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Inactivation methods for human coronavirus 229E on various food-contact surfaces and foods

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ARTICLE INFO

Keywords:
HCoV-229E
Chlorine dioxide
Peroxyacetic acid
UV-C irradiation
Food-contact surfaces
Foods

ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of the COVID-19 outbreaks, is transmitted by respiratory droplets and has become a life-threatening viral pandemic worldwide. The aim of this study was to evaluate the effects of different chemical (chlorine dioxide \([\text{ClO}_2]\) and peroxyacetic acid \([\text{PAA}]\)) and physical (ultraviolet [UV]-C irradiation) inactivation methods on various food-contact surfaces (stainless steel \([\text{SS}]\) and polypropylene \([\text{PP}]\)) and foods (lettuce, chicken breast, and salmon) contaminated with human coronavirus 229E \((\text{HCoV-229E})\). Treatments with the maximum concentration of \(\text{ClO}_2\) (500 ppm) and \(\text{PAA}\) (200 ppm) for 5 min achieved \(>99.9\%\) inactivation on \(\text{SS}\) and \(\text{PP}\). At 200 ppm \(\text{ClO}_2\) for 1 min on lettuce, chicken breast, and salmon, the HCoV-229E titers were 1.19, 3.54, and 3.97 log\(_{10}\) TCID\(_{50}\)/mL, respectively. Exposure (5 min) to 80 ppm \(\text{PAA}\) achieved 1.68 log\(_{10}\) reduction on lettuce, and 2.03 and 1.43 log\(_{10}\) reductions on chicken breast and salmon, respectively, treated with 1500 ppm \(\text{PAA}\). In the carrier tests, HCoV-229E titers on food-contact surfaces were significantly decreased \((p < 0.05)\) with increased doses of UV-C \((0-60 \text{ mJ/cm}^2)\) and not detected at the maximum UV-C dose \((\text{Detection limit: } 1.0 \text{ log}_{10} \text{ TCID}_{50}/\text{coupon}). The UV-C dose of 900 \text{ mJ/cm}^2 proved more effective on chicken breast \((>2 \text{ log}_{10} \text{ reduction})\) than on lettuce and salmon \((>1 \text{ log}_{10} \text{ reduction})\). However, there were no quality changes \((p > 0.05)\) in food samples after inactivation treatments except the maximum \(\text{PAA}\) concentration \((5 \text{ min})\) and the UV-C dose \((1800 \text{ mJ/cm}^2)\).

1. Introduction

The world has been confronting a novel life-threatening challenge since December 31, 2019, when the first case of coronavirus disease 2019 (COVID-19) was identified. The pandemic started in Wuhan, China, and on March 11, 2020, it was declared by the World Health Organization (WHO) as a global public health emergency (World Health Organization, 2020). At the time of this writing, more than 480 million cases of COVID-19 and 6.1 million deaths have been confirmed in 226 countries (CoronaBoard, 2022).

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of COVID-19, is an enveloped RNA betacoronavirus of zoonotic origin. SARS-CoV-2 propagates mainly through contact via airborne routes, including droplet and aerosol transmission from infected individuals (van Doremalen et al., 2020). However, there were several studies that have proven SARS-CoV-2 viability on various foods and environmental surfaces (He et al., 2021; Jia et al., 2022; Ronca et al., 2021; van Doremalen et al., 2020). For that reason, contaminated surfaces may also play a role in the spread of the virus and could also be carriers of the virus (Razzini et al., 2020). Anelich et al. (2020) reported that 10% of SARS-CoV-2 transmissions occur through contact with contaminated surfaces. SARS-CoV-2 can persist in conditions found in frozen food, packaging, and cold-chain products, and index cases in recent outbreaks in China have been linked to the imported cold chain (Yuan et al., 2020). The WHO also reported the possibility of transmission of SARS-CoV-2 through frozen products (Joint WHO—China Study Team, 2021).

Coronavirus is a respiratory virus, not a foodborne virus. Based on existing literature data, there is no evidence that SARS-CoV-2 transmission is related to the consumption of contaminated food and water (Centers for Disease Control and Prevention [CDC], 2020). Nevertheless, food or contaminated food packaging should be considered an important carrier for the indirect transmission of the virus. Therefore, prevention and control should focus not only on humans but also on food, food-contact surfaces, and food packaging. Several vaccines have been developed and administered; however, the spread of COVID-19 is still...
ongoing due to the emergence of new types of variants, such as Delta and Omicron (C. Wang & Han, 2022).

Chemical disinfectants with proven virucidal activity are being investigated for their inactivation of SARS-CoV-2 and other coronaviruses on abiotic food-contact surfaces. In particular, chlorine dioxide (ClO₂) is widely used as a broad-spectrum antimicrobial in the food industry, but it can generate chlorinated byproducts detrimental to the environment and food processing settings (Keremi et al., 2020). Peroxycetic acid (PAA) is also commonly used for the inactivation of a variety of microorganisms in the food industry. It is a very strong oxidizing agent compared to chlorine-based disinfectants; moreover, it is ecofriendly, as PAA is decomposed into acetic acid, water, and oxygen, which already exist naturally in the agroecosystem (Kitis, 2004). One of the intermediate degradation products of PAA is hydrogen peroxide (H₂O₂), which may also contribute to the disinfection action and bacteriostatic effects of PAA.

Ultraviolet (UV) light treatment may be an effective strategy to control coronavirus on food-contact surfaces (Heilingloh et al., 2020; Kariva et al., 2006). Existing data collated by Bisht et al. (2001) suggests that an appropriate dose of UV-C could prove superior to other preservation techniques in maintaining the desired quality, enhancing the nutritive value of the product during storage, and being effective at eliminating COVID-19. Notably, UV-C (wavelength 200–280 nm) radiation does not produce chemical residues, byproducts, or radiation (Liberti et al., 2000; Rajala et al., 2003).

