The complexity of bacterial interactions in cheese ripening

Cleide O. de A. Møller
Assistant Professor

Section of Microbiology and Fermentation

Department of Food Science

cleide@food.ku.dk

UNIVERSITY OF COPENHAGEN

Outline:

- **Introduction to starter (SLAB) and non-starter (NSLAB) lactic acid bacteria in Cheddar cheese**
- Mechanisms involving bacterial interactions in cheese ripening
- Addition of sugars to boost NSLAB growth
- Reducing salt content for developing healthier products
- Increasing ripening temperature to accelerate cheese ripening

Introduction to starter (SLAB) and non-starter (NSLAB) lactic acid bacteria in Cheddar cheese

Starter LAB (SLAB)

Mesophilic LAB able to grow to 8-9 $\log_{10}$ cfu g^{-1} until salting

(but thermophilic cultures also may be added at low levels)

Predominated by **lactococci**

Lc. lactis sub. lactis and *Lc. lactis sub. cremoris*

Intentionally added

Commercially available cultures

"in house" cultures

Premium quality Cheddar cheese:

Moisture = about 38 %

Salt-in-moisture = 4.7-5.7 %

pH = 5.1-5.3

Cheddar cheese

Non-starter LAB (NSLAB)

LAB able to grow under harsh conditions *(**32-39 %** moisture, **4-6 %** salt-in-moisture, pH **4.9-5.3**)*

Predominated by **mesophilic lactobacilli**

(pediococci and micrococci may also be found)

Lb. paracasei, *Lb. plantarum Lb. curvatus and Lb. rhamnosus*

(Lb. brevis, Lb. fermentum, P. pentosaceus and P. acidilactici)

Natural present

Milk

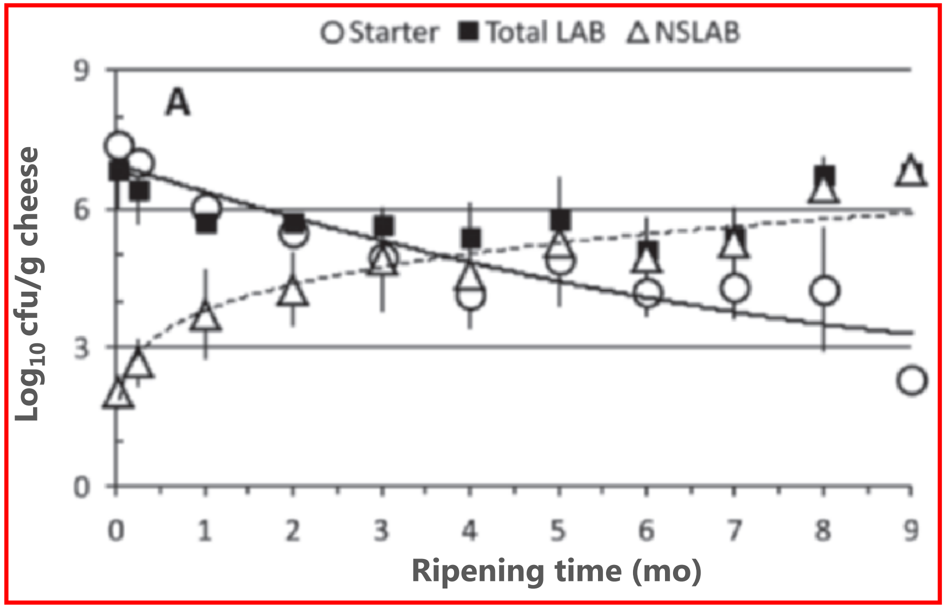
Processing environment

Outline:

- Introduction to starter (SLAB) and non-starter (NSLAB) lactic acid bacteria in Cheddar cheese
- **Mechanisms involving bacterial interactions in cheese ripening**
- Addition of sugars to boost NSLAB growth
- Reducing salt content for developing healthier products
- Increasing ripening temperature to accelerate cheese ripening

Mechanisms involving bacterial interaction in cheese ripening

Ripening of cheese consists of complex microbial interactions between starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB).



mean logarithmic ratios were plotted against storage time (9 mo at 6°C) and a trend line fitted to each set of data for Starter (solid line) and NSLAB (dashed line). Error bars = SE (n = 3)

Source: D.J. McMahon et al. (2014) J. Dairy Sci. 97:4780-98

Energy source?

Related to cheese:
N-acetylgalactosamine
from k-casein and
milk fat globule membrane
Free Amino Acids (FAA)
from breakdown of caseins

Related to SLAB:
N-acetylglucosamine
and N-acetylmuramic acid
from cell wall
Ribose
from DNA and RNA

Nutrients important factors

Availability and accessibility?

DIFFUSION

New Zealand Journal of Dairy Science and Technology, **22**, 215-219 (1987).

Cannibalism Among Bacteria Found in Cheese

TERENCE D THOMAS

New Zealand Dairy Research Institute, Palmerston North, New Zealand

Modelling approach for diffusion:

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

ELSEVIER

Modelling the influence of metabolite diffusion on non-starter lactic acid bacteria growth in ripening Cheddar cheese

Tamás Czárán [a], Fergal P. Rattray [b], Cleide O.de A. Møller [b], Bjarke B. Christensen [c,*]

SLAB to nutrient conversion by decay

Local rate of SLAB decay →

$$\frac{\partial S}{\partial t} = -d_{SLaB}\, S$$

Local rate of nutrient cc. change →

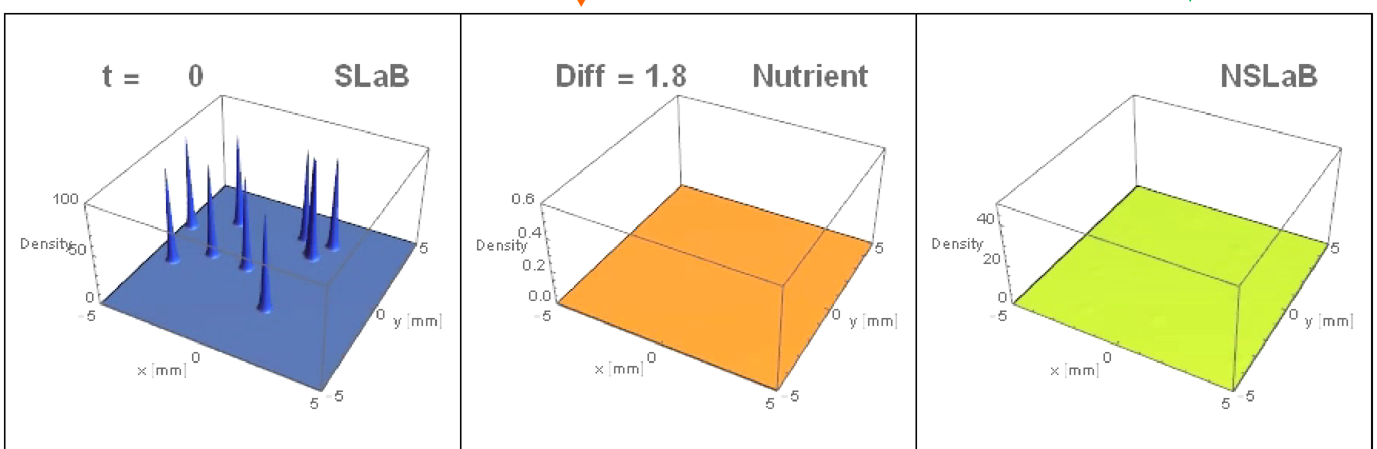
$$\frac{\partial L}{\partial t} = D_{Lys}\left(\frac{\partial^2 L}{\partial x^2} + \frac{\partial^2 L}{\partial y^2}\right) + d_{SLaB}\, S - r_{NSLaB}\frac{L}{L+1}N$$

