Effects of treatments used in food processing on viruses

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Summary

This chapter gives an overview of the effect of different types of food processes (heat, mild food processing and preservation techniques) on foodborne viruses. Inactivation and survival data are presented for the foodborne viruses of major health concern such as *Norovirus*, hepatitis A virus, hepatitis E virus and avian influenza virus, although the latter is not proven to be foodborne. It is important to note that many of these viruses cannot be grown in routine tissue culture and that inactivation data rely on the use of model surrogate viruses. Heat represents the most efficient treatment to inactivate foodborne viruses and as a general rule, the higher the temperature, the higher and the faster the reduction in viral infectivity. Temperatures ≥90 °C are effective in inactivating the most heat resistant enteric viruses, even in complex matrices such as shellfish and meat. Mild food preservation processes such as washing (including shellfish depuration and relaying), acidification, modified atmosphere packaging, ultrasound-based treatments and pulsed electric field were shown to have only a marginal effect on the viral load. Storage in cool conditions, freezing and freeze-drying had no significant inactivation effect. High hydrostatic pressure processing was shown to be a promising technology especially in shellfish, as treatment conditions were found which enabled >3 log reduction while the organoleptic quality could be retained. UV treatment can be useful to inactivate viruses in water, but has limited application in the food industry because UV does not penetrate foods. The use of ionizing radiation appears very limited, as irradiation doses (gamma and electron beam) required to achieve a significant inactivation of enteric viruses are well beyond the current maximum dose allowed by the food and drug administration for fresh produce.

Keywords: foodborne viruses, inactivation, preservation steps, reduction, surrogate virus

1. Introduction

Foodborne viruses are an emerging food safety concern and represent a major cause of outbreaks of gastroenteritis. The characteristics of foodborne viruses present new challenges for the food industry. Viruses need to enter living cells in order to replicate. Unlike bacteria, viruses cannot grow in food nor cause deterioration of the product. Consequently, the organoleptic properties of the food will not change due to viral contamination.

As viruses do not grow in food, it is questionable if control measures aiming at microbial growth inhibition are effective to reduce the level of viruses, if present. Hence there is the

need for the food industry to assess whether control measures in place for bacterial hazards require adjustments to be effective against viruses.

This chapter gives an overview of the resistance of viruses subjected to different types of food processes: heat (Section 2); mild food processing and preservation steps (Section 3) and alternative preservation techniques (Section 4). As the matrix plays an important role in their resistance, a range of matrices such as water, shellfish, fresh produce and meat is discussed.

The effects of treatments used in food processing are considered for viruses that are, or have the potential to be transmitted to humans via food. It mainly focuses on the virus-commodity combinations of highest public health concern, namely *Norovirus* (NoV) and hepatitis A virus (HAV) in water, shellfish and fresh produce. Additionally, human *Rotavirus* (HRV) and other enteroviruses are included. Information is also provided regarding the inactivation through food processing of other viruses, such as hepatitis E virus (HEV) and avian influenza viruses (AIV) in animal products, as food might be a vehicle of these viruses to cause infection in humans.

Since enteric viruses must survive the enzymatic and extreme pH conditions of the gastrointestinal tract to infect a host, they tend to persist in the environment and to resist a wide range of commonly used food processing, preservation and storage treatments. In contrast, enveloped viruses, such as AIV, surrounded by a lipid membrane must remain wet and are generally transmitted in fluids, respiratory droplets, blood and tissue and are less resistant to harsh environmental conditions.

To definitely establish the efficacy of commonly used food processing, preservation and storage treatments on virus infectivity, quantitative assays capable of discriminating viable from non-viable (or inactivated) viruses are necessary. It is important to note that data on inactivation and persistence of NoV strains have to be inferred using model surrogate viruses, such as feline calicivirus (FCV), murine norovirus (MNV) or MS2 bacteriophage, as NoV strains cannot currently be grown in routine tissue culture, making it impossible to test the effects of different processes on their infectivity. Infectivity assays are available for HAV, FCV, MNV, MS2, human adenoviruses, HRV, AIV and many enteroviruses. Consequently, reduction levels presented in the following sections are expressed in $\log_{10}$ reduction of infectious units from inactivation studies using viruses for which infectivity assays are available, except for HEV where a swine bioassay was used.

2. Effect of heat

The effect of heat upon viral reduction is illustrated in this section and sub-divided into the effect of high temperatures (≥90 °C) (Section 2.1), intermediate temperatures (<90 °C and ≥70 °C) (Section 2.2) and low temperatures (<70 °C) (Section 2.3) on viruses.

2.1 High temperatures (≥90 °C)

It is widely accepted that boiling water (rolling boil for 1 minute minimum) has a very high effectiveness (>4 log reduction) in killing viruses which are transmitted by contaminated water such as enteroviruses, HRV, NoV, HAV and HEV (Table 1) (Centers for Disease Control and Prevention, 2009).

For other matrices more time might be needed to achieve a significant kill (>3 log reduction). Shellfish in particular appear to be a protective matrix for viruses. A study using New Zealand greenshell mussels showed that 3 min in boiling water was required to reach a 3.5 log reduction in viable HAV (Table 1) (Hewitt and Greening, 2006). In this study, the mean maximum internal temperature reached on boiling was 97 °C after 4 min of immersion. To achieve and maintain an internal temperature of 90 °C for 90 s, as recommended in the UK Ministry of Fisheries directive (Waterman, 2001) and the Codex Guidelines on the application of general principles of food hygiene to the control of viruses in food (Codex Alimentarius Commission, 2012), the authors showed that mussels would generally need to be cooked in boiling water for an average of 4 min 20 s, consisting of 170 s to achieve 90 °C and a further 90 s maintenance at this temperature.

In comparison, Slomka and Appleton (1998) used FCV as a model virus for heat inactivation of caliciviruses such as NoV in shellfish. Cockles contaminated with FCV were immersed in boiling water. A 2 log reduction of the FCV titer was observed after only 30 s and FCV could not be cultured from cockles which had been immersed in boiling water for 1 min or longer (Table 1).

Table 1. Effect of high temperatures (≥90 °C) on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	High temperature	**Water**		
Enterovirus, HAV, NoV, HRV	Rolling boil for 1 min minimum	Drinking water	>4	CDC, 2009
		Shellfish		
FCV	Boiling for 1 min	Cockles	>4	Slomka and Appleton, 1998
HAV	Boiling for 3 min	Greenshell mussels	>3.5	Hewitt and Greening, 2006
		Fresh produce		
FCV	Steam blanching at 95 °C for	Basil	>4	Butot *et al.*, 2009
HAV	2.5 min		>3	
	Heated to 100 °C for 20 min	Freeze-dried berries	>4	
		Meat/eggs		
HEV	Heated to 95 °C for 5 min	Pig liver	Complete inactivation	Feagins *et al.*, 2008

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

FCV = feline calicivirus; HAV = hepatitis A virus; HEV = hepatitis E virus; HRV = Rotavirus; NoV = Norovirus.

