Mechanisms of Coagulation:
The principles, the science and what they mean to cheesemakers

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What are casein micelles?

Kappa casein

AA 169

{ nonpolar

{ polar
What are casein micelles?

Mechanisms of coagulation

Å Rennet (rapid: 30 - 60 min)
Å Acid (slow: 5 - 24 hr)
Å Acid-rennet (slow: 12 -24 hr)
Å Acid-heat
What is rennet?

Å General term for enzymes used to coagulate milk
Å Technically restricted to enzymes derived from ruminant stomachs
Å All are protein degrading enzymes (proteininase, protease, proteolytic enzyme)
Å All are members of the aspartic proteininase family
All aspartic proteinases have 2 characteristics in common

Kappa casein is uniquely vulnerable to the action of aspartic proteases

AA 105-106 (very vulnerable to aspartic proteinases)

AA 169

nonpolar

polar
Rennet cleavage of kappa casein

chymosin, etc.
Rennet coagulation occurs in two phases

1. Enzymatic
2. Non-enzymatic
1. **Enzymatic phase:**

cleavage of $\kappa$-casein

Rennet enzymes shave off polar surface

*Adapted from: Horn, D. 1998. International Dairy J. 8:171-177*
1. **Enzymatic phase:**

   cleavage of $\kappa$-casein

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**Diagram:**

- **Casein micelle**
- **Kappa-Casein with exposed polar region**
2. Nonenzymatic phase: 
*Ca\(^{++}\)* induced aggregation

Very Nonpolar surface

Casein micelle

Ca\(^{++}\)

CMP/GMP (AA106-169)
2. Nonenzymatic phase: aggregation of casein micelles

Nonpolar micelles + Ca$^+$ → Permanent bonding

(commences after 80-90% cleavage of ⊔-casein)
2. Nonenzymatic phase continued: chain formation/flocculation

visible flocs = rennet clotting time
continuous gel - coagulation
Matrix rearrangement

Fine matrix - small pores, weak gel

Coarse matrix - large pores, firm gel
Repercussions of matrix rearrangement

- Cheese moisture content
- Acid development during cheese making
- Cheese yield
Matrix rearrangement

Fine matrix - small pores, weak gel

Coarse matrix contracts, syneresis pressure ↑
Repercussions of matrix rearrangement

1. Cheese moisture content

Å Cutting early (weak set) enables much rearrangement to occur after cutting; syneresis $\uparrow$, cheese moisture content $\downarrow$
  - ex: alpine cheeses

Å Cutting late (firm set) limits rearrangement after cutting; syneresis $\downarrow$, cheese moisture content $\uparrow$
  - ex: traditional Brie, Camembert

Å Cutting firmness should be consistent from vat-to-vat to reduce moisture variation
Repercussions of matrix rearrangement
2. Acid development

- If the amount of time to the desired cutting firmness varies greatly from day-to-day, the subsequent rate of acidification during the rest of cheese making may be affected:
  - Extended cutting time, ↑ starter culture population, ↑ rate of acidification
  - Reduced cutting time, ↓ starter culture population, ↓ rate of acidification

- Therefore, both cutting firmness and cutting time should be optimized and held constant from day-to-day
**Bottom line**

- Cutting should be initiated at a consistent curd firmness that is optimized for the type of cheese being made.
- The time required to achieve the target cutting firmness should be consistent from vat-to-vat across season.
- In practice, this can be challenging because several factors may influence coagulation and cause curd firmness at cutting and/or cutting time to vary.
Repercussions of matrix rearrangement

- Cheese moisture content
- Acid development during cheese making
- Cheese yield
Repercussions of matrix rearrangement

3. Cheese yield

Å Weak curds are fragile and tend to shatter during cutting
  1. fat and casein losses ↑, cheese yield ↓
  2. however, matrix rearrangement after cutting occurs rapidly, curd particles firm up quickly

Å Firm curds are more forgiving with respect to shattering during cutting
  1. however, matrix rearrangement after cutting occurs slowly
  2. curd particles firm up slowly and remain vulnerable to shattering for longer time after cutting
Mechanisms of coagulation

