The Role of Cultures & Coagulants in Cheese-making

SDT – Summer Syposium – June 2023

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Chr. Hansen (UK) Ltd.

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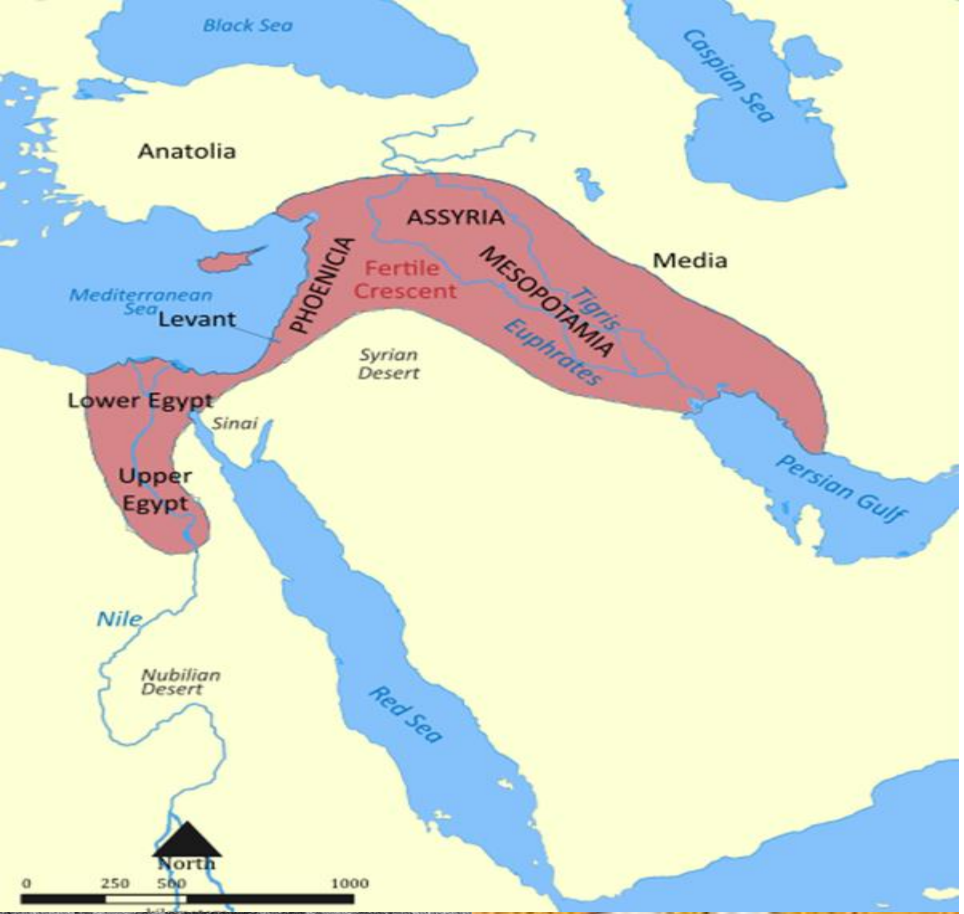
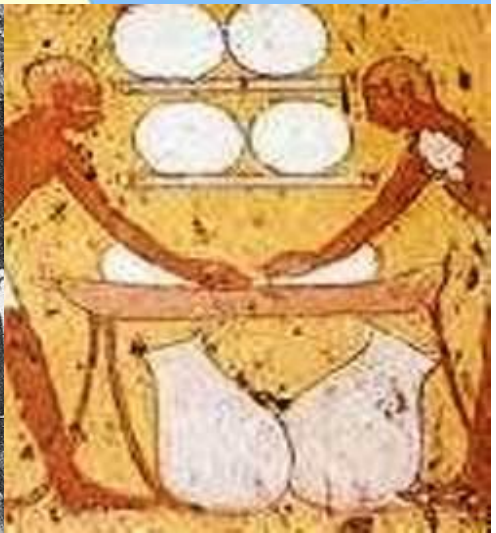
Overview

- Introduction & History
- Snapshot of the Cheddar Market
- Coagulants
- Starter & Adjunct Cultures
- NSLAB - Non-Starter Lactic Acid Bacteria





Origins & History of Cheese Making

- Cheese making is thought to have originated in the 'Fertile Crescent' centered around the Tigris and Euphrates rivers
- The 'Agricultural Revolution' which was the start of domestication of plants and animals (farming and human consumption of animal milks)
- Collection of animal milk + natural environment would have encouraged the acid-coagulation of milk, which on agitation, would have split into curds and whey
- Egyptian Cheese was being made 6000-7000 years ago
- Romans – making and trading cheese - La Luna Brand – 300AD
- Cheddar was recorded as a happy accident in 1100
- First commercial cheese factory was recorded in Rome NY in 1851 and in the UK in Longford, Derbyshire in 1870
- Chr. Hansen was established as an ingredient supplier to the cheese industry in 1874

Briefly about cheddar cheese

DEFINITION AND TRAITS

Cheddar cheese is the most popular type of cheese in the English-speaking world. Cheddar is described as a natural cheese that is relatively hard, off-white (or orange if colorings such as annatto are added) and sometimes sharp tasting. There are a variety of Cheddar types, each one boasting a different texture and flavour profile suitable for applications ranging from commodity ingredient cheese to a premium table cheese.

CURD/FRESH

Young cheddar-type cheese matures for less than 2 months. It is off-white; however, it has a mild, buttery, creamy, grassy and sweet flavor. Varieties include Colby and Monterey Jack and are ideal for ingredient and processed cheese applications,

MILD/MEDIUM

Semi-mature cheddar ripens for 3 to 6 months. This has a stronger more developed flavor than mild cheddar, with a springier and more coherent texture than aged Cheddar. It is suitable for grating, slicing and chunks.

MATURE

Mature cheddar typically ripens for 6 to 12 months. Flavor profile is saltier with an intense flavor which lingers on the palette on the finish. This variety has a drier, crumblier texture Varieties include British Territorials and are suitable as table cheese

VINTAGE

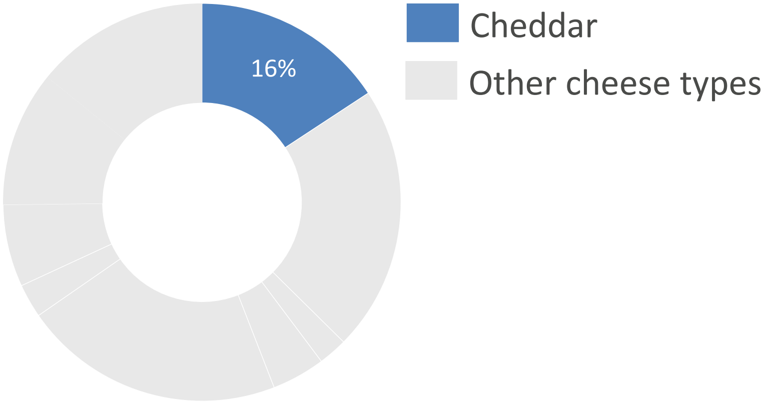
Vintage cheddars are the strongest of the cheddar varieties and are matured for 12 to 24 months. They are very crumbly, bitey and tangy in flavor. They may also have crystals, which develop during the maturation process to give a slightly crunchy texture.

A quick look at the Global, UK and Irish markets

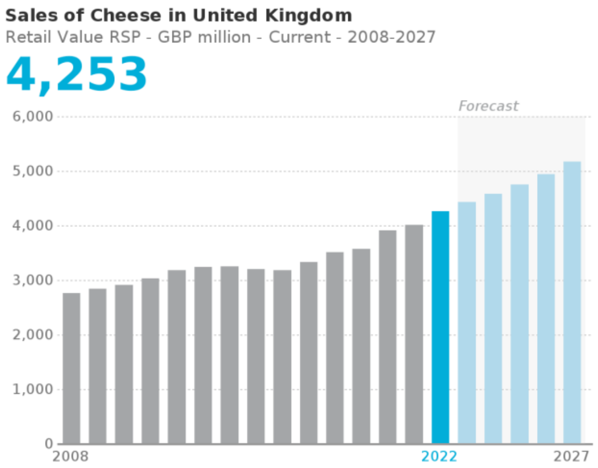
MARKET FACTS

- Cheddar-types include Monterey Jack, Colby, British Territorials and American cheddar. The types vary from each other in terms of flavor contribution and functionality.
- Global production volume: 4.9 M tons annually.
- 2-3% expected annual growth until 2025.
- US manufacturers produce more than 65% of the global production volume.

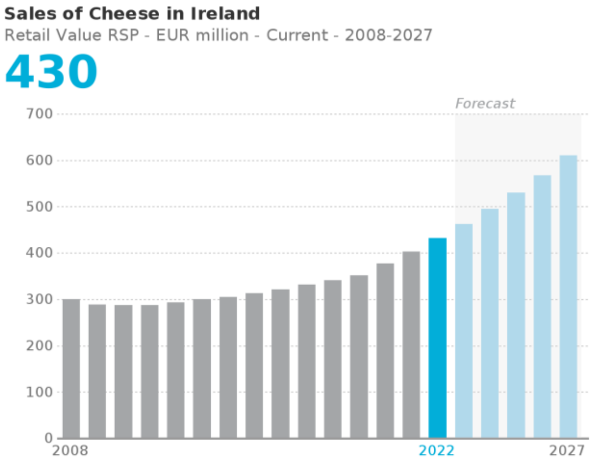
VOLUME SHARE OF GLOBAL CHEESE PRODUCTION



UNITED KINGDOM



IRELAND



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Chr. Hansen mapping, 2021

1 Euromonitor Country Reports 2022

Cheese may be considered as concentrated milk

Milk → Cheese manufacturing process → Cheese

↓

Whey

- Milk quality is key to making high quality cheese – parameters such as salt, moisture and recipe determine the success of the final outcome
- High quality ingredients are also essential to deliver the required flavour and texture and will drive the direction a cheese will take
- Maturation is the process of revealing the initial inputs

	Milk	Cheddar
	%	%
Fat	3.5	34
Protein	3.2	25
Lactose	4.6	0
Ash	0.7	2
Water	88	37
Salt	0	2

•Fat and protein is conc. approx 10 times
•Lactose, whey protein and 95 % of water go to whey

