Assessing the efficacy of control measures against viruses using surrogates

Annette Sansom
Campden BRI

IAFP European Symposium on Food Safety
Session 6
Thursday 5th May 2022

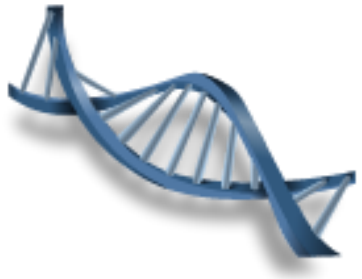
Viruses of concern to food industry

- Virus related foodborne illness is a cause for concern
  - Norovirus (NoV), Hepatitis A (HAV), Hepatitis E (HEV)
- Increasing awareness of role of food in spread of illness
  - Report published by the FSA in 2019 estimated that 380,000 cases of norovirus linked to food occur in the UK per year.
  - Another report by FSA published in 2021 showed norovirus to rank “high” in detriment to society
- Important to have methods to enable us to understand survival and persistence of viruses on foods and in the environment

Campden BRI
food and drink innovation

But are they still infective.....

If I find this



in my product....

Does it mean that there are

Present in the sample

?

Limited information on survival and persistence of infectious viruses

Campden BRI
food and drink innovation

Assessing virus control measures

- In order to assess the effectiveness of control strategies on viruses, need to measure how the strategy affects the infectivity of the virus
- Many viruses that infect humans relevant to food industry are currently unculturable or difficult to culture
  - They may be dangerous to handle and require specialist laboratory conditions.
  - Provide hurdles to assess and validate effectiveness of virus control measures

Campden BRI
food and drink innovation

Use of surrogates

- If we can use other “non-human infectious” viruses, similar in structure, size – provides a method to assess infectivity
- Enabling assessment of virus control measures
- These “surrogate” viruses can be:
  - Non-human mammalian viruses
    - For example - Murine norovirus (MNV), Feline calicivirus
  - Bacteriophage - viruses that infect bacteria
    - For example -MS2, Phi X174, Phi6

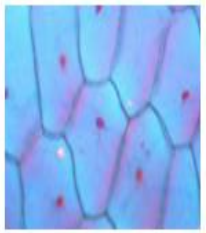
Campden BRI
food and drink innovation

Norovirus surrogates – MS2 & MNV

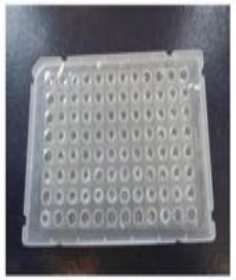
- **MS2 bacteriophage**
  - Non -Enveloped RNA virus
  - Reported to be a useful surrogate for norovirus – similar in size & structure
  - Biosafety level 1 – can be used safely in different environments
  - Infects E.coli– will be part of natural gut microbiome – similar environmental stress
  - Easy to use, get consistent large titers of the virus
- **Murine norovirus**:
  - Non-Enveloped RNA virus
  - Reported to be a useful surrogate for norovirus – similar in size & structure
  - Biosafety level 2
  - Mammalian virus
  - Infects BV2 or RAW cells grown in tissue culture facilities

Infectious assay methods

Mammalian virus - TCID50 Assay - MNV



Grow host cells to specific conditions, & growth stage



Infect host cells

Incubation

Enumeration of infective particles

Dilutions of virus

Bacteriophage Plaque assay – MS2 & ΦX174

Phage
Host
Soft agar

Pre-poured plate

Incubation

Both methods look for the infectious action of the virus

Campden BRI
food and drink innovation

Virus control – Research at Campden BRI

- Increase understanding of the “survival” and “stability” of infective virus particles in food products and environments

- Establish the effects of food safety control measures on the infectivity of viruses
  - Intrinsic (pH, aW)
  - Extrinsic (storage temperature)
  - Decontamination processes (Heat treatments, produce decontamination technologies, environmental decontamination)

