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# Fate of Foodborne Viruses in the "Farm to Fork" Chain of Fresh Produce

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**Abstract:** Norovirus (NoV) and hepatitis A virus (HAV) are the most important foodborne viruses. Fresh produce has been identified as an important vehicle for their transmission. In order to supply a basis to identify possible prevention and control strategies, this review intends to demonstrate the fate of foodborne viruses in the farm to fork chain of fresh produce, which include the introduction routes (contamination sources), the viral survival abilities at different stages, and the reactions of foodborne viruses towards the treatments used in food processing of fresh produce. In general, the preharvest contamination comes mainly from soli fertilizer or irrigation water, while the harvest and postharvest contaminations come mainly from food handlers, which can be both symptomatic and asymptomatic. Foodborne viruses show high stabilities in all the stages of fresh produce production and processing. Low-temperature storage and other currently used preservation techniques, as well as washing by water have shown limited added value for reducing the virus load on fresh produce. Chemical sanitizers, although with limitations, are strongly recommended to be applied in the wash water in order to minimize cross-contamination. Alternatively, radiation strategies have shown promising inactivating effects on foodborne viruses. For high-pressure processing and thermal treatment, efforts have to be made on setting up treatment parameters to induce sufficient viral inactivation within a food matrix and to protect the sensory and nutritional qualities of fresh produce to the largest extent.

**Keywords:** foodborne viruses, fresh produce, HAV, norovirus

## Introduction

Foodborne viruses are overall excreted in high numbers in human feces, and they are transmitted by the fecal-oral route. The 2 most frequently linked viruses with foodborne outbreaks, and as such identified as the foodborne viruses with the highest priority worldwide are norovirus (NoV) and hepatitis A virus (HAV) (FAO/WHO 2008).

Fresh produce has been identified as an important vehicle for the foodborne transmission (Bassett and McClure 2008; FAO/WHO 2008). Vegetable row crops (such as leafy greens) and fruits were responsible for 30% and 21%, respectively, of NoV foodborne outbreaks in the U.S. (2009 to 2012) (Hall and others 2014). Concerning fresh produce outbreaks, NoV was identified as the top cause of outbreaks (40%), according to a comprehensive survey of outbreaks with identified food sources in the U.S. (1990 to 2005) (Dewaal and Bhuiya 2009).

Epidemiologic evidence linking foodborne outbreaks with virus contaminated fresh produce are available in the literature (Table 1). Frequently identified fresh produce items that were implicated in these outbreaks are soft red fruits (including raspberries and strawberries) and leafy greens (such as salads). The overview of the peer-reviewed outbreak investigation literatures gives only a narrow view on the relevance of fresh produce as a vehicle for foodborne viruses, since not every foodborne outbreak is reported in peer-reviewed publications. Mostly the reported outbreaks are from North America or Europe.

In order to determine a basis to identify possible prevention and control efforts, this article reviewed the transmission routes and viral persistence of foodborne viruses (mainly NoVs and HAV) during the farm-to-fork chain of fresh produce, as well as the effect of treatments used in food processing of fresh produce on viruses.

Since it still remains impossible to determine the viral infectivity of human NoVs and most wild-type HAV strains, the detection of foodborne viruses relies mainly on molecular methods, exclusively reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR). In order to understand the stability of human NoVs, surrogates that share pathological and/or biological features with human NoVs (for example, feline calicivirus [FCV], murine norovirus [MNV], and Tulane virus [TV]) have been used (Cromeans and other 2014; Wang and others 2014; Arthur and Gibson 2015; Mormann and others 2015). For HAV, only a laboratory-adapted variant HM175 can be propagated and therefore has been used in the survival and inactivation studies (Shimasaki and other 2009).

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Table 1–Foodborne outbreaks due to NoV or HAV contaminated fresh produce in recent 10 y (2006 to 2015)

| Implicated food | Virus | Year and location (origin of raw material) | Cases (*n*) (attack rates, %) | Contamination | Reference |
|---|---|---|---|---|---|
| Salad | NoV (GII.7) | 2006, Austria | 182 (56%) | FH involvement confirmed | Schmid and others (2007) |
| Mixed salad | NoV (GII.4) | 2007, U.K. | 34 (86% to 95%) | FH was ruled out, presence of different subtypes in stool suspected contamination through exposure to sewage | Showell and others (2007) |
| Salads | NoV (GII.6) | 2007, U.K. | 79 (57% to 73%) | FH involvement confirmed (in presymptomatic phase during salad preparation) | Vivancos and others (2009) |
| Salad buffet vegetables | NoV (GI.3) | 2007, Sweden | 413 (24%) | FH involvement confirmed | Zomer and others (2010) |
| Salad vegetables[a] | NoV (GII.4) | 2007, Japan | 23 | / | Oogane and others (2008) |
| Lettuce salad and soup | NoV (GII.4) | 2008, Portugal | 16 (73%) | / | Mesquita and Nascimento (2009) |
| Salad | NoV (GII.4) | 2009, Germany | 27 | FH involvement confirmed | Wadl and others (2010) |
| Lettuce[a] | NoV (GI and GII) | 2010, Denmark (France) | 260 (54%) | All foodborne outbreaks linked to the same kind of lettuce and the same supplier suggesting contamination at farm-level. Lettuce was believed to be contaminated with multiple nonzoonotic pathogens leading to the speculation that human fecal matter may have been the source of contamination, possibly via contaminated water | Ethelberg and others (2010) |
| Mixed raw vegetables | NoV (GII.1) | 2006, Finland | >400 | Epidemiologic evidence on vegetables originating from a single provider as vehicle, indicating contamination before arrival in the canteens | Makary and others (2009) |

(*Continued*)

Table 1–Continued.

| Implicated food | Virus | Year and location (origin of raw material) | Cases (*n*) (attack rates, %) | Contamination | Reference |
|---|---|---|---|---|---|
| Dried radish salad | NoV (GII.4) | 2008, Korea | 117 | Infected FH suspected | Yu and others (2010) |
| Cabbage kimchi[a] | NoV (GI.3) | 2011, Korea (Korea) | 451 | Groundwater used for processing of the cabbage was identified as source since GI.3 NoV was detected in the water (homology >99.4% with clinical sample and isolate from kimchi) | Cho and others (2014) |
| Frozen raspberries | NoV | 2006, Sweden (China) | 43 (40% to 91%) | / | Hjertqvist and others (2006) |
| Frozen raspberries[a] | NoV (GI.4) | 2009, Finland (Poland) | Approximately 200 | All outbreaks were traced to the same batch of imported raspberries | Maunula and others (2009) |
| Frozen raspberries[a] | NoV (GII.4, GII.b, GII.7, GI.4) | 2009, Finland (Poland) | 900 (49% in one of the 13 outbreaks) | Some foodborne outbreaks were traced back to the same contaminated batch of frozen raspberries | Sarvikivi and others (2012) |
| Frozen mixed berries | HAV (IB) | 2013, U.S.A. | 162 | / | CDC (2013) |
| Frozen mixed berries,[a] fresh berries, mixed berry cake, frozen berry mix cake[a] | HAV (IA) | 2013 to 2014, Italy, Ireland, the Netherlands, Norway, France, Germany, Sweden, U.K., Finland | 1444 | / | Chiapponi and others (2014), ECDC (2014), EFSA (2014b), Guzman-Herrador and others (2014), Rizzo and others (2013) |
| Frozen pomegranate seeds[a] in raw frozen fruit mix | HAV (IB) | 2012, Canada (Egypt) | 6 | / | Swinkels and others (2014) |
| Frozen strawberries | HAV (IB) | 2012 to 2013, Denmark, Finland, Norway, Sweden | 103 | / | Nordic outbreak investigation 2013; Gillesber Lassen and others (2013) |
| Frozen strawberries[a] | NoV (GII.16/II.13[b]; GI.9; GII.6) | 2012, Germany (China) | 10950 | / | Mäde and others (2013), Bernard and others (2014) |

/, data not reported; FH, food handler.
[a] The viral agent was also recovered from the food samples.
[b] A recombinant genotype with combination of genotypes II.16 (viral polymerase) and II.13 (viral capsid).