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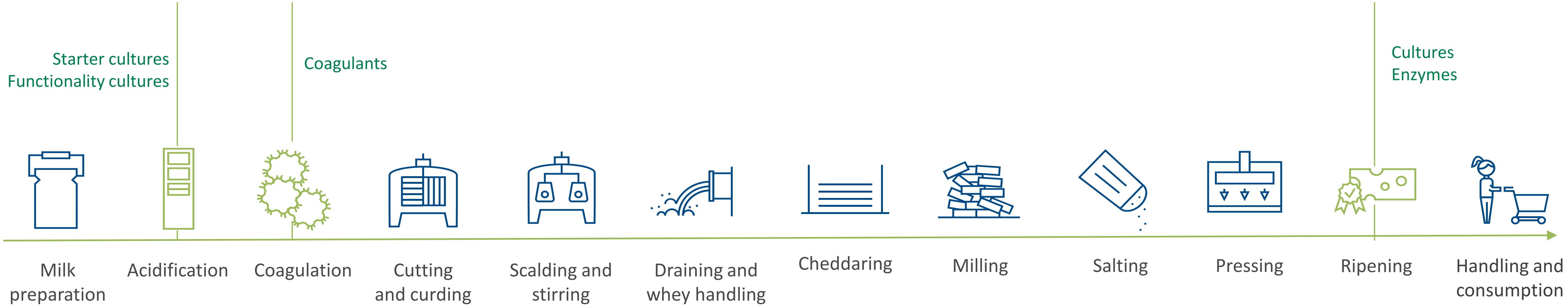
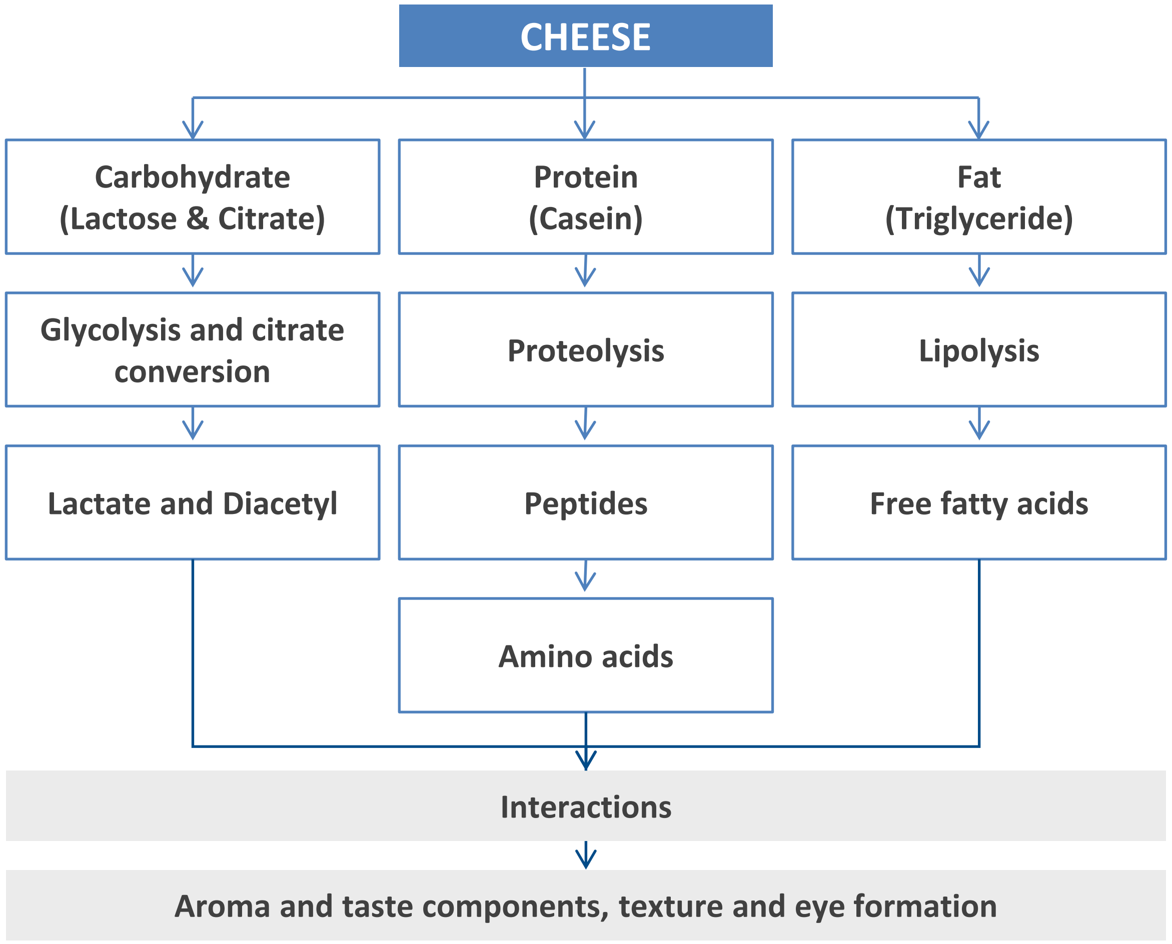
Culture and Coagulants in Cheddar Cheese making



The combination of processing parameters and choice of coagulant, starter and ripening cultures is crucial for the desired characteristics and quality of the cheese product

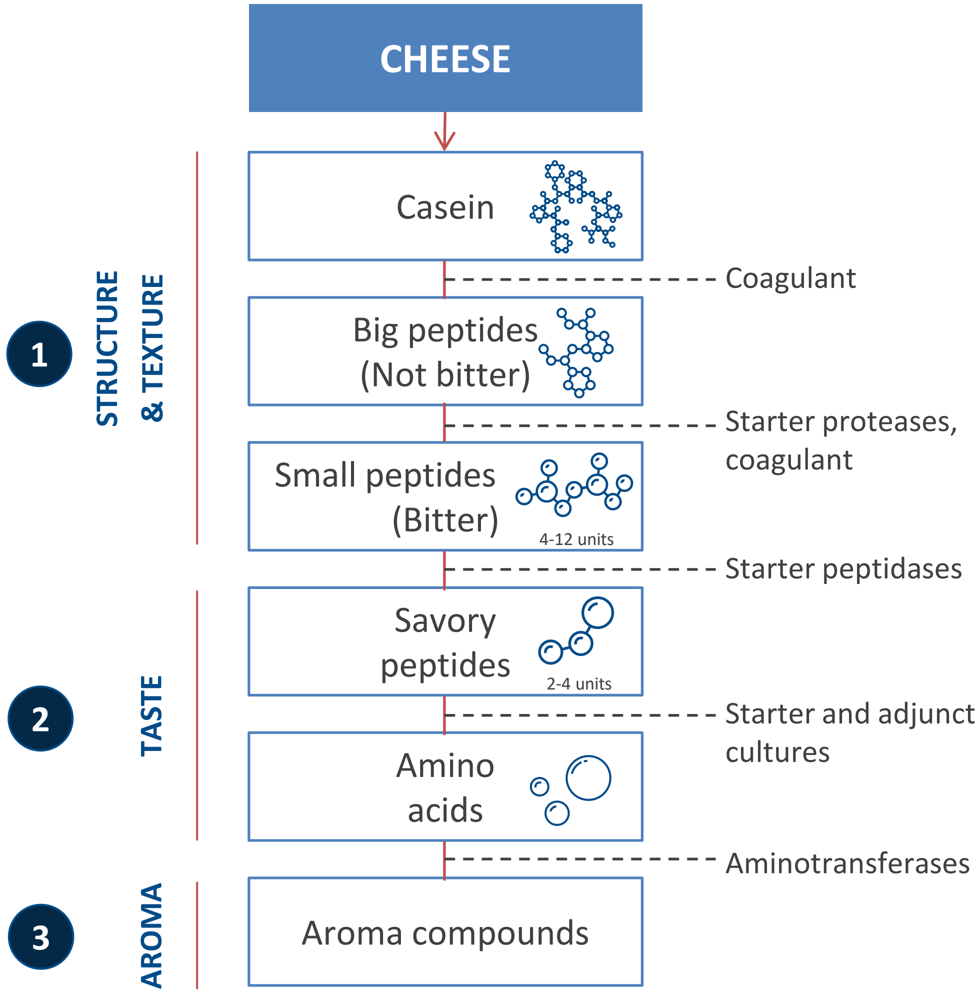
Understanding the primary biochemical pathways of cheese ripening

These processes influence the flavor, surface, appearances and ripening speed



- Starter cultures, coagulants, ripening cultures and lipase enzymes affect pathways that influence the ripening of cheese
- The conversion of lactose, citrate, casein and fat contributes to different outcomes for flavor directions, textures, and the formation of eyes
- **Controlling these pathways** will influence the ripening process and ripening speed across all cheese types

The proteolytic pathway: a summary



1. PRIMARY PROTEOLYSIS: STRUCTURE AND CONSISTENCY(LOW ACTIVITY)

- Indigeneous milk proteases - plasmin
- Proteases from psychrotrophic bacteria
- Coagulant
    - Dosage + C/P-ratio = proteolytic impact
- Starter culture protease

2. SECONDARY PROTEOLYSIS: TASTE (HIGH ACTIVITY)

- Starter, adjunct, NSLAB
    - High Peptidase
- Inoculation rate
- High lysis

3. TERTIARY PROTEOLYSIS: AROMA (BALANCED)

- High amino aminotransferase

Coagulants

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Specificity (C/P-ratio) of coagulants matters as it disclose the performance

THE C/P RATIO[1]	CASEIN SUBSTRATE	EFFECT	IMPACT
C Clotting activity[1]	K	• The higher clotting activity the more precise cutting of Kappa Casein and ability to form strong casein network	• Use of a coagulant with a high C/P ratio results in a stronger casein network and fat and proteins are kept in the cheese
P General proteolytic activity	α, β	• The proteolytic activity impacts the speed of breakdown of casein in bigger and smaller peptides (fast speed gives bitter taste)	• Cheese yield is directly linked to the C/P ratio of the coagulants

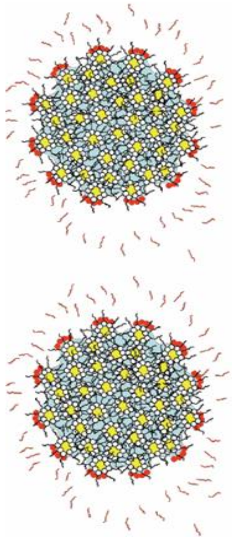
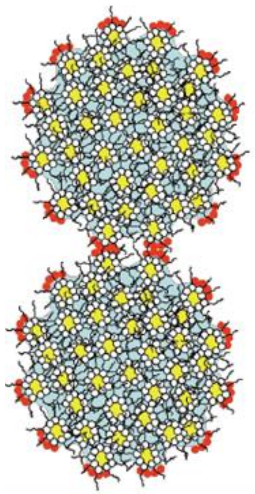
[1] C/P is the ratio between the specific clotting activity and general proteolytic activity. Reference method by E045
1 Analysis method = 50 IMCU/L Milk, pH 6.5. 3 Analysis method = Curd simulation & peptides extraction

A more specific coagulant enables precise cutting of the kappa casein and allow strong network formation

SPECIFICITY

What is specificity?

