# 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

Inventory costs

Capital costs

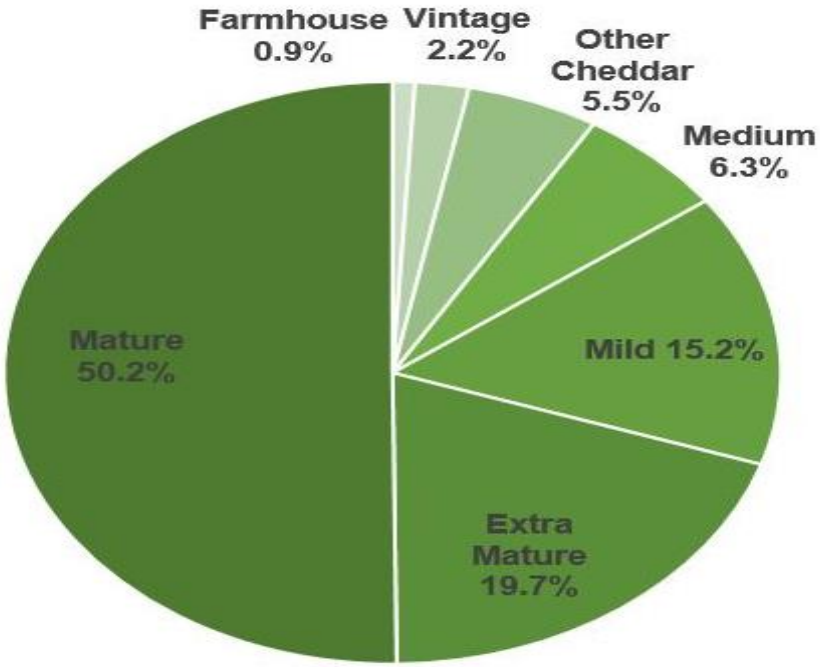

- Subject of much scientific investigation since the 1950s
- Costs approximately €55 (minimum) per tonne of Cheddar per month (Upadhyay and McSweeney, 2003)

# 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

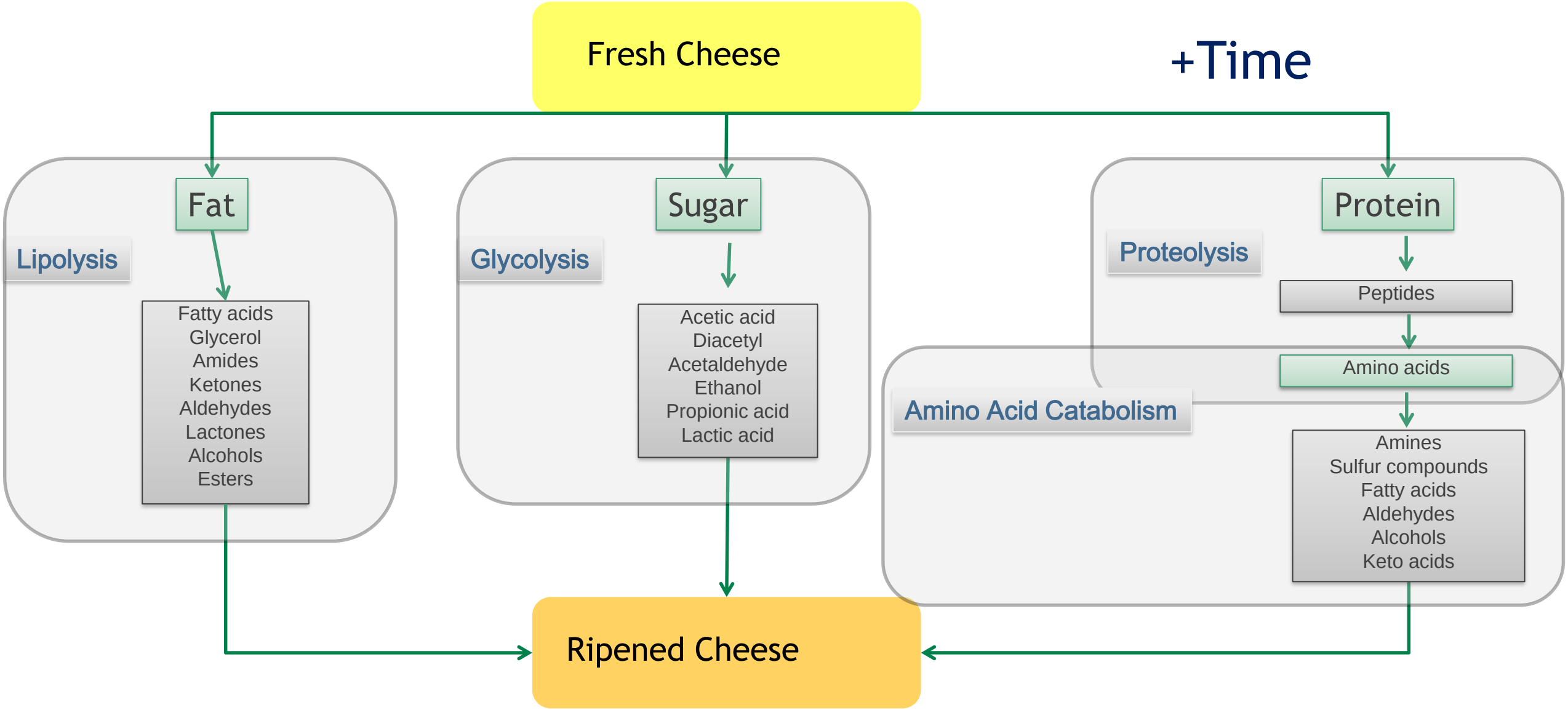
- Extra mature (12 months ripening)
  - 46,000 tonnes x €55 x 12 month
    = €30 million




Total ripening costs €69 million (estimate)

