National Institute for Public Health
and the Environment
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Efficacy of applied processing measures on virus reduction in food

RIVM report 330371007/2013

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Colofon

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This investigation has been performed by order and for the account of the Dutch Food Safety Authority, within the framework of Project V/330371 'Viruses in food'.

Rapport in het kort

De effectiviteit van desinfectieprocessen om virussen in voedsel te verminderen

Verse producten zoals groente en fruit worden tijdens het productieproces behandeld om de aantallen schadelijke micro-organismen te verlagen. Hiermee wordt zowel de houdbaarheid van het product verlengd, als het aantal ziekmakende micro-organismen verlaagd. Deze behandelingen zijn vooral effectief om bacteriën onschadelijk te maken, wat de kans verkleind dat consumenten er ziek van worden. Voor virussen is dit effect echter gering als de behandeling wordt uitgevoerd met de doseringen die de voedselindustrie momenteel gebruikt. Hogere doseringen zijn effectiever, maar tasten de voedselkwaliteit en de kleur en structuur van het product te veel aan. Dit blijkt uit een literatuurstudie van het RIVM naar de effectiviteit van desinfectieprocessen in de voedselindustrie op virussen. De studie is in opdracht van de Nederlandse Voedsel en Warenautoriteit (NVWA) uitgevoerd.

Een van de mogelijke behandelingen is producten wassen in water waaraan desinfecterende stoffen zijn toegevoegd, zoals chloorverbindingen, waterstofperoxide of ozon. Verder wordt op kleine schaal gewerkt met behandelingen waarmee ook de micro-organismen worden bereikt die dieper in het product verscholen zitten, zoals het product behandelen met UV- of gammastralen of onder hoge druk plaatsen. Ze zijn echter het meest effectief bij doseringen die de voedselkwaliteit en het karakter ervan aantasten.

Een veelbelovende oplossing lijkt de combinatie van verschillende behandelingsmethoden (de ‘hurdle-technologie’). Op deze manier kan elke methode onder mildere condities of met een lagere intensiteit worden uitgevoerd, waardoor de structuur en kleur van het product wel behouden blijven. Zorgvuldig onderzoek is echter nodig naar welke combinaties het effectiefst zijn om virussen onschadelijk te maken en die tegelijkertijd de kwaliteit van de verse producten behouden.

Trefwoorden: virusinactivatie, voedsel, desinfectant, bestraling, hoge druk behandeling

Abstract

Efficacy of applied processing measures on virus reduction in food

Fresh produce such as fruit and vegetables are treated during production to decrease the numbers of harmful microorganisms. This will extend the produce's shelf life and will reduce the numbers of pathogenic microorganisms on the food. These treatments are especially effective for the inactivation of bacteria, thereby reducing the risk of consumers becoming ill. However, viruses are not efficiently inactivated when treatment is performed with the doses currently used in the food industry. Higher doses may be more effective but adversely affect the quality, color and texture of the product. This has emerged from a literature review conducted by the National Institute for Public Health and the Environment (RIVM) on the effectiveness of disinfection processes for viruses in the food industry. The study was commissioned by the Dutch Food and Consumer Product Safety Authority (NVWA).

One of the possible treatments is the washing of produce in water containing disinfectants, such as chlorine compounds, hydrogen peroxide or ozone. Other treatments that are applied on a small scale, such as treatment by UV or gamma rays or high-pressure processing, have the advantage that they not only affect the surface of produce but penetrate the produce to inactivate pathogens sheltered in e.g. crevices or seed pockets. However, they are most effective at doses that induce undesirable structural changes and deteriorate food quality.

A promising solution is a combination of treatments ('hurdle technology'). This enables each treatment to be applied at low intensity, thus preserving the freshness and structure of produce but giving it a longer shelf life. The selection of hurdles needs to be made carefully to obtain effective virus inactivation while preserving the quality of the fresh produce.

Keywords: virus inactivation, food, disinfectant, radiation, high pressure processing

Acknowledgements

The authors would like to thank Erwin Duizer (Laboratoy for Infectious Diseases and Perinatal Screening, RIVM) for his close reading of the manuscript and helpful comments.

Contents

Summary

Infectious viruses on foods may be reduced by various decontamination techniques; however, the procedures used in the food industry have not yet been fully evaluated for their effectiveness against viruses. This report describes current and potential intervention measures for the soft fruit and leafy greens production chain. Literature was reviewed in order to assess the ability of currently applied and promising new intervention measures to reduce the infectious virus load on the selected food commodities. Moreover, critical parameters affecting the efficacy of current processing measures in virus reduction are specified.

In general, the persistence of enteric viruses is high at low temperatures, and decay rates increase with increasing temperatures. Since the shelf life of fresh produce is short and only a low reduction of enteric viruses is expected in this time span, additional intervention measures are needed to reduce the levels of contaminating infectious viruses. The efficacy of current treatment processes is influenced by several parameters such as the type of produce, surface morphology, temperature, pH, type of pathogen and microbial attachment to the produce. Pathogen reduction by washing procedures that use disinfectants based on active chlorine is often only marginally greater than when using only potable water. Disinfectants applied in washing waters of lettuce are mostly promising as a disinfectant since they diminish the risk of cross-contamination and infiltration during washing. It is advisable to add the disinfectants to the washing waters prior to the cutting of lettuce to optimize the efficiency of the process and to further minimize the infiltration of pathogens into the produce. Due to the generation of harmful by-products when chlorine interacts with organic matter, the use of hypchlorite-based systems for fresh produce washing is prohibited in various European Union countries. Therefore, the activity of alternative sanitizers, such as chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), peracitic acid (PAA), and ozone (O_3), were compared with that of chlorine washing.

Novel technologies, such as irradiation or high-pressure processing, which not only affect the surface of produce but also penetrate the produce to inactivate pathogens sheltered in e.g. crevices or seed pockets of the produce, are evaluated. In the food industry, radiation has been applied in the form of UV-light (non-ionizing) gamma radiation and electron beam radiation and is effective in the reduction of food spoilage, the reduction or elimination of pathogenic organisms, prevention of sprouting, and delaying of fruit ripening. High-pressure processing shows great potential in the food industry for inactivating microorganisms and extending the shelf life of food products, with little effect on the quality of fresh foods. Literature on the inactivation of viruses in food items by both methods is limited. However, enteric viruses are in general more resistant to radiation and high-pressure processing than bacteria. The main limitation of both methods is their impact on produce quality when the doses required to efficiently inactivate viruses are used. For frozen berries, high-pressure processing or irradiation at higher doses may be feasible, because the structure of berries has already been changed prior to processing during freezing. The hurdle technology approach seems most promising in guaranteeing food safety while maintaining produce quality.

1 Introduction

Disease outbreaks caused by foodborne viruses are increasing, partly because of increases in population, scarcity of clean water and changes in eating habits such as the increase in consumption of fresh produce throughout the year (Goyal 2006, EFSA 2011). Sales of minimally processed ready-to-eat fruits and vegetables have grown rapidly in the last decade (Han et al. 2004), and fruits and vegetables have become a leading vehicle in foodborne outbreaks (Smith 2004). Recently, the US Center for Disease Control and Prevention published a report on the surveillance of foodborne disease outbreaks in the US in 2006, and after poultry (21 per cent), leafy vegetables (17 per cent) and fruits/nuts (16 per cent) were recognized as the most common food commodities associated with foodborne outbreaks, whereby the majority of outbreaks were caused by human norovirus (hNoV) (CDC 2009, Doyle 2008).

