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Review

High hydrostatic pressure as emergent technology for the elimination of foodborne viruses

Katarina Kovač[a], Marta Diez-Valcarce[a], Marta Hernandez[a], Peter Raspor[b] and David Rodríguez-Lázaro[a,*]

[a]Instituto Tecnológico Agrario (ITA), Valladolid, Spain (Instituto Tecnológico Agrario, Subdirección de Investigación y Tecnología, Carretera de Burgos, km. 119, 47071 Valladolid, Spain. Tel.: +34 983 31 73 83; fax: +34 983 414 780; e-mail: ita-rodlazda@itacyl.es)

[b]Biotechnology, Microbiology and Food Safety, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

High hydrostatic pressure is a non-thermal technology that eliminates microorganisms with a milder effect on the quality of the foods than that produced by heat treatment. Consequently it can produce microbiologically safe foods, with an extended commercial shelf life and with better characteristics compared to heat-treated foods. Whereas the effect of this technology on foodborne pathogenic bacteria has been extensively studied, there is less information on pressure inactivation of enteric viruses. In this article, we review recent studies on the elimination of foodborne viral risks, and detail the different parameters which could influence the inactivation.

* Corresponding author.

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Use of HHP in food industry

High hydrostatic pressure (HHP) is a non-thermal process that inactivates pathogenic and spoilage microorganisms as well as endogenous enzymes, preserving the sensorial characteristics and prolonging the shelf life of food products (Considine, Nelly, Fitzgerald, Hill, & Sleator, 2008; Patterson, 2005). However, the response of different types of micoorganisms varies significantly; i.e. vegetative bacteria are the most sensitive group to HHP, followed by yeasts and moulds, while viruses and bacterial spores are the most resistant (Patterson, Linton, & Doona, 2007).

HHP is being used in recent years in the food industry as an alternative to a wide range of food processing technologies, especially to thermal processing (Buckow & Heinz, 2008; Considine *et al*., 2008; Fonberg-Broczek *et al*., 1999, 2005). It is an energy-efficient and rapid process that can allow short processing times (Buckow & Heinz, 2008; Farr, 1990; Knorr, 1995; Patterson *et al*., 2007). It uses pressure of up to 1000 MPa; this is transmitted isostatically and instantaneously, and thus the process is independent on the shape or size of the food, which can often be problematic in thermal processing of large food items (Farr, 1990; Knorr, 1999; Smelt, 1998). Compared to thermal processing, pressure has less detrimental effects on food and therefore the products preserve most of their natural colours and flavours and health-promoting substances (Kingsley, Guan, & Hoover, 2005; Wilkinson, Kurdziel, Langton, Needs, & Cook, 2001).

The first HHP-treated product that appeared on the market was a high acid jam in Japan in the early 1990s. Since then, a wide spectrum of food products have been commercialised, for example fish and seafood products, meat products such as cook or cured ham, fruit products such as guacamole, fruit jellies and juices, and ready-to-eat (RTE) products (Considine *et al*., 2008; Goh, Hocking, Stewart, Buckle, & Fleet, 2007; Murchie *et al*., 2005; Smelt, 1998; Torres & Velazquez, 2005).

Effect of HHP treatment on food

HHP can help to maintain the quality attributes of fresh food, rendering products microbiologically safe with an extended shelf life (Hogan, Kelly, & Sun, 2005; Patterson, 2005). However HHP can sometimes affect the food yield, sensory qualities such as colour and texture, and produce biochemical changes affecting negatively to the food

properties, but these effects are less severe than those experienced using thermal processing techniques (Buckow & Heinz, 2008; Hogan *et al*., 2005). In addition, those side effects on food properties can be attenuated by a suitable selection of the processing parameters: temperature, time and pressure (Buckow & Heinz, 2008).

HHP, in contrast to heat, does not disrupt covalent bonds thus maintaining the primary structure of proteins, but does alter the conformation of proteins by causing irreversible changes to the secondary, tertiary, quaternary, and supramolecular structure (Murchie *et al*., 2005; Palou, Lopez-Malo, Barbosa-Canovas, & Swanson, 1999). The secondary structure of proteins is disrupted only at very high pressures, leading to irreversible denaturation, and finally proteins can aggregate in gel (Cheftel, 1995; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998; Knorr, 1999; Palou *et al*., 1999). HHP can also inactivate protease inhibitors such as phytate and increase *in vitro* protein digestibility (IVPD) of legumes (Han, Swanson, & Baik, 2007).

The secondary structure and function of complex polysaccharides and lipids is also modified by HHP (Ledward, 1995). The application of HHP can affect smaller molecules such as vitamin C and β-carotene or inactivate some enzymes (Butz *et al*, 2002; Bull *et al*., 2004; Cheftel, 1995).

HHP can also alter the food rheological properties (Patterson *et al*., 2007). Whereas the physical structure of most high-moisture foods remains unchanged, colour and texture may change after HPP treatment in gas-containing products due to gas displacement and liquid infiltration, leading shape distortion and physical shrinkage, and finally irreversible compression of whole foods (Hogan *et al*., 2005). However, those modifications can vary in different products; whereas minimal changes in colour, shape and overall appearance can be observed in different fruits such as grapes and blueberries, especially in segments of fruits, similar pressure treatments affected the aspect of green onions and strawberries (Kingsley *et al*., 2005; O'Reilly *et al*, 2002). Moreover, changes in colour are minimal in white or cured meats (Cheftel & Culioli, 1997), but colour can be affected in fresh meat and poultry due to modifications in myoglobin, heme displacement/release or ferrous atom oxidation (Hugas, Garriga, & Monfirt, 2002; Cheftel & Culioli, 1997).

HHP inactivation of foodborne viruses in food

Little information on pressure inactivation of viruses exists in comparison to foodborne pathogenic bacteria. Resistance of viruses to HHP depends principally on their structure (Mañas & Pagán, 2005). Whereas non-enveloped viruses show a wide range of sensitivities to HHP (Grove *et al*, 2006), the viral capsid coat proteins are in general much less stable to HHP than the assembled icosahedral viral particles (Silva, Foguel, Da Poian, & Prevelige, 1996).

The mechanism of virus inactivation by HHP is not well understood. HHP treatment seems not to affect viral nucleic acids as they can still be detected after the HHP treatment (Khadre & Yousef, 2002; Kingsley, Hoover, Papafragkou, & Richards, 2002; Li *et al*., 2009; Tang *et al*., 2010). However, viral nucleic acids can not be detected when an enzymatic treatment is used prior to molecular detection. This enzymatic treatment can eliminate all nucleic acids of naked or disrupted viral particles, and therefore only nucleic acids from intact particles can be detected. It has been suggested that HHP inactivation is produced by the denaturation of the capsid proteins essential for host cell attachment to initiate infection, therefore preventing the binding to host cells (Hogan *et al*., 2005; Khadre & Yousef, 2002, Kingsley *et al*., 2002, Buckow & Heinz, 2008; Li *et al*., 2009; Tang *et al*., 2010).

