Accelerated Cheese Ripening

A review of various approaches

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UNIVERSITY OF COPENHAGEN
Approaches used

• 1. Elevated ripening temperature
• 2. Addition of exogenous enzymes
• 3. Addition of adjunct or attenuated cultures
• 4. High-pressure (HP) treatment
Accelerated cheese ripening

- REDUCE -> Ripening Time
- NO CHANGE -> Flavour, Texture, Safety

➢ Subject of much scientific investigation since the 1950s
➢ Costs approximately €55 (minimum) per tonne of Cheddar per month (Upadhyay and McSweeney, 2003)
Example: *UK Cheddar market*

235,000 tonnes Cheddar produced in 2017

- Mature (6 months ripening)
  - 118,000 tonnes x €55 x 6 months
  - = €39 million

- Extra mature (12 months ripening)
  - 46,000 tonnes x €55 x 12 month
  - = €30 million

**Total ripening costs €69 million (estimate)**

https://dairy.ahdb.org.uk/market-information/dairy-sales-consumption/cheese-market/#.WrOni02otD8
Biochemical changes during ripening

Fresh Cheese

Fat
- Lipolysis
  - Fatty acids
  - Glycerol
  - Amides
  - Ketones
  - Aldehydes
  - Lactones
  - Alcohols
  - Esters

Sugar
- Lipolysis
  - Acetic acid
  - Diacetyl
  - Acetaldehyde
  - Ethanol
  - Propionic acid
  - Lactic acid

Protein
- Proteolysis
  - Peptides
  - Amino acids

- Amino Acid Catabolism
  - Amines
  - Sulfur compounds
  - Fatty acids
  - Aldehydes
  - Alcohols
  - Keto acids

+Time

Ripened Cheese
Approaches used

• 1. Elevated ripening temperature
• 2. Addition of exogenous enzymes
• 3. Addition of adjunct or attenuated cultures
• 4. High-pressure (HP) treatment
1. Elevated ripening temperature (Gouda)

Possible to manufacture Gouda cheese which at the age of 5-10 months already shows the flavour characteristics of 10-20 months old cheese.

Crystals already present at 6-9 months compared to at least 12 months for normal Gouda.

*Patent: EP0281167B1 (FrieslandCampina)*
1. Elevated ripening temperature (Cheddar)

Proteolysis increase with elevated ripening temperature

1. Elevated ripening temperature (Cheddar)

Mature flavour score increased with elevated ripening temperature

1. Elevated ripening temperature (Cheddar)

Preference score decreased with elevated ripening temperature

1. Elevated ripening temperature

➢ ADVANTAGES
  ➢ Technically simple
  ➢ No legal barriers
  ➢ No cost (perhaps saving)

➢ DISADVANTAGES
  ➢ Non-specific
  ➢ Risk of off-flavours
  ➢ Risk of microbial spoilage
    ➢ NSLAB grow faster when temperature is raised above 8°C

➢ Very high quality milk is required
➢ Very careful and frequent (monthly) cheese grading required
What about temperature reduction?

➢ Huge demand at Christmas
  ➢ Product produced in autumn and then frozen at -20ºC for up to 3 months.
  ➢ Thawed prior to market.
Approaches used

• 1. Elevated ripening temperature
• 2. Addition of exogenous enzymes
• 3. Addition of adjunct or attenuated cultures
• 4. High-pressure (HP) treatment
2. Addition of exogenous enzymes

➢ Principle/assumption
➢ Ripening is catalyzed by enzymes rather than viable cells
➢ => add specific enzymes or cocktail of enzymes
➢ => select enzyme(s) for pathway(s) to accelerate (proteolysis, lipolysis, glycolysis, amino acid catabolism)
2. Addition of exogenous enzymes

Table 19.1  Enzyme preparations, other than rennets, commercially available for cheese or enzyme-modified cheese (modified from Wilkinson and Killeawley, 2002)

