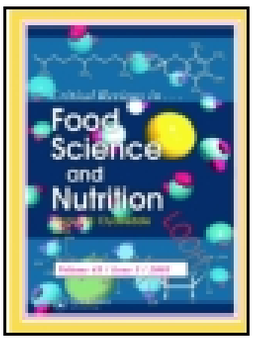

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Norovirus elimination on the surface of fresh foods

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ABSTRACT

Fresh foods like fruits, vegetables and shellfish are potential sources for viral infections such as human norovirus (NoV). Chemical treatment like chlorination is a well-known process for food pathogens and virus elimination. However, with the increase of the consumer demand for less toxic treatment, the use of natural antimicrobials like essential oils from spice or plants, fruit extracts, and cold pasteurization treatments (fermentation, irradiation, ozonation and high pressure) could be considered. The aim of this review is to discuss these technologies and their efficacy to eliminate NoV on the surface of fresh food.

KEYWORDS

Human norovirus; inactivation; feline calicivirus; murine norovirus; fresh food and food surfaces

Introduction

Human norovirus (NoV) is the leading cause of viral gastroenteritis worldwide. Indeed, the annual costs associated with disease are high and include absences from work, hospitalization or death. In fact, according to the United States Department of Agriculture (USDA), food-borne illnesses cost between 10 to 83 billion USD each year (McLinden et al. 2014). NoV is ranked among 5 of the costliest pathogens associated with foodborne infections within the United States of America (USA) (Scharff 2012). The burden of NoV in the USA is estimated to be around 900 deaths, 109 000 hospitalizations and more than 465 000 Emergency Department (ED) visits, annually (Burke et al. 2020). Fruits, vegetables and shellfish are highly contaminated by viruses such as NoV (Berger et al. 2010; Gyawali et al. 2019). They are mainly contaminated before and after harvest in the farm. Indeed, even before harvest, fertilizers and water used for irrigation can contaminate foods (Cheong et al. 2009; Mara and Sleigh 2010; Wei and Kniel 2010). In addition, it only takes less than 100 infectious particles of NoV to cause the disease (Sair et al. 2002).

NoV is a member of the caliciviridae family, containing a positive-sense single-stranded RNA genome (approximately 7.7 kb in size) enclosed in a non-enveloped capsid (Glass, Parashar, and Estes 2009). This type of virus is highly resistant to environmental conditions and antimicrobials in general (Barker, Stevens, and Bloomfield 2001; Watanabe, Miyata, and Sato 1989). NoVs are enteric viruses that are present in feces and vomit of infected individuals (Rzezutka and Cook 2004). Humans are involuntarily infected during their life with NoVs because of the multiple transmission modes. For example, there is direct transmission from person-to-person with contaminated individuals. Plus, there is also transmission through manipulation of surfaces or objects contaminated by NoV (dishes preparation, utensils, handles) and finally the consumption of contaminated foods with NoV such as potable water (Lane et al. 2019; Lopman et al. 2012). The fact that NoV is able to survive on many environmental surfaces for weeks represents a major concern in finding ways to inactive it (Barker, Vipond, and Bloomfield 2004).

People who have been exposed to the virus, usually have symptoms from 24 – 48 hours or within the first 12 hours post-consumption (Fretz-Männel et al. 2005). The most common symptoms include nausea, vomiting, diarrhea and stomach cramps (Ahmed et al. 2014; Patel et al. 2008; Ryu et al. 2015).

Currently, it remains impossible to propagate NoV and to distinguish infectious particles. There are no animal models or cell culture possible for NoV detection. However, there are NoV surrogates such as feline calicivirus (FCV) and murine norovirus (MNV) (Hirneisen, Markland, and Kniel 2011; Lacombe et al. 2017). They represent accurate models since they have the same shape and size as NoV (Wobus, Thackray, and Virgin 2006). Foods such as lettuce are good representative models for research on NoV because of the multipliable leaves and the many inclusions, making it difficult to adequately wash (Fraisse et al. 2011).

In fact, there has been an evolution of consumer's demand in correlation with food technologies. Since the 80's, consumers have desired mass produced, ready-to-eat and individual portions (Cayot 2007). Moreover, there is increasing need to develop safer and healthier food without synthetic additives. There are many different techniques for food decontamination; two of them are ozonation (Dubuis et al. 2020; Hirneisen, Markland, and Kniel 2011; Khadre, Yousef, and Kim 2001) and irradiation (Holley 2014; Lacroix and Vigneault 2007; Molina-Chavarria et al. 2020).

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NoV is highly resistant to chemicals such as alcohol and quaternary ammonium compounds (Donaldson et al. 2008). It is important to notice that nonenveloped viruses such as NoV are resistant to many antimicrobials under various environmental conditions (Gilling et al. 2014b). This contributes into viral spread of NoV during transmission.

The aim of this review is to discuss on the use of natural extracts to eliminate NoV from food surfaces by using essential oils (EOs), natural plant extracts and also various techniques such as fermentation, irradiation, ozonation and high pressure.

Anti-viral treatments

Synthetic chemical compounds

Currently, industries use a washing process for ready-to-eat fruits and vegetables that includes detergents, followed by a rinsing step with water (Fraisse et al. 2011). Detergents have been proven effective for their antimicrobial properties. The inactivation of viral infectivity is mainly due to the damage of the viral genome. For example, H_2O_2 is an oxidizing agent and produces hydroxyl free radicals (•OH) that target essential cell components like lipids, proteins and DNA (Fraisse et al. 2011). Bleach is probably the most common disinfectant used in food industries, because it acts quickly and efficiently and because of its low cost (Fraisse et al. 2011; Seymour and Appleton 2001). Chlorine Dioxide is known for its antiviral properties such as acting on viral nucleic acids and proteins (Li et al. 2004). The following results concerning synthetic chemical agents will be presented in Table 1.

Chlorine (Cl) and chlorine dioxide (ClO_2)

Chlorine is an important chemical for treatment of drinking water. It is also found in disinfectants or in detergents and acts as a bleaching agent (Beuchat et al. 1998). Chlorine Dioxide (ClO_2) and Hydrogen peroxide (H_2O_2) are both oxidizing agents targeting nucleic acids and proteins. For enteric viruses, such as NoV, FCV and MNV, the first target should be the capsid and followed next by the nucleic acid. The gaseous form of these two compounds is more antimicrobial than the aqueous state because of increased diffusibility and the penetration (Montazeri et al. 2017).

Viral FCV suspensions of 20 uL at a concentration of 2.6×10^6 Tissue Culture Infectious Dose/mL ($TCID_{50}$/mL), quantity of pathogen agent that will produce pathological change in 50% of cell culture inoculated) were treated with sodium hypochlorite (NaClO) to determine viral sensitivity to this chemical agent (10 µg/mL) (Urakami et al. 2007). The study showed that the sensitivity of viral FCV increased with a reduction in the amount of cellular host debris. They partially purified the virus from the cellular hosts and demonstrated that FCV infectivity was reduced by more than 4.6 log after a 5 minutes treatment in presence of 300 ng/mL of free chlorine (Urakami et al. 2007) (Table 1).

Thurston-Enriquez et al. (2003) treated FCV in presence of free-chlorine at a concentration of 0.5 mg/L for 0.25 minutes (pH 6.0 and 5 °C) and observed a 4.3 log reduction of FCV in buffered-demand-free (BDF) water (Table 1).

Duizer et al. (2004) studied the effect of 3000 ppm of NaClO on FCV (2×10^5 $TCID_{50}$/mL-1×10^6 $TCID_{50}$/mL) and obtained a reduction of 5.0 $\log_{10}$ $TCID_{50}$/mL after 10 minutes of treatment at pH 7.0 and 22 °C (Table 1). Belliot et al. (2008) observed a reduction of 4.0 $\log_{10}$ PFU/mL of MNV-1 after a treatment with NaClO at 36.3 mM for 0.5 minute (Table 1).

Industrial processes used for ready-to-eat vegetables include a prior washing step with tap water containing a disinfectant such as NaClO followed by a rinsing step. Fraisse et al. (2011) demonstrated that a washing step with or without ultrasounds followed by another washing step, in presence of disinfectant such as NaClO during 2 minutes at 15 ppm, caused a decrease of 2.9 log units of an FCV population on the surface of fresh lettuce. However, MNV-1 virus was shown to be a bit more resistant; a 1.4 log reduction was observed with the same treatment. A simple disinfectant-free wash showed a decrease of 0.7 log for FCV and 1.0 log for MNV-1 (Fraisse et al. 2011) (Table 1).

Chlorine dioxide is a yellow/red gas and represents an oxidized form of chlorine. It is also used as a disinfectant and bleaching agent (Du, Han, and Linton 2002). Thurston-Enriquez et al. (2005) studied the efficiency of ClO_2 against FCV after 0.75 minutes of treatment with ClO_2 at a concentration of 0.90 mg/L at pH 8.0 at a temperature of 5 °C. A

Table 1. Examples of performance of synthetic chemical compounds against virus.

