plasma inactivation of MS2 bacteriophage: Effect of oxygen concentration on virucidal activity. *Journal of Applied Microbiology*, *115*, 1420–1426.

Anang, D. M., Rusul, G., Bakar, J., & Ling, F. H. (2007). Effects of lactic acid and lauricidin on the survival of *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* O157:H7 in chicken breast stored at 4 °C. *Food Control*, *18*, 961–969.

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

Bae, J., & Schwab, K. J. (2008). Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Applied and Environmental Microbiology*, *74*, 477–484.

Basaran, P., Basaran-Akgul, N., & Oksuz, L. (2008). Elimination of *Aspergillus parasiticus* from nut surface with low pressure cold plasma (LPCP) treatment. *Food Microbiology*, *25*, 626–632.

Bidawid, S., Malik, N., Adegbunrin, O., Sattar, S. A., & Farber, J. M. (2003). A feline kidney cell line-based plaque assay for feline calicivirus, a surrogate for Norwalk virus. *Journal of Virological Methods*, *107*, 163–167.

Bogaerts, A., Neyts, E., Gijbels, R., & van der Mullen, J. (2002). Gas discharge plasmas and their applications. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *57*, 609–658.

Chun, H., Kim, J., Chung, K., Won, M., & Song, K. B. (2009). *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. *Meat Science*, *83*, 599–603.

D'Souza, D. H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C., et al. (2006). Persistence of caliciviruses on environmental surfaces and their transfer to food. *International Journal of Food Microbiology*, *108*, 84–91.

D'Souza, D. H., & Su, X. (2010). Efficacy of chemical treatments against murine norovirus, feline calicivirus, and MS2 bacteriophage. *Foodborne Pathogens and Disease*, *7*, 319–326.

Fridman, G., Brooks, A. D., Balasubramanian, M., Fridman, A., Gutsol, A., Vasilets, V. N., et al. (2007). Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria. *Plasma Processes and Polymers*, *4*, 370–375.

Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry*, *64*, 97–112.

Gweon, B., Kim, D., Kim, D. B., Jung, H., Choe, W., & Shin, J. H. (2010). Plasma effects on subcellular structures. *Applied Physics Letters*, *96*, 101501.

Hewitt, J., Rivera-Aban, M., & Greening, G. E. (2009). Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis A virus in heat inactivation studies. *Journal of Applied Microbiology*, *107*, 65–71.

Hong, Y. H., Ku, K. J., Kim, M. K., Won, M. S., Chung, K. S., & Song, K. B. (2008). Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* inoculated on chicken by aqueous chlorine dioxide treatment. *Journal of Microbiology and Biotechnology*, *18*, 742–745.

Hunter, R. S., & Harold, R. W. (1987). *The measurement of appearance* (2nd ed.). New York, NY: Wiley Interscience Publication.

Jean, J., Morales-Rayas, R., Anoman, M. N., & Lamhoujeb, S. (2011). Inactivation of hepatitis A virus and norovirus surrogate in suspension and on food-contact surfaces using pulsed UV light. *Food Microbiology*, *28*, 568–572.

Kayes, M. M., Critzer, F. J., Kelly-Wintenberg, K., Roth, J. R., Montie, T. C., & Golden, D. A. (2007). Inactivation of foodborne pathogens using a one atmosphere uniform glow discharge plasma. *Foodborne Pathogens and Disease*, *4*, 50–59.

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

Ko, J., Ma, Y., & Song, K. B. (2005). Effect of chlorine dioxide treatment on microbial growth and qualities of chicken breast. *Jonrnal of Food Science and Nutrition-New Series*, *10*, 122–129.

Koopmans, M., & Duizer, E. (2004). Foodborne viruses: An emerging problem. *International Journal of Food Microbiology*, *90*, 23–41.

Lee, H. J., Jung, H., Choe, W., Ham, J. S., Lee, J. H., & Jo, C. (2011). Inactivation of *Listeria monocytogenes* on agar and processed meat surfaces by atmospheric pressure plasma jets. *Food Microbiology*, *28*, 1468–1471.

Lee, H. J., Jung, H. S., Ham, J. S., & Jo, C. R. (2012). Effect of atmospheric pressure plasma jet on inactivation of *Listeria monocytogenes*, quality, and genotoxicity of cooked egg white and yolk. *Korean Journal of Food Science and Animal Resources*, *32*, 561–570.

Malek, M., Barzilay, E., Kramer, A., Camp, B., Jaykus, L. A., Escudero-Abarca, B., et al. (2009). Outbreak of norovirus infection among river rafters associated with packaged delicatessen meat, Grand Canyon, 2005. *Clinical Infectious Disease*, *48*, 31–37.

Mattison, M. L., Kraft, A. A., Olson, D. G., Walker, H. W., Rust, R. E., & James, D. (1986). Effect of low dose irradiation of pork loins on the microflora, sensory characteristics and fat stability. *Journal of Food Science*, *51*, 284–287.

Mattison, K., Shukla, A., Cook, A., Pollari, F., Friendship, R., Kelton, D., et al. (2007). Human noroviruses in swine and cattle. *Emerging Infectious Diseases*, *13*, 1184–1188.

