



Lysozyme side effects in Grana Padano PDO cheese: new perspective after 30 years using

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Background (I)

- Grana Padano (GP) PDO cheese is made with partly skimmed raw milk added with natural whey starter and calf rennet
- Curd is cooked up to 53-54°C
- Hence, mostly thermophilic SLAB (*L. helveticus*, *L. delbruekii sub lactis*) and NSLAB (*Lactobacillus casei* group, *Pediococcus acidilactici*) co-operate in cheese ripening
- Biochemical features of GP are well characterized as well

Background and scope

- Lysozyme (LZ) is in use to avoid the cheese blowing defect in Grana Padano as well as in other hard cheeses
- LZ is obtained from hen's egg white, hence it shall be declared on cheese label as a potential allergen
- We have studied how deeply Grana Padano cheese characteristics might change in case of discontinuing the use of LZ

Cheesemaking trials

* Raw bulk milk



- 4 dairies, 8 cheesemakings
- Twin vats: LZ+ / LZ-

Natural creaming to fat=2.2-2.3%

* - Natural whey starter

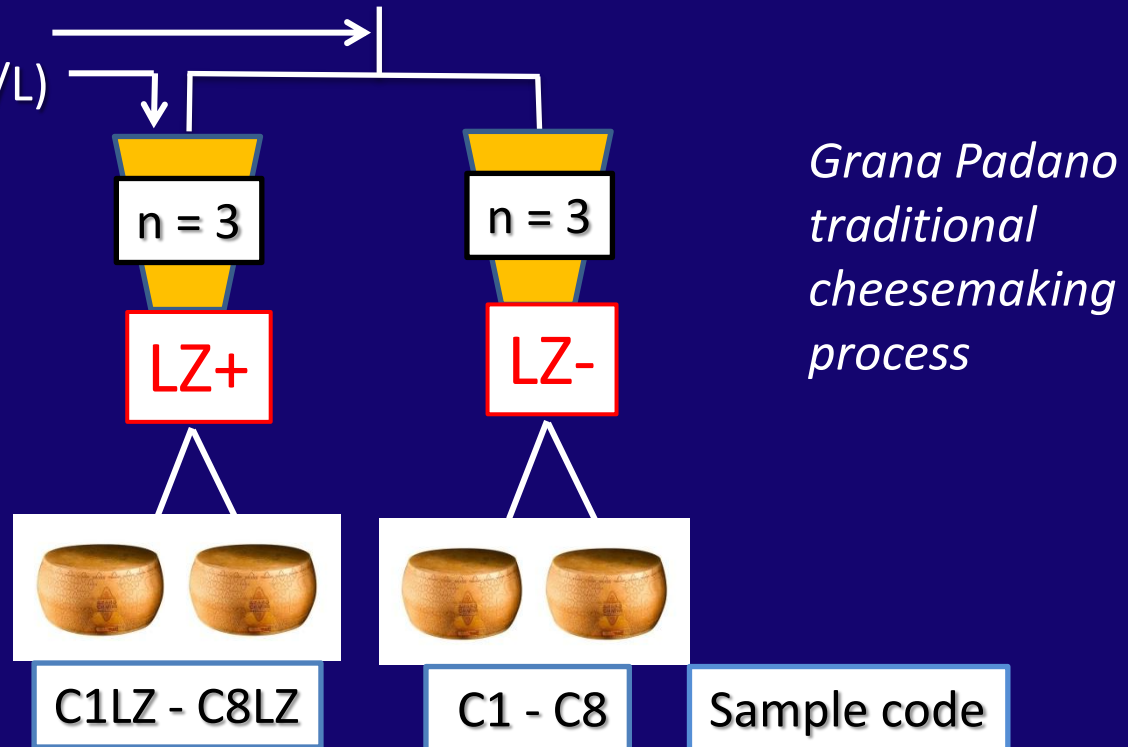
Lysozyme (2mg/L)

* In VAT

- Semiskimmed milk
- Whey

* CHEESE

- (acid curd)
- 9-month ripened
- 16-month ripened



* = sampling step

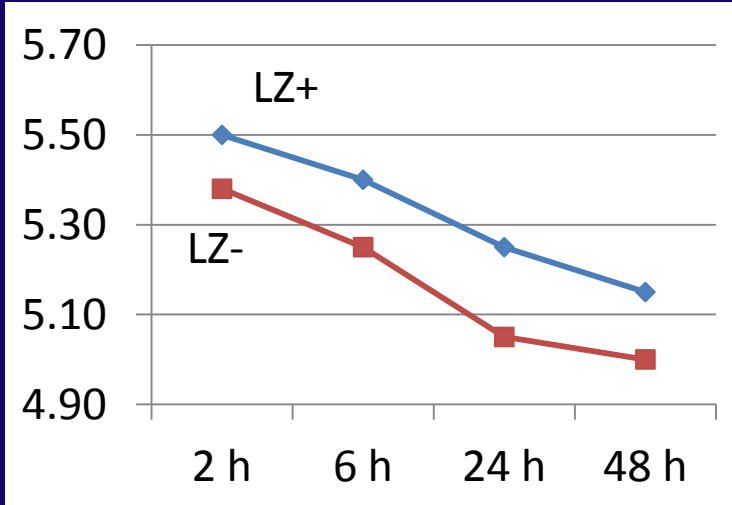
LZ= Lysozyme

9-month ripened cheeses

LZ+

LZ-

C1



Acidification of cheeses in mould is faster and more intense in the absence of LZ