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Free chlorine	300 ng/mL, 5 min	4.6 $\log_{10}$ $TCID_{50}$/mL FCV	Urakami et al. (2007)
Free chlorine	0.5 mg/L, 0.25 min at pH 6 and 5 °C	4.3 $\log_{10}$ MPN/mL FCV	Thurston-Enriquez et al. (2003)
NaClO	3000 ppm, 10 min at RT	5 $\log_{10}$ $TCID_{50}$/mL FCV	Duizer et al. (2004)
NaClO	36.4 mM for 0.5 min	4 $\log_{10}$ PFU/mL MNV-1	Belliot et al. (2008)
Washing step (with or without ultrasound) followed by washing with NaClO	2 min at 15 ppm of active chlorine	2.9 $\log_{10}$ $TCID_{50}$/mL FCV 1.4 $\log_{10}$ $TCID_{50}$/mL MNV	Fraisse et al. (2011)
ClO_2	0.75 min at 0.90 mg/L at pH 8.0 and at a temperature of 5 °C	3.6 $\log_{10}$ MPN/mL FCV	Thurston-Enriquez et al. (2005)
ClO_2	0.25 min at 0.72 mg/L at pH 8.0 and at a temperature of 15 °C	4.15 $\log_{10}$ MPN/mL FCV	Thurston-Enriquez et al. (2005)
$C_2H_4O_3$	100 ppm	3.2 $\log_{10}$ $TCID_{50}$/mL FCV 2.3 $\log_{10}$ $TCID_{50}$/mL MNV	Fraisse et al. (2011)

MPN: most probable number, RT: room temperature.

reduction of 3.6 $\log_{10}$ most probable number/mL (MPN/mL). When a concentration of 0.72 mg/L was used for 0.25 minutes of treatment at pH 8 and at a temperature of 15 °C, a reduction of more than 4.15 $\log_{10}$ MPN/mL was observed (Table 1).

Peracetic acid ($C_2H_4O_3$) is a biocidal agent and its degradation products (acetic acid and oxygen) are less toxic for the environment (McDonnell and Russell 1999). The mode of action of this product results in the disruption of chemical bonds that stabilize the cell membrane of microorganisms (Block 2001).

Fraisse et al. (2011) demonstrated that a concentration of 100-ppm of $C_2H_4O_3$ can reduce the level of FCV by 3.2 log and MNV-1 by 2.3 log. Their results also showed that a simple disinfectant-free water wash showed a decrease of 0.7 log for FCV and 1.0 log for MNV-1 (Table 1).

The washing of fruits and vegetables with chemicals can incur additional costs. For example, sodium-orthophenylphenate-hexamine creates dullness of fruits, which then require wax for polishing (Smith 1962).

Industries are moving toward more natural food-processing pathways, and many new options are being considered, notably the use of natural extracts like plants, spices and fruit extracts. These compounds could be used alone or in combination with cold processes like ozonation, irradiation or high pressure. Fermentation is also considered as an efficient process to eliminate the virus.

Natural compounds used to eliminate virus

The following results concerning the natural compounds used against the viruses will be presented in Table 2.

Plant extracts

Plant extracts are rich in polyphenols. These compounds include amongst others phenolic acids, flavonoids (flavanones, anthocyanidins) and tannins (Shahidi and Ambigaipalan 2015). Flavonoid is the most important category and is subdivided into six classes: Flavones, Flavanones, Flavonols, Isoflavones, Anthocyanidins and Flavanols (Manach et al. 2004). Flavonoids are widely found in fruits, in vegetables and in red wine. A diet rich in fruits and vegetables is correlated with low incidences of coronary heart disease and cancer (Block, Patterson, and Subar 1992). Some phenolic compounds present in fruits and vegetables such as flavonoids (apigenin, myricetin, robinetin), isoflavanoids (biochanin A) and lignans (sesamin) showed protective effects against cancer in animal models (Vercauteren and Chèze 1998).

Carvacrol is a monoterpenic phenol (Ultee et al. 2000). In fact, carvacrol is a powerful bactericidal, antiviral and anti-infectious compound (Nostro and Papalia 2012). This compound is found in many spices like oregano, savory and thyme (Alinkina, Misharina, and Fatkullina 2013) and has been recognized as a natural and economic preservative (Lu and Wu 2010). It was observed by Gilling et al. (2014b) that carvacrol can also inhibit MNV by acting on the capsid first and subsequently on the viral RNA. Sánchez, Aznar, and Sánchez (2015) tested the efficiency of 0.25, 0.50 and 1% carvacrol against FCV, MNV and Hepatitis A in quantities of 6–7 log $TCID_{50}$/mL during 2 hours at 37 °C. Carvacrol at a concentration of 0.5% (w/w) completely inactivated the two NoVs surrogates (FCV and MNV) and carvacrol at a concentration of 1.0% completely inactivated HAV (Table 2). Carvacrol was also tested on lettuce and when added to lettuce rinse, the efficiency of carvacrol against MNV was dependent on the chemical oxygen demand (COD), which is an indicative measure of the amount of oxygen that is needed for the oxidation of all organic substances in the solution or the amount of oxygen that can be consumed by reactions in a measured solution. A complete elimination of FCV at a concentration of 6–7 log $TCID_{50}$/mL was observed when carvacrol 0.5% (w/w) was added on inoculated lettuce. According to Gilling et al. (2014b), the efficiency of carvacrol against MNV-1 can be effective at concentrations $<$ 0.5% (Table 2). After only 15 minutes of treatment with carvacrol at a concentration of 0.25% and 0.5%, a decrease of the viral titer by 1.03 and 1.28 $\log_{10}$ was respectively observed. After 60 minutes of treatment, a decrease of 1.95 $\log_{10}$ and 3.87 $\log_{10}$ of MNV-1 in presence of carvacrol at 0.25% and 0.50% was respectively obtained.

Antiviral activities of flavones (apigenin, baicalein and luteolin) have been also demonstrated. For example, simultaneous application of apigenin with acyclovir showed antiviral effects against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), Poliovirus type-2 and hepatitis C virus (HCV) (Zakaryan et al. 2017). A research on flavonoids showed that among all 10 flavonoids tested on FCV and MNV, kaempferol exhibited the most antiviral activity at 200 uM reducing FCV titers by 69.76% (Seo et al. 2016) (Table 2). The efficiency of kaempferol was explained by the structure-activity relationship showing that kaempferol worked as a neuraminidase inhibitor.

Su and D'Souza (2013) studied the mechanism of action of flavonoids on NoV surrogates. They tested four flavonoids: myricetin, L-epicatechin, tangeretin and naringenin for their antiviral properties. Their results showed that in presence of myricetin at a concentration of 0.5 mM and 1 mM, FCV-F9 at an initial viral titer of 5 $\log_{10}$ Plaque-forming unit (PFU)/mL, was completely inactivated to undetectable levels after 2 hours of treatment at 37 °C (Table 2). Tangeretin and naringenin did not reduce the FCV viral titer. The viral titer of FCV-F9 was also decreased by 1.40 $\log_{10}$ PFU/mL when treated with L-epicatechin at concentration of 0.5 mM. The FCV-F9 (viral titer 7 $\log_{10}$ PFU/mL) after a treatment with myricetin and L-epicatechin at 0.5 mM concentrations, respectively, was decreased by 3.17 and 0.72 $\log_{10}$ PFU/mL and by 1.73 $\log_{10}$ PFU/mL with myricetin at a concentration of 0.25 mM (Su and D'Souza 2013).

Natural extracts could be used under the form of essential oils and are composed of volatile compounds such as terpenes, terpenoids, aldehydes, esters, phenol-derived aromatic components and aliphatic components (Bakkali et al. 2008; Oh and Chung 2014). Steam distillation is one of the most popular methods to extract essential oils from plants (Boutekedjiret et al. 2003). EOs have the potential as natural agents to increase shelf life of foods (Burt and Reinders

Table 2. Examples of performance of natural compounds against virus.

