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The efficacy of different sanitizers against MS2 bacteriophage introduced onto plastic or stainless steel surfaces

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ABSTRACT

The virucidal activities of 11 prepared disinfectant solutions (active ingredients of household sanitizers) and 10 household sanitizers against bacteriophage MS2 on plastic and stainless steel surfaces were studied. Among the prepared sanitizers, 70–90% ethanol and ethanol-based disinfectants resulted in 1–2.5 log PFU/mL reductions on both surfaces. The 70% isopropanol and isopropanol-based formula reduced MS2 by 0.7–1.5 log PFU/mL on both surfaces. Other disinfectants, containing 0.1% benzalkonium chloride (BAC), 0.5% hydrogen peroxide, or 4% acetic acid, showed significant ($P < 0.05$) lower log reductions (−0.17-0.55 log PFU/mL) compared with other treatments. At room temperature, the virucidal activities of 70% ethanol on plastic (1.46–1.64 log PFU/mL reductions) and stainless steel (0.84–0.93 log PFU/mL reductions) surfaces were not significantly ($P > 0.05$) affected by the treatment time (30–600 s). However, 85% ethanol-treated groups showed significant ($P < 0.05$) higher log reductions in 60 and 600 s treated groups (1.69–2.24 log PFU/mL) compared with those in 30 s treated groups (0.92–1.32 log PFU/mL). Their virucidal activities were further examined at low temperatures (4 and 8 °C). We observed that the surface inactivation efficacies were not affected by the low temperatures. In addition, the virucidal activities of household sanitizers revealed that sanitizers with 1.84% (pH = 12.5, ~17,500 ppm free-chlorine concentrations) or 3% (pH = 13.1, ~38,100 ppm free-chlorine concentrations) sodium hypochlorite (NaClO) reduced 4.15–6.23 log PFU/mL MS2 on hard surfaces after 60 s contact time. Furthermore, an approximately 1.5 log PFU/mL reduction was observed in groups treated by sanitizer H (active ingredients: 58% ethanol + 0.1% quaternary ammonium compound). Household products with BAC or organic acid resulted in −0.28-0.33 log reductions on two surfaces after 30 or 60 s treatment. Therefore, the use of ethanol and NaClO-based products should be considered as a potential surface decontamination strategy in the food industry.

1. Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged as a serious threat to public health (Liu et al., 2020). As of December 28th' 2021, more than 279 million cases, including 5.40 million deaths, were reported to the World Health Organization (WHO, 2021). SARS-CoV-2 is generally transmitted via direct contact between individuals, and airborne droplets and aerosols produced when coughing or sneezing (Prather et al., 2020). In addition, recent studies have suggested that indirect contact via contaminated surfaces is a secondary transmission route, as SARS-CoV-2 is able to survive on hard surfaces (plastic, stainless steel, copper, etc.) for up to 72 h (Chin et al., 2020; Van Doremalen et al., 2020; Marquès and Domingo 2020). However, the Centers for Disease Control and Prevention (CDC) suggested that the risk of surface transmission route is low. Surface disinfection is recommended in indoor environment where there has been a suspected/confirmed COVID-19 case (CDC, 2021). The surface stability of SARS-CoV-2 also greatly impacts many value chains in the food supply system and threatens food security and safety on a global scale. The U.S. Food and Drug Administration (FDA) suggested that employers in food industry should clean and disinfect a sick worker's workspace after waiting 24 h (U.S. FDA, 2020).

In addition to SARS-CoV-2, many other viruses also exhibit surface

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stability. For instance, norovirus (NoV), the leading cause of human acute viral gastroenteritis, is able to survive and remain infectious on fomite surfaces for 2 weeks or more (Bolton et al., 2013). Concurrently, hepatitis A virus (HAV) causes the highest number of foodborne invasive viral infections in humans. Its environmental persistence enables contamination of kitchen surfaces (Rajiuddin et al., 2020). Indeed, the most frequent reported foodborne outbreaks resulted from NoV and HAV infections have originated from restaurants or canteens, where the kitchen surface environments and foods were contaminated by infectious food handlers (Boxman et al., 2011; Fankhauser et al., 2002; Franck et al., 2015). To reduce the risk of potential surface contamination and transmission of related viruses under current COVID-19 pandemic, a number of measures, including regular surface disinfection, have been adopted by the food industry (Aday and Aday, 2020; Rajiuddin et al., 2020).

Previous studies have examined the efficacies of various sanitizers on hard surfaces artificially contaminated by viruses (e.g., SARS-CoV-2 and HAV) or their surrogates (e.g., beta human coronaviruses 229 E and OC43, bacteriophages MS2 and ΦX174) (Hoelzer et al., 2013; Meyers et al., 2021). Ethanol (62–80%) and isopropanol (70–80%) solutions were shown to be effective against coronavirus 229 E on porcelain and ceramic tiles, resulting in >4 log reduction (Meyers et al., 2021). Furthermore, household sanitizer with 3% sodium hypochlorite (NaClO) resulted in >3 log reductions of murine norovirus on stainless steel (Girard et al., 2010). The inactive efficacies of sanitizers are affected by various factors, such as disinfectant type, pH, pK_a, presence of metal cations, and physical properties of the viruses (Hoelzer et al., 2013). Generally, the active ingredients of household disinfectants contain alcohol, benzalkonium chloride (BAC), hydrogen peroxide (H_2O_2), acid, or NaClO (Hoelzer et al., 2013; Lin et al., 2020). However, little information about these sanitizers on food contact surfaces is available.