Local rate of NSLAB growth →

$$\frac{\partial N}{\partial t} = r_{NSLaB}\frac{L}{L+1}N$$

Net nutrient influx from nearby locations by diffusion

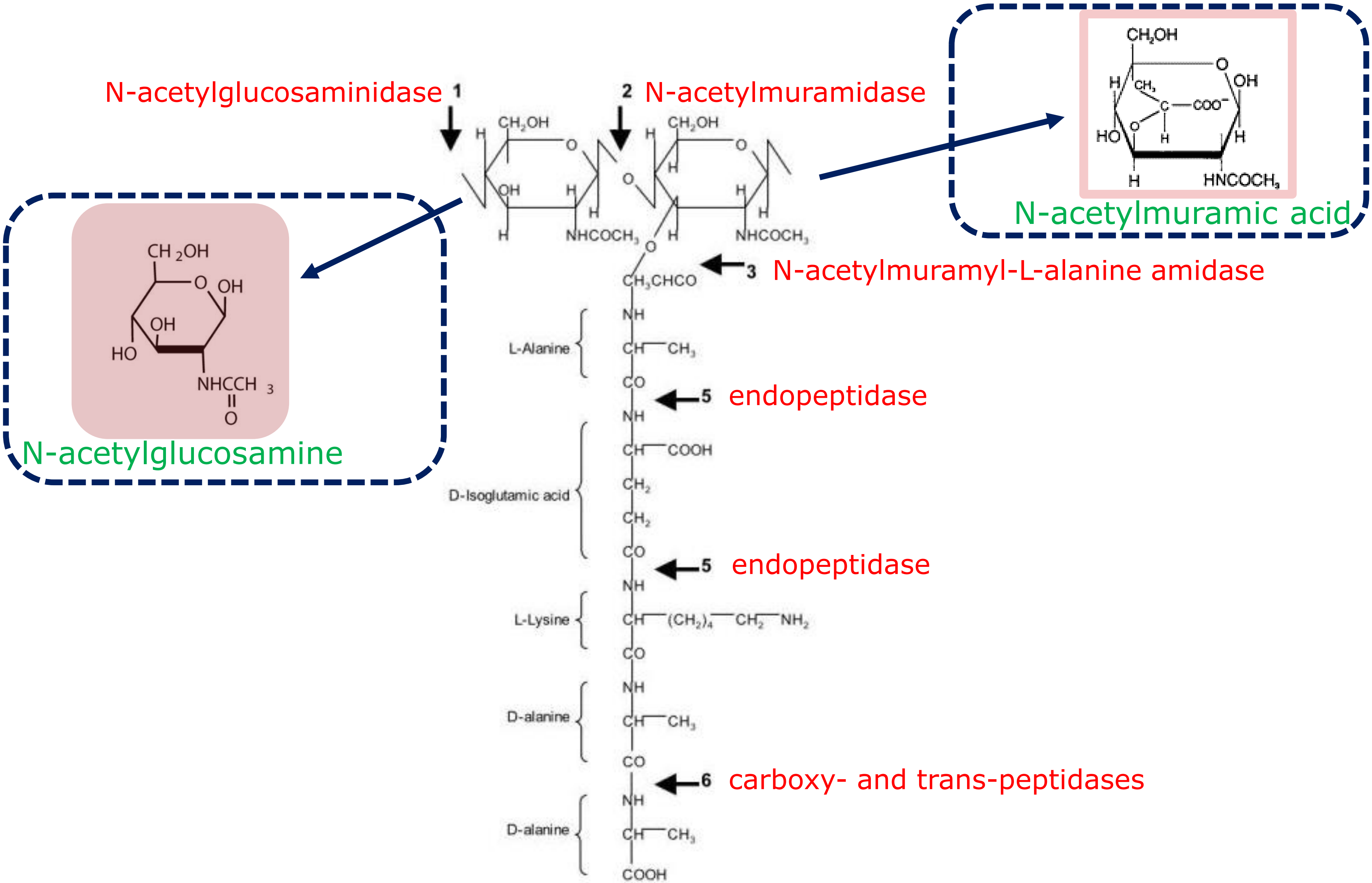
Nutrient to NSLAB conversion by population growth



Outline:

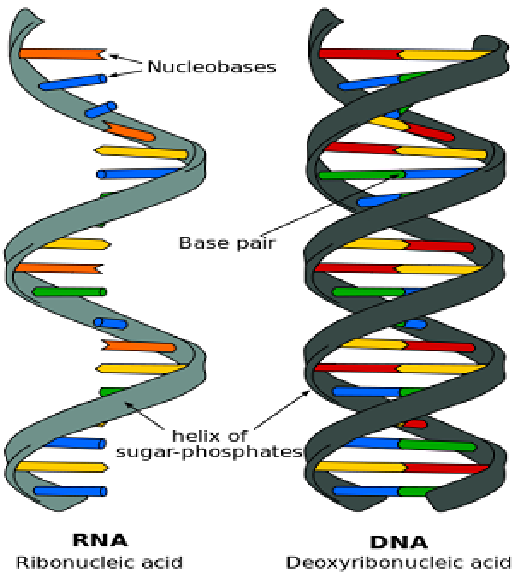
- Introduction to starter (SLAB) and non-starter (NSLAB) lactic acid bacteria in Cheddar cheese
- Mechanisms involving bacterial interactions in cheese ripening
- **Addition of sugars to boost NSLAB growth**
- Reducing salt content for developing healthier products
- Increasing ripening temperature to accelerate cheese ripening

1. N-acetylglucosamine and N-acetylmuramic acid from starter cell-wall

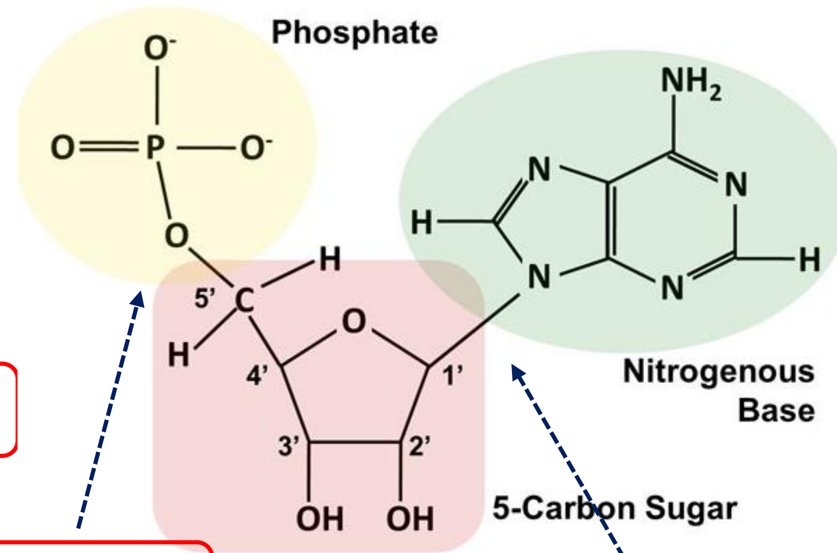


2. Ribose from starter DNA/RNA

RNA & DNA



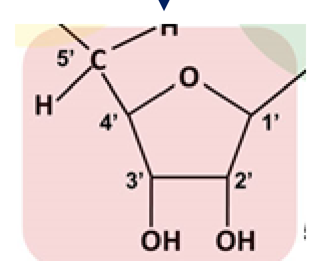
Nucleotide



DNAse & RNAse

Phosphatase

DNA N-glycosidase



Ribose/deoxyribose

Addition of sugars to boost NSLAB growth

Nutrients released from **SLAB lysis** may support growth **NSLAB** and accelerate flavor development