- Specificity is the degree of precision which the Kappa caseins are cut during coagulation
- The specificity varies dependent on the type and generation of coagulant

INSTABILITY	COAGULATION
SPECIFIC CLEAVING 	
Coagulant is added, CMP released after cleaving Kappa casein	Casein network formed as destabilized micelles aggregate into curd

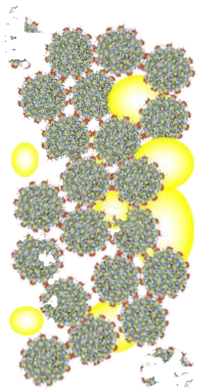
Higher specificity (C/P ratio) leads to faster coagulation and higher yield

BETTER NETWORKS, HIGHER YIELD

Influence of specificity

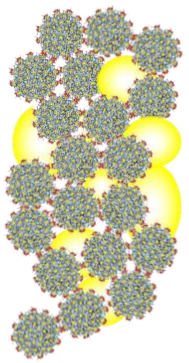
- The more precise the micelles are cut, the better networks they form and the more you keep what you need in the cheese without affecting the quality of the whey
- Using a coagulant with high C/P-ratio gives a superior network capturing fat and retaining intact proteins

LOW C/P RATIO



Casein network is weaker due to unintended cleaving, losing fat and bits of broken protein to the whey

HIGH C/P RATIO

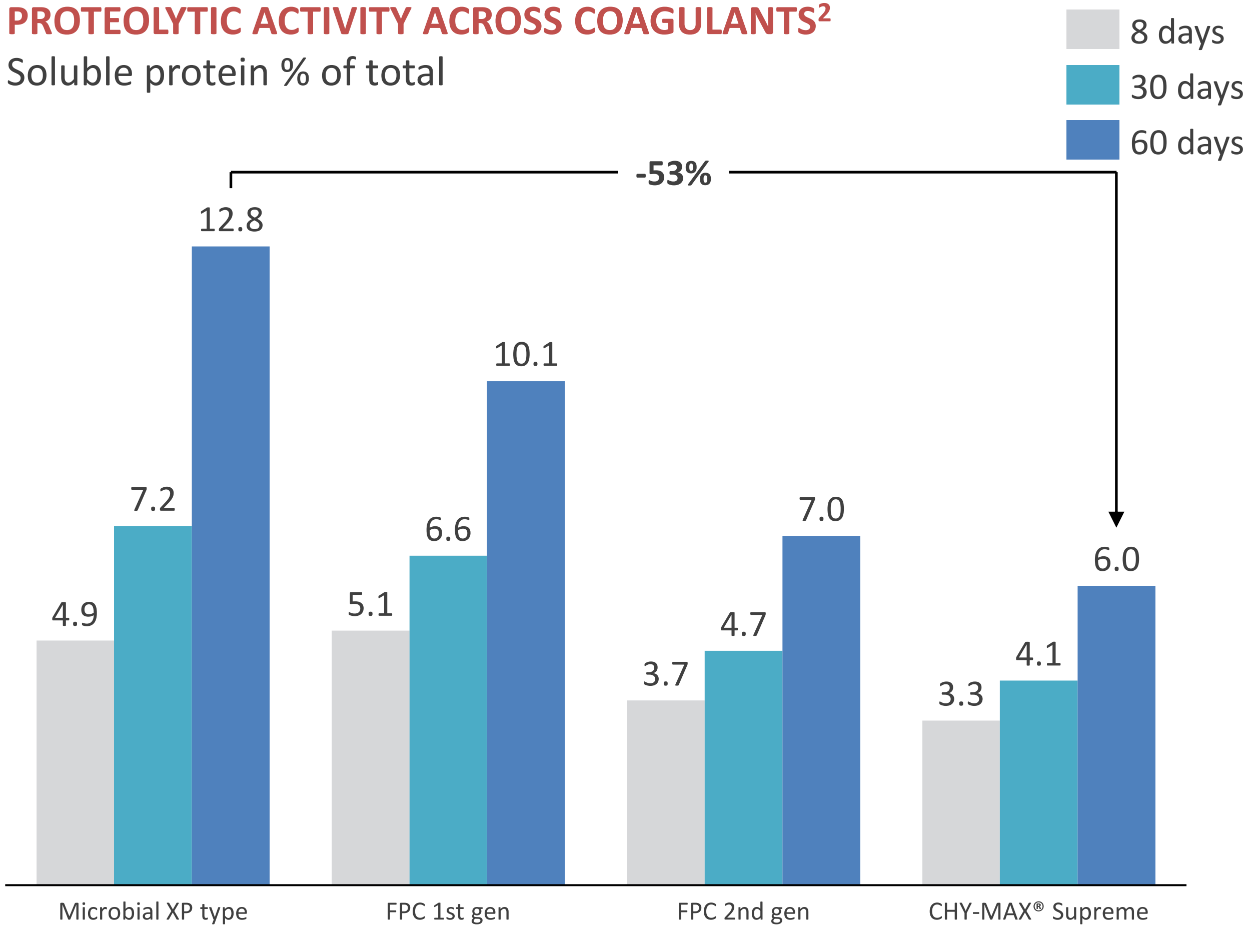


Casein network is strong and captures optimal levels of fat with minimal protein breakdown

Coagulant selection will deliver the desired level of intact caseins[1] throughout shelf-life

PROTEOLYTIC ACTIVITY ACROSS COAGULANTS[2]

Soluble protein % of total



- **CHY-MAX® Supreme** is **53% less proteolytic** after 60 days compared to 1st gen microbial **meaning 53% more soluble proteins** are recovered in the cheese after 60 days
- When stored at 5° C/ 41° F the shelf life can be extended from **2 to 4 months**
- The high level of intact caseins provides a superior **functionality** incl. **texture** of the cheese which enables easy conversion of the cheese with less giveaways
- In addition shelf life and the **window** for conversion is **extended**

1 Compared to other coagulants in the market
2 Trials in Chr. Hansen's facility.

Trends in the market

YIELD AND FUNCTIONALITY

- Continuous focus on maximizing value of milk through higher cheese yield and better whey quality
- Increasing demand for cheese as an ingredient and convenient formats call for functionality improvements during production[1]

PRESERVATIVE-FREE AND ORGANIC

- Big surge towards preservative-free coagulants globally
- Increasing consumer interest for organic and more natural foods with fewer ingredients[2]

SUSTAINABILITY

- UN global goals as a proactive tool to measure business impact. For instance ensuring sustainable consumption and production patterns

1 Euromonitor 2017 - 2020 2 Mintel GNPD 2017 - 2020

Culture

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Starter Cultures - Functionality

PRIMARY

The primary function of a starter culture is to produce lactic acid from lactose in milk

Starters convert lactose into glucose and galactose - then ferment the glucose component to lactic acid and reduce the pH of the milk/curd

The galactose moiety will accumulate in the curd

SECONDARY

The secondary function of a starter culture is to create the flavour and aroma that underpins cheese flavour & aroma producing

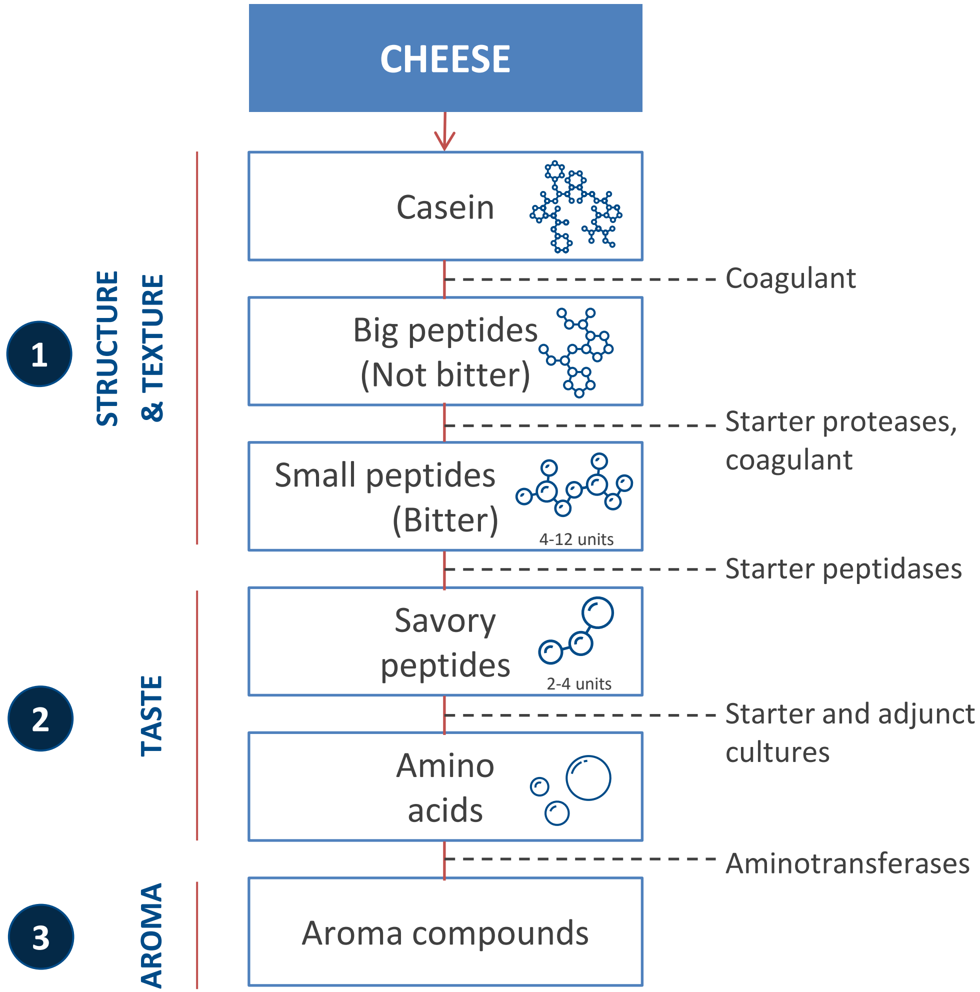
AROMA

- Compounds that interact with olfactory receptors
- Organic compounds
- Compounds with a certain level of volatility

