Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Survival of human coronavirus 229E at different temperatures on various food-contact surfaces and food and under simulated digestive conditions

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ABSTRACT

The COVID-19 pandemic caused by SARS-CoV-2 has had a major impact on human health and the global economy. Various transmission possibilities of SARS-CoV-2 have been proposed, such as the surface of food in the cold chain and food packaging, as well as the fecal-oral route, although person-to-person contact via droplets and aerosols has been confirmed as the main route of transmission. This study evaluated the survivability of HCoV-229E, a SARS-CoV-2 surrogate, in suspension, on food-contact surfaces and on food at various temperatures, and in simulated digestive fluids by $TCID_{50}$ assay. In suspension, HCoV-229E survived after 5 days at 20 °C with a 3.69 log reduction, after 28 days at 4 °C with a 3.07 log reduction, and after 12 weeks at –20 °C with a 1.18 log reduction. On food-contact surfaces, HCoV-229E was not detected on day 3 on stainless steel (SS), plastic (LDPE), and silicone rubber (SR) at 20 °C with a 3.28, 3.24 and 3.28 log reduction, respectively, and survived after 28 days on SS and LDPE at 4 °C with a 3.13 and 2.88 log reduction, respectively, and survived after 12 weeks on SS, LDPE, and SR at –20 °C with a 1.92, 1.32 and 1.99 log reduction, respectively. On food, HCoV-229E was not detected on day 3 on lettuce and day 4 on chicken breast and salmon at 20 °C with a 3.61, 3.26 and 3.08 log reduction, respectively, and on day 14 on lettuce and day 21 on chicken breast and salmon at 4 °C with a 3.88, 3.44 and 3.56 log reduction, respectively. The virus remained viable for 12 weeks in all foods at –20 °C with 2–2.47 log reduction. In addition, in simulated digestive fluid experiments, HCoV-229E was relatively resistant in simulated salivary fluid (SSF; pH 7, 5), fed state simulated gastric fluid (FeSSGF; pH 3, 5, 7), and fasted state simulated intestinal fluid (FaSSIF; pH 7). However, the virus was less tolerant in fasted state simulated gastric fluid (FaSSGF; pH 1.6) and fed state simulated intestinal fluid (FeSSIF; pH 5). Therefore, this study suggested that HCoV-229E remained infectious on various food-contact surfaces and foods; in particular, it survived longer at lower temperatures and survived depending on the pH of the simulated digestive fluid.

1. Introduction

COVID-19, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), emerged in December 2019 in Wuhan, China and spread worldwide (WHO, 2022), causing a major impact on human health and an economic crisis. This virus, which belongs to the Coronaviridae family, has a prolonged viral RNA shedding ability compared to other respiratory viruses, suggesting an increased potential transmission risk (Hikmet et al., 2020). SARS-CoV-2 is also responsible for the COVID-19 pandemic, which has affected more than 500 million people worldwide and caused more than six million deaths (WHO, 2022). COVID-19 cannot only present with respiratory symptoms, but also gastrointestinal (GI) symptoms, such as vomiting, abdominal pain, and diarrhea (Cimolai, 2020; Kariyawasam, Jayarajah, Riza, Abeysuriya, & Seneviratne, 2021).

The main confirmed transmission routes are represented by person-to-person transmission through contaminated respiratory droplets and aerosols formed by the sneezing or coughing of infected individuals (Yekta, Vahid-Dastjerdi, Norouzbeigi, & Mortazavian, 2021). However, other potential transmission routes are suggested as indirect transmission via contaminated fomites, such as commonly used surfaces and food-contact surfaces. According to the report by Bode, Craven, Leopoldseder, Rutten, and Wilson (2020), approximately 90% of SARS-CoV-2 transmissions occur from symptomatic, pre-symptomatic, and asymptomatic individuals, leaving 10% transmissions from the environment, which includes surfaces. Notably, several studies have

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reported the high stability and persistence of SARS-CoV-2 on various inanimate surfaces, especially at low temperatures (Chin et al., 2020; van Doremalen et al., 2020).

Additionally, some researchers have proposed the fecal-oral route as a possible transmission route of SARS-CoV-2 transmission based on the existing literature, although coronaviruses are not known to be food-borne but respiratory viruses. Various recent studies have shown that COVID-19 patients suffered GI symptoms. For example, 61% of COVID-19 patients showed GI symptoms according to a US cohort study (Redd et al., 2020). The viral load in feces can reach up to 10^7 copies/g RNA in some cases (Ding & Liang, 2020; Gao, Chen, & Fang, 2020; Kariyawasam et al., 2021; Wölfel et al., 2020) and the virus persists in feces even longer than in respiratory secretions (Cheung et al., 2020), demonstrating that fecal excretion poses a possibility of SARS-CoV-2 transmission in humans via the fecal-oral route (Guo, Tao, Flavell, & Zhu, 2021). In addition, some patients with GI symptoms have been reported to have higher pH values in their stomach, indicating that the virus may survive gastric conditions and bind to angiotensin converting-enzyme 2 (ACE2), which is predominantly expressed in pulmonary epithelial cells, as well as in the duodenum and small intestine (Hikmet et al., 2020). ACE2 is the main receptor for SARS-CoV-2 binding to target cells (Harlow, Dallner, & Nasheri, 2022). The virus is then processed to release viral contents into the host cell cytosol (Hoffmann et al., 2020). ACE2 is known to be highly expressed in the GI tract.

Theoretically, coronaviruses are enveloped respiratory viruses and are expected to be susceptible to gastric acid and bile; therefore, they should not survive in the lower GI tract and should lose infectivity upon excretion in feces (Hirose et al., 2017). To enable the fecal-oral route of SARS-CoV-2, infectious virus particles should tolerate GI fluids and should be expelled or accumulate through the feces of COVID-19 patients. Meanwhile, many poultry-associated coronaviruses cause GI symptoms in their hosts, such as poultry and swine (Cimolai, 2020). The exact mechanism is unclear; however, it is expected that highly viscous mucus, such as viscous sputum and nasal mucus, may protect virions to retain virus infectivity.

Currently, many authorities have stated that there is no scientific evidence that SARS-CoV-2 is likely to be associated with food products and packaging (Who, 2021; Fda, 2021; Efsa, 2021), as the amount of virus deposited in food and food packaging materials is relatively low and most of the infectivity would be lost in the human stomach environment. However, transmission via food-contact surfaces and food packaging materials cannot be ignored, considering the research results of various routes for SARS-CoV-2 transmission studied recently. Recent reports from China found that at least nine cases of food packing material and storage environments, including frozen raw fish, were found to be contaminated with SARS-CoV-2, suggesting that SARS-CoV-2 can survive on cold chain food and its packaging surface for a long time, which may result in long-distance transmission (Han, Zhang, He, & Jia, 2020). More recently, Feng et al. (2021) found that SARS-CoV-2 on pork, beef, and salmon were remained infectious for at least 9 days at 4 °C and 20 days at −20 °C, indicating potential risk of SARS-CoV-2 transmission from food products in the cold chain.

