5-2013

Chemical Disinfectants for Inactivation of Human Norovirus Surrogates

Cong Cao
ccao2@utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Cong Cao entitled "Chemical Disinfectants for Inactivation of Human Norovirus Surrogates." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Doris H. D'Souza, Major Professor

We have read this thesis and recommend its acceptance:

Svetlana Zivanovic, Irene Hanning

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Chemical Disinfectants for Inactivation of Human Norovirus Surrogates

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Cong Cao

May 2013

Acknowledgments

I would sincerely like to thank my major professor Dr. Doris D'Souza for giving me the opportunity to complete my M.S. degree and appreciate all her time and efforts in supporting me academically and financially through the last two years. Besides my major advisor, I would like to express my appreciation to my committee members, Dr. Svetlana Zivanovic and Dr. Irene Hanning for their intellectual contributions, guidance and feedback. My sincere thanks also go to Dr. Chayapa Techathuvanan, Dr. Xiaowei Su, Hayriye Bozkurt Cekmer, Huaiqiong Chen and Qiumin Ma for their encouragement and friendship. They are remarkable friends that have always been there for me through difficult times, being genuinely happy for my success and giving me hugs, smiles and memories. To the Food Science Department, I would like to thank all the faculty, staff, and students for creating such wonderful studying and working environment. Last, but certainly not least, I would like to thank my family, my parents Mr. Zhihai Cao and Mrs. Zhouping Zhan for their endless love, care and support throughout my life.

ABSTRACT

Human noroviruses (HNoVs) are considered the leading cause of acute non-bacterial gastroenteritis worldwide. Effective chemical disinfectants to inactivate HNoVs are needed. Since HNoVs cannot be cultivated in the lab, cultivable surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1), are used to determine inactivation using infectivity plaque assays. This study aimed to: 1) determine the ability of benzalkonium chloride (BAC) and potassium peroxymonosulfate (KPMS) to inactivate FCV-F9 and MNV-1 *in vitro* using suspension and carrier tests under clean and simulated dirty (5% fetal bovine serum) conditions over 1 h at room temperature; 2) determine inactivation of FCV-F9 and MNV-1 in suspension and carrier tests over 1 h at room temperature by sodium metasilicate (SMS). In suspension tests, BAC caused 1.94 and 2.59 log reductions of low and high FCV-F9 titers after 1 h, respectively. MNV-1 at low and high titers was reduced by > 3 and 1.47 logs with BAC after 1 h, respectively. KPMS at 5 and 10 mg/mL reduced low titers of both viruses to non-detectable levels within 30 s. High FCV-F9 titers were non-detectable after 2 min with 5 mg/mL and within 30 s with 10 mg/mL of KPMS. KPMS at 5 mg/mL had little effect against high titers of MNV-1, but caused a 4.61 log reduction after 5 min with 10 mg/mL of KPMS. Using clean carrier tests, KPMS at 5 and 10 mg/mL reduced both tested viruses at low titers after 30 s and only high FCV-F9 titers after 10 min to undetectable levels. MNV-1 at high titers were reduced to non-detectable levels after 15 min with 10 mg/mL KPMS. BAC reduced low titers of both viruses to undetectable levels after 1 h using carrier tests with no significant reduction of high titers even after 2 h. The antiviral effect of both chemicals decreased under simulated dirty conditions. Both viruses were reduced within 15 s by 5% and 10% SMS using suspension tests. FCV-F9 and MNV-1 at high

titers were reduced to undetectable levels after 2 min and 15 s, respectively with 2% SMS. KPMS and SMS appear suitable for the rapid control of HNoV transmission.

TABLE OF CONTENTS

I. Literature Review....1
Introduction....1
Human Norovirus and its Surrogates....2
Chemical Inactivation of Human Norovirus surrogates....6
References....28
Appendix....38
II. Chemical disinfection of human norovirus surrogates for the prevention of nosocomial human norovirus outbreaks....51
Abstract....52
Introduction....53
Materials and Methods....55
Virus Stocks....55
Chemicals and Treatments....56
Plaque Assays for FCV-F9 and MNV-1....57
Statistical Analysis....57
Results....58
Reduction of FCV-F9 and MNV-1by 0.32 mg/mL BAC in Suspension Tests58
Reduction of FCV-F9 and MNV-1 by 5 and 10 mg/mL KPMS in Suspension Tests....58
Reduction of FCV-F9 and MNV-1 by 0.32 mg/mL BAC in Clean Coupon Tests....58
Reduction of FCV-F9 and MNV-1 by 5 and 10 mg/mL KPMS in Clean Coupon Tests....59
Reduction of FCV-F9 and MNV-1 by 0.32 mg/mL BAC in Dirty Coupon Tests....59
Reduction of FCV-F9 and MNV-1 by 5 and 10 mg/mL KPMS in Dirty Coupon Tests....60
Discussion....60
Conclusions....67
Acknowldegements....67
References....68
Appendix....72
III. Inactivation of Human Norovirus Surrogates by Sodium Metasilicate....80
Abstract....81
Introduction....82
Materials and Methods....84
Viruses and Cell Lines....84
Chemicals....84
Treatments....85
Plaque Assays for FCV-F9 and MNV-1....85
Statistical Analysis....85
Results....86
Effect of 5 and 10% sodium metasilicate against FCV-F9 and MNV-1 at high and low titers in suspension tests....86

Effect of 2% sodium metasilicate against FCV-F9 and MNV-1 in suspension tests ····86
Effect of 2% sodium metasilicate against FCV-F9 and MNV-1 in carrier tests87
Discussion ····87
Conclusions ····90
Acknowledgements ····91
References ····92
Appendix ····96
Vita ····99

LIST OF TABLES

1.1: Examples of studies using chlorine against FCV-F9 and MNV-1 ·····39
1.2: Examples of studies using ethanol, 1-propanol and 2-propanol against FCV-F9 and MNV-1 ·····41
1.3: Examples of studies using glutaraldehyde against FCV-F9 and MNV-1 ·····43
1.4: Examples of studies using organic acids against FCV-F9 and MNV-1 ·····44
1.5: Examples of studies using ozone and hydrogen peroxide against FCV-F9 and MNV-1 45
1.6: Examples of studies using trisodium phosphate against FCV-F9 and MNV-1·····46
1.7: Advantages, disadvantages and applications of disinfectants reviewed in this chapter 47
2.1: Reduction of Low (~ 4 log PFU/mL) and High (~ 6 log PFU/mL) Titers of FCV-F9 and MNV-1 at Room Temperature by 0.32 mg/mL Benzalkonium Chloride (BAC) Using Suspension Tests·····74
2.2: Reduction of FCV-F9 and MNV-1 at High Titers (~ log 6 PFU/mL) by Potassium Peroxymonosulfate (KPMS) at 5 and 10 mg/mL at Room Temperature in Suspension Tests ·····75
2.3: Reduction of FCV-F9 and MNV-1 at high titers on clean (a) and dirty (b) sterile stainless-steel coupons (carrier tests) by 5 mg/mL and 10 mg/mL potassium peroxymonosulfate (KPMS) at room temperature·····76
2.4: Reduction of FCV-F9 and MNV-1 at High Titers on Clean Sterile Stainless-Steel Coupons by 0.32 mg/mL BAC at Room Temperature·····78
2.5: Reduction of FCV-F9 and MNV-1 at high and low titers on sterile stainless-steel coupons with 5% FBS (dirty condition) by 0.32 mg/mL BAC at room temperature 79
2.6: Reduction of FCV-F9 and MNV-1 at low titers on dirty carrier tests by 5 and 10 mg/mL potassium peroxymonosulfate (KPMS) at room temperature ·····80
3.1: The effect of sodium metasilicate at 2% on reduction of FCV-F9 at high titer using suspension tests at room temperature·····98
3.2: The effect of sodium metasilicate at 2% on reduction of FCV-F9 and MNV-1 at high titers using carrier tests at room temperature ·····99

LIST OF FIGURES

Figure 1.1: Chemical structures of disinfectants evaluated in this work. Panel A- benzalkonium chloride; Panel B- potassium peroxymonosulfate; Panel C- Sodium metasilicate·····50

CHAPTER I

LITERATURE REVIEW

Introduction

Foodborne illnesses are regarded as leading public health concerns worldwide as causes of morbidity and mortality. It is estimated that foodborne diseases cause approximately 9.4 million illnesses, 55961 hospitalizations, and 1351 deaths in the United States each year associated with 31 known pathogens (Scallan et al., 2011). The causative agents of foodborne illness include viruses, bacteria, parasites, toxins, metals, and prions (Mead et al., 1999). Among them, viruses are currently considered the leading cause of foodborne illnesses, responsible for 59% (5.5 million) of foodborne disease cases (Scallan et al., 2011).

Enteric viruses that can be transmitted by foodborne or waterborne routes include human caliciviruses (noroviruses, sapoviruses, vesiviruses and lagoviruses), hepatitis A and E virus, human enteroviruses (poliovirus, coxsackie A and B, echovirus, enterovirus serotypes 68-71), astroviruses, other small round viruses, parvoviruses, and rotavirus (Sair et al., 2002). From an epidemiological perspective, the agents that are responsible for the most common cause of acute viral gastroenteritis around the world are human noroviruses. Current estimates are that human noroviruses are responsible for approximately 3.2 million (58%) illnesses, 14,550 (26%) hospitalizations and 287 (11%) deaths annually in the U.S. alone (Scallan et al., 2011). However, these figures can be considered as an underestimate due to the disease being under-reported and cases frequently undetected or outbreaks being uninvestigated. Therefore, it is imperative to develop effective inactivation strategies to control human norovirus spread and prevent their outbreaks in food processing and nosocomial environments.

Human Norovirus and its Surrogates

Human norovirus was first discovered by Albert Kapikian in 1972 using immune electron microscopy (IEM) of stool samples collected from a 1968 outbreak of gastroenteritis in Norwalk, Ohio, USA (Kapikian et al., 1972). Since then, the so-called "Norwalk virus" began to be significantly recognized. Norovirus, the prototype member of small round structured viruses (SRSVs), belongs to the family *Caliciviridae* based on the genetic sequencing work (Jiang et al., 1993; Xi et al., 1990). Currently, this family is comprised of four genera: noroviruses (represented by Norwalk-like viruses, having Norwalk virus prototype strain), sapoviruses (represented by Sapporo-like viruses, having Sapporo virus prototype strain), vesiviruses (represented by vesicular exanthema of swine virus and feline calicivirus) and lagoviruses (represented by rabbit hemorrhagic disease virus and European brown hare syndrome virus), the former two genera being significant to human epidemic gastroenteritis (D'Souza et al., 2007; Sair et al., 2002).

The genus norovirus is comprised of a genetically diverse group of non-enveloped, single-stranded, positive sense RNA viruses. These viruses are icosahedral in shape with a diameter of 27 to 35 nm; and buoyant density of 1.33-1.41 g/cm^3 in CsCl (Kapikian et al., 1972). The norovirus capsid protein consists of 180 capsid protein molecules possessing a molecular weight ranging from 59,000 to 65,000 Da (Jiang et al., 1993; Kapikian et al., 1996). The norovirus genome consists of three open reading frames (ORF) to encode structural and non-structural proteins. ORF1 possesses the longest nucleotides and encodes the non-structural proteins (RNA dependent RNA polymerase); ORF2 encodes the viral capsid protein; and ORF3 encodes a small polypeptide conjectured to package the genome into virions (Donaldson et al., 2008; Jiang et al., 1993).

Based on the sequencing analysis of the RNA-dependent RNA polymerase region and capsid protein region, noroviruses are classified into five genogroups. Genogroups (G) I, GII and GIV are associated with human infections and responsible for foodborne illness outbreaks; they are represented by the prototype Norwalk virus, prototype Snow Mountain agent and prototype Ft. Lauderdale virus, respectively. The other genogroups, GIII and GV are associated with animal infections, represented by prototype, bovine enteric calicivirus and prototype murine norovirus, respectively (D'Souza et al., 2007). Among human infecting genogroups, GII is predominant, responsible for the most human norovirus outbreaks. As reported previously, approximately 73% of human norovirus illnesses are caused by GII viruses (Zheng et al., 2006). The emerging of virulent strains including GII.4 can cause deaths in the elderly and immune-compromised (Siebenga et al., 2010). Norovirus genogroups can be further subdivided into genotypes, also known as genoclusters. Currently, GI is comprised of eight genotypes and GII has at least seventeen genotypes (Zheng et al., 2006).

Human noroviruses, in a similar manner to other enteric viruses, can be transmitted by the fecal-oral route or by direct contact with an infected person as well as by consuming contaminated food or water or touching contaminated surfaces (Kukkula et al., 1999; Weinstein et al., 2008). Aerosolized vomitus is another transmission mode for human noroviruses (Patterson et al., 1997). The difficulty in controlling the spread of human noroviruses is due to their high primary and secondary attack rate, environmental stability and low infectious dose (Caul, 1994; D'Souza et al., 2006; Liu et al., 2009). Outbreaks associated with human noroviruses have occurred in a variety of closed settings, including healthcare facilities, restaurants, cruise ships, schools, hotels, and other institutional settings (CDC, 2011), as evidenced by the environmental persistence of human noroviruses. Further, the survival of

human noroviruses in human gastrointestinal tract indicates their persistence in the presence of degrading enzymes and extreme pH environments (Sair et al., 2002). Previous studies have shown that human noroviruses can be detected on food-contact surfaces for up to 7 days at room temperature; can persist at temperatures ranging from freezing to 60^{o}C (30 min); at pH 2.7 for 3 h at room temperature in stool; with treatment of 20% ether for 18 h at 4^{o}C; and resistant to low concentrations of chlorine (3.75-6.25 mg/L) (D'Souza et al., 2006; D'Souza et al., 2007; Dolin et al., 1972; Patel et al., 2009; Teunis et al., 2008). The infectious dose of human noroviruses is quite low; only 10 infectious units can cause disease. The incubation period of human norovirus infection is typically 12-48 h and characterized by mild to severe gastroenteritis with symptoms include vomiting, nausea, diarrhea, and abdominal pain. The duration of the infection may last 12-72 h; however, fecal viral shedding can be extended for several more days (Grohmann et al., 1981; Cliver et al., 2002; D'Souza et al., 2007). Although, HNoV infection itself is self-limiting in healthy individuals, they can be life-threatening to the elderly and immune-compromised (Donaldson et al., 2010; Siebenga et al., 2009).

To date, the major hurdle for gaining a better knowledge of human noroviruses lies in the fact that human noroviruses cannot be cultivated in cell culture. Therefore, cultivable surrogates are needed to study the basic pathology of human noroviruses, explore the environmental stability of these viruses and determine the efficacy of various control strategies. Several cultivable enteric caliciviruses within the *Caliciviridae* family have been utilized as human norovirus surrogates, including feline calicivirus (FCV-F9), murine norovirus (MNV-1), canine calicivirus (a vesivirus) (Mochizuki et al., 1993), Tulane virus (Farkas et al., 2008) and porcine enteric calicivirus (a sapovirus) (Guo et al., 1999). Currently, the most widely used surrogates are FCV-F9 and MNV-1, which are genetically similar to norovirus because they all belong to

the *Caliciviridae* family. FCV-F9 is positioned in the *Vesivirus* genus, causing acute oral and upper respiratory tract disease in feline populations, typically cats (Green et al., 2000; Radford et al., 2007). This virus has been extensively utilized as a surrogate for human norovirus as a model of evaluating viral persistence and reduction due to its ease of culture and quantification. However, unlike human norovirus, FCV-F9 is transmitted by the respiratory instead of the fecal-oral route (Green et al., 2001). Further, this virus is more vulnerable to environmental stresses, being sensitive to low pH and elevated temperatures as well as chemical treatments (D'Souza and Su, 2010; Doultree et al., 1999; Slomka and Appleton, 1998). MNV-1, a recently cultivable surrogate for human norovirus, belongs to the *Norovirus* genus, which indicates more morphological and genetic similarity to human noroviruses; this virus is currently the only norovirus known in literature that is amenable to cell culture (Wobus et al., 2006). MNV-1 can infect and replicate in mice. Like human norovirus, this virus is transmitted via the fecal-oral route. Further, MNV-1 shares many biochemical and genetic characteristics with human strains, including size, shape and buoyant density (Wobus et al., 2006). With such similarities, MNV-1 shows considerable promise as a surrogate for human noroviruses. However, there are differences. MNV-1 binds to sialic acid on the host cell surface as a receptor, while human norovirus recognizes the histoblood group antigens (HBGAs) (Taube et al., 2009); and the two viruses have different clinical manifestations for MNV-1 does not cause diarrhea or vomiting (Karst et al., 2003). Thus, an effective animal model is still being investigated. Recently, Tulane Virus has been cultivated in the laboratory and is considered an alternate surrogate to MNV-1 by some investigators (Li et al., 2012; Tan and Jiang, 2010). This enteric virus was isolated from stool samples of captive juvenile rhesus macaques, which can replicate *in vitro* in monkey cell lines (Farkas et al., 2008). Tulane virus has a diameter of 36 nm and a buoyant density of 1.37

g/mL; and most importantly, this virus recognizes human HBGAs as a receptor (Farkas et al., 2010). However, Tulane virus belongs to a new calicivirus genus separate from the *Norovirus* genus and the pathogenicity of Tulane virus in rhesus macaques remains unknown (Farkas et al., 2008). Further, Hirneisen and Kniel compared the behavior of MNV-1 and Tulane virus and concluded that MNV-1 is a better surrogate for HNoV, as MNV-1 is more pH (2.0 to 10.0) stable, more resistant to chlorine treatment at 2 ppm and more persistent in tap water at 4 ^{o}C (Hirneisen and Kniel, 2013). Overall, the infectivity studies of surrogates can be used to gain a better understanding of human noroviruses; however, they cannot always model human strains. Hence, it is necessary to use caution when interpreting the results of surrogates. Feeding and challenge studies are deemed more suitable by some investigators.

Chemical Inactivation of Human Norovirus surrogates

To control the spread of human noroviruses and their emerging virulent forms, control strategies have been evaluated including heat inactivation, non-thermal methods, chemical and natural bioactives as alternates. Since human noroviruses can be transmitted via the fecal-oral route, human excretions including stool and vomit can be the main source of human norovirus environmental transmission. The titer of viral particle shed in the feces can be as high as 10^8 /g (Atmar et al., 2008). Therefore, it is imperative to implement control measures to interrupt the transmission of human noroviruses. The use of chemical disinfectants can be considered as an effective intervention strategy to prevent transmission of human noroviruses from contaminated surfaces (Barker et al., 2004).

Chlorine:

Chlorine is an important chemical applied as a disinfectant to kill microorganisms in drinking water and swimming pools. Chlorine is the active ingredient of store-bought household bleach, which normally contains 5.7% available chlorine. The dosage typically used is 4 ppm for disinfecting water.

The antiviral activity of free chlorine is proposed to occur by damaging the capsid as well as RNA (McDonnell and Russell, 1999). Li and others (2002) reported that chlorine at 10 or 20 mg/L concentration could completely inactivate hepatitis A virus (HAV) after 30 min of exposure in a cell culture model. Using an enzyme-linked immunosorbent assay, the disappearance of antigenicity was observed after chlorine treatment/inactivation, which indicates that chlorine may target nucleic acids rather than capsid proteins. It was reported that the inactivation of HAV was due to the damage of the 5' nontranslated regions (5'NTR), as evaluated by long-overlap RT-PCR (Li et al., 2002). Furthermore, Baert and others (2009) suggested that FCV, MS2 and HAV react similarly to chlorine because a similar decline was observed among FCV, MS2 and HAV (Baert et al., 2009). Allwood and others (2004) observed 2.9 log reductions of both FCV and MS2 on leafy vegetables treated with 200 ppm chlorine after 2 min of exposure (Allwood et al., 2004). Similarly, Casteel and others (2008) found that produce treated with 20 ppm chlorine for 10 min enabled a reduction of at least 1.7 log PFU/mL HAV and MS2 (Casteel et al., 2008).

Chlorine was found to be effective against viruses based on the dose, contact time, and the type of viruses (Table 1.1). FCV was reduced by 2.9 $\log_{10}$ PFU/mL on lettuce after a 2 min treatment with 15 ppm chlorine, while MNV-1 was reduced by 1.4 $\log_{10}$ PFU/mL under the same

experimental conditions (Fraisse et al., 2011). Chlorine at 200 ppm reduced FCV-F9 at low titers (~5 log PFU/mL) on produce to undetectable levels after a short contact time of 15 s or 30 s, but only ≤1.4 log reduction of high titers (~7 log PFU/mL) (Su and D'Souza, 2011). MNV-1 reached lower reductions compared to FCV-F9 at both low and high titers under the same treatment and contact time with a 2~3 log-PFU reduction and <1.3 log-PFU reduction, respectively (Su and D'Souza, 2011). Using a surface test, 5000 ppm of chlorine was found to almost completely inactivate FCV-F9 at both low titers (~5 log PFU/mL) and high titers (~7 log PFU/mL) after 30 s or 1 min contact time, respectively at room temperature (D'Souza and Su, 2010). Duizer and others (2004) suggested that chlorine at concentration of ≥3000 ppm could completely inactivate FCV-F9 after a 10 min exposure at room temperature (Duizer et al., 2004). Kitajima and others (2010) found that MNV-1 was inactivated by more than 4 logs after 120 min and 0.5 min contact time with initial free chlorine concentration of 0.1 and 0.5 mg/L, respectively (Kitajima et al., 2010). The findings were similar with Belliot and others (2008), with inactivation of MNV-1 by >4 log after treatment with 0.26% chlorine after a 0.5 min contact time (Belliot et al., 2008). Using a suspension test, 0.3 ppm of free chlorine was found to reduce partially purified FCV-F9 by >4.6 logs after a 5 min exposure (Urakami et al., 2007). However, Doultree and others (1999) observed a 5 log reduction in the infectivity of FCV-F9 after 1 min of exposure to a concentration of 5000 ppm free chlorine (Doultree et al., 1999). It is generally recognized that relatively higher concentrations of chlorine are needed to inactivate human norovirus surrogates on the surfaces. For example, Whitehead and McCue (2010) found that FCV-F9 with 100 ppm chlorine for 1 min resulted in <2.3 log reduction, but was reduced by >3 log with 1,000 ppm chlorine after the same exposure time (Whitehead and McCue, 2010). The results were similar to the reports by Jimenez and Chiang (2006). They observed a 3.2 log reduction treated by 100 ppm

chlorine and a 6.6 log reduction by 1,000 ppm chlorine after 10 min (Jimenez and Chiang, 2006). Gulati and others (2001) reported that, after a range of 1-10 min of exposure to different concentrations of chlorine, a <1 log reduction was observed by <800 ppm chlorine, while a 3.4 log reduction with 5000 ppm chlorine was observed (Gulati et al., 2001). Similarly, Doultree and others (1999) found that <500 ppm and 5000 ppm of chlorine resulted in a <3 log and 5 log reduction, respectively after 1 min of contact time (Doultree et al., 1999). Fewer studies have been conducted with MNV-1. Girard and others (2010) observed that MNV-1 was completely inactivated by 3000 ppm free chlorine after both 5 min and 10 min exposure, as determined by infectivity assay, but with a 4 log reduction using real time RT-PCR (Girard et al., 2010).

It is believed that the effect of chlorine on inactivating human norovirus surrogates is readily decreased by organic matter. For instance, Poschetto and others (2007) observed that FCV-F9 was reduced by >5 log with 4500-5000 ppm of free chlorine after 15 min contact time without feces, while with feces, a 4 log reduction was observed with 5500 ppm after the same exposure time (Poschetto et al., 2007).

Although chlorine is effective against viruses, it may create harmful disinfection by-products including trihalomethanes, which is dangerous to life and health, and produces unfavorable sensory effects. Chlorine can also bleach food and carcinogenic byproducts can be formed by the interaction of chlorine with organic matter (Dunnick and Melnick, 1993). Hence, alternate disinfectant methods are being sought.