Taken together, the results from the two studies indicate that FCV is less heat resistant than HAV in shellfish. However, it is unknown if the different shellfish species (greenshell mussels and cockles) have a different protective effect on viruses and if, as an example, FCV had been tested in mussels, it would have shown a slightly higher heat resistance in mussels compared to cockles.

Another widely used industrial food preparation process involving heat is blanching which consists of scalding fruits, vegetables or herbs in boiling or steaming water for a short time. This process retains flavour, colour and texture of the produce by stopping enzyme action, before freezing or dehydrating the product. Steam blanching of various herbs at 95 °C for 2.5 min showed inactivation of both HAV and FCV in these matrices. As an example, in basil FCV and HAV titres were reduced by 3 and 4 log, respectively (Table 1). Similar results were obtained for chives, mint and parsley (Butot *et al.*, 2009).

Freeze-drying is a mild process widely used for manufacturing high-quality dehydrated products and is discussed in more detail in Section 3.5. Due to the high resistance of HAV on freeze-dried berries, freeze-dried products were subjected to various dry heat treatments. In most freeze-dried berries (blackberries, raspberries and strawberries) the HAV infectious titer was reduced to undetectable levels after heating for 20 min at 100 °C (Table 1), except in blueberries, where heating to 120 °C was required (Butot *et al,.* 2009). These heat treatments appear very harsh compared to the conditions needed to inactivate HAV during blanching and might well reflect the difference between wet and dry heat.

In contrast to other enteric viruses, HEV can be transmitted through contaminated undercooked meat products. A study using HEV-positive pig livers showed that infectious HEV in this matrix could be inactivated by stir-frying at 191 °C for 5 min or boiling in water for 5 min (Feagins *et al.*, 2008). As shown in Table 1, the inactivation of HEV in pig liver cannot be expressed in log reduction, since reliable cell culture assays for HEV propagation are not currently available. The inactivation of HEV is assessed in a swine bioassay measuring seroconversion.

2.2 Intermediate temperatures (<90 °C and ≥70 °C)

Intermediate temperatures are frequently applied in the food industry to blanch vegetables or pasteurize fruit-based products and these processes are often limited to short times and rather low temperatures to maintain flavour, colour and nutritional qualities.

In water, both HAV and MNV showed inactivation of greater than 3.5 log after 1 min at 72 °C (Table 2). Interestingly, a similar reduction in infectivity was observed for both viruses in milk, showing that under the conditions tested no protective effect of milk on viruses could be demonstrated (Hewitt *et al.*, 2009).

The study using New Zealand greenshell mussels showed that to reach an internal temperature of 72 °C in mussels immersed in boiling water and steam, 90 and 190 s were needed, respectively. A combined treatment of 37 s boil and 180 s steam was necessary to achieve a 2.5 log reduction of HAV (Table 2), showing that the time to reach a certain temperature

Table 2. Effect of intermediate temperatures (<90 °C and ≥70 °C) on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	Thermal treatment	**Water**		
HAV	Heated to 72 °C for 1 min	Water	≥3.5	Hewitt *et al.*, 2009
MNV				
		Shellfish		
HAV	Boiling for 37 s followed by steaming for 180 s	Green shell mussels	2.5	Hewitt and Greening, 2006
		Fresh produce		
FCV	Steam blanching at 75 °C for 2.5 min	Basil, chives	4, >4	Butot *et al.*, 2009
HAV			>1.9, >3	
	Heated to 80 °C for 20 min	Freeze-dried berries	<2	
	Heated to 85 °C for 1 min	Strawberry mashes (28° Brix)	1	Deboosere *et al.*, 2004
	Heated to 85 °C for 5 min	Strawberry mashes (52° Brix)		
MNV	Heated to 75 °C for 15 s	Raspberry puree	2.8	Baert *et al.*, 2008a
	Steam blanching at 80 °C for 1 min	Spinach	≥2.4	Baert *et al.*, 2008b
		Meat/eggs		
AIV H5N1	Heated to 70 °C for 5 s or 73.9 °C for 0.8 s	Chicken meat	11	Thomas and Swayne, 2007
HEV	Heated to 71°C for 20 min	Pork meat	Complete inactivation	Barnaud *et al.*, 2012

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AIV H5N1 = avian influenza virus strain H5N1; FCV = feline calicivirus; HAV = hepatitis A virus; HEV = hepatitis E virus; MNV = murine norovirus.

and subsequently a certain virus kill is both matrix and process dependent (Hewitt and Greening, 2006).

Butot *et al.* (2009) showed that steam blanching at 75 °C for 2.5 min led to significantly less inactivation of HAV compared to FCV, especially in basil (Table 2). Blanching of spinach at 80 °C for 1 min was effective in inactivating MNV and resulted in at least 2.4 log reduction of infectious MNV (Baert *et al.*, 2008b).

As already discussed for high temperatures (Section 2.1) a considerable difference was also observed at intermediate temperatures between dry and wet conditions. Significantly more time was needed to inactivate approximately 2 log HAV in freeze-dried berries (20 min) compared to fresh herbs (2.5 min) (Table 2) (Butot *et al.*, 2009).

Investigating the thermal resistance of MNV during mild pasteurization of raspberry puree Baert *et al.* (2008a) demonstrated that MNV heat treated at 75 °C in raspberry puree was readily

inactivated and showed a 2.8 log reduction after 15 s only (Table 2). In comparison, Deboosere *et al.* (2004) studied the thermal inactivation of HAV in strawberry mashes supplemented with different sucrose concentrations. The $D_{85°C}$ value obtained in the presence of 52° Brix of sucrose was approximately eightfold higher than with 28° Brix. Therefore, more time was required to inactivate 1 log HAV in a matrix containing a high concentration of sugar (Table 2), demonstrating the protective effect of sugar on the thermal stability of HAV and probably other enteric viruses.

Intermediate temperatures (~70 °C) are able to inactivate non-enveloped enteric viruses in meat, but only if the temperature is held long enough. Barnaud *et al*. (2012) showed that heating pork to an internal temperature of 71 °C for 20 min was necessary to completely inactivate HEV (Table 2). In the group of four pigs inoculated with the viral suspension heated to 71 °C for 20 min, none excreted HEV. However, in the viral suspension treated at 71 °C for 5 and 10 min, pigs showed viral excretion at 11 and 18 or 20 days post infection, respectively.