The aim of this research was to investigate the efficacies of 21 sanitizers, including 10 household sanitizers, in the inactivation of bacteriophage MS2 on plastic and stainless steel surfaces. MS2 is an *Escherichia coli* bacteriophage, and it is commonly used as a surrogate of human enteric viruses, such as NoV and HAV (Bozkurt et al., 2015). Moreover, MS2 can be used as a highly conservative surrogate for SARS-CoV-2. Similar with SARS-CoV-2, the MS2 is a positive-sense, single-stranded RNA virus. Unlike SARS-CoV-2, the MS2 phage lacks a lipid envelope, making it more resistant to disinfection (Zulauf et al., 2020). In this work, the inactivation potential of 11 sanitizers on MS2 at different exposure times was determined, followed by examining the effects of low temperatures (4 and 8 °C) on the disinfection efficacy of selected sanitizers. Although the risk of SARS-Cov-2 infection from cold chain food is very low, contamination of certain viruses (e.g., NoV and HAV) under low temperature is possible (Bozkurt et al., 2021; Lu et al., 2021; Mormann et al., 2015). Lastly, the disinfection performances of 10 household sanitizers were additionally examined.

2. Material and methods

2.1. Bacteriophage, bacterial host, and disinfectants

Freeze-dried MS2 bacteriophage (phage; ATCC 15597-B1) was purchased from Cedarlane Labs (Burlington, ON, Canada), and *Escherichia coli* (*E. coli*) ATCC 15597 was used as the host organism for phage propagation. Table 1 shows the information on selected disinfectants. Their active ingredients were the major compounds of disinfectants recommended by Health Canada (2021b). Moreover, 10 recommended household sanitizers with different active ingredients (BAC, H_2O_2, alcohol, acid, NaClO, and quaternary ammonium compound) were selected for the surface inactivation test.

2.2. Propagation and quantification of MS2 phage

Lyophilized MS2 phage was rehydrated in 1.5 ml Tryptic Soy Broth (TSB; Difco, Becton Dickinson, NJ, USA) before use. Phage propagation and quantification were performed according to the methods described by Fong et al. (2019) and Wong et al. (2020). Briefly, fresh *E. coli* culture was prepared by colony inoculation into 10 mL TSB, followed by incubation at 37 °C for 16 h at 170 rpm. Reconstituted MS2 was ten-fold serially diluted in salt-magnesium (SM) buffer (0.05 M Tris-HCl, 0.1 M NaCl, and 0.01 M MgSO4; pH 7.5). Then, 50 μL dilutions were spotted on bacterial overlays composed of 300 μL 1:10 (v/v) diluted *E. coli* culture and 4 mL 0.7% (w/v) soft tryptic soy agar (TSA, Becton Dickinson). The overlays were scraped into 5 mL SM buffer after incubating at 37 °C for 4 h. The mixtures were centrifuged at 4500×*g* for 20 min, and the supernatants were filtered through 0.45 μm filters. Obtained phage suspensions were stored at 4 °C before use. Phage enumeration was conducted according to the method described by Fong et al. (2017) with modifications. The filtrates were spotted onto the prepared bacterial overlays as previously described, and the plates were then incubated for 18 ± 2 h at 37 °C before plaques were counted. Phage concentration was expressed as plaque forming units (PFU)/mL. In this study, phage filtrate with high titer (around 4.0×10^{11} PFU/mL) was stored at 4 °C as stock solution.

Table 1
Selected disinfectants used in this study.

Prepared disinfectants	Active ingredient(s)	Household sanitizers	Active ingredient(s)
i	70% isopropanol	Sanitizer A	0.1% alkyl dimethyl benzyl ammonium chloride
ii	0.1% benzalkonium chloride	Sanitizer B	70% isopropanol
iii	0.1% sodium hypochlorite	Sanitizer C	1.84% sodium hypochlorite
iv (modified WHO II formula)	75% (w/w) isopropanol, 0.725 glycerol, 0.125% hydrogen peroxide	Sanitizer D	0.26%, w/w, alkyl dimethyl benzyl ammonium chloride
v	0.5% hydrogen peroxide	Sanitizer E	3% sodium hypochlorite
vi	4% acetic acid	Sanitizer F	0.2% alkyl dimethyl benzyl ammonium chloride, 0.15% octyl decyl dimethyl ammonium chloride, 0.075% didecyl dimethyl ammonium chloride, 0.075% dioctyl dimethyl ammonium chloride
vii	70% ethanol	Sanitizer G	0.19%, w/w, L-lactic acid
viii (modified WHO I formula)	80% (w/w) ethanol, 0.725% glycerol, 0.125% hydrogen peroxide	Sanitizer H	58%, w/w, ethanol, 0.1%, w/w, n-alkyl dimethyl benzyl ammonium saccharinate
ix	85% ethanol	Sanitizer I	0.15% alkyl dimethyl benzyl ammonium chloride, 0.15% alkyl dimethyl ethylbenzyl ammonium chloride
x	85% ethanol, 5% isopropanol	Sanitizer J	1.75%, w/w, glycolic acid
xi	90% ethanol		

