Food Control 150 (2023) 109738

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

# Antiviral capacity of sanitizers against infectious viruses in process water from the produce industry under batch and continuous conditions

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ARTICLE INFO

*Keywords:*
Disinfection
Enteric viruses
Produce
Food safety
Chlorine
Chlorine dioxide
Peracetic acid

ABSTRACT

The presence of human enteric viruses in produce has extensively been reported. However, the significance of the quality of process water (PW) used by the produce industry and the viral inactivation capacity of water disinfection agents used to maintain the microbiological quality of PW has received limited attention. This study evaluates the antiviral disinfection efficacy of chlorine, chlorine dioxide ($ClO_2$) and peracetic acid (PAA) at recommended operational limits in PW using hepatitis A virus (HAV), the cultivable norovirus surrogate, murine norovirus (MNV-1), and MS2 coliphages. Defined commodity representative crops (baby leaves, bell peppers, and the vegetable mix of tomatoes, cucumbers, peppers, and onions) associated with specific water-based processes were studied. Two systems classified as either batch or continuous system were used. The continuous system allows the continuously entrance of sanitizer solution and organic matter added to the washing tank to simulate the conditions of an industry wash tank. Batch scale experiments showed that 20 mg/L chlorine and 3 mg/L chlorine dioxide completely inactivated MNV-1 and MS2 (mean of 5 log) after 1 min contact time regardless of the PW type. However, the infectivity of HAV was reduced only by less than 2 log after 1 min for chlorine and chlorine dioxide and the complete inactivation was not observed even after 10 min. On the contrary, residual viral infectivity/viability of HAV, MNV-1 and MS2 was observed for PAA in the three types of PW. The inactivation kinetic models for MS2 coliphages were developed based on the data obtained under the continuous system comparing the three types of PW. Chlorine (5 mg/L) and chlorine dioxide (2–3 mg/L) avoided the accumulation of MS2 below the detection limit while PAA (80 mg/L) was unable to prevent it independently of the type of PW. In summary, in the washing operation, it is a key objective to reach virus inactivation through the selection of the most effective sanitizer by guaranteeing that sufficient concentration and contact times prevent the risk of viral cross-contamination.

## 1. Introduction

Despite accounting for one of the major causes of foodborne outbreaks, human enteric viruses have received comparatively less attention than other foodborne pathogenic bacteria. Human enteric viruses are the most common etiologic agents identified in produce-associated outbreaks (54%), frequently linked with food-handling issues (Bennett et al., 2018). Fruits and vegetables are susceptible to being contaminated through contaminated soil, or contaminated irrigation waters (Ashbolt, 2015; Li et al., 2018; López-Gálvez et al., 2016, 2018; Randazzo et al., 2016; Tian et al., 2017; Truchado et al., 2021). Among others, the viruses most commonly detected in irrigation waters include human norovirus, astrovirus (HAstV), rotavirus A (RV), and hepatitis A virus (HAV).

Process water (PW) has been defined as water resulting from operations such as washing, rinsing, cooling, or transporting, in packinghouses or processing facilities, which usually accumulates organic matter including microorganisms (Suslow, 1997). Process water in the

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https://doi.org/10.1016/j.foodcont.2023.109738
Received 6 January 2023; Received in revised form 22 February 2023; Accepted 11 March 2023
Available online 13 March 2023
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fruit and vegetable sector is highly variable in terms of quality parameters, such as dissolved and suspended solids, organic matter content, and microbiological quality. Physicochemical parameters, together with classical microbial indicators, such as fecal indicator bacteria, including fecal coliforms, *Escherichia coli*, and enterococci have been widely used to assess the quality of PW used in different postharvest operations. However, studies investigating the presence of human enteric viruses or viral indicators are limited (Cuevas-Ferrando et al., 2021; Dunkin et al., 2017; López-Gálvez et al., 2016; Moreno et al., 2015; Ortiz-Solà et al., 2020). Sanitizers are considered processing aids regulated by the EU (Regulation (EC) No 1333/2008). Their harmonization by the EU should be approved according to Regulation (EU) No 528/2012. Different factors affect the antimicrobial efficacy of sanitizers, including a) pathogen type, population size, and physiological status; b) antimicrobial type, concentration, and activity; and c) the physicochemical characteristics of the water, particularly the organic matter (Gombas et al., 2017). The occurrence of potentially infectious enteric viruses in PW used by the produce industry is possible and thus, the efficacy of sanitizers at concentrations recommended for bacteria inactivation needs to be closely examined and confirmed. Sodium hypochlorite (chlorine), chlorine dioxide ($ClO_2$) and peracetic acid (PAA) are the sanitizers most commonly applied by the produce industry to maintain the microbiological quality of PW (Gil et al., 2009). The operational limits have been already established based on pathogen inactivation and results published showed that maintaining a concentration >3 mg/L for chlorine, 2–3 mg/L for chlorine dioxide and 80 mg/L for PAA reduced microbial population in wash water (Gómez-López et al., 2014; Banach et al., 2021; López-Gálvez et al., 2018). This study aims at evaluating the efficacy of current water disinfection practices to minimize viral cross-contamination. Thus, we investigated viral infectivity/viability of viruses (murine norovirus, MNV-1 and HAV) and MS2 coliphages in response to chlorine, chlorine dioxide and PAA at established operational limits for the disinfection of PW from washing baby leaves, peppers and a vegetable mix of tomatoes, cucumbers, peppers, and onions. To evaluate the inactivation rates of viruses exposed to sanitizers in PW, our approach included batch scale and continuous system experiments (Fig. 1). Data were used to developing predictive viral inactivation models for understanding the impacts of water quality on disinfection efficacy.

## 2. Material & methods

### 2.1. Viruses, phages, cells and bacteria

The cytopathogenic MNV-1 strain, (kindly provided by Prof. H.W. Virgin, Washington University School of Medicine, USA) and HAV, HM-175/18f strain, (ATCC VR-1402) were propagated and assayed in RAW 264.7 (ATCC TIB-71) and FRhK-4 cells (ATCC CRL-1688), respectively. Cell lines were grown according ATCC recommendations. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$/mL) in 96-well microtiter plates with eight wells per dilution and 20 μL of inoculum per well using the Spearman-Karber method (Falcó et al., 2018). Wild-type MS2 bacteriophage DSM 13767 was obtained from the German Collection of Microorganisms and Cell Cultures and enumerated using the host strain *Escherichia coli* CECT 9198 and the double layer agar method (Adams, 1959).

### 2.2. Physicochemical characteristics of process water

Three types of PW from processing lines of baby leaves, peppers and the vegetable mix were collected 4–5 h after the production started and transported to the lab in less than 45 min. The pH, oxidation reduction potential (ORP) and electric conductivity (EC, μS/cm) were measured using a pH and redox multimeter probe (Crison, Barcelona, Spain). Organic matter was measured as chemical oxygen demand (COD) determined by the standard photometric method (APHA, 1998; Shin et al., 2019; Zhang et al., 2010) using the Spectroquant NOVA 60 photometer. Turbidity was measured using the Turbiquant 3000R turbidimeter (Merck, Madrid, Spain).