Enveloped viruses such as AIV are readily inactivated by temperatures close to 70 °C. Thomas and Swayne (2007) predicted the time to obtain 11 log reduction of a H5N1 virus strain in chicken meat to be 5.5 s at 70 °C and 0.8 s at 73.9 °C (Table 2).

2.3 Low temperatures (<70 °C)

At lower temperatures, longer holding times are required to achieve a significant inactivation of enteric viruses. Treatment of MNV and HAV (63 °C, 1 min) resulted in only 1.1 and 1.3 log reductions in infectivity in water, respectively. Respective reductions in milk were 1.4 and 1.6 log (Hewitt *et al.*, 2009). However, 5 and 10 min exposures at the same temperature showed >3 log reduction in infectivity for both viruses (Table 3). As for intermediate temperatures, under the conditions tested no protective effect of milk on viruses could be demonstrated at lower temperatures (Hewitt *et al.*, 2009).

Steaming of mussels for approximately 3 min corresponds to a common cooking practice. However, Hewitt *et al*. (2006) showed that New Zealand Green mussels undergoing this treatment reached an internal temperature of 63 °C only, resulting in a <2 log reduction in infectious HAV titres (Table 3).

Low temperatures are commonly used in the food industry for low heat dehydration processes to preserve both fruits and vegetables. The study by Laird *et al.* (2011) demonstrated the effects of this process on inactivating HAV on contaminated green onions. The 20 h heating at 56.4 and 65.9 °C reduced infectious HAV in onions by 1.6 and >3.9 log, respectively (Table 3) and it was concluded that low heat dehydration using 62.5 °C or above could effectively inactivate HAV on contaminated onions by >3 log.

The study assessing the thermal resistance of MNV during mild pasteurization of raspberry puree showed that MNV heat treated at 65 °C in raspberry puree reached a 1.9 log reduction after 30 s (Table 2) (Baert *et al.*, 2008a). However, compared to the study by Laird *et al*. (2011) on dehydrated green onions, it appears that compared to dry conditions significantly less

Table 3. Effect of low temperatures (<70 °C) on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	Thermal treatment	**Water**		
HAV	Heated to 63 °C for 1or 5 min	Water	1.3, ≥3.5	Hewitt *et al.*, 2009
MNV			1.1, 3.1	
		Shellfish		
HAV	Steaming for 3 min	Green shell mussels	<2	Hewitt and Greening, 2006
		Fresh produce		
HAV	Low-heat dehydration at 56.4 °C or 65.9 °C for 20 h	Green onions	1.6, >3.9	Laird *et al.*, 2011
MNV	Heated to 65 °C for 30 s	Raspberry puree	1.9	Baert *et al.*, 2008a
		Meat/eggs		
HEV	Heated to 62 °C for 2 h	Pig liver	Incomplete inactivation	Barnaud *et al.*, 2012
AIV H5N1	Heated to 61.1 °C for 7.7 min	Chicken meat	11	Thomas and Swayne, 2007
AIV H5N2	Heated to 60 °C for 6.2 min	Whole egg blends	13.7	Swayne and Beck, 2004
	Heated to 56.7 °C for 3.5 min	Fresh liquid egg white		
	Heated to 54.4 °C for 2.6 days	Dried egg white (7.5% moisture)	7	Thomas and Swayne, 2009
	Heated to 57.7 °C for 6.3 min	Fat free egg product (FFPE)	5.7	Chmielewski *et al.*, 2011

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AIV H5N1 = avian influenza virus strain H5N1; AIV H5N2 = avian influenza virus strain H5N2; HAV = hepatitis A virus; HEV = hepatitis E virus; MNV = murine norovirus.

time is needed in wet conditions to achieve a similar inactivation, as already discussed for high and intermediate temperatures (Sections 2.1 and 2.2).

Low temperatures ranging from 60 to 65 °C appear inappropriate to inactivate enteric viruses in meat. HEV contaminated pig liver held for 2 hours at 62 °C remained infectious as shown by Barnaud *et al*. (2012) using the swine bioassay. Conversely, these temperatures achieve very high inactivation of AIV in meat. Thomas and Swayne (2007) predicted that the time needed for 11 log reduction of a H5N1 virus strain in chicken meat was 7.7 min at 61.1 °C (Table 3).

Temperatures below 60 °C used in the pasteurization of various commercial egg products which are subsequently used in a large variety of food manufacturing processes are also very efficient and achieve high inactivation rates of AIV. In liquid egg products such as whole egg blends, fresh liquid egg white and fat free egg products (FFEP) (the latter consisting of >98% egg white) heat inactivation of a H5N2 virus strain was shown to be >5 log when applying time-temperatures combinations according to industry pasteurization standards (Froning *et al.*, 2002). H5N2 heat treated at 60 and 56.7 °C in whole egg blends and fresh liquid egg white, respectively, was predicted to reach a 13.7 log reduction after 6.2 and 3.5 min (Swayne and Beck, 2004). In FFEP heating at 57.7 °C for 3.6 min reduced infectious H5N2 by 5.7 log

(Chmielewski *et al.*, 2011). Time-temperatures combinations applied by the industry to dried egg white are longer and were also effective in the inactivation of H5N2. H5N2 heat treated at 54.4 °C in dried egg white with 7.5% moisture was predicted to reach a 7 log reduction after 2.6 days (Table 3) (Thomas and Swayne, 2009). However, the moisture content of dried egg white affects virus inactivation and previous studies using lower moisture content predicted a slower inactivation time (Swayne and Beck, 2004).

Together these studies show that, although AIV are heat labile, the thermal inactivation of these viruses is influenced by factors such as moisture content of the food product and the process time-temperature. Additionally, the thermal stability of the strain or pathotype may also play a role and needs to be taken into consideration.

3. Effect of mild food processing and preservation steps

Mild food processing and preservation steps are used to minimize health risks linked to pathogenic bacteria and to prolong the shelf life of food products. Viruses differ from bacteria and this section gives an overview of the effects of mild food processing and preservation steps on viral inactivation. The efficacy of each process type (washing including shellfish depuration and relaying, cool storage, freezing, pH and other processes applied to fresh produce) is discussed separately.