TASTE

- Compounds that interact with olfactory receptors
- Organic compounds
- Compounds with a certain level of volatility

The proteolytic pathway: a summary



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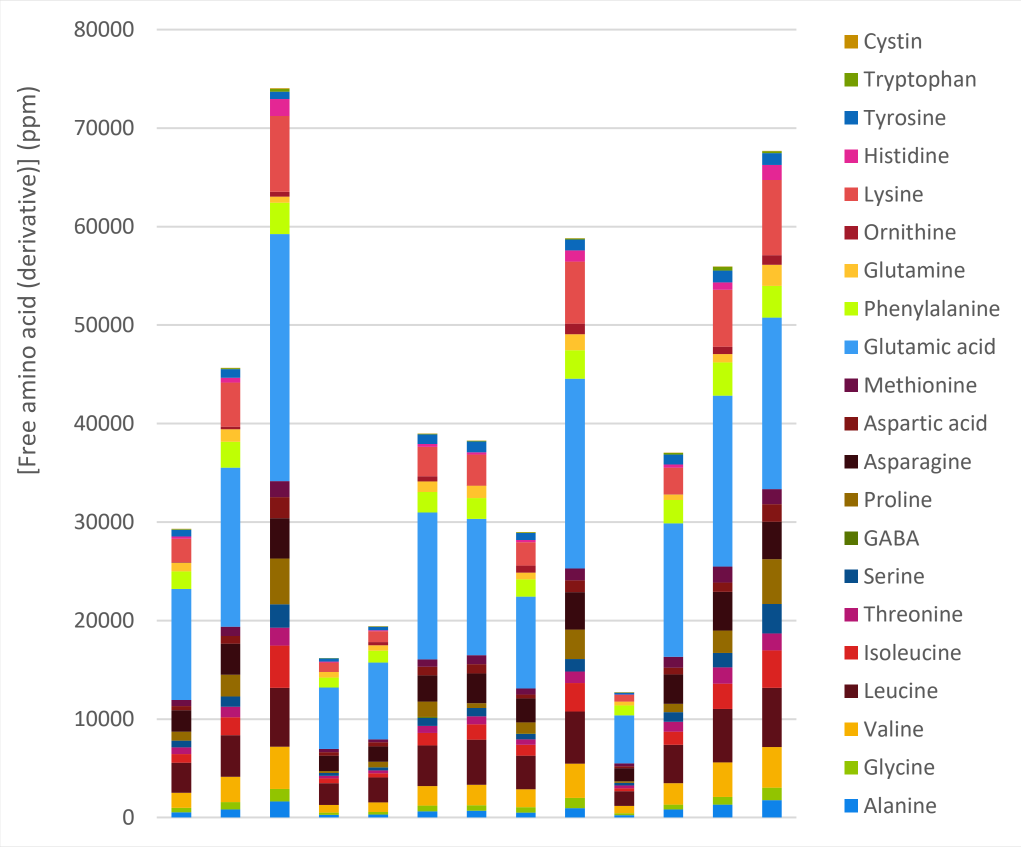
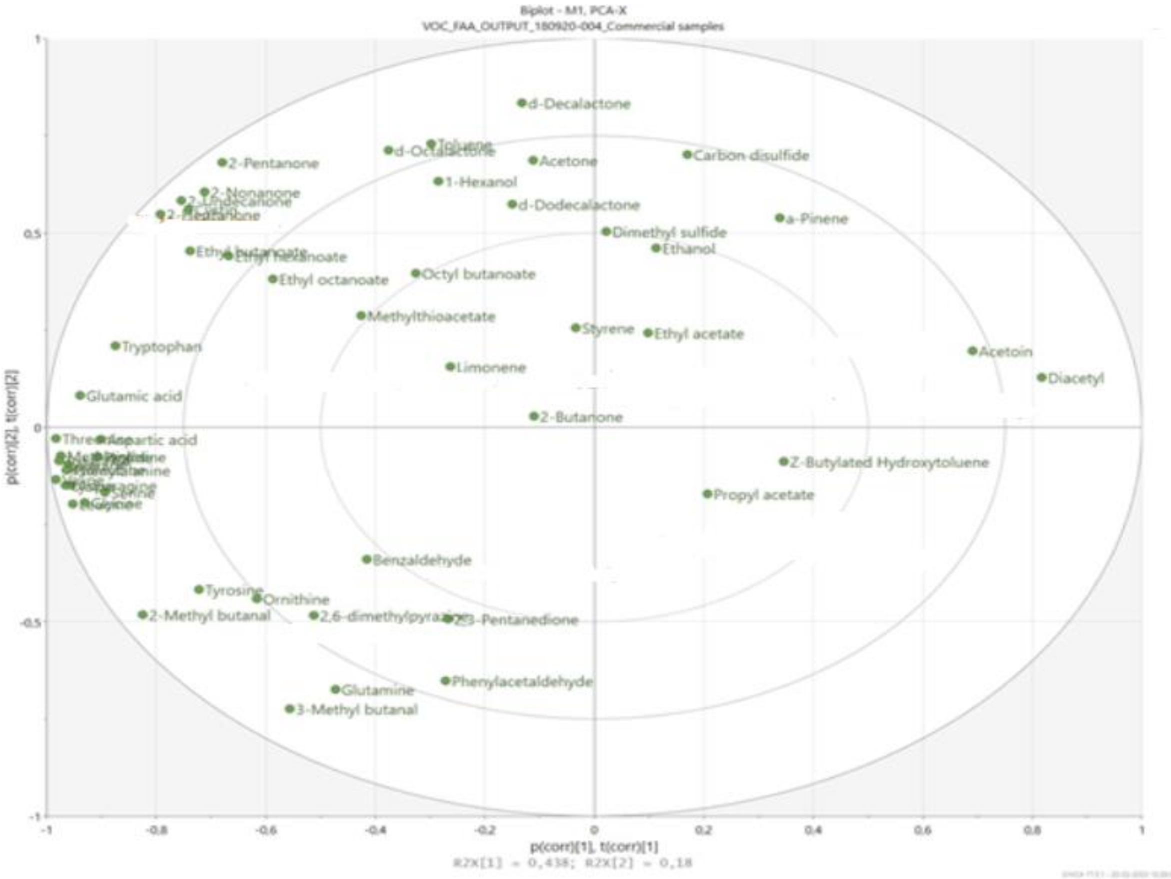
Analysis of Cheddar cheese from AU, NZ and UK markets: *Composition*

No	Origin	Age	Protein	Fat	FDM	MNFS	Moisture	Salt	S/M	pH
7	Australia		29.5	24.8	41.5	53.5	40.2	1.7	4.3	5.24
3	New Zealand	32	24.5	40.1	60.3	56.0	33.5	1.7	4.9	5.03
1	New Zealand	12	24.7	36.6	56.8	56.1	35.6	1.8	5.0	5.13
2	New Zealand	18	24.3	37.4	57.7	56.2	35.2	1.8	5.0	5.18
4	Australia		25.3	34.0	53.8	55.7	36.7	1.8	5.0	5.23
5	Australia		26.2	32.6	51.6	54.7	36.8	1.8	5.0	5.12
6	Australia		26.7	34.5	53.3	53.8	35.3	1.8	5.0	5.18
12	Australia	32	26.1	36.1	55.3	54.5	34.8	1.7	5.0	5.27
11	Australia	20	26.6	35.5	53.8	52.8	34.0	1.8	5.3	5.25
10	Australia		25.2	34.9	53.7	53.6	34.9	1.9	5.4	5.45
8	Australia	5-6	25.7	34.4	52.8	53.2	34.9	2.0	5.8	5.38
13	UK	12-14	25.2	35.7	55.2	55.0	35.4	2.1	6.0	5.16
9	Australia	8-10	25.4	33.5	52.3	53.9	35.9	2.3	6.3	5.42

Analyzing commercial Cheddar cheese from AU, NZ and UK markets: *Free amino acids and organic volatile compounds*

- Large concentration range of total free amino acids spanned with some (small) differences in composition. Absence of GABA.
- Large differences in the relative intensities of volatile compounds with separation of samples into 5 main flavor profiles





The autolysis of starter and ripening cultures greatly enhances cheese ripening, by releasing nutrients and enzymes into the cheese serum

Environmental stress exposure during cheesemaking and ripening (temperature, salt, pH, nutrient starvation) induces cell permeabilization or complete cell autolysis.