SLAB lysate obtained by enzymatic digestion

- Lysozyme + phospholipase (3 h/37 °C)
- dnase + rnase (2.5 h/37 °C)
- Proteinase (overnight/37 °C)
- INACTIVATION (30 min/80 °C)

2 strains of SLAB:

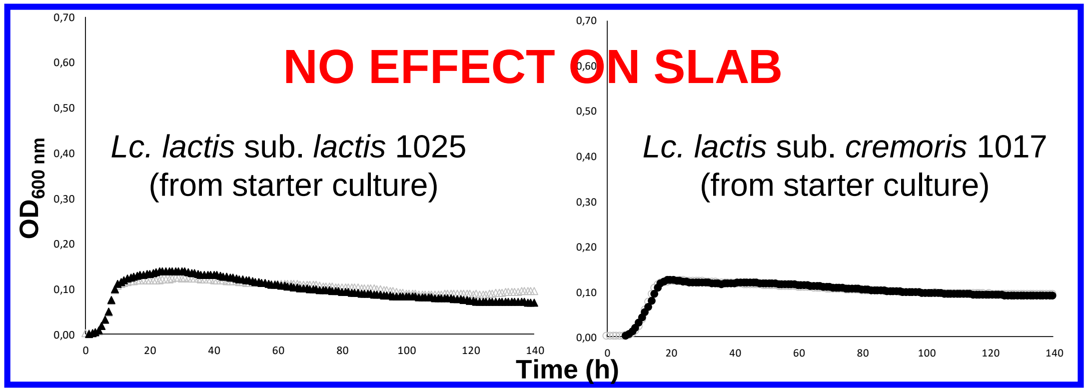
- *Lc. lactis lactis*
- *Lc. lactis cremoris*

15 strains of NSLAB:

- *Lb. brevis (2)*
- *Lb. casei*
- *Lb. coryniformes*
- *Lb. curvatus (2)*
- *Lb. delbrueckii (2)*
- *Lb. fermentum*
- *Lb. helveticus*
- *Lb. parabuchneri*
- *Lb. paracasei*
- *Lb. plantarum*
- *Lb. rhamnosus (2)*

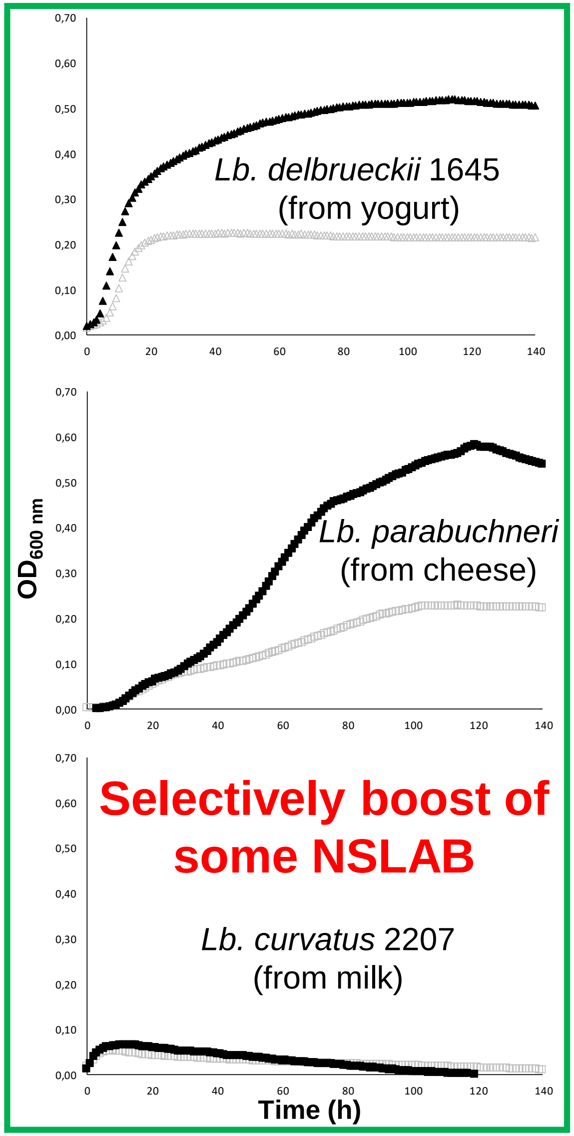
LAB individually inoculated (about 5 log10 cfu mL^{-1}) in M17 broth (no lactose), with and without 10 % SLAB lysate, and incubated at 30 °C for 140 h