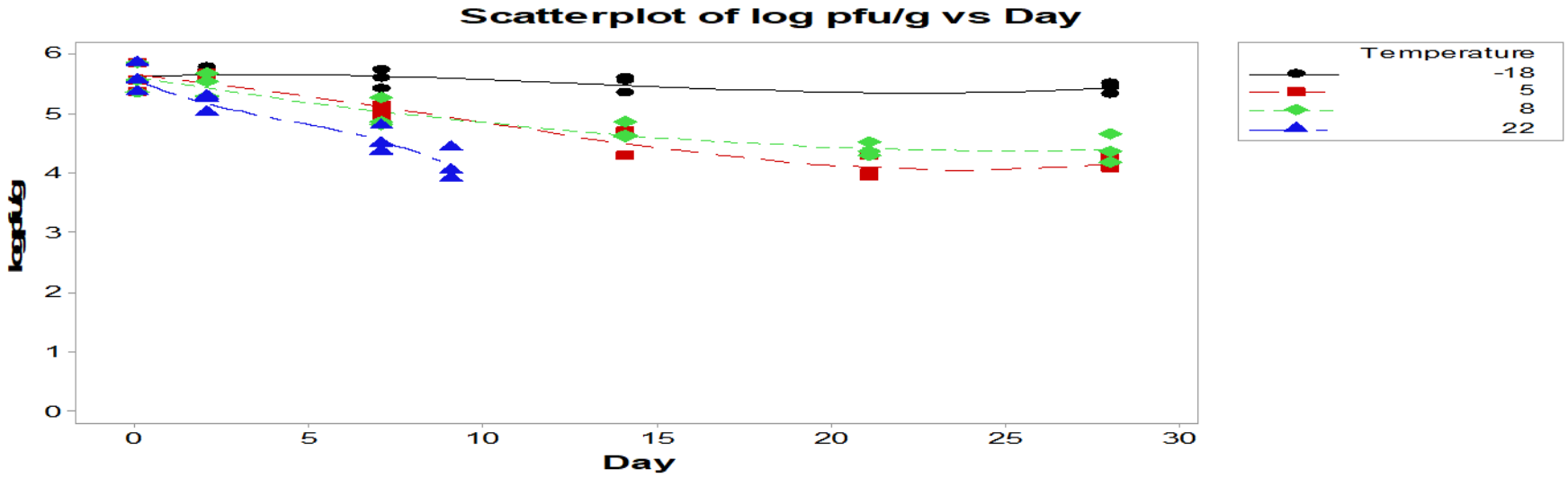
Campden BRI
food and drink innovation

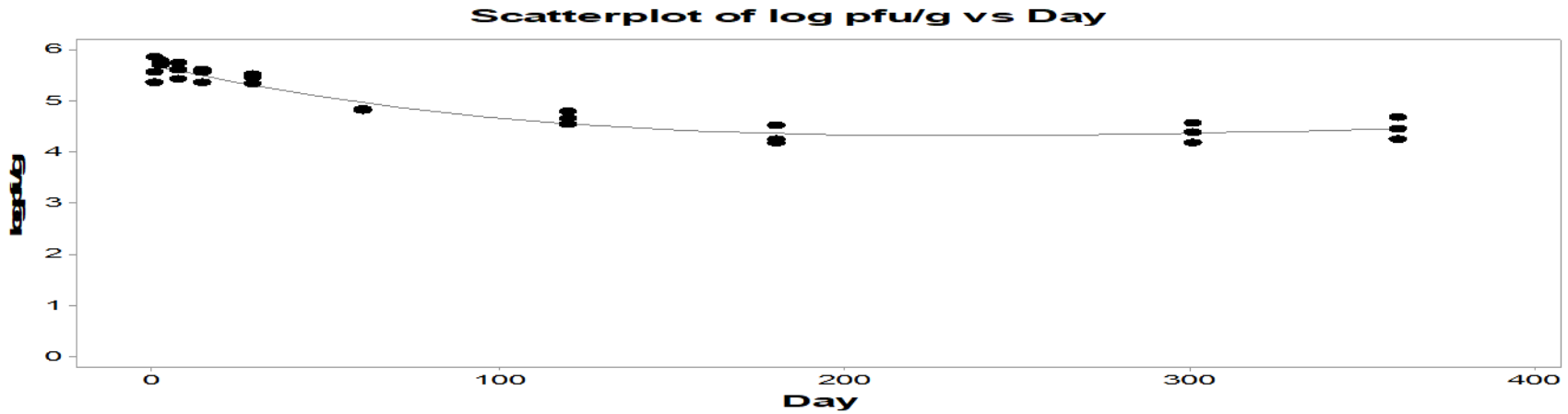
Persistence trials

- Inoculated trials
- Triplicate samples enumerated on ≥4 time points

Parameter	Surrogate	Matrix	Storage temperature (°C)
Temperature	MS2	Parsley	22, 8, 4 & -18
pH	MS2 & ϕX174	NB with HCl, or citric acid, juice	5