To reduce the risk of foodborne viral illness associated with these outbreak-related food commodities, the investigation of efficient food safety strategies to prevent and control virus contamination is highly relevant. Enteric viruses cannot grow in or on food, but food can be contaminated with viruses and serve as a vehicle for transmission. The contamination of produce with human pathogenic viruses is due to human faecal pollution and can occur at several points within the farm-to-fork trajectory. Suspected primary causes of virus contamination of fresh produce are: (i) faecally contaminated water used for irrigation, in the dilution of chemical substances prior to harvesting or during processing and (ii) infected or contaminated people handling the food during production, processing, preparation, and retail or vomiting in production or preparation facilities. Moreover, cross-contamination can occur via contaminated surfaces, processing water, equipment and direct contact between contaminated and non-contaminated produce. Any produce touched by humans is susceptible to human faecal contamination. Also raw or minimally processed food is at risk to be contaminated with enteric viruses. Examples of these are fruits and vegetables, ready-to-eat foods like sandwiches and vegetable salads, and raw or lightly cooked bivalve molluscs (EFSA 2012) and meat.

There are two ways to lower the incidence of foodborne viral illness: prevention of contamination and intervention measures implemented to reduce or eliminate viral contamination (CAC 2012). An overall food safety concept from farm to fork including good practice (e.g. GAP, GHP and GMP) and optimized or novel, more effective intervention measures are needed to minimize the risk of illness from enteric viruses present on fruits and vegetables. The contamination of produce is, however, poorly understood and good practice is often difficult to control; particularly due to the high number of asymptomatic infections of hNoV and other viral disease (De Wit et al. 2001). Efficient intervention measures will reduce the risk of any emerging virus being transmitted by food.

The load of infectious viruses on foods may be reduced by various decontamination techniques; however, the procedures used in the food industry have not yet been fully evaluated for their effectiveness against viruses. So far, the intervention measures used in food industry is validated mainly for bacterial and fungal pathogens. The literature suggests, however, that viruses are often more resistant to treatment.

This report describes current and potential intervention measures for the raspberry and lettuce production chains. Literature was reviewed in order to assess the ability of intervention measures to inactivate and/or remove viruses on the selected food commodities. Critical parameters affecting the inactivation efficacy of those interventions are specified. In addition, the impact of the treatment processes on produce quality is described, because this is often a limiting factor for their application.

2 Natural inactivation

Virus infectivity can be affected by various environmental conditions and factors, such as temperature, humidity, and pH value (Rzeżutka et al. 2004). In addition, time is an important factor for viral persistence; the longer a virus is infectious, the higher the probability of its transmission. The shelf life of fresh produce is short, indicating that only a low reduction in numbers of infectious enteric viruses (virus inactivation) is to be expected if fresh produce is stored at cold temperatures (Verhaelen et al. 2012). Viral persistence in the field may be lower than persistence in the retail chain due to higher temperatures and other factors influencing the virus infectivity such as sunlight (UV). Most of the enteric viruses are single-stranded RNA viruses, which are known to be more susceptible to inactivation by UV radiation than double-stranded DNA viruses such as hAdV (Gerba 2002).

Studies on the persistence of enteric viruses have been performed in several matrices, including fruits (raspberries, strawberries), vegetables (lettuce, parsely, cabbage, bell peppers), stainless steel and other working surfaces, and liquids (buffered solutions, water, milk) (Table 1). In general, the persistence of enteric viruses is high at low temperatures, and decay rates increase with increasing temperatures. Kurdziel et al. (2001) studied the persistence of poliovirus on several soft fruits and salad vegetables over a period of two weeks at 4°C and found no viral decay on green onion and fresh raspberries, while a decline in virus numbers of 90 per cent was obtained for lettuce, white cabbage, and frozen raspberries after 8 to 14 days. Mattison et al. (2007) studied the persistence of a hNoV surrogate, feline calicivirus (FCV), on strawberries and lettuce. They showed that FCV is reduced rapidly on strawberries stored at room temperature, with a sharp initial decrease, which was not the case after storage at 4°C. Verhaelen et al. (2012) demonstrated that noroviruses were less persistent on strawberries than on raspberries. The difference in viral persistence was most pronounced at 21°C. MNV-1 infectivity dropped about 1.5 $\log_{10}$-unit on strawberries after just one day of storage at room temperature, whereas no virus decay was observed on raspberries during the same period. Assuming a similar persistence for MNV-1 and hNoV, hNoV is therefore likely to stay infectious on raspberries during retail under the tested conditions and is also more likely to persist on raspberries in the field than on strawberries.
Relative humidity does not uniformly affect the survival of microorganisms on produce surfaces (Stine et al. 2005). HAV and the coliphage PRD1 were found to have the lowest inactivation rate, whereas FCV tended to become inactivated more rapidly. Although enteric viruses tend to survive better in conditions of high relative humidity, Mbiti et al. (1991) demonstrated that HAV infectivity was inversely proportional to the level of relative humidity and temperature.
Hewitt et al. (2009) and Butot et al. (2008) studied the persistence of hNoV GI and GII under freezing and heating to 72°C, respectively, and demonstrated a high persistence of NoV GI compared with NoV GII, based on PCR detection. Verhaelen et al. (2012) showed different persistence for NoV GI and GII on strawberries at 21°C. These data implied that NoV GII is more resistant then NoV GI.

Table 1: Persistence of enteric viruses and surrogates on fresh produce and food contact surfaces

Virus	Matrix	Conditions	Reference
MNV-1, FCV	Buffer, stainless steel	1. pH 2-10 2. 56°C, 63°C, 72°C 3. organic solvents: Freon, chloroform, vertrel 4. 4°C stainless steel (7 days, wet and dry)	Cannon (2006)
FCV, CaCV	Buffer	pH 2, 37°C, 30 min	Duizer (2004a)
NoV GI, NoV RNA, FCV	Stainless steel, formica, ceramic	1. Room temperature, 7 days 2. transfer: steel to wet/dry lettuce	D'Souza (2006)
FCV	Lettuce, strawberries, ham, stainless steel	1. 4°C, 7d 2. Room temperature, 7 days	Mattison (2007)
MNV-1, HAV, NoV GI/II	Water, milk	63°C and 72°C up to 10 min	Hewitt (2009)
NoV GI/II, FCV, HAV, RV	Blueberries, raspberries, strawberries, basil, parsley	-20°C for 2, 30, 90 days	Butot (2008)
MNV-1	Spinach, onions, water	-21°C, 6 months	Baert (2008)
MS2	Strawberries, lettuce, tomato, parsley and other	4°C, 8°C, 22°C, 7 days	Dawson (2005)
FCV	Cell culture medium, cover slip (dry)	1. 4°C, 20°C, 37°C suspension 2. 4°C, 20°C, 37°C dried state	Doultree (1999)
FCV, E-coli, MS2	Lettuce, cabbage	4°C, 25°C, 37°C for 21 days	Allwood (2004)
E-coli, Shigella, Salmonella enterica, Clostridium perfringens, HAV, FCV, PRDI	Cantaloupe, lettuce, bell peppers	light exposure, humidity, 22–24°C, 14 days	Stine (2005)
HAV	Stainless steel	1. 25%, 55% and 80% humidity, 2. 5°C, 20°C, and 35°C	Mbithi (1991)
PV	Lettuce, green onion, cabbage, raspberries, frozen strawberries	4°C for 15 days	Kurdziel (2001)
NoV GI/II, MNV-1, hAdV	Strawberries, raspberries, buffer	4°C, 10°C, and 21°C for 7 days	Verhaelen (2012)
NoV GI	Ground-, tap- and reagent-grade water	Room temperature, > 3 years	Seitz (2011)

Since no robust cell culture system is available for the replication of hNoV (Duizer et al., 2004), hNoV infectivity can be assessed only using human dose-response experiments. Consequently, there is little information available about the persistence of hNoV and most studies refer to the use of surrogates such as FCV and murine norovirus (MNV-1). Cannon et al. (2006) studied the persistence of both viruses in suspension and on stainless steel and demonstrated that persistence was similar at 4°C. At room temperature in solution, MNV-1 was more stable than FCV. Both the genetic relatedness of MNV-1 to hNoVs and its ability to survive under gastric pH levels make this virus

a promising surrogate to study hNoV persistence. However, Hewitt et al. (2009) showed that caution should be exercised when extrapolating surrogate virus data for hNoV by showing that hNoV may be more resistant to heat than MNV-1. Studies comprising human volunteers showed that hNoV remained infectious after exposure to pH 2.7 for 3 hours at room temperature (Dolin 1972, Greening 2006). Long-term infectivity studies of human norovirus in groundwater performed with eligible volunteers revealed that NoV GI (Norwalk virus) can remain infectious for at least 61 days in the dark at room temperature (Seitz et al., 2011).

