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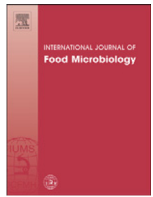

Review

The efficacy of preservation methods to inactivate foodborne viruses

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ABSTRACT

During the last decade an increased incidence of infections and outbreaks attributed to foodborne viruses, in particular noroviruses (NoV), was observed world wide. The awareness of the presence of viruses on food emphasized the need to acquire knowledge regarding the effect of preservation methods upon viruses. Most foodborne viruses cannot be cultured in the laboratory, which hinders studies of their stability in food. Cultivable surrogate viruses, genetically related to the human infecting strains, are taken as a substitute to define inactivation rates. The last years, the number of survival and inactivation studies using various surrogate viruses increased. In this review, state-of-the-art information regarding the efficacy of preservation methods to reduce the level of viruses on food is compiled. In the first place, the effect of preservation methods establishing microbial growth inhibition (chilling, freezing, acidification, reduced water activity and modified atmosphere packaging) upon foodborne viruses is described. Secondly, the use of preservation methods establishing microbial inactivation such as heat treatment, high hydrostatic pressure processing and irradiation to eliminate viruses is discussed. In the third place, the efficacy of decontamination methods on fresh produce and purification procedures applied on live bivalve shellfish to reduce the viral load is included. These studies indicate that viruses persist well on chilled, acidified, frozen foods and foods packed under modified atmosphere or in dried conditions. Intervention strategies inducing microbial inactivation are required to achieve a 3 log reduction of the level of viruses. Decontamination of fresh produce reduces viruses with a maximum of 1 to 2 log while purification of live bivalves is not adequate to prevent viral outbreaks. It was noted that the effect of a particular food preservation method is dependent upon the virus tested and type of food.

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Abbreviations: NoV, norovirus(es); HAV, hepatitis A virus; FCV, feline calicivirus; ss, single stranded; ds, double stranded; PFU, plaque forming units; CaCV, canine calicivirus; MNV-1, murine norovirus 1; MAP, modified atmosphere packaging; RH, relative humidity; VLPs, virus like particles; NoVLPs, norovirus VLPs; HPP, high hydrostatic pressure processing; PBS, phosphate buffered saline; PAA, peroxyacetic acid; RoVLPs, rotavirus VLPs; HBGA, histo-blood group antigen.

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1. Introduction

Viruses are increasingly recognized as an important cause of foodborne outbreaks. In Europe, viral agents were responsible for 10.2% of the foodborne outbreaks during 2006 and were pointed out as the second most common causative agent, after *Salmonella* (EFSA, 2007). It was estimated by Mead et al. (1999) that each year in the US 67% of the foodborne acute gastroenteritis cases are attributed to viruses, representing the leading cause. Noroviruses (NoV), hepatitis A virus (HAV), rotavirus, astrovirus and enteric adenoviruses were depicted as foodborne associated viruses of which NoV was by far the major causative agent (EFSA, 2007; Mead et al., 1999). Foodborne viruses are transmitted via the fecal–oral route. Fruits and vegetables can be contaminated at the pre-harvest stage through contact with fecally contaminated irrigation water or organic-based fertilizer in the field (Carter, 2005). For shellfish, polluted growing/harvesting water is a potential source of viral contamination (Lees, 2000). Fresh produce and shellfish can therefore be considered as high risk foods. At the (post)-harvest stage, infected food handlers not respecting hygienic regulations, play a prominent role (Baert et al., 2009). The latter risk is actually associated with any food item handled manually which is not intended to be heated before consumption.

In contrast to most microbiological agents, viruses cannot grow on food and thus the contamination level cannot increase during processing or storage but survival should be considered due to a low infectious dose (Koopmans and Duizer, 2004; Carter, 2005). Because the majority of the human infecting viruses, present on food, cannot be cultivated in the laboratory, survival data and inactivation rates are mainly obtained by the use of surrogate viruses. Cultivable viral strains and representative surrogate viruses used to estimate the behavior of their related human infecting strains are summarized in Table 1.

Data with regard to survival of viruses on foods and particularly on fresh produce is reviewed by Rzezutka and Cook (2004) and Seymour and Appleton (2001). Nevertheless, Seymour and Appleton (2001) could not find data on the effectiveness of disinfectants and processing aids for the decontamination of fresh produce with respect to viruses. Sair et al. (2002) reported that data on inactivation rates of viruses by industrial food processing techniques were limited. During the latest years, an increase of studies determining inactivation rates of viruses arose. This review aims to give an overview of the generated data with respect to reduction and/or inactivation of viruses by food preservation methods. The efficacy of preservation methods to reduce the level of viruses is subdivided into methods with the objective (i) to hinder microbial growth during storage, (ii) to inactivate micro-organisms and (iii) to reduce the microbial load by decontamination processes.

2. Food preservation methods to establish microbial growth inhibition

Viruses are intracellular parasites infecting only a specific host. Unlike viruses, bacteria, yeasts and also moulds are able to grow on food if intrinsic (water activity, pH, etc.) and extrinsic (temperature, atmosphere, etc.) factors are favorable. To prevent early and fast decay of foods, conditions are modified to inhibit the growth of micro-organisms. Traditional concepts include chilling, freezing, acidification, reduced water activity or packing under modified atmosphere of the food products. The effect upon viruses is questioned for each of these mentioned preservation methods. Other microbial growth inhibiting approaches are available (Manas and Pagan, 2005) but are not considered herein due to a lack of information regarding the effect upon viruses.

2.1. Chilling

Below, recent studies investigating survival of viruses on chilled foods are discussed whereby additional information about survival of enteric viruses on foods can be found in the review of Rzezutka and Cook (2004).

Mattison et al. (2007) investigated the survival of feline calicivirus (FCV) inoculated at a level of 10^5 PFU (plaque forming units) on lettuce and strawberry disks (1 cm in diameter) stored at 4 °C. The inoculum suspension was prepared in a sterile 10% fecal solution with the aim to mimic the contamination of produce by a food handler. Approximately 2 log reduction was observed on lettuce after 7 days at 4 °C. Strawberries showed more than 2.5 log reduction after 6 days at 4 °C (Mattison et al., 2007). Although viral reduction was induced, survival of a significant number of viruses was noted on minimal processed fresh produce during the short shelf life. Dawson et al. (2005) used MS2 as surrogate for NoV to follow up the survival on different types of fresh produce. For each type of produce 10 g was inoculated with 10^8 PFU of MS2 purified stock. At 4 °C, less than 1 log reduction was observed after 7 days storage on produce (tomato, cabbage, carrots, pepper, strawberry and lettuce) and less than 2 log reduction was established before the deterioration of the produce types. Kurdziel et al. (2001) reported 1 log reduction of poliovirus after 11.6 days storage of lettuce and after 14.2 days on white cabbage at 4 °C. No significant decline was observed on green onions within 15 days. The study was performed by inoculating 1 ml purified poliovirus stock onto respectively 30 g of lettuce and 90 g of cabbage while one onion was considered per sample. The initial viral load between 10^4 and 10^5 infectious units on these produce types probably resembled a more realistic viral concentration encountered in natural conditions than the higher level which was used in the study of Dawson

Table 1
Characteristics of representative viral strains used in studies investigating the stability of foodborne viruses.

