UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

Dairy Product Technology 2
NFOK16006U

Notes taken during the course, including lectures, exercises, curriculum, practicals, and group reports

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Link to GitHub reporsitory

Preface

These course notes have been prepared as part of the NFOK16006U course Dairy Product Technology 2 at the University of Copenhagen, covering the period from November 2025 to February 2026.

The notes compile material and reflections relevant to the course and are intended as a resource to enhance the learning experience for students. The content is shared freely and may be used as study material or as a template for structuring individual notes.

All information is provided without responsibility for its correctness, and users are encouraged to verify data, formulas, and interpretations with the original sources and course materials.

Please enjoy reading these notes, and feel free to reach out if you have any questions.

Course Description

Education

MSc Programme in Agriculture

Content

The aim of the course is to give students a detailed knowledge of both theoretical and practical aspects of cheese and fermented milk production, characterisation, technology and biochemistry.

Learning Outcome

The course is targeted to students interested in plant science (Horticulture and Agriculture) and food science students who are particularly interested in fruit and berry crops and the quality and use of the raw materials/food products these crops provide.

Knowledge

- Understand chemistry, biochemistry, microbiology, physics and technology in the production of cheese and fermented milks.
- Understand the characteristics of, and basic differences between, cheese groups and varieties.
- Understand the use and application of dairy enzymes in cheese and fermented milks

Skills

- Apply critical analysis of cheese and fermented milk quality and characteristics.

Competences

- Ability to use and evaluate scientific information and knowledge concerning cheese and fermented milks, incl. all steps of production and ripening.
- Ability to comprehend the technology behind different cheese varieties and fermented milk types.
- Capacity to critically evaluate laboratory results and pilot plant trials with regard to the possible development of innovative cheese and fermented milks and/or processes.

Academic qualifications equivalent to a BSc degree is recommended.

Teaching and Learning Methods

The course involves lectures and laboratory practicals, workshops and oral presentations, literature studies, patent searches and report writing. The student must obtain their own laboratory results and evaluate them in relation to theory given at lectures and found in literature, including critical use of electronic data bases and internet. Results from all students' practicals and literature studies will be merged during oral presentations and discussions in workshops.

Workload

Table 1: *A table with an overview over the workload for the course.*

Category	Hours
Lectures	32
Class Instruction	15
Preparation	75
Practical exercises	75
Project work	8
Exam	1
Total	206

Exam

Table 2: *A table with an overview over the elaborated description of the course*

Credit	7.5 ECTS
Type of assessment	Oral examination, 20 minutter
Type of assessment details	3 weeks before the examination, all examination questions (covering the essential issues dealt with in the course) will be published on Absalon. The students are expected to use all of these questions to be fully prepared for the subsequent oral exam. At the oral exam itself, one of the questions will be drawn by the student, and the examination immediately proceeds. For the question drawn, all aids are allowed during the exam (approx. 15 minutes). The last 5 minutes of the exam will be used for questions in relation to the reports prepared from the laboratory practicals.
Examination prerequisites	Attendance at all laboratory practicals is compulsory. All laboratory reports must have been approved.
Aid	All aids allowed Read about how to use Generative AI on KuNet
Marking scale	7-point grading scale
Censorship form	• No external censorship • Several internal examiner
Re-exam	Same as ordinary exam. Non-approved reports must be handed in three weeks prior to the re-exam and approved 2 weeks prior to the re-exam.

Contents

Chapter 1
Lecture Notes

Introduction

The following lecture notes have been compiled by combining personal notes taken during class, personal highlights from the lecture slides, and targeted use of generative AI. The AI was provided with my notes and the highlighted slides, together with carefully designed prompts, to ensure that it focused on the most relevant and interesting aspects of each lecture. This approach aims to produce a coherent and focused summary that reflects both the course content and my individual learning perspective.

1 Lecture 01: Introduction - 17/11-2025

Dairy Product Technology 2
Anni Bygvrå Hougaard, Associate professor FOOD, 2025/2026

Summary
The course Dairy Product Technology 2 is designed to provide students with detailed knowledge of the theoretical and practical aspects of cheese and fermented milk production, characterisation, technology, and biochemistry. The areas of competence include the chemistry, biochemistry, microbiology, physics, and technology required for production, including the influences of milk quality and treatment. Students are expected to understand the characteristics and basic differences between various cheese groups and varieties, as well as the technology behind different fermented milk types. This involves the ability to use and evaluate scientific information regarding all steps of production, ripening, and packaging.

The curriculum covers a wide range of scientific topics, including principles of manufacture, cheese yield, starter cultures, pH, microflora, and gas production. Further technical lectures focus on cheese proteolysis, amino acid catabolism, cheese flavour, and analytical methods such as peptide, amino acid, and aroma analysis. The course also addresses cheese defects, ripening engineering, curd formation, coagulants, and specific products such as cheese powder and processed cheese. The weekly structure consists of lectures and theoretical workshops on Mondays, followed by pilot plant and laboratory exercises on Wednesdays.

Practicals and workshops are central to the course and consist of several structured components:

1. Laboratory and/or pilot plant work.
2. Critical evaluation of results.
3. Calculations.

4. Discussions and conclusion about reliability of results.
5. Discussion of results in relation to literature and results of the other groups.
6. Report writing.

In the dairy pilot plant, students produce cheddar cheese using both standard recipes and modified versions involving reduced fat, thermophilic cultures, or changed cutting techniques. Yoghurt production involves six samples with varying fat and protein content or post-fermentation processing, which are later analysed for viscosity, water binding, pH, and sensory properties. Cheese analysis practicals require groups to work with specific varieties, such as Feta, Danbo, Havarti, or Grana Padano, to perform analyses on moisture, ashes, salt, fat, protein, pH, protein degradation, lipolysis, and glycolysis.

The course maintains a strict code of conduct, requiring students to be on time, follow technician instructions, and wear lab coats and safety glasses at all times in the laboratory. Students must read guidelines before starting work and perform calculations continuously, as an analysis is not considered finished until results are evaluated and interpreted. Reports for the cheese analysis practical must be approximately 20 pages and include the following contents:

1. Title.
2. Name of all authors & date.
3. Introduction with short background and objectives.
4. Material and Methods.
5. Results and Discussion.
6. Conclusion.
7. Reference list.

The final examination is a 20-minute oral exam where a student randomly draws a question from a list distributed three weeks prior. The session includes a 7-8 minute presentation and a discussion of the question and the laboratory reports. Participation in practicals and the approval of reports are mandatory prerequisites for attending the exam.

2 Lecture 02: Principles of Cheese Manufacture - 17/11-2025

Anni Bygvrå Hougaard, Associate professor FOOD, 2025/2026

Summary
Cheese is defined as coagulated milk and the concentration of milk nutrients, serving to preserve milk nutrients through a low water activity, a low pH, a high salt content, and microbial competition. In 2023, total milk production in the EU-27 was 160.8 million tonnes, of which 96% was cows' milk. In the same year, Denmark produced 517,900 tonnes of cheese, corresponding to about 2% of global production. The value of Danish cheese exports reached 14.74 billion DKK, accounting for 56% of total dairy exports, with per capita consumption in Denmark estimated at 25 - 30 kg/year.

Cheeses are grouped by coagulation method into those coagulated by renneting, those coagulated by acidification, and those made from cheese whey. Rennet-coagulated cheeses are further classified by firmness (MNFS%) and minimum pH, including extra hard varieties like Grana (MNFS < 51%), hard varieties like Emmental and Cheddar,

semi-hard varieties such as Gouda, Danbo, and Havarti, and soft varieties like Brie and Feta. Acid-coagulated cheeses include Tvorog, Quarg, and Skyr, while whey cheeses are produced by precipitating proteins through heating and acidification (e.g., Ricotta) or by concentration through evaporation (e.g., Brunost).

The principles of cheese manufacture follow a specific sequence of technical steps:

1. Cheese milk treatment.
2. Coagulation - Setting.
3. Syneresis - Cutting/Stirring/Washing/Scalding.
4. Moulding & Pressing.
5. Salting.
6. Surface treatment.
7. Ripening.

Cheese milk treatment involves cold storage (< 5°C), which can lead to the loss of β-casein from micelles and poor rennetability. Standardisation is used to achieve a fat to protein ratio that ensures the most economical use of milk components. Heat treatment, commonly 72°C for 15 seconds, destroys pathogenic microorganisms but may cause whey protein denaturation and the transfer of soluble calcium and phosphate to the colloidal phase. Centrifugal bactofugation can remove 98 - 99.5% of anaerobic spores.

