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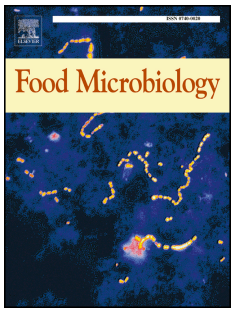

Viability of SARS-CoV-2 on lettuce, chicken, and salmon and its inactivation by peracetic acid, ethanol, and chlorine dioxide

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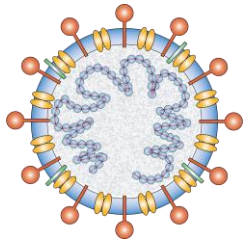
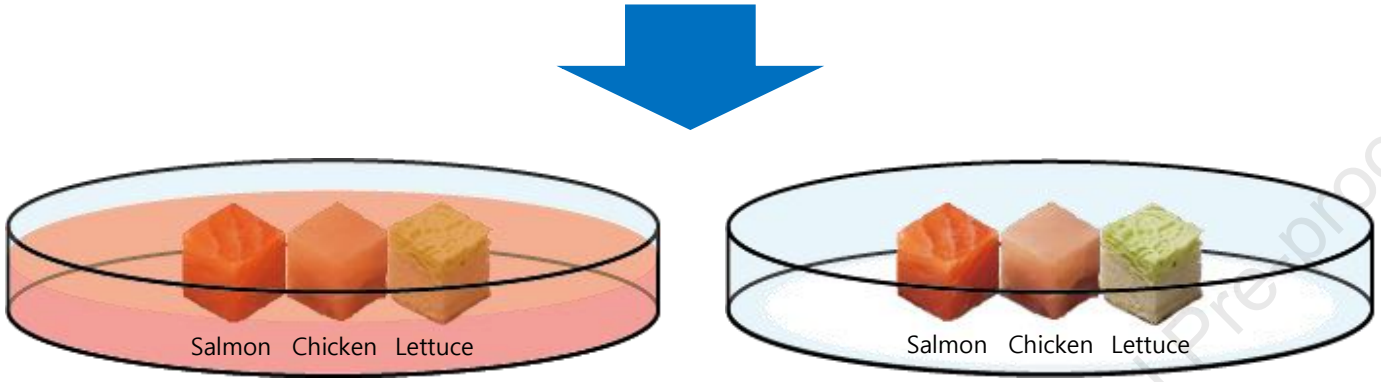
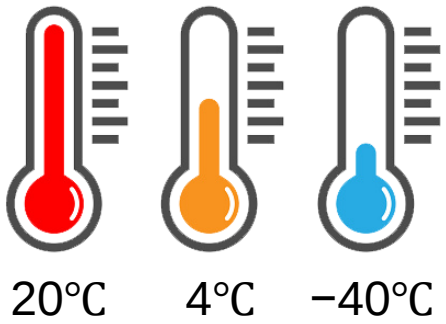


SARS-CoV-2 L

SARS-CoV-2 S





Ethanol

Chlorine dioxide

Peracetic aced

Viability and inactivation test

Title page

Title: Viability of SARS-CoV-2 on lettuce, chicken, and salmon and its inactivation by peracetic acid, ethanol, and chlorine dioxide

Authors information

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Abstract

Since the first SARS-CoV-2 outbreak in Wuhan, China, there has been continued concern over the link between SARS-CoV-2 transmission and food. However, there are few studies on the viability and removal of SARS-CoV-2 contaminating food. This study aimed to evaluate the viability of SARS-CoV-2 on food matrices, depending on storage temperature, and inactivate the virus contaminating food using disinfectants. Two SARS-CoV-2 strains (L and S types) were used to contaminate lettuce, chicken, and salmon, which were then stored at 20 ,4 and −40 °C. The half-life of SARS-CoV-2 at 20 °C was 3–7 h but increased to 24–46 h at 4 °C and exceeded 100 h at −40 °C. SARS-CoV-2 persisted longer on chicken or salmon than on lettuce. Treatment with 70% ethanol for 1 min inactivated 3.25 log reduction of SARS-CoV-2 inoculated on lettuce but not on chicken and salmon. ClO_2 inactivated up to 2 log reduction of SARS-CoV-2 on foods. Peracetic acid was able to eliminate SARS-CoV-2 from all foods. The virucidal effect of all disinfectants used in this study did not differ between the two SARS-CoV-2 strains; therefore, they could also be effective against other SARS-CoV-2 variants. This study demonstrated that the viability of SARS-CoV-2 can be extended at 4 and −40 °C and peracetic acid can inactivate SARS-CoV-2 on food matrices.

Keywords: SARS-CoV-2, Viability, Ethanol, Peracetic acid, Chlorine dioxide, Lettuce, Salmon, Beef

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, which started in 2019, has caused 585 million confirmed cases and 6.42 million deaths by August 10, 2022 (WHO, 2021). Although over 4 billion people have completed vaccination, the pandemic is still ongoing, with the daily number of confirmed cases reaching 1.5 million (Mathieu et al., 2021). Concerns over food safety have been raised since the seafood market in Wuhan was identified as the initial source of SARS-CoV-2. In December 2019, 55% of SARS-CoV-2 infections in China were associated with markets in Wuhan, including the Huanan seafood wholesale market (Bai et al., 2021). In the United States, between April and May 2020, 264 meat and poultry processing facilities in 23 states reported coronavirus disease 2019 (COVID-19) outbreaks, with 17,358 confirmed cases among workers (Birhane et al., 2020). In addition, SARS-CoV-2 RNA has been detected on the surfaces of various foods and their packaging materials, and it has been reported that some of the outbreaks may be related to frozen imported foods and food packaging materials (Liu et al., 2020; Pang et al., 2020; Zhao et al., 2020).

As person-to-person, airborne, and contact with contaminated surfaces is spreading virus, foodborne transmission is the important route as well. Virus can be contaminated on food with two main means (Ceylan et al., 2020). The first can occur during the production and manufacture of food, including using contaminated water during harvest processing and infected food handlers. The second is the consumption of animal-derived foods infected with zoonotic viruses. Although SARS-CoV-2 infection by eating contaminated food was not reported, there have been cases of SARS-CoV-2 infection in foods related facilities, and up to 10^{11} copies of RNA have been detected in human excretions, such as sputum from patients infected with SARS-CoV-2 (Wölfel et al., 2020). Nipah virus is a zoonotic respiratory virus

closely related to infection by contaminated food, and SARS-CoV and MERS-CoV, which are members of SARS-CoV-2, can also be transmitted through food (Cui et al., 2019; WHO, 2018). As such, the foodborne transmission of SARS-CoV-2 seems plausible but unproven. However, there are few studies on the persistence of SARS-CoV-2 contaminating foods or how to eliminate it.

