

INTRODUCTION

Since the last decade, human noroviruses and Hepatitis A virus (HAV) are currently recognized as the main foodborne pathogens. In 2012, the number of reported outbreaks caused in Europe by the pathogenic enteric viruses increased compared to the previous year (9.3% to 13.8%)¹. Human noroviruses and HAV, transmitted by the fecal-oral route, are the main cause of non-bacterial gastroenteritis and enteric hepatitis, respectively. Occurrence of enteric viruses in red fruits is well known, as reported by the RASFF network and literature data⁵. Due to their fragility, red fruits are rarely submitted to technological treatments after harvesting.

Ozone (O₃), a strong oxidizing agent, has been widely used as disinfectant in drinking water treatment plants in Europe. Ozone gas seems to be the best candidate to potentially ensure the food safety of red fruits. **In this study, the virucidal impact of ozone gas has been investigated on fresh raspberries artificially contaminated by enteric viruses.**

MATERIAL AND METHODS

Using the Murine Norovirus (MNV) and the HAV strain HM175/18f (clone B) as current surrogates of pathogenic human noroviruses and HAV, the virucidal efficiency of ozone gas has been assessed as follows:

- Fresh raspberries samples of 25 g were independently spiked on the surface matrix with 100 µL of each viral stock solution at 2.0×10^6 TCID₅₀ (for MNV) and 1.45×10^6 PFU (for HAV).
- Ozone disinfection of samples was performed at different ozone concentration and contact time (three repetitions per condition), using a lab-scale ozonation unit designed by Alphatec Technologies (Figure 1).
- After ozone gas treatment, viruses were extracted from samples by elution-concentration method, as recommended by the ISO TS/XP 15216 standard².
- Virus inactivation (infectious virus) and virus removal (RNA genome) were respectively determined with cell culture and RT-qPCR methods by calculating the log unit N_i/N_0 , where N_0 is the titer of virus recovered on the untreated sample and N_i is the titer of virus recovered on the sample treated with ozone gas (three analyses per sample).

Figure 1: Description of Experiments and Physicochemical Characteristics of Pilot Unit Using Ozone Gas.

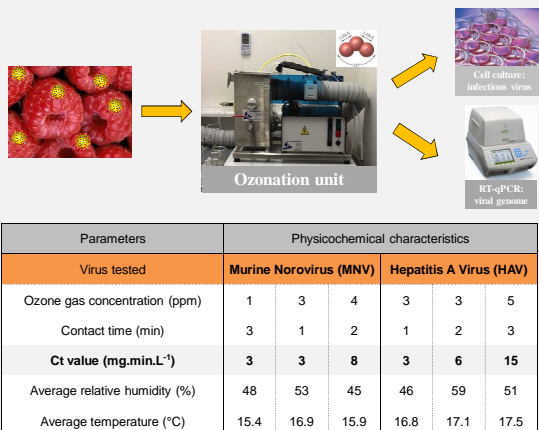
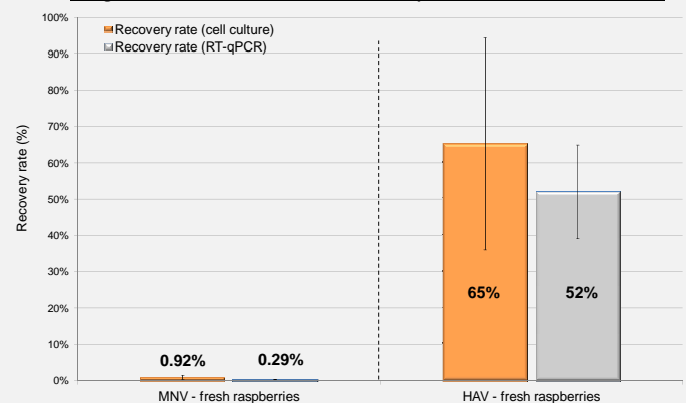


Figure 2: Recovery Efficiency of MNV and HAV Obtained on Fresh Raspberries by Using the Extraction Protocol Recommended by the ISO TS/XP 15216 Standard.