Stability of MS2 on flat leaf parsley

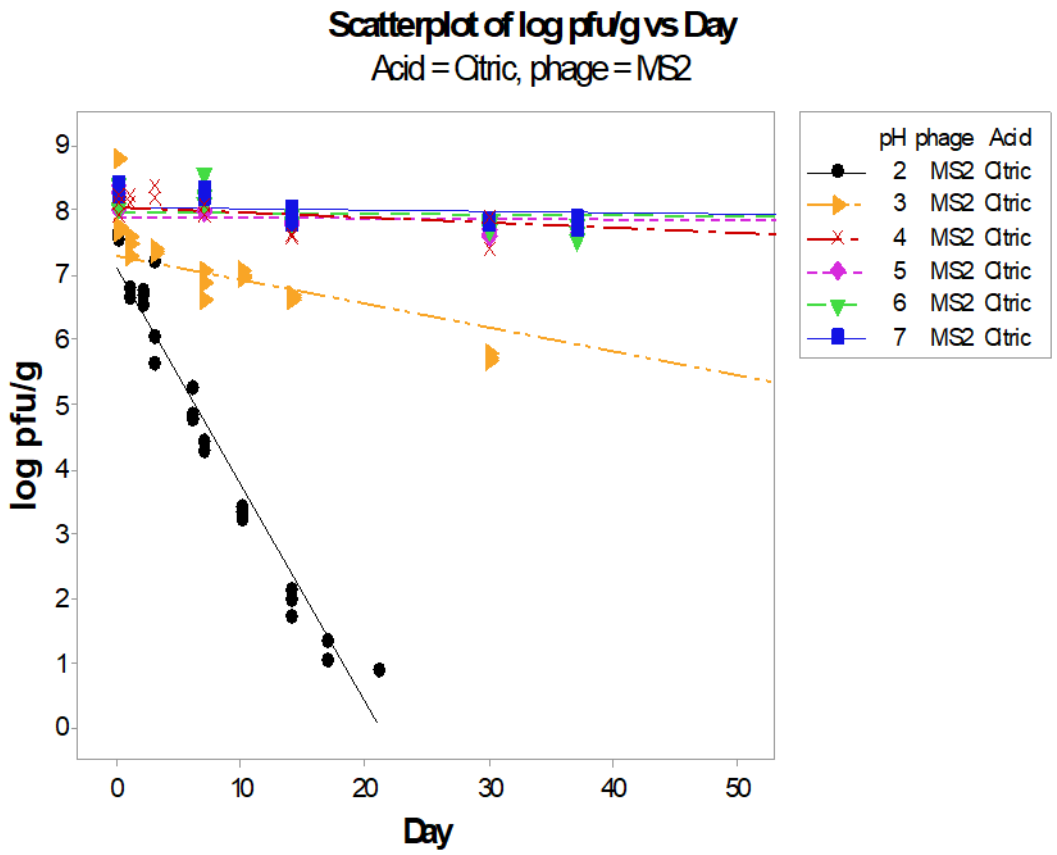




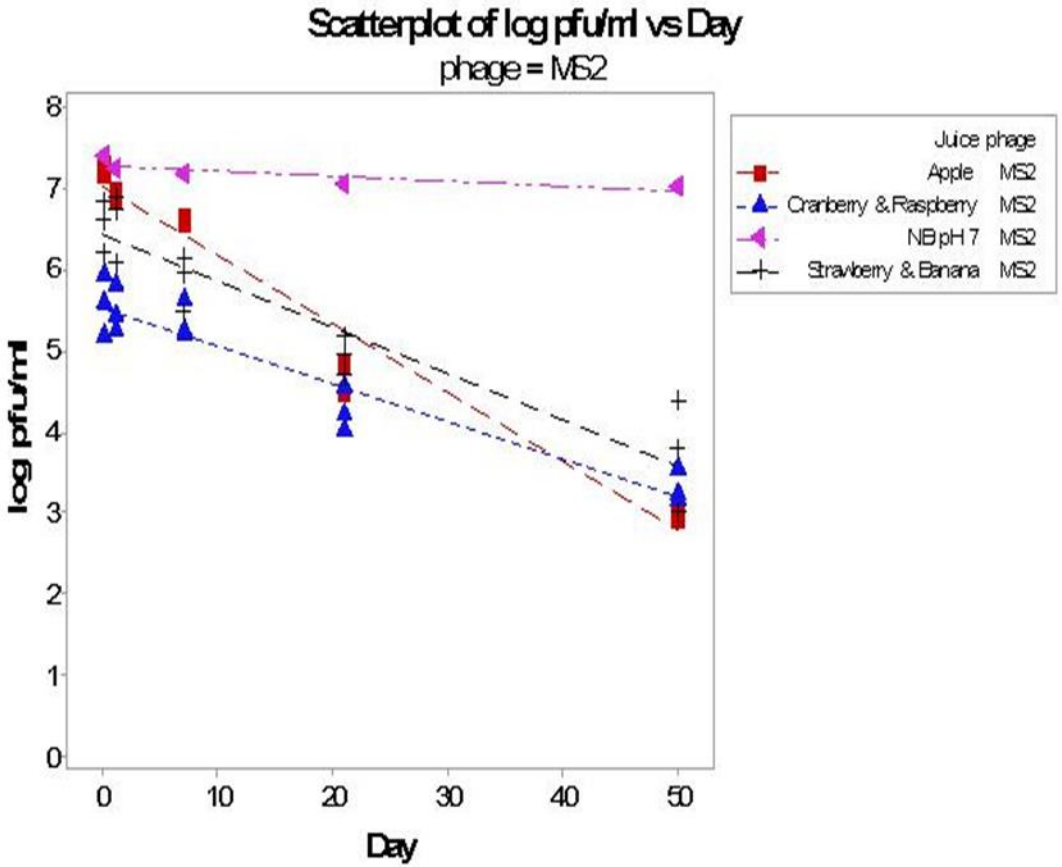
Campden BRI
food and drink innovation

Stability under various pH

Citric acid



Juice



Persistence trials – results summary

- Temperature storage - infective phage remains on parsley for as long as the product life
  - Agrees with other published data that show viruses are stable under frozen & chilled storage conditions
- Above pH 4 no reduction in virus observed over 50 days
  - Little reduction in levels of MS2 is seen at pH 4-7
  - At pH 2 infective phage was found for up to 19 days