3.1 Washing

Shellfish naturally concentrate viruses in their digestive tissues by filtering the surrounding water to feed them. When the level of faecal contaminants is exceeded, shellfish suppliers use processes to purge out microbial contaminants by extending the natural filter-feeding processes in clean seawater (relaying in the natural environment or depuration in tanks). Love *et al*. (2010) studied the efficacy of depuration of HAV on oysters (*Crassostrea virinica*) and hard shell clams (*Mercinaria mercinaria*) at various temperatures (12, 18 and 25 °C). After 44 hours of oyster depuration, a higher reduction rate was obtained at 25 °C compared to 12 and 18 °C and this depuration resulted in a less than 2 log reduction. However, 25 °C did not induce faster viral depuration of hard shell clams than 12 or 18 °C. The maximum depuration rate observed with hard shell clams was below 0.5 log (Table 4), suggesting that the depuration efficacy may be shellfish species dependent. Moreover, Love *et al.* (2010) corroborate that enteric bacteria are poor process indicators for depuration of shellfish containing enteric viruses. Depuration and relaying may be improved by optimizing process parameters to enhance NoV reduction (e.g. depuration times, water temperature), but only limited data are currently available (EFSA, 2012).

Decontamination treatments of fresh produce such as berries, vegetables and herbs need to keep the appearance of produce intact. The most commonly used treatment is washing with tap water or water supplemented with chemical agents such as chlorine. Berries and herbs inoculated with HAV and FCV were washed by stirring for 30 seconds in 200 ml of chlorinated water with 200 ppm, free chlorine followed by a rinsing step with 200 ml of tap water (Table 4) (Butot *et al.*, 2008). The results of this study showed significant reductions of

Table 4. Effect of washing on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	Washing	**Shellfish**		
HAV	44 h depuration at 25 °C	Oysters	<2	Love *et al.*, 2010
		Hard shell clams	<0.5	
		Fresh produce		
FCV	Washing with chlorinated water (200 mg/kg)	Berries	>3.5	Butot *et al.*, 2008
		Parsley	>2.7	
HAV		Raspberries	<1	
		Parsley	1.4	
	Washing with tap water	Lettuce	<1	Croci *et al.*, 2002

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

FCV = feline calicivirus; HAV = hepatitis A virus.

FCV in parsley and berries but less than 1 log and 1.4 log reduction of HAV in raspberries and parsley, respectively. This can be explained by the lower resistance of FCV compared to HAV. Chlorinated water had a limited effect on HAV titres when used to decontaminate raspberries and parsley, probably because of their irregular surface protecting enteric viruses. Similarly, washing of lettuce for 5 min with tap water reduced the HAV load by 1 log (Table 4) (Croci *et al.*, 2002).

3.2 Cool storage

Cool storage is commonly used to preserve food and avoid the growth of pathogenic and spoilage microorganisms. Based on the study of Biziagos *et al.* (1988), HAV in mineral water stored for 9 months seems more resistant at 4 °C with an inactivation of 0.5 log compared to room temperature (4.1 log reduction) (Table 5). However, this storage study was performed using mineral water spiked with HAV in sterile plastic bottles and it has been shown that viruses in plastic PET bottles tend to adhere to the bottle wall (Butot *et al.*, 2007).

The cool storage of oysters at 5 °C for 15 days reduced the load of poliovirus (PV) by no more than 1 log (Table 5) (Di Girolamo *et al.*, 1970). As PV is known to be less resistant to environmental conditions than other enteric viruses (Abad *et al.*, 1994), the storage of shellfish will most likely not contribute to reduce the viral load of contaminated shellfish even if the contamination is low.

Croci *et al.* (2002) observed 2 log reduction of HAV in lettuce and >2.5 log reduction in fennel and carrot when stored at 4 °C for 7 days (Table 5). For carrot, the decrease in viral load happened immediately after the contamination and this might be explained by the presence of specific substances having an antimicrobial effect, as described by Babic *et al.* (1994).

Table 5. Effect of cool storage on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	Cool storage	**Water**		
HAV	4 °C for 9 months	Mineral water	0.5	Biziagos *et al.*, 1988
		Shellfish		
PV	5 °C for 15 days	Oyster	<1	Di Girolamo *et al.*, 1970
		Fresh produce		
HAV	4 °C for 7 days	Lettuce	2	Croci *et al.*, 2002
		Fennel and carrot	>2.5	
		Meat /eggs		
AIV H5N1	4 °C for >100 days	Peptone water	Virus survival	Shahid *et al.*, 2009

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AIV H5N1 = avian influenza virus strain H5N1; HAV = hepatitis A virus; MNV = murine norovirus; PV = poliovirus.

Shahid *et al.* (2009) demonstrated that H5N1 virus survived at 4 °C during more than 100 days in peptone water (Table 5). As it is not possible to cultivate H1N5 virus in cell culture, this virus is propagated in embryonated chicken eggs. For this study, the amnio-allantoic fluid containing the virus was diluted in sterile peptone water in order to apply the different treatments.

3.3 Freezing

Similarly to cool storage, freezing is commonly used to preserve food and avoid the growth of pathogenic and spoilage microorganisms.

PV can survive for a long period of time in frozen Pacific oysters. Only a 1 log reduction was observed after 12 weeks of storage at -17.5 °C (Table 6) (DiGirolamo *et al.*, 1970).

In strawberries, Butot *et al.* (2008) showed that freezing for two days at -20 °C had no effect on the HAV load, however, a 2.7 log reduction of FCV was observed (Table 6). While FCV is frequently used as a surrogate for human NoV it has been reported to be an inappropriate surrogate of NoV in acidic conditions (Cannon *et al.*, 2006; Hewitt and Greening, 2004; Slomka and Appleton, 1998). The rapid decrease of the FCV load seen in frozen strawberries is due to the acid pH of this food matrix, emphasizing that these results cannot be extrapolated to NoV.

The inefficiency of freezing and frozen storage on virus inactivation has also been observed on AIV (Fatunmbi *et al.*, 1993). Freezing allantoic fluid contaminated with H4N2 at -20 °C for 42 months did not reduce the viral load (Table 6), despite the fact that H4N2 is an enveloped virus which is generally known to be less resistant than non-enveloped enteric viruses.

Table 6. Effect of freezing on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	Freezing	**Shellfish**		
PV	-17.5 °C for 12 weeks	Oyster	1	Di Girolamo *et al.*, 1970
		Fresh produce		
FCV	-20 °C for 2 days	Strawberries	2.7	Butot *et al.*, 2008
HAV	-20 °C for 90 days		No reduction	
		Meat/eggs		
AIV H4N2	-20 °C for 42 months	Allantoic fluid	No virus inactivation	Fatunmbi *et al.*, 1993

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AIV H4N2 = avian influenza virus strain H4N2; FCV = feline calicivirus; HAV = hepatitis A virus; PV = poliovirus.

