Comprehensive REVIEWS in Food Science and Food Safety

Processing Strategies to Inactivate Hepatitis A Virus in Food Products: A Critical Review

Gloria Sánchez

Abstract: Hepatitis A infection, caused by hepatitis A virus (HAV), is the leading cause of human viral hepatitis throughout the world and is mainly propagated via the fecal–oral route. Transnational outbreaks of food-borne infections are reported with increasing frequency as a consequence of international food trade. Food-borne outbreaks caused by HAV are mainly associated with bivalve molluscs, produce (soft fruits and leafy greens), and ready-to-eat meals. The purpose of this paper was to conduct a structured and systematic review of the published literature on the current state of knowledge regarding the stability of HAV in foods as well as the efficacy of food processing strategies and to identify and prioritize research gaps regarding practical and effective mechanisms to reduce HAV contamination of foods. In particular, processing and disinfection strategies for the 3 food categories have been compiled in this review, including common and emerging food technologies. Overall, most of these processes can improve food safety; however, from a commercial point of view, none of the methods can guarantee total HAV inactivation without affecting the organoleptic qualities of the food product.

Keywords: foodborne pathogens, food processing, food safety

Introduction

Hepatitis A virus (HAV) is responsible for about half the total number of human hepatitis infections diagnosed worldwide. According to the World Health Organization, there are more than 1.4 million new cases of hepatitis A worldwide every year (WHO 2012). HAV can be transmitted directly from person-to-person, but also indirectly via virus-contaminated food, water, and surfaces. Unlike blood-borne hepatitis B and C, hepatitis A infection does not cause chronic infections. However, the infection may occasionally proceed to a fulminant hepatitis, mainly among patients with underlying chronic liver diseases (Pintó and others 2012).

Hepatitis A infection is caused by a small (27 to 32 nm), nonenveloped, and positive-sense single-stranded RNA virus, which belongs to the *Hepatovirus* genus within the *Picornaviridae* family (Hollinger and Emerson 2001). Immunological evidence has determined the existence of a single serotype of HAV, but 6 genotypes can be differentiated in the VP1×2A region. Genotypes I, II, and III are associated with human infection, whereas genotypes IV, V, and VI cause infections in simians.

Hepatitis A is a self-limited disease that results in death in 0.1% to 0.3% of patients, except in the elderly where this percentage increases to 1.8% (ECDC 2014). The course of hepatitis A infection is extremely variable. However, it is a significant cause of morbidity and socioeconomic losses in many countries. Symptoms develop gradually and include loss of appetite, headache, nausea, fever, and vomiting, followed by jaundice 1 to 2 wk later, with no associated chronic illness. The illness lasts from a few weeks to several months and is typically more severe in adults than in children, in whom it is asymptomatic or subclinical.

Patients suffering from hepatitis A may excrete from 10^6 to10^{11} viruses per gram of feces (Costafreda and others 2006; Pintó and others 2012), even before being symptomatic, and, therefore, infection mainly occurs through the fecal–oral route either by direct contact with an HAV-infected person or by ingestion of contaminated food or (drinking) water.

The incidence of HAV infection is strongly correlated with socioeconomic development, access to safe water, and sanitation. Geographical areas can be characterized by high, intermediate, or low levels of endemicity patterns of HAV infection based on the age-specific serovalence (WHO 2007).

High rates are reported in countries where sewage treatment and hygiene practices are poor (such as some countries of sub-Saharan Africa and South-East Asia). In many of these countries, where HAV infection is endemic, the majority of people are infected in early childhood (>90%) and virtually all adults are immune. Reported disease incidence in these countries may reach 150 per 100000 per year, and outbreaks are rarely reported (WHO 2012; Gossner and others 2015). Intermediate endemicity, defined as at least 50% seroprevalence by age 15 years and less than 90% by age 10, is reported in countries with transitional economies, and some regions of industrialized countries where sanitary conditions are variable (some regions in the southern and eastern parts of Europe, Latin America, northern Africa, China, the Middle East,

MS 20150813 Submitted 15/5/2015, Accepted 2/7/2015. *Author Sánchez is with Dept. of Microbiology and Ecology, Univ. of València, Dr. Moliner, 50. Burjassot, Valencia, Spain. Author Sánchez is with Inst. of Agrochemistry and Food Technology (IATA), Spanish Council for Scientific Research (CSIC), Agustín Escardino, 7. Paterna, Valencia, Spain. Direct inquiries to author Sánchez (E-mail: gloriasanchez@iata.csic.es).*

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and Russia). In these areas, children escape infection during early childhood; however, these improved economic and sanitary conditions may lead to a higher disease incidence because infections occur in older age groups and reported rates of clinically evident hepatitis A are higher. Finally, low endemicity (seroprevalence of at least 50% by age 30 y and less than 50% by age 15) occurs in USA, Canada, northern and western European countries, Australia, New Zealand, and Japan. In these countries, infection rates are generally low, and only very few people are infected in early childhood. Thus, the majority of adults remains susceptible to infection and, paradoxically, the severity of the disease is high. Moreover, in these low-endemicity HAV countries, disease may occur among specific risk groups such as travelers or men-having-sex-with-men group (Pintó and others 2012).

HAV, as well as human noroviruses, has been determined to be a virus of greatest concern from a food safety perspective, based on the incidence of reported food-borne disease, the severity of disease, including mortality, and their potential for transmission via foods. Estimates of the proportion of hepatitis A infection attributed to food are in the range of around 5% (FAO/WHO 2008). Nevertheless, this figure is probably an underestimate because a considerable proportion of cases (~68%) remains uncharacterized. According to an analysis by the Ohio State University (Scharff 2012), there are more than 1500 new food-borne cases of hepatitis A in the USA every year, and the total cost of food-borne illness because of HAV is now estimated at up to 50 million U.S. dollars. Foods are increasingly imported, often from countries with high endemic levels of hepatitis A and, therefore, hepatitis A has recently been considered as a re-emerging food-borne public health threat in Europe (Sprenger 2014).

Food-borne outbreaks caused by HAV are mainly associated with 3 food categories, shellfish, soft fruits and leafy greens (produce), and ready-to-eat meals (Table 1). Shellfish can accumulate HAV if water has previously been contaminated with human fecal material, and they are also at risk because they are often eaten raw, like oysters, or are only slightly cooked or steamed for merely a few minutes. Fresh produce may be contaminated by irrigation water or by virus-infected individuals (such as workers picking berries) (Van Boxstael and others 2013), whereas ready-to-eat meals may be contaminated during preparation through contact with fecal-contaminated hands or environmental surfaces (fomites). For instance, several reported outbreaks have been associated with an IgM-positive food handler who contaminated one or more food items (Weltman and others 1996; Robesyn and others 2009; Schmid and others 2009).

The current knowledge of HAV has been hampered by the lack of an easy cell-culture assay. HAV is characterized by its slow-growth phenotype, and, in general, lack of cytopathic effects in permissive cell cultures. HAV detection by cell culture is mainly based on the formation of cytopathic effects, followed by quantification of the viruses by plaque assay, tissue culture infectious dose 50 ($TCID_{50}$), or the most probable number. Although cell culture propagation of wild-type strains of HAV may be possible, the procedure is complex and tedious because it requires virus adaptation before its effective growth. The use of a cell line that allows the growth of a wild-type HAV isolate from stool has been reported, although its validity for broad-spectrum isolation of HAV is not yet demonstrated (Konduru and Kaplan 2006). In summary, until issues are resolved regarding cell-culture method complexity, cost-effectiveness, and validity for the detection of a broad spectrum of HAV isolates, infectivity is not yet a useful method for detecting HAV in food. Thus, current methods for the detection of HAV naturally present in foods are based on molecular techniques (Bosch and others 2011).