1. The microbiological study

Methods: Microbiological parameters

- Plate count on **MRS** (Log of UFC/g cheese)
- Total amount of bacterial DNA
 - LH-PCR: total peak area
 - extracted from whole cells (**DNA tot w**)
 - extracted from lysed cells (**DNA tot l**)
- Amount of DNA of recognized LAB species
 - LH-PCR profiling associated to the sequencing of 16S rRNA genes

Whole cells

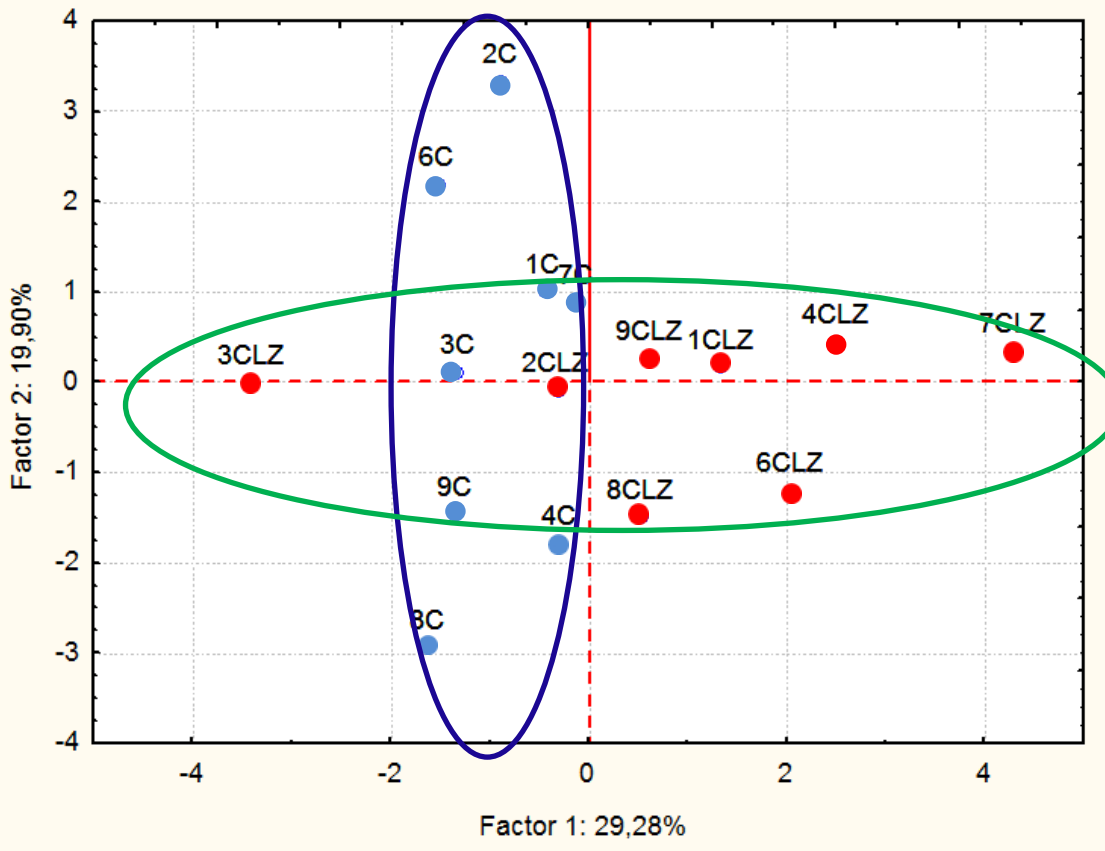
- *Lactobacillus helveticus* (**Lhw**)
- *Lactobacillus delbrueckii* (**Ldw**)
- *Lactobacillus rhamnosus* (**Lrw**)
- *Lactobacillus fermentum* (**Lfw**)
- *Pediococcus acidilactici* (**Paw**)