The HHP processing parameters, i.e. temperature, time and pressure, can affect the elimination of microorganisms in food products. As a general principle, the degree of inactivation of viruses increase as pressure and/or time increase while the effect of the temperature on HHP virus inactivation varies (Calci, Meade, Tezloff, & Kingsley, 2005; Chen, Hoover, & Kingsley, 2005; Kingsley & Chen, 2008). However, the dissociation and denaturation of proteins and viruses by pressure can be promoted by low temperatures (Bonafe *et al*., 1998; Foguel, Teschke, Prevelige, & Silva, 1995; Gaspar, Johnson, Silva, & Da Poian, 1997; Kunugi & Tanaka, 2002; Tian, Ruan, Qian, Shao, & Balny, 2000; Weber, 1993; Calci *et al*., 2005, Chen, Guan, & Hoover, 2006; Kingsley & Chen, 2008). This is due to exposure of non-polar side chains to water at low temperatures. As non-polar interactions are more compressible they are more affected by pressure (Grove *et al*., 2006; Silva & Weber, 1993).

A non-processing parameter which could also influence the virus inactivation by HHP is the local environment or substrate in which the virus is found (Calci *et al*., 2005; Chen *et al*., 2006; Kingsley & Chen, 2008). Food constituents such as proteins, lipids, or carbohydrates can confer a protective effect (Simpson & Gilmour, 1997; García-Graells, Masschalck, & Michiels, 1999; Kingsley & Chen, 2009; Murchie, Kelly, Wiley, Adair, & Patterson, 2007).

Picornaviridae

Viruses from family Picornaviridae are small icosahedral particles which contain a single-stranded positive sense RNA. The family consists of five genera: enteroviruses, rhinoviruses, cardioviruses, aphthoviruses, and hepatoviruses (Lin *et al*., 2009). Genetic variation among the different genera within this family is considerable. Hepatitis A virus (HAV) (*Hepatovirus* family) is the main foodborne virus of this group, but also poliovirus (*Enterovirus* family) and Aichi virus have been related to food- and waterbone outbreaks (Cliver, 1994; Le Guyader *et al*., 2008; Choo & Kim, 2006).

The mechanism of inactivation of HAV using HHP treatment has not been clearly unravelled, but results of RNase

protection assays suggest that the HAV capsid remains intact following inactivation by HHP (Kingsley *et al*., 2002), similarly as observed in rotavirus (Pontes *et al*., 2001). Therefore, the mechanism of HHP inactivation for HAV is presumed to be denaturation of capsid proteins preventing the attachment to the appropriate cellular receptor, or the blockage of the penetration and virion-uncoating mechanisms subsequent to viral attachment (Kingsley *et al*., 2002).

Inactivation of HAV has been amply studied in cell culture and food matrices in different conditions, noting considerable differences of HAV inactivation sensitivity in different environments (Table 1). While HHP treatments of HAV stocks in cell culture with pressures of up to 300 MPa had limited effects on HAV titer, higher pressures resulted in significant reduction (Kingsley *et al*., 2002, 2006; Grove *et al*., 2008). Treatments with at least 400 MPa are in general the most efficient, but the reductions were significantly different when using different processing temperatures or times (Table 1). The inactivation of HAV is strongly influenced by the temperature. It is reduced proportionally to the decrease of the processing temperature, i.e. it is greater at higher temperatures (>30 °C), while HAV is more resistant to inactivation temperatures close to or below 0 °C (Kingsley, Guan, Hoover, & Chen, 2006) (Table 1). Similarly, the interaction of pH and temperature is also significant. The effect of pH is more evident at 20 °C, and HAV reduction is enhanced throughout the pH range (Kingsley & Chen, 2009).

Oscillatory high-pressure processing -i.e. cycles at high and atmospheric pressure- has been suggested to enhance microbial inactivation (Alemán *et al*., 1998; Hurtado, Montero, & Borderías, 1998). Interestingly, it does not substantially increase the inactivation rate of HAV (Kingsley *et al*., 2006). When 2, 4, 6 and 8 cycles were used in treatment with 400 MPa at two different temperatures, 20 and 50 °C, no distinct advantage over continuous high-pressure treatment at the same temperature and pressure conditions was observed (Kingsley *et al*., 2006).

The salinity of the food environment can confer a protective effect to HHP as salt may act to stabilize viral capsid proteins. While NaCl concentrations up to 1 % does not provide any significant protective effect to HAV, higher concentrations are baroprotective (Kingsley & Chen, 2009; Kingsley *et al*., 2006; Grove, Lee, Stewart, & Ross, 2009). Similarly, HAV in high salinity seawater required higher pressures for comparable rates of inactivation as observed in isotonic media (Kingsley *et al*., 2002).

Shellfish is a common source of foodborne viral contamination. A few studies have been carried out on the effect of HHP on HAV in oysters (Table 1). The presence of the shell during commercial bivalve processing does not have any mitigation effects on HHP inactivation as non statistically significant differences are observed when shucked or whole shellfish are treated (Kingsley, Calci, Holliman, Dancho, & Flick, 2009). The salinity of the water where the oysters are harvested is a key aspect, as intracellular ionic strength of oysters varies with the surrounding water (Kingsley, Holliman, Calci, Chen, & Flick, 2007). Comparable inactivation rates are observed in artificially contaminated shellfish from a low-salinity estuary (approximately 5- to 20-ppt-salinity seawater) to those in normal cell culture at 20 °C using the same pressure and time (Calci *et al*., 2005). However, the increase of inactivation of HAV in buffers and salts with higher temperatures was not observed for HAV in oyster homogenates (Kingsley & Chen, 2009). This resistance may be due to the composition of oysters that mitigates the inactivation by pressure and high temperature. Nevertheless, there are discrepancies in the results of HAV inactivation in oysters and in buffers with similar pH and NaCl concentration. Whereas Kingsley and Chen (2009) showed that HAV was more resistant in oyster homogenates suggesting that some oyster components are baroprotective, Grove *et al*. (2009) obtained greater HAV inactivation in oyster homogenate than in buffered medium for several pressure and salt combinations (Table 1). These discrepancies may be explained as homogenization of oyster disrupts tissue and membranes and releases cellular contents, being the homogenized tissue therefore exposed to enzymatic degradation. That degradation in combination with HHP can have contributed to the damage of viral capsid proteins which resulted in greater virus inactivation compared to the inactivation observed in buffered medium (Grove *et al*., 2009).

HAV is less barotolerant in soft fruits and vegetables than in cell culture (Kingsley *et al*., 2005). In addition, the reduction of HAV in strawberry puree is significantly higher than in sliced green onions using the same conditions (Kingsley *et al*., 2005). This difference may be related to the acidic pH of strawberry puree (pH 3.67) as the reduction of the pH can increase the HHP inactivation (Patterson *et al*., 2007; Kingsley & Chen, 2009).

