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Short communication

Survival of hepatitis A virus and Aichi virus in cranberry-based juices at refrigeration (4 °C)

Snigdha Sewlikar, Doris H. D'Souza*

The University of Tennessee-Knoxville, Department of Food Science and Technology, 2600 River Drive, Knoxville, TN 37966, USA

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ABSTRACT

Viral foodborne illness continues to be a health-concern globally, with numerous fruit and juice outbreaks of Hepatitis A virus (HAV) reported worldwide. Aichi virus (AiV) is an emerging pathogen with limited epidemiological data. Both, HAV and AiV are resistant to low pH and can survive under adverse environmental conditions leading to transmission ease. The objective of this study was to evaluate the survival of HAV and AiV in commercially-available cranberry-based juices (Cranberry juice cocktail, CJ and a 100% juice with cranberry, MJ) over 21 days at refrigeration (4 °C). Equal volumes of juice was mixed with each virus individually (final titer of 6 log PFU/mL) and stored at refrigeration over 21 days. At each time interval, the inoculated juices were serially diluted in cell culture media and infectious virus survival was determined by standard plaque assays. Each experiment was carried out in duplicate and replicated thrice. Reductions of 0.72 ± 0.06 (after day 1) to 2.3 ± 0.18 log PFU/mL (after day 21) and 0.63 ± 0.02 (after day 1) to 1.84 ± 0.14 log PFU/mL (after day 21) were obtained for AiV with MJ and CJ, respectively. Reductions ranging from 0.67 ± 0.03 (after day 1) to 1.09 ± 0.1 log PFU/mL (after day 21) and 0.93 ± 0.27 (after day1) to 1.49 ± 0.18 log PFU/mL (after day 21) were obtained for HAV at refrigeration in MJ and CJ, respectively. HAV showed greater survival than AiV in these juices over refrigerated storage. These results provide survival data of HAV and AiV in cranberry-based juices that can be used in risk-modeling and risk assessment studies.

1. Introduction

Foodborne illnesses are a major threat to human health around the globe with 9.4 million cases of foodborne illness occurring every year in the United States, 59% of which are caused by viruses (Scallan et al., 2011). Human noroviruses, hepatitis A virus (HAV), hepatitis E virus, Aichi virus (AiV), sapoviruses, rotaviruses, parvoviruses, astroviruses, other small round viruses and human enteroviruses including polioviruses, echoviruses, and coxsackieviruses play major roles in foodborne and waterborne viral infections (Sair et al., 2002; D'Souza et al., 2007). Scallan et al. (2011) reported that 27% of the ~55,961 estimated hospitalizations and 12% of the 2612 deaths due to foodborne illnesses caused annually in the United States are due to viruses.

HAV is considered an epidemiologically significant virus, due to severity of the disease with typical symptoms that include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine and jaundice, that typically lasts for no more than 2 months, but some (~10%–15%) patients may demonstrate prolonged or relapsing symptoms for up to 6 months (CDC, 2014; Collier et al., 2014; Gillesberg Lassen et al., 2013). HAV is a single-stranded RNA virus of the *Picornaviridae* family and is stable in the gastrointestinal tract after it is excreted from the liver of an infected person into the bile (Lemon, 1997). HAV is very sturdy/resilient to environmental stress, can persist for long durations in fresh and salt water and soil, and temperatures higher than 85 °C are required for inactivation (Sattar et al., 2010; Sobsey et al., 1988; Mbithi et al., 1992). In fact, D-values (time at a given temperature for the reduction of 90% of the target population) for HAV in buffered cell culture medium were reported to be 56.22, 8.40, 2.67, 1.73, and 0.88 min at 50 °C, 56 °C, 60 °C, 65 °C, and 72 °C, respectively (Bozkurt et al., 2014a). When tested in blue mussel homogenate, D-values (min) for HAV were observed to be 54.17, 9.32, 3.25, 2.16, and 1.07 min at 50 °C, 56 °C, 60 °C, 65 °C, and 72 °C, respectively (Bozkurt et al., 2014b). HAV also exhibits resistance to various inactivation methods that include use of detergents, freezing and low pH (pH 2) (Brundage and Fitzpatrick, 2006; Grove et al., 2006). Orange juice contaminated by HAV

* Corresponding author.
E-mail address: ddsouza@utk.edu (D.H. D'Souza).