Lysed cells

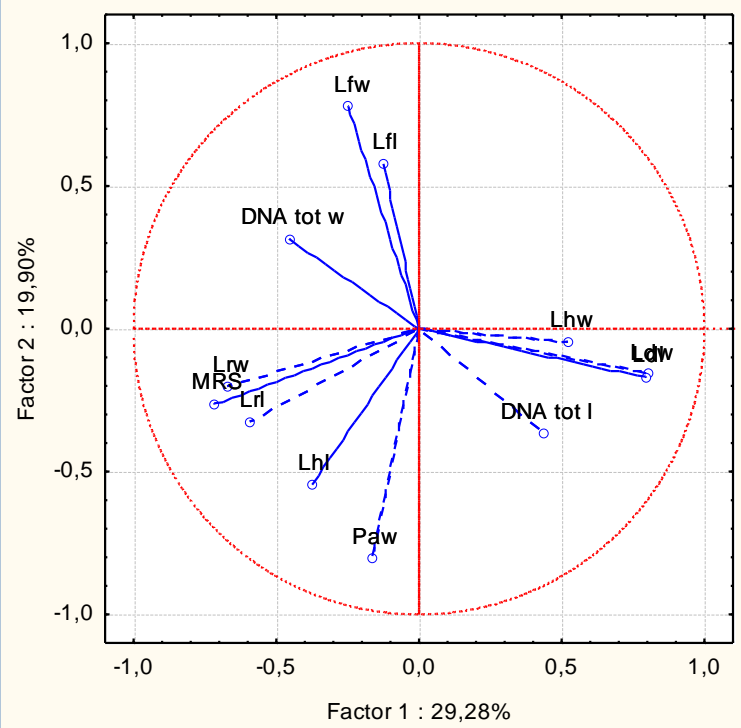
- *Lactobacillus helveticus* (**Lhl**)
- *Lactobacillus delbrueckii* (**Ldl**)
- *Lactobacillus rhamnosus* (**Lrl**)
- *Lactobacillus fermentum* (**Lfl**)
- *Pediococcus acidilactici* (**Pal**)

PCA for microbiological parameters in 9-month ripened cheeses

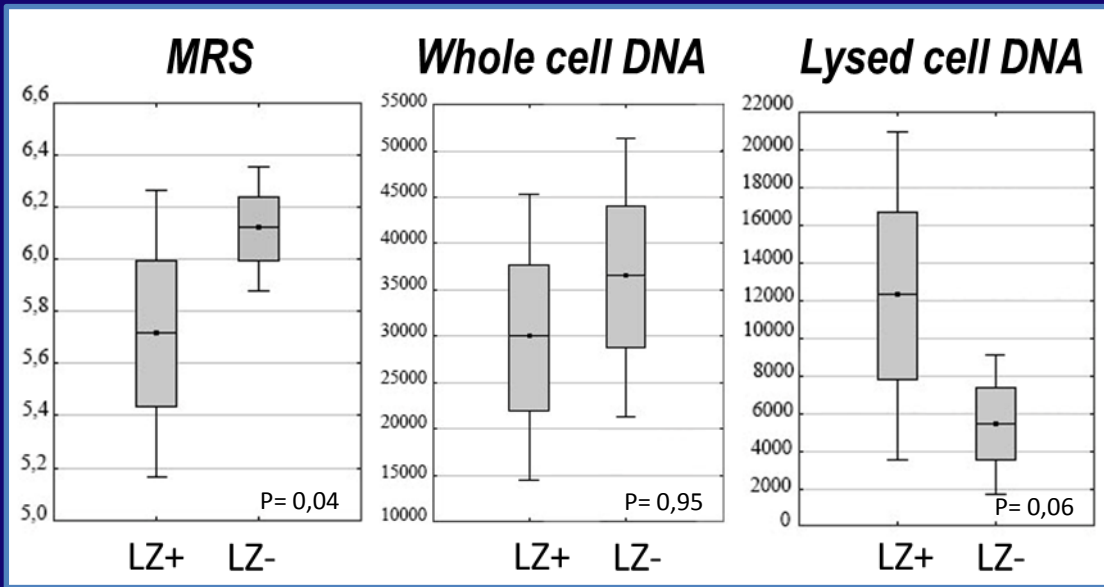
Projection of the cases on the factor-plane (1 x 2)
Cases with sum of cosine square $\geq 0,00$



Projection of the variables on the factor-plane (1 x 2)



