Review paper

Running title: Nonthermal treatments of norovirus

Reduction of norovirus in foods by nonthermal treatments

Ahmed Hany[1], Maunula Leena[2], Korhonen Jenni[1]*

[1] Institute of Public Health and Clinical Nutrition, University of Eastern Finland, 70211 Kuopio, Finland

[2] Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, 00014 Helsinki, Finland

Key words: nonthermal processing, high-pressure processing, irradiation, UV light, norovirus

*Author for correspondence**.** Tel: +358 40 3552948, Fax: Not available, E-mail: jenni.korhonen@uef.fi

ABSTRACT

Human noroviruses are enteric pathogens causing substantial proportion of acute gastroenteritis cases worldwide, irrespective of background variables, such as age, ethnicity or sex. Although person-to-person contact is the general route of transmission, foodborne infections are also common. Thorough cooking eliminates noroviruses, but several food products such as berries, leafy vegetables or mollusks undergo only limited heat treatment, if any, before consumption. Novel applications of nonthermal processing technologies are currently under vigorous research as they can inactivate pathogens in variable degree together with extending product shelf life and varying effect on nutrients and perceivable quality. These technologies have been adopted from several industrial fields, some even approved as food processing methods and applied in the food industry for years. However, majority of the research has been conducted by bacteria and simple matrixes or surfaces. Hence, this review focuses on norovirus elimination in food matrixes by nonthermal technologies based on four distinctive categories; high hydrostatic pressure, light, irradiation and cold atmospheric plasma. We discuss the properties of norovirus, principles of chosen technologies and their inactivation mechanisms and present main findings of relevant studies. Further, we provide an overview of the current status of the research and propose future directions for related work.

HIGHLIGHTS

- High-pressure processing (HPP) is the most promising nonthermal treatment
- HPP, ionizing radiation and UVC can reduce noroviruses (NoV) from foods
- Treatment conditions eliminating viruses can impair product quality
- Optimal strategy should be validated independently for each product

Since its identification in 1972, *Norwalk virus* or norovirus (NoV) has been ubiquitously recorded as the cause of outbreaks and sporadic cases of acute gastroenteritis worldwide, particularly after the development of applicable molecular methods *(82)*. In fact, globally 18 % (95 % CI: 17–20) of all diagnosed cases of acute gastroenteritis result from NoV and the burden is of similar magnitude in developed and developing countries making it a universal health challenge *(5, 62, 64)*. Bartsch et al. *(14)* estimated that NoV infections result in annual total losses of $60.3 billion worldwide which could be further divided to $4.2 billion in health care costs and $56.2 billion in productivity losses. This ratio is somewhat concordant across regions reinforcing the universal magnitude of societal and economic burden of NoV. While person-to-person route is the principal mode of NoV transmission, water- and foodborne routes are also acknowledged as important sources of infection *(61, 100)*. Foodborne infections, causing annually an estimated 14–15 million illnesses and 400 deaths in Europe alone, typically result from a contamination somewhere along the production line, for example, at primary processing the irrigation water and at later stages workers or processing surfaces may be the sources of foodborne pathogens *(104)*. Typical high-risk food for NoV contamination do not go through any heating treatments, are manually processed or live in water environments. Hence, there are common sources of foodborne outbreaks including, but not limited to, vegetables, berries and mollusks *(12, 82)*.

The importance of a diet quality and nutrition in healthy lifestyle have been recognized for decades but only after the beginning of the 21st century they have shaped consumer trends dramatically increasing the demand for nutrient-rich, minimally processed, preservative-free and natural products *(8)*. Expectedly, the food industry operating in the consumer driven market follows the trends closely and responds to the consumers' desires. However, food safety, attractive sensorial properties and long shelf life remain equally important factors for both parties. Currently the only generally recognized method for

eliminating NoV is heating the product thoroughly above 70 ℃ for several minutes as the virus survives for prolonged time in temperature of 60 ℃ *(18)*. Hence, the food industry and research are forced to develop new approaches for ensuring safe products as such thorough heating is not an option for majority of the risk products.

Viruses in general are less susceptible to processing than bacteria, excluding spores, or molds due to their small size and simple structure limiting the effectivity of traditional techniques such as pH or water activity modification in eliminating NoV contaminations *(18)*. Nonthermal processing techniques are potential options for controlling pathogens in products that are delicate and meant to be consumed minimally processed. Although many technologies are still in the pipeline, their perks go beyond pathogen inactivation since product shelf-life can be extended without significant alterations in nutrient composition or sensory attributes *(58)*. Such technologies employ stress factors like pressure, light or irradiation that have already been verified to be effective against various bacteria and used for sterilization or disinfection in numerous industries. Further, the use of applications, for instance HPP, UVC and gamma irradiation have been approved and applied in food products such as salsa, juices and spices. Nevertheless, data is scarcer when it comes to NoV elimination, especially in food matrixes that are complex in terms of composition and structure. Additionally, to overcome the methodological issues of NoV culturing, majority of the studies in food matrixes have used surrogate viruses to model NoV.

This review focuses on the recent findings of NoVs or surrogates' inactivation by nonthermal treatments that are based on the following four methodologies; pressure, light, irradiation and cold plasma. Pressure applications were based on high-pressure processing (HPP). Identified applications utilizing different wavelengths of light were UVC (253–260 nm), blue light (405 nm) and pulsed light (200–1100 nm). Recognized irradiation applications were gamma and electron beam (e-beam) radiation that differ by the source of the radiation.

Finally, cold plasma was generated by plasma jets and dielectric barrier discharge technologies. Among discussing the key findings of the recognized studies, the article explores the properties and proposed mechanisms of inactivation of the included technologies and summarizes virologic and clinical factors of NoV.

NOROVIRUS: VIROLOGY AND RESEARCH

Characteristics of NoV. Taxonomically, NoVs belong to the family *Caliciviridae* in genus *Norovirus* which has only a single species, the *Norwalk virus (102).* NoVs are non-enveloped, single-stranded, positive-sense RNA viruses holding a 7.5–7.7 kb long genome with 3 open reading frames (ORF1, ORF2 and ORF3), except for murine norovirus (MNV) that has 4 ORFs. ORF1 encodes a polyprotein involved in replication cycle, ORF2 the major structural protein VP1, and ORF3 a minor structural protein VP2, together forming the viral capsid. The capsid protecting the viral RNA consists of 180 VP1 proteins organized into 90 dimers with few copies of VP2 protein located in the internal side of the capsid forming a symmetrical icosahedral shape with a diameter of 27–40 nm *(78).* VP1 consists of protruding domain P linked by a flexible hinge to the shell domain (S) surrounding the RNA. Further, P domain can be divided into P1 and P2 which shows great variability between virus species and is likely to be a determinant in the virus-host interaction due to its binding activity. VP2 is likely to have a part in RNA encapsidation and capsid assembly.

The classification of NoVs is based on dual nomenclature utilizing either the amino acid sequence of the complete gene of capsid protein VP1 or the nucleotide sequence of the RNA-dependent RNA polymerase region of ORF1, marked by capital P for 'polymerase' in the label, allowing a more precise typing of types, variants and recombinant forms *(17).* NoVs can be divided into 10 genogroups (GI–GX) and these groups have a variable number, 49 in total, of distinct genotypes such as GI.1 or GII.4. Genotypes that

replicate in humans belong to groups GI, GII, GIV, GVIII and GIX although GII and GIV have genotypes that infect porcine and feline/canine hosts, too. In the other groups, GIII replicates in bovine, GV in murine, GVI and GVII in canines and GX in bats. Some predominant variants responsible for epidemic outbreaks surfacing every 2–3 year due to mutations and recombination may have the geographical location of the first identified outbreak attached into their name. The recently proposed dual-typing nomenclature for NoV strains would first list the genotype followed by P-type in brackets, e.g. GII.4 Sydney [P16].

NoV surrogates. Until now, the research on human norovirus (HuNoV) mechanistic features has been hampered due to the inexistence of reproducible culture system supporting high level of replication regardless of the recently published successful efforts in B cells and stem cell-derived human enteroids *(23, 40)*. However, the viral fold increases in these cultures are relatively modest, ranging from 10^1 to 10^2, compared to 10^5–10^6 in well-established surrogate cultures allowing extensive passaging. Hence, culturable surrogate viruses resembling HuNoVs are likely to remain a mainstay in the studies until the HuNoV culture systems permit production of highly concentrated stocks. Most utilized surrogates are *Feline calicivirus* (FCV), MNV, bacteriophage MS2 and the recently discovered rhesus monkey calicivirus Tulane virus (TV). MNV is genetically closest to HuNoVs whereas FCV belongs to the genus *Vesivirus* and TV to *Recovirus*, both however belonging to *Caliciviridae* family. Bacteriophage MS2 infects bacteria from the family *Enterobacteriaceae* but has also been applied due to structural similarities to NoV. In addition to culturable surrogates, virus-like particles (VLPs) assembled in infected insect cells are also commonly applied for studying capsid stability and interaction *(77)*. The VLPs consist of VP1 proteins forming a capsid that is morphologically and antigenically comparable to HuNoV but lacks the genome and VP2 proteins, both of which could contribute to capsid stability. Despite resemblance to

142 HuNoVs, surrogates rarely have similar survival rates when challenged with physical or
143 chemical treatments that are in industrial use as concluded by a recent systematic review *(46)*.
144 For example, based on RT-qPCR signals, HuNoV is significantly more persistent than MNV-
145 1 and FCV F-9 in heat and chlorine treatments, respectively. Hence, extrapolating results
146 from surrogate studies to HuNoVs is likely to underestimate the measures required for
147 complete inactivation in virus contaminated foods. Due to this and the differences for
148 example in genetic and host-virus interaction properties, no surrogate has been recognized as
149 an ample replica of HuNoV.
150
151 **Assessment of infectivity.** The evaluation of infectivity is the key determinant
152 when investigating the efficacy of an inactivation approach in fresh foods. The real-time qRT-
153 PCR is currently considered as the golden standard due to its high sensitivity and specificity
154 for detecting and assessing the viral loads of HuNoVs from clinical and environmental
155 samples *(102)*. However, genome-based molecular methods are incapable of differentiating
156 between infectious and non-infectious viruses since vital capsid structures for host-virus
157 interactions may be rendered defective without noticeable effect on viral RNA derived from
158 functional, partially or completely degraded viruses. The lack of a well-established HuNoV
159 cell culture model has prevented the evaluation of infectivity via plaque or the 50 % tissue
160 culture infectious dose ($TCID_{50}$) assays which are routinely applied with the surrogates.
161 Hence, auxiliary approaches to discriminate functional from non-functional HuNoVs prior to
162 qRT-PCR is to bind infectious viruses to ligands or prevent the amplification of RNA of the
163 capsid-damaged viruses by nucleic acid intercalators. Among several options, studies with
164 fresh foods have primarily used porcine gastric mucin conjugated magnetic beads (PGM-
165 MBs) containing histo-blood group antigens (HBGA) binding TV and HuNoV strains from
166 the genogroups I and II *(95, 96)*. The PGM-MBs are mixed with contaminated samples

allowing capture of infectious viruses which can be quantified by the following qRT-PCR. Compared to standard detection by RT-PCR, PGM-MBs did show a 2-log increase in sensitivity in PBS medium and in food matrixes such as lettuce, oysters and strawberries *(96)*. Another strength of PGM-MB assay is the presence of sialic acid, an attachment factor for MNV, enabling the parallel use for another surrogate among TV in addition to HuNoV strains within the same experimental model. Observations have suggested that assay specificity could be further improved by pre-treatments such as RNAse or protease K treatment removing inactivated virions with remaining binding activity *(38, 106)*. Recently, results of quantifying FCV inactivation by ethidium monoazide (EMA, nucleic acid intercalator) coupled RT-qPCR were comparable to results with RNAse RT-qPCR method, yet both underestimated inactivation by ~2 logs *(1)*. This underlines the importance of developing a validated cell culturing method for assessing HuNoV infectivity. Apart from a single study utilizing EMA, options for augmenting the performance of nucleic acid-based methods, only RNAse was applied in the reviewed publications.