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Carvacrol	0.50% for 2 h at 37 °C	Complete inactivation of 6–7 $\log_{10}$ $TCID_{50}$/mL FCV and MNV	Sánchez, Aznar, and Sánchez (2015)
Carvacrol	1.0% for 2 h at 37 °C	Complete inactivation of 6–7 $\log_{10}$ $TCID_{50}$/mL HAV	Sánchez, Aznar, and Sánchez (2015)
Carvacrol	0.50%, on lettuce	Complete inactivation of 6–7 $\log_{10}$ $TCID_{50}$/mL FCV	Sánchez, Aznar, and Sánchez (2015)
Carvacrol	0.25%, for 15 min	1.03 $\log_{10}$ $TCID_{50}$/mL MNV-1	Gilling et al. (2014b)
Carvacrol	0.50%, for 15 min	1.28 $\log_{10}$ $TCID_{50}$/mL MNV-1	Gilling et al. (2014b)
Carvacrol	0.25%, for 60 min	1.95 $\log_{10}$ $TCID_{50}$/mL MNV-1	Gilling et al. (2014b)
Carvacrol	0.50%, for 60 min	3.87 $\log_{10}$$TCID_{50}$/mL MNV-1	Gilling et al. (2014b)
Kaempferol	200 μM	69.76% reduction of FCV	Seo et al. (2016)
Myricetin	0.5 mM and 1 mM, for 2 h at 37 °C (initially 5 $\log_{10}$ PFU/mL)	Complete inactivation of 5 $\log_{10}$ PFU/mL FCV	Su and D'Souza (2013)
L-epicatechin	0.5 mM, for 2 h at 37 °C (initially 5 $\log_{10}$ PFU/mL)	1.40 $\log_{10}$ PFU/mL FCV	Su and D'Souza (2013)
Myricetin	0.5 mM, for 2 h at 37 °C (initially 7 $\log_{10}$ PFU/mL)	3.17 $\log_{10}$ PFU/mL FCV	Su and D'Souza (2013)
L-epicatechin	0.5 mM, for 2 h at 37 °C (initially 7 $\log_{10}$ PFU/mL)	0.72 $\log_{10}$ PFU/mL FCV	Su and D'Souza (2013)
Myricetin	0.25 mM, for 2 h at 37 °C (initially 7 $\log_{10}$ PFU/mL)	1.73 $\log_{10}$ PFU/mL FCV	Su and D'Souza (2013)
Zanthoxylum schinifolium (ZSO)	0.01% concentration	70% reduction of FCV-F9	Oh and Chung 2014
Oregano	2%, for 2 h at 37 °C	3.75 $\log_{10}$ $TCID_{50}$/mL FCV	Elizaquível et al. (2013)
Oregano	0.50%, for 2 h at 37 °C	1.04 $\log_{10}$ $TCID_{50}$/mL MNV	Elizaquível et al. (2013)
Oregano	1.0%, for 2 h at 37 °C	1.17 $\log_{10}$ $TCID_{50}$/mL MNV	Elizaquível et al. (2013)
Oregano	2.0%, for 2 h at 37 °C	1.62 $\log_{10}$ $TCID_{50}$/mL MNV	Elizaquível et al. (2013)
Cinnamon	3.0%, for 2 h at 37 °C	2.38 $\log_{10}$ $TCID_{50}$/mL FCV	Azizkhani and Tooryan (2016)
Rosemary	2.5%, for 2 h at 37 °C	3.38 $\log_{10}$ $TCID_{50}$/mL FCV	Azizkhani and Tooryan (2016)
Zataria	0.1%, for 2 h at 37 °C	4.51 $\log_{10}$ $TCID_{50}$/mL FCV	Azizkhani and Tooryan (2016)
Zataria	0.1%, for 2 h at 37 °C	0.25 $\log_{10}$ $TCID_{50}$/mL MNV	Azizkhani and Tooryan (2016)
Rosemary	2.5%, for 2 h at 37 °C	1.44 $\log_{10}$ $TCID_{50}$/mL MNV	Azizkhani and Tooryan (2016)
CJ	30 min at pH 2.7 and 7.0	5 $\log_{10}$ PFU/mL FCV	Su, Howell, and D'Souza (2010)
CJ	1 h at pH 2.6	1.90 $\log_{10}$ PFU/mL MNV	Su, Howell, and D'Souza (2010)
CJ	1 h at pH 7.0	1.66 $\log_{10}$ PFU/mL MNV	Su, Howell, and D'Souza (2010)
CJ-PAC	Immediately, 0.15 mg/mL and 0.30 mg/mL at RT	Complete inactivation FCV	Su, Howell, and D'Souza (2010)
CJ-PAC	0.15 mg/mL, for 1 h at RT	2.24 $\log_{10}$ PFU/mL MNV	Su, Howell, and D'Souza (2010)
CJ-PAC	0.20 mg/mL, for 1 h at RT	2.94 $\log_{10}$ PFU/mL MNV	Su, Howell, and D'Souza (2010)
B-PAC	1, 2 or 5 mg/mL, for 15 min at 37 °C in apple juice at pH 3.6	Complete inactivation of FCV and MNV	Joshi, Howell, and D'Souza (2017)
B-PAC	2 mg/mL, for 24 h in 2% reduced-fat milk	0.4 $\log_{10}$ PFU/mL FCV	Joshi, Howell, and D'Souza (2017)
B-PAC	5 mg/mL, for 24 h in 2% reduced-fat milk	1.09 $\log_{10}$ PFU/mL FCV	Joshi, Howell, and D'Souza (2017)
B-PAC	5 mg/mL, for 24 h in 2% reduced-fat milk	0.81 $\log_{10}$ PFU/mL MNV	Joshi, Howell, and D'Souza (2017)
PP	2 mg/mL, for 20 min at RT	4.02, 0.68 and 0.18 $\log_{10}$ PFU/mL for FCV, MNV and MS2 coliphage	Su, Sangster, and D'Souza (2011)
PP	4 mg/mL, for 20 min at RT	5.09, 1.14 and 0.19 $\log_{10}$ PFU/mL for FCV-F9, MNV-1 and MS2 coliphage	Su, Sangster, and D'Souza (2011)
PJ	pH 7.0, for 20 min at RT	3.12, 0.79 and 0.23 $\log_{10}$ PFU/mL for FCV, MNV and MS2 coliphage	Su, Sangster, and D'Souza (2011)
GSE	FCV at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	3.64, 4.10 and 4.61 $\log_{10}$ PFU/mL FCV	Su and D'Souza (2011)
GSE	MNV at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	0.82, 1.35 and 1.73 $\log_{10}$ PFU/mL MNV	Su and D'Souza (2011)
GSE	MS2 at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	1.13, 1.43 and 1.60 $\log_{10}$ PFU/mL MS2	Su and D'Souza (2011)
GSE	HAV at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	1.81, 2.66 and 3.20 $\log_{10}$ PFU/mL HAV	Su and D'Souza (2011)
GSE	FCV at low titer (5 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	4.98 $\log_{10}$ PFU/mL FCV for each concentration	Su and D'Souza (2011)
Clove extracts	Pretreatment of FCV with non-diluted extract at 4 °C, for 24 h	6 $\log_{10}$ $TCID_{50}$/mL FCV	Aboubakr et al. (2016)
Ginger extracts	Pretreatment of FCV with non-diluted extract at 4 °C, for 24 h	2.7 $\log_{10}$ $TCID_{50}$/mL FCV	Aboubakr et al. (2016)
Camellia sinensis extracts	100 μg/mL	87% of 7 $\log_{10}$ PFU/mL FCV	Seo and Choi (2017)
Ficus carica extracts	100 μg/mL	49% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)
Pleuropterus multiflorus extracts	20 μg/mL	53% of 7 $\log_{10}$ PFU/mL FCV	Seo and Choi (2017)
Alnus japonica extracts	20 μg/mL	50% of 7 $\log_{10}$ PFU/mL FCV	Seo and Choi (2017)
Inonotus obliquus extracts	150 μg/mL	92% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)
Crataegus pinnatifida extracts	50 μg/mL	58% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)
Coriandrum sativum extracts	20 μg/mL	45% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)

RT: room temperature, CJ: Cranberry juice, CJ-PAC: Cranberry juice proanthocyanidins, B-PAC: Blueberry proanthocyanidins, PP: Pomegranate polyphenol, PJ: Pomegranate juice, GSE: Grape seed extract.

2003). There are more than 3000 EOs that are already recognized (Burt 2004) and over 300 are on the market and usable. EOs are known for their antibacterial properties against many pathogenic bacteria such as *Campylobacter jejuni, Listeria monocytogenes, Salmonella, Staphylococcus* and *Escherichia coli* O157:H7 (Elizaquível et al. 2013; Ghabraie et al. 2016). Their actions are mainly on the membrane permeability, which results in a loss of metabolites and enzyme denaturation (Boumail, Salmieri, and Lacroix 2016; Oussalah, Caillet, and Lacroix 2006). So far, the antiviral effects of EOs against human enteric viruses are still mainly unknown (Elizaquível et al. 2013). On the other hand, antiviral effects have been found with *Zanthoxylum schinifolium* (ZSO) essential oil, which contains oleic acids (composition of 35.36%) and linoleic acids (22.6%) as major fatty acids present in ZSO. ZSO at a concentration of 0.01% reduced by 70% the viral titer of FCV-F9. A direct interaction between NoV surrogates and ZSO components has been proposed (Oh and Chung 2014) (Table 2). Another research group has attempted to understand the antiviral mechanisms of action of essential oils against HSV. It was shown that the altered viral multiplication step was just before adsorption or during adsorption itself but not during viral penetration of the cell. This indicates that essential oils may be acting by inhibiting components of virus essential for cell adhesion (Reichling et al. 2009). According to Gilling et al. (2014a) some essential oils like Allspice could act on the viral capsid of MNV to affect its integrity causing the viral genome would then be exposed and targeted.