Given the limited access to biosafety level 3 (BSL-3) laboratories to handle live SARS-CoV-2, surrogates are widely used to study viral replication and survival in modified human GI fluids. Human coronavirus 229E (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, Liang, & Fung, 2021; Warnes, Little, & Keevil, 2015).

Therefore, the aim of this study was to evaluate the persistence of HCoV-229E as a surrogate for SARS-CoV-2 on commonly used food and food-contact surfaces under various temperatures. This study further examines the survivability of HCoV-229E in simulated human digestive conditions (simulated salivary, gastric, and intestinal fluids), where different pH environments are present to investigate the ability to retain infectivity in the human GI tract.

2. Materials and methods

2.1. Cell line and virus

HCoV-229E was provided by ATCC (Rockville, MD, USA). It was propagated in the human fetal lung fibroblast cell line MRC-5 (ATCC), which was cultured in culture media composed of minimum essential medium eagle (MEM, Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), and 1% penicillin–streptomycin (Gibco). When a monolayer of MRC-5 cells in a 75-cm^2 culture flask had a confluence of 90–100%, the cell culture media was removed by aspiration. The cells were washed twice with Dulbecco's phosphate buffered saline (DPBS, Sigma-Aldrich). An appropriate amount of HCoV-229E stock was infected into the cells (multiplicity of infection = 0.1), and the flask was incubated at 33 °C and 5% CO_2 for 2 h to allow virus adsorption. Then, a maintenance medium, consisting of MEM with 1% FBS was added and propagated for 3 to 7 days. When the cytopathic effect (CPE) was observed more than 90%, the virus-infected flask was exposed to three freeze–thaw cycles to release virus particles by cell lysis. The contents were centrifuged at 4,000 × g for 10 min at 4 °C to remove cell debris. The supernatant was filtered using a 0.2 μm pore size filter (Sartorius Stedim Biotech, Göttingen, Germany), and stored at –80 °C in a deep freezer until use.

2.2. $TCID_{50}$ assay

Quantification of the infectious HCoV-229E titer was determined by the 50% tissue culture infectious dose ($TCID_{50}$) assay. Additionally, 1×10^4 MRC-5 cells/100 μL were seeded in cell culture medium per well in 96-well plates and incubated at 37 °C and 5% CO_2 for 24 h until 50% confluence was reached. The cell culture medium was discarded and the cell monolayers were washed once with DPBS. The virus samples were serially diluted 10-fold in MEM supplemented with 1% FBS and 100 μL of viral dilutions were inoculated into each well. The well plates were further incubated at 33 °C and 5% CO_2 for five days. On day 5, cytopathic effects (CPEs) were observed in each well and counted under the microscope. Viral titer was calculated as $TCID_{50}$/mL by the Reed-Muench method (Reed & Muench, 1938) using the following formula. Log reduction values were also determined using the following formula.

$TCID_{50}$/mL = Dilution (where CPEs >50%) + (% positive above 50% – 50%)/(% positive above 50% – % positive below 50%).

Log reduction = Log HCoV-229E titer of control – Log virus titer after treatment.

2.3. Virus survivability at various temperatures

2.3.1. Preparation of food-contact surfaces and inoculation

Stainless steel (SS) measuring 1 cm in diameter and 0.1 cm thick were purchased from a company (Posco Co., ltd., Pohang, Korea). Plastic (LDPE; Cleanwrap, Korea) and silicone rubber (SR; Komax Industrial Co., Goyang, Korea) coupons were cut into 1 × 1 cm square pieces with a sterilized knife. The SS coupons were immersed in 70% ethanol for 1 h and washed with distilled water. After washing, the coupons were autoclaved at 121 °C for 15 min and stored in a drying oven until use. Because LDPE and SR have heat-sensitive properties, they were immersed in 70% ethanol overnight without autoclaving. Each side of all coupons was exposed to ultraviolet (UV) rays for 15 min to eliminate residual microorganisms.

All coupon samples were transferred to sterile Petri dishes. A 20 μL aliquot of HCoV-229E stock (approximately 6.2–6.3 log $TCID_{50}$/mL) was inoculated on the center of the coupon surfaces using a

micropipette. The samples were then dried in a biosafety cabinet for 30 min at room temperature (23–25 °C) and 21–32% relative humidity.

2.3.2. Preparation of foods and inoculation

Before starting the experiment, fresh lettuce, chicken breasts, and salmon were purchased from a local market in Anseong, Korea. The lettuce was washed with tap water and distilled water twice, respectively. It was cut into 1 × 1 cm pieces with a sterilized knife. For chicken breast and salmon, they were cut into 0.5 × 0.5 × 0.5 cm cubes. All food samples were irradiated with UV light for 10 min on each front and back side to remove remaining microorganisms and dried in a laminar flow hood equipped with a fan.

All food samples were transferred to sterile Petri dishes. Moreover, a 30 μL aliquot of the HCoV-229E stock was spread over the lettuce by pipetting. Chicken breast and salmon were inoculated with 40 μL of viral stock (approximately 6.8–7.6 log $TCID_{50}$/mL). The samples were then incubated in a biosafety cabinet for 30 min at room temperature and a relative humidity of 21–32%.

2.3.3. Survivability experiment in suspension, on food-contact surfaces, and on foods

A total of 1 mL of each HCoV-229E suspension (approximately 5.2–6.1 log $TCID_{50}$/mL) in 1.5 mL microtubes was stored in the chamber at 20 °C (room temperature) for 0, 1, 2, 3, 4, 5, 6, and 7 days; in the chamber at 4 °C (refrigerated temperature) for 0, 1, 3, 5, 7, 10, 14, 21, and 28 days; and in the freezer at –20 °C (freezing temperature) for 0, 1, 2, 3, 4, 6, 8, and 12 weeks. At the predetermined time point, samples were drawn in triplicate and stored at –80 °C in a deep freezer until the $TCID_{50}$ assay.

Inoculated SS, LDPE, and SR coupons were stored under the above temperature and time conditions. The coupons were collected in triplicate at the predetermined time point and placed in 50 mL conical tubes. Then, 1 mL of maintenance media (MEM + 1% FBS) was added into the tubes, and the coupons were vortexed for 30 s to recover the virus. The recovered viral suspension was then kept in a deep freezer until virus titration.

Inoculated lettuce, chicken breast, and salmon were placed under the above temperature and time conditions. The food samples were taken out at a predetermined time and deposited into 50 mL conical tubes. For virus recovery, 1 mL of maintenance medium was added to the tubes and the samples were vortexed for 30 s and centrifuged at 1500*g* for 5 min at 4 °C. The supernatant was filtered using 1.2-, 0.8-, 0.45-, and 0.2-μm pore size filters. Then, the recovered viral suspension was stored in a deep freezer until viral titration.