NONTHERMAL PROCESSING METHODS

High-pressure processing. The properties of each technology are summarized in

Processing technology	Characteristics	Mechanism of inactivation	Advantages	Disadvantages
High-pressure processing (HPP)	Hydrostatic pressure transmitted by fluid in pressure vessel Pressures range from 200 to 800 MPa Treatment times are generally below 10 minutes including come up, hold and depressurization times	Disintegrates capsid attachment and binding sites Disrupts capsid structure at higher levels	Pressure is uniformly distributed Preserves nutritional properties Independent of product geometry and size Extends shelf-life	Expensive Requires pre-packing Batch process Large equipment Optimal conditions are product specific

	Sub-ambient temperatures enhance efficacy			
Pulsed, blue and UVC light	Utilizes various wavelengths of light; UVC 240–260 nm, blue 405 nm and pulsed 200–1100 Effective against surface contaminations Appropriate doses are achieved in under 5 minutes or in few seconds with pulsed light	Photodimerization of RNA molecules and disruption of capsid structure (UVC wavelength) Creation of oxidative radicals	Preserves nutritional properties Low cost Easy and safe handling Possibility for continuous process Does not require contact	May impair sensorial properties at high doses Limited penetration depth that is also lost in turbid liquids Effectivity largely attributed to surface structure
Gamma and electron beam (e-beam) irradiation	Electromagnetic radiation with adequate energy for ionizing ^{60}Co or ^{137}Cs are the radioactive sources of gamma rays in food processing E-beam is constituted of electrically accelerated electrons Commonly applied doses in fresh foods are 1–10 kGy, yet doses up to 50 kGy are allowed for sterilization	Disrupts RNA and capsid structure Creation of oxidative radicals	Preserves nutritional properties Extends shelf-life Possibility for continuous process Does not require contact Gamma radiation is highly penetrating E-beams are created rapidly, directed to targets and can be powered off when not used	Expensive Negative consumer perception May impair sensorial properties at high doses E-beams have limited penetration depth Safety and security risks especially with gamma radiation
Cold atmospheric plasma (CAP)	Inert gas or gas mixture ionized by strong electromagnetic fields Plasma is created in atmospheric pressure and generally remains close to ambient temperature Treatment times generally below 20 minutes Can be applied directly, remotely or via activated water on the product	Reactive oxygen and nitrogen species generated by the ionization of gas Capsid protein oxidation and disintegration RNA degradation	Preserves sensorial properties Extends shelf-life Can be applied directly to packed products with modified atmosphere Utilizing air as feed gas is economical	Some setups may cause product heating Large number of technologies with varying properties complicate comparisons Expensive with pure gases Industrial scale food processing equipment still under development

.The essential advantage of HPP is based on the isostatic principle denoting that pressure is uniformly distributed throughout the product independent of the size or geometry which is particularly favorable in high-moisture foods like vegetables and beverages since HPP does not affect covalent bonds but alters non-covalent interactions forming the secondary and tertiary structure of proteins *(58).* Thus, helping to preserve sensorial attributes and low-molecular mass particles contributing to the flavor and nutritional profile. However, optimal

processing parameters for balancing pathogen inactivation with minimal changes in quality are product dependent since colors and texture of berries or vegetables can change and berry purees may lose their thickness *(59)*. The potential and basics of HPP has been reviewed extensively by several authors elsewhere *(13, 15, 103)*.

Pressure inactivation of HuNoVs and surrogates has been studied in an increasing pace since the turn of 21st century, yet only recently the research has shed light on the mechanism of inactivation *(45)*. Tang et al. *(93)* evaluated MNV-1 capsid integrity and antigen capture by RT-PCR and receptor binding by ELISA after HPP treatment of 400 MPa at 0 °C. This resulted in a successful reduction of 8.22 log PFU but viral RNA remained intact and the remaining viruses were still capable of binding to the specific antibody. Binding to the cell receptors on the other hand was significantly affected indicating interference with the binding proteins on viral capsid. Capsid integrity was sustained throughout HPP but when challenged with proteinase K, results implicated that capsid proteins were more prone to enzyme digestion than in untreated controls. Thus, authors concluded that inactivation is primarily transmitted through the changes in the function of capsid binding proteins without significant effect on RNA or capsid integrity. These findings were expanded by Lou et al. *(59)* as transmission electron microscope imaging showed that at 350 MPa, the structure of MNV-1 was partly ruptured and above 500 MPa, protein debris was the primary result indicating complete capsid degradation and loss of infectivity. However, despite of the degradation of capsid structure, RNA integrity remained and capsid proteins VP1 and VP2 retained their form and antigenic properties demonstrated by SDS-PAGE and Western blot analysis. This was expected as HPP doesn't affect covalent bonds of nucleic acids or proteins.

Dancho et al. *(19)* extended studies to HuNoV strains GI.1 and GII.4 by assessing their binding to PGM-MBs after HPP, UVC and heat treatments. They also assessed the binding efficacy by dividing the PGM-MB bound RNA equivalents by the total bound and

unbound RNA equivalents in the sample. The efficacy for untreated GII.4 strain was 69 % whereas for GI.1 strain the range was 68 – 84 %. The binding to PGM-MB was consistently reduced with increasing heat, light or pressure treatments. For example, with HPP at 300 MPa, no considerable reductions in binding affinity were evident whereas 400 MPa proved to be the threshold for significant decline as binding was reduced by over 3 logs. At 500–600 MPa binding was further declined by around 5 logs whereas total RNA decline was around 1.5 log, mirroring the results at 400 MPa. Such reductions showed also clinical relevance in the only clinical human volunteer study conducted by Leon and colleagues *(51)* although reduction rates were not measured. They demonstrated that GI.1 seeded oysters HPP treated in 400 MPa were not rendered safe for consumption while participants who consumed oysters treated in 600 MPa did not suffer from signs of infection. Thus, these findings further promote the hypothesis of primary inactivation mechanism through modification of binding site integrity in viral capsid, rather than degradation of whole capsid structure or viral RNA.

UVC light. UVC light is short-range wavelength (100–280 nm) electromagnetic radiation with mutagenic properties attributed to photodimerization of pyrimidines and formation of other photoproducts such as pyrimidine adducts and DNA-protein cross-linking in the DNA *(33)*. Among the germicidal effect, factors such as low cost, easy handling and absence of toxic residues have resulted in wide industrial applications of UVC for sanitizing food contact surfaces, water and even solid and liquid foods. However, effectivity is limited to surfaces and rapidly lost in turbid liquids. In RNA viruses the nucleic acids are considered to be the primary site of inactivation although at higher levels of over 1000 mJ/cm^2 capsid denaturation can also initiate *(66)*. In the case of FCV, several UVC wavelengths at fluence level below 40 mJ/cm^2 induced over 3 log viral reductions in infectivity *(92)*. Although UVC induced capsid protein oxidation, morphological analysis showed that capsid structures were

not significantly affected. However, RNA copy numbers assessed by RT-PCR decreased by approximately 50 % after treatment. In another study the binding of HuNoV GI.1 to PGM-MBs were reduced by 1.8 and 3.8 log following a 1000 and 2000 mJ/cm^2, respectively, UVC treatment *(19)*. The loss in binding was not followed by similar reduction in RNA copies measured by RT-PCR. Hence, these results demonstrate that the loss of infectivity is due to damage to RNA and capsid proteins.

Pulsed light. Another application utilizing the electromagnetic energy carried by light is pulsed light treatments where lamp units produce short, rapid bursts of light covering a wide scale of wavelengths (200–1100 nm) including UV, visible light and infrared *(67)*. The greatest asset of pulsed light technology is that it delivers high amounts of energy in a matter of seconds. In extended treatments, the heating effect can make it unsuitable for delicate products if a cooling protocol is not adopted in the process *(36)*. The disinfection potential is attributed to the UV and energy content, ranging from 0.1 to 50 000 mJ/cm^2, being markedly greater than in continuous light delivery applications. However, the FDA has ruled that cumulative fluence of 12 000 mJ/cm^2 is the upper limit of pulsed light treatments in foods. The mechanism of virus inactivation by pulsed light was elucidated by Vimont et al. *(101)* who demonstrated with MNV-1 suspension that the main targets of the treatment are capsid structure, major capsid proteins and viral RNA. Imaging showed a mixture of debris, empty and intact particles indicating degradation of the main structure of the capsid. The abundance of VP1 proteins decreased by half suggesting disintegration of the primary protein structure. Additionally, the amount of undamaged RNA and the total RNA were decreased. These effects resulted in a total loss of infectivity and were achieved below the total fluence level of 12 000 mJ/cm^2.

Blue light. Effectivity of visible blue light (405 nm) against multiple microbes such as bacteria and molds is based on the photosensitizers, molecules excited by light wavelengths, inducing production of oxidative agents that damage and kill cell *(98)*. Photosensitizers may be present in the surrounding media as exogenous or endogenous as is the case in bacteria where porphyrin rings absorb light generating singlet oxygen and other reactive oxygen species (ROS). However, viruses contain mainly proteins and nucleic acids that do not directly absorb such wavelengths, hence findings from other micro-organisms should not be extended to viruses as such. Instead, it is proposed that exogenous photosensitizers can damage viral capsids, cause alteration in the DNA-protein interactions and in the DNA secondary structure together predisposing viruses to inactivation *(22)*. Tomb et al. *(97)* showed that blue light had the capacity to inactivate bacteriophages suspended into nutrient broth. A 2.7 to 7.1 log PFU/ml reduction was evident depending on the initial viral count and light dose. However, the reduction was only 0.3 log PFU/ml in simple PBS in otherwise similar parameters. Later on, the same authors provided similar evidence with FCV as a blue light dose of 421 J/cm^2 yielded a 4.8 log PFU/ml reduction in nutrient-rich media compared to a 3.9 log decline in PBS after light dose of 2804 J/cm^2 *(98)*. These findings suggest that the composition of the surrounding media has a critical effect on inactivation and length of the blue light treatment as a 5-hour treatment was required for FCV inactivation in PBS compared to 45 minutes in nutrient-rich media.