Alcohols:

Ethanol is a commonly employed sanitizer and disinfectant used in the general concentration range of 70%-75%. The mechanism of its antiviral activity is proposed to occur by

denaturing capsid proteins and dissolving the lipids of the enveloped viruses (McDonnell and Russell, 1999). Ethanol at 70% concentration has been evaluated most frequently by investigators for its efficacy against human norovirus surrogates. However, no conclusive suggestions/recommendations have been reached, as there are variable results against non-enveloped viruses (Table 1.2). A few studies have reported that ethanol has a strong effect against FCV-F9 and MNV-1. For example, Di Martino and others (2010) found that 70% ethanol was able to inactive FCV-F9 by >4 log PFU/mL after a 10 min exposure, as evaluated by nested RT-PCR (Di Martino et al., 2010). Malik and others (2006), using tissue culture infectious dose 50 ($TCID_{50}$), showed that 70% and 90% ethanol were most effective at killing 99% of FCV-F9 after 1 min of exposure (Malik et al., 2006). Similarly, Gehrke and others (2004), using quantitative suspension assays, suggested that 67% ethanol had a high degree of efficacy on decontaminating FCV-F9 after 1 min contact time (Gehrke et al., 2004). Macinga and others (2008) found that, using an infectivity assay, 70% ethanol could inactivate FCV-F9 by $\geqslant$4.75 $\log_{10}$ PFU/mL after a 30 s contact time (Macinga et al., 2008). Belliot and others (2008) found that MNV-1 was significantly inactivated (>4 log PFU/mL) by 60% ethanol after 30 s exposure, as evaluated by plaque assays and real-time RT-PCR (Belliot et al., 2008). Similarly, Magulski and others (2009) observed $\geqslant$4 log reduction on MNV-1 with 50% ethonal after 5 min of exposure using an infectivity assay (Magulski et al., 2009). Park and others (2010), using plaque assays, also suggested that ≥70% ethanol was able to inactivate MNV-1 by >3.6 log with contact times ranging from 1 min to 5 min (Park et al., 2010).

On the other hand, many studies showed that ethanol was ineffective against HNoV surrogates. For example, D'Souza and Su (2010) using plaque assays found that treatment with 70% ethanol was not effective in reducing the infectivity of both FCV and MNV at low (~5 log

PFU/mL) or high (~7 log PFU/mL) titers after 30 s or 1 min contact times (D'Souza and Su, 2010). Park and others (2010) reported that 70% ethanol resulted in a 2.6 log reduction in FCV after 5 min exposure (Park et al., 2010). The findings of Duizer and others (2004) were similar, with FCV being inactivated <2 log after an 8 min exposure to 70% ethanol, and a 3 log-reduction after a 30 min exposure, as evaluated by infectivity plaque assays (Duizer et al., 2004). Doultree and others (1999) also reported that FCV was not significantly inactivated (1.25 log) at ethanol concentrations of 75% with a contact time of 1 min (Doultree et al., 1999), which were similar to the reports by Whitehead and McCue's (2010), with FCV being reduced approximately 1.3 logs after 1 min of exposure to 60-75% ethanol (Whitehead and McCue, 2010). Lages and others (2008), using fingertips, found that ethanol concentrations of 99.5% was more effective (≥1 log reduction) than 62% ethanol (<1 log reduciton) against FCV-F9 with contact times of 30s or 2 min, respectively (Lages et al., 2008).

Other alcohols, including 1-propanol and isopropanol, were also used to reduce FCV-F9 and MNV-1 (Table 1.2 and 1.3). For example, Gehrke and others (2004) reported that 50% to 70% of 1-propanol was effective at inactivating FCV-F9 within 30 s, using a quantitative suspension assay. They found that 1-propanol at concentrations of 60% was the most effective, and 50% and 70% of 1-propanol could reduce FCV-F9 by 4 log units (Gehrke et al., 2004). Using a surface test, Magulski and others (2009) reported that MNV-1 was inactivated ⩾4 logs after a 5 min exposure to 30% 1-propanol, as evaluated by an infectivity assay (Magulski et al., 2009).

Isopropanol was shown to be effective at inactivating FCV-F9 and MNV-1(Belliot et al., 2008; Gehrke et al., 2004; Malik et al., 2006; Park et al., 2010), although reports by Park and others (2010) showed almost no reduction of FCV-F9 by 50%, 70% or 90% of isopropanol after

1 min or 5 min exposure, as determined by plaque assays (Park et al., 2010). Other studies suggested that isopropanol has a high degree of efficacy in decontaminating FCV-F9. For instance, Gehrke and others (2004) found that 58% of isopropanol was most effective against FCV-F9 after 1 min of exposure, as evaluated by a quantitative suspension assay (Gehrke et al., 2004). Similarly, Malik and others (2006) reported that using $TCID_{50}$, 40% to 60% isopropanol was most effective, which could kill 99% of FCV-F9 within 1 min (Malik et al., 2006). Compared to FCV-F9, MNV-1 showed more resistance to isopropanol. Park and others (2010) reported that ⩾70% of isopropanol reduced the infectivity of MNV-1 by ⩾2.6 log units after 5 min contact time, as evaluated by plaque assay (Park et al., 2010). Belliot and others (2008) observed >3.8 log reduction of MNV-1 after 30 s exposure to 60% isopropanol, as determined by infectivity assay (Belliot et al., 2008). Using surface testing, Magulski and others (2009) found that 60% isopropanol inactivated MNV-1 by 3.02 log units after 5 min contact time using infectivity plaque assays (Magulski et al., 2009).

Glutaraldehyde

Glutaraldehyde is an organic compound used to disinfect medical and dental equipment and also used as a preservative in industrial cleaning agents. Glutaraldehyde has been studied as a disinfectant agent against food-borne viruses and surrogates, including hepatitis A virus (HAV), rotavirus, FCV-F9 and MNV-1.

Glutaraldehyde at low concentration has been shown to inactivate food-borne viruses. Passagot and others (1987) reported that a > 3 log_{10} reduction was achieved after 30 min of exposure using 0.10% glutaraldehyde and within only 3 min using 0.50% glutaraldehyde against HAV using $TCID_{50}$ assays (Passagot et al., 1987). Mbithi and others (1990) found that 2%

glutaraldehyde reduced HAV by 99.9% after 1 min of exposure on contaminated stainless-steel disks (Mbithi et al., 1990). Jean and others (2003) observed that disinfectant containing 5% glutaraldehyde plus 10% quaternary ammonium was effective in inactivating HAV in solution after a 5 min of contact time, which could reduce HAV by 7.25 logs at the final concentration of 3,000 ppm (Jean et al., 2003). Lloyd-Evans and others (1986) reported that 2% glutaraldehyde was also effective against human rotavirus (Lloyd-Evans et al., 1986). Glutaraldehyde was also found to effectively inactivate human norovirus surrogates, FCV-F9 and MNV-1. Using surface tests, D'Souza and Su (2010) found that 2% glutaraldehyde effectively inactivated both FCV-F9 and MNV-1 at both low (~5 log PFU/mL) and high (~7 log PFU/mL) titers after 30 s or 1 min exposure times (D'Souza and Su, 2010). Malik and others (2006) reported that, after a range of 1 to 10 min exposure to 2.6% glutaraldehyde, FCV-F9 was inactivated by more than 99.99% (Malik et al., 2006). Similarly, Magulski and others (2009) observed a $\geqslant$4 log reduction of MNV-1 treated by 2500 ppm glutaraldehyde within 5 min exposure time (Magulski et al., 2009). Furthermore, there were no significant differences in virus reduction under either dirty or clean conditions. Using suspension tests, Doltree and others (1999) observed complete inactivation of FCV-F9 with 0.5% glutaraldehyde after 1 min contact time (Doultree et al., 1999).

Benzalkonium chloride:

Benzalkonium chloride (BAC) belongs to the quaternary ammonium compound (QAC) group, which have been widely utilized in hand sanitizers (Moadab et al., 2001) and disinfectants for hard surfaces (Gradel et al., 2004) as well as for pharmaceutical preservatives (Marple et al., 2004) due to its effectiveness in decontaminating a broad spectrum of bacteria, viruses and fungi in solutions (Bastiani et al., 1974; Belec et al., 2000; Jira et al., 1982). BAC at low concentrations is reported to be non-irritating, non-toxic, and non-corrosive to food contact

surfaces and thus can be safely used as a disinfectant in the food industry as a readily-used antimicrobial agent. As quaternary ammonium compounds are surface acting agents, the antiviral mechanism of BAC may have been due to the alterations in the surface components at, or adjacent to the attachment site, which interacts with the receptor on the surface of the host cell (Wood and Payne 1998).

BAC at 512 ppm (0.512 mg/mL) is reported to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA), *Campylobacter*, *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Pseudomonas aeruginosa* after an exposure of 5 min in both suspension and surface tests (Riazi and Matthews, 2011). In addition to bacteria, studies have also shown that BAC has antiviral activity against canine coronavirus (Pratelli, 2007), respiratory syncytial virus, adenovirus, herpes simplex virus hominis type 2 and cytomegalovirus (Belec et al., 2000). In suspension tests, in the presence of organic load, BAC effectively inactivate enveloped viruses herpes simplex virus type 1 and human immunodeficiency virus type 1, as well as the non-enveloped human coxsackie virus at the concentration of 0.2% w/v after 1 min with initial viral titers of 10^6-10^7 PFU/$TCID_{50}$ or syncytia-forming assay (SFU) (Wood and Payne 1998).

Su and D'Souza (2012) reported that BAC at 0.2, 0.5, and 1 mg/mL reduced FCV-F9 and MNV-1 at low (~5 $\log_{10}$ PFU/mL) titers to undetectable levels after 2 h at room temperature in suspension tests; and high (~7 $\log_{10}$ PFU/mL) titers of FCV-F9 was reduced by 2.87, 3.08, and 3.25 $\log_{10}$ PFU/mL and high titer MNV-1 was reduced by 1.55, 2.32, and 2.75 $\log_{10}$ PFU/mL with BAC at three concentrations, respectively (Su and D'Souza, 2012).

Organic acids:

Organic acids, including lactic acid, tannic acid, and peroxyacetic acid, have been used for inactivating human noroviruses and human enteroviruses and their surrogates. The acid mediated mechanisms leading to a loss of infectivity have not been completely understood, which is believed to result from the alterations of the virus capsid and nucleic acid (Cliver, 2009; Salo and Cliver, 1976). However, whether the antiviral effect was derived from the decrease in pH or the type of acid is still not clear. It appears that the inactivation of non-enveloped viruses is due to the denaturation of capsid proteins due to the decrease in pH (Rodger et al., 1977)

Using suspension tests, Straube and others (2011) found that D, L-lactic acid at the concentration of 0.3% (PH 3.4-3.5) and 0.4% (PH 3.2-3.3) could cause significant titer reductions of FCV-F9 after 7 days of exposure at 20°C (Straube et al., 2011). Using an organic acid-based disinfectant containing 55 to 60% formic acid and 7% glyoxylic acid, Poschetto and others (2007) observed a $\geq$5 $\log_{10}$ reduction of FCV-F9 after 15 min contact time, estimated by cell culture or a 2 to 3 $\log_{10}$ reduction after 15 min or 60 min as determined by RT-PCR (Poschetto et al., 2007). However, the disinfectant efficacy was strongly reduced by the presence of fetal bovine serum (FBS) or feces. Baert and others (2009) reported that 250 mg/L peroxyacetic acid could obtain an additional 1-log reduction for MNV-1 on shredded iceberg lettuce, and its efficacy was not influenced by the presence of organic materials (Baert et al., 2009). Fraisse and others (2011) found that 100 ppm of peroxyacetic-based biocide effectively inactivated FCV-F9 by 3.2 log units and MNV-1 by 2.3 log units on the lettuce leaves after 2 min of contact time, as estimated by cell culture (Fraisse et al., 2011). Whitehead and McCue (2010) reported that one acid-based bathroom disinfectant containing 2.5% citric acid (pH 2.0) could reduce FCV-F9 by >3.17 $\log_{10}$ reduction after 1 min of exposure (Whitehead and McCue,

2010). Recently, Zhang and others (2011) found that tannic acid derived from Chinese medicinal herbs as an inhibitor in binding to histo-blood group antigens (HBGAs) receptors, which indicated that tannic acid may be a promising antiviral against HNoVs (Zhang et al., 2011). Su and D'Souza (2012) reported that tannic acid at 0.2 mg/mL reduced low titers (~5 $\log_{10}$ PFU/mL) of FCV-F9 by 1.95 log and <1 logs of high titers (~7 $\log_{10}$ PFU/mL) of FCV-F9 after 2 h of contact time at room temperature. They also reported that gallic acid at 0.1, 0.2, and 0.4 mg/mL reduced low-titers of FCV-F9 by 2.50, 2.36, and 0.86 $\log_{10}$ PFU/mL, respectively with little effect against high-titer FCV-F9 under the same exposure time (Su and D'Souza, 2012). Oh and others (2012) demonstrated that gallic acid in black raspberry (BRB) juice did not show any antiviral activity against FCV-F9 or MNV-1, though BRB exhibited high efficacy against these viruses. Acetic acid is the typical acid used with chitosan and is known to have antibacterial activity (Oh et al., 2012). Acetic acid has been found to be effective against bacteria such as *Salmonella* Typhimurium, *Enterobacter sakazakii* and *Listeria monocytogenes* (Alvarez-Ordonez et al., 2009; Back et al., 2009; Vasseur et al., 1999). Davis and others (2012) found that chitosan dissolved in 1% acetic acid reduced phiX174 by 1.19-1.29, MS2 by 1.88-5.37, FCV-F9 by 2.27-2.94, and MNV-1 by 0.09-0.28 $\log_{10}$ PFU/mL, respectively after incubation for 3 h at 37 ℃ (Davis et al., 2012). Malic acid is a dicarboxylic acid which was first isolated from apple juice. Malic acid at 10% combined with 1% thiamine dilauryl sulfate was found completely inactivated *E. coli* O157: H7 on 10g of inoculated alfalfa seeds (Fransisca et al., 2012). Malic acid combined with citric acid and sodium laruryl sulfate showed antiviral activity against rhinovirus (Hayden et al., 1985). Humic acid is a principle component of humic substances, which naturally occurs as decomposed constituents of soil. Ansorq and Rochus (1978) found that humic acids inhibited *Staphylococcus epidermidis, S. aureus, Streptococcus pyogenes, S.*

Typhimurium*, P. vulgaris, E. cloacae, P. aeruginosa* and *C. albicans* (Ansorg and Rochus, 1978). Certain modified forms of humic acid have also shown antiviral activities against herpes simplex and human immunodeficiency viruses (Klocking et al., 2002; Schneider et al., 1996; Van Rensburg et al., 2002).

Electrolyzed water

Electrolyzed water (aka electrolyzed oxidizing water or EOW) is produced from the electrolysis of water containing sodium chloride. This procedure generates a solution of sodium hypochlorite, which is the active ingredient in household bleach. EOW has been applied in the food industry and agriculture due to its environmentally friendly nature, high efficiency, and no adverse side effects. EOW is believed to destroy the outer cellular membrane, increase the membrane permeability and decrease the activity of key enzymes due to its physical and chemical properties, including low pH, high oxidation-reduction potential, high dissolved oxygen and free chlorine (Koseki et al., 2001; Kuira and others 2002; Len et al., 2000; Liao et al., 2007; Park et al., 2004; Zeng et al., 2010). However, there is still no definite conclusion on the disinfection targets/and mechanisms of EOW.

The acidic form of EOW was found to effectively inactivate food-borne pathogens on the food surfaces, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*. Park and others (2006) observed 2.41 and 2.65 log CFU reductions per lettuce leaf of *E. coli* O 157:H7 and *L. monocytogene*s respectively using acidic electrolyzed water (AC-EW) (45 ppm residual chlorine) after 3 min of exposure time at 22℃ (Park et al., 2006). Koseki and others (2003) reported 4.6 and 4.4 log CFU/g reductions of *E. coli* O 157:H7 and *S.* Typhimurium respectively with the treatment of spot inoculated lettuce surfaces using 40 ppm

AC-EW for 1 min (Koseki et al., 2003). Guentzel and others (2008) showed that dip treatment of inoculated spinach and lettuce surfaces with 20 and 50 ppm AC-EW for 10 min resulted in 2.14 to 4.97 log CFU/g reductions of *E. coli, S.* Typhimurium, and *L. monocytogene*s (Guentzel et al., 2008). Park and others (2008) reported that *E. coli* O 157:H7, *S.* Typhimurium, and *L. monocytogene*s were reduced below the detection limit (0.7 log) after a 3-min treatment of AC-EW (~37.5 mg/L) at room temperature on the surfaces of lettuce and spinach (Park et al., 2008).

Slightly acidic electrolyzed water (SAEW) was also reported to be effective towards inactivating these food-borne pathogens. Issa-Zacharia and others (2010) reported that SAEW containing 23 mg/L available chlorine effectively reduced the population of *E. coli, S. aureus*, and *Salmonella spp.* by 5.1, 4.8, and 5.2 log CFU/mL, respectively after 60 s of treatment (Issa-Zacharia et al., 2010). However, the efficacy of AC-EW could be decreased by the presence of organic matter. Park and others (2009) evaluated the effects of bovine serum on the bactericidal activity of AC-EW on *E. coli, S.* Typhimurium, and *L. monocytogene*s inoculated surfaces of green onions and tomatoes. They showed that AC-EW (~37.5 mg/l available chlorine concentration) treatment alone achieved the reductions below the detection limit (0.7 log CFU/g) after 3 min, whereas the bactericidal activity decreased with the increase of bovine serum concentration (Park et al., 2009).

EOW was also studied for the removal of inoculated human norovirus from the surfaces of raspberries and romaine lettuce by a simple wash. Tian and others (2011) found that an AC-EW wash enhanced the binding of HNoV to raspberries and lettuce with only ~7.5% and ~4% of HNoV removed, respectively (Tian et al., 2011).

Ozone:

Ozone is a powerful oxidizing agent, which has been used as a disinfectant for killing microorganisms in air and water sources. Ozone applications in the food industry typically include decontamination of product surfaces and water treatment (Kim et al., 1999). The mechanism of inactivation of viruses may be due to both viral RNA and capsid protein destruction (Kim and others, 1999). Roy and others (1981) believed that damage to the viral nucleic acid was the major cause of poliovirus 1 inactivation by ozone (Roy et al., 1981). This is supported by the report of Shin and Sobsey (2003), which showed that virus reductions of poliovirus 1and coliphage MS2 exposed to ozone were similar as evaluated by both RT-PCR and infectivity assays (Shin and Sobsey, 2003). However, others suggested that ozone actually targets the capsid protein. Kim and others (1980) found that ozone breaks the protein capsid into subunits, which leads to the inactivation of bacteriophage f2 (Kim et al., 1980). This is evidenced by the study of Lim and others (2010), who reported that ozone at 1 mg/liter (at pH of 7 or 5.6 and temperature of 20℃ or 5℃） effectively inactivated more than 99% of MNV-1 within 2 min, as measured by plaque assays, but RT-PCR assay significantly underestimated the inactivation of MNV-1 (Lim et al., 2010).

Ozone has shown its efficacy on the inactivation of norovirus surrogates, including FCV-F9 and MNV-1. Hirneisen and others (2011) observed >6 log $TCID_{50}$/mL of FCV reduction in water and ~2 log $TCID_{50}$/mL on green onions and lettuce after 5 min of 6.25 ppm ozone treatment. MNV-1 inoculated onto these produce items produced a >2 log reduction after 1 min of exposure, as determined by plaque assay (Hirneisen et al., 2011). Hudson and others (2007) reported that ozone gas, at the level of 20-25 ppm, was able to reduce the concentration of

infectious FCV by a factor of more than 10^3 within less than an hour of exposure (Hudson et al., 2007).

Hydrogen peroxide:

Hydrogen peroxide (H_2O_2) is also a strong oxidizer, which is often used as a bleaching or cleaning agent. Hydrogen peroxide has shown to be effective against enteric viruses and their surrogates. For example, Li and others (2011) found that liquid H_2O_2 (L-H_2O_2) at concentrations of 2.1% inactivated MNV-1 and coliphage X174 on stainless steel discs by approximately 4 $\log_{10}$ units within 10 min, whereas for *Bacillus fragilis* Phage B40-8, 15% of L-H_2O_2 was needed to obtain a similar reduction in 10 min, with only a <1 $\log_{10}$ unit reduction after 5min of exposure to 2.52% of Vaporized H_2O_2 was achieved for the tested model viruses (Li et al., 2011). Tuladhar and others (2011) reported that hydrogen peroxide vapor at 127 ppm completely inactivated poliovirus, rotavirus, adenovirus and murine norovirus after 1 h treatment at room temperature on carriers (Tuladhar et al., 2011). Bentley and others (2011) observed a 4 log reduction on all tested surfaces within 20 min of exposure to 30% (w/w) H_2O_2 vapour. The reduction was achieved most rapidly on vinyl flooring (10 min), while the longest was observed on stainless steel (20 min). For glass, ceramic tile and plastic surfaces, the desired reduction titer was reached within 15 min (Bentley et al., 2011). Malik and others (2006) found that a combination of 2% sodium bicarbonate with 2% hydrogen peroxide killed FCV-F9 by 99.00% in 1 and 3 min, and 99.68% of the virus was killed after 10 min contact time on stainless steel coupons (Malik and Goyal, 2006).

Potassium monosulfate:

Potassium monosulfate (KPMS) is widely used as a powerful oxidizing agent. Currently, KPMS is routinely used in swimming pools against *Escherichia coli* to keep the water clean and safe (Anipsitakis et al., 2008). It has also been used as a mist in veterinary hospitals to decontaminate *S. aureus* and *S*. Typhiumurium by >99.9999% with the concentration of 4% for 30 min exposure time after misting was completed (Patterson et al., 2005). In a 6-year study, KPMS at concentrations of 1 and 10 mg/mL completely inactivated *S. aureus, MRSA* and *Pseudomonas aeruginosa* after 10 and 2 min exposure times, respectively and no significant variation in resistance to KPMS was observed during the period of the study (Giacometti et al., 2005). However, only a few studies reported the application of KPMS for viral inactivation. The KPMS-based product Virkon S was shown to completely inactivate the poultry enteric mortality syndrome (PEMS)-associated astrovirus in an embryo model (Schultz-Cherry et al., 2001). Also, KPMS was shown to completely inactivate FCV after 10 min of exposure (Eleraky et al., 2002). However, this study used only FCV from animal isolates and not the HNoV surrogate strains FCV-F9 or MNV-1.

Solomon and others (2009) reported that Virkon at 1% containing 5 mg/mL KPMS reduced FCV-F9 from 4-5 $\log_{10}$ PFU/mL to undetectable levels after 10 min (Solomon et al., 2009). Su and D'Souza (2012) reported that KPMS at 5, 10, and 20 mg/mL reduced high and low titers of FCV-F9 and low titers of MNV-1 to undetectable levels, while high titers of MNV-1 were reduced by 0.92 and 3.44 $\log_{10}$ PFU/mL with KMPS at 5 and 10 mg/mL, respectively after 2 h at room temperature (Su and D'Souza, 2012).

Trisodium phosphate

Trisodium phosphate (TSP) is considered a generally recognized as safe (GRAS) chemical by the Food and Drug Admiminstration (FDA: Lindsay 1985), which has a pH of 12 in a 1% solution. TSP has shown efficacy against a wide range of pathogens, including *E. coli* O157: H7, *Salmonella*, *poliovirus* 1 and bacteriophages. Jerzy and others (2003) reported that significant reductions of bacteriophage MS2 and *Poliovirus* 1 were obtained by using 1.0% TSP solution on strawberries (Jerzy et al., 2003). TSP has also been evaluated as a detergent against norovirus surrogates, including FCV-F9 and MNV-1. D'Souza and Su (2010) reported that 5% TSP effectively inactivated high titers of MNV-1 and FCV by ⩾6 log PFU/mL, and with a 5 log reduction of low viral titers after 30s or 1 min of contact time. TSP at 2% inactivated high titers of FCV by ⩾6 log PFU/mL, but only a 1.05 log reduction for MNV-1 after 1 min exposure., while1% TSP reduced FCV by ~2.65 log PFU/mL, with no reduction of high titers of MNV-1 (D'Souza and Su, 2010). Su and D'Souza (2011) found that 2% and 5% TSP reduced low titers of FCV to undetectable levels after 15 s or 30 s. Low titers of MNV-1 was reduced by ~2 - 3 log PFU/mL by 2% TSP and to undetectable levels by 5% TSP. High titers of FCV and MNV-1 were reduced by > 5 or ~ 2- 3.4 log PFU/mL with 2% TSP, respectively, and to undetectable levels with 5% TSP (Su and D'Souza, 2011).

