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Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2

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ABSTRACT

Disinfection of produce is principally used to inactivate spoilage microbes and may also reduce the risk of consumer exposure to enteric pathogens. However, the rate and extent of enteric virus inactivation by free chlorine on produce has not been adequately characterized. Experiments were performed to determine the kinetics of free chlorine inactivation of hepatitis A virus (HAV) and the indicator virus coliphage MS2 on strawberries (SBs), cherry tomatoes (CTs), and head lettuce (HL). The oxidant demand of these produce items also was determined. When produce items were exposed to approximately 20 parts per million (ppm) solution of free chlorine for 5–10 min, HAV and MS2 were inactivated by 90–99% and in some cases virus inactivation was ≥99%. Exposure of strawberries to approximately 200 ppm free chlorine resulted in more rapid and extensive inactivation of both viruses. The produce items tested in this study exhibited a demand for chlorine which varied by produce type, and chlorine residuals declined over time. These results demonstrate the potential for chlorine to reduce the levels of infectious viruses on different produce types, but adequate contact time and chlorine residual are required to achieve maximum virus inactivation. The difference in chlorine demand between SBs, CTs, and HL suggests that varying disinfection practices are needed for the wide variety of processed fruits and vegetables. The inactivation kinetics of MS2 and HAV were similar, suggesting that MS2 and perhaps other similar bacterial viruses may be used as process indicators and surrogates for determining the disinfection efficacy of produce in the laboratory or in actual practice.

1. Introduction

Spraying, washing or immersion of fruits and vegetables (produce) in water is a common practice during postharvest processing. Water has been identified as a critical control point in the farm to fork continuum (Tauxe, 1997), because it can become easily contaminated with feces or other undesirable material, and a small amount of contamination can become widely distributed throughout a water source. Aqueous disinfectants have been used in such procedures to inactivate waterborne microbes, to reduce the total load of spoilage microorganisms on produce and in water, and to wash soil from the surface of produce (Nguyen-the and Carlin, 1994). Surface disinfection of produce using chlorine and other chemical disinfectants and sanitizers has also been identified as a procedure for produce growers, produce processors, consumers, and travelers to reduce the risk of exposure to infectious enteric pathogens (Anonymous, 1998; Beuchat, 1998; Parnell and Harris, 2003; Anonymous, 2008a,b). Chlorine is the most widely used halogen for these purposes, but it is used under widely varying postharvest procedures (Beuchat and Ryu, 1997). Relatively little specific information is available on chlorine and other disinfectant dosages and contact times to achieve maximum inactivation of produce-associated microbes.

Efforts have been made towards the standardization of protocols for assessing produce disinfection efficacy (Beuchat et al., 2001a,b), and chlorine has been shown to inactivate bacteria such as *Shigella*, *Salmonella*, *Escherichia coli*, and *Listeria* on produce (Behrsing et al., 2000; Weissinger et al., 2000; Takeuchi and Frank, 2001). In general, the chlorine dosages (50–200 ppm) and contact times (1–2 min) used by produce processors generally result in 1–2 $\log_{10}$ (90–99%) bacterial inactivation. In contrast, less information is available on the inactivation of viruses on produce, such as hepatitis A virus (HAV). This is despite the fact that foodborne viruses are responsible for appreciable human morbidity (Mead et al., 1999), and diseases such as hepatitis A have been epidemiologically linked to a variety of fruits and vegetables (Ramsay and Upton, 1989; Rosenblum et al., 1990; Niu et al., 1992; Hutin et al., 1999; Dentinger et al., 2001; Calder et al., 2003; Wheeler et al., 2005). Although the majority of information in the area of produce disinfection and sanitization is concentrated on enteric bacteria ambient spoilage microbes, Mariam and Cliver (2000a) reported that washing experimentally contaminated strawberries with 2 ppm chlorine dioxide reduced infectious HAV by less than 70% in 30 s. In addition, others have reported that poliovirus 1 on strawberries was inactivated by about 95% after 2 min of contact with 50 ppm free chlorine (Lukasik et al., 2003). However, little detailed

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information exists on the inactivation kinetics of pathogenic and fecal indicator viruses on the various kinds of produce exposed to various disinfection parameters.

In the present study, experiments were performed to determine the ability of free chlorine to inactivate infectious HAV on produce items. The other virus tested in these experiments was coliphage MS2 (MS2), a bacterial virus used as a process indicator and surrogate for HAV and other human enteric viruses in various environmental virology studies, including produce disinfection studies (Mariam and Cliver, 2000b). Strawberries, cherry tomatoes, and leaf pieces of head lettuce seeded with HAV and MS2 were exposed to approximately 10, 20, or 200 ppm of free chlorine for up to 10 min. The demand for free chlorine from the different produce items also was determined.