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

# Biochemical changes during ripening



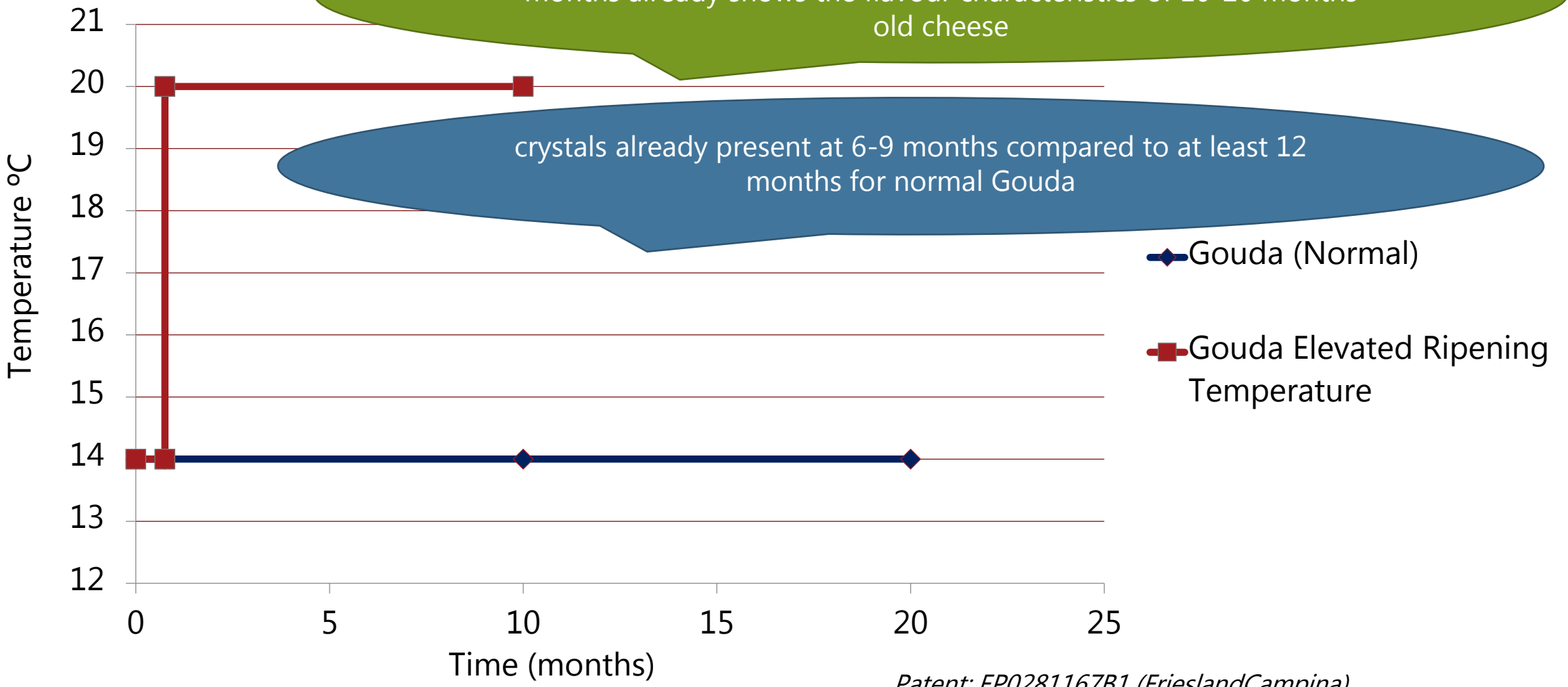
# Approaches used

- 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)

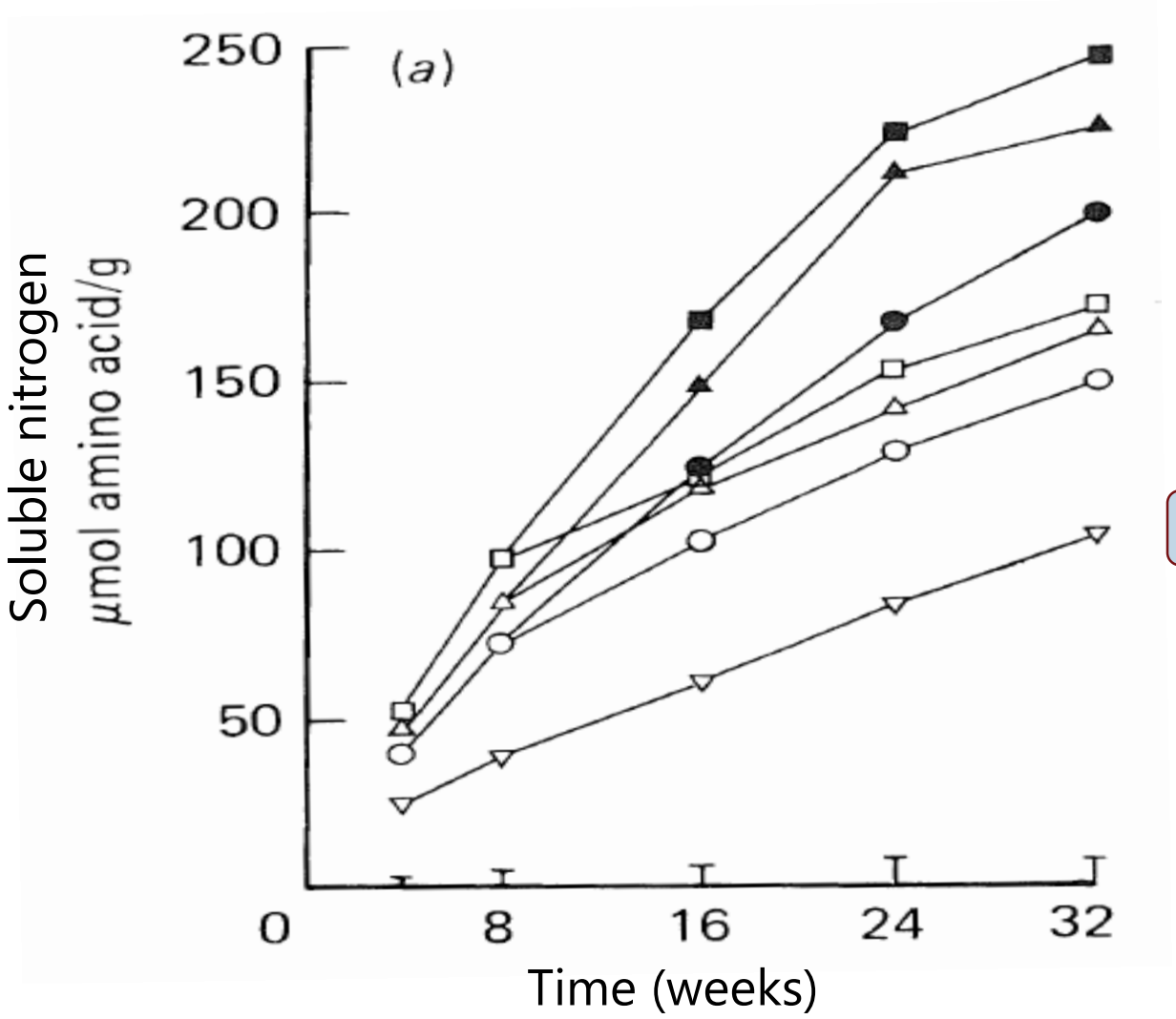
possible to manufacture Gouda cheese which at the age of 5-10 months already shows the flavour characteristics of 10-20 months old cheese

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




*Patent: EP0281167B1 (FrieslandCampina)*

# 1. Elevated ripening temperature (Cheddar)




| | |
|---|---|
| T20-2 | 20°C x 32 weeks |
| T17-2 | 17.5°C x 32 weeks |
| T15-2 | 15°C x 32 weeks |