Gamma irradiation. In food irradiation products are subjected to radiation holding the energy to remove electrons from atoms or molecules thereby ionizing them *(90)*. Rationales for irradiation incorporates delay of ripening, inhibition of sprouting, shelf-life extension, pathogen control and sterilization. Gamma radiation is emitted from a radioactive source, mainly cobalt-60 in food applications, in the process of radioactive decay. Gamma

rays from cobalt-60 carry the energies of 1.17 and 1.33 MeV and are photons without mass permitting high penetrability as they can pass through food matrix of various densities. The mechanism of virus inactivation by gamma radiation was elucidated by Feng et al. *(24)* who demonstrated by MNV-1 that irradiation affects several factors resulting in the loss of infectivity. The major capsid protein VP1 gradually degraded along elevating radiation dose and by 22.4 kGy, VP1 proteins were undetectable. Similar to the VP1 proteins, the amount of intact virion structures reduced along the gamma ray dose and at 22.4 kGy only debris was visible. Doses of 2.8 and 5.6 kGy decreased RNA levels and viral titers to certain levels and complete degradations were shown at 22.4 kGy. Moreover, the capsid stability of HuNoV VLPs were comparable to MNV-1. However, doses of over 11.2 kGy were required to produce significant reductions of over 3 logs in infectivity in buffer solutions.

Currently, adsorbed irradiation doses for assuring microbiological safety of foods may exceed 10 kGy as such doses have been proved to be safe and nutritionally adequate *(6)*. Nevertheless, authorities have underlined that irradiation should not be a substitute for hygienic production protocols, applied doses should be the lowest possible to reach the technological need and products need to be clearly labeled. In the EU, irradiation of "dried aromatic herbs, spices and vegetable seasonings" are authorized in every member state and some member states have temporary admittance for treating additional food stuffs. In the USA, regulations are more permissive for doses in specific food categories and labeling. The EU and USA limits in NoV risk food categories for absorbed irradiation doses (kGy) range from 1 to 4 in the US and 0.15 to 2 in the EU for vegetables or berries and 5.5 in the US and 3 in the EU for shellfish *(6, 74)*.

Electron beam irradiation. In electron beam (E-beams) technology, the irradiation produced electrically by generating electrons and accelerating them by electromagnetic fields *(90)*. The

perks of e-beams are their controllability as they can be targeted to small area and switched off when necessary. Also, accelerated electrons have typically the energy up to 10 MeV being ten times the energy compared to gamma rays. This makes e-beams more cost-efficient and enables rapid treatment times. However, decontamination by e-beams is limited to the outer surfaces as effective penetration depth ranges from 3 to 10 cm depending on the food material. Being ionizing irradiation, the inactivation mechanism is shared among different technologies to produce radiation. DiCaprio et al. *(21)* studied e-beam inactivation in viruses and showed that HuNoV VLPs were reduced by 90 % after e-beam treatment of 28.3 kGy and the remaining particles retained binding potential to PGM-MBs. This suggests that the degradation of tertiary protein structures is the primary method of inactivation. Additionally, compared to HuNoV GII.4, TV seemed to be more susceptible to e-beam but not to gamma radiation although complete degradation of RNA was not achieved. Predmore and colleagues *(80)* also concluded that viral elimination likely result from the degradation of the capsid protein structure and in lesser degree from the breakdown of capsid protein VP1 as shown in e-beam treated MNV-1 and TV. Moreover, a dosage of over 32 kGy rendered the genetic material of MNV-1, but not TV, undetectable.

Cold atmospheric plasma. Cold atmospheric plasma (CAP) is generated by subjecting inert gas, such as a noble gas or a mixture of gases like air, to strong electromagnetic fields *(65)*. Electrically produced microwaves, radio frequency current, direct or alternating current can ionize the gas forming a vast number of excited reactive species determined by the feed gas. CAP devices operate at pressures of one atmosphere at ambient temperatures, are chemical-free and have antimicrobial qualities making them an attractive option for food processing. The technologies that have been applied in the included studies were plasma jet and dielectric barrier discharge (DBD) where plasma is generated directly on

the products with radio frequency electrodes. Further, apart from the antimicrobial properties, these technologies can also have additional value for example in shelf-life extension and food functionalizing *(86)*. The antiviral properties are accredited to the variation of ionic, atomic, radical and molecular species within the plasma *(25)*. Indeed, singlet oxygen and perioxynitrous acid, reactive oxygen and nitrogen species produced by CAP, were shown to inactivate FCV via modification of capsid proteins *(2)*. Their role in FCV inactivation was later affirmed by Yamashiro et al. *(105)* as exposing viruses to similar concentrations of perioxynitrous acid as generated in CAP treatment produced comparable loss in infectivity. Moreover, the presence of scavengers of singlet oxygen and perioxynitrous acid decreased inactivation efficiency. Finally, a 15 s CAP treatment effectively rendered over 6 log $TCID_{50}$ non-infectious but this was not due to capsid degradation *(3)*. Instead, infectivity was lost by oxidization of capsid proteins in the attachment and entry site of the viral capsid. However, a 2-minute CAP treatment disintegrated the majority of viruses' capsid structure and exposed the RNA to damage.

HPP IN FRESH PRODUCTS

Berries. Blue-, rasp- and strawberries have been used as substrates in several studies either in their natural form *(34, 35, 54, 55, 59)* or as pureed or juiced *(20, 35, 47, 59, 68)*. A detailed overview of the effect of HPP on surrogate inactivation in berries and other food commodities is displayed in Table 2. Core features of the treatment for effective inactivation, irrespective of virus model, type or the form of the berry, were observed to be pressure, temperature and pH. While the relationship is linear between pressure magnitude and inactivation, the effect of temperature is inverse favoring inactivation at sub-ambient temperatures of 0–4 °C. For pH, the optimal range was repeatedly observed to be at 6.5–7.4 which enhances inactivation opposed to acidic conditions of 2.5 to 4.0 in surrogates MNV-1

366 and TV *(20, 54, 55, 59)*. This was also observed with HuNoVs GI.1 and GII.4 as the pH of
367 the surrounding waters and viral reductions were significantly lower in more acidic straw- and
368 raspberries compared to blueberries after treatment as displayed in Table 3 *(35)*. However, in
369 addition to the pH, the surface structure of whole blueberries as opposed to straw- and
370 raspberries was also proposed to cause fluctuations in reduction potential as inactivation was
371 significantly higher in whole blueberries *(35)*. While blueberries have a smooth surface,
372 straw- and raspberries irregular surface has shielding cavities and pouches. For instance, GI.1
373 was effectively reduced in blueberries by > 3.2 log genome copies/g in 2-minute handling of
374 550 MPa at 0 ℃ but elevating pressure levels up to 650 MPa showed only a 2.5 log
375 elimination in raspberries and 1.7 log in strawberry quarters. Similar effect was also seen with
376 GII.4, albeit of greater reductions suggesting increased susceptibility towards HPP. Further,
377 there were no differences in inactivation of GI.1 between berry purees but GII.4 was more
378 susceptible to elimination in straw- and raspberry than in blueberry puree. To achieve a
379 reduction of ≥ 3 logs in surrogates MNV-1 and TV the pressures required were on average
380 400 MPa irrespective of the form of the berry matrix *(34, 47, 55, 59, 68)*. Finally, direct
381 contact to water i.e. wet state, was found to be superior over dry state as lower pressures
382 yielded better results regardless of berry or virus type *(34, 54, 55)*. This was discussed to
383 derive from the pressure forcing water into the viral capsid protein structures disrupting the
384 molecular folding of the protein domains associated with binding and attachment .
385 Several factors could account for the significance of matrix composition
386 irrespective of the virus model indicating that matrix protects viruses during HPP. Pressures
387 required for inactivation increase gradually from simple aqueous to more complex medium
388 while being systematically higher in actual food matrixes *(20, 47, 54, 55)*. Degree of the
389 processing also affects as Pan and others *(68)* determined that MNV-1 was more easily
390 inactivated from strawberry juice than puree, a finding in line with studies by Kovač et al.

(47) and Lou et al. *(59)*. Likewise, differences in inactivation that were associated with the berry type in whole berries were absent in purees even with more resistant HuNoV GI.1 *(35)*. HPP has also less impact on the sensorial qualities of purees opposite to whole berries where undesirable changes in the texture and color limits application of pressure levels necessary for achieving satisfactory inactivation levels *(35, 59)*.

Vegetables. Surrogates have been applied in variety of fresh or pickled vegetables, such as lettuce, green onions, salsa and cabbage *kimchi (29, 31, 59, 72, 89)*. Contrary to findings of Sharma et al. *(88)* in deli sausages, FCV was found to be significantly more susceptible in salsa as it was reduced by ~6.5 log $TCID_{50}$/g in 250 MPa *(29)*. To reach over 3 log inactivation of MNV-1, pressures of 300–400 MPa were required in salsa and 400–500 MPa in lettuce, green onions and *kimchi (31, 59, 72, 89)*. Importantly, Hirneisen et al. *(31)* also pointed out that HPP is equally effective against MNV-1 inoculums in internal tissues or on the outer surface underlining the uniformity of pressure treatment. The only results with HuNoVs were provided by Sido and colleagues *(89)* where GI.1 proved to be more resistant in salsa as it required 500 MPa at 1 ℃ for 2 minutes to induce a > 3 log genome copies/g reduction whereas 300 MPa in otherwise equivalent conditions for GII.4 showed a decline of 3.31 logs. Green onions instead required even higher pressures for a decline of similar magnitude as GI.1 and GII.4 needed 600 and 500 MPa, respectively, in otherwise similar parameters. However, the above-mentioned pressure ranges for effective inactivation, excluding FCV, deteriorated sensorial qualities of lettuce, green onion and *kimchi (29, 31, 59, 72)*.

Seafood. Clams or oysters are particularly prone to microbiological hazards as they are filter-feeders that bioconcentrate seaborne human pathogens and can be consumed

raw or lightly cooked. Hence, several studies applying HPP and surrogates have been conducted with shellfish *(7, 52, 55, 91)*, various seafood salad-like products *(30)* and sea squirt *(73)*. On average, inactivation at ambient temperatures was found at 250 MPa but for achieving a 4 log PFU decline in MNV-1 titers, pressure of at least 400 MPa in temperatures close to 0°C were required. To reach such reductions in sub-optimal temperatures of 25–28 °C, a threshold of 500 MPa was observed in both Manila clams *(7)* and sea squirt *(73)* independent of exposure duration. Such losses in infectivity were not followed by declines in viral RNA levels denoting the inadequacy of simple PCR assay *(52)*.

