

Title A single method for recovery and concentration of enteric viruses and bacteria from fresh-cut vegetables

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Abstract

Fresh-cut vegetables are prone to be contaminated with foodborne pathogens during growth, harvest, transport and further processing and handling. As most of these products are generally eaten raw or mildly treated, there is an increase in the number of outbreaks caused by viruses and bacteria associated with fresh vegetables. Foodborne pathogens are usually present at very low levels and have to be concentrated (i.e. viruses) or enriched (i.e. bacteria) to enhance their detection. With this aim, a rapid concentration method has been developed for the simultaneous recovery of hepatitis A virus (HAV), norovirus (NV), murine norovirus (MNV) as a surrogate for NV, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica*. Initial experiments focused on evaluating the elution conditions suitable for virus release from vegetables. Finally, elution with buffered peptone water (BPW), using a Pulsifier, and concentration by polyethylene glycol (PEG) precipitation were the methods selected for the elution and concentration of both, enteric viruses and bacteria, from three different types of fresh-cut vegetables by quantitative PCR (qPCR) using specific primers. The average recoveries from inoculated parsley, spinach and salad, were ca. 9.2%, 43.5%, and 20.7% for NV, MNV, and HAV, respectively. Detection limits were 132 RT-PCR units (PCRU), 1.5 50% tissue culture infectious dose (TCID₅₀), and 6.6 TCID₅₀ for NV, MNV, and HAV, respectively. This protocol resulted in average recoveries of 57.4%, 64.5% and 64.6% in three vegetables for *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* with corresponding detection limits of 10³, 10² and 10³ CFU/g, respectively.

Based on these results, it can be concluded that the procedure herein is suitable to recover, detect and quantify enteric viruses and foodborne pathogenic bacteria within 5 h and can be applied for the simultaneous detection of both types of foodborne pathogens in fresh-cut vegetables.