At the start of the pandemic, much was unknown about SARS-CoV-2 and its survival in food and on food-contact surfaces. Therefore, scientists turned to studies with similar viruses to address concerns about the possibility of transmission via food and food-contact surfaces and whether SARS-CoV-2 poses a risk to food safety. The first two human coronaviruses, HCoV-229E (alphacoronavirus) and HCoV-OC43 (betacoronavirus), were identified in the mid-1960s as generally associated with mild upper respiratory tract infection (Almeida & Tyrrell, 1967) and are estimated to cause up to 15–30% of the common cold in adults (Liu et al., 2021). HCoV-229E is easy to culture and shares a close evolutionary history and important physicochemical characteristics with recently emerged highly pathogenic coronaviruses, such as the Middle East respiratory syndrome-related coronavirus (MERS-CoV), SARS-CoV, and SARS-CoV-2 (Liu, 2021; Warnes et al., 2015). Moreover, live SARS-CoV-2 must be handled in biosafety level 3 (BSL-3) laboratories; therefore, this study evaluates the efficacy of two commonly used disinfectants (PAA and ClO₂) and UV-C irradiation against HCoV-229E, as a surrogate of SARS-CoV-2, on various food items and food-contact surfaces. In addition, a quality evaluation was performed in this study to evaluate the potential of these approaches as a control strategy for coronavirus in the real food industry.

2. Materials and methods

2.1. Cell culture

HCoV-229E and MRC-5 (CCL-171) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). MRC-5, a human fetal lung cell line, was cultured in Eagle’s minimum essential medium (MEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 44 mM sodium bicarbonate (Sigma-Aldrich), and 1% penicillin/streptomycin (Gibco) and incubated at 37 °C in a humidified incubator containing 5% CO₂. Then, the cells were sub-cultured every 3–4 days using trypsin/EDTA (0.25% Gibco).

2.2. Virus preparation

When MRC-5 cell monolayers reached 90–100% confluency in 75-cm² tissue culture flasks, the cells were washed twice with Dulbecco’s phosphate-buffered saline (DPBS; Sigma-Aldrich). Cells were inoculated with 3 mL of HCoV-229E (multiplicity of infection 0.01–0.1) and incubated at 33 °C under 5% CO₂ for 90–120 min to permit virus adsorption. Then, 7 mL MEM containing 1% (v/v) FBS, 44 mM sodium bicarbonate, and 1% penicillin/streptomycin was added for propagation. When the cytopathic effects (CPE) reached more than 90%, the virus-infected culture flasks were exposed to three freeze-thaw cycles to release virus particles by cell lysis. The propagated HCoV-229E solution was centrifuged (4,000 × g, 4 °C for 10 min), and the resultant supernatant was filtrated through a 0.2-μm filter and stored at −80 °C until use.

2.3. Sample preparation and inoculation

2.3.1. Food-contact surfaces

Stainless steel (SS) and polypropylene (PP) were selected as food-contact surfaces commonly used for cooking utensils and food-packaging materials. Coupons (1 cm in diameter) were serially washed with tap water and distilled water and then soaked in 70% ethanol for more than 1 h. After rinsing and drying, coupons were autoclaved at 121 °C for 15 min. For inoculum of food-contact surfaces, a 160-μL soil load (25 μL of 5% bovine serum albumin, 35 μL of 5% yeast extract, and 100 μL of 0.4% mucin) was added to 340 μL of virus suspension (approximately 6.4–6.8 log₁₀ TCID₅₀/mL, where TCID₅₀ denotes 50% tissue culture infectious dose). The virus stock containing soil load was prepared immediately before the experiment, and 50 μL of virus stock was inoculated on the middle of coupons and dried for 1 h (20–25 °C and 18–23% relative humidity [RH]).

2.3.2. Foods

Three different types of food were purchased from a local grocery market in Anseong (Korea) on the day of the experiment. Lettuce was washed with flowing tap water and then rinsed at least three times with distilled water. For chicken breast and salmon, the rinsing step was omitted to prevent microbial growth. All the food samples were dried in a laminar flow hood with UV rays for 10 min. With a sterile knife, each food sample was cut into 1 × 1 cm² for lettuce and 5 × 5 × 5 mm³ for the rest. Samples were inoculated with 50 μL of viral stock (approximately 6.6–7.2 log₁₀ TCID₅₀/mL), and then dried for 1 h (20–25 °C and 18–23% RH).

2.4. Disinfectant treatments

Both disinfectants used in this study are permitted as food additives by the U.S. Food and Drug Administration (FDA) and the Korea Ministry of Food & Drug Safety (MFDS). ClO₂ (0.1% initial concentration; Sungchan Co., Seoul, Korea) treatments were performed at 100, 200, 300, 400, and 500 ppm (for food-contact surfaces) and 25, 50, 100, and 200 ppm (for foods). PAA (16% initial concentration; Daesung C & F, Seoul, Korea) treatments were performed at 100, 200, 300, 400, and 500 ppm (for foods). PAA (16% initial concentration; Daesung C&S, Seoul, Korea) treatments were performed at 50, 100, 150, and 200 ppm for SS and PP; 20, 40, 60, and 80 ppm for lettuce; and 500, 1000, 1500, and 2000 ppm for chicken breast and salmon. Disinfectants were diluted with hard water, and the control (0 ppm) was treated with hard water. Hard water was prepared according to the Organization for Economic Cooperation and Development (OECD) method (OECD, 2013, p. 11).

The inoculated coupons were placed horizontally in 50-μL conical tubes and treated with 100 μL of disinfectant solution for 1 and 5 min. After each treatment, 900 μL MEM (containing 1% FBS), as a neutralizer, was immediately added, and then the samples were vortexed for 30 s to detach virus particles. In the food tests, virus-inoculated samples were immersed in 10 mL of disinfectant solution for 1 and 5 min. After each treatment, the samples were directly transferred to 1 mL of recovery solution (MEM containing 1% FBS). Treated samples with neutralizer were vortexed for 30 s and centrifuged (1500 × g, 4 °C, 5 min). The supernatant was collected through sequential filtration with 0.45- and 0.20-μm filters. Each eluted virus sample was analyzed by a TCID₅₀ assay.
2.5. UV-C irradiation

Samples were irradiated using 10-, 15-, and 30-W low-pressure UV lamps (Sankyo Ultraviolet Co., Seoul, Korea) emitting monochromatic UV irradiation at 260 nm. Prior to the study, UV irradiance was measured with a radiometer (HD 2102.2, Delta OHM, Padova, Italy). Food-contact surfaces were treated with UV-C doses of 15, 30, 45, and 60 mJ/cm², and foods were treated with 60, 300, 600, 900, and 1800 mJ/cm². Target doses were computed as the product of UV irradiance (mW/cm²) and exposure time (s). After predetermined exposure times, HCoV-229E-inoculated samples were removed from the UV irradiation system, vortexed for 30 s with 1 mL MEM containing 1% FBS, and centrifugation and filtration steps were added (for food samples only), as described in section 2.4.