2. Materials and methods

2.1. Viruses and cells

A rapidly-replicating variant of HAV, strain HM175, was grown and assayed via the plaque technique in newly confluent layers of FRhK4 (fetal rhesus kidney-derived) cells as previously described (Cromeans et al., 1987). Coliphage MS2 (MS2; ATCC 15597-B1) was grown and assayed via lysis of an overnight culture of *E. coli* C3000 (ATCC 15597) by the double agar overlay technique (Adams, 1959). Viruses used in disinfection experiments were obtained from fluorocarbon-extracted preparations of crude cell lysates and were further processed by centrifugal ultrafiltration (Centricon-100, Amicon, Inc. Beverly, MA) and resuspension in oxidant demand-free buffer (see below for description) to reduce chlorine demand. Viral aggregates were dispersed by filtering the ultrafiltered portions through Tween-80 treated 0.2 and 0.08 μm pore size polycarbonate filters. Infectivity data are reported as $\log_{10}$ plaque forming units ($\log_{10}$ PFU).

2.2. Strawberries, cherry tomatoes, and head (iceberg) lettuce

Strawberries, cherry tomatoes, and head lettuce used in experiments were purchased from a local area supermarket, transported to the laboratory, and stored in the original container at 4 °C until ready for use (<2 d after purchase). The calyces on strawberries and tomatoes and the outermost leaves of head lettuce were gently removed and discarded. Produce items were rinsed briefly with tap water and then rinsed with distilled, deionized (DI) water. The tomatoes were rinsed with warm tap water to remove the wax coating, followed by a DI water rinse. All produce items were weighed prior to use in experiments.

2.3. Seeding viruses on produce

Produce items were processed as described above and allowed to equilibrate to room temperature before experiments. Selected produce items were placed in plastic weigh boats during the virus seeding process.

Individual produce items were seeded with 6–7 $\log_{10}$ PFU of each virus in small suspension volumes (10 to 20 μL) of 0.15 M phosphate

TEST SAMPLE	BIOLOGICAL CONTROL	CHEMICAL CONTROL
Place produce item that has been seeded with virus in to a specimen cup	Place produce item that has been seeded with virus in to a specimen cup	Add 30 mL of free chlorine solution to specimen cup
↓	↓	↓
Add 30 mL of free chlorine solution; expose for a single time interval (0.5, 1, 3, 5, or 10 min)	Add 30 mL of sterile, ODF H_2O	Immediately after addition, remove 10 mL and measure for residual free chlorine
↓	↓	↓
At end of time interval remove 10 mL and measure for residual free chlorine	Immediately after addition of H_2O, remove and discard 10 mL	Repeat procedure at 10 min interval
↓	↓	↓
Add 10 mL $Na_2S_2O_3$ (final conc., 1%) to 20 mL chlorine solution remaining in specimen cup; decant and store at 4°C (wash volume #1)	Add 10 mL fresh ODF H_2O to specimen cup; decant and store at 4°C (wash volume #1)	Report average concentration of free chlorine
↓	↓	
Add 30 mL of eluent to specimen cup / produce item and utilize virus recovery procedure; decant eluate and store at 4°C (wash volume #2)	Add 30 mL of eluent to specimen cup / produce item and utilize virus recovery procedure; decant eluate and store at 4°C (wash volume #2)	
↓	↓	
Assay wash volumes 1 and 2 separately; calculate total PFU/mL recovered and report as Nt	Assay wash volumes 1 and 2 separately; calculate total PFU/mL recovered	
	↓	
	Repeat entire procedure at 10 min interval	
	↓	
	Calculate average concentration from sample and report as No	

Fig. 1. Flow diagram for produce disinfection.

buffered saline (PBS; pH 7.2), and allowed to dry in an operating biological safety cabinet. Virus suspensions were spotted in small (2 to 4 μL) drops evenly over the surface of the produce item. Negative controls consisted of unseeded produce items manipulated in the same manner as seeded produce items. Seeded produce items were used in disinfection experiments after the drops containing test viruses suspension had visibly dried or disappeared.