Moisan, M., Barbeau, J., Crevier, M. C., Pelletier, J., Philip, N., & Saoudi, B. (2002). Plasma sterilization. Methods and mechanisms. *Pure and Applied Chemistry*, *74*, 349–358.

Moisan, M., Barbeau, J., Moreau, S., Pelletier, J., Tabrizian, M., & Yahia, L. H. (2001). Low-temperature sterilization using gas plasmas: A review of the experiments and an analysis of the inactivation mechanisms. *International Journal of Pharmaceutics*, *226*, 1–21.

Moon, S. Y., Kim, D. B., Gweon, B., Choe, W., Song, H. P., & Jo, C. (2009). Feasibility study of the sterilization of pork and human skin surfaces by atmospheric pressure plasmas. *Thin Solid Films*, *517*, 4272–4275.

Moore, C., Clark, E. M., Gallimore, C. I., Corden, S. A., Gray, J. J., & Westmoreland, D. (2004). Evaluation of a broadly reactive nucleic acid sequence based amplification assay for the detection of noroviruses in faecal material. *Journal of Clinical Virology*, *29*, 290–296.

Niemira, B. A., & Sites, J. (2008). Cold plasma inactivates *Salmonella* Stanley and *Escherichia coli* O157: H7 inoculated on golden delicious apples. *Journal of Food Protection*, *71*, 1357–1365.

Noriega, E., Shama, G., Laca, A., Diaz, M., & Kong, M. G. (2011). Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with *Listeria innocua*. *Food Microbiology*, *28*, 1293–1300.

Park, S. Y., & Ha, S. D. (2015). Ultraviolet-C radiation on the fresh chicken breast: Inactivation of major foodborne viruses and changes in physicochemical and sensory qualities of product. *Food and Bioprocess Technology*, *8*, 895–906.

Park, H., Kim, M., & Ko, G. (2010). Evaluation of various methods for recovering human norovirus and murine norovirus from vegetables and ham. *Journal of Food Protection*, *73*, 1651–1657.

Ragni, L., Berardinelli, A., Vannini, L., Montanari, C., Sirri, F., Guerzoni, M. E., et al. (2010). Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *Journal of Food Engineering*, *100*, 125–132.

Richards, G. P. (2001). Food-borne pathogens enteric virus contamination of foods through industrial practices: A primer on intervention strategies. *Journal of Industrial Microbiology and Biotechnology*, *27*, 117–125.

Robesyn, E., De Schrijver, K., Wollants, E., Top, G., Verbeeck, J., & Van Ranst, M. (2009). An outbreak of hepatitis A associated with the consumption of raw beef. *Journal of Clinical Virology*, *44*, 207–210.

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*, 233–238.

Siebenga, J. J., Vennema, H., Zheng, D. P., Vinjé, J., Lee, B. E., Pang, X. L., et al. (2009). Norovirus illness is a global problem: Emergence and spread of norovirus GII. 4 variants, 2001–2007. *Journal of Infectious Diseases*, *200*, 802–812.

Son, N. R., Seo, D. J., Lee, M. H., Seo, S., Wang, X., Lee, B. H., et al. (2014). Optimization of the elution buffer and concentration method for detecting hepatitis E virus in swine liver using a nested reverse transcription-polymerase chain reaction and real-time reverse transcription-polymerase chain reaction. *Journal of Virological Methods*, *206*, 99–104.

Song, H. P., Kim, B., Choe, J. H., Jung, S., Moon, S. Y., Choe, W., et al. (2009). Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes*. *Food Microbiology*, *26*, 432–436.

Straub, T. M., Honer zu Bentrup, K., Bartholomew, R. A., Valdez, C. O., Bruckner-Lea, C. J., & Gerba, C. P. (2007). In vitro cell culture infectivity assay for human noroviruses. *Emerging Infectious Diseases*, *13*, 396–403.

The Center for Science in the Public Interest (CSPI) (2014). Outbreak alert, 2014. http://www.cspinet.org/food safety/outbreak_report.html.

Turner, E. W., Paynter, W. D., Montie, E. J., Bessert, M. W., Struck, G. M., & Olson, F. C. (1954). Use of the 2-thiobarbituric acid reagent to measure rancidity in frozen pork. *Food Technology*, *8*, 326–330.

Wan, J., Coventry, J., Swiergon, P., Sanguansri, P., & Versteeg, C. (2009). Advances in innovative processing technologies for microbial inactivation and enhancement of food safety—Pulsed electric field and low-temperature plasma. *Trends in Food Science and Technology*, *20*, 414–424.

Wobus, C. E., Thackray, L. B., & Virgin, H. W. (2006). Murine norovirus: A model system to study norovirus biology and pathogenesis. *Journal of Virology*, *80*, 5104–5112.

Yong, H. I., Kim, H. J., Park, S., Alahakoon, A. U., Kim, K., Choe, W., et al. (2015). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma. *Food Microbiology*, *46*, 46–50.

Zhou, G. H., Xu, X. L., & Liu, Y. (2010). Preservation technologies for fresh meat—A review. *Meat Science*, *86*, 119–128.