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# Assessment of cold oxygen plasma technology for the inactivation of major foodborne viruses on stainless steel

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ABSTRACT

This study investigated the efficacy of cold oxygen plasma (COP) for inactivation of murine norovirus-1 (MNV-1; a human norovirus [NoV] surrogate) and hepatitis A virus (HAV) on stainless steel surfaces. Decrease in the MNV-1 and HAV titers resulting from 10 to 300 s of COP were 0.27–3.89 and 0.77–2.02 log PFU/ml, respectively. The Weibull model was used to calculate D-values of 1, 2, and 3-log reductions as the treatment times for MNV-1 ($R^2 = 0.95$, RMSE = 0.08) and HAV ($R^2 = 0.96$, RMSE = 0.05). The D = 2 and D = 3 values for MNV-1 (0.72 min for D = 2, 4.98 min for D = 3) were less than those for HAV (1.43 min for D = 2, 9.99 min for D = 3). However, there was no significant difference in values for D = 1 by COP between MNV-1 (21.65 s) and HAV (25.10 s). COP treatment on food contact surfaces could be effective for inactivation of NoV and HAV.

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## 1. Introduction

Human norovirus (NoV) and hepatitis A virus (HAV) are considered major causes of epidemic non-bacterial gastroenteritis worldwide. The transmission of pathogenic microorganisms to food through contaminated surfaces is a significant problem in food processing, catering, and industrial environments. Among food-contact surfaces, stainless steel is commonly used in food manufacturing and processing industries for manufacture, bulk storage and transportation, preparation, and presentation applications. The use of disinfectants is a key intervention measure to interrupt the transmission of pathogenic microorganisms onto food-contact surfaces.

Plasma comprises partially or wholly ionized gases as a highly energized fourth state of matter.

Cold or non-thermal plasma technology (NTP) is considered an emerging physical intervention for disinfection in the food industry (Stoica et al., 2013). Cold oxygen plasma (COP) is based on the principle of plasma formation using gas, compring photons, electrons, free radicals generated at room temperature, and atmospheric pressure (Niemira, 2012). BioZone Scientific has developed a novel NTP approach in which COP is produced by subjecting atmospheric air to high-energy, deep ultraviolet light with an effective radiation spectrum between 180 and 270 nm. This COP is composed of $O^+$, $O^-$, $O_2$, O, $O_3$, ionized O, metastable excited O, free electrons, and UV-photon. In a study by Terrier et al. (2009), COP treatment was effective in controlling airborne viral contamination.

No attempts have been made to evaluate the efficiency of COP treatment against foodborne enteric viruses on food-contact surfaces. Therefore, this study investigated the effectiveness of COP treatment in controlling the infectivity of NoV, using murine norovirus-1 (MNV-1) as a NoV surrogate, and HAV on a stainless steel surface. The investigation was intended to compare reduction responses of the viruses on the COP treated surface.

## 2. Materials and methods

### 2.1. Virus cell culture

Murine norovirus-1 (MNV-1; a surrogate for NoV) and Hepatitis A virus (HAV) HM-175 were maintained in murine RAW 264.7 cells and monkey FRhK-4 cells, respectively. Cells were cultured following the previously described method by Park et al. (2015).

### 2.2. Virus preparation

Virus was prepared following the previously described method

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by Park et al. (2015). A 200-μl aliquot of the MNV-1 and HAV HM-175 inoculums was added to the flasks, which were then incubated for 30 min to allow virus adsorption. The flasks then received 15 ml of maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotic-antimycotic), and were incubated at 37 °C in a 5% $CO_2$ atmosphere for 3 days (RAW 264.7) or 7 days (FRhK-4). If the observed cytopathic effects were >90%, the virus-infected flasks were frozen and thawed three times. The viruses were released by cell lysis during this step. The above contents were centrifuged at 1500 × *g* for 10 min to remove the cell debris, and the supernatants were subsequently harvested.

### 2.3. Preparation of stainless steel surface material and viral inoculation

The stainless steel coupons (10 mm in diameter, 5 mm in thickness) were soaked in 70% ethanol for 1 h to remove any residue such as oil and then washed with distilled water. After rinsing, the coupons were dried in a desiccator and placed in a sealed bottle to be autoclaved at 121 °C for 15 min. Fifty microliters of each viral suspension, containing approximately 5.0 log plaque-forming units (PFU)/ml of MNV-1 and 6.1 log PFU/ml of HAV, were inoculated onto the surface of the coupons. Inoculated coupons were dried in a laminar flow hood at room temperature for 1 h.

### 2.4. COP device and treatment

The operation of the COP device was previously described by Jahid et al. (2014). Atmospheric air was used to generate COP. The apparatus functions by subjecting air to high-energy, deep-UV light (30 W pressure UV-C lamp). The distance between the sample and the electrode is modifiable; in this study, the electrode was placed above (10 cm) the samples. Ozone production was monitored using the Ozone Analyzer (Excellence in Instrumentation, USA) at a distance of 9.5 cm; approximately 3.0 ppm ozone was generated. Emitted UV light was measured by a photo-radiometer (HD2102.1, Delta Ohm, Italy), with doses of 1210–1250 μW/cm$^2$ at a distance of 9.5 cm. The temperature did not exceed 32.5 °C. The top surfaces of the 3 sample coupons, placed in a sterile Petri dish, were exposed to COP for 0, 10, 30, 60, 90, 120, 180, 240, and 300 s.