Viral strains	Genus	Family	Genome	Host	Surrogate for viruses infecting humans
Feline calicivirus	*Vesivirus*	*Caliciviridae*	ss-RNA positive	Cat	Norovirus
Canine calicivirus				Dog	Norovirus
Murine norovirus 1	*Norovirus*			Mouse	Norovirus
Attenuated hepatitis A virus HM-175	*Hepatovirus*	*Picornaviridae*		Human	Hepatitis A virus
Attenuated poliovirus (Sabin, Lsc-2ab)	*Enterovirus*			Human	Poliovirus
Coxsackieviruses				Human	Coxsackieviruses Aichivirus
Aichivirus	*Kobuvirus*			Human	
MS2	*Levivirus*	*Leviviridae*		*E. coli*	Enteric viruses[a]
Rotavirus SA11	*Rotavirus*	*Reoviridae*	ds-RNA segmented	Simian	Rotavirus

[a] Human enteric viruses such as noroviruses, hepatitis A virus, enteroviruses and rotaviruses.

et al. (2005). Croci et al. (2002) inoculated shredded produce (carrots, fennel, lettuce) by immersion into a suspension containing HAV instead of the drop inoculation method carried out in other studies (Mattison et al., 2007; Dawson et al., 2005; Kurdziel et al., 2001). The authors found by this means a 2 log reduction of HAV on cut lettuce after 9 days storage at 4 °C. Longer survival of HAV was noted on lettuce than on fennel or carrots, probably due to the size and the wrinkled texture of lettuce leaves. Also a faster decline was noted on fennel and carrots. It was suggested that the presence of substances exhibiting antimicrobial activity could be responsible for the rapid decline. Antimicrobial activity of fresh carrot extracts was reported earlier (Babic et al., 1994). Besides, grape juice, apple juice and tea induced inactivation of poliovirus (Konowalchuk and Speirs, 1978). The antiviral effect was assumed to be related to polyphenols that could form complexes with viruses rendering non-infectious units.

Although MS2 was found to be more stable on fresh produce in comparison with FCV, poliovirus and HAV, experimental conditions such as the higher inoculum level or the inoculation procedure could be responsible for the longer persistence.

Besides the persistence on fresh produce, Mattison et al. (2007) examined the survival of FCV on ham disks and observed approximately 1 log reduction after 7 days storage at 4 °C. The better survival on ham compared to fresh produce remained undetermined but the authors suggested that ham protected the virus better against dryness. Contrary to fresh produce, ham is rich in proteins and fats. The presence of these compounds might offer protection of the virus particles.

HAV and FCV, inoculated in commercially prepared marinated mussels, showed a 1.7 log reduction of HAV after 4 weeks storage at 4 °C and 7 log decrease of FCV after 1 week (Hewitt and Greening, 2004). The acid marinade had a pH of 3.75 which might have been responsible for the fast inactivation of FCV. Another study examined the survival of poliovirus in commercial yoghurt (3.5% fat, approximately pH 4) and found infective units after 24 days of storage at 4 °C (Strazynski et al., 2002).

Overall, experimental studies, performed on chilled fresh produce and other chilled foods, ascertained survival of a considerable number of viruses before deterioration of the specified food.

2.2. Freezing

The animal caliciviruses FCV and canine calicivirus (CaCV) revealed a decline in infectivity of respectively 0.34 ± 0.18 log and 0.44 ± 0.12 log after 5 cycles of freeze–thawing (Duizer et al., 2004). Poliovirus showed 1 log reduction in frozen strawberries after 8.4 days (Kurdziel et al., 2001). No significant reduction of murine norovirus 1 (MNV-1) infectivity was observed in deep-frozen onions or deep-frozen spinach during a period of 6 months (Baert et al., 2008b). Butot et al. (2008) reported that the number of rotavirus and HAV remained stable during 90 days on frozen raspberries, blueberries, strawberries, parsley and basil with the exception of rotavirus that was reduced by 1 log on blueberries and basil after 2 days frozen storage. In contrast with HAV and rotavirus, FCV showed a 1 to 2 log reduction after 2 days storage on frozen strawberries and raspberries, probably due to the acid environment. Generally, these studies indicated that freezing will not ensure an adequate reduction of the viral load on foods.

2.3. Acidification

Rotaviruses were rapidly inactivated at a pH of 2 at 37 °C with half-lives of less than 1 min. These acid conditions represent an environment encountered in the stomach (Weiss and Clark, 1985). This could account for the fact that human rotavirus disease occurs in children below the age of 3 as their stomach is characterized by a higher pH. Rotavirus (strain SA11) was also reduced by 1.7 log after 3 h exposure in pineapple juice having a pH of 3.60 at 28 °C (Leong et al., 2008). In contrast with these findings, no significant decrease of rotavirus (strain DS1) in fruit juice (pH 3.01) stored at 4 °C for 3 days was reported (Mahony et al., 2000). The higher stability observed by Mahony et al. (2000) may be attributed to several factors such as strain variant, the lower temperature or the type of juice.

HAV infectious units were present after 5 h exposure to a pH of 1 at room temperature while coxsackieviruses A9 and B1, enterovirus 9 and poliovirus could not be detected anymore within 2 h at the specified conditions (Scholz et al., 1989). At 38 °C, HAV remained infectious for up to 90 min at pH 1 (Scholz et al., 1989). The incubation of FCV and CaCV at a pH of 2 or lower for 30 min at 37 °C induced more than 5 log inactivation (Duizer et al., 2004). Less than 1 log reduction of MNV-1 was observed when exposed to a pH of 2 at 37 °C for 30 min while FCV was reduced by 4.4 log after exposure to the same conditions (Cannon et al., 2006). Infective virus particles were still found when a NoV stool filtrate was subjected to a pH of 2.7 for 3 h (Dolin et al., 1972).

Such low pH levels (pH 1 to 3.5) are not prevailing in foods due to unacceptable sensorial quality. Consequently, enteric viruses such as NoV and HAV will probably survive acidification or fermentation of foods as preservation method. The acid resistance of these viruses might explain their association with outbreaks where acid fruits were implicated, e.g. raspberries (Cotterelle et al., 2005; Hjertqvist et al., 2006).

2.4. Reduced water activity

Reduced water activity and the accompanying low relative humidity of food slows down the growth rate of bacteria and other micro-organisms. The impact of relative humidity (RH) was investigated on viruses by Stine et al. (2005) on the surface of cantaloupe, lettuce and bell peppers. Survival of HAV, FCV and PRD1 (ds-DNA coliphage) differed between produce type and was not uniformly affected by relative humidity (Stine et al., 2005). Another study conducted in 1975 found no decline of enteroviruses (coxsackievirus B5, poliovirus, echovirus 7) on covered vegetables at 4 °C during 8 days while uncovered vegetables showed a virus loss of 30% after 1 day (Konowalchuk and Speirs, 1975). It was assumed that the inoculum onto the covered vegetables was protected against drying.

Studies dealing with the influence of relative humidity (water activity) upon viral persistence on foods are limited although survival on inanimate surfaces under varying conditions of relative humidity is also described. Mbithi et al. (1991) reported that HAV decay rates were higher at a high RH (80%), revealing 34% survival, than at a low RH (25%), showing 52% survival, on nonporous inanimate surfaces during 4 h at 20 °C. However, Abad et al. (1994) reported enhanced HAV survival when RH was high (90%) compared to moderate RH (50%) on nonporous materials during 60 days at 20 °C. A maximum decline of 2 log was induced in a time span of 60 days under these conditions. Rotavirus survival was enhanced at a high RH on porous material (Abad et al., 1994). Anomalous results were noted by Sattar et al. (1986) whereby a better survival of rotavirus was observed on nonporous inanimate surfaces at low or medium RH. Poliovirus was found to survive better at ultrahigh RH (95%) than at low RH on nonporous material (Mbithi et al., 1991). Moce-Llivina et al. (2006) found concordantly a better persistence at ultrahigh RH of poliovirus adsorbed to cellulose ester membrane filters at 4 °C and room temperature during 7 days. According to these studies, virus strain and type of surface are factors contributing to inactivation rates at specific RH conditions.