<table>
<thead>
<tr>
<th>Principal enzymatic activity</th>
<th>Trade name</th>
<th>Host organism/source</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopeptidase</td>
<td>Accelase™&lt;sup&gt;a&lt;/sup&gt;, Savorase&lt;sup&gt;b&lt;/sup&gt;, Debitrase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Lactococcus lactis, Rhizopus oryzae</td>
<td>Rhodia Food</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Acid Protease A</td>
<td>Aspergillus niger</td>
<td>Amano Enzymes</td>
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<tr>
<td>Aminopeptidase</td>
<td>Acid Protease II</td>
<td>Rhizomucor niveus</td>
<td>Amano Enzymes</td>
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<tr>
<td>Aminopeptidase</td>
<td>Bioprotease A conc</td>
<td>Bacillus subtilis</td>
<td>Quest International</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Bioprotease N 100</td>
<td>Aspergillus oryzae</td>
<td>Quest International</td>
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<tr>
<td>Aminopeptidase</td>
<td>Bioprotease P conc</td>
<td>Bacillus subtilis</td>
<td>Quest International</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Fermizyme® B 500</td>
<td>Aspergillus sp.</td>
<td>DSM</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>FlavorAge&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Aspergillus var. strains</td>
<td>Chr Hansen</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Flavorpro 192</td>
<td>Aspergillus oryzae</td>
<td>Biocatalysts</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Flavourzyme</td>
<td>Bacillus subtilis</td>
<td>NOVO</td>
</tr>
<tr>
<td>Protease</td>
<td>Neutrase</td>
<td>Rhizomucor oryzae</td>
<td>NOVO</td>
</tr>
<tr>
<td>Protease</td>
<td>Peptidase ‘R’ Amano</td>
<td>Bacillus subtilis</td>
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<td>Protease</td>
<td>Promod 24L</td>
<td>Aspergillus sojae</td>
<td>Biocatalysts</td>
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<tr>
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<td>Promod 215P</td>
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<td>Amano Enzymes</td>
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<tr>
<td>Lipase</td>
<td>Capalase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Animal</td>
<td>Degussa Bioactives</td>
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<tr>
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<td>Italase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Animal</td>
<td>Degussa Bioactives</td>
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<tr>
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<td>Kid Lipase</td>
<td>Animal</td>
<td>Chr Hansen</td>
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<td>Lipase M ‘Amano’ 10</td>
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<td>Amano Enzymes</td>
</tr>
<tr>
<td>Lipase</td>
<td>Palatase&lt;sup&gt;c&lt;/sup&gt; 20000 L</td>
<td>Rhizomucor miehei</td>
<td>NOVO</td>
</tr>
</tbody>
</table>

<sup>a</sup> Can also be mixed with enzymes from other sources.
<sup>b</sup> Proteinase-lipase preparation.

2. Addition of exogenous enzymes: addition points

1. Milk
2. Vat
3. Salting
4. Moulding
5. Brining
6. Danbo
7. Cheddar
8. Fresh curd
9. Washing water
2. Addition of exogenous enzymes: Cheddar

- VAT 1: CONTROL
- VAT 2: + AM317 (protease, lipase and peptidase from Aspergillus niger)
- VAT 3: + CPG (carboxypeptidase from Aspergillus niger)
- VAT 4: + AHC50 (protease, peptidase and aroma enzyme)

➢ Cheese ripened for 1, 14, 28, 56 and 112 Days at 8ºC.