3 Disinfectants

In this section, the results from studies on the efficacy of disinfectants in virus removal and inactivation are discussed. Both disinfectants that are currently used in the food industry for soft fruits and leafy greens and those that are of potential use in the food industry are included.

3.1 Chlorine solutions

Currently, chlorine is the most widely used sanitizer in the food industry. Seventy-six percent of fresh produce manufacturers use chlorine-based washing systems (Betts 2005). It is, however, reported that in many cases the level of microbial reduction achieved by hypochlorite systems on vegetable and salad crops is only marginally better than the reduction achieved by using potable water alone. Baert et al. (2009), for example, reported that treatment of shredded lettuce with a 200 ppm sodium hypochlorite (NaOCl) suspension resulted in a supplementary 1 log PFU/g reduction of murine norovirus compared with washing with tap water alone – similar to the reductions achieved for *E. coli* O157:H7 and *L. monocytogenes*. Similar results were obtained by (Fraisse et al., 2011), who described a supplementary reduction of 0.4 log units for MNV-1, 1.2 log units for HAV and 2.2 log units for FCV by washing for 2 minutes with 15 ppm active chlorine as compared with washing without disinfectant. It has to be considered, though, that aside from the inactivation of pathogens on the produce, the addition of chlorine reduces the microbial contamination in the flume or washing water, preventing the re-introduction of viruses onto the produce and cross-contamination. Even at a NaOCl concentration of only 20 ppm it is reported that no infectious murine norovirus could be found in the washing water of shredded lettuce. This is especially relevant if washing water is reused. To disinfect produce, chlorine is commonly used at concentrations of 50-200 ppm free chlorine with a contact time of 1–2 minutes (Beuchat 1998, Butot 2008).

The effectiveness of a chlorine washing step is affected by several factors. Beside the pathogen, food items themselves have an impact on the inactivation efficacy due to differences in pH, protein, and lipid content, etc. It is thus necessary to study efficacy in terms of pathogen–product combinations. Butot et al. (2008), for example, investigated the inactivation potential of chlorinated water on NoV GI and NoV GII present on various foods and found that chlorinated water had only a limited effect on enteric virus titers when used to decontaminate raspberries and parsley; probably because of the nature of their surfaces. Raspberries have crevices and hairlike projections, which may shield the viruses (Butot 2008). Washing with chlorinated water (200 ppm free chlorine), however, resulted in significant reductions of NoV GI and GII on blueberries, strawberries, and basil, perhaps due to their smooth surface compared with raspberries. The impact of the food surface on treatment efficiency is further illustrated by Keskinen et al. (2009). They reported different treatment efficiency using iceberg and romaine lettuce as produce for *E. coli* O157:H7 and *L. monocytogenes* testing.

The efficiency of chlorine washing is further dependent on process parameters. It is important to know the critical process parameters in order to be able to control and optimize the process. For chlorine-based washing procedures the amount of free chlorine, and therewith the inactivation properties, is mainly dependent on pH, temperature, and the amount of organic matter in the solution (Beuchat 1998). In order to achieve maximal inactivation the activity of

the chlorine needs to be optimized and controlled. A pH of 6.0 to 7.5 is most appropriate for effective sanitizing activity without damaging equipment surfaces (Beuchat 1998, Parish 2003). Hypochlorous acid (HOCl) is the form of free available chlorine that has the highest bactericidal activity; between 50-80 times higher than hypochlorite (OCl^-) (Betts 2005). The equilibrium between HOCl and OCl^- is pH-dependent, with the concentration of HOCl increasing as pH decreases (Parish 2003). Yet it has been reported that only 20 per cent of the fresh produce industry routinely controls the pH of the solution (Betts 2005). For chlorine washings in the fresh produce industry sodium hypochlorite (NaOCl) is frequently used. Since NaOCl forms HOCl and NaOH when dissolved in water, the solution has a tendency to be alkaline, resulting in suboptimal inactivation. The control of the pH by adding e.g. citric acid (FSAI 2001) is essential for the efficacy of the process.
Temperature is also an important parameter for disinfection efficacy. Chlorine has a maximum solubility in water at about 4°C. The temperature of the chlorinated water should, however, be about 10°C higher than the temperature of the fruit or vegetables to minimize the uptake of washing water through stem tissues and open areas, such as cutting edges in the skin or leaves (Beuchat 1998). This is essential to minimize the infiltration of pathogens. Infiltration occurs because gas inside fruit and vegetables contracts with a decrease in temperature, creating a partial vacuum that draws in water through pores, channels, or punctures, allowing the uptake of human pathogens therewith (Sapers 2003). No literature describing the infiltration of viruses in this manner could be found, but logically this incidence should apply not only to bacteria but also to viruses. In terms of product quality, the use of cold processing water is, however, beneficial and thus in practice water at a temperature of 4°C or lower is used to wash cut lettuce leaves promoting the intake of pathogens. Infiltration should also be kept in mind as a possible means of virus contamination during irrigation, since irrigation is performed in the hot summer months, meaning that the produce has a higher temperature than the irrigation water, which is pumped up from a well.
Moreover, chlorine rapidly loses its effectiveness on contact with organic matter. Chlorine can bind with compounds from organic material, e.g. ammonia or other nitrogenous compounds, to form 'bound' or 'combined' chlorine, which is much less effective as a biocide than free chlorine (HOCL and OCl^-). In the majority of cases, however, it is levels of total chlorine that are controlled by the food industry, which does not provide an accurate measure of the efficacy of the system (Betts 2005).
Combining commonly used sanitizers with surfactants, such as sodium dodecyl sulphate (SDS) and polysorbates, has been shown to enhance the efficiency of inactivation of MNV-1 by chlorine. A reduction of approximately 3 logs was obtained for MNV-1 present on strawberries, lettuce, cabbages and raspberries when treated with 50 ppm of SDS or polysorbates and 200 ppm chlorine, as compared with a reduction of <1.2 logs when treaed with 200 ppm chlorine without surfactant (Predmore and Li, 2011). SDS is an FDA-approved food additive and polysorbates are recognized by the FA as GRAS (generally recognized as safe) products. Therefore, implementation of this novel sanitization strategy would be a feasible approach to the effective reduction of the virus load in fresh produce.

Summarizing, the efficacy of chlorine washing is influenced by several parameters, including type of produce, surface morphology, temperature, pH, type of pathogen, and microbial attachment to the produce. This needs to be considered when evaluating the efficacy of chlorine washing in inactivating viruses.

