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Inactivation of hepatitis A virus and norovirus surrogate in suspension and on food-contact surfaces using pulsed UV light (pulsed light inactivation of food-borne viruses)

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ABSTRACT

This study was conducted to evaluate the inactivation of murine norovirus (MNV-1) and hepatitis A virus (HAV) by pulsed ultraviolet (UV) light. MNV-1 was used as a model for human norovirus. Viral suspensions of about 10^6 PFU/ml were exposed to pulses of UV light for different times and at different distances in a Xenon Steripulse device (model RS-3000C). Inactivation studies were also carried out on 1-cm^2 stainless steel and polyvinyl chloride disks with 10^5 PFU/ml. Inactivation of MNV-1 and HAV at 10.5 cm from the UV source was greater on inert surfaces than in suspension. The presence of organic matter (fetal bovine serum) reduced the effectiveness of pulsed light both in suspension and on surfaces. However, 2-s treatment in the absence of FBS completely inactivated (5 log reduction) the viral load at different distances tested, whether in suspension (MNV-1) or on disks (MNV-1 and HAV). The same treatment in the presence of fetal bovine serum (5%) allowed a reduction of about 3 log. This study showed that short duration pulses represent an excellent alternative for inactivation of food-borne viruses. This technology could be used to inactivate viruses in drinking water or on food-handling surfaces.

1. Introduction

Several groups of viruses may be transmitted to humans through contaminated food, the environment, water, or person-to-person contact (Richards, 2001). Among these, norovirus (NoV) is currently recognized as the most common human food-borne pathogen in terms of the number of outbreaks and persons affected (Mead et al., 1999; Moore et al., 2004). According to epidemiological estimates in the USA, NoV accounted for over 60% of cases, 33% of hospitalizations and 7% of deaths among all diseases attributable to food-borne pathogens (Mead et al., 1999). The study and detection of NoV are based on molecular methods and culturable surrogates. Until 2004, feline calicivirus (FCV) was considered the most suitable model for NoV studies. However, FCV is a respiratory virus with low tolerance to acidic pH, in contrast with enteric viruses (Doultree et al., 1999). Murine norovirus (MNV-1) has therefore emerged as a more suitable model for NoV studies due to its more similar characteristics such as genetic organization (Wobus et al., 2006) and transmission by the fecal-oral route (Wobus et al., 2004).

Together with NoV, hepatitis A virus (HAV) infections constitute one of the leading causes of food-borne disease outbreaks, particularly in developing countries (Halliday et al., 1991; Koopmans and Duizer, 2004; Mead et al., 1999). The availability of vaccine and improvements in sanitation and living conditions have contributed to a significant decrease in the incidence of HAV cases in developed countries and among populations of high socio-economic status in developing countries (Normann et al., 2008). However, international travellers are still under at risk of infection due to the length of stay, living conditions, and the incidence of HAV infection in the region visited (Keystone and Hershey, 2008). In addition, the Centers for Disease Control and Prevention (CDC) recorded 5683 cases of HAV infection including 14 hepatitis A-related deaths in the USA in 2004 (CDC, 2006), many of which were believed due to food consumption behaviours. Ready-to-eat products that have been in contact with contaminated surfaces are among the high-risk products. The prevalence of viruses on different food surfaces has been linked to their high stability in the environment. In a food preparation setting, surfaces can be contaminated by food handlers with poor personal hygiene, which can lead to the transfer of the virus to various food products (D'Souza et al., 2006).

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In terms of food safety, different food preservation processes such as heating, high-pressure processing, dehydration, freezing and the addition of preservatives have been used to reduce the incidence of food-borne illnesses due to viruses and other pathogens. However, many of these preservation techniques affect the organoleptic quality of foods and reduce their nutritional value by degrading certain components (Elmnasser et al., 2007). In this context, pulsed light could represent an excellent alternative or complement to conventional thermal or chemical destruction, thus ensuring safe foods with satisfactory nutritional and organoleptic qualities. The pulsed light method was proposed as a novel non-thermal technology for food preservation or packaging material decontamination by PurePulse Technology Inc. in San Diego, California (Dunn et al., 1995). It is based on short and high peak-energy light pulses with a large spectrum of wavelengths (Elmnasser et al., 2007). As reported by Dunn et al. (1995), pulsed light systems can produce a spectrum that is 20,000 times more intense than sunlight at sea level and contains some ultraviolet wavelengths that are removed from sunlight by the filtering effect of the Earth's atmosphere. Moreover, pulsed light uses relatively low energy input to produce high peak-power dissipation, thus providing an economical inactivation method (Rowan et al., 1999). Some studies have reported the efficacy of pulsed UV light against a broad spectrum of food-related microorganisms including bacteria (Rowan et al., 1999), some viruses (Lamont et al., 2007; Roberts and Hope, 2003) and spores (Jun et al., 2003). However, it has not yet been tested against the most common food-borne viruses such as NoV and HAV. The purpose of the present study was therefore to investigate the effectiveness of pulsed UV treatment for the inactivation of MNV-1 and HAV, in suspension and on food-contact surfaces.