### 2.3. Evaluation of the inactivation efficacy of sanitizers using the batch system

Batch inactivation experiments were conducted at 4 °C in sterile free chlorine demand 250-mL beakers containing an initial volume of 200 mL of PW (Fig. 1). The physicochemical characteristics of PW of baby leaves, bell peppers and the vegetable mix used in the batch trials are shown in Table 1. Two mL of MNV-1, HAV (about ca. 5–6 log $TCID_{50}$/mL) and MS2 phage suspension (about ca. 8–9 log plaque-forming units

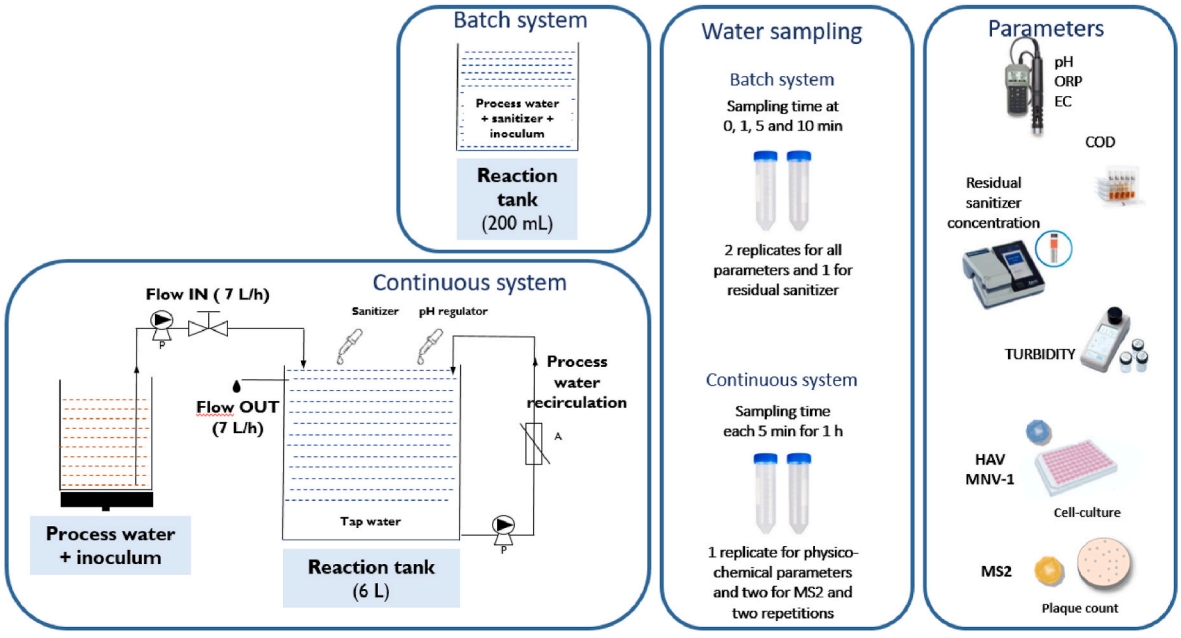


**Fig. 1.** Schematic representation of the batch and continuous systems used for the inactivation studies of viruses exposed to sanitizers in process water. Water sampling, the number of replicates, repetitions and the parameters measured are described.

**Table 1**
Physiochemical characterization of process water of baby leaves, bell peppers and the vegetable mix used in the batch system trials.

| Process water | pH | ORP | EC | COD | Turbidity |
|---|---|---|---|---|---|
| Baby leaves | 8.1 ± 0.3 | 743 ± 15 | 1110 ± 286 | 23.4 ± 7.9 | 19 ± 7 |
| Bell peppers | 3.8 ± 0.2 | 410 ± 39 | 765 ± 54 | 1496 ± 437 | 112 ± 67 |
| Vegetable mix | 7.3 ± 0.3 | 218 ± 51 | 879 ± 155 | 166 ± 16.7 | 19 ± 2 |

ORP, oxidation-reduction potential (mV); EC, electric conductivity (μS/cm); COD, chemical oxygen demand (mg/L); Turbidity (NTU, nephelometric turbidity unit). The values are the mean ± the standard deviation of three repetitions.

or PFU/mL) were added to 200 mL of PW. For each sanitizer, the volume needed to reach an initial concentration of about 10–20 mg/L chlorine, 2 mg/L chlorine dioxide and 80 mg/L for PAA was added. Sanitizer concentrations were determined by using a Kemio™ instrument (Palintest, Gateshead, UK), that uses a chronoamperometry detector and the Kemio™ sensors to measure free chlorine, chlorine dioxide or PAA in PW samples. Beakers were continuously mixed throughout the experiment. Changes in the infectivity/viability of viruses and MS2 coliphages were measured at different time intervals (0, 1, 5 and 10 min). For the inactivation experiments, samples of 1 mL were taken from the treated PW and transferred into tubes containing 9 mL of a neutralizing solution (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum) for viruses, increasing the limit of detection. For MS2, sodium thiosulphate was added for quenching the residual concentration of chlorine and chlorine dioxide, combined with catalase for PAA. The amount of sodium thiosulphate (0.5 M) needed to neutralize chlorine (20 mg/L) was 1 μL/mL, for chlorine dioxide (3 mg/L) was 0.05 μL/mL and for PAA (80 mg/mL) was 2.6 μL/mL. The catalase (2000 U/mg) added for PAA (80 mg/L) was 0.8 μL/mL. Then, samples were ten-fold diluted in PBS and titrated by cell culture using RAW 264.7 and FRhk-4 cells for MNV-1, and HAV, respectively. The MS2 coliphages were enumerated using the host strain *E. coli* CECT9198 and the double layer agar method (Guzmán et al., 2008). To avoid contamination with the PW microbiota, PW samples were decontaminated by sequential filtering through 0.45 and then 0.22 μm (Spin Centrifuge Tube Filters, Corning, Thermo Fisher Scientific Inc., Madrid, Spain). Each sample was evaluated in triplicate and viral inactivation was calculated as $\log_{10}(N_x/N_0)$, where $N_0$ is the initial infectious virus titer in untreated samples and $N_x$ is the infectious virus titer for sanitizer-treated samples at each time point.