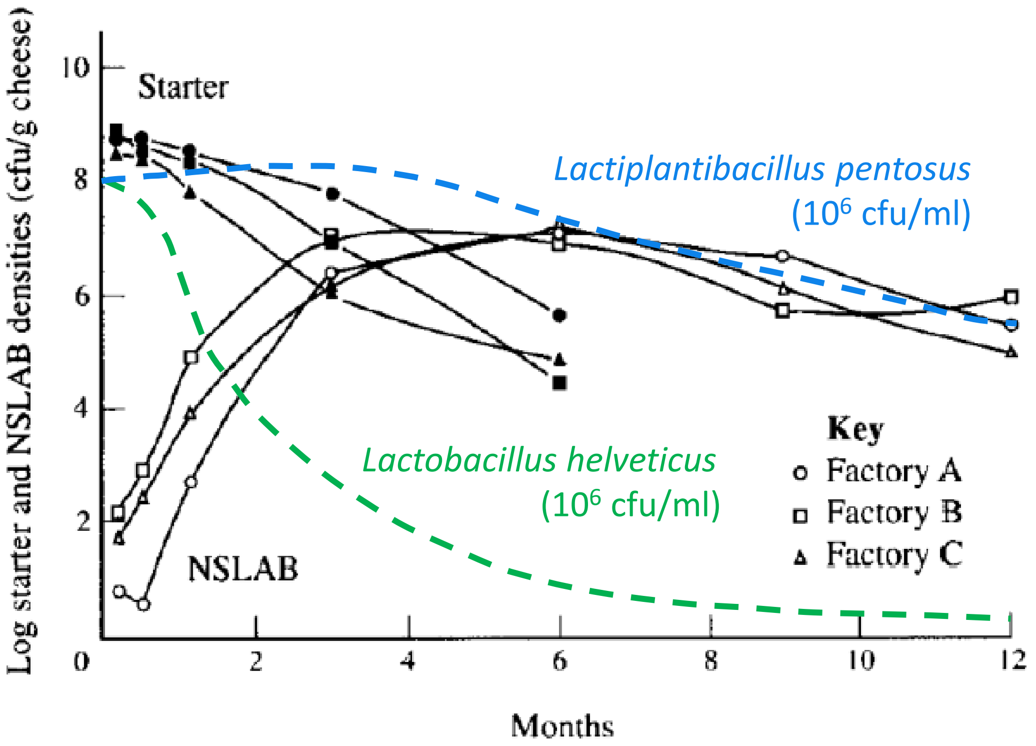


Fig 1: Average starter and non-starter lactic acid bacteria (NSLAB) densities (CFU/g) in Cheddar cheese manufactured with similar composition (pH, S/M, MNFS) from 3 New Zealand factories (Crow et al. 1995)

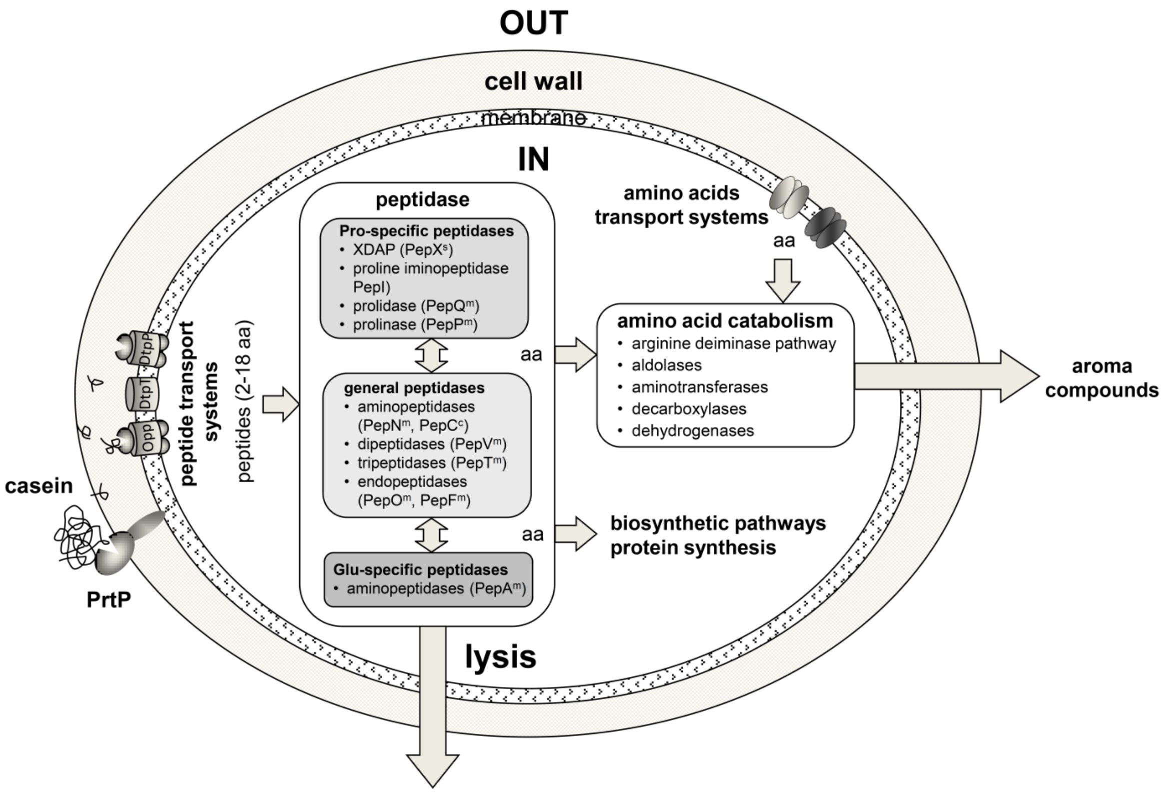
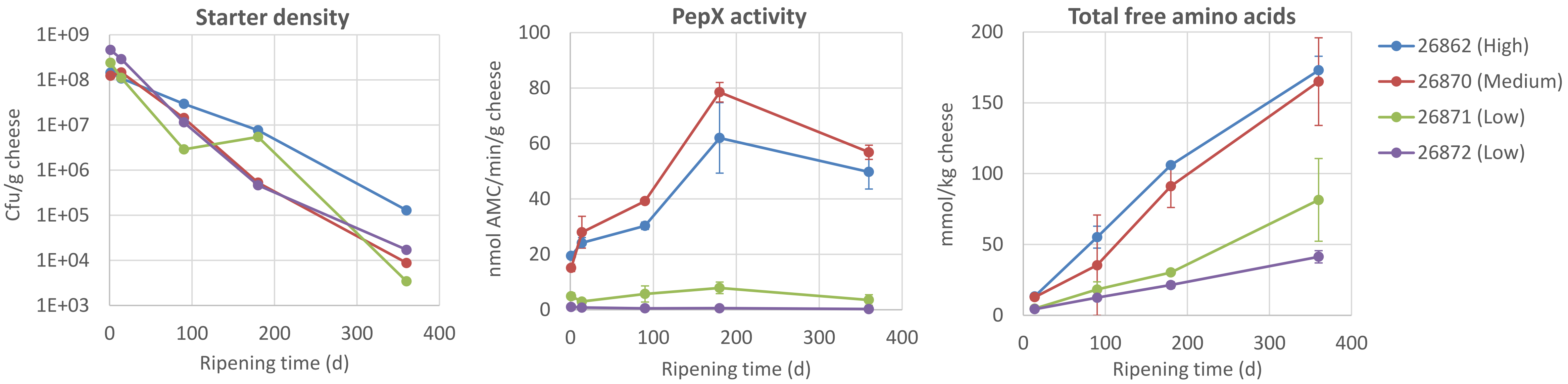


Fig 2: Schematic representation of nitrogen metabolism in lactic acid bacteria. Abbreviations refer to the enzymes of *Lc. lactis* (Parente & Cogan, 2004)

Cheddar cheese made with *Lc. lactis* single strains previously screened to different levels of Pro-specific aminopeptidase activity

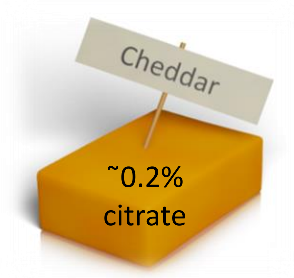


Correlation between temperature-induced lysis screening and Cheddar cheese. Strains with lysis index > 0.25 (Medium and High) demonstrate good flavour properties

Linear correlation between CFU, PepX and total FAA until 6 months of age. After 6 months the starter culture is no longer the dominant bacterial population in the cheese, and PepX no longer a sufficient single marker of the ripening stage

Use of additional enzymatic flavor markers would further complement the analysis of biochemical compounds and elucidate the microbial species and metabolic pathways of interest

Citrate utilization by lactic acid bacteria <u>without</u> associated gas formation: The *Lactiplantibacillus pentosus* example



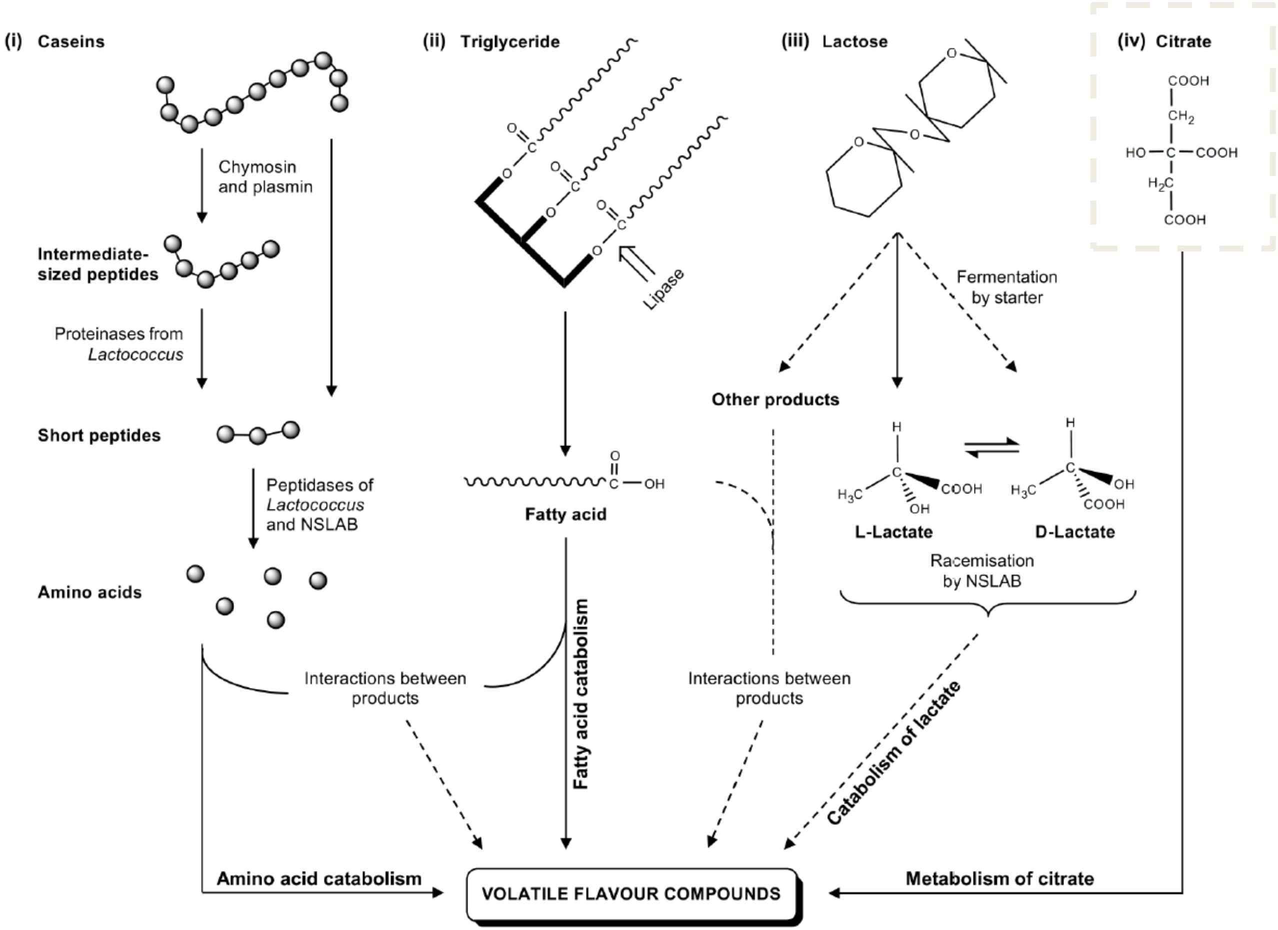


Fig 1: General overview of the biochemical pathways contributing to flavour and texture development during cheese ripening (McSweeney, 2004)