The individual behaviors of different matrixes were revealed by Hirneisen et al. *(30)* as they contaminated cooked seafood samples of cod, tuna, shrimp and clams with or without mayonnaise with FCV and also evaluated the protective effect of mayonnaise alone on FCV and MNV-1. Viral declines after treatment of 200 MPa at 5 °C showed significantly lower susceptibility in cod (−1.15 log $TCID_{50}$/g) than in tuna (−4.54 log) or shrimp (−4.46 log). Addition of mayonnaise to the seafood meats did not show any significant differences in inactivation compared to the meats alone. However, compared to cell culture medium, mayonnaise provided significant protection for FCV and MNV-1 reducing them only by 1.09 log and 1.39 log, respectively. Takahashi and others *(91)* also observed the protecting effect of matrix complexity by growing demand of pressure to reach satisfactory inactivation result from salt and pH matched buffer to oyster homogenate to whole oyster. Additionally, HPP-treatments did not negatively alter, rather they improved the sensorial quality of oysters and sea squirt *(52, 73, 107)*.

Imamura et al. *(39)* assessed the naturally present genogroups GI and GII in aqua-cultured Pacific oysters showing a prevalence of roughly 17 % and an average total RNA count around 2.95 log RNA/g that was inactivated below the theoretical limit of detection of 2.36 log after treatment of 400 MPa at 10 °C (Table 3). Slightly improved, yet

441 modest outcomes were also shown in bioaccumulated oysters where the combined reductions
442 of GII.4 and GII.17 were −1.87 log and −1.99 log for separate batches after 400 MPa at 25 ℃
443 *(38)*. However, these results were also based on RT-PCR after RNAse treatment without
444 information on infectivity or binding potential. Like the bio-accumulation in water tanks, the
445 studies of artificially inoculated oysters or clams have led to significantly higher
446 contamination levels of 4–7 log RNA copies/g or ml and exclusively applied genotypes GI.1
447 and GII.4 *(60, 106, 107)*.
448 Measurements by PGM-MB/RT-PCR assays with prior RNAse treatments are in
449 line with previous findings as GI.1 proved out to be more pressure resistant since 450–500
450 MPa produced ~4 log RNA/g reduction in low temperatures of 1 or 6 ℃ whereas 350–400
451 MPa was sufficient for GII.4, even at 25 ℃ *(106, 107)*. For now, the only human study of
452 pressure-treated oysters spiked with 4 logs of GI.1 8FIIb showed that 5-minutes in 600 MPa,
453 but not in 400 MPa, at 6 ℃ protected volunteers from infection suggesting complete
454 inactivation *(51)*. Lou and others *(60)* expanded research into animal models as they fed
455 HuNoV GII.4 strain 765 -inoculated oyster homogenates with or without HPP treatment to 2-
456 day old gnotobiotic piglets to see whether this protects piglets from norovirus infection during
457 7-day period since swine have similar HBGA phenotypes to humans. The effect of HPP in
458 350 MPa at 35 ℃ (~1 log reduction) or at 0 ℃ (~3.7 log reduction) was verified by PGM-
459 MB/RT-PCR assays. Only piglets fed with homogenate treated with 350 MPa at 0 ℃ were
460 protected from infection as the signs of virus shedding in feces, mild diarrhea, histological
461 lesions and viral antigens in small intestine were absent in this group in contrast to others.
462 These findings highlight the transferability of PGM-MB binding assay results to biological
463 systems when estimating optimal treatment parameters for ensuring food safety.
464

Meat products. Although only 3.3 % of meat and meat product samples were found to be contaminated by NoV in a recent survey, mishandled meat products still represent a risk *(84)*. Sharma et al. *(88)* inoculated pork meat deli sausages by FCV or bacteriophage MS2 after immersing samples in sterile water for 5 minutes and drying. HPP treatment in 500 MPa at around 4 ℃ decreased FCV titers by 2.89 log $TCID_{50}$/ml and MS2 titer by 1.47 log PFU/g but could not completely remove all viral titers as recoveries of FCV and MS2 were 4.00 log $TCID_{50}$/ml and 5.34 log PFU/g, respectively. Compared to sterile water, immersing samples to 100 ppm EDTA or 2 % lactoferrin did not significantly alter virus or phage attachment. Reductions were significant in all samples but there were no differences derived to the additives with FCV. MS2 on the contrary was more resistant to pressure while water or EDTA showed significantly better outcome in the elimination when compared to lactoferrin. The authors speculated that the coarse surface of grinded meat protected the NoV surrogates from HPP as has been discussed previously *(50)*. However, the protein, fat and salt contents in the product and their interactions may also offer protection from inactivation *(30, 44)*.

Overall, HPP has been tested with multiple food matrixes and virus models over the last decade addressing the expectations towards this technology. The use of HuNoV strains, generally GI.1 and GII.4, was a particular asset of HPP research although surrogates, mainly MNV-1 were equally common. Observed outcomes reinforced the high resistance of GI.1 compared to GII.4 based on the binding assay while out of surrogates, FCV is the most susceptible while MNV-1 and TV share similar resistance to pressure. Although the surrogates' comparability to HuNoVs in terms of susceptibility is questionable, they mimicked HuNoVs response to temperature, pH and the presence of water. *(20, 54, 55, 89)*. Out of a wide range of treatment parameters (Table 2 and 3), the best outcomes were associated with temperatures below 5 ℃ and pressures between 400 and 600 MPa with direct water contact augmenting inactivation and limiting matrix damage to some extent in berries

(54, 55). The only product related features observed to favor inactivation were smooth surface and neutral pH concerning both the product itself and the surrounding water *(20, 35)*. However, product composition could overcome the effect of low pH as demonstrated with lemon juice *(59)*. Hence, rather than tracing the matrix features to a single factor, it is more likely that the complexity and interactions between components determine the protective effect *(30)*. The downfalls of HPP are the possible texture changes at high pressures with whole berries and leafy greens making it unsuitable for produces sold fresh in their original form *(35, 59)*. On the other hand, for purees, juices and oysters it is a viable option. Treatment durations can be reduced by assessing the optimal conditions since inactivation is saturated rapidly at adequate pressure levels *(29, 72)*. Finally, the device investments are high as technology is expensive and requires maintenance along its lifespan. Nevertheless, several HPP-treated products like salsa and juices are available in the markets and it is also used in shucking, extending shelf life and reducing *Vibrio* spp. in oysters but the pressure levels currently applied are ≤ 300 MPa *(106)*.

UVC, PULSED AND BLUE LIGHT IN FRESH PRODUCTS

Berries. UVC was the most frequent source of light introduced to inoculated blue-, rasp- or strawberries *(16, 26, 56)* followed by pulsed *(36, 37)* and monochromatic blue light *(43)* as summarized in Table 4. Fino and Kniel *(26)* demonstrated that while UVC fluence of 50–75 mJ/cm^2 inactivated up to 7 log $TCID_{50}$/ml of FCV in cell culture media, reductions reached only 1.13–2.28 log $TCID_{50}$/ml in strawberries following fluences of 40–240 mJ/cm^2. Similar maximum reduction levels were evident with MNV strain S99 in fresh strawberries (1.27 log $TCID_{50}$/g) and raspberries (1.52 log) but significantly higher in blueberries (3.12 log) while fluences administered ranged ~200 to 1300 mJ/cm^2 during 20–120 s period *(16)*. Alterations in sensory characteristics were also noted after extended

exposure to UVC (4000 mJ/cm^2) in frozen strawberries as some panelists noted off-flavors or slightly darker colors, the latter likely due to the degradation of anthocyanin pigments. Concurrent water wash could enhance UVC inactivation as Liu and colleagues *(56)* showed MNV-1 reductions of >3.20 log PFU/ml in blueberries at 600 mJ/cm^2, increasing to > 4.32 log with fluence of 1200 mJ/cm^2, both being more effective than dry conditions. Fluences were substantially higher, 63200 mJ/cm^2 in strawberries and 53900 mJ/cm^2 in raspberries, in pulsed light treatments resulting in MNV-1 reductions of 1.8 log PFU/g in strawberries and 3.6 logs in raspberries *(36)*. Cutting the light fluences to 5900–22500 mJ/cm^2 yielded only a modest MNV-1 inactivation of 0.7 to 0.9 log PFU/sample in strawberries but results with blueberries were again more encouraging as average reductions were 3.1 to 3.8 log *(37)*. The lately applied blue light treatment in blueberries was shown to be ineffective against TV as from total fluences of 1260, 3780 and 7560 mJ/cm^2, only 7560 mJ/cm^2 produced a non-significant reduction of 0.06 log PFU *(43)*.

Observed differences between berries likely stem from the variations in surface topography as strawberries and raspberries have an irregular, cavity-holding exterior compared to blueberries shielding virus particles and the shape permitting shadowing *(16, 36, 37)*. Moreover, the observed saturation effect after UVC fluence of 200 mJ/cm^2 was hypothesized to depend on these factors despite of the application of mirror reflectors *(16)*. The surface-related differences could be compensated by coupling water wash and UVC treatment *(56)*. A 5-minute wash alone reduced viral titers by 1.73 logs but resulted in high viral counts in washing water. The total reduction of >4.36 logs and the absence of virus in the water after water-assisted UVC treatment suggests that virus particles removed by washing are effectively killed by UVC light. Additionally, in the presence of blueberry juice (2 % v/v), but not crushed berries (5 % of sample mass), inactivation outcomes were ~2 log lower compared to the clear water assisted UVC treatment. Differences likely derive from

over 3 times higher water turbidity and over 20 times higher chemical oxygen demand in the water containing blueberry juice. Moreover, the presence of water was vital in pulsed light treatments due to the heating effect of fluence levels up to 63200 mJ/cm^2 deteriorating the visual appearance of fresh blueberries *(36)*. Compared to treatments without water, post-treatment surface temperatures were reduced in raspberries from 59.9 to 31.5 °C and in strawberries from 53.5 to 33.3 °C. In the single trial with blue light, the light alone was ineffective against TV but coupling fluence level of 7560 mJ/cm^2 with riboflavin or rose bengal increased viral reduction by approximately 0.5 and 1.0 log PFU, respectively *(43)*. Riboflavin and rose bengal are enhancers of singlet oxygen formation with structural similarities to porphyrins, hence they absorb light energy and generate ROS that induce inactivation. These compounds alone were effective in inactivating TV as riboflavin reduced viral titers by 0.13 log and rose bengal by 0.66 log. This indicates that blue light had the capacity to induce ROS formation, allegedly the key mechanism of inactivation, from appropriate molecules.

Vegetables. Application of UVC fluences between 40 and 240 mJ/cm^2 reduced the viral counts of FCV in lettuce and green onions by 3.48–4.62 log $TCID_{50}$/ml in the former and 2.44–3.92 log in the latter *(26)*. MNV-1 on the other hand was reduced by 0.2 log PFU/plant in internal and 1.2 log in external parts of green onions following an identical 240 mJ/cm^2 UVC treatment *(31)*. Likewise, Li et al. *(53)* had modest results with MNV-1 inoculated lettuce as reductions of ~0.6–0.8 log PFU were shown following a 5-minute UVC treatment and the combination of UVC and vaporized 2.52 % H_2O_2 did not improve the outcome. However, the authors did not state the total light dosage complicating direct comparisons to other studies. Additionally, despite the similar surface structure to lettuce, green onions contain mucus-like compounds exposed in cutting that can affect virus

attachment or recovery possibly explaining observed differences between matrixes. Moreover, differences between internal and external viral counts in green onions were only logical considering the low penetrability of UVC.