- Rennet (rapid: 30 - 60 min)
- Acid (slow: 5 - 24 hr)
- Acid-reennet (slow: 12 - 24 hr)
- Acid-heat
Acid coagulation

1. Starter culture produces lactic acid, H+ ions accumulate

2. Calcium phosphate dissolves into water phase

3. H+ ions neutralize the negative charges on k-casein

4. Neuralized k-casein collapses
Acid coagulation

H+  
pH 4.6

Polar Surface

Micelle rich in calcium phosphate

Collapsed neutralized k-cn nonpolar surface

Micelle depleted of calcium phosphate
Aggregation of casein micelles

continuous gel - coagulation
Acid coagulation

- Acid gels lack the capacity to contract and synerese
- Therefore, final cheese moisture content is very high (around 70-80%, depending of the fat content)
- In general, acid coagulated cheeses are eaten fresh, not ripened
Mechanisms of coagulation

Å Rennet (rapid: 30 - 60 min)
Å Acid (slow: 5 - 24 hr)
Å Acid-rennet (slow: 12 - 24 hr)
Å Acid-heat
Key parameters of acid-rennet coagulation

1. Amount of rennet added to the milk:
   - Anywhere from 1 - 30% of the level used in rennet coagulation

2. Coagulation temperature:
   - Anywhere from 18 - 32°C
Example 1: Quark

- Lactic fermentation at ca. 30°C for 16 hr
- A small amount of rennet (e.g., 1 - 10% of level used in rennet coagulation) added at around pH 6.3
- Rennet proceeds through enzymatic and non-enzymatic phases as milk pH ↓
- Coagulation occurs at pH 4.8 or 4.9 instead of pH 4.6
- The resulting curd develops hybrid characteristics that fall somewhere between those of rennet curd and acid curd
Advantages of acid-rennet coagulated Quark

- Better draining results in a lower moisture content
- Lower moisture content along with a firmer coagulation result in a firmer cheese body, improved texture
- Higher cutting pH (e.g. from pH 4.6 to 4.8 or 4.9) results in a less acidic flavor
Example 2: soft ripened goat’s milk cheese

- The milk undergoes lactic acidification at around 20°C for 24 hr
- Rennet (about 1/3 the level used in rennet coagulation) is added, often around pH 6.3.
- The rennet proceeds through enzymatic phase but the non-enzymatic phase is strongly impeded at 20°C,
- Coagulation occurs in 24 hr when the pH reaches around pH 5.3
- The resulting curd develops hybrid characteristics that fall somewhere between those of rennet curd and acid curd
Advantages of acid-rennet coagulation for goat’s cheeses

Å Syneresis is improved, resulting in a final cheese with ca. 60-70% moisture.

Å This moisture range is low enough to support controlled ripening.

Å The end result is a group of soft ripened goat’s milk cheeses with a unique lactic acid dominated texture.
Factors that affect rennet coagulation

- Temperature history of milk
- pH of milk during coagulation
- Temperature of milk during coagulation
- $\text{Ca}^{++}$ ion content of milk
- Casein content of milk
Temperature history of milk:
1. Cooling (< 10ºC)

\[
\begin{align*}
\beta - \text{casein} \\
\text{Ca}^{++} \\
\text{HPO}_4^- \\
\text{H}^+ \\
\text{H}^+ \\
\end{align*}
\]

\[\text{pH} = 0.2 - 0.3\]
Repercussions of cooling milk to 4ºC

Å ↑ milk pH
  ï Slower enzymatic phase
  ï ↓ attraction between rennet enzymes and casein micelles, ↑ cleavage of k-casein needed to induce coagulation
  ï Therefore, longer time needed to attain target cutting firmness

Å Altered casein micelle structure
  ï Curd matrix less able to undergo structural rearrangement, contraction
  ï Therefore, weaker set, slower syneresis, higher moisture content in cheese (especially in high moisture types, e.g., bloomy rind)