Besides the impact of RH, inactivation of viruses by drying or survival in dried state was investigated in several studies. After drying for about 4 h on finger pads at room temperature, 7% of rotavirus and between 16% and 30% of HAV could be recovered from the initial number of viruses (Ansari et al., 1988; Mbithi et al., 1992). Poliovirus showed reductions between 3.1 and 3.5 log after drying 3–5 h on nonporous surfaces while a decline between 0.1–0.5 log and 0.3–1.1

Table 2
The efficacy of heat treatment, high hydrostatic pressure processing and irradiation to inactivate foodborne viruses.

Virus		Inactivation method	Matrix	Log_{10} reduction[a]	Reference
Heat treatment					
Reoviridae	Rotavirus	60 °C, 10 min	Cell culture medium	7	Mahony et al. (2000)
Picornaviridae	HAV[b]	85 °C, <0.5 min	Milk	5	Bidawid et al. (2000b)
		71 °C, 6.55 min (skimmed); 8.31 min (homogenized); 12.67 min (cream)	Milk	4; 4; 4	
	HAV	85 °C, 0.96 min (28°Brix); 4.98 min (52°Brix)	1 g strawberry mashes	1; 1	Deboosere et al. (2004)
		80 °C, 8.94 min (52°Brix)		1	
	HAV	60 °C, 10 min; 80 °C, 3 min	4 ml virus suspension	>4.6; >4.6	Croci et al. (1999)
		60 °C, 10 min; 80 °C, 3 min	4 ml shellfish homogenate	2; 2	
	HAV	62.8 °C, 30 min; 71.6 °C, 0.25 min	Milk	3; 2	Parry and Mortimer (1984)
	HAV	63 °C, 30 min	Milk	3	Mariam and Cliver (2000b)
	Poliovirus	72 °C, 0.25 min; 72 °C, 0.5 min	Milk	0.56; >5	Strazynski et al. (2002)
	Poliovirus	Steaming 30 min	Oysters	2	Di Girolamo et al. (1970)
Caliciviridae	FCV[c], CaCV[d]	71.3 °C, 1 min	Cell culture medium	3	Duizer et al. (2004)
	FCV, CaCV	37 °C, 24 h; 56 °C, 8 min	Cell culture medium	3; 3	
	FCV	0.5 min immersion of 6–8 cockles in boiling water	Cockles	1.7	Slomka and Appleton (1998)
	FCV	56 °C, 3 min; 56 °C, 60 min	Cell culture medium	No red[e]; 7.5	Doultree et al. (1999)
		70 °C, 1 min; 3 min; 5 min	Cell culture medium	3; 6.5; 7.5	
		Boiling 1 min	Cell culture medium	7.5	
	FCV	70 °C, 1.5 min	Cell culture medium	6	Buckow et al. (2008)
	FCV	63 °C, 0.41 min; 72 °C, 0.12 min	Cell culture medium	1; 1	Cannon et al. (2006)
	NoV	60 °C, 30 min	Stool filtrate	Incomplete	Dolin et al. (1972)
	MNV-1[f]	80 °C, 2.5 min	Cell culture medium	6.5	Baert et al. (2008c)
	MNV-1	65 °C, 0.5 min; 75 °C, 0.25 min	Raspberry puree (9.2°Brix)	1.86; 2.81	Baert et al. (2008a)
	MNV-1	63 °C, 0.44 min; 72 °C, 0.17 min	Cell culture medium	1; 1	Cannon et al. (2006)
High hydrostatic pressure processing					
Reoviridae	Rotavirus	300 MPa, 25 °C, 2 min	Cell culture medium	8	Khadre and Yousef (2002)
Picornaviridae	HAV	450 MPa, ambient temp[g], 5 min	Cell culture medium	>6	Kingsley et al. (2002)
	HAV	400 MPa, ambient temp, 10 min	Cell culture medium	>2	Grove et al. (2008)
	HAV	400 MPa, 9 °C, 1 min	Oysters	3	Calci et al. (2005)
	HAV	375 MPa, 21 °C, 5 min	Mashed strawberries; sliced green onions	4.3; 4.7	Kingsley et al. (2005)
	HAV	500 MPa, 4 °C, 5 min	Sausages	3.23	Sharma et al. (2008)
	Poliovirus	600 MPa, ambient temp, 5 min	Cell culture medium	No red	Kingsley et al. (2004)
		600 MPa, 20 °C, 60 min		No red	Wilkinson et al. (2001)
	Poliovirus	600 MPa, ambient temp, 5 min	Cell culture medium	No red	Grove et al. (2008)
	Aichivirus	600 MPa, ambient temp, 5 min	Cell culture medium	No red	Kingsley et al. (2004)
	Coxsackievirus B5			No red	
	Coxsackievirus A9			7.6	
Caliciviridae	FCV	275 MPa, ambient temp, 5 min	Cell culture medium	>6	Kingsley et al. (2002)
	FCV	200 MPa, −10 °C or 20 °C, 4 min	Cell culture medium	5 or 0.3	Chen et al. (2005)
	FCV	300 MPa, ambient temp, 3 min	Cell culture medium	5	Grove et al. (2008)
	FCV	500 MPa, 4 °C, 5 min	Sausages	2.89	Sharma et al. (2008)
	MNV-1	400 MPa, 5 °C, 5 min	Oyster tissue	4	Kingsley et al. (2007)
	MNV-1	450 MPa, 20 °C, 5 min	Cell culture medium	6.85	Kingsley et al. (2007)
Leviviridae	MS2	600 MPa, 21 °C, 10 min	Cell culture medium	3.5	Guan et al. (2006)
	MS2	500 MPa, 4 °C, 5 min	Sausages	1.47	Sharma et al. (2008)
Irradiation					
Reoviridae	Rotavirus	2.4 kGy	Oysters; clams	1; 1	Mallett et al. (1991)
Picornaviridae	HAV	UV dose: 40 mW s/cm^2	Lettuce; green onions; strawberries	4.3; 4.2; 1.3	Fino and Kniel (2008)
		UV dose: 120 mW s/cm^2	Lettuce; green onions; strawberries	4.5; 5.3; 1.8	
	HAV	3 kGy	Lettuce; strawberries	1; 1	Bidawid et al. (2000a)
	HAV	2.0 kGy	Oysters; clams	1; 1	Mallett et al. (1991)
	HAV	High intensity broad spectrum pulsed light 1 J/cm^2	PBS + 5% FCS[h]; PBS	4.1; >5.7	Roberts and Hope (2003)
	Aichivirus	UV dose: 40 mW s/cm^2	Lettuce; green onions; strawberries	4.0; 2.4; 1.5	Fino and Kniel (2008)
		UV dose: 120 mW s/cm^2	Lettuce; green onions; strawberries	4.4; 3.7; 1.6	
	Poliovirus	High intensity broad spectrum pulsed light 1 J/cm^2	PBS + 5% FCS; PBS	3.2; >6.7	Roberts and Hope (2003)
	Coxsackievirus B2	7 kGy	Ground beef	1	Sullivan et al. (1973)
Caliciviridae	FCV	UV dose: 12 mW s/cm^2; 200 Gy	Virus suspension with low protein content	3; 1.6	De Roda Husman et al. (2004)
	CaCV	UV dose: 20 mW s/cm^2; 200 Gy		3; 2.4	
	FCV	UV dose: 40 mW s/cm^2	Lettuce; green onions; strawberries	3.5; 2.5; 1.1	Fino and Kniel (2008)
		UV dose: 120 mW s/cm^2	Lettuce; green onions; strawberries	3.8; 3.9; 1.6	
Leviviridae	MS2	UV dose: 65 mW s/cm^2; 200 Gy	Virus suspension with low protein content	3; 7	De Roda Husman et al. (2004)