  - Acid tolerance was not significantly influenced by acid type use
- Juice
  - The results of the juice trials were similar but different to the citric acid pH 2 – 3 results.
  - Immediate drop could possibly be due to ingredients of the juices

Campden BRI
food and drink innovation

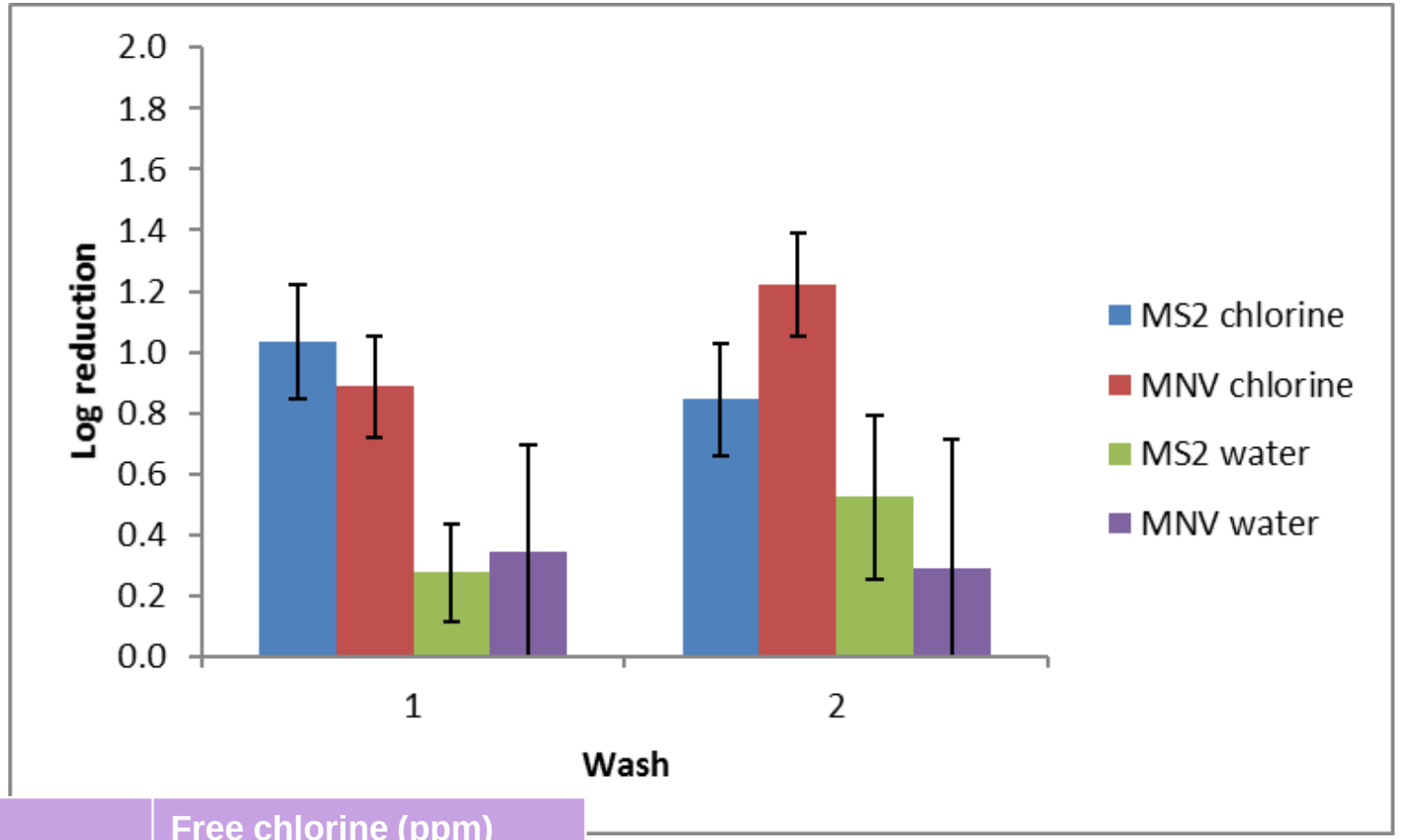
Produce decontamination

- Iceberg lettuce, inoculated with MS2 & MNV
- Washed with water or chlorine – lab scale
  - Duplicate trials
- Washed with chlorine or hydrogen peroxide based wash – pilot scale
  - Triplicate trials
- Triplicate samples tested before and after washing