Although our knowledge of HAV levels in naturally contaminated food samples is far from complete, an increase in publications estimating those levels by real-time RT-PCR (RT-qPCR) has been seen in recent years. This is mainly because of the development of standardized methods, for example the technical specification norm for norovirus and HAV detection in different foods (ISO/TS 15216) or the BAM26 (Detection and Quantitation of Hepatitis A Virus) (FDA 2015).

HAV concentrations detected in food samples varied greatly from less than 100 genomic copies to more than 100000 per gram of food analyzed. Costafreda and others (2006) reported between 1×10^3 to 1×10^5 of HAV genomic copies per gram of coquina clams, originating in Peru and associated with an outbreak in Spain. Benabbes and others (2013) quantified HAV in shellfish collected along the Mediterranean Sea and Atlantic Coast of Morocco, reporting levels around 100 genomic copies per gram digestive tissue. Recently, the presence of HAV was detected in 18.5% of mussels harvested in Spain. Contamination levels ranged from 1.1×10^2 to 4.1×10^6 RNA copies/g digestive tissue (Manso and Romalde 2013). Felix-Valenzuela and others (2012) reported HAV concentrations ranging from 2.8×10^2 to 2.4×10^3 genomic copies per gram of Mexican parsley, green onions, and coriander. These concentrations were by far greater than the infective dose for HAV, estimated to be around 10 and 100 viral particles (Yezli and Otter 2011).

Stability of HAV in Food Products

HAV is a highly stable virus and can persist in the right environment for extended periods, and therefore also in foodstuff (review by Sánchez 2013). This is most probably because of the special codon usage, which prevents direct competition with the host cell system, and concomitantly allows a more stable molecular structure of HAV capsid (Sánchez and others 2003; Pintó and others 2007). HAV strains whose capsids differ in such folding show significant differences in resistance to temperature and acid pH (Costafreda and others 2014).

Most studies to determine the potential of HAV to persist in food matrices have been performed by spiking a known amount of virus into a given sample, determining the reduction in the infectious titer after subjecting the inoculated sample to designated conditions, and applying statistical procedures to determine the significance of virus decay (reviewed by Kotwal and Cannon 2014). Obviously, this implies the use of virus strains that may be propagated in cell culture and enumerated through infectivity, thus greatly restricting the range of strains that are able to be included in these studies. Until now there are described a few cell culture-adapted strains, such as CF53, FG, HAS, and HM-175, and the latter one has been extensively used in most of the studies (Table 2 to 6). Overall, these studies have shown that HAV is capable of surviving in several food products, and it can persist under standard storage conditions beyond the usual periods between purchase and consumption. The most important factors affecting the stability of viruses in food products are temperature, pH, relative humidity, moisture content, sunlight exposure, and type of food, which are discussed below.

Refrigerated storage

Studies evaluating HAV stability on chilled foods (2 to 8 °C) has been reported for several food matrices (Table 2). On lettuce, carrot, and fennel, Croci and others (2002) reported complete HAV

Table 1–Selected large outbreaks (over 100 cases) of hepatitis A virus reported in the last 2 decades.

Implicated food	Exporting country	Importing country	Year	Number of cases	Remark	Reference
Frozen berries		11 European countries	2013	1,315		ECDC 2014
Frozen strawberries		Denmark, Finland, Norway, and Sweden	2013	103		Nordic outbreak investigation team 2013
Frozen pomegranate arils	Turkey	USA	2011	165	Two persons developed fulminant hepatitis, and one needed a liver transplant	Collier and others 2014
Sun-dried tomatoes	Turkey	United Kingdom, The Netherlands, France	2010–2012	308		Gallot and others 2011; Petrignani and others 2010
Sun-dried and semidried tomatoes		Australia	2009	562		Donnan and others 2012
Frozen coquina clams	Peru	Spain	2008	100		Pintó and others 2009
Oysters		France	2007	111		Guillois-Bécel and others 2009
Raw beef		Belgium	2004	269	Food-handler contamination	Robesyn and others 2009
Orange juice		Egypt	2004	351	Poor hygiene during processing	Frank and others 2007
Green onions	Mexico	USA	2003	601	Three deaths	Wheeler and others 2005;
Frozen coquina clams	Peru	Spain	1999	184		Bosch and others 2001
Frozen strawberries	Mexico	USA	1997	242	Possible contamination during harvesting. Hand washing in field was limited.	Hutin and others 1999

Table 2–Survival of HAV in artificially inoculated foods at refrigerated storage.

Temperature (°C)	Time	Food Food type	Initial inoculum level	HAV strain	Inactivation (log reduction)	Remark	Reference
3.4	30 d	Green onions	3.7 log PFU	HM-175	1.0	Samples sealed in a high-density polyethylene (HDPE) produce bag	Sun and others 2012
4	ON	Carrot	5.5 log PFU	HM175/18f	2.7		Wang and others 2013a
4	ON	Cantaloupe	5.5 log PFU	HM175/18f	1.4		Wang and others 2013a
	ON	Celery	5.5 log PFU	HM175/18f	1.0		Wang and others 2013a
4	ON	Honeydew melon	5.5 log PFU	HM175/18f	1.6		Wang and others 2013a
4	17 d	Peppers	5.3 log PFU	–	1.0		Lee and others 2015
4	28 d	Mussels	6.5 log $TCID_{50}$	HM-175	1.7		Hewitt and Greening 2004
4	29 d	Oysters	5.3 log PFU	–	1.0		Lee and others 2015
4	360 d	Bottled water	7.6 log $TCID_{50}$	CF 53	0.7		Biziagos and others 1988
5.4	28 d	Spinach	5.5 log PFU	HM-175	1.0	Moisture- and gas-permeable packages	Shieh and others 2009
6	4 d	Carrot	5.0 log $TCID_{50}$	FG	>5.0		Croci and others 2002
6	7 d	Fennel	5.0 log $TCID_{50}$	FG	>5.0		Croci and others 2002
6	9 d	Lettuce	5.0 log $TCID_{50}$	FG	2.5		Croci and others 2002
10	72 h	Oysters	5.5 log $TCID_{50}$	HM-175	2.4	10% NaCl	Park and Ha 2014
10	5 d	Raw crab in soy sauce	5.1 log $TCID_{50}$	HM-175	1.1	20% NaCl	Park and Ha 2015b
10	5 d	Raw crab in soy sauce	5.1 log $TCID_{50}$	HM-175	0.5	5% NaCl	Park and Ha 2015b
10	16 d	Green onions	3.7 log PFU	HM-175	1.1		Sun and others 2012

ON, overnight; PFU, plaque-forming unit; $TCID_{50}$, tissue culture infectious dose.

inactivation by day 4 and 7 for carrot and fennel, respectively. On lettuce a slight decrease was observed over time. On artificially inoculated green onions, HAV survived more than 20 d in storage at 3 to 10 °C (Sun and others 2012), whereas for pepper 17 d were estimated to reduce by 1 log HAV infectivity at 4 °C (Lee and others 2015). Wang and others (2013a) reported the greatest reduction of infectious HAV (2.7 log reduction) on carrots refrigerated overnight, possibly because of the natural antimicrobial properties of carrots.

Shieh and others (2009) assessed the stability of HAV on fresh spinach leaves in moisture- and gas-permeable packages stored at 5.4 °C, reporting only 1 log reduction of infectious HAV over 4 wk of storage.

