Enteric Viruses in Process Water

Subjects: Virology

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Process water has been defined as water resulting from washing raw materials, rinsing water, or water used for cooling or transport, which usually accumulates organic matter, including micro-organisms. Process water in the fruit and vegetable sector is highly variable in terms of quality parameters, such as dissolved solids, chemical oxygen demand, and microbiological quality. This fact makes it a challenge to implement a standard treatment fit for all purposes. The occurrence of potentially infectious enteric viruses in PW used by the fresh produce industry is likely possible, and thus, it must be closely examined. Several factors must be considered to address this issue: (i) Relatively low levels of enteric viruses introduced will be randomly distributed into large volumes of water and may not be detectable using protocols indicating small volume collection; (ii) sampling points in commercial facilities are critical for pathogen detection; (iii) molecular-based methods, currently used for enteric virus detection in food cannot discriminate between inactivated and potentially infectious enteric viruses; (iv) organic fresh produce market, limiting the use of sanitizers, has tremendously increased in the last years and the food safety perception of consumers must be assured.

Keywords: human enteric viruses ; viral indicator ; bacteriophages ; molecular methods ; infectivity ; produce ; wash water ; food safety

1. Overview

The virological quality of process water (PW) used by the produce industry has received limited attention. As a first step to overcoming technical limitations in monitoring viruses in PW, the analytical performance of ultrafiltration was assessed to concentrate viral particles from 20 L of spiked PW. The selected method used for sample concentration of PW was carefully validated, thus enabling the accurate quantification and estimation of viral titers of human enteric viruses and phages. PW from the produce industry was collected periodically from the washing tanks of commercial facilities. The analysis of coliphages was performed by plaque assay, while the occurrence of enteric viruses and crAssphage was determined by molecular techniques. Significant differences in the physicochemical composition of PW, mostly due to the different nature of fresh produce types and differences in the sanitizer used in commercial operation, were observed. Accumulation of crAssphage and coliphages was observed in PW, but correlation with human enteric viruses was not possible due to the low prevalence of these pathogens in the PW analyzed. The obtained results showed that depending on the type of product washed, the product/water ratio and the residual concentrations of the sanitizers, the prevalence and concentration of bacteriophages changed significantly.

2. Human Enteric Viruses

Despite being one of the major causes of foodborne outbreaks in high-income countries, human enteric viruses have received comparatively less attention than foodborne pathogenic bacteria. Enteric viruses are the most common etiologic agents identified in produce-associated outbreaks (54%), frequently linked with food-handling issues [1]. The presence of human enteric viruses in irrigation waters has been extensively reported [2][3][4][5][6]. Among others, the viruses most commonly detected in irrigation waters include human norovirus, astrovirus (HAstV), rotavirus A (RV), and hepatitis A virus (HAV) [7]. Moreover, the USA included norovirus in a list of water contaminants that must be regulated in drinking water [8]. Physical and chemical parameters, together with classical microbial indicators such as fecal indicator bacteria (FIB), including fecal coliforms, *Escherichia coli* (*E. coli*), and enterococci, have been widely used to assess the quality of process water (PW) used in different postharvest unit operations. However, the presence of human enteric viruses has not been fully implemented for this purpose.

Process water has been defined as water resulting from washing raw materials, rinsing water, or water used for cooling or transport, which usually accumulates organic matter, including micro-organisms [9]. Process water in the fruit and vegetable sector is highly variable in terms of quality parameters, such as dissolved solids, chemical oxygen demand, and microbiological quality. This fact makes it a challenge to implement a standard treatment fit for all purposes [10]. The

occurrence of potentially infectious enteric viruses in PW used by the fresh produce industry is likely possible, and thus, it must be closely examined. Several factors must be considered to address this issue: (i) Relatively low levels of enteric viruses introduced will be randomly distributed into large volumes of water and may not be detectable using protocols indicating small volume collection; (ii) sampling points in commercial facilities are critical for pathogen detection [11]; (iii) molecular-based methods, currently used for enteric virus detection in food [12] cannot discriminate between inactivated and potentially infectious enteric viruses; (iv) organic fresh produce market, limiting the use of sanitizers, has tremendously increased in the last years and the food safety perception of consumers must be assured.

Due to the difficulties associated with direct detection of viral pathogens in water, bacteriophages infecting enteric bacteria, such as coliphages, have been suggested as a viral indicator in irrigation water because they mimic viruses better than any other group of indicators showing moderate resistance to treatments and persistence in the environment [13]. Coliphages are viruses used as viral indicators and can infect *E. coli.* They are split into two categories based on the route of bacterial host infection: somatic coliphages and male-specific (F+) coliphages (F-RNA & F-DNA) [14]. Recently, crAssphage (cross-assembly phage) has been suggested as a novel viral indicator of fecal contamination as it is present in high abundance compared with human enteric viruses. Recent data indicate that crAssphage can be used to detect human fecal contamination on environmental surfaces and hands [15]. However, the usefulness of this indicator in PW is still unknown.

Monitoring and maintaining the quality of PW during postharvest operations is considered important for both the safety and quality of end-products [10].

3. Conclusions

Process water obtained from three different commercially handled and processed lines of fruits and vegetables showed significant differences in their physicochemical composition primarily due to the diverse nature of the product type and the use or non-use of sanitizers (chlorine and PAA). The recoveries and LoD achieved with the method optimized for PW suggested that this procedure can be standardized and used for routine monitoring. This method was suitable for detecting and quantifying (RT)-qPCR of different types of viruses, including enteric viruses and crAssphage, as well as viable bacteriophages, including total coliphages, F-specific RNA phages, and MS2. The obtained results showed that depending on the product, the water ratio, the type of product washed in the water, and the residual concentration of the sanitizer, the prevalence, and concentration of bacteriophages varied significantly. The concentration of coliphages and crAssphage was the highest in PW with a low replenishment rate and no sanitizers. On the contrary, the prevalence and concentrations of bacteriophages were much lower when residual chlorine was constantly maintained. An intermediate situation was illustrated in washing peppers as phages accumulated in PW even though the prevalence of the enteric viruses was very low. Based on the limit of detection for enteric viruses, it may be possible that the viruses were present, but the method’s sensitivity was not adequate for their detection and quantification. More research should be done to lower the detection limit to confirm the low potential risk linked to the accumulation of enteric viruses in PW when a residual sanitizer is present.

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