Non-thermal inactivation of Noroviruses in food

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Abstract. An increased incidence of foodborne illnesses caused by Norovirus and consumer demand for fresh, convenient, and safe foods have prompted research into alternative antiviral processing technologies. Chlorine dioxide, UV treatment and thermal processing are standard antinoroviral technologies that have been employed for a while; however, they tend to be non-effective in modern processing due to residue concerns (ClO₂), shadowing effects (UV) and low-energy efficiency (heat treatment). Alternative technologies have been validated such as ozone treatment, high pressure processing and pulse electric fields. Although these techniques are promising, none of them individually can deem food free of Norovirus. Further research on the effects on Norovirus in various food matrices is required. Good manufacturing practices and proper sanitation procedures remain the “gold” safety tools in food business.

1. Introduction
Noroviruses (NoV) are small, non-enveloped viruses with a single-stranded RNA genome that make up the genus norovirus of the family Caliciviridae. These viruses cause acute diarrhoea with an incubation period of 24 to 48 hours [1]. NoV usually have a low infectious dose of as few as 10 virus copies [2], they are highly infectious, and can be spread by water, aerosol vomitus droplets, person-to-person contact, fomites and food. Outbreak control is hindered by the low infectious dose and environmental persistence of the virus.

When it comes to NoV shedding in human infection, it was determined that the amount of norovirus shed was approximately $9.5 \times 10^9$ genomic copies/g faeces as determined using quantitative reverse transcriptase-PCR (qRT-PCR). Viral shedding usually lasts up to 10 days after infection [3]. In contrast to some of the other enteric viruses, the genetic type of NoV may impact its resistance or sensitivity to inactivation treatments. They are divided into six major genogroups designated GI through GVI. GI and GII contain the majority of NoV strains associated with human illness and are further divided into about 30 genotypes. Genogroup V includes murine norovirus (MNV), which is the most genetically similar laboratory surrogate for human noroviruses.

Since NoV have an RNA genome and replicate rapidly using a polymerase that lacks 3’-5’ proofreading activity, they would be expected to mutate quickly. This was demonstrated by Nilsson et al. [4], who found 32 amino acid changes in the capsid protein of a norovirus shed chronically over 1 year by an immunocompromised patient. A single genotype, GII.4, has been associated with the
majority of worldwide outbreaks since the mid-1990’s, when active surveillance with reverse transcriptase-PCR (RT-PCR) was initiated. Thanks to implementation of these molecular diagnostic techniques during 1990s, an increase in the percentage of all documented laboratory-confirmed outbreaks due to noroviruses increased from more than 10-fold in 2000s [5]. However, regardless of this increase, gastrointestinal illnesses from noroviruses are still grossly underreported. As predicted by Widdowson [5], the CDC [6] confirmed that noroviruses were the cause of >50% of foodborne disease outbreaks. Greater than 56% of norovirus outbreaks are associated with eating salads, sandwiches, or fresh produce indicating that contamination of foods usually requires handling without an intervening heating step [5].

Many industrial techniques for controlling bacterial levels in food, including pH, temperature, and water activity are mostly ineffective barriers against NoV viral transmission to human hosts [7]. Up-to-date food hygiene criteria, which are optimized for the prevention of bacterial growth, may not be effective against NoV [1]. Since enteric viruses must survive the enzymatic and extreme pH conditions of the upper gastrointestinal tract to infect a host, they are resistant to a wide range of commonly used food processing, preservation, and storage treatments. Additionally, ingredients of the food itself may provide protection to virus particles against processing methods and host ingestion.

There has been increasing consumer’s demand for food that is minimally processed, additive free, and that has an extended shelf life. Therefore, there also has been greater interest in the commercial development of non-thermal processing technologies. This interest is motivated by the advantages of a preservative process that effectively inactivates problematic microorganisms and proteinaceous metabolites, increases shelf life while retaining the sensory attributes and nutrient content similar to the raw or fresh product. There are many non-thermal treatments that are in various stages of development that have the potential to destroy pathogens and retain food quality [8]. Key role in NoV inactivation plays either changes in protein capsid layer or damage of NoV RNA. Structural changes in capsid cause failure to recognize receptor on host cell thus disabling virus to enter host, while treatments damaging NoV RNA render virus incapable to enter lysogenic/lytic cycle.

In this paper, we will discuss on three currently employed non-thermal noroviral inactivation treatments in food.

