Occurrence and accumulation of potentially infectious viruses in process water and impact of water disinfection practices to minimize viral cross-contamination

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Summary

Enteric viruses are the major causes of foodborne outbreaks; however, the virological quality of process water used by the produce industry has received limited attention. This project aims to assess the risk of process wash water (PWW) contamination by infectious viruses under several scenarios and the correlation with coliphages. Additionally, this project is investigating the efficacy of the most common disinfection agents used in processing facilities. Operational conditions and critical parameters will be defined for each washing system needed for the inactivation of enteric viruses and coliphages. Results of this project will contribute to get insight whether selected disinfectant conditions are able to control the contamination of enteric viruses in the process wash water and the use of coliphages as viral indicators.

Objectives

1. Detection and quantification of potentially infectious enteric viruses and coliphages in process water used from industrial partners
2. Inactivation studies to evaluate the efficacy of chlorine and non-chlorine based sanitizers
3. Validation of the established water disinfection practices for enteric viruses and coliphages in commercial facilities.
4. Establishment of coliphages as a suitable indicator of enteric viruses in commercial facilities.

Methods

Process wash water (PWW) samples were collected six times from July to December at industrial collaborators' facilities from baby leaves, peppers and veggie fruit mix processing lines. Physiochemical properties of PWW and sanitizer concentrations were determined. A dead-end hollow-fiber ultrafiltration procedure was used to concentrate viruses from 20-L PWW samples. Detection and quantification of coliphages and human enteric viruses was carried out by plaque count and RT-qPCR methods, respectively. Inactivation experiments were performed using the three PWW samples and sodium hypochlorite, peroxyacetic acid (PAA) and chlorine dioxide as disinfectant solutions for murine norovirus (MNV), Tulane virus (TuV), hepatitis A virus (HAV), and MS2 phage. MS2 and MNV were also assessed in a dynamic system. Viral inactivation kinetics were defined by cell-culture.

Results to Date

Physicochemical characteristics and levels of total and F-specific RNA coliphages, crAssphage, and human enteric viruses in PWW of commercial lines of baby leaves, bell pepper and veggie fruit mix were determined. Most samples tested negative for the presence of human enteric viruses (LoD about 1.1–1.7 $\times 10^3$ for norovirus and 4.28 $\times 10^2$ for rotavirus). Detection of crAssphage was observed in 70 and 60% of pre-washing and washing samples, respectively, while coliphages were detected in 50% of samples (**Table 1**). Inactivation experiments performed in PWW showed that 10–20 mg/L of chlorine effectively inactivated enteric viruses and MS2, while 80 mg/L of PAA slightly affected the viral infectivity (**Figure 1** and **Figure 2**). Dynamic system inactivation experiments showed that 5 mg/L of free chlorine completely inactivated MS2 inoculated in PWW (**Figure 3**).

Benefits to the Industry

The main beneficiaries of the project will be the produce industry, which will be guided using evidence-based conditions for the performance of chlorine and non-chlorine based sanitizers for the disinfection of wash water used for washing whole and fresh-cut commodities to prevent viral contamination. Additionally, the output of the project will provide relevant information on the operational limits and quality parameters of the process water used by the fresh produce industry and the potential use of coliphages and crAssphage as viral indicators.