The effect of HHP on HAV attached to pork sausages has been also studied (Sharma *et al*, 2008). After 5 min treatment of HAV inoculated sausages with 500 MPa at 4 °C, titers recovered from HHP-treated samples were significantly lower. In addition, concomitant chemical (chelating) treatment did not increase virus inactivation on sausages (Sharma *et al*., 2008).

The susceptibility of the HHP varies among other members of the family Picornaviridae (Table 1). Poliovirus is extremely resistant to HHP, i.e. treatment of 600 MPa for 1 h does not produce significant virus reduction (Wilkinson *et al*., 2001). Some explanations have been formulated for the poliovirus baroresistance such as the pivotal role of the viral capsid shape (Wilkinson *et al*., 2001) or the high thermodynamic stability inherent in the composition of the poliovirus particle (Oliveira *et al*., 1999). Lowering the temperature has no additional effect in poliovirus infectivity. However, the combination of pressure, low temperature (−15 °C) and urea (2 M) produce a significant reduction (Oliveira *et al*., 1999). This implies a structural change as

Table 1. Effect of HHP treatment on viruses from Picornaviridae family

Virus	Matrix	Pressure	Time	Temp	Reduction[a]	Reference
Hepatitis A virus	Cell culture	300 MPa	5 min	−10 °C	0.6 PFU/mL	Kingsley *et al.*, 2006
				40 °C	1.3 PFU/mL	
				50 °C	0.8 PFU/mL	
		350 MPa	1 min	−10 °C	0.9 PFU/mL	
				50 °C	2.4 PFU/mL	
		400 MPa	18 s	−10 °C	0.0 PFU/mL	
				20 °C	0.4 PFU/mL	
				50 °C	2.8 PFU/mL	
			30 s	50 °C	4.1 PFU/mL	
			1 min	−10 °C	1.0 PFU/mL	
				20 °C	2.5 PFU/mL	
				50 °C	4.7 PFU/mL	
			10 min	50 °C	4.9 PFU/mL	
			20 min	−10 °C	4.3 PFU/mL	
				20 °C	4.1 PFU/mL	
	Cell culture	300 MPa	10 min	Ambient	>1 $TCID_{50}$/mL	Grove *et al.*, 2008; 2009
		400 MPa	5 min		1.8 $TCID_{50}$/mL	
		400 MPa	10 min		>2 $TCID_{50}$/mL	
		500 MPa	5 min		>3.5 $TCID_{50}$/mL (ND)	
		600 MPa	1.5 min			
	Cell culture (4.1 ppt NaCl)	450 MPa	5 min		>6 $TCID_{50}$ (ND)	Kingsley *et al.*, 2002
			15 min			
	Seawater (27.4 ppt NaCl)	450 MPa	5 min		>3 $TCID_{50}$	
	Cell culture	400 MPa	1 min	50 °C	4.0 PFU/mL	Kingsley & Chen, 2009
	Cell culture with 1 % NaCl				4.1 PFU/mL	
	Cell culture with 3 % NaCl				1.3 PFU/mL	
	Cell culture with 6 % NaCl				0.4 PFU/mL	
	Cell culture (3 % salt)	300 MPa	10 min	Ambient	<0.5 $TCID_{50}$/mL	Grove *et al.*, 2009
		400 MPa	5 min		0.2 $TCID_{50}$/mL	
		400 MPa	10 min		<0.5 $TCID_{50}$/mL	
		500 MPa	6 min		>3 $TCID_{50}$/mL (ND)	
	Oyster homogenate (1.5 % salt)	375 MPa	5 min	Ambient	2 $TCID_{50}$/mL	
	Oyster homogenate (3 % salt)	375 MPa	5 min	Ambient	1.7 $TCID_{50}$/mL	
	Oysters	300 MPa	1 min	9 °C	0.2 PFU	Calci *et al.*, 2005
		325 MPa			0.8 PFU	
		350 MPa			1.3 PFU	
		375 MPa			2.3 PFU	
		400 MPa			3.2 PFU[b]	
	Mediterranean mussels	300	5 min	Ambient	0.1 PFU	Terio *et al.*, 2010
		325			0.7 PFU	
		350			1.7 PFU	
		375			2.5 PFU	
		400			2.9 PFU	
	Blue mussels	300	5 min	Ambient	0.8 PFU	Terio *et al.*, 2010
		325			1.0 PFU	
		350			2.1 PFU	
		375			2.7 PFU	
		400			3.6 PFU	
	Strawberry puree	250 MPa	5 min	21 °C	1.2 PFU	Kingsley *et al.*, 2005
		275 MPa			2.1 PFU	
		300 MPa			3.1 PFU	
		375 MPa			4.3 PFU	
	Sliced green onions	250 MPa	5 min	21 °C	0.3 PFU	
		275 MPa			0.7 PFU	
		300 MPa			1.4 PFU	
		375 MPa			4.8 PFU	
	Sausages immersed in water	500 MPa	5 min	4 °C	3.2 $TCID_{50}$/mL	Sharma *et al.*, 2008
	Sausages immersed in 100-ppm EDTA				2 $TCID_{50}$/mL	
	Sausages immersed in 2 % lactoferrin				2.1 $TCID_{50}$/mL	

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Table 1 (*continued*)

Virus	Matrix	Pressure	Time	Temp	Reduction[a]	Reference
Poliovirus	bisTris-propane buffer (pH 7.5)	240 MPa	120 min	Ambient −15 °C	No significant reduction	Oliveira *et al.*, 1999
	bisTris-propane buffer (pH 7.5) + 2 M urea	240 MPa	120 min	−15 °C	>4 PFU/mL	
	Cell culture	200 MPa 400 MPa 600 MPa 600 MPa	15 min	20 °C	No significant reduction	Wilkinson *et al.*, 2001
			60 min			
	Cell culture	600 MPa	5 min	Ambient	No reduction	Kingsley *et al.*, 2002
	Cell culture	600 MPa	5 min	Ambient	<1 $TCID_{50}$/mL	Grove *et al.*, 2008
Aichivirus	Cell culture	400 MPa 500 MPa 600 MPa	5 min	Ambient	No reduction	Kingsley *et al.*, 2004
Coxsackievirus B5	Cell culture	400 MPa 500 MPa 600 MPa	5 min	Ambient	No reduction	
Coxsackievirus A9	Cell culture	400 MPa 500 MPa 600 MPa	5 min	Ambient	3.4 $TCID_{50}$ 6.5 $TCID_{50}$ >7.1 $TCID_{50}$ (ND)	

ND: non-detected.
[a] Results shown are $\log_{10}$ reductions observed or calculated from the text and tables of references.
[b] Virus was not detected in one of three trials, so the detection limit 1.5 $\log_{10}$ was assumed.

urea predominantly affects the secondary and tertiary structure of proteins, primarily by perturbing the hydrogen-bonding network.