## Introduction and Survival of Foodborne Viruses in the Farm-to-Fork Chain of Fresh Produce

Enteric viruses such as NoVs and HAV follow the fecal-oral transmission route. As both human NoV and HAV are currently believed to be nonzoonotic viral pathogens, the primary cause of contamination is contact with (residue of) infected and shedding people. A person infected with NoV can shed up to $10^{12}$ viruses (RT-PCR) per gram of feces (Atmar and others 2008). A person infected with HAV can excrete $10^6$ to $10^8$ particles/g of feces during infection (Sanchez 2013). Next to the high viral load during shedding and the low infectious dose, also environmental persistence facilitates water- and foodborne spread of NoV and HAV. Hence in this section, next to the causes of the contamination of fresh produce, also environmental persistence will be included during each of the stages of the farm-to-fork production chain.

## Preharvest Contamination

### Contaminated seeds

The life cycle of a plant starts with a seed. Viral outbreaks have not yet been linked to sprouted seeds. Nevertheless, to understand whether viral-contaminated seeds could also pose a threat to human health, Wang and others (2013) investigated the persistence of HAV and human NoV surrogates MNV and TV on alfalfa seeds during storage and on sprouts after a 7-d germination period. It was reported that HAV, MNV, and TV remained infectious on the surface of the alfalfa seeds after 50 d. Following a 7-d germination period, viruses were located in all tissues as well as in sprout-spent water sampled on several occasions (Wang and others 2013). As such, good agricultural practices (GAP) during production of seeds and appropriate control measures to prevent cross-contamination due to reuse of water during germination should also focus on enteric viruses as a possible contaminant.

### Contaminated soil

Similarly, although specific foodborne outbreaks due to fresh produce linked to viral-contaminated soil are missing, proof of the concept has been demonstrated in a study by Wei and others (2010). Attachment of MNV was observed upon contact of lettuce with spiked treated sludge and manure. Hence, viral presence in soil may increase the risk of fresh produce contamination (Wei and others 2010).

As NoV and HAV are generally strictly confined to humans as their sole hosts, application of animal manure to the soil as fertilizer does not contribute to viral contamination of the produce. However application of manure or slurry contaminated with excrements of human origin, or the proximity of a latrine may pose a risk. A second source of viral contamination of the soil is the application of sludge. Sludge originates from the process of waste water treatment and, hence, might contain high loads of pathogens (viruses, bacteria, and so on) present in waste waters.

Concerning the persistence of enteric viruses in soil, temperature, and moisture are primary factors influencing persistence. Overall, both relatively short-term (11 d) persistence of poliovirus in soil in Ohio in summer (Tierney and others 1977) and long term persistence (≥6 mo) of coxsackievirus in soil in winter in Denmark (Damgaardlarsen and others 1977) of enteric viruses in (amended) soils have been observed. More detailed reviews are available in the literature (Rzezutka and Cook 2004; Wei and Kniel 2010).

### Contaminated water

Intentional application of water at the farm stage includes the use of water for irrigation, the use of water to dissolve and apply chemicals (such as fungicide, insecticide) to the produce, and the use of water for cleaning of equipment. Links to a major outbreak in the Czech Republic in 1979 (28880 ill persons) specifically linked to HAV-contaminated frozen strawberries due to irrigation with sewage can be found (Legge ÄM 1997). Viral foodborne outbreaks due to possible contamination as a result of vegetable or fruit spraying with insecticides and fungicides mixed with contaminated water were not found in the literature. However, the relevance of these 2 transmission routes for viral pathogens has been proven during experimental field studies (Cheong and others 2009; Brassard and others 2012) and by the use of QMRA (Stine and others 2005b, 2011).

Viral contamination of plants by means of irrigation water may occur in 2 ways, either by direct contact, like by spray or splash, or through internalization into the tissue via the root system of the plant. In studies in which plants are grown in viral-contaminated hydroponic solution, high contamination levels of enteric viruses in edible plant tissue have been demonstrated, such as contamination levels exceeding 4 $\log_{10}$ GC/plant for HAV and MNV have been found in all portions of both green onion and spinach plants, including the edible portions (Hirneisen and Kniel 2013a).

Transfer of organisms from water to produce surfaces via irrigation is influenced by irrigation method and the type of produce. Irrigation method is an important factor as choosing an optimal strategy can minimize the contact of irrigation water with the above-ground portion of the crop and, hence, lower the risk of viral contamination. In a field study by Stine and others (2005b) no viral contamination of lettuce was detected when grown using subsurface drip irrigation practices, while the use of furrow irrigation led to contaminated lettuce. Crops irrigated with sprinkler and furrow systems may have a higher chance of direct contact with viruses and are, hence, considered to be more hazardous for fresh produce crops such as lettuce (Wei and Kniel 2010). Concerning the influence of produce type, leafy vegetables such as lettuce, with high water retention capacity and in close contact with the ground, are identified to be especially vulnerable to viral contamination through irrigation (Hamilton and others 2006).

Presence of enteric viruses has been demonstrated in all sorts of waters generally used for irrigation of produce. As such, NoVs have been detected in ground water wells in the United States (Fout and others 2003), Korea (Cheong and others 2009; Park and others 2010), and Italy (Gabrieli and others 2009); in canal waters in the United States (Kayed 2004); in reclaimed wastewater, and in river water samples all over the world as in Poland (Kozyra and others 2011), in the Netherlands (Westrell and others 2006), in Japan (Haramoto and others 2005), and in South Africa (Mans and others 2013). Sources of irrigation water can be generally ranked by the microbial contamination hazard: in order of increasing risk these are potable or rain water, groundwater from deep wells, groundwater from shallow wells, surface water, and finally raw or inadequately treated wastewater (Pachepsky and others 2011).

The omnipresence of enteric viruses in these waters can be explained by (i) the recalcitrance of enteric viruses such as NoVs toward wastewater treatments, as viruses have been detected in both influent and effluent waters (da Silva and others 2007; Sidhu and Toze 2009; Battistone and others 2014), (ii) the deficient state of current sewage systems and the omnipresence of viral contamination sources such as leaking septic tanks, latrines, combined with

a higher potential for transport in soil (Hijnen and others 2005), and (iii) the high persistence of enteric viruses in these waters. In general, mean inactivation rates of viruses in fresh water are less than 1 $\log_{10}$ per day, indicating that viruses can persist in freshwater sources for prolonged periods (Rzezutka and Cook 2004). Persistence of surrogate virus MNV-1 has also been observed in reconstituted pesticides (Verhaelen and others 2013b). With time, the ratio of infectious particles to genomic copies (molecular detection) has been observed to decrease and, as such, this ratio is partly depending on the "age" of contamination (De Roda Husman and others 2009). The persistence of enteric viruses in water is known to be affected by temperature, virus association with solids, exposure to light (UV), and the presence of indigenous microbiota. These are all factors that are known to be substantially different from one geographical location to another (John and Rose 2005; Bosch and others 2006).