2.6. TCID₅₀ assay

Cell culture plates (96-well) were evenly seeded with MRC-5 cells at a density of 1.0 × 10⁴/100 μL and incubated at 37 °C and 5% CO₂ for 24 h. The maintenance medium was aspirated, and 10-fold serially diluted viruses (100 μL) were inoculated to 8 wells. The plates were subsequently incubated at 33 °C and 5% CO₂ for 5 days with CPE as the endpoint. Virus titers were determined by the Reed–Muench method: (Reed & Muench, 1938).

\[
\text{TCID}_{50} = \frac{\text{log} \text{dilution above 50%}}{(\% \text{ positive above 50%}) - (\% \text{ positive below 50%})}
\]

2.7. Quality measurement

Instrumental color (CIELAB L*, a*, and b*) values for lightness, redness, and yellowness, respectively) of the surface of treated (ClO₂, PAA, UV-C) and control food samples was determined using an UltraScan Pro colorimeter (HunterLab Co., Reston, VA, USA). The instrument was standardized against the calibration tile (L* = 99.49, a* = −0.14, b* = −0.03) before each experiment. The average CIELAB values of at least five spots of each sample were recorded. The overall color difference value (ΔE*) was calculated by the following formula:

\[\Delta E^* = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}\]

Texture measurements of foods were recorded using a texture analyzer (TA-XT Express, Stable Micro Systems Ltd., Surrey, UK). The test conditions for lettuce were set as follows: SMS P/2N probe; pre-test speed of 1.0 mm/s; test speed of 1.0 mm/s; post-test speed of 5.0 mm/s; trigger force of 5.0 g. The test conditions for chicken breast and salmon were as follows: SMS P/0.5S probe; pre-test speed of 3.0 mm/s; test speed of 1.0 mm/s; post-speed of 5.5 mm/s; trigger force of 5.0 g. At least five replications were conducted for each sample, and the results (g/cm²) were analyzed using Exponent Lite Express (version 4.0.8.0; Stable Micro Systems Ltd.).

2.8. Statistical analysis

All experiments were repeated at least three times with triplicate samples. Data analysis was conducted using IBM SPSS Statistics version 26 (IBM Corp., Chicago, IL, USA). Viral titers were expressed as logarithmic functions (log₁₀ TCID₅₀/coupon on food-contact surfaces, and log₁₀ TCID₅₀/mL on foods) of the mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed on the HCoV-229E redution data, and differences between treatment variables were determined using Duncan’s multiple range test at p < 0.05.

3. Results

3.1. Effects of ClO₂ against HCoV-229E on food-contact surfaces and foods

Data illustrating the efficacy of ClO₂ against HCoV-229E on food-contact surfaces (SS, PP) at 100, 200, 300, 400, and 500 ppm for two treatment times (1 and 5 min) are shown in Table 1. After treatment with ClO₂ for 1 min, the HCoV-229E titers diminished by 0.62–4.0 log₁₀. Gradually, reduction values at 5 min increased until no counts were detected (Detection limit: 1.0 log₁₀ TCID₅₀/coupon). The reduction value of HCoV-229E on PP decreased by 0.51–2.31 at 1 min, and 0.51–3.90 at 5 min as the concentration of ClO₂ increased. There were no significant differences (p > 0.05) between 100 and 200 ppm against HCoV-229E on SS, whereas the average viral titers decreased significantly on PP with increasing ClO₂ levels.

For HCoV-229E inactivation on food surfaces (lettuce, chicken breast, salmon), ClO₂ was treated at 25, 50, 100, and 200 ppm for two treatment times (1 and 5 min). As shown in Table 1, the viral titers on lettuce decreased by 2.67, 2.39, and 1.29 log₁₀ TCID₅₀/mL at 25, 50, and 100 ppm, respectively.

| Targets          | Concentration (ppm) | Contact time |
|------------------|---------------------|--------------|
|                  |                     | 1 min | 5 min  |
| Stainless steel (SS) | Control | 5.46 ± 0.03 A,b | 4.86 ± 0.13 A,b |
|                  | 100 | 4.84 ± 0.16 A,b | 4.78 ± 0.09 A,b |
|                  | 200 | 4.64 ± 0.13 A,b | 4.22 ± 0.09 A,b |
|                  | 300 | 3.52 ± 0.15 C,a | 3.08 ± 0.22 D,b |
|                  | 400 | 2.62 ± 0.10 D,a | 1.62 ± 0.10 B,a |
|                  | 500 | 1.46 ± 0.07 E,C | N.C. |
| Polypropylene (PP) | Control | 5.50 ± 0.22 A,a | 5.46 ± 0.07 A,a |
|                  | 100 | 5.14 ± 0.13 A,a | 4.94 ± 0.12 A,a |
|                  | 200 | 4.52 ± 0.15 C,a | 4.39 ± 0.16 C,a |
|                  | 300 | 4.17 ± 0.14 D,a | 3.89 ± 0.25 D,a |
|                  | 400 | 3.52 ± 0.15 C,a | 2.14 ± 0.13 C,a |
|                  | 500 | 3.19 ± 0.05 E,C | 1.56 ± 0.09 E,C |
| Lettuce | Control | 3.69 ± 0.13 A,a | 3.46 ± 0.07 A,a |
|                  | 25 | 2.73 ± 0.09 B,a | 2.67 ± 0.17 A,B,a |
|                  | 50 | 2.64 ± 0.13 A,a | 2.39 ± 0.32 A,B,a |
|                  | 100 | 1.84 ± 0.28 C,a | 1.29 ± 0.26 C,a |
| Chicken breast | Control | 4.70 ± 0.04 A,a | 5.31 ± 0.12 A,a |
|                  | 25 | 4.36 ± 0.22 A,a | 4.08 ± 0.22 A,a |
|                  | 50 | 3.94 ± 0.19 A,a | 3.67 ± 0.19 A,a |
|                  | 100 | 3.67 ± 0.17 C,a | 3.56 ± 0.10 A,a |
|                  | 200 | 3.54 ± 0.19 C,a | 3.38 ± 0.12 A,a |
| Salmon | Control | 5.08 ± 0.22 A,a | 4.61 ± 0.12 A,a |
|                  | 25 | 4.67 ± 0.17 B,a | 4.41 ± 0.26 A,a |
|                  | 50 | 4.57 ± 0.24 B,a | 4.03 ± 0.21 B,a |
|                  | 100 | 4.19 ± 0.34 D,a | 3.78 ± 0.09 D,a |
|                  | 200 | 3.97 ± 0.24 E,a | 3.73 ± 0.09 E,a |

