Review

Inactivation of Foodborne Viruses of Significance by High Pressure and Other Processes†

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ABSTRACT

The overall safety of a food product is an important component in the mix of considerations for processing, distribution, and sale. With constant commercial demand for superior food products to sustain consumer interest, nonthermal processing technologies have drawn considerable attention for their ability to assist development of new products with improved quality attributes for the marketplace. This review focuses primarily on the nonthermal processing technology high-pressure processing (HPP) and examines current status of its use in the control and elimination of pathogenic human viruses in food products. There is particular emphasis on noroviruses and hepatitis A virus with regard to the consumption of raw oysters, because noroviruses and hepatitis A virus are the two predominant types of viruses that cause foodborne illness. Also, application of HPP to whole-shell oysters carries multiple benefits that increase the popularity of HPP usage for these foods. Viruses have demonstrated a wide range of sensitivities in response to high hydrostatic pressure. Viral inactivation by pressure has not always been predictable based on nomenclature and morphology of the virus. Studies have been complicated in part from the inherent difficulties of working with human infectious viruses. Consequently, continued study of viral inactivation by HPP is warranted.

All foodborne viruses that are detrimental to human health emanate from the human intestinal tract (7). Fecal oral transmission can occur by indirect routes or direct personal contact. Those foods and beverages susceptible to fecal contamination and lacking an intervention step prior to consumption, such as cooking, usually carry a greater risk of causing viral illness.

Even though gastroenteritis caused by viruses is generally ranked as the primary cause of foodborne illness in the United States by a very wide margin, viruses are often the least or last studied in process development and are not routinely tested in food and environmental samples because of technical and cost issues associated with their extraction, observation, and culture.

Viruses differ greatly from the bacterial agents of foodborne disease. They have been described as extracellular organelles evolved to transfer nucleic acid from one cell to another (39). Viruses have no cellular structure and contain either RNA or DNA enclosed in a protein coat or capsid (63). The capsid functions as the primary protective barrier for the viral particle or virion. The capsid of some viruses is additionally enveloped in an outer lipid membrane. All human enteric viruses are nonenveloped, for enveloped viruses tend to be susceptible to adverse environmental conditions and are generally destroyed by the low pH and bile found in the gastrointestinal tract of humans (1).

The diameters of virus particles range between 25 and 300 nm, so most cannot be visualized with a light microscope. Furthermore, viruses are obligate intracellular parasites and can only replicate in a suitable living host cell (20). As a result, viruses cannot multiply in the environment or in foods, so the traditional factors used to control bacterial levels in food systems (e.g., acidified pH, lowered temperature, or reduced water activity) are ineffective as barriers to viral hazards (46). The persistence of viruses in foods, coupled with their usually low infectious doses believed to be far less than 100 viral particles (20), means that even a small amount of contamination may result in a significant threat to public health (46).

VIRAL ENTERITIS

Over the last several years, there has been a growing awareness of the significant role viruses play in foodborne disease. Although it is uncertain how many different viral diseases have been or can be spread by contaminated foods...
and beverages, the number of different viruses acknowledged as primary agents of foodborne illness is actually quite short. Noroviruses are now recognized as the most common cause of all foodborne disease in the United States, estimated to be responsible for 23 million cases annually (68). Approximately 40%, or 9.2 million cases per year, are estimated to be foodborne, and this number corresponds to 67% of all cases, 33% of hospitalizations and 7% of deaths due to foodborne illness annually (68). Furthermore, noroviruses appear to be becoming increasingly virulent (31). Outbreaks of hepatitis A continue to occur throughout the world despite an increase in sanitation and hygiene standards (36). The Centers for Disease Control and Prevention ranked hepatitis A virus as the fourth most frequently identified cause of foodborne disease in the United States (46), with an estimated 33,000 acute cases in 2003 (16). Due to the serious nature of the disease it causes, hepatitis A virus is usually ranked second on the list of important foodborne viruses and is described as the only common vaccine-preventable foodborne disease in the United States (28).

**Rotavirus and astrovirus.** Foodborne rotavirus and astrovirus cases are far more difficult to quantify and have milder disease symptoms than hepatitis A. Consequently rotaviruses and astroviruses are considered less of a concern in foods and are usually ranked lower in importance, with neither virus routinely reported. It is believed that the percentages of foodborne illness due to rotavirus and astrovirus are very low, probably less than 1%; however, total cases for rotavirus and astrovirus are each estimated to be 3.9 million cases per year because it is assumed that every child has at least one symptomatic infection and the annual U.S. birthrate is 3.9 million. Thus, the number of foodborne cases caused by rotaviruses and astroviruses are each approximated to be <39,000 per year in the United States (68).

**Foods.** Human enteric viruses are transmitted by the fecal-oral route via contamination with human fecal matter. Filter-feeding shellfish such as oysters are at particular risk of human enteric virus transmission. Oysters feed by filtering small particles such as algae from the surrounding water (84) and are usually cultivated in estuaries where the sheltered waters contain high nutrient levels; however, these shallow inlet waters may also be contaminated with human sewage and other forms of pollution (60). The microbiological profile of oysters is directly related to that in the surrounding water (89). Pathogenic microorganisms bioaccumulated by oysters are often detected in oyster flesh at concentrations greater than that in the surrounding water. Because oysters and other bivalve mollusks are often consumed whole and raw or lightly cooked, their consumption results in an increased risk of illness (35, 60). Fresh produce may also transmit enteric viruses following irrigation with contaminated water, or food may be contaminated by infected food handlers with poor personal hygiene.