2.4. Virus survivability under simulated digestive conditions

2.4.1. Preparation of simulated digestive fluids

Simulated digestive fluids were prepared with simulated salivary fluid (SSF) for the oral phase, simulated gastric fluid (SGF) for the gastric phase, and simulated intestinal fluid (SIF) for the intestinal phase with slight modifications to the method of a previous study (Minekus et al., 2014). SSF, SGF, and SIF were prepared for each pH in the fasted and fed states, respectively. The pH conditions, which were slightly modified from the method of Klein (2010), of each simulated digestive fluid are as follows: fasted state simulated salivary fluid (FaSSSF; pH 7), fed state simulated salivary fluid (FeSSSF; pH 5), fasted state simulated gastric fluid (FaSSGF; pH 1.6), fed state simulated gastric fluid (FeSSGF; pH 3, 5, 7), fasted state simulated intestinal fluid (FaSSIF; pH 7), and fed state simulated intestinal fluid (FeSSIF; pH 5). The base of each simulated digestive fluid is the electrolyte stock solution at a 1.25 × concentration. The pH of the electrolyte stock solution and the concentration and volume of the stock solution components are detailed in Table 1. After mixing all the components of the stock solution, the pH was adjusted and distilled water was added to make the final volume of 100 mL. The final simulated digestive fluid was made by mixing the stock solution and digestive enzyme. The final concentration of α-amylase from human saliva (Type IX-A, 1000–3000 U/mg protein, Sigma-Aldrich) was 75 U/mL in the oral phase, of pepsin from porcine gastric mucosa (≥2,500 U/mg protein, Sigma-Aldrich) was 2,000 U/mL in the gastric phase, and of pancreatin from porcine pancreas (4 × USP, Sigma-Aldrich) was 100 U/mL in the intestinal phase. Digestive enzymes were added to the final mixture of simulated digestive fluid and sample.

Table 1
pH of electrolyte stock solution of SSF, SGF, and SIF, concentration, and volume of components of the stock solution.

Component	Conc. (g/L)	Conc. (mol/L)	SSF pH 7, 5 Vol. (mL)	SGF pH 1.6, 3, 5, 7 Vol. (mL)	SIF pH 7, 5 Vol. (mL)
KCl	37.3	0.5	3.775	1.725	1.7
KH_2PO_4	68	0.5	0.925	0.225	0.2
$NaHCO_3$	84	1	1.7	3.125	10.625
NaCl	117	2	–	2.95	2.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.125	0.1	0.257
$(NH_4)_2CO_3$	48	0.5	0.03	0.125	–
			Conc. (mmol/L)	Conc. (mmol/L)	Conc. (mmol/L)
$CaCl_2(H_2O)_2$	44.1	0.3	0.75*	0.075*	0.3*

SSF: simulated salivary fluid, SGF: simulated gastric fluid, SIF: simulated intestinal fluid.
* $CaCl_2(H_2O)_2$ concentration in the final mixture of simulated digestive fluid and sample.

2.4.2. Survivability experiment with simulated digestive fluids

To make SSF, 1.75 mL of SSF stock solution was mixed with 0.25 mL of α-amylase solution (1500 U/mL) and 12.5 μL of 0.3 M $CaCl_2(H_2O)_2$, and its pH was adjusted (FaSSSF; pH 7, FeSSSF; pH 5) with 1 M HCl and 1 M NaOH; moreover, distilled water was added to achieve a final volume of 2.5 mL. To make SGF, 1.875 mL of SGF stock solution was mixed with 0.4 mL of pepsin solution (25,000 U/mL) and 1.25 μL of 0.3 M $CaCl_2(H_2O)_2$, and its pH was adjusted (FaSSGF; pH 1.6, FeSSGF; pH 3, 5, 7) with 1 M HCl and 1 M NaOH; moreover, distilled water was added to achieve a final volume of 2.5 mL. To make SIF, 1.35 mL of SIF stock solution was mixed with 0.5 mL of pancreatin solution (800 U/mL) and 4 μL of 0.3 M $CaCl_2(H_2O)_2$; moreover, its pH was adjusted (FaSSIF; pH 7, FeSSIF; pH 5) with 1 M HCl and 1 M NaOH, and distilled water was added to achieve a final volume of 2 mL. SSF, SGF, and SIF were preheated for 5 min at 37 °C to activate the enzyme before use. HCoV-229E suspension (approximately 5.8–6.1 log $TCID_{50}$/mL) was mixed with each fluid as treatment group or MEM + 1% FBS as control with a 1:1 ratio (v/v) in 50 mL conical tubes. The samples mixed with SSF were incubated for 2 min, and the samples mixed with SGF and SIF were incubated for 2 h at 37 °C and 180 rpm in a shaking incubator. All samples were frozen in a deep freezer at –80 °C to stop enzyme activity and thawed for the $TCID_{50}$ assay.

2.5. Statistical analysis

All experiments were performed independently at least three times with triplicate samples. The $TCID_{50}$ assay data were represented with logarithmic functions (log $TCID_{50}$/mL in suspension, log $TCID_{50}$/coupon on food-contact surface, and log $TCID_{50}$/g on food). All data were expressed as mean ± standard deviation (SD). Graphs were constructed using SigmaPlot version 10.0 (Systat Software, Inc., San Jose, CA, USA). Statistical analysis was performed using Duncan's multiple range test and one-way analysis of variance (ANOVA) using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Significant differences between control and treatment groups were determined ($p < 0.05$).

3. Results

3.1. Virus survivability at various temperatures

3.1.1. Survivability in suspension

The result of the survivability of HCoV-229E in suspension at various temperatures and storage times is shown in Fig. 1. At 20 °C, HCoV-229E titers decreased from 5.19 log $TCID_{50}$/mL (control) to the detection limit (1.0 log $TCID_{50}$/mL) with a 4.19 log reduction after 6 days. At 4 °C, the control titer of HCoV-229E was 5.5 log $TCID_{50}$/mL. Viral titers remained consistently infectious from 0 to 3 days without significant differences and dropped to 2.43 log $TCID_{50}$/mL, with a 3.07 log reduction after 28 days. At –20 °C, the initial titer on day 0 was 6.06 log $TCID_{50}$/mL. After 12 weeks of storage, the titers decreased slightly to 4.88 log $TCID_{50}$/mL with a reduction of 1.18 log $TCID_{50}$/mL. All results were significantly different ($p < 0.05$) within the same temperature.