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during the manufacturing process, was identified as the cause for an outbreak among 351 tourists from nine European countries visiting Egypt (Frank et al., 2007). HAV outbreaks with fruits including frozen berries used in smoothies (Gillesberg Lassen et al., 2013), have also been reported. A multistate (affecting 10 states) outbreak of HAV (first in ~10 years) traced back to the consumption of frozen pomegranate arils (imported from Turkey) occurred in the United States in May 2013 and resulted in 165 illnesses, 69 hospitalizations, 2 cases of fulminant hepatitis and 1 case in which liver transplant was needed (Collier et al., 2014). While a 90% decrease in HAV cases (mostly attributed to vaccination of children and people at risk for HAV) has been observed in the US over the last 20 years, there are still an estimated 2700 new cases of HAV illness per year (CDC, 2014).

AiV is an emerging foodborne virus of the *Kobuvirus* genus in the *Picornaviridae* family (Reuter et al., 2011) that causes gastroenteritis (symptoms include diarrhea, nausea, vomiting, abdominal pain and fever) in humans (Yamashita et al., 1991, 2001). Similar to HAV, AiV is also a single-stranded positive sense RNA virus, spherical (~30 nm in diameter) in shape and non-enveloped (Yamashita et al., 1991, 2003; Reuter et al., 2009a; Drexler et al., 2011). Shellfish contaminated with water from sewage have been identified as the major source of AiV infections (Yamashita et al., 2000; Ambert-Balay et al., 2008; Le Guyader et al., 2008). AiV, *in vitro*, exhibits stability in acidic conditions as low as pH 2 and is resistant to conventional methods of inactivation including heat, alcohols, chlorine, high hydrostatic pressure, chloroform, ether and non-ionic detergents (Yamashita et al., 1998; Cromeans et al., 2014).

AiV was identified as one of the causative agents in an outbreak of gastroenteritis (205 cases) in France associated with consumption of oysters from a flooded shellfish production lagoon (Le Guyader et al., 2008). Between 1987 and 1998, AiV was detected in 20% of the 268 fecal samples obtained from patients suffering from gastroenteritis due to consumption of oysters in Japan (Yamashita et al., 2000). AiV has shown to be prevalent globally as it has been detected in human stool samples across Asia (Japan, Thailand, Bangladesh, Vietnam, and China), Europe (Germany, France, Hungary, and Finland), South America (Brazil), and Africa (Tunisia) (Yamashita et al., 1991, 1993; Oh et al., 2006; Pham et al., 2007.; Ambert-Balay et al., 2008; Sdiri-Loulizi et al., 2008; Reuter et al., 2009b; Yang et al., 2009; Kaikkonen et al., 2010). However, information on the epidemiology for AiV in the United States is scarce.

Given the fecal-oral route of transmission for these viruses, illness can occur due to the contamination of foods and beverages caused by improper handling. It is hence essential to study the survival of foodborne viruses in fruit juices to understand food safety risk. Juices are generally stored at refrigeration and hence evaluation of the survival of these viruses under refrigeration over time is important. Cranberry juice (CJ) and proanthocyanidins have been reported to exhibit antiviral activity against the influenza virus (Weiss et al., 2005), simian rotavirus SA-11, bacteriophages T4 and T2, bovine reovirus, (Lipson et al., 2007) and human norovirus surrogates, namely murine norovirus (MNV-1), feline calicivirus (FCV-F9), bacteriophage MS2 and bacteriophage φ-X174 (Su et al., 2010a).