During syneresis, the curd is cut into cubes and stimulated by mixing and heating. Moulding and pressing techniques influence texture; pre-pressing under whey results in round-eyed cheese, while air between grains creates an open texture. Salting stimulates syneresis, lowers water activity, and provides preservation. Methods include salt on grains, dry salting, and salting in brine, with brine times ranging from 0.5 - 3 hours for soft cheeses to 3 weeks for extra hard cheeses.

Surface treatments include plastic film, waxed surfaces, smeared surfaces, or mould growth. Ripening involves the growth and metabolism of bacteria, cell lysis, and enzyme activity in the cheese matrix. In semi-hard cheese ripening, 100% of lactose and citrate are decomposed, while 25 - 30% of casein and less than 1% of milk fat are broken down. This process is performed by the coagulant, milk enzymes, starter cultures, and non-starter microorganisms.

3 Lecture 03: Cheese Yield - 17/11-2025

Anni Bygvrå Hougaard, Associate professor FOOD, 2025/2026

Summary
Cheese yield is fundamentally defined by the recovery of milk components, particularly the maximum recovery of casein and fat while minimizing losses in the whey. Scientifically, yield is expressed as the weight of cheese (in kg) of a specific dry matter content produced from a defined quantity of milk with known protein and fat content. Two primary measures are utilized: Actual cheese yield (Ya), which is the weight of the cheese divided by the total weight of milk, starter, and salt, and Moisture-adjusted cheese yield (MACY), which adjusts the actual yield to a reference moisture content to allow for theoretical comparisons between different batches.

Predictive formulae are employed to anticipate labour requirements, equipment needs, and profitability. These formulae are cheese-type dependent, ranging from the Babcock "rule of thumb" (Yield=1.1$\times$% fat+2.5$\times$% casein) to the more complex Van Slyke and Publow formulae. The modified Van Slyke and Publow formula for all cheeses

accounts for fat recovery (Kf), milk fat (F), milk casein (C), moisture (M), salt (SC), and whey solids (WS). Retention of milk solids is critical to these calculations; for instance, approximately 85-95% of fat globules are retained in the casein network, while only 3-5% of lactose is typically retained, most of which is converted into lactic acid. Protein retention is approximately 76.3%, including para-casein and small amounts of denatured whey protein from heat treatments or starter cultures.

Several biological and handling factors significantly influence the final yield:

1. Protein genotypes: The BB genotype of κ-casein is associated with higher casein concentrations, superior renneting properties, and a 3-8% increase in MACY.
2. Somatic cell count (SCC): High SCC indicates poor health status and is associated with casein-degrading proteinases that lead to soluble peptide losses in the whey.
3. Cold storage: Extended cooling (< 5°C) causes the solubilisation of micellar caseins, especially β-casein, making them susceptible to hydrolysis.
4. Milk handling: Excessive pumping or shearing damages the milk fat globule membrane (MFGM), leading to fat hydrolysis and lower recovery.
5. Microbial quality: High levels of psychrotrophic bacteria can produce lipases and proteinases that decrease yield.

To improve production efficiency, several methods to increase cheese yield are implemented:

1. Ultrafiltration (UF): Used at low, medium, or high concentrations to increase whey protein incorporation and improve fat and casein retention.
2. Microparticulated whey protein: Techniques like Leancreme utilize high heat and shear force to create particles similar to fat globules, resulting in a yield increase of 6-10%.
3. Low proteolytic coagulants: Using camel chymosin (CHY-MAX M) provides a high clotting-to-proteolytic (C/P) ratio, reducing protein loss compared to microbial coagulants. item Transglutaminase: This enzyme creates iso-peptide bonds between proteins, increasing moisture retention and yield.
4. Phospholipase: YieldMAX (PLA1) modifies the MFGM by hydrolyzing phospholipids to lysophospholipids, which act as emulsifiers to prevent globule rupture, particularly in pasta filata cheese, increasing yield by $\geq 1\%$.

4 Lecture 04: Starter, pH, and Microflora - 24/11-2025

Henrik Siegumfeldt, Department of Food Science, 2025

Summary

Cheese ripening is characterized by the activity of bacteria immobilized within the cheese matrix. For specific varieties such as Danbo 45+, the gross composition typically comprises Water 48%, Fat 25%, Protein 24%, and others 3%. Culture actions are central to manufacture, involving the conversion of lactose to lactic acid, which facilitates a pH drop, preservation, texture development, and improved coagulation and syneresis. Culture groups are classified based on their optimal growth temperatures and gas production:

1. Mesophilic (20-30°C): Non-gas producers including *Lactococcus lactis* and *L. cremoris* (O), and gas producers including citrate-fermenting *L. lactis* (D) and *Leuconostoc* spp. (L).
2. Thermophilic (35-45°C): Non-gas producers such as *Streptococcus thermophilus* (ST), *Lactobacillus helveticus* (LH), and *Lb. bulgaricus* (LB).
3. Secondary cultures: Includes moulds (*Penicillium roqueforti*, *P. camemberti*), yeasts (*Debaryomyces hansenii*),

and bacteria such as *Propionibacteria* and *Brevibacterium linens*.

Starter systems have evolved from liquid cultures in the 1890s to modern Direct Vat Set (DVS) and Direct Vat Inoculation (DVI) systems. Bulk culture propagation is considered complicated compared to DVS, which uses highly concentrated bacterial cells for direct inoculation into cheese vats of 10,000-20,000 liters. Frozen DVS/DVI pellets (F-DVS/DVI) have concentrations between $1 \times 10^{10} - 5 \times 10^{10}$ cfu/g and require storage at -45°C. Freeze-dried pellets (FD-DVS/DVI) reach concentrations of $5 \times 10^{10} - 1 \times 10^{11}$ cfu/g, are stored at -20°C, and require rehydration.

Cheese segmentation is defined by scalding temperatures, consistency, and traditional culture use. Cottage cheese types use O cultures at 22-32°C, while soft cheeses like Camembert and Brie use O or LD cultures at a maximum of 35°C. Semi-hard Continental types such as Gouda and Danbo are scalded at 35-40°C using LD cultures. Harder varieties, including Pasta Filata and Cheddar, utilize temperatures between 37-43°C. Extra hard Grana types require scalding at 50-55°C with ST, LH, and LB cultures.

During ripening, the microbial flora shifts as Starter LAB (LAB) decrease and Non-Starter Lactic Acid Bacteria (NSLAB), such as *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*, increase. Lactose and citrate metabolism pathways involve homofermentative or heterofermentative processes. Homofermentative citrate-positive LAB produce pyruvate, which is converted to lactate via lactate dehydrogenase (LDH) or into aroma compounds like diacetyl, acetoin, and 2,3 butanediol. Heterofermentative species like *Leuconostoc* reduce acetaldehyde to ethanol via alcohol dehydrogenase.

The rate of acidification and the minimum pH reached after 24 hours are critical for cheese quality, structure, and biochemical reactions. Minimum pH values range from 4.5-4.6 for Quarg and Danablu to 5.2-5.3 for Emmental and Grana. The pH decrease occurs in two phases: Phase 1 before whey separation during heating and stirring, and Phase 2 after whey separation in the fresh cheese. This development is determined by the ratio of lactose content to buffering substances in the curd. During ripening, pH typically increases, particularly in smeared surface cheeses, due to metabolic activities such as lactate utilization.

5 Lecture 05: Gas Production and Eye Formation - 24/11-2025

Henrik Siegumfeldt Department of Food Science, 2025

Summary Eye development in cheese is a physical and biochemical process that is governed by specific parameters regarding gas dynamics and the physical state of the cheese matrix. The formation and growth of eyes depend on:

1. Rate and quantity of gas production.
2. Number and size of air pockets and irregularities.
3. CO_2 pressure and diffusion rate.
4. Cheese structure and elasticity.
5. Temperature.

Cheeses are categorized by their internal texture, including round-eyed varieties like Danbo, Gouda, and Edam, which typically utilize LD-starters or secondary Propionic Acid Bacteria (PAB) cultures. Open-textured cheeses

like Havarti and Esrom also use LD-starters, while closed-texture varieties such as Cheddar and Parmigiano-Reggiano are produced without intended gas openings. In a typical 80 kg Emmental cheese, approximately 120 liters of CO_2 are produced; 60 liters remain dissolved in the cheese mass, 40 liters are lost to the environment, and 20 liters remain to form the eyes. Physically, the pressure in a bubble is defined as two times the surface tension divided by the radius. Consequently, larger eyes grow faster because less gas pressure is required to enlarge an existing large hole compared to maintaining small ones.