3.1.2. Survivability on food-contact surfaces

The result of the survivability of HCoV-229E on various food-contact surfaces is shown in Fig. 2 and Tables 2–4. The initial HCoV-229E titers recovered from SS, LDPE, and SR on day 0 were 4.28, 4.24, and 4.28 log $TCID_{50}$/coupon, respectively. At 20 °C, the viral titers on SS, LDPE, and SR reached the detection limit (1.0 log $TCID_{50}$/coupon) on day 3. Virus reduction values on SS, LDPE, and SR were similar and these were 3.28, 3.24, and 3.28 log $TCID_{50}$/coupon, respectively. At 4 °C, the initial (day 0) HCoV-229E titers recovered from SS, LDPE, and SR were 4.58, 4.46, and 4.61 log $TCID_{50}$/coupon, respectively. On SS, the viral titers decreased to 2.38 log $TCID_{50}$/coupon with a 3.13 log reduction after 28 days. On LDPE, the viral titer dropped to 1.53 log $TCID_{50}$/coupon with a reduction value of 2.88 log $TCID_{50}$/coupon after 28 days. On SR, HCoV-229E on SR was undetectable on day 21 and the reduction value was 3.61 log $TCID_{50}$/coupon. At –20 °C, HCoV-229E infectivity on SS decreased from 5.61 at week 0 to 3.69 log $TCID_{50}$/coupon with a 1.92 log reduction after 12 weeks. There was no significant difference in viral concentration between weeks 3 and 12. On LDPE, the initial (week 0) HCoV-229E titer was 5.38 log $TCID_{50}$/coupon. The viral titer on LDPE dropped to 4.06 log $TCID_{50}$/coupon with 1.32 log after 12 weeks. On SR, the initial (week 0) titer recovered from SR was 5.15 log $TCID_{50}$/coupon and the viral titer reached 3.17 log $TCID_{50}$/coupon with a 1.99 log reduction after 12 weeks. The mean of all HCoV-229E titers decreased significantly ($p < 0.05$), with increasing storage time at each temperature.

3.1.3. Survivability on foods

The result of HCoV-229E survivability on lettuce, chicken breast, and salmon is shown in Figs. 3–5. The mean HCoV-229E titers on each food were significantly different ($p < 0.05$) with the passage of time at each temperature. For lettuce surface (Fig. 3), the initial HCoV-229E titer recovered from lettuce surface was 4.61 log $TCID_{50}$/g and the viral titers achieved the detection limit (1.0 log $TCID_{50}$/g) with the reduction value of 3.61 log $TCID_{50}$/g on day 3. At 4 °C, the recovered HCoV-229E titer on lettuce was reduced from 4.88 log $TCID_{50}$/g to the detection limit (1.0 $TCID_{50}$/g) with a 3.88 log reduction after 14 days. At –20 °C, virus infectivity decreased slightly from 5.06 log $TCID_{50}$/g on week 0 to 2.58 log $TCID_{50}$/g after 12 weeks. The reduction value of the HCoV-229E titer was 2.47 log $TCID_{50}$/g after 12 weeks. For chicken breast surface (Fig. 4), the initial titer from chicken breast was 4.76 log $TCID_{50}$/g, and the HCoV-229E titers dropped to 1.5 log $TCID_{50}$/g, which was the detection limit on day 4, and the reduction value was 3.26 log $TCID_{50}$/g at 20 °C. At 4 °C, the initial titer was 4.94 log $TCID_{50}$/g and the HCoV-229E titers reached the detection limit (1.5 log $TCID_{50}$/g) with a 3.44 log reduction on day 21. At –20 °C, the HCoV-229E titer decreased from 5.54 log $TCID_{50}$/g to 3.46 log $TCID_{50}$/g with a 2.08 log reduction after 12 weeks. For salmon surface (Fig. 5), the recovery titer of HCoV-229E on salmon on day 0 was 4.58 log $TCID_{50}$/g and the viral titers decreased to the detection limit with a 3.08 log reduction on day 4 at 20 °C; the detection limit was 1.5 log $TCID_{50}$/g. At 4 °C, the recovery titer of HCoV-229E on day 0 was 5.06 log $TCID_{50}$/g and the viral titers decreased to the detection limit with a reduction value of 3.56 log $TCID_{50}$/g after 21 days. At –20 °C, the viral titers decreased slightly from 5.28 $TCID_{50}$/g to 3.28 log $TCID_{50}$/g with a 2.0 log reduction after 12 weeks.

3.2. Virus survivability under simulated digestive conditions

The survivability of HCoV-229E in the simulated digestive environment is shown in Fig. 6. HCoV-229E was exposed to SSF for 2 min, SGF for 2 h, and SIF for 2 h at 37 °C. As the pH of digestive fluids can change after food intake, the pH of the simulated digestive fluids was prepared in the fasted state and the fed state (Dressman et al., 1990). In the oral phase, the titer of HCoV-229E mixed with MEM + 1% FBS as a control was 5.83 log $TCID_{50}$/mL. HCoV-229E maintained consistent viability after 2 min treatments in FaSSSF (pH 7) and FeSSSF (pH 5), with a 0.21 and 0.25 log reduction, respectively. In the gastric phase, the control titer was 5.83 log $TCID_{50}$/mL. HCoV-229E infectivity was irreversibly lost after 2 h treatment in FaSSGF (pH 1.6) with a 4.33 log reduction, while the virus survived in FeSSGF (pH 3, 5, 7). The viral titers decreased to 3.39, 4.76, and 4.31 log $TCID_{50}$/mL with a 2.44, 1.07, and 1.53 log reduction at pH 3, 5, and 7 of FeSSGF, respectively. There were no significant differences at pH 5 and 7. In the intestinal phase, the control titer was 5.99 log $TCID_{50}$/mL. HCoV-229E was infectious after 2 h

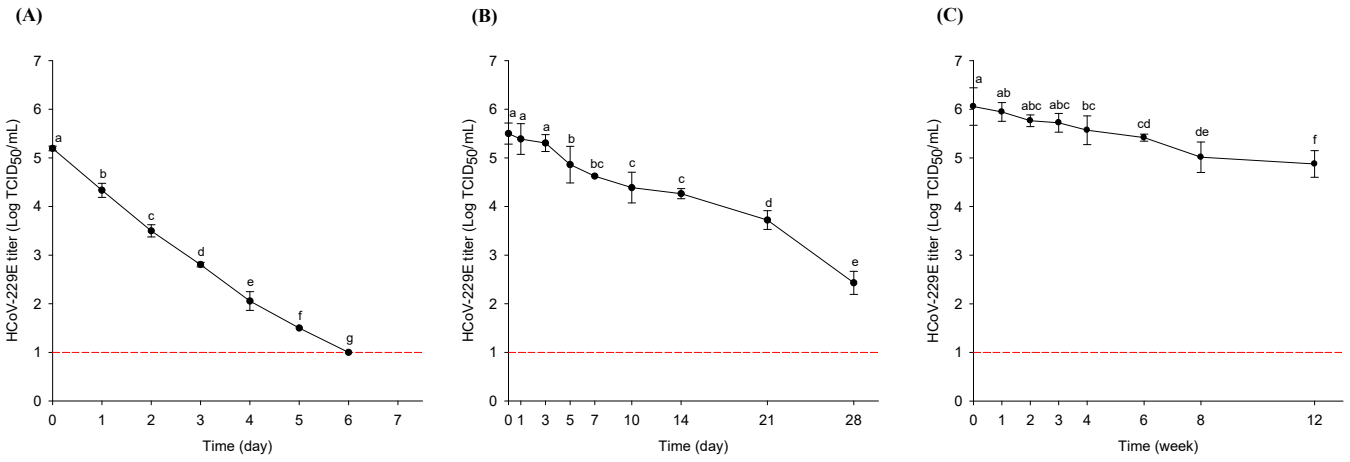

Fig. 1. Survivability of HCoV-229E in suspension at (A) 20 °C, (B) 4 °C, and (C) –20 °C The error bars represent standard deviations (SD) of the mean. The dash line indicates detection limit (1.0 log $TCID_{50}$/mL). a–g indicate significant differences ($p < 0.05$) within the same temperature.