Recent studies have raised concerns about food-related transmission or oral infection. Van Doremalen et al. (2020) reported that depending on the surface (except copper), SARS-CoV-2 can remain infectious for up to several days and survives for hours in aerosols. These results suggest the possibility of transmission of SARS-CoV-2 via fomites on contaminated surfaces. In addition, Dai et al. (2021) reported that SARS-CoV-2 contaminating fish could survive up to a week at 4 ℃. Gastrointestinal symptoms, including diarrhea, loss of appetite, nausea, vomiting, abdominal pain, and bloody stools caused by SARS-CoV-2 infection, have also been reported (Guan et al., 2020; Guo et al., 2021). In a case control study of COVID-19 patients in Baltimore, USA, 73% of patients experienced gastrointestinal symptoms (Chen et al., 2020). SARS-CoV-2 not only caused gastrointestinal symptoms but was also shed in feces. Moreover, SARS-CoV-2 RNA was detected in feces or rectal swabs in 48% (312/650) of the investigated COVID-19 patients. Of these, SARS-CoV-2 RNA was detected in 63.2% (187/296) in fecal/rectal samples but not in respiratory samples (Guo et al., 2021). In addition, SARS-CoV-2 RNA detected in fecal/rectal samples had a faster threshold cycle than respiratory samples or shed high titers over a longer period than respiratory samples, where titers decreased over time (Han et al., 2020; Xu et al., 2020). Animal coronaviruses, already well studied, are enterotropic viruses that infect and cause symptoms in the intestinal tract, and associations with the intestinal tract were also observed with the previous novel coronaviruses SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV)

(Assiri et al., 2013; Leung et al., 2003; Weiss and Leibowitz, 2011). For SARS-CoV-2 to enter cells, angiotensin-converting enzyme 2 and transmembrane serine protease 2 are essential, and they are expressed not only in the lungs but also in the ileum and colon (Hoffmann et al., 2020; Zhang et al., 2020). SARS-CoV-2 is also known to be robust under extremely acidic conditions (Chin et al., 2020).

The viability of SARS-CoV-2 on food matrix have been studied little. As the efficacy of sanitizers could be reduced depending on the characteristics of food matrix, the inactivation of SARS-CoV-2 by disinfectants have been conducted on the various food matrices. SARS-CoV-2 viral RNA was detected in the imported frozen food packages and the surface of the frozen foods including salmon, beef, and chicken wings (Bai et al., 2021). Chicken is the world's most consumed meat, and lettuce is one of the main sources of food-borne viruses (CDC, 2021; OECD/FAO, 2021). Therefore, this study aimed to evaluate the viability of SARS-CoV-2 according to the storage temperature on experimentally contaminated food matrices and to assess the efficacy of disinfectants in inactivating SARS-CoV-2 contaminating food matrices.

Materials and methods

Viruses and cells

Vero E6 (ATCC CL-1586) cells were grown in DMEM (Gibco; Waltham, MA, USA) with 10% FBS and 1% antibiotics-antimycotics (Gibco). Cells were cultured at 37 °C with 5% CO_2 in a humidified incubator. Confluent Vero E6 cells were inoculated with two strains of SARS-CoV-2 (L type, NCCP43326; S type, NCCP43330) at 0.1 multiplicities of infection in DMEM with 2% FBS. SARS-CoV-2 was incubated at 37 ℃ with 5% CO_2 for 4 days. After two freeze/thaw cycles, viral titers were assessed using a 50% tissue culture infectious dose ($TCID_{50}$) and quantified using the Spearman–Karber method (Ramakrishnan, 2016). All experiments related to SARS-CoV-2 were performed at the biosafety level 3 facility of the Zoological Infectious Disease Research Institute, Chonbuk National University.

Viability test on food matrices

SARS-CoV-2 RNA detected in human excreta is 10^6–10^{11} copies, and when converted into infectious particles, it is approximately 10^2–10^7 $TCID_{50}$ (Sender et al., 2021; Wölfel et al., 2020). Therefore, in the present study, the concentration of SARS-CoV-2 to be used for food contamination was determined to be 10^6 log $TCID_{50}$/mL. The three food matrices (lettuce, chicken breast, and salmon) used in the viability test were purchased from a market in Anseong, Korea. Viability tests were performed based on a previous study (Dai et al., 2021). Briefly, chicken and salmon were cut into 125 mm^3 pieces and lettuce into 1 cm^2 pieces, which were then used as food matrices. Thereafter, the food matrices were immersed in SARS-CoV-2 L and S at 6 log $TCID_{50}$/mL and incubated for 15 s. After removing excess virus using filter paper, food matrices were immediately transferred to an individual 1.5 mL tube. Thereafter, individual contaminated food matrices were maintained at 40% relative

humidity (RH) under three temperature conditions (room temperature: 20 °C, refrigerated: 4 °C, and frozen: −40 °C). For each period (room temperature: 0, 8, 24, 48, and 72 h; refrigerated: 0 and 8 h and 2, 3, 7, 10, and 14 d; frozen: 0, 7, 14, 21, and 28 d), 1 mL of DMEM with 2% FBS was added to individual tubes and vortexed for 10 s to recover viruses and then filtered using a 0.45-μm filter. Recovered viruses were immediately diluted 10-fold and viral infectivity was determined using $TCID_{50}$.

Quantitative carrier test using food matrices

A quantitative carrier test to evaluate the inactivation effect of disinfectants on SARS-CoV-2 contaminated food matrices were performed with modifications to OECD guidelines and previous study. (OECD, 2013; Moon et al., 2021). As disinfectants to be evaluated, chlorine dioxide (ClO_2) and peracetic acid (PAA) were selected in consideration of food grade, persistence, and generation of toxic by-products such as trihalomethane (Aieta et al., 1983; Farinelli et al., 2022). As ethanol (EtOH) is known to be effective in inactivating SARS-CoV-2 on various surface materials, food-grade ethanol was used in this study (Jung et al., 2023). The two disinfectants except EtOH were used at half of the recommended concentration, the recommended concentration and twice of the recommended concentration. SARS-CoV-2 contaminated food matrices prepared in the same manner as for the viability test were treated with 50 μL of 30, 50, and 70% EtOH (Sigma-Aldrich, St. Louis, MO, USA) or 20, 40, and 80 ppm ClO_2 (LifeClean, Uddevalla, Sweden) for 1 and 5 min, respectively. In addition, lettuce was treated with 40, 80, and 160 ppm PAA (Daesung C&S, Seoul, Korea), and chicken and salmon were treated with 1,000, 2,000, and 4,000 ppm of the same as recommended (MFDS, 2019). After neutralizing the disinfectant by adding 950 μL of 5% FBS DMEM to the food matrices, the neutralized solutions were immediately evaluated for

152 viral infectivity using $TCID_{50}$.