Growth monitored by optical density at 600 nm



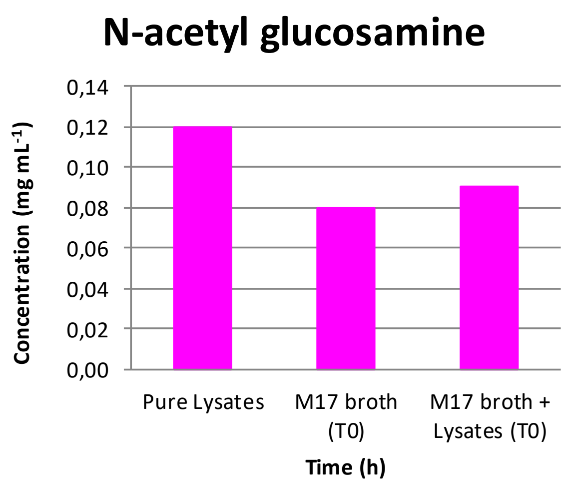
With lysate

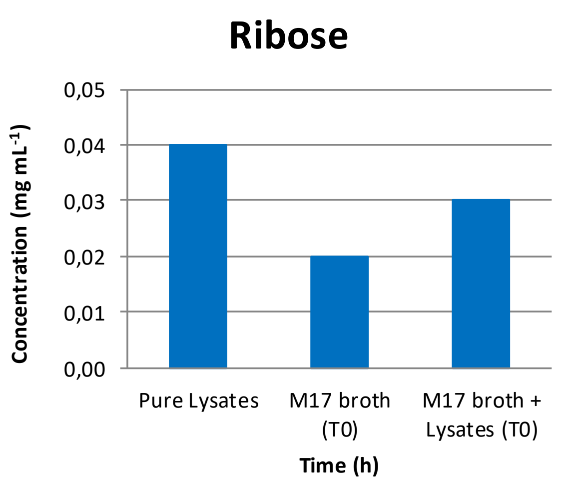
No lysate



Addition of sugars to boost NSLAB growth

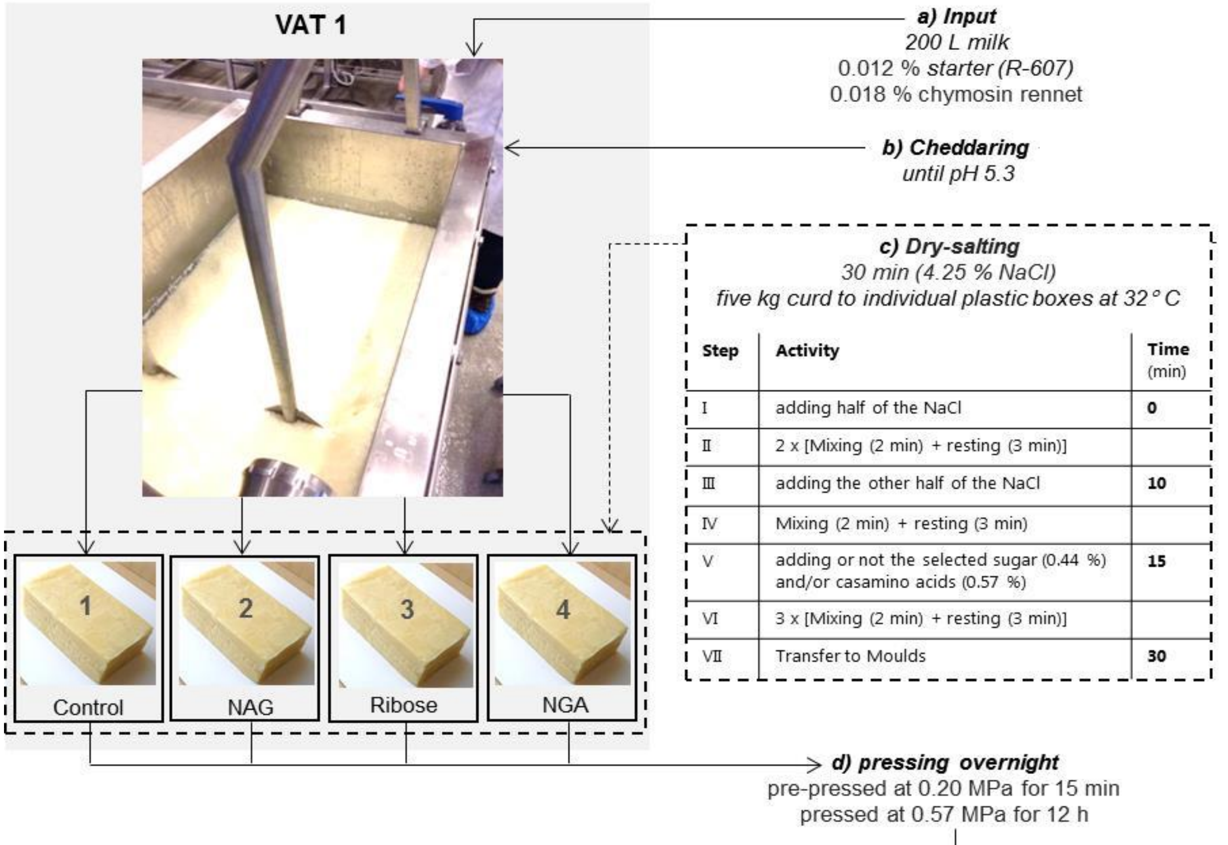
Sugars released from SLAB lysis in the Lysate





Cheese trial I

Effect of sugar in cheese matrices



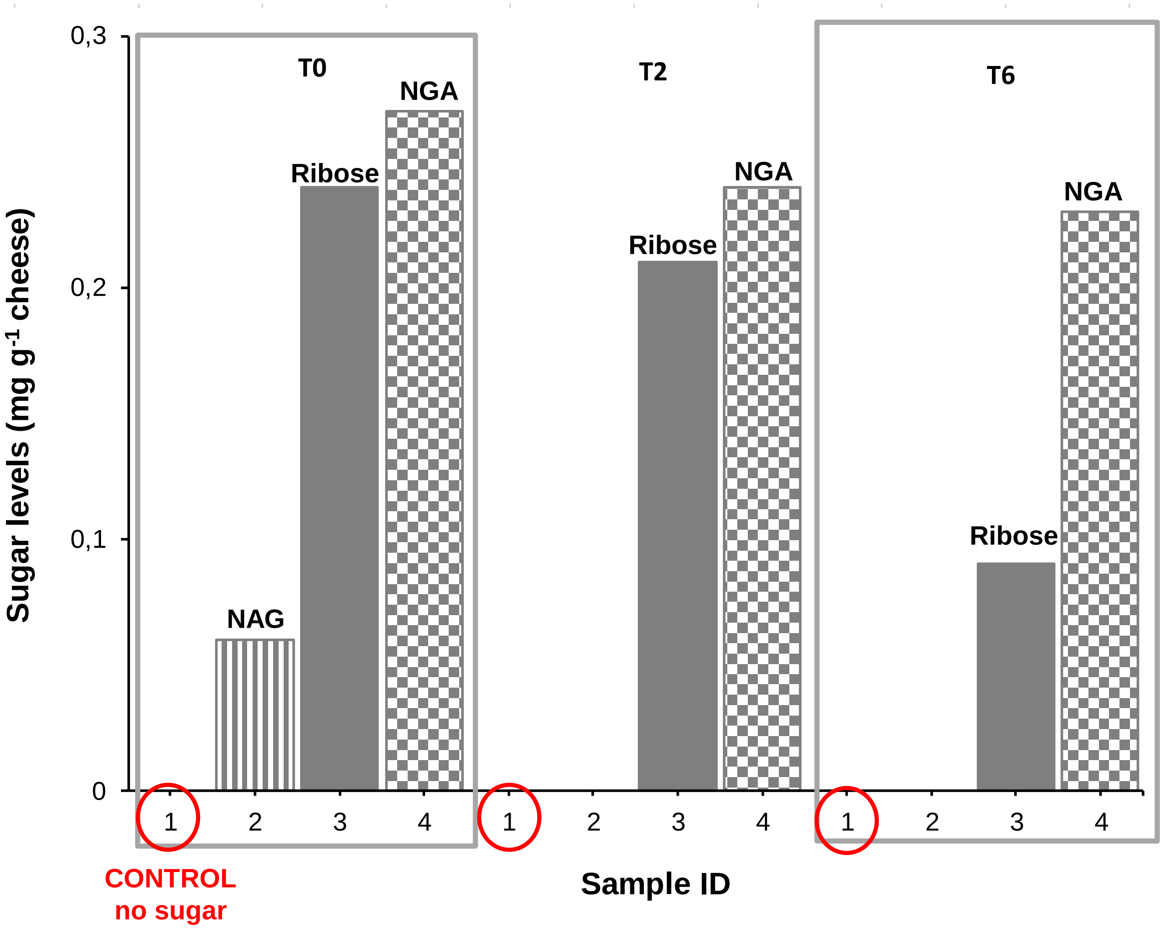
Step	Activity	Time (min)
I	adding half of the NaCl	0
II	2 x [Mixing (2 min) + resting (3 min)]	
III	adding the other half of the NaCl	10
IV	Mixing (2 min) + resting (3 min)	
V	adding or not the selected sugar (0.44 %) and/or casamino acids (0.57 %)	15
VI	3 x [Mixing (2 min) + resting (3 min)]	
VII	Transfer to Moulds	30

Each of the four cheeses (4.6-4.7Kg) → cut into ten blocks → vacuum packed

↓

stored at 10° C for six months or until the moment of analysis

Addition of sugars to boost NSLAB growth



Changes in levels (mg g^{-1}) of sugars, before (T0) and after two (T2) and six (T6) months of ripening, in Cheddar cheese with no addition of sugars (1) or with addition of sugars: N-Acetylglucosamine (NAG, columns with vertical lines), Ribose (solid columns) or N-Acetylgalactosamine (NGA, columns in chess). Vacuum packaged cheeses were ripened at 10° C until sampling.