[a] Log_{10} reduction represents the reduction in infectivity.
[b] HAV: hepatitis A virus.
[c] FCV: feline calicivirus.
[d] CaCV: canine calicivirus.
[e] No red: no reduction.
[f] MNV-1: murine norovirus 1.
[g] Temp: temperature.
[h] FCS: fecal calf serum.

log of respectively HAV and rotavirus was observed on these surfaces (Abad et al., 1994). Desiccation can play an important role in the ability of viruses to survive in the environment. HAV and rotavirus showed to be more stable than poliovirus highlighting the possible transmission of these viruses by fecally contaminated environmental surfaces. Moreover, infectious HAV was found after drying in a fecal suspension for 30 days at 25 °C and 42% RH (McCaustland et al., 1982). Doultree et al. (1999) observed more than 8 log decrease of FCV after 20 days at room temperature in dried state. Despite the decline of FCV, a NoV outbreak on a cruise ship suggested environmental persistence of NoV. Identical sequences were present in subsequent cruises whereby the vehicle was thoroughly cleaned and disinfected in between consecutive cruises (Isakbaeva et al., 2005). The detection of identical NoV sequences on surfaces (table, elevator button) and in patients in another NoV outbreak also assumed that surfaces could act as vehicle for NoV transmission (Wu et al., 2005).

Survival after drying at room temperature might depend on the medium wherein the virus is suspended, as was indicated for rotavirus (Ward et al., 1991). The presence of non-fat dry milk or laboratory medium protected rotavirus better against drying than the presence of fecal matter or water. Nevertheless, other investigators suggested that fecal matter did provide protection when viruses were dried (Sattar et al., 1986; Konowalchuk and Speirs, 1975).

Transfer of NoV and FCV to lettuce decreased in the case the inoculum was dried (D'Souza et al., 2006). Also HAV, dried onto finger pads, was transmitted to stainless steel disks in a lesser extent (Mbithi et al., 1992). Despite the advantage of the observed lower transferability, greater resistance towards a chlorine based disinfectant was reported for MNV-1 and MS2 in dried state compared to the viruses present in suspension (Park et al., 2007).

2.5. Modified atmosphere packaging

Modified atmosphere packaging (MAP) can inhibit spoilage causing bacterial and fungal micro-organisms to extend the shelf life of foods. In food products packed under modified atmosphere, the growth and survival of pathogenic micro-organisms is of major importance. Bidawid et al. (2001) reported that MAP did not influence HAV survival on lettuce incubated at 4 °C. Improvement in virus survival (43% HAV survival) was observed in the presence of higher CO_2-levels (70%) compared to storage in air or lower CO_2-levels (ca. 6% HAV survival) after 12 days at room temperature which may be attributed to the inhibitory effect of CO_2 on the enzymes or micro-flora present in lettuce. MAP conditions would therefore not be a suitable strategy to reduce the number of viruses present on food.

3. Food preservation methods to establish microbial inactivation

Food preservation methods having a larger impact on micro-organisms than chilling, freezing, reduced water activity, acidification or MAP packaging are required due to (i) the long term survival of viruses under these conditions and (ii) the low infectious dose. Heating, high hydrostatic pressure processing and irradiation are preservation methods that can be used to establish microbial inactivation. The efficacy of these techniques to inactivate viruses is discussed herein and is summarized in Table 2. Other alternative non-thermal preservation technologies exist such as the use of high pressure carbon dioxide (Garcia-Gonzalez et al., 2007) but are not described because no data about the efficacy upon viral inactivation is (yet) available.

3.1. Heat treatment

Duizer et al. (2004) observed similar inactivation rates of FCV and CaCV at temperatures ranging from 37 °C to 100 °C. Also similar thermal inactivation rates at 63 °C and 72 °C were noted for FCV and MNV-1 (Cannon et al., 2006). Dispersed reductions of FCV for the same time–temperature combination (Table 2) were achieved by Doultree et al. (1999) and Buckow et al. (2008). The experimental set-up, 100 µl virus suspension heated in Sarstedt tubes (Doultree et al., 1999) or 10 µl virus suspension added to 90 µl preheated medium in 0.2 ml reaction tubes (Buckow et al., 2008), was likely to be responsible for the differences in heat inactivation rates.

Bidawid et al. (2000b) studied heat inactivation of HAV in sterile skimmed milk (0% fat), homogenized milk (3.5% fat) and table cream (18% fat). At 71 °C, exposure of 0.16, 0.18 and 0.52 min were needed in respectively skimmed milk, homogenized milk and cream to reduce HAV by 1 log whereas 4 log reduction required 6.55 (skim), 8.31 (homogenized) and 12.67 (cream) min (Table 2). A longer heat treatment was needed in cream to achieve similar inactivation of HAV compared to milk. The high fat content presumably protected HAV towards heat. Accordingly, the loss of infectivity of poliovirus was lower in milk after 0.25 min exposure to 72 °C compared to the reduction in water proving a protective effect of milk constituents (Strazynski et al., 2002). Besides, a protective effect of the mussel matrix was indicated by Croci et al. (1999). A 2 log reduction of HAV was found in a mussel homogenate after 10 min exposure at 60 °C or after 3 min at 80 °C whereas at least 4.6 log reduction was induced after the same treatments in cell culture medium (Croci et al., 1999).

Bidawid et al. (2000b) noted a nonlinear decline of HAV in milk between 65 °C and 75 °C. The observed rapid inactivation in the initial phase could be explained by freely suspended virus particles along with virus particles present at the outside of viral aggregates. Floyd and Sharp (1979) also stated that a subpopulation of organisms may be more resistant to disinfection because of the formation of aggregates. Virus aggregation is influenced by the ionic composition of the medium, pH and the isoelectric point of the virus. Conditions inducing aggregation will be consequently influenced by the food matrix and virus type.

Thermoresistance of HAV inoculated in synthetic media mimicking chemical characteristics of strawberry mashes was investigated (Deboosere et al., 2004). These experiments showed that sucrose concentration (indicated as Brix value) and pH affected HAV heat inactivation. In 1 g strawberry mash, HAV was lowered by 1 log after a heat treatment consisting of 2 min to reach 85 °C followed by 0.96 min at 85 °C (Deboosere et al., 2004). MNV-1 showed a reduction of 2.81 log after exposure to 75 °C for 0.25 min in 10 g of preheated raspberry puree (Baert et al., 2008a). Although HAV seems to be more resistant than MNV-1, the composition of strawberries, e.g. higher sucrose concentration and possibly other components, the initial warming up period of 2 min and the higher virus inoculum (10^7 PFU/ml HAV versus 10^5 PFU/ml MNV-1), could be partially responsible for this difference in inactivation rate.

Slomka and Appleton (1998) investigated the inactivation of FCV by immersion of cockles in boiling water for 0.5 min and found 1.7 log reduction of FCV. At that time, the internal temperature of the cockles reached approximately 60 °C. After 1 min, the internal temperature reached 78 °C and FCV (initially 4.5 log $TCDI_{50}$/g present) could not be detected anymore. Di Girolamo et al. (1970) demonstrated the survival (7%) of poliovirus in oysters after steaming for 30 min whereby a temperature above 88 °C was reached after 25 min. Millard et al. (1987) reported that when the internal temperature of the cockle meat was raised to 85–90 °C and hold it for 1 min, HAV was inactivated. In contrast with the observations in oysters reported by Di Girolamo et al. (1970), Millard et al. (1987) noted that the internal temperature of more than 88 °C was reached after 2 min steaming of cockles. At this point infectious HAV (initially 10^5 infectious units) was not detected anymore. Since the preparation of shellfish usually involves heating until the shells are opened, which is in clams obtained at 70 °C after 47 ± 5 s (Koff and Sear, 1967), this heating process would be insufficient to inactivate viruses. Remarkably, Dolin et al. (1972) reported remaining infectivity of NoV in stool filtrate

exposed to 60 °C for 30 min. The latter study showed contradictory results compared to studies using NoV surrogates (Table 2) and should be further investigated.