2.3. Cytotoxicity control, interference test, and neutralizer validation

The cytotoxicity of neutralized sanitizers, neutralizer, and SM buffer (control) on host bacterial cells was examined according to the methods described in the American Society for Testing and Materials (ASTM) standard number E2197-17e1 (ASTM International, 2017). The ASTM method was used as the reference since it was recommended by Health

Canada (2021a) for disinfectant testing. The 21 disinfectants were 1:20 (v/v) and 1:200 (v/v) quenched by neutralizer Difco™ D/E (Dey/Engley) neutralizing Broth (BD Biosciences, Mississauga, ON, Canada). The neutralizer was selected according to the method described in ASTM E1054-08 (ASTM International, 2008). Twenty drops (each drop comprised 5 μL solution) of each prepared solution were spotted onto the bacterial overlays and air-dried at room temperature followed by incubation at 37 °C for 16 h. The experiments were performed in triplicate.

The ability of the prepared solutions to interfere with virus infectivity was tested. The bacterial overlays with air-dried neutralized solution spots were prepared as previously mentioned. MS2 phage drops (5 μL each drop) containing 500–1000 log PFU/mL were added onto the air-dried spots, and the plates were further incubated for 16 h at 37 °C. Plaques were counted to determine if the neutralized disinfectants, neutralizer, and SM buffer interfered with plaque formation. SM buffer was used as a control. The experiments were performed in triplicate.

Neutralization validation was conducted by diluting MS2 phage in neutralized sanitizer solutions (1:20 and 1:200 dilutions), neutralizer, and SM buffer (Morin et al., 2015). Twenty drops (5 μL each drop) of each phage solution were spotted onto the bacterial overlays, and the plates were incubated at 37 °C (16 h). Plaques were counted to validate neutralization efficacy of the disinfectants.

2.4. Carrier test on plastic and stainless steel

Plate recovery control was conducted to determine the phage concentration applied on the surface disinfection study. Working stocks of MS2 (previously prepared in 2.2) were ten-fold serially diluted in SM buffer. The phage dilutions (10 μL) were individually spotted onto the test surfaces and air dried in a biosafety cabinet for 30 min. The plastic surface (untreated 24-well polypropylene microplate) and sterilized stainless steel discs (1 cm diameter discs of AISI type 304 stainless steel) were purchased from Pegen Industries Inc. (Ottawa, ON, Canada). SM buffer (50 μL) was subsequently added onto the air-dried phage spots. Then, 1 mL neutralizer was added, and the phage enumeration was further performed (Fong et al., 2017).

The carrier test was conducted to determine the surface inactivation efficacies of selected disinfectants. The methods described in ASTM E2197-17e1 and ASTM E1053-20 (ASTM International, 2017; ASTM International, 2020) were applied with modifications. The phage inoculum was prepared by mixing 340 μL of phage solution with 160 of μL soil load, comprised of 25 μL bovine serum albumin solution (50 mg/mL in PBS, pH 7.2), 35 μL 50 mg/mL yeast extract stock, and 100 μL 4 mg/mL mucin. The mixture was vortexed, and 10 μL suspension drops were spotted on a sterilized plastic plate (untreated 24-well polypropylene microplate) and sterilized stainless steel discs (1 cm diameter discs of AISI type 304 stainless steel, Pegen Industries Inc., Ottawa, ON, Canada). Polypropylene was chosen as the plastic material because it is one of the most common polymers used in the food packaging industry due to its good functionality and relatively low cost (Paiva et al., 2021). The drops were air-dried at room temperature in a biosafety cabinet for 30 min.

The virucidal trials were performed by adding 50 μL of disinfectant onto the phage spots, and allowing appropriate contact time. SM buffer (50 μL) was used as a negative control. At the end of contact, 1 mL of the neutralizer was immediately applied to the droplets. For sanitizer E, 2 mL of the neutralizer was applied due to insufficient neutralization of 1 mL D/E neutralizing broth. The treated viruses were eluted from stainless steel discs by vortexing for 30 s in Nalgene vials, while the samples in plastic wells were pipetted up and down to obtain the eluted solutions. Phage enumeration was performed (as previously described in section 2.2) to determine viral survival.

The effect of temperature on the virucidal activities of selected disinfectants was tested. The MS2 phage inoculum was prepared as mentioned above in section 2.4. The virus drops were spotted on stainless steel and plastic surfaces, and air-dried under room temperature. The carriers and selected disinfectants were incubated at 4, 8, and 21.8 °C before inactivation treatment. The eluted solutions were collected for phage quantification.