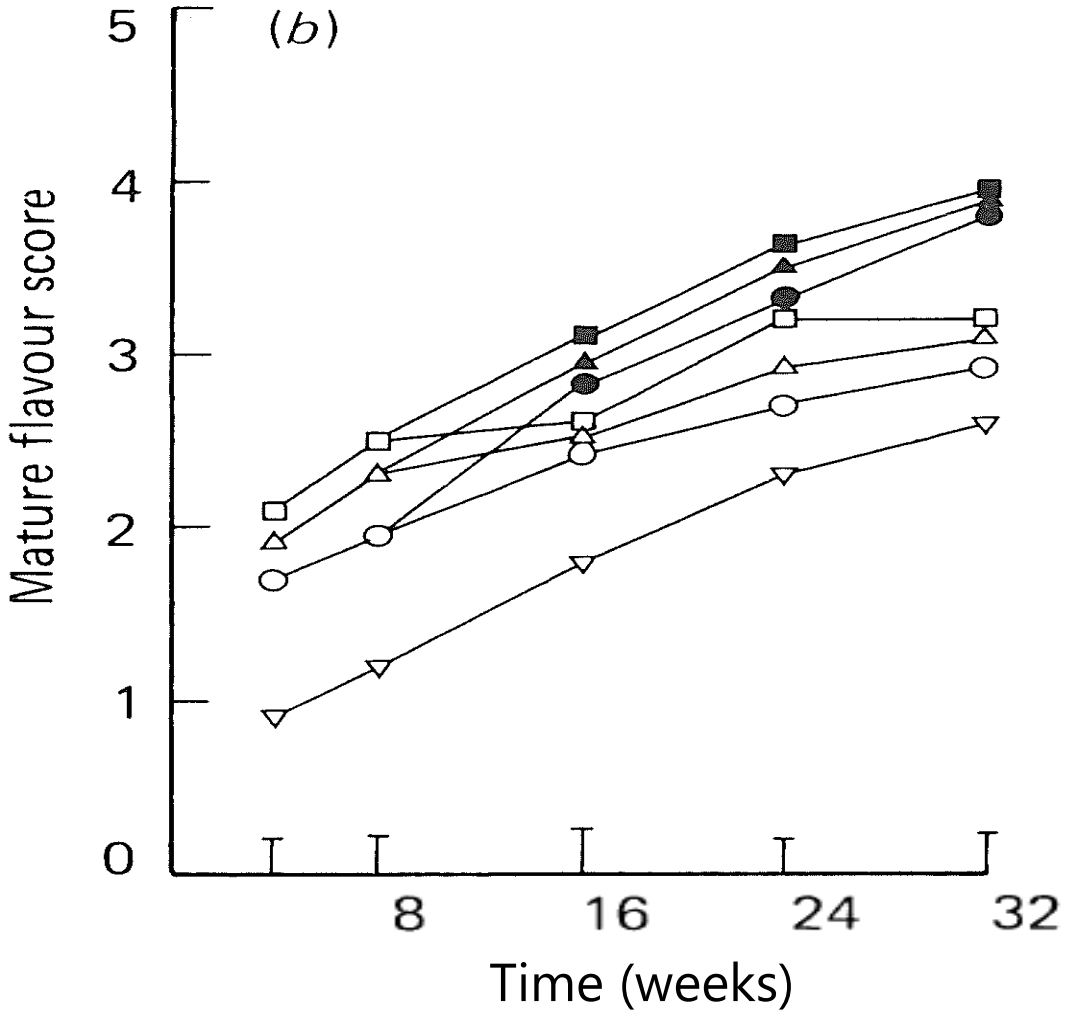
| | |
|---|---|
| T20-1 | 20°C x 8 weeks, 8°C x 24 weeks |
| T17-1 | 17.5°C x 8 weeks, 8°C x 24 weeks |
| T15-1 | 15°C x 8 weeks, 8°C x 24 weeks |

| | |
|---|---|
| Control | 8°C x 32 weeks |

**Proteolysis increase with elevated ripening temperature**

Aston et al., (1985) Journal of Dairy Research,52:565-572

# 1. Elevated ripening temperature (Cheddar)

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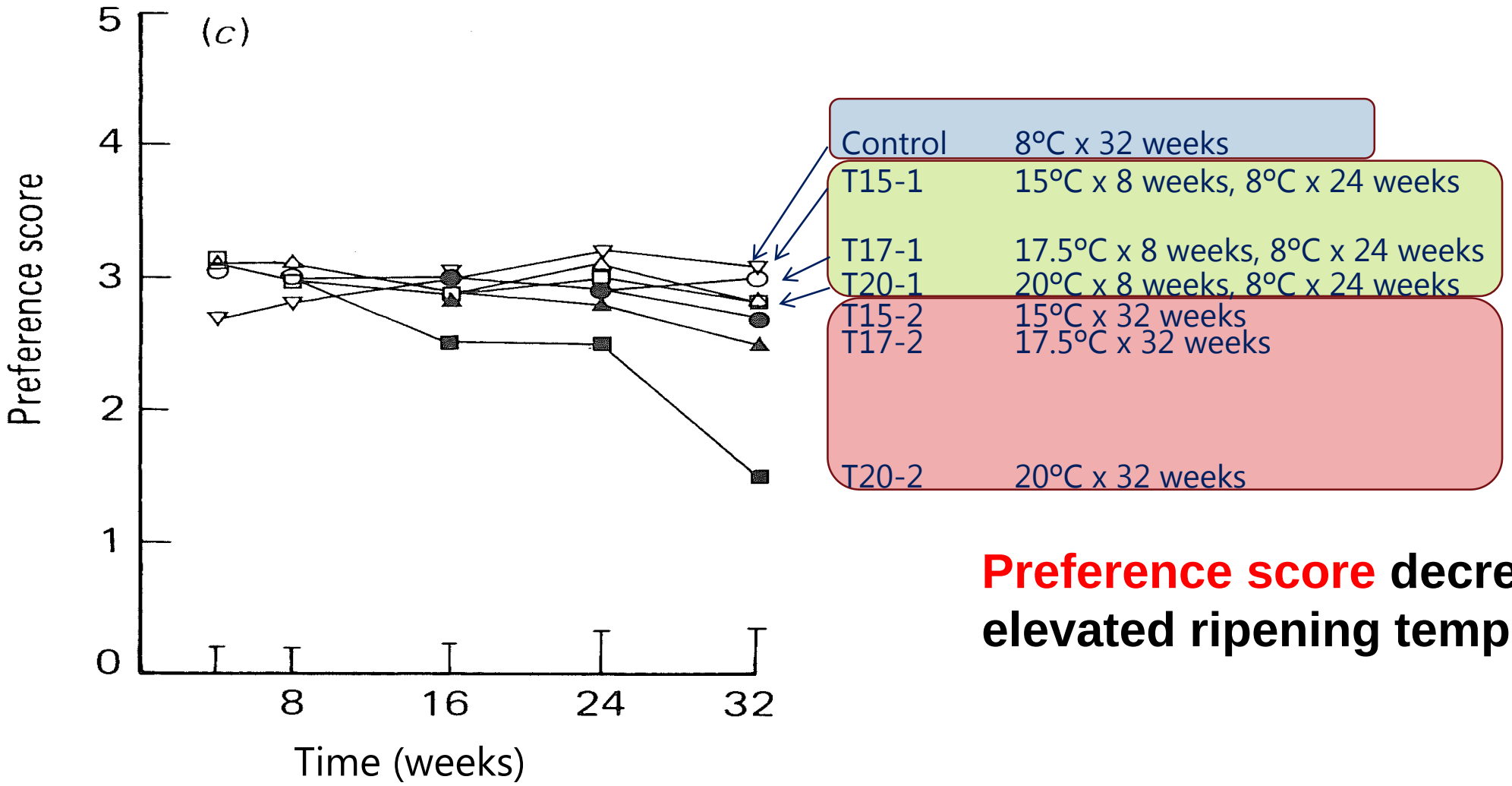
| Control | 8°C x 32 weeks |

**Mature flavour score increased with elevated ripening temperature**

Aston et al., (1985) Journal of Dairy Research,52:565-572

# 1. Elevated ripening temperature (Cheddar)




**Preference score decreased with elevated ripening temperature**

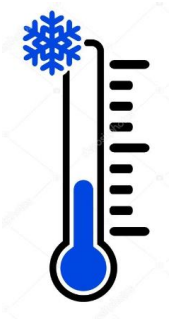
Aston et al., (1985) Journal of Dairy Research,52:565-572