Values are represented with mean ± SD (n = 3). A-F indicate a significant (p < 0.05) difference within a column and a-b indicate a significant (p < 0.05) difference within a row. N.C.: No Cytopathic effect. Detection limits for each sample were 1.0 log₁₀ TCID₅₀/coupon (food contact surfaces), 1.0 log₁₀ TCID₅₀/mL (lettuce), 1.5 log₁₀ TCID₅₀/mL (chicken breast) and 2.5 log₁₀ TCID₅₀/mL (salmon).
3.2. Effects of PAA against HCoV-229E on food-contact surfaces and foods

Viral titers after treatment of food-contact surfaces with PAA (50, 100, 150, and 200 ppm) for 1 and 5 min are shown in Table 2. The efficacy of 200 ppm PAA against HCoV-229E on SS and PP caused reduction values of >1 log_{10} at 1 min, but a >3.8 log_{10} reduction at 5 min. The average viral titers on food-contact surfaces differed significantly (p < 0.05) between treatment times at all treated concentrations, except 50 and 100 ppm on SS.

PAA treatments were performed at 20, 40, 60, and 80 ppm for lettuce, and 500, 1000, 1500, and 2000 ppm for chicken breast and salmon (all for 1- and 5-min exposures), according to the MFDS guideline. As demonstrated in Table 2, the efficacy of PAA against HCoV-229E inoculated on lettuce increased gradually at increased concentration (p < 0.05), estimating 1.43 and 1.68 log_{10} reductions at 1 and 5 min, respectively. Viral counts on chicken breast and salmon treated with 1500 ppm for 5 min were reduced by 2.03 and 1.43 log_{10}, respectively, without significant changes (p > 0.05) in the values of color and texture. Viral titers against HCoV-229E on chicken breast and salmon were not significantly different (p > 0.05) between 50 and 1000 ppm at both 1- and 5-min exposures.

Table 2

| Targets          | Concentration (ppm) | Contact time (p<sub>0.05</sub>) | Contact time (p<sub>0.05</sub>) |
|------------------|---------------------|----------------------------------|----------------------------------|
|                  |                     | 1 min                            | 5 min                            |
| Stainless steel (SS) | Control             | 5.46 ± 0.07<sup>a,b</sup> | 4.86 ± 0.13<sup>a</sup> |
|                  | 50                  | 4.92 ± 0.22<sup>a</sup>          | 4.73 ± 0.09<sup>a</sup>          |
|                  | 100                 | 4.65 ± 0.17<sup>b</sup>          | 4.15 ± 0.28<sup>a</sup>          |
|                  | 150                 | 4.46 ± 0.12<sup>b</sup>          | 1.89 ± 0.25<sup>b</sup>          |
|                  | 200                 | 4.38 ± 0.22<sup>b</sup>          | 1.06 ± 0.10<sup>b</sup>          |
| Polypropylene (PP) | Control             | 5.50 ± 0.22<sup>a</sup>          | 5.46 ± 0.07<sup>a</sup>          |
|                  | 50                  | 5.35 ± 0.17<sup>b</sup>          | 4.70 ± 0.04<sup>b</sup>          |
|                  | 100                 | 5.08 ± 0.22<sup>b</sup>          | 4.08 ± 0.22<sup>b</sup>          |
|                  | 150                 | 4.78 ± 0.05<sup>b</sup>          | 2.42 ± 0.14<sup>b</sup>          |
|                  | 200                 | 4.35 ± 0.17<sup>b</sup>          | N.C                             |
| Lettuce          | Control             | 3.69 ± 0.17<sup>a</sup>          | 3.46 ± 0.07<sup>a</sup>          |
|                  | 20                  | 3.45 ± 0.12<sup>a</sup>          | 2.70 ± 0.04<sup>a</sup>          |
|                  | 40                  | 3.28 ± 0.19<sup>a</sup>          | 2.39 ± 0.19<sup>a</sup>          |
|                  | 60                  | 2.64 ± 0.13<sup>a</sup>          | 2.22 ± 0.05<sup>b</sup>          |
|                  | 80                  | 2.26 ± 0.10<sup>a</sup>          | 1.78 ± 0.09<sup>b</sup>          |
| Chicken breast   | Control             | 4.70 ± 0.04<sup>a</sup>          | 4.31 ± 0.17<sup>a</sup>          |
|                  | 500                 | 3.67 ± 0.17<sup>a</sup>          | 3.46 ± 0.07<sup>a</sup>          |
|                  | 1000                | 3.47 ± 0.29<sup>a</sup>          | 3.17 ± 0.33<sup>a</sup>          |
|                  | 1500                | 2.92 ± 0.22<sup>a</sup>          | 2.28 ± 0.19<sup>b</sup>          |
|                  | 2000                | 2.57 ± 0.24<sup>a</sup>          | 1.72 ± 0.19<sup>b</sup>          |
| Salmon           | Control             | 4.70 ± 0.04<sup>a</sup>          | 4.67 ± 0.29<sup>a</sup>          |
|                  | 500                 | 4.52 ± 0.15<sup>a</sup>          | 4.19 ± 0.05<sup>b</sup>          |
|                  | 1000                | 4.13 ± 0.22<sup>a</sup>          | 4.08 ± 0.22<sup>a</sup>          |
|                  | 1500                | 3.81 ± 0.34<sup>a</sup>          | 3.24 ± 0.35<sup>a</sup>          |
|                  | 2000                | 3.29 ± 0.43<sup>a</sup>          | 2.56 ± 0.10<sup>b</sup>          |

Values are represented with mean ± SD (n = 3). A–D indicate a significant (p < 0.05) difference within a column and a–b indicate a significant (p < 0.05) difference within a row. N.C.: No Cytotoxic effect. Detection limits for each sample were 1.0 log_{10} TCID<sub>50</sub>/coupon (food contact surfaces), 1.0 log_{10} TCID<sub>50</sub>/mL (lettuce), 1.5 log_{10} TCID<sub>50</sub>/mL (chicken breast) and 2.5 log_{10} TCID<sub>50</sub>/mL (salmon).