3.4 pH

Preservation of foods by acidification is a well known and commonly used approach in food microbiology. Limited studies have addressed the effect of acidification on viruses. The study of Cannon *et al.* (2006) compared MNV and FCV, to predict NoV stability towards pH. At low pH values of 2, 3 and 4, MNV infectivity was reduced by less than 1 log while the FCV titer was reduced by 2-4 logs (Table 7). The sensitivity of FCV to pH can be explained by its transmission route. FCV is a respiratory virus and thus transmission through the acid

Table 7. Effect of pH on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	pH treatment	**Shellfish**		
FCV	pH 3.75 for 4 weeks at 4 °C	Liquid acid marinade	>4	Hewitt and Greening, 2004
HAV			<0.5	
		Fresh produce		
HRV	pH 3.01 for 3 days at 4 °C	Fruit juice	<0.5	O'Mahony *et al.*, 2000
FCV	pH 2 at 37 °C for 30 min	Buffer	>4	Cannon *et al.*, 2006
	pH 4 at 37 °C for 30 min		2.3	
	pH 5-9		~2	
MNV	pH 2-4 at 37 °C for 30 min		<1	
		Meat/eggs		
AIV H5N1	pH 3-12 for 10 min at room temperature	Allantoic fluid	No virus inactivation	Wanaratana *et al.*, 2010

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AIV H5N1 = avian influenza virus strain H5N1; FCV = feline calicivirus; HAV = hepatitis A virus; HRV = Rotavirus; MNV = murine norovirus.

environment of the gastro-intestinal system is not required in contrast to enteric viruses. Another enteric virus, HRV (cell culture adapted rotavirus DS1 strain), was shown to survive well (<0.5 log reduction after 3 days) in a filtered sterilized fruit juice having a pH of 3.01 (O'Mahony *et al.*, 2000). Acid resistance is probably crucial for successful infection of enteric viruses at the level of the gastro-intestinal tract.

The effect of a mussel marinating process on FCV and HAV survival was investigated (Hewitt and Greening, 2004). The process consisted of a heat treatment (immersion in boiling water and steaming for 3 min) followed by marinating in an acetic acid-based marinade (pH 3.75). In agreement with other studies, FCV was observed to be pH sensitive; >4 log reduction was observed after 24 h in the acid marinade (no heat treatment applied). HAV titer was not significantly reduced after 4 weeks storage in the acid marinade (no heat treatment applied) (Table 7). The marinating process (both heat treatment and 4 weeks storage in acid marinade) of mussels did not result in a complete inactivation of HAV; 3 of 9 mussels were observed to be HAV positive by cell culture. The short heat treatment, designed to be listericidal, followed by acid marinade may fail to inactivate enteric viruses.

Despite the fact that AIV are known to be viruses that are easily inactivated, H5N1 viruses (strains 2004.1, CUK-2 and 2004.2) were not inactivated by 10 min exposure to pH 3, 5, 7, 9 or 12 (Table 7) (Wanaratana *et al.*, 2010).

3.5 Other processes applied to (fresh) produce

To retain the nutritional value, the 'fresh' sensorial properties and a prolonged shelf life of fresh produce, many mild preservation processes are of interest for the food industry. Alternatives have been investigated to replace the typically used chlorine washes due to the low efficacy of microbial reduction and the reduced reactivity of chlorine in the presence of organic material. Liquid H_2O_2 has been suggested as an alternative to chlorine in the wash water of fresh produce. Liquid H_2O_2 (2.52%) was shown to result in a marginal reduction of MNV (Table 8) (Li *et al.*, 2011). The obtained reduction was not significantly higher compared to washing with water only (Li *et al.*, 2011). A 3.2 log reduction was observed by Xie *et al.*(2008) by the use 2% H_2O_2 (Table 8). The main difference between the two mentioned studies was the virus type; MS2 (Xie *et al.*, 2008) compared to MNV (Li *et al.*, 2011). In addition, the experimental set up may also play an important role in the efficacy of the treatment. The study of Xie *et al.* (2008) consisted of a treatment of 5 cm^2 lettuce sections while the study of Li *et al.* (2011) treated 50 g of lettuce. It is important to mimic as closely as possible the ratio of product and sanitizer as applied by food industries.

Interestingly, Xie *et al.* (2008) observed a significantly higher reduction of MS2 when H_2O_2 was sprayed on lettuce and subsequently exposed to UV-light. An explanation for the higher activity of this combined treatment may be the formation of radicals that were able to effectively penetrate the phage capsid.

Modified atmosphere packaging (MAP) is also commonly used to prolong the shelf life of foods. The gas mixture in the package is modified to inhibit chemical and microbial spoilage. Bidawid *et al.* (2001) studied different MAP conditions applied to lettuce pieces (2×1 cm).

Table 8. Effect of various mild preservation processes on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	Freeze-drying	**Fresh produce**		
HAV	Freeze-dried over 18 h	Raspberries, strawberries	1.5	Butot *et al.*, 2009
	Modified atmosphere packaging (MAP)	**Fresh produce**		
HAV	Packaged[2] 9 days at room temperature	Lettuce	<1	Bidawid *et al.*, 2001
	Packaged[2] for 9 days at 4 °C		<0.5	
	Hydrogen peroxide	**Fresh produce**		
MNV	Liquid H_2O_2 (2.52%) for 5 min	Lettuce	~1	Li *et al.*, 2011
MS2	Spray with liquid H_2O_2 (2%) for 60 s		3.2	Xie *et al.*, 2008
	Spray with liquid H_2O_2 (2%) for 10 s + 0.632 mW/cm^2 UV for 30 s		>4	
	Ultrasound	**Produce**		
MNV	HIUS sonicated at 20 kHz for 30 min	Buffer	>3.5	Su *et al.*, 2010
		Orange juice	1.6	
MS2	Steam-ultrasound for 1 s	Raspberries	~1	Schultz *et al*, 2012
	Pulsed electric field			
HRV	Pulsed at 20 to 29 kV/cm at 25 °C for 145.6 µs	Cell culture medium	No reduction	Khadre and Yousef, 2002

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

[2] At CO_2:N_2 atmospheres of 30:70, 50:50, 70:30 or 100% CO_2.

HAV = hepatitis A virus; HIUS = high intensity ultrasound; HRV = Rotavirus; MNV = murine norovirus; MS2 = MS2 bacteriophage.

None of the conditions tested showed a considerable reduction of HAV (Table 8). Unlike bacteria, viruses are inert particles which might explain the inefficacy of the CO_2 present in MAP (Bidawid *et al.*, 2001).