Seafood. Pilotto et al. *(75)* studied whether UVC as an adjunctive measure could assist in Pacific oyster depuration. The MNV-1 bioaccumulated oysters were placed to water tanks and the standard depuration was compared to UVC assisted (fluence rate 44 mW s/cm^2) where light was constantly applied on circulating water. After 48 hours, the reduction rate was approximately 1 log PFU/g of digestive tissue reaching 1.2 log by the end of the 120-hour period irrespective of the method. Hence, depuration with or without UVC light was not sufficient to ensure product safety in contaminated oysters as virus attachment to oyster digestive tissue averted pathogen release back to the water.

Meat products. Research is also limited for other food commodities of animal-origin as only a single study by Park and others *(70)* has applied UVC on MNV-1 contaminated fresh, raw chicken breast. Fluences between 60 and 3,600 mJ/cm^2 showed declines of 0.14–1.23 log PFU/ml from initial titer of 4.34 log. However, negative impacts on sensorial properties were confirmed after 1,800 mJ/cm^2 deeming products unacceptable for consuming. Hence, fluences up to 1,200 mJ/cm^2 (log reduction of 0.58) were defined as optimal for preserving consumer acceptability together with viral reduction. Nevertheless, this was far from optimal in regards of food safety and requires additional decontamination steps.

Among the studies, UVC was the common choice while pulsed or blue light treatments remained in minor roles. Only two separate studies utilized FCV or TV and none of the studies evaluated HuNoVs leaving MNV as the preferred surrogate. In general, the outcomes of light treatments were mainly below 2 logs except for blueberries and strongly

590 associated with the surface structure *(16, 31, 37, 56).* Irregular surfaces provided better
591 attachment sites and shelter for viruses, shadowing effect and limited to non-existing
592 penetrability in solid products that is also rapidly lost in liquids with increasing turbidity.
593 Inactivation can be augmented by agitation, free floating in water and applying multiple lights
594 instead of single source or reflective surfaces *(26, 56).* Such approaches are also called for
595 since a saturation or plateau of effectivity limits the potential of increasing fluence levels *(16,*
596 *26, 37).* Also, UVC light can potentially induce fat oxidation or mutagenicity but in these
597 treatment ranges, it was observed to be safe *(16, 70).* Much of the above mentioned is based
598 on UVC or pulsed light as only a single study utilized blue light and the results were far from
599 encouraging *(43).* As such, the potential of blue or pulsed light technologies in controlling
600 NoV contaminations in food products remains inconclusive.
601
602 **GAMMA AND ELECTRON BEAM IRRADIATION IN FRESH PRODUCTS**
603 **Berries.** MNV-1 inoculated strawberries required 8 kGy of e-beam radiation for
604 a decline of 1.56 log PFU/g and further raising the dose to 12 kGy yielded a decline of 2.21
605 log from the initial level of 5.37 log as expressed in Table 5 *(85).* Instead, gamma radiation
606 had better outcome on MNV-1 levels in strawberries as 2.8 kGy showed a decline of 1.31 log
607 PFU/ml and gradually raising doses to 11.2 and 16.8 kGy produced reductions of around 4
608 and 5 logs, respectively, from the initial levels of 7 logs *(24).* Correspondingly, Pimenta and
609 others *(76)* estimated that a gamma ray dose of 3.7 kGy reduced viral counts by 2.2 logs in
610 strawberries and a similar reduction in raspberries required a dose of 3.4 kGy while 7 kGy
611 produced roughly a 3-log decline in both berry types. The D_{10} values were estimated to be 3.0
612 kGy in strawberries and 3.2 kGy in raspberries.
613 Outcomes with another surrogate in strawberries, TV, were comparable to
614 MNV-1 as 4.1 kGy delivered by e-beam yielded a decrease of 1.4 log PFU/ml, 8.2 kGy a

615 decrease of 2.6 logs and after 16.3 kGy, no virus was detected *(80)*. DiCaprio and colleagues
616 *(21)* assessed the susceptibility of TV and HuNoV GII.4 in strawberries after e-beam radiation
617 with the PGM-MB assay (Table 3). GII.4 required 12.2 kGy for a decline of 1 log genome
618 copies/g and at 16.3 kGy, the decline was 2.46 log while for TV the decrease was around 2
619 log at same doses indicating comparable resilience to irradiation. After 28.7 kGy, the levels
620 were below detection limit for both viruses. Overall, the doses for efficient inactivation are
621 over the current legal limits and doses over 10 kGy cause notable deteriorations in the
622 sensorial quality making the use of higher doses impractical for such products *(80, 85)*.
623
624 **Vegetables.** In the study of Zhou et al. *(108)* 3 kGy of gamma radiation reduced
625 FCV titers by 1 log/g in lettuce whereas the highest dose of 5 kGy showed a decline of
626 approximately 2 logs. Based on the results, the estimated D_{10}-value was approximately 2.95
627 kGy. In other leafy greens, romaine lettuce and spinach, 2.8 kGy of gamma rays declined
628 titers in spinach by 1.77 logs and 1.40 logs in romaine lettuce, by 5.6 kGy reductions were
629 around 2 logs and at 11.2 kGy around 4 logs for both greens *(24)*. Spinach seemed to protect
630 MNV-1 from gamma rays as after a dosage of 22.4 kGy, elimination was complete in romaine
631 lettuce, but 2.4 logs PFU/ml were still detectable in spinach. Better survival on spinach than
632 on lettuce could be attributed to differences in leaf surface texture or binding to carbohydrate
633 moieties on leaf surface *(27, 32)*. However, in shredded cabbage, e-beam had inferior effect
634 on MNV-1 since 4, 6 and 12 kGy showed reductions of 0.5, over 1.0 and approximately 2.8
635 log PFU/g, respectively *(85)*. A more processed form of cabbage, commercial *kimchi*, was
636 evaluated by Park et al. *(69)* who subjected MNV-1 inoculated samples to gamma irradiation
637 and the results reflected the ones from fresh cabbage. Doses of 5, 7 and 10 kGy were followed
638 by declines of 0.98, 1.45 and of 1.76 log PFU/ml, respectively, while the D_{10} value was 5.75
639 kGy based on the linear regression analysis. Identical doses of gamma rays administered on

MNV-1-contaminated green algae fulvescens and brown algae fusiforme, sea vegetables widely consumed in Eastern Asia, proved to be more effective as 5, 7 and 10 kGy showed a 1.41, 1.94 and 2.46 log PFU/ml reductions in fulvescens and a 1.26, 2.60 and 2.21 log reductions in fusiforme, respectively *(71)*.This was further noticeable in the Weibull model calculated D_{10} values being 2.89 kGy and 3.93 kGy for fulvescens and fusiforme, respectively, both lower than in cabbage *kimchi*. Further, the authors also determined that inactivation levels of 3 log would be reached by 13.83 kGy in green and by 14.93 kGy in brown algae. Also, *kimchi* and algae retained their sensorial qualities under average levels of irradiation of 10 kGy while no changes were observed in the quality of cabbage under doses of 4 kGy *(69, 71, 85)*.

Seafood. Praveen and others *(79)* subjected MNV-1 -contaminated whole oysters and oyster meat homogenates to varying levels of e-beam treatment and estimated the D_{10} values of 4.05 and 4.97 kGy for whole oysters and homogenates, respectively, by linear regression analysis. The required radiation dose for complete inactivation in homogenates from the initial level of 4.898 log PFU/ml was 31.9 kGy, a value almost six times higher than the approved radiation limit of 5.5 kGy in the US for shellfish. In another model of contaminated seafood, abalones were inoculated by MNV-1 and e-beam radiated by Kim et al. *(42)* who used first order model to determine D_{10} values of 6.26 and 5.23 kGy for sliced meat and minced viscera, respectively. At maximum level of 10 kGy, reductions were 1.45 log PFU/ml for meat and 1.56 log for viscera. Likewise, in MNV-1 contaminated *Gwamegi*, a half-dried Pacific herring or saury, and semi-dried squid, the 10 kGy reduced titers by 1.66 log PFU/ml and 1.81 log, respectively *(41)*. A dose of 7 kGy was required in both food samples for a decline of over 1 log. These results propose that the currently applied radiation levels in seafood do not guarantee food safety and increasing the upper limit to 10 kGy would

665 not show marked improvements to the situation. However, up to 10 kGy of radiation did not
666 undermine the product qualities irrespective of the higher risk of lipid peroxidation within the
667 product category *(41, 42)*.

668 Apart from single studies with HuNoV GII.4 and FCV, e-beam and gamma
669 irradiation were mainly applied to surrogates MNV-1 or TV. If doses fell within current
670 limits, inactivation levels were generally 1–2 logs regardless of the virus model or radiation
671 technology. Although debatable, it is proposed that among other reasons, the production of
672 oxidative molecules is reduced by other scavenger components present in food limiting
673 efficacy. Hence, to reach satisfactory results i.e. over 3-log reduction with HuNoVs, it is
674 likely that the applied doses should be well over 10 kGy, although berries could be an
675 exception *(76, 80)*. Unfortunately, even if regulations permit usage of higher dosages, it
676 would be at the expense of organoleptic qualities like texture and flavor *(41, 80, 85)*. Thus,
677 product properties, safety aspects and industrial scale set-up are the factors more likely to
678 determine the choice of irradiation source. For instance, the complexity and structure of the
679 matrix are more important determinants for e-beam effectivity due to the poor penetrability as
680 opposed to gamma radiation. Even delivery of e-beams to irregularly shaped products is
681 challenging as adjacent areas of the same product could receive exposure levels of both
682 extremes *(80)*. E-beam treatment is more rapid but on the other hand, longer exposure to
683 radiation could potentially improve the inactivation result due to extended duration of water
684 radiolysis and ROS generation but this was shown at very high exposure levels *(21)*. The
685 cleavage of water molecules to ROS by ionizing radiation induces oxidative stress that can
686 further augment viral inactivation. Subsequently, the effect of product water activity on
687 outcome could also be a factor that should be investigated in the future *(41)*.