### 2.4. Inactivation experiments in a continuous system

The inactivation experiments using the continuous system were repeated twice with PW obtained on different days from processing facilities of baby leaves, peppers and vegetable mix. The continuous system is used as it represents a better approach to the conditions that happen in a commercial washing tank where the organic matter with the virus inoculum is continually added as well as the sanitizer, assuring the mix at any point in the reaction tank because of the continuous recirculation of the process water (Fig. 1) (Gómez-López et al., 2014). Each PW was inoculated with MS2 at 5–6 log PFU per liter. The inactivation experiments were carried out in a cold room at 5 °C and the process water was stored at 5 °C. Chlorine (5 mg/L), chlorine dioxide (2–3 mg/L) and PAA (80 mg/L) were continuously added to maintain the target concentration while the organic matter and the inoculum were progressively accumulated. In the case of chlorine, the minimum operation limits tested under batch experiments were used to reduce as much as possible the sanitizer needed. Samples were taken every 5 min for 1 h and physicochemical parameters were measured as described above. The accumulation of COD and the changes in EC are shown in Fig. 2 while the mean values of pH and ORP are presented in Table 2. The specific measures taken every 5 min for 1 h are included in Tables S1, S2 and S3 for baby leaves, bell peppers and the vegetable mix, respectively. The inactivation rate was measured after samples were concentrated using polyethylene glycol precipitation (30% v:v) overnight at 4 °C. The final concentrate for MS2 was enumerated as described above.

### 2.5. Models development for the continuous inactivation experiments

Modeling studies were performed using the continuous system to characterize the inactivation kinetics and describe mechanistically the inactivation performance of chlorine, chlorine dioxide and PAA. In the

**Table 2**
Range of pH and oxidation-reduction potential (mV) in the process water of baby leaves, bell peppers, and the vegetable mix in the continuous system without sanitizer (Control) and after adding chlorine (5 mg/L), chlorine dioxide ($ClO_2$) (2–3 mg/L) and peracetic acid (PAA) (80 mg/L). Parameters were measured in 1 replicate each 5 min for 1 h with two repetitions.

| Process water | Sanitizer | pH | ORP |
|---|---|---|---|
| Baby leaves | Control | 8.2–8.3 | 321–623 |
| | Chlorine | 5.3–5.7 | 780–841 |
| | Chlorine dioxide | 7.7–7.9 | 749–775 |
| | PAA | 5.8–6.2 | 324–332 |
| Bell peppers | Control | 7.4–7.8 | 195–509 |
| | Chlorine | 5.0–5.8 | 721–834 |
| | Chlorine dioxide | 6.8–7.6 | 672–771 |
| | PAA | 4.5–5.9 | 311–365 |
| Vegetable mix | Control | 7.4–7.7 | 201–286 |
| | Chlorine | 5.5–5.7 | 812–846 |
| | Chlorine dioxide | 7.1–7.7 | 704–769 |
| | PAA | 5.0–6.1 | 317–350 |

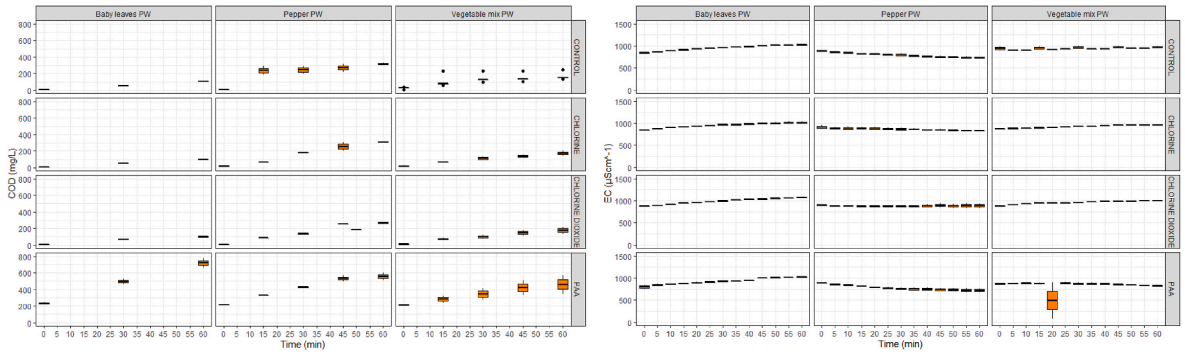


**Fig. 2.** Changes in chemical oxygen demand (COD) and electrical conductivity (EC) in the process water of baby leaves, bell peppers and the vegetable mix without sanitizer (Control) and after adding chlorine (5 mg/L), chlorine dioxide (2–3 mg/L) and peracetic acid (PAA) (80 mg/L). Boxplots show median prevalence with the 25th and 75th percentile values of one replicate per sampling point every 5 min for 1 h. The trial was repeated twice and error bars indicate the SD of two repetitions.

case of PW treated with chlorine and chlorine dioxide as well as the control conditions without sanitizers, data were adjusted using Chick (first order reaction) model, while for PW treated with PAA, the Chick model was modified to include the loss of effectiveness when COD concentration increased.

### 2.6. Statistical analysis

The statistical analysis was carried out by the post-hoc Tukey's method ($p < 0.05$) to determine the difference among sanitizers. The Student's t-test was used to compare average values of sanitizers alone and combined with a continuous system. Statistic software version 10 (StatSoft Inc., Tulsa, OK, USA) was used for statistical analyses.

## 3. Results

### 3.1. Inactivation efficacy of sanitizers in batch scale trials

Results showed the complete inactivation (below limit of detection) of MNV-1 (mean of 4 log reduction) and MS2 (mean of 5 log reduction) after 1 min contact with chlorine (10–20 mg/L) in all PWs except in pepper PW for MS2 which needed a contact time of 5 min to achieve its complete inactivation (Fig. 3). For HAV, the infectivity was reduced by less than 2 log after 1 min, and the complete inactivation (below limit of detection) was not observed even after 10 min (Fig. 3). Chlorine dioxide (2–3 mg/L) was very effective in reducing MNV-1 infectivity and MS2 viability below the limit of detection (Fig. 4). Results indicated that after 1 min contact time with chlorine dioxide (2–3 mg/L), 4 log reduction of MNV-1 and MS2 was achieved in PW of baby leaves, pepper, and vegetable mix and slightly less (3 log reduction) for MNV-1 in PW of baby leaves. When PWs were treated with PAA, the infectivity/viability of HAV, MNV-1 and MS2 was hardly reduced in any PW except 2.3 log reduction observed for MNV-1 titers after 1 min in PW of the vegetable mix (Fig. 5).

### 3.2. Inactivation efficacy of sanitizers in the continuous system trials

To understand the impact of the type of PW on the disinfection efficacy of MS2 coliphages, the inactivation kinetics of the sanitizers were determined and compared without sanitizer. Data of the accumulation of MS2 coliphages in the PWs and the inactivation with chlorine and chlorine dioxide were adjusted using Chick (first order reaction) model, defined by the equation:

$$\frac{dN}{dt} = \frac{F}{V}\left(N^I - N\right) - kNC$$

where N is the virus concentration in the tank, F is the flux incoming in the tank (and equal to the flux outcoming due to overflow), V is the tank volume, C is the sanitizer concentration, $N^I$ is the concentration of virus in the incoming PW into the tank and k is the disinfection rate constant (Fig. 1). Note that the Chick model is the simplest continuous model to describe inactivation dynamics, and is the preferred choice attending to the parsimony principle (García & Cabo, 2018; Gyürék & Finch, 1998).