### Persistence on crops in the field

Generally a faster die-off rate is observed on fresh produce than on or in soil which is considered as a more protective environment from solar radiation and desiccation (Choi and others 2004). However, enteric viruses can persist for several days on fresh produce during preharvest conditions. As such, a *D*-value of 4.8 d was observed for MNV-1 on semisavoy spinach during a persistence study in greenhouse biocontrol chambers (Hirneisen and Kniel 2013c). While an inactivation rate ($k_d$) as low as 0.01, 0.12, and 0.11 per day (corresponding to a *D*-value of 100, 8, and 9 d) was observed for HAV on cantaloupe, lettuce, and bell peppers, respectively, during a persistence study in a controlled environment chamber mimicking relevant growing condition in the United States and Central America (Stine and others 2005a). All in all, these limited studies suggest that enteric viruses persist longer than enteric bacteria and may persist from the time of contamination (such as by means of irrigation) to harvesting (Stine and others 2005a). Persistence can depend on crop type and even crop variety (e.g., survival of MNV-1 and TV on semisavoy spinach versus smooth spinach mentioned by Stine and others 2005a; Carratala and others 2013; Hirneisen and Kniel 2013c). Since the surface texture and structure of vegetables may play an important role in the attachment and persistence of viruses. As such, the rougher or more irregular the surface of produce, the longer viruses are able to persist (Stine and others 2005a; Hirneisen and Kniel 2013c).

There has been evidence for biphasic inactivation of viruses on crops during preharvest conditions (Petterson and others 2001). An important implication of the biphasic inactivation is the possibility for virus accumulation on the crop surface over subsequent irrigations due to the presence of a persistent subpopulation of viruses that decay slowly (Petterson and others 2001). This higher persistence of this subpopulation could be a result of their location in a more protective niche such as stomata, complex wax structures, or cuts. Also, the location of inoculum on the abaxial (lower) leaf surfaces has been observed to result in higher decimal reduction times (*D*-values) compared to viruses present on adaxial (upper) leaf surfaces (Hirneisen and Kniel 2013c).

## Harvest and Postharvest Contamination

In this stage food handlers are identified as critical point or hot spots for the transmission of foodborne viruses. Food handlers in this context include field harvesters, production plant workers, professional chefs and caterers, but also nonprofessionals such as those cooking at home, or at a youth camp preparing food. The risk of contamination posed by an infected food handler can depend on personal factors specific to a food handler, including, for example, phase of clinical infection which impacts the degree of virus-shedding, personal hygiene habits, and a variety of behavioral factors such as the willingness to work when feeling ill (Mokhtari and Jaykus 2009). Note that this transfer by infected food handlers can involve both symptomatic as well as asymptomatic food handlers, as also asymptomatic food handlers can shed similar high loads of virus particles (Ozawa and others 2007). For instance, up to 14% of analyzed feces samples of asymptomatic food handlers working at a none-outbreak-related facility in Japan tested positive for NoV (Okabayashi and others 2008).

### Transmission during harvesting

The harvesting of fresh produce can be either manual or mechanical. As such, contamination can take place due to contaminated food handlers and/or contaminated surfaces.

Food handlers' hands can also get contaminated by the produce and serve as a vehicle for further contamination. This was observed in a study on hand hygiene of pickers of green bell peppers in Mexico where the workers' hands were not contaminated before work (0/36), while 13.9% (5/41) of the pickers' hands were contaminated with NoVs after 3 h of work (Leon-Felix and others 2010). During harvesting, food handlers such as fruit pickers have been suspected as the source of contamination in several reported viral soft red fruit outbreaks (Table 1).

To assess to which extent food handlers and contaminated food contact materials contribute to the introduction and spread of foodborne viruses, transfer experiments are available in the literature that encompass all of the possible transfer combinations with hands, produce, food contact materials as either donor surface or acceptor surface (reviewed in Kotwal and Cannon 2014). In short, mean transfer rates of infectious viruses ranging from 2% to 18% and 0.1% to 2.3% have been found for contact of contaminated finger paths (dry conditions) with lettuce (Bidawid and others 2004; Stals and others 2013) and berries (Verhaelen and others 2013a), respectively. Identified variables that have a major influence on transfer rates are dry time of inoculum on donor surface (Sharps and others 2012), moisture conditions of acceptor surface (D'Souza and others 2006), and pressure and friction applied during transfer (Mbithi and others 1992; Escudero and others 2012).

Enteric viruses have the potential to persist on hands for the better part of a work shift, as in a study by Mbithi and others (1992) a biphasic reduction curve was observed, resulting in a mere 0.5 to 0.8 $\log_{10}$ reduction 4 h after inoculations of HAV on human hands. This illustrates the potential risk when infected food handlers are employed in a food processing/handling environment. Once surfaces are contaminated, these surfaces can function as reservoir for further contamination events, and this for prolonged periods of time, as the relative persistence of enteric viruses in the environment is high. As such, the half-life of HAV on stainless steel under different conditions ($T \leq 20$ °C and $RH < 80\%$) was at least 4 d (Sattar and others 2000). Surrogate MNV-1 has been observed to remain infectious after 28 d on several surfaces (stainless steel, ceramic, rubber, wood, glass, plastic) at room temperature (Kim and others 2014). Exceptionally, it has been found that copper could effectively inactivate MNV-1 by destroying the viral capsid massively (Warnes and others 2015). On inanimate surfaces, the most important factors that affect virus stability are the type of virus and surface, relative humidity, moisture content, temperature, composition of the suspending medium, light exposure, and presence of antiviral chemicals or biological agents (Bosch and others 2006).

Next to these influencing factors, the presence of food residue has been observed to increase the persistence and the resistance of enteric viruses towards chemicals (Takahashi and others 2011).

### Transmission during postharvest handling

After harvest the produce is cooled at the farm or immediately after entering the postharvest processing or distribution stage, depending on the locally available infrastructure.

In the case of raspberries, postharvest processing can consist of the production of individual quick-frozen (IQF) raspberries or raspberry puree. For the production of IQF raspberries, raspberries are frozen after which manual sorting can take place. The presence of NoV shedders is realistic considering the high prevalence of NoV infections in a community. Transmission through contaminated hands is hence realistic since the presence of NoV contamination on the hands of infected individuals has been confirmed during clinical trials (Liu and others 2013).

Since washing is one of the typical processing units in the production process of fresh-cut lettuce, risks concerning washing practices will be discussed for lettuce. In the processing of lettuce toward fresh-cut leafy greens, the produce is cut, washed, and spin-dried before packaging with or without protective atmosphere. This washing process has the potential to reduce the microbial load of the incoming fresh produce but has also the potential to be a direct source of contamination and a vehicle for spreading localized bacterial and viral contamination (cross-contamination) when sanitizers are used inadequately or are lacking (Holvoet and others 2014). As such, in a recent outbreak in Korea the use of contaminated ground water during the processing of cabbage kimchi was identified as the source of viral contamination (Cho and others 2014). Viral transfer from contaminated fresh produce to washing water (without sanitizers) has been documented for both lab-scale and industrial-scale washing units (Baert and others 2009; Casteel and others 2009; Holvoet and others 2014). Persistence of enteric viruses in wash water has been shown to amply exceed common working hours (≥32 h, 10 °C) (Baert and others 2009) endorsing the potential risk for cross-contamination. In-depth study on the consequence of a contaminated wash bath for the processing of several batches of lettuce and resulting quantitative data of transfer rates is available (Holvoet and others 2014).

Next to the washing process, cross-contamination by contaminated machinery (such as by cutters), contaminated surfaces, and leftovers from a previous contaminated batch is also a possibility.