Gas production originates from four primary metabolic pathways: lactose metabolism, citrate metabolism, lactate metabolism, and amino acid catabolism. Citrate fermentation by homofermentative bacteria like L. lactis ssp. lactis biovar diacetylactis is pH-dependent, with optimal growth between 30-35°C. Heterofermentative organisms like Leuconostoc species produce diacetyl and acetoin below pH 5.8 and avoid acetaldehyde accumulation through alcohol dehydrogenase activity. In Swiss-type cheeses, PAB convert three moles of lactic acid into two moles of propionic acid, one mole of acetic acid, and one mole of CO_2. This eye formation process generally requires two weeks at 10°C to stabilize chemistry followed by three weeks at 17-20°C to stimulate growth.

Butyric acid fermentation, caused by anaerobic spore-forming bacteria such as Clostridium tyrobutyricum, is a detrimental process leading to "late blowing." This reaction converts two moles of lactic acid into butyric acid, two moles of CO_2, and two moles of H_2. This defect is stimulated by high pH, high temperature, and low salt content. Prevention strategies include the addition of nitrate or lysozyme to cheese milk, bactofugation, microfiltration, or the use of nisin-producing starters. Furthermore, Non-Starter Lactic Acid Bacteria (NSLAB) can cause racemisation, converting L-lactate to the less soluble DL-lactate, which forms white crystals on the surface or within cheese holes.

Oxidative metabolism also contributes to gas production; for instance, Penicillium species and yeasts in surface-ripened cheeses like Brie consume lactate and oxygen to produce CO_2 and water. This process raises the pH to 7-8, driving a gradient that leads to the precipitation of calcium phosphate. Additionally, specific amino acid catabolism through decarboxylation produces CO_2 and biogenic amines. Finally, urease-positive strains of Streptococcus thermophilus can metabolize urea into CO_2 and NH_3, which can cause curds to float in the cheese vat, a notable problem in cottage cheese production.

6 Lecture 06: Cheese Proteolysis - 01/12-2025

Henrik Siegumfeldt, Department of Food Science, 2021

Summary
Proteolysis is defined as the hydrolysis of peptide bonds by peptide bond hydrolases. These enzymes are classified by the International Union of Biochemistry and Molecular Biology as Hydrolases (EC 3), specifically those acting on peptide bonds (EC 3.4). While the terms protease and proteinase are frequently used interchangeably, this is not absolutely correct. Proteinases are endo-acting peptide bond hydrolases that act at peptide bonds in the inner regions of the polypeptide chain away from the N and C termini; examples include chymosin, pepsin, and cathepsin D. Peptidases are exo-acting hydrolases that act only near the ends of polypeptide chains, such as aminopeptidases and carboxypeptidases.

The substrates for these reactions are milk proteins, primarily caseins (α_{s1}, β, α_{s2}, κ) rather than whey proteins (β-lactoglobulin, α-lactalbumin, bovine serum albumin). Caseins possess a high proportion of proline residues, resulting in an open flexible structure with no rigid tertiary structure, making them highly susceptible to hydrolysis.

The assessment of proteolysis involves cheese fractionation (pH 4.6 soluble and insoluble nitrogen) and methods such as Kjeldahl, Dumas, and Formol titration to measure peptides, free amino acids, and NH_3. Measuring proteolysis is essential as an:

1. Indication of age and type of cheese.
2. Indication of flavour defects, such as bitterness.
3. Indication of culture lysis or the use of adjunct/ripening cultures.
4. Indication of the specific coagulant used.

The mechanisms of cheese ripening are structured into four primary areas:

1. Primary casein breakdown by indigenous milk proteases and coagulants.
2. Starter proteinase activity.
3. Starter peptidase activity and lysis of starter bacteria.
4. Non-starter lactic acid bacteria (NSLAB) in cheese proteolysis.

Indigenous milk proteases include plasmin, a serine proteinase that dissolves fibrin blood clots and is associated with casein micelles. Plasmin survives high cooking temperatures and is most relevant in high-cook varieties like Swiss or Italian cheeses, where it hydrolyzes β-casein into γ-caseins and proteose-peptones. Coagulants, such as animal rennet or chymosin, are aspartic acid proteinases with low pH optima that are partially inactivated at high cooking temperatures. Approximately 2 - 10% of chymosin is retained in the cheese, where it is responsible for the initial hydrolysis of α_{s1}-casein at the $\text{Phe}_{23} - \text{Phe}_{24}$ bond.

Starter culture activity involves cell-envelope associated proteinases (CEP), also known as lactocepins (e.g., PrtP, PrtB, PrtS), which digest casein peptides produced by rennet. Intracellular degradation is carried out by peptidases, which are released upon bacterial lysis—a process correlated with good flavour production. These include endopeptidases (PepO, PepF), aminopeptidases (PepN, PepC, PepA), proline-specific peptidases (PepX, PepR, PepQ, PepI, PepP), and di/tripeptidases. Finally, NSLAB species such as *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum* are excellent survivors that contribute to peptidolysis and introduce specific flavour notes.

7 Lecture 07: Peptide and Amino Acid Analysis - 01/12-2025

René Lametsch, Associate Professor, University of Copenhagen, 2025

Summary

High Pressure Liquid Chromatography (HPLC) is utilized for the analysis of amino acids through a system comprising solvent pumps, a mixer, a sample injection unit, a column, and a detector integrated with a data system. The basic principles of separation within the HPLC column follow a specific methodological procedure:

1. Equilibration (typically over 10 column volumes).
2. Sample injection volume.
3. Gradient elution (utilizing a gradient of Acetonitrile from 0% to 80%).
4. Wash (typically over 5 column volumes).
5. Re-equilibration.

Separation is achieved using specialized HPLC columns, such as C18. The detection of amino acids requires

a derivatization reaction where the amino acid reacts with ortho-phthalaldehyde (OPA) and N-acetylcysteine to form a product measurable by a fluorescence detector. Quantification is performed by identifying individual peaks on a chromatogram based on retention time, allowing for the measurement of compounds such as aspartic acid, glutamic acid, asparagine, serine, and various hydrophobic and charged amino acids. Specific applications of this technique include the analysis of Danbo and Grana Padano cheeses, where the Total Ion Count (TIC) and UV-280nm absorbance are used to characterize the profiles.

Protein and peptide characterization is further conducted using Ultra High Pressure Liquid Chromatography (UHPLC) coupled with Mass Spectrometry (MS), specifically employing equipment such as the Exploris 480. The standard identification workflow includes:

1. Enzyme digestion of the protein sample.
2. 1D or 2D chromatography for separation.
3. MS1 analysis to determine the total ion count and mass-to-charge (m/z) ratios.
4. Gas phase fragmentation of selected peptides.
5. MS2 analysis to generate MS/MS spectra.
6. Peak picking and matching through a search engine against protein and DNA sequence databases.

Peptide identification relies on understanding peptide bond fragmentation, which occurs at specific sites designated as a, b, c (N-terminal) and x, y, z (C-terminal). Comparison of observed b-ion and y-ion sequences against theoretical mass tables allows for precise sequence determination.

Methodological research into plant-based cheese proteolytic activity has investigated the impact of *Lactobacillus helveticus* strains on soy protein isolates (SPI). SDS-PAGE analysis is used to monitor the degradation of soy protein subunits, including lipoxygenase, β-conglycinin, and glycinin, over a fermentation period of 7 days. Results are visualized through peptigrams that map peptide intensity across the protein sequence and 3D structural models of proteins like glycinin G1 to predict cleavage sites. Free amino acid concentrations, particularly alanine, arginine, glutamic acid, and lysine, are quantified to assess the extent of proteolysis during fermentation.

8 Lecture 08: Aroma - 08/12-2025

Sylvester Holt, Department of Food Science, 2025 Summary Aroma is defined as something smelled orthonasally or retronasally, whereas taste depends on basic sensations categorized as sour, salt, sweet, bitter, and umami. Flavour is the combination of retronasal odour, taste, and chemical feeling. Aroma compounds are volatile organic compounds that interact with approximately 300 different odour receptors in the olfactory system and are active at very low quantities. These compounds belong to various classes, including sulfur compounds, aldehydes, ketones, esters, acids, and alcohols. Major biochemical pathways for aroma formation in dairy include proteolysis of casein into peptides and free amino acids, citrate conversion into diacetyl, lipolysis of triglycerides into free fatty acids, and glycolysis of lactose into lactate.