Foodborne transmission of human enteric viruses most frequently occurs in those foods requiring little or no intervention (e.g., heat processing) prior to consumption and/or ready-to-eat foods that are prepared by a food handler immediately before consumption. It is these foods that would benefit from more stringent farming practices and improved education for food handlers as well as an intervention strategy that would ensure the “fresh” qualities of the food.

**NOROVIRUSES**

The norovirus genus refers to those viruses previously classed taxonomically as Norwalk-like viruses, including the type species Norwalk virus (10). Other well-characterized strains include the Snow Mountain virus, Desert Shield virus, and Hawaii virus (36, 43).

Noroviruses are nonenveloped and contain single-stranded positive-sense RNA within capsids constructed of a single polypeptide of 59 kDa. The determination of the composition of this polypeptide was the primary reason for the classification of norovirus as a calicivirus, even though the overall structure of the capsid does not resemble the typical cup-shaped morphology of calicivirus (36). The capsids of noroviruses are approximately 27 to 35 nm in diameter and, because of a lack of any distinct morphology, were previously classified as small round-structured viruses (36; Fig. 1). Norovirus RNA encodes for only three genes or three open reading frames (50).

**Illness and transmission of infection.** Noroviruses cause gastroenteritis in humans, a generally mild disease featuring symptoms that include nausea, vomiting, diarrhea, malaise, abdominal pain, muscle pain, anorexia, headache, and low-grade fever (20, 37). Symptoms generally begin 1 to 2 days following consumption of contaminated foods or water and persist for 1 to 8 days (20, 56, 79). Gastroenteritis caused by norovirus is characterized by the sudden onset of vomiting (36), which is often projectile in infected adults (56).

The actual occurrence of viral gastroenteritis caused by norovirus around the world is probably greatly underreported (2). This is because the majority of norovirus infections are usually self-limiting and mild, so medical attention may not often be sought (2, 66, 70), although hospitaliza-
Inactivation of viruses may be required in a small number of cases (56). There is also substantial strain diversity as a result of multiple genetic and antigenic types. Such a variety in analytical types adds to the underestimation of the prevalence of norovirus due to misdiagnosis.

Noroviruses appear sensitive to heat and are inactivated during normal cooking (84). Virtually any food may be implicated in the transmission of noroviruses, but foods normally implicated in a norovirus outbreak have not undergone heat treatment prior to consumption. Examples of such foods are salads, pastry frostings, boxed lunches, and raw oysters (20). Infected food handlers can also contaminate ready-to-eat foods (20); however, foodborne transmission is not the only possible method for transmitting infection. Fecally contaminated water is a key vector in the spread of the disease.

The predominant symptom of norovirus infection is vomiting, which generates aerosols and causes surface contamination (40, 78). When combined with its low infectious dose (as low as 10 to 100 particles; (14)), the norovirus attack rate (the proportion of infected individuals in relation to those who were at risk of infection during the event) is often high during outbreaks, up to 90 to 100% (20, 60). Vomiting during norovirus infection may release an estimated 30 million viral particles from the body (14). This encourages transmission of infection either by the inhalation and subsequent ingestion of aerosolized virus particles (64) or by the contamination of food or surfaces such as bench tops and sinks in food preparation areas (66, 78).

Outbreaks of interest. An outbreak described by Marks et al. (64) highlighted the ease of norovirus particle transmission by aerosolized vomit. A diner who was not feeling ill, vomited (not projectile) with little warning on the floor of a restaurant. The vomitus was quickly cleaned with a mop and disinfectant. Fifty-two people present at the restaurant that evening reported ill with various symptoms of gastroenteritis in Sydney rock oysters (Saccostrea glomerata), the New South Wales (NSW) state government introduced shellfish-harvesting regulations that included the sampling of oysters for bacterial indicators (37). Excessive fecal coliform and Escherichia coli counts were found in almost 75% of oyster samples taken from the Georges River during the outbreak, but in only one oyster sample were particles resembling norovirus visualized by electron microscopy (70). Subsequently, the NSW government prohibited the sale of oysters sourced from within the state unless a depuration process was applied for at least 2 days (37). In addition, depurated oysters were not permitted in the market unless samples had been test consumed by a panel of volunteers. Within a year of the introduction of this regulation, consumption of depurated oysters resulted in norovirus infection in some panelists (37).

Infection with norovirus may strike any age group and outbreaks are extremely difficult to prevent and control as evidenced by the regular occurrence of viral gastroenteritis on cruise ships in spite of extreme preventative actions (30, 78). The low infective dose and high attack rate ensure that infection is rapidly spread through small communities and establishments, such as kindergartens and schools (79), restaurants (78), hotels (2, 64, 66), nursing homes (51), and cruise ships (40, 67).

Additionally, there is a lack of lasting immunity. Antibody to the virus is produced during infection, but immunity does not usually last beyond a year (20). The disease can occur with reinfection, and it is difficult to develop a vaccine (15). The highly contagious nature of the virus and the short-lived immunity following infection ensures the previously mentioned high attack rates during outbreaks.