For PW disinfected with PAA, the previous equation was modified to include the loss of efficacy when COD concentration increased. This factor was included in the last term of the equation, where the COD is stated.

$$\frac{dN}{dt} = \frac{F}{V}\left(N^I - N\right) - ke^{(-\mu COD)}NC$$

Results are shown in Fig. 6A for baby leaves, Fig. 6B for peppers and Fig. 6C for the vegetable mix. Observational data showed that when PW was disinfected with chlorine (5 mg/L) and chlorine dioxide (2–3 mg/L) the accumulation of MS2 coliphages was avoided as the levels were below the detection limit for the entire duration of the tests, independently of the type of PW. In contrast, the addition of PAA (80 mg/L) was unable to prevent the accumulation of MS2 and only a moderate effect of 2 log reduction was observed in pepper PW, as observed in the batch experiments. The lack of efficacy of PAA on viral accumulation is clearly shown in the model developed and remarks on the relevant differences between sanitizers (Fig. 6). Thus, the potential cross-contamination of viral particles in the washing tank highly depended on the type of sanitizer. When the impact of PW on the inactivation efficacy was studied, it was observed that the volume of the sanitizers to maintain the recommended residual levels varied depending on the type of PW. The consumption of chlorine and chlorine dioxide slightly differed among PW

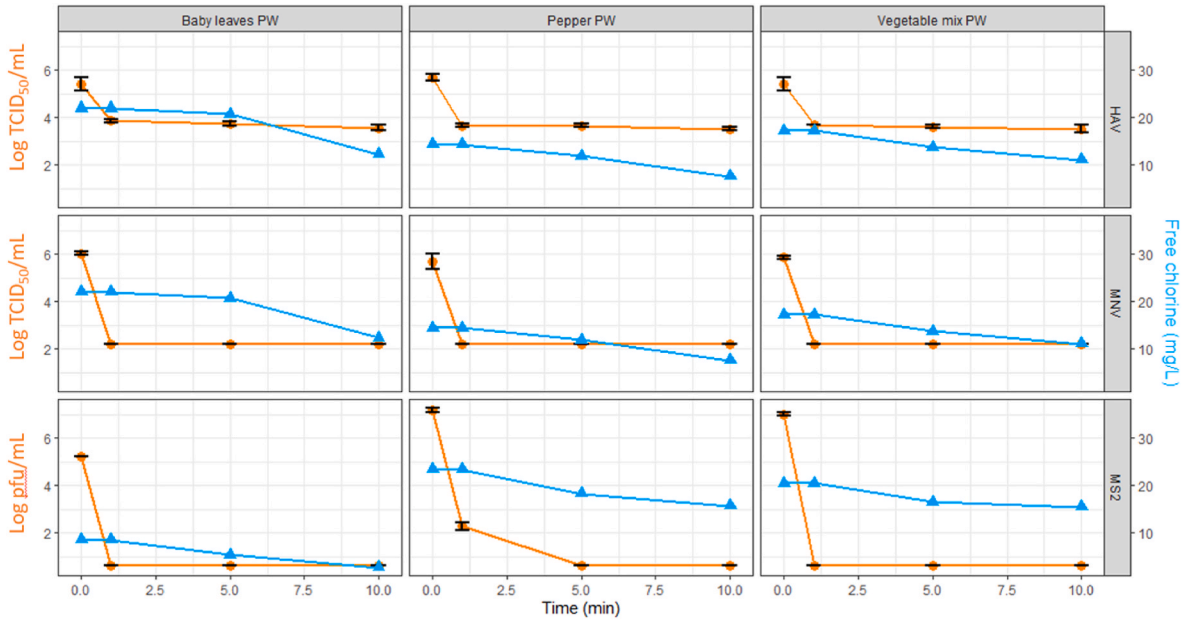


**Fig. 3.** Effect of chlorine on hepatitis A virus (HAV), murine norovirus (MNV-1), and MS2 coliphages infectivity/viability in process water of baby leaves, bell peppers, and the vegetable mix. Chlorine concentration is plotted on the right axes (blue lines), and virus level (orange lines) on the left axes. Error bars indicate the SD of triplicates per sampling time per viruses and coliphages and one replicate for monitoring chlorine.

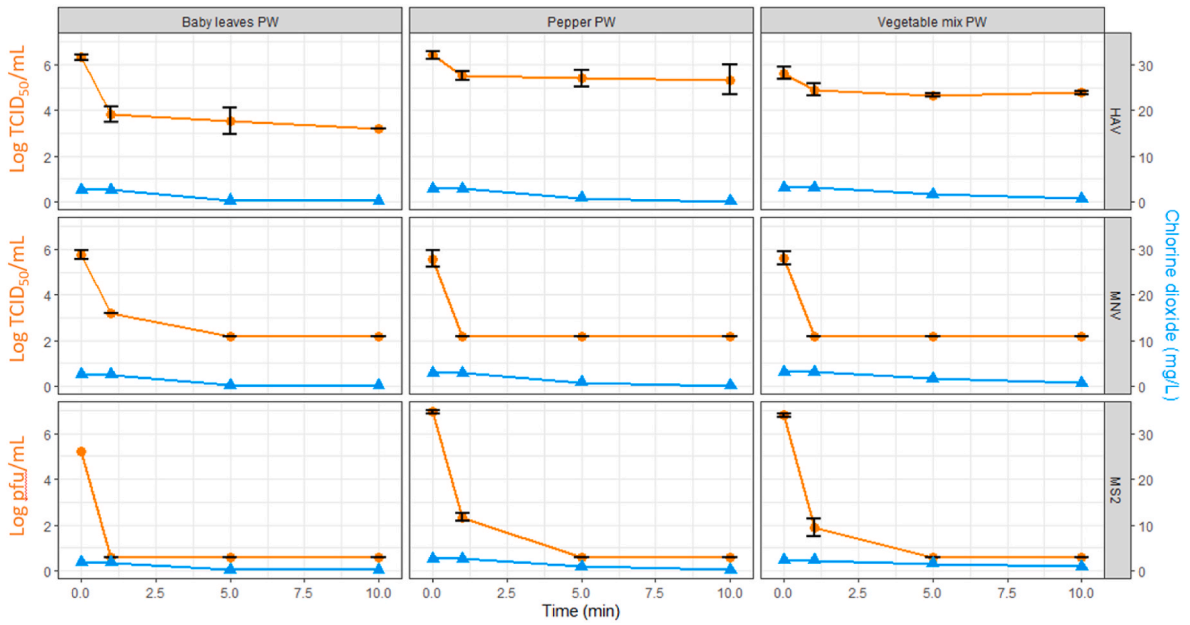


**Fig. 4.** Effect of chlorine dioxide on hepatitis A virus (HAV), murine norovirus (MNV-1), and MS2 coliphages infectivity/viability in process water of baby leaves, bell peppers, and the vegetable mix. Chlorine dioxide concentration is plotted on the right axes (blue lines), and virus level (orange lines) on the left axes. Error bars indicate the SD of triplicates per sampling time per viruses and coliphages and one replicate for monitoring chlorine dioxide.