2. Ozone
Ozone (O3), a strong oxidizing agent which has been widely used as a disinfectant in drinking water treatment plants in many European countries, receiving more attention after the discovery of potentially harmful chlorine by-products [9,10]. Owing to its high effectiveness and lack of residue after disinfection (unlike chlorine dioxide), ozone can be used for both surface and groundwater disinfection.

Ozone is a naturally occurring form of oxygen that exists as a bluish gas with a strong odour. It is moderately soluble in water and is effective at killing a range of microorganisms through the oxidation of cellular membranes [11]. Due to its oxidizing potential is 2.07 mV, it is considered as one of the most powerful common oxidizing agents available [12]. The antimicrobial effectiveness of ozone can be up to 52% stronger than chlorine [13], and it is active over a much wider spectrum of microorganisms than chlorine and other disinfectants.

The potential targets of viral inactivation by ozone treatment are the viral capsid proteins, antigenic sites for host cell receptor attachment and nucleic acid of non-enveloped viruses such as NoV. The interaction of ozone with the viral capsid proteins and antigenic sites may affect the adsorption and penetration of the virus into host cells. However, ozone also does penetrate through the capsid to RNA core inside virus, thus rendering RNA defective and prevent the replication of the virus after infection [14,15]. Typical ozone treatment (0.51 mg/L) at 20°C and pH 7.2 with a 2-min contact time resulted in approximately 75% of viruses still capable of cell attachment but >90% of these were non-infective.

Ozone decomposes in the water phase of foods very fast that which results in expression of its antiviral effect mainly at the surface of solid foods. Main product of O3 disintegration is plain oxygen, so there are no safety issues related to residual hazardous ozone residues in the treated food products.
or surfaces [16]. What also a positive effect of ozone is, the rapid breakdown prevents the accumulation of waste products from the process in the environment [17]. Thanks to its high oxidation potential, ozone quickly kills microorganisms; yet, it also reacts rapidly with complex organic compounds as found in foods. The existence of organic substances can neutralize the ozone required to inactivate microorganisms once being mixed with food.

Ozone should not be used directly on high ozone-demand foods, such as meat products, because the application of ozone may change sensory qualities of the product [18]. Aqueous ozone is applicable on products that have smooth and intact surfaces with low ozone demand such as fruits and vegetables [17,19]. Ozone is applicable in the industry to treat process water, as a fruit and vegetable wash, in fruit and vegetable storage, and in recycled water where fresh fruits and vegetables are first washed by ozonated water [20].

3. High pressure processing

High pressure processing (HPP) has emerged as a novel technology for food processing where foods can maintain their raw character and flavour. Applications of HPP include its use as a “cold pasteurization” method for fruit juices, a means of sanitizing packaged ready-to-eat meats, and inactivation of spoilage enzymes to enhance refrigeration shelf-life of avocados and guacamole. High pressure can also separate raw shellfish meat from its shell [21].

Successful inactivation of NoV varies depending on time, temperature, pressure level, and characteristics of the food matrix. The main mechanisms of noroviral inactivation by HPP is altering the virus capsid or protein coat surrounding the positive-stranded RNA. Enteric viruses are non-enveloped and, by definition, do not contain lipid envelopes. Therefore, HPP inactivation of foodborne virus, unlike foodborne bacteria, has no lipid-specific component. High pressure usually does not disrupt covalent bonds and it is hypothesised that high pressure does not damage the primary structure of nucleic acids, such as the RNA encoded within these viruses. It makes sense, therefore, that HPP inactivation is a function of pressure’s effect on virus protein conformations. Pressure-denaturation of proteins is a complex event and is dependent upon the structure of the protein, pressure range, temperature, pH, and solvent composition (including the presence of sugars, salts, and other additives). Pressure-induced changes to the viral coat can be discreet modifications to capsid proteins or receptor recognition proteins that result in loss of NoV infectivity.

Viewed from a capsid function perspective, the virus must attach to its host cell receptor, penetrate the cell membrane, and then release the RNA into the cytosol of the cell. Once inside the cytosol, the virus RNA genomes of picornaviruses and calciviruses are functional mRNAs that are sufficient to initiate transcription and subsequent virus replication [22]. Thus, high pressure must cause a protein-mediated effect that prevents virus attachment, penetration of the host cell, or uncoating once the virus has entered the cell.

