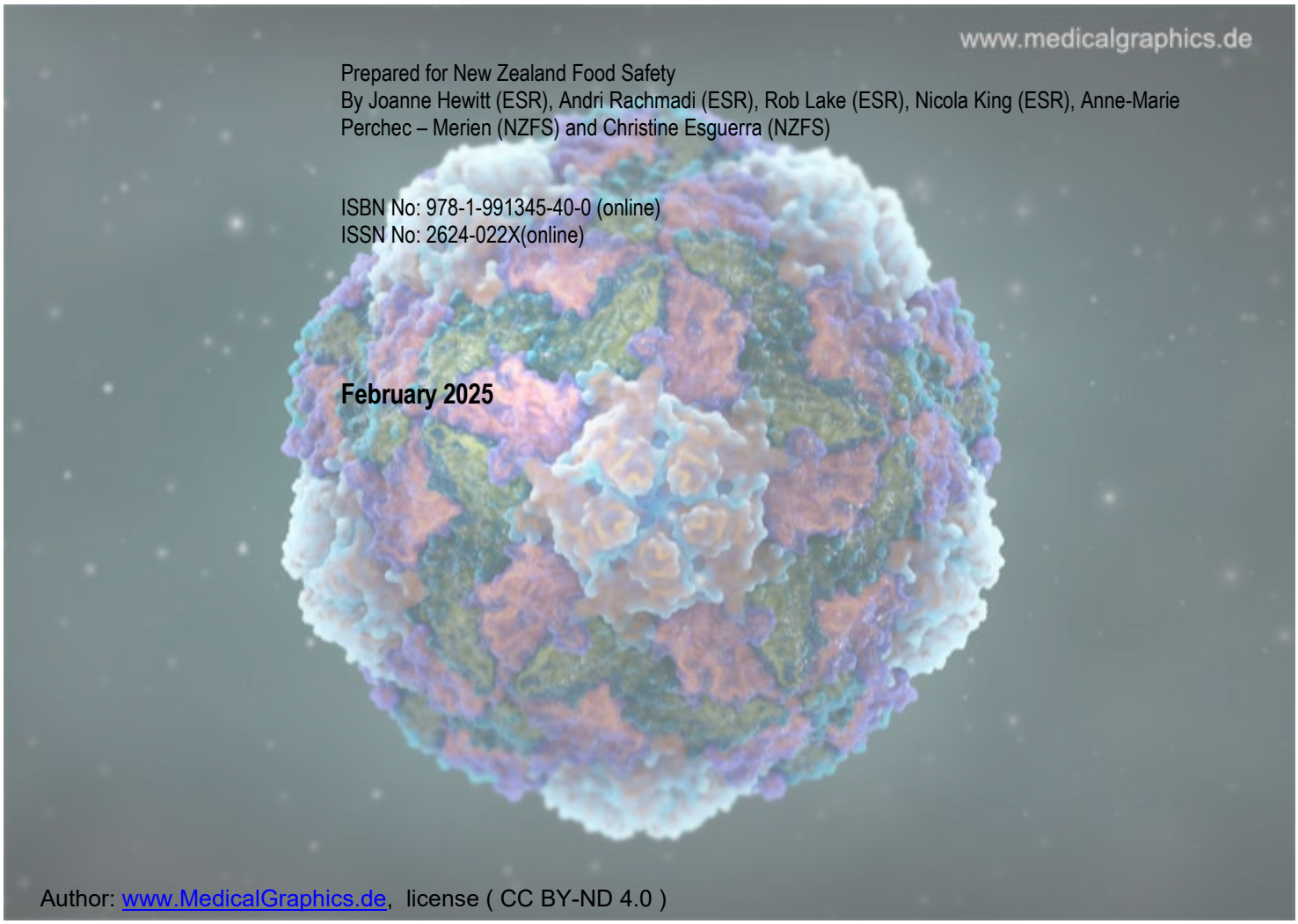
New Zealand Food Safety
Haumaru Kai Aotearoa

Literature review on inactivation of hepatitis A virus in berries

New Zealand Food Safety Technical Paper No: 2025/01



Prepared for New Zealand Food Safety
By Joanne Hewitt (ESR), Andri Rachmadi (ESR), Rob Lake (ESR), Nicola King (ESR), Anne-Marie Perchec – Merien (NZFS) and Christine Esguerra (NZFS)

ISBN No: 978-1-991345-40-0 (online)
ISSN No: 2624-022X(online)

February 2025

Te Kāwanatanga o Aotearoa
New Zealand Government

Ministry for Primary Industries
Manatū Ahu Matua

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry for Primary Industries does not accept any responsibility or liability for error of fact, omission, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526 WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at http://www.mpi.govt.nz/news-and-resources/publications/

Scientific Interpretative Summary

This scientific interpretative summary provides context to the following report prepared by Institute of Environmental Science and Research (ESR) for New Zealand Food Safety (NZFS)
.
Hepatitis A virus (HAV) is an important foodborne pathogen transmitted via the faecal-oral route. In recent years, there have been an increase in norovirus and HAV outbreaks worldwide associated with the consumption of frozen berries, including the occurrence of two HAV outbreaks in New Zealand in 2015 and 2022. This raised concerns within NZFS about the safety of frozen berries imported into New Zealand and the effectiveness of existing import controls. In response to this, a comprehensive review of the import requirements for frozen berries was undertaken 2023, and new requirements were introduced in April 2024.

Currently, Ministry for Primary Industries (MPI) and Food Standards Australia New Zealand (FSANZ) provide guidelines for manufacturers and consumers regarding the heat inactivation of HAV in berries and berry products, based on studies available at the time of their original publication in 2015. The guidelines provide recommended heat treatments for various berry matrices, however, there are some uncertainties about the level of HAV reduction achieved for these matrices.

NZFS risk managers need more information to assist them in their decision-making regarding the adequacy of various inactivation processes that could be applied to berries and berry products imported or further processed in New Zealand. To address this knowledge gap, NZFS commissioned ESR through the New Zealand Food Safety Science and Research Centre (NZFSSRC) to undertake a literature review of the latest research and data on inactivation of HAV in berries, focussing on the effectiveness of thermal and non-thermal methods used to inactivate HAV, norovirus, and surrogate viruses in raw berries and berry products.

The inactivation processes that were reviewed in the current study included technological applications associated with thermal treatments (any processes using heat), High-Pressure Processing (HPP), ultraviolet light, pulsed light, ozone gaseous, irradiation, cold atmospheric plasma, chlorine dioxide gas, steam ultrasound, osmotic dehydration, and air drying. Of these, only thermal and HPP treatments were extensively investigated due to the availability of relevant published studies. While several publications were available for thermal and HPP treatments on berries and berry products, the authors noted that there were not enough studies on the other treatments and, consequently, they were unable to specify the parameters needed to provide sufficient information on the inactivation of HAV in berries with these methods.

For thermal treatments, limited recent data were found in the literature, with only a single study on processes involving freeze-drying. Findings from this study indicate that HAV and norovirus are relatively resistant to freeze-drying and consequently, this method cannot be used as an inactivation strategy. Freeze-drying combined with heating resulted in the inactivation of HAV for freeze-dried berries treated at 100 or 120°C for 20 min, but not for blueberries treated at 100°C. Data from a single study using HPP indicated that effective HAV inactivation in strawberry puree could be achieved with a pressure of at least 375 MPa for 5 minutes at ambient temperature.

Three more recent inactivation models for berry products involving surrogate viruses (mostly non-human pathogenic viruses which are easier to grow than HAV) were identified in the literature. However, the authors of this literature review reported that a high uncertainty remains regarding how the studies using surrogate viruses would effectively reflect the real inactivation of HAV in the same conditions. No new inactivation models using directly HAV were located, consequently, the previous HAV inactivation models are still relevant.

Overall, the information gathered in this report indicates that the current guidance provided by NZFS/MPI and FSANZ regarding HAV inactivation in berries and berry products should still be used by food manufacturers and the general public. This guidance is available at https://www.mpi.govt.nz/dmsdocument/10769/direct and summarized below:

- MPI and FSANZ recommend cooking food to 85°C for 1 minute to inactivate hepatitis A virus but recognise that the extent of virus inactivation is influenced by the food matrix.
- Although MPI and FSANZ provide some D-values and temperature/time equivalents for some berry types as guidelines, manufacturers are advised to conduct a proper risk assessment

taking into consideration their raw materials, characteristics of their own products and the treatment parameters required to inactivate any hazards that are reasonably likely to occur.

Currently, it is not possible to provide additional advice on using alternative methods of heat treatment other than cooking and boiling. For example, not enough robust research has been performed to investigate the effect of microwaving on the inactivation of HAV in berries, and consequently no specific parameters regarding time and microwave power could be included in the guidance.

Unfortunately, uncertainty will remain for new and more unusual inactivation processes for HAV until internationally, new research is undertaken and published. There is currently limited knowledge regarding the processes used by the food industry and food services, noting that a robust method validation should be performed for any specific types of berries and specific food products before using any alternative inactivation process.

New Zealand
FOOD SAFETY SCIENCE
& RESEARCH CENTRE

Literature review on inactivation of hepatitis A virus in berries

NZFSSRC Number 407172
ESR Number FW24006

Authors Joanne Hewitt, Andri Rachmadi, Rob Lake and Nicola King (ESR)

Funding This work was funded by New Zealand Food Safety, Ministry for Primary Industries

Prepared for New Zealand Food Safety (a business unit of the Ministry for Primary Industries) by the Institute of Environmental Science and Research (ESR) Limited

Reviewed by: Cath McLeod (Cawthron), Libby Harrison (NZFSSRC) and Daniel Bohnen (ESR)

Date: 26 July 2024

ESR Science for Communities
He Pūtaiao, He Tāngata

New Zealand Food Safety
Ministry for Primary Industries
Manatū Ahu Matua

New Zealand Food Safety Science & Research Centre
Hopkirk Institute, Massey University, Tennant Drive, Palmerston North 4442
Phone: +64 (0) 6 356 9099

RECOMMENDED CITATION: Hewitt J, Rachmadi A, Lake R, King N (2024) Literature review on inactivation of hepatitis A virus in berries. Prepared for New Zealand Food Safety (Ministry for Primary Industries). NZFSSRC, Palmerston North. Report No. 407172.

New Zealand
FOOD SAFETY SCIENCE
& RESEARCH CENTRE

CONTENTS

New Zealand FOOD SAFETY SCIENCE & RESEARCH CENTRE

LIST OF TABLES

ACKNOWLEDGMENTS

The authors thank Dr Cath McLeod, Daniel Bohnen and Dr Libby Harrison for reviewing this report.

ABBREVIATIONS

ATCC	American type culture collection
CAP	Cold atmospheric plasma
CPE	Cytopathic effect
CRFK	Crandell-Rees Feline Kidney
ESR	Institute of Environmental Science and Research
FCV	Feline calicivirus
FFU	Focus forming unit
FSANZ	Food Standards Australia New Zealand
HAdV	Human adenovirus
HAV	Hepatitis A virus
HBGA	Histo-blood group antigen
HIE	Human intestinal enteroid
HPP	High-pressure processing
kGy	Kilogray
LOQ	Limit of quantitation
MPN	Most probable number
MPa	Megapascal (SI unit, unit of pressure)
MPI	Ministry for Primary Industries
MNV	Murine norovirus
NZ	New Zealand
NZFS	New Zealand Food Safety
O_3	Ozone
ppm	Parts per million
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PFU	Plaque forming units
PBS	Phosphate buffered saline
PGE	Polyethylene glycol
PGM	Porcine gastric mucin
PGM-MB	PGM conjugated magnetic bead
PMA	Propidium monoxide
$PtCl_4$	Platinum chloride
RT-PCR	Reverse transcription-PCR
RT-ddPCR	Reverse transcription-digital droplet PCR
RT-qPCR	Reverse transcription-quantitative PCR
T90	Time to reduce titre by 90%
$TCID_{50}$	Tissue culture infective dose, 50%
TGBE	Tris/glycine/beef extract
UV	Ultraviolet

New Zealand
FOOD SAFETY SCIENCE
& RESEARCH CENTRE

SUMMARY

New Zealand Food Safety (NZFS) requires information on the effectiveness of different methods (thermal and non-thermal) to inactivate hepatitis A virus (HAV) in raw berries and berry products. NZFS will use this information to support the implementation of new import controls for ready-to-eat frozen berries (MPI, 2024) and other risk management activities, such as assessments of treatment methods applied by manufacturers and development of guidance for domestic manufacturers, food service providers or consumers.

This report summarises available data to meet this need. Data were compiled from studies of HAV, norovirus or virus surrogate inactivation in berries and berry products. These data have been compiled from the peer-reviewed scientific literature.

This review includes information on the methodology for conducting experiments on the behaviour of viruses which are important to understand the results and limitations.

The review covers the following treatments:

- Thermal
- High-pressure processing (HPP)
- Ultraviolet light
- Pulsed light
- Ozone gaseous
- Irradiation
- Cold atmospheric plasma
- Chlorine dioxide gas
- Steam ultrasound
- Osmotic dehydration and air drying

The data available on virus inactivation in berries and berry products by different treatments are complex. Results from different studies, even using similar treatments and similar infectivity assays, are rarely similar. While data from inactivation experiments in berry foods using a number of different surrogates have been reported, how well these data reflect behaviour of HAV is uncertain.

We have limited information on how many of these treatments are being used during commercial production of berries and berry products.

Of the treatments considered in this review, only thermal and HPP treatments have been extensively investigated. For the other treatments, only up to four studies of HAV or surrogates in berries or berry products have been identified. We consider that there are insufficient data to define parameters for these other treatments that would provide sufficient inactivation of HAV.

In terms of using the data published on the effect of thermal and HPP treatments, the MPI advice on HAV risk management published in 2015 identified that a “target 6-log reduction would be considered to provide satisfactory assurance for control of the hazard”. The advice provided time and temperature information for berry products with or without added sugar to achieve 6 $\log_{10}$ reductions based on published models. Apart from some data on the effects of heat following a freeze-drying process, no additional data on HAV inactivation from more recent heat treatment

experiments on HAV were found, and so the published models remain the best source for guidance regarding thermal control of HAV in berries and berry products.

Based on the data published for the effect of high-pressure processing (HPP) on HAV in strawberry puree, a pressure of at least 375 MPa for 5 minutes at ambient temperature (4.3 $\log_{10}$ reduction) would be required to approach a 6 $\log_{10}$ reduction. Data from HPP experiments with surrogates appear to be consistent with these parameters, with higher pressures (for a shorter time), lower temperature, and increased pH contributing to greater log reductions.

No new models for HAV inactivation have been identified since those used to support the 2015 FSANZ and MPI advice. These models were for HAV in natural berries (Deboosere *et al.*, 2010) and in berries with added sugar (Deboosere *et al.*, 2004).

More recent models have been identified for:

- Murine norovirus (MNV) and Tulane virus in strawberry and raspberry purees under thermal treatment.
- Human norovirus in strawberry purees under HPP.
- MNV and MS2 bacteriophage in strawberry puree and juice under HPP.

As these models only involve surrogates, we consider that they should not change the advice derived from the earlier models.

1 INTRODUCTION

New Zealand Food Safety (NZFS) requires information on the effectiveness of different methods (thermal and non-thermal) to inactivate hepatitis A virus (HAV) in raw berries and berry products. NZFS will use this information to support the implementation of new import controls for ready-to-eat frozen berries (MPI, 2024) and other risk management activities, such as assessments of treatment methods applied by manufacturers and development of guidance for domestic manufacturers, food service providers or consumers.

This report summarises available data to meet this need. Data were compiled from studies of HAV, norovirus or virus surrogate inactivation in berries and berry products. These data have been compiled from the peer-reviewed scientific literature.

1.1 SCOPE

The scope of this work was agreed through the Request for Service and subsequent discussions with NZFS (**Table 1**).

Table 1. Scope of this review

Topic	Within scope	Specific exclusions
Hazard	- Hepatitis A virus (HAV) - Norovirus, as another pathogenic foodborne virus, member of *Caliciviridae* family - Cultivable, surrogate viruses used for assessing HAV and norovirus behaviour (the single-stranded, non-enveloped RNA viruses in the *Caliciviridae* family such as feline calicivirus, murine norovirus and Tulane virus), and phage	(none identified)
Food (matrix)	- All types of berries (including blackberry, blueberry, boysenberry, cranberry, currants (red, black, white), goji berry, gooseberry, juneberry, logan berry, mulberry, raspberry, rose hip, strawberry) - Raw berries that are fresh, frozen, or dried without a heat treatment sufficient to eliminate pathogens - Berries that are whole, in pieces, crushed, pulped or pureed, with or without added sugars or coatings (e.g., chocolate-coated berries) - Other small fruits, e.g., grapes, kiwiberries, cherries	- Processed food containing berries or berry products, where berries are a minor ingredient (e.g., ice cream, yoghurt and baked goods) - Laboratory media, water or similar (i.e., liquid suspension)
Food/hazard controls	- Thermal inactivation methods, including but not limited to: Boiling, microwaving (including microwave-assisted thermal processing), pasteurisation, heat exchange, ohmic treatment or electro-heating, drying, freeze-drying - Non-thermal inactivation methods, including but not limited to: high-pressure processing, irradiation (ionizing radiation), other forms of radiation (ultra-violet, pulsed light), cold plasma, ozone gas	- Methods that use chlorine or other sanitisers, enzymes or essential oils - Inactivation methods that require the berries to be sprayed or immersed in a liquid, e.g., electrolysed water, washing of any kind

Method to measure inactivation	- Methods that quantify the concentration of infectious viruses - Methods that show presence/absence of infectious viruses - Molecular methods that have been designed to measure the concentrations of infectious vs. non-infectious viruses	- Methods that do not confirm the presence of infectious viruses

1.2 METHOD OVERVIEW AND REPORT STRUCTURE

Relevant scientific journal publications were identified through structured Boolean searches of science citation databases. Additional reports were identified through reference lists, further keyword searches of the citation databases plus website searches of trusted sources. See Appendix A for details. A spreadsheet was used to capture relevant data from scientific reports and then to produce many of the tables in this report.

The first part of this report (Section 2) briefly describes methodological information regarding viruses and experimental details that inform the interpretation of published data. This material is expanded in Appendix B with further detail.

Section 3 describes data from published experiments using different inactivation treatments on viruses in berry foods.

2 EXPERIMENTAL CONTEXT

Studies using HAV, norovirus and a range of surrogates are included in this review, as described in the scope (Section 1.1). The following material provides background information on the viruses used for inactivation studies, as well as aspects of the experimental methods. This information is important for interpreting the results from inactivation studies.

2.1 THE VIRUSES

Generally, laboratory-adapted strains or reference strains are used for virus inactivation studies, although some viruses are obtained from naturally contaminated matrices (e.g., norovirus can be isolated from human faecal specimens). Sometimes, naturally contaminated products can be used for inactivation studies if viral concentrations are adequately high for the required detection assays. However, viral concentrations on foods are generally too low to enable inactivation to be measured, particularly where $\log_{10}$ reductions are to be determined.

HAV, human norovirus and Aichi virus are non-enveloped viruses containing single-stranded RNA and are known human pathogens.[1] Due to difficulties in culturing human norovirus and wild-type HAV (and to an extent even the laboratory-adapted strains), viral surrogates have been used for research, particularly for evaluating treatment and control measures. As well as being culturable, surrogate viruses should have properties in common with the pathogenic virus that it is representing. Ideally, a surrogate virus would have similar physical and/or chemical properties, including the same type of nucleic acid (e.g., single stranded RNA), to the pathogen. With similar properties, the assumption is that their susceptibility to stressors such as heat, pH and chemicals would also be similar. However, that is not always the case, with even closely related viruses having different properties and susceptibilities to stressors (Richards, 2012). For example, murine norovirus (MNV) and feline calicivirus (FCV) are genetically and structurally similar but have different susceptibilities to alcohol, pH and UV (Richards, 2012). The information summarised through this current review shows that HAV can behave differently to surrogate viruses when challenged by inactivation treatments, and also that the surrogate viruses can behave differently to each other.

MNV, FCV and Tulane virus, which are non-enveloped viruses containing single-stranded RNA, are often used as surrogates for HAV and human norovirus. Bacteriophages, which are viruses that infect bacteria, can also be used as surrogates. The bacteriophage MS2 is an example of an HAV surrogate used in research.

2 2.1.1 Hepatitis A virus

HAV belongs to the genus *Hepatovirus* in the family *Picornaviridae*. There are nine species, with human HAV being assigned to the species *Hepatovirus A* (Migueres *et al.*, 2021). There are several genotypes, denoted I to III (with sub-genotypes IA, IB, IIA, IIB, IIIA and IIIB), and multiple strains that infect humans, with a wide degree of heterogeneity observed globally. There is only one serotype (Migueres *et al.*, 2021).

3 2.1.2 Norovirus

Norovirus infecting humans belong to the genus *Norovirus* in the family *Caliciviridae*. Classification is based on a dual typing system based on the capsid VP1 and partial RdRp sequences.

[1] More specifically, HAV is quasi-enveloped since these viruses can obtain an envelope when inside an infected host but when in faeces or in the environment, this envelope is absent.

Genogroups I, II and IV (GI, GII and GIV) are associated with illness in humans (with GII being most commonly reported in outbreaks settings, including in New Zealand).