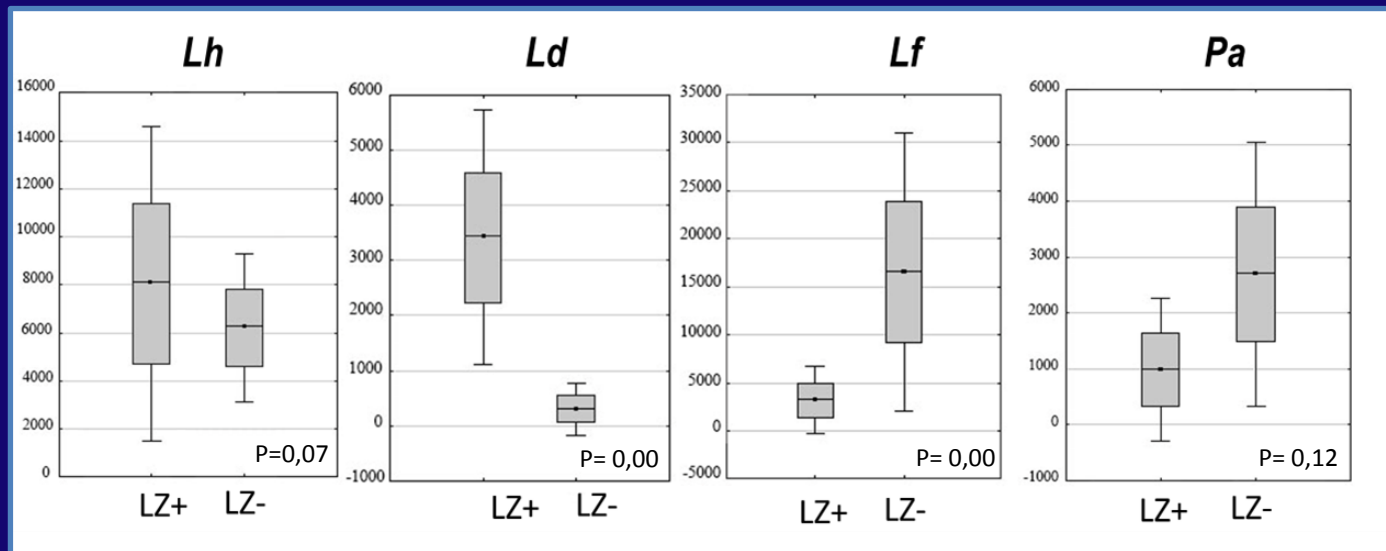
Differences between LZ+ and LZ- cheeses



In the absence of LZ:

LAB total count (MRS) and amount of DNA of whole cells are higher

The amount of DNA of lysed cells is lower



In particular:

SLAB (Lh, Ld) decrease

NSLAB (Lf, Pa) increase

2. The biochemical study

Methods: Biochemical parameters

- Primary proteolysis : $\Sigma\gamma\text{CN}/\Sigma\beta\text{CN}$

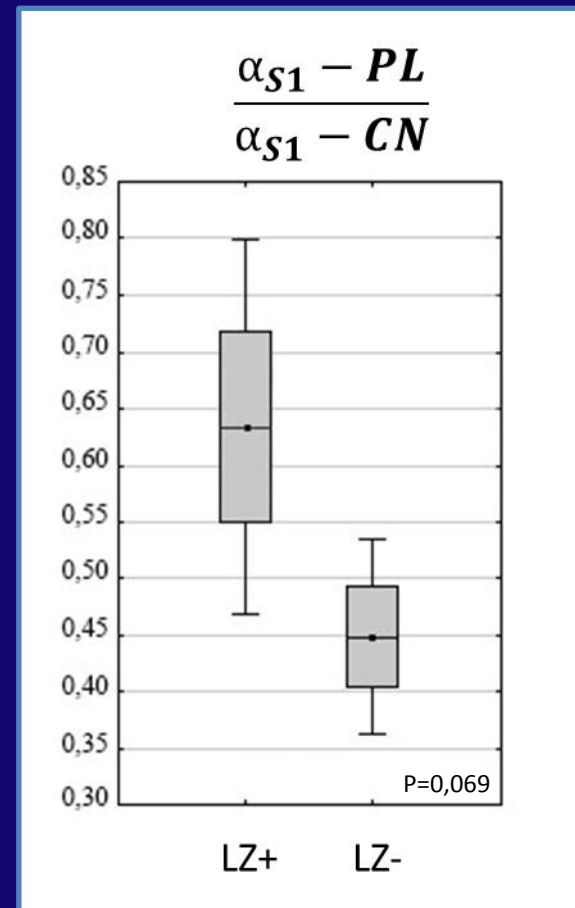
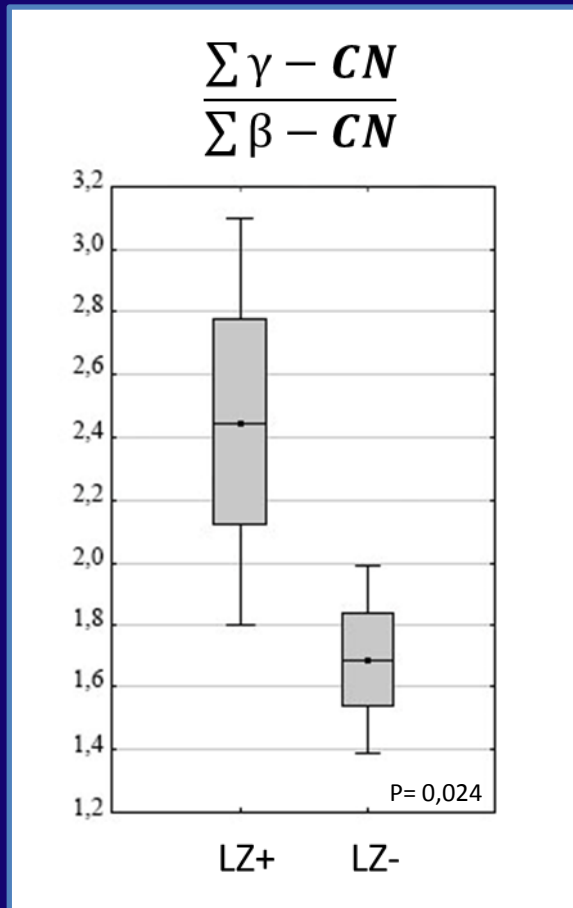
$$\alpha\text{s1CN (f 88-199)}/\alpha\text{s1CN}$$