2.5. Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) in SPSS (Version 22.0, IBM, Armonk, NY, USA). Differences with a P value < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Surrogate phage MS2, cytotoxicity and interference control, and validation

The surface stability of certain viruses (e.g., SARS-CoV-2, NoV, and HAV) has led to concerns of potential surface transmission throughout the food supply chain (Bolton et al., 2013; González et al., 2021; Rajiuddin et al., 2020). In this study, the inactivation efficacies of selected disinfectants against surrogate MS2 on food contact surfaces were tested, to reinforce both personal and food hygiene principles during the COVID-19 pandemic.

None of the neutralized sanitizers nor neutralizers themselves exhibited significant ($P < 0.05$) cytotoxicity to the *E. coli* host cell, compared with the control group. In addition, the interference test revealed that 1:20 diluted disinfectants, such as 70% isopropanol, 0.1% BAC, 0.1% NaClO, 70% ethanol, 85% ethanol, 85% ethanol +5% isopropanol, and 90% ethanol, did not notably ($P > 0.05$) affect the plaque counts, compared with that in the control group. Moreover, neutralization validation showed that a 1:20 dilution of the sanitizers (except household sanitizer E, which was 1:40 diluted) with D/E neutralizing broth effectively quenched the virucidal activity. Therefore, the 21 selected disinfectants and neutralizer were used for subsequent carrier tests.

3.2. Surface inactivation efficacies of prepared disinfectants on a plastic surface

The inactivation efficacies of the selected disinfectants against MS2 on plastic surfaces are shown in Table 2. Application of seven disinfectants (70% isopropanol, modified WHO II formula, 70% ethanol, modified WHO I formula, 85% ethanol, 85% ethanol + 5% isopropanol, and 90% ethanol) significantly reduced ($P < 0.05$) the viral load after 30 s of treatment (Table 2). Virucidal effects were increased by 12.33–80.61% upon treatment for 60 s. Generally, a treatment time of 600 s treatments did not enhance the virucidal activity, although, 85% ethanol +5% isopropanol, and 90% ethanol, further lowered the viral load by 2.78 and 2.53 log PFU/mL, respectively. Moreover, 0.1% NaClO (1000 ppm free-chlorine concentration), which is recommended by the WHO (2020), displayed 0.40 and 0.54 log PFU/mL viral reductions after contact for 60 and 600 s, respectively. Interestingly, application of 70% isopropanol exhibited a significant ($P < 0.05$) lower virucidal activity than 70% ethanol (Table 2). Similar results were also observed by Moorer (2003): ethanol is a better antiviral agent compared with isopropanol.

On the contrary, disinfectants containing BAC, H_2O_2, or acetic acid exhibit limited inactivation efficacies (<0.24 log PFU/mL) against MS2 on plastic surfaces (Table 2). In a previous study, BAC at 0.1–0.5 mg/mL resulted in 1.74–2.00 log PFU/mL MS2 reduction after 2 h treatment in suspension (Su & D'Souza, 2012). These different performances may be a result of different reaction environments (liquid suspension *vs.* plastic surface) and contact time (2 h *vs.* 30–600 s). Generally, longer contact time or higher concentration of disinfectants are required for effective virucidal activity on hard surfaces, compared with those in liquid

Table 2
Inactivation efficacy of prepared disinfectants against MS2 on a plastic surface.

Disinfectant	Active ingredient (s)	Log reduction (30 s)[a]	Log reduction (60 s)[a]	Log reduction (600 s)[a]
i	70% isopropanol	0.88 ± 0.08^{aBC}	0.85 ± 0.30^{aD}	0.98 ± 0.02^{aD}
ii	0.1% BAC[b]	-0.02 ± 0.05^{bD}	0.00 ± 0.13^{bEF}	0.24 ± 0.10^{aEF}
iii	0.1% sodium hypochlorite	0.10 ± 0.09^{bD}	0.40 ± 0.12^{aE}	0.54 ± 0.11^{aE}
iv	Modified WHO II formula[c]	0.79 ± 0.27^{bC}	1.58 ± 0.02^{aC}	1.67 ± 0.03^{aBC}
v	0.5% hydrogen peroxide	0.15 ± 0.10^{aD}	0.12 ± 0.13^{aEF}	0.04 ± 0.16^{aF}
vi	4% acetic acid	0.20 ± 0.16^{aD}	-0.17 ± 0.68^{aF}	0.08 ± 0.43^{aF}
vii	70% ethanol	1.46 ± 0.42^{aA}	1.64 ± 0.04^{aC}	1.58 ± 0.05^{aC}
viii	Modified WHO I formula[d]	1.79 ± 0.06^{bA}	2.74 ± 0.00^{aA}	2.04 ± 0.55^{bB}
ix	85% ethanol	1.32 ± 0.38^{bAB}	2.24 ± 0.33^{aB}	1.74 ± 0.32^{abBC}
x	85% ethanol+5% isopropanol	0.98 ± 0.09^{cBC}	1.78 ± 0.18^{bC}	2.78 ± 0.05^{aA}
xi	90% ethanol	0.93 ± 0.58^{bBC}	1.46 ± 0.16^{bC}	2.53 ± 0.22^{aA}

[a] In each column, values with the same upper letter do not differ significantly at $P < 0.05$; in each row, values with the same lower letter do not differ significantly at $P < 0.05$.
[b] BAC: benzalkonium chloride.
[c] Modified WHO II formula: 75% (w/w) isopropanol, 0.725 glycerol, and 0.125% hydrogen peroxide (H_2O_2).
[d] Modified WHO I formula: 80% (w/w) ethanol, 0.725% glycerol, and 0.125% H_2O_2.