There are no reference strains that can be commercially purchased. Instead, norovirus strains that infect humans are obtained from faecal specimens, often submitted first to clinical laboratories for diagnostic purposes.

4 2.1.3 Aichi virus

Aichi virus belongs in the genus *Kobuvirus* in the family *Picornaviridae*. The virus was first isolated in a stool from a patient with gastroenteritis. Like enteric viruses such as HAV and norovirus, it is stable at low pH. Aichi virus is similar in size (30 nm) to HAV and norovirus, and also contain a single stranded RNA (Rivadulla and Romalde, 2020). As a non-enveloped virus, Aichi virus is resistant to alcohols, chloroform and non-ionic detergents, as well as being relatively resistant to heat, chlorine, and other stressors (Cromeans *et al.*, 2014).

5 2.1.4 Murine norovirus

MNV belongs to the genus *Norovirus* in the family *Caliciviridae*. The MNV-1 strain was first isolated from laboratory mice in 2002 (Karst *et al.*, 2003). In the laboratory, MNV is grown in RAW 264.7 (murine macrophage cells, ATCC 15597-B1). While the MNV-1 strain is mainly used, other strains have been used for inactivation studies, e.g., Strain S99 (Butot *et al.*, 2021). MNV can be quantified using plaque assay and end point titrations. Maximum MNV titres of 7-8 $\log_{10}$ plaque forming units (PFU)/mL or 50% tissue culture infective dose ($TCID_{50}$)/mL can be expected. As a calicivirus, MNV has the same physical properties as human norovirus, also being similar in size (28-35 nm diameter) to HAV (at 27-28 nm).

6 2.1.5 Feline calicivirus

FCV belongs to the genus *Vesivirus* in the family *Caliciviridae*. FCV has been frequently used as a surrogate, but certain properties, including its sensitivity to low pH, mean there are limitations in its usefulness as an enteric virus surrogate. Since the discovery of MNV, FCV has been less frequently used as a surrogate as MNV has been shown to be a better surrogate for human norovirus than FCV.

7 2.1.6 Tulane virus

Tulane virus belongs to the genus *Recovirus* in the family *Caliciviridae* The virus was first isolated from faeces of asymptomatic macaque monkeys (*Macaca mulatta*) and was immediately proposed as a norovirus surrogate (Farkas *et al.*, 2008). As a calicivirus, Tulane virus is most closely related to human norovirus, and shares genetic and biological properties including similar genome organisation and tissue tropism. Like norovirus, the Tulane virus capsid binds to histo-blood group antigens (HBGAs), a property that makes it more structurally similar to human norovirus than MNV or FCV. As such, it has been used as a human norovirus surrogate and model system in the last decade (Farkas, 2015).

8 2.1.7 Bacteriophages

F-specific RNA bacteriophages belonging to the family *Leviviridae*, and that infect bacteria, are divided into four subgroups and have a similar structure to enteric viruses. These include MS-2 (in group I) and Qβ (in group III) that have been used as norovirus surrogates in inactivation studies (Richards, 2012). These viruses are widely available and grow to high titres.

2.2 VIRUS SEEDING METHODS FOR BERRY FOODS

This section, supported by Appendix B.3, provides information on the reported methods used to prepare and inoculate berries and purees with viruses prior to applying inactivation treatments.

When reported, berries were purchased from local markets or shops. For some studies, the berries were chopped into smaller pieces prior to treatment (Chen *et al.*, 2020; Sanglay *et al.*, 2011).

Purees may be purchased as a prepared commercial product (Deboosere *et al.*, 2004) or prepared in the laboratory using an electric blender (Wales *et al.*, 2024). In some studies, the puree was modified prior to treatment, e.g., by adding citric acid to change the pH (Deboosere *et al.*, 2010).

Approaches to viral inoculation of berries included:

- Addition of a virus suspension (in phosphate buffered saline (PBS) or cell culture medium) directly onto the fruit surface, i.e., spot or drop inoculation.
- Aerosolisation (i.e., spraying inoculum onto the fruit using a nebuliser).
- Immersion of the whole fruit in a virus suspension.
- Injection of virus into an individual fruit.

For purees, the virus inoculum is directly added to the liquid, and mixed.

As virus recovery methods usually require at least 20 g of fruit for processing, samples may be pooled to generate enough product to analyse. Viral quantitation can only be done above the limit of quantification (LOQ) for the selected assay, which may be as low as 200 PFU/20 g for example.

2.3 VIRUS RECOVERY METHODS

Virus recovery methods need to be designed in such a way that the integrity of the virus is not disrupted during the process so to ensure that virus infectivity is maintained. A dilution may be required to avoid cell toxicity, interference in counting plaques, and/or inhibition, noting such steps influence the detection limit of the assay (and will affect the ability to detect a reduction in viral numbers due to inactivation treatments). Appendix B.4 contains further information.

2.4 DETECTION ASSAYS TO ASSESS VIRAL INACTIVATION: CELL CULTURE

A range of methods have been used to assess the effect of an inactivation treatment on viruses in berry foods. The studies of interest are those that used methods to measure the presence of infectious viruses after treatment, generally described as infectivity assays.

Infectivity assays include cell-based virus culture assays, viral capsid integrity or binding assays, and volunteer feeding studies. Volunteer feeding studies have been used to assess infectivity for norovirus through the observation of gastroenteritis illness following ingestion. These are few in number due to ethical considerations. Infectivity assays can also be used to determine viral persistence (sometimes referred to ‘survivability’), for instance, persistence at refrigeration temperatures.

Detection of virus by reverse transcription (RT)-PCR alone will not distinguish between infectious and non-infectious viruses. However, there are several pre-treatments before the RT-PCR step that are available to determine virus capsid integrity as a proxy for infectivity. These are referred to as capsid integrity assays (Section 2.5).

Traditionally, virus infectivity has been determined using a cell culture assay. Traditional cell culture assays are utilised for HAV and the virus surrogates, MNV, FCV and Tulane virus.

Infectivity of bacteriophages, such as MS-2, are determined usually by a standard double layer phage plaque assay using a bacterial host.

The following sections introduce assays based on cell culture. Further information is provided in Appendix B.5.

2.4.1 Plaque forming assay

The plaque forming assay is suitable for culturable viruses that show cytopathic effect (CPE). For lytic viruses, CPE manifests as cell lysis when infected with a virus, and this can be used as the measured end point. To perform the assay, susceptible cells are plated in a confluent monolayer (monolayer plate assay), virus added and left to infect for 1-2 hours. The cells may be washed to remove inoculum and then overlaid with semi-solid agar. Alternatively, cells and virus may be mixed in an agarose suspension. In this case, the assay is referred to as an agar cell suspension assay.

For both approaches, plaques (clearer spots in the agar) form over 1-5 days as the virus replicates in the cells, and then lyses them. The number of PFU per volume of measure added to a well (e.g., 6 or 24-well plate format) can be counted by eye. This is often aided through the addition of a cell dye such as crystal violet or trypan blue.

2.4.2 Focus forming assay

The focus forming assay is a modification of the plaque forming assay. This assay is suitable for culturable viruses that infect cells but show no (i.e., non-cytopathic viruses) or minimal cell lysis (minimally cytopathic). When susceptible cells are plated in a confluent monolayer, cells where viral replication is occurring (known as foci) are detected by using immunofluorescent or immunoperoxidase staining. The focus forming assay is not used as much as the plaque forming assay but was used for HAV in early papers on HAV inactivation.

2.4.3 End point dilution assay

Unlike plaque assays, the end point dilution assay involves the viral inoculation of cells in the liquid phase (normally in cell culture maintenance medium). Two different measurements can be reported. These are the $TCID_{50}$, which is a measure of the amount of virus needed to infect 50% of the cells, and the most probable number (MPN). Both are determined using a dilution series (e.g., eight chambers per dilution, or to increase sensitivity using a whole plate (commonly 96-well plates) per dilution). Like the plaque assay, as viruses replicate in the cells, visible lysis occurs and the number of positive (with CPE) and negative (with no CPE) wells are counted.

The titre is calculated using the Spearman-Kärber and/or Reed and Muench method (Kärber, 1931; Reed and Muench, 1938) where $TCID_{50}$ or MPN is calculated from the proportion of positive wells, taking into account the dilution used (Reed and Muench, 1938).

2.4.4 Integrated cell culture-PCR assay

When cell culture is followed by PCR or quantitative PCR (qPCR) to detect viruses (or RT-PCR or RT-qPCR for RNA viruses), often in the absence of CPE, it is referred to as integrated cell culture-PCR (or just cell culture-PCR). This method can be used as a quantitative assay by applying a MPN strategy (i.e., multiple wells/flasks with different dilutions analysed).

2.4.5 Human intestinal enteroid cell culture

Human intestinal enteroid (HIE) cells have been successfully developed for culturing several human norovirus strains (Ettayebi *et al.*, 2016). Virus replication (i.e., the presence of infectious virus) is determined, not by CPE, but by the increase of viral genome copies as determined by RT-qPCR or RT-digital droplet PCR (RT-ddPCR). The quantity of genome copies before and after cell culture is compared. However, the assay is costly, time consuming, has low sensitivity and can only be performed in specialised laboratories. The addition of complex matrices, including food or food concentrates, to the cells to determine infectivity can be problematic. The major issue being toxicity and other interference to the cells from the matrix.

2.5 DETECTION ASSAYS TO ASSESS VIRUS INACTIVATION: CAPSID INTEGRITY ASSAYS

Also called virus integrity assays, capsid integrity assays provide an alternative to cell culture-based methods for estimating the number of infectious viruses in a sample. Instead of assessing the ability of a virus to infect host cells, viral infectivity is inferred through an assessment of the viral capsid integrity, based on the assumption that a virus with an undamaged capsid is potentially infectious. This method also detects non-infectious viruses with an intact capsid, virus infectivity numbers can be overestimated by as much as 2-3 $\log_{10}$ (Chen *et al.*, 2020).

The following sections introduce different approaches to assessing capsid integrity. Further information is provided in Appendix B.5.

2.5.1 Viability dye (nucleic acid binding) assay

Agents that bind to nucleic acid such as platinum chloride ($PtCl_4$), propidium monoxide (PMA) or the PMA derivative, PMAxx™, have mostly been used in viral inactivation studies. These agents bind to nucleic acid and make this unavailable for subsequent PCR. The assumption is that if the viral capsid is damaged, the dye will bind onto nucleic acid. An undamaged capsid, representing an infectious virus, will protect the nucleic acid and this can be detected by subsequent PCR.

2.5.2 RNase assay

RNase is an enzyme that can be used to break up any free viral RNA (and/or DNase to remove free viral DNA). In theory, the RNA of intact viruses will be unaffected and remain available for subsequent PCR detection. This method has been frequently used to measure the effect of heat inactivation studies including in studies of HAV.

2.5.3 Capsid binding assay

If a virus capsid is damaged, its ability to bind to a host cell may be affected. Without the ability to bind, the virus is non-infectious. The capsid binding assay makes use of the specificity of this binding. The norovirus (and the surrogate, Tulane virus) capsid can bind to HBGAs or porcine gastric mucin (PGM). HBGA and PGM bind preferentially to intact virus particles. If HBGA or PGM are also bound to magnetic beads, intact and bound viruses can be captured. Sialic acid will bind MNV but not human norovirus or Tulane virus.

2.6 MAXIMUM MEASURABLE LOG_{10} REDUCTION

Virus inactivation following any treatment is calculated by comparing the number of infectious viruses recovered from the sample *before* treatment is applied (i.e., the N0 value) to the number of

infectious viruses recovered *after* treatment is applied (i.e., Nt value). These values are converted to log base 10, to give $\log_{10}$ reduction (i.e., Nt/N0).

Methodological issues can make it difficult to measure virus inactivation beyond 2-3 $\log_{10}$ for some viruses/assays/matrices, although data presented in Section 3 shows that this has been achieved in some studies. The maximum $\log_{10}$ reduction that can be measured depends on several factors including the initial virus concentration, the percentage of the inoculum that can be recovered (often very low), the LOQ of the infectivity assay and any cell toxicity/assay inhibition. Generally, higher virus concentrations result in better assessment, but other factors are also important.

See Appendix B.6 for further information.

3 TREATMENTS

As described in Appendix A, after review of initial searches the citation set contained 61 references, with publication dates ranging from 2008 to 2024. Additional references were located as these reports were reviewed in detail, resulting in a total of 80 relevant references. Most references related to thermal and high-pressure processing. For most other treatments there were less than five references.

In this report, we have excluded studies where only RT-PCR was used without pre-treatment being applied (as described in Section 2.4). Treatments that involved sanitisers were also excluded (see Section 1.1).

It is important to note that, for many published experiments, the authors only report the concentration of virus inoculated onto the food and the log reduction after treatment. Because the methods used to detect infectious viruses can have low recovery rates (see Section 2.6), the inoculation concentration cannot be considered equivalent to the number of infectious viruses recovered from the sample before the treatment is applied (N0). Where authors have reported a N0 value, this has been included. For all other studies, we have assumed that the authors have adhered to standard protocols and have calculated viral reduction values based on a measured N0 value. This approach accounts for recovery rates.

While some authors provided the inactivation data in text or tables, others presented these data as graphs. For the latter, we have inferred values from the graphs either visually or using specific software.[2]

In the following sections, data values (including $\log_{10}$ reductions) are reported without standard deviations, which were not always reported in the considered studies.

3.1 THERMAL TREATMENT

17 3.1.1 Introduction

The effectiveness of thermal (heating or cooling) processes to inactivate viruses in foods depends on virus type, temperature, treatment duration, food matrix, water activity (a_w), and pH (Johne *et al.*, 2024). Thermal processes can be used as an effective intervention strategy to inactivate viral particles if the appropriate parameters are used.

This section provides data from studies that investigated thermal inactivation of viruses in berry foods. Data were available for microwaving (and microwave-assisted thermal processing), boiling, pasteurisation, heat exchange, ohmic treatment or electro-heating, drying, and freeze-drying.

Early studies showed that heating above 60 °C reduces infectivity of HAV in suspension, for example a 2-3 $\log_{10}$ reduction can be expected at 72 °C for 2 minutes in buffers (Cook *et al.*, 2014; Parry and Mortimer, 1984). Increased temperatures and heating duration results in higher inactivation.

As heat can readily be applied by consumers as well as manufacturers, heat treatments have been recommended as a practical intervention strategy. For example, following a large norovirus outbreak associated with frozen strawberries in Germany in 2012 (Bernard *et al.*, 2014), a recommendation of treating berries at 72 °C for 2 minutes was given if berry products were to be consumed by vulnerable persons (BfR, 2024). In New Zealand, during a 2015 hepatitis A outbreak

[2] automeris.io: AI assisted data extraction from charts using WebPlotDigitizer accessed 5 June 2024

associated with frozen berries, MPI recommended cooking food to 85 °C for 1 minute to inactivate HAV but recognised that the extent of virus inactivation is influenced by the food matrix (MPI, 2015). Although these recommendations may have been initially provided in response to an outbreak, the guidance provided is applicable, especially for vulnerable consumers, at any time.

A detailed 2024 review of studies investigating the heat stability of HAV (and also human norovirus and hepatitis E virus) in a wide range of matrices stated “The effect of the tested matrix greatly influenced the heat stability of HAV” (Johne *et al.*, 2024).

Most inactivation studies, including those reported here, are performed using a single HAV strain (HM175), which creates some uncertainty in applying the findings to wild strains.

There are several studies that have examined the effect of thermal treatments on infectious HAV using a cell culture-based approach (plaque assay or $TCID_{50}$). For some studies, the results have been compared to a direct HAV RT-qPCR and/or to the effect of treatment on norovirus (e.g., Butot *et al.* (2009)). Several thermal inactivation studies on berries and purees have been performed on norovirus, MNV, Tulane virus and MS2. The most recent study explored the inactivation of MS2 bacteriophage on whole strawberries during microwaving (Dolan *et al.*, 2023), as covered in Section 3.1.3.

18 3.1.2 Studies: Heating of berry purees

The effect of heat on laboratory acidified raspberry purees at different pH values (2.5, 3.0 and 3.3) was assessed by Deboosere *et al.* (2010), as pH was considered an important parameter to assess at natural sugar levels (approximately 5% wt/wt). To do this, whole raspberries were first pureed (i.e., mashed), and the pH adjusted by adding citric acid to mimic the pH range of several berry types. This was followed by inoculation with HAV to obtain concentrations of 10^6 to 10^8 PFU/mL. The puree was stored in tubes at room temperature for 3 h prior to heating. The HAV-seeded puree was then heated in a glycerol bath set at 65, 70 and 75 °C for various time periods. The temperature was monitored, and it was determined that the required temperature was achieved at approximately 2 minutes after immersion (temperature within 2-3 °C were achieved after 1 minute). Using a HAV plaque assay, up to a 4 $\log_{10}$ reduction was demonstrated at 65 to 75 °C within approximately 6 minutes after the temperature was reached. However, at 65 °C (and to some extent at 70 °C) there was limited virus inactivation within the first 1 to 4 minutes after the temperature was reached.

Lower pH resulted in increased inactivation (i.e., inactivation at pH 2.5 was greater than at pH 3.3) and this difference was more pronounced at higher temperatures. Non-linear inactivation curves (three-phase) were observed.

An inactivation model was developed (for pH 2.5 to 3.3, and for 65 to 75 °C) for HAV inactivation in red berries and validated using data obtained at 65 to 75 °C on pureed strawberries, raspberries and bilberries (all at their natural pH of 3.35, 3.05 and 2.87, respectively). It was concluded by the authors that the “model could be used to predict relatively reliable heat inactivation of viruses in soft fruit, acidified or not, in regard to pH variation”. It is presumed that the authors meant this to be applied to purees rather than whole soft fruits.

Using the model on temperatures 65, 70 and 75 °C, MPI and FSANZ calculated the times needed to achieve a reduction in HAV infectivity of 6 $\log_{10}$ in pureed strawberries, raspberries and bilberries (**Table 2**). These data were reported in the guidance document separately published by FSANZ and MPI in 2015 (MPI, 2015). Additional data presented showed that virus was still detected after 85 °C for 4, 11 and 30 minutes at 28%, 40% and 52% sugar, respectively (with a starting virus concentration of 10^7 PFU/mL). The T90 value (time to reduce the infectious titre by 90%, or 1 $\log_{10}$ reduction) was 0.8, 1.88 and 6.28 minutes respectively.

Table 2. Time (minutes) required for a 6-$\log_{10}$ reduction in hepatitis A virus (HAV) infectivity in pureed strawberries, raspberries and bilberries at 65, 70 and 75 °C

Fruit	pH	Time required for a 6-$\log_{10}$ reduction in HAV infectivity (minutes)		
		65 °C	70 °C	75 °C
Strawberries	3.35	18	9	6
Raspberries	3.05	9	6	4
Bilberries	2.87	12	7	5

An earlier study by the same group (Deboosere *et al.*, 2004) using the same approach as the 2010 study, showed that high sugar concentrations had a protective effect against thermal inactivation. That study, performed on synthetic strawberry puree (to model pH, sugar and calcium concentration) seeded HAV into puree to give a concentration of 10^7 PFU/mL. Purees with a pH of 3.8 and containing 28% sucrose required more than 5 minutes at 75 °C to reduce the HAV infectivity by 4 $\log_{10}$ (as determined by plaque assay). When the pH was reduced to below 3.3, the time needed was less than 4 minutes.

Baert *et al.* (2008) evaluated MNV infectivity (along with bacteriophage B40-8) in commercially available raspberry puree. Using a plaque assay, this showed that an approximate 1.9 $\log_{10}$ reduction was achieved when heated at 65 °C for 30 seconds. The reduction in infectivity increased to 2.8 $\log_{10}$ when heated at 75 °C for 15 seconds (**Table 3**). It was concluded that phage B40-8 was an ineffective surrogate for norovirus as its behaviour differed from that of MNV under experimental conditions. The results of this study demonstrated that <3 $\log_{10}$ reductions were being achieved with mild heating (pasteurisation temperatures).

Bartsch *et al.* (2019) also determined the effect of heating (50, 56, 63, 72 and 80 °C) on MNV and Tulane virus as surrogates for norovirus in strawberry puree. Results were compared to human norovirus. MNV was assayed using plaque assay, Tulane virus by plaque assay and a capsid integrity assay with RNase, and human norovirus was analysed by capsid integrity assay with RNase. Different recovery workflows were required depending on the analysis.[3] Results for the culture assays are shown in **Table 3**. D-values (minutes) were derived using a primary Bigelow model and are shown in **Table 4**. Temperatures greater than 63 °C for 5 minutes resulted in 5 $\log_{10}$ reduction in infectivity for both MNV and Tulane virus, as determined by plaque assay. For both MNV and Tulane virus, reductions in excess of 7 $\log_{10}$ were observed when exposed to 80 °C for 8 seconds (compared to capsid integrity assays where the reduction was approximately 3.5 $\log_{10}$). These results were similar to that observed by Baert *et al.* (2008) as described above. Bartsch *et al.*, (2019) showed, as others have demonstrated that the capsid integrity results did not align with infectivity, even with the addition of RNase pre-treatment. The results of the study also suggested that human norovirus may have higher heat stability than MNV and Tulane virus (human norovirus D-values were higher than for Tulane virus by RNase/PCR capsid assay, **Table 4**).

