Milk Coagulants

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Vice President – Product Research

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Agenda

- Review of history and chemistry of coagulation
- Properties of coagulants
- Use of coagulants
- So.. What kind of variety of cheese do you want to make and what make procedure are you going to use to make it?
History

- The first commercially available, standardized calf rennet was produced by Christian D.A. Hansen in 1874.

- In 1972, the FDA permitted the use of microbial coagulants as availability of calf abomasums became limited.

- In 1980’s, Pfizer, Hansen/Genencor, and DSM developed Fermentation Produced Chymosin (FPC).

- Between 2000 and 2002, FPC became increasing popular due to mad cow and hoof-and-mouth concerns.

- FPC is currently used in over 90 % of the cheese made in the U.S.

- 2008 – Introduction of ChyMAX M non-bovine chymosin
Nomenclature

- “Rennet” – Generic term for animal derived coagulant. A mixture of chymosin and pepsin.

- “Rennet Paste” – Traditionally made from calves that had just suckled so had both chymosin and pregastric lipase.

- Chymosin – The main gastric protease in young ruminants. (E.C. 3.4.23.4)(M.W.= 35,600)

- FPC – Fermentation Produced Chymosin – Chymosin produced in either yeast or fungi instead of calves.
Nomenclature

- Pepsin – The main gastric protease in adult animals including pigs, cattle, and chickens.
- Microbial – (Mucor types) from *Rhizomucor miehei* or *pusillus*
- Microbial – (Endotheia) – from *Cryphonectria parasitica*
- Cardoon (Vegetable) from Cynara (thistle)
Enzymatic destabilizing of casein micelles

Observation – Once Coagulant is added, cheese will be made.

(Dalgleish, 2007)
3D aggregation of casein micelles ➔ curd

The coagulation reaction is delayed until 60 to 75% of surface k-casein is hydrolyzed in normal milk depending on the casein concentration.
Reactions Occurring During Renneting

- CMP
- VIS
- GEL
- AT
- CT
- GT
- RT

Stages:
- Stable
- Aggregation
- Gelation
- Syneresis
# Coagulant Comparison Chart

<table>
<thead>
<tr>
<th>Coagulant Type</th>
<th>Calf Rennet &amp; Bovine Rennet</th>
<th>Fermentation Produced Chymosin</th>
<th>ChyMAX M (Chymosin, non-bovine)</th>
<th>Microbial (Mucorpepsin)</th>
<th>Microbial (Endothia-pepsin)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Milk Clotting Enzyme</strong></td>
<td>Chymosin &amp; Pepsin</td>
<td>Chymosin</td>
<td>Halal, Kosher, Vegetarian</td>
<td>Halal, Kosher, Vegetarian</td>
<td>Halal, Kosher, Vegetarian</td>
</tr>
<tr>
<td><strong>Certification</strong></td>
<td>Limited Halal</td>
<td>Halal, Kosher, Vegetarian</td>
<td>Halal, Kosher, Vegetarian</td>
<td>Halal, Kosher, Vegetarian</td>
<td>Halal, Kosher, Vegetarian</td>
</tr>
<tr>
<td><strong>Casein Specific Activity</strong></td>
<td>+++ depending on enzyme ratio</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Proteolytic Activity</strong></td>
<td>+++ depending on enzyme ratio</td>
<td>++</td>
<td>+</td>
<td>+++ (depending on type)</td>
<td>++++++</td>
</tr>
<tr>
<td><strong>Concerns</strong></td>
<td>Animal Product</td>
<td>GM Host</td>
<td>GM Host</td>
<td>Reduced Yield / Bitterness</td>
<td>Reduced Yield / Bitterness (Swiss OK)</td>
</tr>
</tbody>
</table>
Effect of Coagulant on Mozzarella

<table>
<thead>
<tr>
<th></th>
<th>Chymosin</th>
<th>Mucor</th>
<th>Endothia</th>
</tr>
</thead>
<tbody>
<tr>
<td>α Casein</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β Casein</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Proteolysis (12% TCA)</td>
<td>8%</td>
<td>7%</td>
<td>14%</td>
</tr>
<tr>
<td>Melt Diameter</td>
<td>50mm</td>
<td>50mm</td>
<td>57mm</td>
</tr>
<tr>
<td>Free Oil</td>
<td>27%</td>
<td>27%</td>
<td>35%</td>
</tr>
</tbody>
</table>

* Drain pH = 6.40 ; Stretch Temperature - Curd = 55°C
## Yield Loss Compared to FPC

<table>
<thead>
<tr>
<th>Coagulant</th>
<th>Difference (kg cheese / 100 kg milk)</th>
<th>Kg Cheese / M kg milk</th>
<th>Difference in Cheese (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation Chymosin</td>
<td>0.00</td>
<td>100,000</td>
<td>0</td>
</tr>
<tr>
<td>Bovine Rennet</td>
<td>- 0.08</td>
<td>99,200</td>
<td>- 800</td>
</tr>
<tr>
<td><em>R. miehei</em></td>
<td>- 0.25</td>
<td>97,500</td>
<td>- 2,500</td>
</tr>
<tr>
<td><em>R. pusillus</em></td>
<td>- 0.26</td>
<td>97,400</td>
<td>- 2,600</td>
</tr>
<tr>
<td><em>C. parasitica</em></td>
<td>- 0.52</td>
<td>94,800</td>
<td>- 5,200</td>
</tr>
</tbody>
</table>
Use considerations
Coagulant Use Instructions - Dilution

- Dilute 1:20 to 1:40 with water in a clean, sanitized container.
  - Prefer distilled or demineralized water
  - 1 quart of clean water per 1000 lb. of milk

- Chlorine
  - Rennet - 2 PPM chlorine - 40 % lost in 3 min.
  - Rennet - 5 PPM chlorine - 60 % lost in 3 min.