153

154 **Statistical analysis**

155 All data are presented as the mean ± SD. To determine virus stability, a Bayesian
156 regression model was used to estimate the decay rate of viable virus titers (Van Doremalen et
157 al., 2020). The posterior samples were drawn using a No-U-Turn Sampler (a form of Markov
158 Chain Monte Carlo). For the quantitative carrier test, a two-sample *t* test was used to analyze
159 differences between strains. The disinfection effect between concentrations on viruses was
160 analyzed using one-way ANOVA and the Tukey post-hoc. Statistical significance was set at p
161 < 0.05. All experiments were performed in triplicate, and statistical analysis was performed
162 using R version 4.1.0.

163

164 **Results**

165 **Viability test on food matrices**

166 At 20 ℃, SARS-CoV-2 did not survive more than 3 days on food matrices (Fig. 1A). On
167 lettuce, the SARS-CoV-2 titers, which were 3.06×10^4 $TCID_{50}$/mL after contamination,
168 decreased by more than 1 log $TCID_{50}$/mL to 7.08×10^2 and 3.98×10^1 $TCID_{50}$/mL for SARS-
169 CoV-2 L and SARS-CoV-2 S at 24 h, respectively. After 48 h, the titers were below the
170 detection limit. Similar to lettuce, the titers of both SARS-CoV-2 strains on chicken were
171 below the detection limit from 48 h. On salmon, SARS-CoV-2 titers decreased by more than
172 1 log at 8 h (SARS-CoV-2 L to 4.80×10^2 $TCID_{50}$/mL and SARS-CoV-2 S to 3.98×10^2
173 $TCID_{50}$/mL), which was faster than on lettuce and chicken, but viruses survived longer, up to
174 48 h. The kinetics of the two SARS-CoV-2 strains on food matrices were somewhat different.
175 On all surfaces, SARS-CoV-2 S was more unstable than SARS-CoV-2 L, and the two strains

significantly differed at 24 h on lettuce ($p < 0.001$), 8 and 24 h on chicken ($p = 0.014$ and $p < 0.001$, respectively), and 48 h on salmon ($p = 0.016$). Virus titers decreased sharply on all food matrices, as indicated by the linear decrease over time (Fig. 1B). The half-life of SARS-CoV-2 differed depending on the food matrix (Fig. 1C and Table S1). The median estimates of half-lives for SARS-CoV-2 L and SARS-CoV-2 S on lettuce were 3.47 and 2.60 h, respectively, and 4.80 and 3.08 h on chicken, respectively, slightly longer than those on lettuce. On the other hand, the median estimate of half-lives on the salmon surface, where infectious virus was detected for up to 2 d, were longest at 7.46 h for SARS-CoV-2 L and 4.65 h for SARS-CoV-2 S.

At 4 °C, the viability of the viruses was prolonged to 10 days (Fig. 2A). The time required for viruses to decrease by 1 log $TCID_{50}$/mL on lettuce was 24 h, which was the same as that at 20 ℃; however, infectious SARS-CoV-2 was detected for up to 10 d. The difference in viability between the two SARS-CoV-2 strains was greatest at 72 h ($p < 0.001$). The differences between the two SARS-CoV-2 strains were most pronounced on chicken. SARS-CoV-2 L, although in small amounts, maintained the infectivity for up to 10 d (3.98×10^1 $TCID_{50}$/mL), whereas SARS-CoV-2 S decreased sharply at 8 h (to 6.26×10^2 $TCID_{50}$/mL) and was not detected from 7 d. The prolongation of the viability of both SARS-CoV-2 strains was particularly pronounced on salmon, where they remained infectious for up to 14 d post contamination. Due to the spoilage of the food samples, the experimental period was limited to 2 weeks. The posterior distribution of the decay model showed that SARS-CoV-L was less scattered than SARS-CoV-2 S (Fig 2B). The half-life of SARS-CoV-2 was also increased 5–9 fold compared with that at 20 °C, except for that of SARS-CoV-2 S on chicken (Fig. 2C and Table S1). On lettuce, median half-life estimates for SARS-CoV-2 L and SARS-CoV-2 S were 25.90 and 23.20 h, respectively, close to 1 d. However, the median half-life estimate for

SARS-CoV-2 L on chicken was increased to 30.1 h, whereas that for SARS-CoV-2 S was only 11.6 h. Similar to the half-life results at 20 ℃, the half-lives of SARS-CoV-2 L and SARS-CoV-2 S on salmon were 46.6 and 38.2 h, respectively, longer than on other foods.

At -40 °C, the SARS-CoV-2 strains were viable for 4 weeks on all food matrices (Fig. 3A). In particular, SARS-CoV-2 L maintained infectivity of more than 2 log $TCID_{50}$/mL (4.80×10^2, 3.16×10^2, and 5.54×10^2 $TCID_{50}$/mL on lettuce, chicken, and salmon, respectively). In addition, the decay model data were smooth on all food matrices until the end of the experiment (Fig. 3B). Accordingly, the half-life of the virus on each surface was significantly increased, but there were differences depending on the strain. (Fig. 3C and Table S1). On the one hand, SARS-CoV-2 L had a half-life greater than 5 d, similar across all foods (129, 123, and 128 h on lettuce, chicken, and salmon, respectively). On the other hand, the half-lives of SARS-CoV-2 S were 72.3, 84.9, and 103 h on lettuce, chicken, and salmon, respectively, up to 56.7 h shorter than those for SARS-CoV-2 L.

Virucidal effect of disinfectants on food matrices

According to the OECD guideline criteria, the standard effect of the carrier test is defined as a ≥3 log reduction; nevertheless, no reduction of more than 3 logs was observed in the experiments on chicken and salmon due to the detection limit. Therefore, only ≥2 log reduction was observed in both foods in this study. EtOH effectively reduced the infectivity of SARS-CoV-2 contaminating food, albeit not completely (Fig. 4). EtOH exposure for 1 min reduced the SARS-CoV-2 strains on lettuce by 1.96 and 2.45 log $TCID_{50}$/mL at 30 and 50%, respectively, and achieved a ≥3 log reduction at 70%. On chicken, the SARS-CoV-2 strains were reduced by only 0.63 and 0.58 log $TCID_{50}$/mL at 30 and 50%, respectively, and did not achieve a 2 log reduction even at 70%. When treated for 5 min, SARS-CoV-2 on lettuce was

reduced by more than 3 log $TCID_{50}$/mL at 50%; nonetheless, SARS-CoV-2 L was not completely inactivated even at 70%, similar to the results for 1 min exposure. Although the amount of infectious SARS-CoV-2 was reduced compared with that for the 1 min exposure, it was still insufficient to reduce SARS-CoV-2 contaminating chicken and salmon. SARS-CoV-2 was reduced by 1.04 and 1.54 log $TCID_{50}$/mL on chicken and 1.13 and 1.38 log $TCID_{50}$/mL on salmon at 30 and 50% concentrations, respectively. Treatment with 70% EtOH for 5 min reduced SARS-CoV-2 by >2 logs on both foods, SARS-CoV-2 S on salmon was completely inactivated, but there was no difference between the strains.