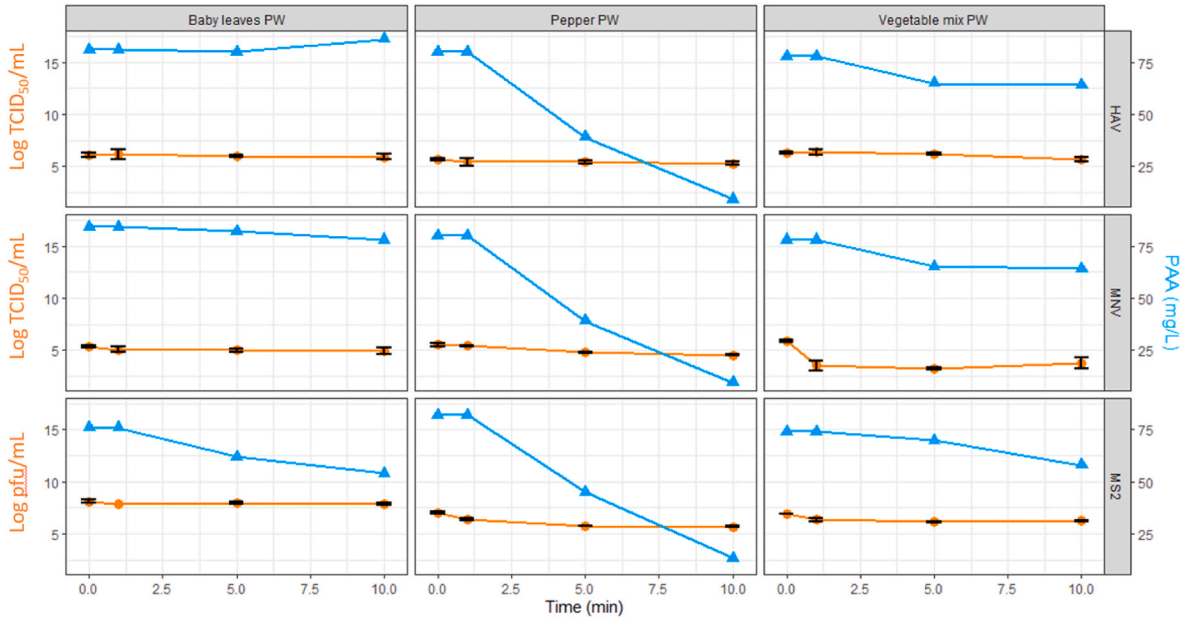


**Fig. 5.** Effect of peracetic acid (PAA) on hepatitis A virus (HAV), murine norovirus (MNV-1), and MS2 coliphages infectivity/viability in process water of baby leaves, bell peppers, and the vegetable mix. PAA concentration is plotted on the right axes (blue lines), and virus level (orange lines) on the left axes. Error bars indicate the SD of triplicates per sampling time per viruses and coliphages and one replicate for monitoring PAA.

but interestingly the volume of PAA added over the 1 h trial differed significantly depending on the type of PW (Fig. 7). Pepper PW showed the highest consumption of PAA to maintain 80 mg/L compared with the vegetable mix PW, which was higher than that of baby leaves PW. The physicochemical parameter measured that differed the most between PWs was the turbidity with 10 times higher for pepper PW than for baby leaves and the vegetable mix.

## 4. Discussion

A comprehensive understanding of virus contamination and the inactivation efficacy of sanitizers to prevent transmission from PW to the product is needed. Previous studies revealed that the relative resistance of viruses contaminating water can be modulated by water quality when treated with chemical oxidants (Dunkin et al., 2017; Kahler et al., 2010). Due to the continuous entrance of organic matter and the fluctuations in the sanitizer concentration, the quality of PW quickly changed during the washing operation. Unfortunately, water quality is often ignored in