Campden BRI
food and drink innovation

Produce decontamination Results – lab scale

- MNV & MS2 reductions similar
- 50ppm free chlorine wash better than water alone
- Chlorine keeps the wash water clean of virus – preventing cross contamination

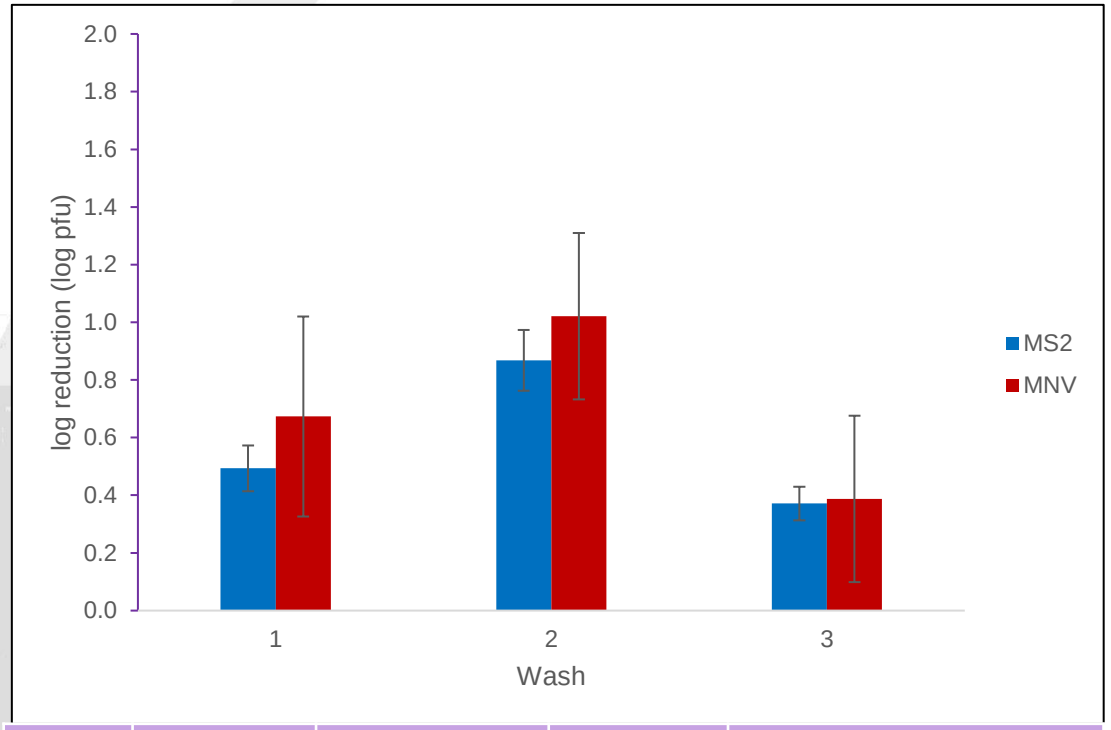


Wash type	Rep	MS2 (pfu/ml)	MNV ($TCID_{50}$/ml)	pH	Free chlorine (ppm)	
		After	After	Before	Before	After
Chlorine	1	<10	<63	6.82	55	50
	2	<10	<63	6.82	55	53
Water	1	$4.1x10^4$	$2.9x10^4$	7.4	NT	NT
	2	$2.9x10^4$	$2.9x10^4$	7.4	NT	NT

