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

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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

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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.

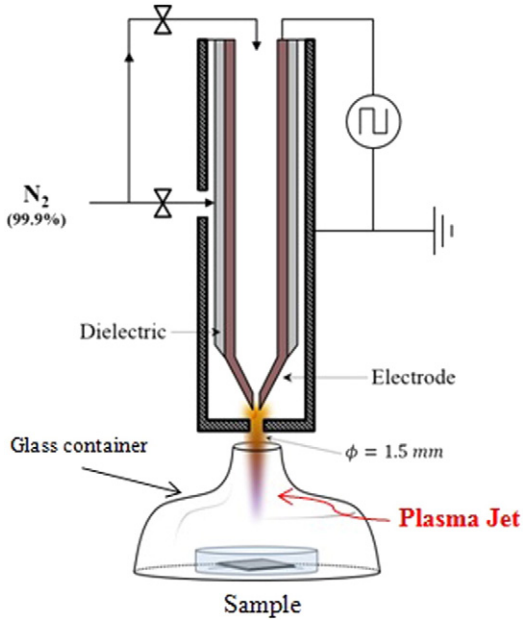

Fig. 1. Schematic diagram of the experimental system of atmospheric pressure plasma jets (APP jets).