The mechanism of inactivation above 65 °C might be caused by large irreversible structural changes presumably due to extensive protein unfolding (Volkin et al., 1997). Nuanualsuwan and Cliver (2003b) suggested that heat treatment did not cause loss of RNA infectivity of poliovirus but that the presumable target might be the viral capsid. In addition, the quaternary structure of NoVLPs (NoV-virus like particles) was found to be unaffected up to 60 °C. Starting from 65 °C particles were irregular in shape with significant disrupture of their icosahedral structure (Ausar et al., 2006).

Because heat affects primarily the viral capsid, a discrepancy between virus infectivity and RT-PCR detection can be observed. MNV-1 exposed to 80 °C for 2.5 min showed 6.5 log reduction of PFU while the RT-PCR signal, representing the number of genomic copies, was not reduced even after 1 h heating at this temperature (Baert et al., 2008c). Accordingly, genomic copies of MNV-1 were detected on blanched spinach by real-time RT-PCR while no infective MNV-1 particles were found by plaque assay (Baert et al., 2008b). RT-PCR signals obtained from heat treated samples should therefore be interpreted with caution because an overestimation of viral infectivity and consequently viral risk would be possible.

3.2. High hydrostatic pressure processing

High hydrostatic pressure processing (HPP) has been applied to raw bivalve shellfish, fruit juices, cider, jams and jellies, drinkable yoghurt, smoothies, avocado products, chopped onions and ready-to-eat meat products (Kingsley et al., 2005). In contrast to heat, HPP does not disrupt covalent bonds thus maintaining the primary structure of proteins and retaining thereby appearance, flavor, texture and nutritional qualities of the unprocessed product (Murchie et al., 2005). Changes in the tertiary structure, maintained by hydrophobic and ionic interactions, are usually observed for proteins in general above 200 MPa (Balny and Masson, 1993).

Kingsley et al. (2005) studied the persistence of HAV in mashed raspberries and sliced green onions. HAV exposed to pressures of 375 MPa at 21 °C for 5 min was reduced by respectively 4.3 and 4.7 log in strawberry puree and on sliced green onions (Table 2). Structural and organoleptic changes were observed for treated whole green onions and strawberries, although sliced green onions or strawberry puree might be accepted by consumers and can be used as flavor enhancers or as ingredient for cream, jams, juices or smoothies. HPP was used to treat oysters with a pressure of 400 MPa for 1 min (9.0 °C) and induced 3 log reduction of HAV (Calci et al., 2005) whereas MNV-1 was reduced by 4 log (5 °C) (Kingsley et al., 2007).

The effect of HPP on other picornaviruses than HAV was investigated in cell culture medium by Kingsley et al. (2004). Aichivirus and coxsackievirus B5 remained fully infectious if 600 MPa was applied for 5 min at ambient temperature whereas coxsackievirus A9 was reduced by 7.6 log under the same conditions. Similarly poliovirus was found to be resistant to 600 MPa for 1 h (Wilkinson et al., 2001).

It can be concluded that the sensitivity towards HPP does not agree between genetically related taxonomic groups or even strains. A possible explanation could be the difference in protein sequence and structure. Members of the genus *Enterovirus*, such as poliovirus, are characterized by large capsid proteins that may protect these virions against HPP (Grove et al., 2008). The aberrant behavior of coxsackievirus A9 by HPP may suggest an atypical protein capsid. Herrmann and Cliver (1973) indicated a distinct viral capsid as well because coxsackievirus A9 was sensitive towards protease activity in contrary to the majority of enteroviruses.

The efficacy of HPP might be influenced by ionic strength. Kingsley et al. (2005) reported an ionic strength of 3.2‰ for strawberry mash, a salt concentration of 2.1‰, 4.1‰ and 5–20‰ for respectively onion extracts, cell culture media and oysters. An inverse correlation between ionic strength and viral inactivation induced by HPP (Table 2) was observed. Moreover, an increasing concentration of sucrose (0% to 40%) or NaCl (0% to 12%) had an increasing protective effect upon FCV in cell culture medium regarding HPP (Kingsley and Chen, 2008). Not necessarily water activity played a role because a sucrose solution decreased FCV in a lesser extent compared to a NaCl solution having an equivalent water activity. The observations of the above mentioned studies assumed protection of viruses by components of the food matrix against HPP. On the other hand, the food matrix can enhance inactivation as was suggested by Sheldon et al. (2008). They noted enhanced inactivation of T7 (ds-DNA phage) in the presence of oyster meat in some HPP conditions while in other conditions the virus was more protected. The mechanism of food components altering virus inactivation rates is not yet elucidated.

Besides, temperature affected the HPP efficacy. FCV was reduced by 4 to 5 log at low temperatures (−10 °C) when treated with a pressure of 200 MPa (4 min) however the same treatment at 20 °C only reduced the titer by 0.3 log (Chen et al., 2005). Also Kingsley et al. (2007) found only 1.15 log reduction when MNV-1 was treated with a dose of 350 MPa (5 min) in propagation medium at 30 °C, while a reduction of 5.56 log was observed at 5 °C.

A disadvantage of HPP is that viral strains could develop resistance to this technology as was assumed by Smiddy et al. (2006). They noticed altered plaques of Qß (ss-RNA coliphage) after HPP treatment whereby the altered shaped plaques persisted by sub-culturing. These phages with unusual plaque morphology might be more pressure resistant but this hypothesis was not further investigated.

3.3. Irradiation

UV light treatment of lettuce at a dose of 40 mW s/cm^2 achieved 4.3, 4.0 and 3.5 log reduction of respectively HAV, aichivirus and FCV (Fino and Kniel, 2008). Inactivation was greatest on lettuce and least effective on strawberries. Viruses were likely sheltered from UV light by the strawberry matrix. A 3 log reduction was achieved for FCV, CaCV and MS2 in tenfold diluted cell culture medium after exposure to UV at a dose of respectively 12, 20 and 65 mW s/cm^2 (De Roda Husman et al., 2004). The protein load present in the viral suspension did not influence inactivation obtained by UV treatment. UV inactivation of micro-organisms is probably due to the absorption of UV by nucleic acids causing dimerization of thymine in DNA or uracil in RNA (Sommer et al., 2001; Nuanualsuwan and Cliver, 2003a). At higher doses (≥1000 mW s/cm^2) UV light can also affect the capsid proteins. The combined effect of size/type of the virion and nucleic acids are thought to be factors determining the resistance/sensitivity of viruses towards UV (Sommer et al., 2001). Surprisingly, Ma et al. (1994) found 4.5 log reduction of infective poliovirus particles by plaque assay after treatment with an UV dose of 22 mW s/cm^2 while only 1 log reduction of the genomic copies, detected by RT-PCR, was observed since UV was thought to break down the genome. Damage of the genomic material after UV treatment might be not sufficient to break down the small target RNA fragment needed for RT-PCR detection. In accordance with heat treated samples, RT-PCR signals obtained from UV treated samples should be interpreted with caution.

In contrast to UV treatment, high intensity broad spectrum pulsed light covers wavelengths between 200 and 1100 nm. The latter treatment involves short pulses of high intensity (at least 1000 times that of conventional UV light; Roberts and Hope, 2003). A dose of 1 J/cm^2 (corresponding with 1 W s/cm^2) reduced HAV and poliovirus by at least 5.7 and 6.7 log in PBS. In the presence of proteins, i.e. 5% fetal calf serum, virus inactivation was less effective (Table 2). Inactivation of viruses in food matrices and the effect of this treatment upon the organoleptic and structural properties of foods should be further examined.