Sodium metasilicate:

Sodium metasilicate is a highly soluble chemical with the pH of a 1% solution ranging from 12.5 to 13.0. Sodium metasilicate is USDA FSIS approved as a processing aid and can be used as an antimicrobial component of marinades for meat and poultry products up to 2% by weight of the marinades and as a carcass rinse or spray on raw beef carcasses, subprimals, and

trimmings up to 6% in solution (USDA FSIS, 2011). Sodium metasilicate has been effective against *E. coli* O157: H7 and *Salmonella* both *in vitro* and in beef trimmings (Carlson et al., 2008; Geornaras et al., 2012; Pohlman et al., 2009; Weber et al., 2004), *Campylobactor jejuni* and *Salmonella* Typhimurium in fresh chicken breast meat (Sharma et al., 2012a, b). Weber and others (2004) found that sodium metasilicate at 0.6% (pH 12.1) resulted in complete inhibition of *E. coli* O157: H7 after 5 to 10 s exposure in water at room temperature (Weber et al., 2004). Carlson and others (2008) reported that 4% sodium metasilicate (23^{o}C, pH 12.94) reduced *E. coli* O157: H7 and *Salmonella* by ~1.9 and ~ 2.6 log CFU/mL, respectively from inoculated beef hides following a 30 s- spray- 2 min- dwell- 30 s - water rinse procedure (Carlson et al., 2008). Pohlman and others (2009) demonstrated that *E. coli* and *Salmonella* Typhimurium were reduced by ~1 log to $\geqslant$1.5 log CFU/mL, respectively, treated with 4% sodium metasilicate from inoculated beef trimmings (Pohlman et al., 2009). Geornaras and others (2012) compared the efficacy of sodium metasilicate against *E.coli* O157:H7 and 6 non-O157 Shiga toxin-producing *E. coli* (nSTEC) serogroups suggesting similar effectiveness. The initial pathogen counts were decontaminated by 1.3 to 1.5 log CFU/cm^2 from inoculated beef trimmings after immersion for 30 s in solution of 4% sodium metasilicate (pH 12.5) (Geornaras et al., 2012). Adler and others (2011) observed that brine solution containing 2.2% sodium metasilicate immediately reduced *E.coli* O157:H7 by $\geqslant$2.4 log CFU/mL (0 h) (Adler et al., 2011).

Sodium metasilicate has also been evaluated for efficacy against viral pathogens, including human rotaviruses and vesicular stomatitis viruses (Springthorpe et al., 1986; Wright, 1970). Springthorpe and others (1986) tested the efficacy of 69 commercial and 7 non-commercial disinfectant formulations against human rotaviruses and found that the efficacy of quaternary ammonium-based disinfectant formulations was greatly improved by adding 0.5 to

5% sodium metasilicate. They believed that quaternary ammonium compounds and sodium metasilicate may act in an additive or synergistic manner to inactivate human rotaviruses (Springthorpe et al., 1986). However, Wright (1970) demonstrated that sodium metasilicate at 5% (pH 12.1) was not viricidal against vesicular stomatitis virus after 10 min contact time (Wright, 1970).

Natural antimicrobial extracts:

As the global consumer's demand for natural ingredients to reduce pathogenic microorganism from foods is steadily increasing, the investigation of the effectiveness of potential natural antimicrobials is necessary. Some natural extracts such as Cranberry juice and cranberry proanthocyanidins, Black raspberry juice, Grape seed extract, Pomegranate juice and pomegranate polyphenols, Hibiscus sabdariffa have shown antibacterial and antiviral activities.

Cranberry extracts have been reported to have antibacterial activity against *E. coli* O157: H7, *Salmonella Typhimurium*, *Listeria Monocytogenes*, *Helicobacter pylori* and *Staphylococcus aureus* (Matsushima et al., 2008; Nogueira et al., 2003; Wu et al., 2008). In addition to antibacterial property, they have also shown antiviral activity against reovirus, bacteriophages T4, phiX-174 and MS2, rotavirus, influenza virus and human norovirus surrogates (Lipson et al., 2007; Su et al., 2010a; Su et al., 2010b; Weiss et al., 2005). The mechanism of cranberry extracts on the antimicrobial activity has not been well established. Wu and others (2008) believed that both low pH and phenolics may cause the antibacterial effect by observing cell wall and cell membrane damage treated with cranberry concentrate, which may make bacteria vulnerable to cranberry concentrate (Wu et al., 2008). Similarly, cranberry extracts may injure the viral capsid structure or viral nucleic acid to interfere with viral replication. Su and others (2010a) reported

that FCV-F9 at ~ 5 $\log_{10}$ PFU/mL was reduced to undetectable levels after 1h of exposure to cranberry juice (CJ) (pH 2.6) or 0.15, 0.30, and 0.60 mg/mL cranberry proanthocyanidins (PAC) at room temperature. MNV-1 was reduced by 2.06 $\log_{10}$ PFU/mL with CJ, and 2.63, 2.75, and 2.95 $\log_{10}$ PFU/mL with the three tested PAC solutions, respectively (Su et al., 2010a). In another time- dependent study by these investigators, FCV-F9 at low viral titers was reduced by ~5 $\log_{10}$ PFU/mL within 30 min treated by CJ (pH 2.6 and pH 7.0) and the two PAC solutions (0.15, 0.30 mg/mL). MNV-1 titers were decreased similarly with CJ at pH 2.6 or 7.0. In most cases, viral reduction within the first 10 min of treatment accounted for 50% of the total reduction (Su et al., 2010b).

Pomegranate juice and pomegranate polyphenols exhibited a wide range of antioxidant, anticancer, anti-inflammatory and antimicrobial properties (Braga et al., 2005; Haidari et al., 2009; Kotwal, 2008; Lee et al., 2010; Neurath et al., 2005; Prashanth et al., 2001; Reddy et al., 2007). Pomegranate extracts have shown antibacterial activities against *Salmonella* Typhimurium, *Staphylococcus aureus, E. coli,* and *Bacillus subtilis* (Braga et al., 2005; Prashanth et al., 2001). They are also reported to have antiviral properties against influenza virus, HIV-1, poliovirus, herpes virus, poxvirus, and human norovirus surrogates (Haidari et al., 2009; Jayaprakasha et al., 2006; Konowalchuk and Speirs, 1976; Kotwal, 2008; Neurath et al., 2005; Su et al., 2010c, 2011). These antiviral effects were attributed to the high content of polyphenols, which were believed to act directly on the viral particles to block the attachment of the viruses to the cell receptors (Haslam, 1974). Pomegranate juice and pomegranate polyphenols were shown to cause 2.56, 1.32, and 0.32 log reduction for low titers of FCV-F9, MNV-1, and MS2, respectively, after treatment by pomegranate juice for 1 h at room temperature. The effects were decreased using high titers of three tested viruses. Pomegranate polyphenols at 8, 16, 32 mg/mL

were shown to reduce both low and high titers of FCV-F9 to undetectable levels within 1 h. MNV-1 at low titers was reported to be reduced by 1.30, 2.31, and 3.61 $\log_{10}$ PFU/ mL and at high titers by 1.56, 1.48, and 1.54 $\log_{10}$ PFU/mL with three tested concentrations of PP, respectively, after 1 h by these investigators (Su et al., 2010c). According to the time-dependent study carried out by Su and others (2011), PJ and PP were found as rather rapid acting, resulting in ≥ 50% reduction within the first 20 min of treatment for all tested viruses. FCV-F9 and MNV-1 at titers of ~ 5 $\log_{10}$ PFU/mL were reduced by 3.12 and 0.79 $\log_{10}$ PFU/mL, respectively, using PJ within the first 20 min. Titer reductions of 4.02 and 0.68 $\log_{10}$ PFU/mL with 2 mg/mL PP and 5.09 and 1.14 $\log_{10}$ PFU/mL with 4 mg/mL PP were reported by these investigators for FCV-F9 and MNV-1, respectively, after 20 min (Su et al., 2011).

Hibiscus sabdariffa are prepared as hot and cold beverages worldwide, and is classified as a GRAS ingredient for the food industry (CDC 2006). In addition, aqueous extracts of *Hibiscus sabdariffa* have been used in folk medicine in the treatment of hypertension (Odigie et al., 2003; Olaleye, 2007; Wang et al., 2000). Hibiscus anthocyanins, a group of phenolic natural pigments present in calyces, have been found to have cardioprotective, hypocholesterolemic, antioxidative and heaptoprotective effects in animals (Chen et al., 2003; Jonadet et al., 1990; Wang et al., 2000). Moreover, the aqueous extracts were reported the presence of hibiscitrin, gossypitrin, sabdaritrin, flavonol glycosides and some organic acids including citric, malic, tartaric and ascorbic (Ali et al., 2005; Cowan, 1999; Tsai et al., 2002). Aqueous extracts of *H. sabdariffa* have exhibited a wide range of antimicrobial properties against *Pasteurella, Pseudomonas, Proteus, Streptococcus, P. aeruginosa, Lactobacillus sp., Bacillus sp., Corynebacterium sp., Salmonella, Escherichia coli O157:H7* And *Listeria monocytogenes* (Oboh and Elusiyan, 2004; Pliego, 2007; Sharaf et al., 1966). The mode of action includes phenols that

inhibit metabolic enzymes of the cells leading to their inability to reproduce. The phenolic compound, anthocyanins, extracted from *Hibiscus sabdariffa* were suggested to complex with nucleophilic amino acids of the cell wall, resulting the loss of function (Cowan, 1999; Tsai et al., 2002). However, phenols may not be the only compound responsible for all the antimicrobial activities of *Hibiscus sabdariffa*. Roman (2006) found that rifamycine, a precursor of the antibiotic rifampicin, was present in *Hibiscus sabdariffa* extracts (Roman 2006).

Based on the current literature, the aims of this research were to (1) investigate the antiviral activity of benzalkonium chloride at 0.32 mg/mL and KPMS against human norovirus surrogates over 1 h at room temperature using suspension and carrier tests and (2) determine the ability of sodium metasilicate to inactivate human norovirus surrogates at RT over 1 h for application in the food industry and prevent human noroviral transmission.

References

Adler, J.M., Geornaras, I., Byelashov, O.A., Belk, K.E., Smith, G.C., Sofos, J.N., 2011. Survival of *Escherichia coli* O157: H7 in meat product brines containing antimicrobials. Journal of food science 76, M478-M485.

Ali, B.H., Wabel, N.A., Blunden, G., 2005. Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. Phytotherapy research 19, 369-375.

Allwood, P.B., Malik, Y.S., Hedberg, C.W., Goyal, S.M., 2004. Effect of temperature and sanitizers on the survival of feline calicivirus, *Escherichia coli*, and F-specific coliphage MS2 on leafy salad vegetables. Journal of Food Protection® 67, 1451-1456.

Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., López, M., 2009. Comparison of acids on the induction of an Acid Tolerance Response in *Salmonella* typhimurium, consequences for food safety. Meat science 81, 65-70.

Anipsitakis, G.P., Tufano, T.P., Dionysiou, D.D., 2008. Chemical and microbial decontamination of pool water using activated potassium peroxymonosulfate. Water research 42, 2899-2910.

Ansorg, R., Rochus, W., 1978. Studies on the antimicrobial effect of natural and synthetic humic acids (author's transl)]. Arzneimittel-Forschung 28, 2195-2198.

Atmar, R.L., Opekun, A.R., Gilger, M.A., Estes, M.K., Crawford, S.E., Neill, F.H., Graham, D.Y., 2008. Norwalk virus shedding after experimental human infection. Emerging infectious diseases 14, 1553.

Back, S.-Y., Jin, H.-H., Lee, S.-Y., 2009. Inhibitory effect of organic acids against *Enterobacter sakazakii* in laboratory media and liquid foods. Food Control 20, 867-872.

Baert, L., Debevere, J., Uyttendaele, M., 2009. The efficacy of preservation methods to inactivate foodborne viruses. International journal of food microbiology 131, 83-94.

Barker, J., Vipond, I., Bloomfield, S., 2004. Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. Journal of Hospital Infection 58, 42-49.

Bastiani L. Antibacterial, antifungal and antiprotozoal action of a preparation based on benzalkonium chloride. Farmaco-Edizione Pratica 1974;29:594-601.Cliver, D.O., Riemann, H.P., 2002. Foodborne Diseases. Academic Press, London.

Bélec, L., Tevi-Benissan, C., Bianchi, A., Cotigny, S., Beumont-Mauviel, M., Si-Mohamed, A., Malkin, J.-E., 2000. *In vitro* inactivation of *Chlamydia trachomatis* and of a panel of DNA (HSV-2, CMV, adenovirus, BK virus) and RNA (RSV, enterovirus) viruses by the spermicide benzalkonium chloride. Journal of Antimicrobial Chemotherapy 46, 685-693.

Belliot, G., Lavaux, A., Souihel, D., Agnello, D., Pothier, P., 2008. Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. Applied and environmental microbiology 74, 3315-3318.

Bentley, K., Dove, B., Parks, S., Walker, J., Bennett, A., 2011. Hydrogen peroxide vapour decontamination of surfaces artificially contaminated with norovirus surrogate feline calicivirus. Journal of Hospital Infection.

Braga, L., Shupp, J., Cummings, C., Jett, M., Takahashi, J., Carmo, L., Chartone-Souza, E., Nascimento, A., 2005. Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. Journal of ethnopharmacology 96, 335-339.

Carlson, B.A., Ruby, J., Smith, G.C., Sofos, J.N., Bellinger, G.R., Warren-Serna, W., Centrella, B., Bowling, R.A., Belk, K.E., 2008. Comparison of antimicrobial efficacy of multiple beef hide

decontamination strategies to reduce levels of *Escherichia coli* O157: H7 and *Salmonella*. Journal of Food Protection® 71, 2223-2227.
Casteel, M.J., Schmidt, C.E., Sobsey, M.D., 2008. Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2. International journal of food microbiology 125, 267-273.
Caul, E. O. 1994. Small structured viruses: airborne transmission and hospital control. Lancet 343:1240–1242.
Centers for Disease Control and Prevention. CDC 2006. Technical information: Division of bacterial and mycotic diseases. Available at: http://www.cdc.gov/ncidod/DBMD/diseaseinfo.
Chen, C.-C., Hsu, J.-D., Wang, S.-F., Chiang, H.-C., Yang, M.-Y., Kao, E.-S., Ho, Y.-C., Wang, C.-J., 2003. Hibiscus sabdariffa extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. Journal of Agricultural and Food Chemistry 51, 5472-5477.
Cliver, D.O., 2009. Capsid and infectivity in virus detection. Food and environmental virology 1, 123-128.
Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical microbiology reviews 12, 564-582.
D'Souza, D.H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C., Jaykus, L.A., 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. International journal of food microbiology 108, 84-91.
D'Souza, D.H., Moe, C.L., Jaykus, L.. 2007. Foodborne viral pathogens. In: Doyle, M.P., Beuchat, L.R. (Eds.), Food Microbiology, Fundamentals and Frontiers. ASM Press, Washington, D.C., pp. 581-607.
D'Souza, D.H., Su, X., 2010. Efficacy of chemical treatments against murine norovirus, feline calicivirus, and MS2 bacteriophage. Foodborne Pathogens and Disease 7, 319-326.
Davis, R., Zivanovic, S., D'Souza, D.H., Davidson, P.M., 2012. Effectiveness of chitosan on the inactivation of enteric viral surrogates. Food Microbiology.
Di Martino, B., Ceci, C., Di Profio, F., Marsilio, F., 2010. *In vitro* inactivation of feline calicivirus (FCV) by chemical disinfectants: resistance variation among field strains. Archives of virology 155, 2047-2051.
Dolin, R., Blacklow, N.R., DuPont, H., Buscho, R.F., Wyatt, R.G., Kasel, J.A., Hornick, R., Chanock, R.M., 1972. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis, Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, NY). Royal Society of Medicine, pp. 578-583.
Donaldson, E.F., Lindesmith, L.C., Lobue, A.D., Baric, R.S., 2008. Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. Immunological reviews 225, 190-211.
Donaldson, E.F., Lindesmith, L.C., LoBue, A.D., Baric, R.S., 2010. Viral shape-shifting: norovirus evasion of the human immune system. Nature Reviews Microbiology 8, 231-241.
Doultree, J., Druce, J., Birch, C., Bowden, D., Marshall, J., 1999. Inactivation of feline calicivirus, a Norwalk virus surrogate. Journal of Hospital Infection 41, 51-57.
Duizer, E., Bijkerk, P., Rockx, B., De Groot, A., Twisk, F., Koopmans, M., 2004. Inactivation of caliciviruses. Applied and environmental microbiology 70, 4538-4543.
Dunnick, J.K., Melnick, R.L., 1993. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. Journal of the National Cancer Institute 85, 817-822.
Eleraky, N.Z., Potgieter, L.N., Kennedy, M.A., 2002. Virucidal efficacy of four new disinfectants. Journal of the American Animal Hospital Association 38, 231-234.

Farkas, T., Cross, R.W., Hargitt, E., Lerche, N.W., Morrow, A.L., Sestak, K., 2010. Genetic diversity and histo-blood group antigen interactions of rhesus enteric caliciviruses. Journal of virology 84, 8617-8625.
Farkas, T., Sestak, K., Wei, C., Jiang, X., 2008. Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. Journal of virology 82, 5408-5416.
Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., Vialette, M., Morin, T., Perelle, S., 2011. Comparison of chlorine and peroxyacetic-based disinfectant to inactivate Feline calicivirus, Murine norovirus and Hepatitis A virus on lettuce. International journal of food microbiology 151, 98-104.
Fransisca, L., Park, H.K., Feng, H., 2012. E. Coli O157: H7 Population Reduction from Alfalfa Seeds with Malic Acid and Thiamine Dilauryl Sulfate and Quality Evaluation of the Resulting Sprouts. Journal of food science.
Gehrke, C., Steinmann, J., Goroncy-Bermes, P., 2004. Inactivation of feline calicivirus, a surrogate of norovirus (formerly Norwalk-like viruses), by different types of alcohol in vitro and in vivo. Journal of Hospital Infection 56, 49-55.
Geornaras, I., Yang, H., Manios, S., Andritsos, N., Belk, K.E., Nightingale, K.K., Woerner, D.R., Smith, G.C., Sofos, J.N., 2012. Comparison of Decontamination Efficacy of Antimicrobial Treatments for Beef Trimmings against *Escherichia coli* O157: H7 and 6 Non‐O157 Shiga Toxin‐Producing E. coli Serogroups. Journal of food science.
Giacometti, A., Cirioni, O., Greganti, G., Fineo, A., Ghiselli, R., Del Prete, M., Mocchegiani, F., Fileni, B., Caselli, F., Petrelli, E., 2002. Antiseptic compounds still active against bacterial strains isolated from surgical wound infections despite increasing antibiotic resistance. European Journal of Clinical Microbiology & Infectious Diseases 21, 553-556.
Girard, M., Ngazoa, S., Mattison, K., Jean, J., 2010. Attachment of noroviruses to stainless steel and their inactivation, using household disinfectants. Journal of Food Protection® 73, 400-404.
Gradel, K., Sayers, A., Davies, R., 2004. Surface disinfection tests with *Salmonella* and a putative indicator bacterium, mimicking worst-case scenarios in poultry houses. Poultry Science 83, 1636-1643.
Green, K., Ando, T., Balayan, M., Berke, T., Clarke, I., Estes, M., Matson, D., Nakata, S., Neill, J., Studdert, M., 2000. Taxonomy of the caliciviruses. Journal of Infectious Diseases 181, S322-S330.
Green, K., Chanock, R., Kapikian, A., 2001. Human caliciviruses. Fields virology 1, 841-874.
Grohmann, G., Murphy, A., Christopher, P., Auty, E., Greenberg, H., 1981. Norwalk virus gastroenteritis in volunteers consuming depurated oysters. Aust. J. Exp. Biol. Med. Sci 59, 219-228.
Guentzel, J.L., Liang Lam, K., Callan, M.A., Emmons, S.A., Dunham, V.L., 2008. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. Food Microbiology 25, 36-41.
Gulati, B.R., Allwood, P.B., Hedberg, C.W., Goyal, S.M., 2001. Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. Journal of Food Protection® 64, 1430-1434.
Guo, M., Chang, K., Hardy, M., Zhang, Q., Parwani, A., Saif, L., 1999. Molecular characterization of a porcine enteric calicivirus genetically related to Sapporo-like human caliciviruses. Journal of virology 73, 9625-9631.

Haidari, M., Ali, M., Ward Casscells III, S., Madjid, M., 2009. Pomegranate (Punica granatum) purified polyphenol extract inhibits influenza virus and has a synergistic effect with oseltamivir. Phytomedicine 16, 1127-1136.
Haslam, E., 1974. Polyphenol–protein interactions (Short Communication). Biochemical journal 139, 285.
Hayden, G.F., Gwaltney, J.M., Thacker, D.F., Owen Hendley, J., 1985. Rhinovirus inactivation by nasal tissues treated with virucide. Antiviral research 5, 103-109.
Hirneisen, K., Markland, S., Kniel, K., 2011. Ozone Inactivation of Norovirus Surrogates on Fresh Produce. Journal of Food Protection® 74, 836-839.
Hirneisen, K.A., Kniel, K.E., 2013. Comparing Human Norovirus Surrogates: Murine Norovirus and Tulane Virus. Journal of Food Protection 76, 139-143.
Hudson, J., Sharma, M., Petric, M., 2007. Inactivation of Norovirus by ozone gas in conditions relevant to healthcare. Journal of Hospital Infection 66, 40-45.
Issa-Zacharia, A., Kamitani, Y., Morita, K., Iwasaki, K., 2010. Sanitization potency of slightly acidic electrolyzed water against pure cultures of *Escherichia coli* and *Staphylococcus* aureus, in comparison with that of other food sanitizers. Food Control 21, 740-745.
Jayaprakasha, G., Jaganmohan Rao, L., Sakariah, K., 2006. Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Food chemistry 98, 720-724.
Jean, J., Vachon, J.-F., Moroni, O., Darveau, A., Kukavica-Ibrulj, I., Fliss, I., 2003. Effectiveness of commercial disinfectants for inactivating hepatitis A virus on agri-food surfaces. Journal of Food Protection 66, 115-119.
Jerzy, L., Bradley, M.L., Scott, T.M., Mabel, D., Andrew, K., Wei-Yea, H., Bartz, J.A., Farrah, S.R., 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157: H7 on strawberries by physical and disinfectant washes. Journal of Food Protection® 66, 188-193.
Jiang, X., Wang, M., Wang, K., Estes, M.K., 1993. Sequence and genomic organization of Norwalk virus. Virology 195, 51-61.
Jimenez, L., Chiang, M., 2006. Virucidal activity of a quaternary ammonium compound disinfectant against feline calicivirus: A surrogate for norovirus. American journal of infection control 34, 269-273.
Jira, T., Panzig, B., Pohloudek-Fabini, R., 1982. On the antimicrobial activity of benzalkonium chloride in potential contact lens fluids]. Die Pharmazie 37, 587.
Jonadet, M., Bastide, J., Bastide, P., Boyer, B., Carnat, A., Lamaison, J., 1990. *In vitro* enzyme inhibitory and in vivo cardioprotective activities of hibiscus (Hibiscus sabdariffa L.). Journal de pharmacie de Belgique 45, 120.
Kapikian, A.Z., Wyatt, R.G., Dolin, R., Thornhill, T.S., Kalica, A.R., Chanock, R.M., 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. Journal of virology 10, 1075-1081.
Kapikian, AZ, Estes MK, Chanock RM. 1996. Norwalk group of viruses. In: Fields BN, KnipeDM, Howley PM, editors. Fields virology, 3rd ed. Philadelphia: Lippincott- Raven Publishers. p 783-810.
Karst, S.M., Wobus, C.E., Lay, M., Davidson, J., Virgin IV, H.W., 2003. STAT1-dependent innate immunity to a Norwalk-like virus. Science Signalling 299, 1575.
Kim, C.K., Gentile, D.M., Sproul, O.J., 1980. Mechanism of ozone inactivation of bacteriophage f2. Applied and environmental microbiology 39, 210-218.