An increase in pressure level has a greater effect on viral inactivation compared to increases in treatment time [23]. Typical pressures used for commercial food processing machines are as high as 600 MPa (1 MPa equals 9.87 bar). Commercial HPP units are quite large with capacities exceeding several hundred litres. Processing is by the batch with machines filled, treated for short intervals (usually less than 5-min), and then emptied. Commercial units are almost exclusively water based, but research units can use water, oil, or alcohol as the pressure application medium. Although HPP is classified as a non-thermal process, an adiabatic heating effect occurs under pressure that can be substantial with increasingly greater adiabatic heating effects observed for water, oil, and alcohol pressure medium [24-26]. Therefore, while the temperature before pressure application of 600-MPa may be at 25°C, the expected temperature achieved under pressure assuming a 3.5°C adiabatic heating per 100-MPa for a water-based unit, would increase to approximately 46°C [21].

Some viruses are extremely pressure-resistant, while others are sensitive; FCV is completely inactivated by pressures as low as 275 Megapascals (MPa) for 5 min [22]. A human volunteer study involving HPP treatment of NoV-contaminated oysters was performed [27]. This was accomplished by injecting $10^4$ RT-PCR units of GI.1 NoV into pressure-shucked
oysters. A 5-min, 400-MPa treatment at 25°C was not sufficient to inactivate the virus. Testing a second volunteer group with 5-min-, 600-MPa-treated virus at 6°C indicated that the virus was completely inactivated. A third group was fed NoV-contaminated oysters after a 5-min, 400-MPa treatment at 6°C. This treatment reduced the numbers of volunteers who became sick, but did not completely protect all volunteers. Therefore, pressures of at least 400-MPa or higher would be required to make human NoV-contaminated shellfish safe for consumption. Based on the reduction of human volunteers, it was postulated that the 400-MPa, 6°C treatment probably inactivates between 3- and 4 log10 of human NoVs [27]. This conclusion is also supported by subsequent research which has shown a dramatic drop in NoV’s ability to bind to virus receptor-like swine mucin glycoproteins after a 5-min, 400-MPa treatment at 5°C [28]. This drop was not observed for a 5-min, 300-MPa treatment at 5°C [28] which is not sufficient to inactivate NoV. The volunteer study also confirmed that colder temperatures did enhance the inactivation of human norovirus as was observed for the NoV surrogates FCV and MNV since complete inactivation of NoV was observed when pressure was applied at 6°C and not 25°C for 400-MPa treatments.

In conclusion, NoV can be inactivated by HPP. However, given the complexities of food matrices (water activity, carbohydrates, ionic strength) and variable response of different viruses, direct validation of HPP conditions within the food or food matrix being produced will be required in future.

4. Pulsed electric fields

Pulsed electric fields (PEFs) are another non-thermal process that can be used to inactivate foodborne pathogens in foods and beverages. Pulsed electric field processing is conducted by passing short high voltage electrical pulses through a fluid. These short electrical pulses may range anywhere between microseconds up to milliseconds, and the food may be processed at either ambient or refrigerated temperatures. The pulse process typically occurs in milliseconds, but the localized heating of the food from this short pulse is significant and should be measured or controlled.

In order to be successful, food preservation requires the inactivation of not only spoilage microorganisms and pathogenic microorganisms, but also enzymes that catalyse unwanted chemical degradation in foods limiting their shelf life. PEF has the advantage of being able to inactivate both enzymes and microorganisms. Although the antibacterial effects of pulsed electric field treatment are well documented, little information is available on its antiviral effects. The few studies carried out on enteric viruses show that pulsed electric fields of strengths up to 29 kV/cm do not produce the minimal 3 log10 cycle reduction of infectious particles required to warrant further attention [18,29].

Mechanism of activity relies upon the “dielectric rupture theory,” proposed by Zimmerman [30]. The external electric field will induce a change in transmembrane potential (TMP) across the cell membrane of a target organism. When this TMP reaches a threshold value, pore formation or electroporation of the cell membrane may occur. This will in turn induce an increase in cell membrane permeability, which can be lethal to the cell. It is hypothesized that the mechanism is somewhat similar in viruses, where PEF affects the outermost protein capsid of the virus, allowing its genetic material to escape the cell making it non-infectious.

5. Conclusion

Traditional thermal processing is highly efficient in inactivation of noroviruses although it may not be the most effective way for optimisation of food safety. Having in mind recent outbreaks in fruit and other RTE food, new technologies should be taken in account in NoV combat. These emerging technologies will help food business operators firmly to hold balance between safe NoV inactivation and the preservation of sensory properties.

For sure, no technology itself can deem complete NoV inactivation. This is due to specificity of non-envelope enteric virus, influence of food matrix, mechanism of inactivation, and virus strain. Further research on the effects on viruses in various food matrices is required. While all these techniques improve antiviral safety of food, good manufacturing practices and proper sanitation procedures remain the “gold” safety tools in food business.
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