2.4. Chlorine solutions and their measurement, oxidant demand-free (ODF) glassware, and water

Bleach (sodium hypochlorite, NaOCl; Clorox Corporation, Oakland, CA) was purchased at a local area supermarket, stored at room temperature and used within <14 d. The morning of an experiment, a working stock of approximately 100–1000 ppm free chlorine was made by diluting the NaOCl in ODF buffered water and glassware. Glassware was made ODF by soaking for 24 h in a 30 ppm free chlorine solution and then rinsed four times with ODF water. The glassware was covered with aluminum foil and heated at 200 °C for 4 h. ODF water used in experiments was twice-deionized and activated carbon-filtered, then passed through a macroreticular scavenging resin bed (Dracor Corp., Durham, NC).

Potassium phosphate buffer stocks of 0.1 M were made in ODF water according to standard protocols (APHA, 1995). Stock buffers used in experiments were diluted in ODF water to make 0.01 M working stocks. Adjustment of buffer pH (to pH 7.0) was made with concentrated (5 M) sulfuric acid or sodium hydroxide. Free chlorine was measured by titration with ferrous ammonium sulfate using *N*,*N*,-diethyl-*p*-phenylenediamine as the indicator (APHA, 1995). Free chlorine solutions of approximately 10, 20, or 200 ppm were prepared from working stocks of diluted sodium hypochlorite.

2.5. Experimental design for produce disinfection

Individual produce items seeded with test viruses were exposed to approximately 10, 20, or 200 ppm solution of free chlorine and then processed and assayed at each exposure time point as shown in Fig. 1 At free chlorine doses of 10 and 20 ppm, time points were 0.5, 1, 3, 5, and 10 min; at a free chlorine dose of 200 ppm, exposure times were 0.5, 1, 3, and 5 min. To initiate an experiment for a single exposure time interval, 30 mL of chlorine-containing solution was added at time=0 to a specimen cup (sterile; 90 mL capacity) containing a produce item seeded with virus. The cup was placed on a shaker platform at 100 RPM for the specified time period. After the specified exposure time, 10 mL of the chlorine solution was removed and diluted with 90 mL ODF water in an ODF volumetric flask for free chlorine analysis. Ten (10) mL of concentrated sodium thiosulfate ($Na_2S_2O_3$) was added to the remaining cup volume to a final concentration of 1% in order to neutralize the chlorine. The 100 mL of diluted disinfectant solution was measured for residual free chlorine concentration. The 30 mL of the neutralized chlorine solution was decanted to a labeled 50 mL conical centrifuge tube and stored (<12 h) at 4 °C until assayed for test viruses.

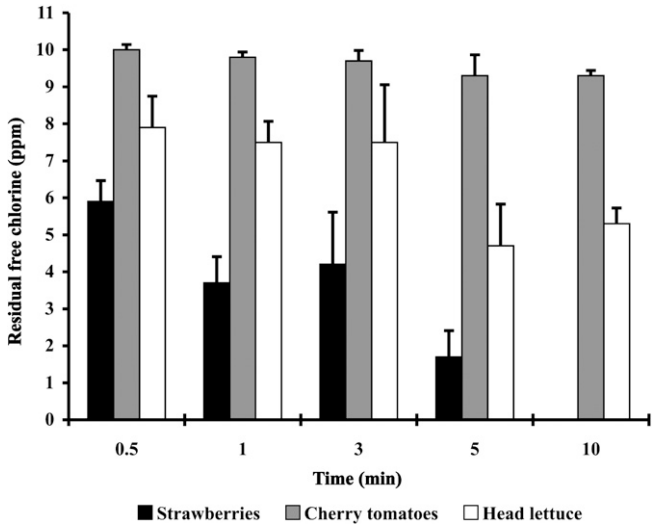

Fig. 2. Average (n=2) consumption of an initial dose of approximately 10 ppm[a] free chlorine by produce[b]. [a]Actual chlorine dosage in trial 1=10.3 ppm; trial 2=10.1 ppm. [b]Average (±SD) weights for strawberries, cherry tomatoes and head lettuce were 22.76±5.07 g, 15.33±2.37 g, and 1.05±0.37 g, respectively. Error bar represents one standard deviation.