Sampled water		Date	Ratio Produce/water (kg/L)	Sanitizer	Sanitizer concentration (mg/L)	COD (mg/L)	Total coliphages (log pfu/L)	F-specific RNA coliphages (log pfu/L)	crAssphage (log gc/L)	Human enteric viruses (log gc/L)
Baby leaves	Pre-Washing	7/21/2020	1.6	Chlorine	19 ± 1	188 ± 39	Nd	Nd	Nd	Nd
		9/30/2020	1.2	Chlorine	0 ± 0	67 ± 8	Nd	Nd	2.18 ± 0.00	Nd
		10/13/2020	1.7	Chlorine	36 ± 1	84 ± 25	Nd	Nd	Nd	Nd
		10/27/2020	1.6	Chlorine	37 ± 2	42 ± 7	Nd	Nd	Nd	Nd
		11/10/2020	1.6	Chlorine	57 ± 6	32 ± 17	Nd	Nd	2.97 ± 0.06	Nd
		11/24/2020	1.8	Chlorine	59 ± 0	71 ± 8	Nd	Nd	2.46 ± 0.04	Nd
	Washing	7/21/2020	1.6	Chlorine	12 ± 1	75 ± 26	Nd	Nd	Nd	Nd
		9/30/2020	1.2	Chlorine	3 ± 0	23 ± 3	Nd	Nd	Nd	Nd
		10/13/2020	1.7	Chlorine	96 ± 8	35 ± 3	Nd	Nd	Nd	Nd
		10/27/2020	1.6	Chlorine	72 ± 1	19 ± 3	Nd	Nd	1.66 ± 0.00	Nd
		11/10/2020	1.6	Chlorine	120 ± 3	14 ± 12	Nd	Nd	2.93 ± 0.04	Nd
		11/24/2020	1.8	Chlorine	128 ± 5	25 ± 10	Nd	Nd	2.62 ± 0.14	Nd
Bell peppers	Pre-Washing	7/21/2020	31.0	None	-	845 ± 106	4.4 ± 0.0	4.4 ± 0.0	Nd	Nd
		9/29/2020	32.0	None	-	301 ± 14	3.4 ± 0.0	3.1 ± 0.0	3.08 ± 0.02	Nd
		10/13/2020	4.0	None	-	414 ± 10	3.8 ± 0.1	3.9 ± 0.0	1.18 ± 0.0	Nd
		10/27/2020	137.7	None	-	281 ± 8	3.8 ± 0.1	3.9 ± 0.0	Nd	Nd
		11/10/2020	88.0	None	-	548 ± 0	4.3 ± 0.0	4.0 ± 0.0	3.02 ± 0.04	Nd
		11/24/2020	136.0	None	-	341 ± 20	3.5 ± 0.0	3.3 ± 0.0	3.14 ± 0.20	Nd
	Washing	7/21/2020	31.0	PAA	416 ± 96	1020 ± 35	Nd	Nd	Nd	Nd
		9/29/2020	32.0	PAA	233 ± 2	1541 ± 96	2.9 ± 0.0	3.0 ± 0.0	1.18 ± 0.0	Nd
		10/13/2020	4.0	PAA	326 ± 9	2158 ± 43	3.3 ± 0.1	3.8 ± 0.0	Nd	Nd
		10/27/2020	137.7	PAA	330 ± 7	1207 ± 46	3.7 ± 0.2	3.6 ± 0.0	Nd	Nd
		11/10/2020	88.0	PAA	370 ± 9	1900 ± 0	2.7 ± 0.1	3.1 ± 0.1	2.94 ± 0.09	5.55*
		11/24/2020	136.0	PAA	332 ± 9	1162 ± 26	Nd	Nd	2.73 ± 0.77	Nd
Veggie fruit mix	Pre-Washing	9/29/2020	5.6	None	-	171 ± 8	3.9 ± 0.1	4.1 ± 0.0	Nd	Nd
		10/14/2020	10.4	None	-	144 ± 2	2.0 ± 0.0	2.0 ± 0.0	Nd	Nd
		10/27/2020	2.9	None	-	147 ± 8	Nd	Nd	2.11 ± 0.08	Nd
		11/24/2020	9.1	None	-	169 ± 16	3.8 ± 0.1	3.7 ± 0.0	Nd	Nd
		12/9/2020	7.8	None	-	179 ± 8	3.7 ± 0.1	3.6 ± 0.1	2.92 ± 0.04	Nd
		12/22/2020	13.1	None	-	185 ± 1	2.7 ± 0.1	2.0 ± 0.0	2.39 ± 0.21	Nd

*Positive rotavirus detection; Nd: non-detected

Table 1. Physicochemical characteristics, including sanitizer concentration and chemical oxygen demand (COD) and levels of total and F-specific RNA coliphages, crAssphage and human enteric viruses in pre-washing and washing water of commercial lines of baby leaves, bell peppers and veggie fruit mix.

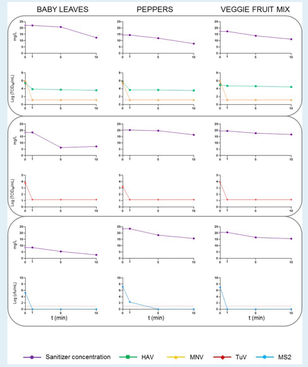

Figure 1. Effect of chlorine (10–20 mg/L) on murine norovirus (MNV), hepatitis A virus (HAV), Tulane virus (TuV) and MS2 infectivity/viability in wash water from baby leaves, peppers and veggie fruit mix.

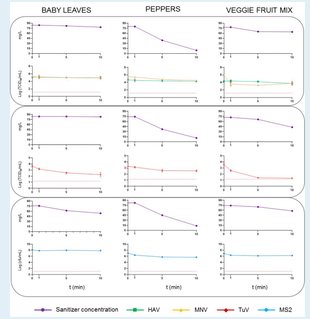

Figure 2. Effect of peroxyacetic acid (80 mg/L) on murine norovirus (MNV), hepatitis A virus (HAV), Tulane virus (TuV) and MS2 infectivity/viability in wash water from baby leaves, peppers and veggie fruit mix.

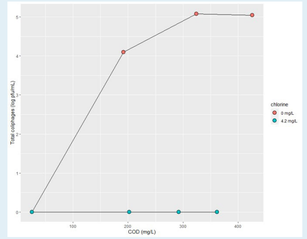

Figure 3. Effect of chlorine dioxide (4.2 mg/L) on the inactivation of total coliphages in process water from washing baby leaves when increasing chemical oxygen demand (COD).