# 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?

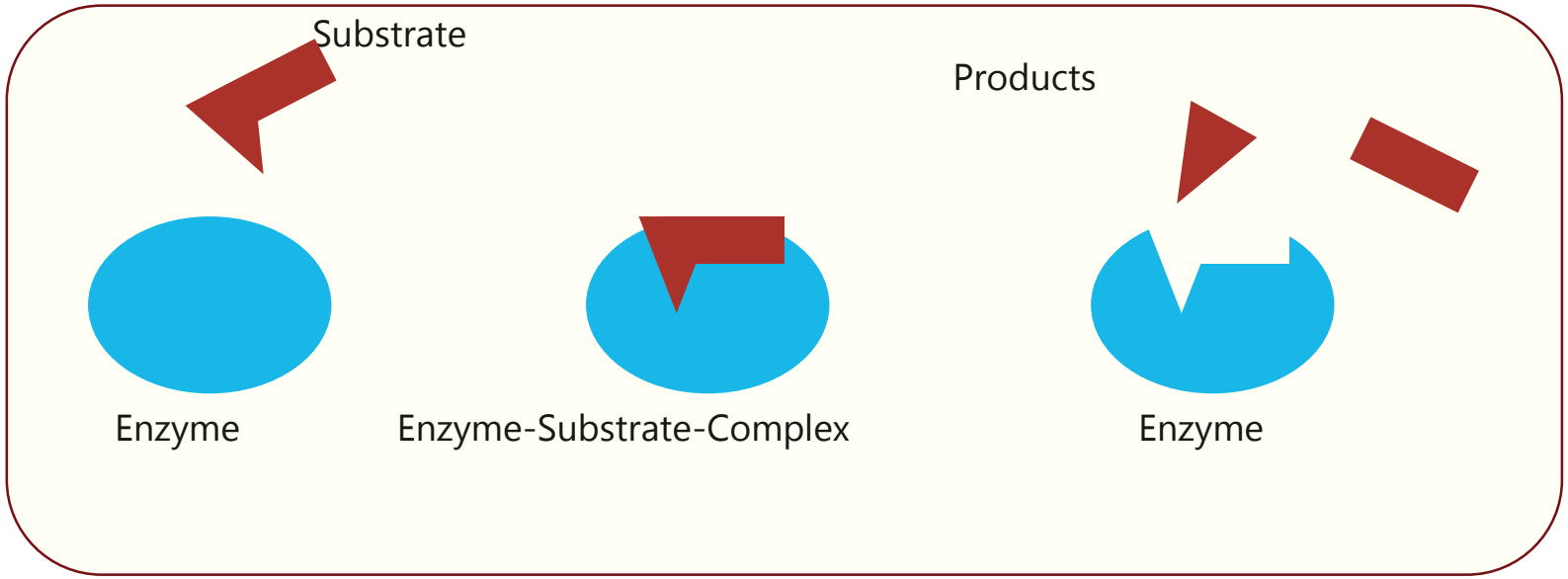
- Huge demand at Christmas
  - Product produced in autumn and then frozen at -20°C for up to 3 months.
  - Thawed prior to market.

# 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

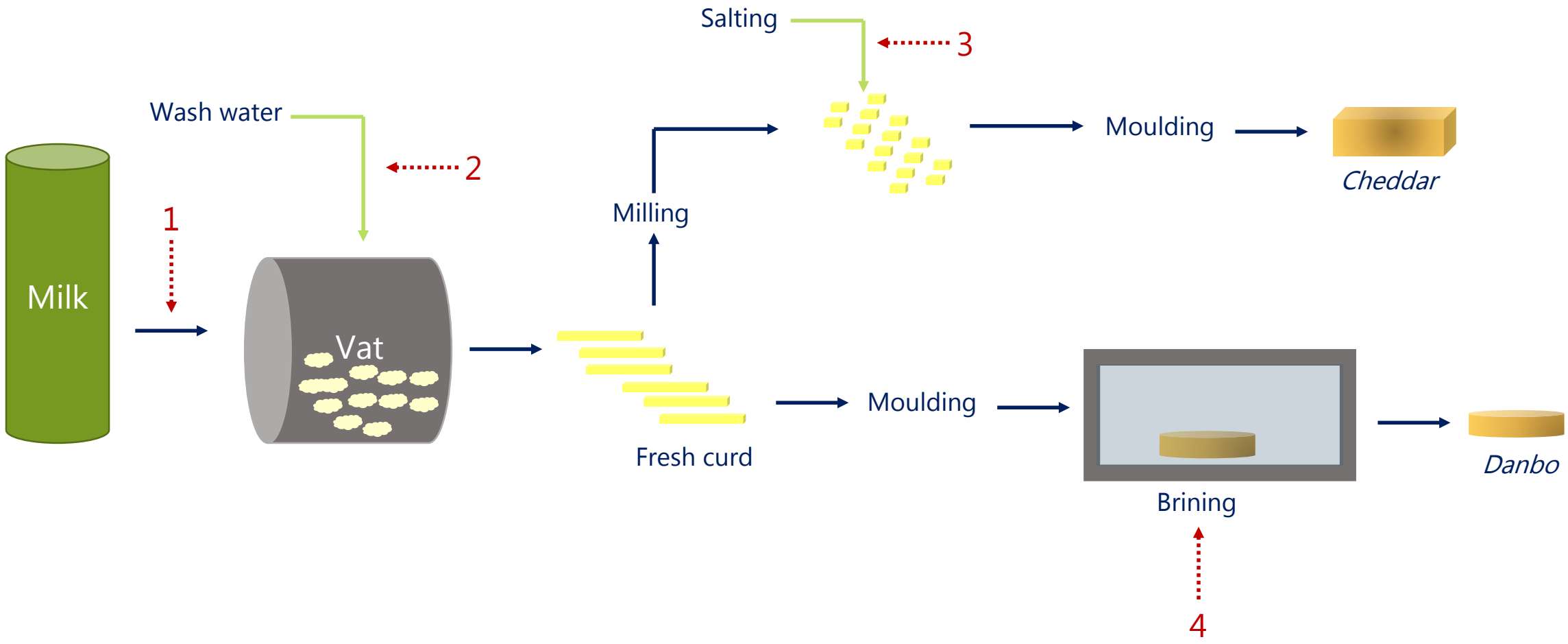
**Table 19.1** Enzyme preparations, other than rennets, commercially available for cheese or enzyme-modified cheese (modified from Wilkinson and Kilcawley, 2002)

| Principal enzymatic activity | Trade name | Host organism/source | Company |
|---|---|---|---|
| Aminopeptidase | Accelase™[a], Savorase®, Debitrase® | *Lactococcus lactis* *Rhizopus oryzae* | Rhodia Food |
| Aminopeptidase | Acid Protease A | *Aspergillus niger* | Amano Enzymes |
| Prote(in)ase | Acid Protease II | *Rhizomucor niveus* | Amano Enzymes |
| | Bioprotease A conc | *Aspergillus niger* | Quest International |
| | Bioprotease N 100 | *Bacillus subtilis* | Quest International |
| | Bioprotease P conc | *Aspergillus oryzae* | Quest International |
| | Fermizyme® B 500 | *Bacillus subtilis* | DSM |
| | FlavorAge[b] | *Aspergillus* sp. | Chr Hansen |
| | Flavorpro 192 | *Aspergillus* var. strains | Biocatalysts |
| | Flavourzyme | *Aspergillus oryzae* | NOVO |
| | Neutrase | *Bacillus subtilis* | NOVO |
| | Peptidase 'R' Amano | *Rhizomucor oryzae* | Amano Enzymes |
| | Promod 24L | *Bacillus subtilis* | Biocatalysts |
| | Promod 215P | *Aspergillus sojae* | Biocatalysts |
| | Protease 'A' Amano 2 | *Aspergillus oryzae* | Amano Enzymes |
| | Protease M | *Aspergillus oryzae* | Amano Enzymes |
| | Protease N | *Bacillus subtilis* | Amano Enzymes |
| | Prozyme 6 | *Aspergillus melleus* | Amano Enzymes |
| | Sternzyme B5021 | *Aspergillus niger* | Stern-Enzyme |
| | Sternzyme B5026 | *Aspergillus oryzae* | Stern-Enzyme |
| Lipase | Capalase® | Animal | Degussa Bioactives |
| | Italase® | Animal | Degussa Bioactives |
| | Kid Lipase | Animal | Chr Hansen |
| | Lipase M 'Amano' 10 | *Rhizomucor javanicus* | Amano Enzymes |
| | Palatase® 20000 L | *Rhizomucor miehei* | NOVO |

[a] Can also be mixed with enzymes from other sources.
[b] Proteinase-lipase preparation.

Upadhyay and McSweeney (2003) Dairy Processing: Improving Quality. Boca Raton, CRC Press: 419–447.