688

689 **COLD ATMOSPHERIC PLASMA IN FRESH PRODUCTS**

690 **Berries.** As shown in Table 3Table 6, only 4 studies have assessed the potential
691 of CAP in food products contaminated with NoV or surrogates. In the study of Lacombe and
692 colleagues *(49)* TV and MNV-1 -inoculated blueberries were subjected to CAP jet for 0–120
693 s. Product temperatures rose to 70 ℃ when treatment durations were over 60 s. To mitigate
694 the thermal effect, an ambient-temperature air stream was channeled on the berries during
695 treatment limiting the rise in berry temperatures to roughly 47 ℃ by the end of the 120 s
696 treatment. MNV-1 was significantly more susceptible to CAP jet as the log reduction after 15
697 s handling was 0.5 log PFU/g and at 90 s the reduction reached 5 logs and the limit of
698 detection. TV on the contrary achieved significant decreases at 45, 90 and 120 s being
699 approximately 1.5, 2.5 and 3.5 log PFU/g, respectively. Such differences were discussed to
700 result from intrinsic factors between MNV-1 and TV, such as the latter's HBGA binding
701 properties. Although the effects on sensorial qualities were not evaluated in this study,
702 another study from the authors with comparable setup showed that blueberry texture softened
703 significantly after 60 s, anthocyanins degraded significantly after 90 s and negative darkening
704 of color was apparent after 120 s treatments *(48)*.
705
706 **Vegetables.** 2 individual studies have used lettuce as food matrix and generated
707 CAP with the DBD technology *(1, 63)*. In the first one by Min et al. *(63)*, TV-inoculated
708 lettuce was either treated in closed Petri dish or in modified atmosphere packaging with 5 or
709 10 % O_2 (balance N_2) for 5 minutes. In the Petri dish, outcomes indicated total inactivation of
710 1.3±0.2 log PFU/g while in the 5 % and 10 % O_2 modified atmosphere packaging reductions
711 were 0.7±0.3 and 0.2±0.2 logs, respectively. Variations in effectivity may be connected to the
712 concentration of available oxygen as the low levels of oxygen in modified atmosphere
713 packaging can limit the generation of ROSs contributing to inactivation outcomes.
714 Additionally, treatment did not induce any significant changes in the colors or appearance of

715 the lettuce. Sample temperatures remained under 29 ℃ throughout the treatment and no
716 significant weight loss was observed. In the second study lettuce was inoculated with HuNoV
717 GII.4 or FCV and subjected to CAP treatment produced by two-dimensional array of
718 integrated coaxial-microhollow DBD *(1)*. HuNoV GII.4 inactivation efficacy on lettuce was
719 determined by EMA-RT-qPCR method while FCV by cell culturing method. After 1-, 3- and
720 5-min treatments reductions were approximately 0.5, 1.8 and 2.6 log genome copy
721 number/sample for GII.4. After 1-, 2- and 3-min FCV was reduced by approximately 2.7, 3.9
722 and >5 log $TCID_{50}$/sample. No observable changes were detected in lettuce after handling.
723 Outcomes did not differ from inactivation on stainless-steel surface which is surprising since
724 pathogen elimination is usually impaired in the presence of organic material. Nevertheless,
725 observed discrepancies should be compared cautiously as methodological approaches differ
726 and the EMA-RT-qPCR method was shown underestimate FCV inactivation by about 2 logs
727 compared to the cell culture assay.
728
729 **Meat products.** The final research applying CAP jet on MNV-1 -contaminated
730 food products used fresh beef loin, pork shoulder and chicken breast as model matrix *(11)*.
731 Inactivation from the initial inoculum of 5 log PFU/ml after 5 min treatments for beef, pork
732 and chicken were 2.05, 2.11 and 2.01 log, respectively. Extending duration to 20 min did not
733 produce additional benefit as outcomes were 2.09 log in beef, 2.15 log in pork and 2.07 log in
734 chicken. No statistical differences were detected between meats or the 5- and 20-minute
735 treatments. From sensorial characteristics, changes in the surface colors and moisture content
736 in the under 5-minute, but not over, treatments remained insignificant. Lipid peroxidation
737 increased gradually with treatment duration but remained under the threshold of critical
738 change. Hence, a 5-minute treatment with CAP jet in meats preserved the quality and
739 produced significant inactivation, but 3 log viral titers remained in the products.

Overall, the number of publications of HuNoV or surrogate decontamination by CAP technologies on food products is sparse for the time being. Hence, the effect of matrix composition and form on the virus inactivation remains inconclusive. Also, the appropriate virus model, technology and application are yet to be determined. Out of the several options, CAP was generated either by jet or DBD technology and treatment durations ranged from 0.5 to 20 minute. Interestingly, a time-dependent increase in inactivation was evident in all studies when handling duration was <5 min, but no additional benefit was exerted by over 5-minute treatment in meats *(11)*. Significant reductions of surrogates or HuNoV were produced in all applied matrixes with minimal impact to sensory characteristics. However, plasma jet did increase the temperature of blueberry samples to around 70 °C without parallel cooling, but no heating effect was reported with plasma jet treatment in meats or DBD CAP in lettuce *(1, 11, 49, 63)*. Nevertheless, differences between technological characteristics, such as gas type, power and distance complicate direct comparisons.

CONCLUSIVE REMARKS AND FUTURE PERSPECTIVES

A recent review by Hall et al. *(28)* concluded that annually in the US alone, NoV results into 19–21 million illnesses, 1.7–1.9 million outpatient visits, 400,000 emergency visits, 56,000–71,000 hospitalizations and 570–800 deaths while on a global scale, it is estimated to cause on average 684 million diseases leading to a total of 212,000 deaths yearly *(57)*. As an important route of transmission, foodborne infections warrant for the development of new approaches fitted for delicate fresh products overrepresented in NoV outbreaks. Novel technologies are also called for due to the resistance of NoVs against traditional food safety measures. HPP, ionizing radiation and light-based applications were chosen as the approaches of interest due to their well-established background, industrial implementations and minor effects on nutritional value and perceived freshness (

Processing technology	Characteristics	Mechanism of inactivation	Advantages	Disadvantages
High-pressure processing (HPP)	Hydrostatic pressure transmitted by fluid in pressure vessel Pressures range from 200 to 800 MPa Treatment times are generally below 10 minutes including come up, hold and depressurization times Sub-ambient temperatures enhance efficacy	Disintegrates capsid attachment and binding sites Disrupts capsid structure at higher levels	Pressure is uniformly distributed Preserves nutritional properties Independent of product geometry and size Extends shelf-life	Expensive Requires pre-packing Batch process Large equipment Optimal conditions are product specific
Pulsed, blue and UVC light	Utilizes various wavelengths of light; UVC 240–260 nm, blue 405 nm and pulsed 200–1100 Effective against surface contaminations Appropriate doses are achieved in under 5 minutes or in few seconds with pulsed light	Photodimerization of RNA molecules and disruption of capsid structure (UVC wavelength) Creation of oxidative radicals	Preserves nutritional properties Low cost Easy and safe handling Possibility for continuous process Does not require contact	May impair sensorial properties at high doses Limited penetration depth that is also lost in turbid liquids Effectivity largely attributed to surface structure
Gamma and electron beam (e-beam) irradiation	Electromagnetic radiation with adequate energy for ionizing ^{60}Co or ^{137}Cs are the radioactive sources of gamma rays in food processing E-beam is constituted of electrically accelerated electrons Commonly applied doses in fresh foods are 1–10 kGy, yet doses up to 50 kGy are allowed for sterilization	Disrupts RNA and capsid structure Creation of oxidative radicals	Preserves nutritional properties Extends shelf-life Possibility for continuous process Does not require contact Gamma radiation is highly penetrating E-beams are created rapidly, directed to targets and can be powered off when not used	Expensive Negative consumer perception May impair sensorial properties at high doses E-beams have limited penetration depth Safety and security risks especially with gamma radiation
Cold atmospheric plasma (CAP)	Inert gas or gas mixture ionized by strong electromagnetic fields Plasma is created in atmospheric pressure and generally remains close to ambient temperature Treatment times generally below 20 minutes	Reactive oxygen and nitrogen species generated by the ionization of gas Capsid protein oxidation and disintegration RNA degradation	Preserves sensorial properties Extends shelf-life Can be applied directly to packed products with modified atmosphere Utilizing air as feed gas is economical	Some setups may cause product heating Large number of technologies with varying properties complicate comparisons Expensive with pure gases

	Can be applied directly, remotely or via activated water on the product	Industrial scale food processing equipment still under development

765). A total of 47 original research articles published after 2008 were retrieved
766 through database searches and article reference of which approximately half were based on
767 HPP and the rest on light and irradiation, Owing to the technical limitations of HuNoV
768 cultivation, only a fifth of the studies utilized HuNoV strains while majority applied
769 surrogates MNV, FCV or TV. Hence, surrogates were also incorporated into the review
770 compensating the limited number of HuNoV inactivation studies in actual food matrixes.
771 Common traits determining the effectiveness among processing methods were
772 observed to be the food matrix and surface topography as both shield viral particles from
773 inactivation. In the case of the former, more research is needed to establish the role of
774 different matrix components and their interactions in regards of food safety. Although the
775 surrogates share some commonalities with HuNoV, none has proven to be a convincing
776 substitute. Thus, when assessing the parameters for effective treatments, the primary choice of
777 model viruses should be HuNoV strains. More precisely the circulating variants from
778 genogroups GI and GII that have been predominantly identified in the outbreaks globally
779 *(99)*. The pre-treatments, such as binding to PGM-MBs or nucleic acid intercalators, for
780 distinguishing infectious viral particles from non-infectious are welcomed practices for
781 evaluating HuNoVs, but they serve as a proxy for infectivity at best as observed with the lack
782 of infectivity in plaque assays concurrent with sustained RT-PCR readings *(1, 20, 21)*.
783 Nevertheless, it should be noted that the results of binding assays are in line with the only
784 human and animal studies conducted with actual food matrixes *(51, 60)*. As a result, in the
785 absence of clinical human trials due to their ethical, practical and financial challenges, the
786 development of validated HuNoV culture propagation methodology and application of animal
787 models, such as the gnotobiotic piglets, is crucial for studying the true infectivity and the
788 potential of nonthermal treatments against NoV contaminations in food commodities.

Out of the approaches, HPP proved to be most promising showing to reduce viral titers despite their location due to the uniformity of pressure *(31)*. A desirable outcome with HPP is likely to require a pressure of ≥ 500 MPa, wet state in packaging and a temperature close to 0 °C. The combination is expected to yield a sufficient reduction in viral titres averaging between 2 to 4 logs, a range observed in naturally contaminated berry purees, whole blueberries and oysters *(12, 39)*. However, there are no universal treatment parameters that could be applied to all food products since the outcomes depend on product properties and applied treatment. It is impossible to draw any conclusions about the potential of irradiation, light or CAP technologies due to the limited number of publications and utilization of HuNoVs in the research. Also, the inoculation levels were consistently unnaturally high which could over- or underestimate the treatment outcomes. In the future, the application of biologically relevant inoculums and the harmonization of reporting, such as detection limits, D_{10} and exact log values is encouraged. In fact, rather than relying on single technology, the potential of hurdle technologies with the reviewed methods should be assessed carefully due to the possible synergistic effects. Lastly, the amount of product in individual samples remained in experimental scale in majority of the publications so the effects remain to be validated on pilot and industrial scales. Especially for light applications where the proximity of products and uneven distribution of light is likely to cause mitigation of inactivation. Altogether, industrial applications require vigorous testing and the safety should be verified carefully with appropriate methods for each product before large-scale implementations.

ACKNOWLEDGMENTS

This work was funded by EU, European Regional Development Fund, Regional Council of Pohjois-Savo, Improving shelf-life and ensuring quality in food using new technologies –development project.

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1124 Table 1. Reviewed processing technologies, their characteristics, mechanism of inactivation, advantages and disadvantages.