3.2 Other sanitizers

There is a need to investigate alternative sanitizers, because chlorine is known to interact with the organic matter present in washing water for produce, generating a spectrum of harmful by-products including trihalomethanes (Beuchat 1998). Therefore, the use of hypochlorite-based systems for fresh produce washing is already prohibited in various European Union countries. In addition, it is of interest to study the virus inactivation efficacy of other potential sanitizers and compare it with that of chlorine. Chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), peracitic acid (PAA), and ozone (O_3) are possible alternatives to NaOCl. The primary inactivation action of chemical sanitizers is oxidation. Comparing oxidation potential therefore gives an indication of the inactivation efficacy of a sanitizer. NaOCl has the lowest oxidation potential of those listed and ozone has by far the greatest. In theory, the strongest oxidizer should inactivate pathogens most rapidly. However, the influence on the reduction efficiency of e.g. pH and organic matter in the washing water needs to be considered.
Chlorine dioxide (ClO_2) received attention as a alternative to chlorine largely because its efficacy is less affected by pH and organic matter and it does not react with ammonia to form chloramines (Butot 2008, Beuchat 1998), resulting in less formation of toxic or mutagenic by-products (Rodgers 2004). Butot et al. (2008) tested HAV, hNoV, FCV and Rotavirus reduction on produce. Norovirus GII appeared to be more susceptible to inactivation by a ClO_2 treatment on raspberries and parsley than hNoV GI, based on RT-PCR results. Efficiency on raspberries and parsley was found to be lower than on other produce, likely due to crevices and hair-like projections, which suggest a poor ability of ClO_2 to penetrate the protective surfaces of produce. Under the used conditions, a similar hNoV reduction was obtained using 200 ppm NaOCl instead of a concentration of 10 ppm chlorine, showing a higher efficacy of ClO_2 at lower concentrations. Kim et al. (2008) reported no colour change in lettuce treated with concentrations ranging from 10 to 50 ppm ClO_2; a concentration as high as 200 ppm ClO_2 results, however, in noticeable bleaching at the cut edges of lettuce (Keskinen 2009). As for NaOCl, the decontamination of washing water for lettuce was reported to be successful using low ClO_2 concentrations (Singh 2002). Further investigation of the efficacy of ClO_2 treatment for lettuce at concentrations not affecting the produce quality is worthwhile, because of the expected higher efficacy at lower concentrations in comparison with NaOCl and less formation of toxic by-products. A drawback of this sanitizer is its instability and the associated necessity for onsite production (Beuchat 1998).

Another potential disinfectant is hydrogen peroxide (H_2O_2). Li et al. (2011) demonstrated that both liquid H_2O_2 and a combination of vaporized H_2O_2 and UV light can be used for norovirus inactivation on surfaces, resulting in a 4 log reduction of MNV titer. However, only about a 1 log reduction was achieved if the virus was spiked on lettuce. No significant change of appearance or sensorial quality for fresh-cut lettuce was observed after H_2O_2 (2-3 per cent) treatment (Li et al. 2011, McWatters et al. 2002). It is reported that exposure to H_2O_2 vapour causes bleaching of anthocyanins in strawberries and raspberries. The U.S. Food and Drug Administration mandates to limit the use of H_2O_2 to fresh produce containing endogenous catalase (Burnett 2001).

Peracitic acid (PAA) is a mixture of acetic acid and H_2O_2 in an aqueous solution. It outranges the oxidation potential of chlorine and ClO_2. Moreover, it is shown to be hardly influenced by organic compounds present in lettuce washing water (Baert et al. 2009). When PAA dissolves in water, it decomposes to hydrogen

peroxide and acetic acid, which decomposes to water, oxygen, and carbon dioxide. PAA degradation products are thus non toxic and can easily dissolve in water (Lenntech). Additionally, levels of pesticide residues on food surfaces can be reduced (Hwang 2001). Baert et al. (2009) showed that PAA used in a concentration of 250 ppm results in a similar reduction of MNV-1 to that achieved with 200 ppm free chlorine on shredded lettuce. This result does not confirm the expected higher inactivation efficacy due to the greater oxidation potential of PAA in comparison with NaOCl. Furthermore, MNV-1 seems to be more resistant to PAA than FCV and MS2 (Baert et al. 2009). Fraisse et al. (2011) also reported that MNV-1 was more resistant to PAA treatment (100 ppm for 2 min.) than FCV, since FCV was inactivated by 3.2 by log units compared with 2.3 log units for MNV-1. With a reduction of 0.7 log units, inactivation of HAV was less efficient than the hNoV surrogates studied (Fraisse et al. 2011). A disadvantage of the use of PAA could be discoloration of berries, which is reported at concentrations of > 100 ppm (Lukasik 2003). Furthermore, consumers that were offered produce that was treated with several sanitizers only detected the use of 80 ppm PAA on chopped lettuce (Rodgers et al. 2004). Currently, PAA is mainly used as a sanitizer for food processing equipment, where it is particularly effective against biofilms (Beuchat 1998).

Ozone is a powerful antimicrobial. Although one of the most effective sanitizers known, yet ozone leaves no hazardous residues on food or food-contact surfaces (Khadre 2001). Ozone decays quickly in air and water, indicating that no residual ozone is present on the food upon consumption (Sharma 2005). Since 1997, ozone has been declared GRAS. The efficacy of ozone in inactivating viruses in water is tested in several studies. Data on virus inactivation on foods is, however, lacking. Doses for virus inactivation using water vary between 0.1 and 4.1 mg/L ozone with treatment times of 0.02 to 29 minutes (Kim 1999a). Shin and Sobsey (2008) investigated the inactivation of norovirus in water using a dose of 0.37 mg of ozone/litre and achieved a 3 log reduction within a contact time of 10 seconds. Thurston-Enriquez et al. (2005) found a 4 log reduction of FCV for 0.01 to 0.03 mg/l ozone within one minute of treatment with ozone. Overall, the results of these studies indicate that viruses can be reduced rapidly and extensively by ozone disinfection. Nevertheless, no direct conclusions as to the efficacy of ozone inactivation for viruses present on foods can be drawn, because ozone reacts with organic compounds present on foods, such as proteins and fats, thereby lowering the inactivation potential of ozone on the microorganisms (Seydim 2004). Additionally, the inactivation of microflora on food by ozone depends greatly on the composition of the food surface, the type of microbial contaminant, and the degree of attachment or association of the microorganism with the food. Indeed, greater treatment times of ozone were needed in fresh produce to achieve the same level of inactivation of FCV and MNV-1 in water (Hirneisen et al., 2011). To achieve a 2- to 3-log inactivation of FCV on green onions and lettuce, 5 to 10 minutes of ozone (6.25 ppm) was needed, whereas in water 1 minute of ozone treatment was required for similar inactivation of FCV. The same was observed for MNV, where after 0.5 minutes of ozone in water, approximately 2 log MNV was inactivated. To achieve a similar reduction on lettuce and green onions, a 1-minute treatment was necessary (Hirneisen et al. 2011). Kim et al. (1999b) compared the efficacy of different ozone applications on shredded lettuce and found that bubbling gaseous ozone into water in combination with high-speed stirring is the most effective application of ozone. In addition to concentration of ozone, product, and treatment time, the impact of stirring, bubble size (smaller bubbles provide a larger surface area and thereby greater inactivation of microorganisms) and temperature influence inactivation efficacy. Furthermore, the application of

pressure might facilitate penetration of ozone into the inaccessible cracks and crevices of foods and thereby enhance inactivation (Sharma 2005). Baur et al. (2004) studied the effects of chlorinated and ozonated water on the quality of shredded iceberg lettuce during storage. No relevant differences between organoleptic properties were found after two and five days of treatment. Only after seven days did chlorine washing result in a slightly better evaluation by consumers.

3.3 Electrolyzed water

In recent years electrolyzed oxidizing water (EOW) has been regarded as a new sanitizer. In Japan this novel antimicrobial agent has already been in use for several years. It is an effective disinfection method, easy to operate, relatively inexpensive and environmentally friendly (Huang 2008). Data on virus inactivation using electrolyzed water on food produce could not be found in the literature.