![Fig. 1. Effects of UV-C treatment against HCoV-229E on stainless steel (a) and polypropylene (b). Data represent mean ± standard deviation. A–D indicate a significant difference between different doses of UV (p < 0.05). Detection limit (1.0 log_{10} TCID<sub>50</sub>/coupon) is presented by a dotted line.](image)

3.3. Effects of UV-C against HCoV-229E on food-contact surfaces and foods

The virucidal activity of UV-C irradiation (15, 30, 45, and 60 mJ/cm<sup>2</sup>) against HCoV-229E on food-contact surfaces is illustrated in Fig. 1. The viral titers decreased as the dose of UV-C increased, and viral particles were not detected on both SS and PP treated at 60 mJ/cm<sup>2</sup> (Detection limit: 1.0 log_{10} TCID<sub>50</sub>/coupon). Reduction values at UV-C doses between 45 and 60 mJ/cm<sup>2</sup> on SS and 30–60 mJ/cm<sup>2</sup> on PP were not significantly different (p > 0.05).

To examine the virucidal effect of UV-C irradiation (60, 300, 600, 900, and 1800 mJ/cm<sup>2</sup>) against HCoV-229E on foods, the reduction of...
3.4. Quality measurement

The physicochemical effects of disinfectants (ClO₂ and PAA) and UV-C on foods are shown in Tables 3-5. Quality measurements (surface color, texture) were determined after 5-min treatments with disinfectants. No quality changes were observed in lettuce irrespective of the chemical and physical treatments compared to the control sample (p > 0.05). ClO₂ treatment did not affect the physicochemical quality of chicken breast, but L* value increased significantly (p < 0.05) at 2000 ppm PAA. Hardness, L* value, and b* value of chicken breast were changed significantly (p < 0.05) at the UV-C dose of 1800 mJ/cm². PAA treatment (2000 ppm) and UV-C irradiation (1800 mJ/cm²) significantly affected the physicochemical (hardness, L* value, and a* value) properties of salmon (p < 0.05), whereas ClO₂ treatment caused no significant influence (p > 0.05). Total color differences (ΔE*) of all treatments were <1.2, indicating no perceptible change (Mokrzycki & Tatol, 2011).

Table 3
Quality measurement of chlorine dioxide treatment (5 min) on foods.

| Targets          | Concentration (ppm) | Hardness (g/cm²) | L* value | a* value | b* value | ΔE*       |
|------------------|---------------------|------------------|----------|----------|----------|-----------|
| Lettuce          | Control             | 66.47 ± 0.76     | 77.57 ± 0.38 | -9.41 ± 0.22 | 28.77 ± 0.27 | 28.74 ± 0.06 |
|                  | 100                 | 67.09 ± 0.64     | 77.20 ± 0.44 | -9.35 ± 0.44 | 28.94 ± 0.56 | 0.82 ± 0.08 |
|                  | 200                 | 66.24 ± 0.77     | 77.04 ± 0.41 | -9.26 ± 0.29 | 28.47 ± 0.36 | 0.81 ± 0.06 |
| Chicken breast   | Control             | 348.24 ± 0.68    | 49.59 ± 0.36 | -0.89 ± 0.05 | 6.27 ± 0.23 | 0.23 ± 0.04 |
|                  | 100                 | 339.83 ± 0.73    | 49.89 ± 0.26 | -0.82 ± 0.17 | 6.15 ± 0.31 | 0.18 ± 0.06 |
|                  | 200                 | 339.51 ± 0.82    | 49.84 ± 1.05 | -0.78 ± 0.10 | 6.17 ± 0.07 | 0.34 ± 0.07 |
| Salmon           | Control             | 321.58 ± 0.42    | 41.66 ± 0.60 | 7.59 ± 0.35 | 4.87 ± 0.19 | 0.44 ± 0.01 |
|                  | 100                 | 321.05 ± 0.56    | 41.72 ± 0.46 | 7.49 ± 0.14 | 4.82 ± 0.12 | 0.27 ± 0.06 |
|                  | 200                 | 320.93 ± 0.71    | 42.35 ± 0.68 | 7.33 ± 0.45 | 4.78 ± 0.46 | 1.14 ± 0.09 |

Values are represented with mean ± SD (n = 5). Values without any remarks are not significantly different (p > 0.05). L* = lightness (0 = dark, 100 = bright), a* = redness/greenness (+ = red, - = green), b* = yellowness/blueness (+ = yellow, - = blue), ΔE* = overall color difference. The formulator provided in the table is ΔE* = \sqrt{ΔL^2 + Δa^2 + Δb^2}.

Fig. 2. Effects of UV-C treatment against HCoV-229E on lettuce (a), chicken breast (b), and salmon (c). Data represent mean ± standard deviation. A–E indicate a significant difference between different doses of UV (p < 0.05). Detection limit in (a) 1.0 log₁₀ TCID₅₀/mL, (b) 1.5 log₁₀ TCID₅₀/mL, and (c) 2.5 log₁₀ TCID₅₀/mL is presented by a dotted line.

4. Discussion

The existing literature indicates that the overall potential hazard from contaminated food and water, food-contact surfaces, or food packaging is likely to be low, leading to the conclusion that there is no evidence that SARS-CoV-2 is a food safety risk (FDA, 2021; World Health Organization, 2020). However, it is well-known that respiratory infections, particularly those caused by coronaviruses, may be transmitted from contaminated food and water, food-contact surfaces, or food packaging.
through indirect contact (Centers for Disease Control and Prevention, 2022) if someone touches a surface contaminated with the virus and then consequently touches the respiratory tracts, such as the mouth, nose, or eyes (Sohrabi et al., 2020). Therefore, it is important to find optimal methods for sanitizing and chemically treating food-contact surfaces and food to decrease the COVID-19 pandemic. Not many studies have yet evaluated the efficacies of ClO₂, PAA, and UV treatment against coronaviruses, such as SARS-CoV-2, on food-contact surfaces and foods. Therefore, the efficacy results of this study were compared with similar studies conducted with other viruses or bacteria.

Chlorine-based disinfectants, such as sodium hypochlorite (NaOCl) and ClO₂, are reasonably priced, easy to use, and widely applied in the food industry to control microorganisms or viruses (Farahmandfar et al., 2021). Viruses consist of an inner nucleic acid core and an outer protein, and the outer protein is divided into structure (spike) protein, envelope protein, and membrane protein (E. Kim & Lee, 2020). In general, free chlorine damages the viral genome or envelope and weakens the binding between the spike protein (receptor-binding domain) of the virus and host receptors (Quevedo et al., 2020; Young, 2016). The spike protein of the SARS-CoV-2 envelope is coated with glycans, which afford it greater protection compared to other viruses; nonetheless, ClO₂ damages glycans on the viral envelope and allows to weaken the binding of the spike protein with receptors (Eduardo et al., 2021).