Bartsch *et al*. (2019) also produced a tertiary model[4] for human norovirus to predict the average reduction in 'capsid-protected' RNA copies (i.e., presumptive infectious viruses). At 50 °C for 90 minutes, 56 °C for 30 minutes, 63 °C for 15 minutes, 72 °C for 40 seconds and 80 °C for 8 seconds, the reductions were 0.2, 0.4, 1.8, 1.4 and 3.6 $\log_{10}$, respectively. The model can be used to predict human norovirus inactivation within the range of 50-80 °C and up to 1.5 hours of treatment for strawberry puree. For example, for a 4 $\log_{10}$ reduction the model predicts that 2 minutes would be required at 72 °C, and 10 seconds at 80 °C.

[3] Samples for plaque assay underwent ultrafiltration, plus, for Tulane virus, further concentration using PEG precipitation to aid in recovery. For the capsid integrity assay, PEG precipitation followed by RNase treatment and a chloroform/butanol wash was done on a 15 mL aliquot, prior to RNA extraction.
[4] The model archives are available from https://data.mendeley.com/datasets/zmdvzy2pyg/1 (accessed 26 July 2024).

Table 3. Effect of heating on murine norovirus (MNV), Tulane virus, hepatitis A virus (HAV) and human norovirus on fresh berries and purees

Virus	Fruit	Temperature (°C)	Time (minutes)	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
MNV	Raspberry puree	65	0.5	1.9	Added as liquid	Plaque (PFU)	Baert *et al*. (2008)
		75	0.25	2.8			
MNV	Strawberry puree	50	15	2.3	Added as liquid	Plaque (PFU)	Bartsch *et al*. (2019)
			30	2.6			
			45	2.7			
			60	3.1			
			90	3.5			
		56	5	2.2			
			10	2.7			
			20	5.3			
			30	6			
			40	8			
		63	1	1.5			
			2	2.8			
			5	5.1			
			10	5.8			
			15	9			
		72	0.7	8			
		80	0.15	9			
Tulane virus	Strawberry puree	50	15	1	Added as liquid	Plaque (PFU)	Bartsch *et al*. (2019)
			30	1.6			
			45	3			
			60	3.5			
			90	4			
		56	10	2.5			
			20	4.3			
			30	7.2			
			40	7.5			
		63	1	1			
			2	3			
			5	7.3			
			11	7.4			
			15	7.5			
		72	0.7	7.2			
		80	0.13	7.4			
HAV	Raspberries	60	15	1.1	Surface, spot	RT-qPCR PMAxx	Chen *et al*., (2019)
		72		1.9			
		95		>3.5			
	Strawberries	60	15	0.3			
		72		1.9			
		95		2.8			
Norovirus GI	Raspberries	60	15	1.9			
		72		1.2			
		95		1.8			
	Strawberries	60	15	1.5			
		72		1.6			
		95		2.0			
Norovirus GII	Raspberries	60	15	1.2			
		72		1.1			
		95		>3.8			
	Strawberries	60	15	1.1			
		72		1.9			
		95		>3.5			

Table 4. D-values (minutes) of murine norovirus (MNV), Tulane virus and human norovirus in strawberry puree at different temperatures

Virus	Assay	Temperature (°C)				
		50	56	63	72	80
MNV	Plaque	31.4 ± 4.8	5.9 ± 0.7	2.2 ± 0.3	0.1 ± 0.0	0.0 ± 0.0
Tulane virus	Plaque	23.2 ± 2.6	5.4 ± 0.3	0.7 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
	RNase/PCR	126.3 ± 18.8	19.5 ± 2.6	4.6 ± 0.6	0.2 ± 0.0	0.0 ± 0.0
Human norovirus	RNase/PCR	584.1 ± 189.5	59.8 ± 8.1	8.0 ± 1.0	0.6 ± 0.2	0.0 ± 0.0

Modified from Bartsch *et al.* (2019)

19 3.1.3 Studies: Heating of whole berries

An assessment of the usefulness of two capsid integrity markers, PMAxx™ and $PtCl_4$ as pretreatment viability markers for HAV and human norovirus infectivity assessment has been published. Following preliminary experiments that showed that PMAxx performed better than $PtCl_4$, an evaluation of PMAxx (100 µM) following different thermal treatments (60, 72 and 95 °C for 15 minutes) using whole berries was done (Chen *et al.*, 2020). No HAV culture was performed to compare to the PMAxx results. Whole raspberries and strawberries (25 g) were seeded with both viruses, and following heating, and then with and without PMAxx™ treatment, samples were tested by RT-qPCR. Results of PMAxx treatment are summarised in **Table 3**. At the most extreme temperature (95 °C for 15 minutes), using PMAxx™ treatment, HAV and norovirus GII were not detected (>3.5 $\log_{10}$ reduction), while norovirus GI reduced by 1.8 $\log_{10}$. Similar results were observed for strawberries. Reductions were less at lower temperatures as may be expected. The authors concluded that while PMAxx may still overestimate virus infectivity, this approach was an improvement over RT-qPCR alone.

20 3.1.4 Studies: Microwaving of whole frozen berries

The infectivity of bacteriophage MS2 on frozen strawberries following microwaving was determined in one study where four power settings with time periods up to 4 minutes were assessed (Dolan *et al.*, 2023). The strawberries were commercially packaged frozen berries. To inform on experimental design, the authors reviewed microwave package instructions for frozen strawberries and concluded that these varied by food manufacturer. Instructions ranged from microwaving for 1 to 6 minutes, using low or defrost settings. The instructions intend to produce a thawed product, rather than to heat/cook the product.

The results of the experiment are summarised in **Table 5**. High concentrations of bacteriophage MS2 (10 $\log_{10}$ PFU/g berry) were seeded on either three or five frozen whole strawberries, to evaluate the impact of different sample sizes (each berry weighed approximately 15 g). The temperature of the inoculated berries prior to microwaving was -2.3 °C. MS2 infectivity was evaluated using a F-RNA bacteriophage plaque assay. The average MS2 recovered with no treatment (T0) was 8.9 ± 0.5 PFU $\log_{10}$ /g berry with an assay detection limit of 2 $\log_{10}$ PFU/g. A 1300-Watt (max power) microwave with a frequency of 2450 MHz was used, at 30, 50, 70 and 100% power setting for 60-300, 30-240, 15-150 and 15-120 seconds respectively. The temperature was monitored throughout the experiment. For maximum power, temperatures reached 80 °C after 1 minute for five berries treated (the time was less for three berries).

Table 5. Effect of microwave (30-100% power) on MS2 bacteriophage on whole strawberries, pH 3.65 (Dolan *et al.*, 2023).

Power	Time (seconds)	3 strawberries[a]			5 strawberries[a]		
		Initial (log_{10})	After (log_{10})	Reduction (log_{10})	Initial (log_{10})	After (log_{10})	Reduction (log_{10})
30%	60	8.0	7.0	1.0	8.3	7.4	0.9
	120		5.2	2.9		6.5	1.8
	180		4.1	3.9		5.4	2.9
	240		2.8	5.2		4.2	4.1
	270		-	-		not reported	
	300		not reported			3.3	5.0
	300		not reported			not detected	
50%	30	8.6	8.2	0.4	9.2	8.8	0.4
	60		7.1	1.5		7.9	1.3
	90		6.0	2.6		7.3	1.9
	120		5.0	3.6		5.5	3.6
	150		4.3	4.3		4.3	4.9
	180		2.9	5.3		4.1	5.1
	210		not detected			not reported	
	240		not reported			3.4	5.9
	270		not reported			not detected	
70%	15	8.8	8.5	0.3	9.3	8.5	0.8
	30		7.1	1.6		8.1	1.2
	45		6.6	2.2		7.4	1.8
	60		5.7	3.1		5.6	3.6
	75		5.0	3.8		5.2	4.1
	90		4.5	4.3		4.7	4.6
	105		3.3	5.4		4.3	5.0
	120		not detected			3.4	5.8
	150		not reported			2.9	6.3
	180		not reported			not detected	
100%	15	9.3	8.4	0.9	9.3	8.7	0.6
	30		7.7	1.6		7.8	1.5
	45		6.7	2.6		7.3	2.0
	60		5.5	3.8		6.5	2.8
	75		4.5	4.8		5.5	3.8
	90		3.6	5.7		4.6	4.7
	105		not detected			4.4	4.9
	120		not reported			3.0	6.3
	135		not reported			not detected	

[a]Data provided from author (D Schaffner, personal communication)

MS2 infectivity reduced with increasing power (watts) and exposure time. The lowest power setting (30% power, 390 watts) for 1 minute resulted in approximately 1 log_{10} reduction in infectivity, while full power (100%, 1300 watts) for 1 minute, resulted in a 3.8 log_{10} reduction (**Table 5**). No MS2 infectivity could be detected when exposed for 105 seconds, and for 135 seconds for three and five berries respectively at 100% power– representing approximately a 6 log_{10} or more reduction. D values of 21.4 and 10.6 seconds at 10 and 60 °C were determined, respectively. It is presumed that 10 °C was used to represent the desired temperature of a berry product that is just thawed but not heated. The results indicated that the D was linear (rather than exponential), dependent on temperature, postulating that microwave inactivation may be different to other types of thermal treatment, and warranted further investigation.

21 3.1.5 Studies: Freeze-drying and heating of freeze-dried berries

Freeze-drying is a common method used to preserve berries. To produce freeze-dried berries, fresh product is frozen, and then placed under reduced pressure/vacuum to sublimate the ice. Heating may be applied to facilitate the sublimation process. Freeze-dried products can be used as a component of other products including bakery goods, ice creams and cereals.

Freeze-drying berries

One study has investigated the effect on HAV and norovirus on berries of freeze-drying (not used as an inactivation strategy but to preserve the berries). For this experiment, HAV and human norovirus were spot inoculated (six spots of 30 µL virus suspension) on fresh berries to give a total of 10^6 $TCID_{50}$ HAV, 1.2 $x10^5$ PCR units norovirus GI and $2x10^6$ PCR units norovirus GII (Butot *et al.*, 2009). The effect on HAV and norovirus of freeze-drying (combination of vacuum freeze drying at -20 °C and applied heat: 115 °C for 3 hours, 60 °C for 13 hours, and then 60-115 °C for 2 hours resulting in a maximum internal temperature of 55 °C for approximately 10 hours) was determined by endpoint cell culture ($TCID_{50}$) for HAV, and by RT-qPCR for HAV and norovirus.

This freeze-drying process reduced infectious HAV titres by 1.4 to 2.4 log_{10}, while reductions in the HAV genome copy number by RT-qPCR were less than 1 log_{10} (**Table 6**) (Butot *et al.*, 2009). Freeze-drying reduced the RT-qPCR titre of norovirus GI to a similar extent as HAV. However, the reductions in norovirus GII genome levels were approximately 1 log_{10} greater than for norovirus GI and HAV (**Table 6**), suggesting that HAV and GI were more resistant to freeze-drying than norovirus GII.

Overall, virus reductions following freeze-drying were higher on blueberries (2.4 log_{10}) than the other berries (1.4-1.8 log_{10} reduction), which the authors suggested was possibly due to the "crevices and hair like projections" on the blackberries, raspberries and strawberries that may protect against stressors to a certain degree (Cook *et al.*, 2014).

Table 6. Effect of freeze-drying berries on hepatitis A virus (HAV) (by culture, $TCID_{50}$ and RT-qPCR) and human norovirus genogroup I and II (GI and GII) (by RT-qPCR) (Butot *et al.*, 2009)

Virus	Fruit	Reduction (log_{10}) Culture, $TCID_{50}$[a]	Reduction (log_{10}) RT-qPCR[a]
HAV	Blackberries	1.8	0.6
	Blueberries	2.4	1.0
	Raspberries	1.5	-0.3
	Strawberries	1.4	0.9
Norovirus GI	Blackberries	NA	0.7
	Blueberries		1.3
	Raspberries		0.6
	Strawberries		0.8
Norovirus GII	Blackberries	NA	1.7
	Blueberries		2.7
	Raspberries		1.2
	Strawberries		1.5

Heating freeze-dried berries

The effect on HAV of heating freeze-dried berries (blackberries, blueberries, raspberries and strawberries) at 80, 100 and 120 °C for 20 minutes was also assessed by the same authors (Butot *et al.*, 2009). To do this, 15 g berries were first spot inoculated with virus and freeze-dried as described above. The berries were then placed in an oven (80, 100 and 120 °C) for 20 minutes. The reduction was determined by comparing the virus titre recovered from the freeze-dried product to the virus titre recovered from the freeze-dried berries heated.

The heat step to 80 °C (reaching an internal temperature of 55 °C) applied to freeze-dried product, resulted in a moderate (1.6 to 2.0 log_{10}) reduction in HAV infectivity in all berries tested (and < 1 log_{10} using RT-qPCR). No infectious virus could be detected following heating to 100 and 120 °C for most fruits (the exception was blueberries, where infectious HAV could still be detected following 100 °C, which could be considered an anomaly which was not discussed in the paper). Viral RNA reductions were less than as determined by the cell culture assay (**Table 7**). The same experiments using RT-qPCR were done on norovirus GI and GII showing that their persistence was similar to HAV by the same method (data not shown).

Table 7. Effect of oven heat at 80, 100 and 120 **(°C)** for 20 minutes on hepatitis A virus on freeze-dried berries (by $TCID_{50}$ and RT-qPCR) (Butot *et al.*, 2009)

Fruit	Oven Temperature (°C)	Inoculation method	Reduction (log_{10}) Culture, $TCID_{50}$	Reduction (log_{10}) RT-qPCR
Blackberries freeze-dried	80	Surface, spot	1.9	0.7
	100		not detected	1.1
	120		not detected	not detected
Blueberries freeze-dried	80	Surface, spot	1.6	0.3
	100		2.0	1.2
	120		not detected	not detected
Raspberries freeze-dried	80	Surface, spot	1.9	0.8
	100		not detected	1.3
	120		not detected	not detected
Strawberries freeze-dried	80	Surface, spot	1.6	1.0
	100		not detected	0.7
	120		not detected	2.4

3.2 HIGH-PRESSURE PROCESSING

22 3.2.1 Introduction

High-pressure processing (HPP), also described as high-hydrostatic pressure or ultra-high-pressure processing, is a treatment that typically subjects liquid and solid foods, with or without packaging, to elevated pressures. Commercial equipment is usually capable of applying pressures up to 600 MPa.[5]

HPP has been applied to a wide range of foods including fruit juices, fresh-cut fruits and vegetables, raw milk, ready-to-eat meats, guacamole, salad dressings, jams and fruit sauces, and oysters, mostly for shelf-life extension. A review of parameters associated with pathogen inactivation by HPP, principally in milk, was conducted by ESR for MPI in 2018 (Horn *et al.*, 2018). Many HPP-treated products are commercially available in different countries. HPP can be performed on unpackaged liquids prior to packaging but pre-packaging of foods (such as sliced ready-to-eat deli meat products and some juices) offers the advantage of preventing recontamination of food after pressurisation.

A review article, published in 2021 (Govaris and Pexara, 2021) stated that the effectiveness of HPP to inactivate viruses in foods depends on processing parameters (pressure, temperature, and duration time) and non-processing parameters (virus type, food matrix, water activity (a_w), and pH). HPP renders viruses non-infectious by disrupting the capsid and binding sites.

HPP processing can affect the quality properties of foods in both positive and negative ways, depending on the food. For example, discolouration of raw meat after HPP treatment has been noted. The antioxidant activity of HPP-treated berry purees has been found to be higher than thermally processed products (Patras *et al.*, 2009).

23 3.2.2 Studies

The log reduction data and HPP conditions for the relevant studies are summarised in **Table 8**.

A laboratory-based study of the effect of HPP processing on inactivation of HAV in strawberry puree used pressures of up to 375 MPa at ambient temperature for 5 minutes (Kingsley *et al.*, 2005). HAV was added to puree to give a concentration of approximately 10^5 PFU/mL and subjected to different HPP conditions for 5 minutes. Remaining infectious virus was determined by plaque assay. The detection limit was determined to be 0.5 log_{10}. The pH of the samples was 3.49

[5] The unit of pressure frequently used associated with food HPP is the Pascal (Pa) or mega Pascal (MPa; 1,000,000 Pa).

(the natural pH of strawberry puree is approximately 3.5 (Lou *et al.*, 2011)). These experiments showed that $\log_{10}$ reductions of up to 4.3 (i.e., beyond the detectable limits) could be achieved at the maximum pressure of 375 MPa. The authors commented that as only a 10^5 HAV PFU/mL puree could be achieved, this restricted the ability to determine larger log reductions.

A conference paper reported reductions of HAV following HPP of strawberries and blueberries (Ye *et al.*, 2018). HAV was spot inoculated (3.5 to 4.0 $\log_{10}$ PFU/sample) onto whole fresh and frozen berries which were then vacuum sealed for treatment at up to 600 MPa for 3 minutes at 4 °C. Virus reductions were determined by plaque assay. Detailed results were not given apart from a >2 $\log_{10}$ reduction at 400 MPa of strawberries, and reduction to below detection limit (not given) at 500 MPa of both fresh and frozen strawberries. Reductions were significantly lower on fresh and frozen blue berries, with reductions at 400 MPa of 1.1 $\log_{10}$ and 1.2 $\log_{10}$, respectively (lower reductions for norovirus on blueberries were also found in a study on thermal treatment (Cook *et al.*, 2014).

These are the only HPP studies using HAV found in the literature. Most other studies have used MNV as a surrogate for human norovirus, and more recently human norovirus itself.

A study of the behaviour of MNV in strawberry puree showed that $\log_{10}$ reductions from HPP were dependent on pH and temperature, as well as pressure (Lou *et al.*, 2011). Using an inoculum of 10^6 PFU/g puree, log reductions in strawberry puree increased with increasing pH (2.5 to 6.5), for the same pressure and time. Note that this is in contrast to the effect of thermal treatment, where lower pH resulted in greater reductions (Section 3.1.2). Log_{10} reductions at 4 °C were greater than those at 20 °C, for the same pressure and time, both in strawberry puree and on strawberry pieces (where 10^7 MNV PFU/g was inoculated).

The previous two studies determined virus numbers using a plaque assay. In contrast, another study also using MNV, determined both genome copy numbers (as determined by RT-qPCR) and virus infectivity using the $TCID_{50}$ culture assay (Kovač *et al.*, 2012). Complete inactivation (>99.9%, presumably 3 $\log_{10}$ reduction) of infective virus in strawberry puree was achieved by 400 MPa for 2.5 minutes or longer. Reduction in the number of viral genomes was more modest (up to 2 $\log_{10}$ reductions) and inconsistent, indicating that the viral genome is stable under HPP and RT-qPCR is not useful as a measure of effectiveness.

The finding that virus reductions were greater at lower temperatures was supported by studies using MNV in strawberry puree (inoculum of 10^6 PFU/g puree) using 350 MPa for 2 minutes at temperatures from 0 °C to 20 °C (Huang *et al.*, 2014). The greatest reduction (4.4 $\log_{10}$) was at 0 °C.

An extensive series of experiments by the same group examined reductions in the titres of human norovirus GI.1 and GII.4 in a range of whole berries and purees (Huang *et al.*, 2016). Experiments done at 0, 4 and 20 °C consistently showed lower reductions at higher temperature. From the range of matrices, blueberries and three berry purees (strawberry, blueberry, raspberry) were evaluated for colour, pH and viscosity, which were found to be largely unchanged following treatment. Sensory evaluation of the three berry purees after HPP treatment of 550 MPa for 2 minutes at 0 °C showed no significant reduction in sensory quality (appearance, aroma, colour and overall acceptability). However, significantly higher scores were given for untreated blueberries over HPP treated blueberries for appearance, colour, and overall acceptability.

An extensive study of the effect of HPP on MNV and MS2 bacteriophage used both laboratory prepared and commercial strawberry puree, as well as strawberry juice (Pan *et al.*, 2016). A series of experiments using increasing pressures for the same time, and the same time and pressure for different temperatures, enabled the derivation of linear regression equations. These equations showed the relationship between reductions ($\log_{10}$) versus temperature for 3 minutes at 300 MPa, and log reduction and pressure for 3 minutes at 20 °C.

One study investigated the potential of Tulane virus as an alternative surrogate to MNV (Li *et al.*, 2013). Some of these experiments compared the behaviour of Tulane virus and MNV on blueberries in both the dry and wet state (i.e., immersed in PBS). Pressures used were 400 or 600 MPa (dry state) and 300, 350, or 400 MPa (wet state), and temperatures were 4, 21 and 35 °C. Under dry conditions reductions in infectivity were negligible (<1 $\log_{10}$) for both viruses. Under wet conditions reductions in MNV infectivity were consistent with other experiments. Tulane virus reductions in infectivity were lower for experiments at 4 °C, but higher at 21 and 35 °C, for the same conditions. This is in contrast to other studies which have shown greater reductions at lower temperatures, for the same pressure and time.

A more recent study compared the reduction of human norovirus GII.4 strain and Tulane virus in strawberry puree under HPP (DiCaprio *et al.*, 2019). The effect of HPP on virus infectivity was assessed by a PGM-MB assay followed by RT-qPCR. Reductions in norovirus infective viral RNA copy numbers in strawberry puree after HPP (400 MPa for 2 minutes) were significant at pH 7 but not pH 4, whereas reductions in norovirus suspended in PBS were significant at both pH values. Reductions were greater for experiments conducted at 4 °C than at 20 °C. After HPP treatment at 600 MPa for 4 minutes at 4 °C and at natural pH (4), no detectable infective virus remained. The overall conclusion, consistent with previous studies, was that norovirus reductions through HPP treatment were enhanced at lower temperature and higher pH.