To summarize, stability rates differed according to produce type. HAV contaminating the surfaces of green onions, lettuce, pepper, and spinach at refrigeration temperatures of <10 °C remains infectious for periods exceeding the shelf-life of the abovementioned vegetables.

The stability of HAV inoculated in bottled water was evaluated by Biziagos and others (1988). Infectious HAV was detected after 1 y of storage at 4 °C, with less than 1 log reduction. This study also reported that HAV stability was dependent on the protein concentration added to the water.

In marinated mussels, infectious titers of HAV were reduced by 1.7 logs after 4 wk at 4 °C (Hewitt and Greening 2004), whereas in raw oysters HAV stability significantly decreased ($P < 0.05$) on

Table 3–Relevant examples of thermal treatments applied to shellfish-based products.

Temperature (°C)	Time (min)	Shellfish type	Initial level	HAV strain	Inactivation (log reduction)	Remark	Reference
40	1,140	Oysters	5.3 log PFU	–	1.0		Lee and others 2015
50	54.1	Mussels	4.7 log PFU	HM-175	1.0		Bozkurt and others 2014
60	3.2	Mussels	4.7 log PFU	HM-175	1.0		Bozkurt and others 2014
60	5.0	Dried mussels	5.5 log $TCID_{50}$	HM-175	1.3		Park and Ha 2015a
60	10	Mussels	5.0 log $TCID_{50}$	FG	2.0		Croci and others 1999
60	10	Manila clams	5.9 log $TCID_{50}$	HM-175	2.0		Cappellozza and others 2012
63	3.0	Green mussels	4.0 log $TCID_{50}$	HM-175	1.5		Hewitt and Greening 2006
65	2.1	Mussels	4.7 log PFU	HM-175	1.0		Bozkurt and others 2014
70	2.4	Mussels	6.5 log $TCID_{50}$	HM-175	< 2.0	Marination	Hewitt and Greening 2004
70	10	Manila clams	5.9 log $TCID_{50}$	HM-175	4.2		Cappellozza and others 2012
72	1.0	Mussels	4.7 log PFU	HM-175	1.0		Bozkurt and others 2014
80	3.0	Mussels	5.0 log $TCID_{50}$	FG	2.0		Croci and others 1999
80	10	Mussels	5.0 log $TCID_{50}$	FG	4.0		Croci and others 1999
80	10	Manila clams	5.9 log $TCID_{50}$	HM-175	4.4		Cappellozza and others 2012
90	1.5	Soft-shell clams	5.4 log PFU	HM-175	2.7		Sow and others 2011
90	3.0	Soft-shell clams	5.4 log PFU	HM-175	> 4.0		Sow and others 2011
90	10	Manila clams	5.9 log $TCID_{50}$	HM-175	> 4.0		Cappellozza and others 2012
92	3.0	Green mussels	4.0 log $TCID_{50}$	HM-175	> 4.0		Hewitt and Greening 2006
98	9.0	Mussels	4.0 log $TCID_{50}$	FG	> 3.0	Mussels hors-d'oeuvre	Croci and others 2005
100	8.0	Mussels	4.0 log $TCID_{50}$	FG	> 4.0	Mussels in tomato sauce	Croci and others 2005
250	5.0	Mussels	4.0 log $TCID_{50}$	FG	> 3.0	Mussels au gratin	Croci and others 2005

PFU: Plaque-forming unit
$TCID_{50}$: Tissue culture infectious dose

Table 4–Relevant examples of thermal treatments applied to non-shellfish food products.

Temperature (°C)	Time	Food type	Initial level	HAV strain	Inactivation (log reduction)	Remark	Reference
40	7.2 h	Pepper	5.3 log PFU	–	1.0		Lee and others 2015
50	34.4 min	Spinach	5.1 log PFU	HM-175	1.0	Blanching	Bozkurt and others 2015
56	8.4 min	Spinach	5.1 log PFU	HM-175	1.0	Blanching	Bozkurt and others 2015
60	4.5 min	Spinach	5.1 log PFU	HM-175	1.0	Blanching	Bozkurt and others 2015
65	2.3 min	Spinach	5.1 log PFU	HM-175	1.0	Blanching	Bozkurt and others 2015
71.7	15 s	Milk, ice cream	6.3 log PFU	HM-175	< 2.0	High-temperature short-time (HTST) and immediate packing	Bidawid and others 2000c
72	0.9 min	Spinach	5.1 log PFU	HM-175	1.0	Blanching	Bozkurt and others 2015
75	2.5 min	Basil, chives, mint, and parsley	6.2 log $TCID_{50}$	HM175/18f	2.0	Blanching	Butot and others 2009
80	5 min	Strawberry mashes	7.0 log PFU	HM175/18f	4	Sucrose concentration above 28%, pH 3.8	Deboosere and others 2004
95	2.5 min	Basil, chives, mint, and parsley	6.2 log $TCID_{50}$	HM175/18f	> 3.0		Butot and others 2009
120	5 min	Freeze-dried berries	6.2 log $TCID_{50}$	HM175/18f	> 4.0		Butot and others 2009

PFU, plaque-forming unit; $TCID_{50}$, tissue culture infectious dose.

increasing NaCl concentrations and storage time (Park and Ha 2014). Salted oysters containing 3% to 10% NaCl showed only marginal effects on HAV infectivity over 72 h of storage at 10 °C (Park and Ha 2014).

Frozen storage

The occurrence of numerous HAV outbreaks linked to the consumption of shellfish and berries that had been frozen for several months clearly indicates that if food is contaminated before freezing, substantial percentages of the viruses will remain infectious during frozen storage (Table 1). For instance, in the last decade, several outbreaks of hepatitis A associated with frozen foods of foreign origin have been reported in industrialized countries (Collier and others 2014; Gossner and Severi 2014; Guzman-Herrador and others 2014; Wenzel and Allerberger 2014). Additionally, some studies have been able to sequence HAV strains from frozen shellfish and berries. For example, HAV was detected and typed from imported frozen coquina clams involved in hepatitis (shellfish-borne) outbreaks (Bosch and others 2001; Sánchez and others 2002; Pintó and others 2009). Similarly, HAV was detected and typed from oysters associated with a multistate outbreak (Shieh and others 2007). Those oysters were refrigerated for 12 d and then frozen for 7 wk before analysis. On berries, HAV was recently detected and typed from samples of mixed frozen berries linked to an Italian hepatitis A outbreak in 2013 (Chiapponi and others 2014).

Butot and others (2008) extensively assessed the stability of HAV in frozen raspberries, strawberries, blueberries, parsley, and basil, concluding that frozen storage for 3 mo was ineffective (less than 0.5 log reduction) to inactivate HAV. As anticipated, these data showed that freezing is ineffective for the inactivation of HAV if present in foods.

Storage at room temperature

Butot and others (2007) reported that HAV inoculated into bottled water can become adsorbed to the container wall after 62 d of storage at room temperature. This attachment was demonstrated to be independent of the presence of autochthonous microbiota. Moreover, HAV stability in alfalfa seeds, pepper, and oysters during storage at room temperature (RT) has been investigated. Following 50 d of storage at 22 °C, HAV remained infectious with HAV titers reduced by 3.6 log (Wang and others 2013b). HAV titers declined by 1 log in the digestive gland of oysters after 4.5 and 8.8 d of storage at 25°C and at 50% and 70% relative humidity (RH), respectively. Under the same conditions, 1 log reduction of infectious HAV was estimated after 1.4 and 2.1 d of storage (Lee and others 2015).