as peak area ratios, determined by Capillary Zone Electrophoresis

- Secondary proteolysis : **Free Amino Acids**

as mMoles/Kg cheese protein, determined by AA analyzer

Primary proteolysis



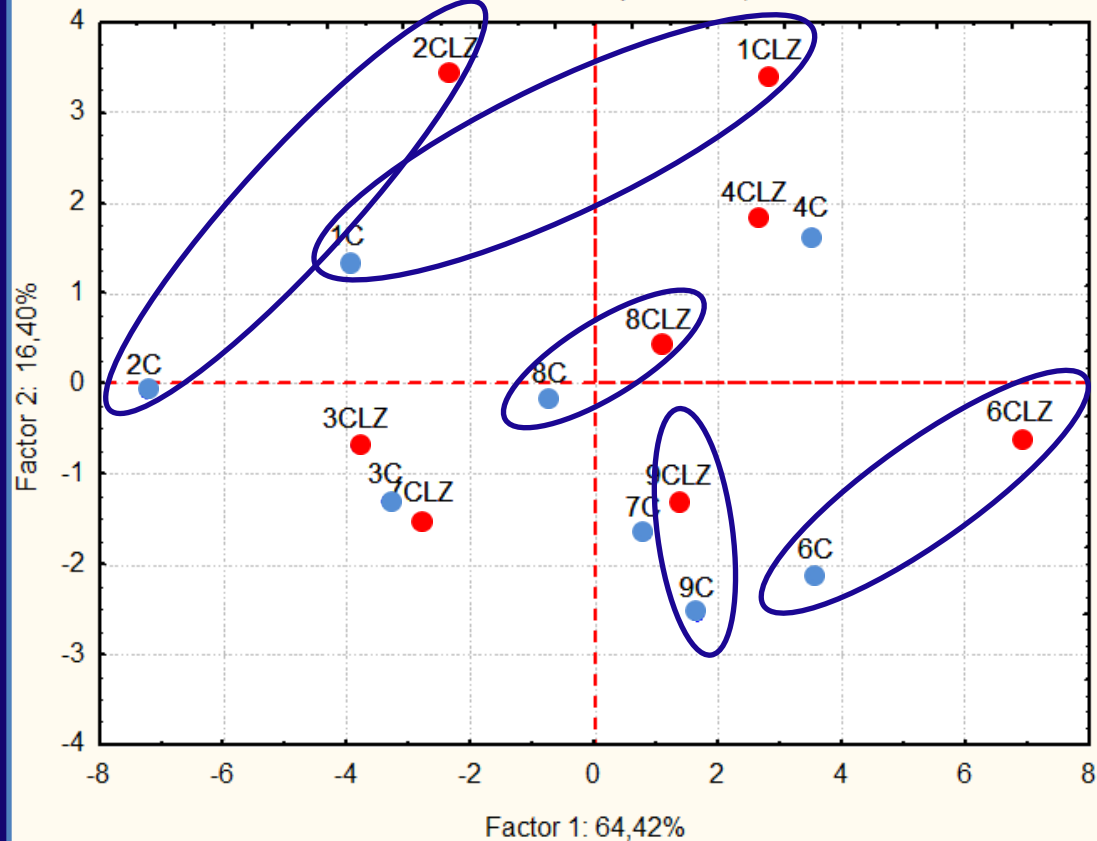
- In the absence of LZ, proteolysis of both β -CN and α S1-CN is less intense