In 2024, two studies on the effect of HPP on human norovirus infectivity as determined by HIE culture in berry purees were published.

One study compared the assessment of norovirus infectivity using either capsid integrity RT-qPCR or using HIE cells (Wales *et al.*, 2024). Part of this study involved using both assays to assess infectivity/viability of human norovirus in strawberry puree treated using HPP at 300, 400, and 450 MPa for 5 minutes. The puree was prepared in the laboratory from blending fresh strawberries from a local market. Overall, the three types of capsid viability RT-qPCR results lacked correlation with results from the HIE assay, and it was concluded that PCR was not suitable for assessing infectivity. The authors did not report log reduction values as measured by the HIE culture assay, so data are not included in **Table 8**. Instead, they show which HPP conditions allow for an increase (or not) in viral RNA titre following HIE culture. An increase in viral RNA signals that infectious viruses were present in the treated sample. On this basis, norovirus GI.3 remains infectious in berry puree treated at 450 MPa, although the quantity of viral RNA was lower compared with other HPP treatments (possibly indicating that less infectious virus particles survived the treatment- although the correlation between input and output is not clear). Norovirus GII.4 Sydney appeared to be more sensitive to HPP treatment, with no increase in norovirus RNA titre detected after the 450 MPa treatment, and only a low increase in norovirus RNA titre after the 400 MPa treatment.

The second study examined the effect of HPP on several viruses in strawberry puree: human norovirus GI (GI.3[P13]) and GII (GII.4 Sydney[P16]), along with MNV and Tulane virus serving as surrogates (Pandiscia *et al.*, 2024). HIE was used for human norovirus, and endpoint ($TCID_{50}$) assays for MNV and Tulane virus. Strawberries were obtained from a local market and blended in the laboratory. The purpose of the study was to validate operating parameters (300, 400 and 450 MPa over different times) for effective control of human norovirus contamination in berry puree. Infectivity was still detectable for human norovirus GI.3, in strawberry puree, after 450 MPa for 5 minutes. Infectious human norovirus GII.4 was not detected when exposed to 400 MPa for 7.5 minutes (but was still detected following shorter time periods). At 450 MPa, infectious norovirus GII was not detected after 5 minutes exposure. By comparing the results to PBS, some protective effect was observed. MNV and Tulane virus in strawberry puree behaved similarly when treated with HPP at different pressure/time combinations. However, the results are reported as figures displaying the $\log_{10}$ MNV and Tulane virus $TCID_{50}$/mL values measured, rather than showing the reduction of infectious viruses, and were shown on a $\log_{10}$ scale. This creates ambiguity, so

individual data points are not included in **Table 8**. Using the control as a comparator, some general trends were evident. HPP at the highest pressure applied (450 MPa) and the longest time at this pressure (5 minutes) reduced the MNV and Tulane virus concentrations below the limit of detection (estimated as a 4 $\log_{10}$ reduction, i.e., approximately 6 $\log_{10}$ $TCID_{50}$/mL to 2 $\log_{10}$ $TCID_{50}$/mL). At 350 MPa, treatment for 5 minutes reduced the viral concentration by a relatively small amount compared to other pressure/time treatments and the control (the concentration of Tulane virus after HPP treatment was not significantly different to the untreated control).

While the authors of the above studies described any non-detections by HIE as 'complete inactivation', given the fact that the HIE assay has low sensitivity and that log reductions cannot be readily determined for HIE, the actual reductions in terms of infectivity on human norovirus are not known. However, it was postulated that conditions ≥450 MPa (for example for 5 minutes) would be sufficient to damage the viral capsid enough to render viruses non-infectious. By comparing the norovirus results to that obtained for Tulane virus and MNV, it was concluded that these were conservative surrogates for norovirus GII (for HPP in strawberry puree).

Table 8. Effect of high-pressure processing on hepatitis A virus (HAV), murine norovirus (MNV), MS2 and human norovirus for fresh/frozen berries, purees and/or juices

Virus	Fruit	Pressure (MPa)	Time (minutes)	Temperature (°C)	pH[a]	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
HAV	Strawberry puree	225	5	Ambient	3.49	0.4	Added as liquid	Plaque (PFU)	Kingsley *et al*., 2005
		250				1.2			
		275				2.1			
		300				3.1			
		325				3.8			
		350				4.2			
		375				4.3			
HAV	Strawberries (fresh and frozen) Blueberries (fresh) Blueberries (frozen)	400	3	4		>2	Spot	Plaque (PFU)	Ye *et al*., 2018
		500				not detected[b]			
		400				1.1			
		400				1.2			
MNV	Strawberry puree	400	2	4	2.5	2.8	Added as liquid	Plaque (PFU)	Lou *et al*., 2011
		400			3.5	3.6			
		400			4.5	3.8			
		400			5.5	4.3			
		400			6.5	4.8			
		350		4		2.2			
		400				4			
		450				4.7			
		350		20		0.8			
		400				1.6			
		450				4			
MNV	Strawberry pieces	350		4		2.2	Added to bag with fruit	Plaque (PFU)	Lou *et al*., 2011
		400				4.7			
		450				5.9			
		350		20		0.2			
		400				1.8			
		450				4.8			
MNV	Strawberry puree	200	2.5	4		1.0	Added as liquid	End point ($TCID_{50}$)	Kovac *et al*., 2012
		300				1.2			
		400				not detected[b]			
		600				not detected[b]			
		200	5	4		1.1			
		300				2.6			
		400				not detected[b]			
		600				not detected[b]			
		200	10	4		3.2			
		300				2.8			
		400				not detected[b]			

Virus	Fruit	Pressure (MPa)	Time (minutes)	Temperature (°C)	pH[a]	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
		600				not detected[b]			
MNV	Strawberry puree	350	2	0		4.4	Added as liquid	Plaque (PFU)	Huang et al., 2014
				5		3.2			
				10		1.5			
				20		0.5			
Norovirus GI.1	Strawberry puree	450	2	0		1.4		RT-qPCR	Huang et al., 2016
		500				2.5			
		550				>3.0[c]			
	Blueberry puree	450	2	0		1.6			
		500				2.4			
		550				>2.9[c]			
	Raspberry puree	450	2	0		2.7			
		500				2.7			
		550				>2.9[c]			
	Strawberry quarters	450	2	0		0.6			
		500				0.9			
		550				1.4			
		600				1.9			
		650				1.7			
	Blueberries	450	2	0		2.7			
		500				>3.2[c]			
		550				>3.2[c]			
	Raspberries	450	2	0		1.5			
		500				1.9			
		550				2.1			
		600				2.2			
		650				2.5			
Norovirus GII.4	Strawberry puree	250	2	0		0.1			
		300				0.7			
		350				2.4			
		400				>4.2[c]			
		450				>4.2[c]			
		500				>4.2[c]			
	Blueberry puree	300	2	0		1.1			
		350				1.6			
		400				2.1			
		450				2.6			
		500				3.5			
		550				>4.4			
		600				>4.4			
	Raspberry puree	300	2	0		3.7			
		350				>4.2[c]			
		400				>4.2 [c]			

Virus	Fruit	Pressure (MPa)	Time (minutes)	Temperature (°C)	pH[a]	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
	Strawberry quarters	550	2	0		2.8			
		600				3.0			
		650				3.1			
	Blueberries	250	2	0		3.2			
		300				>4.1[c]			
	Raspberries	500	2	0		3.5			
		550				>4.1[c]			
		600				>4.1[c]			
Norovirus GI.1	Strawberry puree	450	2	0		1.4		RT-qPCR	Huang *et al.*, 2016
				4		1.3			
				20		0.9			
		500	2	0		2.5			
				4		2.2			
				20		1.0			
		550	2	0		3.0			
				4		2.9			
				20		1.5			
MNV	Strawberry puree	250	3	20		0.2		Plaque (PFU)	Pan *et al.*, 2016
		300				0.7			
		350				2.0			
		400				4.3			
		424	3	20		5.0[d]		NA[c]	
MNV	Strawberry juice	300	1.5	20		0.9		Plaque (PFU)	
			3	10		1.7			
				20		1.2			
				30		0.3			
MS2	Strawberry puree	600	3	15		<1.0 (48%)[e]		Plaque (phage, PFU)	
				20		<1.0 (58%)[e]			
				38		<1.0 (68%)[e]			
				37		<1.0 (76%)[e]			
				37		<1.0 (87%)[e]			
				37		<1.0 (94%)[e]			
MNV	Blueberries (example)	350	2	4		4.7		Plaque (PFU)	Li *et al.*, 2013
				21		0.3			
				35		0			
		400		4		5.6			
				21		2.3			
				35		0			
Human norovirus	Strawberry puree	400	2	4	4	not significant		PGM-MB	DiCaprio et al., 2019

[a]Not always reported; [b]No virus detected, and no minimum log_{10} reduction provided; [c] More than the detection limit, so the log reduction may be equal to or greater than this value; [d]Predicted reduction based on regression equation; [e]Percentage recovery provided as reduction was less than 1 log_{10}.

3.3 ULTRAVIOLET LIGHT

24 3.3.1 Introduction

Ultraviolet (UV) light, with a germicidal range typically between 200-300 nm (UV-C), has been USFDA-approved for controlling microorganisms on surfaces and in juice products (USFDA, 1996b). This form of radiation damages nucleic acids in cells and viruses, primarily through the formation of pyrimidine dimers and other photoproducts, leading to their inactivation (Augsburger *et al.*, 2021). Studies have demonstrated the effectiveness of UV light in reducing bacteria and viruses on various food surfaces like blueberries, strawberries, lettuce, and onions, with minimal impact on the nutritional content, and sensory qualities (colour, odour and pH) of the food compared with heat treatments (Butot *et al.*, 2018; Fino and Kniel, 2008; Pala and Toklucu, 2013). In addition, UV is low cost, with no toxic by-products generated (Butot *et al.*, 2018).

A significant limitation of UV treatment is its shallow penetration depth on opaque food surfaces, making it challenging to inactivate viruses attached to rough surfaces or in crevices, such as within or along seed pockets in berry fruit. Viruses may also be internalised, possibly through contaminated irrigation water, and these would also not be susceptible to UV treatment. Microorganisms on food surfaces must directly face the UV lamp for inactivation to occur, further restricting its efficacy. To our knowledge there are no guidelines available for virus inactivation in berries using UV.

25 3.3.2 Studies

The log reduction data and UV conditions for the relevant studies are summarised in **Table 9**. UV inactivation studies have been performed on Aichi virus, MNV and FCV, in addition to HAV.

Fino and Kniel (2008) seeded HAV and surrogates (i.e., Aichi virus and FCV) on fresh strawberries. The seeded berries were treated by UV light at 253.7 nm wavelength. The effect of UV on HAV on inoculated strawberries was determined by cell culture ($TCID_{50}$ assay). Using an inoculum of 10^7 to 10^9 $TCID_{50}$, reductions of infectious viruses were 1.3, 1.8 and 2.6 log_{10} after UV dose exposure of 40, 120 and 240 mW s/cm^2, respectively. This was also performed for FCV and Aichi virus. For FCV, the same UV doses achieved reductions of 1.1, 1.6 and 2.3 log_{10}, respectively, while for Aichi virus infectivity reductions were 1.5, 1.6 and 1.9 log_{10}, respectively.

Butot *et al.* (2018) evaluated the efficacy of UV-C light to inactivate viral pathogens on fresh and frozen berries (strawberries, raspberries and blueberries). UV-C average fluence of 1331 mJ cm^{-2} was used for 2 minutes. HAV and MNV (as a surrogate for human norovirus) were spot inoculated on the fruit surface and the titre determined using endpoint $TCID_{50}$ assay. HAV on frozen berries showed a slightly higher sensitivity to UV-C treatment compared to fresh strawberries. Mean HAV reductions of 1.3 and 1.7 log_{10} were determined for fresh strawberries and frozen strawberries, respectively. These reductions compared to 2.4 and 2.6 for fresh and frozen blueberries, and to 1.6 and 1.5 log_{10} for fresh and frozen raspberries, respectively (**Table 9**). Inactivation experiments were also done with MNV. In general, MNV and HAV were shown to have similar sensitivities to UV-C treatment at the same fluence. MNV reductions were 1.3 and 0.8 log_{10} for strawberries and frozen strawberries, respectively, with reductions of 3.1, 2.1, 1.5, and 0.6 log_{10} for blueberries, frozen blueberries, raspberries, and frozen raspberries, respectively.

A novel water-assisted UV treatment was assessed by immersing MNV-seeded fresh, whole blueberries in agitated water during the UV treatment (Liu *et al.*, 2015). The method was developed to address the limitation of shallow penetration and the need of the food surface to face the UV light. Before seeding with MNV, the berries were UV-treated to reduce background microflora. A

MNV titre of 7 log_{10} PFU was spotted on the fruit surface and the virus was measured using a plaque assay (initial virus numbers were 6.8 log_{10} PFU/sample). UV intensity of 10 mW/cm^2 and water depth of 6.5 cm, and incubation time of 1, 2, and 5 minutes were used for the inactivation conditions. A dry UV treatment (without water immersion) was also conducted. The results showed 3.2, >4 and >4 log_{10} reduction for UV water-assisted inactivation with 1, 2 and 5 minutes, respectively. The reduction was higher compared to UV dry treatment which showed a 2.5, 2.5 and 3.2 log_{10} reduction for 10 mW/cm^2 fluence and 1, 2 and 5 minutes, respectively. The treatment was also more effective compared to using water only. A 5-minute treatment with water reduced MNV by 1.7 log_{10} PFU/sample. The UV inactivated virus particles both on the fruit and in the wash water.

Water-assisted UV-C treatment effects on MNV on whole and fresh cut strawberries were part of a larger study that included bacterial pathogens (Ortiz-Solà *et al.*, 2022). The UV-C was applied at 10.5 W m^{-2} for 2 minutes (1.3 kJ m^{-2}). Log reductions were modest: 1.4 log_{10} for conventional dry UV-C, 1.7 log_{10} for hypochlorite solution (200 mg/L), 1.3 log_{10} for water-assisted UV-C, and 1.6 log_{10} for water-assisted UV-C plus peracetic acid (40 mg/L).

Table 9. Effect of UV treatment on Aichi virus, feline calicivirus (FCV), hepatitis A virus (HAV), and murine norovirus (MNV) on fresh and/or frozen berries

Virus	Fruit	Condition	Time (minute)	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
Aichi virus	Strawberries	40 mW s/cm^2		1.5	Surface, spot	Endpoint ($TCID_{50}$)	Fino and Kniel, 2008
		120 mW s/cm^2		1.6			
		240 mW s/cm^2		1.9			
FCV		40 mW s/cm^2		1.1			
		120 mW s/cm^2		1.6			
		240 mW s/cm^2		2.3			
HAV		40 mW s/cm^2		1.3			
		120 mW s/cm^2		1.8			
		240 mW s/cm^2		2.6			
HAV	Strawberries	1331 mJ cm^2	2	1.3	Surface, spot	Endpoint ($TCID_{50}$)	Butot *et al*., 2018
	Frozen strawberries	1331 mJ cm^2	2	1.7			
	Blueberries	1331 mJ cm^2	2	2.4			
	Frozen blueberries	1331 mJ cm^2	2	2.6			
	Raspberries	1331 mJ cm^2	2	1.6			
	Frozen raspberries	1331 mJ cm^2	2	1.5			
MNV	Blueberries	10 mW/cm^2	1	2.5	Surface, spot	Plaque (PFU)	Liu *et al*., 2015
			2	2.5			
			5	3.0			
	Blueberries	10 mW/cm^2 (water-assisted)	1	3.2			
			2	>4[a]			
			5	>4[a]			
	Strawberries	1331 mJ cm^2	2	1.3	Surface, spot	Endpoint ($TCID_{50}$)	Butot *et al*., 2018
	Frozen strawberries	1331 mJ cm^2	2	0.8			
	Blueberries	1331 mJ cm^2	2	3.1			
	Frozen blueberries	1331 mJ cm^2	2	2.1			
	Raspberries	1331 mJ cm^2	2	1.5			
	Frozen raspberries	1331 mJ cm^2	2	0.6			

[a] More than the detection limit, so the log reduction may be equal to or greater than this value.

3.4 PULSED LIGHT

26 3.4.1 Introduction

Pulsed light is a disinfectant technology intended to avoid degradation of delicate food products. This treatment involves exposing the sample to a burst of high intensity white light (wavelength from 200 to 1100 nm, hence UV is included) for microseconds (μs) (Jubinville *et al.*, 2022). This method has been approved by the USFDA for the treatment of food since 1996 with conditions that limit pulse duration (2000 μs maximum) and the total fluence or energy absorbed per unit of product surface to 12 J/cm^2 (USFDA, 1996a). Despite being approved since 1996, large scale use by the food industry has not occurred (Rowan, 2019). This is attributed to the absence of international harmonisation and consensus on what constitutes priority experimental methods and exposure conditions. Rowan (2019) provides recommendations for reporting of experimental data and parameters.

Pulsed light has been applied to juice, syrup (Rowan, 2019), milk, water and berries (Huang and Chen, 2015; Jubinville *et al.*, 2022).

27 3.4.2 Studies

The studies that assess virus inactivation on whole (or halved) berry fruit with pulsed light are shown in **Table 10**.

The effect of pulsed light treatment, with an intensity of 830 J and 2700 volt to obtain a fluence of 11.78 J/cm^2, on HAV and MNV on strawberries, raspberries and blackberries was evaluated (Jubinville *et al.*, 2022). To do this, HAV and MNV were spot inoculated (approximately 4.0 $\log_{10}$ PFU per sample, with each sample consisting of three berries) and left to dry for 90 minutes before treatment. For HAV, 2.1, 2.0 and 1.3 $\log_{10}$ reductions for strawberries, raspberries, and blackberries, respectively, were demonstrated. Reductions for MNV were similar, including lower reduction in blackberries. This study commented that pulsed light did not change the appearance of strawberries, raspberries and blackberries. Blueberries, which were not tested for virus inactivation, did show some colour change (darkening). Heat was minimised by an icepack.

As previous studies had shown significant temperature increases during pulsed light treatment of fresh produce, which affected sensory perception, a water-assisted pulsed light inactivation was developed. Huang and Chen (2015) spot-contaminated berries with MNV and treated them with pulsed light while being agitated in water. The concentration of the MNV inoculum was 4.5 $\log_{10}$ PFU/g berries. MNV reductions of 1.8 and 3.6 $\log_{10}$ after 63.2 J/cm^2 and 53.9 J/cm^2 of water-assisted pulsed light for strawberries and raspberries, respectively, were shown. Agitating the berries in water with 1% hydrogen peroxide increased the MNV reduction (2.2 $\log_{10}$) for strawberries but reduced it for raspberries (2.5 $\log_{10}$). These reductions were greater than those achieved by simply washing in 10 parts per million (ppm) chlorinated water (1.3 $\log_{10}$ and 2.2 $\log_{10}$ for strawberries and raspberries respectively).

Another study evaluated the effect of pulsed light inactivation on MNV on berries (Huang *et al.*, 2017). The initial concentration for MNV for the spot inoculation was 150 μL of 6 $\log_{10}$ PFU/mL as determined by plaque assay. With pulsed light fluence of 5.9 J/cm^2 and 6 seconds exposure, there were 0.7 and 3.1 $\log_{10}$ reductions of MNV on strawberries and blueberries, respectively. Higher exposure fluence of 11.4 J/cm^2 for 12 seconds and 22.5 J/cm^2 for 24 seconds caused MNV infectivity to reduce by 0.9 $\log_{10}$ on strawberries, and by 3.2 and 3.8 $\log_{10}$ on blueberries.

A study using Tulane virus to assess the effect of 405-nm visible light as a treatment on blueberries has been reported (Kingsley *et al.*, 2018a). In the study, five blueberries were seeded

with 2 x 10^7 PFU total and dried for 1 hour prior to treatment with 405 nm light for 5, 15 and 30 minutes, emitting 4.2 mW/cm^2. During the experiment, the surface temperature did not exceed 32 °C. Little or no change in titres following 5, 15 and 30 minute exposure was observed (i.e., authors reported average $\log_{10}$ changes of -0.2, -0.02 and +0.06 $\log_{10}$, respectively).

Riboflavin and rose bengal interact with light radiation to form reactive oxygen species (oxygen radicals), which can inactivate viruses. When 0.1% riboflavin or 0.1% rose bengal was used to coat the blueberries prior to 4.2 mW/cm^2 light exposure for 30 minutes, inactivation in Tulane virus infectivity was 0.5 and 1.0 $\log_{10}$, respectively. It was noted though that rose bengal alone (with no light treatment) reduced infectivity by 0.7 $\log_{10}$. Overall, it was concluded that Tulane virus was resistant to 405nm light treatment.