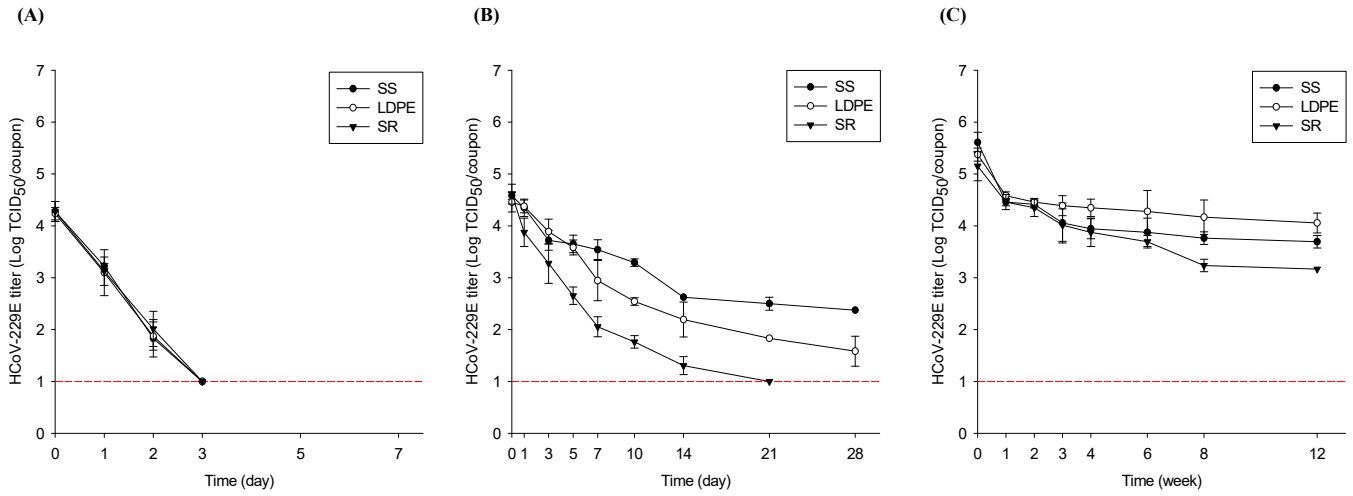

Fig. 2. Survivability of HCoV-229E on various food-contact surfaces at (A) 20 °C, (B) 4 °C, and (C) –20 °C The error bars represent standard deviations (SD) of the mean. The dash line indicates detection limit (1.0 log $TCID_{50}$/coupon). SS: stainless steel, LDPE: plastic, SR: silicone rubber.

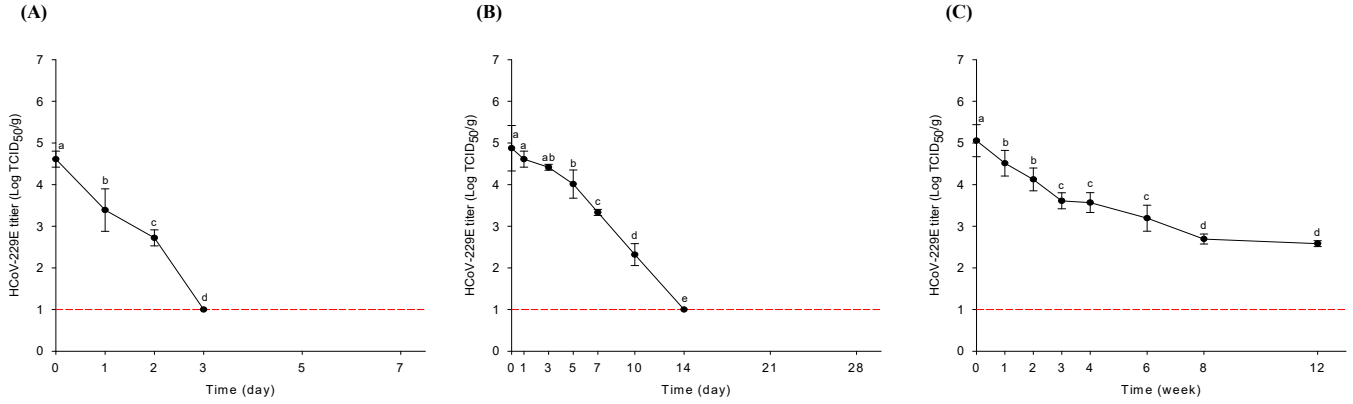

Fig. 3. Survivability of HCoV-229E on lettuce at (A) 20 °C, (B) 4 °C, and (C) –20 °C The error bars represent standard deviations (SD) of the mean. The dash line indicates detection limit (1.0 log $TCID_{50}$/g). a–e indicate significant differences ($p < 0.05$) within the same temperature.

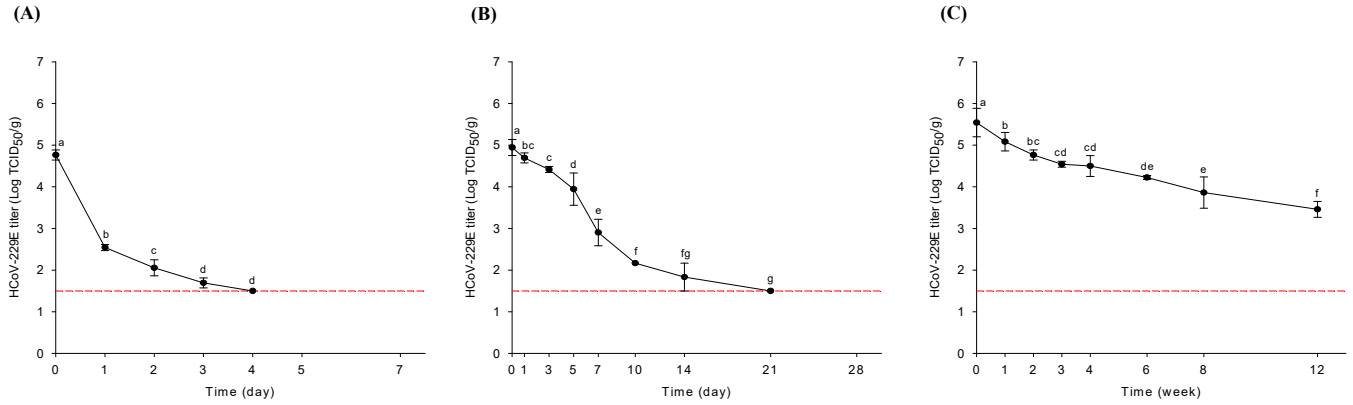

Fig. 4. Survivability of HCoV-229E on chicken breast at (A) 20 °C, (B) 4 °C, and (C) –20 °C The error bars represent standard deviations (SD) of the mean. The dash line indicates detection limit (1.5 log $TCID_{50}$/g). a–g indicate significant differences ($p < 0.05$) within the same temperature.