Kim, J.-G., Yousef, A.E., Dave, S., 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. Journal of Food Protection® 62, 1071-1087.
Kitajima, M., Tohya, Y., Matsubara, K., Haramoto, E., Utagawa, E., Katayama, H., 2010. Chlorine inactivation of human norovirus, murine norovirus and poliovirus in drinking water. Letters in applied microbiology 51, 119-121.
Klöcking, R., Helbig, B., Schötz, G., Schacke, M., Wutzler, P., 2002. Anti-HSV-1 activity of synthetic humic acid-like polymers derived from p-diphenolic starting com-pounds. Antiviral chemistry & chemotherapy 13, 241-249.
Konowalchuk, J., Speirs, J.I., 1976. Virus inactivation by grapes and wines. Applied and environmental microbiology 32, 757-763.
Koseki, S., Yoshida, K., Isobe, S., Itoh, K., 2001. Decontamination of lettuce using acidic electrolyzed water. Journal of Food Protection® 64, 652-658.
Koseki, S., Yoshida, K., Kamitani, Y., Itoh, K., 2003. Influence of inoculation method, spot inoculation site, and inoculation size on the efficacy of acidic electrolyzed water against pathogens on lettuce. Journal of Food Protection® 66, 2010-2016.
Kotwal, G.J., 2008. Genetic diversity-independent neutralization of pandemic viruses (eg HIV), potentially pandemic (eg H5N1 strain of influenza) and carcinogenic (eg HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCs). Vaccine 26, 3055-3058.
Kukkula, M., Maunula, L., Silvennoinen, E., von Bonsdorff, C.-H., 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. Journal of Infectious Diseases 180, 1771-1776.
Lages, S., Ramakrishnan, M., Goyal, S., 2008. In-vivo efficacy of hand sanitisers against feline calicivirus: a surrogate for norovirus. Journal of Hospital Infection 68, 159-163.
Lee, C.-J., Chen, L.-G., Liang, W.-L., Wang, C.-C., 2010. Anti-inflammatory effects of Punica granatum Linne *in vitro* and *in vivo*. Food chemistry 118, 315-322.
Len, S.-V., Hung, Y.-C., Erickson, M., Kim, C., 2000. Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. Journal of Food Protection® 63, 1534-1537.
Li, D., Baert, L., De Jonghe, M., Van Coillie, E., Ryckeboer, J., Devlieghere, F., Uyttendaele, M., 2011. Inactivation of Murine Norovirus 1, Coliphage φX174, and Bacillus fragilis Phage B40-8 on Surfaces and Fresh-Cut Iceberg Lettuce by Hydrogen Peroxide and UV Light. Applied and environmental microbiology 77, 1399-1404.
Li, J., Predmore, A., Divers, E., Lou, F., 2012. New interventions against human norovirus: progress, opportunities, and challenges. Annual review of food science and technology 3, 331-352.
Li, J.W., Xin, Z.T., Wang, X.W., Zheng, J.L., Chao, F.H., 2002. Mechanisms of inactivation of hepatitis A virus by chlorine. Applied and environmental microbiology 68, 4951-4955.
Liao, L.B., Chen, W.M., Xiao, X.M., 2007. The generation and inactivation mechanism of oxidation–reduction potential of electrolyzed oxidizing water. Journal of Food Engineering 78, 1326-1332.
Lim, M.Y., Kim, J.-M., Lee, J.E., Ko, G., 2010. Characterization of ozone disinfection of murine norovirus. Applied and environmental microbiology 76, 1120-1124.
Lindsay, R.C. 1985. Food additives. In Food Chemistry, 2nd Ed., (O.R.Fennema, ed.) pp. 629–687, Marcel Dekker, New York.

Lipson, S.M., Cohen, P., Zhou, J., Burdowski, A., Stotzky, G., 2007. Cranberry cocktail juice, cranberry concentrates, and proanthocyanidins reduce reovirus infectivity titers in African green monkey kidney epithelial cell cultures. Molecular nutrition & food research 51, 752-758.
Liu, P., Chien, Y.-W., Papafragkou, E., Hsiao, H.-M., Jaykus, L.-A., Moe, C., 2009. Persistence of human noroviruses on food preparation surfaces and human hands. Food and environmental virology 1, 141-147.
Lloyd-Evans, N., Springthorpe, V.S., Sattar, S.A., 1986. Chemical disinfection of human rotavirus-contaminated inanimate surfaces. Epidemiology and infection 97, 163-173.
Macinga, D.R., Sattar, S.A., Jaykus, L.-A., Arbogast, J.W., 2008. Improved inactivation of nonenveloped enteric viruses and their surrogates by a novel alcohol-based hand sanitizer. Applied and environmental microbiology 74, 5047-5052.
Magulski, T., Paulmann, D., Bischoff, B., Becker, B., Steinmann, E., Steinmann, J., Goroncy-Bermes, P., Steinmann, J., 2009. Inactivation of murine norovirus by chemical biocides on stainless steel. BMC infectious diseases 9, 107.
Malik, Y.S., Goyal, S.M., 2006. Virucidal efficacy of sodium bicarbonate on a food contact surface against feline calicivirus, a norovirus surrogate. International journal of food microbiology 109, 160-163.
Malik, Y.S., Maherchandani, S., Goyal, S.M., 2006. Comparative efficacy of ethanol and isopropanol against feline calicivirus, a norovirus surrogate. American journal of infection control 34, 31-35.
Marple, B., Roland, P., Benninger, M., 2004. Safety review of benzalkonium chloride used as a preservative in intranasal solutions: an overview of conflicting data and opinions. Otolaryngology-Head and Neck Surgery 130, 131-141.
Matsushima, M., Suzuki, T., Masui, A., Kasai, K., Kouchi, T., Takagi, A., Shirai, T., Mine, T., 2008. Growth inhibitory action of cranberry on *Helicobacter pylori*. Journal of gastroenterology and hepatology 23, S175-S180.
Mbithi, J.N., Springthorpe, V.S., Sattar, S.A., 1990. Chemical disinfection of hepatitis A virus on environmental surfaces. Applied and environmental microbiology 56, 3601-3604.
McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clinical microbiology reviews 12, 147-179.
Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. Emerging infectious diseases 5, 607.
Moadab, A., Rupley, K.F., Wadhams, P., 2001. Effectiveness of a nonrinse, alcohol-free antiseptic hand wash. Journal of the American Podiatric Medical Association 91, 288-293.
Mochizuki, M., Kawanishi, A., Sakamoto, H., Tashiro, S., Fujimoto, R., Ohwaki, M., 1993. A calicivirus isolated from a dog with fatal diarrhoea. The Veterinary record 132, 221.
Neurath, A.R., Strick, N., LI, Y.Y., Debnath, A.K., 2005. Punica granatum (Pomegranate) Juice Provides an HIV−1 Entry Inhibitor and Candidate Topical Microbicide. Annals of the New York Academy of Sciences 1056, 311-327.
Nogueira, M.C., Oyarzabal, O.A., Gombas, D.E., 2003. Inactivation of *Escherichia coli* O157: H7, *Listeria monocytogenes*, and *Salmonella* in cranberry, lemon, and lime juice concentrates. Journal of Food Protection® 66, 1637-1641.
Oboh, G., Elusiyan, C., 2004. Nutrient composition and antimicrobial activity of sorrel drinks (soborodo). Journal of medicinal food 7, 340-342.

Odigie, I., Ettarh, R., Adigun, S., 2003. Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. Journal of ethnopharmacology 86, 181-185.
Oh, M., Bae, S.Y., Lee, J.-H., Cho, K.J., Kim, K.H., Chung, M.S., 2012. Antiviral Effects of Black Raspberry (Rubus coreanus) Juice on Foodborne Viral Surrogates. Foodborne Pathogens and Disease.
Olaleye, M.T., 2007. Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. Journal of Medicinal Plants Research 1, 9-13.
Park, C.M., Hung, Y.C., Doyle, M., Ezeike, G., Kim, C., 2006. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. Journal of food science 66, 1368-1372.
Park, E.-J., Alexander, E., Taylor, G.A., Costa, R., Kang, D.-H., 2009. The decontaminative effects of acidic electrolyzed water for *Escherichia coli* O157: H7, *Salmonella* typhimurium, and *Listeria monocytogenes* on green onions and tomatoes with differing organic demands. Food Microbiology 26, 386-390.
Park, E.J., Alexander, E., Taylor, G., Costa, R., Kang, D.H., 2008. Effect of electrolyzed water for reduction of foodborne pathogens on lettuce and spinach. Journal of food science 73, M268-M272.
Park, G.W., Barclay, L., Macinga, D., Charbonneau, D., Pettigrew, C.A., Vinje, J., 2010. Comparative efficacy of seven hand sanitizers against murine norovirus, feline calicivirus, and GII. 4 norovirus. Journal of Food Protection® 73, 2232-2238.
Park, H., Hung, Y.-C., Chung, D., 2004. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes*. International journal of food microbiology 91, 13-18.
Passagot, J., Crance, J., Biziagos, E., Laveran, H., Agbalika, F., Deloince, R., 1987. Effect of glutaraldehyde on the antigenicity and infectivity of hepatitis A virus. Journal of virological methods 16, 21-28.
Patel, M.M., Hall, A.J., Vinje, J., Parashara, U.D., 2009. Noroviruses: A comprehensive review. Journal of clinical virology 44, 1-8.
Patterson, G., Morley, P.S., Blehm, K.D., Lee, D.E., Dunowska, M., 2005. Efficacy of directed misting application of a peroxygen disinfectant for environmental decontamination of a veterinary hospital. Journal of the American Veterinary Medical Association 227, 597-602.
Patterson, W., Haswell, P., Fryers, P., Green, J., 1997. Outbreak of small round structured virus gastroenteritis arose after kitchen assistant vomited. Communicable disease report. CDR review 7, R101.
Pliego, M.P.C., 2007. Effect of natural antimicrobials against *Salmonella*, *Escherichia coli* O157: H7 and *Listeria monocytogenes*. Texas A&M University.
Pohlman, F., DIAS‐MORSE, P., Quilo, S., Brown Jr, A., Crandall, P., Baublits, R., Story, R., Bokina, C., Rajaratnam, G., 2009. Microbial, instrumental color and sensory characteristics of ground beef processed from beef trimmings treated with potassium lactate, sodium metasilicate, peroxyacetic acid or acidified sodium chlorite as single antimicrobial interventions. Journal of Muscle Foods 20, 54-69.
Poschetto, L.F., Ike, A., Papp, T., Mohn, U., Böhm, R., Marschang, R.E., 2007. Comparison of the sensitivities of noroviruses and feline calicivirus to chemical disinfection under field-like conditions. Applied and environmental microbiology 73, 5494-5500.

Prashanth, D., Asha, M., Amit, A., 2001. Antibacterial activity of Punica granatum. Fitoterapia 72, 171-173.
Pratelli, A., 2007. Action of Disinfectants on Canine Coronavirus Replication In Vitro. Zoonoses and public health 54, 383-386.
Radford, A.D., Coyne, K.P., Dawson, S., Porter, C.J., Gaskell, R.M., 2007. Feline calicivirus. Veterinary research 38, 319-335.
Reddy, M.K., Gupta, S.K., Jacob, M.R., Khan, S.I., Ferreira, D., 2007. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. Planta Medica-Natural Products and Medicinal Plant Research 73, 461-467.
Riazi, S., Matthews, K.R., 2011. Failure of foodborne pathogens to develop resistance to sanitizers following repeated exposure to common sanitizers. International Biodeterioration & Biodegradation 65, 374-378.
Rodger, S.M., Schnagl, R.D., Holmes, I., 1977. Further biochemical characterization, including the detection of surface glycoproteins, of human, calf, and simian rotaviruses. Journal of virology 24, 91-98.
Roman E. 2006. Personal communication. Universidad Veracruzana, Orizaba, Ver., México
Roy, D., Wong, P., Engelbrecht, R., Chian, E., 1981. Mechanism of enteroviral inactivation by ozone. Applied and environmental microbiology 41, 718-723.
Sair, A., D'Souza, D., Jaykus, L., 2002. Human enteric viruses as causes of foodborne disease. Comprehensive reviews in food science and food safety 1, 73-89.
Salo, R., Cliver, D., 1976. Effect of acid pH, salts, and temperature on the infectivity and physical integrity of enteroviruses. Archives of virology 52, 269-282.
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17.
Schneider, J., Weis, R., Manner, C., Kary, B., Werner, A., Seubert, B.J., Riede, U.N., 1996. Inhibition of HIV-1 in cell culture by synthetic humate analogues derived from hydroquinone: mechanism of inhibition. Virology 218, 389-395.
Schultz-Cherry, S., King, D.J., Koci, M.D., 2001. Inactivation of an astrovirus associated with poult enteritis mortality syndrome. Avian Diseases, 76-82.
Sharma, C., Williams, S., Schneider, K., Schmidt, R., Rodrick, G., 2012a. Sodium metasilicate affects growth of *Campylobacter jejuni* in fresh, boneless, uncooked chicken breast fillets stored at 4 degrees Celsius for 7 days1, 2. Poultry Science 91, 2324-2329.
Sharma, C., Williams, S., Schneider, K., Schmidt, R., Rodrick, G., 2012b. Sodium metasilicate affects growth of *Salmonella* Typhimurium in fresh, boneless, uncooked chicken breast fillets stored at 4° C for 7 days. Poultry Science 91, 719-723.
Sharaf A, Geneidi A, Negm S. 1966. Further study on the antibacterial effect of H. sabdariffa. Path Microbiol 29(1):120-25
Shin, G.-A., Sobsey, M.D., 2003. Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. Applied and environmental microbiology 69, 3975-3978.
Siebenga, J.J., Lemey, P., Pond, S.L.K., Rambaut, A., Vennema, H., Koopmans, M., 2010. Phylodynamic reconstruction reveals norovirus GII. 4 epidemic expansions and their molecular determinants. PLoS pathogens 6, e1000884.
Siebenga, J.J., Vennema, H., Zheng, D.-P., Vinjé, J., Lee, B.E., Pang, X.-L., Ho, E.C., Lim, W., Choudekar, A., Broor, S., 2009. Norovirus illness is a global problem: emergence and spread of norovirus GII. 4 variants, 2001–2007. Journal of Infectious Diseases 200, 802-812.

Slomka, M., Appleton, H., 1998. Feline calicivirus as a model system for heat inactivation studies of small round structured viruses in shellfish. Epidemiology and infection 121, 401-407.
Solomon, E.B., Fino, V., Wei, J., Kniel, K.E., 2009. Comparative susceptibilities of hepatitis A virus, feline calicivirus, bacteriophage MS2 and bacteriophage ΦX-174 to inactivation by quaternary ammonium and oxidative disinfectants. International journal of antimicrobial agents 33, 288-289.
Springthorpe, V.S., Grenier, J.L., Lloyd-Evans, N., Sattar, S.A., 1986. Chemical disinfection of human rotaviruses: efficacy of commercially-available products in suspension tests. Epidemiology and infection 97, 139-161.
Straube, J., Albert, T., Manteufel, J., Heinze, J., Fehlhaber, K., Truyen, U., 2011. In vitro influence of d/l-lactic acid, sodium chloride and sodium nitrite on the infectivity of feline calicivirus and of ECHO virus as potential surrogates for foodborne viruses. International journal of food microbiology 151, 93-97.
Su, X., D'Souza, D.H., 2011. Trisodium phosphate for foodborne virus reduction on produce. Foodborne Pathogens and Disease 8, 713-717.
Su, X., D'Souza, D.H., 2012. Inactivation of Human Norovirus Surrogates by Benzalkonium Chloride, Potassium Peroxymonosulfate, Tannic Acid, and Gallic Acid. Foodborne Pathogens and Disease.
Su, X., Howell, A.B., D'Souza, D.H., 2010a. The effect of cranberry juice and cranberry proanthocyanidins on the infectivity of human enteric viral surrogates. Food Microbiology 27, 535-540.
Su, X., Howell, A.B., D'Souza, D.H., 2010b. Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–A time dependence study in vitro. Food Microbiology 27, 985-991.
Su, X., Sangster, M.Y., D'Souza, D.H., 2010c. *In vitro* effects of pomegranate juice and pomegranate polyphenols on foodborne viral surrogates. Foodborne Pathogens and Disease 7, 1473-1479.
Su, X., Sangster, M.Y., D'Souza, D.H., 2011. Time-dependent effects of pomegranate juice and pomegranate polyphenols on foodborne viral reduction. Foodborne Pathogens and Disease 8, 1177-1183.
Tan, M., Jiang, X., 2010. Norovirus gastroenteritis, carbohydrate receptors, and animal models. PLoS pathogens 6, e1000983.
Taube, S., Perry, J.W., Yetming, K., Patel, S.P., Auble, H., Shu, L., Nawar, H.F., Lee, C.H., Connell, T.D., Shayman, J.A., 2009. Ganglioside-linked terminal sialic acid moieties on murine macrophages function as attachment receptors for murine noroviruses. Journal of virology 83, 4092-4101.
Teunis, P.F., Moe, C.L., Liu, P., E Miller, S., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon, R.L., 2008. Norwalk virus: How infectious is it? Journal of medical virology 80, 1468-1476.
Tian, P., Yang, D., Mandrell, R., 2011. Differences in the binding of human norovirus to and from Romaine lettuce and raspberries by water and electrolyzed waters. Journal of Food Protection 74, 1364-1369.
Tsai, P.-J., McIntosh, J., Pearce, P., Camden, B., Jordan, B.R., 2002. Anthocyanin and antioxidant capacity in Roselle (Hibiscus Sabdariffa L.) extract. Food Research International 35, 351-356.
Tuladhar, E., Terpstra, P., Koopmans, M., Duizer, E., 2012. Virucidal efficacy of hydrogen peroxide vapour disinfection. Journal of Hospital Infection 80, 110-115.

Urakami, H., Ikarashi, K., Okamoto, K., Abe, Y., Ikarashi, T., Kono, T., Konagaya, Y., Tanaka, N., 2007. Chlorine sensitivity of feline calicivirus, a norovirus surrogate. Applied and environmental microbiology 73, 5679-5682.
USDA FSIS (United States Department of Agriculture Food Safety and Inspection Service). 2011. FSIS Directives. Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products. 7120.1, Revision 5. Accessed Aug. 22, 2011. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.pdf.
Van Engelenburg, F., Terpstra, F., Schuitemaker, H., Moorer, W., 2002. The virucidal spectrum of a high concentration alcohol mixture. Journal of Hospital Infection 51, 121-125.
Vasseur, C., Baverel, L., Hebraud, M., Labadie, J., 2001. Effect of osmotic, alkaline, acid or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. J. Appl. Microbiol. 86, 469-476.
Wang, C.-J., Wang, J.-M., Lin, W.-L., Chu, C.-Y., Chou, F.-P., Tseng, T.-H., 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association 38, 411.
Weber, G.H., Obrien, J.K., Bender, F.G., 2004. Control of *Escherichia coli* O157: H7 with sodium metasilicate. Journal of Food Protection® 67, 1501-1506.
Weinstein, R.A., Said, M.A., Perl, T.M., Sears, C.L., 2008. Gastrointestinal flu: norovirus in health care and long-term care facilities. Clinical infectious diseases 47, 1202-1208.
Weiss, E., Houri-Haddad, Y., Greenbaum, E., Hochman, N., Ofek, I., Zakay-Rones, Z., 2005. Cranberry juice constituents affect influenza virus adhesion and infectivity. Antiviral research 66, 9-12.
Whitehead, K., McCue, K.A., 2010. Virucidal efficacy of disinfectant actives against feline calicivirus, a surrogate for norovirus, in a short contact time. American journal of infection control 38, 26-30.
Wobus, C.E., Thackray, L.B., Virgin, H.W., 2006. Murine norovirus: a model system to study norovirus biology and pathogenesis. Journal of virology 80, 5104-5112.
Wood, A., Payne, D., 1998. The action of three antiseptics/disinfectants against enveloped and non-enveloped viruses. Journal of Hospital Infection 38, 283-295.
Wright, H.S., 1970. Inactivation of Vesicular stomatitis virus by disinfectants. Applied microbiology 19, 96-99.
Wu, V.C.-H., Qiu, X., Bushway, A., Harper, L., 2008. Antibacterial effects of American cranberry (Vaccinium macrocarpon) concentrate on foodborne pathogens. LWT-Food Science and Technology 41, 1834-1841.
Xi, J., Graham, D.Y., Wang, K., Estes, M.K., 1990. Norwalk virus genome cloning and characterization. Science (New York, NY) 250, 1580.
Zeng, X., Tang, W., Ye, G., Ouyang, T., Tian, L., Ni, Y., Li, P., 2010. Studies on disinfection mechanism of electrolyzed oxidizing water on E. coli and *Staphylococcus aureus*. Journal of food science 75, M253-M260.
Zhang, X.-F., Dai, Y.-C., Zhong, W., Tan, M., Zhou, Y.-C., Jiang, X., 2011. Tannic acid inhibited norovirus binding to HBGA receptors, a study of 50 Chinese medicinal herbs. Bioorganic & medicinal chemistry 20, 1616-1623.
Zheng, D.-P., Ando, T., Fankhauser, R.L., Beard, R.S., Glass, R.I., Monroe, S.S., 2006. Norovirus classification and proposed strain nomenclature. Virology 346, 312-323.