EOW is created by electrolysis of diluted sodium chloride solutions in an electrolysis chamber, having free chlorine as the major disinfection factor (Gomez-Lopez 2006). To produce it, a two-cell chamber consisting of anode and cathode separated by a membrane through which a diluted salt-water solution can pass is used. Subjecting the electrodes to direct current voltages results in two types of water, which have different characteristics: an acidic EOW (AcEW) and an alkaline EOW (AlEW). AcEW is more studied, because of its microbicidal properties: it has been shown to reduce the numbers of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* on lettuce and spinach (Park 2008). It is produced from the anode side and is characterized by a low pH (about 2 to 3), a high oxidation reduction potential (ORP), and the presence of hypochlorous acid, whereas AlEW has a high pH (about 11) and a low ORP. Furthermore, it is possible to produce a single stream of neutral EOW (NEW) in a single-cell system (Gomez-Lopez 2006, Huang 2008). AlEW lacks antimicrobial properties and, unlike AcEW, could not effectively remove bacterial pathogens from lettuce or spinach (Park 2008). Regarding viral decontamination, pre-washing the produce by AlEW prior to washing with AcEW may be beneficial. The combination of AlEW and AcEW will reduce bacterial pathogens, and the high pH of the AlEW is reported to favor viral release from fruits and vegetables (Dubois 2002), which may boost the reduction of virus particles. This favourable virus release induced by washing with AlEW could not be demonstrated for hNoV present on lettuce and green onions (Tian et al., 2011). The use of AcEW showed a significant decrease in the removal of hNoV from contaminated produce compared with regular water rinses. Results were similar for NoV GGI and GGII.

Literature on bacterial inactivation suggest that the use of EOW results in equivalent bacterial reduction on fresh-cut produce to sodium hypochlorite washing, while using less free chlorine (Abadias 2008, Koide 2009, Udompijitkul 2007). Therefore, the formation of toxic by-products derived from chlorination reactions is expected to be lower (Gomez-Lopez 2006, Udompijitkul 2007).

3.4 Gaseous vs. aqueous application of sanitizers

Several sanitizers, including as ClO_2, O_3, and H_2O_2, can be applied in gaseous form or dissolved in water. The literature suggests promising results for the application of ClO_2 in reducing non-viral pathogens contaminating fresh produce such as *Escherichia coli* O157:H7 or *Salmonella* on lettuce (Lee 2004). *Cyclospora cayatenensis*, a parasite associated with foodborne outbreaks with raspberries, is, however, not affected by gaseous ClO_2 treatment (Ortega 2008).

The gaseous application of sanitizers has several advantages over aqueous application. One advantage is better penetrability of gaseous sanitizers to protective sites of produce to reduce pathogens harboured in e.g. hydrophobic pockets or folds in the leaf surface in comparison with aqueous sanitizers (Lee 2004, Sy 2005a). The ability of gaseous sanitizers to penetrate inaccessible areas of produce can additionally be enhanced by pressurising the sanitizer. Bialka et al. (2007) investigated the efficiency of continuous and pressurised O_3 on raspberries and strawberries and found a 2.2 log CFU/g reduction of *Salmonella* on raspberries O_3 at 83 kPa, whereas continuous O_3 treatment resulted only in a 0.9 log CFU/g reduction after the same treatment time. However, the application of pressure may allow or increase infiltration of viral particles into the food matrix. A second advantage of gaseous sanitizers is that the problem of residual moisture promoting the growth of molds does not apply (Sy 2005b). This is of particular relevance to soft fruits, because they are susceptible to fungal contamination (Tournas 2005). Moreover, gaseous decontamination prevents cross-contamination, which may occur during washing.
Gaseous sanitizers also have drawbacks, such as the high cost of purchasing and operating complex machinery for continuous application of the sanitizer and the required technical expertise (Lee 2004, Han 2004). Additionally, gaseous sanitizers such as ClO_2 leave undesirable chlorite residues on the surface of produce (Han 2004). A complete decontamination of produce cannot be guaranteed, because even if the penetration ability of gaseous sanitizers might be higher, internalized pathogens cannot be removed.

A direct comparison of the aqueous and gaseous applications of sanitizers on lettuce based on published data is difficult, because the set-up of experiments varies widely. Kim et al. (2008) studied the efficiency of aqueous ClO_2 on lettuce using 1, 10, and 50 ppm of ClO_2. The highest concentration resulted in an additional reduction of *Salmonella* of 1.95 log CFU/g on shredded lettuce compared with washing solely with water. Sy et al. (2005b) found a reduction of about 1.5 log CFU/g on shredded lettuce using 4.1 mg/L of ClO_2. These results do not confirm the expected higher efficacy of gaseous application of sanitizers, even though results cannot be compared directly because the use of different *Salmonella* strains, inoculation procedures, sanitizer concentrations and microbiological analyses. Also for the human NoV surrogates MNV-1 and bacteriophage φX174, the expected higher efficiency of gaseous sanitizers could not be confirmed (Li et al. 2011). The inactivation of hNoV surrogates treated with liquid hydrogen peroxide (L-H_2O_2) was compared with inactivation using vaporized hydrogen peroxide (V-H_2O_2). L-H_2O_2 (2.1 per cent) was able to inactivate MNV-1 and bacteriophage φX174 on stainless steel discs by approximately 4 logs within 10 minutes of exposure. Treatment with V-H_2O_2 (2.52 per cent) resulted only in a marginal reduction (< 1 log after 5 min.) of these model viruses. Similar trends were observed for the inactivation of those viruses on shredded iceberg lettuce.

Unlike aqueous decontamination, gaseous decontamination is a potential intervention measure for the reduction of pathogens on soft fruits like raspberries (Bialka 2007, Han 2004, Sy 2005a). However, the treatment efficiency of gaseous sanitizers for berries like raspberries seems to be much lower than for lettuce. Sy et al. (2005a) investigated the reduction of Salmonella on blueberries, strawberries, and raspberries; Lee at al. (2004) for lettuce, using a similar set-up and similar ClO_2 concentration. Whereas on lettuce a reduction of about 4 log could be achieved using approx. 4 mg/L ClO_2, treatment of blueberries, strawberries, and raspberries resulted in a 3-log, 2.3-log and 0.5-log reduction, respectively. The authors related the low reduction on raspberries to the high

respiratory rate of raspberries in comparison with strawberries and blueberries. The high evolution of CO_2 associated with the high respiration rate was assumed to protect the surface from contact with gaseous ClO_2, thereby potentially reducing the lethality of ClO_2. Furthermore, the reduced access of ClO_2 between the drupelets of raspberries and harbourage sites in tissue juice released from broken trichomes were viewed as a possible explanation for the low efficacy of the treatment on raspberries (Sy 2005a). However, in the same study the highest reduction of yeast and molds due to ClO_2 treatment was on average achieved for raspberries, followed by strawberries and blueberries. These examples show once more the importance of investigating pathogen–produce combinations. ClO_2 is reported to be in general less effective on rough-textured produce items than on those with a smoother surface, such as lettuce (Ortega 2008). For lettuce the treatment is reported to be more effective on whole lettuce than on cut lettuce (Sy et al. 2005b). Beside type of produce and pathogen, treatment efficiencies are further influenced by humidity, temperature, the composition of the surrounding environment, e.g. shipping container, and light intensity (Lee 2004).

The concentration of a treatment is primarily determined by its effect on the produce quality and by legislative restrictions. Data about the deteriorating effect of gaseous sanitizers on lettuce quality varies widely depending on the source and the used method. Lee et al. (2004) stored lettuce leaves after treatment with 4, 7, and 9 mg/L ClO_2 for 18 days and detected no visible quality difference between untreated and treated lettuce leaves. Mahmoud et al. (2008), on the contrary, reported an immediate color change from yellow-green to white-brown after treatment with 5.0 mg/L ClO_2 for 10 minutes. Vandekinderen et al. (2009) stated that treatment with 1.5 mg/L gaseous ClO_2 did not affect the sensory quality of fresh-cut iceberg compared with washing with water alone, based on a triangle test. A significant change in product color was, however, observed by use of a spectrophotometer. Also, Sy et al. (2005b) reported that subjective evaluation of fresh-cut lettuce following a treatment with 1.4 mg/L ClO_2 revealed slight browning of the lettuce. Browning of lettuce can be inhibited by submerging the lettuce in a cysteine solution before ClO_2 treatment, though it has to be taken into account that the cysteine can also decrease the decontamination efficacy of the treatment (Gomez-Lopez 2008). Discoloration of berries is a problem attributed to the use of high concentrations of gaseous sanitizers (Lukasik 2003). A treatment of approx. 2 h with 5 per cent (wt/wt) ozone gas is reported to have no negative impact on fruit color (Bialka 2007). Sy et al. (2005b) reported that the sensory attributes (appearance, color, aroma, and overall quality) of untreated raspberries and raspberries treated with 4.1 mg/L ClO_2 were not significantly different when stored for 3, 7, or 10 days.