Table 10. Effect of pulsed light exposure on hepatitis A virus (HAV), murine norovirus (MNV) and Tulane virus on berries

Virus	Fruit	Condition (Dose/Fluence)	Time (seconds)	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
HAV	Strawberries	11.78 J/cm^2	not reported	2.1	Surface, spot	Plaque (PFU)	Jubinville *et al*., 2022
	Raspberries			2.0			
	Blackberries			1.3			
MNV	Strawberries	11.78 J/cm^2	not reported	1.6			
	Raspberries			1.9			
	Blackberries			1.4			
MNV	Strawberries	63.2 J/cm^2	60	1.8	Surface, coating	Plaque (PFU)	Huang and Chen, 2015
	Raspberries	53.9 J/cm^2	60	3.6			
MNV	Strawberries[a]	5.9 J/cm^2	6	0.7	Surface, spot	Plaque (PFU)	Huang *et al*., 2017
		11.4 J/cm^2	12	0.9			
		22.5 J/cm^2	24	0.9			
	Blueberries	5.9 J/cm^2	6	3.1			
		11.4 J/cm^2	12	3.2			
		22.5 J/cm^2	24	3.8			
Tulane virus	Blueberries	405 nm light 4.2 mW/cm^2	300 (5 min)	<0.2	Surface, coating	Plaque (PFU)	Kingsley *et al*., 2018a
			900 (15 min)	<0.2			
			1800 (30 min)	0.0			

[a]Strawberry halves

3.5 OZONE – GASEOUS

28 3.5.1 Introduction

Ozone (O_3), applied in an aqueous or gaseous form, can be used as an effective and safe disinfectant agent to preserve foods (i.e., quality and shelf-life). As an oxidising agent, ozone disrupts virus capsid integrity by degrading proteins, which affects infectivity. The effectiveness of viral inactivation depends on ozone concentration, exposure time, temperature and pH, as well as the characteristics of the virus (Xue *et al.*, 2023). Ozone does not affect nucleic acid, so RT-qPCR output would remain unaffected following treatment (Predmore *et al.*, 2015a). Studies in water show that ozone can inactivate HAV by 4 $\log_{10}$ at 1 ppm for 1 minute (Khadre *et al.*, 2001). Ozone causes changes to the texture and colour of strawberries (Predmore *et al.*, 2015a).

29 3.5.2 Studies

There are two studies that assessed virus inactivation on whole berry fruit with ozone. The results are shown in **Table 11**.

Predmore *et al.* (2015a) assessed MNV and Tulane infectivity on fresh strawberries following exposure to gaseous ozone. The viruses were seeded onto fresh strawberries via surface pipetting (as spots), or unusually, the virus was injected into the flesh. Plaque assays were used for experiments using both viruses. The results showed that Tulane virus was more sensitive to ozone than MNV. For example, after 10 minutes with a concentration of 6% wt/wt, MNV infectivity reduced by approximately 2 $\log_{10}$ compared to a 3.7 $\log_{10}$ reduction for Tulane virus. The injected viruses were protected from the gaseous ozone treatment. A reduction of <2 $\log_{10}$ was measured after an ozone treatment lasting 40 minutes.

In a later study, Brié *et al.* (2018) assessed the effect of exposure to gaseous ozone on HAV and MNV infectivity seeded onto fresh raspberries via surface spot inoculation. Plaque assay was used for HAV, and end point assay ($TCID_{50}$) for MNV. An ozone concentration of 4 ppm with a contact time of 2 minutes, caused a loss of MNV infectivity of at least 3.3 $\log_{10}$ (same as measured for 3 ppm, 1 minute). A reduced ozone concentration of 1 ppm for 3 minutes resulted in a reduction of approximately 2 $\log_{10}$. The MNV result contrasted with HAV, where 5 ppm for 3 minutes resulted in minimal loss (0.3 $\log_{10}$ reduction). Loss of virus infectivity on fruit was greater than in PBS. Inactivation was also measured using RT-qPCR, which did not detect significant changes in the number of viral genome copies after the ozone treatments. While this study showed that ozone had no effect on the appearance of the food following treatment, the results indicated that gaseous ozone at a concentration of 5 ppm for 3 minutes was ineffective for HAV inactivation on whole berry fruit (in this case raspberries).

Table 11. Effect of ozone on murine norovirus (MNV), Tulane virus and hepatitis A virus (HAV) on whole strawberries and raspberries

Virus	Fruit	Condition	Time	Initial[a] (log_{10})	After (log_{10})	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
MNV	Whole strawberries	6% wt/wt	10 min	6.0	3.8	2.2	Surface, spots	Plaque (PFU)	Predmore *et al.,* 2015[a]
			20 min		4.0	2.0			
			30 min		3.5	2.5			
			40 min		2.5	3.5			
			10 min	6.3	6.2	0.1	Injection		
			20 min		5.3	1.0			
			30 min		5.7	0.6			
			40 min		4.7	1.6			
Tulane virus	Whole strawberries	6% wt/wt	10 min	5.8	2.1	3.7	Surface, spots		
			20 min		1.7	4.1			
			30 min		1.5	4.3			
			40 min		0.3	5.5			
			10 min	4.7	4.7	0.0	Injection		
			20 min		4.1	0.6			
			30 min		4.1	0.6			
			40 min		2.8	1.9			
HAV MNV	Whole raspberries[b]	5 ppm	3 min	not reported		0.3	Surface, spots	Endpoint ($TCID_{50}$)	Brie *et al.*, 2018
		1 ppm	3 min	not reported		1.8			
		3 ppm	1 min	not reported		>3.3[c]			
		4 ppm	2 min	not reported		>3.3[c]			

[a]The value is the virus titre at time 0 (T0; i.e., no treatment) following virus recovery from the matrix.
[b]Total of 6 log_{10} PFU (HAV) or 6 $log_{10}TCID_{50}$ (MNV) per 25 g fruit added, stored at 20 hours prior to ozone treatment. The T0 value was not described.
[c] More than the detection limit, so the log_{10} reduction may be equal to or greater than this value.

3.6 IRRADIATION

30 3.6.1 Introduction

Irradiation is a widely used technology for the prevention of food spoilage and to reduce or eliminate microbiological hazards in food (IFST, 2022). Irradiation (both gamma and e-beam irradiation) causes viral proteins and nucleic acid to degrade.

Gamma radiation from the radioactive isotopes Cobalt-60 (^{60}Co) or Caesium-137 (^{137}Cs) can be used for food irradiation.[6] Irradiation is measured in kilograys (kGy), with, for example doses of 2-4 kGy used for food products to maintain organoleptic properties. Gamma radiation can penetrate foods, as well as being active on the surface. A study on strawberry cells (i.e., homogenised and sieved) showed that 10 kGy radiation, considered a high dose, did not significantly affect cell appearance but 20 kGy did affect the texture and quality (Molina-Chavarria *et al.*, 2020).

31 3.6.2 Studies: Gamma irradiation

Published studies describe the assessment of virus inactivation on whole berry fruit using gamma radiation (**Table 12**). These experiments used a Cobalt-60 experimental chamber with a dose rate of 1.6 kGy/hour (Pimenta *et al.*, 2019) generating doses from 0.175 to 22.4 kGy (Feng *et al.*, 2011) or a Gamma cell 220 irradiator that delivered 4 kGy/hour (0.4 Mrad/hour) using a cobalt (^{60}Co) source (Bidawid *et al.*, 2000).

Bidawid *et al.* (2000) applied gamma radiation at ambient temperature to HAV inoculated onto the surface of strawberries. By applying doses from 1 to 10 kGy, a linear reduction in infectivity was observed. Based on this relationship, a dose between 2.7 and 3.0 kGy caused a 1 $\log_{10}$ reduction in HAV. The maximum dose tested, 10 kGy, reduced infectious HAV by almost 3 $\log_{10}$ (**Table 12**).

The effect of gamma irradiation using a range of doses on MNV on strawberries was determined (Feng *et al.*, 2011). MNV was relatively resistant to gamma irradiation, with doses of more than 5 kGy required to achieve a 2 $\log_{10}$ reduction in infectivity, and 11.2 kGy required for a 4.1 $\log_{10}$ reduction (**Table 12**). No infectious MNV was detected after irradiation at 22.4 kGy. This study also included an assessment of persistence of human norovirus virus-like particles (capsid protein analysis) and infectivity of vesicular stomatitis virus, but as this was not carried out on produce, results are not reported here.

In another study, whole raspberries and whole strawberries inoculated with MNV or human adenovirus (HAdV) type 5 were subjected to gamma radiation between 0.9 and 7.6 kGy (Pimenta *et al.*, 2019). To better explore the effect of higher power gamma irradiation, a separate experiment was done using between 3.6 and 11.3 kGy for raspberries. Virus reductions for MNV and HAdV were similar for both raspberries and strawberries. For example, on strawberries, a 2.1 and 2.2 $\log_{10}$ reduction was achieved at 3.7 kGy for HAdV and MNV respectively. Reductions were similar for raspberries where 2.0 and 2.2 $\log_{10}$ reduction was achieved at 3.4 kGy for HAdV and MNV respectively. Approximately 3 $\log_{10}$ reduction was achieved with a dose of approximately 7 kGy – similar levels to that reported by Bidawid *et al.* (2000). D_{10} values (kGy required to reduce infectivity by 1 $\log_{10}$) of MNV and HAdV confirmed the resistance of these viruses to gamma radiation. D_{10} values of between 2.8 and 3.4 kGy for HAdV, and 2.6 and 3.2 for MNV were calculated (Pimenta *et al.*, 2019). Values were similar for experiments using strawberries and raspberries.

[6] Standard 1.5.3 of the Australia New Zealand Food Standards Code sets out permissions for food traded in New Zealand (https://www.foodstandards.gov.au/food-standards-code/legislation, accessed 23 July 2024).

Other studies have assessed gamma radiation on norovirus and Tulane virus, but these experiments were performed on viruses in liquid suspension only (DiCaprio *et al.*, 2016) and so are not reported here.

Table 12. Effect of gamma irradiation on hepatitis A virus (HAV), murine norovirus (MNV) and human adenovirus (HAdV) type 5 on whole strawberries and raspberries

Virus	Fruit	Condition (kGy)	Initial (log_{10})[a]	After (log_{10})	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
HAV	Whole strawberries	1	6.7	6.6	0.1	Surface, spread	Plaque (PFU)	Bidawid *et al*., 2000
		2		6.4	0.3			
		3		6.4	0.3			
		4		5.9	0.8			
		5		5.0	1.7			
		6		4.8	1.9			
		7		4.5	2.2			
		8		4.3	2.4			
		9		3.9	2.8			
		10		3.8	2.9			
MNV	Whole strawberries	0.1-0.7	7.3	6.8-7.0	0.3-0.5	Surface, spread	Plaque (PFU)	Feng *et al.,* 2011
		2.8		6.0	1.3			
		5.6		4.9	2.4			
		11.2		3.2	4.1			
		22.4		not detected[b]				
HAdV type 5	Whole raspberries	1	4.7	3.8	1.0	Surface, spots	Plaque (PFU)	Pimenta *et al.,* 2019
		3.4		2.7	2.0			
		7		1.7	3.0			
	Whole strawberries	1	4.6	3.4	1.3			
		3.7		2.5	2.1			
		7.6		1.5	3.1			
MNV	Whole raspberries	1	4.3	3.1	1.2	Surface, spots	Plaque (PFU)	
		3.4		2.1	2.2			
		7		1.1	3.2			
	Whole strawberries	1	4.5	3.3	1.2			
		3.7		2.3	2.2			
		7.6		1.0	3.5			

[a] The value is the virus titre at time 0 (T0; i.e., no treatment) following virus recovery from the matrix.
[b]Not detected means that the detection limit was reached for 22.4 kGy but was not specified.

32 3.6.3 Studies: Electron beam (e-beam) irradiation

Electron beams are generated by passing electrons through a high voltage electrostatic field, achieving energies up to 10 MeV. Compared to gamma radiation, e-beams do not penetrate as far (3-10 cm compared to 1 m for gamma) into the product. DiCaprio *et al.* (2016) showed that e-beam irradiation degraded viral capsid structure and nucleic acid of viruses. Studies reviewed for this report noted changes in the physical appearance and texture such as 'gumminess' of strawberries at doses greater than 8 kGy (DiCaprio *et al.*, 2016; Predmore *et al.*, 2015b). Inactivation results are summarised in **Table 13**.

Strawberries subjected to e-beam irradiation showed that doses of 16 kGy or higher reduced Tulane virus infectivity (as determined by culture) to non-detectable levels. Lower doses decreased infectivity by 1-2 $\log_{10}$. The reduction of Tulane virus and human norovirus titres as determined by PGM-MB binding assay was less (approximately 2 $\log_{10}$ and >1 $\log_{10}$ respectively at 12.2 kGy), than observed for culture. This difference decreased with increasing dosages (DiCaprio *et al.*, 2016).

Butot *et al.* (2021) investigated the effect of e-beams on HAV and MNV (plus MS2, Qβ and other targets) on freeze-dried raspberries and frozen blueberries. Poor recovery (<1%) of HAV and MNV affected the maximum measurable $\log_{10}$ reduction, and thus the ability to determine any reductions greater than 2 or 3 logs. On frozen blueberries, HAV reduced by 0.5, 1.4 and 2.4 $\log_{10}$ after treatment with 4, 8 and 16 kGy, respectively. The blueberry experiments suggested that HAV was more susceptible to inactivation by e-beam irradiation, but the MNV results were affected by the poor recovery (the reduction values for five of the nine MNV replicates were below the maximum measurable reduction).

Sanglay *et al.* (2011) showed that a 4 kGy e-beam dose was associated with a small reduction (0.4 $\log_{10}$) in MNV infectivity (on strawberries). This was less than that observed by Predmore *et al.* (2015b) for Tulane virus where a reduction of 1.4 $\log_{10}$ was observed at the same kGy dose. Tulane virus was not detected at all after a dose of 16 kGy or more, suggesting that Tulane virus is more sensitive to e-beams than MNV. Overall, viral reductions are low at e-beam doses of 4 kGy or less. While higher doses are more effective in terms of reduction, there are detrimental effects on the food such as discoloration, loss of firmness, and changes to sensory properties at these levels.

Table 13. Effect of e-beam radiation on hepatitis A virus (HAV), Tulane virus, human norovirus, and/or murine norovirus (MNV) on whole and chopped strawberries, freeze dried raspberries and/or whole frozen blueberries

Virus	Fruit	Condition (kGy)	Initial[a] (log_{10})	After (log_{10})	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
MNV	Chopped strawberries	2	5.4	5.3	0.1	Surface, liquid added in bag	Plaque (PFU)	Sanglay *et al*., 2011
		4		5.0	0.4			
		6		4.4	1.0			
		8		3.8	1.6			
		10		3.5	1.9			
		12		3.2	2.2			
Tulane virus	Whole strawberries	4	4.4	3.0	1.4	Surface, liquid added in bag	Plaque (PFU)	Predmore *et al*., 2015
		8		1.8	2.6			
		16		not detected				
		25		not detected				
		30		not detected				
Tulane virus	Whole strawberries	1.7	4.9	4.9	0.0	Injection	Plaque (PFU)	DiCaprio *et al*., 2016
		9.8		3.6	1.3			
		12.2		2.8	2.1			
		16.3		not detected				
		28.7		not detected				
		1.7	5.3	5.3	0.0		PGM binding assay	
		9.8		5.0	0.3			
		12.2		3.2	2.1			
		16.3		3.1	2.2			
		28.7		not detected				
Human norovirus	Whole strawberries	1.7	5.5	5.0	0.5		PGM binding assay	
		9.8		5.0	0.5			
		12.2		4.3	1.2			
		16.3		3.0	2.5			
		28.7		not detected				
HAV	Freeze-dried raspberries	4		not reported[b]	1.3	Surface, spots	Endpoint, ($TCID_{50}$)	Butot *et al*., 2021
		8			≥1.6[c]			
		16			≥1.7[c]			
	Frozen, whole blueberries	4		not reported[b]	0.5			
		8			1.4			
		16			≥2.4[c]			
MNV	Frozen, whole blueberries	4		not reported[b]	0.4			
		8			≥0.8[c]			
		16			≥1.2[c]			

[a]The value is the virus titre at time 0 (i.e., no treatment) following virus recovery from the matrix; [b] From separate trials to measure recovery efficiencies, an example of a T0 value reported for HAV inoculated on freeze-dried raspberries was 3.04 log_{10} $TCID_{50}$/5 g (recovered from 5.45 log_{10} $TCID_{50}$/5 g); [c] Due to low recovery rates and the detection limit quantification threshold, the log reduction may be equal to or greater than this value. In these experiments, poor recovery (<1%) was noted.

3.7 COLD ATMOSPHERIC PLASMA

33 3.7.1 Introduction

Cold atmospheric plasma (CAP) is a non-thermal technology used in many applications including for surface decontamination and increasing shelf-life of foods. CAP is generated by applying an electromagnetic or electric field to a gas. While it has been shown to have an impact on virus infectivity, the mode of action is unclear.

34 3.7.2 Studies

Velebit *et al.* (2022) determined that 1 minute of CAP treatment reduced the number of infectious HAV particles on whole raspberries, with the largest reduction being 4.1 $\log_{10}$ after a 10 minute treatment. MNV was more sensitive to treatment than HAV, with less time required to reduce the infectivity by approximately 4 $\log_{10}$ (5 minutes for MNV compared to 10 minutes for HAV) (**Table 14**). The authors proposed that CAP would be suitable to inactivate viruses during production and processing of berries. However, they measured changes to raspberry colour in CAP treatments exceeding 7 minutes, with the berries becoming darker, less red and less yellow.

In another laboratory-scale CAP test, blueberries inoculated with MNV or Tulane virus (added to the fruit, vortexed and dried for 1 hour prior to treatment) were subjected to CAP treatment for up to 2 minutes (Lacombe *et al.*, 2017). It was shown that when treated for 60 seconds, the temperature of the product reached 70 °C, so a separate set of experiments were performed by adding air steam (force air cooling) to reduce the temperature (to 47 °C after 2 minutes) to ensure thermal inactivation was not a factor. The experiment showed that the CAP treatment was not thermal (i.e., virus inactivation was due to the cold plasma and not heat). The maximum reduction measured for Tulane virus was 3.5 $\log_{10}$ after 2 minutes exposure. MNV was more sensitive than Tulane virus to CAP. For example, with a 1-minute CAP treatment (with cooling), the concentration of infective MNV reduced by 5.4 $\log_{10}$ compared to 1.8 $\log_{10}$ for Tulane virus (**Table 14**).

Table 14. Effect of cold atmospheric plasma on hepatitis A virus (HAV), murine norovirus (MNV) and Tulane virus on whole raspberries and blueberries

Virus	Fruit	Condition Time (min)	Initial (log_{10})	After (log_{10})	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
HAV	Whole raspberries	1	not reported	not reported	0.6	Surface, aerosol	Plaque (PFU)	Velebit *et al*., 2022
		3			2.1			
		5			3.1			
		7			3.5			
		10			4.1			
MNV		1	not reported	not reported	0.7	Surface, aerosol	Plaque (PFU)	
		3			2.2			
		5			4.2			
		7			4.7			
		10			4.8			
Tulane virus	Whole blueberries	**Cold plasma only**						
		0.25	5.4	4.9	0.5	Surface, vortex	Plaque (PFU)	Lacombe *et al.,* 2017
		0.5		4.8	0.6			
		0.75		4.0	1.4			
		1		3.3	2.1			
		1.5		not done				
		2		not done				
		Cold plasma with air (to cool)						
		0.25	4.7	4.3	0.5			
		0.5		4.0	0.8			
		0.75		3.2	1.5			
		1		3.0	1.8			
		1.5		2.2	2.6			
		2		1.3	3.5			
MNV	Whole blueberries	**Cold plasma only**						
		0.25	5.9	5.3	0.5	Surface, vortex	Plaque (PFU)	Lacombe *et al*., 2017
		0.5		4.3	1.7			
		0.75		3.2	2.8			
		1		3.1	2.9			
		1.5		not done				
		2		not done				
		Cold plasma with air (to cool)						
		0.25	6.6	5.5	1.1			
		0.5		5.0	1.6			
		0.75		3.7	2.9			
		1		1.2	5.4			
		1.5		0.1	6.5			
		2		0.1	6.5			

3.8 CHLORINE DIOXIDE GAS

35 3.8.1 Introduction

Gaseous chlorine dioxide (ClO_2) is a strong oxidising agent and is recognised as an effective treatment for microorganism inactivation while preserving food quality. Limitations to its use include difficulties in generating ClO_2 at large-scale, costs, and environmental and worker safety.

36 3.8.2 Studies

There are limited data on the use of gaseous ClO_2 against HAV and other enteric viruses/surrogates on fresh and frozen berries. HAV and Tulane virus have been used across three studies.

A study of the effect of gaseous ClO_2 on HAV on blueberries, strawberries, blackberries, and raspberries was described by Annous *et al.* (2021). Individual berries were inoculated through 'coating' with 10 mL HAV suspension volume at 10^6 PFU/mL in sample cups/beakers for approximately 1 minute. The excess liquid was then discarded as the berries were removed and left to dry for 1 hour before treatment (on 100 g for strawberries, and 50 g for the other berry types). Gaseous ClO_2 concentrations delivered were 1 mg/L or 2 mg/L and ranged from 8.5 hours at 1 mg/L, to 1.15 hours at 1 mg/L, to give a total treatment of 1.00 to 6.27 ppm-h/g ClO_2. The berries were stored at 5 °C overnight prior to analysis by plaque assay. These experiments showed that at least a 2-$\log_{10}$ reduction could be achieved for any conditions or berry fruit tested, with HAV inactivation being the greatest on blueberries, and least on raspberries. Using 1.00 ppm-h/g ClO_2, HAV infectivity reductions ranged from 2.4 to 3.5 $\log_{10}$. For a treatment of 6.27 ppm-h/g ClO_2, reductions ranged from 3.2 to 4.4 $\log_{10}$ (**Table 15**).