Elizaquível et al. (2013) tested three essential oils including *Origanum compactum*, *Eugenia caryophyllus* and *Zataria multiflora Boiss* against NoV surrogates, FCV and MNV. The essential oils were individually mixed with each virus at virus titers of 7–8 log $TCID_{50}$/mL and incubated for 2 hours at 4 °C and 37 °C. Results showed that a presence of 2% oregano decreased the FCV titers by more than 3.75 log $TCID_{50}$/mL at 37 °C. A decrease in viral titers of 1.04, 1.17 and 1.62 log $TCID_{50}$/mL of MNV at respective concentrations of 0.5%, 1% and 2% of oregano, was observed. Cloves and zataria followed the same trends as oregano, and maximum viral reduction was achieved with 0.1% zataria at 37 °C to eliminate FCV (Table 2).

Azizkhani and Tooryan (2016) investigated the antiviral effects of cinnamon (1, 2 and 7%), rosemary (1, 1.5, 2 and 2.5%) and zataria (0.01, 0.04, 0.08 and 0.1%) against FCV and MNV. The essential oils were individually mixed with viruses at titers between 7–8 log $TCID_{50}$/mL and incubated 2 hours at 4 and 37 °C. In samples incubated at 37 °C, cinnamon at a concentration of 3% reduced the FCV titer by 2.38 log $TCID_{50}$/mL. A concentration of 2.5% rosemary reduced the titer by 3.38 log $TCID_{50}$/mL and finally, a concentration of 0.1% of zataria reduced the FCV titer by 4.51 log $TCID_{50}$/mL. For MNV, results showed a reduction of 0.25 log $TCID_{50}$/mL when treated with zataria 0.1% and a reduction of 1.44 log $TCID_{50}$/mL in presence of rosemary (2.5%) at 37 °C (Azizkhani and Tooryan 2016) (Table 2).

Fruit extracts

Fruit extracts are rich in polyphenols (Block, Patterson, and Subar 1992). Polyphenols from cranberries are known for their antioxidant (Côté et al. 2011a; Heinonen 2007), antibacterial (Côté et al. 2011c), anti-inflammatory (Vorsa et al. 2007), anti-mutagenic (Dixon, Xie, and Sharma 2005), antiviral (Scalbert 1991) and pharmacological properties (Wu et al. 2009). These phytochemicals compounds include flavanols and cinnamic acid derivatives like gallic acid. Gallic acid is a phenolic acid that is present in flavonoids (Scalbert 1991). Tannins are distributed in two groups: proanthocyanidins and hydrolyzable tannins (Scalbert 1991). In fact, tannic acid was found to have more antiviral activity against poliovirus than gallic acid (Konowalchuk and Speirs 1976). Many compounds extracted from plants (simple or complexed) can inactivate enteric viruses (Gilling et al. 2014a). In addition, many organic acids are found in fruit extracts such as malic acid and citric acid that were detected as main organic acids in commercial pomegranate juice (Tezcan et al. 2009). Organic acids have been used to inactivate NoV surrogates and its mechanism of action is not completely understood but can be attributed to alterations of the virus capsid and the nucleic acid (Cao 2013). It was suggested that the inactivation of non-enveloped viruses (such as NoV) is due to the denaturation of capsid proteins due to acidity of organic acids (Cao 2013). According to Lipson et al. (2010), the anti-viral mechanism of action of cranberry polyphenols or polyphenols originating from cranberries (CP) acts by inhibiting viral penetration in cells. Joshi, Howell, and D'Souza (2016) showed that lingonberry proanthocyanidins (PACs) affect viral penetration and PAC from an African resurrection plant inhibits viral attachment and penetration.

Su, Howell, and D'Souza (2010) tested the effects of cranberry juice (CJ) on MNV-1, FCV-F9, bacteriophage MS2 (ssRNA) and bacteriophage φ X-174 (ssDNA) at exposure times ranging from 0 to 60 minutes at room temperature. Among all these surrogates of food viruses, FCV-F9 was the viral titer that displayed the most rapid and abundant decrease. They observed 5 $\log_{10}$ PFU/mL reduction after 30 minutes of treatment with CJ (pH 2.7 and 7.0). The total reduction in titer for MNV-1 was 1.90 and 1.66 $\log_{10}$ PFU/mL after 1 hour of treatment with CJ at pH 2.6 and CJ at pH 7.0, respectively (Su, Howell, and D'Souza 2010) (Table 2). Similar results were observed by Côté et al. (2011b) where cranberry juice containing water-soluble phenolic compounds inhibited pathogens like *Listeria monocytogenes*.

Su, Howell, and D'Souza (2010) tested the effect of cranberry Proanthocyanidins (PAC) on MNV-1, FCV-F9, bacteriophage MS2 (ssRNA) and bacteriophage φ X-174 (ssDNA) at exposure times ranging from 0 to 60 minutes at room temperature. CJ-PAC at both 0.15 mg/mL and 0.30 mg/mL (CJ-PAC concentration) decreased the FCV-F9 titer to an undetectable level immediately. For MNV-1, a reduction of 2.24 and 2.94 $\log_{10}$ PFU/mL at 0.15 mg/mL and 0.20 mg/mL concentration of CJ-PAC was observed respectively, after one hour of treatment (Table 2).

Blueberry Proanthocyanidins (B-PACs) are also able to decrease viral titers of NoV surrogates (Joshi, Howell, and D'Souza 2017). The authors assessed the benefits of B-PAC in apple juice (AJ) and in 2% reduced fat milk in simulated gastrointestinal fluids infected with FCV-F9 and MNV-1 and treated at 37 °C for 24 hours. The viruses were inactivated after 15 minutes in AJ at pH 3.6 in presence of 1, 2 or 5 mg/mL B-PAC. In contrast, B-PAC did not display much virucidal activity in 2% partly-skim milk. In milk, FCV-F9 was reduced by only 0.4 log PFU/mL in presence of 2 mg/mL B-PACs and by 1.09 log PFU/mL in presence of 5 mg/mL of B-PACs after 24 hours. MNV-1 was reduced by 0.81 log PFU/mL in presence of 5 mg/mL of B-PACs after 24 hours. This loss of the antiviral properties against MNV-1 was associated with the presence of carbohydrates, lipids and proteins in the milk matrix and then induced loss of effectiveness (Table 2).

Antiviral effects of Pomegranate Polyphenols (PP) were evaluated in presence of 2 mg/mL and 4 mg/mL of PP against FCV-F9, MNV-1 and bacteriophage MS2 at room temperature for up to 1 hour (Su, Sangster, and D'Souza 2011) (Table 2). Each virus (5 $\log_{10}$ PFU/mL) was mixed with an equivalent volume of PP at 4 or 8 mg/mL and incubated for 0, 10, 20, 30, 45 and 60 minutes at room temperature. A decrease of 4.02, 0.68 and 0.18 $\log_{10}$ PFU/mL for FCV-F9, MNV-1 and MS2, was respectively observed after 20 minutes' exposure in the presence of 2 mg/mL of PP (Su, Sangster, and D'Souza 2011). Concentration of 4 mg/mL PP resulted in a decrease of 5.09, 1.14 and 0.19 $\log_{10}$ PFU/mL for FCV-F9, MNV-1 and MS2, respectively after 20 minutes of treatment.

Polyphenolic compounds represent the most studied group of plant phenolics. Pomegranate juice (PJ) contains mainly anthocyanins and anthoxanthins (Aviram and Rosenblat 2012). Among the most anti-NoV plant polyphenols, anthocyanins, proanthocyanins and catechins are the most characterized. In fact, anthocyanin's glycoside may inhibit MNV-1 during internalization or during an early replication step (Ryu et al. 2015) and anthoxanthins can act as antioxidants (Pate et al. 2017). Su, Sangster, and D'Souza (2011) tested the antiviral effects of PJ at pH 7 against FCV-F9, MNV-1 and bacteriophage MS2 at room temperature for up to 1 hour. Each virus (5 $\log_{10}$ PFU/mL) was mixed with equivalent volumes of PJ and incubated for 0, 10, 20, 30, 45 and 60 minutes at room temperature. In all cases of viral surrogates, viral titers were reduced by more than 50% during only the first 20 minutes' post-treatment. A decrease of 3.12, 0.79 and 0.23 $\log_{10}$ PFU/mL in only 20 minutes of exposure with PJ for FCV-F9, MNV-1 and MS2 was observed, respectively.