### 2.5. Sample processing for virus recovery

Following COP radiation, 50 μl of maintenance medium were deposited on the center of the COP treated coupons. The virus-contaminated coupons bearing the medium were soaked in 450 μl of the same medium in 15-ml conical tubes. The samples were vortexed for 2 min to elute the virus. Each eluted viral suspension was serially diluted in DMEM. Viral titers were determined using a plaque assay (Park et al., 2015).

### 2.6. Determination of D = 1, 2, and 3 values of COP using the Weibull model

The Weibull model can be expressed as:

$$\mathrm{Log}\left(\frac{Nt}{N0}\right) = bt^n \quad (1)$$

Here, *Nt* is the virus concentration after an COP exposure time *t*, *No* is the initial concentration of virus, *t* is the exposure time, *b* and *n* are the scale (a characteristic time) and the shape parameter as a behavior index, respectively. The *b* value represents the time required to reduce the population by one log unit. The *n* parameter indicates the shape of the survival curve. For the calculation of D from the Weibull parameters, equation (2) was used (Buzrul and Alpas, 2007).

$$\mathrm{D} = 1,\ 2,\ \text{or}\ 3\left(\frac{a}{b}\right)^{1/n} \quad (2)$$

Here, the D-value indicates the COP exposure times required to reduce virus by 1, 2, or 3 log ($a$ = 1, 2, or 3).

## 3. Results

### 3.1. Effects of COP on MNV-1 inactivation and D-values on stainless steel surfaces

To assess the effects of COP against MNV-1 virus on stainless steel surface, the log reductions of MNV-1 with diverse exposure times of COP were analyzed. The $\log_{10}$ reductions of MNV-1 gradually increased ($P < 0.05$) with longer exposure times of COP (10–300 s, Table 1). More than 1 log-reduction were observed after all exposure times of COP, except that for 10 s. Specifically, > 3 log-reduction were demonstrated after 90–300 s of COP.

Data for MNV-1 survival on the stainless steel surface treated by COP were fitted using the Weibull model; the D = 1, D = 2, and D = 3 values of the COP exposure times were predicted using the model (Table 2). The Weibull model fitted the experimental data well for all the tested COP- times (10–300 s), as reflected by an $R^2$ (0.95) and RMSE (0.08) (Table 2). The D = 1, D = 2, and D = 3 value was 21.65 s, 43.27 s and 89.89 s (1.43 min) of COP. That is, the values were increased approximately 2-fold on the surface in line with the stepwise 1-log increase in D-value.

### 3.2. Effects of COP on HAV inactivation and D-values on stainless steel surfaces

The log reductions of HAV gradually increased ($P < 0.05$) at greater COP exposure times (10–300 s, Table 1). However, there were no significant differences in HAV log reduction for COP at 60–90 or 120–240 s. In addition, 1–2 log-reductions of HAV-1 were mostly observed at 30–300 s of COP. More than 2 log-reductions were observed only after 300 s of COP.

The HAV survival data from the COP-treated surfaces was also fitted using the Weibull model; the D-values of the exposure times were predicted using the model (Table 2). The model fitted the experimental data well for all the COP-tested times (10–300 s), as evident from an $R^2$ (0.96) and RMSE (0.05) (Table 2). The D = 2

**Table 1**
The reduction of MNV-1 and HAV titers ($\log_{10}$ PFU) on a stainless steel surface by COP treatment.

| COP (Second) | $\log_{10}$ reduction of MNV-1 | $\log_{10}$ reduction of HAV |
|---|---|---|
| 10 | 0.65 ± 0.07 $^{a, G}$ | 0.77 ± 0.01 $^{a, E}$ |
| 30 | 1.32 ± 0.12 $^{a, F}$ | 1.27 ± 0.06 $^{a, D}$ |
| 60 | 2.86 ± 0.06 $^{a, E}$ | 1.70 ± 0.11 $^{b, C}$ |
| 90 | 3.11 ± 0.04 $^{a, D}$ | 1.73 ± 0.01 $^{b, C}$ |
| 120 | 3.20 ± 0.03 $^{a, CD}$ | 1.84 ± 0.02 $^{b, B}$ |
| 180 | 3.39 ± 0.01 $^{a, BC}$ | 1.89 ± 0.02 $^{b, B}$ |
| 240 | 3.54 ± 0.08 $^{a, B}$ | 1.92 ± 0.02 $^{b, B}$ |
| 300 | 3.89 ± 0.27 $^{a, A}$ | 2.02 ± 0.03 $^{b, A}$ |

The data presents means of three samples with standard deviations (3 samples/treatment).
Within the same row, virus log reduction means with different letters (a or b for each duration time of COP) differ significantly ($P < 0.05$) by t-test.
Within the same column, virus log reduction means with different letters (A ~ G for MNV-1 or A ~ E for HAV) differ significantly ($P < 0.05$) by Duncan's multiple range test.

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