Secondary proteolysis

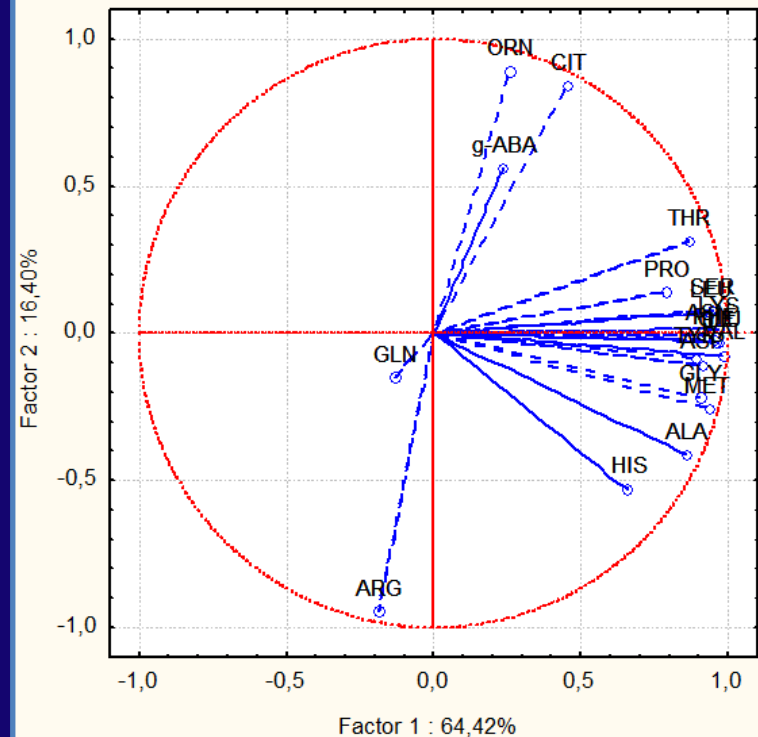
PCA for FAA in 9-month ripened cheeses

Projection of the cases on the factor-plane (1 x 2)

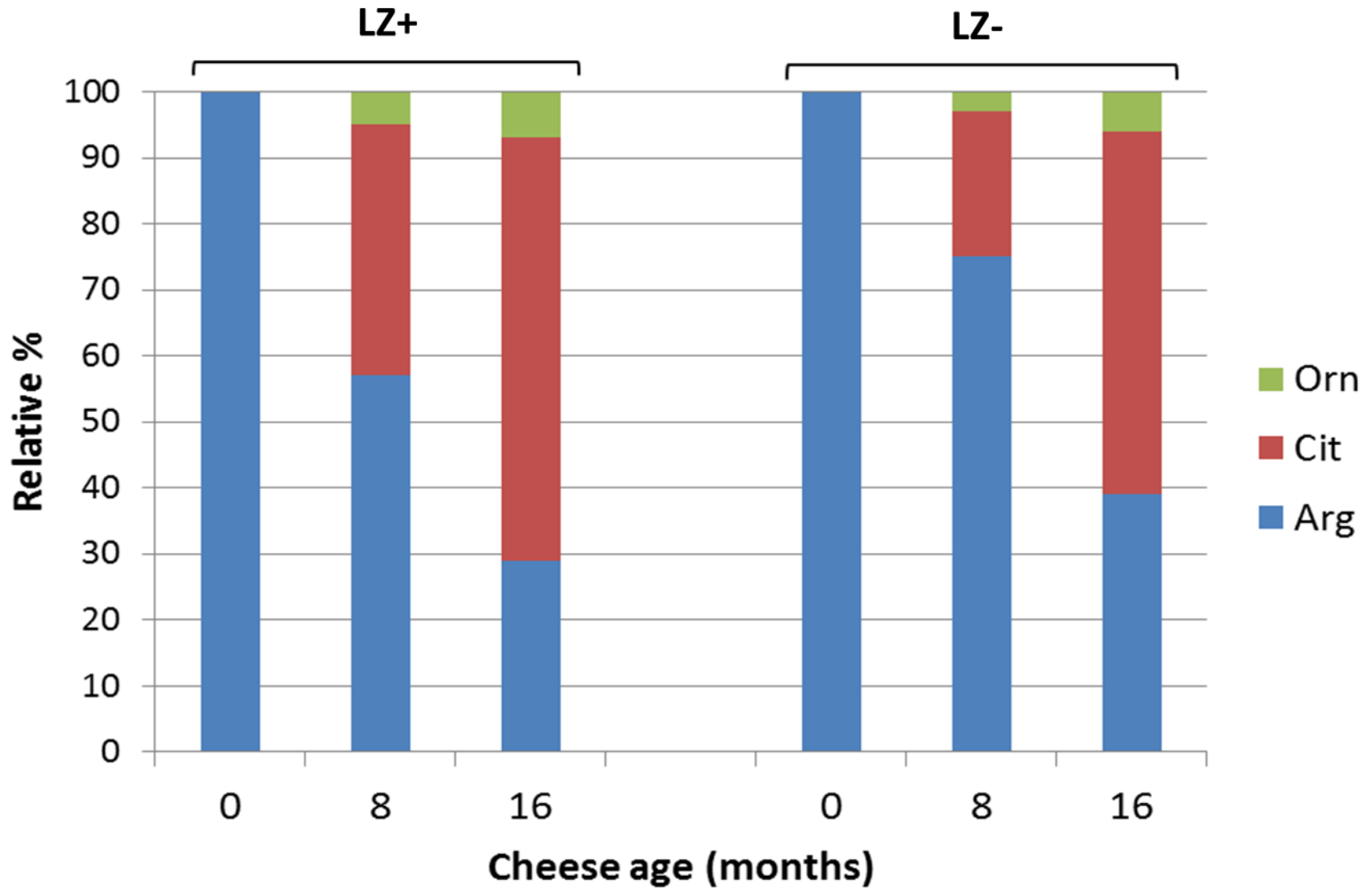
Cases with sum of cosine square $\geq 0,00$



