Influence of Water Mobility of the Persistence of Salmonella in Dry Foods

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Known factors that influence the survival of *Salmonella* in dry foods

- Temperature
- Water activity
- Food composition
Impact of Composition on Survival of Salmonella

Peanut butter
Levels of fat and sugar (Burnett et al 2000)

Chocolate
Milk vs Dark (Tamminga et. al, 1976)
Water Activity is an imperfect descriptor of the nature of water in dry foods

It is a good predictor of microbial behavior in high and intermediate moisture foods, but less useful for dry foods.

Hysteresis loops of moisture sorption isotherms indicate that the assumption of equilibrium may not hold for water activity measurements.

Solutes affect survival differently at equivalent water activities.

Moisture sorption isotherm showing hysteresis

Andrade, Lemus, Perez 2011
A challenge for predictive modeling for dry foods is the lack of knowledge of controlling physico-chemical factors in food that affect *Salmonella* survival.

**Long Term Research Goal:**
Identify additional physico-chemical factors could allow development of useful predictive models for survival of *Salmonella* in dry foods and allow for safer product formulations.
**Role of water in microbial inactivation**

- Required for physiological activity to maintain cell functions
- Required for protein denaturation leading to cell death
  - Lack of water results in less effective heat inactivation
**Water activity:** Vapor pressure exerted by water in the food, resulting equilibrium with the atmosphere.

**Water mobility:** The ability of water molecules to move at the molecular level.
Water Activity

Closed System

Macroscopic translation

Water Mobility

Food

Rotational motion

Molecular rotation and translation

Modified from Schmidt, S. (2007)
Research objective

Determine if the influence of molecular mobility of water on survival of Salmonella in dry foods differs from the effect of water activity.

Determine if water mobility when used in conjunction with water activity and temperature will improve predictions of Salmonella survival in dry foods.
Experimental Approach

• Use a simple fat-free model food system to simplify data interpretation
• Manipulate model food to achieve different water mobilities at adjusted water activity levels.
• Inoculate water activity adjusted foods with Salmonella and determine survival at various temperatures.
Whey protein powder was selected as the model food system

Simple composition (95% protein)
Protein configuration (tertiary structure) can be manipulated to achieve different water-protein interactions (surface hydrophobicities) by pH adjustment.

This was achieved by adjustment of pH to 1, 5, and 7 followed by heating to 80°C, cooling and holding for 16 hours to fix the structure.

pH was then readjusted to 7.0 and solution was freeze dried

(Alizadeh-Pasdar and Li-Chan, J. Agric Food Chem 2000)
Water activity adjustments

Protein of each configuration adjusted to water activities of

0.19 ±0.007
0.29 ±0.004
0.36 ±0.005
0.43 ±0.008
0.54 ±0.014

Equilibrated in dessicators with appropriate salt solutions at 25 °C.
Water mobility measurement

Indicated by the effective spin-spin relaxation time \( (T_2^*) \) determined by using proton NMR

Employed spectral width of 300 kHz

Measurements done at 25 °C in triplicate

(Kou et al, 2004)

H- NMR spectra fitted using Mnova software.
H-NMR spectra for protein equilibrated to 17% ERH (left) and 49% ERH (right).

Narrow peak: mobile water; Broad peak: immobile water
(Peak width decreases as EHR increases)

Spin-spin relaxation time $T_2^*(s) = 1/\pi \nu_{1/2}$ (Hz)

$\nu_{1/2}$ (Hz) Represents the peak width at half-height
$T_2^*$ values vs $a_w$ for protein powders equilibrated to different water activities. Water mobility (effective proton spin-spin relaxation time) loses correlation with $a_w$ as $a_w$ increases. Each point represents the average of three replicates.
Preparation of inoculum

Four strain cocktail included: Typhimurium, Agona, Montevideo, and Tennessee serotypes.

--grown at 37°C for 24 hours
--Centrifuged and pellet suspended in 2 ml of 1% peptone to achieve $10^{10}$ cfu/ml
--Cell suspension dried over anhydrous calcium sulfate for 3 days
--Dried cells (0.5 g) mixed with 0.95 g of equilibrated test powder.
    Re-equilibration of mixture was not necessary
    Vacuum packaged
--Whole samples were used in analysis to avoid sampling error
Packaging samples for treatment

Packaging considerations
- moisture impermeable
- no head space so moisture retained in powder when heated

Reproducible geometry for consistent heat penetration

Packaging material
- Standard retort pouches
- Vacuumed in Food Saver pouches
Packaging inoculated protein powders
## Survival treatments

<table>
<thead>
<tr>
<th>Storage temperatures (°C)</th>
<th>Storage time</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>168 d</td>
</tr>
<tr>
<td>35</td>
<td>168 d</td>
</tr>
<tr>
<td>50</td>
<td>672 h (28 d)</td>
</tr>
<tr>
<td>60</td>
<td>672 h</td>
</tr>
<tr>
<td>70</td>
<td>2880 min (48 h)</td>
</tr>
<tr>
<td>80</td>
<td>2880 min</td>
</tr>
</tbody>
</table>
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 35 °C during 168 days.
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 35 °C during 168 days.

- $a_w = 0.42 \pm 0.004$
- $a_w = 0.52 \pm 0.002$
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 50 °C during 672 hours (28 days)
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 50 °C during 672 hours (28 days)

$\text{a}_w = 0.46 \pm 0.01$

$\text{a}_w = 0.58 \pm 0.01$
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 70 °C during 2880 min (48 hours)

\[ a_w = 0.43 \pm 0.004 \]

\[ a_w = 0.56 \pm 0.006 \]
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 80°C during 60 minutes.

\[ a_w = 0.18 \pm 0.01 \]

\[ a_w = 0.29 \pm 0.01 \]

\[ a_w = 0.36 \pm 0.01 \]
Salmonella survival in three modified whey protein powders adjusted to different water activities and held at 80°C during 60 minutes
Salinity studies 80°C

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Configuration</th>
<th>Volume Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity 1</td>
<td>configuration 2</td>
<td>9ml/1.5 L</td>
</tr>
<tr>
<td>Salinity 2</td>
<td>configuration 1</td>
<td>10.3 ml/1.5 L</td>
</tr>
<tr>
<td>Salinity 3</td>
<td>configuration 3</td>
<td>10.9 ml/1.5 L</td>
</tr>
</tbody>
</table>

Whey + salt powder NaCl 1M
Whey powder NaOH 10M
**Future studies**

- Develop a predictive model based on the data set for low fat dry foods including dairy powders and flours.
- Determine predictive value of the data set for determining the influence of solutes and amphiphilic food components on water mobility and *Salmonella* survival.
Conclusions

• Water mobility as indicated by effective proton spin-spin relaxation time may have an influence on inactivation of *Salmonella* in dry protein powder at $a_w$ values of with heating at 80 $^\circ$C. The effect decreases as temperature is lowered and $a_w$ decreases, but the effect is not consistent with a greater amount of mobile water in the heated sample.

• Measures of water mobility in room temperature protein samples may not correlate with water mobility of heated samples.
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