

Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Survival factors affecting the infectivity of hepatitis A virus isolated from Egypt

Abdallah S Ragab*, Ahmed Ahmed Hmed, Ahmed Ramadan Sofy

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Egypt

ARTICLE INFO

Article history:

Received 18 September 2023

Received in revised form 23 October 2023

Accepted 29 October 2023

Keywords:

HAV
Temperature
PH
Salt
Disinfectants

ABSTRACT

Background: The physicochemical properties of viruses affect both how well they spread and how long they may survive in different environments. It would be helpful to have a complete understanding of the tools to break or limit the chain of virus transmission. In addition to choosing safe circumstances for preventing infections and the spread of these viruses. **Methods and results:** The data obtained demonstrated that hepatitis A viruses (HAV) were stable at freezing (-20°C). HAV's infectivity was considerably impacted by the temperature increase (70°C), with a $4.77 \log_{10}\text{TCID}_{50}/\text{ml}$ titer drop. HAV was resistant to various pH levels. At pH 4 the virus titer decreased by $0.88 \log_{10}\text{TCID}_{50}/\text{ml}$. However, the virus was entirely inactivated at pH 12. The minor titer decreased by $0.54 \log_{10}\text{TCID}_{50}/\text{ml}$ at 3% salt conc., indicating that HAV was salt stable. Virus deactivation by disinfectants (ethanol 20, 70 and 95%), phenol (1, 2 and 3), chlorine (5, 10 and 20 mg/l). Iodine (0.5, 1 and 3%) was performed. The exposure to 70% ethanol, a partial inactivation of $4.16 \log_{10}\text{TCID}_{50}/\text{ml}$ was detected. In contrast, HAV was completely inactivated at 3% phenol, while at 1 and 2% it maintained its infectivity. The virucidal efficiency of chlorine was detected at 20 mg/l, while at 5 and 10 mg/l, the titer was decreased by 0.86 and $6.19 \log_{10}\text{TCID}_{50}/\text{ml}$, respectively. Iodine at all measured concentrations did not show a complete ability to fully inactivate HAV. **Conclusion:** HAV is very stable and somewhat resistant to environmental influences, acidic pH, high temperature and chemicals.

Introduction

Every year, millions of people around the world suffer from foodborne illnesses, and it is expected that this number will rise in direct proportion to global warming [1,2]. Viruses are thought to be the cause of 12% of fatalities, 27% of hospitalizations, and 59% of all foodborne diseases. In less developed nations, enteric viruses found in food and water are a significant cause of baby and young child mortality [3]. Food, water, touch

surfaces, and people are all polluted as a result of inadequate sanitation measures by infected food handlers, and enteric viruses, in particular, are frequently disseminated by the fecal-oral route. People who have eaten contaminated food have the ability to spread foodborne viruses to others, whether they exhibit symptoms or not. Hepatitis A virus (HAV), one of many intestinal pathogenic viruses, is thought to be the most significant [4-6].

The only species of the hepatovirus genus, which belongs to the picornaviridae family, is HAV.

DOI: 10.21608/MID.2023.237037.1619

* Corresponding author: Abdallah Shaban Ragab

E-mail address: abdallahshaban005@gmail.com

© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

HAV particles have a diameter of around 28 nm and an icosahedral capsid that encloses the single-stranded positive-sense RNA genome. The 7.5 kb HAV genome consists of four structural and seven non-structural genes. The HAV infectious dosage may be extremely low, possibly even just one particle in some circumstances [4]. Following ingestion, the virus travels to the liver via the digestive system [5]. The immunological response to the virus-infected cells may be the main cause of liver damage [6]. Released virions go through the digestive tract, the bile duct, and the intestines before being eliminated in feces. Infected individuals typically experience no symptoms while the virus is actively replicating. Infected individuals can have the virus in their stools prior to becoming aware of any illness, and peak virus shedding occurs approximately 4-6 weeks after ingesting the agent, just before the onset of acute symptoms [which include jaundice, fever, malaise, nausea, abdominal discomfort, and dark urine]. According to **Hollinger et al.**, virus excretion can often continue for up to 8 days following the start of symptoms. In comparison to adults, infants and young children typically experience lesser symptoms, although fecal excretion lasts longer [7]. According to **Issa et al.** there are about 0.3% fatalities and people over 40 are more prone to experience major difficulties [8].

So, the current study aimed to study the main factors that directly affect the infectivity of hepatitis A virus in order to control these factors to break the virus's ability to infect.

Materials and methods

Virus source

Isolation of HAV from water samples collection of water samples

One hundred and sixty (160) sewage water samples (10 liters / samples) were collected in sterile container from four wastewater treatment plants (wwtps), (Kafr El-Sheikh, El Hamool, Sidi Salem and Desouk). The four sites are located in Kafr El-Sheikh (N 30° 56' 45"E"42'06°31) governorate, which lies in the northern part of Egypt, along the western branch of the Nile in the Nile Delta. These particular areas were chosen due to the increase in water pollution in these areas as a result of water pollution from the two most dangerous sources of pollution in Kafr El-Sheikh are the main Gharbia drain known as "Kitchener" and the "Number 8" drain. This pollution is due to the presence of industrial, health and agricultural pollutants in these

two banks." The total of 160 samples were divided into 40 samples from each plant (20 influent and 20 effluent samples). Ten liters of each water sample in sterile plastic container were added to ten ml of aluminum chloride (AlCl₃) (1.1/ 100 ml water) (v/v) (El Nasr Pharmaceutical Chemical Co., Egypt) to increase the stability of the viruses in the samples and kept at 4°C during transportation for laboratory (plant viruses and bacteriophage Lab. of Botany and micro. Dept., Fac. of Sci., Al-Azhar Univ. Cairo, Egypt) analysis on the next day according to **Lodder et al.** [9]

Concentration of water samples

Primary concentration

Adsorption / elution technique was used for concentration of water samples [10]. Virus particles are negatively charged at pH 7.0, and for the water passing through an electronegatively charged filter nitrocellulose membranes (Shleicher and Schuell, 0.45Mm pore size and 142 mm diameter filter series, Whatman® filters, Sigma, Germany), [11]. The water samples were acidified (approximately pH 3.5) to alter the charge of the viral particle before filtration so that the virus will be absorbed to the filter and to enhance the viral adsorption. Aluminum chloride (AlCl₃) was added (if it had not been added in the sample transfer step) and mixed to each sample (1.1 mL of a 4% AlCl₃/100 ml sample) before acidification and filtration. An elite 3% beef extract (Lab-Limco powder, Oxoid, USA) at alkaline pH 9.5 was left in contact with the filter to allow the viruses to return into solution.

Secondary concentration

All samples were secondary re-concentrated using an organic flocculation method according to USEPA and **Katzenelson et al.** [10, 12]. Briefly, the elute was acidified to pH 3.5 using HCL (5N), (Merck-Schuchardt, Germany) and centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded, and the pellet was dissolved in 1ml of Na₂HPO₄ (El Nasr Pharmaceutical Chemical Co. Egypt) (0.14N, pH 9) for water samples. The samples were kept at -70°C until used.

