Shedding some light on inactivation of foodborne viruses

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Foodborne viruses

- **Virion**: non enveloped
- **Capsid**: icosahedral
- **Diameter**: 27 - 38 nm
- **Genome**: single-stranded RNA

Norovirus (NoV)  
- Cannot replicate in food, in very low number
- Low infective dose (10-100)
- Difficult to work since no culture system

Hepatitis A virus (HAV)
Transmission

Symptomatic (70%)  Asymptomatic (30%)

Infected individual

Intestinal disease

Shedding viruses

Feces  Vomitus

Present (40%)  Absent (60%)

Protected  Susceptible

Previously acquired immunity

Non-shedding (20%)  Shedding (80%)

Resistant  Susceptible

Exposed population

Transmission vectors

Person  Food  Water  Environment

Hall et coll. 2013
Inactivation strategies

- Individual
- Water
- Food
- Environment

Chemical and Physical Approaches
Inactivation: *chemical* approach

- **✓** Chlorine
- **✗** Alcohol
- **✗** Quaternary Ammoniums
  - × Chlorine dioxide
  - × Ozone
  - × Peracetic Acid

Does not seem to be affected by organic matter

3-log reduction MNV; 2 ppm, 15 min
3-log reduction MNV; 6 ppm, 30 min
1-log reduction MNV; 20 ppm, 1 min
Disinfection: *physical approach*

Compared to antibacterial activities, very few antiviral studies have been conducted. The effectiveness of different equipment (static vs dynamic) and the difference in data (UV amount vs fluence) have been noted. Promising results show a reduction of several log in short time.

Sow et al. FPD 2011; Jean et al. FM 2011; Vimont et al. AEM 2015
Specific objectives

1. Evaluate the anti-norovirus activity:
   - in suspension
   - on inert surfaces
   - in artificial biofilm

2. Identify the viral targets involved in mechanism of action

Peracids

Pulsed light

60% : acid
40% : $\text{H}_2\text{O}_2$
What’s a peracid?

Organic acid + Hydrogen peroxide → Peroxide liaison

\[
\text{R-COOH} + \text{HO-OH} \rightleftharpoons \text{HO-OH} + \text{H}_2\text{O}
\]

FREE RADICALS
highly reactive
with high antimicrobial activity

Sanchez and Myers 2000
Inactivation : suspension

- According to ASTM E 1052-96
- MNV : $10^7$ PFU mL$^{-1}$

**SOLUTIONS** : peracetic / perpropionic / perlactic / percitric

**CONCENTRATIONS** : 50 / 250 / 500 / 1000 mg L$^{-1}$

**CONTACT TIME** : 1 / 5 / 10 min
Inactivation: suspension

**PERACETIC ACID**

**Good candidate**

Viral reduction (log_{10})

<table>
<thead>
<tr>
<th>Peracetic acid concentration (mg L^{-1})</th>
<th>1 min</th>
<th>5 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>c</td>
<td>ab</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>c</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>500</td>
<td>a</td>
<td>a</td>
<td>bc</td>
</tr>
<tr>
<td>1000</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>
Inactivation : suspension

PERACETIC ACID

\[ \text{H}_3\text{C}–\text{COOOH} \]

PERPROPIONIC ACID

\[ \text{H}_3\text{C}–\text{CH}_2–\text{COOOH} \]

Viral reduction (\(\log_{10}\))

![Bar chart for PERACETIC ACID](image)

![Bar chart for PERPROPIONIC ACID](image)
Inactivation: suspension

PERACETIC ACID

\[ \text{H}_3\text{C}–\text{COOOH} \]

Viral reduction \((\log_{10})\) vs. [peracide] (mg L\(^{-1}\))

PERLACTIC ACID

\[ \text{H}_3\text{C}–\text{CH}–\text{COOOH} \]

\[ \text{OH} \]

deb
Inactivation: suspension

**PERACETIC ACID**

H$_3$C–COOOH

**PERCITRIC ACID**

HOOOC–H$_2$C–CH$_2$–COO–OH

Viral reduction (log$_{10}$) vs. [peracide] (mg L$^{-1}$)

- **1 min**
- **5 min**
- **10 min**
Inactivation: suspension

Reversible reaction

\[
\text{Organic acid} + \text{Hydrogen peroxide} \rightleftharpoons \text{Peracid} + \text{Water}
\]

Viral reduction \((\log_{10})\):

- Peracetic acid
- Acetic acid
- Hydrogen peroxide
Inactivation: surfaces and biofilm

Peracetic et perpropionic acids: 50 mg L\(^{-1}\); 5 min

**SURFACES**

ASTM E 2197-02

Stainless steel, PVC (clean and soiled)