Campden BRI
food and drink innovation

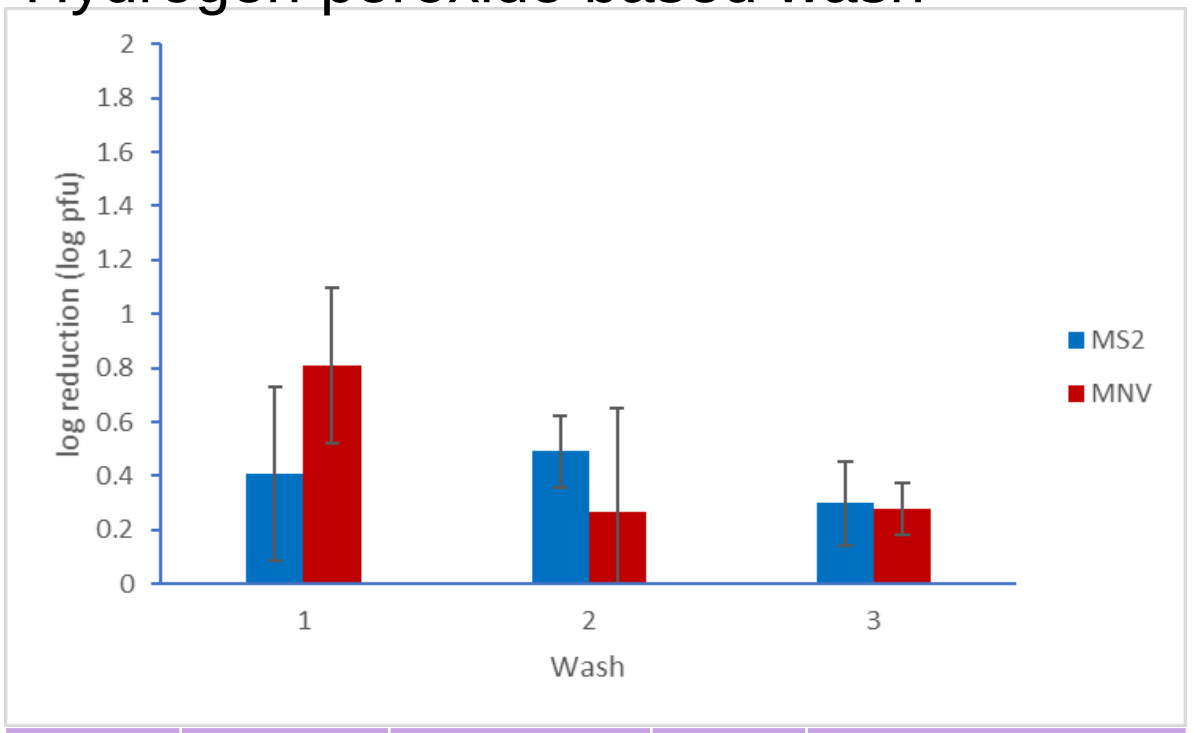
Produce decontamination Results – Pilot scale

Chorine wash



Rep	MS2 (pfu/ml)	MNV ($TCID_{50}$/ml)	pH	Free chlorine (ppm)	
	After	After	Before	Before	After
1	<10	<63	7.16	50	28
2	<10	<63	7.01	50.4	35.8
3	<10	<63	7.14	54.4	52.8

Hydrogen peroxide based wash



Rep	MS2 (pfu/ml)	MNV ($TCID_{50}$/ml)	pH	Hydrogen peroxide (ppm)	
	After	After	Before	Before	After
1	<10	<63	7.79	50	50
2	<10	<63	8.04	50	50
3	<10	<63	8.27	50	50

UV trials with MNV & MS2

- Various Matrices
  - Stainless steel squares (5cm^2)
  - Blueberries
  - Water
- Inoculated with MS2 or MNV
  - Blueberries and stainless steel had overnight attachment step
  - Water inoculated on treatment day