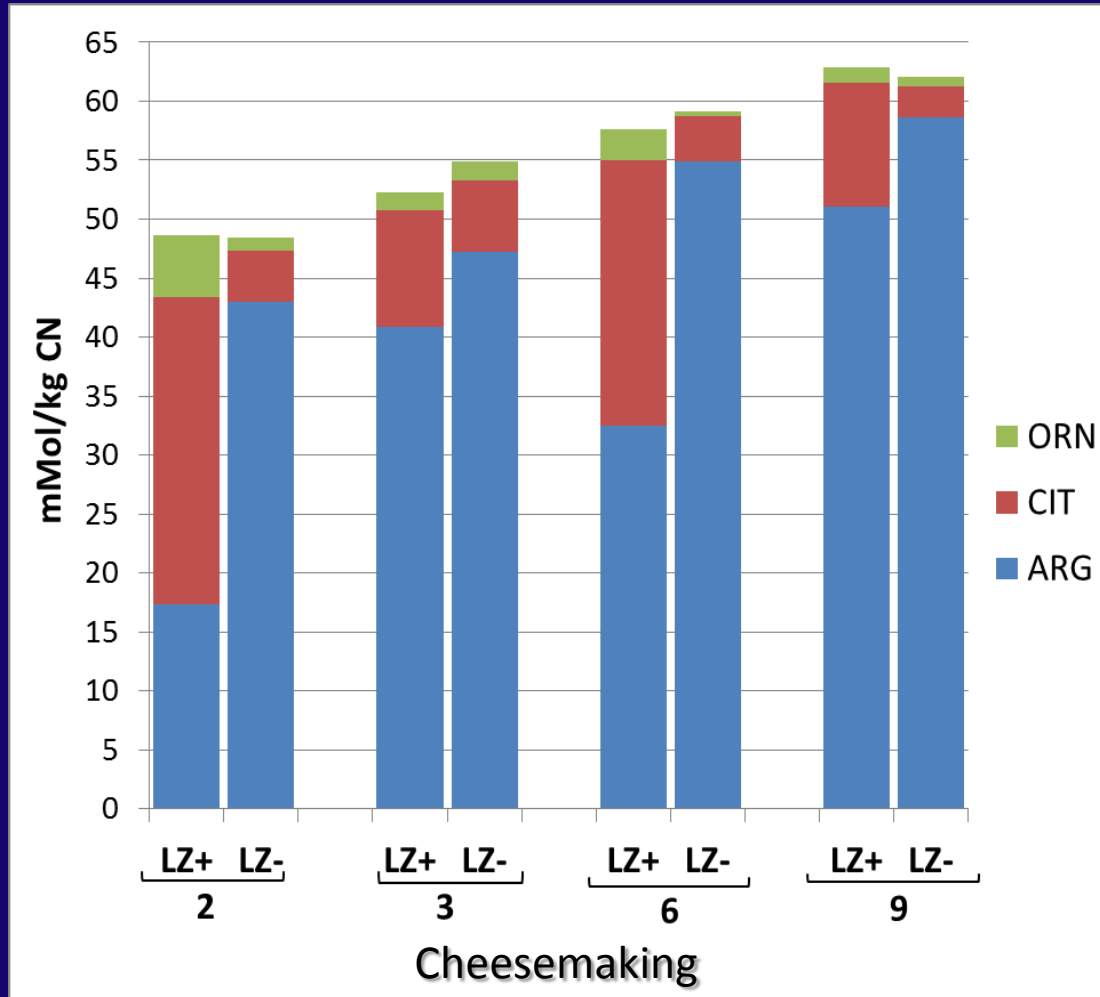
Projection of the variables on the factor-plane (1 x 2)