At the level of caterers and professional food handlers the same risk factors exist as at the processing level. The bare-hand contact with food is likely the most prominent way of transmission (Hall and others 2014). Therefore, a "no bare hands" rule was included in the U.S. Food and Drug Administration's model food code and has been already adopted by many states. Such legislation is not commonly found in other parts of the world. However, while gloves may provide an important barrier against food contamination, it has to be noted that they cannot be used as a stand-alone hygienic measure. The combination of hand washing with an extra intervention measure—hand gloving and hand gloving/disinfectant—is advised to prevent virus spread during food preparation (Stals and others 2015). In addition, infected food handlers can also indirectly contaminate the food by contaminating the environment. Besides, cross-contamination with naturally contaminated fresh produce or other food commodities such as seafood is also a risk factor. For example, the cross-contamination of salad by seafood was identified as the probable cause of an outbreak of NoV illness in 1979 (Griffin and others 1982).

## Effect of Treatments used in Food Processing of Fresh Produce on Foodborne Viruses

This section will focus on treatments applicable to fresh produce that allow the retention of fresh-like organoleptic properties and the data will focus on effect of processing on the viral load of fresh produce. Special attention will be reserved for the 2 NoV—fresh produce commodities frequently linked to viral foodborne outbreaks, lettuce and raspberries. However, as frozen raspberries are frequently linked to viral outbreaks, leading to the recommendation to heat frozen berries before consumption, as in several North European countries, both freezing and heat treatment will also be included in this section.

## Effect of Storage Conditions

Low-temperature storage immediately upon harvest is recommended to preserve the quality of fresh produce, primarily by lowering the respiration and metabolism rates. Ideal storage temperatures for berries and leafy greens are 3 to 5 °C and 0 to 5 °C, respectively (EFSA BIOHAZ Panel 2014a,b). Temperature has been identified as the major factor influencing virus persistence. However, in contrast to bacterial pathogens, maintaining the cold-chain cannot be considered as a mitigation strategy for viral pathogens on fresh produce, as persistence of enteric viruses is higher at low temperatures, and decay rates generally increase with increasing temperatures (RIVM 2013). In Table 2, $\log_{10}$ reduction data are presented for the persistence of enteric viruses or their surrogates on soft red fruits and leafy greens. Next to temperature, persistence has been found to depend on other factors: type of fresh produce (Croci and others 2002; Verhaelen and others 2012), different environmental factors, such as relative humidity (RH), presence of feces, and aggregation (Konowalchuk and Speirs 1975), and the virus type (Rzezutka and Cook 2004). The presence of fecal material strongly enhances virus persistence (Escudero and others 2012). The effect of RH is less unambiguous given that MNV and MS2 persisted better at low RH, while HAV persisted better at higher RH in a study by Kim and others (2012). In a recent study it was suggested that absolute humidity (AH, a measure of the actual amount of water vapor in a particular sample of air) rather than RH (the ratio of the actual amount of water vapor present in a sample to that amount that would be needed to saturate that particular sample) is the critical factor for keeping NoV infectious. The data also suggested that when the atmosphere was not entirely saturated (as 100% RH), low AH values (below 0.007 kg water/kg air) are favorable to NoV persistence. This possibly explains the seasonality of NoV infections since low winter AH conditions (96.3% of the day with AH <0.007 kg water/kg air) in a temperate climates as in Paris, France provides favorable conditions for keeping human NoV infectious (de la Noue and others 2014).

Since the shelf-life of fresh produce, and especially for case-studies lettuce and raspberries, is short, only a low reduction in the numbers of infectious viral particles is expected when stored at cold temperatures. Overall, persistence of enteric viruses can be expected during the time between purchase and consumption.

In case of fresh-cut lettuce, modified-atmosphere packaging (MAP) is generally adopted. MAP is a food-packaging method in which the proportions of carbon dioxide, nitrogen, and oxygen in a sealed container are different from those in the normal (ambient) air to enhance the food's shelf life. Next to functions such as the control of the respiration and reduction of enzymatic browning reactions, MAP conditions have also been designed to reduce the growth of spoilage microorganisms and pathogens. However, in

 

Table 2–Summary table of selected persistence studies on soft red fruits and leafy greens.

| Matrix | Virus[a] | Storage condition | $Log_{10}$ reduction (95% CI) | Reference |
|---|---|---|---|---|
| Strawberry | MNV | 4 °C, 7 d | 0 | Verhaelen and others (2012) |
| | | 10 °C, 7 d | 0.9 (0.7 to 1.0) | |
| | | 21 °C, 3 d | 1.4 (1.2 to 1.5) | |
| | FCV | 4 °C, 6 d | >1.5 | Mattison and others (2007) |
| Raspberry | MNV | 4 °C, 7 d | 0 | Verhaelen and others (2012) |
| | | 10 °C, 7 d | 0.5 (0.3 to 0.6) | |
| | | 21 °C, 3 d | 1.1 (0.8 to 1.4) | |
| | PV | 4 °C, 9 d | 0 | Kurdziel and others (2001) |
| Lettuce | HAV | 4 °C, 7 d | 2.0 | Croci and others (2002) |
| | MNV-1 | 4 °C, 11 d | Approximately 1 | Escudero and others (2012) |
| | PV-1 | 4 °C, 8 d | 0.36 | Yepiz-Gomez and others (2013) |
| | FCV | 4 °C, 7 d | Approximately 2 | Mattison and others (2007) |
| | | RT, 4 d | >2.7 | |
| Spinach | HAV | 5.4 ± 1.2 °C, 14 d | 1.0 | Shieh and others (2009) |

CI, confidence interval; RT, room temperature.
[a]Infectivity was assessed using cell culture.

a study on the persistence of HAV in packaged lettuce, a modified atmosphere did not influence the persistence when incubated at 4 °C. Even a slight improvement in virus persistence on lettuce was observed in the presence of high $CO_2$ levels (70% $CO_2$, 43% persistence) at room temperature (RT) compared to when stored in bags with normal atmospheric conditions (6% persistence) (Bidawid and others 2001). MAP is also applied on berries, however mainly on berries intended to ship fresh for long distances, and not applied in final consumer packages (EFSA BIOHAZ Panel 2014a).

Next to MAP packaging, the antiviral activity of active packaging material consisting of silver-infused polylactide (PLA) films has also been explored on virally contaminated vegetables. However, the efficiency of active packaging based on silver depends very much on the food type, on environmental factors, and on the pathogen. For instance, on paprika no antiviral activity of the packaging towards FCV was observed, while reductions >3.5 $log_{10}$ were observed for FCV on lettuce (Martinez-Abad and others 2013).

By far, the most popular method for storing berries is freezing. In the 2 largest European raspberry producing countries Serbia and Poland, the majority of raspberries (>70% and >90%, respectively), is exported frozen (Djurkovic 2012). Freezing, however, has no pronounced influence on the viral load of fresh produce as no reduction was noted of MNV-1 surrogate on frozen onions and spinach after 6 mo of storage (Baert and others 2008a), and frozen storage for 3 mo had limited effects on HAV and RV persistence in berries and herbs (Butot and others 2008). Cryostability of NoV (GII.4) to freezing and thawing was also observed in a recent study by Richards and others (2012). In general, freezing is actually used as a method for long-term storage of fecal and lysate stocks of enteric viruses in research. Also, during outbreak investigations, according to the CDC updated NoV outbreak management and disease prevention guidelines, food samples strongly suspected as the source of an outbreak of acute gastroenteritis should be stored frozen at −20 °C before analysis for optimal preservation (Hall and others 2011). In conclusion, enteric viruses such as NoV and HAV are expected to persist during the shelf-life (up to 24 mo and more) of frozen fruit and vegetable products and have been implicated in several foodborne outbreaks due to frozen berries (Table 1).