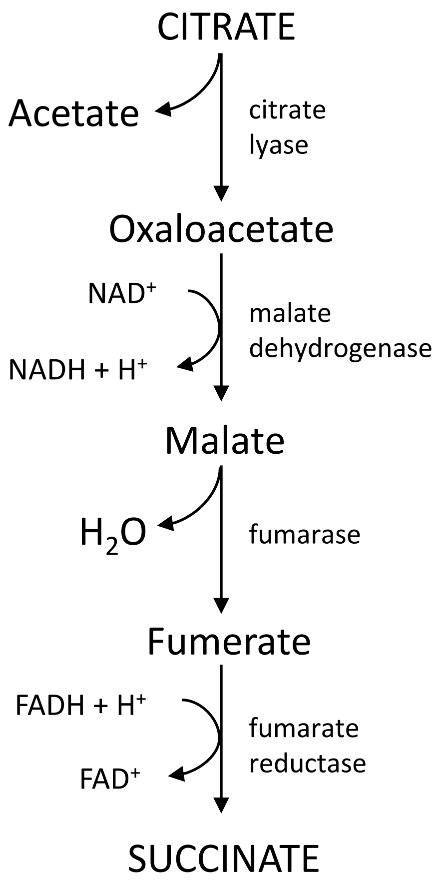


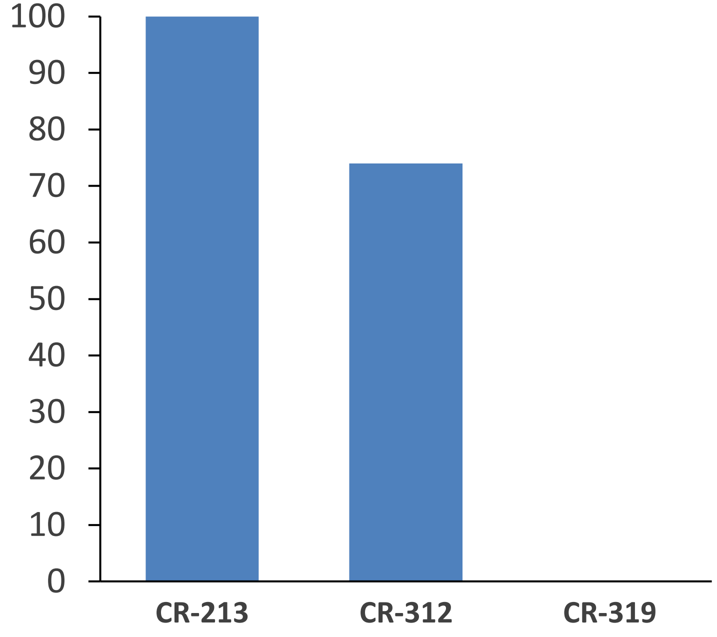
Fig 2: Conversion of citrate to succinate via the reductive TCA pathway by *Lactiplantibacillus* as first suggested Chen & McFeeters, 1986

CR-200 & CR-300 – debittering strength

Lactococcus lactis & *cremoris* efficiently reduce bitterness by limiting primary proteolysis and boosting peptide breakdown.

PROTEINASE ACTIVITY

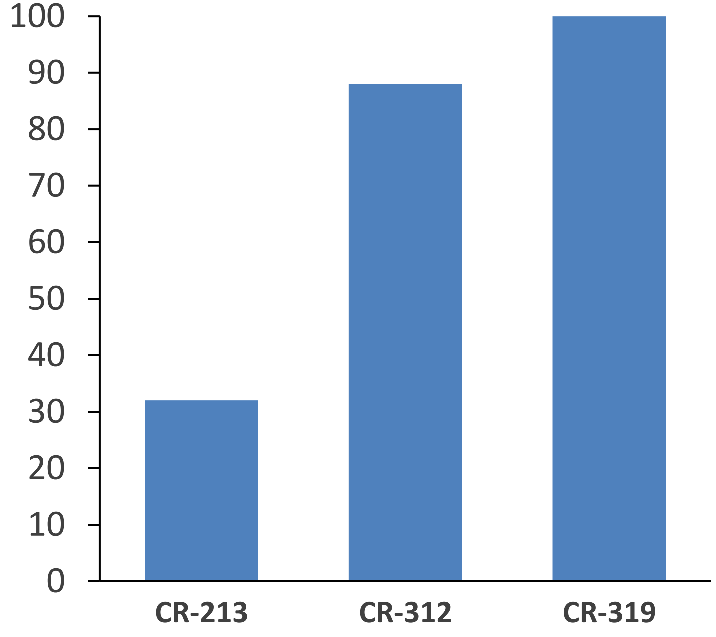
Relative %



Primary proteolysis - the ability to break down intact proteins and larger peptides.

PEPTIDASE ACTIVITY

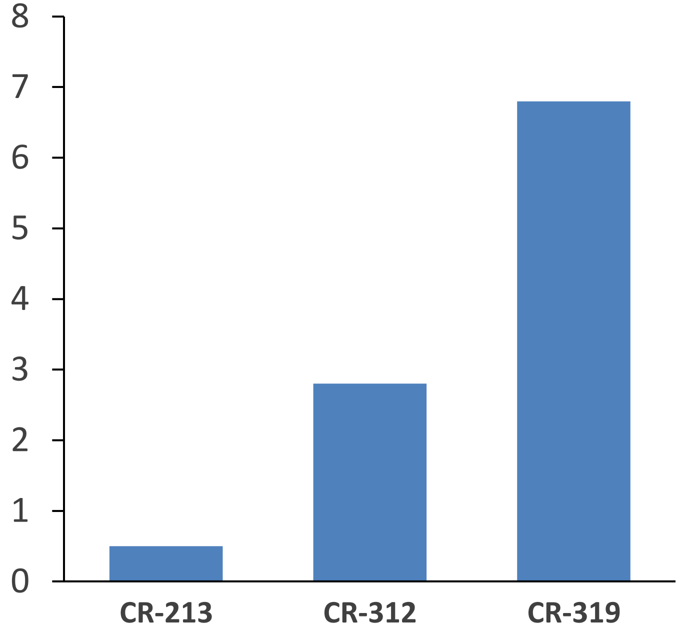
Relative %



Secondary proteolysis – the ability to break down peptides to free amino acids

AUTOLYSIS

Autolysis index (PepX activity)



Autolysis – the ability of the cells to lysis helps to speed the ripening process

Data can be compared between the cultures presented in the graphs above but cannot be compared to data from other culture ranges.

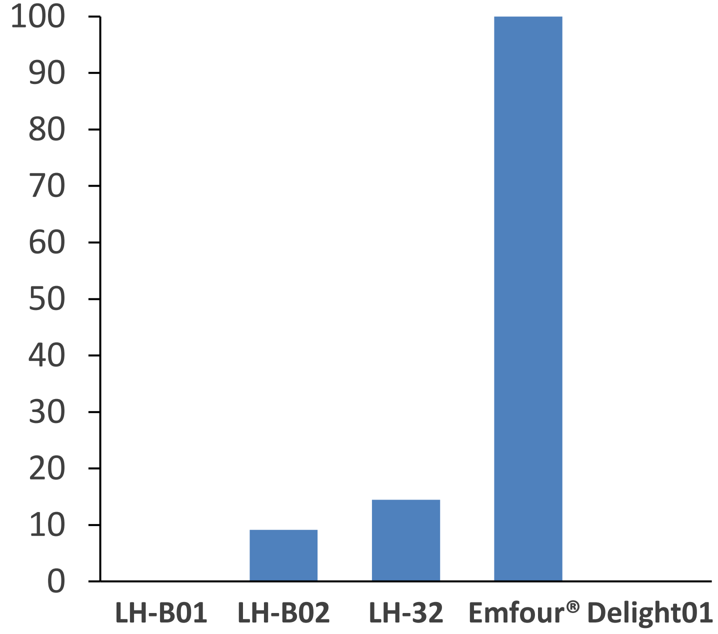
Lactobacillus helveticus – Solutions for Flavour Development

Lactobacillus helveticus has a highly efficient proteolytic system that breaks down milk proteins to peptides and free amino acids.

The good balance between proteinase and aminopeptidase activities is responsible for the good action on bitterness control.