ARG metabolism during cheese ripening



ARG metabolism in different cheeses

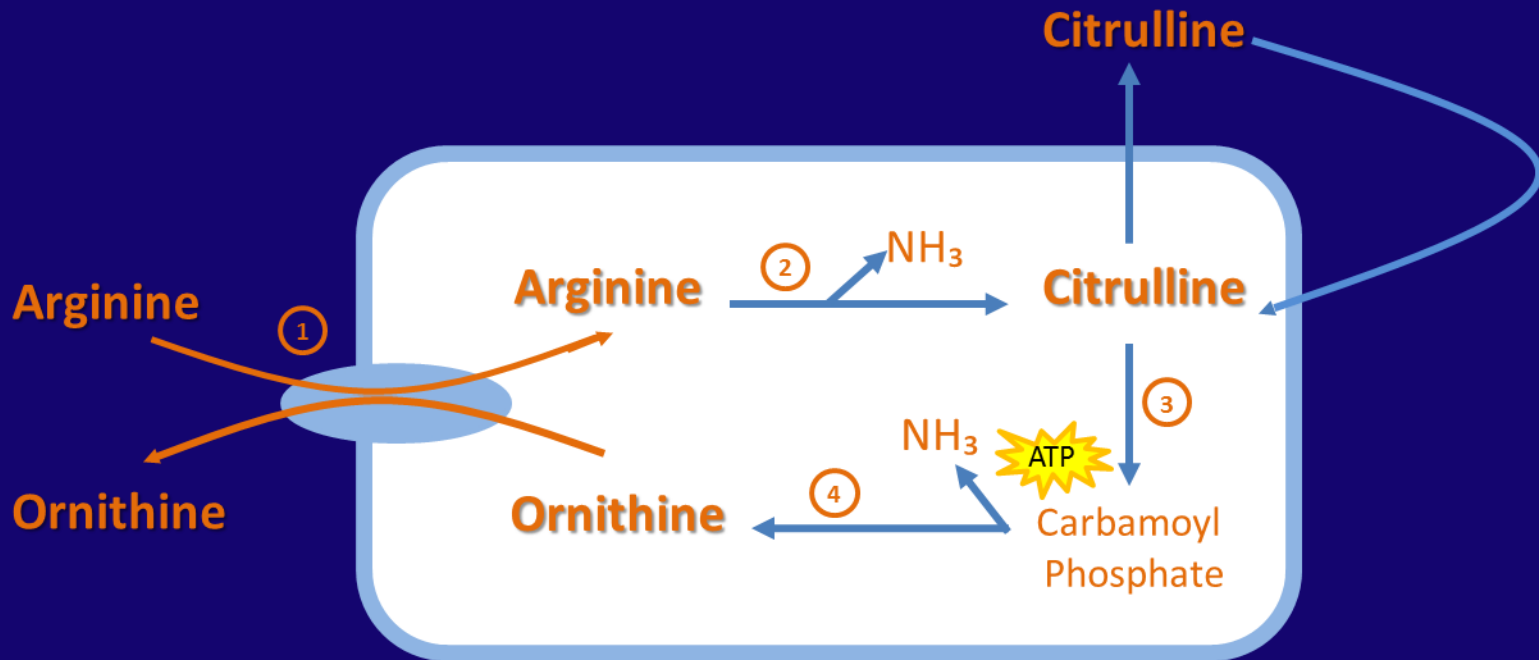


The **total amount of ARG** split from casein (i.e. free ARG+CIT+ORN) is characteristic of the dairy but is not LZ-dependent

In the absence of LZ: lower amounts of CIT and ORN

3. Combining microbiological and biochemical results

Microbial metabolism of ARG



1: arginine/ornithine antiporter

2: arginine deiminase (ADI)

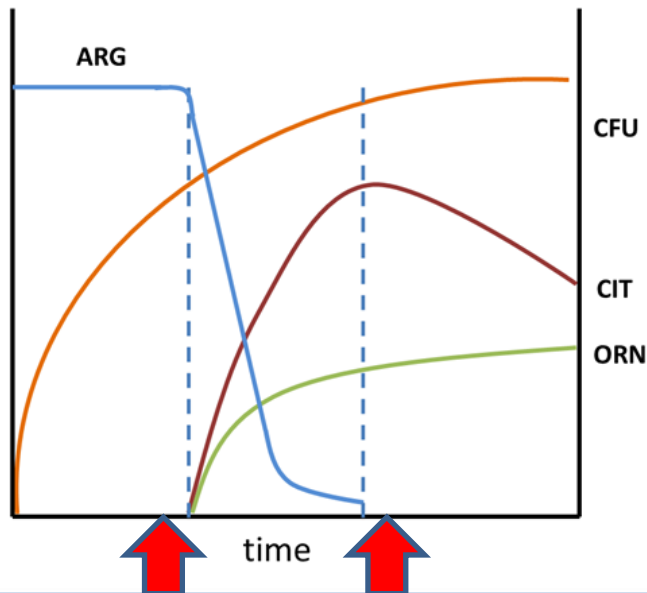
3: ornithine transcarbamoylase

4: carbamate kinase

Adapted from Vrancken et al. 2009

- The **ADI pathway** is widespread in Lactic Acid Bacteria
- It is a temperature, pH, osmotic stress-response and an energy production way
- Strain specific

Overall scheme of ADI pathway



According to literature:

- Uptake of ARG begins at a critical cell density, CIT and ORN are concomitantly released
- ARG conversion rate and final CIT/ORN ratio (arrows) depend on the level of cell stress conditions
- Narrow ranges of temperature and pH values are stressing to cells

In GP cheese:

- ARG is made progressively available by proteolysis (no LZ-dependent)
- Cell stressing conditions (temperature, pH) occur in early ripening, when growth of SLAB prevails
- NSLAB growth takes place when stressing conditions are over but sources of energy become scarce



- The ADI pathway is adopted throughout ripening by different species, with different purposes and to a different extent
- LZ itself does not represent a cell stress factor, it probably indirectly affects stressing conditions or interferes with ADI pathway expression

Conclusions

- The LZ+ and LZ- cheeses show minor systematic differences
- These interesting differences can be interpreted only by combining microbiological and biochemical evidences
- Cheeses made without LZ fall into the overall variability which is typical of cheeses made with raw milk, undefined starter and traditional technology
- The sensory properties were not distinguished from those of the GP cheese made with LZ

Acknowledgments

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Thank you for your attention

