Protease Inactivation in Milk by Thermosonication and Impact on Milk Characteristics

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Outline

- Ultrasound in the Food and Dairy Industries
- Research Objective
- Experimental Design
- Results
- Implications
- Questions
Ultrasound in the Food Industry

- **Activation/inactivation of microorganisms**
  (Chemat and Hoarau, 2004; Knorr et al., 2004)
- **Activation/inactivation of enzymes**
  - Peroxidase inactivated when sonicated over 3 hrs
    (Mason et al., 1996)
- **Extraction processes**
  - Enhanced mass transfer in sugar extraction
    (Chendke and Fogler, 1975)
- **Quality Control**
  - Measured extent of crystallization and melting in emulsion
    (Mason et al., 1996)
Ultrasound in the Dairy Industry

- Increasing cheese yield (Muller, 1992)
- Decrease in time for yogurt production (Mason et al., 1996)
- Homogenization (Gaffney, 1997)
- Inactivation of microorganisms (Mason et al., 1999)
- Inactivation of spoilage enzymes (Raviyan et al., 2005)
- Freezing
  - Promote nucleation and reduce ice crystal size in ice cream (Zheng and Sun, 2006)
Fluid Milk Limitation

- Shelf-life
  - How long does it stay good in your refrigerator?
  - What do you observe at the end of the milk’s shelf-life?
- Is it safe to drink the milk after its shelf-life?
  - Pasteurization (72°C, 15s)
    - Destroys all pathogens and most spoilage microbes
- So what is the shelf-life of fluid milk based on?
  - Remaining spoilage microorganisms
  - Enzymes
Proteases in fluid milk

- Native and/or produced by spoilage microbes
- Are heat stable (survive pasteurization)
- Cause age gelation (Proteolysis)
- Limit shelf life
- Can be inactivated at Ultra-High Temperatures (quality is reduced)

Can proteases be inactivated by ultrasound??
(Vercet et al., 2000)
Limitations to ultrasound

- Insufficient for considerable inactivation
  - Needs to be combined with heat, pressure etc.
    (Earnshaw et al., 1995; Vercet et al., 2002)

- Qualitative implications
  - Can cause whey protein denaturation
    (Villamiel and de Jong, 2000)
  - Can affect sensory properties of milk
    (Riener et al., 2009)

What about thermosonication??
Our Research Objectives

• Study the effect of ultrasound treatment (different amplitude and time combinations) in combination with heat on
  ▫ Protease activity in milk
  ▫ Rheological properties of milk
  ▫ Sensory properties of milk
Experimental Design

- Protease – Source: *Staphylococcus aureus*
- Milk – pasteurized skim, reduced-fat (2%) and whole milk
- Preheating – to 60°C
- Sonication (20kHz) amplitude – 160, 170, & 180 µm
- Sonication time – 1, 2, and 2.5 min
Protease Activity
Azocasein method
(Christen and Marshall, 1984)
Effect of Amplitude and Time

Skim milk

Reduction in protease activity, Units/100ml vs. sonication amplitude, µm.

Reduced-fat milk

Whole milk

Protease activity, Units/100ml vs. sonication amplitude, µm.

All are significantly different (p<0.05)
Effect of Fat Content of Milk

![Bar Chart]

- **Protease Activity, Units/100ml**
  - Y-axis: 0 to 100

- **Fat content of milk, %**
  - X-axis: 1min, 2min, 2.5min

- **3.35% Fat content**
  - 160 µm: Blue
  - 170 µm: Red
  - 180 µm: Green

- **2% Fat content**
  - 160 µm: Blue
  - 170 µm: Red
  - 180 µm: Green

- **0.05% Fat content**
  - 160 µm: Blue
  - 170 µm: Red
  - 180 µm: Green

* No significant difference
Rheological Properties
(Rheometer)
## Effect of Sonication on Rheological Properties

<table>
<thead>
<tr>
<th>Milk-treatment (180µm, 2.5 min)</th>
<th>Average viscosity, Ns/m²</th>
<th>Consistency coefficient, Pa.s^n</th>
<th>Flow behavior index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim control</td>
<td>0.0011</td>
<td>0.0013</td>
<td>0.98</td>
</tr>
<tr>
<td>Skim sonicated</td>
<td>0.0011</td>
<td>0.0014</td>
<td>0.96</td>
</tr>
<tr>
<td>Reduced-fat control</td>
<td>0.0013</td>
<td>0.0017</td>
<td>0.95</td>
</tr>
<tr>
<td>Reduced-fat sonicated</td>
<td>0.0014</td>
<td>0.0019</td>
<td>0.96</td>
</tr>
<tr>
<td>Whole control</td>
<td>0.0013</td>
<td>0.0020</td>
<td>0.94</td>
</tr>
<tr>
<td>Whole sonicated</td>
<td>0.0013</td>
<td>0.0015</td>
<td>0.97</td>
</tr>
</tbody>
</table>

No significant differences (P<0.05)
Sensory Evaluation
**Effect of Sonication on Sensory Properties (n=2; expert dairy judging panelists)**

<table>
<thead>
<tr>
<th>Milk: sonicated at 180µm 2.5min</th>
<th>Sensory Odor Attribute (defective)</th>
<th>Consumer Accept. (odor attributes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plastic /burnt /rubbery</td>
<td>Cooked (custard)</td>
</tr>
<tr>
<td>Skim</td>
<td>S</td>
<td>D</td>
</tr>
<tr>
<td>Reduced-fat</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Whole</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

**Pyrolysis of volatile and non-volatile organic compounds at the collapsing bubble? (Neppolian et al., 2004)**

S-Slight  D-Definite  P-Pronounced
Summary of Results

- Thermosonication (60°C, 180µm, 2.5min) treatment
  - decreased protease activity in skim, 2%, and whole milk
  - did not affect rheological properties of milk
  - caused undesirable odors in 2% and whole milk
Further Research

- Modify treatments
  - Higher amplitude, shorter time
- Sensory
  - Larger panel, untrained panelists
- Extended storage study
- Compare proteases from different sources
- Investigate impact of fat content on aroma compounds
- Investigate the effect on raw milk
- Evaluate cost/energy efficiency of optimized conditions
Conclusions

- The food and dairy industries have a variety of uses for ultrasound.
- Thermosonication may inactivate protease and extend the shelf-life of milk.
- Conditions must be optimized to reduce off aromas/flavor before commercialization is an option.
Thank you!!!

Got milk?? Got Questions?
Effect of Preheating (no holding time) – enzyme in skim milk

![Graph showing the effect of temperature on protease activation in skim milk.](image-url)