When SARS-CoV-2 was treated with 24 ppm ClO₂ and NaOCl for 1 min each in the presence of 0.5% FBS, ClO₂ proved more effective than NaOCl, decreasing the viral titer by > 4 log and <2 log, respectively (Hatanaka et al., 2021). Furthermore, Miura and Shibata (2010) reported that ClO₂ was 10-fold more antiviral than NaOCl against influenza A virus. Similarly, Han et al. (2021) found that ClO₂ had more virucidal effects than NaOCl at lower concentrations in murine norovirus 1 (MNV-1) suspension and MNV-1-contaminated clam meat. NaOCl at 5000 ppm for 1 min reduced HCoV-229E on SS by more than 3 log_{10} PFU/mL, and NaOCl at 600 ppm for 1 min against transmissible gastroenteritis virus (TGEV, a diarrheal pathogen of swine and a member of the group I coronaviruses) and mouse hepatitis virus (MHV, a pathogen of laboratory mice and a member of group II coronaviruses) on SS achieved reductions of 0.62 and 0.35 log_{10} PFU/mL, respectively (Hulkower et al., 2011; Sattar et al., 1989). However, 500 ppm ClO₂ for 1 min yielded an HCoV-229E reduction of 4.0 log_{10} for 5 min, the population of S. Enteritidis on chicken skin was reduced by 1.93 log_{10} CFU/g (Byun et al., 2021). However, under the same condition, the present study obtained viral reduction values on SS, PP (plastic), and chicken breast of 0.88, 0.51, and 0.93 log. In addition, 30 ppm ClO₂ for 1 min on pathogen-inoculated lettuce inactivated 88% of Escherichia coli and 90% of S. enterica after 2 min compared with the untreated control (Hassenberg et al., 2021). Under a similar condition in the present study, 25 ppm ClO₂ for 1 min on lettuce reduced the HCoV-229E titer by 0.97 log_{10} TCID₅₀/mL. ClO₂ at 200 ppm for 5 min recorded a 1.07 log_{10} reduction in the natural microflora on salmon (J. Kim et al., 1999), and the present study achieved a 0.88 log_{10} reduction in the HCoV-229E titer under the same condition, indicating that viruses are more resistant to ClO₂ disinfection than bacteria.

Reduction values at all ClO₂ concentrations were similar on the three foods (p > 0.05) between the 1- and 5-min treatments, except for the chicken and salmon controls and the 50-ppm treatment of salmon. These results are similar to those from a study by Singh et al. (2002) in which the population of E. coli O157:H7 on lettuce and baby carrots was not

| Targets | Concentration (ppm) | Hardness (g/cm²) | L* value | a* value | b* value | Δ E* value |
|---------|---------------------|-----------------|----------|----------|----------|------------|
| Lettuce | Control             | 72.26 ± 0.56    | 83.27 ± 0.21 | -6.38 ± 0.29 | 21.70 ± 0.26 | 0.88 ± 0.28 |
|         | 40                  | 72.52 ± 0.36    | 82.97 ± 0.60 | -6.34 ± 0.31 | 21.87 ± 0.66 | 0.88 ± 0.30 |
|         | 80                  | 71.99 ± 0.68    | 83.63 ± 0.59 | -6.40 ± 0.13 | 21.72 ± 0.37 | 0.63 ± 0.42 |
| Chicken breast | Control             | 414.94 ± 0.43   | 54.44 ± 0.72  | -0.92 ± 0.24 | 5.36 ± 0.67  | 0.65 ± 0.16  |
|         | 1000                | 414.85 ± 0.57   | 55.02 ± 0.15  | -0.99 ± 0.15 | 5.30 ± 0.27  | 0.65 ± 0.16  |
|         | 1500                | 414.29 ± 0.45   | 55.08 ± 0.29  | -0.97 ± 0.28 | 5.24 ± 0.37  | 0.76 ± 0.54  |
|         | 2000                | 414.64 ± 0.29   | 55.41 ± 0.74  | -1.20 ± 0.12 | 5.18 ± 0.18  | 1.15 ± 0.48  |
| Salmon  | Control             | 444.35 ± 0.33   | 41.70 ± 0.68  | 7.61 ± 0.25  | 4.69 ± 0.34  |
|         | 1000                | 444.99 ± 0.52   | 41.84 ± 0.52  | 7.53 ± 0.41  | 4.47 ± 0.42  | 0.70 ± 0.31  |
|         | 1500                | 445.13 ± 0.60   | 41.87 ± 0.32  | 7.64 ± 0.32  | 4.58 ± 0.46  | 0.57 ± 0.16  |
|         | 2000                | 445.25 ± 0.78   | 42.54 ± 0.33  | 8.05 ± 1.00  | 4.22 ± 0.58  | 3.18 ± 0.97  |

Values are represented with mean ± SD (n = 5). A-B indicate a significant (p < 0.05) difference within a column. Values without any remarks are not significantly different (p > 0.05), L* = lightness (0 = dark, 100 = bright), a* = redness/greenness (+ = red, − = green), b* = yellowness/blueness (+ = yellow, − = blue), Δ E* = overall color difference. (V = Δ L² + Δ a² + Δ b²)

| Targets | UV-C dose (mJ/cm²) | Hardness (g/cm²) | L* value | a* value | b* value | Δ E* value |
|---------|-------------------|-----------------|----------|----------|----------|------------|
| Lettuce | Control           | 63.01 ± 0.60    | 81.50 ± 0.40 | -7.71 ± 0.21 | 24.76 ± 0.21 | 0.82 ± 0.55 |
|         | 900               | 62.40 ± 0.37    | 81.01 ± 0.68 | -7.68 ± 0.18 | 25.06 ± 0.50 | 0.77 ± 0.45 |
|         | 1800              | 62.77 ± 0.30    | 80.84 ± 0.39 | -7.59 ± 0.22 | 24.93 ± 0.39 | 0.46 ± 0.14 |
| Chicken breast | Control           | 553.34 ± 0.41   | 46.52 ± 0.29 | -0.14 ± 0.49 | 5.81 ± 0.43  |
|         | 900               | 553.11 ± 0.44   | 46.25 ± 0.37 | -0.12 ± 0.15 | 5.84 ± 0.19  |
|         | 1800              | 556.57 ± 0.46   | 45.79 ± 0.41 | 0.24 ± 0.07  | 6.35 ± 0.43  |
| Salmon  | Control           | 392.14 ± 0.63   | 40.49 ± 0.43 | 7.31 ± 0.29  | 4.66 ± 0.26  |
|         | 900               | 392.86 ± 0.53   | 40.04 ± 0.36 | 7.52 ± 0.29  | 4.74 ± 0.22  |
|         | 1800              | 395.35 ± 0.57   | 39.84 ± 0.46 | 7.76 ± 0.31  | 4.83 ± 0.45  |