Subsequently, Su and D'Souza (2011) attempted to evaluate the efficiency of grape seed extract (GSE) against hepatitis A virus (HAV) and against surrogates of NoV (FCV-9, MS2 and MNV-1). High titers and low titers of virus inoculated at 7 $\log_{10}$ PFU/mL and 5 $\log_{10}$ PFU/mL titer were used, respectively (Table 2). Equal part of viruses (7 $\log_{10}$ or 5 $\log_{10}$ PFU/mL) and GSE were mixed at different concentrations (0.5, 1 and 2 mg/mL) of GSE and incubated for 2 hours at room temperature. For FCV-F9 at high titers, reductions of 3.64, 4.10 and 4.61 $\log_{10}$ PFU/mL in presence of three respective concentrations (0.5, 1 and 2 mg/mL) was obtained. For the high titer of MNV-1 (7 $\log_{10}$ PFU/mL), in presence of the three respective concentrations (0.5, 1 and 2 mg/mL), a reduction of 0.82, 1.35 and 1.73 $\log_{10}$ PFU/mL was attained, respectively. MS2 phage, at high titer (7 $\log_{10}$ PFU/mL) was reduced by 1.13, 1.43 and 1.60 $\log_{10}$ PFU/mL as the concentration of GSE increased from 0.5 to 1 and 2 mg/mL, respectively (Su and D'Souza 2011). For HAV virus at high titer (7 $\log_{10}$ PFU/mL), the reductions were 1.81, 2.66 and 3.20 $\log_{10}$ PFU/mL in presence of 0.5, 1 and 2 mg/mL of GSE, respectively. At low titer (5 $\log_{10}$ PFU/mL), FCV-F9 was always reduced by 4.98 $\log_{10}$ PFU/mL (Su and D'Souza 2011).

Spice and herbal extracts

Herbal extracts contain some antimicrobial components such as terpenoids, alkaloids and phenolic compounds that are interacting with enzymes or proteins (Mostafa et al. 2018). The antiviral mechanism of herbal extracts (ex: thiophenes, polysaccharides, polyacetylenes and furyl compounds) is due to the inhibition of viral enzymes, viral replication and protein synthesis (Seo et al. 2017). It has been shown that combining herbal extracts with other conditions such as low pH, storage temperature, irradiation, bacteriocins or organic acids causes synergistic antimicrobial activities (Perumalla and Hettiarachchy 2011).

Aboubakr et al. (2016) tested aqueous extracts of flower of clove, fenugreek seeds, garlic and onion bulbs, ginger rhizomes, and jalapeno peppers against FCV (Table 2). Results showed that non-diluted clove and ginger extracts inactivated 6.0 log $TCID_{50}$ and 2.7 log $TCID_{50}$, respectively of the initial viral titer. Eugenol, a phenylpropene (29.5%), was the major component of clove extract and R- (-)-1,2-propanediol (10.7%) was the major component of ginger extract. Eugenol tested alone showed antiviral activities. These findings confirmed that eugenol is the component most likely responsible for viral elimination in cloves.

Extract of *Camellia sinensis* and *Ficus carica* have also been evaluated. A significant inhibition of FCV (~7 $\log_{10}$ PFU/mL) and MNV (~7 $\log_{10}$ PFU/mL) by 87% and 49% was observed in presence of these respective extract (Seo and Choi 2017). In addition, FCV showed 53% inhibition by *Pleuropterus multiflorus* extracts (20 µg/mL) and 50% with *Alnus japonica* extracts (20 µg/mL) (Seo and Choi 2017). MNV was also inhibited by more than 92% in the presence of 150 µg/mL of *Inonotus obliquus* extracts and 58% in presence of 50 µg/mL of *Crataegus pinnatifida* extracts. In addition, 20 µg/mL of *Coriandrum sativum* extracts reduced MNV titer about 45% (Seo and Choi 2017). They attributed the antiviral mechanism of action of bioactive substances to the anti-adhesion of virus on cells, direct virucidal action or immune enhancement (Seo and Choi 2017).

Non-thermal treatments

The following results concerning the fermentation treatments against the viruses will be presented in Table 3.

Table 3. Examples of performance of fermentation against virus.

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Fermentation of dongchimi	20 days	4.12 $\log_{10}$ PFU/g FCV	Lee et al. (2012)
Fermentation of dongchimi	20 days	2.12 $\log_{10}$ PFU/g MNV	Lee et al. (2012)
Fermentation of oysters	5% salt at 18 °C for 15 days	3.0 $\log_{10}$ PFU/g FCV	Seo et al. (2014)
Fermentation of oysters	5% salt at 18 °C for 15 days	1.6 $\log_{10}$ PFU/g MNV	Seo et al. (2014)
Fermentation of kimchi	4 °C for 28 days	1.31 $\log_{10}$ genomic copies/reaction NoV GII.4	Lee et al. (2017)
Coccus-shaped pretreatment with LAB from kimchi	5 °C for 21 days *Pediococcus pentosaceus* and *Weissella cibaria*	1.93 to 3.49 $\log_{10}$ PFU/mL MNV-1	Seo et al. (2020)
Rod-shaped pretreatment with LAB from kimchi	5 °C for 21 days *Lactobacillus sakei* and *Lactobacillus curvattus*	1.42 to 1.70 $\log_{10}$ PFU/mL MNV-1	Seo et al. (2020)
Pretreatment with LAB-free filtrates	5 °C for 21 days	0.26 to 0.50 $\log_{10}$ PFU/mL MNV-1	Seo et al. (2020)
Co-treatment MNV-1 with LAB	5 °C for 21 days	0.56 to 0.60 $\log_{10}$ PFU/mL MNV-1	Seo et al. (2020)

Fermentation

The antimicrobial potential of fermented foods is due to their acidity which is caused by the production of lactic acid and bacteriocins, but there are also important effects caused by high salinity, temperature and ripening (Dortu and Thonart 2009; Seo et al. 2014). Fermentation with lactic acid bacteria (LAB) reduces the risk of foodborne illness by inhibiting the growth of pathogens (Kim, Zheng, and Shin 2008; Ross, Morgan, and Hill 2002). Known antimicrobial factors produced by fermentation include acidity, organic acid production, bacteriocins, CO_2, hydrogen peroxide, ethanol, diacetyl production, and low redox potential (Adams and Nicolaides 1997). Lactic acid fermentation can inactivate and inhibit the growth of pathogenic bacteria (Lee et al. 2017). For example, pathogens such as *Salmonella* Typhimurium and *Staphylococcus aureus* have been eliminated with lactic acid fermentation (Nout, Rombouts, and Havelaar 1989). The antiviral mechanism of action of fermented food is still mainly unknown but can be attributed to high acidity production (Lee et al. 2012). Also, according to Aboubakr et al. (2014), *Lactococcus lactis,* a lactic bacteria used in fermentation, possesses an anti-FCV mechanism of action that could be attributed to 1) denaturation of capsid proteins due to acidity, 2) trapping viral particles by the membrane peptidoglycans of lactic acid bacteria, 3) production of metabolites that may disturb viral penetration in cells and 4) competition between bacteria and virus for cell attachment. Fermentation has been shown to be highly effective against NoV on the surface of foods but do not keep the product entirely fresh (Adams and Nicolaides 1997; Lee et al. 2017).

In Korea, it's a common practice to ferment foods such as cabbage, green onions, carrots, cucumbers, etc. (Lee 1997). Kimchi is a dish made from fermented vegetables and there are many different types such as Dongchimi (Lee et al. 2012). Dongchimi is prepared with sliced radish, 1% green onions, 0.5% garlic, and 0.3% ginger. Saltwater (2.5%) is added to the vegetable mix at a ratio of 1: 1.5 (W/V). It was observed that the viral titer of FCV was significantly reduced from 5.69 $\log_{10}$ PFU/g to 1.57 $\log_{10}$ PFU/g after 20 days of fermentation (Lee et al. 2012) (Table 3). The viral titer of the MNV was reduced less significantly than FCV, from 5.63 to 3.51 $\log_{10}$ PFU/g after 20 days of fermentation.

Seo et al. (2014) evaluated the effects of FCV and MNV-1 survival during oyster fermentation. Lactic acid fermentations in presence of 5% and 10% salt at 18° C for 15 days were made. In fermented oysters containing 5% salt, the FCV titer decreased by 3 log and the MNV titer decreased by 1.6 log after 15 days of lactic acid fermentation (Table 3). It was concluded that the antimicrobial compounds generated during the lactic acid fermentation contributed to the reduction of food viruses (Seo et al. 2014).

Lee et al. (2017), also evaluated the level of NoV on kimchi stored at 4 °C and 10 °C during lactic acid fermentation. Although, the amount of NoV, originally at 3 $\log_{10}$ copies/200 µL was reduced by 1.31 $\log_{10}$ genomic copies/reaction, it was detected in all samples tested after 28 days of fermentation (Table 3).