Appendix

Table 1.1： Examples of studies using chlorine against FCV-F9 and MNV-1

Viruses	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	15ppm (lettuce)	2 min	10^7 $TCID_{50}$/mL	2.9	Plaque assay and qRT-PCR	Fraisse et al. 2011
	3,000ppm (suspension)	10 min	2 x 10^5~ 1 x 10^6 $TCID_{50}$/mL	>5 log units	RT-PCR and qRT-PCR	Duizer et al. 2004
	200 mg/L= 200 ppm (lettuce and pepper)	15 s or 30 s	~5 log_{10} PFU/mL	To undetectable levels	Plaque assay	Su and D'Souza, 2011
			~7 log_{10} PFU/mL	≤1.4		
	5,000ppm (surface)	30 s or 1 min	~5 log_{10} PFU/mL	~5	Plaque assay	D'Souza and Su 2010
			~7 log_{10} PFU/mL	>6		
	300 ng/mL= 0.3ppm (suspension)	5 min	2.6 x 10^6 TCID	>4.6 log units	$TCID_{50}$	Urakami et al. 2007
	100ppm, 1000ppm (surface)	1 min	NR	<2.27, >4.2	Plaque assay	Whitehead and McCue, 2010
	100, 1000 ppm (surface)	10 min	NR	3.2, 6.6	Plaque assay	Jimenez and Chiang, 2006
	200, 400, 800, 5000ppm (stainless steel, lettuce and strawberry)	1-10 min	NR	<800 ppm <1 (ss) 5000 ppm 3.4 (ss) 800 ppm 1.5 (ls)	Plaque assay	Gulati et al., 2001
	100, 250, 500, 1000 and 5000 ppm (suspension)	1 min	NR	<500 ppm <3 5000 ppm 5	$TCID_{50}$	Doultree et al., 1999
	4500-5000 ppm (suspension)	15 min	NR	Without feces: >5	Plaque assay and RT-PCR	Poschetto et al., 2007
	5500 ppm	15 min	NR	With feces: 3		
	5000 ppm (fecally soiled stainless steel surface)	1.9 min	NR	3	Plaque assay	Park and Sobsey, 2011

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction
$TCID_{50}$= Tissue culture infectious dose 50%
ss = stainless steel; ls = lettuce and strawberry

Table 1.1：Examples of studies using chlorine against FCV-F9 and MNV-1 (Continued)

Viruses	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
MNV-1	15ppm (lettuce)	2 min	3 x 10^6 $TCID_{50}$/mL	1.4	Plaque assay and qRT-PCR	Fraisse et al. 2011
	0.1 mg/L	120 min	NR	>4	Plaque assay and real-time RT-PCR	Kitajima et al. 2010
	0.5 mg/L	0.5 min	NR	>4		
	0.26%= 2600 ppm (suspension)	0.5 min	NR	>4	Plaque assay and real-time RT-PCR	Belliot et al. 2008
	200mg/L (lettuce and pepper)	15 s or 30 s	~5 log_{10} PFU/mL	2~3	Plaque assay	Su and D'Souza 2011
			~7 log_{10} PFU/mL	<1.3		
	3000ppm (surface)	5 min or 10 min	NR	Complete inactivation	Plaque assay and real-time RT-PCR	Girard et al., 2010
				4		
	5000ppm (fecally soiled stainless steel surface)	3.2 min	NR	3	Plaque assay	Park and Sobsey, 2011

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction

Table 1.2: Examples of studies using ethanol, 1-propanol and 2-propanol against FCV-F9 and MNV-1

Viruses	Chemical	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	**Ethanol**	70%	10min	4.5 log_{10} PFU/mL	>4	nested RT-PCR	Martino et al. 2010
		70%	30min	2 x 10^5~ 1 x 10^6 $TCID_{50}$/mL	3	RT-PCR and qRT-PCR	Duizer et al. 2004
		70%	30s or 1min	~5 log_{10} PFU/mL ~7 log_{10} PFU/mL	Not effective	Plaque assay	D'Souza and Su, 2010
		70% and 90%	1min	10^7 $TCID_{50}$/mL	Most effective, killing 99% of FCV	$TCID_{50}$	Malik et al. 2006
		67%	1min	NR	Most effective	Plaque assay	Gehrke et al. 2004
		70%	30s	NR	≥4.75	Plaque assay	Macinga et al. 2008
		75%	1min	~5 log_{10} PFU/mL	1.25	$TCID_{50}$	Doultree et al. 1999
		60% (surface)	1min	NR	1.3	Plaque assay	Whitehead and McCue 2010
		50% or 70%	5min	NR	≥2.2	Plaque assay	Park et al., 2010
		99.5%, 62% (fingertips)	30s or 2min	NR	≥1, <1	Fingerpad and Plaque assay	Lages et al. 2008
MNV-1		70%	30s or 1min	~5 log_{10} PFU/mL ~7 log_{10} PFU/mL	0	Plaque assay	D'Souza and Su. 2010
		70%, 90%	5min	NR	≥3.6	Plaque assay	Park et al., 2010
		60%	30s, 1min or 3min	NR	>4	Plaque assay and RT-qPCR	Belliot et al. 2008
		50% (surface)	5 min	NR	≥4	Plaque assay	Magulski et al. 2009

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction
$TCID_{50}$= Tissue culture infectious dose 50%

Table 1.2: Examples of studies using ethanol, 1-propanol and 2-propanol against FCV-F9 and MNV-1 (Continued)

Viruses	Chemical	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	**1-propanol**	50% and 70%	30s	NR	4	Plaque assay	Gehrke et al. 2004
		60% (suspension)	30s		Most effective		
MNV-1		30% (surface)	5 min	NR	≥4	Plaque assay	Magulski et al. 2009
FCV-F9	**2-propanol**	40% to 60%	1 min	10^7 $TCID_{50}$/mL	most effective, killing 99%	$TCID_{50}$	Malik et al. 2006
		50%, 70% or 90%	1 min or 5 min	NR	almost no	Plaque assay	Park et al., 2010
		58% (suspension)	1 min	NR	reduction	Plaque assay	Gehrke et al. 2004
					Most effective		
MNV-1		≥70%	5 min	NR	>2.6	Plaque assay	Park et al., 2010
		60%	30 s, 1 min or 3 min	NR	3.86 (30 s), >4	Plaque assay and qRT-PCR	Belliot et al. 2008
		60% (surface)	5 min	NR	3.02	Plaque assay	Magulski et al. 2009

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction
$TCID_{50}$= Tissue culture infectious dose 50%

Table 1.3: Examples of studies using glutaraldehyde against FCV-F9 and MNV-1

Viruses	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	2% (surface)	30 s or 1 min	~5 log_{10} PFU/mL	≥4	Plaque assay	D'Souza and Su 2010
			~7 log_{10} PFU/mL	≥6		
	0.5% (suspension)	1 min	~5 log_{10} PFU/mL	~5	Plaque assay	Doultree et al. 1999
	2.6% (fabrics and carpets)	1-10 min	NR	>99.99%	$TCID_{50}$	Malik et al., 2006
MNV-1	2% (surface)	30 s or 1 min	~5 log_{10} PFU/mL	≥4	Plaque assay	D'Souza and Su 2010
			~7 log_{10} PFU/mL	≥6		
	2500ppm (stainless steel)	5 min	NR	≥4	Plaque assay	Magulski et al., 2009

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction
$TCID_{50}$= Tissue culture infectious dose 50%

Table 1.4: Examples of studies using organic acids against FCV-F9 and MNV-1

Viruses	Chemical	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	**Formic acid and glyoxylic acid**	55~60% (FA) + 7% (GA)	15 min 15 min or 60 min	NR	≥5 2 to 3 log units	Plaque assay RT-PCR	Poschetto et al., 2007
	Peroxyacetic acid (lettuce leaves)	100 ppm	2 min	NR	3.2	Plaque assay	Fraisse et al., 2011
	Citric acid	2.5%	1 min	NR	>3.17	Plaque assay	Whitehead and McCue, 2010
	Tannic acid	0.2 mg/mL	2 h	~5log_{10}PFU/mL ~7log_{10}PFU/mL	1.95 <1	Plaque assay	Su and D'Souza, 2012
	Gallic acid	0.1, 0.2, and 0.4 mg/mL		~5log_{10}PFU/mL ~7log_{10}PFU/mL	2.50, 2.36 and 0.86 Little effect		
MNV-1	**Peroxyacetic acid (lettuce leaves)**	100 ppm	2 min	NR	2.3	Plaque assay	Fraisse et al., 2011

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction
RT-PCR= Reverse transcription- polymerase chain reaction

Table 1.5: Examples of studies using ozone and hydrogen peroxide against FCV-F9 and MNV-1

Viruses	Chemical	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	**Ozone**	6.25 ppm (water)	5 min	NR	>6 log $TCID_{50}$/mL	$TCID_{50}$	Hirneisen et al. 2011
		6.25 ppm (produce)			~2 log $TCID_{50}$/mL		
		20- 25 ppm	< 1 h	NR	>3	qRT-PCR and plaque assay	Hudson et al. 2007
MNV-1		1 mg/liter	2 min	NR	>99%	$TCID_{50}$	Lim et al. 2010
		6.25 ppm (produce)	1 min	NR	>2 log TCID50/mL	$TCID_{50}$	Hirneisen et al. 2011
FCV-F9	**Hydrogen peroxide**	V- H_2O_2 30% (w/w) (carriers)	20 min	NR	4	Plaque assay	Bentley et al. 2011
MNV-1		L-H_2O_2 2.1% (stainless steel)	10 min	NR	~4	Plaque assay	Li et al. 2010
			5 min		<1	Plaque assay	
		V- H_2O_2 2.52% V- H_2O_2 127 ppm (carriers)	1 h	NR	To undetectable levels	Plaque assay	Tuladhar et al. 2012

NR= Not Reported
qRT-PCR= Quantitative reverse transcription- polymerase chain reaction
$TCID_{50}$= Tissue culture infectious dose 50%

Table 1.6: Examples of studies using trisodium phosphate against FCV-F9 and MNV-1

Viruses	Concentration	Contact time	Initial titer	Reduction in log_{10} PFU/mL	Method	Reference
FCV-F9	1%	30 s or 1 min	~ 7 log_{10} PFU/mL	~ 2.65	Plaque assay	D'Souza and Su, 2010
	2%	1 min		≥6		
	5%			≥6		
	1%		~ 5 log_{10} PFU/mL	~ 5		
	2%			~ 5		
	5%			~ 5		
	2%	15 s or 30 s	~ 7 log_{10} PFU/mL	>5		
	5%			To undetectable levels		
	2%		~ 5 log_{10} PFU/mL	To undetectable levels		
	5%			To undetectable levels		
MNV-1	1%	30s or 1 min	~7 log_{10} PFU/mL	0	Plaque assay	D'Souza and Su, 2010
	2%	1 min		1.05		
	5%			≥6		
	1%		~ 5 log_{10} PFU/mL	1.69		
	2%			~ 5		
	5%			~ 5		
	2%	15 s or 30 s	~ 7 log_{10} PFU/mL	~ 2 – 3.4	Plaque assay	Su and D'Souza, 2011
	5%			To undetectable levels		
	2%		~ 5 log_{10} PFU/mL	~ 2 - 3		
	5%			To undetectable levels		

NR= Not Reported

Table 1.7: Advantages, disadvantages and applications of disinfectants reviewed in this chapter

Chemicals	Advantages	Disadvantages	Applications	References
Chlorine	Low cost; easily available; broad spectrum against bacteria, viruses, fungi, algae and mycoplasmas	Bleach food; carcinogenic byproducts; inactivated by organic matter rapidly; caustic to tissues and equipment; skin irritation; potential safety problems	Household cleaning products; public sanitation, disinfection and antisepsis	Allwood et al., 2004
Alcohols	Effective against gram-positive and gram-negative bacteria	Long contact time required; not effective against some viruses and bacterial spores; evaporate quickly	Skin antiseptic; alcohol-based hand sanitizers	D'Souza and Su, 2010
Glutaraldehyde	Non corrosive to metal; effective against bacteria, viruses, fungi, spores and parasites; remains effective in the presence of organic matter	Toxic to human and animals; long contact time; must be used in a well ventilated area	Disinfecting medical and dental equipment; industrial water treatment; preservertive	Doltree et al., 1999

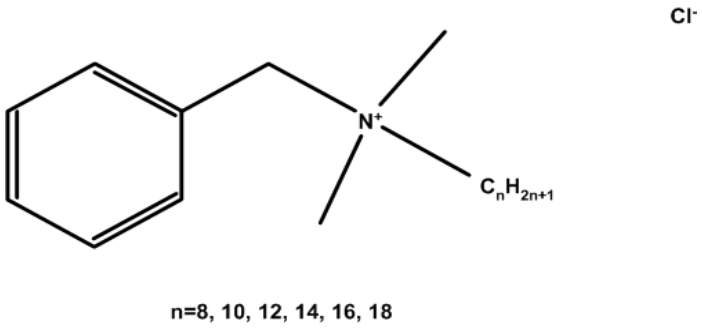
Table 1.7: Advantages, disadvantages and applications of disinfectants reviewed in this chapter (Continued)

Chemicals	Advantages	Disadvantages	Applications	References
Benzalkonium chloride	Non-irritating to skin; not corrosive; has been used for decades in hospital environment	Slow- acting; not effective against gram-negative bacteria or spores	Non-alcohol-based hand sanitizers; hard surface disinfectants; surgical instrument sterilizing solutions; clinical settings disinfectants; pharmaceutical preservatives	Moadab et al., 2001
Organic acids	Less reaction with organics; wider pH range; no toxic residues	Not effective against spores	Disinfecting surfaces; red meat carcass spray; fruits and vegetables	Fraisse et al., 2011
Electrolyzed water	Kill spores and many viruses and bacteria; On-site production of disinfectant; can be applied directly on fresh food products	Lose potency fairly quickly; equipment costly	Disinfectant on food contact surfaces, fruits and vegetables	Koseki et al., 2001
Ozone	Broad spectrum against bacteria, viruses and fungi	Inactivation by organic matter; 10 ppm can be harmful to human health	Sanitizers; process water; products (fruits, vegetables and juices); gas (control mold in storage)	Kim et al., 1999

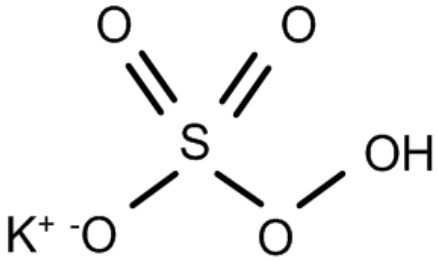
Table 1.7: Advantages, disadvantages and applications of disinfectants reviewed in this chapter (Continued)

Chemicals	Advantages	Disadvantages	Applications	References
Hydrogen peroxide	A valuable bleaching, cleansing and deodorizing agent; no toxic residues	not effective against viruses, bacterial spores, fungal spores, Cryptosporidia, Giardia, mycobacteria; damaging to tissues; moderately corrosive; ineffective in the presence of organic matter	Skin antiseptic; disinfectant; chemical sterilant	Li et al., 2011
Potassium peroxymonosulfate	Fast- acting	Corrosive	Pool and spa disinfectants	Anipsitakis et al., 2008
Trisodium phosphate	Approved by FDA to be a GRAS chemical;	Relatively high cost compared to chlorine	Cleaning agent; approved for treatment of beef carcasses	Lindsay 1985
Sodium metasilicate	Approved by USDA FSIS to be used in food; no adverse effect in food	Relatively high cost compared to chlorine	Processing aid as an antimicrobial in marinades for meat products; a carcass rinse or spray on beef carcasses, subprimals, and trimmings	USDA FSIS, 2011

Panel A



Panel B



Panel C

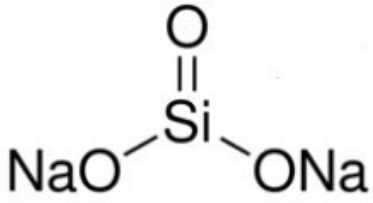


Figure 1.1: Chemical structures of disinfectants evaluated in this work. Panel A- benzalkonium chloride; Panel B- potassium peroxymonosulfate; Panel C- Sodium metasilicate

CHAPTER II

Chemical disinfection of human norovirus surrogates for nosocomial outbreak prevention using benzalkonium chloride and potassium peroxymonosulfate

C. Cao and D. H. D'Souza*

Department of Food Science and Technology, University of Tennessee-Knoxville, Knoxville, TN, USA, 37996-4591

Running Title: *Decontamination of human norovirus surrogates*

KEYWORDS: Disinfection, benzalkonium chloride, potassium peroxymonosulfate, human norovirus surrogates

*Corresponding author: Tel.: 865-974-2753; Fax: 865-974-7332; E-mail: ddsouza@utk.edu

Abstract

Human noroviruses, the major cause of acute nonbacterial gastroenteritis worldwide, are easily transmitted via person-to-person and contaminated food-contact surfaces. This research determined inactivation of human norovirus surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1), by benzalkonium chloride (BAC) and potassium peroxymonosulfate (KPMS) over 1 h at room temperature using suspension and carrier (stainless-steel coupons) tests. Virus recovery after triplicate treatments was evaluated using infectious plaque assays and compared to controls. In suspension tests, 0.32 mg/mL BAC caused 1.94 and 2.59 log PFU/mL reductions of FCV-F9 at low and high titers after 1 h, respectively; and > 3 and 1.47 log reduction of MNV-1 at low and high titers, respectively. KPMS at 5 and 10 mg/mL reduced both viruses at low titers within 30 s and high titers of FCV-F9 to undetectable levels after 2 min and 30 s, respectively. KPMS at 5 mg/mL had little effect against high titers of MNV-1, though 10 mg/mL KPMS caused a 4.61 log reduction after 5 min. Using carrier tests, KPMS at 5 and 10 mg/mL reduced both viruses at low titers after 30 s. High titers of FCV-F9 and MNV-1 required 10 mg/mL KPMS for 10 and 15 min, respectively for complete inactivation. Carrier tests with 0.32 mg/mL BAC reduced low titers of both viruses to undetectable levels after 1 h without significant reduction of high titers even after 2 h; simulated dirty conditions showed decreased viral reduction. These results indicate that KPMS causes viral reduction within short contact times, while BAC requires longer contact.

Introduction

Human noroviruses (HNoVs) are considered as the most common cause of acute non-bacterial gastroenteritis in the world, that are currently responsible for approximately 90% of non-bacterial enteric outbreaks (Scallan et al., 2011; Siebenga et al., 2009; Lindesmith et al., 2003). These viruses can be transmitted via contaminated water, food, surfaces, or through direct contact with infected persons (Goodgame et al., 2006). HNoVs are quite persistent in the environment being detected on food-contact surfaces for up to 7 days at room temperature (D'Souza et al., 2006; Liu et al., 2009). They can persist in water and foods, such as marinated mussels, lettuce, green onions, strawberries and ready-to-eat products for several days (Bidawid et al., 2004; Green et al., 1998, 1999; Hewitt and Greening, 2004). Although, HNoV infection itself is self-limiting in healthy individuals, they can be life-threatening to the elderly and immunocompromised (Siebenga et al., 2009; Donaldson et al., 2010). HNoV outbreaks result in economic losses from product recalls or public health costs, as well as loss of labor due to inability of employees to report to work. Outbreaks related to cruise-ships, nursing homes, and elderly care facilities continue to be reported annually (Cheesbrough et al., 2000; Anderson et al., 2001; Center for Disease Control and Prevention, 2002; Scallan et al., 2011).

Since HNoVs cannot be cultivated in the lab, cultivable surrogates including feline calicivirus, FCV-F9 and murine norovirus, MNV-1, are used to determine efficacy of inactivation using infectivity plaque assays. Currently, chemical disinfectants that are utilized for HNoV decontamination include chlorine, sodium hypochlorite (household bleach), glutaraldehyde, and other quaternary ammonium compounds (Jimenez and Chiang, 2006; Jean et al., 2003; Solomon et al., 1998). These chemicals are reported to be effective on contact surfaces against FCV-F9 and MNV-1 (Park and Sobsey, 2011; Duizer et al., 2004; D'Souza and Su,

2010). However, some of the disadvantages of these chemicals include their corrosive nature that can cause damage to equipment including rust and staining of surfaces and are harmful during handling. Also, carcinogenic byproducts can be formed by the interaction of chlorine with organic matter (Dunnick and Melnick, 1993). Therefore, it is important to explore alternative chemical disinfectants to effectively inactivate HNoVs that have a broad range of antimicrobial activity against nosocomial infections.

Benzalkonium chloride (BAC) is a chemical that has been used in hospital environments for surface disinfection and decontamination against a wide range of pathogenic bacteria. BAC at 512 ppm (0.512 mg/mL) is reported to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA), *Campylobacter, Salmonella, Escherichia coli* O157:H7*, Listeria monocytogenes* and *Pseudomonas aeruginosa* after an exposure time of 5 min in both suspension and surface tests (Riazi and Matthews, 2011). In addition to bacteria, studies have also shown that BAC has antiviral activity against canine coronavirus (Pratelli, 2007), respiratory syncytial virus, adenovirus, herpes simplex virus hominis type 2 and cytomegalovirus (Belec et al., 2000). Potassium peroxymonosulfate (KPMS) is another chemical that is routinely used in swimming pools to keep the water clean, and is effective against *Escherichia coli* after 1 h at a dose of 25 ppm (0.025 mg/mL) (Anipsitakis et al., 2008). Previous studies have also shown its effectiveness against veterinary isolates of FCV and the poultry enteritis mortality syndrome-associated astrovirus (Eleraky et al., 2002; Schultz-Cherry et al., 2001). Therefore, routinely used chemical disinfectants for bacterial disinfection in these facilities may also have the potential to inactivate HNoVs and/or HNoV surrogates to prevent their spread.

With the alarming increase in HNoV outbreaks and their emerging virulent forms such as GII.4 that are known to cause death in the elderly and immune-compromised (Siebenga et al.,

2010), it has become imperative to implement improved control strategies in high risk areas including hospitals, elderly-care facilities, cruise-ships, and the food environment.

Previously, using suspension tests, it was shown that BAC (0.2, 0.5, and 1 mg/mL) and KPMS (5, 10, and 20 mg/mL) can reduce the titers of FCV-F9, MNV-1, and bacteriophage MS2 after 2 h at room temperature (Su et al., 2012). In this study, the time-dependence effects of benzalkonium chloride (BAC at 0.32 mg/mL) and potassium peroxymonosulfate (KPMS at 5 and 10 mg/mL) against FCV-F9 and MNV-1 over 1 h at room temperature were compared *in vitro* using suspension tests and carrier tests on stainless-steel coupons under clean and simulated dirty conditions. The infectivity after treatment was evaluated by using infectious plaque assays and compared to control (water-treated) viruses.

Materials and Methods

Virus Stocks

Feline calicivirus (FCV-F9) and its host, Crandell Reese Feline Kidney (CRFK) cells, were obtained from American type culture collection (ATCC) (Manassas, VA). Murine norovirus (MNV-1) was kindly provided by Dr. Skip Virgin (Washington University, St. Louis, MO), and its host, RAW 264.7 cells were obtained from the University of Tennessee cell culture collection. Viral stocks were obtained as previously described using their respectively confluent cell lines in 175cm^2 flasks (D'Souza et al., 2011; Su et al., 2011). Viral titer was quantified using infectious plaque assays as described below.

Chemicals and Treatments

Benzalkonium chloride (BAC), obtained from MP Biomedicals (Illkirch, France), was diluted with sterile de-ionized water to reach a concentration of 0.32 mg/mL and then filter-sterilized with a 0.2 µ m filter (Fisher Scientific, GA, USA). Potassium peroxymonosulfate (KPMS) (Tokyo Chemical Industry Co., Japan) was prepared in sterile de-ionized water to reach final concentrations of 5 and 10 mg/mL and filter-sterilized with a 0.2 µ m filter (Fisher Scientific, GA, USA).

For the suspension test, one volume (167 µl) of BAC and KPMS were mixed with an equal volume of FCV-F9 or MNV-1 at high and low titers of ~7 $\log_{10}$ PFU/mL and ~5 $\log_{10}$ PFU/mL to obtain a final concentration of 0.16 mg/mL BAC or 2.5 mg/mL and 5 mg/mL KPMS, respectively, and incubated for specific time intervals of 0, 5, 10, 15, 30 and 60 min at room temperature (22^{o}C). For the carrier test, stainless steel coupons sterilized by autoclaving were used as a surface model (fomite). Under clean conditions, MNV-1 and FCV-F9 were inoculated on sterile coupons at high and low titers of ~7 $\log_{10}$ PFU/mL and ~5 $\log_{10}$ PFU/mL and allowed to dry for 1 h at room temperature in a BioSafety Level 2 hood. After drying, the coupons were treated with BAC or KPMS and sampled at specific time intervals of 0, 5, 10, 15, 30 and 60 min at room temperature (RT). Heat-inactivated fetal bovine serum (5% FBS, HyClone Laboratories, Inc, Logan, Utah) was added into the virus stocks to simulate an organic load to test the efficacy of chemicals against HNoV surrogates in the presence of dirty conditions using the same procedures as described above for clean conditions.

Sterile water was used as non-treated controls to test the viral recovery and to compare with treated samples. All the chemical treatments were neutralized after their appropriate treatment contact times by serially diluting the virus-chemical mix in Dulbecco's Modified

Eagle's Medium/Ham's F-12 (HyClone Laboratories, Inc, Logan, Utah) containing 10% heat-inactivated FBS followed by serial dilutions in DMEM-F12 containing 2% FBS and plaque assayed as described below.

Plaque Assays for FCV-F9 and MNV-1

Serial dilutions (0.5 mL) of control and treated viruses were inoculated onto the confluent CRFK cells for FCV-F9 and RAW 264.7 cells for MNV-1 in 6-well plates, and incubated for 2.5 h at 37℃ in a CO_2 incubator. Plaque assays were performed as previously described (D'Souza et al., 2006; D'Souza et al., 2009; Su et al., 2011). Briefly, overlay medium containing 0.75% noble agar (Sigma-Aldrich, St. Louis, MO) was added to each infected or control well and incubated for 2~3 d at 37^{o}C under 5% CO_2. Finally, overlay media containing neutral red (Sigma-Aldrich, St. Louis, MO) was added for visualization of plaques after incubation.

Statistical Analysis

All treatment and controls were replicated thrice in duplicate. A one-way analysis of variance (ANOVA) was used to test for differences in means of plaque counts among treatments and means were analyzed using a completely randomized design at $p<0.05$ in SAS program (version 9.3, SAS Institute, Cary, NC, USA). If statistically significant differences were found using the ANOVA, the Tukey's Post Hoc test was used to analyze differences in means between treatments.