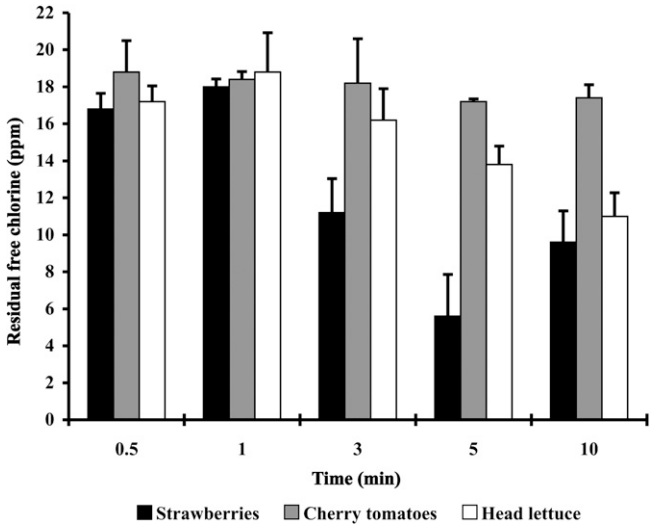

Fig. 3. Average (n=2) consumption of an initial dose of approximately 20 ppm[a] free chlorine by produce[b]. [a]Actual chlorine dosage in trial 1=20.5 ppm; trial 2=20.8 ppm. [b]Average (±SD) weights for strawberries, cherry tomatoes and head lettuce were 21.67±4.66 g, 14.03±1.28 g, and 1.27±0.25 g, respectively. Error bar represents one standard deviation.

As shown in Fig. 1, surviving viruses on produce were recovered by elution using 30 mL of 3% beef extract/0.1% Tween-80 (pH 8). The beef extract eluent was allowed to equilibrate to room temperature before use. Specimen cups were placed on a shaker platform set at 100 RPM for 60 min, followed by decanting the beef extract eluate from the produce item into a labeled 50 mL conical centrifuge tube and storage at 4 °C until assayed (<12 h) for test viruses. Serial dilutions of the eluate were made in PBS or appropriate media for mammalian cell infectivity assays. Replicate portions of each dilution, positive controls (titrated stocks of viruses used in experiments), and negative controls (produce items not seeded with viruses) were assayed for each trial. Assays also were performed for two biological controls, which consisted of virus-seeded produce items not exposed to the disinfectant-containing water and which were sampled immediately after addition of the eluent and at the end of an experiment. Two chemical controls, consisting of disinfectant-containing water only, were sampled immediately after addition to a specimen cup and after 5 or 10 min (Fig. 1).

2.6. Determination of chlorine demand from individual produce items

The free chlorine demand of individual produce items in 30 mL of chlorinated water (about 10 or 20 ppm) was determined at time points of 0.5, 1, 3, 5 and 10 min. Produce items were individually placed in specimen cups, 30 mL of a chlorine solution was added, and specimen cups were placed on a shaker platform at 100 RPM until the end of the desired contact time. Chlorine solutions were decanted and diluted with ODF water. Free chlorine residuals were measured and

Table 1
Mean (±SD) numbers (log_{10}) of HAV and MS2 on produce remaining at time (min) and log_{10} reductions after exposure to doses of approximately 10 ppm free chlorine (n=3)

Time (min)	Strawberries[a]			Cherry tomatoes[a]			Head lettuce[a]		
	Free chlorine (ppm)	MS2	HAV	Free chlorine (ppm)	MS2	HAV	Free chlorine (ppm)	MS2	HAV
		Log_{10} remaining virus			Log_{10} remaining virus			Log_{10} remaining virus	
0	10.6±1.1	4.3±0.5[b]	3.6±0.3[b]	10.9±1.5	1.9±0.6[b]	2.7±0.3[b]	10.3±0.2	2.2±0.4[b]	2.6±0.6[b]
0.5	5.4±2.7	4.0±0.5	2.9±0.3	10.6±0.4	1.5±0.5	2.1±0.2	10.0±0.3	1.3±0.3	1.8±0.5
1	0[c]	3.5±0.6	2.2±0.8	10.0±0.8	1.1±0.8	1.4±0.2	10.2±0.3	1.5±0.4	1.3±0.5
3	3.5±0.6	2.9±0.8	1.9±0.4	10.3±0.2	<0.7[d]	1.6±0.3	10.0±2.5	1.0±0.7	1.2±0.3
5	1.7±0.8	2.8±0.3	2.1±0.3	10.3±0.8	<0.7[d]	<0.4[d]	5.8±2.3	<0.7[d]	<0.4[d]
10	0[c]	2.4±0.8	1.4±0.2	10.6±1.9	<0.7[d]	<0.4[d]	7.3±2.4	<0.7[d]	<0.4[d]
		Log_{10} virus inactivation			Log_{10} virus inactivation			Log_{10} virus inactivation	
0	–	0	0	–	0	0	–	0	0
0.5	–	0.3	0.7	–	0.4	0.6	–	0.9	0.8
1	–	0.8	1.4	–	0.8	1.3	–	0.7	1.3
3	–	1.4	1.7	–	≥1.2	1.1	–	1.2	1.4
5	–	1.5	1.5	–	≥1.2	≥2.3	–	≥1.5	≥2.2
10	–	1.9	2.2	–	≥1.2	≥2.3	–	≥1.5	≥2.2