Acidification

Preserving some food products such as sauces, dressings, and marinades by acidification is a common practice to prevent spoilage. They may consist of naturally acidic foods, such as fruit juices or tomatoes, or they may be formulated by combining acidic foods with other foods to achieve the desired acidity. Some foods, such as vinegar and certain pickled vegetables, may develop acidity from microbial fermentation. HAV is highly resistant at acid pH (Siegl and others 1984), therefore acidification of food is not a suitable procedure to control HAV in foods. For instance, HAV remained infectious at treatments of pH 1 and 38 °C for 90 min (Scholz and others 1989). Moreover, HAV had a high residual infectivity after 2 h of exposure to pH 1 at RT, remaining infectious for up to 5 h. Therefore, acidification does not seem to be a suitable strategy to reduce the infectivity of HAV if present in food.

Drying

HAV stability in the dried state has basically been evaluated on or in environmental surfaces or fomites (Mbithi and others 1991; Abad and others 1994). As for other parameters, the impact of drying on the infectivity of HAV on foods has scarcely been investigated. The HAV outbreak associated with the consumption of dried tomatoes indicates that if food is contaminated before drying, a significant fraction of HAV will still remain infectious (Petrignani and others 2010; Gallot and others 2011).

Relative humidity

Stine and others (2005) evaluated the stability of HAV in lettuce, bell pepper, and cantaloupe stored at 22 °C under high (85.7% to 90.3%) and low (45.1% to 48.4%) relative humidity. HAV survived significantly longer ($P < 0.05$) in cantaloupe than in lettuce, and high inactivation rates were observed under high humidity conditions. Recently, the influence of RH on HAV inactivation was not observed in pepper and oysters stored at 4, 15, 25, and 40 °C (Lee and others 2015).

Modified atmosphere packaging (MAP) is a well-established packaging technique which, when used in conjunction with refrigeration, helps to keep certain foods safe and fresh. MAP is usually applied to slow down the respiration rate of produce, reducing their metabolism and maturation, and is a way of extending the shelf-life of fresh produce by inhibiting spoilage bacteria and fungi. This technology replaces the atmospheric air inside a package with a selected combination of gases (usually oxygen, carbon dioxide, and nitrogen). There have been limited studies investigating the stability of HAV in produce stored under modified atmospheres, but what has been published suggests there is little impact on HAV infectivity. Bidawid and others (2001) assessed the effect of various modified atmospheres on the stability of HAV in produce. Lettuce samples were stored in heat-sealed bags at different percentages of gas mixtures (CO_2:N_2): 30:70, 50:50, 70:30, and 100% CO_2 and stored at RT and 4 °C for up to 12 d. A significant decline ($P < 0.05$) in HAV stability was only reported at 70% CO_2 at RT. As most commercially distributed produce is stored under lower CO_2 concentrations and in refrigerated areas, standard MAP packaging will most likely not provide protection against HAV transmission.

The Effect of Common Food Manufacturing Processes on HAV

As a nonenveloped virus, HAV tends to be more resistant to inactivation than food-borne bacteria to commonly used food manufacturing processes. The efficacy of common food manufacturing processes to inactivate or eliminate HAV (such as by thermal processing, low-heat dehydration, shellfish depuration, blanching, and freeze-drying) has been reported for HAV suspensions and certain foods.

The risks of consuming products that may have become contaminated with enteric viruses have been categorized as high, medium, low, and negligible depending on whether food manufacturing processes reduced the infectivity of common food-borne viruses of at least 1, 2, 3, and 4 log units, respectively (Koopmans and Duizer 2004). Until now, most of the reported studies have evaluated the efficacy of these treatments on HAV suspensions using the HM-175 strain. However, from the published literature no much information of their efficacy is available on food samples. Overall, it is difficult to draw any general conclusions from these studies because of differences in the experimental set-up and methods used to recover viruses from foods. This section summarizes the existing reports evaluating HAV stability under common food processing technologies, to illustrate their efficacy if raw materials are contaminated, which enable establishment of risk.

Heat-processing remains one of the most important methods of food preservation in the food industry. Thermal processing is a very effective technology for bacteria and yeast inactivation; however, heat inactivation of enteric viruses, particularly HAV, in food, has been poorly explored (Bertrand and others 2012).

Inactivation of HAV by heat is influenced by factors such as the presence of organic matter, such as fecal material, the food matrix, the initial level of virus contamination, and the process time-temperature (Table 3 and 4). For instance, the presence of fats or proteins in shellfish plays a protective role (Millard and others 1987; Croci and others 1999).

Overall, conventional bulk pasteurization (63 °C for 30 min or 70 °C for 2 min) seems more effective than high-temperature short-time pasteurization (71.7 °C for 15 to 20 s); nevertheless, HAV is unlikely to be completely inactivated by these treatments. For example, heating at 60 °C for 10 min contaminated mussel homogenates or manila clams reduced HAV titers by 2 logs. Furthermore, infectious HAV was still detected when heat treatment was performed at 80 °C for 10 min (Croci and others 1999; Cappellozza and others 2012).

The occurrence of HAV outbreaks associated with the consumption of fried, grilled, stewed, and steamed shellfish indicates that standard cooking procedures do not guarantee complete inactivation of HAV (Koff and Sear 1967; Lees 2000). Light cooking of shellfish usually involves heating until the shell opens, usually achieved at temperatures below 70 °C during approximately 47 s, which is unable to completely inactivate HAV (Abad and

others 1997). In the same line, other reports have shown the persistence of infectious HAV after shellfish steaming (Croci and others 1999, 2005; Hewitt and Greening 2006). These authors have shown that complete inactivation of HAV in shellfish is achieved after heating the shellfish to an internal temperature of 85 to 90 °C for 1.5 min, which may not be acceptable from an organoleptic point of view (Lees 2000). This is the thermal treatment recommended by the UK Ministry of Fisheries (Waterman 2001) and the Codex Guidelines on the application of general principles of food hygiene to the control of viruses in food (Codex Alimentarius Commission 2012).

As for shellfish, the effect of fat content has an impact on HAV heat resistance in other food matrices. For instance, Bidawid and others (2000c) investigated HAV heat resistance in milk with different fat contents, reporting that routine pasteurization temperatures are insufficient to inactivate HAV in dairy products (Table 4). Moreover, it has been shown that increasing the concentration of fat has a protective role and may further contribute to increased heat stability of HAV in food products during heating.

Sucrose content (from 28 to 52 °Brix) also has been described to have a big effect on HAV heat resistance in berries (Deboosere and others 2004). Limited thermal inactivation data exist for HAV in soft fruits and leafy greens because these types of products are usually consumed fresh or frozen. A French research group defined the heat inactivation kinetics of HAV in a fruit model system (Deboosere and others 2004). Later, the same research group developed a predictive mathematical model for the inactivation of HAV in raspberry puree (5 °Brix) without supplemented sugar and with different pH values (Deboosere and others 2010). The authors concluded that pH exerted a significant effect on HAV thermoresistance in berry-based products and that adjustment to acidic pH values, less than pH 3.3, could improve the thermal inactivation of HAV without supplemented sugar.

In addition, Butot and others (2009) evaluated the effect of heating at 80, 100, or 120 °C for 20 min on different types of freeze-dried berries artificially contaminated with HAV. The authors showed that heating for 20 min at 120 °C completely inactivated infectious HAV from freeze-dried berries.