Virus cultivation and purification

Fetal rhesus monkey kidney cells (frhk-4), obtained from the Holding Company for Biological Products & Vaccines (Vacsera, Cairo, Egypt), were used to replicate HAV (isolated from sewage samples) in Dulbecco's Modified Eagle Medium (DMEM),

(Oxoid, USA) with 8% fetal bovine serum (FBS), (Oxoid, USA) 100 U/ml penicillin, 100 U/ml streptomycin, 1% L-glutamine, and 1% non-essential amino acids. Frhk-4 cells were then infected with the virus in 80–90% confluent monolayers until complete CPE was seen. By freezing and thawing infected cells three times, the virus was freed from the cells. Centrifugation was used to precipitate cellular debris for 15 min. at 2,000 g. Before being used, the supernatant fluid was stored in 2 ml aliquots at -80°C after being filtered using a 0.2 m membrane filter (Millipore, Billerica, MA, USA).

Assessment of HAV infectivity

For assessment of the infectious titer of the concentrated HAV from water samples. The, frhk-4 were cultured in 96-well plates, and after that, ten-fold serial dilutions of a concentrated virus in Eagle's minimal essential medium (MEM-E) were injected into each of the ten wells. The tissue culture infective dose (TCID₅₀) was used to quantify the viral titer during an incubation period of 4–7 days at 37 °C in a CO₂ environment. Reed and Muench estimated the TCID₅₀/ml [13]. The infectious titres of HAV were around 108 TCID₅₀ [13].

Stability assay of HAV under physical and chemical conditions

Fresh produce [lettuce] with uniform sizes and weights was acquired from neighborhood grocery stores and then crushed in 9 ml of sterile distilled water (DW) in three distinct tubes [one for each treatment]. All tubes were vortexed for 30 s for good distribution. Tubes were exposed to UV (254 nm, 100µw/cm²) for 30 min before inoculation. Produce items were kept under the laminar flow hood (local manufacture in Egypt, with standard specification) at room temperature until the liquid of each inoculum was visibly dry [~ 1 hr]. Subsequently, produce items were sealed in sterile containers to prevent contamination as well as, accumulation of humidity and stored overnight at 4°C until processing. A 25 µl of HAV (108 TCID₅₀ /ml) inoculum was dispensed in one item of produce sample [13].

Heat stability

Different tubes infected with the HAV virus were shaken and simulated to various storage conditions, eight different temperature settings (-20, 4, RT, 40, 50, 60, 70 and 80 °C) for 60 minutes using a water bath. At the time of inoculation [minute 0], the virus was counted using TCID₅₀/ml at room temperature

immediately and used as a control for viral reduction. The samples were immediately cooled (5 min) on ice to prevent further inactivation. The virus dilutions were stored at pH7 for up to 60 min and then tested by TCID₅₀. All experiments were done in triplicate to calculate levels of virus reduction [14].

pH stability

To verify buffer stability during the experimental circumstances, the pH of the citrate, phosphate, and carbonate buffers was tested using a pH meter both before and after the injection of the virus inoculum. Tubes from both produce items were brought to a range of pH values using several buffers, including concentrate HCl of 1.5 M, NaOH of 1 M, and 2, 4, 7, and 12 [carbonate buffer], before being injected with virus. The virus dilutions were stored at room temperature for up to 60 min and then tested by TCID₅₀. All experiments were done in triplicate to calculate levels of virus reduction [15].

Salt [NaCl] stability

Salo and Cliver, as well as **Kingsley et al.** examined the effect of NaCl concentration on HAV inactivation at room temperature for a period of one hour. By dissolving salt in DMEM-FBS and NaCl, the virus stock item was transformed into a virus stock. The viral stock was then proportionately mixed with NaCl in DMEM-FBS to produce concentrations [1, 3, 6, and 10 % (wt/vol)], [15,16]. The virus dilution was assessed by TCID₅₀ after being kept at pH7 (0.1M phosphate buffer) for up to 60 min. To determine the amounts of viral decrease, each experiment was performed three times. As a control, a virus in DMEM-FBS devoid of extra NaCl was employed.

Exposure to disinfectants

For experiments involving disinfectant inactivation of HAV. Virus in a separated panel of different tubes was diluted in each disinfectant (0.2 ml of virus stock was added to 1.8 ml of disinfectant and vortexed briefly) at different concentrations of ethanol (20, 70, and 95%), phenol (1, 2%, and 3%), chlorine (5, 10 and 20 mg/L) and iodine (0.5, 1 and 3%) at room temperature for one hour of contact at pH 7. The disinfectant was immiscible with the virus preparation and then mechanically agitated after treatment. The virus-chemical mixture was eluted with maintenance medium, and the first 1.0 ml of each mixture was then titrated after one hour to assess post treatment infectivity using TCID₅₀. All experiments were done in triplicate to calculate

levels of virus reduction. Experiments on HAV inactivation by disinfectants were done according to **Kramer et al.** and **Fraisse et al.** [17,18].

Results

Isolation of HAV from water samples

The results presented in **table (1)** and illustrated in **figure (1)** exhibited a high surveillance of HAV in influent sewage water samples. Whereas HAV was detected in 51 (63.75%) out of 80 of influent samples, on the other hand 22 (27.5%) out of 80 effluent samples were positive for HAV. A higher distribution rate of HAV was detected at Sidi Salem WWTP (62.5%) followed by Desouk (47.5%) and El Hamool (45%) WWTPs, while Kafr El-Sheikh WWTP showed the lowest detection rate (27.5%).

Temperature stability

The speed at which enteric viruses inactivate in the environment, including water and food, is influenced by a variety of physical and chemical parameters. One of the physical parameters most understood to affect virus stability or loss of infectivity is temperature. After 60 minutes of exposure at pH 7, the data obtained demonstrated that HAV was stable at low temperatures (-20°C) and (4°C). However, compared to the initial 8-log₁₀ TCID₅₀/ml, there was a very slight loss of infectivity titers by 0.4 reductions at (-20°C). At 4°C HAV was affected by 0.6 log titer reduction. It was concluded that freezing and low temperature have little effect on HAV survival, which were quite stable with nearly (100%) infectivity (**Figure 1**). At room temperature (RT) there is no effect on the survival of the virus with constant log₁₀ titers showing complete infectivity.

Although the tested virus survived best at the lowest temperature, as expected their infectivity decreased gradually with increasing the temperature more than 40°C. In contrast, complete inactivation at the highest temperature 80°C in HAV infectivity titer has occurred.

At 40°C and 50°C, a faint decline occurred in the infectious titer of HAV with reducing 0.27 and 0.53 log₁₀TCID₅₀/ml, respectively.