An earlier study by the same group evaluated the effect of ClO_2 on Tulane virus infectivity (Kingsley and Annous, 2019). The same approach was taken as Annous *et al.* (2021), using blueberries, strawberries, blackberries, and raspberries, but the ClO_2 concentrations applied to the berries were lower. Tulane virus was inactivated by at least 3.0 $\log_{10}$ and up to 4.8 $\log_{10}$ with 1.25 ppm-h/g (1 mg/L). No significant differences were noted on the level of inactivation between the different fruits tested (**Table 15**).

This followed from a previous study on blueberries with Tulane virus that looked at the feasibility of using gaseous ClO_2 on blueberries (Kingsley *et al.*, 2018b). For that experiment, five individual blueberries per treatment were added to a 50 mL tube, 0.1 mL solution with Tulane virus was added (to give a total amount of 4 x 10^6 PFU), and the sample allowed to dry for 1 hour. By using sodium chlorite (up to 10 mg) and 10% HCl, gaseous ClO_2 was generated. The study showed that 2.5, 5 and 10 mg acidified sodium chlorite (ACS) for 30 minutes reduced Tulane virus infectivity by at least 2.7 $\log_{10}$ (i.e., to numbers below the detection limit). It was noted that the higher concentration adversely affected the appearance of the fruit. Less virus inactivation was evident at 0.1 and 1 mg ACS when exposed for 15 minutes, where 0.6 and approximately 2 $\log_{10}$ reductions were determined respectively.

Together these studies support the use of gaseous ClO_2 at levels (i.e., such as < 1 mg) that wouldn't affect the physical properties of the berry fruit, as an effective intervention strategy for HAV (and norovirus), particularly if a 3 $\log_{10}$ reduction in viral infectivity is sufficient, due to the low concentration present when naturally contaminated.

Table 15. Effect of gaseous chlorine dioxide on hepatitis A virus (HAV) and Tulane virus on whole berries

Virus	Fruit	Condition ClO_2 ppm-h/g (concentration applied)	Initial[a] (log_{10})	After (log_{10})	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
HAV	Whole blueberries	1 (1 mg/L)	5.0	1.8	3.2	Surface, coating	Plaque (PFU)	Annous *et al*., 2021
		2 (1 mg/L)	4.8	1.5	3.3			
		6.27 (1 mg/L)	5.0	0.7	4.3			
		2 (2 mg/L)	4.8	1.2	3.6			
		6.27 (2 mg/L)	5.0	0.6	4.4			
	Whole strawberries	1 (1 mg/L)	4.9	2.4	2.4			
		2 (1 mg/L)	5.4	2.3	3.2			
		6.27 (1 mg/L)	4.8	0.8	4.0			
		2 (2 mg/L)	5.0	2.3	2.7			
		6.27 (2 mg/L)	5.1	1.6	3.5			
	Whole blackberries	1 (1 mg/L)	4.4	0.9	3.5			
		2 (1 mg/L)	4.5	2.0	2.6			
		6.27 (1 mg/L)	4.7	1.5	3.2			
		2 (2 mg/L)	4.4	1.6	2.8			
		6.27 (2 mg/L)	4.7	1.4	3.2			
	Whole raspberries	1 (1 mg/L)	4.9	2.4	2.5			
		2 (1 mg/L)	4.9	2.8	2.2			
		6.27 (1 mg/L)	4.9	1.7	3.2			
		2 (2 mg/L)	4.3	1.9	2.5			
		6.27 (2 mg/L)	5.4	1.5	4.0			
Tulane	Whole blueberries	0.63 (1 mg/L)	5.0	1.2	3.8	Surface, coating	Plaque (PFU)	Kingsley and Annous, 2019
		1.25 (1 mg/L)	5.9	1.8	4.1			
		2.35 (1 mg/L)	5.5	0.8	4.6			
		3.01 (1 mg/L)	4.2	1.2	3.0			
		3.03 (2 mg/L)	5.6	1.0	4.6			
		4.40 (2 mg/L)	5.6	2.0	3.6			
	Whole strawberries	0.63 (1 mg/L)	5.3	2.3	3.0			
		1.25 (1 mg/L)	6.0	1.8	4.2			
		2.35 (1 mg/L)	5.5	1.8	3.8			
		3.01 (1 mg/L)	4.9	1.2	3.7			
		3.03 (2 mg/L)	5.7	1.0	4.7			
		4.40 (2 mg/L)	5.2	1.2	4.0			
	Whole blackberries	0.63 (1 mg/L)	5.0	0.8	4.2			
		1.25 (1 mg/L)	6.0	1.6	4.4			
		2.35 (1 mg/L)	6.2	2.4	3.8			
		3.01 (1 mg/L)	4.7	1.1	3.6			
		3.03 (2 mg/L)	5.4	0.8	4.6			
		4.40 (2 mg/L)	5.7	1.5	4.2			
	Whole raspberries	0.63 (1 mg/L)	5.2	1.8	3.4			
		1.25 (1 mg/L)	6.0	1.2	4.8			

Virus	Fruit	Condition ClO_2 ppm-h/g (concentration applied)	Initial[a] (log_{10})	After (log_{10})	Reduction (log_{10})	Inoculation method	Detection assay (unit)	Reference
		2.35 (1 mg/L)	5.6	2.7	2.9			
		3.01 (1 mg/L)	5.1	1.4	3.7			
		3.03 (2 mg/L)	5.8	2.1	3.7			
		4.40 (2 mg/L)	6.0	1.3	4.7			
Tulane	Whole blueberries	0.1 mg (30 min)[b]	not reported		0.6	Surface coating	Plaque (PFU)	Kingsley *et al.*, 2019
		1 mg (30 min)			2.5			
		2.5 mg (30 min)			>2.7[c]			
		5 mg (30 min)			>2.7[c]			
		10 mg (30 min)			>2.7[c]			
		0.1 mg (5 min)	not reported		0			
		0.1 mg (15 min)			0.6			
		0.1 mg (30 min)			1.2			
		0.1 mg (60 min)			1.2			
		0.1 mg (180 min)			1.2			
		0.1 mg (330 min)			1.2			
		1 mg (5 min)	not reported		1.0			
		1 mg (15 min)			2.2			
		1 mg (30 min)			2.9			
		1 mg (60 min)			2.8			
		1 mg (180 min)			>3.3[c]			
		1 mg (330 min)			>3.3 [c]			

[a] The value is the virus titre at time 0 (i.e., no treatment) following virus recovery from the matrix; [b] The concentrations in ppm-h/g were not provided. ClO_2 was generated in a mason jar chamber using 1mL of a sodium chlorite ($NaClO_2$) solution at concentrations ranging from 0.1 to 10 mg/mL, with exposure for the time indicated in brackets; [c] More than the detection limit, so the log reduction may be equal to or greater than this value.

3.9 STEAM-ULTRASOUND

37 3.9.1 Introduction

This treatment involves applying wet heat using steam through special nozzles which generate ultrasound, with the underlying theory being that this disrupts the laminar layer of air on the surface of fruits to facilitate instant heat transfer. This is intended to have a biocidal effect (Rajiuddin *et al.*, 2020). Steam ultrasound has been used to inactivate bacteria on skin and meat products (Morild *et al.*, 2011).

38 3.9.2 Studies

Only one study was located that investigated viral inactivation by steam-ultrasound on berries. In this study, approximately 10^6 PFU HAV or MNV were spot inoculated onto the surface of raspberries, allowed to dry for an hour, and then treated with steam-ultrasound (Rajiuddin *et al.*, 2020). Each nozzle in the steam-ultrasound equipment delivered 25 kg/steam per hour at 2.7 Bar(g) pressure, with ultrasound in the range 20-40 kHz. The steam-ultrasound treatments investigated were combinations of temperature (85, 90 or 95 °C) and exposure time (1-4 seconds).

A reduction in infectious HAV (using plaque assay), was measured under all treatments. Compared to controls, there was little difference between the treatment temperatures, but HAV inactivation was greater with longer treatment times. At 85 °C, HAV infectivity decreased by <0.5 $\log_{10}$ PFU after 1 second of treatment but decreased overall by 1.5 $\log_{10}$ PFU after 4 seconds. The largest reduction of infectious MNV was 3.3 $\log_{10}$ PFU after 4 seconds of steam-ultrasound treatment at 95 °C. For 1-4 seconds of treatment at 85 and 90 °C lower mean reductions of 1.1–2.5 $\log_{10}$ PFU were achieved.

3.10 OSMOTIC DEHYDRATION

39 3.10.1 Introduction

Osmotic dehydration is a process that uses concentrated sugar solutions to remove a large amount of water from the fruit through osmosis, either without heat or by applying mild temperature to the sugar solution and the fruit (Shi *et al.*, 2009). The process is also known as sugar infusion or candying. To remove excess moisture from the osmotic dehydration, an additional drying process can be applied, such as hot air, drum, microwave or infrared drying.

40 3.10.2 Studies

Only one study was located that investigated the effectiveness of osmotic drying on viruses in berries. In this study, HAV, MNV or MS2 were spot inoculated onto thawed, previously frozen blueberries, and after the inoculum was allowed to dry the berries were subjected to osmotic treatments through soaking in a sugar solution at selected temperatures (Bai *et al.*, 2020). The osmotic treatments were designed to represent commercial processes, which aim for a sucrose content in berries of 34-45%, corresponding to 26-31 °Brix. This required use of a sugar solution of 75% sucrose and a 15 h soak period.

The inoculum concentrations of HAV and MNV were 2.6 and 4.5 $TCID_{50}$/g, respectively, and was 9.1 $\log_{10}$ PFU/g for MS2. Virus recovery from the surface of blueberries was measured at 4.7%, 3.2% and 52.1% for HAV, MNV and MS2, respectively.

Different temperatures were selected for the blueberry/sugar solution soaking period. All three viruses were tested at 23 °C. Based on preliminary thermal resistance studies, an additional three temperatures were selected for each virus:

- HAV: 45, 55 and 65 °C.
- MNV: 35, 45 and 55 °C.
- MS2: 35, 40 and 45 °C.

At 23 °C, there were 0.4, 0.2, and 1 $\log_{10}$ reductions observed for HAV, MNV and MS2, respectively. Greater reductions were measured at higher temperatures, although the following was observed:

- A mean reduction of more than 2 $\log_{10}$ was not measured for HAV, even at 65 °C. This is a result of the low starting inoculum and recovery rates.
- Due to the same issues affecting HAV (above), the largest reduction of MNV was measured at 45 °C (3.4 $\log_{10}$ $TCID_{50}$/gram), and this was not increased with treatment at 55 °C.
- The experiments with MS2 showed increasing inactivation with increasing temperature, with no survivors detected after treatment at 45 °C (>7.6 $\log_{10}$ PFU/g reduction).

All viruses could be detected in the sugar solutions after treatment, although the concentrations decreased with increasing treatment temperature. This shows potential for cross-contamination via the sugar solution.

In a second round of experiments, berries that were osmotically treated at 23 °C were subjected to an additional oven drying step of 100 °C for 1 h. This combined osmotic-heat drying treatment increased the overall viral inactivation, with final mean reductions of 2.6, >3.4, and 7.2 $\log_{10}$ for HAV, MNV and MS2, respectively. These are considerably higher than the values observed after the osmotic treatment at 23 °C alone (above). However, HAV was still quantifiable after this combined treatment, signalling that it is more tolerant than the surrogate viruses tested.

4 DISCUSSION

The data available on HAV inactivation in berries and berry products by different treatments are complex. Results from different studies, even using similar treatments and similar infectivity assays, are rarely comparable. While data from inactivation experiments in berry foods using different surrogates have been reported, how well these data reflect behaviour of HAV is uncertain (see Section 4.1).

We have limited information on how many of these treatments are being used during commercial production of berries and berry products.

Of the treatments considered in this review, only thermal and HPP treatments have been extensively investigated. With the widespread availability of microwave ovens in consumer homes and food service operations, there is the potential for this form of heating to be used when heat treatment is advised. The effect of microwaving on HAV infectivity requires further research to provide guidance.

The guidance document produced by FSANZ and MPI in 2015 on the thermal inactivation of HAV in berries, stated that a “target 6-log reduction would be considered to provide satisfactory assurance for control of the hazard” (MPI, 2015). The guidance provided time and temperature processing information for berry products with or without added sugar to achieve 6 $\log_{10}$ reductions based on published models (Deboosere *et al.*, 2004; Deboosere *et al.*, 2010). Apart from some data on the effects of heat following a freeze-drying process, no additional data on HAV inactivation from more recent heat treatment experiments on HAV were found, and so the published models remain the best source for guidance regarding thermal control of HAV in berries and berry products.

Based on the data published by Kingsley *et al.* (2005) for the effect of HPP on HAV in strawberry puree, a pressure of at least 375 MPa for 5 minutes at ambient temperature (4.3 $\log_{10}$ reduction) would be required to approach a 6 $\log_{10}$ reduction. Data from HPP experiments with surrogates appear to be consistent with these parameters, with higher pressures for a shorter time, lower temperature, and increased pH contributing to greater log reductions.

For treatments other than thermal and HPP, only up to four studies of HAV or surrogates in berries or berry products were identified. We consider that there are insufficient data to define parameters for these other treatments that would provide sufficient inactivation of HAV. With further validation, UV and pulsed light could be useful for virus inactivation on a commercial scale although issues with heat generation and fruit surface coverage need resolving. Water assisted UV appears promising, but this introduces a wet post-harvest process that may negatively affect berry quality. Treatments using ozone or chlorine dioxide need to be investigated for issues such as residual chemicals resulting in unfavourable changes to organoleptic properties. Consumer acceptance of such chemical treatments may also be a barrier to their commercial implementation.

Gamma irradiation could be a safe treatment for viral inactivation on fresh and frozen berries, but the available data suggest this has limited efficacy for virus inactivation unless high doses are applied. Consumer resistance to irradiated food may also be a barrier to commercial application. Cold atmospheric plasma and steam ultrasound treatments are more technologically complex and require specialised equipment, so their efficacy needs to be weighed against implementation costs and effects on berry quality.

Very few studies have evaluated organoleptic qualities and/or consumer acceptance post-treatment.

4.1 SURROGATES

Due to the limited data for HAV, this review has included relevant data from experiments in berry foods using viruses with similar characteristics. However, these viruses can behave differently to each other and to HAV. Some studies located for this current review tested HAV under the same conditions as other viruses. Mainly, these experiments enable comparisons to be made between HAV and MNV on berry surfaces. The results are not consistent. HAV was more sensitive compared with MNV when treated with pulsed light or e-beam radiation (Butot *et al.*, 2021; Jubinville *et al.*, 2022). HAV was more tolerant to treatment with ozone or cold atmospheric plasma than MNV (Brié *et al.*, 2018; Velebit *et al.*, 2022). For most of these experiments, the number of infectious HAV and MNV differed by <2 $\log_{10}$, but not always (e.g., under ozone treatment the data indicates that the difference exceeds 3 $\log_{10}$). In another experiment, HAV, FCV and Aichi virus inoculated onto the surface of strawberries were all inactivated to similar numbers after treatment with UV (Fino and Kniel, 2008). Surrogates can also behave differently to each other. For example, MNV generally survived better than Tulane virus when heat-treated in strawberry puree (Bartsch *et al.*, 2019). Further to the above, a review of data on viral thermal inactivation in a range of matrices showed that changes in experimental conditions also affected viral survival (Bozkurt *et al.*, 2015). These findings mean that caution is required when using non-HAV experimental data to inform on HAV infectivity/survival.

4.2 LIMITATIONS AND DATA GAPS

There are few data from experiments using HAV, and these biased towards HAV laboratory adapted strains, so may not reflect the behaviour of wild-type strains. Studies on non-food matrices have showed that even laboratory-adapted HAV strains can behave differently to each other in inactivation studies (Shimasaki *et al.*, 2009).

For the studies collated in this report, some experimental parameters were not always reported. This particularly related to characteristics of the experimental berry food matrix such as pH, water activity, or sugar content.

In some publications, the log reduction achieved by the inactivation method was only reported as a figure in the form of a graph, and/or only selected data quoted. To obtain the required data for this current review, numerical data were extracted from the figures using the open-source web-based software ImageJ and WebPlotDigitizer[7] or were inferred visually. In one case (Dolan *et al.*, 2023), raw data was provided by a co-author to J. Hewitt (ESR).

4.3 MODELS

The models used to support the 2015 FSANZ and MPI guidance (MPI, 2015) were for HAV inactivation on natural berries (Deboosere *et al.*, 2010) and in berries with added sugar (Deboosere *et al.*, 2004). No new models were identified for HAV inactivation on berries.

More recent models have been identified for:

- MNV and Tulane virus in strawberry and raspberry purees under thermal treatment (Bartsch *et al.*, 2019).
- Human norovirus in strawberry purees under HPP, based on data using MNV and Tulane virus (Pandiscia *et al.*, 2024).

[7] automeris.io: AI assisted data extraction from charts using WebPlotDigitizer accessed 5 June 2024

- MNV and MS2 bacteriophage in strawberry puree and juice under HPP (Pan *et al.*, 2016).

As these models only involve surrogates, we consider that they should not change the advice derived from the earlier models used in the FSANZ and MPI guidance (MPI, 2015).

41 REFERENCES

Annous B A, Buckley D A and Kingsley D H (2021) Efficacy of chlorine dioxide gas against hepatitis A virus on blueberries, blackberries, raspberries, and strawberries. *Food and Environmental Virology*; 13: 241-247. doi: 10.1007/s12560-021-09465-1

Augsburger N, Rachmadi A T, Zaouri N, Lee Y and Hong P Y (2021) Recent update on UV disinfection to fulfill the disinfection credit value for enteric viruses in water. *Environmental Science & Technology*; 55: 16283-16298. doi: 10.1021/acs.est.1c03092

Baert L, Uyttendaele M, Van Coillie E and Debevere J (2008) The reduction of murine norovirus 1, *B. fragilis* HSP40 infecting phage B40-8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. *Food Microbiology*; 25: 871-874. doi: 10.1016/j.fm.2008.06.002

Bai X, Campagnoli M, Butot S, Putallaz T, Michot L and Zuber S (2020) Inactivation by osmotic dehydration and air drying of *Salmonella*, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, hepatitis A virus and selected surrogates on blueberries. *International Journal of Food Microbiology*; 320: 108522. doi: 10.1016/j.ijfoodmicro.2020.108522

Bartsch C, Plaza-Rodriguez C, Trojnar E, Filter M and Johne R (2019) Predictive models for thermal inactivation of human norovirus and surrogates in strawberry puree. *Food Control*; 96: 87-97. doi: 10.1016/j.foodcont.2018.08.031

Bernard H, Faber M, Wilking H, Haller S, Höhle M, Schielke A, Ducomble T, Siffczyk C, Merbecks S S, Fricke G, Hamouda O, Stark K and Werber D (2014) Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. *Eurosurveillance*; 19: 20719. doi: 10.2807/1560-7917.es2014.19.8.20719

BfR (2024) [Safely fed. Particularly sensitive groups of people in communal facilities] In German. Bundesinstitut für Risikobewertung, Berlin. Available at: https://www.bfr.bund.de/cm/350/sicher-verpflegt-besonders-empfindliche-personengruppen-in-gemeinschaftseinrichtungen.pdf. Accessed 31 May 2024.

Bidawid S, Farber J M and Sattar S A (2000) Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. *International Journal of Food Microbiology*; 57: 91-97. doi: 10.1016/S0168-1605(00)00235-X

Bozkurt H, D'Souza D H and Davidson P M (2015) Thermal inactivation of foodborne enteric viruses and their viral surrogates in foods. *Journal of Food Protection*; 78: 1597-1617. doi: 10.4315/0362-028x.Jfp-14-487

Brié A, Boudaud N, Mssihid A, Loutreul J, Bertrand I and Gantzer C (2018) Inactivation of murine norovirus and hepatitis A virus on fresh raspberries by gaseous ozone treatment. *Food Microbiology*; 70: 1-6. doi: 10.1016/j.fm.2017.08.010

Butot S, Cantergiani F, Moser M, Jean J, Lima A, Michot L, Putallaz T, Stroheker T and Zuber S (2018) UV-C inactivation of foodborne bacterial and viral pathogens and surrogates on fresh and frozen berries. *International Journal of Food Microbiology*; 275: 8-16. doi: 10.1016/j.ijfoodmicro.2018.03.016

Butot S, Galbusera L, Putallaz T and Zuber S (2021) Electron beam susceptibility of enteric viruses and surrogate organisms on fruit, seed and spice matrices. *Food and Environmental Virology*; 13: 218-228. doi: 10.1007/s12560-021-09463-3

Butot S, Putallaz T, Amoroso R and Sánchez G (2009) Inactivation of enteric viruses in minimally processed berries and herbs. *Applied and Environmental Microbiology*; 75: 4155-4161. doi: 10.1128/Aem.00182-09

Butot S, Putallaz T and Sánchez G (2008) Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology*; 126: 30-35. doi: 10.1016/j.ijfoodmicro.2008.04.033

Chen J Y, Wu X Y, Sánchez G and Randazzo W (2020) Viability RT-qPCR to detect potentially infectious enteric viruses on heat processed berries. *Food Control*; 107: 106818. doi: 10.1016/j.foodcont.2019.106818

Chhabra P, de Graaf M, Parra G I, Chan M C, Green K, Martella V, Wang Q, White P A, Katayama K, Vennema H, Koopmans M P G and Vinjé J (2019) Updated classification of norovirus genogroups and genotypes. *The Journal of General Virology*; 100: 1393-1406. doi: 10.1099/jgv.0.001318

Cook N, Bertrand I, Bosch A, Gantzer C, Maul A, Pinto R, van der Poel W and Stein J (2014) A critical review of the effect of heat, pH and water activity on the survival of Hepatitis A and E viruses. A Report to the United Kingdom Food Standards Agency FSA Project FS101074. United Kingdom Food Standards Agency, London. Available at: https://www.food.gov.uk/research/foodborne-disease/a-critical-review-of-the-effects-of-heat-ph-and-water-activity-on-the-survival-of-hepatitis-a-and-e-viruses. Accessed 17 June 2024.