[a] Average (±SD) weights for strawberries, cherry tomatoes and head lettuce were 21.93+4.09 g, 17.21+2.51 g, and 1.33±0.40 g, respectively.
[b] Initial concentration of microbes recovered from produce.
[c] Free chlorine was not detectable in 3 of 3 trials.
[d] Detection limit of assay.

the results from replicate (n=2) experiments are reported as an average (±standard deviation, SD) ppm free chlorine. These values were compared to the chemical controls (chlorine solution only, Fig. 1).

2.7. Calculation and presentation of virus inactivation data

Virus inactivation from produce disinfection experiments was calculated by dividing the mean number of viruses (as PFU) surviving at time t (Nt) by the original virus titer (No) and expressing as a log_{10} value, log_{10} (Nt/No), or as a percent reduction. The results from replicate (n=3) experiments were used to calculate an average log_{10} Nt/No value. The average (+SD) numbers of remaining infectious viruses, and the log_{10} inactivation values are presented in tabular form.

3. Results

The free chlorine demand profiles of strawberries, cherry tomatoes, and head lettuce are shown in Figs. 2 and 3, and the experimental data from produce disinfection experiments are shown in Tables 1–3.

3.1. Chlorine demand of strawberries, cherry tomatoes, and head lettuce

The rate and extent of free chlorine demand by strawberries, cherry tomatoes, and leaf pieces of head lettuce (without test viruses) was determined by exposure of single produce items to free chlorine concentrations of approximately 10 or 20 ppm in water for up to 10 min. As shown in Figs. 2 and 3, all produce types exhibited a demand for free chlorine in water, in which the residual concentration of the oxidant decreased over time. At an initial dosage of about 10 ppm, there was a rapid consumption of free chlorine by strawberries within 30 s, which disappeared to below the limits of detection (<0.4 ppm) in 10 min. Cherry tomatoes and head lettuce also exhibited a demand after exposure to about 10 ppm free chlorine in water, but these produce items appeared to exhibit less demand compared to strawberries.

Higher free chlorine residuals for strawberries and head lettuce were observed for an initial dosage of about 20 ppm when compared to the 10 ppm experiments. This phenomenon also was observed at a higher initial dose of about 200 ppm chlorine, in which more than 90% of the disinfectant remained at 3 min after contact with all produce

Table 2
Mean (±SD) numbers (log_{10}) of HAV and MS2 on produce remaining at time (min) and log_{10} reductions after exposure to doses of approximately 20 ppm free chlorine (n=3)

Time (min)	Strawberries[a]			Cherry tomatoes[a]			Head lettuce[a]		
	Free chlorine (ppm)	MS2	HAV	Free chlorine (ppm)	MS2	HAV	Free chlorine (ppm)	MS2	HAV
		Log_{10} remaining virus			Log_{10} remaining virus			Log_{10} remaining virus	
0	20.3±1.0	4.5±0.5[b]	3.7±0.6[b]	20.5±0.5	2.3±0.3[b]	2.8±0.6[b]	20.8±0.7	2.5±0.5[b]	2.1±0.7[b]
0.5	16.9±1.3	4.0±0.7	3.1±0.4	20.0±1.1	2.1±0.4	2.0±0.7	18.4±1.2	2.2±0.6	1.3±0.5
1	18.6±0.7	3.3±0.4	3.0±0.3	20.0±1.3	1.7±0.2	1.4±0.8	19.7±1.2	1.4±0.8	1.1±0.4
3	12.0±0.6	3.5±0.5	2.7±0.5	20.3±0.8	1.0±0.9	<0.4[c]	17.3±0.7	<0.7[c]	1.3±0.3
5	5.5±0.6	2.8±0.7	2.5±0.5	19.2±0.3	<0.7[c]	<0.4[c]	13.5±2.1	<0.7[c]	<0.4[c]
10	10.4±0.4	2.4±1.1	1.4±0.6	19.1±0.3	<0.7[c]	<0.4[c]	11.0±1.1	<0.7[c]	<0.4[c]
		Log_{10} virus inactivation			Log_{10} virus inactivation			Log_{10} virus inactivation	
0	–	0	0	–	0	0	–	0	0
0.5	–	0.5	0.6	–	0.2	0.8	–	0.3	0.8
1	–	1.2	0.7	–	0.6	1.4	–	1.1	1.0
3	–	1.0	1.0	–	1.3	≥2.4	–	≥1.8	0.8
5	–	1.7	1.2	–	≥1.6	≥2.4	–	≥1.8	≥1.7
10	–	2.1	2.3	–	≥1.6	≥2.4	–	≥1.8	≥1.7

[a] Average (±SD) weights for strawberries, cherry tomatoes and head lettuce were 18.95±2.25 g, 16.57±3.61 g, and 1.23±0.32 g, respectively.
[b] Initial concentration of microbes recovered from produce.
[c] Detection limit of assay.