Despite this, HAV remained stable with little effect at 60°C, whereby the titer reduced by only 1.89 log₁₀TCID₅₀/ml, (**Figure 1**). When the temperature increased to 70°C and 80°C, the infectivity of HAV was strongly affected, by 4.77 log₁₀TCID₅₀/ml titer reduction at 70°C and 7.2 log₁₀TCID₅₀/ml at 80°C (**Figure 2**).

pH stability

The pH level is a crucial factor that influences how effectively viruses are inactivated. As a result, we assessed HAV's ability to survive in several pH ranges, including extremely acidic and alkaline situations. The outcomes, which are clearly shown in **figure (3)**, showed that HAV retained infectivity following treatment at a wide range of pHs at room temperature and for one hour of exposure.

Moreover, its infectivity was slightly altered after treatment at pH 4 with a very low declined titer by 0.88 log₁₀TCID₅₀/ml from the initial dose. The inactivation at pH 4 was not significantly different from that at a neutral pH. On the other hand, at pH 2 the virus loses 3.35 log₁₀TCID₅₀/ml from its infectious titer (**Figure 3**). As well as HAV strongly inactivated at pH 9 with a marked drop in its infectious titers by 5.46 log₁₀TCID₅₀/ml (**Figure 3**). At pH 12 the infectious titer of the virus was not detectable and completely inactivated (100% reduction of initial dose) (**Figure 3**).

Salt stability

Salinity is another important factor affecting virus persistence in the environment. In some cases, increased salt concentrations are virucidal. Different NaCl concentration [1, 3, 6, and 10 % (wt/vol)] on HAV inactivation at room temperature and pH 7 was also determined.

The obtained results illustrated in **figure (4)** exhibited that HAV was best survived under different NaCl concentrations, especially at low salt conditions. Infectivity of the virus at 1% completely not affected. While minor titer decreased by 0.54 log₁₀TCID₅₀/ml at 3% salt concentration (**Figure 4**). Faintly, titer inactivation was obtained at 6% NaCl concentration by reducing 1.55 log₁₀TCID₅₀/ml against the stability of the tested virus (**Figure 4**). Although NaCl concentration was increased to 10%, HAV appeared to be resistant even if, with higher NaCl concentrations, the maximum inactivation of HAV was 3.62 log₁₀TCID₅₀/ml reduction regardless of the NaCl concentration (**Figure 4**).

Effect of disinfectants

Disinfectants inactivation studies were conducted for the most widely used disinfectants which are relatively cheap and easy to use in household and institutional settings, including different types of chemicals with more than one concentration ethanol (20, 70, 95%), phenol (1, 2, 3%), chlorine (5, 10, 20 mg/l) and iodine (0.5, Iodine 1 and 3%).

Ethanol

The results demonstrated in **figure (5)** clearly, showed that the effect of the treatments using 20 and 95 % ethanol against HAV is different in the inhibition force according to the concentration. At a concentration of 20 % ethanol there is no effect on viral activity with complete titer stability, whereas at 95% ethanol, there is no titer detected and the virus was completely inactivated (**Figure 5**). On the other hand, at 70% ethanol, HAV was partially inactivated by 4.16 log₁₀TCID₅₀/ml (**Figure 5**).

Phenol

HAV was completely inactivated only at 3% phenol, while did not lose its ability to be infectious at concentrations 1 and 2%, the titer reduction was 1.1 and 5.5 log₁₀TCID₅₀/ml respectively (**Figure 6**).

Chlorine

Complete virucidal efficacy of chlorine was recorded at 20 mg/L against both HAV, with no titer detection after one hour of exposure (**Figure 7**). While the virus was inactivated most but not all at 5 and 10 mg/L of chlorine concentration with titer reduction by 0.86 and 6.19 log₁₀TCID₅₀/ml (**Figure 7**).

Iodine

The data presented in **figure (8)** showed that the Iodine at all measured concentrations did not show a complete ability to fully integrate against HAV. At 0.5, 1, and 3% of iodine HAV titer was reduced by 0.2, 0.78, and 2.48 log₁₀TCID₅₀/ml respectively, (**Figure 8**).

Table 1. Detection of HAV from samples at different WWTPs influent and effluent sewage

Samples (160)		No. of HAV positive samples in four WWTPs				Total
		Kafr El-Sheikh	El Hamool	Sidi Salem	Desouk	
Influent (20)	No.	9	13	16	13	51/80
	%	45%	65%	80%	65%	(63.75%)
effluent (20)	No.	2	5	9	6	22/80
	%	10%	25%	45%	30%	(27.5%)
Total		11/40 (27.5)	18/40 (45%)	25/40 (62.5%)	19/40 (47.5%)	73/160 (45.6)

Figure 1. Influent and effluent HAV positive samples from different WWTPs.

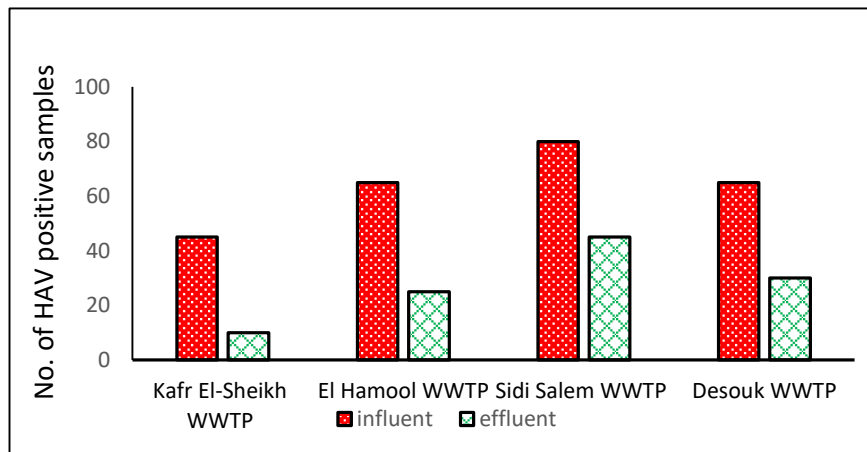


Figure 2. Stability of HAV in fresh produce items under different temperature settings for 1h of exposure at pH 7. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.