Results

Reduction of FCV-F9 and MNV-1 by 0.32 mg/mL BAC in suspension tests

BAC at 0.32 mg/mL concentration for 5, 10 and 15 min contact time caused <1 $\log_{10}$ PFU/mL reduction for FCV-F9 and MNV-1 at low titers, with ~1 $\log_{10}$ PFU/mL of reduction after 30 min, and with greater reduction of 1.94 $\log_{10}$ PFU/mL or ⩾3 $\log_{10}$ PFU/mL for FCV-F9 and MNV-1, respectively, after 1 h at RT. Using high viral titers, FCV-F9 and MNV-1 were reduced by ~1 $\log_{10}$ PFU/mL after 10 min of exposure time. FCV-F9 titer was reduced by 2.59 $\log_{10}$ PFU/mL after 1 h, while a 1.47 log reduction was observed for MNV-1 at RT (Table 2.1).

Reduction of FCV-F9 and MNV-1 by 5 and 10 mg/mL KPMS in suspension tests

FCV-F9 and MNV-1 were undetectable after exposure to 5 or 10 mg/mL of KPMS after 10 min (data not shown). Therefore shorter contact times for 0, 30 s, 1 min, 2 min and 5 min were further evaluated at the same concentrations of KPMS. Low titers of FCV-F9 and MNV-1 were undetectable within 30 s. However, KPMS at 5 mg/mL reduced high titers of FCV-F9 by ≥5 $\log_{10}$ PFU/mL after 2 min, but caused little reduction (~0.5 log) of high titers of MNV-1 after 5 min of exposure time. Increasing the concentration of KPMS to 10 mg/mL for 30 s of contact time resulted in ≥5 $\log_{10}$ PFU/mL reduction for high titers of FCV-F9 and also had a significant effect on high titers of MNV-1 resulting in a 4.61 log reduction after 5 min at RT (Table 2.2).

Reduction of FCV-F9 and MNV-1 by 0.32 mg/mL BAC in clean coupon tests

Since little reduction was achieved in the suspension test after 1 h of exposure and it is generally recognized that chemical efficacy is higher in suspension than carrier tests, longer contact times for BAC at a concentration of 0.32 mg/mL on stainless steel coupons were needed

for testing. Low titers of FCV-F9 and MNV-1 were reduced to undetectable levels after 1 or 2 h of exposure at RT. High titers of FCV-F9 were reduced by 1.45 and 1.05 $\log_{10}$ PFU/mL with the treatment of BAC after 1 h and 2 h, respectively. However, lower reductions of ~0.7 $\log_{10}$ PFU/mL for high titers of MNV-1 were achieved after 1 or 2 h of exposure to 0.32 mg/mL of BAC at RT (Table 2.4).

Reduction of FCV-F9 and MNV-1 by 5 and 10 mg/mL KPMS in clean coupon tests

KPMS at 5 and 10 mg/mL concentrations reduced FCV-F9 and MNV-1 at low titers to undetectable levels within 30 s contact time. KPMS at 10 mg/mL concentration reduced high titers of FCV-F9 and MNV-1 by 1.92 and 1.56 $\log_{10}$ PFU/mL after 5 min, respectively; and complete inactivation was obtained after 10 min and 15 min for FCV-F9 and MNV-1, respectively. Lowering the concentration of KPMS to 5 mg/mL for 5 min of contact time only resulted in a <1 log reduction for high titers of FCV-F9 and MNV-1. FCV-F9 at high titers were reduced to undetectable levels after 10 min of exposure time to 5 mg/mL KPMS, while only 2.46 $\log_{10}$ PFU/mL reduction was reached for MNV-1 after 1 h at RT (Table 2.3a).

Reduction of FCV-F9 and MNV-1 by 0.32 mg/mL BAC in dirty coupon tests

The effect of BAC at 0.32 mg/mL concentration was decreased significantly on FCV-F9 and MNV-1 titer reduction after 1 or 2 h contact times under dirty conditions. Almost no reduction was observed on high titers of FCV-F9 and MNV-1 after 2 h of contact time. Furthermore, <0.5 $\log_{10}$ PFU/mL reduction was observed on low titers of FCV-F9 and MNV-1 after 1 or 2 h of exposure (Table 2.5).

Reduction of FCV-F9 and MNV-1 by 5 and 10 mg/mL KPMS in dirty coupon tests

KPMS at 5 mg/mL for 2 min effectively inactivated low titers of FCV-F9 and reduced MNV-1 to undetectable levels (Table 2.6). FCV-F9 at high titer was reduced to undetectable levels after 15 min of exposure time by 5 mg/mL of KPMS. High titers of MNV-1 were reduced by 2.29 logs after 1 h of contact time at RT, which was similar to the clean condition carrier tests. Increasing the concentration of KPMS to 10 mg/mL reduced FCV-F9 and MNV-1 at low titers to undetectable levels after 1 min and 30 s, respectively. High titers of FCV-F9 were reduced to undetectable levels after 10 min. However, the effect of KPMS on high titers of MNV-1 was decreased compared to the clean conditions, where only a 2.52 $\log_{10}$ PFU/mL reduction was achieved after 15 min of contact time and complete reduction was observed only after 1 h of exposure at RT (Table 2.3b).

Discussion

In this study, the effect of 0.32 mg/mL BAC and 5 and 10 mg/mL KPMS on the titer reduction of FCV-F9 and MNV-1 was evaluated. The virus reduction was found to be related to the contact time, contact surfaces, the type of virus and chemical disinfectant used. Generally, longer contact times resulted in increased reductions for both tested viruses. BAC required longer contact times to effectively inactivate both viruses, while KPMS was found to be a fast-acting agent causing rapid reduction of viral titers within short contact times. Both chemicals had decreased efficacy for both tested viruses using the stainless-steel coupon tests compared to suspension tests that could be attributed to the interference of the physical properties of the surface with contact between the microorganisms and disinfectants (Best et al., 1988). Not

surprisingly, the effect of both chemical disinfectants was decreased in the presence of organic loads.

Current chemical disinfectants routinely used include alcohols including ethanol, 1-propanol, and 2-propanol, glutaraldehyde and sodium hypochlorite. Using suspension tests, 70% ethanol was most frequently tested by investigators, but without any conclusive suggestions on its effectiveness against HNoV surrogates (Martino et al., 2010; Malik et al., 2006a; Park et al., 2010; D'Souza and Su, 2010; Duizer et al., 2004; etc.). Iso-1-propanol at 50% and 70% was reported to have >4 log reduction after 30 s of contact on FCV-F9, whereas 2-propanol was effective only after 3 min of contact time (Gehrke et al., 2004). In another study, 1-propanol at only 30% was shown to be the most effective virucidal agent among the three alcohols tested on stainless steel for MNV-1 with 99.99% reduction, followed by (50-55%) ethanol and then (60%) 2-propanol (Magulski et al., 2009). Furthermore, this study showed the efficacy of 1000 ppm peracetic acid and 2500 ppm glutaraldehyde to inactivate 99.99% of MNV on the carriers within 5 min of exposure time (Magulski et al., 2009). Using surface disinfection tests, 70% ethanol was reported to be ineffective against high as well as low titers of MNV-1 and FCV-F9 after 1 min of contact time (D'Souza and Su, 2010). In comparison to ethanol, the results reported with BAC show higher effects though requiring longer contact time of 1 h. Typical sodium hypochlorite wash solutions were reported to be effective only at >300 ppm for HNoV inactivation, where complete inactivation of FCV-F9 was reported at chlorine levels of 3,000 ppm (or higher) after 10 min at room temperature (Duizer et al., 2004). On the other hand, 2% glutaraldehyde was found to effectively reduce FCV-F9 and MNV-1 by ~6 $\log_{10}$ PFU after 30 s and 1 min of contact time, while 10% household bleach containing 5000 ppm free chlorine resulted in effective reductions of FCV-F9 by >6 $\log_{10}$ PFU/mL in 1 min, but failed to

completely inactivate MNV-1 (D'Souza and Su, 2010). These results are consistent with reports showing that MNV-1 is a sturdy and resilient virus, resistant to most treatments and processing conditions compared to FCV-F9. One surface disinfection test was carried out on fabrics and carpets, to show that an activated dialdehyde-based product containing 2.6% glutaraldehyde could reduce more than 99.99% of FCV in 1-10 min on all types of material tested (Malik et al., 2006b).

Recently, Japanese investigators found that 1000 μg/mL copper iodide nanoparticles had an extremely high antiviral activity reducing FCV by 7 orders of magnitude after 1 h of contact time (Shionoiri et al., 2012). Furthermore, investigators suggested tannic acid derived from Chinese medicinal herbs inhibited binding to histo-blood group antigens (HBGAs) receptors in HNoVs, which indicated tannic acid a promising antiviral (Zhang et al., 2012). A novel MNV strain, MT30-2, was isolated from the feces of mice in Japan and two antiseptics, povidone-iodine and sodium hypochlorite, revealed strong virucidal activity against MNV, which inactivated MNV at low concentrations and in short exposure time (Matsuhira et al., 2012).

Quaternary ammonium compounds (QAC) have been studied as disinfectants against human NoVs and its surrogates including FCV-F9 and MNV-1. Previous studies using commercial cleansers that contain the active ingredient n-alkyl dimethyl benzyl ammonium chloride at 0.08%, showed less than 1 log of reduction for MNV-1 was obtained after 10 min at RT (Girard el at., 2010), and similar results were reported by others showing that QAC disinfectants were not effective against FCV-F9 (Doultree et al., 1999; Eleraky el at., 2002). Another commercial disinfectant with 1:10 final concentrations of QAC showed no reduction against FCV after 1 min (Doultree et al., 1998). QAC disinfectants containing 0.14% BAC was also shown to be ineffective at virolysis of human GII.4 norovirus (Nowak el at., 2011).

Similarly, our study showed that both high and low titer of FCV-F9 and MNV-1 reached $\leqslant 1$ $\log_{10}$ reduction by treatments with 0.32 mg/mL BAC after 10 min at RT, but required longer exposure/contact times for improved effects.

BAC belongs to the QAC group, which have been widely utilized in hand sanitizers (Moadab et al., 2001) and disinfectants for hard surfaces (Gradel et al., 2004) and as pharmaceutical preservatives (Marple et al., 2004) due to its effectiveness in decontaminating a broad spectrum of bacteria, viruses and fungi in solutions (Bastiani et al., 1974; Belec et al., 2000; Jira et al., 1982). BAC at low concentrations is reported to be a non-irritant, non-toxic, and non-corrosive to food contact surfaces and thus can be safely used as a disinfectant in the food industry as a readily-used antimicrobial agent. However, studies showed that BAC requires longer exposure time to inactivate non-enveloped viruses. Furthermore, in our study, longer incubation times resulted in higher reduction irrespective of initial titers. Therefore, longer contact times could be explored to estimate the effectiveness of BAC in decontaminating HNoV surrogates. However, the long contact time of 1 h may not be suitable for all applications.

Recently, alternatives including trisodium phosphate (TSP) and hydrogen peroxide vapor (HPV) are being studied as antiviral sanitizers. TSP at the concentrations of 1%, 2% and 5% were tested on surfaces and produce. It appears to be suitable for titer reductions of FCV, MNV-1 and MS2 bacteriophage on surfaces, and FCV and MNV-1 on produce. Studies showed that higher concentration of TSP resulted in higher titer reduction with short contact times (30 s and 1 min) and 5% TSP achieved similar efficacy to 5000 ppm chlorine (D'Souza and Su, 2010; Su and D'Souza, 2011). Though, the adverse effects of high concentrations of alkaline treatments such as TSP on the environment, if any, may need to be explored. HPV appears to be a promising virucide, showing that poliovirus, rotavirus, adenovirus and murine virus can be

completely inactivated by 127 ppm HPV for 1 h at room temperature on stainless steel and framing panel carriers (Tuladhar et al., 2012). Human norovirus GII.4 appeared to be most resistant to HPV treatment, with a reduction of 0.5 $\log_{10}$ PCR units on stainless steel carriers. Furthermore, their study indicated that low levels of feces (1%) did not significantly influence the effectiveness of HPV (Tuladhar et al., 2012). However, the initial cost of the equipment to generate HPV may be a limiting factor for some small scale industries or communities.

Therefore, other commonly used sanitizers and disinfectants need to be evaluated to determine their effectiveness against human noroviruses. KPMS is widely used as a powerful oxidizing agent. Currently, KPMS is routinely used in swimming pools against *Escherichia coli* to keep the water clean and safe (Anipsitakis et al., 2008). It has also been used as a mist in veterinary hospitals to decontaminate *S. aureus* and *S*. Typhiumurium by >99.9999% with the concentration of 4% for 30 min exposure time after misting was completed (Patterson et al., 2005). In a 6-year study, KPMS at concentrations of 1 and 10 mg/mL completely inactivated all the bacteria-*S. aureus, MRSA* and *Pseudomonas aeruginosa* after 10 and 2 min exposure times, respectively and no significant variation in resistance to KPMS was observed during the period of study (Giacometti et al., 2002). However, only a few studies report the application of KPMS for viral inactivation. The KPMS-based product Virkon S was shown to completely inactivate the poultry enteric mortality syndrome (PEMS)-associated astrovirus in an embryo model, while quaternary ammonium or benzalkonium chloride-based products had no effect against the virus (Schultz-Cherry et al., 2001). Also, KPMS was shown to be effective against veterinary isolates of FCV (Elarky et al., 2002).

In our study, KPMS at 5 mg/mL and 10 mg/mL reduced both viruses at low titers within 30 s and high titers of FCV-F9 after 2 min with 5 mg/mL while within 30 s with 10 mg/mL of

KPMS to undetectable levels. High titers of FCV-F9 were reduced to undetectable levels after 2 min of treatment with 5 mg/mL KPMS. This is consistent with earlier research showing that KPMS completely inactivated FCV after 10 min of exposure (Eleraky et al., 2002). However, this study used only FCV from animal isolates and not the FCV-F9 strain or MNV-1. KPMS appears to be a more suitable disinfectant among the tested chemicals. Though KPMS is fast-acting, it could be corrosive to surfaces and equipment as a strong oxidative disinfectant, which may not be proper for use in hand sanitizers. The applicability of KPMS as an alternative strategy to decontaminate foodborne viruses on food contact surfaces (stainless steel) and under soiled conditions was investigated in this study as well.

Solomon and others (2009) reported that 0.8 mg/mL QAC reduced FCV by 1.9 $\log_{10}$ PFU/mL after 10 min of exposure, while Virkon at 1% containing 5 mg/mL KPMS reduced FCV from 4-5 $\log_{10}$ PFU/mL to undetectable levels after 10 min (Solomon et al., 2009). In comparison, our results showed that 5 mg/mL KPMS reduced FCV from ~6 $\log_{10}$ PFU/mL to undetectable levels after 2 min; and 0.32 mg/mL BAC decreased FCV at ~ 4 $\log_{10}$ PFU/mL by 1.9 $\log_{10}$ PFU/mL after 1 h. Su and D'Souza (2012) reported that BAC at 0.2, 0.5, and 1 mg/mL reduced FCV-F9 and MNV-1 at low (~5 $\log_{10}$ PFU/mL) titers to undetectable levels after 2 h at room temperature in suspension tests; and high (~7 $\log_{10}$ PFU/mL) titers of FCV-F9 were reduced by 2.87, 3.08, and 3.25 $\log_{10}$ PFU/mL and high titers of MNV-1 were reduced by 1.55, 2.32, and 2.75 $\log_{10}$ PFU/mL, respectively. KPMS at 5, 10, and 20 mg/mL reduced high and low titers of FCV-F9 and low titers of MNV-1 to undetectable levels, while high titers of MNV-1 were reduced by 0.92 and 3.44 $\log_{10}$ PFU/mL with KMPS at 5 and 10 mg/mL, respectively after 2 h at room temperature (Su and D'Souza, 2012). In comparison, our study showed that BAC at 0.32 mg/mL reduced FCV-F9 and MNV-1 at low (~5 $\log_{10}$ PFU/mL) titers by 1.94 and $\geqslant$3.14

$\log_{10}$ PFU/mL after 1 h at room temperature in suspension tests, respectively; and FCV-F9 and MNV-1 at high (~7 $\log_{10}$ PFU/mL) titers were decreased by 2.59 and 1.47 $\log_{10}$ PFU/mL, respectively after 1 h. KPMS at 5 and 10 mg/mL reduced both low and high titers of FCV-F9 and MNV-1 to undetectable levels after 1 h

Since HNoV is transmitted via fecal-oral route, human excretions such as vomit and stools can be the major sources of transmission of HNoV in the environment. Therefore, in previous studies, simulated natural conditions have been carried out by adding organic compounds into the virus stocks to determine the effect of disinfectants against HNoV and its surrogates. In one study, investigators simulated field conditions using an organic acid, an aldehyde, a halogen compound, and a peroxide as chemical disinfectants to show that the efficacy of disinfectants against FCV and noroviruses could be greatly reduced in the presence of organic impurities (25% feces) (Poschetto et al., 2007). However, another study showed that organic loads (0.3% BSA plus 0.3% erythrocytes) did not change the effectiveness of the biocides (Magulski et al., 2009). In our study, the effect of both chemicals against FCV-F9 and MNV-1 decreased under simulated dirty conditions (5% FBS). BAC at 0.32 mg/mL reduced low titers of FCV-F9 and MNV-1 by <0.5 $\log_{10}$ PFU/mL under dirty conditions, while undetectable levels were achieved under clean conditions after 1 or 2 h exposure time at RT. Moreover, BAC resulted in 1.45 and 1.05 log reductions against high titers of FCV-F9 after 1 and 2 h, respectively, and ~0.7 log reduction against high titers of MNV-1 after 1 or 2 h under clean conditions. However, almost no reduction of FCV-F9 and MNV-1 at high titers after 1 or 2 h was observed under dirty conditions. The effect of KPMS at 5 and 10 mg/mL was diminished somewhat in the presence of organic loads where 5 mg/mL of KPMS reduced FCV-F9 and MNV-1 at low titers to undetectable levels within 30 s and after 2 min under clean and dirty

conditions, respectively. KPMS at 10 mg/mL showed no difference against FCV-F9 at both high and low titers and MNV-1 at low titer under clean or dirty conditions; however, effects significantly decreased against high titers of MNV-1, resulting in complete inactivation after 15 min and 1 h under clean and dirty conditions, respectively.

Conclusion

This study provides data on alternative methods for HNoV surrogate reduction. Further studies using higher concentrations of BAC may be necessary to determine the efficacy of its antiviral effects against HNoV surrogates for use in food processing, hospital or industrial settings. Our results with KPMS show its strong potential for application in decontaminating enteric viruses in a rapid and effective manner, though caution is advised as it is a strong oxidizing agent.

Acknowledgements

Funding for this research was provided by the TN Agricultural Experiment Station (UT-HATCH TEN-00391) and is gratefully acknowledged. The use of trade names does not imply endorsement by the University of Tennessee-Knoxville.

References

Anderson AD, Garrett VD, Sobel J, Monroe SS, Fankhauser RL, Schwab KJ, Bresee JS, Mead PS, Higgins C, Campana J, Glass RI, and the Outbreak Investigation Team. 2001. Multistate outbreak of Norwalk-like virus gastroenteritis associated with a common caterer. *Am. J. Epidemiol.* 154:1013–1019.

Anipsitakis GP, Tufano TP, Dionysiou DD. 2008. Chemical and microbial decontamination of pool water using activated potassium peroxymonosulfate. *Water Research* 42: 2899–2910.

Bastiani L. 2002. Antibacterial, antifungal and antiprotozoal action of a preparation based on benzalkonium chloride. Farmaco-Edizione Pratica 1974;29:594-601.Cliver, D.O., Riemann, H.P., 2002. *Foodborne Diseases*. Academic Press, London.

Belec L, Tevi-Benissan C, Bianchi A. 2000. *In vitro* inactivation of Chlamydia trachomatis and of a panel of DNA (HSV-2, CMV, adenovirus, BK virus) and RNA (RSV, enterovirus) viruses by the spermicide benzalkonium chloride. *J. Antimicrob Chemother* 46: 685-693.

Best, M., S. A. Sattar, V. S. Springthorpe, and M. E. Kennedy. 1988. Comparative mycobactericidal efficacy of chemical disinfectants in suspension and carrier tests. *Appl. Environ. Microbiol*. 54:2856-2858.

Bidawid S, Malik N, Adegbunrin O, Sattar SA, and Farber JM. 2004. Norovirus cross-contamination during food handling and interruption of virus transfer by hand antisepsis: experiments with feline calicivirus as a surrogate. *Journal of Food Protection* 67: 103–109.

Center for Disease Control and Prevention. Outbreaks of gastroenteritis associated with noroviruses on cruise ships-United States, 2002. *JAMA* 2003b; 289: 167-169.

Cheesbrough JS, Green J, Gallimore CI, Wright PI, and Brown DW. 2000. Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiol. Infect.* 125:93–98.

Donaldson EF, Lindesmith LC, LoBue AD, and Baric RS. 2010. Viral shape-shifting: norovirus evasion of the human immune system. *Nature Reviews Microbiology* 8: 231-241.

Doultree JC, Druce JD, Birch CJ, Bowden DS, and Marshall JA. 1999. Inactivation of feline calicivirus, a Norwalk virus surrogate. *J. Hospital Infect.* 41: 51-57.

D'Souza DH, Su X, and Harte FM. 2009. High pressure homogenization for the inactivation of human enteric virus surrogates. *J. Food Prot.* 72:2418-22.

D'Souza DH and Su X. 2010. Efficacy of chemical treatments against Murine Norovirus, Feline Calicivirus, and MS2 bacteriophage. *Foodborne Pathogens and Diease* 7: 319-325.

D'Souza DH, Sair A, Williams K, Papafragkou E, Jean J, Moore C, Jaykus L. 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. *International J Food Microbiol* 108: 84–91.

Duizer E, Bijkerk P, Rockx B, Groot A, Twisk F, and Koopmans M. 2004. Inactivation of Caliciviruses. *Applied and Environmental Microbiology* 70: 4538–4543.

Dunnick JK and Melnick RL. 1993. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. *Journal of the National Cancer Institute* 85: 817-822.

Eleraky NZ, Potgieter LND, and Kennedy MA. 2002. Virucidal efficacy of four new disinfectants. *J. Am. Anim. Hosp. Assoc.* 38: 231-234.

Gehrke C, Steinmann J, and Goroncy-Bermes P. 2004. Inactivation of feline calicivirus, a surrogate of norovirus (formerly Norwalk-like viruses), by different types of alcohol in vitro and in vivo. *J. Hospital Infect.* 56: 49–55.

Giacometti A, Cirioni O, Greganti G, Fineo A, Ghiselli R, Del Prete MS, Mocchegiani F, Fileni B, Caselli F, Petrelli E, Saba V, and Scalise G. 2002. Antiseptic Compounds Still Active Against Bacterial Strains Isolated from Surgical Wound Infections Despite Increasing Antibiotic Resistance. *Eur J Clin Microbiol Infect Dis* 21:553–556.

Girard M, Ngazoa S, Mattison K, And JEAN J. 2010. Attachment of Noroviruses to Stainless Steel and Their Inactivation, Using Household Disinfectants. *J. Food Production* 73: 400-404.

Goodgame R. 2006. Norovirus gastroenteritis. *Curr Gastroenterol* 8 (5): 401–408.

Gradel, K., Sayers, A., Davies, R., 2004. Surface disinfection tests with *Salmonella* and a putative indicator bacterium, mimicking worst-case scenarios in poultry houses. *Poultry Science* 83: 1636-1643.

Green J, Wright PA, Gallimore CI, Mitchell O, Morgan-Capner P, Brown DWG, 1998. The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse-transcriptase polymerase chain reaction assay. *J. Hospital Infect.* 39: 39– 45.

Green, J., Brown, D., Cheesbrough, J., Wright, P., 1999. The detection of environmental small round structured virus by nested reverse transcriptase polymerase chain reaction. Abstract Book of the International Workshop on Human Caliciviruses, March 29– 31, Atlanta, GA. No. S3-8.

Hewitt J and Greening GE. 2004. Survival and persistence of norovirus, hepatitis A virus, and feline calicivirus in marinated mussels. *J Food Prot.* 67:1743-50.

Jean J, Vachon J-F, Moroni O, Darveau A, Kukavica-Ibrulj I, and Fliss I. 2003. Effectiveness of Commercial Disinfectants for Inactivating Hepatitis A Virus on Agri-Food Surfaces. *J. Food Prot.* 66: 115-119.