Blanching is a mild heat treatment that consists of scalding vegetables in boiling or steaming water for a short time. This procedure helps retain the color, flavor, and texture of vegetables by stopping enzyme action. Blanching is recommended for almost all vegetables before freezing and dehydration. Blanching parameters vary, and temperatures are generally between 75 and 105 °C. Moreover, industrial blanching for leafy vegetables includes the use of steam for 120 to 180 s. Until now, only a couple of reports have evaluated this technology on herbs (including parsley, basil, chives, and mint) and spinach (Butot and others 2009; Bozkurt and others 2015). Butot and others (2009) reported that HAV titers were significantly reduced ($P < 0.05$) immediately following steam blanching at 95 °C for 2.5 min, and no residual HAV was detected. Appreciably less inactivation of HAV was observed when the temperature of steam-blanching was reduced to 75 °C, and the infectious HAV titers were reduced by an average of 2 logs, except for chives. For spinach, the reported *D*-values of HAV were 34.4, 8.4, 4.5, 2.3, and 0.9 min at temperatures 50, 56, 60, 65, and 72 °C, respectively (Table 4). These results indicate that industrial blanching conditions for vegetables (100 °C for 120–180 s) should provide >6 log HAV reduction.

Low-heat dehydration is a common processing method for dehydrating fruits and vegetables which consists of a low-heat treatment between 40 and 60 °C for 10 to 24 h. Efficacy of low-heat dehydration of green onions has recently been investigated (Laird and others 2011; Sun and others 2012). Heating at 47.8, 55.1, and 62.4 °C for 20 h reduced infectious HAV in green onions by 1, 2, and 3 logs, respectively. The authors concluded that low-heat dehydration using 62.5 °C or higher could effectively inactivate HAV in contaminated onions by >3 logs.

Vacuum freeze-drying is the most common food technology for manufacturing high-quality dehydrated products, because such dehydrated foods maintain the color, flavor, and most types of their antioxidants. The production of freeze-dried food products typically involves freezing fresh food, then removing almost all the moisture in a vacuum chamber, and finally sealing the food in an air-tight wrap or container. Freeze-dried foods can be easily transported at room temperature, stored for a long time, and consumed with minimal preparation. One of the major disadvantages of freeze-drying is its high cost because of the equipment investment, and the process itself is time-consuming and intensive work. Some foods are extremely well-suited to the freeze-drying process, including vegetables and fruits. The latter are commonly used by the food industry in cereals, granola bars, chocolate products, and bakery goods. Butot and others (2009) evaluated the efficacy of freeze-drying on HAV inoculated onto the surface of different types of berries and herbs. The authors showed that freeze-drying caused inactivation rates between 1.2 and 2.4 logs.

Shellfish depuration

Unlike most food products, in which handling is often the source of contamination, shellfish are most commonly contaminated by fecally polluted water in the harvest area. Shellfish are filter feeders that ingest and pass out large quantities of water to filter and thus consume microscopic particles. As part of this process, they may accumulate HAV if the water has been contaminated with human sewage. Shellfish are also at risk as they are often eaten raw, like oysters, or improperly cooked, like most other molluscs, or steamed for only a few minutes. Shellfish depuration is a commercial processing option used worldwide, where shellfish are placed in tanks containing clean seawater and allowed to purge the contaminants during several days (Richards and others 2010). Shellfish depuration rapidly removes bacterial pathogens and indicator organisms, however it is barely effective for HAV (Bosch and others 1994; Abad and others 1997; Lees 2000). HAV persistence was demonstrated in oysters and responsible for outbreaks, even though depurated for several days and compliant with the EU standards, based on the numbers of *Escherichia coli* in shellfish meat, which must be below 230 most probable numbers per 100 g of flesh to allow their market distribution (Guillois-Bécel and others 2009). Additionally, HAV was detected at the same frequency in oysters and mussels before and after commercial depuration in 4 European countries (Formiga-Cruz and others 2002).

Despite the demonstrated persistence of HAV in depurated molluscan shellfish, there are very limited data directly quantifying the extent of any HAV reduction during depuration. For instance, commercial depuration conditions used by the shellfish industry were evaluated by Abad and others (1997), showing that infectious HAV were recovered from bivalves after 96 h of immersion in a continuous flow of ozonated marine water. More recently, Polo and others (2014a,b) showed an average reduction of bioaccumulated HAV levels in clams and mussels of less than 1.5 logs after 7 d of depuration. At the international level, the European Food Safety Authority (2012) clearly states that currently used methods of shellfish depuration are ineffective to remove NoV, which most likely will behave as HAV.

Table 5–Relevant examples of HAV inactivation rates by use of HPP.

Pressure (MPa)	Processing conditions (time, temperature)	Food type	Initial level	HAV strain	Inactivation (log reduction)	Reference
250	5 min, 9 °C	Fresh salsa	8.0 log $TCID_{50}$	HM175/18f	1.9	Hirneisen and others 2014
250	5 min, 21 °C	Strawberry puree, sliced green onions	6.2 log PFU	HM175/18f	1.2, 0.3	Kingsley and others 2005
250	10 min, 9 °C	Fresh salsa	8.0 log $TCID_{50}$	HM175/18f	2.6	Hirneisen and others 2014
275	5 min, 21 °C	Strawberry puree, sliced green onions	6.2 log PFU	HM175/18f	2.1, 0.7	Kingsley and others 2005
300	1 min, 9 °C	Oysters	7.1 log PFU	HM-175	0.2	Calci and others 2005
300	5 min, 18–22 °C	Mediterranean mussels, blue mussels	7.0 log PFU	HM175/18f	0.1, 0.8	Terio and others 2010
300	5 min, 21 °C	Strawberry puree, sliced green onions	6.2 log PFU	HM175/18f	3.1, 1.4	Kingsley and others 2005
325	1 min, 9°C	Oysters	7.1 log PFU	HM-175	0.8	Calci and others 2005
325	5 min, 18–22 °C	Mediterranean mussels, blue mussels	7.0 log PFU	HM175/18f	0.7, 1.0	Terio and others 2010
350	1 min, 9 °C	Oysters	7.1 log PFU	HM-175	1.3	Calci and others 2005
350	5 min, 18–22 °C	Mediterranean mussels, blue mussels	7.0 log PFU	HM175/18f	1.7, 2.1	Terio and others 2010
375	1 min, 9 °C	Oysters	7.1 log PFU	HM-175	2.3	Calci and others 2005
375	5 min, 18–22 °C	Mediterranean mussels, blue mussels	7.0 log PFU	HM175/18f	2.5, 2.7	Terio and others 2010
375	5 min, 21 °C	Strawberry puree, sliced green onions	6.2 log PFU	HM175/18f	4.3, 4.7	Kingsley and others 2005
400	1 min, 9 °C	Fresh salsa	8.0 log $TCID_{50}$	HM175/18f	7.0	Hirneisen and others 2014
400	1 min, 9 °C	Oysters	7.1 log PFU	HM-175	3.2	Calci and others 2005
400	5 min, 18–22 °C	Mediterranean mussels, blue mussels	7.0 log PFU	HM175/18f	2.9, 3.6	Terio and others 2010
500	5 min, 4 °C		6.7 log $TCID_{50}$		3.2	Sharma and others 2008

An alternative treatment to shellfish depuration is ultraviolet (UV) irradiation. De Abreu Corrêa and others (2012) reported a 3 log reduction of HAV titers in a shellfish depuration system with UV treatment using a 36 W lamp for 120 h. HAV titers in mussels were significantly reduced by a closed-circuit depuration system that used both ozone and UV light for disinfecting water, although a residual amount of infectious HAV was still detected (De Medici and others 2001) after 120 h treatment. Corrêa and others (2012) recently reported that HAV was not detected in oysters after UV treatment lasting 96 h using a 18 W lamp.