# 2. Addition of exogenous enzymes: addition points



# 2. Addition of exogenous enzymes: Cheddar

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

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

Kilcawley et al., (2012) International Dairy Journal 26 :50-57

# 2. Addition of exogenous enzymes: Cheddar

## Proteolysis and Lipolysis

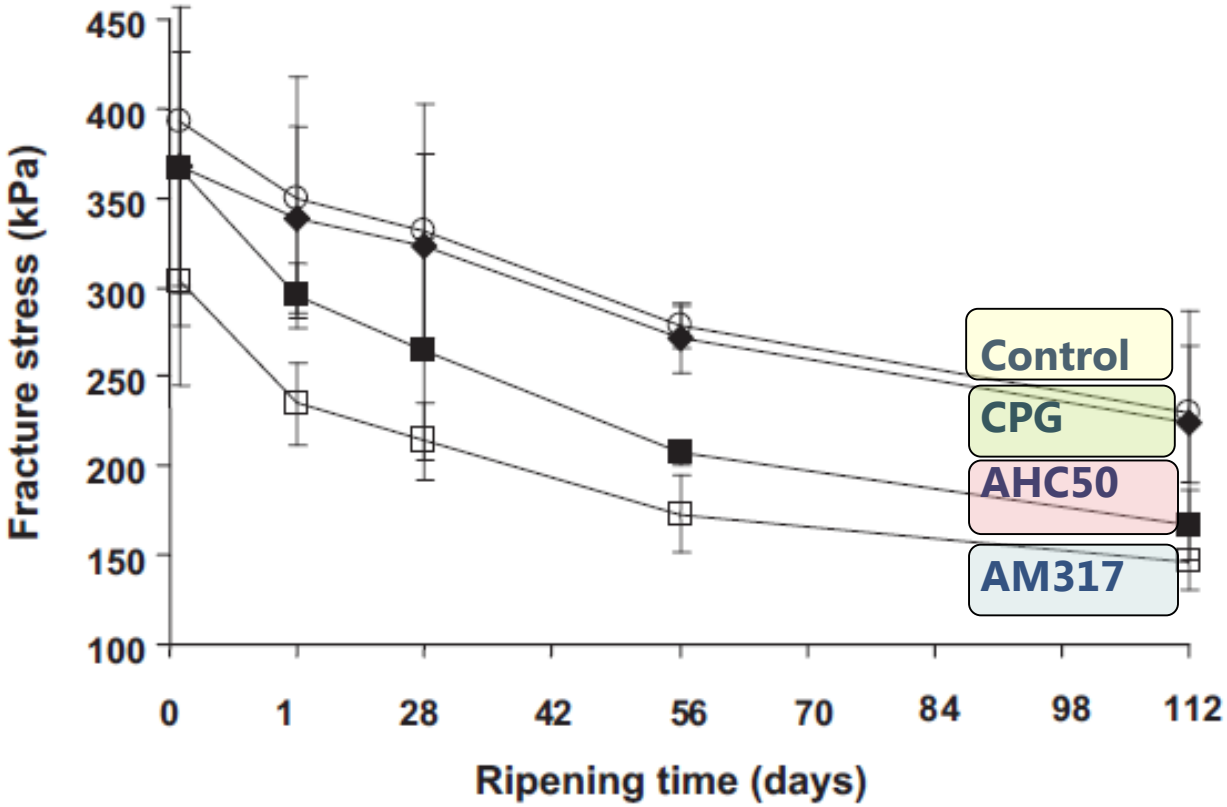
| Age (days) | Control | AM317 | CPG | AHC50 |
|---|---|---|---|---|
| pH4.6-SN | | | | |
| 1 | 3.93 (0.82)a | 4.81 (1.03)a | 3.3 (0.24)a | 4.09 (0.27)a |
| 14 | 6.49 (0.61)a | 10.54 (0.68)b | 6.24 (0.24)a | 9.34 (1.77)b |
| 28 | 8.29 (0.86)a | 12.93 (0.24)b | 7.94 (0.13)a | 12.77 (1.47)b |
| 56 | 11.72 (0.96)a | 18.11 (0.36)b | 11.04 (0.06)a | 18.46 (1.87)b |
| 112 | 16.40 (1.28)a | 24.16 (0.64)b | 15.49 (0.29)a | 26.38 (0.55)b |
| PTA-SN | | | | |
| 1 | 0.74 (0.07)a | 0.76 (0.07)a | 0.66 (0.10)a | 0.75 (0.10)a |
| 14 | 1.19 (0.18)a | 1.36 (0.02)a | 1.24 (0.04)a | 1.53 (0.34)a |
| 28 | 1.48 (0.26)a | 1.60 (0.10)a | 1.49 (0.05)a | 1.77 (0.18)a |
| 56 | 2.13 (0.28)a | 2.46 (0.07)a | 2.13 (0.13)a | 2.65 (0.34)a |
| 112 | 3.47 (0.56)ab | 3.89 (0.09)b | 3.29 (0.05)a | 4.57 (0.33)c |
| TFAA | | | | |
| 1 | 905 (260)a | 1037 (116)a | 980 (104)a | 1069 (37)a |
| 14 | 2153 (390)a | 2878 (581)a | 2727 (337)a | 3175 (722)a |
| 28 | 3013 (134)a | 3454 (385)a | 3669 (320)a | 4208 (165)a |
| 56 | 4609 (460)a | 5662 (445)ab | 5397 (563)ab | 6384 (245)b |
| 112 | 7995 (983)a | 9862 (914)b | 8966 (778)a | 11035 (281)b |
| TFFA | | | | |
| 1 | 752 (88)a | 747 (91)a | 745 (21)a | 763 (72)a |
| 14 | 724 (45)a | 750 (83)a | 723 (38)a | 745 (55)a |
| 28 | 759 (37)a | 761 (17)a | 761 (17)a | 761 (17)a |
| 56 | 729 (7)a | 712 (28)a | 734 (51)a | 733 (44)a |
| 112 | 808 (50)a | 811 (78)a | 813 (58)a | 829 (33)a |