Jimenez L and Chiang M. 2006. Virucidal activity of a quaternary ammonium compound disinfectant against feline calicivirus: A surrogate for norovirus. *Association for Professionals in Infection Control and Epidemiology* 34: 269-273.

Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, and Baric R. 2003. Human susceptibility and resistance to Norwalk virus infection. *Nat. Med.* 9: 548–53.

Jira, T., Panzig, B., Pohloudek-Fabini, R., 1982. On the antimicrobial activity of benzalkonium chloride in potential contact lens fluids. *Die Pharmazie* 37: 587.

Liu P, Chien Y, Papafragkou E, Hsiao H, Jaykus L, and Moe C. 2009. Persistence of Human Noroviruses on Food Preparation Surfaces and Human Hands. *Food and Environmental Virology* 1: 141-147.

Magulski T, Paulmann D, Bischoff B, Becker B, Steinmann E, Steinmann J, Goroncy-Bermes P, and Steinmann J. 2009. Inactivation of murine norovirus by chemical biocides on stainless steel. *BMC Infectious Diseases* 9:107.

Malik YS, Maherchandani S, and Goyal SM. 2006a. Comparative efficacy of ethanol and isopropanol against feline calicivirus, a norovirus surrogate. *American Journal of Infection Control* 34: 31-35.

Malik YS, Allwood PB, Hedberg CW, and Goyal SM. 2006b. Disinfection of fabrics and carpets artificially contaminated with calicivirus: relevance in institutional and healthcare centres. *Journal of Hospital Infection* 63: 205-210.

Marple, B., Roland, P., Benninger, M., 2004. Safety review of benzalkonium chloride used as a preservative in intranasal solutions: an overview of conflicting data and opinions. *Otolaryngology-Head and Neck Surgery* 130: 131-141.

Martino BD, Ceci C, Profio FD, and Marsilio F. 2010. In vitro inactivation of feline calicivirus (FCV) by chemical disinfectants: resistance variation among field strains. *Arch Virol* 155: 2047–2051.

Matsuhira T, Kaji C, Murakami S, Maebashi K, Oka T, Takeda N, and Katayama K. 2012. Evaluation of Four Antiseptics Using a Novel Murine Norovirus. *Exp. Anim.* 61: 35–40.

Mbithi JN, Springthorpe VS, and Sattar SA. 1990. Chemical disinfection of hepatitis A virus on environmental surfaces. *Appl. Environ. Microbiol.* 56: 3601-3604.

Moadab, A., Rupley, K.F., Wadhams, P. 2001. Effectiveness of a nonrinse, alcohol-free antiseptic hand wash. *Journal of the American Podiatric Medical Association* 91: 288-293.

Nowak P, Toppinga JR, Fotheringham V, Gallimore CI, Gray JJ, Iturriza-Gómara M, and Knight AI. 2011. Measurement of the virolysis of human GII.4 norovirus in response to disinfectants and sanitisers. *J. Virological Methods* 174: 7-11.

Park GW, Barclay L, Macinga D, Charbonneau D, Pettigrew CA, and Vinje J. 2010. Comparative Efficacy of Seven Hand Sanitizers against Murine Norovirus, Feline Calicivirus, and GII.4 Norovirus. *Journal of Food Protection* 73: 2232–2238.

Park GW and Sobsey MD. 2011. Simultaneous comparison of murine norovirus, feline calicivirus, coliphage MS2, and GII.4 norovirus to evaluate the efficacy of sodium hypochlorite against human norovirus on a fecally soiled stainless steel surface. *Foodborne Pathogens and Disease* 8: 1005-1010.

Patterson G, Morley PS, Blehm KD, Lee DE, and Dunowska M. 2005. Efficacy of directed misting application of a peroxygen disinfectant for environmental decontamination of a veterinary hospital. *JAVMA* 227: 597-602.

Poschetto LF, Ike A, Papp T, Mohn U, Bohm R, and Marschang RE. 2007. Comparison of the sensitivities of noroviruses and feline calicivirus to chemical disinfection under field-like conditions. *Appl. Environ. Microbiol.* 73: 5494.

Pratelli A. 2007. Action of Disinfectants on Canine Coronavirus Replication *In Vitro*. *Public Health.* 54: 383–386.

Riazi S and Matthews KR. 2011. Failure of foodborne pathogens to develop resistance to sanitizers following repeated exposure to common sanitizers. *International Biodeterioration & Biodegradation* 65: 374-378.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, and Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.

Schultz-Cherry S, King DJ, and Koci MD. 2001. Inactivation of an astrovirus associated with poult enteritis mortality syndrome. *Avian Diseases* 45: 76-82.

Siebenga JJ, Vennema H, Zheng DP, Vinje J, Lee BE, Pang XL, Ho ECM, Lim W, Choudekar A, Broor, S, Halperin T, Rasool NBG, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, and

Koopmans M. 2009. Norovirus illness is a global problem: Emergence and spread of Norovirus GII.4 variants, 2001-2007. *Journal of Infectious Diseases* 200: 802-812.

Siebenga JJ, Lemey P, Pond SLK, Rambaut A, Vennema H, and Koopmans M. 2010. Phylodynamic Reconstruction Reveals Norovirus GII.4 Epidemic Expansions and their Molecular Determinants. *Plos Pathogens* 6: 1-13.

Shionoiri N, Sato T, Fujimori Y, Nakayama T, Nemoto M, Matsunaga T, and Tanaka T. 2012. Investigation of the antiviral properties of copper iodide nanoparticles against feline calicivirus. *Journal of Bioscience and Bioengineering* 113: 580-586.

Solomon C, Casey P, Mackne C, and Take A. 1998. Chlorine disinfection. A technical overview. Project funded by U.S. Environmental Protection Agency Under Assistance Agreement No cx 824652. National Small Flows Clearinghouse.

Su X and D'Souza DH. 2011. Trisodium phosphate for foodborne virus reduction on produce. *Foodborne Pathogens and Disease* 8: 713-717.

Su X and D'Souza DH. 2012. Inactivation of human norovirus surrogates by benzalkonium chloride, potassium peroxymonosulfate, tannic acid, and gallic acid. *Foodborne Pathog Dis*. 9(9): 829-834.

Tuladhar E, Terpstra P, Koopmans M, and Duizer E. 2012. Virucidal efficacy of hydrogen peroxide vapour disinfection. *J. Hospital Infection* 80: 110-115.

Zhang XF, Dai YC, Zhong W, Tan M, Lv ZP, Zhou YC, and Jiang X. 2012. Tannic acid inhibited norovirus binding to HBGA receptors, a study of 50 Chinese medicinal herbs. *Bioorganic and Medicinal Chemistry* 20: 1616–1623.

Appendix

Table 2.1: Reduction of low and high titers of FCV-F9 and MNV-1 at room temperature by 0.32 mg/mL benzalkonium chloride (BAC) using suspension tests.

	FCV-F9 (log_{10} PFU/mL) at Low titer		MNV-1 (log_{10} PFU/mL) at Low titer		FCV-F9 (log_{10} PFU/mL) at High titer		MNV-1 (log_{10} PFU/mL) at High titer	
Contact time (min)	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction
Control	4.66 ± 0.13^{A}	0.00	4.41 ± 0.17^{A}	0.00	6.42 ± 0.14^{A}	0.00	6.24 ± 0.06^{A}	0.00
5	4.10 ± 0.17^{B}	0.56	4.09 ± 0.25^{AB}	0.32	5.76 ± 0.09^{B}	0.66	5.40 ± 0.31^{AB}	0.84
10	4.04 ± 0.06^{B}	0.62	3.84 ± 0.21^{BC}	0.57	5.39 ± 0.08^{C}	1.03	5.33 ± 0.32^{AB}	0.91
15	3.85 ± 0.15^{BC}	0.81	3.68 ± 0.24^{CD}	0.73	5.16 ± 0.09^{C}	1.26	5.01 ± 0.36^{B}	1.23
30	3.55 ± 0.09^{C}	1.11	3.35 ± 0.12^{D}	1.06	4.43 ± 0.10^{D}	1.99	4.93 ± 0.46^{B}	1.31
60	2.73 ± 0.25^{D}	1.94	$<10^{E}$	≥3.41*	3.83 ± 0.09^{E}	2.59	4.77 ± 0.39^{B}	1.47

Different letters denote significant differences when compared within each column alone ($p<0.05$).
*Indicates inactivation values at the detection limit of the assay since BAC fixed the cell monolayers after 1 log serial dilution but not the lower dilution of the virus-treatment combination.

Table 2.2: Reduction of FCV-F9 and MNV-1 at high titers by potassium peroxymonosulfate (KPMS) at 5 and 10 mg/mL at room temperature in suspension tests.

	FCV-F9 (log_{10} PFU/mL) with 5 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 5 mg/mL KPMS		FCV-F9 (log_{10} PFU/mL with 10 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 10 mg/mL KPMS	
Contact time (min)	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction
Control	6.41 ± 0.15^{A}	0.00	6.45 ± 0.12^{A}	0.00	6.00 ± 0.08^{A}	0.00	6.16 ± 0.07^{A}	0.00
0.5	3.71 ± 0.06^{B}	2.70	6.04 ± 0.10^{B}	0.41	$<10^{B}$	≥5.00*	3.98 ± 0.10^{B}	2.18
1	2.10 ± 0.19^{C}	4.31	5.87 ± 0.20^{B}	0.58	$<10^{B}$	≥5.00*	2.91 ± 0.15^{C}	3.25
2	$<10^{D}$	≥5.41*	5.98 ± 0.05^{B}	0.47	$<10^{B}$	≥5.00*	2.48 ± 0.16^{D}	3.68
5	$<10^{D}$	≥5.41*	5.97 ± 0.09^{B}	0.48	$<10^{B}$	≥5.00*	1.55 ± 0.23^{E}	4.61

Different letters denote significant differences when compared within each column alone ($p<0.05$).
*Indicates inactivation values at the detection limit of the assay since KPMS fixed the cell monolayers after the first-log serial dilution but not the lower dilutions of the virus-treatment combination.

Table 2.3: Reduction of FCV-F9 and MNV-1 at high titers on clean (a) and dirty (b) sterile stainless-steel coupons (carrier tests) by 5 mg/mL and 10 mg/mL potassium peroxymonosulfate (KPMS) at room temperature.

a.

	FCV-F9 (log_{10} PFU/mL) with 5 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 5 mg/mL KPMS		FCV-F9 (log_{10} PFU/mL) with 10 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 10 mg/mL KPMS	
Contact time (min)	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction
Control	7.51 ± 0.08^{A}	0.00	5.83 ± 0.07^{A}	0.00	7.51 ± 0.08^{A}	0.00	5.83 ± 0.07^{A}	0.00
0.5	7.25 ± 0.06^{AB}	0.26	5.79 ± 0.03^{A}	0.04	6.36 ± 0.07^{B}	1.15	5.02 ± 0.18^{B}	0.81
1	6.95 ± 0.09^{B}	0.56	5.69 ± 0.07^{AB}	0.14	6.40 ± 0.05^{B}	1.11	5.12 ± 0.04^{B}	0.71
2	6.86 ± 0.11^{B}	0.65	5.58 ± 0.13^{B}	0.25	5.70 ± 0.18^{B}	1.81	4.44 ± 0.08^{B}	1.39
5	7.03 ± 0.14^{B}	0.48	5.16 ± 0.09^{C}	0.67	5.59 ± 0.40^{B}	1.92	4.27 ± 0.23^{B}	1.56
10	$<10^{C}$	≥6.51*	4.19 ± 0.06^{B}	1.64	$<10^{C}$	≥6.51*	3.11 ± 0.06^{B}	2.72
15	$<10^{C}$	≥6.51*	4.18 ± 0.10^{B}	1.65	$<10^{C}$	≥6.51*	$<10^{C}$	≥4.83*
30	$<10^{C}$	≥6.51*	3.63 ± 0.12^{C}	2.20	$<10^{C}$	≥6.51*	$<10^{C}$	≥4.83*
60	$<10^{C}$	≥6.51*	3.37 ± 0.21^{C}	2.46	$<10^{C}$	≥6.51*	$<10^{C}$	≥4.83*

Different letters denote significant differences when compared within each column alone ($p<0.05$).

*Indicates inactivation values at the detection limit of the assay since KPMS fixed the cell monolayers after the first-log serial dilution but not the lower dilutions of the virus-treatment combination.

Table 2.3: Reduction of FCV-F9 and MNV-1 at high titers on clean (a) and dirty (b) sterile stainless-steel coupons (carrier tests) by 5 mg/mL and 10 mg/mL potassium peroxymonosulfate (KPMS) at room temperature (Continued).

b.

	FCV-F9 (log_{10} PFU/mL) with 5 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 5 mg/mL KPMS		FCV-F9 (log_{10} PFU/mL) with 10 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 10 mg/mL KPMS	
Contact time (min)	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction
Control	7.98 ± 0.03^{A}	0.00	5.85 ± 0.09^{A}	0.00	7.98 ± 0.03^{A}	0.00	5.85 ± 0.09^{A}	0.00
10	2.71 ± 0.08^{B}	5.27	4.59 ± 0.03^{B}	1.26	$<10^{B}$	≥6.98*	3.51 ± 0.09^{B}	2.34
15	$<10^{C}$	≥6.98*	4.43 ± 0.09^{BC}	1.42	$<10^{B}$	≥6.98*	3.33 ± 0.06^{B}	2.52
30	$<10^{C}$	≥6.98*	4.26 ± 0.02^{C}	1.59	$<10^{B}$	≥6.98*	2.83 ± 0.13^{C}	3.02
60	$<10^{C}$	≥6.98*	3.56 ± 0.13^{D}	2.29	$<10^{B}$	≥6.98*	$<10^{C}$	≥4.85*

Different letters denote significant differences when compared within each column alone ($p<0.05$).

*Indicates inactivation values at the detection limit of the assay since KPMS fixed the cell monolayers after the first-log serial dilution but not the lower dilutions of the virus-treatment combination.

Table 2.4: Reduction of FCV-F9 and MNV-1 at high titers on clean sterile stainless-steel coupons by 0.32 mg/mL BAC at room temperature.

	FCV-F9 (log_{10} PFU/mL)		MNV-1 (log_{10} PFU/mL)	
Contact time	Recovered titer	Reduction	Recovered titer	Reduction
Water 1 h	7.88 ± 0.05^{A}	0.00	5.60 ± 0.12^{A}	0.00
1 h	6.43 ± 0.08^{B}	1.45	4.84 ± 0.11^{BC}	0.76
Water 2 h	6.78 ± 0.32^{B}	0.00	5.41 ± 0.33^{AB}	0.00
2 h	5.73 ± 0.05^{C}	1.05	4.71 ± 0.24^{C}	0.70

Different letters denote significant differences when compared within each column alone ($p<0.05$).

Table 2.5: Reduction of FCV-F9 and MNV-1 at high and low titers on sterile stainless-steel coupons with 5% FBS (dirty condition) by 0.32 mg/mL BAC at room temperature.

	FCV-F9 (log_{10} PFU/mL) at Low titer		MNV-1 (log_{10} PFU/mL) at Low titer		FCV-F9 (log_{10} PFU/mL) at High titer		MNV-1 (log_{10} PFU/mL) at High titer	
Contact time (min)	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction
Water 1 h	6.03 ± 0.02^{A}	0.00	4.38 ± 0.07^{A}	0.00	8.14 ± 0.06^{A}	0.00	5.90 ± 0.08^{A}	0.00
1 h	5.85 ± 0.18^{AB}	0.18	4.17 ± 0.06^{B}	0.21	8.14 ± 0.03^{A}	0.00	5.01 ± 0.04^{C}	0.89
Water 2 h	6.10 ± 0.03^{A}	0.00	4.40 ± 0.08^{A}	0.00	8.19 ± 0.05^{A}	0.00	5.99 ± 0.06^{A}	0.00
2 h	5.56 ± 0.11^{B}	0.54	3.94 ± 0.08^{C}	0.46	8.01 ± 0.02^{B}	0.18	5.62 ± 0.02^{B}	0.36

Different letters denote significant differences when compared within each column alone ($p<0.05$).

Table 2.6: Reduction of FCV-F9 and MNV-1 at low titers on dirty carrier tests by 5 and 10 mg/mL potassium peroxymonosulfate (KPMS) at room temperature.

	FCV-F9 (log_{10} PFU/mL) with 5 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 5 mg/mL KPMS		FCV-F9 (log_{10} PFU/mL) with 10 mg/mL KPMS		MNV-1 (log_{10} PFU/mL) with 10 mg/mL KPMS	
Contact time (min)	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction	Recovered titer	Reduction
Control	6.03 ± 0.07^{A}	0.00	3.67 ± 0.09^{A}	0.00	5.93 ± 0.09^{A}	0.00	3.67 ± 0.09^{A}	0.00
0.5	4.09 ± 0.06^{B}	1.94	1.76 ± 0.31^{B}	1.91	2.43 ± 0.09^{B}	3.50	$<10^{B}$	≥2.67*
1	3.72 ± 0.04^{C}	2.31	1.46 ± 0.22^{B}	2.21	$<10^{C}$	≥4.93*	$<10^{B}$	≥2.67*
2	$<10^{C}$	≥5.03*	$<10^{C}$	≥2.67*	$<10^{C}$	≥4.93*	$<10^{B}$	≥2.67*

Different letters denote significant differences when compared within each column alone ($p<0.05$).
*Indicates inactivation values at the detection limit of the assay since KPMS fixed the cell monolayers after the first-log serial dilution but not the lower dilutions of the virus-treatment combination.

CHAPTER III

Inactivation of human norovirus surrogates by sodium metasilicate

C. Cao and D. H. D'Souza*

Department of Food Science and Technology, University of Tennessee-Knoxville, Knoxville, TN, USA, 37996-4591

Running Title: *Sodium metasicilate inactivates human norovirus surrogates*

KEYWORDS: Inactivation, sodium metasilicate, human norovirus surrogates

*Corresponding author: Tel.: 865-974-2753; Fax: 865-974-7332; E-mail: ddsouza@utk.edu

Abstract

Sodium metasilicate is USDA FSIS approved as a processing aid for meat and poultry products with known antibacterial properties and anti-rotaviral activity. However, its efficacy against human noroviruses, the leading cause of non-bacterial gastroenteritis, has not been reported. Concentration-dependent effects of sodium metasilicate at 2%, 5%, and 10% against cultivable human norovirus surrogates, (feline calicivirus (FCV-F9) and murine norovirus (MNV-1)), at room temperature for up to 1 h using both suspension and carrier tests were evaluated. Each virus at high (6~8 $\log_{10}$ PFU/mL) or low (4~6 $\log_{10}$ PFU/mL) titers was mixed with equal volumes of sodium metasilicate and incubated for 15 s, 30 s, 1 min, 2 min, 5 min, 10 min, 15 min, 30 min and 1 h. Each treatment was replicated thrice, recovered viruses were plaque assayed in duplicate, and data was statistically analyzed. Virus reduction by 5 and 10% sodium metasilicate was rapid, with complete reduction within 15 s for both tested viruses at both titers using suspension tests. FCV-F9 and MNV-1 at high titers were reduced to undetectable levels with 2% sodium metasilicate after 2 min and 15 s, respectively; with complete reduction of both viruses at low titers within 15 s. For carrier tests, under dry conditions, complete reduction of FCV-F9 at high titers was achieved by 2 and 5% sodium metaslicate after 10 min and 15 s, respectively; high titers of MNV-1 were reduced to undetectable levels by 2% sodium metasilicate after 2 min; and complete reduction of both tested viruses at low titers were observed within 15 s. This research shows the potential application of the processing aid sodium metasilicate as a 2% solution for the control of human norovirus transmission.

Introduction

Human noroviruses are non-enveloped, single-stranded RNA viruses that belong to the *Caliciviridae* family. They are currently considered the leading cause of foodborne illness outbreaks worldwide. In the United States, over half of the foodborne disease outbreaks reported by the Centers for Disease Control and Prevention from 2006 to 2008 were attributed to human noroviruses (Scallen et al., 2011; Siebenga et al., 2009; CDC, 2011). The difficulty involved in controlling human norovirus outbreaks are due to their ease of transmission, high attack rates, environmental stability and low infectious doses of 10 to 100 viral particles (Caul, 1994; D'Souza et al., 2006; Liu et al., 2009). The virus can be transmitted by person to person, or via the fecal-oral route (Goodgame et al., 2006). In addition, people may be infected by consuming contaminated food or water and touching inanimate objects contaminated with the virus. Outbreaks have occurred in a variety of closed settings, including long-term care and other healthcare facilities, restaurants and catered events, cruise ships, schools, hotels, and other institutional settings and also involve ready-to-eat food items that do not undergo further processing before consumption (CDC, 2011). The symptoms include nausea, vomiting, diarrhea and abdominal pain. Although the infection is self-limiting, the emerging virulent strains can be life-threatening to the elderly and the immuno-compromised (Siebenga et al., 2009; Donaldson et al., 2010). Routine sanitation and hygienic practices need to be followed to prevent outbreaks. Commonly used sanitizers including chlorine containing compounds have been found to be efficacious for surface decontamination (D'Souza and Su, 2010).

Sodium metasilicate is a commonly used USDA FSIS approved processing aid in the food industry that is highly soluble with the pH of a 1% solution ranging from 12.5 to 13.0 (Sharma et al., 2012a). It is approved for use as an antimicrobial component of marinades for

meat and poultry products at up to 2% by weight of the marinades and also as a carcass rinse or spray on raw beef carcasses, subprimals, and trimmings at up to 6% in solutions (USDA FSIS, 2011). Sodium metasilicate has been shown to be effective against foodborne bacterial pathogens including *Escherichia coli* O157: H7 and *Salmonella* species both *in vitro* and in beef trimmings (Carlson et al., 2008; Geornaras et al., 2012; Pohlman et al., 2007; Weber et al., 2004), and also *Campylobacter jejuni* and *Salmonella* Typhimurium in fresh chicken breast meat (Sharma et al., 2012a, b). Weber et al. (2004) found that sodium metasilicate at 0.6% (pH 12.1) resulted in complete inhibition of *E. coli* O157: H7 after a 5 to 10 s exposure in water at room temperature (Weber et al., 2004). Carlson et al., (2008) reported that 4% sodium metasilicate (23^{o}C, pH 12.94) reduced *E. coli* O157: H7 and *Salmonella* by ~1.9 and ~ 2.6 log CFU/mL, respectively from inoculated beef hides following a 30 s- spray- 2 min- dwell- 30 s - water rinse procedure (Carlson et al., 2008). Pohlman et al. (2009) demonstrated that *E. coli* and *Salmonella* Typhimurium were reduced by ~1 log to $\geqslant$1.5 log CFU/mL, respectively, after treatment with 4% sodium metasilicate on inoculated beef trimmings (Pohlman et al., 2009). Geornaras et al. (2012) found similar effectiveness of sodium metasilicate against *E.coli* O157:H7 and 6 non-O157 Shiga toxin-producing *E. coli* (nSTEC) serogroups. After immersion for 30 s in a solution of 4% sodium metasilicate (pH 12.5), initial pathogen counts were found to be reduced by 1.3 to 1.5 log CFU/cm^2 on inoculated beef trimmings from an initial count of 3 to 4 log CFU/cm^2 (Geornaras et al., 2012). Adler et al. (2011) observed that brine solution containing 2.2% sodium metasilicate immediately reduced *E.coli* O157:H7 by $\geqslant$2.4 log CFU/mL at 4 or 15 ^{o}C(Adler et al., 2011).

Besides these foodborne bacterial pathogens, sodium metasilicate has also been evaluated for its efficacy against viral pathogens, including human rotaviruses and vesicular stomatitis

viruses (Springthorpe et al., 1986; Wright 1970). Springthorpe et al. (1986) tested the efficacy of 69 commercial and 7 non-commercial disinfectant formulations against human rotaviruses and found that the efficacy of quaternary ammonium-based disinfectant formulations was greatly improved by adding 0.5 to 5% sodium metasilicate, being effective against human rotaviruses. They believed that quaternary ammonium compounds and sodium metasilicate may act in an additive or synergistic manner to inactivate human rotaviruses (Springthorpe et al., 1986). However, information is currently lacking on the antiviral activity of sodium metasilicate against human norovirus surrogates *in vitro*. The objective of this study was to determine the ability of 2%, 5%, and 10% sodium metasilicate to inactivate human norovirus surrogates, FCV-F9 and MNV-1, in suspension and using carrier tests (stainless steel coupons) at room temperature.