Processing technology	Characteristics	Mechanism of inactivation	Advantages	Disadvantages
High-pressure processing (HPP)	Hydrostatic pressure transmitted by fluid in pressure vessel Pressures range from 200 to 800 MPa Treatment times are generally below 10 minutes including come up, hold and depressurization times Sub-ambient temperatures enhance efficacy	Disintegrates capsid attachment and binding sites Disrupts capsid structure at higher levels	Pressure is uniformly distributed Preserves nutritional properties Independent of product geometry and size Extends shelf-life	Expensive Requires pre-packing Batch process Large equipment Optimal conditions are product specific
Pulsed, blue and UVC light	Utilizes various wavelengths of light; UVC 240–260 nm, blue 405 nm and pulsed 200–1100 Effective against surface contaminations Appropriate doses are achieved in under 5 minutes or in few seconds with pulsed light	Photodimerization of RNA molecules and disruption of capsid structure (UVC wavelength) Creation of oxidative radicals	Preserves nutritional properties Low cost Easy and safe handling Possibility for continuous process Does not require contact	May impair sensorial properties at high doses Limited penetration depth that is also lost in turbid liquids Effectivity largely attributed to surface structure
Gamma and electron beam (e-beam) irradiation	Electromagnetic radiation with adequate energy for ionizing ^{60}Co or ^{137}Cs are the radioactive sources of gamma rays in food processing E-beam is constituted of electrically accelerated electrons Commonly applied doses in fresh foods are 1–10 kGy, yet doses up to 50 kGy are allowed for sterilization	Disrupts RNA and capsid structure Creation of oxidative radicals	Preserves nutritional properties Extends shelf-life Possibility for continuous process Does not require contact Gamma radiation is highly penetrating E-beams are created rapidly, directed to targets and can be powered off when not used	Expensive Negative consumer perception May impair sensorial properties at high doses E-beams have limited penetration depth Safety and security risks especially with gamma radiation
Cold atmospheric plasma (CAP)	Inert gas or gas mixture ionized by strong electromagnetic fields	Reactive oxygen and nitrogen species generated by the ionization of gas	Preserves sensorial properties Extends shelf-life	Some setups may cause product heating

	Plasma is created in atmospheric pressure and generally remains close to ambient temperature Treatment times generally below 20 minutes Can be applied directly, remotely or via activated water on the product	Capsid protein oxidation and disintegration RNA degradation	Can be applied directly to packed products with modified atmosphere Utilizing air as feed gas is economical	Large number of technologies with varying properties complicate comparisons Expensive with pure gases Industrial scale food processing equipment still under development

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1127 Table 2. Summary of applied model viruses, food matrixes, treatment parameters and inactivation outcomes in studies evaluating the effect of

1128 HPP treatment on NoV surrogates. [a]

Model virus	Food matrix	Treatment	Log reduction	Unit	Reference
MNV-1	Strawberries, diced (wet state)	300 MPa, 2 min, 0 ℃	2.9	PFU/g	*(34)*
	Strawberries, diced (dry state)	300 MPa, 2 min, 0 ℃	<1		
	Strawberries, pureed	350 MPa, 2 min, 0 ℃	4.4		
MNV-1	Strawberry puree	200 MPa, < 10 min, 16 ℃	<1.0	$TCID_{50}$/ml	*(47)*
		200 MPa, 10 min, 16 ℃	3.21		
		300 MPa, 2.5 min, 19 ℃	1.21		
		300 MPa, 5 min, 19 ℃	2.63		
		300 MPa, 10 min, 19 ℃	2.75		
		400/600 MPa, 2.5 min, 22 ℃	3.33		
MNV-1	Strawberry, juice	300 MPa, 1.5 min, 10 ℃	1.68	PFU/sample	*(68)*
MS2	Strawberry, juice	600 MPa, 3 min, 38 ℃	<1		
MNV-1	Strawberry, puree	400 MPa, 3 min, 20 ℃	4.33		
MS2	Strawberry, puree	600 MPa, 3 min, 38 ℃	<1		
MNV-1	Pomegranate, juice	300 MPa, 1.5 min, 10 ℃	1.69		
MNV-1	Blueberries (wet state)	400 MPa, 2 min, 4 ℃	>5.6	PFU/sample	*(55)*
TV	Blueberries (wet state)	300 MPa, 2 min, 4 ℃	>3.8		
MNV-1	Blueberries (dry state)	400/600 MPa, 2 min, 4/21/35 ℃	<1		
TV	Blueberries (dry state)	400/600 MPa, 2 min, 4/21/35 ℃	<1		
MNV-1	Oysters (*Crassostrea virginica*)	400 MPa, 2 min, 4 ℃	>4.0		
TV	Oysters (*Crassostrea virginica*)	350 MPa, 2 min, 4 ℃	>4.5		
MNV-1	Strawberries, diced	450 MPa, 2 min, 4 ℃	6.0	PFU/g	*(59)*
	Strawberries, puree	450 MPa, 2 min, 4 ℃	4.7		
	Lemon, puree	350 MPa, 2 min, 4 ℃	5.4		
	Watermelon, puree	400 MPa, 2 min, 4 ℃	6.8		
	Tomato puree	400 MPa, 2 min, 4 ℃	5.8		
	Lettuce	450 MPa, 2 min, 4 ℃	7.1		
	Carrot, puree	400 MPa, 2 min, 4 ℃	6.8		
	Carrot, juice	400 MPa, 2 min, 4 ℃	7.3		
MNV-1	Cabbage *Kimchi*	300 MPa, 5 min, 25 ℃	0.3	PFU/ml	*(72)*
		400 MPa, 5 min, 25 ℃	1.5		
		500 MPa, 5 min, 25 ℃	>4.7		
MNV-1	Green onions (*Allium fistulosum x cepa*)	500 MPa, 5 min, 20 ℃	4.7[b]	PFU/plant	*(31)*
			6.4[c]		

MNV-1	Green onions (wet state)	300 MPa, 2 min, 10 ℃	1.2	PFU/g	*(89)*
	Green onions (wet state)	300 MPa, 2 min, 1 ℃	2.8		
	Green onions (wet state)	350 MPa, 2 min, 4 ℃	4.3		
	Green onion (dry state)	350 MPa, 2 min, 4/20 ℃	1.7		
	Salsa	300 MPa, 1 min, 1 ℃	~2.0		
	Salsa	300 MPa, 2.5 min, 1 ℃	~4.0		
FCV	Salsa	250 MPa, 1 min, 9℃	6.48±1.74	$TCID_{50}$/g	*(29)*
MNV-1		400 MPa, 5 min, 9 ℃	~4	PFU/g	
MNV-1	Manila clams (*Ruditapes philippinarum*)	500 MPa, 1 min, 20 ℃	ND from initial value of 4.56	PFU/g	*(7)*
FCV	Tuna	200 MPa, 5 min, 5 ℃	4.54±0.001	$TCID_{50}$/g	*(30)*
FCV	Cod	200 MPa, 5 min, 5 ℃	1.15±0.38		
FCV	Shrimp	200 MPa, 5 min, 5 ℃	4.46±0.001		
FCV	Clam	200 MPa, 5 min, 5 ℃	3.15±1.15		
FCV	Tuna+mayonnaise	200 MPa, 5 min, 5 ℃	3.84±2.00		
FCV	Cod+mayonnaise	200 MPa, 5 min, 5 ℃	2.22±0.23		
FCV	Shrimp+mayonnaise	200 MPa, 5 min, 5 ℃	4.71±0.001		
FCV	Clam+mayonnaise	200 MPa, 5 min, 5 ℃	5.08±0.001		
FCV	Mayonnaise	200 MPa, 5 min, 5 ℃	1.09±0.60		
MNV-1	Mayonnaise	200 MPa, 5 min, 5 ℃	1.39±0.60		
MNV-1	Oysters (*Crassostrea gigas*)	400 MPa, 5 min, 0 ℃	ND from initial value of 4.13	PFU/oyster	*(52)*
MNV-1	Oysters (*Crassostrea gigas*)	275 MPa, 5 min, 0 ℃	2.0±1.6	PFU/sample	*(91)*
		300 MPa, 5 min, 0 ℃	>3.0±1.2		
		350 MPa, 5 min, 0 ℃	>3.0±0.0		
MNV-1	Sea squirt (*Halocynthia roretzi*)	400 MPa, 5 min, 25 ℃	ND from initial value of ~5.6	PFU/ml	*(73)*
FCV	Pork sausage	500 MPa, 5 min, 4 ℃	2.89	$TCID_{50}$/ml	*(88)*
MS2	Pork sausage	500 MPa, 5 min, 4 ℃	1.47	PFU/g	

[a]FCV, feline calicivirus; MNV-1, murine norovirus; MPa, megapascal; MS2, bacteriophage MS2; ND, not detected; $TCID_{50}$, median tissue culture infectious dose; TV, Tulane virus.

[b]Contamination by root uptake where inoculums are in internal tissues.

[c]Contamination by spot inoculation uptake where inoculums are in external surface.

1134 Table 3. HuNoV or TV reductions based on RT-PCR assays following high-pressure processing, electron-beam or atmospheric cold plasma

1135 treatments in different food matrixes. [a]

Technology	Model virus	Food matrix	Treatment	Log reduction	Method	Reference
HPP	GII.4	Strawberries, pureed	500 MPa, 2 min, 4 ℃	<1	PGM-MB/RT-qPCR	*(20)*
			600 MPa, 2 min, 4 ℃	ND from initial value of 5.46±0.28		
	TV		500 MPa, 2 min, 4 ℃	1.67		
			600 MPa, 2 min, 4 ℃	ND from initial value of 6.10±0.39		
HPP	GI.1	Strawberries, pureed	550 MPa, 2 min, 0 ℃	>3.0±0.1	RNAse + PGM-MB/RT-qPCR	*(35)*
	GII.4	Strawberries, pureed	400 MPa, 2 min, 0 ℃	>4.2±0.2		
	GI.1	Strawberries, quarter	650 MPa, 2 min, 0 ℃	1.7±0.4		
	GII.4	Strawberries, quarter	650 MPa, 2 min, 0 ℃	3.1±0.3		
	GI.1	Blueberries, pureed	550 MPa, 2 min, 0 ℃	>2.9±0.2		
	GII.4	Blueberries, pureed	550 MPa, 2 min, 0 ℃	>4.4±0.1		
	GI.1	Blueberries, whole	500 MPa, 2 min, 0 ℃	>3.2±0.1		
	GII.4	Blueberries, whole	300 MPa, 2 min, 0 ℃	>4.1±0.2		
	GI.1	Raspberries, pureed	550 MPa, 2 min, 0 ℃	>2.9±0.4		
	GII.4	Raspberries, pureed	350 MPa, 2 min, 0 ℃	>4.2±0.3		
	GI.1	Raspberries, whole	650 MPa, 2 min, 0 ℃	2.5±0.2		
	GII.4	Raspberries, whole	550 MPa, 2 min, 0 ℃	>4.1±0.3		
HPP	GI.1	Blueberries (wet state)	500 MPa, 2 min 1℃	2.7±0.3	RNAse + PGM-MB/RT-qPCR	*(54)*
		Blueberries (wet state)	600 MPa, 2 min, 21 ℃	>3.0±0.0		
		Blueberries (dry state)	600 MPa, 2 min, 1 ℃	0.5±0.2		
		Blueberries (dry state)	600 MPa, 2 min, 21 ℃	0.9±0.2		
HPP	GI.1	Green onions	500 MPa, 2 min, 1 ℃	>3	RNAse + PGM-MB/RT-qPCR	*(89)*
	GII.4	Green onions	500 MPa, 2 min, 1 ℃	>3		
	GI.1	Salsa	600 MPa, 2 min, 1 ℃	>3		
	GII.4	Salsa	300 MPa, 2 min, 1 ℃	>3		
HPP	GII.4 and GII.17	Oysters (*Crassostrea gigas*)	400 MPa, 5 min, 25 ℃	1.87[b]	RNAse + RT-PCR	*(38)*
				1.99[b]		
HPP	GI and GII	Oysters (*Crassostrea gigas*)	400 MPa, 5 min, 10 ℃	>0.6	RNAse + RT-PCR	*(39)*
HPP	GI.1	Oysters (*Crassostrea virginica*)	450 MPa, 2 min, 0 ℃	3.2±0.2	RNAse + PGM-MB/RT-PCR	*(107)*
	GI.1		500 MPa, 2 min, 0 ℃	>4.3		
	GII.4		350 MPa, 2 min, 25 ℃	3.6±0.1		
	GII.4		350 MPa, 2 min, 0 ℃	>4.2		