ClO_2 has a weaker virucidal effect on SARS-CoV-2 contaminating food than EtOH and did not achieve the standard effect on any food matrix under all exposure time conditions (Fig. 5). Although the virucidal effect on SARS-CoV2 contaminating lettuce was superior to that of other foods, it was reduced by only 2.25 log $TCID_{50}$/mL even at 80 ppm and a 5 min exposure time. Exposure for 1 min showed only a 0.67–1.25 log reduction on chicken and salmon, and the effect was not satisfactory, with a 1.00–1.33 log reduction in 5 min of exposure. Moreover, the virucidal effect of ClO_2 did not appear to be concentration or exposure time dependent, and there was no significant difference between the two virus strains.

SARS-CoV-2 contaminating food matrices was sensitive to PAA treatment (Fig. 6). The virucidal effect of PAA against SARS-CoV-2 contaminating lettuce was significantly reduced by 2.04 and 2.46 logs at 40 and 80 ppm PAA, respectively (p <0.05), although it did not achieve the standard effect. Treatment with 160 ppm PAA for 1 min completely inactivated SARS-CoV-2 L, although SARS-CoV-2 S remained slightly infectivity (0.58 log $TCID_{50}$/ml). When lettuce was exposed for 5 min, it showed an approximately 3 log reduction from 80 ppm, and both SARS-CoV-2 strains were completely reduced at 160 ppm. On chicken and

salmon, which were minimally affected by EtOH and ClO_2, infectious SARS-CoV-2 was no longer detected when PAA was applied at 4,000 ppm for 1 min. Exposure for 5 min reduced SARS-CoV-2 above the standard effect (≥ 2 log reduction) from 2,000 ppm. As with other foods, there were no differences between SARS-CoV-2 strains.

Discussion

SARS-CoV-2 is a respiratory virus that infects the upper respiratory tract and causes respiratory symptoms, such as coughing and sore throat, and has received little attention in terms of food or oral transmission (WHO, 2020). The viability of SARS-CoV-2 was dependent on storage temperature and the contaminated food. At room temperature, SARS-CoV-2 was short-lived on all tested foods, particularly lettuce and chicken. The SARS-CoV-2 contaminated food samples were stored under 40% relative humidity, but the food gradually dried over time. The viability of SARS-CoV-2 may be related to dried food matrices. SARS-CoV-2 is generally less viable on porous than non-porous surfaces (Bueckert et al., 2020). As the food dries, the surface becomes rough, and tends to become more porous (Xiao and Gao, 2012). In particular, SARS-CoV-2 viability was rapidly reduced in cellulose based material, consistent with other enveloped viruses (Bueckert et al., 2020; Chin et al., 2020; Grinchuk et al., 2021; Van Doremalen et al., 2020). SARS-CoV-2 viability may also be associated with the wettability of surfaces. SARS-CoV-2 survives longer on hydrophobic surfaces, such as plastics and glass, than on hydrophilic surfaces, such as stainless steel (Bueckert et al., 2020; Grinchuk et al., 2021; Van Doremalen et al., 2020). In addition, the high water activity in food makes the virus more sensitive to heat (Bosch et al., 2018). Moreover, contrary to expectations that the viability of the two SARS-CoV-2 strains would be similar, the difference was significant. Genetically, SARS-CoV-2 S is more ancient than SARS-CoV-2 L,

272 but the initial outbreak was responsible for SARS-CoV-2 L, and SARS-CoV-2 S spread more
273 slowly (Awadasseid et al., 2021; Tang et al., 2020). This may have made it easier for SARS-
274 CoV-2 L to spread to humans from contaminated food as it could survive longer on food.
275 The persistence of SARS-CoV-2 on food matrices was inversely proportional to storage
276 temperature. In the present study, the half-life of SARS-CoV-2 increased 8.9 and 37.2 fold at
277 refrigeration and freezing temperatures, respectively, compared with that at room
278 temperature, depending on the surface. Chin et al. (2020) conducted a study on the stability of
279 SARS-CoV-2 suspensions at different temperatures. They reported a 0.7 log decrease at 4 °C
280 for 14 days; however, the decrease became faster with increasing temperature, maintaining
281 stability for 7 d at 22 °C and 2 d at 37 °C. In previous studies by Biryukov et al. evaluating
282 the viability of SARS-CoV-2 on stainless steel, SARS-CoV-2 was inactivated significantly
283 faster at 35 °C than at 24 °C (Biryukov et al., 2021; Biryukov et al., 2020). In fact, the
284 correlation between temperature and virus viability is not limited to SARS-CoV-2. For
285 instance, in the coronavirus family, which includes animal and human coronavirus, increases
286 in temperature and the time it takes for decimal reduction (1 log reduction in virus infectivity;
287 D-value) are linearly inversely proportional (Guillier et al., 2020). On the other hand, Kratzel,
288 Steiner, et al. (2020) reported that there was no significant difference in the stability of
289 SARS-CoV-2 on a metal surface at 30 ℃, room temperature, and at 4 ℃. It should be noted
290 that the aforementioned experiments were all conducted at above 0 °C temperatures. In the
291 present study, SARS-CoV-2 persisted for longer in the order of salmon > chicken > lettuce,
292 which seems to be related to food constituents. Although there have been no direct studies on
293 the effect of food constituents on SARS-CoV-2 stability, it can be partially explained by
294 previous studies. The protein and fat content of food increases the stability of viruses under
295 heat treatment. For example, the D-value of a SARS-CoV suspension increased from 1.9 to

296 5.0 when protein (20% FBS) was added (Rabenau et al., 2005). Moreover, the D-value of the
297 hepatitis A virus in milk composed of 1 and 18% fat doubled from 1.6 to 3.1 (Bozkurt et al.,
298 2015). The protein contents of lettuce, chicken breast, and salmon are 1.4, 31, and 20 g per
299 100 g, respectively, and the fat contents are 0.2, 3.6, and 13 g, respectively. Although we did
300 not heat treat our food samples, the present study results suggest that protein and fat content
301 is associated with virus stability. In particular, it seems that fat content affects the stability of
302 SARS-CoV-2 surrounded by a lipid envelope more than protein content
303 PAA inactivated SARS-CoV-2 stains effectively on any of three food matrices. Although
304 its mechanism for inactivating bacteria or viruses is not entirely understood, PAA is a highly
305 effective biocide used in various fields, including wastewater and clinical and food industries
306 (Rutala and Weber, 2008). PAA is a potent oxidizing agent speculated to denature proteins,
307 disrupt cell wall permeability, and oxidize sulfhydryl and sulfur bonds of proteins, enzymes,
308 and other metabolites (Finnegan et al., 2010). The Korean Food and Drug Administration
309 recommends using PAA at 2,000 ppm for poultry and 80 ppm for fruits and vegetables
310 (MFDS, 2019), while the FDA recommends using 350, 2,000, and 230 ppm of PAA in fruits
311 and vegetables, poultry, and fish, respectively (FDA, 2017). Since no recommendations have
312 been made for salmon by The Korean Food and Drug Administration, the recommendations
313 for poultry were applied for salmon in the present study. PAA inactivated most SARS-CoV-2
314 when applied at the recommended concentration for 5 min; however, the recommended
315 concentration was insufficient to completely inactivate the virus. Given the potential for
316 SARS-CoV-2 transmission by imported salmon, it may be necessary to consider adjusting the
317 recommended PAA concentrations for fish. Overall, PAA is a suitable disinfectant for food
318 safety as it inactivates gram-positive and gram-negative bacteria, molds, and yeasts in less
319 than 5 min at <100 ppm and is also effective against non-enveloped viruses, such as

poliovirus and adenovirus, at > 400 ppm (Becker et al., 2017; Rutala and Weber, 2015).