Gamma irradiation at doses of 2 to 4 kGy are often used for bacterial control in particular food products and its effect on foodborne viruses was reported by several investigators. Bidawid et al. (2000a) found that 3 kGy was needed in order to achieve 1 log reduction of HAV on lettuce or strawberries. Mallett et al. (1991) reported that 2.0 kGy was able to

Table 3
The efficacy of decontamination procedures of fresh produce to reduce the level of viruses.

Virus		Decontamination procedure	Matrix	Log_{10} reduction[a]	Reference
Reoviridae	Rotavirus	Water 0.5 min	15 g strawberries/200 ml	1.5	Butot et al. (2008)
		NaOCl 200 ppm, 0.5 min		> 1.5[b]	
Picornaviridae	HAV[c]	Water 5 min	10 g lettuce/fennel/carrot/ 100 ml water	0.1; 1; 0.9	Croci et al. (2002)
	HAV	NaOCl 200 ppm, 0.5 min	15 g strawberries/200 ml	1.0[b]	Butot et al. (2008)
	HAV	20 ppm chlorine 10 min	1.2 g lettuce/30 ml	> 1.7	Casteel et al. (2008)
Caliciviridae	FCV[d]	PAA[e] 300 ppm; 150 ppm 10 min	100 g strawberries/100 ml	3[b]; 1[b]	Gulati et al. (2001)
		PAA 300 ppm; 150 ppm 10 min	10 g lettuce/100 ml	3[b]; 2[b]	
		Water 10 min	100 g strawberries/100 ml; 10 g lettuce/100 ml	2; 2	
		NaOCl 200 ppm; 800 ppm 10 min	100 g strawberries/100 ml	0[b]; 1[b]	
		NaOCl 200 ppm; 800 ppm 10 min	10 g lettuce/100 ml	0[b]; 1.5[b]	
	FCV	Bleach 50 ppm; 100 ppm; 200 ppm;	3 cm^2 disks of lettuce in 5 ml	2.2; 2.6; 2.9	Allwood et al. (2004)
		PAA 80 ppm	Sanitizer solution, 2 min	2.9	
		3% H_2O_2		2.8	
	FCV	NaOCl 200 ppm, 0.5 min	15 g strawberries/200 ml	> 1.6[b]	Butot et al. (2008)
	FCV	NaOCl 300 ppm, 10 min	Cell culture medium	<2	Duizer et al. (2004)
	CaCV[f]	NaOCl 300 ppm, 10 min	Cell culture medium	>3	
	MNV-1[g]	Water 0.42 min	2 onion bulbs/100 ml	0.4	Baert et al. (2008c)
		Water 2 min	10 g spinach leaves/350 ml	1.0	
		Water 5 min	50 g lettuce/500 ml	1.1	Baert et al. (in press)
		NaOCl 200 ppm, 5 min	50 g lettuce/500 ml	1.0[b]	
		PAA 80 ppm; 250 ppm, 5 min	50 g lettuce/500 ml	0.8[b]; 1.4[b]	
Leviviridae	MS2	Chlorine 100 ppm, 5 min	100 g lettuce /1 l	0.7	Dawson et al. (2005)
	MS2	Chlorine 20 ppm, 10 min	1.2 g lettuce/30 ml	> 1.8	Casteel et al. (2008)
	MS2	Bleach 50 ppm; 100 ppm, 200 ppm	3 cm^2 disks of lettuce in 5 ml	1.9; 2.7; 2.9	Allwood et al. (2004)
		PAA 80 ppm	Sanitizer solution, 2 min	2.8	
		3% H_2O_2		2.6	
	MS2	10 s H_2O_2 (2%) followed by 30 s UV (0.63 mW s/cm^2), 50 °C	5 cm^2 sections of lettuce	4.1	Xie et al. (2008)
		$Ca(ClO)_2$ 200 ppm 3 min	Cut lettuce (5 cm^2)/400 ml	1.7	

[a] Log10 reduction represents the reduction in infectivity.
[b] Compared to water.
[c] HAV: hepatitis A virus.
[d] CaCV: canine calicivirus.
[e] PAA: peroxyacetic acid.
[f] FCV: feline calicivirus.
[g] MNV-1: murine norovirus 1.

reduce HAV by 1 log in oysters and clams but 2.4 kGy was needed to achieve this for rotavirus. Coxsackievirus B2 was reduced by 1 log in ground beef when treated with 7 kGy (Sullivan et al., 1973). A dose of 200 Gy reduced CaCV and FCV respectively by 2.4 and 1.6 log (De Roda Husman et al., 2004). The same conditions induced 7 log reduction of MS2. Gamma irradiation was found to be greatly affected by the presence of proteins (De Roda Husman et al., 2004). The authors assumed that free OH radicals, induced by gamma irradiation, which normally interact with nucleic acids and the virus coat were scavenged and induced therefore less inactivation.

4. Food preservation methods to reduce the microbial load

As fruits and vegetables grow, contact with soil, water, fertilizer is inevitable. These ways can introduce micro-organisms on fresh produce. Effort to reduce the microbial load is needed but should be kept minimal to retain the "fresh" status when this type of food is intended to be consumed raw. Similar to fresh produce, raw bivalve shellfish is preferred "live and untreated" by consumers. Purification procedures are required by legislation for bivalve shellfish not complying with the microbiological standards. Decontamination of fresh produce and purification of live bivalve shellfish is discussed herein as preservation methods with the aim to reduce the viral load.

4.1. Decontamination of fresh produce

Consumers prefer pre-cut bagged vegetables or fruits due to their convenience and the broad variety available on the market. A prolonged shelf life of fresh produce complying with microbiological regulations can be achieved by reducing the microbial load. The efficacy of washing fresh produce with tap water, water supplemented with chlorine or other chemical agents to decrease the level of viruses is presented in Table 3.

4.1.1. Washing with water

Washing of cut lettuce, carrot and fennel in potable water for 5 min resulted in a decrease between 0.1 and 1 log of HAV (Croci et al., 2002). Gulati et al. (2001) reported a reduction of FCV by 2 log when lettuce leaves or strawberries were washed in water for 10 min. MNV-1 inoculated on onion bulbs and spinach leaves was reduced by respectively 0.4 and 1.0 log after 5 min washing (Baert et al., in press). The reduction of MS2 obtained by washing cucumber, tomatoes, pepper, lettuce, spring onion, carrot, cabbage, parsley and strawberries with tap water ranged from 0.08 to 0.79 log (Dawson et al., 2005). Apart from tomatoes and strawberries in the latter study, reductions were all below 0.4 log. The authors stated that in natural contaminated raw produce, viruses may be complexed with soil particles or protein material which could result in more difficult and variable decontamination efficiencies than on artificially contaminated produce.

It is clear that removal of viruses by washing depends on produce type. In general, a maximum of 1–2 log removal of micro-organisms could be achieved by washing produce with water (Beuchat, 1998) which is in accordance with the reported decline of viruses.

4.1.2. Chlorination

Dawson et al. (2005) observed reductions between 0.3 and 2.1 log of MS2 after treatment of leafy vegetables with 100 ppm chlorine. A treatment of 200 ppm chlorine rendered an additional 1.0 log reduction of MNV-1 present on lettuce compared to washing in tap water (Baert et al., in press). The application of 200 ppm chlorine to

treat strawberries and lettuce did not result in an additional reduction of FCV compared to washing with tap water (Gulati et al., 2001). The low efficacy of chlorine reported by Gulati et al. (2001) could be due to the presence of organic matter in the inoculum solution. Drop inoculation of FCV onto lettuce or immersion of strawberries into a glycine buffer (pH 3.5) containing FCV probably introduced organic material onto the produce as well. Similarly, Baert et al. (in press) reported a significant lower decline by chlorination in the case MNV-1 lysate was directly spotted onto lettuce than when the inoculum was tenfold diluted in tap water. Less free chlorine was available in the first case showing that chlorine reacted with the organic matter originating from the inoculum suspension.