Biofilm + MNV-1

10\(^7\) PFU g\(^{-1}\)

Treatment

5 min

Neutralisation

**BIOFILMS**

MNV-1

10\(^4\) PFU coupon\(^{-1}\)

Dissolution in sodium citrate
Inactivation: surfaces and biofilm

<table>
<thead>
<tr>
<th></th>
<th>Stainless steel</th>
<th>PVC</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clean</td>
<td>Soiled</td>
<td>Clean</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>94.0 ± 1.8</td>
<td>99.9 ± 0.1</td>
<td>97.5 ± 3.0</td>
</tr>
<tr>
<td>Peracetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perpropionic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total reduction \( \approx 4 \log_{10} \)}
Mechanism of action: possible targets

- Antigens
- Structure

RNA

Host-cell

Multiplication
Mechanism of action: samples

Control
PBS

8.6 \( \log_{10} \) PFU mL\(^{-1} \)

Moderate treatment
50 mg L\(^{-1} \); 5 min

4.9 \( \log_{10} \) PFU mL\(^{-1} \)

Harsh treatment
1000 mg L\(^{-1} \); 10 min

< 1 \( \log_{10} \) PFU mL\(^{-1} \)

MNV-1
9.0 \( \log_{10} \) PFU mL\(^{-1} \)
Morphology

Control
8,6 log$_{10}$ PFU mL$^{-1}$

50 mg L$^{-1}$
4,9 log$_{10}$ PFU mL$^{-1}$

1000 mg L$^{-1}$
< 1 log$_{10}$ PFU mL$^{-1}$
Viral proteins

Control
8.6 log_{10} PFU mL^{-1}

50 mg L^{-1}
4.9 log_{10} PFU mL^{-1}

1000 mg L^{-1}
< 1 log_{10} PFU mL^{-1}

180 copies of VP1

Viral Protein 1 (VP1)

SDS-PAGE
RNA

Control
8,6 log_{10} PFU mL^{-1}

50 mg L^{-1}
4,9 log_{10} PFU mL^{-1}

1000 mg L^{-1}
< 1 log_{10} PFU mL^{-1}

Bioanalyzer
RNA pico chips
Proposed mechanism of action

Moderate treatment

- Fragmentation of RNA
- Compromised replication

Compromised proteins?

Non-recognition by the host-cell
Conclusion - Peracids

**Solutions**: peracetic and perpropionic acids

**Regulation (FDA)**: 80 mg L$^{-1}$

**Possible applications** (reduction of at least 3 log$_{10}$):
- Suspension
- Clean surfaces
- Soiled surfaces
- Biofilm (artificial)

**Viral targets affected**: RNA and proteins
What is pulsed light?

Innovative technology

Non-thermal pasteurization for food preservation and decontamination of air or packaging material

Very short high-intensity pulse

Approval by FDA (200-1000 nm, 2 ms, 12 J cm$^{-2}$)

- Treatment chamber
- Control panel
- Xenon lamp
- Sample

- 8 cm
- 0.69 J cm$^{-2}$ (max : 12 J cm$^{-2}$)
- 0.02 J cm$^{-2}$ UV
- 830 J pulse$^{-1}$
Inactivation: suspension

MNV: $10^5$ PFU mL$^{-1}$

**COMPOSITION**

- Hardness: 0 to 400 mg L$^{-1}$
- Turbidity: 0 to 1000 NTU
- Wastewater
- Mineral water

**ENERGY**

1 to 7 impulsions
(0.7 to 4.8 J cm$^{-2}$)
Inactivation: suspension

Viral reduction (log_{10})

PBS

Fluence (J cm^{-2})

- 0.7 pulse, 0.7 sec
- 2.1 pulses, 1.6 sec
- 3.5 pulses, 2.7 sec
- 4.8 pulses, 3.9 sec
Inactivation: suspensions

PBS

HARD WATER

$P > 0.05$

Pulsed light does not seem to be affected by the hardness of water.
Inactivation: suspensions

**PHOSPHATE BUFFER**

- Viral reduction (log10)
  - Fluence (J cm\(^{-2}\))
    - 0.7
    - 2.1
    - 3.5
    - 4.8

**TURBID WATERS**

- Viral reduction (log10)
  - Fluence (J cm\(^{-2}\))
    - 0.7
    - 2.1
    - 3.5
    - 4.8

*P < 0.05*

Pulsed light affected by the water turbidity

- 50 NTU
- 100 NTU
- 200 NTU
- 500 NTU
- 1000 NTU
Inactivation: suspensions

**PBS**

Viral reduction (log$_{10}$)

0.7  2.1  3.5  4.8

Fluence (J cm$^{-2}$)