Difficulties of laboratory study. Study of the norovirus has been hampered by the lack of viral particles available for research. Since norovirus particles from the 1968 outbreak in Norwalk, Ohio, were first detected by immune
electron microscopy, study of the virus has been limited by the inability to grow it in cell culture as well as the absence of an animal model (48). Historically, diagnosis of norovirus infection has been achieved by clinical presentation (36) or by visualization with electron microscopy (4), which is a relatively insensitive technique in this instance, requiring approximately $10^5$ to $10^6$ virus particles per milliliter of stool for detection (67, 87). An additional hurdle to detection is caused by norovirus often being shed in feces at a lower concentration than this detection limit (4). Furthermore, electron microscopy examinations of fecal specimens obtained more than a day after symptom onset are rarely positive (2, 66, 79), the reasons for which are unknown.

Although noroviruses have not yet been routinely cultured in the laboratory, methods for the detection of the virus from fecal specimens have improved. The sequencing of the norovirus genome has enabled the sensitive and rapid technique, RT-PCR, to be used to detect noroviruses in environmental and clinical samples (33, 36). RT must be applied to the single-stranded RNA genomes of noroviruses prior to the PCR to produce cDNA for amplification (36). Real-time quantitative PCR is a method for enumerating unculturable and difficult-to-culture viruses. It is a popular tool for enumerating within hours viruses purified from environmental or food samples (36); however, it and other PCR assays are at a disadvantage because these methods cannot discriminate between inactivated viruses and whole infectious viral particles, potentially delivering false-positive results from foods that are actually safe (87). Molecular biological detection and enumeration techniques continue to be investigated and improved to provide public health authorities a greater chance of protecting the public from enteric viral diseases. In the meantime, a culturable surrogate virus, feline calicivirus (FCV; Fig. 2), which has similar properties to the norovirus, has provided an alternative method of studying the norovirus.

**FCV—a norovirus surrogate.** FCV causes a variety of diseases in cats, such as conjunctivitis, limping syndrome, and ulcers in the oral cavity (92). Like other caliciviruses, FCV shares similarities in primary sequence and genomic organization with human noroviruses (48), but FCV can be easily grown in cell culture (23). As a result, FCV has been used in several studies as an indicator of the survival of noroviruses in the environment and following disinfection procedures required for their elimination (23, 73, 87).

For example, Doutltree et al. (23) investigated the survival of FCV following a 1-min exposure to a range of commercial disinfectants. A concentration of 1,000 ppm available chlorine in freshly reconstituted granular hypochlorite was required to inactivate FCV, compared to 5,000 ppm available chlorine of a pre-reconstituted retail brand of liquid hypochlorite. Although iodine- and glutaraldehyde-based disinfectants effectively inactivate FCV, iodine-based disinfectants discolor some surfaces, and glutaraldehyde-based disinfectants can be highly toxic (23). The authors subsequently recommended that during an outbreak of norovirus, reconstituted hypochlorite solutions should be prepared daily and applied to contaminated surfaces for at least 10 min to ensure adequate disinfection.

To investigate the survival of FCV in the environment, Doutltree et al. (23) suspended 9.25 log 50% tissue culture infective dose units (TCID$_{50}$) per milliliter of the virus in cell culture supernatant at 4°C. After 60 days, a 4-log loss of titer was observed, highlighting the potential for norovirus to remain infectious during an extended storage period in refrigerated foods. At room temperature, infectious FCV was detectable for up to 21 days; when dried at room temperature on coverslips and stored at room temperature, infectious FCV was detectable for 28 days (23).

If the survivability of noroviruses is similar to that of other caliciviruses, noroviruses may potentially be infective for up to a month in the environment, with high concentrations of disinfectant necessary to eliminate their transmission (23). An outbreak of norovirus infection aboard a cruise ship demonstrated the survivability and highly infectious nature of norovirus particles on surfaces (67). Available evidence indicated infection was transmitted person to person and not through food or water (67). The outbreak lasted for four successive cruises, each of a 1-week duration, and infected a total of 378 passengers. Further transmission was eliminated by the thorough disinfection of all surfaces of passenger and crew bathrooms and living quarters on the ship with a chlorine-based disinfectant.

Although heat inactivation studies with noroviruses are difficult to perform because of their nonculturable nature, the thermal resistance of FCV has been investigated previously, both in vitro (23) and in vivo (87). FCV was eliminated in pure culture following 1 min of boiling (23) and was not recovered from cockle meat following immersion in boiling water for 1 min (87), confirming that current heating recommendations in the United Kingdom are adequate to protect shellfish consumers from viral shellfish-borne illness (87). At lower temperatures in pure culture, FCV was eliminated only after exposure to 56°C for 1 h or 70°C for 5 min (23). Assuming the heat resistance of norovirus is similar to that of FCV, it would be advisable that shellfish prone to contamination are cooked sufficiently prior to consumption; however, because of the popular consumption of raw shellfish worldwide, the adoption of these recommendations in restaurants and households is unlikely.

Although FCV has been used as a surrogate virus for norovirus previously (23, 73, 87), similarity between the stability of the two viruses in the environment and resistance to cooking or cleaning regimes cannot be established with certainty. The difficulty of obtaining stocks of norovirus particles adds to the complexity of performing these studies, since historically the only source of norovirus particles has been from stools of infected individuals, which, as noted earlier, characteristically contain low concentrations of the virus (4).