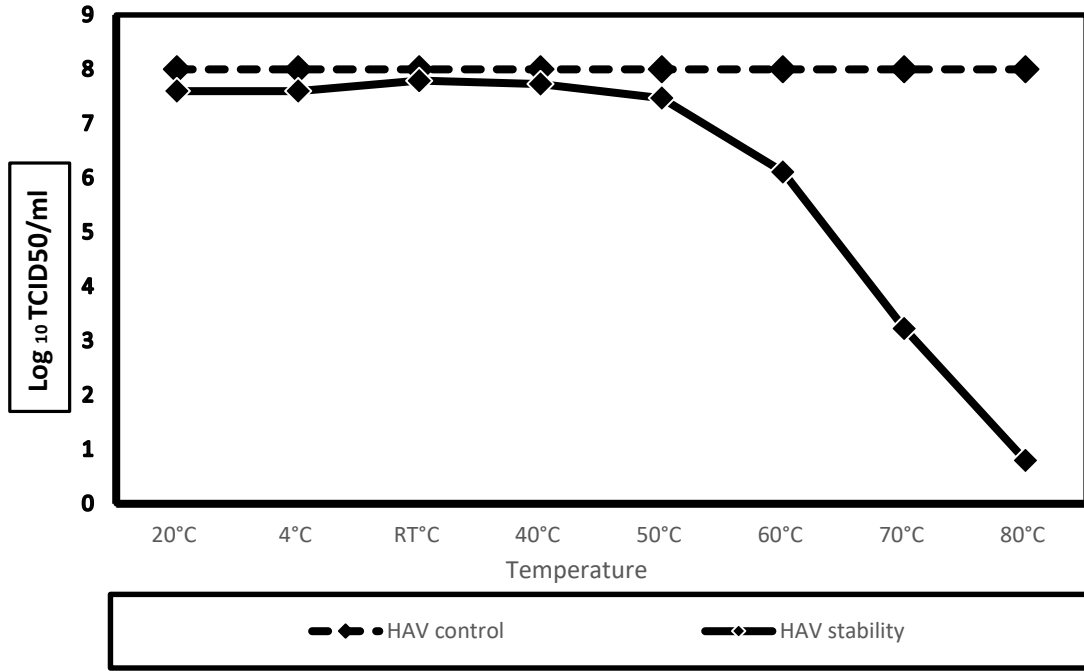


Figure 3. Stability of HAV in fresh produce items under different pH values for 1h of exposure at RT. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.

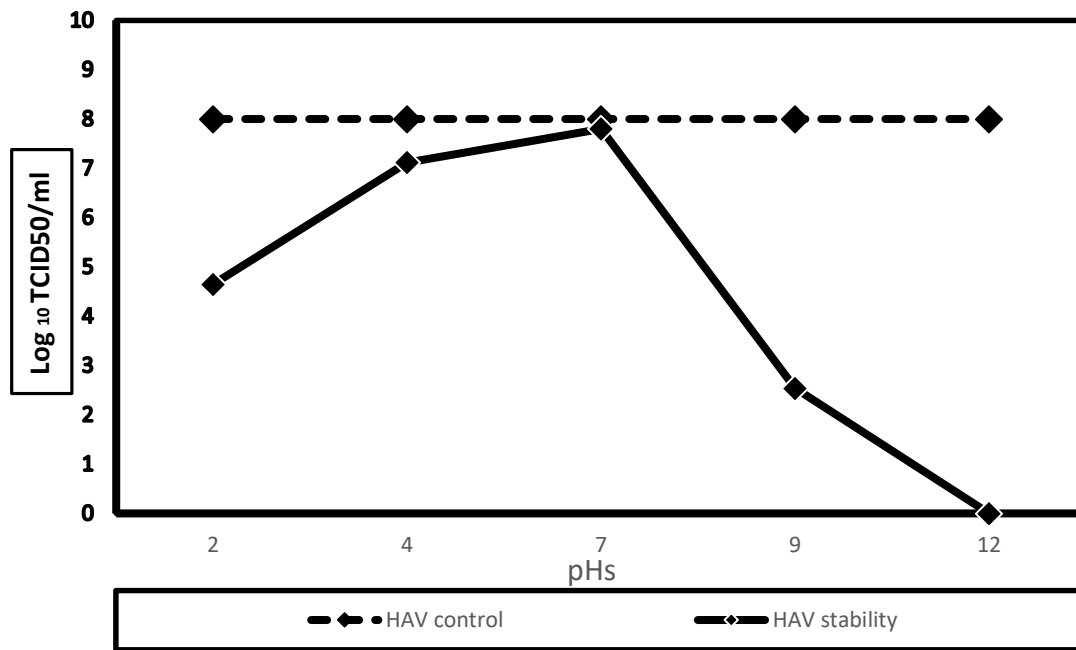


Figure 4. Stability of HAV in fresh produce items under different NaCl% concentrations for 1h of exposure at RT and pH7. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.

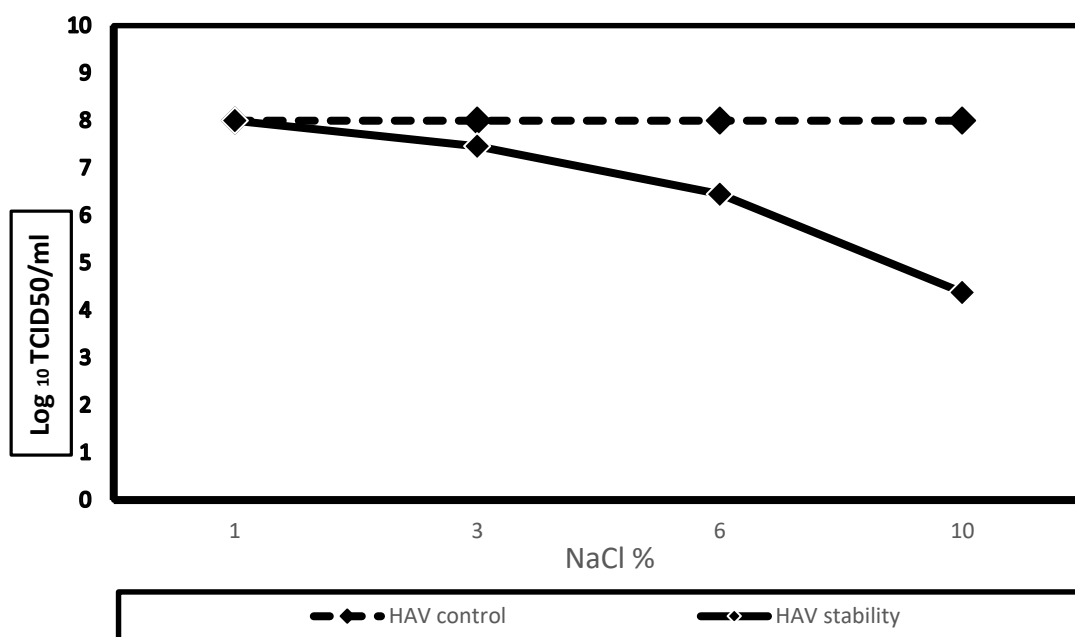


Figure 5. Stability of HAV in fresh produce items under different ethanol concentrations (%) for 1h of exposure at RT and pH7. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.