Table 3
Inactivation efficacy of prepared disinfectants against MS2 on a stainless steel surface.

Disinfectant	Active ingredient (s)	Log reduction (30 s)[a]	Log reduction (60 s)[a]	Log reduction (600 s)[a]
i	70% isopropanol	0.22 ± 0.05^{aEF}	0.16 ± 0.07^{aFG}	0.12 ± 0.03^{aG}
ii	0.1% BAC[b]	-0.01 ± 0.02^{cF}	0.06 ± 0.03^{bFG}	0.16 ± 0.01^{aG}
iii	0.1% sodium hypochlorite	$0.37 \pm 0.12b^{DE}$	0.42 ± 0.10^{bEF}	0.71 ± 0.09^{aEF}
iv	Modified WHO II formula[c]	0.70 ± 0.11^{aBC}	0.63 ± 0.05^{aDE}	0.75 ± 0.06^{aEF}
v	0.5% hydrogen peroxide	0.19 ± 0.07^{aEF}	0.15 ± 0.09^{aFG}	0.22 ± 0.07^{aG}
vi	4% acetic acid	0.04 ± 0.13^{bEF}	-0.06 ± 0.08^{bG}	0.55 ± 0.11^{aF}
vii	70% ethanol	0.84 ± 0.12^{aABC}	0.93 ± 0.14^{aCD}	0.86 ± 0.04^{aD}
viii	Modified WHO I formula[d]	1.08 ± 0.16^{aA}	1.40 ± 0.57^{aAB}	1.47 ± 0.31^{aC}
ix	85% ethanol	0.92 ± 0.14^{bAB}	1.69 ± 0.18^{aA}	1.89 ± 0.00^{aB}
x	85% ethanol+5% isopropanol	0.59 ± 0.43^{cCD}	1.75 ± 0.01^{bA}	2.35 ± 0.20^{aA}
xi	90% ethanol	0.10 ± 0.21^{bEF}	1.21 ± 0.29^{aBC}	1.58 ± 0.19^{aC}

[a] In each column, values with the same upper letter do not differ significantly at $P < 0.05$; in each row, values with the same lower letter do not differ significantly at $P < 0.05$.
[b] BAC: benzalkonium chloride.
[c] Modified WHO II formula: 75% (w/w) isopropanol, 0.725 glycerol, and 0.125% hydrogen peroxide (H_2O_2).
[d] Modified WHO I formula: 80% (w/w) ethanol, 0.725% glycerol, and 0.125% H_2O_2.

suspension (Mileto et al., 2021).

Acetic acid had a limited decontamination effect against MS2. Inactivation rates of other organic acids have been tested on MS2. Very limited reductions (0.00–0.06 log PFU/mL) of MS2 and murine norovirus were observed after treatment with tannic and gallic acid (room temperature, 2 h exposure) (Su & D'Souza, 2012). However, gallic acid demonstrated antiviral activities against herpes simplex virus type B. Its 50% inhibitory concentration (IC_{50}) was 33.56 μM, which was much lower than the 50% cytotoxic concentration (CC_{50}), 1000 μM (Kratz et al., 2008). The antiviral effects of organic acids are affected by various factors, including type and nature of virus and organic acid, treatment time and method. Based on our study, it should be noted that many of the disinfectants that incurred a > one log PFU/ml reduction are isopropanol or ethanol-based (Table 2). Alcohol-based products have historically been shown to be effective against various viruses. Maillard, Beggs, Day, Hudson, and Russell (1994) reported that 70% ethanol reduced MS2 by 3.68 log PFU/mL in suspension, while 100% ethanol demonstrated a reduction of 1.53 log PFU/mL: the inclusion of water in the alcohol biocidal system increases the disinfectant efficacy, as water facilitates a faster denaturation of proteins (Lin et al., 2020). Furthermore, 100% isopropanol inactivated MS2 by 1.50 log PFU/mL after contact for 20 min. Overall, alcohol-based products appear to demonstrate consistent inactivation rates in the decontamination of viruses in a variety of systems, including plastic surfaces.

3.3. Surface inactivation efficacies of prepared disinfectants on a stainless steel surface

The sanitizing efficacies of 11 prepared disinfectants against MS2 on a stainless steel surface were tested (Table 3). Most of the sanitizers resulted in a <1 log PFU/mL reduction after contact for 30 s. Four sanitizers (modified WHO I formula, 85% ethanol, 85% ethanol + 5% isopropanol, and 90% ethanol), inactivated >1 log PFU/mL MS2 on stainless steel surface following 60 s treatment. After contact for 600 s, the sanitizing effects of 85% ethanol +5% isopropanol was further significantly ($P < 0.05$) increased to 2.35 log PFU/mL. Alcohols, specially isopropanol and ethanol, are capable of inactivating a wide spectrum of microorganisms through disruption of the cell membrane and denaturation of intracellular proteins. Viruses are particularly susceptible to this mode of action (Boyce, 2018).