Leafy vegetables (lettuce and cabbage) treated with 200 ppm chlorine enabled a reduction of 2.9 log MS2 and FCV whereas *E. coli* was reduced by 5.5 log (Allwood et al., 2004). Allwood et al. (2004) used one lettuce disk to determine the efficacy of a sanitizer treatment. Although, the efficiency obtained in this way is indicative, the efficiency of these sanitizers should be further evaluated in industrial processes. Therefore, a produce/treatment ratio equivalent to the fresh produce industry is preferred above using individual pieces of produce.

Casteel et al. (2008) observed at least 1.7 log reductions of both MS2 and HAV on strawberries, tomatoes and lettuce treated with 20 ppm chlorine. However, the actual effect of chlorination is not known in the latter study because the effect of treating inoculated produce solely with water was not mentioned. Inactivation rates of MS2 on lettuce differed significantly between the study of Dawson et al. (2005) and the study of Casteel et al. (2008). The experimental set-up (Table 3) seems therefore of importance in defining decline rates. Factors such as produce:treatment solution ratio, the presence of organic matter, inoculation method and produce type are reported to influence the efficacy of chlorination towards bacterial pathogens as well (Francis and O'Beirne, 2002; Lang et al., 2004; Beuchat et al., 2004).

Because chlorination of lettuce induced a similar decline of FCV and MS2 investigated in one study (Allwood et al., 2004) and of HAV and MS2 tested in another study (Casteel et al., 2008), it might indicate that FCV, MS2 and HAV react similar to chlorine. Although Butot et al. (2008) found a significantly higher inactivation of FCV after treatment of blueberries, strawberries, raspberries, basil and parsley with 200 ppm chlorine compared to HAV and rotavirus, suggesting the influence of produce type. In general, small fruits e.g. strawberries and raspberries destined for the fresh market are not washed because they deteriorate rapidly. Nevertheless, berries can be disinfected if they are used for further processing (Mariam and Cliver, 2000a).

High chlorine levels would be required to achieve a 2 to 3 log reduction of viruses on fresh produce. The application of higher concentrations is limited due to sensorial aspects. Prolonging the chlorine treatment would not be useful to increase the efficacy of chlorination since two studies showed that a contact time beyond 10 min made little difference in antiviral activity towards FCV (Gulati et al., 2001; Duizer et al., 2004).

Chlorine is described to be a highly oxidizing agent whereby hypochloric acid is considered to be the active moiety (McDonnell and Russell, 1999). Nuanualsuwan and Cliver (2003a,b) reported that hypochlorite affected both the RNA and capsid of poliovirus, FCV and HAV. Evidence of total breakdown of the genomic material is given by Ma et al. (1994). They noted a good correlation between the decline of genomic copies detected by RT-PCR and infective poliovirus particles detected by cell culture after 1 ppm free chlorine treatment.

4.1.3. The use of other chemical agents

Gulati et al. (2001) found 1 and 2 log reduction of FCV on respectively strawberries and lettuce treated with a 150 ppm peroxyacetic acid (PAA) solution. This study observed no reduction by 200 ppm chlorine utilizing the same experimental set-up. Baert et al. (in press) noted that PAA was not affected by the presence of organic matter as it was demonstrated for chlorine and could explain the observations of Gulati et al. (2001). In practice, fresh produce can contain organic matter originating from the field or due to recycling of water. Consequently sanitizers that do not react with organic material are advantageous.

Allwood et al. (2004) showed a comparable decline of MS2 and FCV in the case 200 ppm chlorine, 3% H_2O_2 and 80 ppm PAA were used as sanitizers. The latter concentration achieved a 4.7 log reduction of *E. coli*. MNV-1 inoculated on lettuce showed an additional decline of 1.4 log compared to water after treatment with a solution containing 250 ppm PAA whereas 1.0 and 1.3 log reduction of *L. monocytogenes* and *E. coli* O157:H7 was achieved (Baert et al., in press). Although, MS2 and FCV were reported to be more resistant than *E. coli*, MNV-1 was more sensitive compared to bacterial pathogens *L. monocytogenes* and *E. coli* O157:H7. Further studies comparing the stability of bacterial and viral agents on foods is needed.

The mechanism of H_2O_2 and PAA to inactivate micro-organisms is not yet clear but these chemical agents probably denature proteins, enzymes and disrupt sulfhydryl (–SH) and (S–S) sulfur bounds. The advantage of PAA is that it is not decomposed by peroxidases like H_2O_2 (McDonnel and Russell, 1999). Furthermore, it was observed that spraying H_2O_2 at 50 °C onto lettuce followed by a further illumination period with UV improved the inactivation of MS2 (Xie et al., 2008). The generation of hydroxyl radicals in the vapor phase and the temperature at 50 °C was suggested to enhance the penetration into the phage capsid. Visual characteristics of treated lettuce were preserved which can be attributed to the inactivation of enzymes responsible for discoloration. Research is needed to evaluate the efficacy of other non-aqueous based decontamination procedures upon elimination of viruses. Gaseous chlorine dioxide and gaseous ozone would be of interest because these treatments were reported to be useful to decontaminate strawberries and raspberries with respect to bacterial pathogens (Sy et al., 2005; Bialka and Demirci, 2007).

Furthermore, internalization of pathogens in fresh produce is of great concern as these micro-organisms are inaccessible by means of washing treatments. Li et al. (2008) demonstrated the infiltration of *E. coli* O157:H7 during vacuum cooling into lettuce tissue. A stringent chlorine treatment or subsequent washing steps could not eliminate the internalized pathogens. Infiltration of *Salmonella* Montevideo into tomatoes was shown when tomatoes, having a temperature of 25 °C, were dipped into a solution of 10 °C (Zhuang et al., 1995). It is therefore recommended to maintain a higher temperature of the washing solution than the temperature of the produce to avoid a negative temperature differential. It is likely that viruses can internalize in a similar way as was observed for bacterial pathogens. Studies are needed to further elucidate this issue.

Overall, the addition of sanitizers (chlorine, PAA, H_2O_2) established a marginal reduction of viruses on fresh produce. Nevertheless, Baert et al. (in press) reported the usefulness of sanitizers to keep microbiological and viral quality of the wash water in order to avoid cross-contamination between wash water and fresh produce.