The Effect of alternative food processing technologies on HAV

The food industry is challenged to broaden the spectrum of nonthermal preservation processes, applicable to a wide range of food matrices, that is able to control microbiological hazards, have less environmental impact, and maintain the organoleptic qualities of food products. High-hydrostatic-pressure processing (HPP), irradiation, active packaging, or ultrasound-based treatments are the most relevant alternative preservation technologies, although some concerns as to current consumer acceptability and regulation have limited their applications.

High-pressure processing (HPP) is an increasingly popular nonthermal food technology which can inactivate food-borne pathogens while only minimally degrading the quality of the product being treated (Rodrigo and others 2007). Inactivation of HAV by HPP has been extensively studied using virus suspensions, and HAV has been classified as a moderately resistant virus (reviewed by Kingsley 2013). In contrast, reports on its efficacy in food matrices are somewhat limited (Table 5). Treatments at 400 MPa or higher pressures are generally very effective for HAV inactivation in foods; however, it is assumed that current commercial HPP conditions applied at 275 or 300 MPa for a few minutes would not have a substantial effect on HAV. Inactivation rates observed are significantly influenced by the applied processing conditions (time and temperatures), pH, salt concentration, and food type (Table 5), and in addition, higher inactivation rates at an acid pH and lower efficiencies at increasing salt concentrations (Kingsley and Chen 2006; Kingsley 2013; D'Andrea and others 2014; Pavoni and others 2015).

The actual mechanism of HAV inactivation by HPP has recently been elucidated. Initially, results based on propidium monoazide and RNase treatments indicated that HAV capsid remains relatively intact after HPP treatment (Kingsley and others 2002; Sánchez and others 2012). Moreover, D'Andrea and others (2014) recently reported that HPP efficacy depends on HAV capsid conformation. Therefore, the mechanism of HPP inactivation for HAV is most likely because of subtle alterations of viral capsid proteins preventing attachment to the cellular receptor or blockage of the penetration and virion-uncoating mechanisms subsequent to viral attachment (Kingsley and others 2002).

UV light is electromagnetic radiation with wavelengths shorter than visible light. It can induce damage to a wide range of food-borne pathogens, thus the U.S. FDA has approved the use of UV-C on food products for controlling surface microorganisms (FDA 2007). The efficacy of UV light (at 40, 120, and 240 mWs/cm^2) was initially evaluated on HAV inoculated into fresh produce. Results varied depending on UV dose and food product (Fino and

Kniel 2008). For example, HAV titers in strawberries were only reduced by 2.6 logs at 240 mWs/cm^2, whereas >4.5 log reduction was reported on lettuce and green onions (Fino and Kniel 2008). Recently, treatments of green onions with UV at 240 mJ s/cm^2 reduced internalized HAV by 0.4 log, whereas the surface-inoculated HAV was inactivated by 5.2 logs (Hirneisen and Kniel 2013). Park and Ha (2015c) recently observed a 1.7 log reduction of HAV inoculated on chicken breast meat when the virus was irradiated with 3600 mWs/cm^2 of UV-C (260 nm) causing deleterious changes to the physicochemical and sensory qualities of the meat surface. Moreover, the authors calculated that *D*-values for HAV titers fell in the range of 2854.12 to 3076.92 mWs/cm^2. As described for other technologies, the topography of food surface has the greatest impact on HAV inactivation, smoother surfaces being the easiest to decontaminate. Crevices or presence of hair-like projections on the produce surface protect viruses from UV light.

Pulsed UV light consists of short- and high-peak-energy light pulses with a large spectrum of wavelengths and has been proposed as a novel nonthermal technology for food preservation. According to the U.S. FDA, pulsed UV light is recommended for the decontamination of food-contact surfaces and food by using a xenon lamp emitting wavelengths between 200 and 1000 nm, pulse durations not exceeding 2 milliseconds, and cumulative intensity not exceeding 12 J/cm^2 (FDA 2013).

HAV suspensions treated with pulsed UV light operated at 50 mWs/cm^2 achieved a reduction of 4.8 logs (Jean and others 2011). The authors observed that HAV inactivation was greater on inert surfaces than in suspension. Moreover, the presence of organic matter reduced the effectiveness of pulsed light both in suspension and on surfaces.

Ionizing radiation can be generated using either radioactive isotopes or linear accelerators to generate electron beams. Radioactive isotopes generate gamma rays, whereas electron beams consist of a beam of high-energy electrons.

Gamma radiation is an alternative nonthermal technology used as a sanitization treatment for shellfish and produce. Nowadays, WHO standards which are based on nutritional, toxicological, and microbiological criteria indicate that the maximum absorbed dose delivered to food should not exceed 10 kGy. Moreover, the U.S. FDA amended the food additive regulations in 2008 to provide for the safe use of ionizing radiation to control food-borne pathogens and extend the shelf-life of fresh iceberg lettuce and fresh spinach at a maximum absorbed dose not exceeding 4 kGy (reviewed by Goodburn and Wallace 2013). Studies investigating gamma radiation efficacy on HAV infectivity are somewhat limited. Bidawid and others (2000b) evaluated its effect at doses ranging between 1 and 10 kGy on strawberries and lettuce. Overall, the authors observed a gradual decrease in HAV titer with increasing irradiation doses for both food products. At 10 kGy, HAV was inactivated by more than 3 logs for both lettuce and strawberries. Furthermore, gamma irradiation at 2 kGy applied to oysters and clams reduced HAV titers while not affecting the sensory quality (Mallett and others 1991).

Electron beam (e-beam) irradiation is another alternative technology evaluated for HAV inactivation in oysters (Praveen and others 2013). The E-beam dose required to achieve a reduction of 1 log of HAV titers in oysters was 4.83 kGy.

Pulsed electric field (PEF) processing is a novel technology for pasteurizing liquid foods, using short bursts of electricity which can inactivate spoilage and food-borne pathogens at or near atmospheric temperatures (Puértolas and others 2012). Several studies have reported the efficacy of PEF treatments on a wide range of food-borne pathogens (Haughton and others 2012; Mukhopadhyay and Ramaswamy 2012); however, we are unaware of any studies published on HAV treated with PEF.

High-power ultrasound (HPU) at lower frequencies (20 to 100 kHz) is also considered a promising emerging technology for the food industry (Bilek and Turantaş 2013). It has been successfully applied to inactivate bacteria in liquid food products and the fresh produce industry. Application of HPU treatment (0.56 kW/L, 20 kHz) for 60 min, however, was ineffective for murine norovirus (MNV) inactivation in lettuce wash water (Sánchez and others 2015b) and in orange juice where only 1.5 log reduction of MNV titers were achieved after a 30-min treatment (Su and others 2012). As for other food technologies, no information is available about their effects on HAV.

Antimicrobial packaging

As a means of preventing recontamination with food-borne pathogens and extending the shelf-life of foods, antimicrobial packaging is one of the most promising technologies in the food area (Appendinia and Hotchkiss 2002). The incorporation of antimicrobial agents in food packaging can be used to control the microbiota and even target-specific food-borne pathogens to provide greater safety and to enhance food quality. However, reports on the evaluation of materials with antiviral properties are somewhat limited. Martínez-Abad and others (2013) synthetized active renewable packaging materials with virucide properties. In this pioneering study, polylactide films satisfactorily incorporated silver ions showing strong antiviral activity on a norovirus surrogate using the Japanese industrial standard (JIS Z 2801). When films were applied to food samples, antiviral activity was very much dependent on the food type and temperature. Moreover, Bright and others (2009) evaluated the antiviral activity of active packaging, reporting that feline calicivirus infectivity was reduced by 5 logs when in contact with plastic coupons impregnated with 10% silver–copper zeolites.