RESULTS AND DISCUSSION

- Better recovery of infectious particles and viral genome of HAV (> 50%) compared to MNV (< 1%) on surface of raspberries, using the extraction method recommended by the ISO TS/XP 15216 standard (Figure 2). These results could highlight i) differences in viral behavior during the elution-concentration process and/or, ii) specific interactions between the surface properties of enteric virus and the outside surface of food matrix.
- Ozone gas is an effective technological treatment for inactivating MNV on raspberries (Figure 3). The viral genome of MNV is not affected by the oxidation effect of ozone gas under the conditions tested. RT-qPCR assays significantly underestimate the inactivation of MNV, compared with that measured by cell culture assays. These results are in agreement with Lim *et al.* (2010)⁴.
- Ozone gas used for the viral decontamination of HAV on raspberries is not efficient in terms of viral inactivation (< 0.6 log) and virus removal (< 0.2 log) under the conditions tested (Figure 4). These results indicate **the resistance of HAV compared to MNV during ozone gas treatment**. Resistance of infectious HAV to oxidizing agents like peroxyacetic-based biocide has been also described by Fraisse *et al.* (2011)².
- Reduction of Aerobic Mesophilic Flora on fresh raspberries is below 0.7 log, using 5 ppm of ozone gas for 3 min. **Organoleptic quality of raspberries** is not lowered by ozone gas treatment (data not shown).

Figure 3: Log Inactivation of Infectious MNV and Log Removal of MNV Genome on Fresh Raspberries by Ozone Gas Treatment at Pilot Scale.

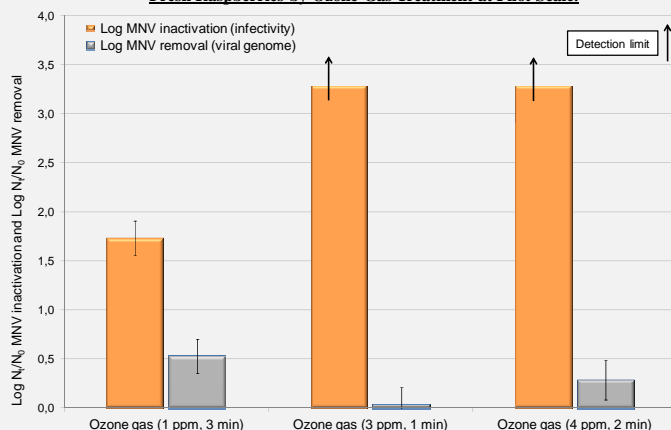
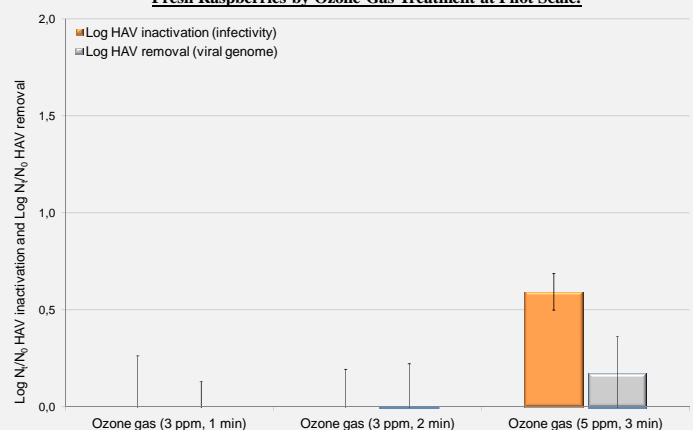


Figure 4: Log Inactivation of Infectious HAV and Log Removal of HAV Genome on Fresh Raspberries by Ozone Gas Treatment at Pilot Scale.



CONCLUSION

Ozone gas may be considered as a **useful and innovative technological treatment** for the viral decontamination of fresh raspberries (fragile fruit). Further research is underway to ensure at least 4.0 log HAV inactivation, especially by increasing ozone concentration and contact time.

From a fundamental viewpoint, mechanisms involved in the **loss of infectivity for enteric viruses** and their surrogates will be investigated in order to accurately assess the virucidal effectiveness of ozone treatment.

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