When the survival of MNV-1 at 5 log PFU/ml in pomegranate juice and juice blends at refrigeration was studied, moderate reduction (1.4 log) in pomegranate juice and reduction to non-detectable levels in an orange and pomegranate juice blend after 7 days was observed (Horm and D'Souza, 2011). These researchers also reported that FCV-F9 at 6 log PFU/ml was completely reduced after 14 days in pomegranate juice and after 1 day in the orange and pomegranate juice blend at 4 °C, while MS2 was reduced by <1 log in pomegranate juice or juice blends after 21 days at 4 °C. Horm et al. (2012) also found that FCV-F9 at ~5 log PFU/mL and MS2 at ~6 log PFU/mL were undetectable after 1 and 7 days, respectively, while MNV-1 at~4 log PFU/ml was reduced by only 1.95 log PFU/mL after 21 days in blueberry juice at 4 °C.

However, the survival of HAV and AiV in CJ at refrigeration temperature needs to be determined. Cranberry juice blends can be a good model matrix to understand if the synergistic effect of a combination of fruit juices (including cranberry, grape, apple and pear) can enhance viral reduction over time. Hence, the objective of this study was to determine the survival of HAV and AiV at refrigeration temperatures over 21 days in cranberry-based juices. This would mimic household conditions of consumption and storage and in addition, provide data for input in risk-modeling and risk assessment studies to understand the risk associated with the consumption of contaminated juices or from cross-contaminated juices.

2. Materials and methods

2.1. Viruses and cell lines

HAV (strain HM175) and fetal rhesus monkey kidney (FRhK4) cells were obtained from our collaborator, Dr. Kalmia Kniel (University of Delaware). Aichi Virus (AiV) was kindly provided by Dr. David Kingsley (USDA ARS, Delaware) for use with the Vero host cells.

2.2. Propagation of viruses

FRhK4 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM-F12; HyClone Laboratories, Logan, UT) supplemented with 10% heat inactivated fetal bovine serum (FBS; HyClone Laboratories, Logan, UT) and 1% Anti-Anti (Antibiotic-Antimycotic, Invitrogen, Grand Island, NY), while Vero cells were grown in DMEM-F12 with 2% FBS and 1% Anti-Anti and incubated in an atmosphere containing 5% CO_2 at 37 °C in 175 cm^2 flasks as described earlier (Su et al., 2010a; Fino and Kneil, 2008). Viral stocks for the study were made by inoculating HAV or AiV stocks (300 infectious particles/cell) onto monolayers of host FRhK4 or Vero cells, respectively and incubated under 5% CO_2 until >90% cell lysis was observed (7 days for HAV and 3 days for AiV). The viral suspensions obtained were freeze-thawed thrice and centrifuged for 10 min at 5000*g*. The supernatants were then filtered through 0.2 μm membrane filters, aliquoted and stored at −80 °C until use as described earlier (Su et al., 2010a).

2.3. Infectious plaque assays

Standard protocols were followed for HAV and AiV plaque assays as described earlier (Su and D'Souza, 2011; Fino and Kniel, 2008). Briefly, FRhK4 or Vero cells in 6-well multidish plates (Biolite, ThermoFisher Scientific, Rochester, NY,USA) were infected with 0.5 ml of serially diluted virus in juice or juice blends or control samples of HAV or AiV in phosphate buffered saline (PBS, pH 7.2), respectively and incubated for 2–3 h to allow for infection. Viruses were then aspirated and the cells were overlaid with 2 ml complete DMEM containing 0.75% agarose for AiV, (Noble Agar, Difco BD, Sparks, MD, USA) or 1% agarose (for HAV) and incubated at 37 °C under 5% CO_2. After an incubation period of three days for AiV and eight days for HAV, the cell monolayers were stained using neutral red and further incubated at 37 °C until plaques were visible and then enumerated.