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A comparison of the thermal inactivation kinetics of human norovirus surrogates and hepatitis A virus in buffered cell culture medium

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ABSTRACT

Human noroviruses and hepatitis A virus (HAV) are considered as epidemiologically significant causes of foodborne disease. Therefore, studies are needed to bridge existing data gaps and determine appropriate parameters for thermal inactivation of human noroviruses and HAV. The objectives of this research were to compare the thermal inactivation kinetics of human norovirus surrogates (murine norovirus (MNV-1), and feline calicivirus (FCV-F9)) and HAV in buffered medium (2-ml vials), compare first-order and Weibull models to describe the data, calculate Arrhenius activation energy for each model, and evaluate model efficiency using selected statistical criteria. The *D*-values calculated from the first-order model (50–72 °C) ranged from 0.21–19.75 min for FCV-F9, 0.25–36.28 min for MNV-1, and 0.88–56.22 min for HAV. Using the Weibull model, the $t_{D=1}$ (time to destroy 1 log) for FCV-F9, MNV-1 and HAV at the same temperatures ranged from 0.10–13.27, 0.09–26.78, and 1.03–39.91 min, respectively. The *z*-values for FCV-F9, MNV-1, and HAV were 9.66 °C, 9.16 °C, and 14.50 °C, respectively, using the Weibull model. For the first order model, *z*-values were 9.36 °C, 9.32 °C, and 12.49 °C for FCV-F9, MNV-1, and HAV, respectively. For the Weibull model, estimated activation energies for FCV-F9, MNV-1, and HAV were 225, 278, and 182 kJ/mol, respectively, while the calculated activation energies for the first order model were 195, 202, and 171 kJ/mol, respectively. Knowledge of the thermal inactivation kinetics of norovirus surrogates and HAV will allow the development of processes that produce safer food products and improve consumer safety.

1. Introduction

In recent years, viruses have been increasingly recognized as important causes of foodborne disease. In particular, human noroviruses and hepatitis A virus (HAV) are the most important human foodborne viral pathogens with regard to the number of outbreaks and people affected. Scallan et al. (2011) reported that an estimated 80–90% of all non-bacterial outbreaks of gastroenteritis reported each year are due to human noroviruses and HAV. These viruses are generally environmentally stable, survive adverse conditions and are resistant to extreme pH conditions and enzymes of the gastrointestinal tract (D'Souza et al., 2007; D'Souza et al., 2006). They have low infectious doses; as few as 10 infectious particles can cause illness (CDC, 2012; Teunis et al., 2008). Even though viruses, unlike bacteria, cannot grow in or on foods, foodborne illnesses result via contamination of the fresh produce or processed food by fecal material containing viruses (Atreya et al., 2004). Thus, proper inactivation of foodborne enteric viruses in foods prior to consumption is essential to protect public health.

Despite its importance in public health, there is little information on the thermal inactivation characteristics of human noroviruses because these viruses are currently non-culturable in the laboratory and their infectivity can only be assessed using human dose experiments (i.e., feeding studies). Cultivable surrogates, such as murine norovirus (MNV-1) and feline calicivirus (FCV-F9), have been used as human norovirus surrogates in inactivation studies based on the assumption that they can mimic characteristics of human noroviruses (Hewitt and Greening, 2004; Richards, 2012). For HAV, there are a few strains (HM-175, HAS-15, MBB 11/5) that are cell-culture adaptable and can be maintained using fetal rhesus monkey kidney (FRhK-4) and/or human fetal lung fibroblast (MRC-5) cells. These strains have been used for inactivation studies (Martin and Lemon, 2006; Reiner et al., 1992).

Thermal processing is still one of the most effective methods for inactivating microorganisms (Silva and Gibbs, 2012). Heat is used to inactivate pathogens to produce safer foods with longer shelf life (Lee and Kaletunc, 2002). In the current literature, limited studies

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have been performed to investigate thermal inactivation of MNV-1 (Cannon et al., 2006; Hewitt et al., 2009; Gibson and Schwab, 2011; Bozkurt et al., 2013), FCV-F9 (Duizer et al., 2004; Cannon et al., 2006; Bozkurt et al., 2013), and HAV (Croci et al., 1999; Hewitt and Greening, 2004) in buffered cell culture media (Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and supplemented with antibiotics). Among these studies, only one was related to the determination of the thermal inactivation kinetics of human norovirus surrogates in capillary tubes (Bozkurt et al., 2013), and HAV was not considered. To our knowledge, there are no studies established to compare the thermal inactivation kinetics of human norovirus surrogates and HAV. To characterize the effect of heat treatment on inactivation behavior, mathematical modeling has been used to predict the number of survivors during thermal processing and to give detailed information about inactivation kinetics during treatments. Choice of the most appropriate model is crucial to gather correct information about thermal inactivation kinetic behavior. Recent studies conducted on thermal inactivation of human norovirus surrogates (Bozkurt et al., 2013, 2014a, 2014b; Seo et al., 2012; Tuladhar et al., 2012) revealed that the Weibull model was statistically superior in describing the thermal inactivation kinetics of human norovirus surrogates than the first-order model. A precise understanding of thermal inactivation kinetics is potentially useful for optimizing thermal treatments to eliminate the risk associated with foodborne pathogens while avoiding over-processing of the food material and thus optimal energy utilization. Therefore, generation of correct thermal process data and establishment of proper thermal processes for inactivating human norovirus surrogates and HAV are important both for consumers and industry. Therefore, the purpose of this study was (i) to characterize and compare the thermal inactivation behavior of human norovirus surrogates and HAV in buffered cell culture medium in 2 ml vials, (ii) to compare first-order, and Weibull models in describing the data in terms of selected statistical parameters, and (iii) to calculate *z*-values and activation energy for each model.