Table 3
Mean (±SD) numbers (log_{10}) of HAV and MS2 on strawberries[a] remaining at time (min) and log_{10} reductions after exposure to doses of approximately 200 ppm free chlorine ($n=3$)

Time (min)	Free chlorine (ppm)	MS2	HAV
		Log_{10} remaining virus	
0	202.2±2.8	4.3±0.3[b]	4.0±1.2[b]
0.5	199.5±1.7	2.9±0.5	3.5±1.1
1	198.0±2.6	2.4±1.2	3.4±0.9
3	197.0±4.3	2.0±0.6	2.8±1.4
5	195.0±4.4	1.1±0.3	1.4±0.5
		Log_{10} virus inactivation	
0	–	0	0
0.5	–	1.4	0.5
1	–	1.9	0.6
3	–	2.3	1.2
5	–	3.2	2.6

[a] Average (±SD) weight of strawberries was 22.84±4.37 g.
[b] Initial concentration of viruses recovered from produce.

items (results not shown). As shown in Fig. 3, approximately 50% of free chlorine remained after a strawberry was exposed to about 20 ppm for 10 min. In contrast, 0% remained after 10 min of contact of strawberries with an initial dose of about 10 ppm (Fig. 2). Some variability occurs in the determination of chlorine remaining in the presence of produce items at 10 or 20 ppm, as it appears that the concentration fluctuates by increasing at later time points compared to earlier time points. However, this is probably an artifact from using stock solutions of chlorine prepared at different times, and from using individual containers of chlorine solution for each time point. The initial dosages of free chlorine for the experiments shown in Fig. 2, for example, were 10.3 ppm and 10.1 ppm, and were 20.5 ppm and 20.8 ppm in the higher dose (~20 ppm) demand trials (Fig. 3). In addition, there was some variation in the weights of produce items, and the weights of strawberries and cherry tomatoes were about 15–20 times greater than head lettuce pieces. For example, the average weights of strawberries, cherry tomatoes, and head lettuce exposed to about 10 ppm free chlorine were 22.76+5.07 g, 15.33+2.37 g, and 1.05+0.37 g, respectively (Fig. 2). Similar weights were recorded for exposure of produce items to 20 ppm (Fig. 2).

While the differences in oxidant demand between the produce items (and at the two different chlorine dosages) may be explained by variation in produce weights and/or stock chlorine solutions, a consistent pattern was observed, in which residuals for strawberries rapidly decreased at both dosages. In contrast, tomato chlorine residuals remained relatively constant despite the initial dosage, and the demand for free chlorine by lettuce appeared to be intermediate between the other produce types. Compared to strawberries and head lettuce, cherry tomatoes appeared to exhibit the lowest free chlorine demand, with >87% of the initial dose of free chlorine remaining after exposure to approximately 10 ppm or 20 ppm free chlorine for 10 min. In free chlorine solutions without produce (chemical controls), the free chlorine concentrations were stable over 10 min, with essentially all (99–100%) of the initial amount of free chlorine remaining (results not shown). Because there was a measurable free chlorine residual at most time points with the lowest initial dose tested (10 ppm), it was determined that it was possible to measure the inactivation of HAV and MS2 on produce at dosages of >10 ppm free chlorine.

3.2. 10 and 20 ppm free chlorine disinfection experiments — strawberries, cherry tomatoes, and lettuce

Average results for strawberry, cherry tomato, and lettuce disinfection experiments at initial dosages of approximately 10 and 20 ppm free chlorine are shown in Tables 1 and 2, respectively. As shown in Tables 1 and 2, there were measurable decreases in the levels of infectious HAV and MS2 on produce after exposure to the free chlorine dosages tested. There was no evidence for background levels of human enteric viruses or coliphages on unseeded produce (negative control) items in these experiments, nor was there evidence for background levels of viruses in subsequent experiments (data not shown). Average weights and standard deviations of produce items used in disinfection experiments were similar to the weights of individual produce items used in demand experiments. For example, average weights of strawberries used in 10 ppm, 20 ppm and 200 ppm disinfection experiments ranged between about 19 and 23 g (Tables 1–3), compared to average strawberry weights of about 22–23 g in demand experiments (Figs. 2 and 3). Similar results were obtained with tomatoes and lettuce in all trials.