4.2. Purification of live bivalve shellfish

Bivalve molluscan shellfish (oysters, mussels, clams, cockles) feed themselves by filtering out solid particles from surrounding water and may in this way also accumulate bacterial and viral pathogens (Lees, 2000). Raw consumption of these products, or only lightly cooked, may pose a risk for human health. The microbiological quality of live bivalve molluscs is evaluated on the basis of the level of *E. coli* and fecal coliforms (EEC Council Directive, 1991; FDA, 2003). Despite the quality of shellfish relying on bacterial indicators, no correlation between bacterial and viral contamination of shellfish was found (Abad et al., 1997; Chironna et al., 2002; Romalde et al., 2002). Besides, FRNA phages and HAV were concentrated up to 100-fold in bivalve shellfish while the accumulation of fecal coliforms and *E. coli* was not equivalent (Enriquez

et al., 1992; Burkhardt and Calci, 2000). Purification procedures (depuration or relaying) are required before shellfish can be marketed as live molluscs, if they do not meet microbiological standards. Depuration involves transferring shellfish in tanks of seawater that has been sterilized by physical or chemical means, mainly by ozone or UV treatment (Son and Fleet, 1980). Depuration rapidly purged out *E. coli* and other bacterial pathogens whereas considerable levels of viral units remained (Son and Fleet, 1980; Schwab et al., 1998). Son and Fleet (1980) reported acceptable purification after 48 h of depuration with regard to *E. coli*, *Salmonella*, *B. cereus* and *C. perfringens* present in oysters. Depuration of oysters during 48 h reduced *E. coli* by 95% while a minimal decrease (7%) of NoV was established (Schwab et al., 1998). Moreover, human pathogenic viruses were detected at the same frequency in oysters with or without the application of commercial depuration practices in four European countries (Formiga-Cruz et al., 2002). Chironna et al. (2002) reported the presence of HAV genomic copies in 11.1% depurated mussels, marketed in Puglia (South Italy), and 4.4% contained infectious HAV units. Nevertheless, a remarkable decrease in the number of contaminated mussels was observed after depuration. Evidence is given by these studies that depuration, according to current legislation, is not adequate. FRNA phages were therefore suggested as viral indicator in addition to *E. coli*. Dore et al. (2000) found that elevated levels of FRNA phages correlated with the presence of NoV contamination in depurated oysters and the incidence of gastroenteritis associated with these products. Additionally, depuration dynamics of FRNA phages in naturally polluted mussels were significantly related with human adenoviruses and enteroviruses (Muniain-Mujika et al., 2002). However, another study using rotavirus like particles (RoVLPs) recorded 1 log reduction after 7 days oyster depuration at 22 °C while MS2 was reduced by 2 log (Loisy et al., 2005). These results indicated that FRNA phages do not correlate with all human enteric viruses in bivalve shellfish.

HAV and rotavirus showed less than 2 log reduction after 4 days depuration of experimentally contaminated mussels while adenovirus and poliovirus were reduced by at least 3 log (Bosch et al., 1995; Abad et al., 1997). The discrepancy observed between different types of viruses regarding the efficiency of depuration raised questions about the possible selective retention or persistence of viruses in shellfish. Specific retention of NoV was observed by Ueki et al. (2007) observing no decline of NoV genomic copies in artificially contaminated oysters after depuration for 10 days whereas FCV could not be detected anymore after 3 days. It is currently demonstrated that NoVLPs bind to histo-blood group antigen-like (HBGA) carbohydrates in the digestive tissue of shellfish and may account for the inefficiency of depuration practices (Le Guyader et al., 2006). Besides the type of virus strain, other factors such as the initial contamination level, depuration system, physiological state of the shellfish, seasonal conditions, water temperature and salinity might have an influence on the depuration dynamics of contaminants (Dore and Lees, 1995; Lees, 2000; De Medici et al., 2001; Kingsley and Richards, 2003).

Because shellfish can be held in depuration tanks only for a relatively short period, relaying could be a good alternative for heavily polluted shellfish (Lees, 2000). Relaying implies transferring polluted shellfish to natural, pollution free marine environments (Son and Fleet, 1980). Humphrey and Martin (1993) reported that FRNA phages were not detected anymore after 2–3 weeks of relaying while somatic phages were still detected after 5 weeks. RoVLPs could be detected up to 37 days of relaying when an initial concentration of 10^5 VLPs/oyster was present (Loisy et al., 2005). Information regarding the efficacy of relaying shellfish to purge out viral contaminants is scarce but the legal requirement of 2 months for heavily contaminated shellfish seems not to be excessive (EEC Council Directive, 1991).

Alternative purification systems or decontamination technologies are required to decrease the viral load in bivalve shellfish. For instance, Tian et al. (2007) suggested the application of HBGA analogs, e.g. pig stomach mucin, in depuration systems to reverse the binding of NoV to oyster tissue.

5. Conclusions

Evidence is given by several studies that depending on the food matrix, viruses can decline during chilling. Yet, persistence of a considerable number of viruses is mostly ascertained during the shelf life period of chilled foods. Studies investigating the stability of viruses in foods, did not focus on the factors which could be responsible for the observed decline. It was suggested that some types of fresh produce showed antiviral activity. Further research is needed to characterize antiviral compounds or micro-organisms exhibiting antiviral activity. Viruses can also survive in acidified, dry or MAP conditions. The long term survival of viruses in combination with the low infectious dose indicates that food preservation methods establishing microbial growth inhibition will not be sufficient to prevent foodborne viral infections.

Preservation methods establishing microbial inactivation such as heating, high hydrostatic pressure processing and irradiation are therefore considered as intervention strategies to reduce the level of viruses. The heat inactivation data obtained in several studies suggested that high temperature, short time pasteurization (e.g. 72 °C, 15 s) would accomplish less than 1 log reduction for some enteric viruses and that at least conventional pasteurization (e.g. 63 °C–30 min, 70 °C–2 min) is needed to achieve more than a 3 log reduction. Additionally, the required time–temperature combination depends upon the food matrix.

Non-thermal preservation technologies are often preferred to retain nutritional and sensorial aspects of foods e.g. raw bivalve shellfish, lettuce, raspberries and strawberries. High hydrostatic pressure might be able to reduce the level of HAV and NoV by more than 3 log, whereas strains of the genus *Enterovirus* are shown to be very resistant to HPP. Investigation of UV and gamma irradiation to eliminate viruses is limited. More data is required to determine the influence of food matrices. Additionally, the possibility of foodborne viruses to acquire resistance or other mutations needs to be examined.

Decontamination procedures on fresh produce were shown to have a reduction of approximately 1 to 2 log reductions. The efficacy of sanitizers varied between viral strains whereby the explanation for the different rate in decline is difficult to define. A different approach by investigators regarding the initial virus titer, inoculation procedure and produce: treatment ratio influences the outcome. Additionally, alternative decontamination treatments shown to be of great value to decrease bacterial pathogens should be evaluated with respect to viruses. Especially procedures which can be applied on perishable produce such as raspberries and strawberries are of interest. The possibility of viruses to internalize fresh produce and the effect upon decontamination should be elucidated.

If inactivation/decontamination studies with regard to viruses would be conducted in the future, it is recommended to include different viral strains in the same experiment. In this way, it will be feasible to compare the reduction levels between viruses which is not obvious with the present reported data.

Depuration and relaying would occasionally be inadequate to remove viruses from live bivalve shellfish within a practical achievable time period. Moreover, the operative microbiological criteria for live bivalve shellfish are not linked to viral contamination. Viral indicators should be included as complementary parameters in addition to the mandatory bacterial indicators. More data showing that a proposed viral indicator such as FRNA phages correlates well with the human enteric viruses in shellfish is needed.

Due to the lack of culturing methods for human enteric viruses, detection of human viral strains in environmental samples and food products is based on molecular methods such as RT-PCR. The discrepancy observed between the detection of viral genomic copies and infectivity after heat or UV treatment involves that the presence of genomic copies is not necessarily associated with infectious virus particles. Novel methodologies enabling the differentiation between infectious and non-infectious virus particles need to be developed. In this way, a more

reliable correlation between the detection of viruses in foods or the environment and the risk for public health could be established.

Detection of genomic copies indicates unequivocal contact with the considered viral pathogen. Good agricultural practices on the field and good hygienic practices throughout the whole food chain can prevent viral contact with food and will eventually decrease foodborne outbreaks attributed to viruses.

Research is needed in evaluating the efficacy of manure treatments and water treatments establishing viral inactivation since water and treated manure can come into contact with shellfish and fresh produce. In this way effective preventive measures toward viral contamination on the primary level can be introduced rather than being solely dependent upon preservation methods to establish a viral decline on foods.

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