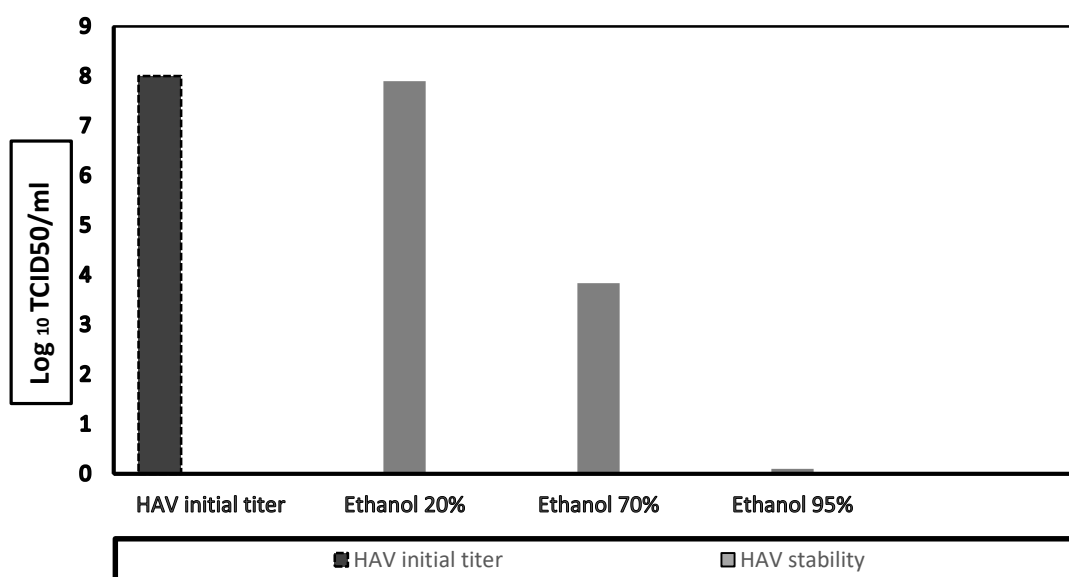


Figure 6. Stability of HAV in fresh produce items under different phenol concentrations % for 1h of exposure at RT and pH7. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.

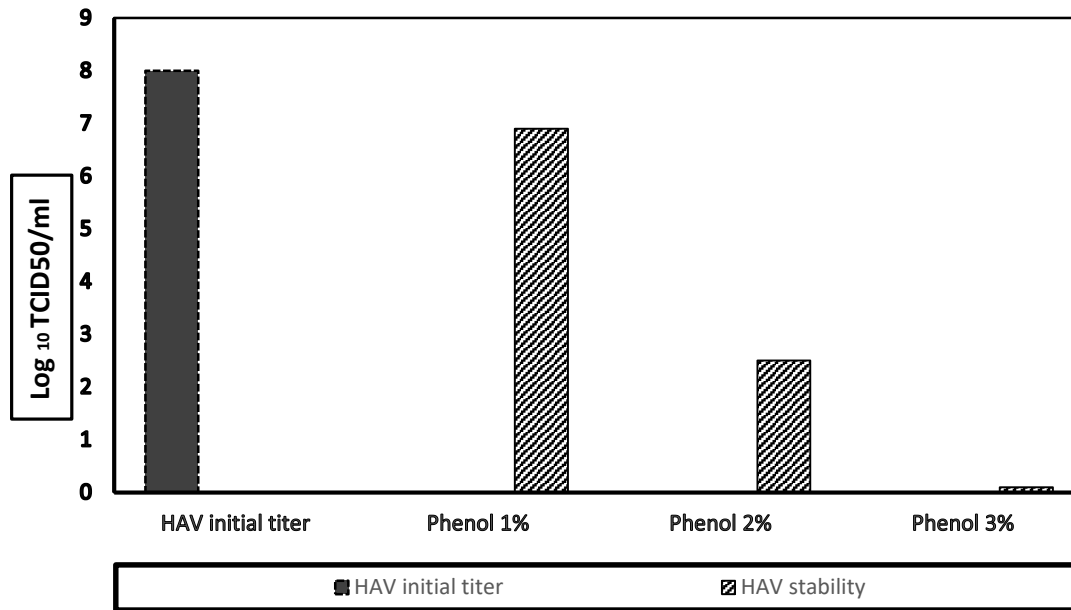


Figure 7. Stability of HAV in fresh produce items under different chlorine concentrations % for 1h of exposure at RT and pH7. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.

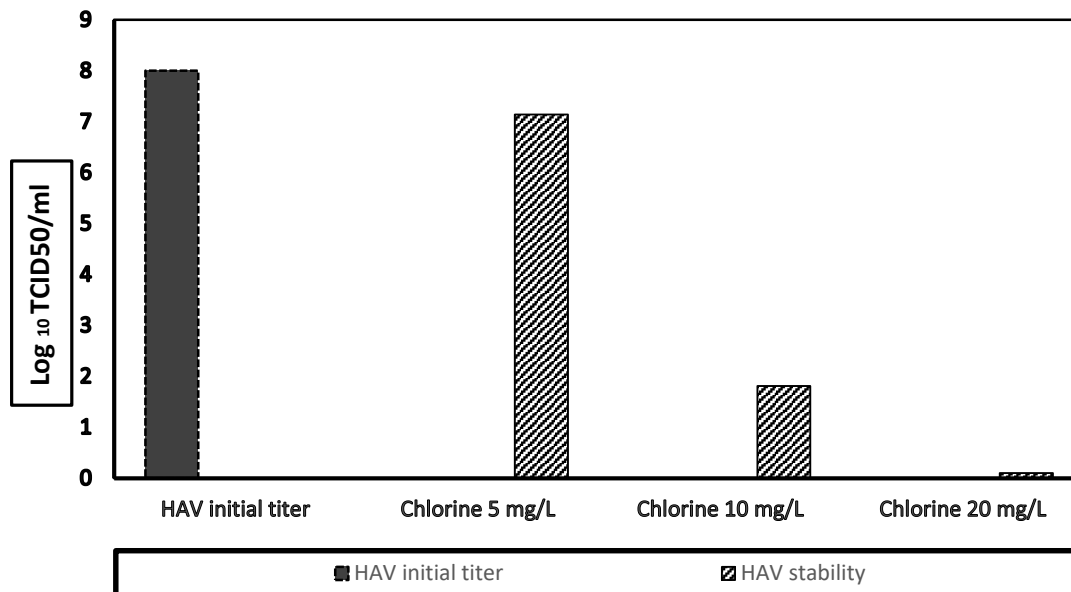
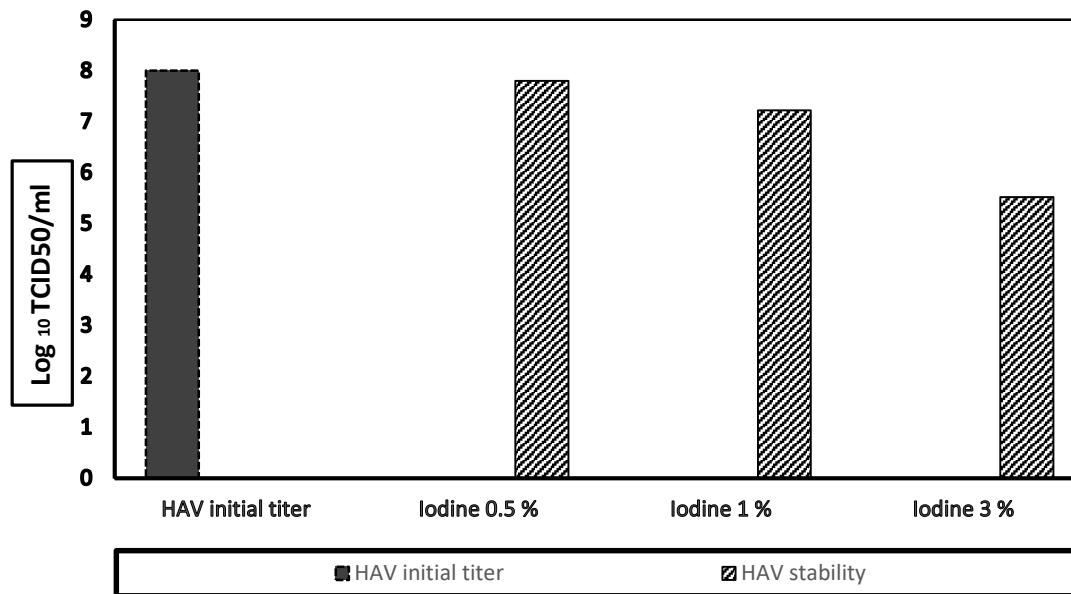