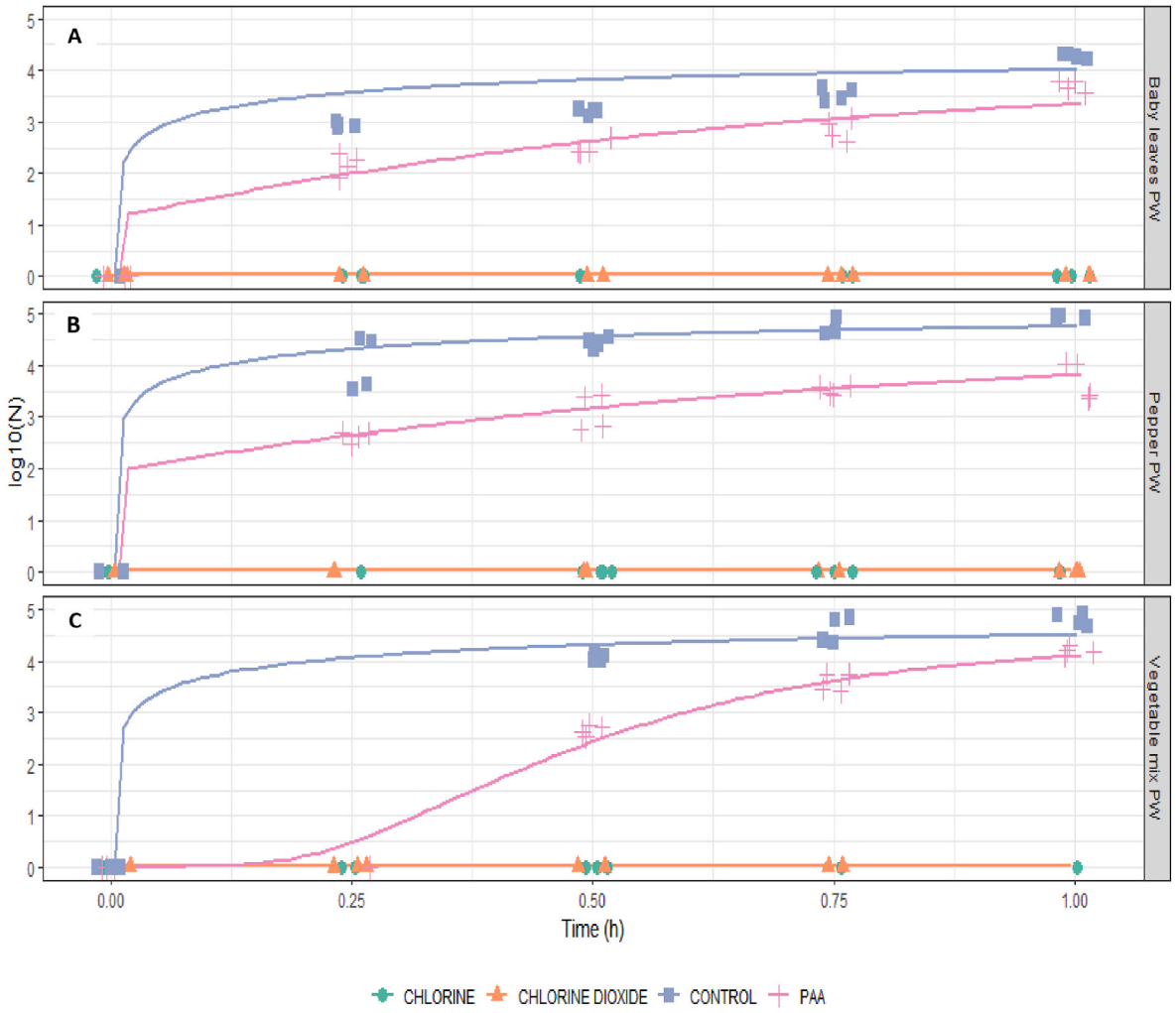


**Fig. 6.** Inactivation kinetics of MS2 coliphages in process water of baby leaves (A), peppers (B) and the vegetable mix (C). Lines represent the best fit for the model and the data (dots) the observations of chlorine (5 mg/L), chlorine dioxide (2–3 mg/L), peracetic acid (PAA) (80 mg/L), compared with process water without sanitizer (Control), when increasing the chemical oxygen demand (0–500 mg/L) over 1 h.

studies of product disinfection, and most of the published data represent standardized processes generated in controlled laboratory conditions using water models, such as buffers, water enriched in protein extracts or vegetable purees (Dunkin et al., 2017; Kahler et al., 2010). One aim of this study was to characterize the antiviral efficacy of chlorine and non-chlorinating agents (e.g., chlorine dioxide and PAA) in PW from three processing operations over time. There is only limited information on the effects of organic matter present in PW on the disinfection performance of human enteric viruses and phages (Cuevas-Ferrando et al., 2021). Some PW characteristics, e.g. the soluble matter and the suspended solids, can increase the virus resistance to sanitizers. Quantitative prevalence data are needed to determine the appropriate log reduction with PW quality to prevent cross-contamination. In wastewater, the impact of the water matrix effect on chemical disinfection kinetics has been widely recognized for PAA (Falsanisi et al., 2006; Kitis, 2004; Ragazzo et al., 2020; Zhang et al., 2020). The efficacy of PAA in reducing foodborne bacteria in PW has been reported at both laboratory and industrial scale conditions (Banach et al., 2021). However, the contribution of water quality to disinfection efficacy is not well known. In the present study, we performed observations and developed kinetics models to mechanistically describe the inactivation performance for chlorine, chlorine dioxide and PAA on PW from three commercial operations. Previous studies in wastewater have described that suspended solids impact the disinfection efficacy and strongly reduced the bacteria inactivation by PAA (Domínguez Henao et al., 2018; Elhalwagy et al., 2021). Suspended solids affecting PAA efficacy have been related to two main mechanisms: (i) consumption of PAA entailing a reduction of the available concentration for disinfection and, thus, a lower PAA exposure dose for viral inactivation which is not our case as we maintained the residual concentration by pumping PAA through the trials, and (ii) shielding of viruses against the action of the sanitizer as microbial aggregates and microorganisms attached to or embedded into particles demonstrated increased resistance to inactivation by PAA compared to non-attached (Domínguez Henao et al., 2018). The contribution of dissolved solids present in some PW such as fresh-cut lettuce has a strong linear relationship with the sanitizer decay (Munther et al., 2015). Previous studies addressed the PAA decay due to suspended solids that can significantly influence the PAA efficacy (Elhalwagy et al., 2021). Pretreatments for the removal of suspended solids decrease the required sanitizer dosage (Domínguez Henao et al., 2018). Our results showed the effect of suspended solids indirectly measured as turbidity on PAA decay and disinfection losses.

Fresh produce may be contaminated by direct contact with fecal contaminated PW during the washing operation. With the occurrence of large foodborne hepatitis A outbreaks, data presented in this study confirmed the survival of HAV and the persistence of infectivity as complete inactivation by any of the sanitizers studied was not observed. The survival of HAV and the relevance of PW as a vehicle for its direct

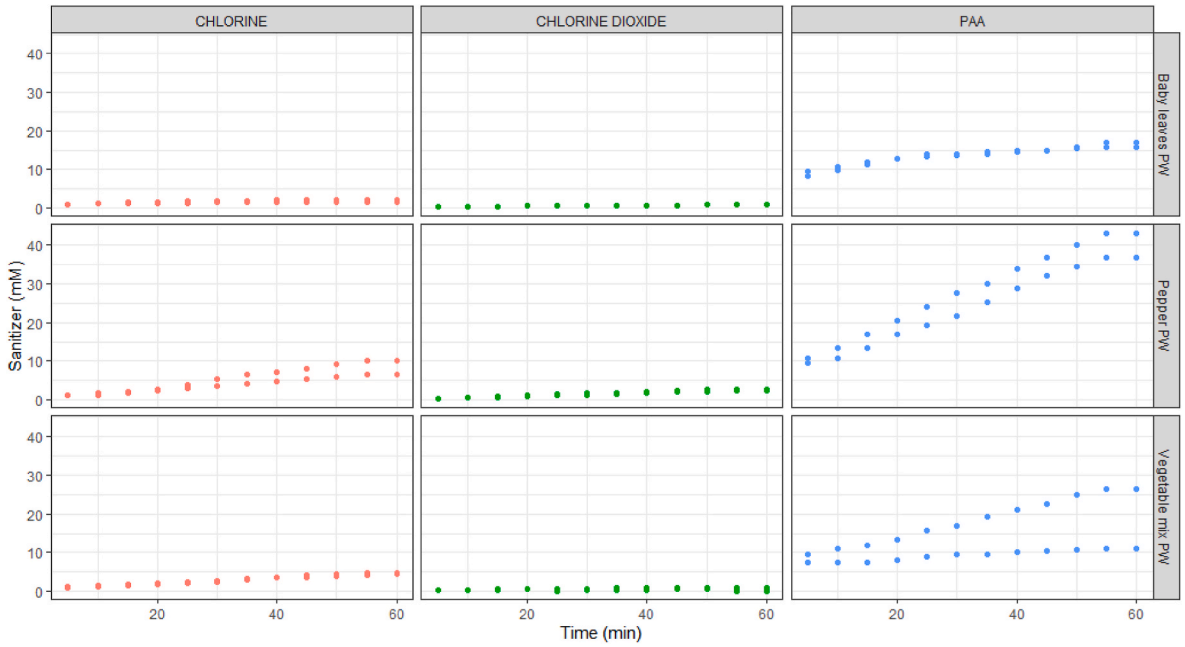


**Fig. 7.** Consumption of chlorine, chlorine dioxide, and peracetic acid (PAA) to maintain residual levels of 5, 2–3 and 80 mg/L, respectively when increasing the chemical oxygen demand (0–500 mg/L) over 1 h.