Materials and Methods

Virus stocks and cell lines: Feline calicivirus (FCV-F9) and its host, Crandell Reese Feline Kidney (CRFK) cells, were obtained from American type culture collection (ATCC) (Manassas, VA). Murine norovirus (MNV-1) was kindly provided by Dr. Skip Virgin (Washington University, St. Louis, MO), and its host, RAW 264.7 cells were obtained from the University of Tennessee cell culture collection. Viral stocks containing ~6 $\log_{10}$ PFU/mL of MNV-1 and ~8 $\log_{10}$ PFU/mL of FCV-F9 were obtained as previously described using their respectively confluent cell lines in 175cm^2 flasks (D'Souza et al., 2011; Su et al., 2011).

Chemicals: Sodium metasilicate obtained from MP Biomedicals (Solon, OH, USA) was dissolved in de-ionized water and then filtered through 0.2 µ m filters (Fisher Scientific, GA, USA) to reach concentrations of 10% (w/v), 5% (w/v), and 2% (w/v).

Treatments: For suspension tests, one volume (167 µ l) of sodium metasilicate was mixed with the same volume of virus to reach final concentrations of 5% (w/v), 2.5% (w/v), and 1% (w/v) and incubated for 15 s, 30 s, 1 min, 2 min, 5 min, 10 min, 15 min, 30 min and 1 h at room temperature. Sterile de-ionized water was used as a control to compare with the treated samples. At each time point, treatments were neutralized by serially diluting in Dulbecco's Modified Eagle's Medium/Ham's F-12 (DMEM, HyClone Laboratories, Inc, Logan, Utah) containing 10% heat-inactivated Fetal Bovine Serum (FBS, HyClone Laboratories, Inc, Logan, Utah), followed by serial dilutions in DMEM containing 2% heat-inactivated FBS.

For the carrier test, stainless steel coupons sterilized by autoclaving were used as a surface model (fomite) to simulate natural conditions. MNV-1 and FCV-F9 were inoculated on sterile coupons at high and low titers and allowed to dry for 1 h at room temperature in a BioSafety Level 2 hood. After drying, the coupons were treated with 2% sodium metasilicate for various time intervals from 0, 15 s, 30 s, 1, 2, 5, 10, 15, 30 and 60 min at room temperature (RT).

Plaque Assays: Treated samples and controls were inoculated onto the confluent CRFK cells for FCV-F9 and RAW 267.4 cells for MNV-1 in 6-well plates, and incubated for 2.5 h at 37 ℃ under 5% CO_2. Then overlay media containing 0.75% (w/v) noble agar was added to each well and incubated for 2 to 3 days at 37℃ in a CO_2 incubator. Finally, neutral red (Sigma-Aldrich, St. Louis, MO) containing overlay media was added and incubated for 3 to 24 h to visualize the plaques.

Statistical Analysis: All treatments and controls were replicated three times in duplicates. A one-way analysis of variance (ANOVA) was used to test for differences in means of plaque counts among treatments and means were analyzed using a completely randomized

design at $p<0.05$ in SAS program (version 9.3, SAS Institute, Cary, NC, USA). If statistically significant differences were found using the ANOVA, the Tukey's Post Hoc test was used to analyze differences in means between treatments.

Results

Effect of 5 and 10% sodium metasilicate against FCV-F9 and MNV-1 at high and low titers in suspension tests: Both 5 and 10% sodium metasilicate reduced high and low titers of FCV-F9 and MNV-1 to undetectable levels within 15 s at room temperature (data not shown).

Effect of 2% sodium metasilicate against FCV-F9 and MNV-1 in suspension tests: Sodium metaslicate at 2% reduced FCV-F9 at low titers and MNV-1 at both high and low titers to undetectable levels within 15 s at room temperature. The reduction caused by 2% sodium metasilicate on high titers of FCV-F9 in suspension tests are shown in Table 3.1. FCV-F9 at high titers were reduced by 5.42, 6.02, and 6.18 log PFU by 2% sodium metasilicate after 15 s, 30 s, and 1 min, respectively; and complete reduction was observed after 2 min contact time at room temperature.

Effect of 2% sodium metasilicate against FCV-F9 and MNV-1 in carrier tests: Low titers of FCV-F9 and MNV-1 were reduced to undetectable levels within 15 s at room temperature using stainless steel coupons. The effects of 2% sodium metasilicate on high titers of FCV-F9 and MNV-1 in carrier tests are shown in Table 3.2. Compared to the titer reduction of the tested viruses at high titers in suspension tests, the titer reduction of FCV-F9 and MNV-1 in carrier tests appeared to be slightly lower, where 2% sodium metasilicate decreased the FCV-F9 at high titers by 2.28, 3.19, 3.59 and 4.80 $\log_{10}$ PFU/mL, respectively after 30 s, 1 min, 2 min and 5 min of exposure time. High titers of MNV-1 were decreased by 1.04 and 2.02 $\log_{10}$ PFU/mL

after 30 s and 1min, respectively with 2% sodium metasilicate. FCV-F9 and MNV-1 were reduced to non-detectable limits (based on the detection limit of the plaque assays 1 $\log_{10}$ PFU/mL) by 2% sodium metasilicate after 10 min and 2 min of contact times at room temperature, respectively.

Discussion

In the present study, the effect of sodium metasilicate at concentrations of 2%, 5%, and 10% on inactivation of FCV-F9 and MNV-1 was evaluated. Sodium metasilicate was found to cause rapid reduction in titers of the tested human noroviral surrogates. FCV-F9 and MNV-1 at low titers were reduced to undetectable levels within 15 s by 2% sodium metasilicate in both suspension and carrier tests at room temperature. Complete reduction of FCV-F9 and MNV-1 at high titers was achieved by 2% sodium metasilicate after 2 min and within 15 s in suspension tests, respectively. In carrier tests, high titer FCV-F9 and MNV-1 were reduced to undetectable levels after 10 min and 2 min at room temperature, respectively. Increasing the concentrations of sodium metasilicate to 5 and 10% were found to reduce FCV-9 and MNV-1 at high titers to undetectable levels within 15 s in both suspension and carrier tests. The results revealed the potential suitability of using sodium metasilicate to prevent the transmission of human noroviral outbreaks.

Chemicals other than sodium metasilicate used include trisodium phosphate (TSP), a common household cleanser, which is also an alkaline detergent with the pH of 12 in 1% solution, and considered as GRAS by the FDA (Lindsay 1985). TSP is reported to be effective against a wide range of pathogens, including *E. coli* O157: H7, *Salmonella*, *Poliovius* 1 and bacteriophages. Jerzy and others (2003) reported that significant reductions of bacteriophage

MS2 and poliovirus1 were obtained using 1.0% TSP solution on strawberries (Jerzy et al., 2003). D'Souza and Su (2010) reported that 5% TSP effectively inactivated high titers of MNV-1 and FCV-F9 by ⩾6 log PFU/mL, and with ~ 5 log reduction of low viral titers after 30s or 1 min contact time. TSP at 2% were found to inactivate high titers of FCV-F9 by ⩾6 log PFU/mL, but only a 1.05 log reduction for MNV-1 after 1 min was obtained. TSP at 1% was also reported to reduce FCV-F9 by ~2.65 log PFU/mL, while no reduction was observed for high titers of MNV-1 (D'Souza and Su, 2010). In this study, sodium metasilicate at the lower concentrations of 2% appears to be more effective than TSP for the titer reduction of these human noroviral surrogates.

The mechanism of antibacterial activity of TSP is believed to be due to a combination of high pH (~12), ionic strength and detergent effects that can cause cell wall and membrane disruption of Gram-negative foodborne pathogens (Mendonca et al., 1994; Sampathkumar et al., 2003; Yuk and Marshall, 2006). However, the mechanism of virucidal activity has not been elucidated. The mechanism of antimicrobial effect of sodium metasilicate could be similar to TSP in that they are both strongly alkaline. Further research is needed to explore the antiviral mechanism of action.

The application of sodium metasilicate in food industry can be broader than TSP, as sodium metasilicate has been approved to be directly added in food as a processing aid to wash fruits, vegetables, and nuts. It can also be applied in poultry and meat products as an antimicrobial (US FDA 2003). Furthermore, previous studies have shown that sodium metasilicate can maintain the quality of fresh commercial poultry, having no negative effects on sensory, physical or chemical characteristics. These investigators found that 1-4% sodium metasilicate did not adversely affect sensory characteristics, color, texture or pH of fresh

commercial chicken breast meat at 4°C after 9 days (Huang et al., 2011). Pohlman et al., (2009) also reported little impact of sensory odor and color characteristics was observed by 4% sodium metasilicate in beef trimmings (Pohlman et al., 2009). Additionally, Quilo et al. (2010) revealed that better quality were perceived by using 4% sodium metasilicate in beef trimmings, resulting in much juicier, lower Warner-Bratzler shear force value for texture, and less cooking loss than the control samples (Quilo et al., 2010).

Besides alkaline chemical treatments, acidic solutions, including lactic acid and peroxyacetic acid, also have a long history of commercial use for the inactivation of foodborne pathogens. The mode of action of these organic acids and its salts on foodborne bacteria pathogens appears to be the result of the diffusion of the undissociated form of the acid into the cell, leading to decreasing intracellular pH. In order to sustain the pH inside the cell, ATPase pumps out protons at the expense of ATP. Eventually, cells die due to the lack of cellular energy (Bogaert and Naidu, 2000). The acid mediated mechanisms leading to a loss of infectivity of foodborne viruses has not been completely understood, which is believed to result from the alterations of the virus capsid and nucleic acid (Cliver, 2009; Salo and Cliver, 1976). However, whether the effect was derived from the decrease in pH or the type of acid is still not clear. It is appeared that the inactivation of non-enveloped viruses is due to the denaturation of the capsid proteins due to the decrease in pH (Rodger et al., 1977).

Previous research has shown the effect of lactic acid and peroxyacetic acid against human norovirus surrogates. Using suspension tests, Straube et al., (2011) found that D, L-lactic acid at the concentration of 0.3% (pH 3.4-3.5) and 0.4% (pH 3.2-3.3) could reach a significant titer reduction of FCV-F9 after 7 days at 20°C (Straube et al., 2011). Baert et al., (2009) reported that the application of 250 mg/L peroxyacetic acid to washing solution could obtain a supplementary

1-log reduction of MNV-1 on shredded iceberg lettuce (compared to tap water), and its efficacy was not influenced by the presence of organic materials (Baert et al., 2009). Fraisse et al., (2011) found that 100 ppm of peroxyacetic-based biocide effectively inactivated FCV-F9 by 3.2 log units and MNV-1 by 2.3 log units on the lettuce leaves after 2 min of contact time, as estimated by cell culture (Fraisse et al., 2011).

Other effective cleansers, including chlorine have been commonly used to disinfect foodborne pathogens. Chlorine has been tested for antiviral activity against human norovirus surrogates and found to be very effective in inactivating FCV-F9 and MNV-1 in suspension, on surfaces, and on produce (Belliot et al., 2008; D'Souza and Su, 2010; Doultree et al., 1999; Duizer et al., 2004; Fraisse et al., 2011; Kitajima et al., 2010; Urakami et al., 2007). However, due to the corrosive nature of chlorine, bleaching of food and unfavorable sensory effects, it has limitations for use. Furthermore, by-products including trihalomethanes may be generated due to interaction with organic matters, which can cause carcinogenic products and potential risks to public health (Dunnick and Melnick, 1993). Overall, based on the findings of this study, sodium metasilicate may be suitable and applicable for the food industry to decrease foodborne human noroviral transmission risk.

Conclusion

Sodium metasilicate at 2% is found to be very suitable for inactivating human norovirus surrogates, FCV-F9 and MNV-1 in both suspension and carrier tests, indicating its potential use as an antiviral component for surface disinfection or could be potentially used in marinades. This chemical is already approved as a processing aid in meat and poultry products and being fast-acting has great promise to reduce human norovirus surrogate transmision. The application of

sodium metasilicate as an alternative coating or strategy to decontaminate foodborne viruses in foods, such as meat and fresh produce, needs to be investigated.

Acknowledgements

Funding for this research was provided by the TN Agricultural Experiment Station (UT-HATCH TEN-00391) and is gratefully acknowledged. The use of trade names does not imply endorsement by the University of Tennessee-Knoxville.

References

Adler, J.M., Geornaras, I., Byelashov, O.A., Belk, K.E., Smith, G.C., Sofos, J.N., 2011. Survival of *Escherichia coli* O157: H7 in meat product brines containing antimicrobials. Journal of food science 76, M478-M485.

Baert, L., Debevere, J., Uyttendaele, M., 2009. The efficacy of preservation methods to inactivate foodborne viruses. International journal of food microbiology 131, 83-94.

Belliot, G., Lavaux, A., Souihel, D., Agnello, D., Pothier, P., 2008. Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. Applied and environmental microbiology 74, 3315-3318.

Bogaert, J.-C., and A. S. Naidu. 2000. Lactic acid, p. 613–636. In A. S. Naidu (ed.), Natural food antimicrobial systems. CRC Press, Boca Raton, Fla.

Carlson, B.A., Ruby, J., Smith, G.C., Sofos, J.N., Bellinger, G.R., Warren-Serna, W., Centrella, B., Bowling, R.A., Belk, K.E., 2008. Comparison of antimicrobial efficacy of multiple beef hide decontamination strategies to reduce levels of *Escherichia coli* O157: H7 and *Salmonella*. Journal of Food Protection 71, 2223-2227.

Caul, E. O. 1994. Small structured viruses: airborne transmission and hospital control. Lancet 343:1240–1242.

Cliver, D.O., 2009. Capsid and infectivity in virus detection. Food and environmental virology 1, 123-128.

D'Souza, D.H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C., Jaykus, L.A., 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. International Journal of Food Microbiology 108, 84-91.

D'Souza, D.H., Su, X., 2010. Efficacy of chemical treatments against murine norovirus, feline calicivirus, and MS2 bacteriophage. Foodborne Pathogens and Disease 7, 319-326.

D'Souza, D.H., Su, X., Harte, F., 2011. Comparison of reduction in foodborne viral surrogates by high pressure homogenization. Journal of Food Protection 74, 1840-1846.

Donaldson, E.F., Lindesmith, L.C., LoBue, A.D., Baric, R.S., 2010. Viral shape-shifting: norovirus evasion of the human immune system. Nature Reviews Microbiology 8, 231-241.

Doultree, J., Druce, J., Birch, C., Bowden, D., Marshall, J., 1999. Inactivation of feline calicivirus, a Norwalk virus surrogate. Journal of Hospital Infection 41, 51-57.

Duizer, E., Bijkerk, P., Rockx, B., De Groot, A., Twisk, F., Koopmans, M., 2004. Inactivation of caliciviruses. Applied and environmental microbiology 70, 4538-4543.

Dunnick, J.K., Melnick, R.L., 1993. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. Journal of the National Cancer Institute 85, 817-822.

Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., Vialette, M., Morin, T., Perelle, S., 2011. Comparison of chlorine and peroxyacetic-based disinfectant to inactivate Feline calicivirus, Murine norovirus and Hepatitis A virus on lettuce. International journal of food microbiology 151, 98-104.

Geornaras, I., Yang, H., Manios, S., Andritsos, N., Belk, K.E., Nightingale, K.K., Woerner, D.R., Smith, G.C., Sofos, J.N., 2012. Comparison of Decontamination Efficacy of Antimicrobial Treatments for Beef Trimmings against *Escherichia coli* O157: H7 and 6 Non‐O157 Shiga Toxin‐Producing *E. coli* Serogroups. Journal of food science 77, 539-544.

Huang, H., Williams, S., Sims, C., Simmone, A., 2011. Sodium metasilicate affects antimicrobial, sensory, physical, and chemical characteristics of fresh commercial chicken breast meat stored at 4° C for 9 days. Poultry Science 90, 1124-1133.

Jerzy, L., Bradley, M.L., Scott, T.M., Mabel, D., Andrew, K., Wei-Yea, H., Bartz, J.A., Farrah, S.R., 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157: H7 on strawberries by physical and disinfectant washes. Journal of Food Protection 66, 188-193.

Kitajima, M., Tohya, Y., Matsubara, K., Haramoto, E., Utagawa, E., Katayama, H., 2010. Chlorine inactivation of human norovirus, murine norovirus and poliovirus in drinking water. Letters in applied microbiology 51, 119-121.

Kukkula, M., Maunula, L., Silvennoinen, E., von Bonsdorff, C.-H., 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. Journal of Infectious Diseases 180, 1771-1776.

Lindsay, R.C. 1985. Food additives. In Food Chemistry, 2nd Ed., (O.R.Fennema, ed.) pp. 629–687, Marcel Dekker, New York.

Liu, P., Chien, Y.-W., Papafragkou, E., Hsiao, H.-M., Jaykus, L.-A., Moe, C., 2009. Persistence of human noroviruses on food preparation surfaces and human hands. Food and environmental virology 1, 141-147.

Mendonca, A.F., Amoroso, T.L., Knabel, S.J., 1994. Destruction of Gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Applied and environmental microbiology 60, 4009-4014.

Pohlman, F., DIAS‐MORSE, P., Quilo, S., Brown Jr, A., Crandall, P., Baublits, R., Story, R., Bokina, C., Rajaratnam, G., 2009. Microbial, instrumental color and sensory characteristics of ground beef processed from beef trimmings treated with potassium lactate, sodium metasilicate, peroxyacetic acid or acidified sodium chlorite as single antimicrobial interventions. Journal of Muscle Foods 20, 54-69.

Quilo, S., Pohlman, F., Dias-Morse, P., Brown, A., Crandall, P., Story, R., 2010. Microbial, instrumental color and sensory characteristics of inoculated ground beef produced using potassium lactate, sodium metasilicate or peroxyacetic acid as multiple antimicrobial interventions. Meat science 84, 470-476.

Rodger, S.M., Schnagl, R.D., Holmes, I., 1977. Further biochemical characterization, including the detection of surface glycoproteins, of human, calf, and simian rotaviruses. Journal of virology 24, 91-98.

Salo, R., Cliver, D., 1976. Effect of acid pH, salts, and temperature on the infectivity and physical integrity of enteroviruses. Archives of virology 52, 269-282.

Sampathkumar, B., Khachatourians, G.G., Korber, D.R., 2003. High pH during trisodium phosphate treatment causes membrane damage and destruction of *Salmonella enterica* serovar Enteritidis. Applied and environmental microbiology 69, 122-129.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17, 7-15.

Sharma, C., Williams, S., Schneider, K., Schmidt, R., Rodrick, G., 2012a. Sodium metasilicate affects growth of *Campylobacter jejuni* in fresh, boneless, uncooked chicken breast fillets stored at 4 degrees Celsius for 7 days1, 2. Poultry Science 91, 2324-2329.

Sharma, C., Williams, S., Schneider, K., Schmidt, R., Rodrick, G., 2012b. Sodium metasilicate affects growth of *Salmonella* Typhimurium in fresh, boneless, uncooked chicken breast fillets stored at 4° C for 7 days. Poultry Science 91, 719-723.

Siebenga, J.J., Vennema, H., Zheng, D.-P., Vinjé, J., Lee, B.E., Pang, X.-L., Ho, E.C., Lim, W., Choudekar, A., Broor, S., 2009. Norovirus illness is a global problem: emergence and spread of norovirus GII. 4 variants, 2001–2007. Journal of Infectious Diseases 200, 802-812.

Springthorpe, V.S., Grenier, J.L., Lloyd-Evans, N., Sattar, S.A., 1986. Chemical disinfection of human rotaviruses: efficacy of commercially-available products in suspension tests. Epidemiology and infection 97, 139-161.

Straube, J., Albert, T., Manteufel, J., Heinze, J., Fehlhaber, K., Truyen, U., 2011. *In vitro* influence of d/l-lactic acid, sodium chloride and sodium nitrite on the infectivity of feline calicivirus and of ECHO virus as potential surrogates for foodborne viruses. International journal of food microbiology 151, 93-97.

Su, X., D'Souza, D.H., 2011. Trisodium phosphate for foodborne virus reduction on produce. Foodborne Pathogens and Disease 8, 713-717.

Urakami, H., Ikarashi, K., Okamoto, K., Abe, Y., Ikarashi, T., Kono, T., Konagaya, Y., Tanaka, N., 2007. Chlorine sensitivity of feline calicivirus, a norovirus surrogate. Applied and environmental microbiology 73, 5679-5682.

U.S. Food and Drug Administration, Code of Federal Regulations. 2003. Sodium metasilicate. 21 CFR 184.1769a.

U.S. Food and Drug Administration, Code of Federal Regulations. 2003. Use of food ingredients and sources of radiation. 9 CFR 424.21.

USDA FSIS (United States Department of Agriculture Food Safety and Inspection Service). 2011. FSIS Directives. Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products. 7120.1, Revision 5. Accessed Aug. 22, 2011. http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1.pdf.

Weber, G.H., Obrien, J.K., Bender, F.G., 2004. Control of *Escherichia coli* O157: H7 with sodium metasilicate. Journal of Food Protection 67, 1501-1506.

Weinstein, R.A., Said, M.A., Perl, T.M., Sears, C.L., 2008. Gastrointestinal flu: norovirus in health care and long-term care facilities. Clinical infectious diseases 47, 1202-1208.

Wright, H.S., 1970. Inactivation of Vesicular stomatitis virus by disinfectants. Applied microbiology 19, 96-99.

Yuk, H.-G., Marshall, D.L., 2006. Effect of trisodium phosphate adaptation on changes in membrane lipid composition, verotoxin secretion, and acid resistance of *Escherichia coli* O157: H7 in simulated gastric fluid. International journal of food microbiology 106, 39-44.

Appendix

Table 3.1: The effect of sodium metasilicate at 2% on reduction of FCV-F9 at high titer using suspension tests at room temperature.

FCV-F9 (log_{10} PFU/mL)		
Contact time	Recovered titer	Reduction
Control	7.86 ± 0.08^{A}	0.00
15 s	2.45 ± 0.16^{B}	5.41
30 s	1.84 ± 0.34^{BC}	6.02
1 min	1.68 ± 0.23^{C}	6.18
2 min	$<10^{D}$	≥6.86*
5 min	$<10^{D}$	≥6.86*

Different letters denote significant differences when compared within each column alone ($p<0.05$).

*Indicates inactivation values at the detection limit of the assay since sodium metasilicate fixed the cell monolayers after the first-log serial dilution but not the lower dilutions of the virus-treatment combination.

Table 3.2: The effect of sodium metasilicate at 2% on reduction of FCV-F9 and MNV-1 at high titers using carrier tests at room temperature.

	FCV-F9 (log_{10} PFU/mL)		MNV-1 (log_{10} PFU/mL)	
Contact time	Recovered titer	Reduction	Recovered titer	Reduction
Control	7.97 ± 0.08^{A}	0.00	5.74 ± 0.11^{A}	0.00
30 s	5.69 ± 0.17^{B}	2.28	4.70 ± 0.04^{B}	1.04
1 min	4.79 ± 0.12^{C}	3.18	3.72 ± 0.06^{C}	2.02
2 min	4.38 ± 0.16^{D}	3.59	$<10^{D}$	≥4.74*
5 min	3.17 ± 0.15^{E}	4.80	$<10^{D}$	≥4.74*
10 min	$<10^{F}$	≥6.97*	$<10^{D}$	≥4.74*
15 min	$<10^{F}$	≥6.97*	$<10^{D}$	≥4.74*

Different letters denote significant differences when compared within each column alone ($p<0.05$).

*Indicates inactivation values at the detection limit of the assay since sodium metasilicate fixed the cell monolayers after the first-log serial dilution but not the lower dilutions of the virus-treatment combination.

Vita

Cong Cao was born in Zhejiang, China on September 14, 1988. She grew up in the main island of Zhoushan Islands and graduated from Zhoushan High School in 2007. She continued her education at Hunan Agricultural University where she earned a B.S. degree majoring in Food Science and Engineering in 2011. In 2013, she earned an M.S degree in Food Science and Technology from the University of Tennessee, Knoxville.