Similar to what we observed on plastic surfaces, BAC, H_2O_2, and acetic acid, exhibited a relatively low range of activity (<0.55 log PFU/mL reduction) on the stainless steel surface (Table 3). Interestingly, the tested disinfectants on stainless steel exhibited lower effectiveness compared with those on plastic surfaces. Similar results were observed in a previous study: electrochemical oxidants could reduce MS2 more effectively on plastic compared with that on stainless steel surface (Julian et al., 2014). Longer treatment (steam-ultrasound) time was also required to reduce murine norovirus on stainless steel to below the theoretical limit of detection, compared with that on plastic (Rajiuddin et al., 2020). It is apparent that surface materials (plastic and stainless steel) may interfere with the sanitizing effects of disinfectants (Møretrø et al., 2012), but, the mechanistic basis for this interference is unknown and warrants further investigation.

In summary, ethanol and ethanol-based disinfectants showed the most effective virucidal activity against MS2 on both plastic and stainless steel surfaces. These results agree with previous observations where ethanol with concentrations ranging from 60% to 95% showed effective inactivation abilities (>3.2 log reductions) against various viruses, such as SARS-CoV, mouse hepatitis virus, etc. (Singh et al., 2020).

3.4. Effect of low temperature on the surface inactivation efficacy of ethanol

Factors, such as sanitizer concentration, contact time, interfering

matter, and pH, affect the inactivation effectiveness to various degrees. Temperature is a key factor affecting effectiveness of virucides in practical use (Pinto et al., 2010; Vong et al., 2018). Unlike bacterial pathogens, low temperature is not a mitigation strategy for viral pathogens, as persistence of enteric viruses (e.g., norovirus) is higher at low temperatures (Bozkurt et al., 2015). It raises questions about disinfectant efficacy in low temperature environments. In the carrier tests on plastic and stainless steel surfaces, ethanol and ethanol-based disinfectants showed good (>0.84 log PFU/mL reduction) virucidal activity against MS2 (Tables 2 and 3), therefore, the effect of low temperature (i.e., 4 and 8 °C) on the surface inactivation efficacy of ethanol was assessed.

Overall, low temperatures did not affect the efficacies of 70% ethanol against MS2 on plastic nor stainless steel (Table 4), compared with those under room temperature (21.8 °C). Approximately one log PFU/mL reduction was observed on a plastic surface, while lower reductions (approximately 0.17–0.64 log PFU/mL) were observed on stainless steel. The data were consistent with the results in Tables 2 and 3 It has been shown that MS2 aggregates at low temperature (<20 °C), leading to similar log PFU/mL reductions at 10 and 20 °C upon treatment (Pinto et al., 2010).

Table 5 describes the effect of 85% ethanol against MS2 on hard surfaces for 1 min at 4, 8, and 21.8 °C. Higher reductions (1.36–2.10 log PFU/mL and 1.04–1.38 log PFU/mL on plastic and stainless steel surfaces, respectively) were observed with 85% ethanol treatments, compared with those in the 70% ethanol treated groups (Tables 4 and 5), in accordance with the data obtained in Tables 2 and 3 In line with our results, it was reported the increased concentration of ethanol from 80% to 95% led to an increase in the reduction factors against SARS coronavirus from 4.25 to 5.5 log PFU/mL (Rabenau et al., 2005). On a plastic surface, treatment with 85% ethanol resulted in an approximately 1.4 log PFU/mL reductions of MS2 at 4 and 8 °C. However, the reduction (2.10 log PFU/mL) at 21.8 °C was significantly ($P < 0.05$) higher than those at 4 and 8 °C. Moreover, approximately 1 log PFU/mL MS2 was inactivated when using this treatment on stainless steel at all test temperatures. It can be concluded that low temperatures 4 and 8 °C, did not negatively affect (except 85% ethanol treatment on a plastic surface) the virucidal activities of 70% and 85% ethanol against MS2 on plastic and stainless steel surfaces. In contrast, other sanitizers have been shown to be unstable at low temperature, for instance, the QACs at high concentration (0.05%, w/v) had no virucidal effect on equine herpesvirus type 1 after 10 min reaction time at 0 °C (Tsujimura et al., 2015).

3.5. Surface inactivation efficacies of household sanitizers

Ten household sanitizers were tested against MS2 on plastic and stainless steel in this study (Table 6). Sanitizers, containing BAC (0.1–0.26%), QAC (0.3–0.5%), or organic acid (0.19% lactic acid and 1.75% glycolic acid), showed limited virucidal activities (−0.28-0.33 log PFU/mL reductions) against MS2. Three sanitizers, in particular, named sanitizer C, E, and H, exhibited promising surface inactivation efficacies. Sanitizer C, with 1.84% NaClO, reduced >4 and 5 log PFU/mL of MS2 on plastic and stainless steel surface, respectively. Sanitizer E, with 3% NaClO, also resulted in >5 log PFU/mL MS2 reductions.