2. Materials and methods

2.1. Viral propagation and titration

MNV-1 was kindly provided by Dr. Christiane E. Wobus at the University of Michigan Medical School, USA and HAV strain HM-175 was obtained from S. Bidawid, Bureau of Microbial Hazards, Health Canada, Ottawa, Ontario. HAV strain HM-175 was propagated in FRhK-4 cells as described by Mbithi et al. (1992, 1991) and MNV-1 was propagated in RAW 264.7 macrophage-like cells according to Wobus et al. (2004). Both cell lines were grown in Dulbecco's Modified Eagle's Medium (Cat. no. 319-030-CL, Wisent Inc., St-Bruno, Québec) supplemented with 10% (v/v) fetal bovine serum (FBS, cat. no. 0800150, Wisent Inc.), 1% (v/v) 200 mM L-glutamine solution (Cat. no. 609-065-EL, Wisent Inc.), 1% (v/v) non-essential amino acids solution (Cat. no. 321-01-EL, Wisent Inc.), 1% (v/v) 1 M HEPES (Cat. no. 330-050-EL, Wisent Inc.) and 1% (v/v) penicillin/streptomycin solution (Cat. no. 450-201-EL, Winsent Inc.) at 37 °C and 5% CO_2. Determination of viral titters for viral stocks and after inactivation tests was done as described by Mbithi et al. (1991) and Wobus et al. (2004) respectively for HAV and MNV-1. Briefly, MNV-1 titer was determined by assaying serial dilutions in duplicate in 12-well tissue culture plates containing 1.5×10^6 cells per well. After inoculation with 150 µl of viral dilution, plates were incubated at 37 °C and shaken gently every 15 min for 90 min. 1 ml of overlay agar (1.5% ultra-pure agarose, cat. no. 15510-027, Invitrogen, Burlington, Ontario) supplemented with 2% (v/v) FBS was then added and the culture plates were incubated at 37 °C with 5% CO_2. After 48 h, the overlay was removed carefully and the monolayer was fixed with 3.7% formaldehyde in 0.85% saline overnight. Finally, the monolayer was stained with 0.1% crystal violet in 0.85% saline for plaque counting (Sattar et al., 1989). The HAV plaque assay was similar to the MNV-1 assay, with minor modifications. Briefly, 200 µl of inoculum were added to a cell monolayer and plates were incubated 90 min at 37 °C with rocking every 10 min. Agarose supplemented with 2% FBS was then overlaid (2 ml) and plates were incubated at 37 °C in the presence of 5% CO_2 for eight days. Plaque counting was carried out after staining with crystal violet as described for MNV-1 (Sattar et al., 1989).

2.2. Pulsed UV treatment

Pulsed light treatments for HAV and MNV-1 inactivation were carried out in a SteriPulse-RS-3000C sterilization research benchtop system (Xenon Corp., MA, USA). The device consists of an electrical unit, a lamp (converting electrical energy into radiant energy generating pulses of light), a cooling system, a control module and a treatment chamber. The xenon lamp emitted a wavelength spectrum ranging from 200 to 1100 nm. The system provides three pulses per second with an irradiance of 1.27 J/cm^2 and electrical power of 505 J per pulse. Samples were set up in the treatment chamber and given pulses for 0.1, 0.6, 1 and 2 s (corresponding respectively to 1, 2, 3 and 6 pulses) at distances of 6, 8 and 10.5 cm from the lamp. Some 3-s treatments were also done where indicated. The theoretical fluence F received by each sample was calculated as follows: For disks, F = fluence rate × exposure time × divergence factor (the divergence factor being $L^2/[L + r]^2$ where L is the perpendicular distance from the UV lamp to the disk surface and r is the position of the disk relative to line L) and for studies in suspension, F = fluence rate × exposure time × divergence factor × reflection factor (0.975) (Bolton and Linden, 2003).

2.3. Inactivation studies

Inactivation of viruses using pulsed UV light was determined in suspension and on food-contact surfaces. Viral suspensions (approximately 10^6 PFU/ml) were prepared in PBS and in PBS containing 5% FBS as organic matter. An absorption spectrum for PBS and PBS-5% FBS were determined with an Agilent 8453A UV–Vis spectrophotometer (Agilent Technologies Inc., Mississauga, ON) as described by Uesugi and Moraru (2009). The absorbance measurements were performed in triplicate for the entire spectral range of the pulsed light treatment (200–1100 nm). For exposure in suspension, 1 ml of viral dilution (in PBS with or without protein) was placed in a 24-well microplate (Sarstedt Inc., USA). Unexposed viral solutions of MNV-1 and HAV were included in each treatment as positive control samples. After treatment, samples were kept refrigerated at 4 °C until the titration step by plaque assay, which was carried out the same day.

For studies on inert surfaces, two types of surfaces widely used in homes and in food processing industries were selected, stainless steel and polyvinyl chloride (PVC). Discs 11.5 mm in diameter and 2.2 mm thick were cleaned with 10% sodium hypochlorite, rinsed thoroughly with sterile water, cleaned with 70% ethanol and rinsed again with sterile water. Finally, stainless steel discs were autoclaved at 121 °C for 20 min while each side of the PVC disks was decontaminated by conventional UV light for 10 min in a laminar flow hood before inoculation. The viral suspension (50 µl) was deposited on the center of each disk at a titer of approximately 10^5 PFU/ml for both MNV-1 and HAV. The experimentally contaminated surfaces were then allowed to dry for 10 min at room temperature in a laminar flow hood and placed in 12-well tissue culture plates (Sarstedt Inc., USA). After treatment with pulsed UV, each disk was placed in a tube containing 500 µl of 0.05 M glycine/10% tryptose phosphate (pH 9) and sonicated (40 kHz, Ultrasonic Cleanser Branson 200, Danbury, CT, USA) for 10 min to elute the virus. After sonication, surfaces were then rinsed repeatedly using