Vacuum freeze-drying is the reference process for manufacturing high-quality dehydrated products to maintain the color, flavor, and most types of antioxidants. The production of freeze-dried produce involves preliminary freezing of fresh produce, followed by placing the produce under reduced pressure with sufficient heat to sublimate ice. In the study of Butot and others (2009), an optimized freeze-drying treatment decreased the HAV infectivity and the human NoV RNA presence on strawberries, raspberries, blackberries, blueberries, parsley, and basil with varied effects (0.6 to 3.5 $log_{10}$ reduction).

Next to temperature, pH has been identified as a principal determinant for the growth of bacteria on fresh produce. Berries have a relative acidic internal pH varying between 2.7 and 4.5, depending on the berry species (Knudsen and others 2001). However, enteric viruses are engineered to survive stomach acid and, hence, long-term persistence (MNV-1, refrigeration temperatures) has been observed in acid conditions such as fruit juices (>21 d) (Horm and D'Souza 2011) and on berries (exceeding the shelf-life) (Verhaelen and others 2012). MNV-1 has proved to be a suitable surrogate for human NoV in acid conditions (Cannon and others 2006; Horm and others 2012a; Seo and others 2012). However, not all surrogate viruses of human NoV are unaffected by low pH. FCV has been observed to be more sensitive to low pH values and, hence, is considered to be a less appropriate surrogate for NoV in acid conditions as in berries and fruit juices (Cannon and others 2006; Duizer and others 2004; Butot and others 2009; Horm and D'Souza 2011).

## Effect of Washing and Sanitation

Besides the removal of dirt, foreign materials and tissue fluids from cut surfaces, washing, rinsing, and spraying are used to reduce the microbial load on fresh produce, while allowing the retention of fresh-like organoleptic properties. Often chemical sanitizers are added to the wash solution in order to maintain the water quality and to increase the reducing effect of the treatment. In this part, efficiency of general washing practices and effect of chemical sanitizers will be discussed. However, comparing the outcome of different studies is not always relevant as several process parameters concerning the experimental set-up, such as treatment time and doses, produce:water ratio, organic load, pH of washing water, and type of produce and virus, can have an influence on the effectiveness of decontamination treatments (Gil and others 2009). An experimental set-up that mimics industrial practices as realistic as possible should be the intention.

### Washing with water

Generally, washing results in ≤1 logarithm decrease (tenfold decrease) in the quantity of viruses detected (Dawson and others 2005; Baert and others 2008b; Butot and others 2008; Li and

others 2011). Minor adaptations to the classic washing step by immersion such as use of bubbling (Fraisse and others 2011) or warm water (43 °C) (Lukasik and others 2003; Butot and others 2008), or the inclusion of hand rubbing (Lukasik and others 2003) did not significantly improve viral reduction on the produce. Household practices such as the addition of salt (2.0% NaCl), liquid dishwashing detergent (0.05%), or use of the consumer-oriented produce wash Fit (Procter and Gamble, contains ethanol, sunflower oil, glycerin, potassium hydroxide, and grape fruit oil) did not have any significant added value for reducing the viral load on strawberries in a study by Lukasik and others (2003).

In Table 3, a selection of available reduction data is given when using tap water or the commonly used chlorine and peroxyacetic acid (PAA) solutions.

## Washing with chlorine solutions

Hypochlorite is, despite its corrosive nature, the most commonly used sanitizing agent and widely applied in food processing. Chlorine preparations are available as solid (calcium hypochlorite: $Ca(ClO)_2$), aqueous solution (sodium hypochlorite: NaOCl), and chlorine gas ($Cl_2$). Chlorine solutions can be either applied by immersion of the food or by spraying. After application of a sanitizer, in spray or in the form of a bath, rinsing or a final wash of the fresh produce in potable water is compulsory to remove any residual chemical and/or by-products. In order to maximize the efficacy of chlorine disinfection, the concentration of free chlorine (FC), the pH (ideal pH 6 to 7), and the organic load (COD level) of the wash water must be controlled.

Generally applied chlorine dosages and contact times by produce processors are 50 to 200 ppm (mg/L) for a maximal contact time of 1 to 2 min, leading to typical $\log_{10}$ reductions of 1 to 2 logs for bacteria and viruses on fresh produce (Casteel and others 2008; Predmore and Li 2011; Goodburn and Wallace 2013). The effectiveness of chlorine in virus inactivation on produce can vary according to the virus under study (Butot and others 2008; Fraisse and others 2011) and according to the type of produce (Butot and others 2008). In spite of the rather modest viral reductions on fresh produce obtained using chlorine, chlorine is much more effective for inactivation of viral pathogens in suspension (wash water) than for removal of these pathogens from fresh produce (Dawson and others 2005). This reasoning also applies to bacteria. Hence, despite the general idea that sanitizers are used to reduce the microbial population on the produce, their main effect is maintaining the microbial quality of the water (Gil and others 2009). Hence, the use of wash water sanitizers is highly valuable to reduce cross-contamination from one contaminated crop/batch to the other crops/batches present in the washing bath.

The drawback for the use of chlorine is that this biocide is highly corrosive for the stainless steel surfaces commonly used in the food industry, and its efficacy is negatively influenced by the organic load of the wash water. Also, the formation of by-products in the wastewater, such as trihalomethanes (THMs), has been frequently cited as the downside of using chlorine and is the reason for the continuous search for new alternatives for disinfection (Fraisse and others 2011). These by-products are formed by reaction of the chlorine disinfectant with organic matter in the wash bath. Despite the occurrence of the formation of THMs in the process wash water, no residue can be found in vegetable tissue after rinsing with tap water (Lopez-Galvez and others 2010; Gomez-Lopez and others 2013). When good practices are applied (hence control of COD, FC, and regular refreshing of washing water), chlorine-based sanitizers such as chlorine gas, sodium hypochlorite, and calcium hypochlorite can be safely used to wash fresh produce, in spite of the formation of THMs in the water. As such, suggestions that the industry should move away from this traditional disinfection agent are unreasonable (Gil and others 2009).

Another chlorine-containing disinfectant used in food production and processing is chlorine dioxide ($ClO_2$). Advantages of $ClO_2$ in comparison to the classic chlorine-containing disinfectants is that no formation of THM compounds occurs in the presence of organic matter (Lopez-Galvez and others 2010); and this sanitizer is little affected in its effectiveness by pH and the presence of high amounts of organic matter (Hirneisen and others 2010). However, application of $ClO_2$ in the United States is restricted for use in washing whole fruits and vegetables and, hence, not permitted for disinfection of fresh-cut fruits and vegetables (Hirneisen and others 2010). Other restrictions for the use of chlorine dioxide are that it must be generated on site due to its instability and that it can be explosive when concentrated. Concentrated solutions of sodium chlorite are on the market, for example, Carnebon 200 (Intl. Dioxide Inc., Clark, N.J., U.S.A.) and Oxine (Bio-Cide Intl., Inc., Norman, Okla., U.S.A.), that upon acidification generate "stabilized chlorine dioxide" (Lukasik and others 2003). However, the effectiveness of $ClO_2$ at the recommended low concentrations for usage by the FDA (max. 5 mg/L or ppm), is rather low (only about 1 $\log_{10}$ reduction) for FCV and HAV, even at the rather extensive contact times (10 min) tested by Butot and others (2008).