The analysis of aroma compounds in cheese involves several critical methodological steps:

1. Sample preparation (cutting, grating, mixing with solvent).
2. Isolation of aroma compounds.
3. Separation of aroma compounds via GC - gas chromatography.
4. Identification of aroma compounds via MS - mass spectrometry.

Isolation techniques include headspace methods such as static headspace sampling (SHS), which mimics direct smelling by analysing the equilibrium between the sample and the headspace. Dynamic headspace sampling (DHS) continuously removes the headspace so that equilibrium is never reached, providing higher sensitivity than solid phase micro extraction (SPME). SPME relies on the partition of volatiles between the headspace and a thin polymeric fiber. Other methods include solvent assisted flavour evaporation (SAFE) and liquid/liquid extraction. Key parameters affecting the concentration of volatiles in the headspace include time, temperature, salts, and the mixture of solvents.

Separation is achieved through Gas Chromatography (GC), which is used for thermally stable volatile compounds based on their boiling points and interactions with a stationary phase. Identification is primarily performed using Mass Spectrometry (MS) through charged molecular fragments and library matching. Challenges such as the co-elution of compounds like 2-methylbutanal and 3-methylbutanal can be resolved using multidimensional PARAFAC2 modelling, which requires MS data. Additionally, Gas Chromatography-Olfactometry (GC-O) utilizes the human nose as a detector to identify odour-active compounds.

Quantification is typically conducted via internal standards or standard addition. Because aroma compounds are present in low concentrations (ppb-ppm), they must be concentrated prior to analysis. Absolute quantification is often tedious, and raw peak areas are frequently used as relative measures of concentration. Experimental results indicate that standard curves can vary significantly depending on the cheese matrix, such as Dubliner, Kadett, or Samsø, due to differences in fat, moisture, and amino acid content. For the course report, students must utilize phenotypic clustering heatmaps to identify compounds at high levels in their specific cheese and describe their biochemical formation pathways.

9 Lecture 09: Amino Acid Catabolism - 15/12-2025

Henrik Siegumfeldt, Department of Food Science, 2025

Summary

Amino acid catabolism in dairy products is a fundamental process for flavour development in both fermented milk and ripened cheese. This metabolic activity involves a diverse range of microorganisms, including LAB such as *Lactococcus lactis* and *Streptococcus thermophilus*, as well as other bacteria like *Brevibacterium linens* and *Propionibacterium*, and various yeasts and moulds. LAB require amino acids for four primary functions:

1. Protein and peptide synthesis.
2. Energy production (ATP and proton-motive force).
3. Maintenance of internal pH in acid environments.
4. NADH/NAD^+ (NADPH/$NADP^+$) regeneration.

The catabolism of amino acids, which exist as 20 basic types in proteins primarily in their L-isomeric form, is facilitated by five main groups of enzymes. Lyases include Threonine aldolase, which converts threonine into glycine and acetaldehyde; the latter is a critical component of yoghurt flavour. Other lyases, such as cystathionine β-lyase (CBL) and methionine γ-lyase (MGL), are responsible for producing volatile sulphur compounds like methanethiol and dimethyldisulfide, which are found in high concentrations in surface-ripened cheeses. Dehydratases act anaerobically on amino acids containing hydroxy or sulphur groups, such as serine, threonine, and cysteine.

Decarboxylases convert free amino acids into CO_2 and biogenic amines. Significant reactions include the conver-

sion of histidine to histamine, tyrosine to tyramine, and glutamate to γ-amino butyrate (GABA). Biogenic amine formation is important due to potential food intoxication effects, such as histamine intolerance (HIT), which can trigger symptoms like facial flushing, headache, and abdominal pain. Deaminases, particularly the arginine deiminase (ADI) pathway, allow for the direct production of ATP from amino acids and help regulate internal pH, increasing bacterial survival in acidic conditions.

Aminotransferases catalyze the substitution of an amino group with an oxo-group, typically utilizing α- ketoglutarate as an acceptor to produce glutamate and an α-keto acid. Specific aminotransferases exist for branched-chain (Leu, Ile, Val), aromatic (Phe, Tyr, Trp), and sulphur-containing (Met) amino acids. Glutamate dehydrogenase (GDH) is essential in this process because it regenerates α-ketoglutarate from glutamate, ensuring that aminotransferase reactions can continue even when α-ketoglutarate is limited in the cheese. These pathways result in various flavour compounds; for instance, leucine is converted to 3-methylbutanal (isovaleraldehyde), while aspartic acid catabolism leads to the formation of diacetyl, acetoin, and butanediol.

Methodological approaches to screen for flavour-producing strains include:

1. Genotyping through PCR methods and sequencing to test for the presence of specific genes. item Enzymatic analysis using permeabilised cells, crude extracts, or pure preparations.
2. Physicochemical analysis, including GC-MS for volatiles and HPLC for amino acids and carboxylic acids.

10 Lecture 10: Cheese Flavour - 15/12-2025

Henrik Siegumfeldt, Department of Food Science

Summary
Flavour formation in cheese involves complex biochemical, chemical, and physical processes during manufacture and ripening. The development of flavour is categorized into seven primary metabolic pathways:

1. Lactose and citrate metabolism - Acid and buttery flavour.
2. Peptides - Bitter and umami tastes.
3. Free amino acids - Salt, sweet, acid, bitter & umami.
4. Amino acid catabolic products - Specific cheese flavours.
5. Esters - Fruity flavours.
6. Thioesters - Cauliflower and cabbage flavours.
7. Fatty acids and methyl ketones - Mouldy and Italian cheese flavours.

Lactose and citrate fermentation is conducted by homofermentative citrate-positive LAB, such as *L. lactis* subsp. *lactis* biovar *diacetylactis*, which produces diacetyl and acetoin in a pH-dependent manner with an optimum growth temperature of 30-35°C. In heterofermentative citrate-positive LAB, such as Leuconostoc species, production occurs below pH 5.8, and acetaldehyde does not accumulate due to alcohol dehydrogenase activity.

Proteolysis results in peptides and free amino acids which contribute to background flavours and taste. Many amino acid sequences in casein can become bitter peptides. Properties that increase the bitter flavour of peptides include:

1. Hydrophobicity.
2. Content of proline residues which makes the molecule bulky.
3. Positively charged amino end.
4. A size of about 2 - 12 amino acids.

5. Large peptides may also be bitter-astringent.

The risk of bitterness is generated by the coagulant type, specifically microbial rennet with a low clotting (C) to proteolytic (P) ratio, and starter culture characteristics such as PI type proteinase and low peptidase activity. Conversely, di- and tri-glutamyl peptides contribute to savoury, umami, and kokumi tastes, showing synergy with glutamic acid and salt.

Amino acid catabolism produces specific cheese aromas including ketones, aldehydes, acids, alcohols, and sulfur compounds. Enzymes involved include decarboxylases, aminotransferases, deaminases, lyases, and dehydratases. Aminotransferases require α-ketoglutarate, while glutamate dehydrogenase (GDH) is crucial for regenerating α-keto-glutarate in the cheese. Non-starter lactic acid bacteria (NSLAB) play a vital role because α-keto acid decarboxylase is rarely found in dairy starter cultures.

Lipolysis involves the breakdown of triglycerides into fatty acids, methyl ketones, and lactones. Methyl ketones, especially 2-heptanone and 2-nonanone, are present at high concentrations in blue mould cheese and are produced by Penicillium roqueforti. Esterification is a reaction between an alcohol and a free fatty acid, primarily occurring in long-ripened cheeses with low water activity. Thioesters, responsible for cauliflower and cabbage flavours, are formed through metabolic cooperation on cheese surfaces between microflora like Brevibacterium linens and Staphylococcus. Fat serves both as a source of aroma components and as a solvent for aroma compounds derived from other metabolic pathways. Over 600 volatile compounds have been identified in cheese using methods such as GC-Olfactometry, GC-MS, and the Nasal Impact Frequency (NIF) method.

11 Video Lecture: Fermented Milks - 22/12-2025

11.1 Introduction and Process Examples

Anni Hougaard, Department of Food Science, University of Copenhagen

Summary
Milk and butter-based culture has been central to the Nordic diet since the Middle Ages. Due to short summers and grazing periods, Nordic countries developed a storage culture to preserve food for winter, leading to the traditional consumption of milk as fermented products, including cheese. These traditional products are primarily based on mesophilic cultures, with the exception of skyr. Fermented milk products offer several advantages, including increased shelf life, improved digestibility and bioavailability of nutrients, utility for individuals with lactose malabsorption, and use as functional foods containing probiotics.