In recent years the 59-kDa norovirus capsid protein has been successfully produced in a baculovirus expression system (47). The baculovirus transfer vectors containing norovirus capsid genes co-transfect insect cells with wild-type baculovirus DNA. The recombinant baculovirus derived
from this co-transfection can subsequently infect an insect cell culture and simultaneously produce vast quantities of the norovirus capsid protein capable of self-assembling into immunoreactive virus-like particles. A virtually unlimited supply of purified viral capsids will encourage study into cell-virus interactions that may lead to discovery of a suitable cell line for Norwalk-like virus propagation (47) and increase knowledge of the survival of norovirus in the environment.

HEPATITIS A VIRUS

Hepatitis A virus (Fig. 3) is a member of the family Picornaviridae. Its positive-sense (i.e., translatable) single-stranded RNA (22) is surrounded by a protein capsid approximately 28 to 30 nm in diameter without distinctive surface features (20). Hepatitis A virions possess notable stability to environmental conditions, in particular to heat and drying (20), and are more resistant to low pH (pH 2.0), gamma rays, UV light, and low levels of chlorine and ozone than other picornaviruses (36).

Illness and transmission of infection. Hepatitis A virus is shed exclusively in human feces; therefore, infection is initiated when the virus is ingested, commonly via contaminated water or food (20). After penetrating and replicating in the intestinal epithelial cells, the virus infects the liver where replication may also take place inside hepatocytes (22). When an immune response is evoked, cytotoxic T cells destroy infected liver cells, severely disrupting regular body functions controlled by the liver (20). Following secretion of the virus from the liver in bile, virions of hepatitis A are excreted from the body in feces. Consequently, if the treatment processes are inadequate to eliminate hepatitis A virus in sewage prior to release into the environment, a significant risk is posed to drinking water, waters in which shellfish are harvested, and waters for recreational use (20).

The incubation period of hepatitis A averages 28 to 30 days, and during this time the virus is shed from the body. Virions continue to be shed in low numbers for up to 2 weeks following the onset of symptoms, which include fever, loss of appetite, nausea, and abdominal discomfort, often followed by jaundice lasting several days (20). In infants and children younger than five years of age, infection is often mild or asymptomatic. Immunity to hepatitis A is usually lifelong and death is rare (36).

From 1987 to 1997, an average of 120,000 acute hepatitis A cases were estimated to have occurred annually in the United States, despite the availability of an effective vaccine and the lifelong immunity following infection (16, 31). Since 1997, cases of hepatitis A have decreased, with almost 8,000 reported and 33,000 estimated cases in 2003. In Australia, occurrence of hepatitis A has declined over the last 30 years; however, in indigenous areas the occurrence remains high (61). Between 1991 and 1999, the annual number of hepatitis A infections in Australia averaged 2,115, but in 2000, the number of hepatitis A cases declined by 48% from the previous year. This drop was thought to be due to effective control measures such as vaccinations of susceptible populations and improvements in hygiene (61).

Hepatitis A can cause particular public health problems in areas lacking proper sewage treatment facilities as well as in locations where an adequate level of hygiene may be difficult to maintain, such as child care centers, prisons, and campgrounds (36). The disease is endemic in developing areas such as Southeast Asia, the Indian subcontinent, and Africa (56). In such areas, many children under the age of six acquire a mild or subclinical disease and, thus, immunity to reinfection. Consequently, the occurrence of hepatitis A outbreaks in these areas is rare (56); however, in developed countries, improving sanitary conditions have resulted in fewer cases of hepatitis A and left large populations susceptible to infection with a greater risk of large-scale epidemics (21, 60).

Outbreaks of interest. In 1988, an outbreak of hepatitis A linked to contaminated raw clams in China killed 32 people and infected nearly 300,000 (38). To date, this outbreak is the largest reported viral food poisoning outbreak in the world (60). Clams were contaminated by the release of untreated sewage from a nearby residential area that had reported an epidemic of hepatitis A in the preceding months and from boats dumping human waste overboard in the vicinity of harvesting areas.

Although far smaller in scale, a hepatitis A outbreak that occurred primarily in the Pittsburgh, Pa., region in 2003 is more well known in the United States. Four people died in this outbreak, with a total of over 650 people demonstrating symptoms (65). Green onions or scallions imported from Mexico were confirmed as the vector. Apparently the scallions were grown under conditions that exposed them to human feces, and the virus particles were taken up by the plant so that sauteing the onions prior to consumption did not inactivate the virus.

Difficulties of laboratory study. Tissue culture is an effective method for the growth and quantification of a variety of viruses, including those pathogenic to humans, but growth of hepatitis A virus in tissue culture can best be described as only moderately successful (56), especially for environmental isolates or wild-type strains, which can prove exceptionally difficult to propagate. Tissue culture assays develop plaques and cytopathic effects in infected cells, enabling enumeration of viable viruses. Although virus particles are released from infected cells into the surrounding liquid medium where they may be recovered for further analysis, only a relatively small proportion of hepatitis A virus is released from infected tissue culture cells. Instead, most of the infectious virus particles remain in the cell cytoplasm (6). Tissue culture methods for analyzing and enumerating hepatitis A remain lengthy and labor-intensive, thereby encouraging the use of PCR techniques.