Some fresh produce such as raspberries are fragile and therefore post harvest decontamination treatments, not affecting the texture and nature of the produce, are scarce. Vacuum freeze-drying is commonly applied by the food industry to such berries. This process dehydrates berries while maintaining colour, flavour and antioxidants (Butot *et al.*, 2009). Freeze-drying of raspberries for 18 h (115 °C for 3 h, decrease to 60 °C for 13 h and heating up to 115 °C in 2 h) induced a 1.5 log reduction of HAV titer. The freeze-drying process heated up the berries until a maximum of 55 °C for approximately 10 h. An additional dry heat treatment for 20 min at 100 °C after freeze-dying of strawberries and raspberries enabled total inactivation (>3 log) of HAV (Table 8 and Section 2.1).

Schultz *et al.* (2012) investigated the steam-ultrasound technique on these fragile berries. Pressurized steam and high power ultrasound are combined and are thought to allow the steam to access irregularities on the surfaces. Steam-ultrasound treatment for 1 min had

only a marginal effect (~1 log reduction) on MS2 inoculated on raspberries (Table 8). It was assumed that matrix protective effects and the potential presence of water condensed on the raspberries caused the inefficacy of the treatment. One day after the treatment of 1 s, fluid leakage and a soft texture was observed, illustrating the limited applicability on raspberries to maintain freshness. However, steam-ultrasound was found be more efficient on plastic surfaces as MS2 and FCV titres were reduced by >4 log and the MNV titer was reduced by 3.7 log.

For less fragile produce-based foods such as apple juice/cider, high intensity ultrasound (HIUS) has been investigated against foodborne bacteria, viruses and fungi (Su *et al.*, 2010). HIUS causes damage to the cell wall, cell membrane and nucleic acids. HIUS treatment for 30 min at 20 kHz inactivated MNV in PBS, a saline buffer, by more than 3.5 log from an initial inoculum of ~4 log plaque forming units (PFU) /ml (Table 8). No reduction of MNV was observed when a high initial titer (~6 log) was examined. It may be that viruses at high titer absorb less ultrasound energy per viral particle than viruses at low titres. Besides MNV, FCV and MS2 were investigated and this comparative study indicated that MNV was the most resistant of these three viruses to HIUS. The reduction effect of HIUS was decreased in orange juice compared to PBS buffer. The authors suggested that the lower efficacy of the treatment was due to the protection effect of food particles and the higher viscosity reducing the velocity of the cavitation bubbles and shear effects.

Another mild preservation technology aimed at producing safe food and maintaining the 'freshness' is pulsed electric field (PEF). HRV (Wa strain) was examined at low and high levels against PEF treatment (20 to 29 kV/cm at 25 °C for 145.6 µs) in cell culture medium but no reduction in virus titer was observed (Khadre and Yousef, 2002) (Table 8). In this study PEF was shown to be not effective for (enteric) viral inactivation in contrast to other studies that investigated bacterial contaminants.

4. Effect of alternative preservation techniques

Food technologists are challenged to broaden the spectrum of non-thermal preservation techniques applicable on a wide range of food matrices that is able to control microbiological hazards and maintain the nutritional and sensorial quality. High hydrostatic pressure processing (HPP) can be considered an alternative preservation technique. The HPP exposes packed food in a pressure vessel to pressures generally in a range of 100 to 600 MPa. Radiation (UV radiation and ionizing irradiation) is another example of alternative preservation technology, although current consumer acceptability and regulation constraints have limited its application.

4.1 High hydrostatic pressure processing

Sufficient cooking of shellfish is a reliable mitigation method to inactivate enteric viruses that might be present in/on shellfish. However, many consumers prefer to eat raw shellfish because cooking changes the organoleptic quality. As a result, alternative technologies such as HPP are of interest to decontaminate raw shellfish.

Arcangeli *et al.* (2012) indicated that HPP treatment at a pressure of 400 MPa for 1 min on Manila clams induced less than a 1 log reduction of MNV (Table 9). However, Kingsley *et al.* (2007) showed complete inactivation of MNV when HPP was applied at 400 Mpa for 5 min at 5 °C. Complete inactivation was also achieved after treatment of 500 MPa for 1 min. The higher resistance observed in the study of Arcangeli *et al.* (2012) may be due to the species of shellfish tested, the equipment used for treatment or the temperature during treatment. Calci *et al.* (2005) showed 3 log reduction of the HAV titer in shucked oysters after a treatment of 400 MPa for 1 min. Again, comparison between different studies of the resistance of HAV with MNV towards HPP in shellfish is difficult, but is seems that HPP seems applicable to decrease the viral load of shellfish.

HAV inactivation was also investigated in matrices other than shellfish such as blended strawberries and green onions (Kingsley *et al.*, 2005). A 4 log reduction of HAV titer was observed when 375 MPa HPP was applied (Table 9). Kingsley *et al.* (2005) reported that there is an inverse relationship between ionic strength and HPP effectiveness for HAV inactivation. Additionally, a baroprotective effect of $CaCl_2$, used to increase the firmness of foods, was observed against MNV (Table 9) (Sanchez *et al.*, 2011). It seems that the composition of different foods influences virus inactivation by HPP.

Studies have indicated that HPP temperature plays an important role on the inactivation potential of the treatment (Kingsley *et al.*, 2007; Sanchez *et al.*, 2011). Generally, at low temperatures higher inactivation levels were observed.

The effect of HPP on the AIV of subtype H7N7 was investigated by Isbarn *et al.* (2007) (Table 9). In contrast to enteric viruses, for H7N7 inactivation in chicken meat the increase in

Table 9. Effect of high hydrostatic pressure processing on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	High hydrostatic pressure processing	**Shellfish**		
HAV	400 MPa for 1 min at 9 °C	Oysters	>3	Calci *et al.*, 2005
MNV	500 Mpa for 1 min at 20 °C	Manila clams	>4	Arcangeli *et al.*, 2012
MNV	400 MPa for 5 min at 5 °C	Oyster tissue	4.1	Kingsley *et al.*, 2007
		Fresh produce		
HAV	375 MPa for 5 min at 21 °C	Strawberry puree	>4	Kingsley *et al.*, 2005
	350 MPa for 5 min at 21 °C	Sliced green onions		
MNV	400 MPa for 15 min at 45 °C	Culture medium	>5	Sanchez *et al.*, 2011
	Same + 10 mM $CaCl_2$		<0.5	
		Meat/eggs		
AIV H7N7	500 MPa at 15 °C for 90 s	Cell culture medium	>5	Isbarn *et al.*, 2007
	500 MPa at 15 °C for 25 s	Chicken meat		

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AIV H7N7 = avian influenza virus strain H7N7; HAV = hepatitis A virus; MNV = murine norovirus.

temperature during treatment from 15 to 30 °C significantly reduced the pressure needed to achieve similar inactivation rates. Interestingly, the presence of chicken meat had only a minor effect on the pressure stability of H7N7 and inactivation rates were shown to be slightly faster compared to cell culture medium. The mechanism responsible for virus inactivation through HPP is poorly understood but apparently pressure sensitivity varies little irrespective of whether a lipid envelope or a protein capsid surrounds the virus.