The manufacture of yoghurt, which includes both set and stirred varieties, follows a standardized preliminary treatment. The process requires standardizing the fat content to <0.5-3.23 g 100 g^{-1} and increasing the protein content by 1-3 g 100 g^{-1} SMP, 1-2 g 100 g^{-1} WPC, or through evaporation or UF retentate. The processing stages are:

1. Homogenise the milk base at 60-70°C and 15-20 MPa pressure.
2. Heat to 80-85°C for 30 min or 90-95°C for up to 5 min.
3. Cool to 37-45°C and inoculate with starter culture.
4. For set-type: add flavouring, fill containers, and incubate until pH ≈4.6.
5. For stirred-type: fill fermentation tank, incubate until pH ≈4.6, pre-cool to 20-25°C, mix with fruit and package.

6. Blast cool to <5°C, transfer to cold store and dispatch.

Alleged nutritional additions to yoghurt may include prebiotics, dietary fibre, phytosterols, dairy derived ingredients (lipids, proteins, peptides), fatty acids, minerals, and various vitamins.

Kefir is produced through traditional, industrial, or Russian/European methods using kefir grains (2-10%) or commercial cultures. Its complex microbial composition includes *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*, and various *Lactobacillus* species such as *L. kefiri* and *L. acidophilus*. Yeasts such as *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* are present, alongside other species like *Acetobacter aceti* and the mould *Geotrichum candidum*.

Buttermilk was traditionally a by-product of butter manufacture from fermented 40% cream using mesophilic starters in churns. While continuous butter-making led to buttermilk produced from fermented skim milk, "traditional buttermilk" is produced from 6-8% fat cream in churns to maintain quality and minimize oxidation risk. Koldskål is a Danish sweet cold dairy beverage made from buttermilk with eggs, sugar, cream, tykmælk, vanilla, and lemon, traditionally served with crispy biscuits called kammerjunker.

Concentrated fermented milks, such as Skyr and Ymer, utilize different concentration methods including cloth filtration, nozzle separators, or ultrafiltration. Skyr is an ancient product, at least 1000 years old, traditionally involving Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Traditional Nordic products like *Tettemelk*, *Långfil*, and *Viili* utilize ropy cultures that produce exopolysaccharides (EPS) to achieve texturizing effects. Combining rennet and acid gelation results in stiffer gels with a higher propensity for syneresis.

11.2 Changes In the Micelles

Richard Ipsen, Department of Food Science, University of Copenhagen

Summary
The acidification of skim milk results in an initial decrease of the average micellar mass and radius alongside a redistribution of mass within the micelles. This reduction is primarily caused by the dissolution of small units of material from individual micelles, triggered by the loss of colloidal calcium phosphate (CCP) at lower pH levels. At neutral pH, approximately 30% of the calcium in milk is in ionic form, with the remainder bound to the micelles as CCP. As pH decreases, there is a gradual increase in ionic "free" calcium, while colloidal calcium and phosphate exhibit a concomitant decrease until pH 5.5 is reached. Following this point, more calcium is removed from the micelle than phosphate. After reaching a pH of about 5.5, previously dissolved casein molecules, which gradually lose their charge and become more hydrophobic, reassemble onto the micelle.

The stability of the casein micelle is maintained by the glycomacropeptide (GMP) part of κ-casein, which is hydrophilic and flexible, sticking out into the serum phase to provide steric stabilization. As the pH approaches the isoelectric point (pI), this protective layer collapses and steric stabilization disappears, making interactions between individual micelles possible. This transition is steep and irreversible below a certain pH minimum, approximately 5 at 20°C. The resulting aggregation of casein micelles leads to an increase in viscosity and particle size. Measurement of the zeta potential confirms that the negative charge of the micelle decreases as pH levels drop.

The dissociation and re-association of caseins are governed by several factors:

1. Temperature and the hydrophobic effect: Much dissociation occurs at 4°C, while only slight dissociation

occurs at 30°C, with most casein remaining in the micelles above 25°C.
2. Composition: Mainly β-casein and κ-casein dissociate independently rather than as constant complexes.
3. Isoelectric precipitation: When pH approaches the isoelectric point, casein is associated back onto the micelles, meaning the original structure is not reformed.

Heat treatment significantly modifies these processes by causing whey proteins to denature when temperatures exceed ∼60°C. The free thiol group of β-lactoglobulin is activated, leading to the formation of micelle-bound complexes through covalent disulphide bonds to κ-casein or the formation of soluble aggregates in the serum phase. These complexes are formed through hydrophobic interactions and inter-molecular SS bonds between whey proteins, κ-casein, and traces of α_s-caseins. In heated milk, the associated whey proteins affect the microstructure of the resulting acid milk gel, increasing the storage modulus (G') and the pH of gelation.

Structure formation in these systems follows a defined sequence:
1. Initial gel network is formed as a result of whey proteins binding to the casein micelles and/or forming soluble aggregates.
2. Loosening of casein micelles occurs due to the continued loss of calcium.
3. Casein micelles fuse to form the final network as pH approaches the isoelectric point of casein (∼4.8).

11.3 Processing and Technologies

Richard Ipsen, Department of Food Science, University of Copenhagen

Summary
The production of fermented milk products begins with the selection of a substrate for fermentation, which is influenced by milk type, cattle breed, genetic variants, and seasonal variations. To prepare the milk base, manufacturers may increase the dry matter percentage through boiling, evaporation, or ultrafiltration, followed by standardization and the potential addition of stabilizers, emulsifiers, or fat replacers. Flavoring ingredients, such as vegetables, fruit, or cereals, may also be incorporated into the substrate.

The manufacture of these products follows a sequence of basic production steps designed to ensure safety and desired physical properties:
1. Homogenization (50-70°C, 10-20 MPa) to improve the viscosity of the final product.
2. Heat treatment to destroy pathogenic organisms, produce stimulating or inhibitive factors for the starter culture, and induce physical-chemical changes.
3. Fermentation, where temperature, time, and pH are controlled using specific starter organisms, often in a Direct Vat Set (DVS) state.
4. Whey drainage for specific varieties such as ymer or labneh.
5. Cooling and stirring, which determine the final pH and viscosity.
6. Further processing, which may include drying, freezing, or additional heat treatment before filling and packaging.

Technological variations define specific regional and traditional products. In Scandinavia and Denmark, traditional buttermilk was historically a byproduct of butter manufacture from fermented 40% cream. This process utilized mesophilic starters, specifically *Lactococcus lactis* ssp. *lactis* biovar *diacetilactis* and various *Leuconostoc* subspecies, resulting in a product with a high content of phospholipids derived from the milk fat globule membrane.

Concentrated fermented milk products are characterized by the removal of whey. Skyr, an Icelandic product with a 1000-year history, is produced from skimmed milk using a thermophilic starter and the addition of cheese rennet, with the whey traditionally drained in cotton bags. Ymer, developed in Denmark in 1937, is produced by heating mesophilic fermented skimmed milk to approximately 50°C, causing the curd to rise due to CO_2 production. After the whey is drained, cream is mixed back in to reach a final fat content of 3.5%.

Yoghurt manufacture is categorized into stirred yoghurt, where fermentation occurs in tanks, and set yoghurt, where fermentation takes place in the final package. Across all varieties, heat treatment is considered crucial to the resulting texture and consistency. These fermented milks serve as effective carriers for probiotic bacteria, making them common functional foods that can be successfully industrialized while maintaining traditional characteristics.

11.4 Structure Formation

Richard Ipsen, Department of Food Science, University of Copenhagen

Summary
Structure formation in fermented milks is fundamentally dependent on heat treatment and the resulting effects on the whey proteins. The development of the gel network during acidification is categorized into specific pH intervals that dictate the physical state of the protein matrix:

1. pH 5.8-5: The number of protein aggregates decreases in the vicinity of the gel point as the network forms. In heat-treated milk, the initial network is formed as a consequence of whey protein associated with the micelle, whereas in unheated milk, the solubilization of Ca^{2+} continues after the gel point.
2. pH 5-4.6: Gel stiffness increases most significantly in this interval, independent of heat treatment. The network contracts and pore size increases due to rearrangements. Changes in this regime are primarily a consequence of micellar interactions.
3. pH 4.6-4.2: The network achieves its final structure. Ionic interactions have a maximum near pH 4.6, while hydrophobic interactions, hydrogen bonds, and disulphide bonds also exert effects.

Protein content and dry matter (DM) enrichment are essential for texture development. It is the protein content that primarily exerts an effect on texture, and the type of dry matter used is important. The addition of skim milk powder or evaporation results in less protein concentration than ultrafiltration. Manufacturers often use tailormade ingredients, typically a mix of casein/caseinates and whey protein.