**Increased pH4.6 soluble N**

**Increased free amino acids**

**No change free fatty acids**

## Texture




**AHC50 and AM317 cheeses too soft and brittle**

Kilcawley et al., (2012) International Dairy Journal 26 :50-57

# 2. Addition of exogenous enzymes

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

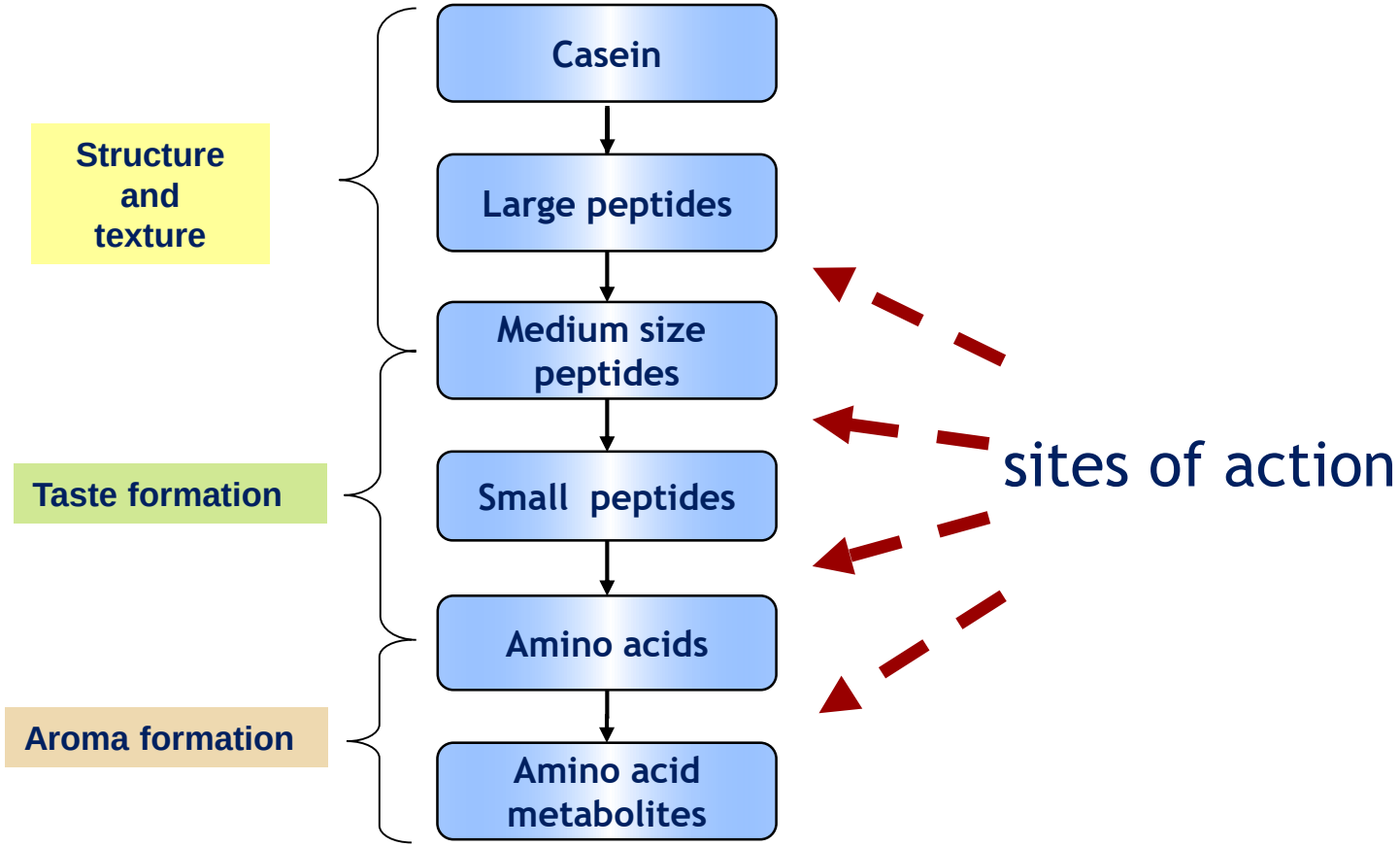
- DISADVANTAGES
  - Difficulty of uniform distribution
  - Off flavours
  - Limited range of suitable enzymes
  - Certain enzymes require cofactors
    - Especially amino acid converting enzymes
  - Possible side activities
  - Cost
  - Legal constraints

# 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

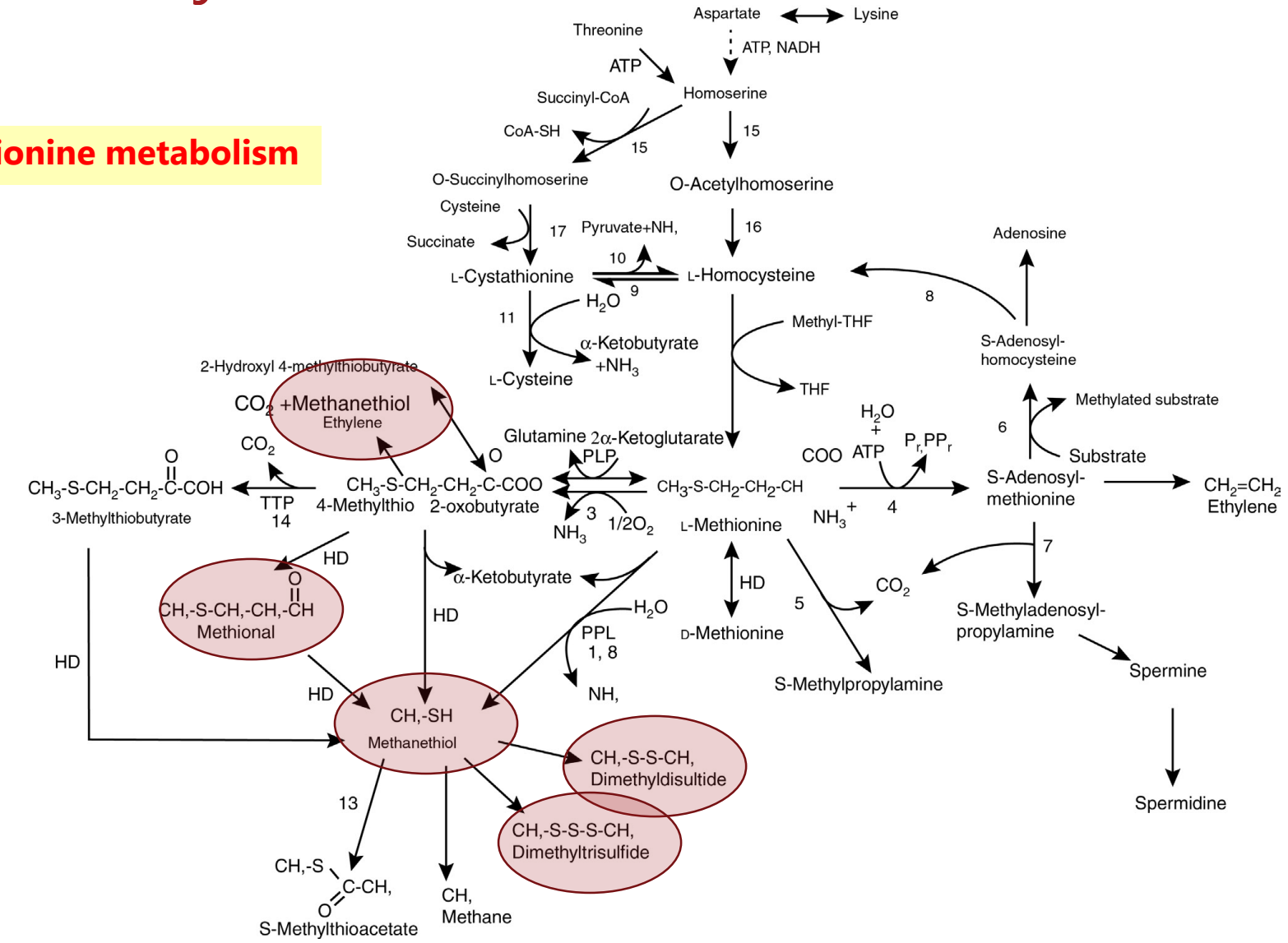
- Principle/theory
  - Use the full enzyme complement of Lactic Acid Bacteria (LAB) cultures to simultaneous increase
    - Proteolysis
    - Petidolysis
    - Amino acid catabolism