Addition of sugars to boost NSLAB growth

Too high concentration of sugars?

No release of key enzymes or amino acids?

Absence of NSLAB?

sugars supported SLAB growth?

NSLAB species?

Lesson learned:
To add an adjunct culture

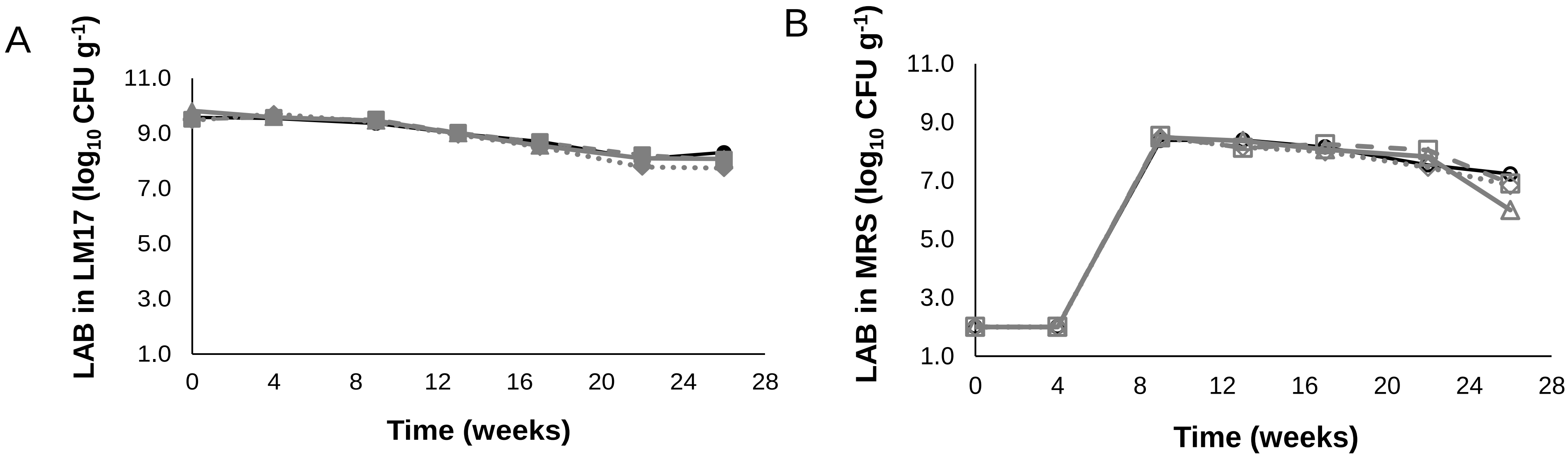


Figure 1 Changes in levels (log^{10} CFU g^{-1}) of lactic acid bacteria, under aerobic conditions on LM17 agar (closed symbols) and under anaerobic conditions on MRS (open symbols), in cheeses with no addition of sugars (in black, ○) or in cheeses made with addition of sugars (in grey): N-Acetylglucosamine (∆), Ribose (◇) or N-Acetylgalactosamine (□). Vacuum packaged cheeses without (A, B) or with (C, D) casamino acids were ripened at 10° C for six months.

Outline:

- Introduction to starter (SLAB) and non-starter (NSLAB) lactic acid bacteria in Cheddar cheese
- Mechanisms involving bacterial interactions in cheese ripening
- Addition of sugars to boost NSLAB growth
- **Reducing salt content for developing healthier products**
- **Increasing ripening temperature to accelerate cheese ripening**

Cheese trial (II) – testing effect of different salt content and temperature of ripening in Cheddar cheese matrices

Healthier product

The reduction of salt levels

To lower the risk of **cardiovascular diseases**, **stomach cancer**, **osteoporosis** and **renal stones**

Source: WHO, 2008; World Cancer Research Fund, 2007; Cappuccio et al., 2000

Increasing demand for

Cost reduction

The use of elevated temperature ripening

To accelerate ripening

Source: Møller, 2012

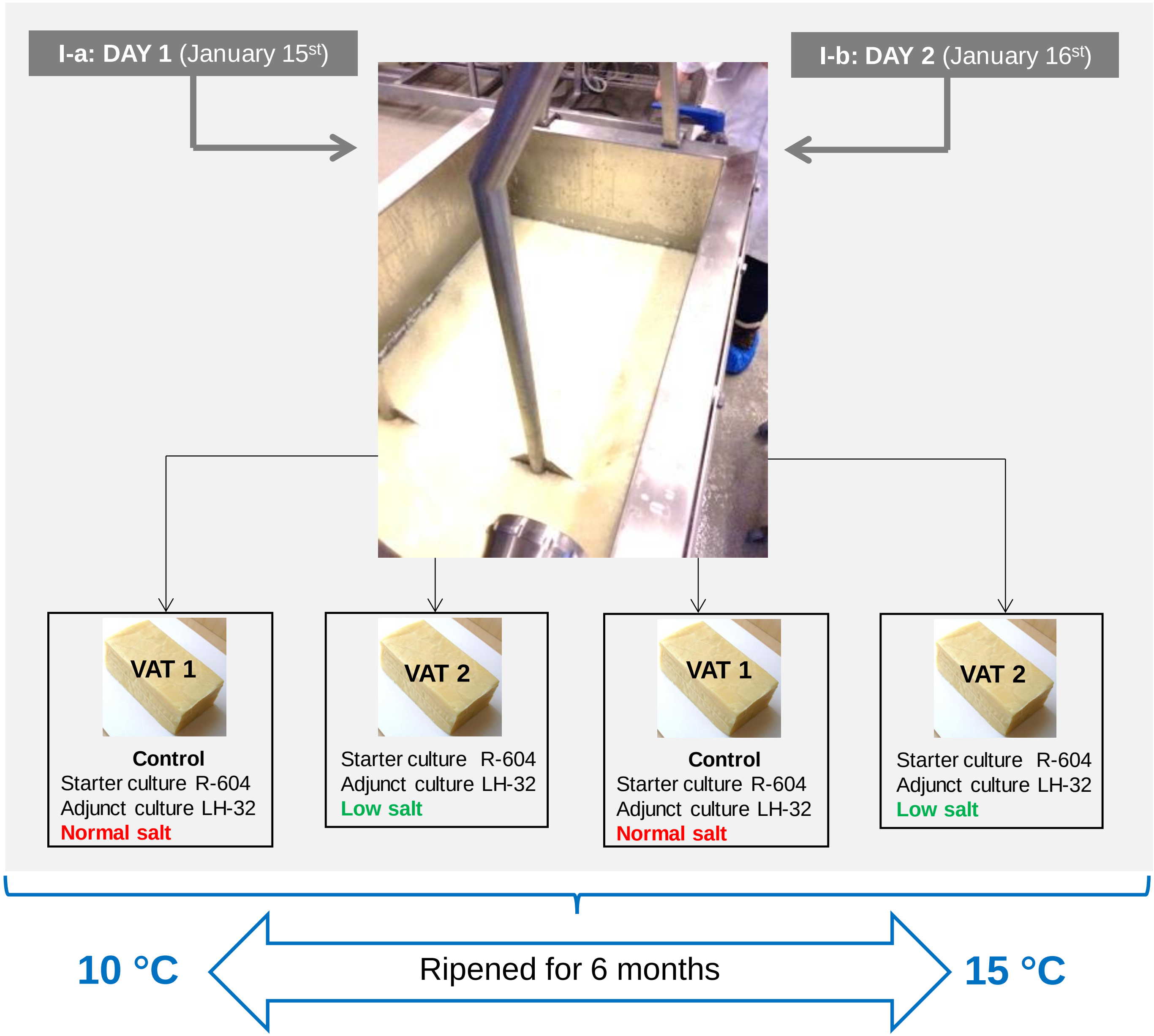
- Maintaining of high product quality
- Food safety assurance