Heat treatment intensity significantly impacts the final network. Unheated milk produces a coarse network with large pores and casein aggregates between 0.5-1 μm, while heat-treated milk produces a finer network with smaller pores and aggregates approximately half that size. The optimum temperature for stirred yoghurt is in the range of 82-93°C for 12-25 min; however, heat treatment can be too intensive, resulting in a coarse network. Homogenization improves texture through the direct incorporation of fat globules into the protein network as active fillers, where casein covers the fat globules so they act as building blocks.

The best texture and lowest whey separation are provided by an intermediate to high inoculation rate (e.g., 2% bulk starter) and a low incubation temperature of approximately 40°C. Temperature and mechanical treatment after fermentation are also crucial. Stirred yoghurt cooling is carried out via agitation in the vat or using plate/tubular coolers, which can result in structural damage. Filling at 20-22°C is considered a compromise that allows for the rebuilding of mechanically damaged structures while avoiding the risk of acidification proceeding too far in the package. Final cooling results in increased firmness and viscosity due to the increased size and contact area of

casein aggregates.

12 Lecture 11: Cheese Defects - 05/01-2026

Anni Bygvrå Hougaard, University of Copenhagen, 2025-26

Summary
Cheese defects are broadly categorized into rind, body, taste, and color defects resulting from milk composition, inappropriate use of starters or coagulants, brine and salting issues, or ripening and packaging conditions. Early blowing is a defect characterized by the appearance of holes after salting but before ripening, typically within 24 to 48 hours in continental cheeses. This is caused by microbial contamination from coliforms, heterofermentative bacteria such as *Leuconostoc* and *Lactobacillus* species, or yeasts, which ferment lactose into lactic acid, acetic acid, ethanol, CO_2, and H_2. Prevention strategies for early blowing include:

1. Finding and cleaning the contamination source (pipes, tanks, tools, and hygiene).
2. Implementing pasteurization.
3. Using highly active starter cultures to ensure pH falls below 6.0 as quickly as possible.
4. Monitoring phage pressure in the plant.

Late blowing appears after several weeks of ripening, ranging from 15 days to 2 months, due to the detrimental activity of anaerobic spore-forming, lactate-fermenting bacteria such as *Clostridium tyrobutyricum*, *Cl. sporogenes*, *Cl. butyricum*, and *Cl. beijerinckii*. This reaction converts two moles of lactic acid into butyric acid, two moles of CO_2, H_2O, and two moles of H_2. Growth is stimulated by high pH, high temperature, low salt content, and high spore numbers. Prevention of late blowing involves adding nitrate or lysozyme to cheese milk, bactofugation, microfiltration, using nisin-producing starters, or employing anticlostridial *Lactobacillus* from cheese microflora.

Texture defects such as cracks and slits are most frequently reported in Cheddar cheese when *Streptococcus thermophilus* is part of the starter. Possible mechanisms include the fermentation of residual galactose by non-starter lactic acid bacteria (NSLAB), decarboxylation of amino acids like glutamic acid, histidine, and tyrosine, or inadequate pressing. Color defects include pink discoloration, which can be caused by the carotenoid-producing thermophile *Thermus thermophilus* in cheeses without colorants. Browning in blue cheese is associated with *Yarrowia lipolytica*, where tyrosine degradation leads to the accumulation of brown pyomelanin pigments, a process stimulated by high manganese content.

Crystal defects arise from the racemisation of L-lactic acid to DL-lactic acid by NSLAB, as DL-lactate is less soluble and forms small white crystals near surfaces and in holes. Tyrosine crystals, known as "pearls," typically form in long-ripened, low-water-content cheeses like Parmigiano-Reggiano due to the low water solubility of tyrosine. Bitterness is caused by "unbalanced" proteolysis resulting in a buildup of hydrophobic peptides, particularly from the C-terminal region of β-casein. The risk of bitterness is influenced by:

1. Coagulant type: Low Clotting/Proteolytic (C/P) ratios, such as in bovine pepsin or certain microbial rennets, increase risk.
2. Quantity and retention: High rennet levels and lower pH at whey drainage retain more chymosin.
3. Starter culture: PI "bitter" strains, slow cell lysis, or low peptidase activity.

Biogenic amine formation, particularly histamine levels exceeding 400 ppm, is a concern in long-ripened cheeses. The highest risks are associated with raw milk, poor hygiene, low salt, and extended ripening times. There is currently no regulatory control of biogenic amines in cheese, and biogenic amine-negative starter cultures do not

guarantee a defect-free product.

13 Lecture 12: Engineering of Cheese Ripening - 05/01-2026

Anni Bygvrå Hougaard, Associate professor FOOD, 2025-26

Summary
The engineering of cheese ripening involves the manipulation of specific parameters, including ripening time, fat and salt levels, fat composition, and lactose levels, to address production costs and consumer health. Accelerated cheese ripening (ACR) aims to reduce ripening time while maintaining flavour, texture, and safety. ACR focuses on proteolysis and amino acid catabolism as essential prerequisites for flavour development. There are five primary approaches used to achieve ACR:

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

Elevated ripening temperature is the simplest and most frequently used method, technically simple with no legal barriers, though it requires high microbiological quality milk to avoid off-flavours and microbial spoilage. Addition of exogenous enzymes, such as proteases and lipases, offers specific action but is limited by high costs and difficulties in achieving uniform distribution. Adjunct or attenuated cultures—which may be wild type, mutant, or heat/freeze shocked—utilize the full enzyme complement of lactic acid bacteria to increase proteolysis and amino acid catabolism simultaneously. High-pressure treatment (300–600 MPa) promotes bacterial lysis and makes the casein matrix more susceptible to proteolysis.

Reduced fat cheese manufacture involves decreasing fat levels and compensating with increased moisture and protein. This results in a dense protein matrix, leading to a rubbery texture and higher concentrations of bitter peptides. To produce these cheeses, manufacturers must limit syneresis through:

1. Lower acidification rates and higher pH at whey drainage.
2. Decreased coagulant addition.
3. Lower cooking temperatures.
4. Shorter holding and stirring times.

Reduced salt cheese addresses the correlation between sodium consumption and hypertension. Sodium chloride acts as a hurdle against pathogens and modulates the activity of rennet, plasmin, and starter cultures. Salt reduction typically decreases starter lysis and free amino acid levels while increasing the levels of bitter peptides such as β-CN(f193-209). Favourable flavour formation in salt-reduced cheeses requires additional actions, such as selecting highly autolytic starter cultures or using low-proteolytic coagulants like camel chymosin.

Other specialized engineering includes vegetable fat cheeses, where saturated milk fat is replaced by oils such as corn or wheat germ oil to reduce cholesterol. Finally, lactose-free cheese is produced through lactose hydrolysis using lactase. This can be conducted for 1-2 hours at 37°C or 12-14 hours at 4°C, with the latter providing a lower risk of off-flavours. Products like Kees Extra Gerijpt contain 60% less saturated fat and 30% less salt than

standard varieties.

14 Lecture 13: Cheese Curd Formation - 07/01-2025

Anni Bygvrå Hougaard, Associate professor FOOD, University of Copenhagen, 2025-26

Summary
The process of cheese curd formation is systematically categorized into four primary stages:

1. Enzymatic reaction.
2. Aggregation.
3. Gelation.
4. Syneresis.

The enzymatic phase involves the interaction between a coagulant (rennet) and the casein micelles. Coagulants are derived from animal, microbial, fungal, or vegetable sources, or produced via fermentation (FPC), with activity measured in International Milk Clotting Units (IMCU) per ml. Chymosin specifically targets κ-casein, which contains 169 amino acids. The glycomacropeptide (GMP) portion, representing 36-37% of the κ-casein molecule, is negatively charged and provides steric and electrostatic stabilization to the micelle. During this phase, chymosin "shaves" the hairy layer of the micelle, reducing its diameter by approximately 12 nm. Hydrolysis of κ-casein follows first-order kinetics; however, aggregation of micelles does not begin until approximately 85% of the total κ-casein has been hydrolyzed. For a single micelle to aggregate, approximately 97% of its κ-casein must be hydrolyzed.