The investigation of gaseous sanitizers in this report is of particular interest, because it is one of the few intervention measures applicable to fresh raspberries. In the case of ready-to-eat-lettuce it is advisable to accomplish the treatment before cutting, to increase treatment effectiveness (Sy et al. 2005b). Moreover, the gaseous application of sanitizers is promising as a hurdle in lettuce production. Applied during transport or storage, before cutting and washing of the lettuce, the treatment could lower enteric virus concentrations on the raw produce and thereby cross-contamination and possible infiltration of viruses during cutting and washing. A subsequent washing step will further wash off residual chlorite. Currently, chemical sachets are on the market that allow the generation of ClO_2 by breaking a permeable membrane, allowing a convenient and continuous sanitation during the storage and delivery of food products (Lee 2004).

4 Radiation

Radiation in the food industry has various applications, including the reduction or prevention of food spoilage and pathogenic organisms, disinfestation, the prevention of sprouting, and delaying of fruit ripening. Radiation is a non-thermal physical process for preserving food and has been established as a safe and effective method of food processing and preservation (Farkas 1998). The main advantages of this technology apart from its efficacy in inactivating microorganisms are: a small temperature increase, absence of residues, effectiveness of treatment of pre-packed food (prohibiting recontamination during distribution) (Korkmaz 2005), and the ability to penetrate foods, allowing the inactivation of pathogens sheltered in plant materials (Lynch 2009). Radiation can, however, cause a change of texture or color in fruit and vegetable tissues, this being one of the main limiting factors in its use on fresh produce (Han 2004). The comparatively high cost and the absence of complete consumer acceptance are additional drawbacks of food irradiation technology (Korkmaz 2005). When applicable, radiation is, however, one of the most powerful methods of decontamination of foods (Beuchat 1998). It has been shown that vegetative organisms could be easily destroyed by radiation; however, additional data is needed on the effect of ionizing radiation on viruses and spore-forming microorganisms (Tewari 2003). In general, literature about the inactivation of viruses on food items is scarce, since most studies are directed toward bacterial inactivation. Viruses are, however, in general more resistant to radiation treatment (de Roda Husman et al. 2004, Fino et al. 2008, Shea 2000).

4.1 Types of radiation

In the food industry radiation in the form of UV-light (non-ionizing), gamma radiation, X-rays, and electron beam radiation can be applied. X-rays have, however, never found application in commercial food irradiation (Diehl 1995 p.18) and will not be further discussed. The impact of UV light is limited to the surface and its efficacy is strongly dependent on the surface topography. Fino and Kniel (2008) studied the inactivation of FCV using UV radiation on lettuce and strawberries, detecting a 4.62- and 2.28-log $TCID_{50}$/ml reduction, respectively, at 240 MW/cm^2. The low inactivation on strawberries in comparison with lettuce is explained by the different surface topography; viruses are likely buried and sheltered from UV light within or along the seed pockets of strawberries. The reduction of FCV infectivity on lettuce is promising, however. In this study a piece of salad leaf was directly exposed to UV radiation, whereas in practise it is not possible to radiate each leaf of lettuce separately, but rather the whole lettuce or the bulk of the leaves. An efficient inactivation of pathogens in crevices and creases or layers of lettuce leaves cannot be achieved with this technique. In comparison to UV light, the efficacy of gamma radiation is not only limited to the surface, but it can penetrate the product and eliminate microorganisms present in crevices and creases, which is significant for vegetables like lettuce (Han 2004). Besides, Chancellor et al. (2006) suggested the uptake of HAV through the roots of green onions as a potential mechanism for HAV contamination. Considering the uptake of virus into the produce by internalization or infiltration, as previously described (Hirneisen et al., 2012), UV radiation cannot result in an efficient, reliable decontamination.

Gamma rays are produced by radioactive elements and artificially induced radioactive isotopes using mainly ^{60}Co or ^{137}Cs. ^{137}Cs is, however, about one-third less efficient than ^{60}Co due to its higher self-absorption of radiation and therefore less attractive as a radiation source (Diehl 1995). The mode of action for virus inactivation is mainly based on the reaction of OH free radicals with nucleic acid strands. The virus coat may also play a role (de Roda Husman et al. 2004). Moreover, direct cleavage of DNA or RNA can also occur (Feng et al., 2011).

In contrast to gamma rays, electron beam rays are not inherently radioactive, which may result in greater consumer acceptance. They are generated from accelerators operating at or below an energy level of 10 MeV, to ensure that no observable radioactivity is introduced. It is generally accepted that with a maximum energy of 10 MeV and normal trace heavy metal content, any radioactivity induced in irradiated foods is no greater that the natural radioactivity of the foodstuffs caused by ^{14}C and ^{40}K. Furthermore, the induced activity decays rapidly during the first 24 hours (Korkmaz et al. 2005). An advantage of E-beam technology over gamma rays is that, in contrast to an isotope source, an electron accelerator can be switched on and off. Additionally, gamma ray sources provide a low dose rate (100–10,000 Gy/h) in comparison with an electron beam (10^4-10^9 Gy/sec). Therefore, radiation using gamma rays generally requires longer exposure in order to provide a specified absorbed dose, whereas electron beams require considerably shorter exposure, typically seconds instead of minutes (Niemira 2003, Diehl 1995). Whereas a gamma source emits light in every direction, an E-beam can be directed along the food (Diehl 1995). A disadvantage of this technology is that it lacks the penetration depth of gamma rays. Electron beams are capable only of limited penetration into food. The depth of penetration of an electron beam in most food stuffs is 5 mm per MeV, which means 5 cm or 10 cm if radiated from two sides, whereas gamma rays can penetrate food to 40 cm (Diehl 1995, Niemira 2003). For irradiation of e.g. pre-packed salads the penetration depth of electron beams might, however, be sufficient, if the produce is irradiated from two or more sites. The total investment and per unit cost are about the same for the two technologies (Niemira 2003).

4.2 Legislation

Many countries have authorized the irradiation of a number of food products. In practise, the use of this technique is, however, rather limited. So far, the list of products authorized for irradiation within the whole EU contains only a single food category: 'dried aromatic herbs, spices and vegetable seasonings', which can be irradiated with a maximum average dose of 10 kGy. At community level irradiated foods and food ingredients are regulated by Framework Directive 1999/2/EC and the Implementing Directive 1999/3/EC. An expert committee of the World Health Organization, the Food and Agriculture Organization, and the International Atomic Energy Agency considers food irradiation with gamma rays up to a maximum overall dose of 10 kGy as a safe and effective processing technology. For use on fruits and vegetables, irradiation is recommended at a maximum level of 1.0 kGy (Niemira 2003). Recently the Food Safety Authority amended food additive regulations to permit the irradiation of fresh iceberg lettuce and fresh spinach, and it is evaluating the irradiation of other fresh produce (McGlynn 2009). Even though gamma radiation up to 10 kGy is considered to be safe, radiation lacks consumer acceptance, because of consumers' fear of induced radioactivity and the consequent unwholesomeness of irradiated foods (Resurreccion et al. 1995). Consumer and media education may, however, be able to change this attitude. Another issue connected to radiation is the fear of mutations induced by it, which could lead to more virulent or resistant pathogens. There is scientific consensus

that radiation, as it is used in the food industry, does not generate microbiological problems different from other preservation processes (Farkas 1989). This conclusion is, however, based on studies on bacteria, and additional research on viruses with respect to the appearance of more resistant or virulent mutants is necessary.