transmission to fresh produce are clearly shown. When comparing the decay rates of the viruses among sanitizers, chlorine and chlorine dioxide decreased the viral load while PAA was not able to reduce the virus load for any PW studied. Additionally, HAV manifests higher resistance to sanitizers than norovirus surrogates. Different viruses are inactivated by PAA via different mechanisms. Fuzawa et al. (2020) demonstrated that PAA disinfection in Tulane virus was due to protein damage while the mechanism for rotavirus was due to genome damage.

## 5. Conclusions

The efficacy of the sanitizer's chlorine, chlorine dioxide and PAA on viral inactivation was studied. HAV was able to survive in an infectious state independently of the sanitizer compare to the inactivation of MNV and MS2 for chlorine and chlorine dioxide. Virus inactivation was tested by exposing MS2 in the three types of PW over 1 h using a continuous system that simulates operating conditions. When sanitizers were compared, the inactivation models based on the exposure dose calculated as concentration and contact time showed that the viral accumulation was not prevented by PAA despite being an oxidizing agent while viral inactivation was observed for chlorine and chlorine dioxide. When the three types of PW were compared, the resistance of viruses to PAA disinfection was observed to be related to suspended solids and virus aggregation.

## CRediT authorship contribution statement

**Irene Falcó:** Methodology, Formal analysis, Writing – review & editing. **Juan A. Tudela:** Methodology, Formal analysis. **Natalia Hernández:** Methodology. **Alba Pérez-Cataluña:** Methodology, Formal analysis. **Míriam R. García:** Conceptualization, Formal analysis, Writing – review & editing. **Pilar Truchado:** Methodology, Formal analysis, Writing – review & editing. **Agustín Garrido:** Methodology. **Ana Allende:** Conceptualization, Formal analysis, Resources, Writing – review & editing. **Gloria Sánchez:** Conceptualization, Formal analysis, Resources, Writing – review & editing. **Maria Isabel Gil:** Formal analysis, Resources, Writing – review & editing, All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## 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

Data will be made available on request.

## Acknowledgements

The authors are thankful for the financial support from Center for Produce Safety (Award No. 2020CPS09). IF is supported by a post-doctoral contract grant for the requalification of the Spanish university system from the Ministry of Universities of the Government of Spain, financed by the European Union (NextGeneration EU), P. Truchado is holding a Ramón y Cajal contract from the Ministerio de Ciencia e Innovación and AP-C is supported by a postdoctoral fellowship (APOSTD/2021/292). IATA-CSIC is a Centre of Excellence Severo Ochoa (CEX2021-001189-S MCIN/AEI/10.13039/501100011033). The technical assistance of Silvia Andújar, Andrea López de Mota and Macarena Moreno-Candel is very much appreciated.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2023.109738.

## References

Adams, M. H. (1959). *Bacteriophages*. New York (& London: Inter-science Publishers. https://doi.org/10.1002/ange.19620740437

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed.). Washington DC: American Public Health Association, American Water Works Association and Water Environmental Federation https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=1909322.

Ashbolt, N. J. (2015). Microbial contamination of drinking water and human health from community water systems. *Current Environmental Health Reports, 1*(2), 95–106.

Banach, J. L., Hoffmans, Y., Appelman, W. A. J., van Bokhorst-van de Veen, H., & van Asselt, E. D. (2021). Application of water disinfection technologies for agricultural waters. *Agricultural Water Management, 244*, Article 106527. https://doi.org/10.1016/j.agwat.2020.106527

Bennett, S. D., Sodha, S. V., Ayers, T. L., Lynch, M. F., Gould, L. H., & Tauxe, R. V. (2018). Produce-associated foodborne disease outbreaks, USA, 1998–2013. *Epidemiology and Infection, 1*(146), 1397–1406. https://doi.org/10.1017/S0950268818001620

Cuevas-Ferrando, E., Allende, A., Pérez-Cataluña, A., Truchado, P., Hernández, N., Gil, M. I., & Sánchez, G. (2021). Occurrence and accumulation of human enteric viruses and phages in process water from the fresh produce industry. *Foods, 10*. https://doi.org/10.3390/FOODS10081853

Domínguez Henao, L., Cascio, M., Turolla, A., & Antonelliet, M. (2018). Effect of suspended solids on peracetic acid decay and bacterial inactivation kinetics: Experimental assessment and definition of predictive models. *Science of the Total Environment, 643*, 936–945. https://doi: 10.1016/j.scitotenv.2018.06.219.

Dunkin, N., Weng, S. C., Jacangelo, J. G., & Schwab, K. J. (2017). Inactivation of human norovirus genogroups I and II and surrogates by free chlorine in postharvest leafy green wash water. *Applied and Environmental Microbiology, 83*. https://doi.org/10.1128/AEM.01457-17

Elhalwagy, M., Biabani, R., Bertanza, G., Wisdom, B., Goldman-Torres, J., McQuarrie, J., Straatman, A., & Santoro, D. (2021). Mechanistic modeling of peracetic acid wastewater disinfection using computational fluid dynamics: Integrating solids settling with microbial inactivation kinetics. *Water Research, 201*, Article 117355. https://doi.org/10.1016/j.watres.2021.117355

Falcó, I., Randazzo, W., Gómez-Mascaraque, L. G., Aznar, R., López-Rubio, A., & Sánchez, G. (2018). Fostering the antiviral activity of green tea extract for sanitizing purposes through controlled storage conditions. *Food Control, 84*. https://doi.org/10.1016/j.foodcont.2017.08.037

Falsanisi, D., Gehr, R., Santoro, D., Dell'Erba, A., Notarnicola, M., & Liberti, L. (2006). Kinetics of PAA demand and its implications on disinfection of wastewaters. *Water Quality Research Journal of Canada, 41*, 398–409. https://doi.org/10.2166/wqrj.2006.043

Fuzawa, M., Bai, H., Shisler, J. L., & Nguyen, T. H. (2020). The basis of peracetic acid inactivation mechanisms for rotavirus and Tulane virus under conditions relevant for vegetable sanitation. *Applied and Environmental Microbiology, 86*. https://doi.org/10.1128/AEM%20.01095-20. e01095-20.