Values are represented with mean ± SD (n = 5). A-B indicate a significant (p < 0.05) difference within a column. Values without any remarks are not significantly different (p > 0.05), L* = lightness (0 = dark, 100 = bright), a* = redness/greenness (+ = red, − = green), b* = yellowness/blueness (+ = yellow, − = blue), Δ E* = overall color difference. (V = Δ L² + Δ a² + Δ b²)
significantly different (p > 0.05) between 1- and 5-min treatments at all applied ClO₂ concentrations. Extending the contact time between foods and ClO₂ seems to improve the virucidal efficacy of ClO₂ treatment, as demonstrated in this study and the work of Singh et al. (2002).

The antiviral mechanism of PAA is still unclear. However, studies suggest that PAA oxidizes –SH and –NH groups, which may damage viral proteins of the envelope and the capsid, causing viral inactivation (Stampi et al., 2001; Wutzler & Sauerbrei, 2000). Schmitz et al. (2021) observed the low reactivity of RNA nucleotides compared to capsid proteins during PAA exposure, indicating that the capsid protein is the major target for the antiviral activity of PAA.

In the present study, 50–200 ppm PAA for 5 min on SS achieved HCoV-229E reduction values of 0.13–3.81 log₁₀ TCID₅₀/coupon. Furthermore, when treated for 5 min, the viral titers on PP were reduced by 0.76 log₁₀ TCID₅₀/coupon at 50 ppm PAA, and no CPE was observed at 200 ppm (Detection limit: 1.0 log₁₀ TCID₅₀/coupon). The titers of MNV-1 on SS diminished by 0.8 log₁₀ PFU/coupon at 50 ppm PAA for 5 min, and a >3 log₁₀ Reduction was achieved at 200 ppm for 5 min (Moon et al., 2021), showing a similar reduction range to the present study. However, at 200 ppm PAA for 1 and 5 min, the hepatitis A virus (HAV) log₁₀ reductions on SS were 0.04 and 0.1, respectively (Song et al., 2022). On SS and ultra-high molecular weight polyethylene (a kind of plastic), 50 ppm PAA for 5 min reduced S. enterica serovar Enteritidis biofilms by 2.76 and 2.32 log₁₀ CFU/cm², respectively (Byun et al., 2022), which were higher than the results of the present study.

The maximum concentrations of PAA applied to foods in this study were determined based on the MFDS guideline (MFDS, 2020). According to the present study, the viral titers on lettuce decreased significantly (p < 0.05) with the increased PAA concentration, and at 80 ppm for 1 and 5 min, a 1.43 log₁₀ reduction and no CPE was achieved, respectively (Detection limit: 1.0 log₁₀ TCID₅₀/mL). In addition, the log₁₀ reduction values of PAA treatments on lettuce were significantly different between 1 and 5 min (p < 0.05), except for the untreated samples. Likewise, the MNV-1 titer on lettuce was under the detection limit (Detection limit: 1.48 log₁₀ PFU/g) when treated with PAA at 75 ppm for 5 min, and significant differences were observed between the 1- and 5-min treatments at all PAA concentrations (Moon et al., 2021). PAA at 80 ppm for 5 min reduced the E. coli O157:H7 counts by 1.5 and 1.0 log on fresh-cut apple and cantaloupe rind, respectively (H. Wang et al., 2006). Furthermore, PAA at 80 ppm for more than 132 s on fresh produces (apples, lettuce, strawberries, and cantaloupe) contaminated with E. coli O157:H7 and L. monocytogenes achieved a 1 log₁₀ reduction (Rodgers et al., 2004). In conclusion, the application of 80 ppm PAA for 5 min reduced more than 1 log of microorganisms on contaminated fruits and vegetables. In the present study, PAA at 500–2000 ppm for 1 and 5 min on chicken breast led to HCoV-229E reduction values of 1.03–2.13 and 0.85–2.58 log₁₀ TCID₅₀/mL, respectively. Cap et al. (2019) showed that the Shiga toxin-producing E. coli population on beef samples decreased by 0.21 log₁₀ CFU/g after exposure to 200 ppm PAA for 15 s. At 200 and 400 ppm for 30 s, the E. coli O157:H7 counts on beef chops were reduced by 0.71 and 0.84 log₁₀ CFU/g, respectively (Visvalingam & Holley, 2018). Moreover, Salmonella reductions on chicken skin were 2.08 and 2.59 log₁₀ CFU/g following treatment with PAA at 700 and 1400 ppm for 15 s, respectively (Laranja et al., 2021). In the present study, PAA at 500–2000 ppm for 1 and 5 min produced viral reduction values on salmon of 0.19–1.41 and 0.47–2.11 log₁₀ TCID₅₀/mL, respectively. Zhao et al. (2021) found that PAA at 200 ppm for 10 min on more effectively achieved 5.4 log₁₀ CFU/g reduction against Listeria innocua, E. coli, and Pseudomonas fluorescens. The results obtained from the present study cannot be compared to these previous works, but it can be inferred that surface disinfection of meat and fish with PAA is more effective against bacteria than viruses.

UV irradiation is a well-known and effective approach for inactivating viruses as it offers benefits over chemicals and other physical methods, particularly on surfaces (Anelich et al., 2020). It is energy-efficient, leaves no chemical residual, is environmentally friendly, and has little or no adverse effects on food quality (Linden et al., 2019). Moreover, it has been proved to be the most effective for virus inactivation (Biasin et al., 2021; Sabino et al., 2020; Walker C & Ko, 2007) and is widely applied to the disinfection of viruses on environmental surfaces. The maximum absorption wavelength of DNA molecules is around 260 nm (Quevedo et al., 2020). Thus, UV-C light destroys genetic materials, such as RNA and DNA, and other cell membrane components of viruses, critical mechanisms for the viricidal effect of UV at wavelengths near 253.7, with the potential to cause cell death and disable replication without generating residues and harmful toxins in the process (Gidari et al., 2021).