Table 4
Effect of temperature on the inactivation efficacy of 70% ethanol (60 s) against MS2 on plastic and stainless steel surfaces.

Surface	Temperature	Log reduction[a]
Plastic	4 °C	0.89 ± 0.31^{A}
	8 °C	0.77 ± 0.32^{A}
	21.8 °C	1.03 ± 0.16^{A}
Stainless steel	4 °C	0.64 ± 0.10^{B}
	8 °C	0.77 ± 0.44^{A}
	21.8 °C	0.17 ± 0.13^{B}

[a] In each column, values with the same upper letter do not differ significantly at $P < 0.05$.

Table 5
Effect of temperature on the inactivation efficacy of 85% ethanol (60 s) against MS2 on plastic and stainless steel surfaces.

Surface	Temperature	Log reduction[a]
Plastic	4 °C	1.36 ± 0.40^{B}
	8 °C	1.44 ± 0.42^{B}
	21.8 °C	2.10 ± 0.07^{A}
Stainless steel	4 °C	1.38 ± 0.11^{B}
	8 °C	1.17 ± 0.12^{B}
	21.8 °C	1.04 ± 0.10^{B}

[a] In each column, values with the same upper letter do not differ significantly at $P < 0.05$.

Table 6
Inactivation efficacy of household disinfectants against MS2 on plastic and stainless steel surfaces.

Disinfectant	Active ingredient (s)	Surface	Log reduction (30 s)[a]	Log reduction (60 s)[a]
Sanitizer A	0.1% BAC[b]	Plastic	0.09 ± 0.06^{aEF}	0.25 ± 0.23^{aD}
		Stainless steel	0.25 ± 0.23^{aEF}	-0.02 ± 0.20^{aD}
Sanitizer B	70% isopropanol	Plastic	0.66 ± 0.06^{aDE}	0.55 ± 0.36^{aD}
		Stainless steel	0.29 ± 0.11^{aEF}	0.41 ± 0.13^{aD}
Sanitizer C	1.84% sodium hypochlorite	Plastic	4.64 ± 2.43^{aB}	4.15 ± 1.80^{aB}
		Stainless steel	5.95 ± 0.22^{aA}	5.90 ± 0.41^{aA}
Sanitizer D	0.26% BAC[b]	Plastic	-0.28 ± 0.26^{aF}	-0.27 ± 0.27^{aD}
		Stainless steel	0.01 ± 0.38^{aEF}	0.32 ± 0.26^{aD}
Sanitizer E	3% sodium hypochlorite	Plastic	5.81 ± 0.19^{aA}	5.60 ± 0.48^{aA}
		Stainless steel	5.72 ± 0.52^{aA}	6.23 ± 0.06^{aA}
Sanitizer F	0.5% QAC[c]	Plastic	-0.08 ± 0.25^{aEF}	-0.01 ± 0.37^{aD}
		Stainless steel	0.09 ± 0.02^{aEF}	0.08 ± 0.08^{aD}
Sanitizer G	0.19% L-lactic acid	Plastic	-0.11 ± 0.29^{aEF}	0.16 ± 0.52^{aD}
		Stainless steel	0.16 ± 0.18^{aEF}	-0.02 ± 0.44^{aD}
Sanitizer H	58% ethanol, 0.1% QAC[c]	Plastic	1.62 ± 0.25^{aC}	1.59 ± 0.30^{aC}
		Stainless steel	1.26 ± 0.13^{aCD}	1.48 ± 0.38^{aC}
Sanitizer I	0.3% QAC[c]	Plastic	0.15 ± 0.36^{aEF}	-0.04 ± 0.48^{aD}
		Stainless steel	0.06 ± 0.07^{aEF}	0.25 ± 0.38^{aD}
Sanitizer J	1.75% glycolic acid (1:64 dilution)	Plastic	0.06 ± 0.34^{aEF}	0.33 ± 0.23^{aD}
		Stainless steel	-0.12 ± 0.60^{aEF}	0.06 ± 0.59^{aD}

[a] In each column, values with the same upper letter do not differ significantly at $P < 0.05$; in each row, values with the same lower letter do not differ significantly at $P < 0.05$.
[b] BAC: benzalkonium chloride.
[c] QAC: quaternary ammonium compound.

Furthermore, around 1.5 log PFU/mL reductions were recorded in sanitizer H treated groups (Table 6). The 30 s and 60 s contact times produced comparable results.

NaClO solution has been widely used to inactivate pathogens on surfaces in the food industry, because of its effectiveness and low cost. NaClO is a strong oxidizing agent, and it can form hypochlorous acid when dissolved in water. The ratios of hypochlorous acid, Cl_2, and OCl^- in solution are pH dependent. The hypochlorous acid can further

interact with peptide bonds and thiol groups, resulting in oxidization of proteins and other biomolecules (Fukuzaki, 2006). The WHO (2020) recommended a conservative concentration (0.1%) of hypochlorite-based products in the context of COVID-19. Moreover, its virucidal efficacy against various viruses also has been widely reported (Chen et al., 2019; Martin et al., 2013). Generally, NaClO solutions with higher concentrations present increased disinfectant efficacies (Meyers et al., 2021). The NaClO-based (1.84–3%) household sanitizers (sanitizer C and E) showed particularly good virucidal properties and outperformed that 0.1% NaClO (Tables 2 and 3). Therefore, NaClO solutions with higher concentrations (1–3%) should be tested in the future.