As shown in Table 1, initial levels of HAV on strawberries were reduced by about 1.4 log_{10} (96%) in 1 min, and inactivation levels did not change appreciably until the 10 min time point, by which time HAV was inactivated by 2.2 log_{10} (99.4%). Coliphage MS2 on strawberries also was inactivated after contact with water containing an initial dose of 10.6 ppm free chlorine, with an average reduction in 10 min of 1.9 log_{10} (98%). Although the levels of free chlorine diminished rapidly throughout the course of the strawberry experiments, falling to below detectable concentrations at 1 and 10 min, the average residual free chlorine level was 3.5 ppm. Also shown in Table 1 is the inactivation of HAV and MS2 on cherry tomatoes and head lettuce exposed to average initial dosages of 10.9 and 10.3 ppm free chlorine, respectively. Compared to the inactivation of viruses on strawberries, the inactivation of HAV and MS2 on cherry tomatoes and head lettuce appeared to be more extensive. As shown in Table 1, MS2 was not detected on tomatoes at 3 min, with a level of inactivation of ≥1.2 log_{10} (>94%), and HAV was inactivated to below the limits of detection (by ≥2.3 log_{10}) at 5 min. In contrast to strawberries, the average level of residual free chlorine was 10.5 ppm, or 96% of the original dose for cherry tomatoes, and 8.9 ppm (87%) for head lettuce.

Results for strawberry, cherry tomato, and lettuce disinfection experiments at 20 ppm free chlorine are shown in Table 2. As with exposure to 10 ppm free chlorine, there were measurable decreases in the concentrations of infectious HAV and MS2 on all produce items. Initial levels of HAV on strawberries were reduced by 90% in 3 min and by 1.2 log_{10} (94%) in 5 min. Coliphage MS2 on strawberries also was reduced by about 90% in 1 min, and was more extensively inactivated in 5 min (1.7 log_{10} or 98%). Reductions of MS2 were somewhat greater (by approximately 0.5 log_{10}) than HAV in 5 min, but the levels of inactivation at 10 min were generally similar (by about 99%). In contrast to strawberries, inactivation of HAV and MS2 on tomatoes and lettuce exposed to approximately 20 ppm free chlorine was more extensive, which suggests that viruses on different produce items will exhibit varying inactivation kinetics when disinfectant dosages are similar. As shown in Table 2, levels of inactivation of HAV at 3 min were ≥2.4 log_{10} on cherry tomatoes and were ≥1.7 log_{10} on head lettuce in 5 min, but were 1–1.2 log_{10} (90%–94%) on strawberries in 3–5 min. As shown in Tables 1 and 2, the limits of detection for HAV and MS2 on produce exposed to chlorinated water were reached in several experiments; hence, the levels of inactivation may have been greater than observed in those instances.

3.3. 200 ppm free chlorine disinfection experiments — strawberries

Experiments were performed to determine if increasing the initial free chlorine dose to about 200 ppm would result in more rapid and extensive inactivation of MS2 and HAV on strawberries. Exposure of strawberries to chlorinated water was for a maximum contact time of 5 min in these experiments. As shown in Table 3, inactivation of HAV on strawberries was 0.5 log_{10}, 0.6 log_{10}, and 1.2 log_{10} in 0.5, 1, and 3 min of exposure, respectively. These inactivation levels of HAV on strawberries are similar to those achieved by 20 ppm free chlorine at

the same contact times (0.6, 0.7, and 1.0 $\log_{10}$ at 0.5, 1, and 3 min, respectively (Table 2)). In contrast, HAV was inactivated by 2.6 $\log_{10}$ (99.7%) in 5 min of exposure to about 200 ppm free chlorine, compared to 1.2 $\log_{10}$ (94%) inactivation in 5 min of exposure to about 20 ppm free chlorine. Although the inactivation of HAV on strawberries at 200 ppm was similar to 20 ppm for contact times of 3 min or less, MS2 was reduced by 1.4 $\log_{10}$ (96%) in 0.5 min of exposure to 200 ppm free chlorine, compared to 0.5 $\log_{10}$ after exposure to 20 ppm free chlorine for 0.5 min (Table 3). Overall, MS2 was inactivated more rapidly and extensively on strawberries exposed to 200 ppm free chlorine compared to 20 ppm free chlorine.