**POWER AND BALANCE**

# 3. Addition of adjunct or attenuated cultures

**Volatiles from methionine metabolism**

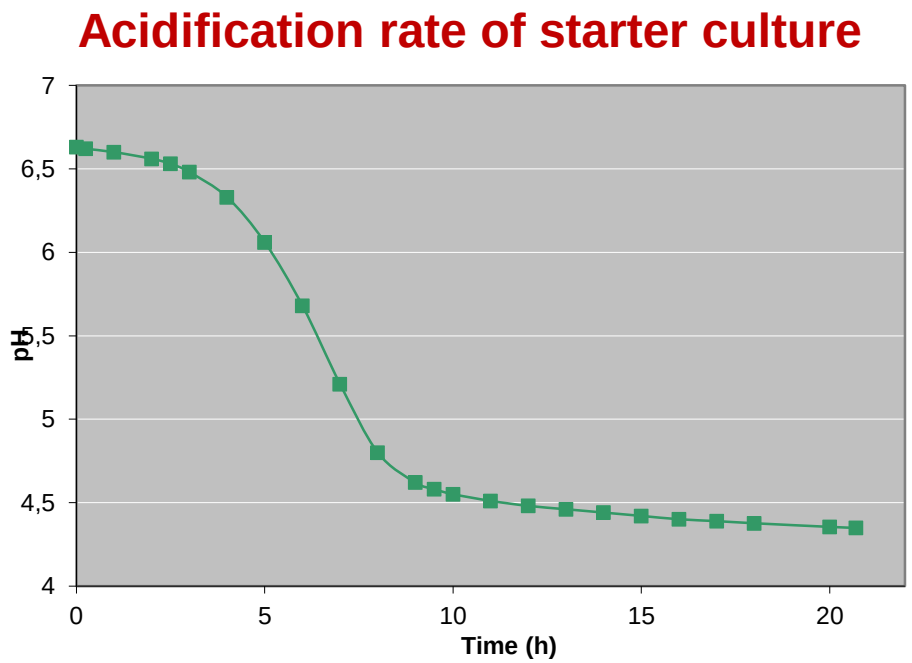



Gganesan and Weimer (2017): Cheese, pp.483-516

# 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

Principal suppliers of Dairy Cultures

| Company | Market share (estimate) | Country | Founded |
|---|---|---|---|
| Chr. Hansen A/S | 1 | Denmark | 1870 |
| DuPont Danisco | 2 | USA | 1802 |
| DSM | 3 | Netherlands | 1902 |
| CSK Food Enrichment | 4 | Netherlands | 1905 |
| Sacco | 5 | Italy | 1872 |





# 3. Addition of adjunct or attenuated cultures

- 8 Gouda-type cheeses with 8 different culture combinations

| Culture combination | | *Lc. lactis* (O) | *Lb. casei* (C) | *Lb. helveticus* (H) | *Lb. rhamnosus* (R) |
|---|---|---|---|---|---|
| O[a] | Control | + | - | - | - |
| OC | Single | + | + | - | - |
| OH | Single | + | - | + | - |
| OR | Single | + | - | - | + |
| OCH | Multiple | + | + | + | - |
| OCR | Multiple | + | + | - | + |
| ORH | Multiple | + | | + | + |
| OCRH | Multiple | + | + | + | + |

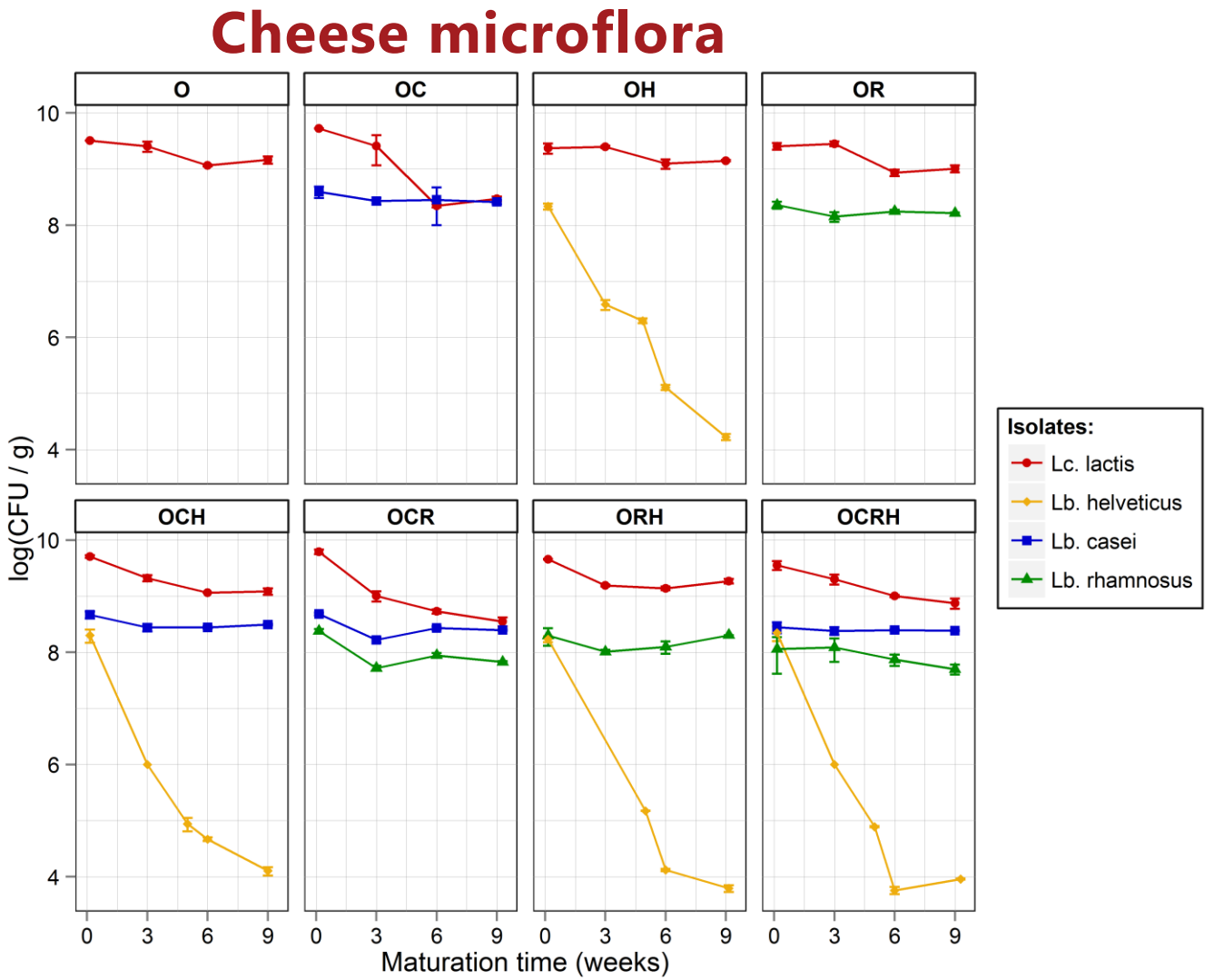
[a] Used as control, + Bacterial culture is included in the culture combination, - Bacterial culture is not included.

O OC OH OR OCH OCR ORH ORCH

Peter Beierholm and Jeppe Frans Steendahl Sørensen, (2017), MSc thesis, KU

# 3. Addition of adjunct or attenuated cultures

- Cheeses ripened for 1 week at °C, 4 weeks at 13 °C, for 4 weeks at 9 °C.
- Microbiological enumeration
- Aroma analysis (DHS-GC-MS)