HPP	GII.4 strain 765	Oyster (*Crassostrea virginica*) homogenates	350 MPa, 2 min, 0 ℃ 400 MPa, 2 min, 0 ℃ 450 MPa, 2 min, 0 ℃ 350 MPa, 2 min, 25 ℃ 400 MPa, 2 min, 25 ℃ 450 MPa, 2 min, 25 ℃ 350 MPa, 2 min, 35 ℃	3.7±0.3 4.0±0.3 4.2±0.2 3.0±0.1 3.8±0.2 4.0±0.1 1.0±0.3	RNAse + PGM-MB/RT-PCR	*(60)*
HPP	GI.1 GI.1 GII.4	Oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*) homogenates	450 MPa, 5 min, 1 ℃ 500 MPa, 5 min, 1/6 ℃ 400 MPa, 5 min, 6 ℃	4.3±0.5 4.0±0.5 3.6±0.4	RNAse + PGM-MB/RT-PCR	*(106)*
Electron beam	GII.4 TV 	Strawberries	12.2 16.3 28.7 12.2 16.3 28.7	~1.0 2.46 ND from initial value of 5.53±0.39 ~2 ~2 ND from initial value of 5.57±0.09	PGM-MB/RT-qPCR	*(21)*
Two-dimensional array of integrated coaxial-microhollow DBD CAP	GII.4	Romaine lettuce	1 min 2 min 3 min 4 min 5 min	~0.4 ~1.4 ~2.0 ~2.2 ~2.6	EMA + RT-qPCR	*(1)*

[a] CAP, cold atmospheric plasma; DBD, dielectric barrier discharge; EMA, ethidium monoazide; HPP, high-pressure processing; MPa, megapascal; ND, not detected; PGM-MB, porcine gastric mucin-conjugated magnetic beads; TV, Tulane virus.

[b] Outcomes from two independent batches of 30 oysters.

1140 Table 4. Overview of utilized model viruses, food matrixes, fluence levels and inactivation outcomes in the studies applying UVC, blue or pulsed

1141 light on NoV surrogates.[a]

Light technology (nm)	Model virus	Food matrix	Fluence (mJ/cm^2)	Log reduction	Unit	Reference
UVC (254)	MNV S99	Blueberries (fresh)	212 – 1331	3.12	$TCID_{50}$/g	*(16)*
		Blueberries (frozen)		2.07		
		Raspberries (fresh)		1.52		
		Raspberries (frozen)		0.62		
		Strawberries (fresh)		1.27		
		Strawberries (frozen)		0.78		
UVC (254)	MNV-1	Blueberries (dry)	600	2.43±0.32	PFU/sample	*(56)*
			1200	2.48±0.56		
			3000	3.04±0.23		
		Blueberries (wet)	600	3.23±0.61		
			1200	>4.32		
			3000	>4.36		
UVC (253.7)	FCV	Strawberries	40	1.13±0.27	$TCID_{50}$/ml	*(26)*
		Strawberries	120	1.57±0.24		
		Strawberries	240	2.28±0.53		
		Green onions	40	2.46±0.38		
		Green onions	120	3.92±0.64		
		Green onions	240	3.88±0.77		
		Lettuce	40	3.48±0.03		
		Lettuce	120	3.82±0.01		
		Lettuce	240	4.62±0.00		
UVC (254)	MNV-1	Lettuce	-	0.6–0.8	PFU	*(53)*
UVC (253.7)	MNV-1	Green onions	240	0.2±0.2[b]	PFU/plant	*(31)*
		(*Allium fistulosum x cepa*)		1.2±0.6[c]		
UVC (254)	MNV-1	Oysters (*Crassostrea gigas*)	44[d]	1.2	PFU/g of digestive tissue	*(75)*
UVC (260)	MNV-1	Chicken breast	60	0.14±0.03	PFU/ml	*(70)*
			300	0.27±0.03		
			600	0.42±0.05		
			1200	0.58±0.01		
			1800	0.69±0.01		
			2400	0.90±0.04		
			3600	1.23±0.07		
Pulsed light (200–1100)	MNV-1	Raspberries	53900	3.6±1.1	PFU/g	*(36)*
		Strawberries	63200	1.8±0.3		

Pulsed light (200–1100)	MNV-1	Blueberries	5900	3.1±0.3	PFU/sample	*(37)*
			11400	3.2±0.3		
			22500	3.8±0.6		
		Strawberries	5900	0.7±0.0		
			11400	0.9±0.2		
			22500	0.9±0.2		
Blue light (405)	TV	Blueberries	7560	0.06	PFU	*(43)*

[a]FCV, feline calicivirus; MNV-1, murine norovirus; $TCID_{50}$, median tissue culture infectious dose; TV, Tulane virus.
[b]Contamination by root uptake where inoculums are in internal tissues.
[c]Contamination by spot inoculation uptake where inoculums are in external surface.
[d]Applied on circulating depuration water for up to 120 h.

1146 Table 5. Summary of model viruses, food matrixes, radiation dosages and inactivation outcomes in the studies employing gamma radiation or

1147 electron beams on NoV surrogates. [a]

Radiation technology	Model virus	Food matrix	Dose (kGy)	Log reduction	Unit	Reference
Gamma rays	MNV-1	Raspberries	3.4	2.2	PFU/g	*(76)*
			7.0	~3.0		
		Strawberries	3.7	2.2		
			7.0	~3.0		
Gamma rays	MNV-1	Strawberries	2.8	1.31	PFU/ml	*(24)*
			5.6	2.4		
			11.2	4.1		
			22.4	~8.0		
		Romaine lettuce	2.8	1.40		
			5.6	1.7		
			11.2	3.6		
			22.4	~8.0		
		Spinach	2.8	1.77		
			5.6	2.1		
			11.2	3.7		
			22.4	5.6		
Gamma rays	MNV-1	Cabbage *kimchi*	1	0.34	PFU/ml	*(69)*
			3	0.71		
			5	0.98		
			7	1.45		
			10	1.76		
Gamma rays	MNV-1	Green algae (*Capsosiphon fulvescens*)	3	1.16	PFU/ml	*(71)*
			5	1.41		
			7	1.94		
			10	2.46		
		Brown algae (*Hizikia fusiforme*)	3	0.37		
			5	1.26		
			7	1.60		
			10	2.21		

Gamma rays	MNV-1	*Gwamegi* (half-dried Pacific herring or saury)	3	0.66	PFU/ml	*(41)*
			5	0.88		
			7	1.31		
			10	1.66		
		Semi-dried squid	3	0.59		
			5	0.88		
			7	1.36		
			10	1.81		
Electron beams	MNV-1	Strawberries, diced	2	0.12	PFU/ml	*(85)*
			4	0.37		
			6	0.94		
			8	1.56		
			10	1.87		
			12	2.21		
		Cabbage, shredded	2	0.40		
			4	0.70		
			6	1.17		
			8	1.65		
			10	2.18		
			12	2.82		
Electron beams	TV	Strawberries	4.1	1.4±0.5	PFU/ml	*(80)*
			8.2	2.6±0.3		
			16.3	ND from initial value of 4.4±0.6		
		Romaine lettuce	3.9	1.3±0.6		
			8.7	ND from initial value of 3.7±0.5		
Electron beams	FCV	Lettuce	1	0.33	$TCID_{50}$/g	*(108)*
Electron beams	MNV-1	Oyster (*Crassostrea virginica*) homogenate	4.90	1.33	PFU/ml	*(79)*
			9.38	2.54		
			23.64	4.54		
			31.90	ND from initial value of 4.90		
Electron beams	MNV-1	Abalone (*Haliotis discus hannai*) meat	1	0.31	PFU/ml	*(42)*
			3	0.64		
			5	0.93		
			7	1.19		
			10	1.45		
		Abalone (*Haliotis discus hannai*) viscera	1	0.41		
			3	0.66		
			5	1.06		
			7	1.30		
			10	1.56		

[a]FCV, feline calicivirus; MNV-1, murine norovirus; ND, not detected; $TCID_{50}$, median tissue culture infectious dose; TV, Tulane virus.

Table 6. Summary of technologies, model viruses, food matrixes, treatment durations and inactivation outcomes in studies applying cold atmospheric plasma treatment on surrogate inoculated food products. [a]

CAP technology	Model virus	Food matrix	Duration (min)	Log reduction	Unit	Reference
Plasma jet	TV	Blueberries	0.75	1.5	PFU/g	*(49)*
			2	3.5		
	MNV-1		0.25	0.5		
			1.5	>5.0		
DBD	TV	Romaine lettuce	5	1.3 ±0.2	PFU/g	*(63)*
				0.7 ±0.3[b]		
				0.2 ±0.2[c]		
Two-dimensional array of integrated coaxial-microhollow DBD	FCV	Romaine lettuce	1	~2.7	$TCID_{50}$/sample	*(1)*
			2	~3.9		
			3	>5.0		
Plasma jet	MNV-1	Beef loin	5	2.05	PFU/ml	*(11)*
			20	2.09		
		Pork shoulder	5	2.11		
			20	2.15		
		Chicken breast	5	2.01		
			20	2.07		

[a]CAP, cold atmospheric plasma; DBD, dielectric barrier discharge; FCV, feline calicivirus; MNV-1, murine norovirus; $TCID_{50}$, median tissue culture infectious dose; TV, Tulane virus.

[b]Lettuce prepacked in a modified atmosphere packaging containing 5 % O_2 (balance N_2).

[c]Lettuce prepacked in a modified atmosphere packaging containing 10 % O_2 (balance N_2).