PROTEINASE ACTIVITY

Relative%



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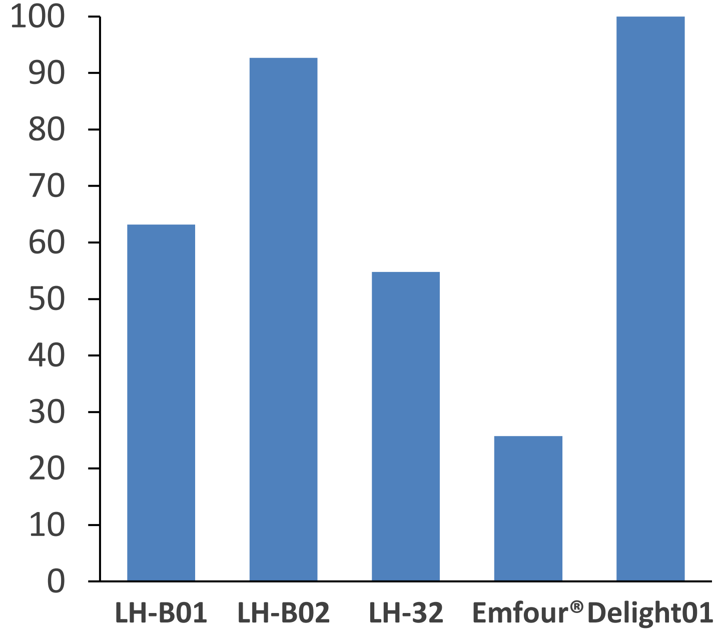
AMINOPEPTIDASE ACTIVITY

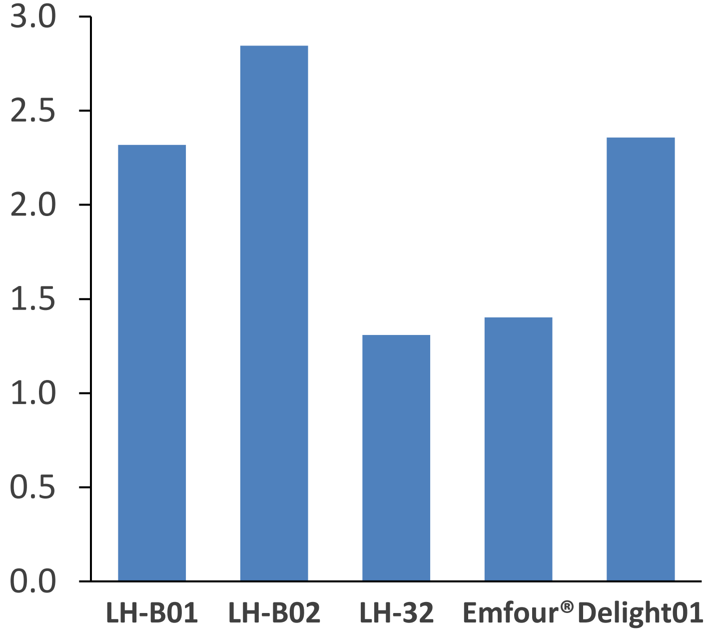
Relative%, pepN



Secondary proteolysis – the ability to break down peptides to free amino acids

TOTAL AMINO TRANSFERASE ACTIVITY

Value/OD



Tertiary proteolysis – the ability to transform free amino acids into volatile compounds

Emfour®: Restricted in use: Contact Marketing.

Delight01: Heat attenuated.

Data can be compared between the cultures presented in the graphs above but cannot be compared to data from other culture ranges.

Culture - NSLAB

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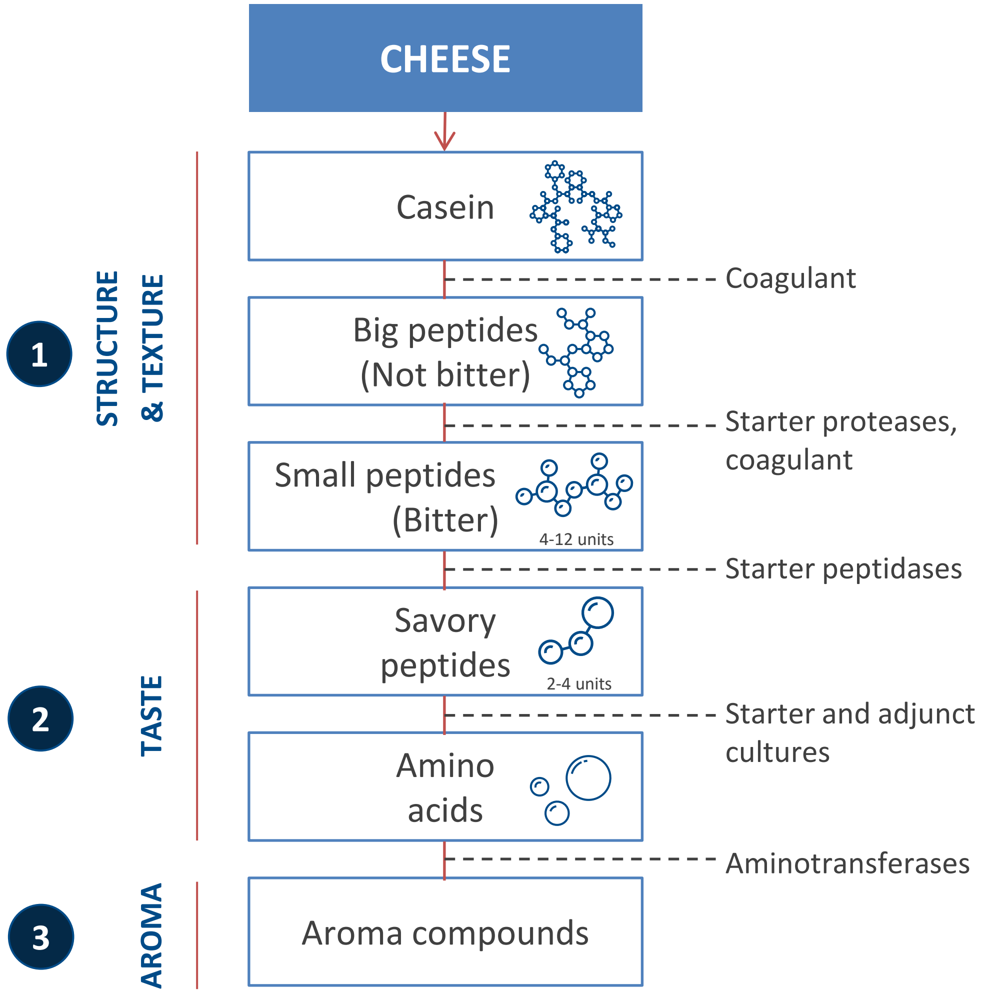
The proteolytic pathway: a summary



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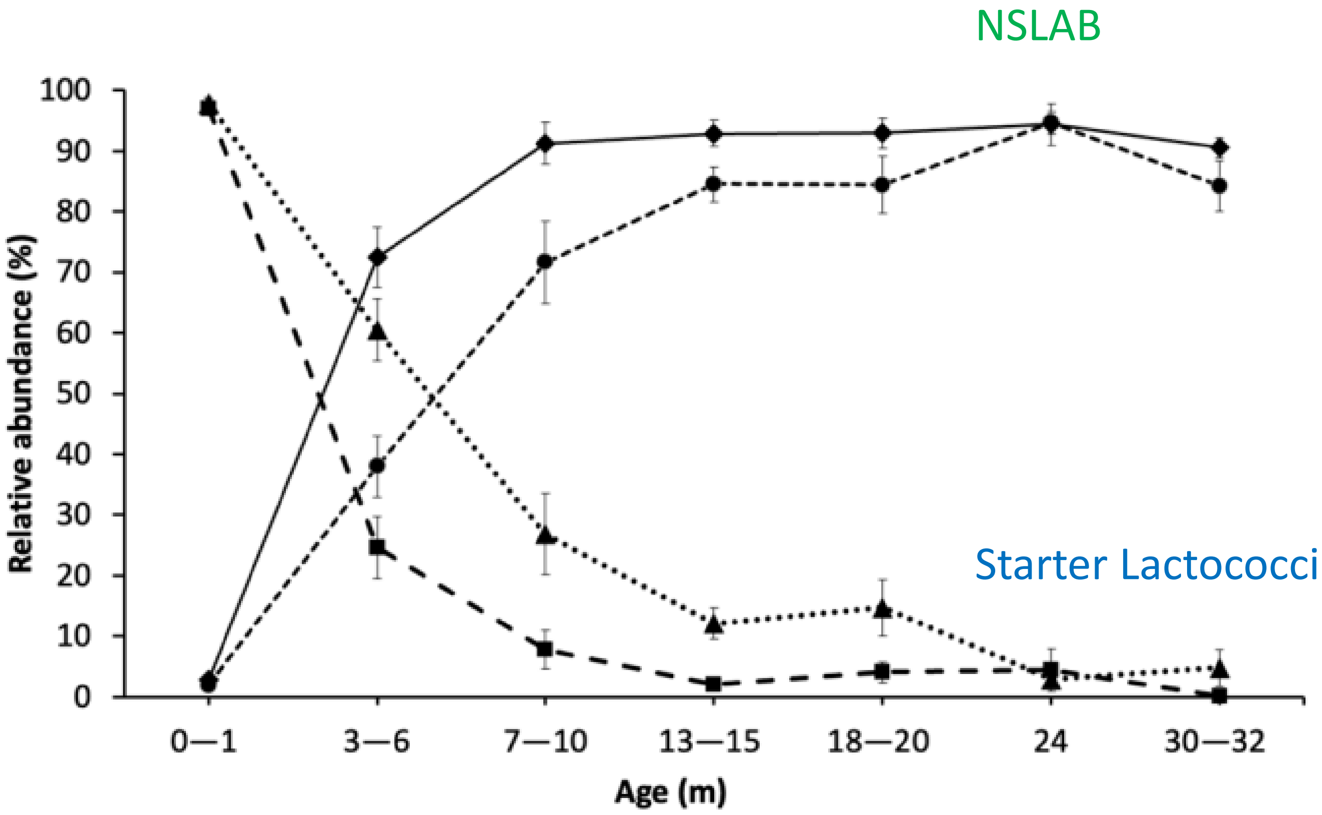
Variation in NSLAB Levels in Mature Cheddar Samples