Although not recommended for direct use in food, ethanol effectively removed SARS-CoV-2 contaminating food. Ethanol inactivates bacteria by coagulating or denaturing proteins and enzymes in the cell wall, cell membrane, and cytoplasm of bacteria (Huffer et al., 2011). The action of ethanol in enveloped viruses is not clearly understood, but it is assumed to be similar to that in bacteria and is generally effective (Dev Kumar et al., 2020). Alcohol-based disinfectants were effective against SARS-CoV-2 in suspension as it was completely inactivated with only 30% ethanol and 2-isopropanol applied for 30 s (Kratzel, Todt, et al., 2020). In the present study, virus-contaminated lettuce was effectively treated in 70% ethanol for 1 min, but for chicken and salmon, this treatment was insufficient. The virucidal effect of ethanol was lower in food with higher protein content, suggesting that the protein denaturation and coagulation action of ethanol may be competitive between the virus and food matrix.

Chlorine-based disinfectants, especially sodium hypochlorite (NaClO), are most widely used on water and food contact surfaces. However, the effect of NaClO on coronavirus is ambiguous. Exposure to 600 ppm NaClO for 1 min reduced the infectivity of animal coronavirus by less than 1 log, significantly lower than that of 70% ethanol, which reduced infectivity by > 3 logs (Hulkower et al., 2011). Although there are differences depending on the type of coronavirus, NaClO concentrations above 1,000 ppm were required to observe a 3 log reduction in infectivity, which is too high for food applications (Kampf et al., 2020). ClO_2 is a chlorine-based disinfectant that acts as an oxidizing agent similar to NaClO; however, ClO_2 has better oxidation ability because it can accept five electrons, whereas NaClO can only accept two electrons (Warf, 2019). Moreover, the solubility of chlorine per molar weight in ClO_2 is 263%, which is remarkably higher than that in NaClO at 95.2%. Therefore, the

present study attempted to remove SARS-CoV-2 contaminating food using ClO_2, which was expected to be more effective. However, even under conditions of the highest concentration and exposure time used in the experiment, SARS-CoV-2 decreased by only 2 logs on lettuce and 1 log on chicken and salmon. Wang et al. (2005) reported that SARS-CoV contaminating wastewater was completely inactivated by treatment with 40 ppm ClO_2 for 5 min. It seems that the action of ClO_2 was also affected by food constituents similar to the EtOH treatment. To reuse processed water for fresh products, it is necessary to prevent cross contamination of pathogens that can threaten food safety and remove organic compounds that encourage microorganism growth (Meneses et al., 2017). However, these organic compounds rapidly deplete free chlorine, inducing a high chlorine demand (CLD). According to a study by Teng et al. (2018), the factors that increased CLD in cabbage wash water included proteins, phenols, organic acids, and sugars, and among them, protein contributed the most to the increase in CLD. Similarly, chicken and salmon are high in protein, which may have increased the chlorine demand to inactivate the SARS-CoV-2.

The results of this study clearly show how well SARS-CoV-2 can survive on different foods. However, it is still unknown whether infection is caused by ingesting food contaminated with SARS-CoV-2. In addition, although SARS-CoV-2 can survive for a long time at low pH, which has been well documented in intestinal infections, there is no direct study on whether it can tolerate the intestinal environment (e.g., bile salt and various digestive enzymes). Therefore, future studies should evaluate the viability of SARS-CoV-2 in a mimicked intestinal environment. In addition, this study presented methods to effectively remove SARS-CoV-2 contaminating food; however, this should be sufficiently studied using food applicable disinfectants, such as ozonated water.

Conclusions

While the viability of SARS-CoV-2 can be maintained in refrigeration and freezing condition, SARS-CoV-2 was rapidly inactivated in food matrices at room temperature. Therefore, the potential for food-borne transmission of SARS-CoV-2 appears to be low, consistent with the FDA opinion. Among the variant strains, SARS-CoV-2 L survived more than SARS-CoV-2 S on the food matrices. When PAA is used two times higher than recommended concentration, SARS-CoV-2 L and S strain can be completely inactivated. Treating food with such an effective disinfectant minimizes the threat of SARS-CoV-2 even in group meals, such as in schools and medical facilities. However, chlorine-based disinfectants are insufficient to inactivate SARS-CoV-2 on food matrices.