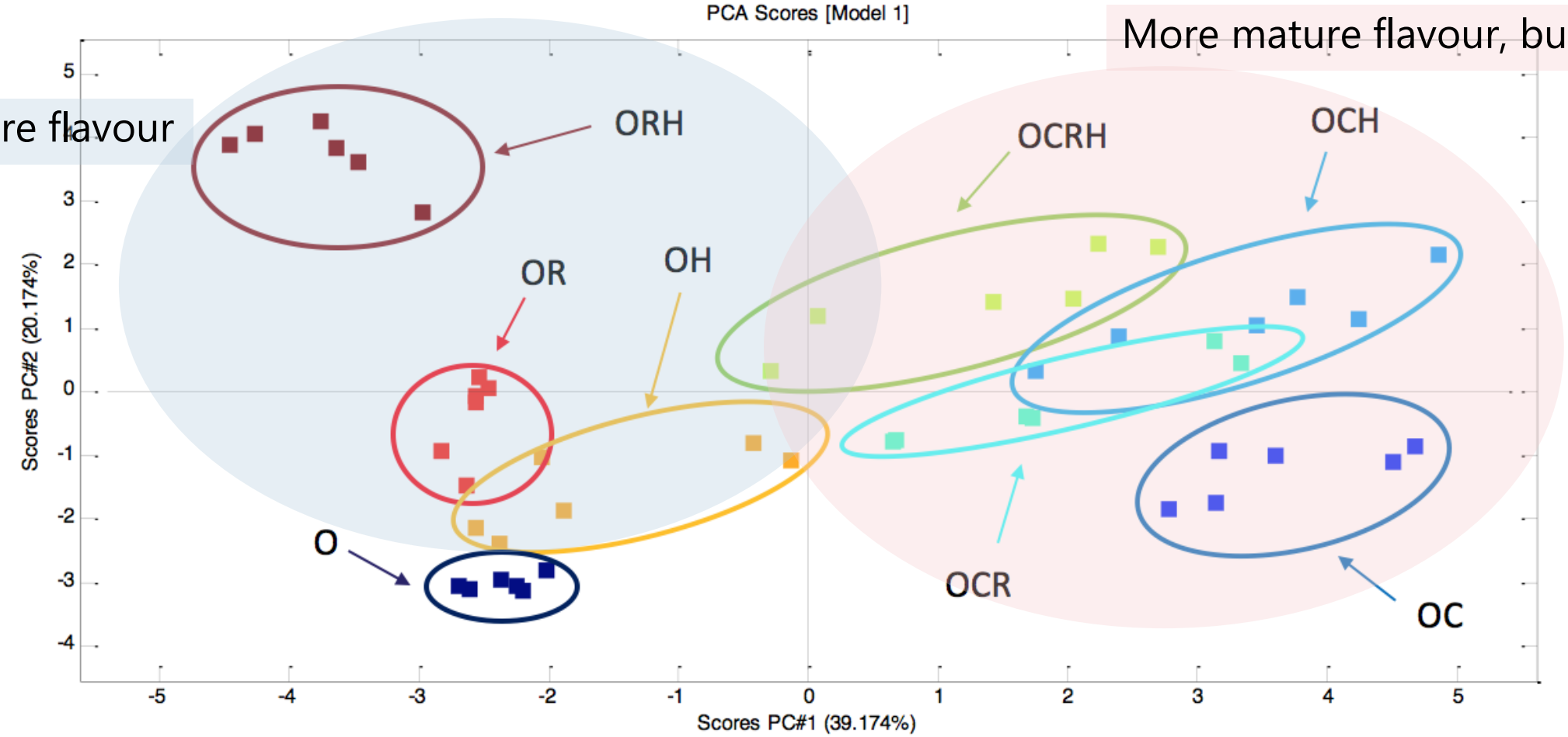

Peter Beierholm and Jeppe Frans Steendahl Sørensen, (2017), MSc thesis, KU

# 3. Addition of adjunct or attenuated cultures

## Gouda-type Cheeses – GC-MS Aroma Analysis

More mature flavour

More mature flavour, but atypical

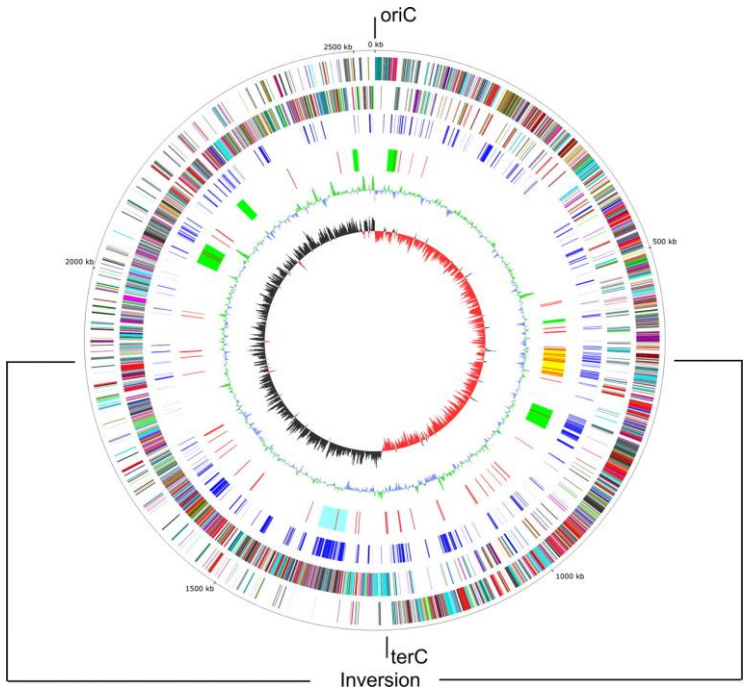



Peter Beierholm and Jeppe Frans Steendahl Sørensen, (2017), MSc thesis, KU

# 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

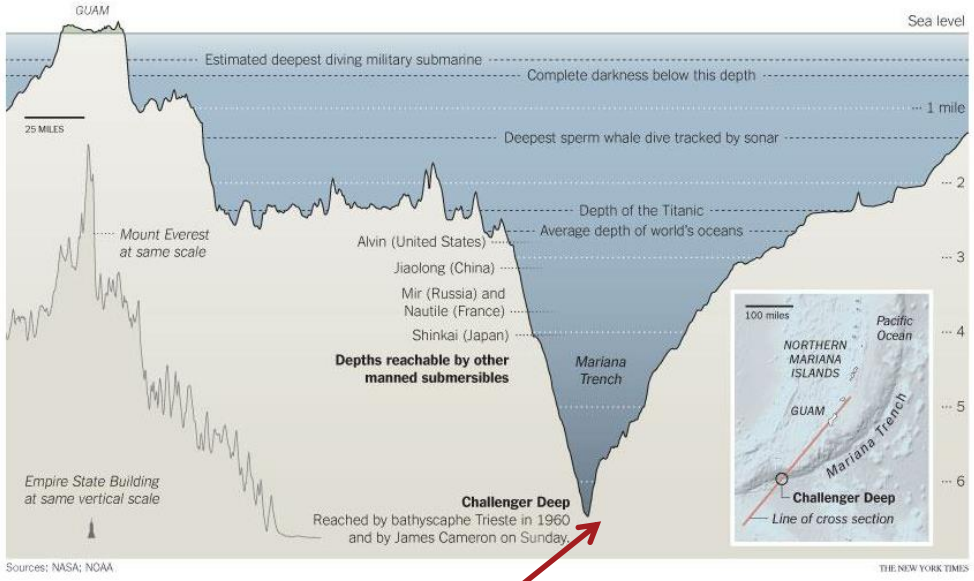
# Approaches used

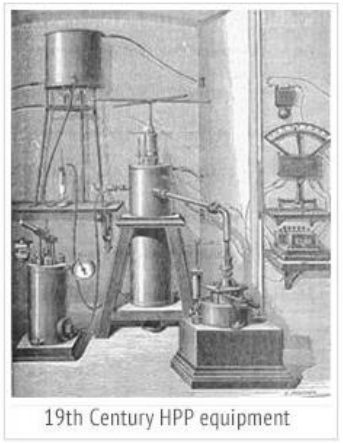
- 1. Elevated ripening temperature
- 2. Addition of exogenous enzymes
- 3. Addition of adjunct or attenuated cultures
- 4. High-pressure (HP) treatment

# 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





19th Century HPP equipment

*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
  - Relase of intracelluar enzymes
- 4. HP modify water distribution and increase pH
  - Enhances enzymatic activities

# 4. High-pressure (HP) treatment: Cheddar

| | Experimental Cheeses | | | | | Commercial cheese (6 months) |
|---|---|---|---|---|---|---|
| **Pressure (MPa)** | **50** | **100** | **500** | **2000** | **3000** | **None** |
| Free amino acids (mg/g) | 16.2 | 20.3 | 26.5 | 25.3 | 5.2 | 21.3 |
| Taste | Insufficient | Superior | Considerably superior | Superior | Insufficient | Considerably superior |

**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 25°C
- 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

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