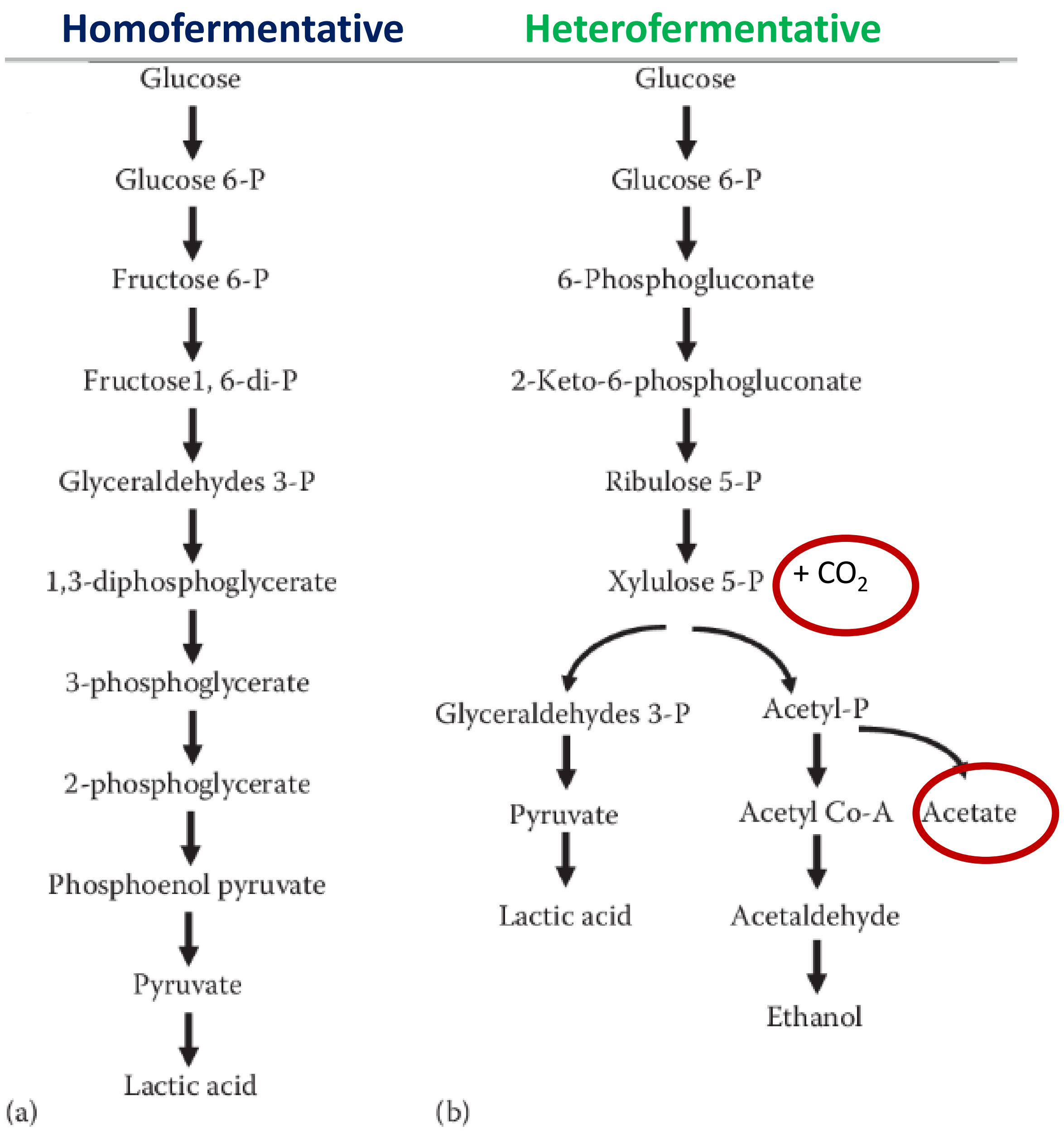


- NSLAB (Non-Starter Lactic Acid Bacteria) are principally facultatively heterofermentative lactobacilli
- *E.G. Lb. brevis, Lb. curvatus, Lb. casei, Lb. plantarum, Lb. fermentum*
- Present as opportunistic organisms in the milk and dairy (biofilms)
- They tend to present at very low numbers initially, but will build up during maturation

REF: microorganisms-10-01669-v2.pdf

Heteroferm. Lb - Metabolism

- Heterofermentative lactobacilli have additional capacity to produce other metabolic end-products, e.g. acetate
- This pathway also produces lactic acid, ethanol and CO_2
- Carbon dioxide produced by this pathway can contribute to open texture development in Cheddar
- Fruity flavour notes can arise from heteroferm. pathway especially through the breakdown of ethanol to esters, which are associated with fruity notes



Crystal Formation in Cheddar

• Calcium Lactate

- •D- and L-Lactate – 2 isomers present in cheese
- •The majority of **L-lactate** in Cheddar is produced by the starter cultures primarily on the day of manufacture (typically 1.2-1.5% lactic acid)

- **•D-Lactate has a lower solubility and is produced by the NSLAB population either by**

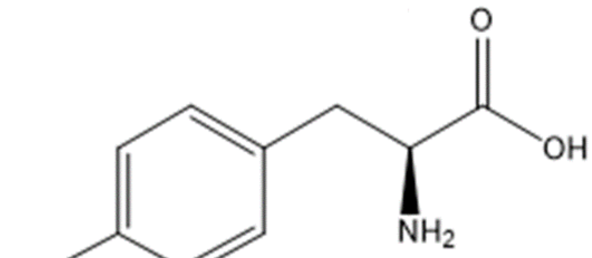
Ca^{2+} + 2 $Lactate^{-}$ ⟷ Ca $Lactate_2$ (Soluble) ⟷ Ca $Lactate_2$ (Nucleated crystals) ⟷ Ca $Lactate_2$ (Visible crystal formation)

• Tyrosine

•Tyrosine is highly insoluble in water/serum phase in cheese

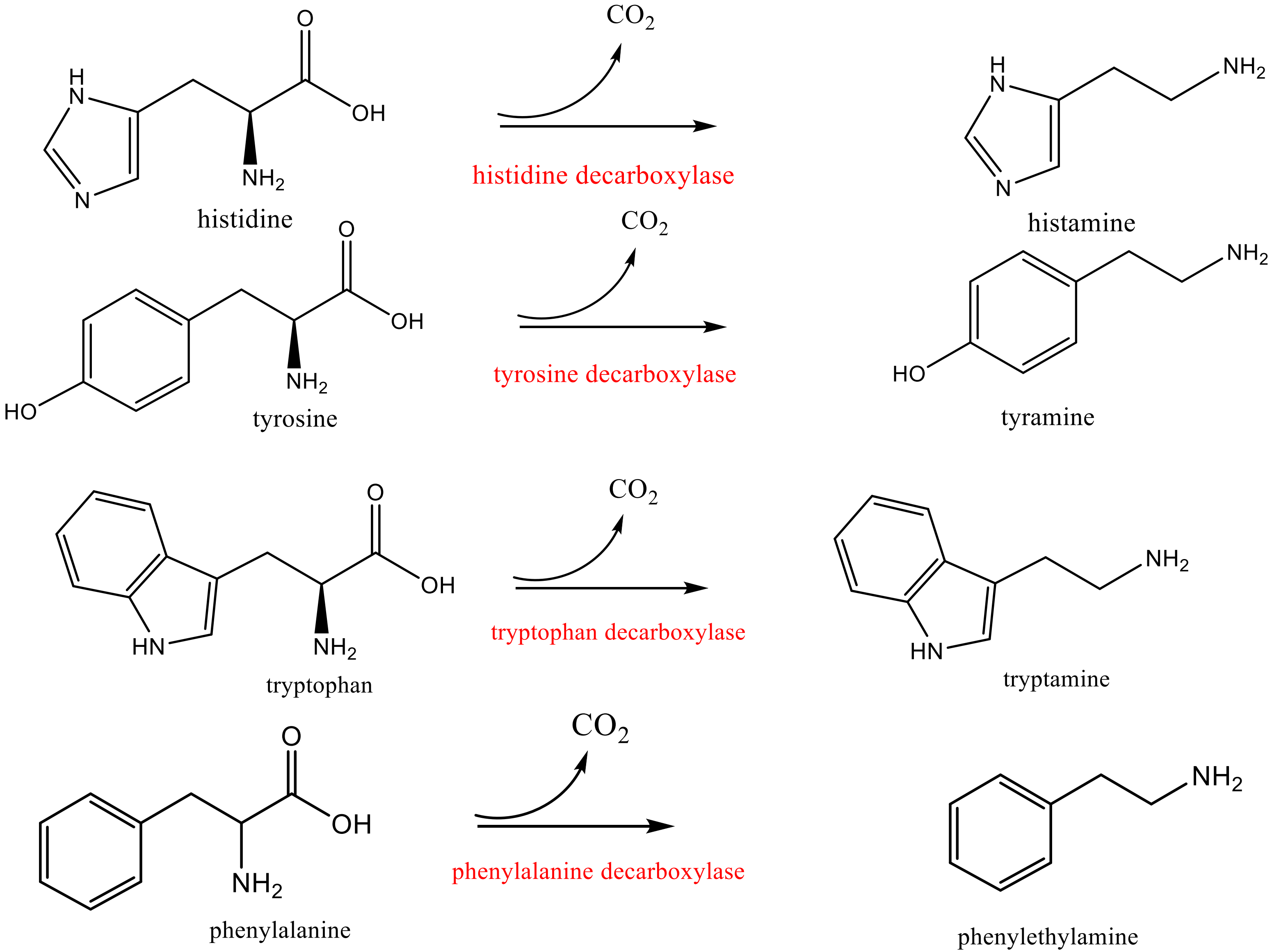
•Therefore, it has a propensity to crystalise within the cheese matrix – this occurs in the later stages of maturation because it first requires proteolysis to have released tyrosine as a free amino acid

•Once tyrosine has exceeded it solubility level, it will begin to form crunchy crystals



tyrosine

Decarboxylation of Amino Acids



Free amino acid → **Biogenic amine**

Please let our experts help you to find the optimal and complete solution for your unique cheese and to get most out of your milk

Differentiate by combining DVS® Cheddar cheese starter cultures with the perfect choice of coagulant, surface ripening, bioprotection and services from Chr. Hansen

COAGULANTS

- CHY-MAX® Special or M
- CHY-MAX® SUPREME
- Microlant® classic
- Microlant® supreme

To master texture and shelf life

RIPENING

DVS® CR adjuncts

DVS® LH adjuncts

To impart a unique flavor

BIOPROTECTION

FRESHQ

BIOSAFE

To keep your final product great

SERVICES/TOOLS

- PhageWatch®
- Coagusens™
- TRIALS & SIX SIGMA
- EASY-DOSE™

Simply to stay ahead

Thank you

FOLLOW CHR. HANSEN