Declarations of interest

None

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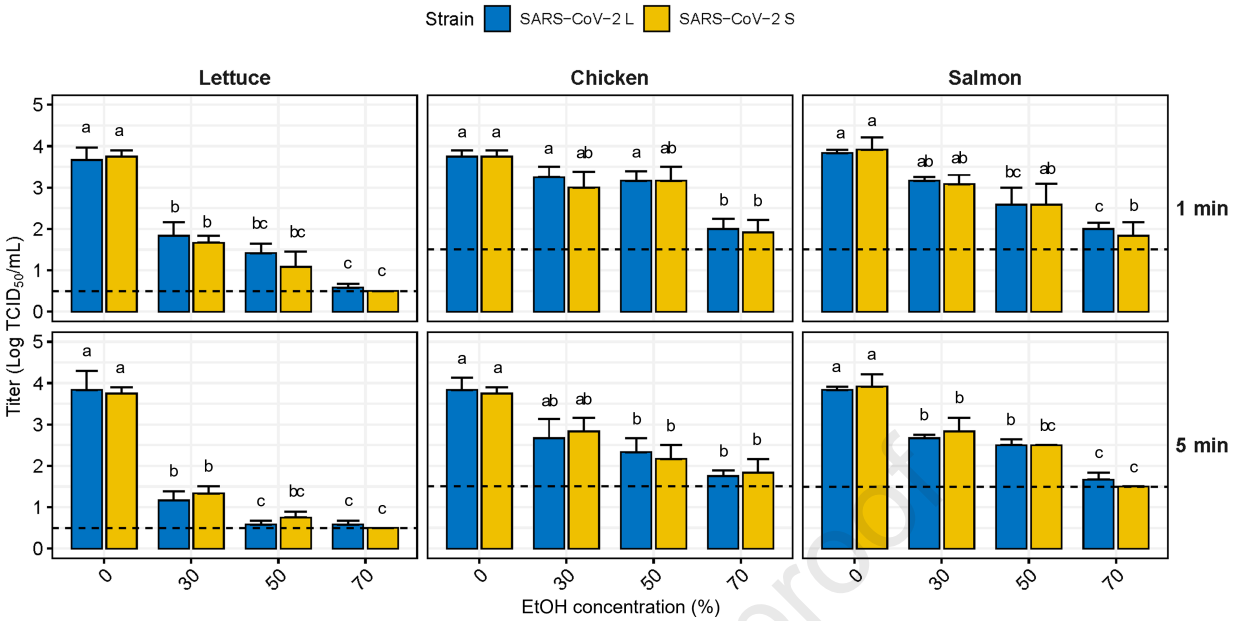
570 **Fig. 1 Viability of SARS-CoV-2 on food matrices at 20 °C.** (A) Virus titer recovered from
571 the surface by timepoint. (B) Bayesian regression plots showing the predicted decay of virus
572 titers over time. The dots are slightly jittered to avoid overlapping. Lines show exponential
573 decay rates and were randomly drawn at 150 per panel from the joint posterior distribution.
574 (C) Violin plot representing the half-life of viruses. The dots represent the median estimates,
575 and the lines are the 95% confidence intervals. The dashed lines in (A) and (B) indicate the
576 limit of detection. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p <$
577 0.005)
578
579 **Fig. 2 Viability of SARS-CoV-2 on food matrices at 4 °C.** (A) Virus titers recovered from
580 the surface by timepoint. (B) Bayesian regression plots showing the predicted decay of virus
581 titers over time. The dots are slightly jittered to avoid overlapping. Lines show exponential
582 decay rates and were randomly drawn at 150 per panel from the joint posterior distribution.
583 (C) Violin plot representing the half-life of viruses. The dots represent the median estimates,
584 and the lines are the 95% confidence intervals. The dashed lines in (A) and (B) indicate the
585 limit of detection. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p <$
586 0.005)
587
588 **Fig. 3. Viability of SARS-CoV-2 on food matrices at −40 °C.** (A) Virus titers recovered
589 from the surface by timepoint. (B) Bayesian regression plots showing the predicted decay of
590 virus titers over time. The dots are slightly jittered to avoid overlapping. Lines show
591 exponential decay rates and were randomly drawn at 150 per panel from the joint posterior
592 distribution. (C) Violin plot representing the half-life of viruses. The dots represent the
593 median estimates, and the lines represent the 95% confidence intervals. The dashed lines in

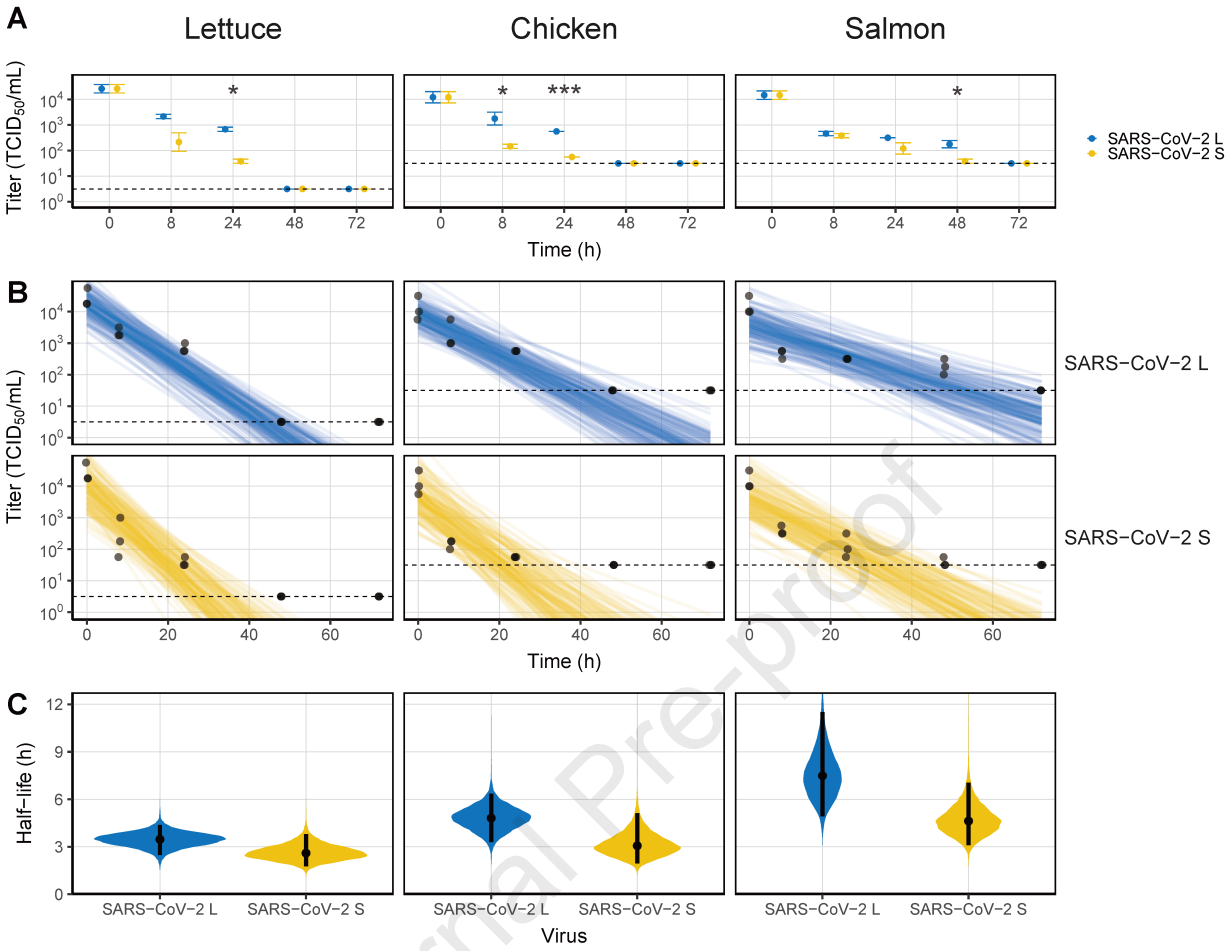
(A) and (B) indicate the limit of detection. Asterisks indicate statistical significance (*p < 0.05, **p < 0.01, ***p < 0.005).