Campden BRI
food and drink innovation

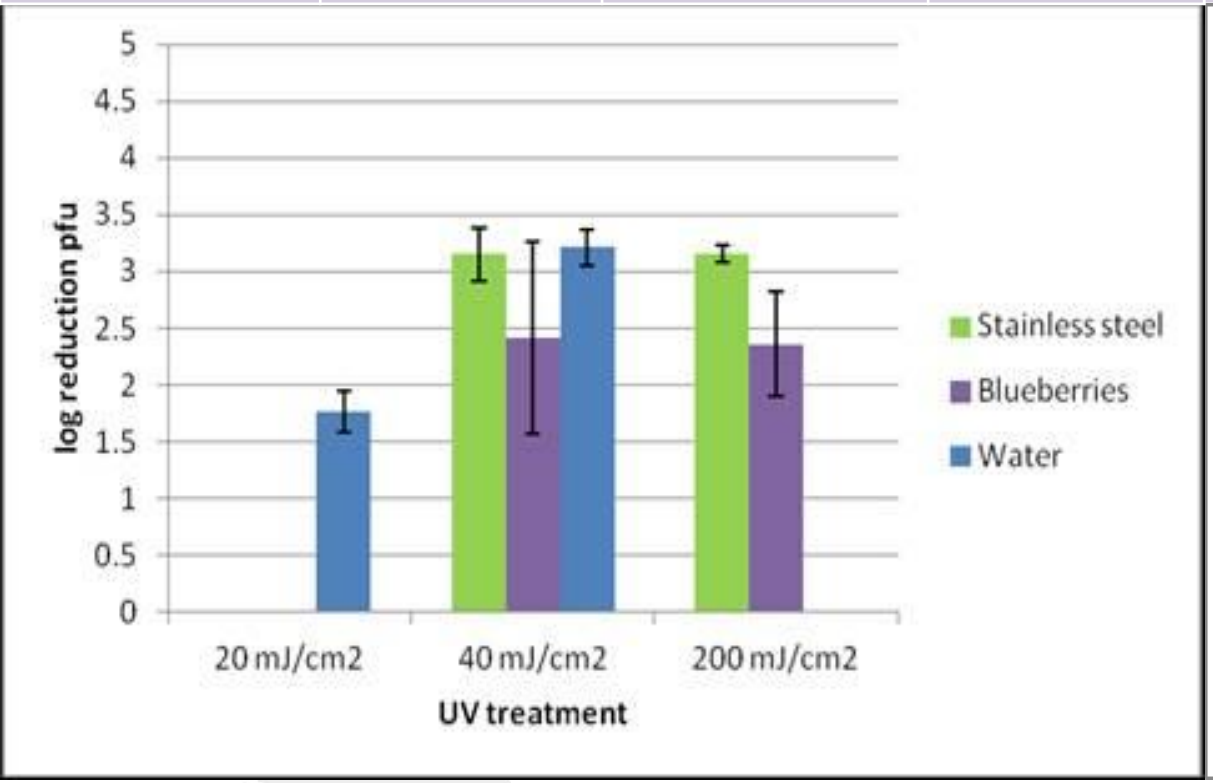
UV-C treatments

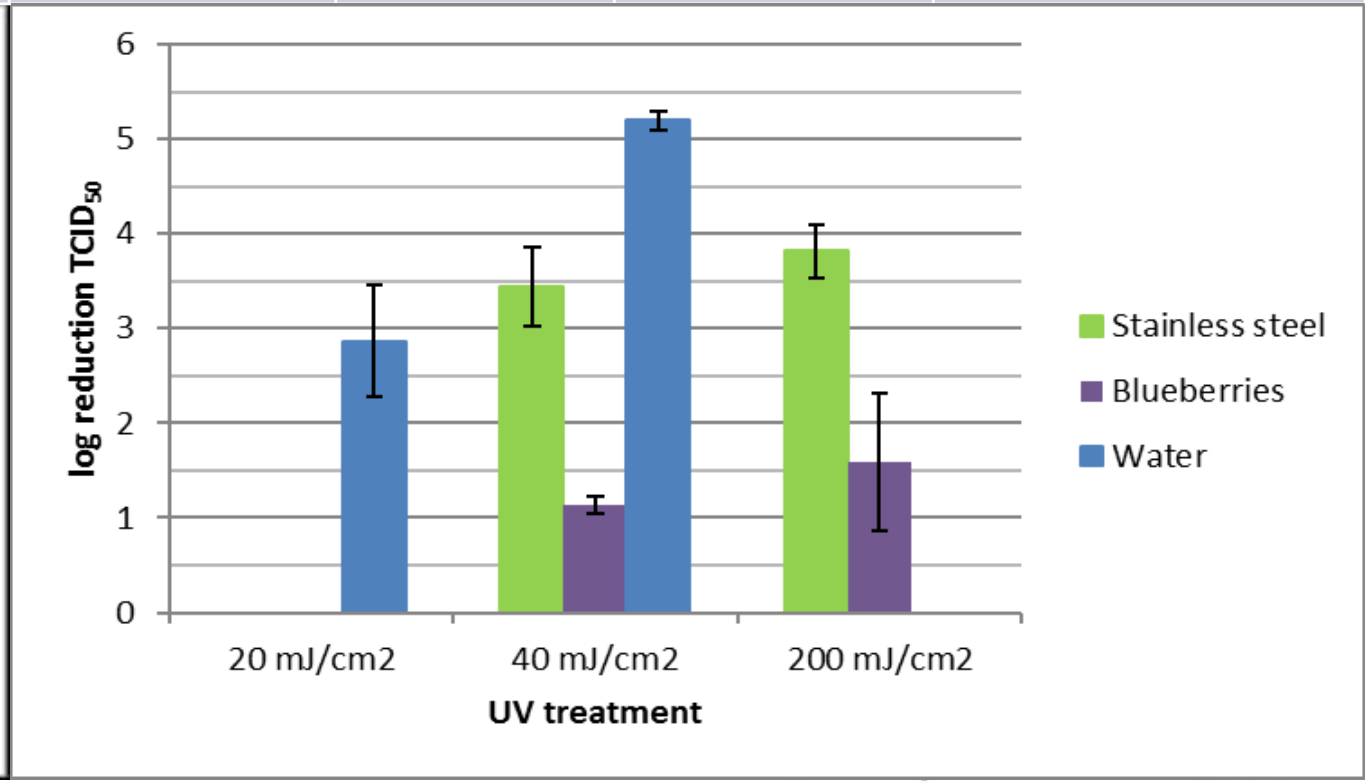
Matrix	UV-C treatment method – UV-C 595nm	UV-C dose (mJ/cm^2)
Stainless steel (5cm^2)	UV tunnel with only top lamps. Held on UV-C transparent support material	40 or 200 Passed through tunnel at 2 different speeds.
Blueberries (100g)	UV tunnel with only top lamps. Held on UV-C transparent support material. Passed through tunnel twice with 180º rotation.	40 or 200 Passed through tunnel at 2 different speeds.
Water (20 ml)	Static box setup with holes and shutter door. Treated in petri dishes with magnetic stirrer, positioned under holes.	20 or 40 Exposure controlled by length of time shutter door was open.