Aggregation is a second-order bimolecular process involving particle collisions. Micelle stability is maintained by overlapping hairy layers that provide steric repulsion (entropic and osmotic models) and electrostatic repulsion. High-tech measurement techniques include Dynamic Light Scattering (DLS) to monitor hydrodynamic radius and ζ-potential (Zeta potential) to measure electrical potential at the slipping plane. Theoretical interpretations of clotting time often utilize the Holter-Foltmann relation, while the Energy Barrier Model describes the kinetics of aggregation by linking the enzymatic reaction to a linear reduction in the energy barrier. Fractal aggregation simulations indicate that the fractal dimension (D) for rennet-induced aggregates is generally between 2.2 and 2.6.

Gelation is studied using Small Amplitude Oscillatory Rheology to determine the elastic modulus (G') and viscous modulus (G"). The storage modulus continues to increase long after κ-casein hydrolysis is complete due to the continuing incorporation of additional casein into the network, the fusion of junctions creating more bonds, and the physical rearrangement of strands. Factors influencing gelation include:

1. Casein and enzyme concentration: Higher concentrations shorten coagulation time and increase firming rates.
2. Calcium levels: Increased soluble and colloidal calcium (typically 33% and 66% of total Ca, respectively) improves gel strength. item pH and Temperature: Optimum firming occurs near pH 6.25, while temperatures above 40°C can inactivate chymosin.
3. Heat treatment: Strong heat treatment denatures whey proteins, which associate with the micelle surface and slow gelation.

Syneresis is the spontaneous expulsion of whey from the porous casein network, which regulates moisture, lactose, and mineral content in cheese. The process is driven by microsyneresis (junction rearrangements) creating endogenous syneresis pressure. Local whey transport is described by Darcy's equation, where flow velocity de-

pends on the permeability coefficient (B), whey viscosity (η), pressure (p), and path length (l). Smaller curd grain sizes (ranging from 2-5 mm for hard cheese to 15 mm for soft cheese) provide more surface area and faster syneresis. Other variables increasing syneresis include lower pH, higher temperatures (enhancing hydrophobic interactions), and mechanical stirring, whereas homogenization and whey protein denaturation reduce it.

15 Lecture 14: Cheese Varieties - 07/01-2026

Anni Bygvrå Hougaard, Associate professor FOOD, University of Copenhagen, 2025-26

Summary
The recognition and standardization of cheese varieties began with the International Dairy Federation (IDF) in 1903 and the Stresa Convention in 1951. The Stresa Convention served as the first international agreement on cheese names and established two levels of protection through distinct appendices:

1. Appendix A: Protection of names that can only be produced in a specific place or origin, including Roquefort, Gorgonzola, Parmigiano-Reggiano, and Pecorino Romano.
2. Appendix B: Mutual permission to use 30 cheese names on domestic and international markets based on product characteristics rather than origin.

National standards in Denmark subsequently renamed several varieties, such as changing Steppeost to Danbo and Dansk Schweizer to Samsø. The Codex Alimentarius, created in 1963 by the FAO and WHO, develops international standards for generic cheese varieties relevant to global trade. It maintains five horizontal cheese standards, including a general standard and specific standards for unripened, brine, extra hard grating, and whey cheeses. Individual standards, such as for Cheddar, define permitted ingredients like starter cultures, rennet, and sodium chloride, while regulating food additives including colors (e.g., Annatto) and preservatives like sodium nitrate and nisin.

Geographical Indications (GIs) in the European Union are justified by the concept of terroir, which posits that a product's qualities are derived from the natural and human factors of its specific territory. The EU system, which came into effect in 1992, identifies three designations:

1. Protected Designation of Origin (PDO): The product must be produced, processed, and prepared in the geographical area, with characteristics essentially due to that area.
2. Protected Geographical Indication (PGI): The product must be produced, processed, or prepared in the area, with a reputation or quality attributable to it.
3. Traditional Speciality Guaranteed (TSG): The product must be traditional (25 years) or established by custom but has no link to a specific geographical area.

Specific examples include Danbo (PGI), which must be produced and matured in Denmark, and Mozzarella (TSG), which requires a particular recipe involving a natural starter and bovine rennet with 20-30% pepsin. Feta (PDO) is produced from a mixture of sheep (min. 70%) and goat milk and ripened for at least two months. Cabrales (PDO) is ripened in caves for at least two months using wild complex microflora.

While GIs provide competitive advantages and added value for producers, they have become a contentious international trade issue, specifically between the US and the EU. Additionally, varieties like Cabrales present safety considerations, as tyrosine decarboxylase activity can lead to tyramine levels of 1000 mg/kg, resulting in facial flushing and headaches. Similarly, West Country Farmhouse Cheddar (PDO) requires milk from specific UK counties and maturation for at least 9 months. Swiss classification uses the AOP system, where Emmentaler AOP

requires production in cooperative dairies using raw milk sourced within a 30 km radius without additives.

Chapter 2
Literature résumés

This section of the course notes is designed to streamline access to the key findings from each reading material (RM), providing a concise and accessible overview of essential information. Created through experimentation with various AI platforms, this chapter also serves to enhance my prompt engineering skills, exploring diverse methods of note-taking for maximum efficiency and clarity. The procedures for creating these summaries have varied, but all methods share a common approach: each RM has been fully read, with summaries and notes prepared after completing each respective subsection. By using these AI-co-op'ed approaches, these notes aim to be both a reliable reference and a resource for continuous improvement in capturing complex concepts.

1 RM 01: BCPP - Chapter 01

Ylva Ardö and E. Waagner Nielsen, 2017, Department of Food Science University of Copenhagen, Denmark

Summary
Cheese is produced as a concentrate of milk nutrients through the coagulation of milk by rennet enzymes or acidification. The resulting coagulum undergoes syneresis, which is the contraction of the cheese curd and the expelling of whey consisting of water and water-soluble milk compounds. Syneresis is accelerated by cutting the gel, heating, and stirring to produce firm curd grains that may be pressed and formed into various shapes. Preservation is maintained through low moisture content, acidification by lactic acid bacteria, and salting. Spoilage by moulds on the surface is prevented by drying, cleaning, oxygen exclusion through brine or coatings, or by stimulating a controlled surface microflora.

Cheese milk treatment involves keeping milk at low temperatures to prevent the growth of detrimental bacteria. Pasteurization at 72°C for 15 seconds typically kills about 99% of raw milk bacteria and inactivates 95% of milk lipase, which contributes to why raw milk cheeses often develop a richer flavour. More intense heating denatures whey proteins, causing them to precipitate with caseins and increase total protein retention, though it may result in slower renneting and a weaker coagulum. Spore-forming bacteria like *Clostridium tyrobutyricum* are removed via bactofugation, which typically removes more than 90% of spores, or microfiltration, which can remove more than 99.9% from the skim milk part. Ultrafiltration concentrates casein micelles and whey proteins into a retentate, allowing for higher cheese yields since whey proteins are retained.

In renneted cheese production, starter cultures produce lactic acid from lactose. Mesophilic starters are used when the curd is heated up to approximately 40°C, while thermophilic cultures are used for cheeses scalded at higher temperatures. Following acidification, syneresis is controlled through specific moulding methods:

1. Curd grains are pressed under whey before moulding to avoid air between the cheese grains (round eyed cheeses).
2. Curd grains are pressed under whey before moulding and then boiled in whey (Halloumi cheese).
3. Grains are separated from the whey before moulding so that air is trapped in the curd to create an open texture (Havarti).
4. Grains are separated from the whey and the curd is left for continued acidification (cheddaring) and then milled, salted and moulded (Cheddar).
5. Concentrated retentate from ultrafiltration is cast directly in moulds or packaging (white salad cheese).
6. After acidification to pH 4.6, the whey is separated from the precipitated curd (Quarg, Skyr).
7. Grains of acid precipitated coagulum are separated from the whey and packed as loose grains (Cottage cheese).

Salting is essential for preservation, taste, and consistency, with most semi-hard cheeses requiring 1 to 2% NaCl. Salting occurs by adding salt to the curd before moulding or by diffusion into the cheese through immersion in 20 to 22% NaCl brine at 10 to 15°C. During ripening, which can last weeks or years, casein, fat, lactose, and citrate are catabolised. Proteolysis breakdown significantly influences structure and produces peptides and amino acids that contribute to the background flavour, while lipolysis hydrolyzes milk fat. Final finishing includes vacuum packing, waxing, or wrapping in foil to prevent moisture loss.

2 RM 02: BCPP - Chapter 06

Ylva Ardö and E. Waagner Nielsen, 2017

Summary
The recovery of milk solids and the resulting yield of cheese are fundamentally determined by the gross composition of the milk source and the efficiency of the coagulation process. When milk is coagulated by rennet or acid, caseins form a three-dimensional network that encloses other constituents. As the coagulum shrinks during syneresis, water and soluble compounds are squeezed out as whey, while larger particles like fat globules and bacteria are retained.