## Washing with other chemical agents

Next to chlorine-containing solutions other chemical agents have been tested for their effectiveness in reducing the viral load of fresh produce during washing: peroxyacetic acid solutions (PAA) (equilibrium mixture of hydrogen peroxide and acetic acid) (as in Allwood and others 2004; Baert and others 2009; Fraisse and others 2011), the use of liquid or vaporized hydrogen peroxide ($H_2O_2$) (as in Lukasik and others 2003; Li and others 2011), ozone ($O_3$) (as in Hirneisen and others 2011; Hirneisen and Kniel 2013b), trisodium phosphate (TSP; $Na_3PO_4$) (as in Lukasik and others 2003; Su and D'Souza 2011), and surfactants (as SDS in Predmore and Li 2011). Next to chemicals, also the antiviral properties of natural biochemical substances such as grape seed extract (GSE) in wash water (Li and others 2012; Su and D'Souza 2013) and sprays of essential oils (Azizkhani and others 2013) have been explored. Each of these tested substances has its own merits, limitations, and drawbacks. The effects of some promising combinations of different chemicals or inactivation strategies have been studied on fresh produce. As such, the use of the combination of the surfactant SDS (50 ppm) and chlorine (200 ppm) enhanced the efficiency of virus removal and inactivation (MNV) resulting in a reduction of up to 3 $\log_{10}$ for lettuce, strawberries, and raspberries (2 min, RT) (Predmore and Li 2011). Also, a synergism was reported between the use of vaporized $H_2O_2$ and UV light on lettuce (Xie and others 2008; Li and others 2011).

In conclusion, good practices for the washing of fresh produce require the use of a sanitizer. Sanitizers (such as Chlorine or PAA), however, are generally more effective in viral reduction in suspension (wash water) than on surfaces such as on fresh produce (Dawson and others 2005) as only marginal reductions of the viral load are obtained on fresh produce (1 to 2 $\log_{10}$). Even the introduction of multiple washing steps performed in series (Baert and others 2008b), extended contact times (Xie and others 2008), or the use of increasing concentration of disinfectants (Butot and others 2008), will not necessarily lead to significantly higher

 

Table 3–Subselection of available literature presenting the effectiveness of commonly used decontamination processes on the viral load of soft red fruits and leafy greens.

| Decontamination procedure (produce, g: water, mL ratio) | | Virus | Fresh produce | $Log_{10}$ reduction | Reference |
|---|---|---|---|---|---|
| Tap water | 0.5 min (15 g : 200 mL) | HAV | Strawberry, raspberry<br>Basil, parsley | 0.8, 0.6<br>1.1, 0.5 | Butot and others (2008) |
| | 2 min (10 g : 350 mL) | MNV-1 | Spinach leaves | 1.0 | Baert and others (2008b) |
| | 5 min (50 g : 500 mL) | MNV-1 | Lettuce | 1.1 | Baert and others (2009) |
| | 2 min, RT (50 g : 2000 mL) | MNV-1 | Strawberry, raspberry | 0.8, 1.2 | Predmore and Li (2011) |
| | 2 min, RT (50 g : 4000 mL) | | Cabbage, lettuce | 0.6, 0.2 | |
| Chlorine solutions | NaOCl 200 ppm, 0.5 min (15 g : 200 mL) | HAV | Strawberry, raspberry<br>Basil; parsley | 1.8, 0.6<br>2.4, 1.4 | Butot and others (2008) |
| | $ClO_2$ 5 ppm, 10 min (15 g : 200 mL) | HAV | Raspberries<br>Parsley | 1.0<br>1.1 | |
| | NaOCl 200 ppm, 5 min (50 g : 500 mL) | MNV-1 | Lettuce | 2.1 | Baert and others (2009) |
| | Chlorine 200 ppm, 2 min, RT, (50 g : 2000 mL) | MNV-1 | Strawberry, raspberry | 1.0, 1.5 | Predmore and Li (2011) |
| | Chlorine 200 ppm, 2 min, RT, (50 g : 4000 mL) | MNV-1 | Cabbage, lettuce | 1.3, 1.1 | |
| PAA | 80 ppm; 250 ppm, 5 min (50 g : 500 mL) | MNV-1 | Lettuce | 1.9, 2.5 | Baert and others (2009) |
| | 100 ppm, 2 min (25 g : 500 mL) | MNV-1<br>HAV | Lettuce | 2.4<br>0.7 | Fraisse and others (2011) |
| | 100 ppm, 2 min, 43 °C (150 g : 2000 mL) | MNV-1 | Strawberry | 1.8 | Lukasik and others (2003) |

PAA, peroxyacetic acid; RT, room temperature.

reductions of the viral load of fresh produce. Similar to bacteria, viruses can be located in protective sites on the produce, such as the stomata or the cut edges, not accessible during washing procedures and most decontamination processes. As such, the use of sanitizers during the wash process is primarily to maintain the microbial quality of the wash water and hence to limit the possibility of cross-contamination. Nonetheless, reporting of the effectiveness of the sanitizer under study in reducing the viral load in the resulting wash water is not always included. However, the latter is important to judge the utility for any sanitizers as mitigation strategy for cross-contamination. Good practices are also required to limit internalization of pathogens by avoiding influx of potentially contaminated wash water into the produce. Therefore, a higher temperature of the washing solution than the temperature of the produce is demanded, as if the reverse is true, air bubbles inside the fresh produce will shrink upon contact with the cold water, resulting in a partial vacuum causing wash water to enter the tissue through pores, channels, or punctures (Holvoet 2014).

## Effect of Alternative Strategies for Decontamination

In this section the effect of radiation, both nonionizing and ionizing radiation, and high-pressure processing (HPP) will be discussed as nonthermal inactivation treatment options for enteric viruses in fresh produce. Both irradiation with ionizing radiation and appropriate use of HPP effectively inactivate both surface and internalized viruses.

### Effect of radiation

Both ionizing and nonionizing radiation have been tested as disinfection strategies for vegetables contaminated with viruses. The most widespread used form of nonionizing radiation for decontamination is the *UV light* (100 to 400 nm). UV disinfection primarily occurs due to the germicidal action of UV-B (280 to 315 nm) and UV-C light (200 to 280 nm) on microorganisms (US EPA 2006). Most studies use low-pressure (LP) mercury lamps with a major wavelength output (85%) at 253.7 nm (monochromatic UV radiation) (Hijnen and others 2006; Eischeid and others 2011). Inactivation by this ultraviolet range is based on the damage caused to the nucleic acids (DNA/RNA) of the cell or virus, for which the UV absorbance peaks near 260 nm. Among food- and waterborne pathogens, viruses are generally more resistant than protozoa, such as Cryptosporidium and Giardia, and the bacterial pathogens (Hijnen and others 2006). Adenoviruses are the most UV-resistant class of viruses presently known and are, therefore, used as a standard for viral inactivation requirements in, for example, water disinfection (Eischeid and others 2011). Concerning fresh produce, UV light (dose: 40 to 120 mW s/cm²) was shown to be effective in the reduction of HAV and FCV on lettuce and green onions, resulting in reduction of 4 to 5 $log_{10}$ for HAV and 2.5 to 4 $log_{10}$ for FCV. However, on strawberries significantly lower reductions were observed for both viruses (<2 $log_{10}$). In a study by Hirneisen and Kniel (2013b), however, MNV-1 proved to be more resistant to UV light, as a dose of 240 mW s/cm² resulted in a mere reduction of about 1.2 $log_{10}$ on green onions. As such, the food matrix and surface typography play an obvious role (Fino and Kniel 2008). Also, a wide variation in viral sensitivity to UV has been recognized (Eischeid and others 2011), making it impossible to estimate the possible influence on human NoV.

Another disinfection strategy using nonionizing radiation is the use of pulsed light (PL) treatment. PL is a modified and possible improved version of delivering UV-C to bodies, using xenon lamps to deliver short-time pulses of an intense broad spectrum (200 to 1100 nm) rich in UV-C light. PL treatment is a relatively new technology and only one study was identified applying PL treatment on produce. In this study the effectiveness of 10 to 30 pulses (300 $\mu$s each, fluence of 0.94 J/cm²/pulse) was tested on MS2 inoculated on black pepper, chopped mint, and garlic powder. However, only marginal reductions were obtained (generally <0.5 $log_{10}$ reductions, except for mint 1.3 $log_{10}$ reduction) in comparison to the reductions obtained in viral suspension after merely 4 pulses (>8 $log_{10}$ reduction) (Belliot and others 2013).