Inactivation of human enteric viruses. Enteric viruses can persist in the environment for weeks and months and are generally acid resistant. Hepatitis A virus is quite resistant to drying (21). Most viruses are inactivated by heating typically used in cooking, and strong oxidizing agents, such
as chlorine, ozone, and UV light, will inactivate viruses in water or on surfaces (7).

As noted by Jay (45), viruses will survive a radiation 12D process for Clostridium botulinum in meat products unless previously damaged by other methods such as heating. Viruses are considered to represent a small target for ionizing radiation and will commonly survive conditions of radappertization (i.e., “radiation’s commercial sterility” or approximately 100 kGy).

The viral capsid is vital in protecting the viral genome from degradation by environmental extremes and the harsh conditions found in the human intestinal tract (73). Inactivation by exposure to UV, hypochlorite (1.2 to 1.25 ppm), or 72°C is due to conformational change of capsid proteins affecting the function of antigenic sites and receptor attachment sites. Virus inactivation almost always accompanies loss of virus attachment (73). Therefore, in order to inactivate viruses in foods by a processing method, either the protective capsid layer must be denatured or disrupted so the virion cannot attach to a host cell, or the nucleic acid contained in the particle must be damaged to an extent preventing replication in a host cell.

NONTHERMAL PRESERVATIVE PROCESSES

It is difficult to educate a population on the health benefits of changing their eating habits from traditionally consuming raw shellfish to thoroughly cooking shellfish before consumption (38, 81). An epidemiological survey conducted in Naples, Italy, concluded that the common consumption of shellfish by the population was not affected by the awareness of the high incidence of hepatitis A infection in the region and knowledge of its route of transmission and that the cooking of shellfish prior to consumption was frequently insufficient to inactivate viruses (81). Thus, an alternative nonthermal or low-heat preservative process would be valuable to reliably improve the margin of safety associated with consumption of raw product. Because of the traditional consumption of oysters raw or minimally cooked, this process must not only ensure a microbiologically safe food but also provide a product that is almost identical to the raw product in organoleptic quality. To ensure that water quality and good manufacturing practices are not compromised, the introduction of such a process must add to, and not replace, current standard procedures. The application of heat has long been recognized as a process that prolongs the shelf life of foods while improving food safety, but in some products heating can cause undesirable changes that affect product organoleptic and nutritional qualities. Food textures are usually altered in some manner, and some vitamins are known to degrade during thermal processing. Vegetable tissues are often softened by heat, and application of chemicals may be required to regain firmness (83).

Consumers are increasingly demanding food products that are fresh tasting, nutritious, and convenient. At the same time, consumer concerns about food safety have steadily increased as the incidence of reported foodborne illnesses has continued to rise. These trends have fueled interest in nonthermal processing technologies, such as high hydrostatic pressure processing, irradiation, pulsed electric fields (PEF), and high-intensity pulsed light. As compared with traditional thermal processing methods, which are often harsh and cause detrimental changes in foods, new preservation technologies are attractive in causing limited detrimental effects on food quality and, thus, can potentially be used to minimize or eliminate extensive thermal processing and the use of chemical preservatives. Preservation of freshness and protection of flavor, appearance, and nutritional value results in a high-quality food product, often with extended shelf life. For these reasons, nonthermal processing technologies offer the ability to produce foods with improved quality, increased consumer appeal, and a value-added premium price. Although commercialization of these technologies has been slow to date, the above trends plus improvements in efficiency and reductions in cost mean that the rate of adoption of nonthermal processes is likely to increase.

High-pressure processing (HPP). Today, high-pressure pasteurization has become a commercial reality with several fruit- and vegetable-based refrigerated food products currently on the international market, including a range of juices and fruit smoothies, jams, applesauce-fruit blends, guacamole and other avocado products, tomato-based salsas and fajita meal kits containing acidified sliced capsicum and onions, and heat-and-serve beef or chicken slices (pre-cooked). Additionally, ready-to-serve meat products and seafood, including oysters, are on the market in the United States and Europe (88, 90). A batch system is typically used where the product is placed into a final flexible consumer package before pressurization. The packages are loaded into a basket and placed into the pressure vessel where they are submerged in a liquid of low compressibility (typically potable water). Once loaded and closed, pressures ranging from 100 to 700 MPa are normally generated by pumping additional water into the vessel. The process is relatively energy efficient, requiring approximately the same energy to raise the pressure to 400 MPa as required to heat to 30°C (18). Once the desired target pressure is obtained, no further energy is required to sustain that pressure (27). Unlike thermal processing, pressure is distributed instantaneously and uniformly throughout foods, ensuring a homogenous treatment regardless of the size or shape of the product (41).

The treatment of foods with HPP is based on compressing the water surrounding the food (5). Although its compressibility is low, the volume of water is decreased by 15% at 600 MPa and 22°C (27). The compression of water causes a moderate increase in temperature (commonly referred to as adiabatic heat or the heat of compression), the extent of which is dependent on the initial temperature of the vessel and the rate of compression. When HPP is conducted at ambient or lower temperatures, there is no substantial rise in the temperature of the treated food. Decompression of the vessel reverses this effect at an equivalent rate (18).