4. Discussion

In the present study, the demand for free chlorine in water by strawberries, cherry tomatoes and lettuce was determined. The chlorine concentrations evaluated in this study (10 to 200 ppm) are reflective of what we have observed in actual practice and are similar to values reported in the literature (Beuchat, 1998). A disinfectant's residual profile compared to the rate and extent of microbial inactivation will determine the most efficient combination of contact time and initial dose to employ, and the measurement of such parameters must be determined for assessing and comparing disinfection efficacy (Haas et al., 1995; Haas and Finch, 2001). This has further implications since differences exist between the major classes of microbes in their response to disinfectants (Sobsey, 1989). However, existing produce disinfection studies, most of which examined bacterial inactivation on produce, did not explore the relationship between disinfectant type, concentration, contact time, and oxidant demand exhibited by different fruits and vegetables. In the present study, strawberries, cherry tomatoes, and leaf pieces of head lettuce consumed chlorine, with the demand for chlorine decreasing with increasing initial dose. This effect appears to be more pronounced for strawberries compared to tomatoes or lettuce. These results also imply an upper limit of the demand of produce for disinfectants by several ppm, beyond which the disinfectant residual appears to stabilize. Similar phenomena are observed for chlorine disinfection processes in water; for example, chlorine in water is consumed to a point after which there is a return to a stable residual (Culp, 1974; Haas, 1999). The different rates of consumption of chlorine by the produce items used in this study suggests that free chlorine and other oxidant demand profiles be determined for the wide variety of processed fruits and vegetables that are now available.

Measurement of infectious viruses and other microbes recovered from disinfected produce or wash water at various contact times is important, because microbial inactivation is a kinetic process. Little or no useful data is currently available to design disinfectant dose and contact times for maximum viral inactivation on produce. In this study, there were measurable decreases of both HAV and MS2 on produce after exposure to 10, 20, or 200 ppm free chlorine. Increasing the initial dose of free chlorine from about 20 to 200 ppm resulted in more extensive inactivation of HAV and MS2 on strawberries at the end of contact periods (5 and 10 min for 20 and 200 ppm, respectively). Hence, adequate contact time, in addition to the maintenance of a chlorine residual, are among the necessary factors to ensure maximum inactivation of viruses on produce. Despite the initial dosage of disinfectant evaluated in this study, the levels of inactivation for both HAV and MS2 on produce were generally similar at contact times of 3 min or less, which may be longer than washing systems used by some processors. However, investigators have reported longer contact times (*e.g.*, 5 to 10 min), both in actual washing systems (Crowe et al., 2005) and in bench-scale studies (Beuchat, 1998). Hence, opportunities may exist to customize washing systems to achieve specific quality and safety objectives. The data presented here may also be useful to modify existing procedures for consumers (Parnell and Harris, 2003) and travelers (*e.g.*, washing produce in 140–205 ppm chlorine for 15–20 min (Anonymous, 2008a,b)).

In conclusion, the use of a surrogate virus indicator such as coliphage MS2 and perhaps other bacteriophages may be used to monitor viral disinfection efficacy of produce at the bench or in the field. Such viruses are relatively easy to measure and correlate reasonably well with the presence of human enteric (fecal) viruses. Chlorine can inactivate HAV on different kinds of produce items, and increasing chlorine concentrations and prolonging contact times results in a more rapid and extensive inactivation of both HAV and MS2 on produce. However, chlorine disinfection itself may not be relied upon to ensure that produce is free from viruses, and there are several issues to consider when using disinfectants for produce. First, disinfection procedures are capable of only a finite level of inactivation, and some disinfectants are more effective against some microbes compared to others (Sobsey, 1989). Second, disinfection procedures have only recently been examined for their efficacy in the inactivation of human enteric viruses and other pathogens on produce, and most studies were performed in the laboratory under relatively clean conditions. Third, the use of chlorine in the presence of organic material, and the subsequent formation of potentially carcinogenic disinfection by products, has not yet been addressed in these applications. Lastly, the disinfection of produce may not be economically feasible, desirable, or practical in all situations. Given these issues, a multiple barrier approach is recommended, like the one practiced by members of the California strawberry industry (Anonymous, 2005). Members of this group routinely monitor chlorine and other disinfectant levels, and many growers and processors also use a combination of good agricultural practices, worker training, and HACCP methods. Commodity-specific procedures also are employed, such as removing strawberry calyces (*i.e.*, "hulling" or "capping") using stainless steel tools instead of workers' fingernails. Combined, such procedures may work effectively to reduce the risk of morbidity and mortality associated with fecally contaminated produce.

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