García, M. R., & Cabo, M. L. (2018). Optimization of *E. coli* inactivation by benzalkonium chloride reveals the importance of quantifying the inoculum effect on chemical disinfection. *Frontiers in Microbiology, 9*, 1259. https://doi.org/10.3389/fmicb.2018.01259

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology, 134*, 37–45. https://doi.org/10.1016/J.IJFOODMICRO.2009.05.021

Gombas, D., Luo, Y., Brennan, J., Shergill, G., Petran, R., Walsh, R., Hau, H., Khurana, K., Zomorodi, B., Rosen, J., Varley, R., & Deng, K. (2017). Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables. *Journal of Food Protection, 80*, 312–330. https://doi: 10.4315/0362-028X.JFP-16-258.

Gómez-López, V. M., Lannoo, A. S., Gil, M. I., & Allende, A. (2014). Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control, 42*, 132–138. https://doi.org/10.1016/J.FOODCONT.2014.01.034

Guzmán, C., Mocé-Llivina, L., Lucena, F., & Jofre, J. (2008). Evaluation of *Escherichia coli* host strain CB390 for simultaneous detection of somatic and F-specific coliphages. *Applied and Environmental Microbiology, 74*, 531–534. https://doi.org/10.1128/AEM.01710-07

Gyürék, L. L., & Finch, G. R. (1998). Modeling water treatment chemical disinfection kinetics. *Journal of Environmental Engineering, 124*, 783–793. https://doi.org/10.1061/(ASCE)0733-9372, 1998)124:9(783.

Kahler, A. M., Cromeans, T. L., Roberts, J. M., & Hill, V. R. (2010). Effects of source water quality on chlorine inactivation of adenovirus, coxsackievirus, echovirus, and murine norovirus. *Applied and Environmental Microbiology, 76*, 5159–5164. https://doi.org/10.1128/AEM.00869-10/ASSET/338FA77C-1154-441B-81E5-039119DC301D/ASSETS/GRAPHIC/ZAM9991012310004 (JPEG).

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: A review. *Environment International, 30*, 47–55. https://doi.org/10.1016/S0160-4120(03)00147-8

Li, M., Baker, C. A., Danyluk, M. D., Belanger, P., Boelaert, F., Cressey, P., Gheorghe, M., Polkinghorne, B., Toyofuku, H., & Havelaar, A. H. (2018). Identification of biological hazards in produce consumed in industrialized countries: A review. *Journal of Food Protection, 81*, 1171–1186. https://doi: 10.4315/0362-028X.JFP-17-465.

López-Gálvez, F., Randazzo, W., Vásquez, A., Sánchez, G., Tombini Decol, L., Aznar, R., Gil, M. I., & Allende, A. (2018). Irrigating lettuce with wastewater effluent: Does disinfection with chlorine dioxide inactivate viruses? *Journal of Environmental Quality, 47*. https://doi.org/10.2134/jeq2017.12.0485

López-Gálvez, F., Truchado, P., Sánchez, G., Aznar, R., Gil, M. I., & Allende, A. (2016). Occurrence of enteric viruses in reclaimed and surface irrigation water: Relationship with microbiological and physicochemical indicators. *Journal of Applied Microbiology, 121*, 1180–1188. https://doi.org/10.1111/JAM.13224

Moreno, L., Aznar, R., & Sánchez, G. (2015). Application of viability PCR to discriminate the infectivity of hepatitis a virus in food samples. *International Journal of Food Microbiology, 201*, 1–6. https://doi.org/10.1016/j.ijfoodmicro.2015.02.012

Munther, D., Luo, Y., Wu, J., Magpantay, F. M. G., & Srinivasan, P. (2015). A mathematical model for pathogen cross-contamination dynamics during produce wash. *Food Microbiology, 51*, 101–107. http://doi: 10.1016/j.fm.2015.05.010.

Ortiz-Solà, J., Abadias, M., Colás-Medà, P., Sánchez, G., Bobo, G., & Viñas, I. (2020). Evaluation of a sanitizing washing step with different chemical disinfectants for the strawberry processing industry. *International Journal of Food Microbiology, 334*, Article 108810. https://doi.org/10.1016/j.ijfoodmicro.2020.108810

Ragazzo, P., Chiucchini, N., & Piccolo, V. (2020). Wastewater disinfection: Long-term laboratory and full-scale studies on performic acid in comparison with peracetic acid and chlorine. *Water Research, 184*, Article 116169. https://doi.org/10.1016/j.watres.2020.116169

Randazzo, W., López-Gálvez, F., Allende, A., Aznar, R., & Sánchez, G. (2016). Evaluation of viability PCR performance for assessing norovirus infectivity in fresh-cut vegetables and irrigation water. *International Journal of Food Microbiology, 229*, 1–6. https://doi: 10.1016/j.ijfoodmicro.2016.04.010.

Shin, H., Park, H., Seo, D. J., Jung, S., Yeo, D., Wang, Z., Park, K. H., & Choi, C. (2019). Foodborne viruses detected sporadically in the fresh produce and its production environment in South Korea. *Foodborne Pathogens and Disease, 16*, 411–420. https://doi.org/10.1089/fpd.2018.2580

Suslow, T. V. (1997). Postharvest chlorination: Basic properties and key points for effective sanitation. Oakland. In *University of California division of agriculture and natural Resources, publication*, 8003. Available at: http://anrcatalog.ucdavis.edu/pdf/8003.pdf Accessed March, 2016.

Tian, P., Yang, D., Shan, L., Wang, D., Li, Q., Gorski, L., Lee, B. G., Quiñones, B., & Cooley, M. B. (2017). Concurrent detection of human norovirus and bacterial pathogens in water samples from an agricultural region in central California Coast. *Frontiers in Microbiology, 21*(8), 1560. https://doi.org/10.3389/fmicb.2017.01560

Truchado, P., Garre, A., Gil, M. I., Simón-Andreu, P. J., Sánchez, G., & Allende, A. (2021). Monitoring of human enteric virus and coliphages throughout water reuse system of wastewater treatment plants to irrigation endpoint of leafy greens. *Science of the Total Environment, 782*, Article 146837. https://doi.org/10.1016/j.scitotenv.2021.146837

Zhang, L., Wang, G., Hou, W., Li, P., Dulin, A., & Bonkovsky, H. L. (2010). Contemporary clinical research of traditional Chinese medicines for chronic Hepatitis B in China: An Analytical Review. *Hepatology*. https://doi.org/10.1002/hep.23384

Zhang, T., Wang, T., Mejia-Tickner, B., Kissel, J., Xie, X., & Huang, C.-H. (2020). Inactivation of bacteria by peracetic acid combined with ultraviolet irradiation: Mechanism and optimization. *Environmental Science and Technology, 54*, 9652–9661. https://doi.org/10.1021/acs.est.0c02424