2. Addition of exogenous enzymes: Cheddar

Proteolysis and Lipolysis

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Control</th>
<th>AM317</th>
<th>CPG</th>
<th>AHC50</th>
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<tbody>
<tr>
<td>pH4.6-SN</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>3.93 (0.82)a</td>
<td>4.81 (1.03)a</td>
<td>3.3 (0.24)a</td>
<td>4.09 (0.27)a</td>
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<tr>
<td>14</td>
<td>6.49 (0.61)a</td>
<td>12.69 (2.69)b</td>
<td>7.54 (1.23)a</td>
<td>12.77 (1.47)b</td>
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<tr>
<td>28</td>
<td>8.29 (0.86)a</td>
<td>13.14 (2.14)b</td>
<td>7.91 (1.35)b</td>
<td>12.77 (1.47)b</td>
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<tr>
<td>56</td>
<td>11.72 (0.96)a</td>
<td>18.11 (0.36)b</td>
<td>11.04 (0.06)a</td>
<td>18.46 (1.87)b</td>
</tr>
<tr>
<td>112</td>
<td>16.40 (1.28)a</td>
<td>24.16 (0.64)b</td>
<td>15.49 (0.29)a</td>
<td>26.38 (0.55)b</td>
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<tr>
<td>PTA-SN</td>
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<td></td>
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<tr>
<td>1</td>
<td>0.74 (0.07)a</td>
<td>0.76 (0.07)a</td>
<td>0.66 (0.10)a</td>
<td>0.75 (0.10)a</td>
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<tr>
<td>14</td>
<td>1.19 (0.18)ab</td>
<td>1.36 (0.02)a</td>
<td>1.24 (0.04)a</td>
<td>1.53 (0.34)a</td>
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<tr>
<td>28</td>
<td>1.48 (0.26)b</td>
<td>1.60 (0.10)a</td>
<td>1.49 (0.05)a</td>
<td>1.77 (0.18)a</td>
</tr>
<tr>
<td>56</td>
<td>2.13 (0.28)b</td>
<td>2.46 (0.07)a</td>
<td>2.13 (0.13)a</td>
<td>2.65 (0.34)a</td>
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<tr>
<td>112</td>
<td>3.47 (0.56)abc</td>
<td>3.89 (0.09)b</td>
<td>3.29 (0.05)a</td>
<td>4.57 (0.33)c</td>
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<tr>
<td>TF AA</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>905 (260)a</td>
<td>1037 (116)a</td>
<td>980 (104)a</td>
<td>1069 (37)a</td>
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<tr>
<td>14</td>
<td>2153 (390)a</td>
<td>2878 (581)a</td>
<td>2727 (327)a</td>
<td>2775 (722)a</td>
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<tr>
<td>28</td>
<td>3013 (134)a</td>
<td>3087 (182)a</td>
<td>2778 (238)a</td>
<td>3178 (165)a</td>
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<tr>
<td>56</td>
<td>4609 (460)a</td>
<td>5662 (445)abc</td>
<td>5397 (563)abc</td>
<td>6384 (245)b</td>
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<td>112</td>
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<td>9862 (914)b</td>
<td>8966 (778)a</td>
<td>11035 (281)b</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>752 (88)a</td>
<td>747 (91)a</td>
<td>745 (21)a</td>
<td>763 (72)a</td>
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<tr>
<td>14</td>
<td>724 (45)a</td>
<td>750 (83)a</td>
<td>723 (38)a</td>
<td>745 (55)a</td>
</tr>
<tr>
<td>28</td>
<td>759 (37)a</td>
<td>712 (28)a</td>
<td>734 (51)a</td>
<td>733 (44)a</td>
</tr>
<tr>
<td>56</td>
<td>729 (7)a</td>
<td>712 (28)a</td>
<td>734 (51)a</td>
<td>733 (44)a</td>
</tr>
<tr>
<td>112</td>
<td>808 (50)a</td>
<td>811 (78)a</td>
<td>813 (58)a</td>
<td>829 (33)a</td>
</tr>
</tbody>
</table>

Increased pH4.6 soluble N
Increased free amino acids
Increased proteolysis
Increased lipolysis
AHC50 and AM317 cheeses too soft and brittle

2. Addition of exogenous enzymes

➢ ADVANTAGES
  ➢ Can be extremely powerful
  ➢ Specific action (for some enzymes)
  ➢ Flavour direction

➢ DISADVANTAGES
  ➢ Difficulty of uniform distribution
  ➢ Off flavours
  ➢ Limited range of suitable enzymes
  ➢ Certain enzymes require cofactors
    ➢ Especially amino acid converting enzymes
  ➢ Possible side activities
  ➢ Cost
  ➢ Legal constraints
Approaches used

• 1. Elevated ripening temperature
• 2. Addition of exogenous enzymes
• 3. Addition of adjunct or attenuated cultures
• 4. High-pressure (HP) treatment
3. Addition of adjunct or attenuated cultures

- **Principle/theory**
  - Use the full enzyme complement of Lactic Acid Bacteria (LAB) cultures to simultaneous increase
  - Proteolysis
  - Petidolysis
  - Amino acid catabolism

- **Structure and texture**
  - Casein
  - Large peptides
  - Medium size peptides
  - Small peptides
  - Amino acids
  - Amino acid metabolites

- **Taste formation**
  - Sites of action

- **Aroma formation**

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POWER AND BALANCE
3. Addition of adjunct or attenuated cultures

Volatile from methionine metabolism

Ganesan and Weimer (2017): Cheese, pp.483-516
3. Addition of adjunct or attenuated cultures

- 1. Adjunct cultures
  - Live
    - Wild type cultures
    - Selected classical mutants
    - GMO

- 2. Attenuated cultures
  - Partially inactivated/dead
    - Heat shocked
    - Freeze shocked

- Critical that the culture does not affect normal acidification rate
3. Addition of adjunct or attenuated cultures

Principle suppliers of Dairy Cultures

<table>
<thead>
<tr>
<th>Company</th>
<th>Market share (estimate)</th>
<th>Country</th>
<th>Founded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr. Hansen A/S</td>
<td>1</td>
<td>Denmark</td>
<td>1870</td>
</tr>
<tr>
<td>DuPont Danisco</td>
<td>2</td>
<td>USA</td>
<td>1802</td>
</tr>
<tr>
<td>DSM</td>
<td>3</td>
<td>Netherlands</td>
<td>1902</td>
</tr>
<tr>
<td>CSK Food Enrichment</td>
<td>4</td>
<td>Netherlands</td>
<td>1905</td>
</tr>
<tr>
<td>Sacco</td>
<td>5</td>
<td>Italy</td>
<td>1872</td>
</tr>
</tbody>
</table>
3. Addition of adjunct or attenuated cultures