Sanitizer H was the only sanitizer containing ethanol (58%). Its surface disinfection performance was generally similar to the prepared ethanol solutions. In a previous study, a household sanitizer (active ingredients: 79% ethanol + 0.1% QAC) resulted in >3 log PFU/mL reductions of mouse hepatitis virus on a Petri dish (Dellanno et al., 2009). The present study showed lower (~1.5 log PFU/mL) reductions of MS2 with Sanitizer H, which may be reflective of the different ethanol concentrations tested. In addition, viral structures might also contribute to the different virucidal activities. MS2 is a non-enveloped virus, which is more resistant to sanitizers compared with the enveloped virus, such as mouse hepatitis virus and SARS-CoV-2 (Ahmed et al., 2020; Kumar et al., 2020).

Based on our data, NaClO solutions with the appropriate concentrations (in this study, we tested a concentration of 1.84%) appear to be the most effective against MS2 on food packing and contact materials, particularly plastic and stainless steel (Fig. 1). The strong oxidizing property of NaClO solution contributes to the virucidal activity (Fukuzaki, 2006). Ethanol-based disinfectants also exhibited good inactivation performance on both plastic and stainless steel surfaces, where it has been shown to effectively denature the proteins and further disrupt the capsid (Lin et al., 2020). Lastly, isopropanol-based disinfectants ranked third, with higher efficacy on plastic surfaces. It has been shown previously that its lipophilic properties are more suitable for inactivation of enveloped viruses (McDonnell and Russell, 1999).

In this study, the efficacies of different sanitizers against MS2 on plastic and stainless steel surfaces were tested. The surface inactivation test of MS2, a traditional surrogate of human enteric viruses (e.g., NoV and HAV), provide valuable information for hygiene regulation in food industry. Moreover, it could serve as a conservative surrogate for SARS-CoV-2. It provided a facile system for rapid quantitative evaluation of respirator disinfection. Because of its higher resistance to sanitizers compared to SARS-CoV-2, the substantial reduction in viable MS2 by certain sanitizers (e.g., sanitizers with 1.84–3% NaClO) gives confidence in an appropriate safety margin for SARS-CoV-2 surface decontamination.

4. Conclusion

To reinforce the hygienic operations in the food industry during the COVID-19 pandemic, the virucidal effects of frequently used sanitizers against MS2 on food contact surfaces (plastic and stainless steel) were examined. Overall, our results showed that isopropanol and isopropanol-based modified WHO II formula reduced 0.7–1.5 log PFU/mL MS2 on plastic and stainless steel surfaces. Moreover, ethanol and ethanol-based disinfectants exhibited promising inactivation effectiveness, resulting in around 1–2.5 log PFU/mL reductions. The effect of low temperature (4 and 8 °C) showed that low temperature generally did not affect the surface inactivation efficacies of 70% and 85% ethanol solutions, emphasizing its utility in the Agri-Food sector. Lastly, the disinfectant activities of 10 household sanitizers were determined to provide consumer reference information. We observed that sanitizers containing appropriate NaClO concentrations (1.84–3%) presented excellent surface decontamination performance of 4.15–6.31 log PFU/mL reductions of MS2. In conclusion, our data provide valuable information for hygiene regulation on a variety of surfaces in the Agri-Food sector.

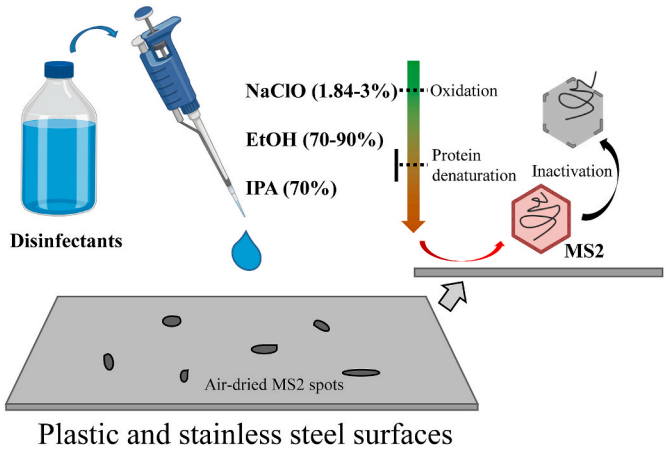

Fig. 1. Illustration of MS2 disinfection using sodium hypochlorite (NaClO), ethanol (EtOH), and isopropanol (IPA) solutions.

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CRediT authorship contribution statement

Lin Chen: Data curation, Formal analysis, Visualization, Writing – original draft. **Win-ju Lee:** Investigation. **Yvonne Ma:** Investigation, Project administration. **Sung Sik Jang:** Funding acquisition, Methodology, Writing - review & editing. **Karen Fong:** Funding acquisition, Methodology, Writing - review & editing. **Siyun Wang:** Conceptualization, Supervision, Methodology, Funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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