Seo et al. (2020) also studied the effects of LAB against MNV-1. RAW264.7 cells were used to propagate the virus and they were either pretreated with LAB, pretreated with LAB-free filtrate or co-treated with LAB and MNV-1 at 5 °C for 21 days (Table 3). The results showed that among the 56 coccus-shaped LAB, the pretreatment with *Pediococcus pentosaceus* and *Weissella cibaria* reduced MNV-1 titer by 1.93 to 3.49 $\log_{10}$ PFU/mL. Also, among the rod-shaped LAB, the pretreatment with *Lactobacillus sakei* and *Lactobacillus curvattus* reduced the MNV-1 titer by 1.42 to 1.70 $\log_{10}$ PFU/mL. MNV-1 co-treated with LAB reduced the viral titer by 0.56 to 0.60 $\log_{10}$ PFU/mL. LAB-free filtrates pretreatment reduced MNV-1 titer by 0.26 to 0.50 $\log_{10}$ PFU/mL. Table 3. Examples of performance of fermentation against virus.

The following results concerning the ozonation treatments against the viruses will be presented in Table 4.

Ozonation

The effectiveness of ozone (O_3) on viruses can be attributed to the fact that non-enveloped viruses are more sensitive to the presence of O_3. This could facilitate the access of O_3 to nucleic acids. Plus, O_3 is a strong oxidizing agent and disturbs the reproduction cycle of the virus by disrupting the contact with cells (Elvis and Ekta 2011). O_3 makes the cells susceptible to viral infection and make them susceptible to oxidation and then elimination (Elvis and Ekta 2011). Ozonation is an O_3 generation process, producing an

Table 4. Examples of performance of ozonation against virus.

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on lettuce for 1 min	1.2 log $TCID_{50}$/mL FCV	Hirneisen, Markland, and Kniel (2011)
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on green onions for 1 min	0.5 log $TCID_{50}$/mL FCV	Hirneisen, Markland, and Kniel (2011)
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on green onions for 10 min	3.78 log PFU/g MNV	Hirneisen, Markland, and Kniel (2011)
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on lettuce for 10 min	3.09 log PFU/g MNV	Hirneisen, Markland, and Kniel (2011)
Gaseous O_3	6%, 15 psi, at 25 °C and RT in liquid stock for 10 min	4.1 log PFU/g MNV	Predmore et al. (2015)
Gaseous O_3	6%, 15 psi, at 25 °C and RT on surface of fresh strawberries for 40 min	3.3 log PFU/g MNV	Predmore et al. (2015)

unstable form of oxygen (highly reactive, making it a powerful oxidizing agent). O_3 is produced by a high source of energy breaking the O_2 molecules. Once the bond is broken, these oxygen atoms (O) bind to a molecule of O_2 and then form O_3. This process induces the reactive oxygen molecules specifically damage cellular DNA, usually DNA strand breakage or chemical modification of bases, which usually leads to cell death (Boumail, Salmieri, and Lacroix 2016). Ozonation is a cleaner process than chlorination because O_2 is the only residue at the end of the process and there is no manipulation of chemicals needed (Rice, Graham, and Lowe 2002). O_3 might also react on cell walls of bacteria by oxidizing major components like proteins and unsaturated lipids (Beuchat 1992; Komanapalli and Lau 1998). In addition, chlorination has the disadvantage of forming toxic and carcinogenic chlorinated compounds (Kim, Yousef, and Khadre 2003; Kirk and Mitchell 1980). There are many advantages using this method. It requires only oxygen, is faster and more efficient than chlorine. It is a non-thermal and non-chemical process and can be applied in aqueous or gaseous state (Guzel-Seydim, Greene, and Seydim 2004; Khadre, Yousef, and Kim 2001; Kim, Yousef, and Khadre 2003; Rice, Graham, and Lowe 2002). Furthermore, it is a safe method because it leaves no residue while it reduces microorganisms in food (Trindade et al. 2012).

A study evaluated the effectiveness of ozonated water to inactivate FCV and MNV inoculated respectively at concentrations of 10^7 $TCID_{50}$/mL and 10^6 PFU/mL on green onions and lettuce (Hirneisen, Markland, and Kniel 2011) (Table 4). The lettuce and green onion samples were submerged in gaseous ozonized water from an O_3 generator (0.9 g of ozone/h at a flow rate of 2.4 litters/min, 6.25 ppm). The samples were ozonized for 0.5, 1, 5 and 10 minutes. Viral inactivation was increased as duration of treatment increased. With regard to the removal of FCV from surface of lettuce and green onions, regardless of duration of the treatment, the virus inactivation was always greater on the surface of the lettuce compared to other vegetables. For example, after one minute of treatment with 6.25 ppm of ozonated water, a reduction of 1.2 log $TCID_{50}$/mL on surface of treated lettuce was observed as compared to a reduction of only 0.5 $TCID_{50}$/mL on surface of green onions (Hirneisen, Markland, and Kniel 2011) (Table 4). In the case of MNV on the surface of the same two foods, viral elimination was always more effective on the surface of lettuce, except at the 10-minutes exposure time where there was a higher viral reduction of 3.78 log PFU/g on green onions and only a 3.09 log PFU/g reduction on lettuce (Hirneisen, Markland, and Kniel 2011). In conclusion, viral inactivation by O_3 was lower in green onions in most cases. There is a possibility that this difference in the inactivation is due to the different organic composition of the two items. O_3 reacts with complex organic compounds because of the high oxidation potential (Hirneisen, Markland, and Kniel 2011).

Predmore et al. (2015) studied the effects of gaseous O_3 on fresh strawberries. Samples were ozonized with 6% (w/w O_3) in oxygen during 0, 10, 20, 30 and 40 minutes. Experiments were done at room temperature at 25 °C and gaseous O_3 was pumped into the chamber until 15 psi was reached. After 10 minutes of treatment in presence of gaseous O_3, MNV-1 at a concentration of 10^8 PFU/mL demonstrated a 4.1 log PFU/g reduction in simple liquid virus stock. After 40 minutes of O_3 treatment, the viral titer decreased by 3.3 log PFU/g on the surface of strawberries, but there was still 2.5 log PFU/g remaining, indicating that MNV-1 is partially resistant to O_3 (Table 4).

The following results concerning the High Pressure Homogenizer (HPH) and High Hydrostatic Pressure (HHP) treatments against the viruses will be presented in Table 5.

High pressure homogenizer (HPH) and high hydrostatic pressure (HHP)

Homogenization by high-pressure valve is a recent, non-thermal process where a fluid feed is forced through small orifices. High-pressure processes include high hydrostatic pressure (HHP) and high-pressure homogenization (HPH) (D'Souza et al. 2009). HPH is a continuous process at low pressure (< 400 MPa) and shorter exposure than HHP (D'Souza et al. 2009). The HPH process has proven effective for bacterial, enzymatic and emulsion enhancement (Diels et al. 2004; Dybowska 2005; Picart et al. 2006).

Chen, Hoover, and Kingsley (2005) demonstrated that FCV at a concentration of $\sim 10^6$ PFU/mL was reduced by 1.7 log PFU/mL in culture with an HHP treatment (250 MPa) of 2 minutes (21 °C). In other conditions (200 MPa), this research also showed an FCV reduction of 5.0 log PFU/mL (-10 °C) and of 0.3 log PFU/mL (20 °C) after 4 minutes of treatment (Table 5). Li and Chen (2015) also demonstrated that the same HHP treatment reduced MNV-1, from a viral concentration of 1×10^7 to 10^8 PFU/mL by only 0.1 log PFU/mL. Therefore, it can be concluded

Table 5. Examples of performance of high-pressure homogenizer (HPH) and high hydrostatic pressure (HHP) against virus.

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
HHP	250 MPa for 2 min at 21 °C	1.7 $\log_{10}$ PFU/mL FCV	Chen, Hoover, and Kingsley (2005)
HHP	250 MPa for 2 min at 21 °C From 1×10^7 to 10^8 PFU/ml	0.1 $\log_{10}$ PFU/mL MNV	Li and Chen (2015)
HHP	200 MPa at −10 °C for 4 min	5.0 $\log_{10}$ PFU/mL FCV	Chen, Hoover, and Kingsley (2005)
HHP	200 MPa at 20 °C for 4 min	0.3 $\log_{10}$ PFU/mL FCV	Chen, Hoover, and Kingsley (2005)
HHP	350 MPa at 4 °C for 2 min and pH 4.0	6.0 $\log_{10}$ PFU/mL MNV-1	Lou et al. (2011)
HHP	350 MPa at 4 °C for 2 min and pH 7.0	8.1 $\log_{10}$ PFU/mL GI.1 HuNoV	Lou et al. (2011)
HPH	300 MPa at 75 °C	3 $\log_{10}$ PFU/mL MS2	D'Souza et al. (2009)
HPH	300 MPa at 75 °C	0.8 $\log_{10}$ PFU/mL MNV	D'Souza et al. (2009)
HPH	300 MPa for 2 min at 25 °C	8 $\log_{10}$ $TCID_{50}$/mL rotavirus	Khadre and Yousef (2002)

