Noroviruses (NoV) & Hepatitis A

>50% NoV; 1/6 ill every year; shedding a trillion NoV virions/day; vomit is infectious

Shellfish: US 4.4% EU >70%

HAV uncommon in US… Developing world high; Foodborne exposure through global trade
High Pressure Processing & Shellfish

- Shucks the shellfish-separates meat/shell
- Enhances shelf-life
- Kills *Vibrio sp.*
- Commercial pressures 275-300 MPa

- 1 MPa = approx 10 atm or 150 psi
HPP & Virus: Universal Themes

- HPP is effective against HuNoV and HAV in Foods! Oysters do taste good...must be fresh!
- Different viruses have variable sensitivity with pressure (first order)
- Time under pressure (Wiebull/log-logistic curves)
- Matrix effects: pH is an issue
Theme Exceptions

- Exception is **Acidic pH** (HAV; HuNoV)

- Second exception: **Temperature** (cold) (HuNoV; HAV)
Time: MNV

325 MPa and 5°C

375 Mpa and 20°C

AEM 73:581
Effect of Temperature on Pressure Inactivation of MNV

350 MPa  5 min

AEM 73:581
Pressure Treatment of HAV *within* Oysters

![Graph showing the effect of pressure on HAV reduction in oysters. The x-axis represents MegaPascals (MPa), and the y-axis represents Log_{10} HAV Reduction. The graph indicates that 1 min within oysters at 9°C and 5 min in DMEM at rt significantly reduce HAV.](image-url)
MNV-1 contaminated oysters

In vivo mice confirmed inactivation (JFP 74:209)
HPP Human volunteer study w GI.1 Norwalk virus

Whole live commercial oysters shucked with 400 MPa then $10^4$ RT-PCR units (total) injected into 3 oysters
Sealed in bags and pressure-treated with 400-600 MPa
Fed to human volunteers
Stools tested for GI.1 NoV
Volunteer Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature</th>
<th>Count</th>
<th>Protect Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>7/15</td>
<td>47%</td>
</tr>
<tr>
<td>400 MPa</td>
<td>25°C</td>
<td>3/5</td>
<td>60%</td>
</tr>
<tr>
<td>400 MPa</td>
<td>6°C</td>
<td>3/14</td>
<td>21%</td>
</tr>
<tr>
<td>600 MPa</td>
<td>6°C</td>
<td>0/10</td>
<td>0%</td>
</tr>
</tbody>
</table>

400 MPa 25°C did not protect
400 MPa 6°C ??
600 MPa did protect

Applied and Environmental Microbiology
(2011) 77:5476-5482
Infectious Assay by RT-PCR

>3 log drop at 400 MPa at 5°C (5 min)
PGM Assay for HPP and HuNoV

Chen (UD) report:
GI.1 norovirus 450 MPa 2 min 1°C inactivates 3 log stock virus
For GII.4: 250 MPa 2 min 1°C inactivates 2.9 log stock virus

Oyster Homogenate
GI.1 400 MPa 5 min at 25, 6, and 1°C = 1.0, 1.3, 2.9 log rd
GII.4 400 MPa 5 min at 25 and 6°C = 1.3 and 3.6 log rd

Clam Homogenate=similar results

IJFM 167: 138
AEM 80:2248
HPP for HuNoV best at neutral pH

GI.1 500 MPa 21°C 2 min pH 4= ~ 0.4   pH 7= ~2.3 log rd
550 MPa 21°C 2 min pH 4= ~ 1.5   pH 7= ~3.0 log rd

GII.4 300 MPa 21°C 2 min pH 4= ~ 0   pH 7= ~1.3 log rd
350 MPa 21°C 2 min pH 4= ~ 1.2   pH 7= ~3.8 log rd

(PGM assay; IJFM 167:138)
HPP Oysters…How do they taste!?

- Literature says good to 400 MPa… others say no!
- Temp for HPP may matter for taste
- Oyster taste test HPP at two temps: RT and Fridge
- 5 criteria using 1-7 likeability score (appearance, texture, flavor, aroma, acceptability)
- triploid small/medium size summer oysters from Cape May NJ (high salt)
Mean values for hedonic scale ratings for each characteristic and each sample
(N=56-61, depending on sample)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>300 MPa 22°C</th>
<th>400 MPa 22°C</th>
<th>500 MPa 22°C</th>
<th>400 MPa 6°C</th>
<th>500 MPa 6°C</th>
<th>600 MPa 6°C</th>
<th>F value (Sig P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>4.11±1.6</td>
<td>5.46±1.5</td>
<td>5.39±1.4</td>
<td>5.20±1.6</td>
<td>5.39±1.5</td>
<td>5.45±1.4</td>
<td>5.22±1.7</td>
<td>5.78 (0.000)</td>
</tr>
<tr>
<td>Texture</td>
<td>4.54±1.9</td>
<td>5.23±1.7</td>
<td>5.36±1.8</td>
<td>5.55±1.6</td>
<td>5.20±1.6</td>
<td>5.47±1.5</td>
<td>5.43±1.7</td>
<td>2.46 (0.024)</td>
</tr>
<tr>
<td>Flavor</td>
<td>4.64±1.7</td>
<td>5.04±1.8</td>
<td>5.05±1.7</td>
<td>5.13±1.7</td>
<td>4.86±1.6</td>
<td>5.35±1.6</td>
<td>5.27±1.6</td>
<td>1.24 (0.287)</td>
</tr>
<tr>
<td>Aroma</td>
<td>4.90±1.4</td>
<td>5.27±1.3</td>
<td>5.04±1.2</td>
<td>5.30±1.3</td>
<td>5.27±1.4</td>
<td>5.33±1.3</td>
<td>5.33±1.4</td>
<td>0.94 (0.469)</td>
</tr>
<tr>
<td>Acceptability</td>
<td>4.64±1.6</td>
<td>5.14±1.6</td>
<td>5.13±1.6</td>
<td>5.28±1.6</td>
<td>5.02±1.5</td>
<td>5.53±1.4</td>
<td>5.38±1.6</td>
<td>2.05 (0.058)</td>
</tr>
</tbody>
</table>

Rating scale: 7 – Like very much; 1 – Dislike very much
Medium triploid summer high salt oysters

AJFT 9: 209
High Pressure Processing and Viruses: Take Home Messages

• HPP kills HAV, HuNoV
• Pressure level is predominate; time secondary
• Temperature is important: For HuNoV-cold kills
• Food matrix effects are important
• HPP is a viable intervention for raw oysters (*taste good*)
• Must be fresh!
Collaborators

H. Chen and D. Hoover: Univ of DE
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Calci: US FDA, Dauphin Island AL
C. Moe: Emory