Table 10. Effect of irradiation on viruses.

Virus	Treatment	Matrix	Inactivation[1]	Reference
	UV	**Water**		
AIV H5N1	25 mWs/cm^2	Clarified ozonated water	5.5	Lenes *et al.*, 2010
AD40	109.6 mWs/cm^2	BDF (buffered demand free	2	Thurston-Enriquez *et al.*, 2003
FCV	36 mWs/cm^2	water) at pH7 (22-25 °C)	4	
		Fresh produce		
FCV	40 mWs/cm^2	Lettuce	>3	Fino and Kniel, 2008
		Strawberries	1.5	
HAV		Lettuce	>4	
		Strawberries	1.3	
HAV	50 mWs/cm^2 pulsed UV light for 2 s	PBS	4.8	Jean *et al.*, 2011
	Ionizing radiation	**Water**		
CaCV	0.2 kGy gamma	Tap water at pH 7.6	2.4	De Roda Husman *et al.*, 2004
FCV			1.6	
		Shellfish		
PV	2.84 kGy gamma	Oyster	1	Jung *et al.*, 2009
		Fresh produce		
HAV	2.72 kGy gamma	Lettuce	1	Bidawid *et al.*, 2000
FCV	2-95 kGy electron (e)-beam			Zhou *et al.*, 2011
MNV	11.2 kGy gamma	Spinach	>3.5	Feng *et al.*, 2011
		Lettuce		
		Strawberries		
	12 kGy e-beam	Cabbage	~2	Sanglay *et al.*, 2011
		Strawberries		
		Meat/eggs		
AIV H5N3	2.6 kGy e-beam	Ground turkey meat	1	Brahmakshatriya *et al.*, 2009
	1.6 kGy e-beam	Egg-white		

[1] Inactivation is expressed in $\log_{10}$ reduction of infectious units.

AD40 = adenovirus type 40; AIV H5N1 = avian influenza virus strain H5N1; AIV H5N3 = avian influenza virus strain H5N3; CaCV = canine calicivirus; FCV = feline calicivirus; HAV = hepatitis A virus; MNV = murine norovirus; PV = poliovirus.

4.2 Irradiation

Irradiation is another non-thermal approach to inactivate foodborne viruses. UV light is commonly used to inactivate microorganisms in various types of water. A study by Thurston-Enriquez *et al.* (2003) showed that the doses of UV required to achieve a 2 log inactivation of enteric adenovirus type 40 (AD40) in water was 109.6 mWs/cm^2. In comparison, a 4 log reduction of FCV was achieved using a lower UV dose of 36 mWs/cm^2. A similarly low UV dose was readily effective for the inactivation of an AIV H5N1 strain in water (>5 log inactivation) (Table 10) (Lenes *et al.*, 2010).

UV light treatment at a dose of 40 mWs/cm^{2}achieved a reduction of >3 log on lettuce, but ≤1.5 log on strawberries for both FCV and HAV, respectively (Table 10) (Fino and Kniel, 2008). The produce matrix and especially the surface topography has a large impact on the effectiveness of UV treatments on fresh produce and it is thought that uneven surfaces shield some virus particles from the light (Doyle, 2010).

Pulsed UV light has been proposed as a novel non-thermal technology for food preservation. It is based on short and high peak-energy light pulses with a large spectrum of wavelengths (Elmnasser *et al.*, 2007). Jean *et al.*(2011) observed a 4.8 log reduction of HAV in PBS when treated with pulsed UV light at a dose of 50 mWs/cm^2 (Table 10). Overall, the authors observed a gradual decrease in viral load with increasing fluency corresponding to an increase in the number of pulses of light generated. Based on their findings the authors suggest that the use of pulsed UV light for inactivation of enteric viruses could be feasible, especially for the decontamination of food-contact surfaces. In addition, compared to conventional (continuous) UV light, pulsed light treatment had the advantage of being fast and more efficient at converting electrical energy into photon energy (Jean *et al.*, 2011).

UV light has limited applications in the food industry because it does not penetrate beyond the surface of foods, hence no values on virus inactivation in shellfish, meat or eggs using UV are given in Table 10.

A dose of 0.2 kGy gamma irradiation reduced canine calicivirus (CaCV) and FCV in water by 2.4 and 1.6 log, respectively (Table 10) (De Roda Husman *et al.*, 2004). Gamma irradiation was found to be greatly affected by the presence of proteins which are presumed to scavenge free radicals induced by the irradiation and necessary for viral inactivation. Consequently, it is not surprising that approximately 10 times higher irradiation doses of 2.84 and 2.72 kGy were necessary to inactivate PV and HAV by 1 log in oysters and lettuce, respectively (Table 10) (Bidawid *et al.*, 2000; Jung *et al.*, 2009). Similarly, Feng *et al.* (2011) showed that MNV was resistant to gamma irradiation in different matrices. At a dose of 11.2 kGy, 3.6 to 4.1 log virus reductions were achieved in all fresh produce samples (Table 10), but only a <2 log reduction was achieved at the food and drug administration (FDA)-approved dose (4 kGy).

Electron beam radiation has been proposed as an alternative irradiation technology for the inactivation of pathogens on food. Electron-beam generators (e-beams) concentrate and accelerate electrons to 99% of the speed of light in the creation of e-beams, but in comparison with gamma irradiation, e-beam irradiation has a relatively small depth of penetration

(Hirneisen *et al.*, 2010). Similar to gamma irradiation, the dose of e-beam irradiation required to reduce the titer of FCV on lettuce by 1 log was 2.95 kGy (Zhou *et al.*, 2011). Another study using MNV demonstrated that e-beam irradiation of inoculated cabbage resulted in a 1 log reduction at 4 kGy and less than a 3 log reduction at 12 kGy. On strawberries, less than a 1 log reduction occurred at doses up to 6 kGy with a maximum reduction of 2.2 log at 12 kGy (Sanglay *et al.*, 2011) (Table 10). Brahmakshatriya *et al.* (2009) determined the effect of varying e-beam radiation doses on the viability of an AIV H5N3 strain. Analogous to a D-value, the doses of irradiation required to reduce the viral titer by 1 log (90%) were 2.6 kGy for ground turkey meat and 1.6 kGy for egg-white (Table 10). Based on these results it was estimated that 10.4 kGy and 8.2 kGy are required to achieve a 4 log reduction in viable AIV in contaminated meat and egg-white, respectively.