treatment in FaSSGF (pH 7). The viral titer decreased to 3.83 log $TCID_{50}$/mL with a 2.15 log reduction. However, virus after 2 h treatment in simulated fed state intestinal fluid (FeSSGF, pH 5) reached the detection limit (1.5 log $TCID_{50}$/mL) with a 4.49 log reduction. HCoV-229E titers were significantly reduced in the same phase ($p < 0.05$).

4. Discussion

4.1. Virus survivability at various temperatures

Temperature is widely recognized as a major factor of virus survival in the environment, and has been applied to inactivate microorganisms in the food industry (Bertrand et al., 2012). In this study, we investigated

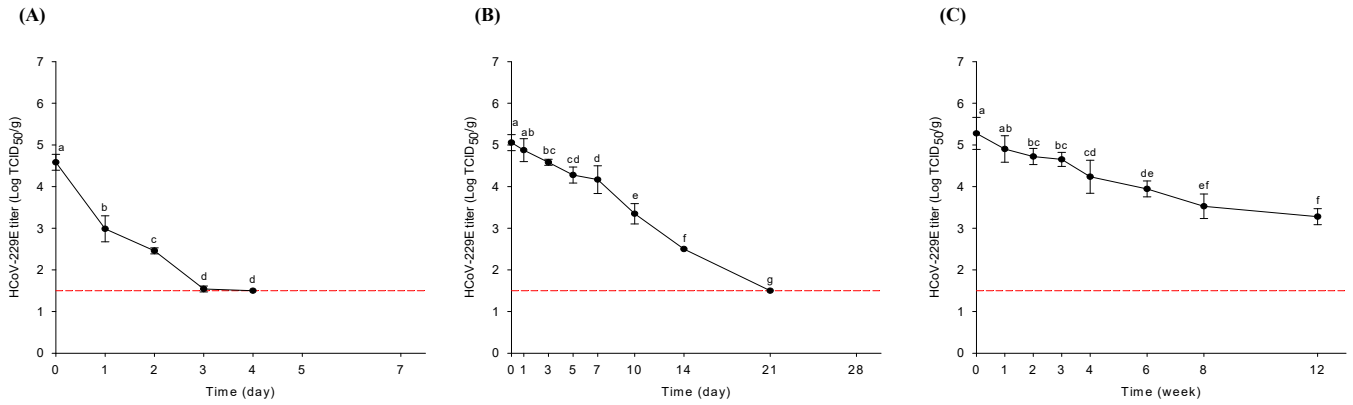

Fig. 5. Survivability of HCoV-229E on salmon at (A) 20 °C, (B) 4 °C, and (C) –20 °C The error bars represent standard deviations (SD) of the mean. The dash line indicates detection limit (1.5 log $TCID_{50}$/g). a–g indicate significant differences ($p < 0.05$) within the same temperature.

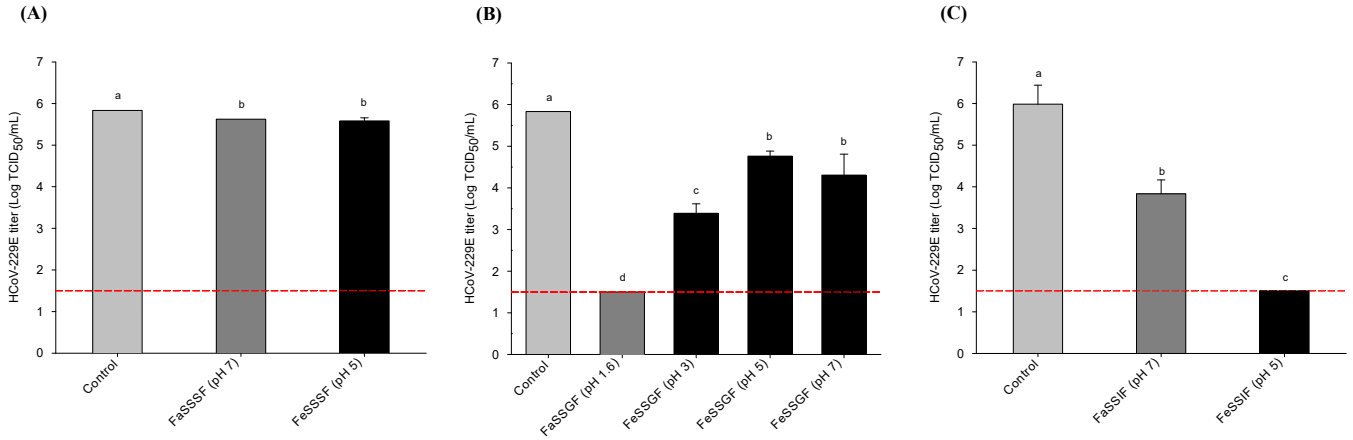

Fig. 6. Survivability of HCoV-229E in simulated digestive fluids in (A) oral phase (2 min, 37 °C), (B) gastric phase (2 h, 37 °C), and (C) intestinal phase (2 h, 37 °C) The error bars represent standard deviations (SD) of the mean. The dash line indicates detection limit (1.5 log $TCID_{50}$/mL). Different letters (a–d) indicate significant differences ($p < 0.05$) within the same phase. FaSSSF: fasted state simulated salivary fluid, FeSSSF: fed state simulated salivary fluid, FaSSGF: fasted state simulated gastric fluid, FeSSGF: fed state simulated gastric fluid, FaSSIF: fasted state simulated intestinal fluid, FeSSIF: fed state simulated intestinal fluid.