Coxsackievirus and Aichi virus display variable sensitivities to HHP (Table 1). While coxsackievirus A9 can be significantly reduced with moderate treatments, coxsackievirus B5 and Aichi virus were completely resistant to HHP treatments at 600 MPa during 5 min (Kingsley, Chen, & Hoover, 2004).

Caliciviridae

The Caliciviridae family includes two genera that infect humans: noroviruses and sapoviruses. Noroviruses are the most common cause of outbreaks and sporadic cases of acute gastroenteritis (Noda, Fukuda, & Nishio, 2008). They posses a single-stranded, positive sense RNA genome, surrounded by an icosahedral capsid (Greening, 2006). Viruses from the *Norovirus* genus were recently classified in 29 genetic clusters within five genogroups (Zheng *et al.*, 2006) from which most of the human noroviruses belong to the genogroups I and II (Patel, Hall, Vinjé, & Parashar, 2009). Outbreaks caused by viruses from these groups are often a primary result of exposure to contaminated food or water, while further propagation is normally spread among contacts with primary cases (Becker, Moe, Southwick, & MacCormack, 2000). As human noroviruses have not been reliably propagated in cell cultures and there are no suitable animal models for their propagation, two surrogate viruses belonging to the family Caliciviridae, feline calicivirus (FCV) and more recently murine norovirus (MNV), are normally used in inactivation studies (Doultree, Druce, Birch, Bowden, & Marshall, 1999; Hewitt, Rivera-Aban, & Greening, 2009) (Table 2). However, the interpretation of the results of inactivation studies using surrogates must be carefully considered, as for example MNV is more resistant to HHP than FCV; e.g. total FCV inactivation and only 1.8 PFU/mL MNV reduction with ∼ 300 MPa for 5 min (Kingsley *et al.*, 2002, 2007; Murchie *et al.*, 2007).

FCV inactivation in cell culture by HHP increases in parallel with the increase of pressure and/or time of application, and low combinations are enough to inactivate FCV to undetectable levels (Chen *et al.*, 2005; Kingsley *et al.*, 2002; Grove *et al.*, 2008) (Table 2). However, the pressure levels have a more dramatic effect on virus inactivation than the operational time, and the same level of inactivation can be obtained with a minimum increase of pressure and a severe reduction of treatment time, e.g. an increase of just 50 MPa and 90.8 % reduction of operational time (Chen *et al.*, 2005).

The effect of temperature has been also studied in the FCV inactivation by HHP. The inactivation rate at temperatures close to ambient is acceptable, however higher (above 50 °C) and lower (below 0 °C) temperatures are more effective (Chen *et al.*, 2005; Buckow, Isbarn, Knorr, Heinz, & Lehmacher, 2008) (Table 2). Interestingly, the effects of the temperature on HHP inactivation of FCV differs from those observed in HAV, where the inactivation only increases at temperatures above 30 °C (Kingsley *et al.*, 2006). Another interesting finding is that the inactivation of FCV is more efficiently inactivated in mineral water than in cell culture (Buckow *et al.*, 2008).

Higher acidity, ionic concentration (NaCl), and sucrose concentration, which can be found in some foods, can have a substantial effect on inactivation of FCV by HHP.

Table 2. Effect of HHP on viruses from Calciviridae family

Virus	Matrix	Pressure	Time	Temp.	Reduction[a]	Reference
Feline calicivirus	Cell culture	200 MPa	5 min	Ambient	3.4 $TCID_{50}$	Kingsley *et al.*, 2002
		225 MPa			4 $TCID_{50}$	
		250 MPa			5.7 $TCID_{50}$	
		275 MPa			>6.6 $TCID_{50}$ (ND)	
		300 MPa			>6.6 $TCID_{50}$ (ND)	
	Cell culture	200 MPa	4.5 min	ambient	1 $TCID_{50}$	Grove *et al.*, 2008
		265 MPa	3 min		>5 $TCID_{50}$	
		300 MPa	2 min		3.6 $TCID_{50}$	
		300 MPa	3 min		>5 $TCID_{50}$ (ND)	
		450 MPa	0.5 min		>5 $TCID_{50}$ (ND)	
		600 MPa				
	Cell culture	200 MPa	4 min	−10 °C	5 PFU/mL	Chen *et al.*, 2005
				0 °C	4.4 PFU/mL	
				20 °C	0.3 PFU/mL	
				50 °C	4 PFU/mL	
			20 min	21 °C	2.8 PFU/mL	
			76 min	21 °C	3.7 PFU/mL	
		250 MPa	7 min	21 °C	3.9 PFU/mL	
	Cell culture	200 MPa	6 min	5 °C	3 PFU	Buckow *et al.*, 2008
		250 MPa	4 min	5 °C	5 PFU	
		300 MPa	0.5 min	10 °C	2 PFU	
		450 MPa	2 min	75 °C	>7 PFU/mL	
	Cell culture	150 MPa	5 min	20 °C	0.3 $TCID_{50}$	Murchie *et al.*, 2007
		200 MPa			1.3 $TCID_{50}$	
		250 MPa			3.8 $TCID_{50}$	
		300 MPa			>5.4 $TCID_{50}$ (ND)	
		350 MPa			>5.4 $TCID_{50}$ (ND)	
	Cell culture pH 6	250 MPa	1 min	20 °C	4.1 PFU/mL	Kingsley & Chen, 2008
	Cell culture		5 min	20 °C	5.0–5.3 PFU/mL	
	Cell culture + 12 % NaCl				0.7 PFU/mL	
	Cell culture + 6 % NaCl				1.9 PFU/mL	
	Cell culture + 20 % sucrose				4.0 PFU/mL	
	Cell culture + 40 % sucrose				0.9 PFU/mL	
	Cell culture + 6 % NaCl and 20 % sucrose				0.9 PFU/mL	
	Cell culture	200 MPa	5 min	4 °C	4.7 PFU/mL	
	Cell culture + 6 % NaCl				2.7 PFU/mL	
	Cell culture + 20 % sucrose				3.3 PFU/mL	
	Cell culture + 6 % NaCl and 20 % sucrose				1.7 PFU/mL	
	Mineral water	200 MPa	6 min	5 °C	5 PFU	Buckow *et al.*, 2008
		300 MPa	0.5 min	10 °C	6 PFU	
		450 MPa	1 min	15 °C	>7 PFU/mL	
	Seawater	150 MPa	5 min	20 °C	−0.3 $TCID_{50}$	Murchie *et al.*, 2007
		200 MPa			0.5 $TCID_{50}$	
		250 MPa			3.5 $TCID_{50}$	
		300 MPa			>4.1 $TCID_{50}$ (ND)	
		350 MPa			>4.1 $TCID_{50}$ (ND)	
	Mussels	150 MPa			0.6 $TCID_{50}$	
		200 MPa			1.0 $TCID_{50}$	
		250 MPa			1.4 $TCID_{50}$	
		300 MPa			>3.2 $TCID_{50}$	
		350 MPa			>4.2 $TCID_{50}$ (ND)	
	Oysters	150 MPa			0.1 $TCID_{50}$	
		200 MPa			0.6 $TCID_{50}$	
		250 MPa			1.6 $TCID_{50}$	
		300 MPa			>3.8 $TCID_{50}$ (ND)	
		350 MPa			>3.8 $TCID_{50}$ (ND)	
	Sausages immersed in water	500 MPa	5 min	4 °C	2.9 $TCID_{50}$/mL	Sharma *et al.*, 2008
	Sausages immersed in 100-ppm EDTA				2.4 $TCID_{50}$/mL	
	Sausages immersed in 2 % lactoferrin				2 $TCID_{50}$/mL	