Cromeans T, Park G W, Costantini V, Lee D, Wang Q, Farkas T, Lee A and Vinjé J (2014) Comprehensive comparison of cultivable norovirus surrogates in response to different inactivation and disinfection treatments. *Applied and Environmental Microbiology*; 80: 5743-5751. doi: 10.1128/aem.01532-14

Deboosere N, Legeay O, Caudrelier Y and Lange M (2004) Modelling effect of physical and chemical parameters on heat inactivation kinetics of hepatitis A virus in a fruit model system. *International Journal of Food Microbiology*; 93: 73-85. doi: 10.1016/j.ijfoodmicro.2003.10.015

Deboosere N, Pinon A, Delobel A, Temmam S, Morin T, Merle G, Blaise-Boisseau S, Perelle S and Vialette M (2010) A predictive microbiology approach for thermal inactivation of Hepatitis A virus in acidified berries. *Food Microbiology*; 27: 962-967. doi: 10.1016/j.fm.2010.05.018

DiCaprio E, Phantkankum N, Culbertson D, Ma Y M, Hughes J H, Kingsley D, Uribe R M and Li J R (2016) Inactivation of human norovirus and Tulane virus in simple media and fresh whole strawberries by ionizing radiation. *International Journal of Food Microbiology*; 232: 43-51. doi: 10.1016/j.ijfoodmicro.2016.05.013

DiCaprio E, Ye M, Chen H Q and Li J R (2019) Inactivation of human norovirus and Tulane virus by high pressure processing in simple mediums and strawberry puree. *Frontiers in Sustainable Food Systems*; 3: 26. doi: 10.3389/fsufs.2019.00026

Dolan K D, Miranda R and Schaffner D W (2023) Estimation of bacteriophage MS2 inactivation parameters during microwave heating of frozen strawberries. *Journal of Food Protection*; 86: 100032. doi: 10.1016/j.jfp.2022.100032

Ettayebi K, Crawford S E, Murakami K, Broughman J R, Karandikar U, Tenge V R, Neill F H, Blutt S E, Zeng X L, Qu L, Kou B, Opekun A R, Burrin D, Graham D Y, Ramani S, Atmar R L and Estes M K (2016) Replication of human noroviruses in stem cell-derived human enteroids. *Science*; 353: 1387-1393. doi: 10.1126/science.aaf5211

Farkas T (2015) Rhesus enteric calicivirus surrogate model for human norovirus gastroenteritis. *Journal of General Virology*; 96: 1504-1514. doi: 10.1099/jgv.0.000020

Farkas T, Sestak K, Wei C and Jiang X (2008) Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. *Journal of Virology*; 82: 5408-5416. doi: 10.1128/JVI.00070-08

Feng K, Divers E, Ma Y M and Li J R (2011) Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*; 77: 3507-3517. doi: 10.1128/aem.00081-11

Fino V R and Kniel K E (2008) UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. *Journal of Food Protection*; 71: 908-913. doi: 10.4315/0362-028x-71.5.908

Govaris A and Pexara A (2021) Inactivation of foodborne viruses by high-pressure processing (HPP). *Foods*; 10: 215. doi: 10.3390/foods10020215

Gust I D, Lehmann N I, Crowe S, McCrorie M, Locarnini S A and Lucas C R (1985) The origin of the HM175 strain of hepatitis A virus. *Journal of Infectious Diseases*; 151: 365-367. doi: 10.1093/infdis/151.2.365

Hollinger F B and Emerson S U (2001). Hepatitis A virus. *In*: Fields Virology, 4th edition, Ed: D. M. Knipe and P. M. Howley, pp799–840. Lippincott Williams & Wilkins: New York.

Horn B, Pattis I and Rivas L (2018) Review of high pressure processes (HPP) applied as an alternative to thermal pasteurisation. Client report FW18012 for Ministry for Primary Industries. Institute of Environmental Science and Research (ESR), Christchurch. Available at: https://www.mpi.govt.nz/dmsdocument/34659/direct. Accessed 5 April 2024.

Huang R Z, Li X H, Huang Y X and Chen H Q (2014) Strategies to enhance high pressure inactivation of murine norovirus in strawberry puree and on strawberries. *International Journal of Food Microbiology*; 185: 1-6. doi: 10.1016/j.ijfoodmicro.2014.05.007

Huang R Z, Ye M, Li X H, Ji L, Karwe M and Chen H Q (2016) Evaluation of high hydrostatic pressure inactivation of human norovirus on strawberries, blueberries, raspberries and in their purees. *International Journal of Food Microbiology*; 223: 17-24. doi: 10.1016/j.ijfoodmicro.2016.02.002

Huang Y X and Chen H Q (2015) Inactivation of *E. coli* O157:H7, *Salmonella* and human norovirus surrogate on artificially contaminated strawberries and raspberries by water-assisted pulsed light treatment. *Food Research International*; 72: 1-7. doi: 10.1016/j.foodres.2015.03.013

Huang Y X, Ye M, Cao X N and Chen H Q (2017) Pulsed light inactivation of murine norovirus, Tulane virus, *Escherichia coli* O157:H7 and *Salmonella* in suspension and on berry surfaces. *Food Microbiology*; 61: 1-4. doi: 10.1016/j.fm.2016.08.001

IFST (2022) Information statement on food irradiation. Institute of Food Science and Technology, London. Available at: https://www.ifst.org/resources/information-statements/food-irradiation. Accessed 2 June 2024.

Johne R, Scholz J and Falkenhagen A (2024) Heat stability of foodborne viruses - Findings, methodological challenges and current developments. *International Journal of Food Microbiology*; 413: 110582. doi: 10.1016/j.ijfoodmicro.2024.110582

Jubinville E, Trudel-Ferland M, Amyot J and Jean J (2022) Inactivation of hepatitis A virus and norovirus on berries by broad-spectrum pulsed light. *International Journal of Food Microbiology*; 364: 109529. doi: 10.1016/j.ijfoodmicro.2021.109529

Kärber G (1931) Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie*; 162: 480-483. doi: 10.1007/BF01863914

Karst S M, Wobus C E, Lay M, Davidson J and Virgin H W t (2003) STAT1-dependent innate immunity to a Norwalk-like virus. *Science*; 299: 1575-1578. doi: 10.1126/science.1077905

Khadre M A, Yousef A E and Kim J G (2001) Microbiological aspects of ozone applications in food: A review. *Journal of Food Science*; 66: 1242-1252. doi: 10.1111/j.1365-2621.2001.tb15196.x

Kingsley D H and Annous B A (2019) Evaluation of steady-state gaseous chlorine dioxide treatment for the inactivation of Tulane virus on berry fruits. *Food and Environmental Virology*; 11: 214-219. doi: 10.1007/s12560-019-09382-4

Kingsley D H and Annous B A (2021) Evaluation of SDS and GRAS liquid disinfectants for mitigation of hepatitis A virus contamination of berries. *Journal of Applied Microbiology*; 131: 2586-2591. doi: 10.1111/jam.15123

Kingsley D H, Chen H and Hoover D G (2004) Inactivation of selected picornaviruses by high hydrostatic pressure. *Virus Research*; 102: 221-224. doi: 10.1016/j.virusres.2004.01.030

Kingsley D H, Guan D S and Hoover D G (2005) Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. *Journal of Food Protection*; 68: 1748-1751. doi: 10.4315/0362-028x-68.8.1748

Kingsley D H, Perez-Perez R E, Boyd G, Sites J and Niemira B A (2018a) Evaluation of 405-nm monochromatic light for inactivation of Tulane virus on blueberry surfaces. *Journal of Applied Microbiology*; 124: 1017-1022. doi: 10.1111/jam.13638

Kingsley D H, Pérez-Pérez R E, Niemira B A and Fan X (2018b) Evaluation of gaseous chlorine dioxide for the inactivation of Tulane virus on blueberries. *International Journal of Food Microbiology*; 273: 28-32. doi: 10.1016/j.ijfoodmicro.2018.01.024

Kovač K, Diez-Valcarce M, Raspor P, Hernández M and Rodríguez-Lázaro D (2012) Effect of high hydrostatic pressure processing on norovirus infectivity and genome stability in strawberry puree and mineral water. *International Journal of Food Microbiology*; 152: 35-39. doi: 10.1016/j.ijfoodmicro.2011.10.005

Lacombe A, Niemira B A, Gurtler J B, Sites J, Boyd G, Kingsley D H, Li X H and Chen H Q (2017) Nonthermal inactivation of norovirus surrogates on blueberries using atmospheric cold plasma. *Food Microbiology*; 63: 1-5. doi: 10.1016/j.fm.2016.10.030

Lemon S M, Whetter L, Chang K H and Brown E A (1992) Why do human hepatitis viruses replicate so poorly in cell cultures? *FEMS Microbiology Letters*; 100: 455-459. doi: 10.1111/j.1574-6968.1992.tb14076.x

Li X H, Ye M, Neetoo H, Golovan S and Chen H Q (2013) Pressure inactivation of Tulane virus, a candidate surrogate for human norovirus and its potential application in food industry. *International Journal of Food Microbiology*; 162: 37-42. doi: 10.1016/j.ijfoodmicro.2012.12.016

Liu C H, Li X H and Chen H Q (2015) Application of water-assisted ultraviolet light processing on the inactivation of murine norovirus on blueberries. *International Journal of Food Microbiology*; 214: 18-23. doi: 10.1016/j.ijfoodmicro.2015.07.023

Lou F F, Neetoo H, Chen H Q and Li J R (2011) Inactivation of a human norovirus surrogate by high-pressure processing: Effectiveness, mechanism, and potential application in the fresh produce industry. *Applied and Environmental Microbiology*; 77: 1862-1871. doi: 10.1128/Aem.01918-10

Maks N, Ye M, Swanson S, Lee A, Freeman B B and Deng K P (2019) Evaluation of inactivating norovirus, hepatitis A, and *Listeria monocytogenes* on raspberries by sanitizer spray. *Journal of Food Protection*; 82: 869-877. doi: 10.4315/0362-028x.jfp-18-415

Marti E, Ferrary-Américo M and Barardi C R M (2017) Detection of potential infectious enteric viruses in fresh produce by (RT)-qPCR preceded by nuclease treatment. *Food and Environmental Virology*; 9: 444-452. doi: 10.1007/s12560-017-9300-x

Migueres M, Lhomme S and Izopet J (2021) Hepatitis A: Epidemiology, high-risk groups, prevention and research on antiviral treatment. *Viruses*; 13: 1900. doi:

Molina-Chavarria A, Félix-Valenzuela L, Silva-Campa E and Mata-Haro V (2020) Evaluation of gamma irradiation for human norovirus inactivation and its effect on strawberry cells. *International Journal of Food Microbiology*; 330: 108695. doi: 10.1016/j.ijfoodmicro.2020.108695

Moreno L, Aznar R and Sánchez G (2015) Application of viability PCR to discriminate the infectivity of hepatitis A virus in food samples. *International Journal of Food Microbiology*; 201: 1-6. doi: 10.1016/j.ijfoodmicro.2015.02.012

Morild R K, Christiansen P, Sørensen A H, Nonboe U and Aabo S (2011) Inactivation of pathogens on pork by steam-ultrasound treatment. *Journal of Food Protection*; 74: 769-775. doi: 10.4315/0362-028X.JFP-10-338

MPI (2015) Guidance for thermal inactivation of Hepatitis A virus in berries. MPI Technical Paper 2015/40. Prepared for MPI by MPI Food Risk Assessment & FSANZ Risk Assessment (Microbiology). Ministry for Primary Industries, Wellington. Available at: https://www.mpi.govt.nz/dmsdocument/10769-Guidance-for-thermal-inactivation-of-Hepatitis-A-virus-in-berries. Accessed 4 June 2024.

MPI (2024) Food Notice: Requirements for registered food importers and imported food for sale. Ministry for Primary Industries, Wellington. Available at: https://www.mpi.govt.nz/dmsdocument/61807-Food-Notice-Requirements-for-Registered-Food-Importers-and-Imported-Food-for-Sale-effective-from-1-August-2024. Accessed 22 July 2024.

Ortiz-Solà J, Valero A, Abadias M, Nicolau-Lapeña I and Viñas I (2022) Evaluation of water-assisted UV-C light and its additive effect with peracetic acid for the inactivation of *Listeria monocytogenes*, *Salmonella enterica* and murine norovirus on whole and fresh-cut strawberries during shelf-life. *Journal of the Science of Food and Agriculture*; 102: 5660-5669. doi: 10.1002/jsfa.11913

Pala Ç U and Toklucu A K (2013) Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *LWT - Food Science and Technology*; 50: 426-431. doi: 10.1016/j.lwt.2012.09.001

Pan H, Buenconsejo M, Reineke K F and Shieh Y C (2016) Effect of process temperature on virus inactivation during high hydrostatic pressure processing of contaminated fruit puree and juice. *Journal of Food Protection*; 79: 1517-1526. doi: 10.4315/0362-028x.Jfp-16-004

Pandiscia A, Falcó I, Terio V, Martínez A, Sánchez G, Rodrigo D and Randazzo W (2024) Survival modelling of infectious human norovirus and surrogates for high-pressure inactivation in strawberry puree. *Innovative Food Science & Emerging Technologies*; 94: 103702. doi: 10.1016/j.ifset.2024.103702

Parry J V and Mortimer P P (1984) The heat sensitivity of hepatitis A virus determined by a simple tissue culture method. *Journal of Medical Virology*; 14: 277-283. doi: 10.1002/jmv.1890140312

Patras A, Brunton N P, Da Pieve S and Butler F (2009) Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*; 10: 308-313. doi: 10.1016/j.ifset.2008.12.004

Pérez-Rodríguez F J, D'Andrea L, de Castellarnau M, Costafreda M I, Guix S, Ribes E, Quer J, Gregori J, Bosch A and Pintó R M (2016) Improving virus production through quasispecies genomic selection and molecular breeding. *Scientific Reports*; 6: 35962. doi: 10.1038/srep35962

Pimenta A I, Margaça F M A and Verde S C (2019) Virucidal activity of gamma radiation on strawberries and raspberries. *International Journal of Food Microbiology*; 304: 89-96. doi: 10.1016/j.ijfoodmicro.2019.05.011

Predmore A, Sanglay G, Li J R and Lee K (2015a) Control of human norovirus surrogates in fresh foods by gaseous ozone and a proposed mechanism of inactivation. *Food Microbiology*; 50: 118-125. doi: 10.1016/j.fm.2015.04.004

Predmore A, Sanglay G C, DiCaprio E, Li J R, Uribe R M and Lee K (2015b) Electron beam inactivation of Tulane virus on fresh produce, and mechanism of inactivation of human norovirus surrogates by electron beam irradiation. *International Journal of Food Microbiology*; 198: 28-36. doi: 10.1016/j.ijfoodmicro.2014.12.024

Rajiuddin S M, Vigre H, Musavian H S, Kohle S, Krebs N, Hansen T B, Gantzer C and Schultz A C (2020) Inactivation of hepatitis A virus and murine norovirus on surfaces of plastic, steel and raspberries using steam-ultrasound treatment. *Food and Environmental Virology*; 12: 295-309. doi: 10.1007/s12560-020-09441-1

Reed L J and Muench H (1938) A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*; 27: 493-497. doi:

Richards G P (2012) Critical review of norovirus surrogates in food safety research: rationale for considering volunteer studies. *Food and Environmental Virology*; 4: 6-13. doi: 10.1007/s12560-011-9072-7

Rivadulla E and Romalde J L (2020) A comprehensive review on human Aichi virus. *Virologica Sinica*; 35: 501-516. doi: 10.1007/s12250-020-00222-5

Rowan N J (2019) Pulsed light as an emerging technology to cause disruption for food and adjacent industries – Quo vadis? *Trends in Food Science & Technology*; 88: 316-332. doi: 10.1016/j.tifs.2019.03.027

Sanglay G C, Li J, Uribe R M and Lee K (2011) Electron-beam inactivation of a norovirus surrogate in fresh produce and model systems. *Journal of Food Protection*; 74: 1155-1160. doi: 10.4315/0362-028x.Jfp-10-405

Sewlikar S and D'Souza D H (2017) Survival of hepatitis A virus and Aichi virus in cranberry - based juices at refrigeration (4 °C). *Food Microbiology*; 62: 251-255. doi: 10.1016/j.fm.2016.10.003

Shi J, Pan Z, McHugh T H and Hirschberg E (2009) Effect of infusion method and parameters on solid gain in blueberries. *Food and Bioprocess Technology*; 2: 271-278. doi: 10.1007/s11947-008-0116-4

Shimasaki N, Kiyohara T, Totsuka A, Nojima K, Okada Y, Yamaguchi K, Kajioka J, Wakita T and Yoneyama T (2009) Inactivation of hepatitis A virus by heat and high hydrostatic pressure: variation among laboratory strains. *Vox Sanguinis*; 96: 14-19. doi: 10.1111/j.1423-0410.2008.01113.x

Tian P, Yang D, Quigley C, Chou M and Jiang X (2013) Inactivation of the Tulane virus, a novel surrogate for the human norovirus. *Journal of Food Protection*; 76: 712-718. doi: 10.4315/0362-028x.Jfp-12-361

USFDA (1996a) Code of Federal Regulations. § 179.41 Pulsed light for the treatment of food. United States Food and Drug Administration, Available at: https://www.ecfr.gov/current/title-21/section-179.41. Accessed 17 June 2024.

USFDA (1996b) Code of Federal Regulations. § 179.39 Ultraviolet radiation for the processing and treatment of food. United States Food and Drug Administration, Available at: https://www.ecfr.gov/current/title-21/section-179.41. Accessed 17 June 2024.

USFDA (2022) Bacteriological Analytical Manual Chapter 26 - Concentration, extraction and detection of enteric viruses from food. United States Food and Drug Administration, Silver Spring, MD. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-26-and-appendices-concentration-extraction-and-detection-enteric-viruses-food. Accessed 17 June 2024.

Velebit B, Milojevic L, Baltic T, Grkovic N, Gummalla S, Velebit M, Skoko I, Mojsova S and Putnik P (2022) Efficacy of cold atmospheric plasma for inactivation of viruses on raspberries. *Innovative Food Science & Emerging Technologies*; 81: 103121. doi: 10.1016/j.ifset.2022.103121

Wales S Q, Pandiscia A, Kulka M, Sanchez G and Randazzo W (2024) Challenges for estimating human norovirus infectivity by viability RT-qPCR as compared to replication in human intestinal enteroids. *International Journal of Food Microbiology*; 411: 110507. doi: 10.1016/j.ijfoodmicro.2023.110507

Xu S, Wang D, Yang D, Liu H and Tian P (2015) Alternative methods to determine infectivity of Tulane virus: a surrogate for human nororvirus. *Food Microbiology*; 48: 22-27. doi: 10.1016/j.fm.2014.12.004

Xue W, Macleod J and Blaxland J (2023) The use of ozone technology to control microorganism growth, enhance food safety and extend shelf life: A promising food decontamination technology. *Foods*; 12: 814. doi: 10.3390/foods12040814

Ye M, Zhang Y, Rolfe C and Lee A (2018) Inactivation of hepatitis A virus on strawberries and blueberries by high-pressure processing [Conference poster presentation]. IAFP Annual Meeting, 8-11 July 2018, Salt Lake City, Utah, United States.

https://iafp.confex.com/iafp/2018/meetingapp.cgi/Paper/18347.
Accessed 17 June 2024.

LITERATURE SEARCH

PubMed[8] and Web of Science[9] are two citation search engines that together index scientific publications spanning agriculture and human health. EndNote[10] was used to manage citations. A core set of starting references was established through searches of PubMed and Web of Science (both Topic and Title) during April 2024. Only publications after the year 1990 were included in our literature search. The key words were as follows:

Hazard 1	Hepatitis OR Hep A OR Hepatitis A virus
Hazard 2	Norovirus
Hazard 3	Surrogate for norovirus (Murine norovirus, Tulane virus, Feline calicivirus)
Treatment 1	High-pressure processing OR HPP
Treatment 2	Thermal inactivation
Treatment 3	Inactivation or Disinfection (Ozone)
Treatment 4	Irradiation (UV OR Pulse light or Pulsed light)
Food 1	Fruits
Food 2	Berry OR Strawberry OR Blueberry OR Raspberry
Food 3	Grapes OR Kiwiberries OR Cherries

Initial searching generated a list of more than 1000 citations, most of which were irrelevant. More specific keyword searches were conducted resulting in an initial set of 619 citations. After removal of duplicates, each citation was assessed for relevance based on the title and abstract. The full texts of these core references were obtained, and their reference lists were used to identify further reports of interest. The initial citation set contained 61 references, with publication dates ranging from 2008 to 2024. Additional references were located as these reports were reviewed in detail, resulting in a total of 80 relevant references.