The present study evaluated the efficacy of UV-C irradiation to inactivate HCoV-229E on selected food-contact surfaces and foods. The selected HCoV-229E population of 6.4–6.8 log₁₀ TCID₅₀/coupon for food-contact surfaces and 6.6–7.2 log₁₀ TCID₅₀/mL for foods provided a reasonable amount of virus but also encompassed the SARS-CoV-2 concentrations in swab samples taken from infected patients (Schiijven et al., 2020). As a result, a UV-C dose of 60 mL/cm² for 60 s was sufficient for HCoV-229E inactivation on both food-contact surfaces (SS and PP). In addition, the UV-C dose of 1800 mL/cm² achieved more than 1 log₁₀ reduction on lettuce and salmon, but a 2.6 log₁₀ reduction on chicken breast, probably due to the differences between the food matrices and food surface profiles or porosities. Fino and Kniel (2008) found that UV-C treatment (40, 120, 240 mW/s/cm²) resulted in 4.5–4.6 log₁₀ TCID₅₀/ml for lettuce, 2.5–5.6 log₁₀ TCID₅₀/ml for green onions, and 1.9–2.6 log₁₀ TCID₅₀/ml for strawberries contaminated with HAV, Aichi virus, and feline calicivirus, respectively. Similarly, UV-C irradiation was more effective for reducing bacteria contamination on SS than on chicken meat (T. Kim et al., 2002). UV-C is generally more effective on smooth and even surfaces than on rough and uneven surfaces, which harbor many cracks and crevices (T. Kim et al., 2002).

To the best of our knowledge, no previous study has investigated the inactivation activity of UV irradiation against isolates of SARS-CoV-2 on food, although some studies have targeted various commonly used materials. Biasin et al. (2021) and Crisculo et al. (2021) described UV-C irradiation as an efficient and sustainable method for inactivating SARS-CoV-2 on solid surfaces, such as glass and gauze. Similar results were observed by Crisculo et al. (2021), showing that short-wavelength UV light treatment was sufficient for the inactivation of SARS-CoV-2 adsorbed to different materials and at a distance of 20 cm from the light source. Complete inactivation (>99.9%) was observed on glass, plastic, and gauze and 90.0–94.4% on fabrics, but no more than 93.3% inactivation was recorded on wood even after 30 min of treatment, highlighting the need for proper sanitizing in food processing and preparation (Crisculo et al., 2021). In addition, various factors, such as surface structure or matrix (smooth/tough and porous/nonporous), RH of the ambient environment, and temperature, could affect the efficacy of UV-C on surfaces as they influence the shadowing effects (Tseng & Li, 2007). Therefore, it is essential to adjust the optimal conditions for inactivation by UV-C, such as exposure time and dose, and perform a robust validation before applying the technique to inactivate coronaviruses in the food industry.

Potential changes in the quality characteristics (surface color, hardness) were also measured after each disinfectant and UV-C treatment. Notably, no quality changes (p > 0.05) were observed in all treatments on lettuce. Previous studies also mentioned that treatment with chlorine-based disinfectants, PAA, and UV-C on fresh produce did not cause noticeable color changes (Ko et al., 2019; Li & Hung, 2019). All selected concentrations of ClO₂ did not affect the quality (p > 0.05) of the foods (lettuce, chicken breast, salmon) in contrast to the maximum treatments of PAA and UV-C, which caused slight quality differences (p < 0.05) in chicken breast and salmon compared to the control samples. It has already been shown that PAA at 700 ppm (5 min) and 1000 ppm (10 s) and UV-C irradiation at 500 mL/cm² on chicken skin or meat did not cause any substantial color changes (p > 0.05) (Chun et al., 2010; Laranja et al., 2021; Moore et al., 2022).
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2017). In the present study, applying PAA increased the brightness (L*-value) of chicken breast and salmon, and similar results were found in other research (Bauermeister et al., 2008; Moore et al., 2017). Chen et al. (2020) identified the epoxidation of the double bonds present in color compounds. Quality changes in chicken breast and salmon (p < 0.05) exposed to UV-C doses of >900 mJ/cm² were observed in the present study, including an increase in hardness and a decrease in the L*-value in both foods. Park and Ha (2015) recommended UV-C irradiation doses of 60–1200 mJ/cm² but not more than 1800 mJ/cm² for chicken breast.

5. Conclusion

The COVID-19 pandemic is an exceptionally growing concern worldwide, and uncertainties regarding additional modes of transmission, such as fomite transmission, remain unresolved. Especially, the transfer rate of SARS-CoV-2 via fomites needs to be established for various types of food-contact surfaces and foods to evaluate the potential risk and understand the importance of fomite transmission in the ongoing pandemic. This study assessed the efficacy of different chemical (ClO₂ and PAA) and physical (UV-C irradiation) inactivation methods on various food-contact surfaces (SS and PP) and foods (lettuce, chicken breast, and salmon) contaminated with HCoV-229E as a surrogate of SARS-CoV-2. All acquired findings from this study, together with existing data from the literature, suggest the important role of a powerful and efficient disinfection approach to control the spread of the virus in the food industry. Considering food safety issues, 5-min exposure to ClO₂ (500 ppm for food-contact surfaces and 200 ppm for foods) and PAA (200 ppm for food-contact surfaces, 80 ppm for lettuce, and 1500 ppm for chicken breast and salmon) could be applied in the food industry. UV-C irradiation at 60 and 900 mJ/cm² on food-contact surfaces and foods, respectively, also offers an alternative approach to control the virus. However, inactivation doses may vary depending on the food or surface type; therefore, further study is required to find the optimal condition for applying UV-C in the food industry.

CRediT authorship contribution statement

Eun Seo Choi: Conceptualization, Investigation, Writing – original draft, Visualization, Data curation. Sangha Han: Software, Writing – original draft, Writing – review & editing. Jeong won Son: Investigation. Gyeong Bae Song: Resources. Sang-Do Ha: Funding acquisition, Supervision.

Declaration of competing interest

None.

Data availability

The data that has been used is confidential.

Acknowledgments

Funding: This work was supported by the Ministry of Food and Drug Safety in 2021 (grant number 20162MFD5039); and by the Ministry of Food and Drug Safety in 2022 (grant number 21153MFD605).

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