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Table 2 (*continued*)

Virus	Matrix	Pressure	Time	Temp.	Reduction[a]	Reference
Murine norovirus	Cell culture	350 MPa	5 min	5 °C	5.6 PFU/mL	Kingsley *et al.*, 2007
				10 °C	4.8 PFU/mL	
				20 °C	1.8 PFU/mL	
				30 °C	1.2 PFU/mL	
		450 MPa		20 °C	6.9 PFU/mL	
		400 MPa		0 °C	8.2 PFU	Tang *et al.*, 2010
	Oyster tissue	200 MPa	5 min	0 °C	0.5 PFU	Li *et al.*, 2009
		300 MPa			0.9 PFU	
		400 MPa			>4.1 PFU (ND)	
	Oyster tissue	400 MPa	5 min	5 °C	4.1 PFU/mL	Kingsley *et al.*, 2007

ND: non-detected.
[a] Results shown are $\log_{10}$ reductions observed or calculated from the text and tables of references.

A low pH environment *per se* results in a substantial reduction of FCV in non-pressurized samples, but there is almost no additional reduction at pH $\leq$ 5.2 when samples are pressured (Kingsley & Chen, 2008). Conversely, substantial reduction occurs when FCV is pressured at pH $\geq$ 6, with the highest reduction at pH 6 (Kingsley & Chen, 2008) (Table 2). As was observed for HAV, the effect of NaCl on FCV is baroprotective. The increasing protection effect is evident from 0 to 12 % NaCl, but no significant increase of the protection is observed when samples are supplemented with concentrations higher than 12 % or up to 21 % (Table 2). Similar effects are observed for sucrose. An enhanced protection effect is observed when the sucrose concentration is increased up to 40 % (Table 2). However, higher sucrose concentrations (i.e. up to 70 %) do not significantly increase the baroprotective effect. Interestingly, when both sucrose and NaCl are added, the baroprotective effect on FCV is additive (Table 2). A reduction in water activity generally results in greater pressure resistance, however, similar water activities for NaCl and sucrose solution result in different levels of FCV baroprotection meaning the degree of pressure inactivation of FCV was not simply a function of water activity.

The HHP effect on FCV has been shown to be different in the different foods studied. Whereas no significant viral reduction was observed regardless of the matrix studied with a mild HHP treatment (i.e. 150 MPa for 5 min at 20 °C), a moderate increase in pressure (up to 250 MPa) produced an obvious difference in inactivation, which was lower in mussels and oysters in comparison to seawater or cell culture (Murchie *et al.*, 2007) (Table 2). These results are in agreement with those obtained using HAV, which suggests that some components in oysters can be baroprotective (Kingsley & Chen, 2009). However, 300 MPa was enough for total reduction of virus in all matrices, but it should be considered that the initial viral concentrations were not the same in the matrices (Table 2). The effect of HHP on FCV attached to pork sausages has been also studied (Sharma *et al.*, 2008). As for HAV, a significant reduction was observed after 5 min treatment with 500 MPa at 4 °C without any additional effect of a concomitant chemical (chelating) treatment (Sharma *et al.*, 2008).

Another human norovirus surrogate has been used in the latest years: murine norovirus (MNV). The results of the inactivation studies using MNV may be more relevant for human norovirus as the two viruses share biochemical and molecular similarities, and an identical route of infection (Wobus, Thackray, & Virgin, 2006). The effect of processing parameters (operational pressure, time and temperature) on MNV inactivation is similar to other viruses (Table 2): significant reductions using increasing pressures (from 325 MPa to 450 MPa) or times or at low temperatures (Kingsley *et al.*, 2007). The inactivation increases in parallel to the increase of operational pressure and/or time, with a rapid initial reduction followed by tailing at longer treatment times as also observed for other viruses.

The effect of HHP inactivation on MNV has been also studied in foods, such as oysters (Kingsley *et al.*, 2007; Li *et al.*, 2009) (Table 2). A slight MNV reduction was obtained in oysters treated with mild pressures (200 and 300 MPa), but a significant reduction (above 4 log reduction) is observed at 400 MPa (Kingsley *et al.*, 2007; Li *et al.*, 2009) (Table 2).

Binding of MNV to RAW 264.7 cells declined remarkably after HHP treatment indicating that the attachment of MNV is affected by HHP. Those results show that HPP primarily affects the receptor-binding site of the MNV capsid protein, suggesting a possible means for development of a NoV vaccine that contains virus inactivated with HHP treatment, if human NoV has similar susceptibilities to HHP as MNV (Tang *et al.*, 2010).

Reoviridae

Rotaviruses are members of Reoviridae family, and are non-enveloped viruses with ichosahedral capsid of 60–80 nm in diameter and possess linear segmented double stranded RNA genome. They are involved in acute food and waterborne gastroenteritis, especially in children (Greening, 2006). Khadre and Yousef (2002) used a come-up time strategy for HHP inactivation of rotavirus,

Table 3. Effect of HHP on viruses from Reoviridae family

Virus	Matrix	Pressure	Time	Temp	Reduction[a]	Reference
Rotavirus	Cell culture	0–300 MPa	1.2 min[b]	25 °C	5 $TCID_{50}$/mL	Khadre & Yousef, 2002
			2 min		8 $TCID_{50}$/mL	
			4 min		9 $TCID_{50}$/mL	
		300 MPa	6 min		8 $TCID_{50}$/mL	
			8 min		9 $TCID_{50}$/mL	
			10 min		9 $TCID_{50}$/mL	
		500 MPa	10 min		9 $TCID_{50}$/mL	
		800 MPa	10 min		9 $TCID_{50}$/mL	

[a] Results shown are log_{10} reductions observed or calculated from the text and tables of the referenced article.
[b] Pressure come-up time.

and 5 log $TCID_{50}$/mL reduction was observed when 300 MPa at 25 °C during the come-up time, 70 s, was used (Table 3). With 300, 500 and 800 MPa treatments for 2–10 min at 25 °C, 8 or 9 log $TCID_{50}$/mL reduction was observed (Table 3). Interestingly, times longer than 2 min did not provide any additional decrease of rotavirus titer.