[8] National Center for Biotechnology Information, US National Library of Medicine, https://www.ncbi.nlm.nih.gov/pubmed/ (accessed 21 October 2020).
[9] Clarivate Analytics, https://www.webofknowledge.com (accessed 21 October 2020).
[10] Clarivate Analytics, https://endnote.com/ (accessed 21 October 2020).

EXPERIMENTAL CONTEXT ADDITIONAL DETAILS

PATHOGENIC VIRUSES

42 B.1.1 Hepatitis A virus

All wild-type HAV strains replicate slowly in cell lines, and without obvious cytopathic effect (CPE) (Hollinger and Emerson, 2001; Lemon *et al.*, 1992). CPE is observed when the structure of the cultured host cell is altered due to viral infection. One wildtype strain of HAV, HM-175 (a IB genotype) was adapted to grow in cell culture through serially passaging (Gust *et al.*, 1985). The HM-175/18f strain (ATCC VR-1402) can be considered the prototype strain. This virus multiplies and causes CPE in FrHK-9 cells (ATCC CRL-1688) or BS-C-1 cells, showing characteristic cell degeneration and cell rounding. This strain is almost exclusively used for HAV inactivation experiments including on food products and water. As such, data may be biased and may not reflect what would apply to wildtype strains.

HM-175 strains generally replicate slowly in cell culture, with at least a 5-10-day incubation period required to observe CPE. Indeed, up to 14 days incubation post-inoculation is recommended before it can be concluded that viral replication has not occurred. For HM-175, HAV titres of approximately 7 $\log_{10}$ $TCID_{50}$ or PFU/mL can be expected to be produced in cell culture. Other HM-175 strains have been developed (such as HM-175-HP: HP referring to high productivity) that grow faster and can produce a higher virus yield than the original HM-175 (Pérez-Rodríguez *et al.*, 2016). However, to our knowledge, this strain has not been used in inactivation experiments.

Other laboratory-adapted strains have been used for inactivation experiments, although not with food as a matrix. This includes strains such as KRM238, KRM003 (IIIB), KRM031 (IA), and TKM005 (IB) that have been used in heat inactivation and HPP experiments with an immune-focus assay used to determine infectious viruses (Shimasaki *et al.*, 2009). As well as differences in persistence being identified (i.e., KRM238 and TKM005 being less susceptible to 60 °C heat over a 10-hour period, and KRM031 (IA) being the most susceptible to HPP at 420 MPa), the study by Shimasaki *et al.* (2009) showed that one strain (KRM238) replicated better than the other strains tested, illustrating some HAV strains may be more suitable for inactivation studies than others.

43 B.1.2 Norovirus

As introduced in Section 2.1.2, norovirus classification is based on a dual typing system based on the capsid VP1 and partial RdRp sequences. Genogroups I, II and IV (GI, GII and GIV) are associated with illness in humans, with GII most commonly identified from human cases. The genotype is based on the VP1 amino acid diversity, and the P-type is based on the nucleic acid diversity of the RdRp. The nomenclature is shown as the genotype (G-type e.g., GII.3 or GI.1) followed by the P-type in square brackets e.g., GII.3[P12] or GI.1[P1]. There are >50 genotypes and >74 P-types (Chhabra *et al.*, 2019).

Human norovirus strains used in laboratory studies are obtained exclusively from faecal specimens collected from infected persons. There are no commercially available strains. Norovirus prepared from faecal samples may be stored long-term at -80 °C in laboratories.

There have been ongoing challenges associated with human norovirus culture *in vitro*. HIE cells can now be used for the propagation of norovirus (Ettayebi *et al.*, 2016). However, of

the strains that will grow in HIE cell culture, increases in the titre are generally low (i.e., range from 10 to 1000 times). The assay also has low sensitivity and due to the nature of the methodology, infectivity titres are not quantitative.

Because norovirus culture is challenging, molecular assays with pre-treatments designed to inform on norovirus infectivity have been also used to assess inactivation on berry products.

44 B.1.3 Aichi virus

In the laboratory, Aichi virus (such as strain A846/88 as used by Fino and Kniel (2008) in UV light studies) grows rapidly in Vero cells (ATCC CCL-81) with characteristic cell degeneration evident 3-5 days after inoculation. The virus is usually quantified using end point titrations. Titres of Aichi virus of 7-8 $\log_{10}$ $TCID_{50}$ can be expected. The use of Aichi virus as a surrogate in inactivation studies is limited. One study demonstrated its resistance to HPP (<600 MPa) but this wasn't performed on berry products (Kingsley *et al.*, 2004). Aichi virus infectivity (along with HAV) was assessed in cranberry-based juices at refrigeration (4 °C), with both viruses maintaining infectivity over 21 days (Sewlikar and D'Souza, 2017).

SURROGATE VIRUSES

45 B.2.1 Murine norovirus

As stated in Section 2.1.4, MNV is grown in the laboratory using murine macrophage cells and the MNV-1 strain is mainly used.

MNV has been used as a surrogate to assess a range of inactivation treatments in berries and/or berry products, including chlorine spray (Maks *et al.*, 2019), cold atmospheric plasma (Velebit *et al.*, 2022), gamma radiation (Pimenta *et al.*, 2019), electron beam (Butot *et al.*, 2021), HPP (Huang *et al.*, 2014; Kovač *et al.*, 2012; Li *et al.*, 2013; Lou *et al.*, 2011; Pan *et al.*, 2016), osmotic dehydration/air drying (Bai *et al.*, 2020), ozone (Brié *et al.*, 2018), pulsed light (Huang *et al.*, 2017), steam-ultrasound (Rajiuddin *et al.*, 2020), thermal (Bartsch *et al.*, 2019) and UV (Liu *et al.*, 2015).

46 B.2.2 Feline calicivirus

The FCV strains most commonly used for laboratory studies are F9 (ATCC VR-782) and the type strain ATCC-651. In the laboratory, FCV grows rapidly in Crandell-Rees Feline Kidney (CRFK) cells with characteristic cell degeneration evident 2-5 days after inoculation. The virus is usually quantified using end point titrations. Titres of FCV of 8-9 $\log_{10}$ $TCID_{50}$ /mL can be expected.

Due to MNV being more suited as a surrogate than FCV (Section 2.1.5), there are few studies published after 2007 where FCV was used.

47 B.2.3 Tulane virus

In the laboratory, Tulane virus grows rapidly with characteristic cell rounding CPE in LLC-MK-2 cells (rhesus macaque kidney cell line, ATCC CCL-7). The virus can be quantified using conventional culture methods (plaque assay or end point titration assay) but only to relatively low titres ($10^{5\text{-}6}$ $TCID_{50}$ or PFU/ml) (Farkas *et al.*, 2008). Alternative approaches to determining Tulane virus infectivity are RNase exposure assay (Xu *et al.*, 2015) and the PGM binding assays that utilise the property of Tulane virus to recognise the human HBGA (as for human norovirus).

Tulane virus has been used as a surrogate for inactivation studies (Tian *et al.*, 2013), including on berries and/or berry products challenged with gaseous chlorine dioxide (Kingsley and Annous, 2019), ionising radiation (DiCaprio *et al.*, 2016), HPP (DiCaprio *et al.*, 2019; Li *et al.*, 2013), thermal (Bartsch *et al.*, 2019) and pulsed light (Huang *et al.*, 2017).

48 B.2.4 Bacteriophages

Titres of F-RNA bacteriophages of 10-11 $\log_{10}$ PFU/mL can be expected, which is higher than achievable with enteric viruses, and MNV and Tulane virus surrogates. MS2 bacteriophage has been used for assessing electron beam (Butot *et al.*, 2021), osmotic dehydration/air drying (Bai *et al.*, 2020), thermal (microwave) (Dolan *et al.*, 2023). Qβ bacteriophage (ATCC 23631-B1) has been used for virus inactivation studies using electron beams (Butot *et al.*, 2021).

Other bacteriophages have also been used as surrogates. The bacteriophage B40-8 that infects *B. fragilis* HSP40 was used a surrogate for human norovirus in one study on raspberry puree (Baert *et al.*, 2008).

VIRUS SEEDING METHODS FOR BERRY FOODS

49 B.3.1 Whole berries

Further to the overview provided in Section 2.2, inoculation of viruses on to whole fruit is usually through spot inoculation. Spot inoculation consists of either applying multiple small volumes (e.g., 10 spot aliquots of 10 to 50 µL) over the fruit surface (Huang *et al.*, 2017; Pimenta *et al.*, 2019), or a single spot (Fino and Kniel, 2008).

As an example of spray inoculation, Velebit *et al.* (2022) used a nebuliser to seed 25 g raspberries with 100 µL of 2 x 10^9 PFU/mL MNV or 4 x 10^{10} PFU/mL HAV.

One study described an alternative approach, which involved injection of virus into the strawberry fruit (Predmore *et al.*, 2015a).

Following inoculation, any liquid is normally allowed to dry (typically for 30 minutes to 2 hours, but can be as long as 20 hours) to facilitate viral attachment prior to the inactivation process (Brié *et al.*, 2018; Butot *et al.*, 2008; Huang *et al.*, 2017). Prolonged periods may encourage a strong attachment that affects virus recovery from the matrix, which in turn influences the maximum measure $\log_{10}$ reduction calculated (see below).

In one study that compared spot inoculation to immersion, the inoculation method was considered to be more efficient than immersion, and to better mimic contamination (Fino and Kniel, 2008).

50 B.3.2 Liquid product (e.g., purees)

Examples of where virus has been added to berry puree are:

- The addition of 10 µL viral suspension to 10 mL puree (Deboosere *et al.*, 2004; Huang *et al.*, 2016).
- Adding human norovirus at a ratio of 1:30, i.e., 1 part norovirus to 30 parts puree (Wales *et al.*, 2024).

As for whole/chopped fruit, seeded purees may be left at room temperature for a number of hours (e.g., 3 hours) as a virus aggregation or adhesion step (Deboosere *et al.*, 2010).

VIRUS RECOVERY METHODS

51 B.4.1 Whole berries

Several approaches to recovering infectious enteric viruses from whole soft berries have been reported. These include:

1. Addition of tris/glycine/beef extract (TGBE) buffer, pH 9.5, and pectinase, then elution, followed by concentration with polyethylene glycol (PEG) 6000 /NaCl precipitation, and further purification by chloroform–butanol. Typically, viruses from 10-25 g fruit would be eluted in 1-2 mL PBS.
2. Addition of TGBE buffer, pH 9.5, and pectinase, followed by centrifugation/ ultracentrifugation, and further purification by chloroform. Typically, viruses from 50 g fruit would be eluted in 2 mL PBS (USFDA, 2022).
3. Addition of media containing 2% (wt/wt) foetal bovine serum, and virus recovery by washing.
4. Addition of phosphate-buffered saline and virus recovery by washing.

52 B 4.2 Liquid product (e.g., purees)

Infectious virus recovery from purees can be achieved by simply diluting the puree post treatment, (e.g., 50 or 100-fold in cell culture medium) prior to testing (Deboosere *et al.*, 2004; Deboosere *et al.*, 2010).

However, methods similar to that used for whole fruit have also been used for purees. For example, Huang *et al.* (2016) added TGBE buffer to puree (pH 9.5), followed by mixing and centrifugation. PEG precipitation was then carried out to concentrate viruses into a smaller volume (5 mL).

Baert *et al.* (2008), studying MNV and B40-8 bacteriophage, used a similar approach to Huang *et al.* (2016) but for purees, pectinase was also added to the TGBE buffer. Baert *et al.* (2008) concentrated viruses from 10 g puree to 2 mL PBS. Testing for MNV and B40-8 bacteriophage was performed on 1/100 and 1/10 dilutions, respectively, due to interference on the plaque assays.

DETECTION ASSAYS TO ASSESS VIRUS INACTIVATION

53 B.5.1 Virus cell culture

For culturable viruses (not including bacteriophages), susceptible cells are inoculated with sample containing the virus, and incubated to allow replication. Infectious viruses that replicate in an adherent cell line can be measured by plaque and focus forming assays, or by the liquid end point dilution assay. Infectivity measurements depend on the cell culture approach method and sensitivity of the cell line used. Compared to the plaque assays, the end point dilution assay is generally less time consuming, more economical and capable to higher throughput. Specialised skills are required for cell culture maintenance and virus cell culture.

54 B.5.2 Plaque forming assay

It is assumed that one PFU is formed through the progeny of one infectious virus particle. However, an aggregation of viruses may appear as one plaque which can underestimate the virus count. Plaque forming assays are not suitable for viruses that develop CPE very slowly,

fail to produce plaques despite infecting the cells, or when the virus does not grow in the cells.

The plaque forming assay has been used for numerous virus inactivation studies on berry foods, including:

- HAV treated with chlorine dioxide gas (Annous *et al.*, 2021), cold plasma (Velebit *et al.*, 2022), disinfectants (Kingsley and Annous, 2021), gamma radiation (Bidawid *et al.*, 2000), heat (Deboosere *et al.*, 2004; Deboosere *et al.*, 2010), HPP (Kingsley *et al.*, 2005), ozone (Brié *et al.*, 2018) and pulsed light (Jubinville *et al.*, 2022).
- MNV treated with cold plasma (Lacombe *et al.*, 2017; Velebit *et al.*, 2022), gamma radiation (Pimenta *et al.*, 2019), HPP (Huang *et al.*, 2014; Li *et al.*, 2013; Lou *et al.*, 2011), pulsed light (Huang and Chen, 2015; Jubinville *et al.*, 2022), heat (Bartsch *et al.*, 2019), and UV (Liu *et al.*, 2015).
- Human adenovirus treated with gamma irradiation (Pimenta *et al.*, 2019).
- Tulane virus treated with cold plasma (Lacombe *et al.*, 2017), e-beam ionising radiation (DiCaprio *et al.*, 2016), gaseous chlorine dioxide (Kingsley and Annous, 2019) and heat (Bartsch *et al.*, 2019).

55 B.5.3 Focus forming assay

These are also called immune-focus assays. The focus forming unit (FFU) per volume of measure added to a well (e.g., 6, 24, 96-well plate format) is determined, usually a manual process, but can be automated, particularly when in a 96-well format. While the focus forming assay has been used for HAV inactivation studies, there are no published reports of it being used for experiments involving berries and berry products.

56 B.5.4 End point dilution assay

Titres determined from an endpoint assay can be greater than those calculated by plaque assays but are dependent on the relative sensitivity of the virus to the culture approach and volumes used. These factors may be considered when selecting a suitable method for inactivation studies.

End point dilution assays are particularly useful for viruses that develop CPE very slowly, and/or fail to produce plaques despite infecting the cells. The output is read by eye (for cytopathic viruses), or immunoperoxidase or immunofluorescent staining (for non-or minimally cytopathic viruses). Cells generally remain viable for longer (up to 14-28 days for some cell lines) than those in a plaque assay, so the end point dilution assay been utilised for studies of slower growing HAV strains. Because the number of cells required is much less than that of the plaque assay, and the method is less time consuming, end point dilution assays have also been utilised for faster growing viruses such as MNV and FCV.

The $TCID_{50}$ MPN assay has been used for inactivation studies on berries and/or berry products including for HAV treated with heat/freeze drying (Butot *et al.*, 2009; Butot *et al.*, 2008) or UV (Butot *et al.*, 2018; Fino and Kniel, 2008), MNV treated with HPP (Kovač *et al.*, 2012), ozone (Brié *et al.*, 2018) or UV (Liu *et al.*, 2015), FCV treated with UV (Fino and Kniel, 2008) and Aichi virus treated with UV (Fino and Kniel, 2008).

57 B.5.5 Cell culture-PCR assay

In a quantitative approach, multiple replicates, with optional dilutions where required are used at the cell culture stage, and PCR detection is used to determine presence/absence of viruses in each well by comparing the titres pre- and post-incubation. An increase in PCR

titre indicates the presence of infectious virus. Wells (e.g., 6 or 24-well plate format) are used for the cell culture step. This approach can take less time than cell culture assays alone.

58 B.5.6 Human intestinal enteroid cell culture

While the development of HIE culture methodology has been successful, culture has not been successful for all norovirus strains from faecal samples. Screening is needed to ensure viral replication is successful and reproducible. Generating norovirus progeny to be used in subsequent infection assays has been difficult to replicate. Despite the challenges, there are a few studies that have utilised HIE culture for inactivation experiments. These include HPP studies on berry puree (Pandiscia *et al.*, 2024; Wales *et al.*, 2024). Improvements are required to increase sensitivity and usability of the assay.

59 B.5.7 Capsid integrity assays (viability PCR)

This method has mostly been used in norovirus studies because these viruses are difficult to culture but has been applied in HAV studies since 2015 (Moreno *et al.*, 2015).

There are different approaches to this test, but all involve pre-treatment with a nucleic acid-binding agent prior to nucleic acid extraction and RT-qPCR or RT-ddPCR.

As introduced in Section 2.5, and detailed further below, the pre-treatments used most often are:

- Viability dye.
- RNase to remove free viral RNA (and/or DNase to remove free viral DNA).
- Capsid binding assay.

60 B.5.7.1 Viability dye (nucleic acid binding) assay

PMA was used for a study examining the effect of cold atmospheric plasma on HAV and MNV (Velebit *et al.*, 2022). PMAxx (100 µM) and $PtCl_4$ (2.5 mM) pre-treatment was assess for heat treatment studies (Chen *et al.*, 2020).

61 B.5.7.2 RNase assay

RNase pre-treatment has been applied during studies of the effect of heat on Tulane virus and human norovirus in strawberry purees (Bartsch *et al.*, 2019), and an assessment to detect inactivation (through heating to 95 °C and UVC for 30 minutes) of HAV, MNV and HAdV on strawberries (Marti *et al.*, 2017).

62 B.5.7.3 Capsid binding assay

A PGM conjugated magnetic bead (PGM-MB) binding assay, followed by RT-qPCR, has been used to assess virus infectivity. DiCaprio *et al.* (2019) applied a PGM-MB binding assay on norovirus (GII.4) for the study on HPP treatment of strawberry puree. HPP inactivation of human norovirus on strawberries, blueberries and raspberries (and purees) was assessed using a PGM-MB binding assay with RT-qPCR (Huang *et al.*, 2016). If other treatments are included, such as RNase to remove free RNA, the assay can more selectively detect infectious viruses. This was demonstrated by Huang *et al.* (2016). Generally, this approach will also overestimate infectivity by 1 to 4 $\log_{10}$, and will not detect loss of infectivity due to genome damage alone.

MAXIMUM MEASURABLE LOG_{10} REDUCTION

As introduced in Section 2.6, the maximum measurable log_{10} reduction depends on a number of factors. This section provides further information to illustrate this point.

HAV HM-175 can be successfully grown to titres ranging from 10^6 to 10^8 PFU/mL as measured by plaque assay, although concentrations up to 10^{10} PFU/mL have been reported (Velebit *et al.*, 2022). A concentration of 10^8 PFU/mL would be similar to that achieved through the culture of surrogate viruses such as MNV. Low concentrations can restrict the ability to calculate log reductions, especially considering detection limits of the assays. In addition, a 100% virus recovery from a sample would also be unexpected. An acceptable recovery is usually set at 10%, but can be much less, such as <0.1% (Butot *et al.*, 2021).

By way of an example, seeding 5 g fruit with a total of 100 µL HAV stock at 10^7 PFU/mL will result in a total virus load of 10^6 PFU per 5 g. If the recovery is 1%, this results in a 10^4 PFU/5 g before treatment (i.e., the N0 value). Assuming a LOQ of 1.0 log_{10} PFU/5 g, then the maximum reduction that could be measured is 3.0 log_{10}. A recovery of 0.1% is not unusual when the viruses are in complex matrices (Butot *et al.*, 2021), and such a recovery would reduce the measurable log reduction to a maximum of 2.0 $log_{10.}$ This is an issue when a viral inactivation method needs to achieve a 3-4 log_{10} reduction.

Cell culture assays can also be affected by cytotoxic agents in the sample, which can further reduce the maximum measurable log reduction. If the sample inoculated onto the cells is cytotoxic, the volume of the sample (e.g., a food extract) to be tested has to decrease, usually through dilution using PBS or cell culture medium (Wales *et al.*, 2024). Dilutions may need to be a high a 1/1000 to counteract toxicity to cells. This issue also negatively impacts the LOQ, (i.e., less sensitive assay). A similar effect occurs if the sample (e.g., food concentrate) is too viscous for cell culture inoculation and needs to be diluted. This applies to all cell culture assays, including the HIE assay for human norovirus. In one study that examined the effect of electron beams on MNV (and HAV) infectivity (Butot *et al.*, 2021), a combination of a very low virus recovery from freeze-dried raspberries, and the toxicity of the concentrate on RAW 264.7 cells, resulted in log_{10} reduction data not being able to be determined for MNV.

Bacteriophages such as MS2 and Qβ can grow to titres that are 2-3 log_{10} higher than viruses, so the maximum measurable reduction can be higher. In addition, less interference from the matrix can occur with the bacteriophage assay, as described by Baert *et al.* (2008); although a 1/100 dilution was needed for MNV analysis, only a 1/10 dilution was required for the phage analysis.