The primary advantages of HPP over thermal processing are the minimal chemical and physical effects exerted on most foods while imparting a microbial kill step. High
pressure does cause a range of effects on the molecular interactions in foods. Ionic bonds and at least a proportion of hydrophobic interactions are broken or distorted by high pressure, whereas hydrogen bonds are strengthened (42) and covalent bonds are unaffected (59). As a result of the pressure-induced changes to ionic bonds and hydrophobic interactions, proteins start to denature at room temperature above pressures of 100 to 200 MPa, initiating protein denaturation (18). Oligomeric structures dissociate into their subunits, monomeric structures partially unfold and denature, and proteins aggregate and gel. The conformation of proteins is altered by an increase in pressure due to irreversible changes to the secondary, tertiary, quaternary, and supramolecular structures (75). Denaturation may result when proteins are exposed to pressure beyond that of the individual protein-specific pressure threshold (18). The structure and function of lipids and polysaccharides are altered by HPP (59); however, pressure effects on lipids are usually reversible, which is often not the case for polysaccharides and proteins. Smaller molecules such as vitamin C and β-carotene are not affected (11, 18). Oxidative reactions in foods and enzymatic browning in some fruits are reportedly enhanced by HPP, whereas partial discoloration has been reported in treated red meats (18, 59).

**Pressure effects on microorganisms.** The required pressure treatment for microbiologically safe and stable products is dependent on the target microorganism to be inactivated. Bacterial vegetative cells, yeasts, molds, and some viruses are sensitive to pressures between 200 and 700 MPa; bacterial spores can survive pressurization above 1,000 MPa (3, 17, 82). Spoilage of food and/or food safety issues due to the outgrowth of bacterial spores can be controlled via complementary means, such as refrigeration and acidification. Various common factors influence the pressure resistance of microorganisms, including the target microorganism and its physiological state, the intrinsic properties of the menstruum, the processing temperature, and the time and magnitude of pressure treatment (42). Considerable variation in susceptibility to high pressure has been observed among various microbial species and strains and on microorganisms in different substrates (77). Certain foods provide microorganisms protection from inactivation or injury from high pressure. For example, milk is said to be more protective to bacteria during HPP than a buffer solution (18) or meat (77).

The critical site of pressure damage leading to inactivation of bacteria and fungi is the cytoplasmic membrane. Cell permeability is altered and ion exchange is disrupted (97) due to crystallization of membrane phospholipids (18) and protein denaturation. Pressure-sensitive bacteria begin to lose viability at approximately 180 MPa (58). Between 200 and 400 MPa, irreversible changes such as cell leakage, which leads to cell death, has been demonstrated by the release of UV-absorbing material from *E. coli* (27, 44, 88).

**Oyster processing.** The application of HPP to whole oyster processing has been attractive for a variety of reasons. Oysters (and other shellfish) are high-value foods traditionally consumed raw throughout the world (55). Pathogens associated with raw oysters, notably *Vibrio* spp., and hepatitis A virus, are sensitive to inactivation by HPP (12, 55, 91). The refrigerated shelf life of harvested oysters is limited, so any extension of the shelf life without altering sensory quality is highly desirable. An extension of oyster shelf life can be achieved by pressure treatment. Additionally, Lopez-Caballero et al. (62) described pressure-treated oysters as “slightly more voluminous with a very pleasant appearance” and reported that oysters were more appealing following treatment at chilled temperatures than at room temperature and above. Flavor may be enhanced, possibly by pressure infusion of the salty liquor within the oyster shell into the meat (42). Oysters are killed by high-pressure treatment (66), and the adductor muscles holding the shell tightly closed are cleaved from the shell. As a result, pressure treatment ensures convenient manual shucking of the whole oyster without the need for shucking knives (55). This allows for higher yields, for there is both a full release of the muscle from the shell and no damage to the tissue from the shucking knife. At 275 MPa, nearly 100% of whole shell oysters open. Usually a hold time of 1 to 2 min is used. Once shucked, the oyster meat can be manually shaken off the shell and further processed in semirigid containers at 415 MPa for several minutes, which extends the refrigerated shelf life to 3 weeks (25). Pressure treatment has been shown to speed removal of lobster meat from its surrounding shell as well.

**Pressure effects on viruses.** The work of Giddings et al. (34) was the first documented attempt to estimate the pressure sensitivity of viruses by studying tobacco mosaic virus. Tobacco mosaic virus was found to be extremely resistant to pressure; pressurization at 920 MPa was necessary to show any measurable inactivation. Fortunately, the pressure resistance of most human and animal viruses is lower than that of tobacco mosaic virus. Most of these viruses can be inactivated at pressures <450 MPa (Table 1).

Nakagami et al. (72) found pathogenic herpes simplex type 1 virus and human cytomegalovirus to be eliminated after 10-min exposures above 300 MPa at 25°C. Kingsley et al. (55) found 450 MPa for 5 min at ambient temperature reduced hepatitis A virus in tissue culture medium to nondetectable levels from initial concentrations of 10^7 infectious units. For the human and animal viruses studied thus far, poliovirus appears to be the most resistant to pressure, capable of surviving an hour at 600 MPa with only modest reductions in infectivity (96).