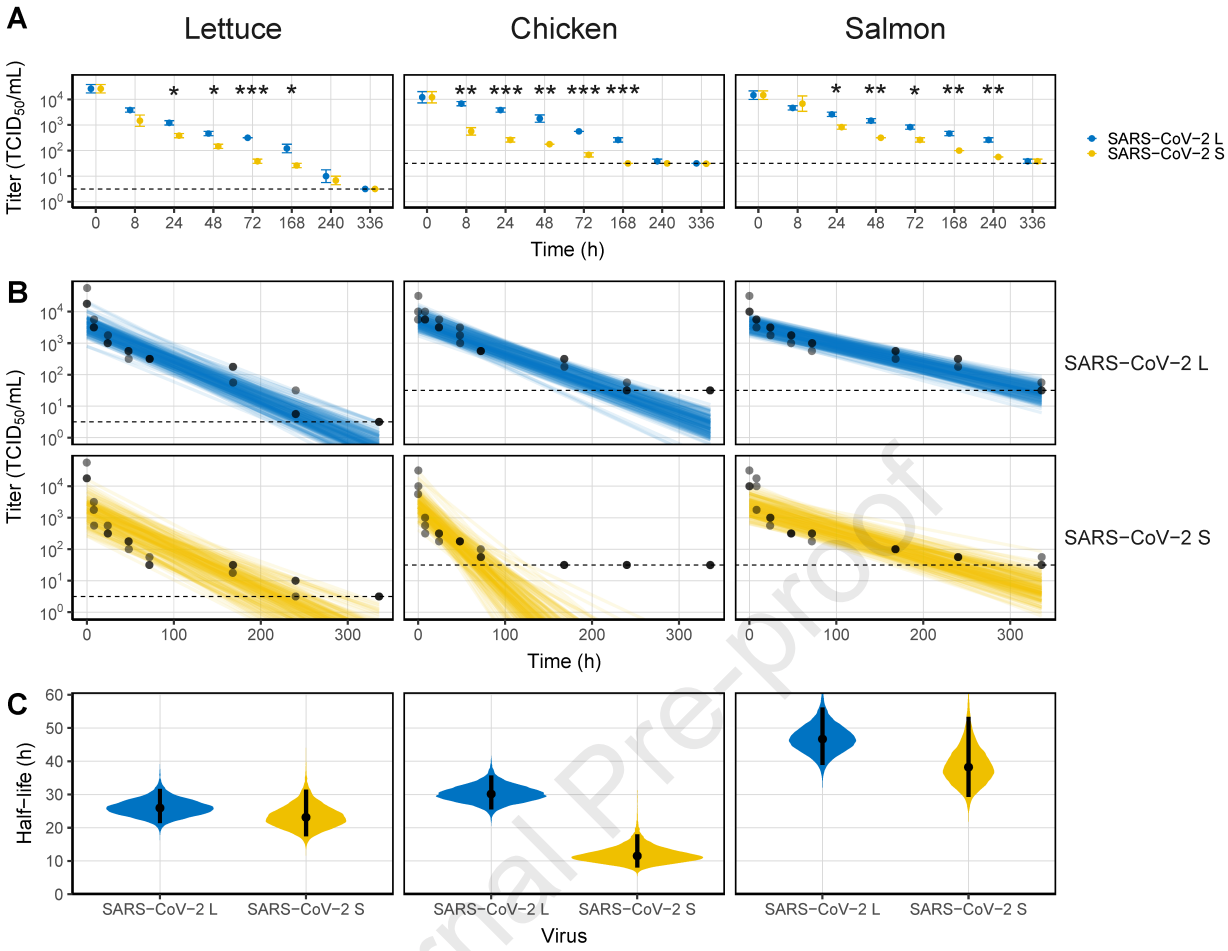
Fig. 4. Virucidal effect of ethanol against SARS-CoV-2 contaminating food matrices. The dashed line indicates the limit of detection at 0.5 $TCID_{50}$/mL for lettuce and 1.5 $TCID_{50}$/mL for chicken and salmon. Lowercase letters indicate significant difference between the disinfectant treated group and the control within the same virus strain (p <0.05). EtOH, ethanol.

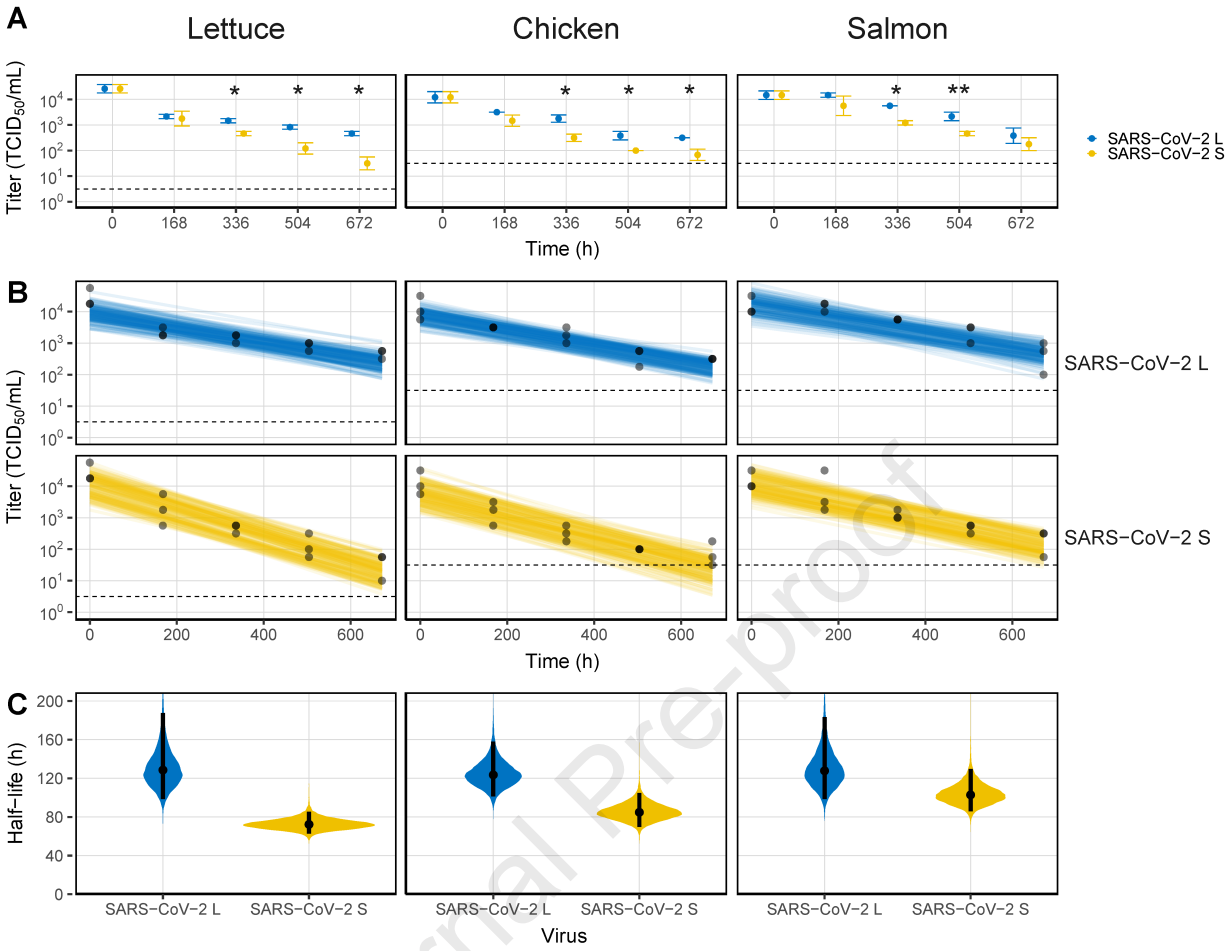
Fig. 5. Virucidal effect of sodium hypochlorite against SARS-CoV-2 contaminating food matrices. The dashed line indicates the limit of detection at 0.5 $TCID_{50}$/mL for lettuce and 1.5 $TCID_{50}$/mL for chicken and salmon. Lowercase letters indicate significant difference between the disinfectant treated group and the control within the same virus strain (p <0.05). ClO_2, Chlorine dioxide.