MS2

MNV

Virus	Stainless steel	Blueberries	Water	Virus	Stainless steel	Blueberries	Water
MS2	$4.8x10^6$ pfu/cm^2	$5.7x10^4$ pfu/g	$8.7x10^5$pfu/ml	MNV	$2.1x10^5$ $TCID_{50}/cm^2$	$5.5x10^4$ $TCID_{50}/g$	$4.1x10^5$ $TCID_{50}/ml$





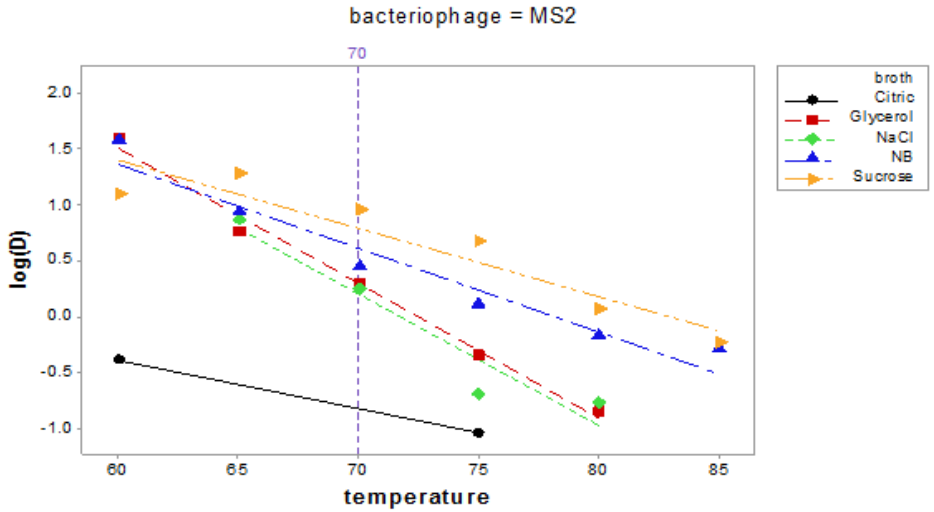
Decontamination summary

- Produce decontamination - chlorine and hydrogen peroxide based
  - Very little reduction of the viruses tested was achieved.
  - Sanitisers keep the wash solution clean
- UV treatment – Reductions of
  - 1.7 – 3.2 log pfu for MS2
  - 1.1 – 5.2 log TCID50 for MNV
  - Greater reduction on water and stainless steel for MNV
  - Greater reduction on blueberries for MS2
  - Higher dose did not necessarily increase the reduction
- UV gives good reduction of virus surrogates in different matrices
- However, reduction will be limited by where the light reaches

Other trials

Have also carried out trials assessing the effects of:

- aW – various solutes
- Heat including drying
- Persistence on reusable containers
- Air cleaning systems

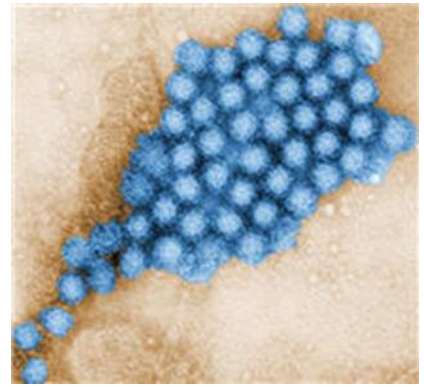


On bacteriophage used as surrogates.

Ongoing trials assessing the effects on mammalian surrogate viruses

Campden BRI
food and drink innovation

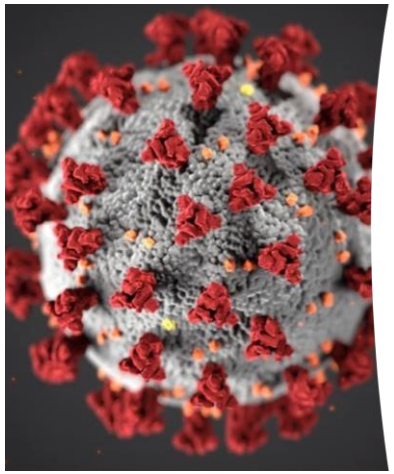
Challenges to assessing control measures



- Standardised method for evaluating decontamination strategies for foods would be useful
- Ideal to have culturable target strains to compare surrogates to.
- Target reduction level – what is necessary???
  - 1log – low reduction
  - 3 log – high reduction
  - is this enough?

Campden BRI
food and drink innovation

By using a range of surrogates, we can build up data. This will provide information on the affect different control strategies can have on viruses.



Campden BRI
food and drink innovation

Thank you for listening!

Questions?

Campden BRI
food and drink innovation