May lead to cheese defects

Disturbance in the normal cheese microflora

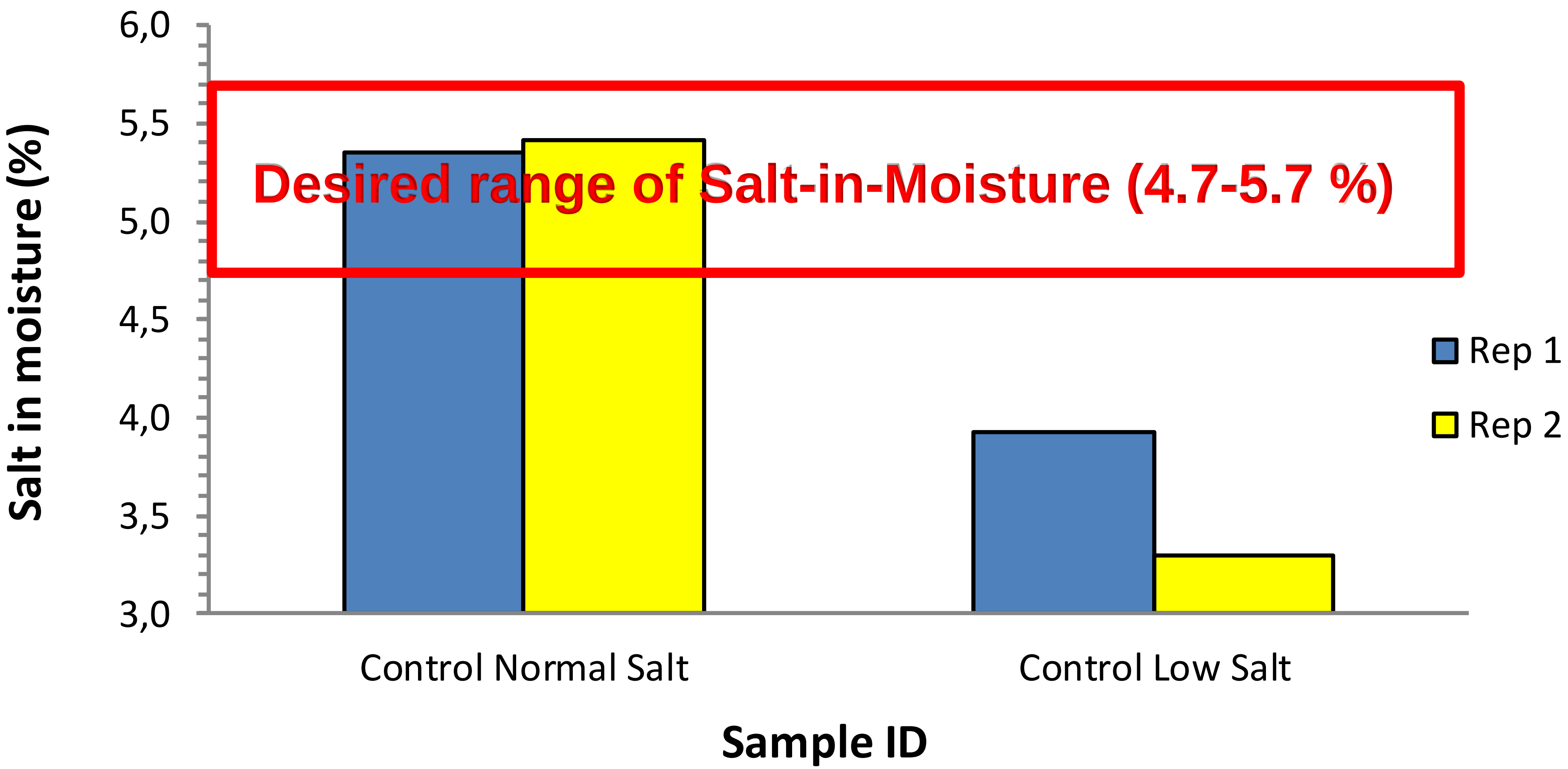
Evolution of an unfavourable detrimental microflora

Cheese trial (II) – effect of different salt content and temperature on ripening of Cheddar cheese matrices



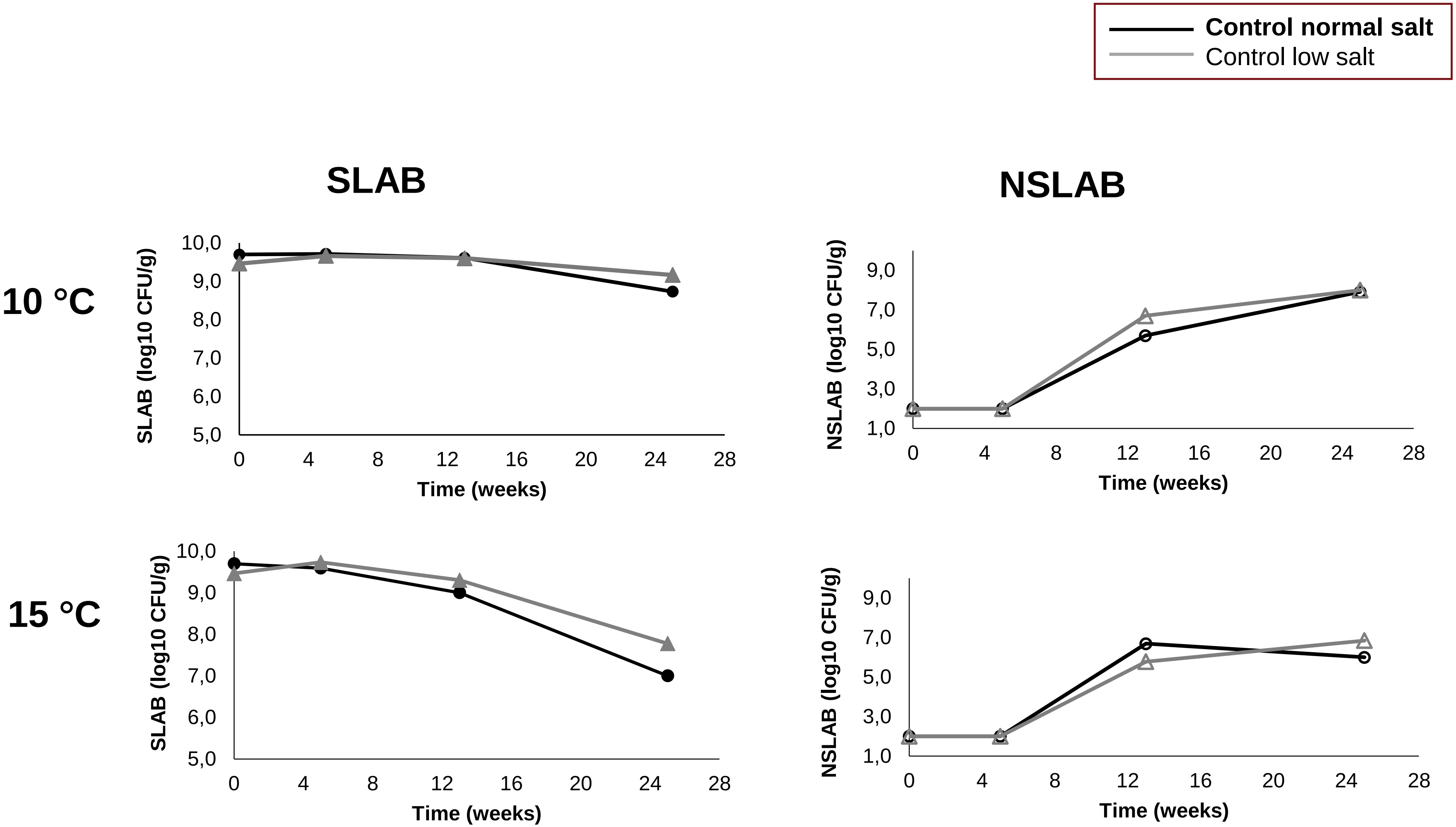
Each of the cheeses was divided into 12 blocks