**WASTE AND MINERAL WATER**

$P > 0.05$

0.7  2.1  3.5  4.8

Fluence (J cm$^{-2}$)

- Wastewater after grit removal
- Wastewater after digestion
- Mineral water
Inactivation: surfaces and biofilm

× 1 to 13 pulses (0.7 to 9.0 J cm$^{-2}$)

SURFACES

Stainless steel, PVC (clean et soiled)

MNV-1 $10^5$ PFU coupon$^{-1}$

Treatment

BIOFILMS

Biofilm + MNV-1 $10^5$ PFU g$^{-1}$

Treatment

Dissolution
Inactivation: surfaces and biofilm

**STAINLESS STEEL**

\[ P < 0.001 \]

Soil could protect MNV by pulsed light on surfaces
Inactivation: surfaces and biofilm

**STAINLESS STEEL**

- Viral reduction (log$_{10}$)
- Fluence (J cm$^{-2}$)
- Clean condition
- Soiled condition

- $P < 0.001$

**PVC**

- Viral reduction (log$_{10}$)
- Fluence (J cm$^{-2}$)
- Clean condition
- Soiled condition

- $P < 0.001$
Inactivation: surfaces and biofilm

Biofilm does not affect the viral inactivation by pulsed light
Mechanism of action: samples

MNV-1
9.0 log_{10} PFU mL^{-1}

Control
0.0 J cm^{-2}

Moderate treatment
2.1 J cm^{-2}

Harsh treatment
9.0 J cm^{-2}

9.0 log_{10} PFU mL^{-1}

3.9 log_{10} PFU mL^{-1}

< 1 log_{10} PFU mL^{-1}
## Morphology

<table>
<thead>
<tr>
<th>Condition</th>
<th>Energy Density</th>
<th>CFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.0 J cm(^{-2})</td>
<td>(3.9 \log_{10} \text{PFU mL}^{-1})</td>
</tr>
<tr>
<td>Moderate</td>
<td>2.1 J cm(^{-2})</td>
<td>(&lt; 1 \log_{10} \text{PFU mL}^{-1})</td>
</tr>
<tr>
<td>Harsh</td>
<td>9.0 J cm(^{-2})</td>
<td>(&lt; 1 \log_{10} \text{PFU mL}^{-1})</td>
</tr>
</tbody>
</table>
Viral proteins

Control
9.0 \( \log_{10} \) PFU mL\(^{-1} \)

Moderate
2.1 J cm\(^{-2} \)
3.9 \( \log_{10} \) PFU mL\(^{-1} \)

Harsh
9.0 J cm\(^{-2} \)
< 1 \( \log_{10} \) PFU mL\(^{-1} \)

180 copies of VP1

SDS-PAGE

kDa
250 150 100 75 50 37 25 20

VP1
RNA

**Control**

9.0 $\log_{10}$ PFU mL$^{-1}$

**Moderate**

2.1 J cm$^{-2}$

3.9 $\log_{10}$ PFU mL$^{-1}$

**Harsh**

9.0 J cm$^{-2}$

< 1 $\log_{10}$ PFU mL$^{-1}$

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**Bioanalyzer**

RNA pico chips
Proposed mechanism of action

Absorption of photons (UV) especially by nucleic acids

= excitation state → relaxation

Photochemical effect
→ Cleavages of viral RNA

Photothermical effect
→ Energy in internal content; evaporation of water
→ Increase of internal pressure
→ Structure explosion
Conclusion – Pulsed light

Viral inactivation increases with fluence but affected by turbidity and presence of organic matter

Regulation (FDA) : 12 J cm\(^{-2}\)

Possible applications (reduction of at least 3 log\(_{10}\)):

- **Hard waters** : 2,1 J cm\(^{-2}\) (1,6 sec)
- **Turbid waters (200 NTU)** : 3,5 J cm\(^{-2}\) (2,7 sec)
- **Waste and mineral water** : 2,1 J cm\(^{-2}\) (1,6 sec)
- **Clean surfaces** : 3,5 J cm\(^{-2}\) (2,7 sec)
- **Plastic soiled surfaces** : 7,6 J cm\(^{-2}\) (6,1 sec)
- **Biofilm (artificial)** : 0,7 J cm\(^{-2}\) (0,5 sec)

Viral targets achieved : capsid and RNA
General conclusion

Vectors of transmission

<table>
<thead>
<tr>
<th>Person</th>
<th>Food</th>
<th>Water</th>
<th>Environment</th>
</tr>
</thead>
</table>

- Fast
- No toxic residue
- No heat

60% : acid
40% : H₂O₂

Peracids

Pulsed light
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