Å May cause problems when switching from warm milk fresh from the animal to cold stored milk
Compensating for cooling milk to 4ºC

• The changes can be largely reversed by normal pasteurization before cheese making
• These changes can be partly overcome by adding calcium chloride.
• If necessary, increase cutting time to restore the target curd firmness
• If necessary, adjust starter usage to restore target acifidification schedule
• If necessary, take action during cheese making to enhance syneresis/draining to reduce cheese moisture content
Factors that affect rennet coagulation

- Temperature history of milk
- pH of milk during coagulation
- Temperature of milk during coagulation
- Ca++ ion content of milk
- Casein content of milk
pH of milk during coagulation

\( pH \downarrow \text{from 6.7 - 6.0} \)
Repercussions of milk pH

As milk pH ↓

- Rennet enzyme activity increases
- Rennet enzymes are more strongly attracted to casein micelles
- Therefore, the enzymatic phase occurs more rapidly

Furthermore:

- Rennet enzymes adsorb more tightly onto the casein micelle surface, resulting in "patches" of k-casein cleavage
- Therefore, the amount of k-casein cleavage needed to induce micelle aggregation ↓
- Also, higher Ca++ ion concentration speeds up the aggregation of casein micelles
- Therefore, Non-enzymatic phase occurs more rapidly

Consequently, rennet clotting time ↓, cutting time ↓, and curd firmness ↑
Factors that affect rennet coagulation

- Temperature history of milk
- pH of milk during coagulation
- Temperature of milk during coagulation
- Ca++ ion content of milk
- Casein content of milk
As coagulation temperature ↑ from ca. 25° - 40°C

- Enzymatic phase occurs more rapidly:
  - Rennet enzyme activity increases with ↑ temperature
- Non-enzymatic phase occurs more rapidly
  - Rennet enzymes adsorb more tightly onto the casein micelle surface
  - Therefore, the amount of k-casein cleavage needed to induce micelle aggregation ↓
- Consequently, rennet clotting time and cutting time ↓, and curd firmness ↑
As coagulation temperature ↓ from 
c. 25° - 15°C

- Enzymatic phase gradually slows down but the non-enzymatic phase fails catastrophically
  - At 20°C, the non-enzymatic phase is severely impeded and casein aggregation/curd formation occurs very slowly (many hours)
  - This principle is exploited in the production of acid-rennet coagulated cheeses
  - At 15°C, the nonenzymatic phase is completely prevented; casein aggregation cannot occur even when k-casein has been completely cleaved from the micelle surface

- Bottom line: coagulation temperature should be tightly controlled
Factors that affect rennet coagulation

- Temperature history of milk
- pH of milk during coagulation
- Temperature of milk during coagulation
- Ca$^{++}$ ion content of milk
- Casein content of milk
Ca^{++} ion content of the milk

Very Nonpolar surface

CMP/GMP (AA106-169)
Calcium chloride addition

- Supplies Ca\(^{++}\) ions, enables nonezymatic phase (aggregation of casein micelles) to proceed
- Decreases the milk pH, thereby stimulating the enzymatic phase
- End result: rennet clotting time ↓, cutting time ↓, and curd firmness ↑
Factors that affect rennet coagulation

• Temperature history of milk
• pH of milk during coagulation
• Temperature of milk during coagulation
• Ca++ ion content of milk
• Casein content of milk
As casein content $\uparrow$:

- The frequency of collisions between casein micelles increases dramatically.
- This causes micelles to aggregate at much lower levels of k-casein cleavage.
- End result: the nonezymatic phase is greatly accelerated: rennet clotting time $\downarrow$, cutting time $\downarrow$, and curd firmness $\uparrow$.
General rules of thumb

• Cut curd at a consistent firmness (for moisture control)
• Cut curd at a consistent time from rennet addition (for maintaining acidification schedule)
• If necessary:
  - Add calcium chloride (up to 0.02% maximum)
  - adjust cutting time to hold cutting firmness constant
  - adjust starter usage to maintain target acidification schedule
Thank You!