Cheese trial (II) – effect of different salt content and temperature on ripening of Cheddar cheese matrices



Levels of Salt in Moisture of the four Cheddar cheeses manufactured in trial II, testing effect of different salt content, and analysed seven days after production.

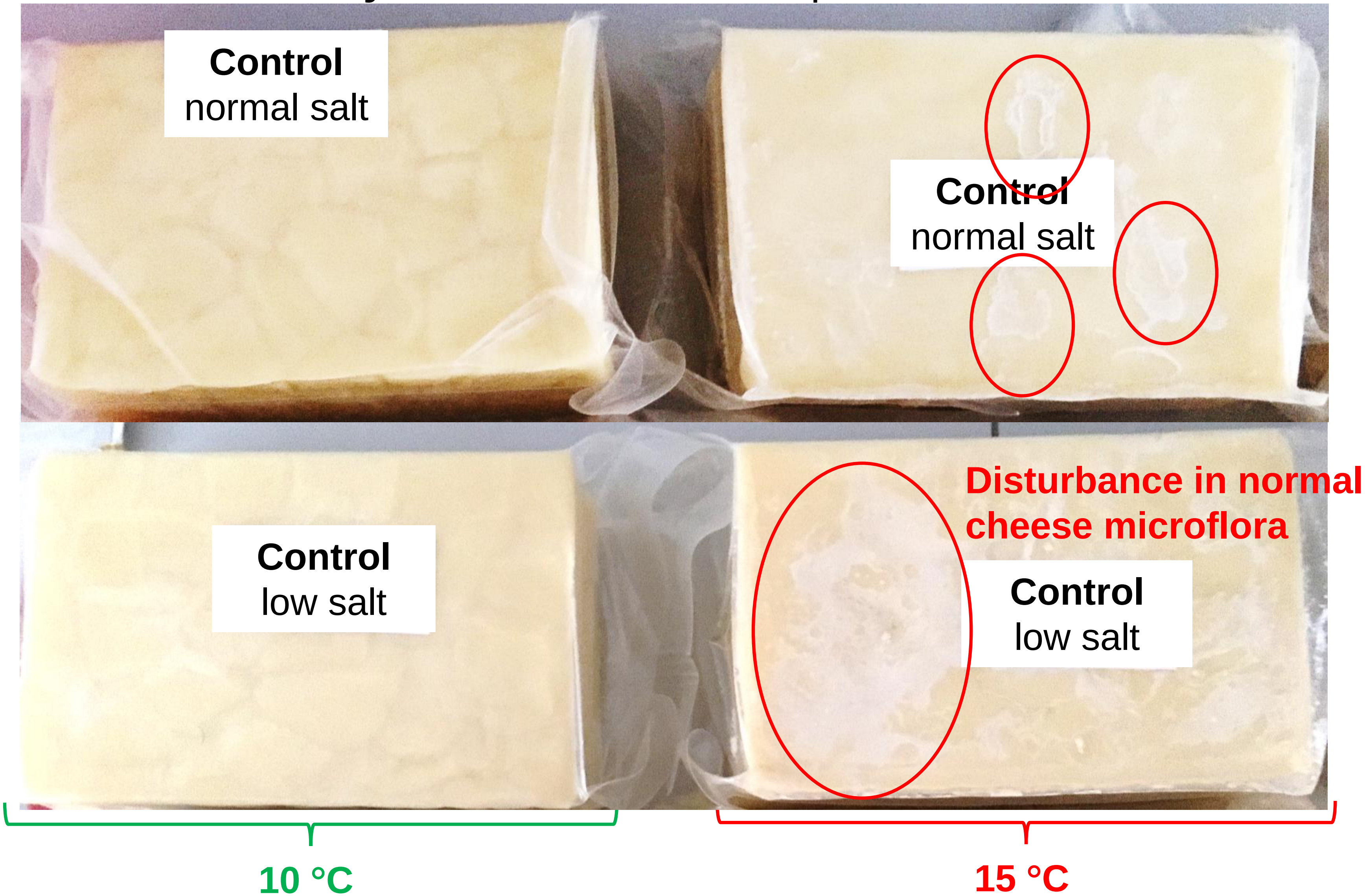
Cheese trial (II) – effect of different salt content and temperature on ripening of Cheddar cheese matrices



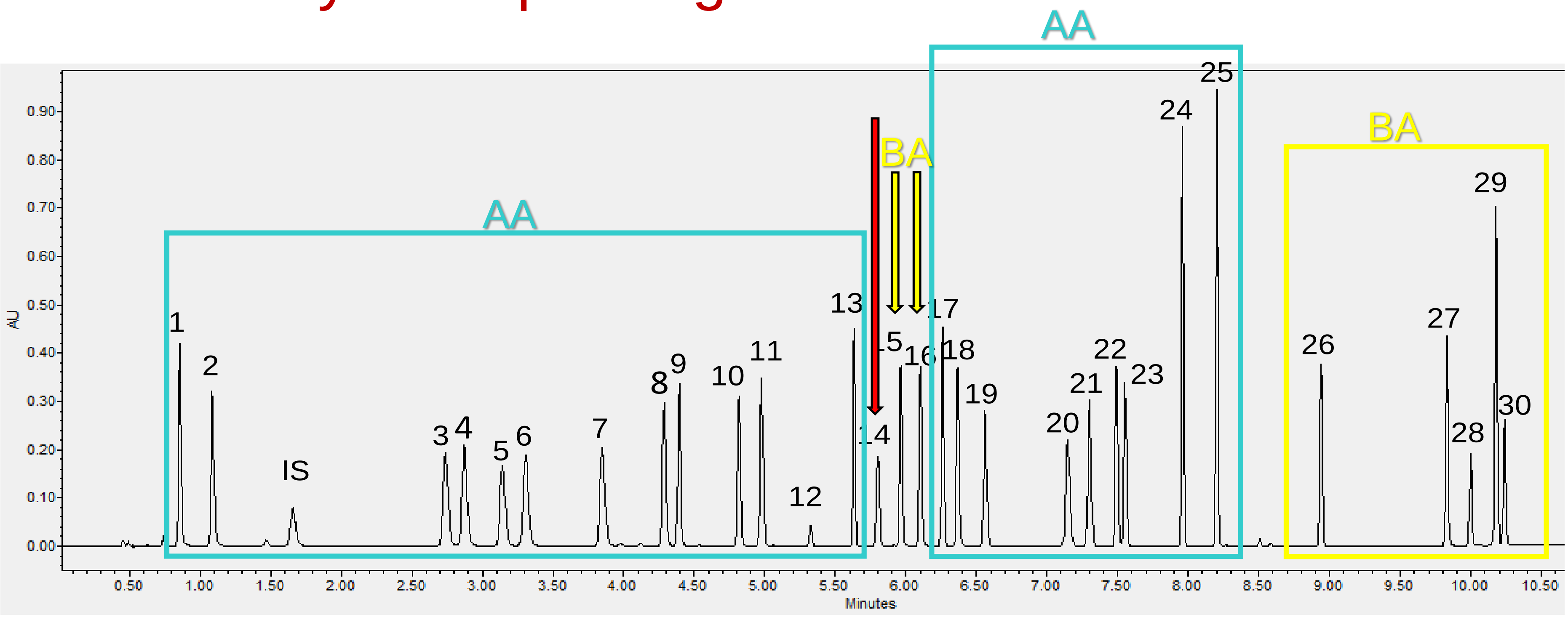
SLAB counts in M17 agar under aerobic conditions (to the left) and NSLAB counts in MRS agar pH 5.4 under anaerobic conditions (to the right) from Cheddar cheeses, ripened at 10 °C and 15 °C