the survival of HCoV-229E in suspension at various temperatures. The results showed that HCoV-229E maintained infectivity at 20 °C (room temperature) after 5 days with a 3.69 log reduction, at 4 °C (refrigerated temperature) after 28 days with a 3.07 log reduction, and at –20 °C (freezing temperature) after 12 weeks with a 1.18 log reduction. Similarly, previous studies have reported that the persistence of liquid-suspended coronaviruses was greater at low temperatures. One study showed that suspended SARS-CoV-2 in suspension survived for 14 days at 4 °C with around a 0.7 log reduction, 7 days at 22 °C with around a 3.3 log reduction, and 1 day at 37 °C with around a 3.5 log reduction. At high temperatures of 56 °C and 70 °C, the viral titer decreased sharply, and the virus lost infectivity after 30 min and 5 min with around a 4.8 log reduction, respectively (Bertrand et al., 2012; Chin et al., 2020). Another study indicated that the reduction value of SARS-CoV-1 in MEM was 0 log $TCID_{50}$/mL at 4 °C, while the reduction value was more than 5 log $TCID_{50}$/mL at 57 °C after 30 min of storage (Rabenau et al., 2005). Lamarre and Talbot (1989) indicated that HCoV-229E was stable for at least 14 days at 4 °C with a decrease of <1 log reduction, but the virus lost infectivity after 14 days at 22 °C with a decrease of more than 4 log and on day 5 at 37 °C with a decrease of more than 3.2 log. The infectivity of Turkey coronavirus was lost after 10 days at room temperature and after 40 days at 4 °C with a 2.8 log reduction (Guionie et al., 2013). Enveloped viruses, such as coronaviruses, are susceptible to heat and, as temperature increases, protein denaturation can occur and change the virion structure (Aboubakr, Sharafeldin, & Goyal, 2021; Lelie, Reesink, & Lucas, 1987; Schlegel, Immelmann, & Kempf, 2001). In the case of SARS-CoV-2, as temperature increased, the spike protein that binds to the host cell receptor shifted to an inactive state. This result indicated that the infectivity of SARS-CoV-2 decreased at higher temperature (Rath & Kumar, 2020). Therefore, we suggest that the survival period of SARS-CoV-2 may be relatively longer at refrigeration and freezing temperatures.

In this study, the survivability of HCoV-229E was evaluated at different temperatures on food-contact surfaces. The result showed that HCoV-229E on SS and LDPE was undetectable at 20 °C after 3 days (3.28 and 3.24 log reduction, respectively), survived at 4 °C after 28 days (3.13 and 2.88 log reduction, respectively), and –20 °C even after 12 weeks (1.92 and 1.32 log reduction, respectively). On SR, the virus lost viability at 20 °C after 3 days and 4 °C after 21 days with a 3.28 and 3.61 log reduction, respectively, and survived at –20 °C even after 12 weeks with a 1.99 log reduction. These findings indicated that HCoV-229E survived longer at low temperatures. According to previous studies, MERS-CoV on SS and plastic was undetectable after 4 days at 20 °C with around 5 log reduction, while the virus lost its viability after 2 days at 30 °C and 30% RH with a more than 5 log reduction (van Doremalen, Bushmaker, & Munster, 2013). Infectivity of SARS-CoV-2 on polypropylene accelerated dramatically with increasing temperature, with decay occurring at 27 °C approximately 5–10 times faster than at 10 °C (Morris et al., 2020). Mouse hepatitis virus (MHV) on SS survived up to 28 days with a 0.25 log reduction at 4 °C and 20% RH. At 20 °C, the virus

also survived up to 28 days, but the titer decreased by 2 log (Casanova, Jeon, Rutala, Weber, & Sobsey, 2010).

SR maintained a lower virus survivability compared to SS and LDPE in the present study. SS, LDPE, and SR are non-porous surfaces. Differences in virus survival between these materials have not been precisely demonstrated in the literature. However, some studies indicated that virus survivability on surfaces may depend on several virus-surface interactions, including hydrophobicity and electrostatic attraction, which depend on pH, virus surface charge concentration, and isoelectric properties of surface proteins (Dika, Ly-Chatain, Francius, Duval, & Gantzer, 2013; Fuhs, Chen, Sturman, & Moore, 1985; Owen, Shivkumar, Cross, & Laird, 2022). Among the food-contact surfaces commonly used in the food industry, there are porous surfaces like paper, wood, and fabric; additionally, other non-porous surfaces are also used. Moisture evaporation and drying occur more easily on porous surfaces (Chatterjee, Murallidharan, Agrawal, & Bhardwaj, 2021). Also, virus recovery from porous surfaces is lower than from non-porous surfaces because of absorption of virus (Castrica et al., 2021; Terio et al., 2021). Therefore, survivability of virus on porous surfaces may be lower than on non-porous and non-porous surfaces may be better carriers of SARS-CoV-2 transmission. In another study, infectious SARS-CoV-2 was not detected on porous surfaces (wood and cloth) on day 2 at room temperature with a 5.66 and 4.84 log reduction, respectively. In addition, SARS-CoV-2 was not detected on non-porous surfaces (SS and plastic) on day 7 with a 5.8 and 5.81 log reduction, respectively (Chin et al., 2020). Another study indicated that SARS-CoV-2 on SS and plastic was viable for up to 4 days with <4 log reduction, while viruses on cardboard were not viable after 1 day at room temperature with around 2 log reduction (van Doremalen et al., 2020). SARS-CoV-2 on non-porous surfaces, including SS, polymer note, paper note, glass, and vinyl, survived at 20 °C for 28 days with approximately 3–5 log reduction, while the virus on cotton cloth, which is a porous surface, was undetectable on day 14 with approximately 2 log reduction at the same temperature (Riddell, Goldie, Hill, Eagles, & Drew, 2020). Therefore, we suggest that food-contact surfaces, especially non-porous surfaces under refrigeration or freezing conditions can potentially spread SARS-CoV-2.

HCoV-229E on lettuce lost infectivity at 20 °C after 3 days with a 3.61 log reduction, and at 4 °C after 14 days with a 3.88 log reduction in the present study. At –20 °C, the virus remained viable even after 12 weeks with a 2.47 log reduction. Moreover, HCoV-229E on chicken breast and salmon was undetectable on day 4 at 20 °C with a 3.26 and 3.08 reduction, respectively, on day 21 at 4 °C with a 3.44 and 3.56 log reduction, respectively, and the virus remained viable after 12 weeks at –20 °C with 2.08 and 2 log reduction, respectively. Consistent with this study, SARS-CoV-2 on beef, pork, and salmon maintained viability for up to 20 days at –20 °C, while the virus was detected on all foods at 4 °C after 3 days (Feng et al., 2021). Another study showed that MERS-CoV in dromedary camel milk survived at 4 °C for 72 h with approximately 0.5 log reduction, while infectivity was lost at 22 °C after 48 h with approximately 0.5–1 log reduction (van Doremalen, Bushmaker, Karesh, & Munster, 2014). Yepiz-Gomez, Gerba, and Bright (2013) investigated the survival of HCoV-229E stored at refrigerated temperature (4 °C). The virus was reduced by 0.2 log after 2 days and was no longer detectable after 4 days. These findings indicated that SARS-CoV-2 infectivity in food remains longer at lower temperatures.