2. Material and methods

2.1. Viruses and cell lines

Murine norovirus (MNV-1) was obtained from Dr. Skip Virgin (Washington Univ., St. Louis, MO) and its host RAW 264.7 cells were obtained from the University of Tennessee, Knoxville. Feline calicivirus (FCV-F9) and its host cells (Crandell Reese Feline Kidney, (CRFK)) were obtained from ATCC (Manassas, VA). Hepatitis A virus (HAV, strain HM175) and fetal monkey kidney (FRhK4) cells were kindly provided by Dr. Kalmia Kniel (University of Delaware).

CRFK, RAW 264.7, and FRhK4 cells were maintained in Dulbecco's modified Eagle's medium/Ham's F-12 (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) at 37 °C in an atmosphere containing 5% CO_2.

2.2. Propagation of viruses

CRFK, RAW 264.7, and FRhK4 cells with ~90% confluence in cell culture flasks were washed with phosphate-buffered saline (PBS; pH 7.4) twice before adding FCV-F9, MNV-1, and HAV stocks to their respective cell monolayers. The infected cells were then incubated until >90% cell lysis in a water jacketed CO_2 incubator at 37 °C. All three viruses were recovered by centrifugation at 5000 × g for 10 min, followed by filtration through 0.2-μm filters, aliquoted, and stored at −80 °C until use as described before (Su et al., 2010).

2.3. Thermal treatment

Heat treatment was carried out in a circulating water bath (Haake model V26, Karlsruhe, Germany) in 2 ml screw-capped vials. Sterilized (121 °C, 15 min) vials were carefully filled with 2 ml buffered cell culture medium (Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic–antimycotic) containing virus by using sterile pipettes in a biosafety cabinet. The filled vials were surface rinsed in 70% ethanol before immersion in a thermostatically controlled water bath. Water bath temperature was confirmed with a mercury-in-glass (MIG) thermometer (Fisher Scientific, Pittsburgh, PA) and by placing type-T thermocouples (Omega Engineering, Inc., Stamford, CT) in the geometric center of the water-bath. Another thermocouple probe was placed at the geometric center of a vial through the lid to monitor the temperature of the buffered media. Thermocouples were connected to MMS3000-T6V4 type portable data recorder (Commtest Inc., New Zealand) to monitor temperature. Samples were heated at 50, 56, 60, 65, and 72 °C for varying treatment times (0–60 min). The treatment time began (and was recorded) when the target internal temperature reached the designated temperature as described earlier (Bozkurt et al., 2013, 2014a, 2014b). Triplicate tubes were used for each temperature and time-point. After the thermal treatment, sample vials were immediately cooled in an ice water bath for 15 min to stop further thermal inactivation. The contents were transferred into a tube which contained 1.5 ml maintenance medium (Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, and 1% antibiotic–antimycotic) using a micro pipette. Un-heated virus suspensions were enumerated as controls.

2.4. Enumeration of survivors by infectious plaque assays

Thermally treated and control viral suspensions were diluted 1:10 in Dulbecco's Modified Eagle Medium (DMEM-F12) containing fetal bovine serum (10% for MNV-1 and 2% for FCV-F9 and HAV) and 1% antibiotic–antimycotic. Infectivity of each treated virus was evaluated using standardized plaque assays in comparison to untreated virus controls following the previously described procedures (Su et al., 2010). Viral survivors were enumerated as plaque forming units/ml (PFU/ml).

2.5. Modeling of inactivation kinetics

2.5.1. First-order kinetics

The traditional approach to describe the change in number of survivors over time for first-order kinetic model can be written as follows:

$$\log_{10}\frac{N_{(t)}}{N_0} = -\frac{t}{D} \quad (1)$$

where $N_{(t)}$ is the number of survivors after an exposure time (t) in PFU/ml and the initial population is N_0 (PFU/ml). D is the decimal reduction time in min (time required to kill 90% of viruses) and t is the treatment time (min).

The relationship between reaction constant (k) and the D value for the first order model can be expressed in the following equation:

$$D = \frac{\ln(10)}{k} = \frac{2.303}{k} \quad (2)$$