The downside of the use of nonionizing radiation compared to ionizing radiation is the superficial character of UV treatment. The light should be able to reach all surfaces of the product, but internalized microorganisms would be unaffected due to the light absorption by the surface. Therefore, this treatment option is quite impractical for decontamination of lettuce on an industrial scale.

Ionizing radiation is radiation that carries enough energy to liberate electrons from atoms or molecules, thereby ionizing them. Ionizing radiation used in food processing can be electromagnetic radiation (gamma rays and X-rays) or particulate radiation

(electron beam). With the first type, $\gamma$-rays are produced from a radioactive source (Co-60 or Cs-137), the other 2 (X-rays and e-beams) require specific equipment converting other energy sources, such as an electric current, without the involvement of any radioactive substance. As such, in the latter 2 cases the producing equipment can be switched on or off depending on the need. The application of X-rays will not be further discussed as X-rays never found application in commercial food irradiation (RIVM 2013). Electron beam irradiation is a relatively new technology. In contrast to $\gamma$-rays and X-rays, an electron beam's main disadvantage is poor penetration power. However, for irradiation of, for example, prepacked salads the penetration depth might be sufficient if the produce is irradiated from 2 or more sites (Niemira 2003). Studies using electron beam (Sanglay and others 2011; Espinosa and others 2012) and $\gamma$-rays (Bidawid and others 2000; Hsu and others 2010; Feng and others 2011) for decontamination of viral contaminated fresh produce are available in the literature.

Viruses, having relatively little nuclear material and being small "targets," are relatively resistant to radiation compared to most vegetative bacteria ($D_{10}$ values of 0.14 to 0.80 kGy) (EFSA 2011). Reported $D_{10}$ values for enteric viruses/surrogates are, for example, 2.97 kGy for HAV on strawberries ($\gamma$-rays) (Bidawid and others 2000) and 2.95 kGy for FeCV on lettuce (e-beam) (Zhou and others 2011). However, $D_{10}$ values are affected by a number of factors including temperature, water activity, and chemical composition of the food (EFSA 2011).

For Europe, the Scientific Committee on Food (SCF) has expressed several opinions on irradiated foods and acceptable doses for specific food classes/commodities (including 1986, 1992, and 1998). As such, for vegetables and for fruits, overall average radiation doses (kGy) of up to 1 and up to 2 kGy, respectively, were evaluated as acceptable. However, as regulated in the EU by Framework Directive 1999/2/EC and Implementing Directive 1999/3/EC, so far only "dried aromatic herbs, spices and vegetable seasoning" at the maximum overall absorbed radiation dose of 10 kGy are allowed (EFSA 2011). Currently, the U.S. FDA approves doses up to 4 kGy to control foodborne pathogens in fresh iceberg lettuce and spinach (FDA 2015) as a response to 3 multistate outbreaks of *E. coli* O157:H7 traced to spinach and lettuce (CDC 2008). However, this irradiation dose proved impractical for the inactivation of viruses on fresh produce as only $<2$ $\log_{10}$ virus reduction of MNV-1 (4 kGy, $\gamma$-rays) was achieved on spinach, romaine lettuce, and strawberries in a study by Feng and others (2011), and a mere reduction of $\leq 0.70$ $\log_{10}$ of MNV-1 was achieved on cabbage and strawberries in a study of Sanglay and others (2011) (4 kGy, e-beam). Hesitant consumer acceptance toward irradiated food, the doses required for a meaningful reduction of viruses typically exceed legally approved doses, and what most produce will tolerate in terms of changes in appearance, flavor, color, and texture (Fan and others 2008) are still drawbacks that must be addressed.

## Effect of high-pressure processing

High-pressure processing (HPP) is a nonthermal operation that inactivates pathogenic and spoilage microorganisms as well as endogenous enzymes and has been used as a "cold pasteurization" method for fruit juices, fruit desserts, avocado-based products, sliced onions, and ready-to-eat vegetable dishes (Kingsley 2013; RIVM 2013). Pressures up to 1000 MPa are used that are instantaneously and uniformly transmitted throughout a sample, thus making this process independent of the shape or size of the food (Kovac and others 2010; Kingsley 2013).

In research the effectiveness of HPP for viral inactivation has been tested on fresh produce matrices such as green onion slices (Kingsley and others 2005), carrot juice, lettuce, blueberries (Li and others 2013b), blueberry juice (Horm and others 2012a), orange juice (Horm and others 2012b), and different purees such as strawberry puree, lemon puree, tomato puree, watermelon puree, and carrot puree (Lou and others 2011). Although the resulting characteristics of the treated products are superior compared to heat-treated products, still HPP has been shown to affect sensory qualities such as color, texture, shape, and rheological properties. However, these variable effects on the sensor quality of fresh produce are depending on the pressure level and type of product (Kovac and others 2010; Lou and others 2011). As such, HPP has been recommended for the processing of fruits intended for frozen storage, since freezing causes similar and more severe texture damage (Lou and others 2011; Li and others 2013b). Fresh produce-related products such as purees, sauces, and juices are also fit for usage of HPP, as compared to intact fresh produce as they lack the presence of intercellular air spaces that can be severely compressed during pressure treatment, inducing physical damage to the tissue (Li and others 2013b).

Next to the treatment parameters such as pressure levels and treatment time, the matrix also can have a significant influence on the effectiveness of HPP (Kingsley and others 2005; Lou and others 2011; Kovac and others 2012). Some parameters, such as temperature and pH (Kingsley and Chen 2009; Lou and others 2011; Li and others 2013b), were shown to influence the HPP inactivation of different types of viruses, in a contradictory way. For example, colder initial temperatures of the product enhanced the inactivation of human NoV (GI.1) (Leon and others 2011) and surrogates MNV-1, TV, and FCV (Chen and others 2005; Li and others 2013b). In contrast, HAV, a picornavirus, is more resistant to HPP at a lower temperature than at room temperature (Kingsley and Chen 2009). The same for the parameter pH, where human NoV (surrogates) tend to be more sensitive to HPP at neutral pH than at acidic pH (Lou and others 2011; Li and others 2013a). Whereas for HAV the opposite is true (Kingsley and Chen 2009). As such, direct validation of HPP conditions within the food or food matrix will be required, given the complexity of food matrices and the variable response of different viruses (Kingsley 2013).

Among enteric viruses a high variability in pressure resistance has been noted, even different virus strains can behave differently under pressure (Shimasaki and others 2009). As such, it is conceivable that different human NoV genogroups, and perhaps different clusters within a human NoV genogroup, would exhibit varied sensitivities to HPP (Leon and others 2011). During testing, human NoV surrogates FCV and TV proved to be more susceptible to HPP compared to MNV-1 (Horm and others 2012a; Li and others 2013b). In the study by Lou and others (2011) on the application of HPP in the fresh produce industry, the optimal condition for MNV-1 inactivation by HPP in diverse fresh produce matrices was determined to be refrigeration temperature with a treatment pressure of 450 MPa and a holding time of 2 min. Using these conditions viral reductions between 4.7 and 7.0 $\log_{10}$ were obtained without significantly altering the physical quality of the food samples (Lou and others 2011). However, when the inactivation kinetics of MNV-1 (cell-culture) are compared to human NoV GI.1 (obtained during a human feeding study using infected high-pressure processed oysters), human NoV might be more

 

resistant to HPP than MNV-1. As in the human feeding study by Leon and others (2011) treatment of oyster (seeded by injection) by HPP at 400 MPa, for 5 min at 6 °C was insufficient to prevent NoV infection in human volunteers, suggesting that 4 $\log_{10}$ genome equivalent reduction was not achieved. While a 5 min, 400 MPa treatment at 5 °C was sufficient to inactivate 4.1 $\log_{10}$ PFU MNV-1 in oyster tissue (Kingsley and others 2007). In the human volunteer study a 600 MPa treatment for 5 min at 6 °C was successful to inactivate human NoV GI.1 within raw oysters (Leon and others 2011). This higher resistance of human NoV to HPP was also observed when binding assays, using porcine gastric mucin-conjugated magnetic beads followed by RT-qPCR assays, were used for discriminating potentially infectious human NoVs GI.1 (Dancho and others 2012) and GII.4 (Li and others 2013a) following HPP.