The extent of virus inactivation is dependent upon treatment pressure duration and temperature. Usually, the degree of virus inactivation is more dependent on variations in treatment pressure than duration, as was indicated by Jurkiewicz et al. (49), who studied the pressure sensitivity of simian immunodeficiency virus. The infectivity of simian immunodeficiency virus was reduced by 5 log infectious units after a 1-h exposure to 250 MPa at 21.5°C. Treatments at 200 and 150 MPa required 3 and 10 h, respectively, to attain equivalent reductions of 5 log infectious units.

A number of reports have indicated that the dissociation and denaturation of proteins and viruses by pressure is
TABLE 1. Pressure inactivation of viruses

<table>
<thead>
<tr>
<th>Name</th>
<th>Enveloped?</th>
<th>Pressure (MPa)</th>
<th>Time (min)</th>
<th>Temp (°C)</th>
<th>Loss in infectivity (log)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aichivirus A846/88</td>
<td>No</td>
<td>600</td>
<td>5</td>
<td>21</td>
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<tr>
<td>Coxackievirus A9</td>
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<tr>
<td>Feline calicivirus</td>
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<td>275</td>
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<td>Foot-and-mouth disease virus</td>
<td>No</td>
<td>240</td>
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<td>−15</td>
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<td>Hepatitis A virus</td>
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<td>55</td>
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<td>Herpes simplex virus type 1</td>
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<td>400</td>
<td>10</td>
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<td>7</td>
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<tr>
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<td>72</td>
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<tr>
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<tr>
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<td>Simian immunodeficiency virus</td>
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<td>Sindbis virus</td>
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<td>Vesicular stomatitis virus</td>
<td>Yes</td>
<td>260</td>
<td>720</td>
<td>20</td>
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promoted by low temperatures (8, 29, 32, 57, 93, 95). The explanation for this phenomenon is that low temperatures promote the exposure of nonpolar side chains to water. The nonpolar interactions are more affected by pressure because they are more compressible. Oliveira et al. (74) examined the combined effect of pressure and low temperature on the stability of foot-and-mouth disease virus, an animal virus that can cause devastating losses in the meat and dairy industries. Foot-and-mouth disease virus was found to be sensitive to pressure: exposure to 240 MPa for 2 h resulted in loss of infectivity of 4 log infectious units at room temperature and 6 log units at −15°C.

The effect of treatment temperature on the inactivation of λ phage, an E. coli phage, under high pressure was studied by Bradley et al. (9). A 3- to 4-log decrease in titer was observed when λ phage in human phage was pressurized at 275 MPa for 7.5 min at temperatures ranging from −62 to 44°C; however, below −30°C, the phage was only slightly inactivated by pressure, suggesting that there exists an optimal temperature for pressure inactivation of λ phage.

Rotavirus titer was found to decline by 5 log TCID$_{50}$/ml within a 70-s exposure to 300 MPa at 25°C, but 1 log TCID$_{50}$/ml remained after a 10-min treatment (52). Herpes simplex virus and human cytomegalovirus could not be recovered following 10 min of treatment at the same pressure. These enveloped viruses were prevented from binding to host cells and subsequently initiating infection as a result of damage to viral envelopes sustained by HPP.

Kingsley et al. (55) suggested that the hepatitis A virus capsid remains intact following inactivation by HPP. HPP may therefore denature the capsid proteins essential for host cell attachment to initiate infection (52) but not release RNA from virus particles (55). RT-PCR performed on the RNA from noninfectious virus particles still yielded a positive result, demonstrating its unreliability for determining the viability of pressure-treated virus.

A 7-log TCID$_{50}$/ml culture of the norovirus surrogate, FCV, was completely inactivated in isotonic tissue culture medium after a 5-min exposure to 275 MPa or more (55). This may give hope of inactivating norovirus with HPP but cannot be relied on to guarantee the susceptibility of norovirus to the process. For example, hepatitis A virus and poliovirus are both members of the picornavirus family but have largely differing susceptibilities.

Increased salinity has been found to protect hepatitis A virus from high pressure. The pressure required to inactivate hepatitis A virus within 5 min increased when treated in seawater of 27.4 ppt salinity, as compared with an isotonic tissue culture medium (55). Salt may act to stabilize viral capsid proteins at high pressure, an observation which may have important implications for future applications of HPP to shellfish products. Hepatitis A virus was also found susceptible to HPP in contaminated strawberry puree and sliced green onions (54).

**EFFECT OF OTHER NONTHERMAL PROCESSING METHODS ON VIRUSES**

**Irradiation.** Although food irradiation has been shown to be an effective nonthermal means of preserving foods for the marketplace, its effectiveness against viruses is dependent on the size of the virus, the suspension medium, product characteristics, and the exposure temperature (26, 76). Most viruses are far more resistant to irradiation than vegetative bacteria, parasites, or fungi, which may be due in part to their smaller size and even smaller genome size (often single-stranded RNA; 26). Additionally, as noted earlier, the treatment levels necessary to minimize organoleptic deterioration in irradiated foods has little effect on viruses, thus rendering irradiation impractical for the inactivation of viruses in commercial food products. The resistance of viruses to irradiation is comparable to that of bacterial endospores and extremely resistant vegetative types. For example, Monk et al. (69) summarized pressure resis-
tances in $D_{10}$-values (kGy) for important food microorganisms. Viruses had $D_{10}$-values ranging from 2 to 8 compared with varieties of E. coli with $D_{10}$-values of approximately 0.3. Radiation-resistant bacterial spores had $D_{10}$-values of 2 to 10, and the supremely resistant Micrococcus radiodurans (now Deinococcus radiodurans) showed $D_{10}$-values of 12 to 14.