An emerging application for antimicrobial packaging is the incorporation of active natural compounds. Grape seed and green tea extracts have recently been incorporated into edible chitosan films and its antiviral activity on MNV has been successfully reported (Amankwaah 2013). Taking these studies together, future research is required in this area to evaluate the use of antimicrobial packaging on relevant food-borne viruses, such as HAV, and explore its efficacy in food applications.

Other alternative food processing technologies

Atmospheric pressure plasma (APP) is an emerging nonthermal food technology. It uses a neutral ionized gas that comprises highly reactive species including positive and negative ions, free radicals, electrons, excited or nonexcited molecules, and photons (Wan and others 2009). As for other emerging technologies, no information is available about its efficacy on HAV. Recently, Ahlfeld and others (2015) investigated the impact of nonthermal or cold APP (CAPP) on the inactivation of a human NoV showing that increased plasma treatment times led to decreased copy numbers of NoV in inoculated surfaces. Similar trends are applied for electrolyzed water (EW), whereas no information is available on HAV and only information on human noroviruses have been published so far. Tian and others (2011) showed that there are minimal or no improvements gained by use of EW instead of tap water wash in the removal of human norovirus from produce.

Efficacy of Washing Procedures to Eliminate or Inactivate HAV on Produce

There is increasing consumer demand for fresh-cut fruits and leafy greens because consumers generally consider them "healthy"

and safe (Lynch and others 2009). However, produce, in particular leafy green vegetables consumed raw, are obtaining increasing recognition as important vehicles for the transmission of foodborne pathogens. HAV can contaminate produce through contact with incorrectly treated sewage or sewage-polluted water in the fields. Contamination may also occur during processing, storage, distribution, or final preparation. This might happen because HAV-infected people, contaminated water, or fomites come into contact with foods. In fact, experimental studies have shown that approximately 9.2% of infectious HAV can be transferred to lettuce from the contaminated hands of handlers (Bidawid and others 2000a).

Produce, including various types of salads, green onions, and berries have repeatedly been associated with outbreaks of hepatitis A (Rosenblum and others 1990; Dentinger and others 2001). For example, a hepatitis A outbreak because of consumption of contaminated green onions affected at least 601 individuals and resulted in 3 deaths (Wheeler and others 2005). HAV has also been detected in market lettuce (Monge and Arias 1996; Hernandez and others 1997; Pebody and others 1998), in 1.32% of salad vegetable samples collected from European countries (Kokkinos and others 2012), and 28.2% of the Mexican samples (Felix-Valenzuela and others 2012). Moreover, in recent years, an increasing number of HAV outbreaks has been associated with foods of foreign origin in industrialized countries (Table 1).

This section is a compilation of current knowledge available to date on the effect of the most common washing procedures applied in the food industry to eliminate or inactivate HAV when present in leafy greens and berries.

Tap water

Vigorous produce washing is common practice post-harvest, which removes soil, and hopefully pesticide residues and many microorganisms. Washing of produce with clean potable water typically reduces the number of microorganisms by 1 to 2 logs and is often as effective as treatment with 100 mg/L of chlorine, the current industry standard (Seymour and Appleton 2001). Croci and others (2002) observed less than 1 log reduction of HAV concentration when lettuce, carrots, and fennel were washed with potable water for 5 min. Butot and others (2008) also evaluated tap-water washing, demonstrating that this process reduced inoculated HAV titers in different types of produce by less than 1.5 logs. Furthermore, the authors observed that the use of warm water (43 °C) did not improve HAV removal from produce. In line with these results, lettuce washes with water with bubbles, or bubbles and a sonication step (at 35 kHz for 2 min), resulted in slight reductions in HAV infectivity with a maximum reduction of less than one log (Fraisse and others 2011). To summarize, these reports clearly show that washing with tap water alone is ineffective in removing HAV from produce. More recent research into the use of scrubbing under running water with a nylon brush, or scouring pad for fresh produce (Wang and others 2013a), showed that this decontamination procedure presents advantages over tap-water washing. Scrubbing produce (honeydew melons, cantaloupes, and carrots) initially inoculated with 5.5 logs resulted in significant ($P < 0.05$) levels of HAV removal, ranging from 1.15 to 2.85 logs.

Chlorine

The use of chlorine-based sanitizers is widespread throughout the fresh produce industry, with the intention to maintain microbial safety of produce, avoid cross-contamination, and recycle water. Butot and others (2008) explored the efficacy of washing different types of berries and vegetables with chlorinated water (200 ppm free chlorine), showing that HAV inactivation varied according to food type, with a maximum of 2.4 log reduction in blueberries. Chlorinated water had a limited effect on HAV titers when used to decontaminate parsley and raspberries, probably because of their complex surface topography. For instance, raspberries have hair-like projections and crevices which may shield the viruses against environmental conditions.

Fraisse and others (2011) investigated the efficacy of 15 ppm of free chlorine, determined as the most representative chlorine concentration used in the food industry. The study reported a 1.9 log reduction of HAV infectivity in lettuce. Casteel and others (2008) reported at least 1.2 log reduction of HAV titers in lettuce samples, strawberries, and tomatoes treated with 20 ppm chlorine for 5 min. All the above-mentioned studies were performed on surface-inoculated produce, without considering the internalization of HAV. Recently, the efficacy of sprayed calcium hypochlorite (150 ppm) was evaluated on HAV internalized in green onions (Hirneisen and Kniel 2013). HAV internalized within the green onions were inactivated by only 0.4 logs, whereas the surface-inoculated HAV was inactivated by 2.6 logs.

Although chlorine is relatively cheap and easy to use, some countries have limited its use since it may generate by-products, such as trihalomethanes and other chemical compounds. Moreover, chlorine is highly corrosive for the stainless steel surfaces used in the food industry. As a result, this has led to the search for new alternative sanitizing treatments to water chlorination to ensure fresh produce quality and safety (Van Haute and others 2013).

Chlorine dioxide has emerged as an alternative to chlorine as its efficacy is little affected by pH and organic matter. Moreover, it does not react with ammonia to form chloramines. The effect of chlorine dioxide has been mainly investigated on HAV suspensions; Zoni and others (2007) evaluated its efficacy at doses from 0.4 to 1.5 mg/L, reporting complete HAV inactivation at 1 mg/L and 30 s of contact. Bigliardi and Sansebastiano (2006) reported 4 log reduction of HAV suspensions treated with 0.6 mg/L of chlorine dioxide for 2 min. Despite that, chlorine dioxide at 10 ppm for 10 min inactivate HAV by less than 2 log in raspberries and parsley (Butot and others 2008).

Peroxyacetic acid (PAA) is considered a potent biocide and has been able to inactivate bacterial food-borne pathogens without the persistent toxic or mutagenic residuals or by-products. Reports evaluating the effects of PAA to inactivate HAV in produce are somewhat limited. Fraisse and others (2011) evaluated the efficacy of a peroxyacetic-based biocide (100 ppm) for lettuce decontamination, reporting only about 0.7 log reduction of HAV.

Ozone (O_3) is widely used as an antimicrobial agent to disinfect water and is active against a broad range of food-borne pathogens. Ozone is also one of the most effective sanitizers, with the advantage of leaving no hazardous residues on food or food-contact surfaces and can be used effectively in its gaseous or aqueous state (Kim and others 2003). The effect of ozone has been mainly investigated on HAV suspensions. Although some tolerance to lower ozone residuals (0.1 to 0.5 mg/L) was reported, the exposure of HAV to ozone concentrations of 1 mg/L or greater resulted in its complete (5 log) inactivation within 60 s (Vaughn and others 1990). Herbold and others (1989) observed that HAV inactivation by ozone was more effective at 10 °C than at 20 °C. At 20 °C, 0.25 to 0.38 mg of O_3 per liter was required for complete HAV inactivation.