Figure 8. Stability of HAV in fresh produce items under different Iodine concentrations % for 1h of exposure at RT and pH7. Control titers for HAV at zero time = 8 log₁₀ TCID₅₀ / ml.



Discussion

A virus's ability to spread depends on both how it interacts with its host and how it interacts with the environment outside of the host [19]. The possibility for transmission of a virus increases with the length of time it can persist without a host. The potential for survival of viruses or the impact of measures intended to eradicate them will be influenced by a variety of environmental conditions and factors, including many physical and chemical factors that allow them to remain infectious or not [20]. HAV remains infectious after refrigeration and freezing, accordingly, frozen fruits can and have been intertwined in hepatitis A outbreaks. HAV is destroyed by conventional cuisine processes but retains infectivity after heating to 60 °C for 30 min [21]. Additionally, our findings are in line with several earlier investigations, hypothesis that low temperatures had little impact on HAV survival was confirmed by their findings, which revealed that the freezing of HAV-contaminated berries and sauces had no impact on HAV survival [22]. **Katz et al.** in 2007 found that HAV dropped by less than 1 log when stored at 4 °C or 28 °C for 24 hours, which is consistent with our findings [23]. In a different experiment, HAV was suspended in milk and incubated at 62.5 °C for 30 min [24], as well as numerous former studies showed that the hepatitis A virus is resistant to harsh environmental conditions of moderate heating, low pH, and desiccation [24].

It can retain its integrity and infectivity after low-temperature heating for a long time (i.e., 60 °C for 60 min, 56 °C for 10- 12 hours), [25]. Treatment at 60 °C for 30 min couldn't completely inactivate the hepatitis A virus in mussels [26]. Lower than a 4 log₁₀ Plaque Forming Units) PFU (reduction of hepatitis A virus passed in dairy products when exposed to 65 s°C for 30min [27]. Our results showed that HAV was able to tolerate the acidic condition and was mostly stable at low pH [28]. Our pattern of results is supported by a number of earlier investigations established that HAV can tolerate the low pH of berry fruits and there is no loss in infectivity if the virus is suspended in a buffer at pH 1, 2, or 3 for 30 min at 37 °C [29]. A previous study by **Scholz et al.** found that HAV was highly stable at low/acidic pH)1.0(at ambient and physiological temperatures) 24 °C and 38 °C ([29]. In contradiction, **Wei J et al.** reported that HAV degraded with adding pH conditions [30]. Also, the results attained in this study confirm and extend former findings on the stability of HAV and give further information about its inactivation by acidic and/ or alkaline conditions. Results of treatment of the strong infectivity of the HAV with high pH also supported our results, which showed that after treatment at pH10, the structure of HAV was altered [31]. While many enteric viruses may endure pH values as low as 3 or 4 and as high as 9 or 10, the ability of the infection to tolerate low pH conditions

for survival may allow the infection to stay inside the acidic environment of the stomach [32]. HAV was shown to stop being infectious at pH 1 after 90 minutes [1]. Foodborne pathogens must endure passage through the stomach's acid. When stored in meals like ketchup and salads, pH 4.5–5.5 showed minimal to no effect on the titers of the majority of enteric viruses [33]. Mechanistically speaking, for pH dependent enveloped viruses, it's known that strong acidity can spark conformational changes in face glycoproteins associated with membrane emulsion and virus entry rather of low pH operation. Although HAV isn't a pH dependent enveloped virus, as part of the infectious process, the HAV capsid must suffer low pH touched off conformational changes after entering the acidic endosome/ lysosome to release its RNA into the cytosol of the cell and initiate replication [34]. Likewise, our findings were analogous to former studies conducted with several different viruses including enteric viruses, which showed that low attention of 1 NaCl] didn't give any inactivation against enteric viruses, still, our results were in diversity with their findings at advanced attention [6 NaCl] whereas, a defensive effect attained against inactivation of HAV [35]. The reduced inactivation of HAV in the presence of NaCl is harmonious with results reported for Feline Calicivirus (FCV), [34]. Also, our results were firmly attached to data from a study by **Park et al**, they found that salted oysters containing 3% to 10% NaCl showed only marginal effects on HAV infectivity over 72 h of storage at 10 °C [36].

Regarding the use of disinfectants in the inactivation of HAV, our results indicated that ethanol at higher concentrations showed a reduction in the infectivity effect of the HAV. These findings did not show a complete similarity with a previous study conducted on HAV, which showed a significant resistance to treatment with 70% ethanol, according to **Abad et al**. [37]. Infectivity against HAV in suspension decreased by less than 0.5 log after one minute. However, in our opinion, shorter exposure time may be to blame for this. This disagreement was attributed by the researchers to variations in viral aggregation and particle association across various trials, as well as variations in the viral suspension medium. 1.85% less of HAV infectivity was observed after being exposed for 20 minutes to 70% ethanol. Alcohol-based hand sanitizers' capacity to render non-

enveloped viruses like HAV inactive may be limited [17].

In contrast to enveloped viruses, non-enveloped viruses, which include the norovirus, rotavirus, and human hepatitis A virus (HAV), are composed of a nucleocapsid without an envelope. Non-enveloped viruses are more resistant than enveloped viruses and are not inactivated by alcohol. However, several reports have suggested that alcohols at high concentrations reduce the viral titers of relatively large non-enveloped viruses such as rotavirus [38], adenovirus [39], rhinovirus [39], and hepatitis A virus (HAV) [40].

It has been demonstrated that phenolic chemicals have antiviral properties. In another study, it was found that a commercial formulation of phenolic compounds was found to be capable of inactivating up to 3 log infectious viruses when tested on HAV in suspension [41]. More recently, **Su X et al**. found that after 1 minute of contact, phenolic chemicals inactivate 1log HAV on lettuce and jalapeno peppers [42]. On the contrary, other previous studies reported that phenolic compound, has been shown to inactivate enveloped viruses such as influenza but not non-enveloped viruses such as bovine rotavirus and fowl adenovirus [43]. The findings suggest that the mechanism behind the antiviral effect of phenolic compounds may require the presence of a viral envelope [43].

