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. The main purpose of this project is to assess the risk of infectious viruses in process water under several scenarios and correlate them with the presence of coliphages. Additionally, this project will investigate the efficacy of the most common disinfection agents used in processing facilities. Operational conditions and critical parameters will be established for each washing system needed for the inactivation of enteric viruses and coliphages. Results of this project will contribute to insight whether selected disinfectant conditions are able to control the presence of enteric viruses in the process wash water and the use of coliphages as viral indicators.

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, this 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 of using 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 on human enteric viruses and coliphages
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

Optimization of the concentration of process water samples for virus detection was performed. The physico-chemical properties of process wash water (PWW) from washing shredded lettuce were determined. Initially, PWW was artificially inoculated with mengovirus and the MS2 bacteriophage. A primary concentration protocol was the dead-end hollow-fiber ultrafiltration (DEUF) of 20 L of PWW with a Rexeed 25AX cartridge (**Figure 1**), followed by a second concentration step using polyethylene glycol precipitation and the extraction of viral RNA in the final concentrate. In parallel, another protocol was an aluminum hydroxide $Al(OH_3)$ adsorption–precipitation method for concentration of 1 L of artificially inoculated PWW.

To establish the detection limit and recoveries of the procedures after process selection, two PWWs from washing shredded lettuce—Lab PWW (obtained in the lab) and Commercial PWW (from the commercial partner)—were artificially inoculated with norovirus GI, norovirus GII, and rotavirus.

Results to Date

Mengovirus recoveries were 23.73% ± 0.21% for DEUF and 23.56% ± 0.53% for $Al(OH)_3$, while MS2 recoveries were 31.72% ± 11.47% for DEUF and 47.91% ± 19.5% for $Al(OH)_3$.

- The DEUF protocol was selected as the concentration process for determination of the limit of detection of the procedure since a higher volume of PWW was able to be studied.

PWW physico-chemical properties are shown in **Table 1**.

DEUF ultrafiltration combined with PEG precipitation resulted in the mean recovery of 27.1, 27.31 and 36.56% for norovirus GI, GII, and rotavirus (**Figure 2**). An average recovery rate of 19% for mengovirus was achieved from all procedures, validating the results. For the DEUF procedure, the LoD95% in process water was 1.09×10^3, 1.71×10^3 and 4.28×10^2 for norovirus GI, GII, and rotavirus.

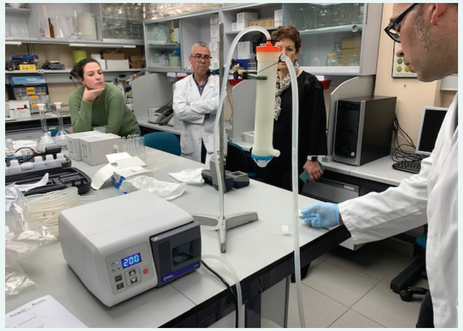

Figure 1. Dead-end hollow-fiber ultrafiltration (DEUF) of process wash water with a Rexeed 25AX cartridge.

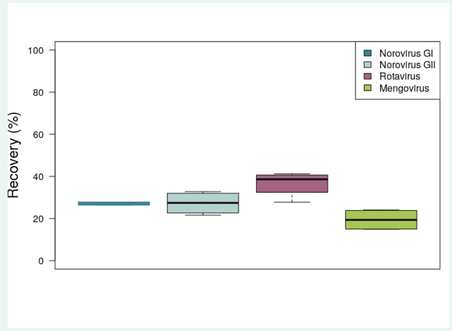

Figure 2. Recoveries of different human enteric viruses in process water by Rexeed 25AX ultrafiltration followed by precipitation with polyethylene glycol.

Type of water	pH	ORP (m/V)	COD (mg/L)	EC (µS cm^{-1})
Lab PWW	5.8±0.5	374±4	892±78	733±104
Commercial PWW	7.1±0.1	279±14	1429±184	955±317

Table 1. Physico-chemical parameters (pH, oxidation-reduction potential [ORP], chemical oxygen demand [COD], and electrical conductivity [EC]) of process wash water (PWW) generated in the lab and obtained from commercial processing line from washing shredded lettuce.