Assessing the efficacy of control measures against viruses using surrogates

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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
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.
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
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
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
- Dilutions of virus
- Infect host cells
- Incubation
- Enumeration of infective particles

Bacteriophage Plaque assay – MS2 & ΦX174

- Pre-poured plate
- Phage
- Host
- Soft agar
- Incubation

Both methods look for the infectious action of the virus
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)
### Persistence trials

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surrogate</th>
<th>Matrix</th>
<th>Storage temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>MS2</td>
<td>Parsley</td>
<td>22, 8, 4 &amp; -18</td>
</tr>
<tr>
<td>pH</td>
<td>MS2 &amp; φX174</td>
<td>NB with HCl, or citric acid, juice</td>
<td>5</td>
</tr>
</tbody>
</table>
Stability of MS2 on flat leaf parsley
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
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
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

<table>
<thead>
<tr>
<th>Wash type</th>
<th>Rep</th>
<th>MS2 (pfu/ml)</th>
<th>MNV (TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>pH</th>
<th>Free chlorine (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Chlorine</td>
<td>1</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>6.82</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>6.82</td>
<td>55</td>
</tr>
<tr>
<td>Water</td>
<td>1</td>
<td>4.1x10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.9x10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7.4</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.9x10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.9x10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7.4</td>
<td>NT</td>
</tr>
</tbody>
</table>
Produce decontamination Results – Pilot scale

Chorine wash

<table>
<thead>
<tr>
<th>Rep</th>
<th>MS2 (pfu/ml)</th>
<th>MNV (TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>pH</th>
<th>Free chlorine (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After</td>
<td>After</td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>7.16</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>7.01</td>
<td>50.4</td>
</tr>
<tr>
<td>3</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>7.14</td>
<td>54.4</td>
</tr>
</tbody>
</table>

Hydrogen peroxide based wash

<table>
<thead>
<tr>
<th>Rep</th>
<th>MS2 (pfu/ml)</th>
<th>MNV (TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>pH</th>
<th>Hydrogen peroxide (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After</td>
<td>After</td>
<td>Before</td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>7.79</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>8.04</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>&lt;10</td>
<td>&lt;63</td>
<td>8.27</td>
<td>50</td>
</tr>
</tbody>
</table>
UV trials with MNV & MS2

• Various Matrices
  – Stainless steel squares (5cm²)
  – Blueberries
  – Water

• Inoculated with MS2 or MNV
  – Blueberries and stainless steel had overnight attachment step
  – Water inoculated on treatment day
## UV-C treatments

<table>
<thead>
<tr>
<th>Matrix</th>
<th>UV-C treatment method – UV-C 595nm</th>
<th>UV-C dose (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless steel (5cm²)</td>
<td>UV tunnel with only top lamps. Held on UV-C transparent support material</td>
<td>40 or 200 Passed through tunnel at 2 different speeds.</td>
</tr>
<tr>
<td>Blueberries (100g)</td>
<td>UV tunnel with only top lamps. Held on UV-C transparent support material. Passed through tunnel twice with 180° rotation.</td>
<td>40 or 200 Passed through tunnel at 2 different speeds.</td>
</tr>
<tr>
<td>Water (20 ml)</td>
<td>Static box setup with holes and shutter door. Treated in petri dishes with magnetic stirrer, positioned under holes.</td>
<td>20 or 40 Exposure controlled by length of time shutter door was open.</td>
</tr>
<tr>
<td>Virus</td>
<td>Stainless steel</td>
<td>Blueberries</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MS2</td>
<td>4.8x10^6 pfu/cm²</td>
<td>5.7x10^4 pfu/g</td>
</tr>
</tbody>
</table>

**Graphs:**

- Left graph: Log reduction pfu
  - UV treatment: 20 ml/cm², 40 ml/cm², 200 ml/cm²
  - Legend: Stainless steel, Blueberries, Water

- Right graph: Log reduction TCID<sub>50</sub>
  - UV treatment: 20 ml/cm², 40 ml/cm², 200 ml/cm²
  - Legend: Stainless steel, Blueberries, Water

Food and drink innovation
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.
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???
  – 1 log – low reduction
  – 3 log – high reduction
  – is this enough?
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.
Thank you for listening!

Questions?