There is no exact evidence on how food affects the survival of human coronaviruses. However, like foodborne viruses, it may be affected by food components such as protein, fat, sugar, minerals, water activity, and pH. Based on several studies, virus survival is prolonged with a higher protein and fat content (Bidawid, Farber, Sattar, & Hayward, 2000; Rabenau et al., 2005). Similar findings indicated that SARS-CoV-2 in foods (meat, seafood, turkey, and cheese), which have high protein and fat, was detectable after 14 days at 4 °C with around 3–4 log reduction, which is consistent with the findings of this study. High moisture was also shown to aid the survival of SARS-CoV-2 (Jia, Taylor, Senger, Ovissipour, & Bertke, 2022). Moreover, heparan sulfate aids the binding of SARS-CoV-2 to host cell receptors, and is abundant in meat and seafood (Clausen et al., 2020). In addition, both meat and seafood are stored at refrigerated or freezing temperature. Therefore, meat and seafood could be potential carriers of SARS-CoV-2. In the case of fresh produce, such as lettuce, they are usually consumed unheated. Therefore, the virus cannot be inactivated, and residual virus may be present on the food. Thus, fresh produce is also a potential carrier of SARS-CoV-2.

4.2. Virus survivability under simulated digestive conditions

In the oral phase, this study showed that the HCoV-229E titer only decreased to <0.3 log compared to the control after a 2-min treatment with SSF. Furthermore, there was no difference in virus reduction according to pH. Similarly, a previous study showed that SARS-CoV-2 survived up to 90 min in simulated saliva with a reduction of <1 log $TCID_{50}$/L (Smither, Eastaugh, Findlay, & Lever, 2020). Although one of the main transmitting carriers of SARS-CoV-2 to humans is saliva droplets, it is not exactly clear what factors in saliva determine the survivability of SARS-CoV-2. However, the virus-saliva interaction may be influenced by biologically active components such as proline-rich proteins and mucins (Almeida, Gregio, Machado, De Lima, and Azevedo, 2008; Li et al., 2020). We showed that HCoV-229E was less resistant in highly acidic FaSSGF (pH 1.6) than in FeSSGF (pH 3, 5, 7). Consistent with this study, other previous studies reported that human coronaviruses, including SARS-CoV-2 and HCoV-229E, were less tolerant in FaSSGF, while human coronaviruses were relatively resistant in FeSSGF (Lee et al., 2020; Zhou et al., 2017). The optimal pH of pepsin activity is approximately 2 and activated pepsin can induce the inactivation of enveloped viruses (Piper & Fenton, 1965; Torgeman et al., 2017). Therefore, HCoV-229E inactivation in FaSSGF may be due to the low pH and the effect of pepsin. However, SARS-CoV-2 survives in a wide pH range (3–10) compared to other human coronaviruses. Thus, SARS-CoV-2 may be more stable in gastric fluid (Aboubakr et al., 2021; Chin et al., 2020). Moreover, another study evaluated the survivability of human coronavirus OC43 (HCoV-OC43) in suspension and on cucumber after FeSSGF treatment (pH 3, 4.5, 6). HCoV-OC43 survived at all FeSSGF pHs as in this study. Additionally, the presence of food (cucumber) in FeSSGF significantly increased the survival of HCoV-OC43 (Harlow et al., 2022). This study showed that HCoV-229E maintained its infectivity in FaSSIF (pH 7), whereas the virus was inactivated in FeSSIF (pH 5) containing pancreatin. A previous study also demonstrated that the infectivity of SARS-CoV-2 and HCoV-229E was maintained in FaSSIF but lost in FeSSIF containing bile salt (Lee et al., 2020; Zhou et al., 2017). Other studies showed that HCoV-OC43 and MERS-CoV were resistant to FaSSIF and FeSSIF (Harlow et al., 2022; Zhou et al., 2017). In another study, residual SARS-CoV-2 mNeonGreen reporter virus was present after 1 h of treatment with simulated small intestinal fluid (Zang et al., 2020). Therefore, SARS-CoV-2 entering through the mouth has a chance of survival depending on the change in pH of the GI fluid. In addition, ACE2, which is a host cell receptor that binds to the S protein of SARS-CoV-2, is abundantly expressed in the glandular cells of the gastric, duodenal, and rectal epithelium. In particular, the highest level of ACE2 is expressed in the small intestine (Li et al., 2020; Qi, Qian, Zhang, & Zhang, 2020; Xiao et al., 2020). TMPRSS2 and TMPRSS4, which cleave protein S to allow invasion of SARS-CoV-2 into the host, increased viral infectivity in intestinal epithelial cells (Zang et al., 2020). A study showed that SARS-CoV-2 nucleocapsid proteins were observed in the cytoplasm of gastric, duodenal, and rectal epithelial cells (Xiao et al., 2020). In another study, after intragastric inoculation with SARS-CoV-2 in rhesus monkeys, infectious viral RNA was detected in the mesenteric lymph and pancreas (Jiao et al., 2021). Lamers et al. (2020) demonstrated that when organoids in the human small intestine were exposed to SARS-CoV-2, the virus easily replicated in abundant cell types of the intestinal lining, producing large amounts of infectious viral particles in the intestine.

These findings indicated that SARS-CoV-2 infection can occur in the GI tract. Therefore, SARS-CoV-2 entering through the mouth can survive in the GI fluid depending on the pH change, and the surviving virus has the potential to cause infection in the GI tract. However, there are not many survival studies in digestive fluids; therefore, more studies are needed to understand the potential for a complete and precise fecal-oral route of transmission for this virus.

5. Conclusions

The survivability of HCoV-229E, as a surrogate for SARS-CoV-2, in suspension, on food-contact surfaces and on foods at various temperatures and in simulated human digestive fluids was evaluated. The results of this study indicated that the virus survived up to 28 days with 2.88–3.61 log reduction depending on the type of food-contact surface and lost infectivity on day 21 with 3.44–3.88 log reduction depending on the type of food at refrigerated temperature. At freezing temperature, HCoV-229E on all tested materials and foods survived even after 12 weeks with 1.32–2.47 log reduction, indicating that HCoV-229E remains infectious on various food-contact surfaces and foods and survives longer at a lower temperature. This study also showed that HCoV-229E had little effect on viability after SSF (pH 7, 5) treatment. In addition, although viral infectivity was lost in FaSSGF (pH 1.6) and FeSSIF (pH 5), the virus retained infectivity in FeSSGF (pH 3, 5, 7) and FaSSIF (pH 7), suggesting that the virus could remain infectious in human digestive fluids depending on pH conditions. All the findings acquired in this study, together with existing data from the literature, suggest a careful food safety strategy including proper management of temperature and pH for the prevention of any potential for SARS-CoV-2; particularly, in the cold chain production during food storage and transportation. In addition, further studies are required to investigate how replication of this virus might affect the clinical symptoms of infection in the GI tract and the transmission dynamics of COVID-19 patients to investigate another possible route of SARS-CoV-2 infection.

CRediT authorship contribution statement

Eun Ji Lee: Investigation, Visualization, Data curation. **Sangha Han:** Software, Investigation. **Seok-Woo Hyun:** Investigation. **Gyeong Bae Song:** Resources. **Sang-Do Ha:** Conceptualization, Funding acquisition, Supervision.

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.

Data availability

The data that has been used is confidential.

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