## Effect of Thermal Treatment

In food processing thermal processing is a classic inactivation strategy which involves heating of a food product at a temperature that ranges from 50 to 150 °C, primary to inactivate foodborne pathogens and to inactivate endogenous enzymes. In light of the main identified food commodities of concern, namely soft red fruits and leafy greens, this section will be restricted to the effect of classic heat treatment (pasteurization) and blanching, since heating of frozen berries has been regularly communicated as a mitigation strategy for foodborne outbreaks due to viral-contaminated berries (Guzman-Herrador and others 2014). The possible effectiveness of this measure was illustrated during the German outbreak in 2012 due to contaminated frozen strawberries. During the outbreak investigation it was noted that not all kitchens which used the implicated batch of frozen strawberries were linked to disease cases. This was a result of the different ways of preparing the strawberry compote among kitchens. As such, it was observed that those places receiving meals from kitchens where the strawberry compote was stewed (thoroughly cooked) were not affected by outbreaks. The schools and childcare facilities that received the compote from kitchens that did not sufficiently heat the compote were indeed obviously associated with disease cases (Task Force gastroenteritis 2012).

In the literature, an overall lower virus sensitivity to temperature change has been noted in complex matrices (such as dairy and other food products) compared to simple matrices (for example, drinking water and synthetic media) at the high temperature range (>50 °C) (Bertrand and others 2012). Hence, validation of a specific heat treatment in the relevant food matrix is well-considered. However, in the literature only a limited number of heat inactivation studies are available for produce matrices. Relevant matrices used for traditional heat inactivation experiments of enteric viruses/surrogates are restricted to purees of soft red fruits (raspberry, strawberry, bilberry) (Deboosere and others 2004, 2010; Baert and others 2008a) and spinach (Bozkurt and others 2014a).

The risk of NoV infection remains associated with mildly pasteurized (30 s at 65 °C and 15 s at 75 °C) raspberry puree, since reductions of less than 3 $\log_{10}$ were obtained for MNV-1 (Baert and others 2008a). The inadequacy of mild heat treatment steps at low temperatures can also be confirmed by a human challenge study in which human NoV was found to remain infectious for volunteers after 30 min at 60 °C (Dolin and others 1972). Next to the virus type, matrix factors such as pH and sugar content have been confirmed to have a significant effect on heat inactivation of enteric viruses and surrogates. As such, studies are available that observed a rise in inactivation time of HAV (in strawberry mashes) and MNV-1 (in PBS), with increasing sucrose concentration and observed a moderate rise in inactivation time of HAV (in strawberry mashes) with increasing pH. Attempts have been made to model the heat inactivation of HAV in berry mashes as a function of temperature and product characteristics, such as pH and sugar concentration (Deboosere and others 2004; Deboosere and others 2010). However, validation of the model by Deboosere and others (2004) in fruit-based products failed. Weaknesses of the latter model of Deboosere and others (2010) are the limited temperature range (65 to 75 °C) and the inclusion of the come-up time (about 2 min) in the treatment time. This practice assumes that the temperature during this come-up time was constant and at the target temperature, and possibly explains the occurrence of a shoulder in the inactivation curves and the very low log reduction estimates when the model was used to calculate the effect of short heat treatments (0.02 and 0.16 $\log_{10}$ reduction at 30 s and 1 min at 75 °C and pH 2.5, respectively). In contrast, in-house data on MNV-1 heat inactivation in raspberry puree (75 °C, 30 s) suggest a reduction of ≥4.29 $\log_{10}$ (unpublished data). As such, there is a need for additional studies that take into account heat-inactivation kinetics during the phase of temperature increase to reach the target temperature (Deboosere and others 2010) or models that do not include the preheating step at all. As in validation of time/temperature treatments in food processing, generally preheating and longer exposure to these temperatures during cooling down are not included to assume a worst case scenario in which the reduction solely originates from the actual heat treatment (Baert and others 2008a).

Another relevant thermal treatment process is blanching. Blanching is a heat pretreatment (between 75 and 105 °C) that is generally conducted prior to freezing and canning to inactivate microorganisms and enzymes and to remove entrapped air. Both the hot water bath blanching process and steam blanching have been proven to be effective. As such, a reduction of ≥2.44 log MNV-1 (detection limit of assay was reached) was observed when fresh spinach was treated for 1 min in a hot water bath of either 80 °C or 90 °C (Baert and others 2008b). Confirmation of the effectiveness of (steam) blanching was provided in a study on fresh herbs such as parsley, basil, mint, and chives. Generally >3 $\log_{10}$ reductions were observed for HAV and FCV on fresh herbs when blanched at 95 °C for 2.5 min. When blanching was performed at 75 °C for 2.5 min, more variation in heat resistance of enteric viruses was observed, varying depending on the herb (e.g., HAV reduction on mint and chives was 1.7 and >3.0 $\log_{10}$, respectively) (Butot and others 2009).

In general, heat inactivation studies indicate that mild thermal inactivation methods (such as pasteurization) may not be stringent enough to eliminate human NoV (Baert and others 2008a; Escudero-Abarca and others 2014). However, cooking procedures in which an internal temperature of the food reaches at least 90 °C for 90 s are considered adequate treatments to destroy viral infectivity in most foods (FAO/WHO 2012). Following the recommendation for heat treatment of shellfish (90 °C for 90 s), HAV was successfully inactivated in shellfish (Hewitt and Greening 2006). Several other thermal studies suggest that high-temperature, short-time treatments (90 °C, 30 s) should suffice for inactivation (>4 $\log_{10}$ reduction) of human NoV (surrogates) (Bozkurt and others 2014b; in-house data on MNV-1 reduction in raspberry puree: ≥4 $\log_{10}$ for 95 °C, 30 s). Nevertheless, data concerning heat treatments of produce at temperatures >75 °C are scarce and only available for a limited number of surrogates (Deboosere and others 2004 for HAV). Hence additional relevant heat inactivation

studies for this high-temperature range in relevant produce matrices and for several (surrogate) viruses should be conducted to obtain more insight.

## Conclusions

Overall, human NoVs are introduced in the fresh produce chain by human fecal pollution, and food handlers are believed to play a significant role. The high persistence of NoV in the environment combined with high resistance of NoV to commonly used decontamination practices (washing) of fresh produce, ensures the persistence of NoV between contamination and consumption due to the relative short shelf-life of fresh produce. As such, effective control strategies need to focus on prevention of contamination and to limit cross-contamination. The most important routes identified in this review are contaminated food handlers, justifying the need for creating awareness on the issue of NoV and HAV and education of food handlers in good hygienic practices. In addition, contaminated irrigation water and process water have been shown to be relevant viral contamination routes of fresh produce. This introduces the need for assessment of the risk associated with the irrigation water source used, the implementation of proper water treatment options, and, in the case of washing of fresh produce, the inclusion of good practices, including the correct use of sanitizers in the wash process.

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## Author Contributions

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