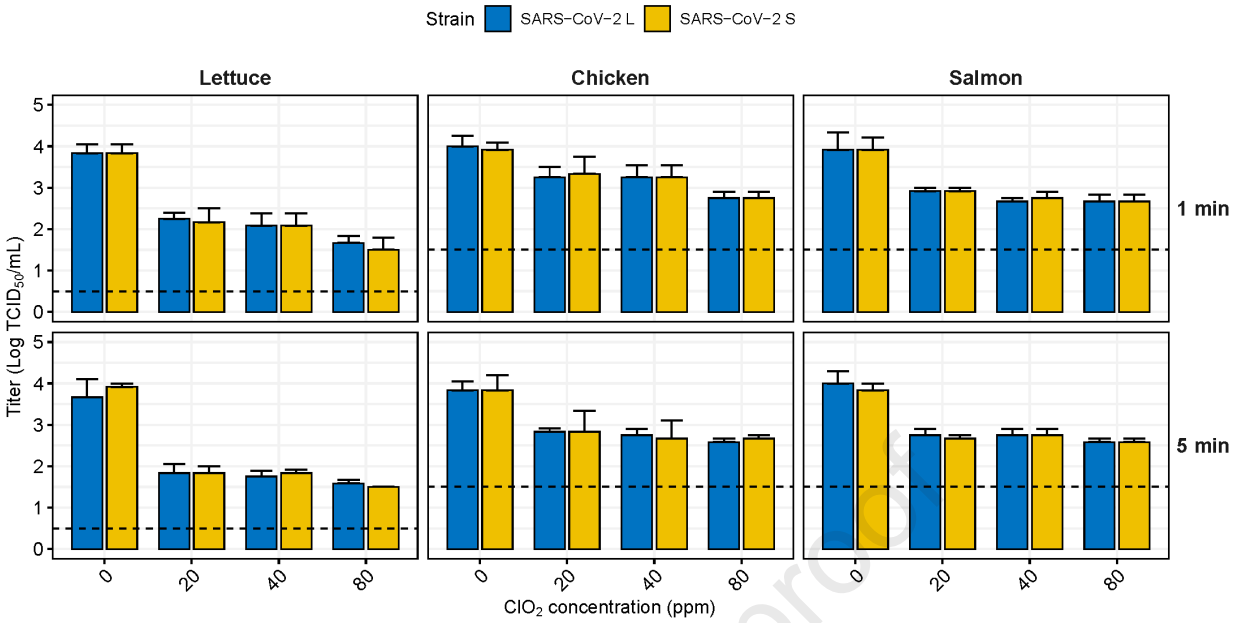
Fig. 6. Virucidal effect of peracetic acid against SARS-CoV-2 contaminating food matrices. The dashed line indicates the limit of detection at 0.5 $TCID_{50}$/mL for lettuce and 1.5 $TCID_{50}$/mL for chicken and salmon. Lowercase letters indicate significant difference between the disinfectant treated group and the control within the same virus strain (p <0.05). PAA, peracetic acid.

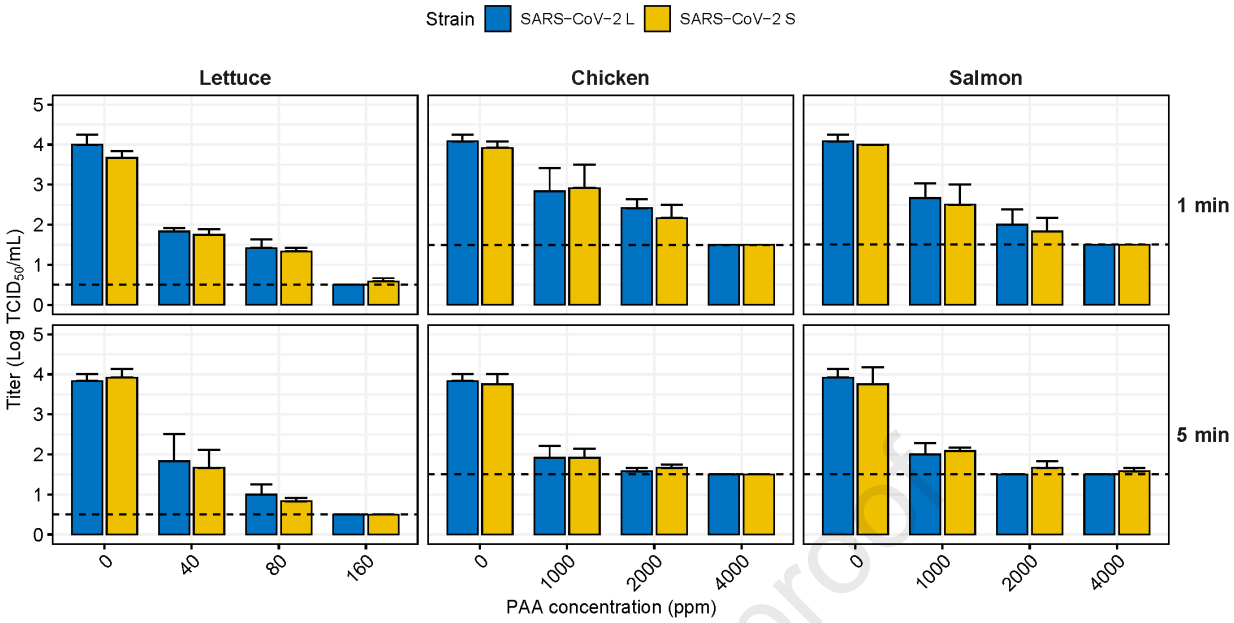












Highlights

- The viability of SARS-CoV-2 L and SARS-CoV-2 S were evaluated on food matrices
- The viability of SARS-CoV-2 at -40 °C was increases 30 times rather than at 20 °C.
- Peracetic acid at twice the recommended concentration inactivated SARS-CoV-2 on food matrices.
- Ethanol and chlorine dioxide were not effective to inactivate SARS-CoV-2 on food matrices

Conflict of Interest

Author declares no financial or personal conflict of interest with any person or organizations reported in this work.