4.3 Irradiation of fresh produce

Only little information is available in the scientific literature on the effects and efficacy of electron beam radiation, since most research is done using gamma or UV radiation. Several studies on the inactivation of norovirus and feline and canine calicivirus using gamma radiation in water have been conducted. However, no direct conclusions from these results can be drawn for the inactivation of norovirus on food, because gamma radiation is less effective in the presence of scavengers, present in foods, which react with OH free radicals (e.g. proteins, cellulose, and polysaccharides). Sullivan et al. (1973), for instance, studied the resistance of Coxsackievirus A9 to gamma irradiation in water and ground beef and reported that D_{10} values varied between 1.4 kGy and 7.6 kGy, respectively. Feng et al. demonatrated that gamma radiation at the FDA-approved dose of 4.0 kGy to control foodborne pathogens in fesh iceberg lettuce and spinach does not effectively inactivate MNV-1 virus in fresh produce. For MNV-1, a 1.7–2.4-log virus reduction was achieved in spinach, lettuce, and strawberries at a dose of 5.6 kGy. Bidawid et al. (2000) studied the effect of gamma radiation on hepatitis A virus present on lettuce and strawberries. Doses of 2.72 and 2.97 kGy were necessary to reduce HAV populations by 1 log on lettuce and strawberries, respectively. Furthermore, no noticeable deterioration in the texture and appearance of either the lettuce or the strawberries, even at 10 kGy, was observed. These results are, however, contradictory to others. Han et al. (2004) reported that only low doses of electron beam radiation (1 kGy) did not alter the overall quality of roman heart lettuce, whereas a dose of 1.5 kGy already resulted in unacceptable quality losses. Berries were sensitive to radiation and only strawberries were found to present adequate potential to utilize radiation for shelf life extension. Some species tolerate up to 4 kGy. The irradiation of strawberries is industrially applied, with doses of 1–3 kGy, and they are sold with acceptance of the consumer in, for example, the USA and France (WHO 1999, ICGFI 1999). Raspberries, however, were found to tolerate only up to 1 kGy. Blueberries were found to be slightly more resistant to irradiation than raspberries, with a radiation dose of 1.6 kGy maintaining the overall fruit quality attributes, using electron beam radiation (Moreno et al. 2007).

Beside types of pathogen and food commodity, temperature has a great impact on microorganism inactivation efficacy because of the higher activity of produced radicals (Krämer 2002). Furthermore, pH and temperature determine the radiolytic products formed during irradiation (Diehl 1995). The aggregate status of the food commodity is also of relevance. Resistance of vegetative bacteria on frozen produce is crucially higher, and the effect of irradiation on solids is less destructive (SCF 2003, Diehl 1995). Therefore, the irradiation of frozen raspberries might have a less detrimental impact on food quality than the irradiation of fresh raspberries. Urbain (1989) reported that irradiation of food in the frozen state increases the threshold doses before off-flavour occurs, meaning that considerably higher radiation doses can be applied to the food than in the fresh state. In practise, food commodities such as shrimps, frogs' legs, and de-boned chicken are irradiated in a frozen state (Diehl 1995). Further parameters, such as moisture content and the presence or absence of oxygen, also have impact on treatment efficacy (Farkas 2006).

5 High-pressure processing

High-pressure processing (HPP) shows great potential in the food industry for inactivating microorganisms, denaturing proteins and extending the shelf life of food products, while maintaining the quality of fresh foods, with little effect on flavor and nutritional values. Drawbacks of this technology are the high investment and processing costs, resulting in slow commercial development (Ludikhuyze 2002), and possible changes to the organoleptic properties of food commodities.
The isostatic rule states that pressure is instantaneously and uniformly transmitted throughout a sample. Thus, the technique is independent of the size, shape, and composition of the product (Norton 2008). The inactivation mechanism is a combination of the breakdown of non-covalent bonds and the puncturing or permeabilization of the cell membrane of the microorganism. In contrast to the irradiation of food, HPP is accepted by the consumer. HPP is industrially applied to fruit juices, jams, fruit coatings, fruit jellies, fruit desserts, avocado-based products, sliced onions, tofu, and ready-to-eat vegetable dishes. Depending on the product, pressures between 400 and 600 MPa are applied, with time ranging from 3 to 30 minutes (for jams). In the meat industry HPP is used, for instance for cooked sliced ham, pork meat products, Parma ham, Serrano ham, and Chorizo, with pressure ranges between 400 and 600 MPa for 2 to 10 minutes (Nchyperbaric 2011). The efficacy of HPP in inactivating pathogens is mainly studied for bacterial and fungal pathogens or for the degradation of enzymes. Only some studies have been made on the inactivation of norovirus surrogates. Kingsley et al. (2007), for instance, studied the susceptibility of MNV-1 to high-pressure processing. He found that five minutes of treatment at 450 MPa at 20°C was sufficient to inactivate 6.85 $\log_{10}$ PFU of MNV-1. Sanchez et al. (2011) reported that a treatment of 450 MPa for 15 minutes at 45°C reduced infectious MNV-1 by 6.5 $\log_{10}$. These results suggest good prospects for inactivation of human norovirus strains in foods. However, the efficacy of virus inactivation is highly dependent on the matrix in which the virus is present (Kovac 2012a, 2012b). The pH and salt content of the matrix affected the inactivation rates by HPP of HAV (Kingsley and Chen, 2009) (Table 2). HAV inactivation is enhanced in acidic matrices, whereas MNV-1 and hAdV2 were shown to be more senstitive to HPP at neutral pH than at acidic pH (Kovac et al. 2012a, Lou et al. 2011). Temperature also has a great impact on the efficacy of HPP. Chen et al. (2005) reported that temperatures above and below 20°C significantly increased HPP inactivation of FCV, and Lou et al. (2011) reported that MNV-1 was more effectively inactivated at 4°C than at 20°C. Considering the positive effect of lower temperatures on the shelf life of food products, the influence of low temperatures on the efficacy of virus inactivation is of interest.

Recently, a few studies on the inactivation of enteric viruses using HPP on soft fruits and fresh produce have been published (Kovac et al., 2012a; Kovac et al., 2012b; Leon et al., 2011; Lou et al., 2011; Sanchez et al., 2011) (Table 2). Lou et al. (2011) studied the inactivation of MNV-1 by HPP (350 MPa, 4°C and 2 min.) on fresh-picked strawberries and concluded that MNV-1 on strawberries was reduced by 2.2 and >6 $\log_{10}$, at 350 and 450 MPa, respectively, at 4°C. MNV-1 was significantly more sensitive to HPP at 4°C than at 20°C, where reductions were 0.5–2 $\log_{10}$ lower (Lou et al. 2011). Reduction of MNV-1 infectivity on lettuce by HPP treatment was comparable to that of strawberries, ranging from 2.2 to >6 $\log_{10}$, at 350 and 450 MPa, respectively, at 4°C (Lou et al. 2011).