that FCV is more sensitive to this type of treatment than MNV-1 (Table 5). Another group tested the effects of HHP treatment against caliciviridaes (Lou et al. 2011). After a treatment of 350 MPa at 4 °C for 2 minutes against MNV-1 (pH 4.0) and GI.1 HuNoV (pH 7.0), viral titers reduction of 6.0 log PFU/mL and 8.1 PFU/mL were respectively found in aqueous medium (Lou et al. 2011) (Table 5). They also studied the viral inactivation of MNV-1 in strawberry puree, strawberry and fresh-cut lettuce after HHP treatment. The authors noticed that the viral inactivation was always greater at 4 °C compared to the reduction at 20 °C in all three fresh products. For example, at 4 °C (450 MPa), reductions between 4.7–7.0 $\log_{10}$ PFU/g were found in all three products compared to 4.1–4.9 $\log_{10}$ PFU/g at 20 °C. Authors also suggested that the food matric plays a role in viral inactivation. MNV-1 was more sensitive to HHP treatment at high pH in aqueous medium than all food samples. The mechanism of action of the viral inactivation by HHP treatment is still mainly misunderstood but authors suggested that viral proteins may be damaged. HHP affects noncovalent bonds. The first and the second protein structure are not affected, suggesting that HHP may alter tertiary and quaternary structures of proteins (Lou et al. 2011). An indirect treatment at 600 MPa at 17 °C for 3–5 min induced enzyme inactivation while keeping sensorial properties of fresh fruit purees (Buzrul and Alpas 2012).

D'Souza et al. (2009) evaluated the efficiency of HPH at different pressures (0, 100, 200, 250 and 300 MPa) against two NoV surrogates; MNV-1 at a concentration of 5.78 log PFU/mL and MS2 coliphage at a concentration of 6.52 log PFU/mL (D'Souza et al. 2009) and found that only HPH at pressures of 300 MPa at 75 °C showed virus inactivation by more than 3 log PFU/mL for MS2 with plaque-assay (Table 5). In addition, at the same pressure, viral titers decrease of more than 0.8 log PFU/mL for MNV-1.

Khadre and Yousef (2002) attempted to compare antiviral effects of ozonation and high-pressure treatments on human rotavirus. O_3 (25 µg/mL) decreased viral titers of about approximately 8–9 $\log_{10}$ $TCID_{50}$/mL with a high initial viral titer of $\sim 10^{11}$ $TCID_{50}$/mL (Table 5). The high-pressure treatment was shown to be extremely effective against rotavirus. Treatment at 300 MPa for 2 minutes at 25 °C decreased viral titers about 8 $\log_{10}$ $TCID_{50}$/mL. Both treatments appear to have similar effects on viruses. The mechanism of action of high pressures can be explained by protein denaturation leading to changes in conformation. It might be possible that this denaturation may occur in viral attachment proteins, thereby preventing viral infection and spread (Khadre and Yousef 2002).

The following results of the γ-irradiation treatments against the viruses will be presented in Table 6.

Irradiation (γ- rays, electron beam and X- rays)

Gamma irradiation has been studied extensively for its effectiveness in eliminating pathogens on food. It can lead to the production of oxygen or hydroxyl radicals (Park and Ha 2017). Gamma rays can interact with nucleic acids because of the DNA modifications and the free radicals (Park and Ha 2017). Among the different damages on nucleic acid, there are double and single-strand breaks, cross-linkage breaks and nucleotide degradation (Park and Ha 2017). The expression of the MNV-1 major capsid protein (VP1) gene decreases as the irradiation dose increases (Park and Ha 2017). VP1 was the only protein studied in order to seek alterations after irradiation of MNV-1 during this research (Park and Ha 2017). Furthermore, gamma irradiation can break covalent and non-covalent bonds (hydrogen bonds, ionic bonds, van der Waals forces and hydrophobic interactions) that are essential for protein structure (Park and Ha 2017). It has been shown that irradiation applied alone or in combination with other treatments such as using antimicrobial coatings can reduce bacterial contamination in food (Boumail, Salmieri, and Lacroix 2016; Rahimi et al. 2013). It is also a cold process that is usually applied on packaged products (Boumail, Salmieri, and Lacroix 2016). The maximum dose that can be applied on fresh fruits and vegetables should not exceed 1 kGy (Komolprasert, Bailey, and Machuga 2008). However, foodborne viruses can only be eliminated by irradiation at doses ranging from 2 to 8 kGy (Monk, Beuchat, and Doyle 1995). Therefore, combining this treatment with antimicrobial coatings or negative air ionization with O_3 can decrease the needed dose (Boumail, Salmieri, and Lacroix 2016). This technique is generally used to assure food safety, disinfection, extend the shelf life of foods and for delaying the germination of seeds (Farkas and Mohácsi-Farkas 2011; Lacroix and Ouattara 2000; Lacroix and Vigneault 2007).

To date, food irradiation is a public concern, but is considered safe by the World Health Organization (WHO), the United Nations International Atomic Agency (IAEA), and the Food Agriculture Organization (FAO). The use of irradiation in combined treatments has the advantage of reducing

Table 6. Examples of the performance of γ-irradiation against virus.

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
γ-irradiation	Fresh produce, 2.8 kGy	1.77 $\log_{10}$ PFU/g for spinach, 1.40 $\log_{10}$ PFU/g for romaine lettuce and 1.31 $\log_{10}$ PFU/g strawberries MNV-1	Feng et al. (2011)
γ-irradiation	Fresh produce, 5.6 kGy	1.7 to 2.4 $\log_{10}$ PFU/g in fresh produce MNV-1	Feng et al. (2011)
γ-irradiation	Kimchi, 1, 3, 5, 7 and 10 kGy	0.34, 0.71, 0.98, 1.45 and 1.76 $\log_{10}$ PFU/mL MNV-1	Park and Ha (2017)
γ-irradiation	Strawberries and raspberries, 1 to 11 kGy	2 log PFU/g reduction of MNV-1 and HAdV at 4 kGy on both fruits	Pimenta, Margaça, and Verde (2019)

the dose required for the elimination of an organism. In the case of resistant organisms such as viruses and Gram (+) bacteria, reducing the required dose to ensure the food safety, also helps to better preserve the nutritional and physicochemical quality of food (Lacroix and Ouattara 2000). Irradiation could be done using electron-beam equipment, a X-rays machine or a source of γ-irradiation. However, electron beams are much less penetrating and require higher levels of dose rate (Woo and Sandford 2002). X-ray irradiation is an electricity-based process and rays are produced by an electron transition phenomenon that is an electron passage to another energy level (Cleland and Stichelbaut 2013).

Feng et al. (2011) have evaluated the effects of γ-irradiation against NoV surrogates such as MNV-1, NoV virus-like particles (VLPs) and vesicular stomatitis (VSV). MNV-1 and VLPs are both resistant to gamma irradiation. A reduction of 1.7 to 2.4 log was observed on the surface of fresh products inoculated with MNV-1 and treated at 5.6 kGy. In contrast, VSV appears to be more susceptible to gamma irradiation, a reduction of 3.3 log was observed after the treatment with 5.6 kGy (Feng et al. 2011) (Table 6). Authors attributed this sensitivity to γ-irradiation to the genome size. In fact, MNV-1 has a genome of 7.4 Kbp and VSV has a bigger genome with 11 Kbp. There are studies showing an inverse relationship between the size of the genome and the inactivation gamma-irradiation dose (Feng et al. 2011). Also, there is another hypothesis suggesting that enveloped viruses are more sensitive to gamma irradiation (Feng et al. 2011).

Park and Ha (2017) also showed that gamma irradiation reduced the MNV-1 viral titer in kimchi as the γ-irradiation dose increased. For irradiation doses of 1, 3, 5, 7 and 10 kGy, a respective reduction of 0.34, 0.71, 0.98, 1.45 and 1.76 $\log_{10}$ was observed (Table 6). In conclusion of this study, γ-irradiation with $\geq$5.75 kGy doses could be effective against NoV contamination in the kimchi industry.

A recent study on gamma irradiation against enteric viruses was carried out on fresh fruits, such as strawberries and raspberries (Pimenta, Margaça, and Verde 2019). First, the fruits were both inoculated with MNV-1 and human adenovirus type 5 (HAdV) alone at a concentration of 10^6 PFU/100 µL or combined in a viral pool of these two viruses at a concentration of 10^6 PFU/mL of both viruses in a 1: 1 ratio. The fruits were then irradiated ($Co\text{-}^{60}$ equipment) at a dose ranging from 1 to 11 kGy. At the surface of both fruits and for both viruses, a reduction of 2 log PFU/g was observed after an irradiation at 4 kGy. Even after an irradiation dose of 11 kGy, viral particles from both viruses were still observed on the surface of both fruits. According to the authors, the irradiation dose required to make the food safe for consumption is approximately 7 kGy (Pimenta, Margaça, and Verde 2019) (Table 6).