Conclusions

HHP is a promising processing technique for food industry as it offers numerous opportunities for developing novel applications. Besides efficient disinfection, a selection of minimally processed safe foods can be foreseen. HHP produced food items are safe for consumers with extended shelf life, high nutritional value and excellent sensorial characteristics (Fonberg-Broczek *et al.*, 1999, 2005; Buckow & Heinz, 2008). Besides HHP applications in food safety area, other applications are indicated. HHP is currently used, for example, for shucking of oysters or to facilitate the removal of the shell of crustacean shellfish such as lobster, crab and shrimp (Terio *et al.*, 2010). HHP has also been successfully evaluated as a potential method for preparation of vaccines and it can be used in some industrial processes such as modulation of microbial fermentations, or it can influence biosynthesis pathways and thus lead to the formation of product variants with novel functional properties (Aertsen, Meersman, Hendrickx, Vogel, & Michiels, 2009).

Although it is accepted that HHP can inactivate foodborne viruses, there are some important technological aspects that must be considered. The balance between food safety and food quality must be considered for each particular food and virus. It is neccessary to apply the correct pressure conditions that efficiently eliminate viruses (and other pathogens) without affecting the food quality. In addition short treatment times are desired for economical and nutritional reasons. HHP conditions must be determined independently for each type of virus as the response and susceptibility is heterogeneous, i.e. from a severe (e.g. hepatitis A or murine norovirus) to a slight reduction (e.g. poliovirus). Identical HHP conditions do not produce similar reductions in members of the same virus family. Therefore, the definition of standard HHP processing criteria, i.e. selection of horizontal processing parameters such as combination of pressure, temperature and time, must be taken carefully in order to assure safe products for final consumers.

Another important aspect in disinfection studies is that some of the main enteric viruses can not grow in cell culture, so there is a lack of direct evaluation of inactivation. In order to overcome these problems, the use of non-pathogenic virus surrogates of similar structural characteristics has been suggested. Mengo virus MC_0 (Costafreda, Bosch, & Pintó, 2006) and feline calicivirus and murine NoV-1 (Cannon *et al.*, 2006) have been proposed as ideal surrogates for HAV and human NoV, respectively. However, it is still under question if the results generated using viral surrogates can be precisely extrapolated to the target viruses.

Responses of foodborne viruses to HHP can vary; however they follow similar kinetic models of inactivation. The development of exact mathematical models for prediction of HHP virus inactivation can be beneficial for the food industry, as they would be useful for optimizing process conditions and constructing hazard analysis critical control point programs to guarantee food safety (Chen *et al.*, 2005). Different models have been used to predict the HHP inactivation of viruses, such as linear or non-linear (Weibull or log-logistic) models. They differ in the assumption that the cells in a population have the same (linear model) or different (Weibull model) resistance to lethal treatments. The non-linear models have been recognized as more appropriate to describe pressure inactivation of viruses (Kingsley *et al.*, 2006, 2007; Grove *et al.*, 2009; Chen *et al.*, 2005). However, poor functional relationships with pressure and temperature in the secondary model approach have been also noticed (Buckow *et al.*, 2008).

In conclusion, although HHP is shown to be a promising strategy for inactivation of microorganisms, it is still in an initial stage for foodborne viruses. Important aspects must be clearly addressed such as the reasons underpinning the differences in resistance of foodborne viruses to HHP in different food products, or the definition of new strategies for

the evaluation of the inactivation results for viruses that can not grow in cell culture. Consequently, more inactivation studies using a range of processing and technological parameters for different food products and viruses are needed to clearly determine the conditions for efficient removal of foodborne viruses. This will also contribute to elucidate the mechanisms of HHP inactivation on viruses. Finally, it will help to define and develop predictive inactivation models for practical application in modern food processing.

References

Aertsen, A., Meersman, F., Hendrickx, M. E. G., Vogel, R. F., & Michiels, C. W. M. (2009). Biotechnology under high pressure: applications and implications. *Trends in Biotechnology, 27*(7), 434–441.

Alemán, G. D., Ting, E. Y., Farkas, D. F., Mordre, S. C., Hawes, A. C. O., & Torres, J. A. (1998). Comparison of static and step-pulsed ultra-high pressure on the microbial stability of fresh cut pineapple. *Journal of the Science of Food and Agriculture, 76*(3), 383–388.

Becker, K. M., Moe, C. L., Southwick, K. L., & MacCormack, J. N. (2000). Transmission of Norwalk virus during football game. *The New England Journal in Medicine, 343*(17), 1223–1227.

Bonafe, C. F., Vital, C. M., Telles, R. C., Gonçalves, M. C., Matsuura, M. S., Pessine, F. B., et al. (1998). Tobacco mosaic virus disassembly by high hydrostatic pressure in combination with urea and low temperature. *Biochemistry, 37*(31), 11097–11105.

Buckow, R., & Heinz, V. (2008). High pressure processing – a database of kinetic information. *Chemie Ingenieur Technik, 80*(8), 1081–1095.

Buckow, R., Isbarn, S., Knorr, D., Heinz, V., & Lehmacher, A. (2008). Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Applied and Environmental Microbiology, 74*(4), 1030–1038.

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., et al. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science & Emerging Technologies, 5*(2), 135–149.

Butz, P., Edenharder, R., Fernandez Garcia, A., Fister, H., Merkel, C., & Tauscher, B. (2002). Changes in functional properties of vegetables induced by high pressure treatment. *Food Research International, 35*(2–3), 295–300.

Calci, K. R., Meade, G. K., Tezloff, R. C., & Kingsley, D. H. (2005). High-pressure inactivation of hepatitis A virus within oysters. *Applied and Environmental Microbiology, 71*(1), 339–343.

Cannon, J. L., Papafragkou, E., Park, G. W., Osborne, J., Jaykus, L. A., & Vinjé, J. (2006). Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *Journal of Food Protection, 69*(11), 2761–2765.

Cheftel, J. C. (1995). Review: high pressure, microbial inactivation and food preservation. *Food Science and Technology International, 1*(2–3), 75–90.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: a review. *Meat Science, 46*(3), 211–236.

Chen, H., Guan, D., & Hoover, D. G. (2006). Sensitivities of foodborne pathogens to pressure changes. *Journal of Food Protection, 69*(1), 130–136.

Chen, H., Hoover, D. G., & Kingsley, D. H. (2005). Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection, 68*(11), 2389–2394.

Choo, Y. J., & Kim, S. J. (2006). Detection of human adenoviruses and enteroviruses in Korean oysters using cell culture, integrated cell culture-PCR, and direct PCR. *Journal of Microbiology, 44*(2), 162–170.

Cliver, D. O. (1994). Other foodborne viral diseases. In R. Gorham, K. D. Murrell, & D. O. Cliver (Eds.), *Foodborne disease handbook. Diseases caused by viruses, parasites and fungi, Vol. 2* (pp. 137–143). New York: Marcel Dekker.