Table 2: Log10 inactivation of enteric viruses and surrogates by high-pressure processing of fresh produce

Virus	Matrix	Pressure (MPa)	Time (min)	Variables	Reduction (log_{10})	Reference
MNV-1	DMEM	450	5	20°C	6.85	Kingsley (2007)
MNV-1	DMEM	450	15	45°C	6.5	Sanchez (2011)
hAdV2	DMEM	600	4 sec	4°C	6.5	Kovac (2012a)
HAV	DMEM	400	1	1%-3% NaCl	5 - 6.2	Kingsley (2009)
MNV-1	Lettuce, strawberry	350	2	4°C	2.2 – 2.4	Lou (2011)
MNV-1	Lettuce, strawberry	450	2	4°C	4.7 - 7	Lou (2011)
MNV-1	Lettuce, strawberry	450	2	20°C	4.1 – 4.9	Lou (2011)
MNV-1	Strawberry puree	400	2	4°C pH 2.5	2.8	Lou (2011)
MNV-1	Strawberry puree	400	2	4°C pH 6.5	4.8	Lou (2011)
MNV-1	Carrot juice	400	2	4°C pH 6.5	6.8	Lou (2011)
MNV-1	Lemon juice	400	2	4°C pH 2.5	5.4	Lou (2011)
MNV-1	Strawberry puree	200	<10	<25°C	1	Kovac (2012b)
MNV-1	Strawberry puree	200	10	<25°C	3.2	Kovac (2012b)
MNV-1	Strawberry puree	600	2.5	<25°C	>3.3	Kovac (2012b)
FCV	Bivalve molluscs	275	5	5°C	>6	Chen (2005)
MNV-1		400	5	5°C	4.1	Kingsley (2007)
hNoV	Oyster	400	5	6°C	4	Leon (2011)

Kovac et al. (2012b) determined the effect of HPP on virus inactivation in strawberry puree at pressures ranging from 200 to 600 MPa. At 200 MPa, MNV-1 inactivation was approximately 1 log_{10} for treatments shorter than 10 minutes, and 3.2log_{10} when treated for 10 minutes. At higher pressures (400 and 600 MPa), MNV-1 was reduced within 2.5 minutes to undetectable levels, indicating that reduction was at least 3.3 log_{10}. Lou et al. (2011) compared the effectiveness of MNV-1 inactivation by HPP in different purees with a pH ranging from 2.5 (lemon juice) to 6.3 (carrot juice). With the exception of lemon puree, the effectiveness of virus inactivation appeared to be correlated with the natural pH in purees. The higher the pH of the food, the greater the log_{10} reduction of virus infectivity observed. At 350 MPa at 4°C for 2 minutes, 4.3 and 4.7 log_{10} MNV-1 reductions were achieved in carrot puree (pH 5.8) and carrot juice (pH 6.3), respectively, while only 3.4 log_{10} reductions were observed in strawberry puree (pH 3.5). Surprisingly, a 5.4 log_{10} reduction was achieved in lemon puree (pH 2.5) (Lou et al. 2011). MNV-1 reduction in lemon puree was higher than that of strawberry puree, of which the pH was artificially lowered to pH 2.5. This indicates that, aside from pH, the food matrix also plays an important role in protection of MNV-1 from inactivation by HPP.

However, also in lettuce undesirable structural changes due to HPP treatment may occur, namely browning of the leaves and a more glassy appearance, which is reported for pressures of 300–400 MPa (Lou et al. 2011, Rastogi 2007). Crispiness of the leaves was mostly preserved after HPP treatment at 350 MPa at 4°C for 2 minutes.

Virus inactivation by HPP has also been studied in bivalve molluscs. Several surrogates for human NoV were studied. FCV showed to be the most sensitive: > 6 $\log_{10}$ reduction at 275 MPa for 5 minutes, whereas infectious MNV-1 was 4 $\log_{10}$ reduced after HPP treatment at 5°C at 400 MPa for 5 minutes (Chen et al., 2005; Kingsley et al., 2007). However, the treatment of oysters by HPP at 400 MPa for 5 minutes was insufficient to prevent HuNoV infection in human volunteers, suggesting that 4 $\log_{10}$ genome equivalent reduction was not achieved (Leon et al. 2011). None of the volunteers that consumed oysters treated by 600 MPa at 6°C for 5 minutes became infected, indicating that 600 MPa is needed to successfully inactivate HuNoV within raw oysters.

Although HPP appears to be effective in reducing enteric viruses, its application to soft fruits, fresh produce, and bivalve molluscs remains questionable on account of organoleptic changes in the products. It was observed that strawberries displayed a slight color change and the inner white tissue appeared more translucent (Lou et al. 2011). The influence of treatment on food texture was product-dependent. Blueberries, raspberries, and strawberries underwent a minor textural change with slight softening of the tissue. For frozen raspberries it might be an option to combine the freezing step and HPP (high-pressure freezing), resulting in virus inactivation together with better produce quality, since smaller ice crystals would be formed. However, it is reported that the ice crystals regrow during product storage, that processing times on an industrial scale are too long for this approach, making it uneconomical. For shellfish, 400 MPa-treated oysters have been shown to be acceptable to consumers. However, it is uncertain whether an uncooked 600-MPa treated oyster is also acceptable to consumers (Leon et al. 2012).

6 Hurdle technology

Human pathogenic viruses are typically more resistant to intervention measures than bacteria and fungi, resulting in high doses being required to achieve meaningful virus reductions; which often leads to unacceptable quality losses of the food produce (Niemira 2003). Thus, the negative effect of intervention methods on product quality is often the limiting factor. Hurdle technology implies the intelligent use of combinations of intervention measures (hurdles) in order to achieve multi-target, mild, but reliable preservation effects (Leistner et al. 1995). Thereby, this technology allows both consumer demands for nutritious, tasty, and minimally processed products and food safety requirements to be met. By combining intervention measures, each measure can be applied at low intensity, to obtain products with quality attributes reminiscent of the fresh or native state of a given food but with a longer shelf life. There are more than 60 potential hurdles for minimally processed products (Leistner 2000), which will not be discussed in detail. The selection of hurdles needs to be tailored to the product. A promising hurdle approach for the production of ready-to-eat salads is e.g. irradiation used as a post-packaging terminal control step combined with a chemical washing step. It has been verified that it is feasible to combine chlorination with irradiation at 0.15-0.5 kGy to produce fresh-cut lettuce with a reduced microbial population (Han 2004). Another promising approach is the use of advanced oxidation processes (AOP) where two or more oxidants are used simultaneously, e.g. a combination of ozone and UV or UV and H_2O_2 (Li et al. 2011, Sharma 2005, Selma et al. 2008); however, applications of AOPs in food are yet to be developed (Khadre 2001). Selma et al. (2008) investigated the disinfection potential of ozone, UV-C, and a combination of the two in washing water for fresh-cut onions, escarole, carrot, and spinach and discovered that the highest microbial reductions were achieved by combining the two methods. Li et al. (2011) studied treatment of MNV-1 and bacteriophage φX174 as human NoV surrogates with a combination of vaporized hydrogen peroxide (V-H_2O_2) and UV light. The combination increased virus inactivation on stainless steel discs by >3 log within 5 minutes of treatment. Similar trends were observed for the decontamination of shredded iceberg lettuce, although less pronounced. Effective applications of AOPs for inactivating enteric viruses in food matrices are yet to be exploited.

7 Conclusions

Numerous processing measures have been described for the reduction, be it inactivation and/or removal, of viruses in and on foods. However, only some of these intervention measures are currently applied in the food industry for the selected food commodities. Moreover, produce integrity and public perception of the safety of processing measures, among other aspects, also contribute to the usability of the different existing interventions. And, since the virus reduction achieved by most processes is low, the hurdle technology approach seems most promising in guaranteeing food safety while maintaining produce quality.

Washing procedures using sanitizers are generally not capable of reducing pathogens in harbourage sites of produce, and pathogen reductions are often only marginally better than reductions achieved by using potable water alone. Therefore, disinfectants applied in washing waters for lettuce are promising because they decontaminate the washing waters and thereby diminish the risk of cross-contamination and infiltration. Furthermore, they can be used as a hurdle in combination with other intervention measures. For both aqueous and gaseous sanitizers, accomplishing the intervention before the cutting of lettuce may optimize the efficiency of the process and may further minimize infiltration of pathogens into the produce.

Novel technologies such as irradiation or HPP, which not only affect the surface of produce but penetrate the produce to inactivate pathogens sheltered in e.g. crevices or seed pockets, need to be considered. The main restriction of both the methods mentioned is, however, their impact on produce quality. Irradiation with a dose that results in an acceptable quality loss for lettuce and raspberries gives only limited reductions in virus infectivity. The product quality of raspberries for fresh consumption is not acceptable after treatment with high pressure processing and a detrimental effect on produce quality of lettuce is reported at 300 Mpa. For frozen berries HPP or irradiation in higher doses might, however, be feasible, because the structure of berries is changed during freezing by the disruption of cell walls anyway.

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