8 Gouda-type cheeses with 8 different culture combinations

<table>
<thead>
<tr>
<th>Culture combination</th>
<th>Lc. lactis (O)</th>
<th>Lb. casei (C)</th>
<th>Lb. helveticus (H)</th>
<th>Lb. rhamnosus (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O§</td>
<td>Control</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OC</td>
<td>Single</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>OH</td>
<td>Single</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>OR</td>
<td>Single</td>
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<td>Multiple</td>
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<td>+</td>
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<tr>
<td>OCRH</td>
<td>Multiple</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Used as control, + Bacterial culture is included in the culture combination, - Bacterial culture is not included.*
3. Addition of adjunct or attenuated cultures

- Cheeses ripened for 1 week at 9°C, 4 weeks at 13°C, for 4 weeks at 9°C.
- Microbiological enumeration
- Aroma analysis (DHS-GC-MS)
3. Addition of adjunct or attenuated cultures

Gouda-type Cheeses – GC-MS Aroma Analysis

More mature flavour, but atypical

More mature flavour

Peter Beierholm and Jeppe Frans Steendahl Sørensen, (2017), MSc thesis, KU
3. Addition of adjunct or attenuated cultures

➢ ADVANTAGES
   ➢ Balanced flavour
   ➢ Powerful effect
   ➢ Flavour direction
   ➢ Technically simple
   ➢ Flexible
   ➢ No legal constraints

➢ DISADVANTAGES
   ➢ Cost

Wegmann et al. (2009), J. Bacteriol. 191:22 7142-7143
Approaches used

- 1. Elevated ripening temperature
- 2. Addition of exogenous enzymes
- 3. Addition of adjunct or attenuated cultures
- 4. High-pressure (HP) treatment
4. High-pressure (HP) treatment

- 1. HP used to accelerate cheese ripening
- 2. HP treatment on the functional and rheological properties of Mozzarella cheese
- 3. HP used to control *C. tyrobutyricum* in late blowing in semi-hard cheese
- 4. HP treatment for reduced-fat Cheddar cheese
- 5. HP to control *Listeria monocytogenes* in fresh cheese
- 6. HP viability of *Streptococcus thermophilus* bacteriophages
4. High-pressure (HP) treatment

Mariana Trench
depth of 11,000 meters
=> 110 MPa

Cheese application

- High pressure-Short time: 300-600 MPa x 5-20 min
- Low/Moderate pressure-Long time: 50-200 MPa x 4-72 hours

https://www.hiperbaric.com
4. High-pressure (HP) treatment: what happens in cheese?

- 1. HP alters enzyme structure
- 2. HP causes conformational changes in the casein matrix
  - More susceptible to proteolysis
- 3. HP promotes bacterial lysis
  - Release of intracellular enzymes
- 4. HP modify water distribution and increase pH
  - Enhances enzymatic activities
4. High-pressure (HP) treatment: Cheddar

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>50</th>
<th>100</th>
<th>500</th>
<th>2000</th>
<th>3000</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free amino acids (mg/g)</td>
<td>16.2</td>
<td>20.3</td>
<td>26.5</td>
<td>25.3</td>
<td>5.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Taste</td>
<td>Insufficient</td>
<td>Superior</td>
<td>Considerably superior</td>
<td>Superior</td>
<td>Insufficient</td>
<td>Considerably superior</td>
</tr>
</tbody>
</table>

Similar taste and free amino acid content as a 6 month cheese obtained after 3 days

Cheese technology
- 1. High pressure treatment for 72 hours
- 2. Held at 25ºC
- 3. A 10-fold higher starter inoculation level

Patent: US005180596A (Fuji Oil Co., Ltd.)
Accelerated Cheese Ripening: Perspectives

➢ 1. Elevated ripening temperature simplest and frequently used method
   ➢ requires milk of good microbiological quality.

➢ 2. Exogenous enzymes are not in widespread use
   ➢ high cost and over-ripening tendency

➢ 3. Adjunct or attenuated cultures
   ➢ perhaps offers the best method
   ➢ frequently used
   ➢ real benefit to cheese producer

➢ 4. High Pressure (HP)
   ➢ Significant capital costs involved and batch nature of unit operation
Thanks for your attention