**PEF.** PEF can be used as a food-processing tool for the destruction of microorganisms in liquids and pumpable foods (86). Food is pumped through a treatment chamber between positive and negative electrodes subjected to electric fields generated in time pulses ranging from a few microseconds to milliseconds (90). The process is continuous, and the fluids must be aseptically packaged immediately following treatment to prevent postprocess contamination (24). Examples of foods that may be treated with PEF include fruit juices, milk, liquid egg and soups (94), pasta sauces, tomato salsas, and various yogurt-based foods (24). PEF increases bacterial and fungal membrane permeability by destabilizing membrane proteins and the lipid bilayer structure, thus forming transmembrane pores (13, 98, 99); however, to date PEF has shown little effectiveness against viruses. Rotavirus is resistant to PEF (52). PEF may be less effective against protein capsids as compared with lipid-rich membranes.

**High-intensity pulsed light.** High-intensity pulsed light involves electrical ionization of a xenon lamp to emit a broadband white light with a spectrum resembling that of sunlight, containing wavelengths that include a large component (45%) of UV light; UV irradiation will inactivate viruses (73). The intensity of pulsed white light required to inactivate viruses is estimated to be about 20,000 times the intensity of sunlight. Roberts and Hope (80) investigated the potential of high-intensity broad-spectrum white light to inactivate viruses. Enveloped viruses (e.g., Sindbis and herpes simplex virus type 1) and nonenveloped viruses (e.g., encephalomyocarditis, poliovirus type 1, hepatitis A, bovine parvovirus, and canine parvovirus) were diluted in phosphate-buffered saline and placed in small plastic samples. Following treatment to prevent postprocess contamination (24), examples of foods that may be treated with PEF include fruit juices, milk, liquid egg and soups (94), pasta sauces, tomato salsas, and various yogurt-based foods (24). PEF increases bacterial and fungal membrane permeability by destabilizing membrane proteins and the lipid bilayer structure, thus forming transmembrane pores (13, 98, 99); however, to date PEF has shown little effectiveness against viruses. Rotavirus is resistant to PEF (52). PEF may be less effective against protein capsids as compared with lipid-rich membranes.

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The food safety hazards posed by noroviruses and hepatitis A virus, especially in raw shellfish, are well known. As the human population on Earth continues to build in number, especially in coastal areas, one can expect an increase in the amount of wastewater discharge into waterways, representing a greater risk to our food supply. Raw shellfish will continue to pose a significant risk of enteric viral transmission to the population, but minimally processed fruits and vegetables are also at ever-increasing risk as the demand for all types of foods continues to rise throughout the world. Additional hurdle steps in food processing, such as implementation of an appropriate nonthermal process into the production sequence, will reduce the risk of viral foodborne disease but only in combination with good manufacturing and food hygiene practices.

The application of HPP to commercial oyster processing appears to be a success story. HPP of shellfish provides a value-added product with the benefits of shelf life extension (nearly double), an increased level of safety, and improved sensory quality generated by pressure-shucking of the shellfish. The capital cost of HPP equipment is high, and the cost of training employees in the operation of the equipment must be considered, but costs would be expected to drop somewhat due to the simplified shucking procedure. These considerations are obviously beneficial to a processor aiming to export its product.

Although HPP may improve the safety level of the product, it can never replace sound harvesting practices and common sense with regard to sanitation and hygiene. Shellfish destined for human consumption must only be harvested from waters containing levels of fecal coliforms within regulatory limits, and regulations concerning the depuration of filter-feeding bivalves should continue to be enforced. Similarly, fresh produce must be grown within regulatory requirements of irrigation water and harvesting methods using good agricultural practices.

Incorporation of an additional preservative step, such as high hydrostatic pressure, to an existing process line should assist in the minimizing of microbial hazards from shellfish, other seafoods, and other foods, such as minimally processed produce. The incidence of foodborne illness may be effectively reduced, especially if combined with an effective viral indicator; however, to reliably realize this benefit, research must continue to evaluate the sensitivity of human enteric viruses to HPP, especially in combination with other processing technologies in pure culture and in relevant at-risk foods. At this time, neither HPP nor any other nonthermal process used singly can claim complete elimination of problematic viruses from treated foods (unless aided by other protocols). Therefore, the mechanisms of microbial inactivation require further investigation for process optimization. The need for such mechanistic studies is further supported by the broad range of sensitivities of viruses to high pressure; for example, the infectivities of aichivirus and poliovirus are hardly affected by relatively high magnitudes of HPP. It is important that suitable treatment parameters be identified to ensure delivery of high-quality and safe products to the marketplace.

In the absence of regulatory routine virus testing procedures in environmental and food samples, fecal bacterial counts, known to be unsuitable for enteric virus detection, are currently relied upon. In this regard, determination of a reliable indicator organism or organisms for human infectious viruses would be quite advantageous; however, it is anticipated that qualitative and quantitative methodologies will continue to improve. Development of rapid molecular methods to accurately detect the presence of viruses in foods without having to test directly for infectivity would be most welcome, and research to date has not identified a reliable and suitable viral indicator for all environments.

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