Considine, K. M., Nelly, A. L., Fitzgerald, G. F., Hill, C., & Sleator, R. D. (2008). High-pressure processing – effects on microbial food safety and food quality. *FEMS Microbiology Letters, 281*(1), 1–9.

Costafreda, M. I., Bosch, A., & Pintó, R. M. (2006). Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. *Applied and Environmental Microbiology, 72*(6), 3846–3855.

Doultree, J. C., Druce, J. D., Birch, C. J., Bowden, D. S., & Marshall, J. A. (1999). Inactivation of feline calicivirus, a Norwalk virus surrogate. *The Journal of Hospital Infection, 41*(1), 51–57.

Farr, D. (1990). High pressure technology in the food industry. *Trends in Food Science & Technology, 1*, 14–16.

Foguel, D., Teschke, C. M., Prevelige Jr., P. E., & Silva, J. L. (1995). Role of entropic interactions in viral capsids: single amino acid substitutions in P22 bacteriophage coat protein resulting in loss of capsid stability. *Biochemistry, 34*(4), 1120–1126.

Fonberg-Broczek, M., Arabas, J., Kostrzewa, E., Reps, A., Szczawiński, J., Szczepek, J., et al. (1999). High pressure treatment of fruit, meat, and cheese products: equipment, methods and results. In F. A. R. Oliveira, & J. C. Oliveira (Eds.), *Processing foods. Quality optimisation and process assessment* (pp. 281–300). New York: CRC Press.

Fonberg-Broczek, M., Windyga, B., Szczawiński, J., Szczawińska, M., Pietrzak, D., & Prestamo, G. (2005). High pressure processing for food safety. *Acta Biochimica Polonica, 52*(3), 721–724.

García-Graells, C., Masschalck, B., & Michiels, C. W. (1999). Inactivation of *Escherichia coli* in milk by high-hydrostatic pressure treatment in combination with antimicrobial peptides. *Journal of Food Protection, 62*(11), 1248–1254.

Gaspar, L. P., Johnson, J. E., Silva, J. L., & Da Poian, A. T. (1997). Partially folded states of the capsid protein of cowpea severe mosaic virus in the disassembly pathway. *Journal of Molecular Biology, 273*(2), 456–466.

Goh, E. L. C., Hocking, A. D., Stewart, C. M., Buckle, K. A., & Fleet, G. H. (2007). Baroprotective effect of increased solute concentrations on yeast and moulds during high pressure processing. *Innovative Food Science & Emerging Technologies, 8*(4), 535–542.

Greening, G. E. (2006). Human and animal viruses in food (Including taxonomy of enteric viruses). In S. M. Goyal (Ed.), *Viruses in foods* (pp. 5–42). New York, USA: Springer.

Grove, S. F., Forsyth, S., Wan, J., Coventry, J., Cole, M., Stewart, C. M., et al. (2008). Inactivation of hepatitis A virus, poliovirus and a norovirus surrogate by high pressure processing. *Innovative Food Science & Emerging Technologies, 9*(2), 206–210, Food Innovation: emerging Science, Technologies and Applications (FIESTA) Conference, April 2008.

Grove, S. F., Lee, A., Lewis, T., Stewart, C. M., Chen, H., & Hoover, D. G. (2006). Inactivation of foodborne viruses of significance by high pressure and other processes. *Journal of Food Protection, 69*(4), 957–968.

Grove, S. F., Lee, A., Stewart, C. M., & Ross, T. (2009). Development of a high pressure processing inactivation model for hepatitis A virus. *Journal of Food Protection, 72*(7), 1434–1442.

Han, I. H., Swanson, B. G., & Baik, B. K. (2007). Protein digestibility of selected legumes treated with ultrasound and high hydrostatic pressure soaking. *Cereal Chem., 84*, 518–521.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., & Weemaes, C. (1998). Effects of high pressure on enzymes related to food quality. *Trends in Food Science & Technology, 9*(5), 197–203.

Hewitt, J., Rivera-Aban, M., & Greening, G. E. (2009). Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis

A virus in heat inactivation studies. *Journal of Applied Microbiology*, *107*(1), 65–71.
Hogan, E., Kelly, A. L., & Sun, D.-W. (2005). High pressure processing of foods: an overview. In S. Da Wen (Ed.), *Emerging technologies for food processing* (pp. 3–31). Academic Press.
Hugas, M., Garriga, M., & Monfirt, J. M. (2002). New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, *62*(3), 359–371.
Hurtado, J. L., Montero, P., & Borderías, J. (1998). Effect of high pressure on muscle of octopus (*Octopus vulgaris*). In: *International Conference on high pressure Bioscience & Biotechnology, 4th joint meeting of Japanese and European Seminars on high pressure Bioscience and Biotechnology*. Heidelberg, Germany: University of Heidelberg. 30 August – 3 September, 1998.
Khadre, M. A., & Yousef, A. E. (2002). Susceptibility of human rotavirus to ozone, high pressure and pulsed electric field. *Journal of Food Protection*, *65*(9), 1441–1446.
Kingsley, D. H., & Chen, H. (2008). Aqueous matrix compositions and pH influence feline calicivirus inactivation by high pressure processing. *Journal of Food Protection*, *71*(8), 1598–1603.
Kingsley, D. H., & Chen, H. (2009). Influence of pH, salt, and temperature on pressure inactivation of hepatitis A virus. *International Journal of Food Microbiology*, *130*(1), 61–64.
Kingsley, D. H., Calci, K., Holliman, S., Dancho, B. A., & Flick, G. (2009). High pressure inactivation of HAV within oysters: comparison of shucked oysters with whole in shell meats. *Food and Environmental Virology*, *1*(3–4), 137–140.
Kingsley, D. H., Chen, H., & Hoover, D. G. (2004). Inactivation of selected picornaviruses by high hydrostatic pressure. *Virus Research*, *102*(2), 221–224.
Kingsley, D. H., Guan, D., & Hoover, D. G. (2005). Pressure inactivation of hepatitis a virus in strawberry puree and sliced green onions. *Journal of Food Protection*, *68*(8), 1748–1751.
Kingsley, D. H., Guan, D., Hoover, D. G., & Chen, H. (2006). Inactivation of hepatitis A virus by high-pressure processing: the role of temperature and pressure oscillation. *Journal of Food Protection*, *69*(10), 2454–2459.
Kingsley, D. H., Holliman, D. R., Calci, K. R., Chen, H., & Flick, G. J. (2007). Inactivation of a norovirus by high-pressure processing. *Applied and Environmental Microbiology*, *73*(2), 581–585.
Kingsley, D. H., Hoover, D. G., Papafragkou, E., & Richards, G. P. (2002). Inactivation of hepatitis A virus and calicivirus by high hydrostatic pressure. *Journal of Food Protection*, *65*(10), 1605–1609.
Knorr, D. (1995). Hydrostatic pressure treatment of food: microbiology. In G. W. Gould (Ed.), *New methods of food preservation* (pp. 159–172). Glasgow: Academic and Professional.
Knorr, D. (1999). Novel approaches in food-processing technology: new technologies for preserving foods and modifying function. *Current Opinion in Biotechnology*, *10*(5), 485–491.
Kunugi, S., & Tanaka, N. (2002). Cold denaturation of proteins under high pressure. *Biochimica et Biophysica Acta*, *1595*(1–2), 329–344.
Le Guyader, F. S., Le Saux, J. C., Ambert-Balay, K., Krol, J., Serais, O., Parnaudeau, S., et al. (2008). Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *Journal of Clinical Microbiology*, *46*(12), 4011–4017.
Ledward, D. A. (1995). High pressure processing-the potential. In D. A. Ledward, D. E. Johnston, R. G. Earnshaw, & A.P.M.. (Eds.), *Hasting, high pressure processing of foods* (pp. 1–6). Leicestershire, UK: Nottingham University Press.
Li, D., Tang, Q., Wang, J., Wang, Y., Zhao, Q., & Xue, C. (2009). Effects of high-pressure processing on murine norovirus-1 in oysters (*Crassostrea gigas*) in situ. *Food Control*, *20*(11), 992–996.
Lin, J. Y., Chen, T. C., Weng, K. F., Chang, S. C., Chen, L. L., & Shih, S. R. (2009). Viral and host proteins involved in picornavirus life cycle. *Journal of Biomedical Science*, *16*(1), 103.
Mañas, P., & Pagán, R. (2005). Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology*, *98*(6), 1387–1399.
Murchie, L. W., Cruz-Romero, M., Ferry, J. P., Linton, M., Patterson, M. F., Smiddy, M., & Kelly, A. L. (2005). High pressure processing of shellfish: a review of microbiological and other quality aspects. *Innovative Food Science & Emerging Technologies*, *6*(3), 257–270.
Murchie, L. W., Kelly, A. L., Wiley, M., Adair, B. M., & Patterson, M. (2007). Inactivation of a calicivirus and enterovirus in shellfish by high pressure. *Innovative Food Science & Emerging Technologies*, *8*(2), 213–217.
Noda, M., Fukuda, S., & Nishio, O. (2008). Statistical analysis of attack rate in norovirus foodborne outbreaks. *International Journal of Food Microbiology*, *122*(1–2), 216–220.
O'Reilly, C. E., Murphy, P. M., Kelly, A. L., Guinee, T. P., Auty, M. A. E., & Beresford, T. P. (2002). The effect of high pressure treatment on the functional and rheological properties of Mozzarella cheese. *Innovative Food Science & Emerging Technologies*, *3*(1), 3–9.
Oliveira, A. C., Ishimaru, D., Gonçalves, R. B., Smith, T. J., Mason, P., Sá-Carvalho, D., & Silva, J. L. (1999). Low temperature and pressure stability of picornaviruses: implications for virus uncoating. *Biophysical Journal*, *76*(3), 1270–1279.
Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., & Swanson, B. G. (1999). High-pressure treatment in food preservation. In M. S. Rahman (Ed.), *Handbook of food preservation* (pp. 533–576). New York: Marcel Dekker, Inc.
Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., & Swanson, B. G. (1999). High-pressure treatment in food preservation. In M. S. Rahman (Ed.), *Handbook of food preservation* (pp. 533–576). New York: Marcel Dekker, Inc.
Patterson, M. F. (2005). Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, *98*(6), 1400–1409.
Patterson, M. F., Linton, M., & Doona, C. J. (2007). Introduction to high pressure processing of foods. In C. J. Donna, & F. E. Feeherry (Eds.), *High pressure processing of foods* (pp. 1–14). USA, Iowa: Blackwell Publishing Proffesional.
Pontes, L., Cordeiro, Y., Giongo, V., Villas-Boas, M., Barreto, A., Araújo, J. R., & Silva, J. L. (2001). Pressure-induced formation of inactive triple-shelled rotavirus particles is associated with changes in the spike protein Vp4. *Journal of Molecular Biology*, *307*(5), 1171–1179.
Sharma, M., Shearer, A. E. H., Hoover, D. G., Liu, M. N., Solomon, M. B., & Kniel, K. E. (2008). Comparison of hydrostatic and hydrodynamic pressure to inactivate foodborne viruses. *Innovative Food Science & Emerging Technologies*, *9*(4), 418–422.
Silva, J. L., & Weber, G. (1993). Pressure stability of proteins. *Annual Review of Physical Chemistry*, *44*, 89–113.
Silva, J. L., Foguel, D., Da Poian, A. T., & Prevelige, P. E. (1996). The use of hydrostatic pressure as a tool to study viruses and other macromolecular assemblages. *Current Opinion in Structural Biology*, *6*(2), 166–175.
Simpson, R. K., & Gilmour, A. (1997). The effect of high hydrostatic pressure on the activity of intracellular enzymes of *Listeria monocytogenes*. *Letters in Applied Microbiology*, *25*(1), 48–53.
Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, *9*(4), 152–158.
Tang, Q., Li, D., Xu, J., Wang, J., Zhao, Y., Li, Z., & Xue, C. (2010). Mechanism of inactivation of murine norovirus-1 by high pressure processing. *International Journal of Food Microbiology*, *137*(2–3), 186–189.
Terio, V., Tantillo, G., Martella, V., Di Pinto, P., Buonavoglia, C., & Kingsley, D. H. (2010). High pressure inactivation of HAV within mussels. *Food and Environmental Virolology*, *2*(2), 83–88.
Tian, S. M., Ruan, K. C., Qian, J. F., Shao, G. Q., & Balny, C. (2000). Effects of hydrostatic pressure on the structure and biological activity of infectious bursal disease virus. *European Journal of Biochemistry*, *267*(14), 4486–4494.

Torres, J. A., & Velazquez, G. (2005). Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering, 67*(1–2), 95–112.
Weber, G. (1993). Thermodynamics of the association and the pressure dissociation of oligomeric proteins. *The Journal of Physical Chemistry, 97*(27), 7108–7115.
Wilkinson, N., Kurdziel, A. S., Langton, S., Needs, E., & Cook, N. (2001). Resistance of poliovirus to inactivation by high hydrostatic pressures. *Innovative Food Science & Emerging Technologies, 2*(2), 95–98.
Wobus, C. E., Thackray, L. B., & Virgin 4th, H. W. (2006). Murine norovirus: a model system to study norovirus biology and pathogenesis. *Journal of Virology, 80*(11), 5104–5112.
Zheng, D. P., Ando, T., Fankhauser, R. L., Beard, R. S., Glass, R. I., & Monroe, S. S. (2006). Norovirus classification and proposed strain nomenclature. *Virology, 346*(2), 312–323.