- pH of Hard Water > 7.00
  - Coagulants are unstable above pH 6.5.
  - CaCl$_2$ Solutions and Annatto are above pH 7

- Add enzyme to water, minimally but completely mix, add immediately to milk
Coagulant Use Instructions – Addition to the vat

- Add diluted coagulant to the vat with sufficient agitation to ensure proper distribution.
  - Typically 5 minutes

- Stop all agitation and let vat set.

- Test vat before cutting.
Coagulant Use Instructions – Cutting the vat

- Methods of Testing
  - Time
  - Finger or Knife Method
  - Rennet Cup
Factors that Effect Rate of the Reactions

- Use level
- Temperature
- pH
- Calcium Concentration - Summer milk slower firming
- Casein Concentration
- Inhibitors to coagulation – Heat Treatment & Calcium
Use considerations - coagulant strength

- FPC is typically packaged at 600 IMCU / gm. (3 mg protein / gm.). Double the strength of calf rennet.

- Coagulants are typically used at:

  ~ 1.5 to 3 oz. / 1000 lbs. Milk (45 to 90 gm. / 500 kg milk) @ single strength equivalent.
Temperature of Storage

- **Recommended 40 - 45 °F in package**
  - Contamination risk on re-use

**% Loss in One Month**

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Rennet</th>
<th>Microbial</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 °F</td>
<td>0 %</td>
<td>0 %</td>
</tr>
<tr>
<td>70 °F</td>
<td>1 %</td>
<td>1 %</td>
</tr>
<tr>
<td>85 °F</td>
<td>3 %</td>
<td>2 %</td>
</tr>
</tbody>
</table>

Barbano, 1986
Effect of Temperature on Coagulant Activity

![Graph showing the effect of temperature on coagulant activity for Chymosin and R. miehei. The graph displays a curve where the relative activity increases with temperature up to a certain point, after which it decreases. The x-axis represents degrees Fahrenheit, ranging from 70 to 130, while the y-axis represents relative activity, ranging from 0 to 200. The graph includes markers for Chymosin (red diamonds) and R. miehei (blue squares).]
Chymosin

- Minimum Temperature for Coagulation = 50° F
- Optimum** Temperature for Coagulation = 112° F
- Maximum Temperature for Coagulation = 122° F
- 99+% inactivated in whey/ 162° F/15 sec./ pH >6.0
Effect of pH on Enzyme Activity
High Solids Milk

- All protease reactions are dependent on the enzyme to substrate ratio.

- In high solids milk (UF / MPC / Condensed) the hydrolysis of kappa casein and the coagulation reaction happen much quicker than with normal solids milk.

- In ageing of cheese made with high solids milk, the hydrolysis reactions are often slowed since the protease to casein ratio is lower than normal cheese.
## The effect of breed on protein in milk

Table 1. The effects of breed on the content and yield of milkfat and milk protein.*

<table>
<thead>
<tr>
<th>Breed</th>
<th>Milk Content (%)</th>
<th>Milk Yield (lb/lactation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat</td>
<td>Protein</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>3.99</td>
<td>3.34</td>
</tr>
<tr>
<td>Brown Swiss</td>
<td>4.16</td>
<td>3.53</td>
</tr>
<tr>
<td>Holstein</td>
<td>3.40</td>
<td>3.32</td>
</tr>
<tr>
<td>Guernsey</td>
<td>4.87</td>
<td>3.62</td>
</tr>
<tr>
<td>Jersey</td>
<td>5.13</td>
<td>3.80</td>
</tr>
<tr>
<td>Milking Shorthorn</td>
<td>3.60</td>
<td>3.20</td>
</tr>
</tbody>
</table>

Wisconsin CDR Pipeline vol. 23 # 1, 2011
Heat-Induced Changes in Milk Proteins

Native whey protein (folded) → heat → Denatured whey protein (unfolded)

Disulfide bond -s-s- → Colloidal calcium phosphate (CCP)
Syneresis

- Weaker set = faster shrink
- Cutting - Smaller curd = faster whey expulsion
- Acidification - Lower pH = faster shrink
- Cook - Higher temperature = faster shrink
Much of the data presented was courtesy of Chr. Hansen, Inc.
Acid Formation & Whey Expulsion vs. Temperature

Temperature (F)

pH at 6 Hours

Whey Expulsion

SCO 238  SCO 273  SCO 291  Whey separation

Dairy Research Institute

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Effect of Temperature on Coagulant Activity

![Graph showing the effect of temperature on coagulant activity. The x-axis represents degrees Fahrenheit (80 to 100), and the y-axis represents relative activity (40 to 160). Two lines are plotted: one for Chymosin (red triangles) and one for R. miehei (blue squares). The activity increases with temperature for both enzymes.](image-url)
Effect of pH on Enzyme Activity
Thank you