5. Conclusions

5.1 What has been achieved?

A wide range of studies examining the inactivation of different viruses through different processes in various matrices are presented. In general, the composition of the food matrices (especially pH, fat and sugar content) and the food processing technique influence virus survival and should be taken into consideration when evaluating the viral risk.

Cooking procedures commonly used in food preparation, where 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 (Codex Alimentarius Commission, 2012). However, the heat effects on enteric viruses differ significantly. Temperatures ≥90 °C are very effective in inactivating the most heat resistant enteric viruses, even in complex matrices such as shellfish and liver meat.

Mild food preservation processes which are used to reduce bacterial load such as washing (including shellfish depuration and relaying), MAP, ultrasound-based treatments and PEF were shown to have only a marginal effect upon the viral load. Storage in cool conditions, freezing and freeze-drying, which are commonly used to prevent bacterial growth had no significant inactivation effect on enteric viruses. The substitution of chlorine by H_2O_2 in combination with UV treatment seemed to have promising reduction effect on viruses but a process-scale validation of such a treatment is required.

Enteric viruses and H5N1 are stable in acidic conditions (~ pH 2-3) and therefore acidification cannot be considered as a virucidal treatment.

HPP was shown to be a promising technology to decrease the viral load in shellfish as treatment conditions was found which enabled >3 log reduction while the organoleptic quality could be retained. Irradiation doses required to achieve a significant inactivation of enteric viruses are well beyond the current maximum dose allowed by the FDA for fresh produce. Additionally, if higher doses of radiation were used to inactivate viruses, losses in colour, texture and flavour are likely to occur in treated fresh produce.

At international level, the European Food Safety Authority (2012) clearly states that currently used methods of depuration and relaying of shellfish contaminated with NoV are ineffective to remove the viral risk. The Codex Guidelines have the potential to be translated into national guidelines and legislations to assist countries in their efforts to protect consumer health from foodborne viral illness (Codex Alimentarius Commission, 2012).

5.2 What has been neglected?

To date, NoV cannot be grown in routine tissue culture systems making it impossible to test the effects of the control measures on their infectivity. Scientists have tried to use quantitative reverse-transcription polymerase chain reaction () to compare the NoV response to heat, UV and other processes with that of surrogate viruses such as MNV and FCV. Several studies using different viruses showed that the reduction in the number of infectious viruses did not correlate with the number of genomes detected by RT-qPCR (Baert *et al.*, 2008b; Butot *et al.*, 2009; Hewitt *et al.*, 2009). Together these studies suggest that RT-qPCR cannot be reliably used to predict the inactivation of enteric viruses and emphasize that a reliable method to assess the inactivation of NoV is currently lacking.

Some food processes (e.g. extrusion, spray drying, mild pasteurization) have not been validated, especially in complex matrices (e.g. fruit purees with a high sugar content, pet food with a high fat content), making it difficult for Hazard Analysis Critical Control Point (HACCP) studies to take the virus risk into consideration. Indeed, up until now viruses are rarely taken into consideration in HACCP studies. Additionally, most if not all virus inactivation studies were conducted at laboratory scale, neglecting the likely impact of physical factors on the inactivation of viruses at factory-scale, such as the time needed to heat up a large batch of food matrix, the velocity of the product flow and the shearing forces.

For certain matrices at certain temperatures, only a very limited number of data points are available, often using only one surrogate virus. As described in Bertrand *et al.* (2012), viruses and bacteriophages used in heat inactivation experiments are not homogeneously and significantly represented in the different categories of temperature, matrix and detection method. Especially data for temperatures in the range of 40-60 °C were neglected and studies of the same virus over a wide range of temperature are missing.

Finally, comprehensive risk assessments are not available for matrices other than shellfish (Dore *et al.*, 2010; Lowther *et al.*, 2010; Pinto *et al.*, 2009). Especially risk assessments of mildly processed high risk matrices such as herbs and berries are lacking. The food industry needs such studies to address the level of viral inactivation necessary for reducing the probability of infection. A bottleneck is that prevalence data are limited and mainly available as RT-qPCR units and the link with infectivity is not known.

5.3 What needs to be done?

The primary interest of food companies is to know the effect of their currently applied processes on the inactivation of human pathogenic viruses, especially the unculturable NoV strains, as they are involved in the majority of viral foodborne outbreaks. Consequently,

research should continue efforts to culture NoV and in parallel focus on the development of new methods or the extension of existing RT-qPCR-based methods to better differentiate intact infectious virus particles from damaged non-infectious ones. Methods for detecting human NoV and predicting their infectivity has been reviewed recently (Knight *et al.*, 2012).

For the time being and as long as easy-to-use cell culture assays are unavailable, validation of preservation processes on virus inactivation relies inevitably on surrogate viruses. It is recommended to compare different surrogate viruses in one single study to get a more realistic picture of the viral reduction levels which can be expected for the unculturable viruses. Up until now it was difficult to compare data obtained in independent studies as different viruses using very diverse experimental set-ups were investigated.

Larger scale inactivation experiments e.g. at pilot plant scale using non-pathogenic surrogate viruses or bacteriophages would be very valuable for the food industry to determine the contribution of the additional physical factors mentioned above (time needed to heat up a voluminous batch of food matrix, the velocity of the product flow and the shearing forces) towards viral inactivation. Additionally, a broad range of treatment conditions should be tested per matrix to express D-values (decimal reduction times), which is the times required to kill 1 log (90%) of an organism at a certain temperature. These values can be more easily used by food industries in their HACCP plans than single point measurements.

Another interesting approach might be the investigation of the hurdle concept that is commonly applied for pathogenic and spoilage bacteria. The effect of combining treatments on viruses is of interest, but there is not always an additive effect of the different treatments, as viruses are intracellular parasites and can therefore behave quite differently compared to foodborne or spoilage micro-organisms. Related to this, studies on the disparity in virus resistance/sensitivity are needed. This may offer new idea for potential strategies to reduce the viral load.

As most mild food preservation processes show only marginal effects on the viral load and intermediate temperatures in complex matrices may not be sufficient to inactivate enteric viruses sufficiently to reach a safe level for the consumer, more emphasis into effective control measures at primary production are also needed. HACCP studies especially need to address good aquacultural and agricultural practices, especially the origin and quality of water used along the food chain which comes into contact with critical raw materials such as shellfish and fresh produce. Although microbiological quality of water is currently determined by testing for *Escherichia coli* only, the fact remains that the absence of *E. coli* does not necessarily correlate with the absence of enteric viruses (Health Canada, 2011). For this reason, efficient viral methods need to be implemented and efforts towards the application of viral indicators such as bacteriophages or human adenoviruses could be useful for certain applications.

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