Ozonated water has been successfully applied to produce washes, reducing bacterial populations (Perry and Yousef 2011)

Table 6–Inactivation rates of HAV suspensions by natural compounds.

Natural compound compound	Initial level	Temperature, contact time	HAV Strain	Inactivation (log reduction)	Reference
Carrageenans (1 mg/mL)	–	Cell pretreatment[a]	CF53	1.0	Girond and others 1991
Carvacrol (0.25, 0.5%, 1%)	6.2 log $TCID_{50}$	37 °C, 2 h	HM175/18f	0.16, 0.14, 0.97	Sánchez and others 2015a
Grape seed extract (0.25, 0.50, 1 mg/mL)	6.6 log PFU	37 °C, 2 hRT, 2 h	HM175	1.81, 2.66, 3.200.86, 1.22, 1.90	Su and D'Souza, 2011
Korean red ginseng (5, 6.7, 10 μg/mL)	–	Cell pretreatment[a]	HM175/18f	0.45, 0.55, 0.66	Lee and others 2013
Oregano essential oil (0.5, 1, 2%)	6.2 log $TCID_{50}$	37 °C, 2 h	HM175/18f	0.37, 0.12, 0.12	Aznar and Sánchez 2015
Quercetin enriched lecithin (20, 40 μg/mL)	4.0 log PFU	40 °C, 1 h	–	0.35, 0.66	Ramadan and Asker, 2009
Thymol (0.5, 1, 2%)	6.2 log $TCID_{50}$	37 °C, 2 h	HM175/18f	0.12, 0.21, 0.01	Aznar and Sánchez 2015
Zataria essential oil (0.01, 0.05, 0.1%)	6.2 log $TCID_{50}$	37 °C, 2 h	HM175/18f	0.1, 0.0, 0.42	Aznar and Sánchez 2015

[a]Cells were exposed at various concentrations of compounds before titrating the virus concentration.
PFU, plaque-forming unit; $TCID_{50}$, tissue culture infectious dose; RT, room temperature.

and norovirus surrogates on produce (Hirneisen and others 2011); however, its efficacy regarding HAV inactivation has yet to be explored.

Natural additives for possible use as a biocide for washing treatments have been proposed as potential alternatives to chemical additives because some of them are categorized as Generally Recognized as Safe (GRAS) and because of increasing consumer demands for safe and "healthy" natural products. So far, natural additives, such as essential oils, chitosan, polyphenols, and juice extracts have mainly been evaluated on norovirus surrogates (reviewed by Li and others 2013; D'Souza 2014; Ryu and others 2015) and information about their efficacy on HAV is somewhat limited (Table 6). Until now, grape seed extract (Su and D'Souza 2011), carvacrol (Sánchez and others 2015a), oregano and zataria essential oils, thymol (Aznar and Sánchez 2015), and Korean red ginseng extracts and ginsenosides (Lee and others 2013) have been evaluated on HAV suspensions in a tissue culture medium or buffer solutions without much success, except for grape seed extract (GSE; Table 5). GSE reduced HAV infectivity at 37 °C and room temperature in a dose-dependent manner and depending on the initial virus concentration; however, these conditions cannot be mimicking practical scenarios for their potential use in food industry applications as a sanitizing agent for reconditioning of water or disinfecting food contact surfaces in the food processing environment (Li and others 2013). So far, the efficacy of natural additives in food applications has hardly been explored. The efficacy of GSE washes on HAV was assessed on lettuce and jalapeno peppers. The study reported that after 1 min of contact, 0.25 to 1 mg/mL GSE caused a reduction of 0.7 to 1.1 and 1 to 1.3 $\log_{10}$ PFU for high and low HAV titers, respectively, on both food products (Su and D'Souza 2013). Considering that most of the evaluated natural compounds slightly affect HAV infectivity, and that GSE washes had a limited effect, future research must look into new natural compounds or combinations thereof.

Conclusions and Future Perspectives

Hepatitis A has recently been considered as a re-emerging food-borne public health threat in developed countries (Sprenger 2014) because of increasing numbers of food-borne outbreaks associated with imported foods (Collier and others 2014; Gossner and Severi 2014; Guzman-Herrador and others 2014; Wenzel and others 2014). As a reflection of the seriousness of HAV outbreaks, extensive attention must be given to the intervention strategies to reduce consumer risk.

To estimate the risk of consuming products, which may have become contaminated with HAV prior to processing, many publications have actually been exploring the stability of HAV in foods as well as the efficacy of treatments usually applied to food products. The use of different virus titers, inoculum-suspending matrices, and virus recovery procedures really complicates comparisons among studies documented in this review.

HAV inactivation rates by common and alternative food manufacturing processes differ according to the technology applied (processing conditions), food (moisture, fat, pH, and sugar content), and surface type. Additionally, the thermal and pressure stability of different HAV strains may also play a role. Shimasaki and others (2009) reported that heating and high-hydrostatic-pressure processing inactivate suspensions differently according to different cell-adapted HAV strains. Similarly, differences in inactivation after pasteurization were also seen for different HM175 variants (Farcet and others 2012). These differences need to be taken into consideration because most studies have been performed using only the HM-175 strain.

Studies on the efficacy of food manufacturing processes have been particularly focused on some relevant food matrices (mussels, oysters, lettuce, green onions, and strawberries). However, there is a definite need for further research using a wider range of food products because of the recent HAV outbreaks associated with unusual food products (sun-dried tomatoes, dates, and pomegranate arils; Petrignani and others 2010; Boxman and others 2012; Wenzel and others 2014).

Overall, there is still much to be learnt about the efficacy of food manufacturing processes and the parameters required to inactivate HAV in foods. In general, HAV is much more resistant to food manufacturing processes and decontamination procedures than are bacterial cells. Overall, some of the processes (mild thermal treatment, HPP, and chlorine sanitization) can inactivate or remove significant numbers of HAV from foods. As the infectious dose of HAV is around 10 to 100 viral particles, those processes could prevent hepatitis A infection when the contamination level is below 2 to 3 log. However, these procedures would be insufficient to inactivate/decontaminate foods contaminated with

higher virus concentrations. Moreover, the efficacy of many of the alternative food technologies against HAV in the context of reducing infection risks is still relatively limited or unknown.

As recently suggested, a wide range of thermal treatment conditions should be tested on each food type to express *D*-values (decimal reduction times), which is the time required to inactivate 1 log (90%) of HAV at a certain temperature. These values can be more easily used by food industries in their HACCP plans than single-point measurements (Zuber and others 2013).

For some food products, such as produce, food manufacturing processes that inactivate HAV cannot be applied without adversely affecting food quality. Therefore, effective prevention of contamination could reduce virus numbers and thereby decrease consumer risks of HAV infections. For this purpose, the use of model-based quantitative risk assessments will provide a valuable tool to the development of targeted intervention measures (Bouwknegt and others 2015).

Mild manufacturing processes show only marginal effects on the viral load, but when the processes are combined, the synergy effect may enhance the level of HAV inactivation. An interesting future approach might be research into the effect of combining food processes on HAV. Moreover, striking a balance between inactivation and maintaining the nutritional and organoleptic characteristics of foods is an ongoing challenge for food technologists.

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