Irradiation treatments in combination with heat processing at temperature above 55 °C reduce considerably the risk of viral contamination on food because of their high heat sensitivity (Farkas 1989).

UV irradiation

Ultraviolet (UV) radiation represents electromagnetic irradiation and has wavelengths of below 450 nm ($\nu \approx 10^{15}$ Hz) and a quantum energy of 3–5 eV (Adams, Moss, and McClure 2016), which is shorter than the wavelengths of visible light at 400–700 nm (Dai et al. 2012). UV-A comprises wavelengths ranging from 320 to 400 nm (Cadet and Douki 2018), UV-B ranging from 280 to 320 nm (Cadet and Douki 2018) and UV-C have wavelengths ranging from 200 nm to 280 nm (Dai et al. 2012). The mechanism of action of UV-C against viruses is linked to the attack of the viral nucleic acids (Dai et al. 2012). The most antimicrobial wavelength is between 250–270 nm, because the UV-C spectrum is strongly absorbed by nucleic acid of the microorganisms (Dai et al. 2012). The UV-C type rays have a low wavelength (200–280 nm) and are therefore less energetic and are longer than x-rays who have wavelengths $<$ 100 nm (Dai et al. 2012). UV-C is normally used for the sterilization of surfaces and transparent objects (Silindir and Ozer 2009). Among the mutations observed after UV treatment, UV-A ray's genotoxic actions involve oxidative processes by the production of reactive oxygen species (ROS) after exciting photosensitizers. The ROS damage major components of cells such as proteins and lipids (Wondrak, Jacobson, and Jacobson 2006). UV-B rays are able to create cyclobutane pyrimidine dimers (CPD) and 6–4 photoproducts (6–4 PPs) and these modifications in DNA strongly affect the functioning of the cell and can induce its death (Cadet and Douki 2018; Lankinen, Vilpo, and Vilpo 1996). UV-C rays create cyclobutane pyrimidine and pyrimidine pyrimidone dimers, blocking the elongation of nucleic acid transcripts (Vreeswijk et al. 1994). UV radiation at 253.7 nm wavelength (UV-C) was tested against FCV and a 3-log reduction of viral titer was achieved with a fluence of 120 J/m^2 (De Roda

Husman et al. 2004). It was shown that the first target to UV radiation is the capsid of the virus using capsid epitope binding experiments (Nuanualsuwan and Cliver 2003).

Combined treatments

Combining irradiation and EOs could decrease the amount of radiation required to reduce food pathogens, while weeping all nutrients, flavors and physico-chemical aspects of the fresh products (Lacroix 2005; Lacroix and Follett 2015; Ndoti-Nembe et al. 2015). As an example, the combination of γ-irradiation with other processes have shown to enhance the preservation of fruits, nuts, vegetables and spices (Lacroix et al. 2002). The combination of the use of UV-C with γ-irradiation against food viruses. However, a research team evaluated the effects of this combination against viruses present in fetal calf serum (FCS), a pharmaceutical asset in vaccine research). The UV-C irradiator had a rate of 15 L/h with four UV lamps (40 Watt). The results showed that parvoviruses present in FCS were highly inactivated by the combination of UV-C and γ-irradiation. The same viruses weren't easily inactivated with γ-irradiation used alone (Hermann, Burian, and Waldmann 2005).

UV irradiation combined with free chlorine/ monochloramine

Shang, Cheung, and Liu (2007) investigated the inactivation of MS2 coliphage with different types of combined treatments. Among the treatments, they tested sequential ultraviolet (UV-C) light/free chlorine exposure, sequential UV-C light/monochloramine exposure, simultaneous UV-C light/free chlorine exposure, and simultaneous UV-C light/monochloramine exposure using either low-pressure (LP) at 254 nm (UV-C) or medium-pressure (MP) ranging from 220 nm (UV-C) to 580 nm UV lamps. All tests were conducted at pH 7 and at a temperature of $20 \pm 2\,^{\circ}C$. Sequential exposure to UV-C or simultaneous exposition (UV-C dose at 51 mJ/cm^2) in the primary disinfection stage, increased MS2 inactivation during the second disinfection stage by monochloramine about 4 to 5 times and with chlorine, by 1.5–2.7 times, compared to the rates by free chlorine.

UV-assisted TiO_2 photocatalysis combined with high hydrostatic pressure

Kim et al. (2017) tested the effects of single and combined UV-C (254 nm) assisted TiO_2 photocatalysis (UVTP) and HHP against MNV-1. UVTP (4.5 mW/cm^2) for 10 minutes decreased the MNV-1 titer by 2.9 $\log_{10}$ post-treatment. The use of HHP at 500 MPa during 5 minutes at room temperature reduced the MNV-1 titer by 3.5 $\log_{10}$. When UVTP was followed by HHP, the titer was decreased by 5.5 $\log_{10}$ (under detection limit). The study showed a synergistic effect between those 2 treatments (Kim et al. 2017).

UV-C light in combination with hydrogen peroxide (H_2O_2)

Xie et al. (2008) tested the combination of UV-C and hydrogen peroxide at a concentration of 2% vol/vol (H_2O_2) against MS2 coliphage with an initial concentration of 7.63 log PFU/mL, an analogue of NoV, on the surface of iceberg lettuce. UV-C light treatment alone for 60 seconds reduced the viral titer by 1.31 log PFU/mL. The H_2O_2 treatment alone for 60 seconds reduced the viral titer by 3.19 log PFU/mL and finally, the treatment combining UV-C light with H_2O_2 for 60 seconds reduced the viral titer by more than 3.54 log PFU/mL. The study showed that the combination of UV-C and H_2O_2 against MS2 coliphage could represent an alternative to hypochlorite-based washes on fresh food products (Xie et al. 2008).

Conclusion

Many chemical treatments, such as peracetic acids and chlorine compounds have been shown to be effective in the elimination of many different type of viruses. On the other hand, as consumers are increasingly looking for greener and less toxic alternatives for the environment and for health, researchers are turning to natural and non-thermal physical treatments. EOs such as: oregano, clove, zataria, cinnamon, rosemary, tea tree, Japanese alder, hasuo and fig tree were shown to be effective against NoV surrogates. Fruit extracts, including their polyphenols, like pomegranate, cranberry and grape seed showed high efficiency in viral elimination. Finally, non-thermal physical treatments like fermentations, ozonation and high-pressure treatments have also been shown to display noticeable antiviral properties. Furthermore, high-pressure treatments and ozonation seems to be effective against enteric viruses. Combined treatments against NoV are very recent and seem to be very promising. Combined treatments of irradiation or ozonation with the utilization of natural antimicrobials such as EOs alone or in combination could bring benefits to the food industry and could decrease the required dose to eliminate viruses while maintaining nutritional and organoleptic quality of food products. The hope of finding effective treatments might be in the path of combining treatments. Further studies are needed to find a cleaner, more cost-effective combination of treatments to meet consumer demand. Also, fruits and vegetables eaten raw represent an additional challenge since treatments with heat must be avoided. We should therefore think of alternatives such as gamma-irradiation, the use of natural extracts and the use of ozone alone or in combination in order to meet the needs while respecting the current issues on the potentially more harmful treatments and the environment.

Abbreviations

AJ	Apple Juice
B-PACs	Blueberry Proanthocyanidins
Cl	Chlorine
ClO_2	Chlorine Dioxide
COD	Chemical Oxygen Demand
CJ	Cranberry Juice
CJ-PACs	Cranberry Juice Proanthocyanidins
CP	Cranberries Polyphenols

$C_2H_4O_3$	Peracetic Acid
EOs	Essential oils
FAO	Food Agriculture Organization
FCV or FCV-F9	Feline Calicivirus (Strain F-9)
GSE	Grape Seed Extract
HAV	Hepatitis-A Virus
HCV	Hepatitis-C Virus
HHP	High Hydrostatic Pressure
HPH	High-Pressure Homogenization
HSV-1 and HSV-2	Herpes Simplex Virus
H_2O_2	Hydrogen Peroxide
IAEA	United Nations International Atomic Agency
Kbp	Kilo base pair
LAB	Lactic Acid Bacteria
MNV or MNV-1	Murine Norovirus (Strain 1)
NaClO	Sodium Hypochlorite
NoV	Human Norovirus
O_2	Dioxygen
O_3	Ozone
PACs	Proanthocyanidins
PFU/mL	Plaque-forming unit/mL
PJ	Pomegranate juice
PP	Pomegranate Polyphenols
ssRNA	single-stranded RNA
ssDNA	single-stranded DNA
TCID50/mL	Tissue Culture Infectious Dose 50/mL
USD	United State Dollars
USDA	United States Department of Agriculture
UV	Ultraviolet light
UVTP	TiO_2 photocatalysis
VLPs	NoV virus-like particles
VSV	Vesicular Stomatitis
WHO	World Health Organization
ZSO	*Zanthoxylum schinifolium*

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