Chlorine is the most commonly used sanitizing agent [44]. Enteric viruses including HAV display a wide range of susceptibilities to chlorine inactivation. There is agreement with our findings by **Hirneisen et al**. found that internalized HAV was more resistant to chlorination than HAV that was on the surface of the green onions (0.4 log internalized virus versus 2.6 log surface virus inactivation), [45].

Research on the mechanisms of chlorine inactivation of enteric viruses has been inadequate. **Li et al**. [46] they discovered that a part of the 5' non-translated regions (5'NTR) could not be detected after exposure, indicating that the effect of chlorine was largely exerted on the genome. The fact that other regions of the genome could be amplified suggests that chlorine had a specific impact on the 5'NTR. It also suggests that chlorine was able to enter the virus capsid while still keeping it intact and able to shield the remainder of the genome. According to **Nuanualsuwan et al**. [47] chlorine appears to inactivate HAV principally

through causing damage to the area of the capsid that interacts with cellular receptors that control infectivity. To more precisely locate the specific sites of chlorine action on HAV nucleic acid plays an important role in the inactivation of virus infectivity using chlorine [48].

Regarding iodine, our results were in agreement with **Baert et al.** (2008) assessed various disinfectants including a 1 % iodine-based disinfectant on Murine Norovirus 1 (MNV-1) surrogate for enteric viruses particularly, norovirus produced >4 log₁₀ reduction in infectious viral titer but viral RNA was detected after iodine treatment. As well as iodine was ineffectual against FCV **Baert et al.** [49].

It is for the most part acknowledged that an enveloped virus is less steady than a non-enveloped virus since the lipid envelope is vulnerable to heat, pH changes, disinfectants, and numerous other stresses. In any case, a few reports have proposed that alcohols at high concentrations diminish the viral titers of non-enveloped viruses such as rotavirus [38]. In the long run, HAV could be a highly steady virus and can continue within the right environment for extended periods, and thus too in foodstuffs [50]. Typically, most likely since of the extraordinary alcohol utilization, which interact with the competition of the virus, and react with the atomic structure of HAV capsid [51]. According to **Costafreda et al.** HAV strains with different capsids exhibit stark differences in how resistant they are to high temperatures and acidic pH levels [52].

In light of this study, we recommend that, not only room temperature together with other most right conditions be used for good survive environment to contamination with HAV. This pathogenic virus remains infectious after harsh conditions like acidic and low salted medium. As well as, at low treatments using most popular disinfectants, therefore, refrigeration and freezing; consequently, frozen fruits, acidic fruit and vegetable products can and have been implicated in this virus' outbreaks.

Funding

Self-funded.

Conflict of interest

There is no conflict of interest.

References

- 1-**Baert L, Debevere J, Uyttendaele M.** The efficacy of preservation methods to inactivate foodborne viruses. *Int. J Food Microbiol* 2009;131, 83–94.
- 2-**Kim SJ, Si J, Lee JE, Ko G.** Temperature and humidity influence the inactivation kinetics of enteric viruses on surfaces. *Environ Sci Technol* 2012; 46:13303–13310.
- 3-**Bernstein DI.** Rotavirus overview. *Pediatr. Infect. Dis. J.* 28:S50-3. Desselberger U 2014. Rotaviruses. *Virus Res* 2009; 190: 75-96.
- 4-**Cliver DO.** Virus transmission via food. *Food Technol.-Chicago.* 1997; 51:71-78.
- 5-**Ciocca M.** Clinical course and consequences of hepatitis A infection. *Vaccine* 2000;18:S71-S74.
- 6-**Pintó R, Bosch A, Kaplan G.** Hepatitis A: immune response and virus evolution. In: *Liver Immunology*, Gershwin ME, Vierling JM & Manns MP, eds, Springer International Publishing. Pp 2014 :173-189.
- 7-**Hollinger FB, Emerson SU.** 'Hepatitis A virus', in **Fields B N, Howley P M, Griffin D E eds**, *Fields Virology* 4th ed, New York, Lippincott Williams & Wilkins 2001:799-840.
- 8-**Issa IA, Mourad FH.** Hepatitis A: an updated overview. *J Med Liban* 2001;49: 61-65.
- 9-**Lodder WJ, de Roda, Husman AM.** . Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands. *Appl Environ Microbiol* 2005; 71: 1453–1461.
- 10-**USEPA Manual of methods for virology.** In: EPA/600/4- 84/013 USEPA, Cincinnati 2001; pp 6–62.
- 11-**Hamza IA, Jurzik L, Stang A, Sure K, Uberla K, Wilhelm M.** Detection of human viruses in rivers of a densely-populated area in Germany using a virus adsorption elution

- method optimized for PCR analyses. *Water Res* 2009;43:2657–2668
- 12-Katzenelson E, Fattal B, Hostovesky T.** Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. *Appl. Environ. Microbiol.* 1976;32:638–639.
- 13-Reed LJ, Muench H.** A simple method of estimating fifty percent endpoints. *Amer. J. Hyg* 1938;27, 493–497.
- 14-Bertrand I, Schijven JF, Sánchez G, Wyn-Jones P, Ottoson J, Morin T, et al.** The impact of temperature on the inactivation of enteric viruses in food and water: a review. *Journal of Applied Microbiology* 2012: 112: 1059-1074.
- 15-Kingsley DH, Chen H.** Influence of pH, salt, and temperature on pressure inactivation of hepatitis A virus. *Int J Food Microbiol* 2009;130:61–4.
- 16-Salo R, Cliver D.** Effect of acid pH, salts, and temperature on the infectivity and physical integrity of enteroviruses. *Arch. Virol.* 1976: 52:269–282.
- 17-Kramer A, Galabov AS, Sattar SA, Doehner L, Pivert A, Payan C, et al.** Virucidal activity of a new hand disinfectant with reduced ethanol content: comparison with other alcohol-based formulations. *Journal of Hospital Infection* 2006: 62: 98-106.
- 18-Fraisse A, Temmam S, Deboosere N, Guillier L, Delobel A, Maris P, et al.** Comparison of chlorine and peroxyacetic-based disinfectant to inactivate Feline calicivirus, Murine norovirus, and Hepatitis A virus on lettuce. *International Journal of Food Microbiology* 2011;151: 98-104.
- 19-Lopman BA, Reacher MA, van Duynhoven Y, Hanon FX, Brown D.** and Koopmans, M. Viral gastroenteritis outbreaks in Europe. *Emerg. Inf. Dis* 2003: 9, 90–96.
- 20-Nuanualsuwan S, Cliver DO.** Inactivation of picornaviruses and caliciviruses; Part 2: Inactivation. *Thai Journal of Veterinary Medicine* 2003;33: 19-33.
- 21-Kim SJ, Si J, Lee JE, Ko G.** Temperature and humidity influence the inactivation kinetics of enteric viruses on surfaces. *Environ Sci Technol* 2012;46:13303–13310.
- 22-Butot S, Putallaz T, Sanchez G.** Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology* 2008;126[1-2]:30–35
- 23-Katz BD, Margolin AB.** Inactivation of hepatitis A HM-175/18f, reovirus T1 Lang and MS2 during alkaline stabilization of human biosolids. *Journal of Applied Microbiology* 2007: 103, 2225–2233.
- 24-Croci LD, De Medici C, Scalfaro A, Fiore L Toti.** The survival of hepatitis A virus in fresh produce. *Int. J. Food Microbiol* 2002;73:29-34.
- 25-Goyal SM.** Viruses in foods. In M.P. Doyle [ed.], *Food microbiology and food safety series*. Springer Science & Business Media, New York, NY 2006.
- 26-Croci LM, Ciccozzi D, De Medici S, Di Pasquale A, Fiore A, Mele L, Toti.** Inactivation of hepatitis A virus in heat-treated mussels. *J. Appl. Microbiol.* 1999: 87:884-888.
- 27-Bidawid SJM, Farber SA, Sattar S, Hayward.** Heat inactivation of hepatitis A virus in dairy foods. *J. Food Prot* 2000: 63:522-528.
- 28-Provost K, Dancho B, Ozbay G, Anderson R, Richards G, Kingsley D.** Hemocytes are sites of enteric virus persistence within oysters.