Cheese trial (II) – effect of different salt content and temperature on ripening of Cheddar cheese matrices

Calcium lactate crystal formation in the produced Cheddar Cheeses



Amino acids (AA) and biogenic amines (BA) standards at University of Copenhagen



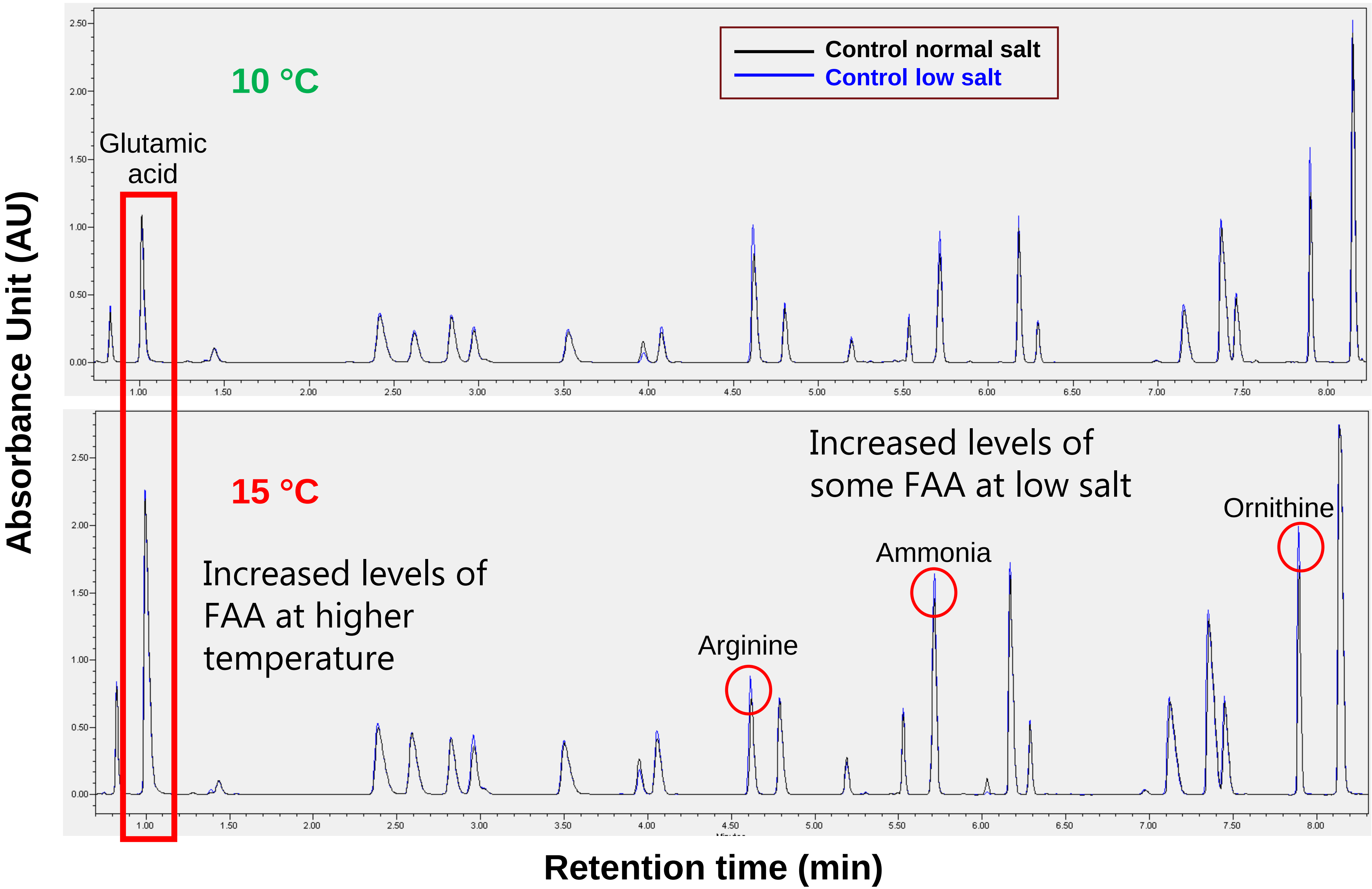
Mix 30 – 1.0 mM

1. Aspartic acid	11. GABA (from glutamic acid)	21. Isoleucine
2. Glutamic acid	12. Proline	22. Leucine
3. Asparagine	13. Tyrosine	23. Phenylalanine
4. Serine	14. Ammonium ion	24. Ornithine
5. Glutamine	15. Agmatine (from arginine)	25. Lysine
6. Histidine	16. Histamine (from histidine)	26. Tyramine (from tyrosine)
7. Glycine	17. Valine	27. Putrescine (from ornithine/arginine)
8. Alanine	18. Methionine	28. Tryptamine (from triptophane)
9. Threonine	19. Cysteine	29. Cadaverine (from lysine)
10. Arginine	20. Tryptophane	30. Phenylethylamine (from phenylalanine)

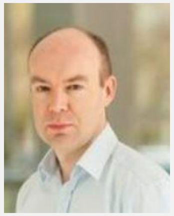
IS = Internal Standard (2 µl of 2 % L-amino adipic acid, made in 0.1 N HCl with 3,30-thiodipropionic acid-TDPA (0.2%, W/V)

Cheese trial (II) – effect of different salt content and temperature on ripening of Cheddar cheese matrices

Free Amino acids (FAA)



Acknowledgments



Fergal P. Rattray
Professor

Bjarke Bak Christensen
Institutdirektør

Tamás Czárán
Postdoc

Kim Sneppen
Professor

Food Microbiology
University of Copenhagen

DTU - Bioengineering

Niels Borh Institut - University of Copenhagen

Mejeriteknisk Selskab

UNIVERSITY OF COPENHAGEN

Arla
Closer to Nature™

InnovationsFonden
FORSKNING, TEKNOLOGI & VÆKST I DANMARK

Mejeribrugets ForskningsFond

Mange tak ☺