- Applied and Environmental Microbiology 2011; 77: 8360-8369.
- 29-Scholz E, Heinricy U. and Flehmig B** Acid Stability of Hepatitis A Virus. *J. gen. Virol* 1989;70, 2481-2485.
- 30-Wei J, Jin Y, Sims T, Kniel KE.** Survival of murine norovirus and hepatitis A virus in different types of manure and biosolids. *Foodborne Pathogens and Disease* 2010; 7: 901-906.
- 31-Harakeh M, Butler M.** Inactivation of human rotavirus, SA11, and other enteric viruses in effluent by disinfectants. *J. Hyg* 1984; 93:157-163.
- 32-FAO/WHO [Food and Agriculture Organization of the United Nations/World Health Organization].** Microbiological hazards in fresh leafy vegetables and herbs: Meeting Report. 2008:Microbiological Risk Assessment Series No. 14. Rome. 151pp.
- 33-Mormann S, Dabisch M, Becker B.** Effects of technological processes on the tenacity and inactivation of norovirusgenogroup II in experimentally contaminated foods. *Appl Environ Microbiol* 2010;76,536-545.
- 34-Kingsley DH, Chen HQ.** Aqueous matrix compositions and pH influence feline calicivirus inactivation by high-pressure processing. *Journal of Food Protection* 2008; 71, 1598–1603.
- 35-Kingsley D, Chen H.** Influence of pH, salt, and temperature on pressure inactivation of hepatitis A virus. *Int. J. Food Microbiol* 2009;130:61–64.
- 36-Park SY, Ha SD.** Ultraviolet-C Radiation on the Fresh Chicken Breast: Inactivation of Major Foodborne Viruses and Changes in Physicochemical and Sensory Qualities of Product 2014; 8:895–906.
- 37-Abad FX, Pinto RM & Bosch A** Disinfection of human enteric viruses on fomites. *FEMS Microbiology Letters* 1997; 156: 107-111.
- 38-Ansari, S. A.; Springthorpe, V. S.; Sattar, S. A.; Tostowaryk, W.; Wells, G. A.** Comparison of cloth, paper, and warm air drying in eliminating viruses and bacteria from washed hands. *Am. J. Infect. Control* 1991; 19, 243-249.
- 39-Sattar, S. A.; Abebe, M.; Bueti, A. J.; Jampani, H.; Newman, J.; Hua, S.** Activity of an alcohol-based hand gel against human adeno-, rhino-, and rotaviruses using the fingerpad method. *Infect. Control Hosp. Epidemiol* 2000; 21, 516-519.
- 40-Wolff, M. H.; Schmitt, J.; Rahaus, M.; Konig, A.** Hepatitis A virus: a test method for virucidal activity. *J. Hosp. Infect.*, 48 Suppl A 2001: S18-22.
- 41-Su X & D'Souza DH.** Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology* 2011; 77: 3982-3987.
- 42-Su X & D'Souza DH** Grape seed extract for foodborne virus reduction on produce. *Food Microbiology* 2013; 34: 1-6.
- 43-Yamada K, Ogawa H, Hara A, Yoshida Y, Yonezawa Y, Karibe K, et al.** The mechanism of the antiviral effect of hydroxytyrosol on the influenza virus appears to involve a morphological change of the virus. *Antiviral Research* 2009; 83, 35-44.
- 44-Fonseca JM.** 2006. Postharvest handling and processing: sources of microorganisms and Grabow, W.O.K., P. Coubrough, C. Hilner and B.W. Bateman Inactivation of hepatitis A virus, other enteric viruses and indicator organisms in water by chlorine, *Wat. Sci. Tech.*, 17, Amsterdam, 1984: 657-664.

- 45-Hirneisen KA, Kniel KE.** Inactivation of internalized and surface contaminated enteric viruses in green onions. *International Journal of Food Microbiology* 2013;166: 201–206.
- 46-Li JW, Xin ZT, Wang XW, Zheng JL, Chao FH.** Mechanisms of inactivation of hepatitis A virus by chlorine. *Applied and Environmental Microbiology* 2002;68: 4951-4955.
- 47-Nuanualsuwan S, Cliver DO.** Capsid functions of inactivated human picornaviruses and feline calicivirus. *Applied and Environmental Microbiology* 2003; 69: 350-357.
- 48-Jia XYM, Tesar DF, Summers, Ehrenfeld E.** Replication of hepatitis A virus with chimeric 5' nontranslated regions. *J. Virol.* 1996;70:2861–2868.
- 49-Baert L, Uyttendaele M, Debevere J.** Evaluation of viral extraction methods on a broad range of Ready-To-Eat foods with conventional and real-time RT-PCR for Norovirus GII detection. *International Journal of Food Microbiology* 2008; 123(1–2), 101–108.
- 50-Sanchez G.** Hepatitis A virus in food: detection and Inactivation methods. In *Springerbriefs in Food, health, and Nutrition* 2013.
- 51-Pintó RM, Aragonés L, Costafreda MI, Ribes E, Bosch A.** Codon usage and replicative strategies of hepatitis A virus. *Virus Res* 2007; 127[2]:158–63.
- 52-Costafreda MI, Perez-Rodriguez FJ, D'Andrea L, Guix S, Ribes E, Bosch A, et al.** Hepatitis A virus adaptation to cellular shutoff is driven by dynamic adjustments of codon usage and results in the selection of populations with altered capsids. *J Virol* 2014; 88:5029–41.