Retention of protein is primarily focused on caseins, which constitute 75 to 80% of milk proteins and are defined by their insolubility at pH 4.6. In rennet-induced coagulation at pH 6.6, the enzyme catalyzes the hydrolysis of κ-casein into para-κ-casein and glyco-macro-peptide (GMP). While para-κ-casein remains in the micelles, the soluble GMP follows the whey. Typical protein retention in rennet cheese is 76%, though actual values range from 75 to 78%. Total retention of nitrogen compounds is influenced by several factors:

1. Heat treatment of milk: This causes denaturation of whey proteins, which then precipitate with caseins to increase retention.
2. Ultrafiltration: This method concentrates casein micelles and whey proteins, leading to higher cheese yields.
3. Biological quality: Inflammation of the udder (mastitis) or the growth of proteolytic psychrotrophic bacteria can decrease protein retention.
4. Fines in the whey: Small particles of precipitated proteins lost during cutting represent a loss of para-casein.

The concept of excluded water is critical for calculating retention; approximately 2.6 g of water per g of cheese protein is unavailable as a solvent for whey proteins, and 0.3 to 0.5 g per g of protein is unavailable for lactose. Retention rates for other milk solids are as follows:

1. Fat and bacteria: Retention is high, typically between 85 and 95%, depending on the size of the grains and homogenization.
2. Lactose: Retention is low (3 to 5%) as it is dissolved in the water phase.
3. Ash compounds: Hard and semi-hard rennet cheeses retain 35 to 40% of ash, whereas acid-precipitated cheeses retain only 10 to 15% because minerals like calcium and phosphate dissolve as pH decreases.
4. Citrate: Retention is approximately 10%, depending on the moisture content and acidification during syneresis.

The calculation of cheese yield and composition is based on these retention coefficients applied to the standardized milk solids. For a semi-hard cheese like Danbo or Gouda, the total milk solids in the curd are combined with added NaCl to determine the final dry matter and moisture content. The final weight of the cheese may decrease during ripening due to evaporation unless moisture loss is prevented by waxing or plastic film.

3 RM 03: BCCP - Chapter 07

Ylva Ardö and E. Waagner Nielsen, 2017

Summary
The world production of cheese comprises thousands of different varieties that are classified and grouped through various methods, most commonly by fat content, firmness, or processing parameters such as the method of coagulation. Motivation for classification lies in international trading, the protection of names and trademarks, and the standardisation of cheese. The International Dairy Federation (IDF), founded in 1903, developed a vast number of codes and standards that were agreed upon by European governments at the Stresa Convention in 1951. This convention established two levels of protection for cheese names:

1. Annex A: Protection of four names to be used only on specific cheeses produced in their original countries (Roquefort, Gorgonzola, Parmigiano-Reggiano, and Pecorino Romano).
2. Annex B: Mutual permission to use 30 cheese names on domestic and international markets, including Danablu, Danbo, Havarti, Samsø, Gouda, Edam, and Camembert.

As a result of this convention, Danish cheeses were renamed from their original descriptors; for example, Danish Schweizer became Samsø and Steppeost became Danbo.

Codex Alimentarius establishes generic cheese standards agreed in international cooperation and administered by the World Trade Organisation (WTO). Standards are defined for 16 cheese types classified according to principal ripening procedure (ripened, mould-ripened, in brine, or unripened) and firmness. Firmness is classified by the moisture in non-fat substance (MNFS%):

1. soft cheese: > 67%.
2. firm cheese: 54-69%.
3. semi-soft cheese: 61-69%.
4. semi-hard cheese: 54-63%.
5. hard cheese: 49-56%.
6. extra hard cheese: < 51%.

The classes of semi-soft and semi-hard were merged in recent updates because production variations often cause cheese varieties to fall into both categories. Traditional cheeses are further protected through geographical designations adopted by the European Commission in 1993, which recognise the specific heritage of a particular region.

There are three levels of protection:

1. PDO (Protected Designation of Origin): Foodstuffs which are produced, processed, and prepared in a given geographical area using recognised know-how.
2. PGI (Protected Geographical Indication): Products where the geographical area has an impact on at least one of the stages of production, processing, or preparation.
3. TSG (Traditional Speciality Guaranteed): Highlights traditional character in composition or means of production without referring to geographical origin.

Additionally, companies or organisations may use registered trademarks, which are national or European brands where the owner decides on branding and marketing programs.

4 RM 04: Technology of Cheese Making; Chapter 01

Edited by Barry A. Law and A.Y. Tamime, 2010

Summary

World production of milk in 2008 is estimated at $\approx 576 \times 10^6$ tonnes, with cows' milk accounting for ≈84.0% of the total. Milk quality for cheese manufacture is defined as its suitability for conversion into cheese to deliver cheese of the desired quality and yield. Milk consists of protein, lipid, lactose, minerals, minor components, and water. The casein fraction coexists with insoluble minerals as a calcium phosphate-casein complex, while the water and its soluble constituents are referred to as serum. Casein is heterogeneous, comprising four main types—α_{s1} ,α_{s2},β and κ—which represent ≈38, 10, 35 and 15 g 100 g^{-1} of the total casein, respectively. Individual caseins vary in their calcium-binding properties and sensitivity to calcium precipitation.

Different models have been proposed for the structure of the casein micelle based on the location of individual caseins and calcium phosphate:

1. A sub-micelle model in which sub-micelles are 'cemented' together by colloidal calcium phosphate (CCP).
2. A dual bonding model where the interior is composed of α_s - and β-caseins forming a lattice through interactions between hydrophobic regions and hydrophilic phosphoserine clusters.
3. A tangled, cross-linked web model comprising rheomorphic casein chains cross-linked by calcium phosphate nanoclusters.
4. An interlocked lattice model featuring anchoring calcium phosphate nanoclusters that bind the phosphoserine domains of α_s - and β-caseins.

The gelation of milk may be induced by selective hydrolysis of κ-casein at the phenylalanine 10^5 -methionine 10^6 peptide bond by rennets, by acidification to a pH close to 4.6, or by a combination of acid and heat. Rennet-induced gelation involves the liberation of caseinomacropeptide into the milk serum and a reduction in the negative surface charge to ≈-10 mV. Acid-induced gelation occurs as forces promoting dispersion are overtaken by reductions in negative charge and hydration. Milk quality requirements are defined by safety, compositional, microbiological, sensory, and ethical criteria. Factors affecting quality include composition, microbiology, somatic cell count (SCC), enzymatic activity, and chemical residues. Higher values for casein number, total casein, and calcium are positively correlated with enhanced rennet coagulation properties and cheese yield. The BB genotypes of κ-casein and β-lactoglobulin are associated with higher concentrations of casein and superior rennet coagulation properties.

Increasing SCC reduces lactose, fat, and casein contents in milk and results in a marked increase in γ-caseins

due to the hydrolysis of β- and α_{s2}-caseins by plasmin. Native milk contains proteinases from several sources, including indigenous plasmin, lysosomal proteinases of somatic cells, and bacterial proteinases. Excessive proteolytic activity is undesirable as it hydrolyses caseins to water-soluble peptides that are lost in whey. Lipolysis in milk is broadly classified into induced lipolysis, promoted by mechanical damage and temperature alterations, and spontaneous lipolysis.

The key elements of good milk production management are outlined as:

1. Breeding and selecting for target cheesemaking properties.
2. Maintaining a high plane of animal nutrition.
3. Minimising bacterial count and SCC in milk.
4. Minimising enzymatic activity associated with somatic cells and contaminating bacteria.
5. Minimising chemical residues, contaminants, fat damage, and levels of free fatty acids.

5 RM 05: Technology of Cheese Making; Chapter 02

M. Johnson and B.A. Law, 2010

Summary

Cheesemaking is a concentration process that has transitioned from a cottage industry to a high-technology fermentation sector. The global market in 2008 was dominated by the European Union and the United States, which together represented approximately 80% of world consumption. The primary goals of cheese technology are to establish parameters for desirable flavour, body, and texture, and to develop protocols that routinely reproduce these attributes. The physical and rheological characteristics of the product are governed by interactions between casein molecules, which are influenced by pH, the dissolution of colloidal calcium phosphate (CCP), proteolysis, temperature, and cheese composition.

Milk coagulation is achieved through three primary methods depending on the variety of cheese being produced:

1. The addition of coagulating enzymes, such as rennets, which destabilise the micelle suspension to form a gel network.
2. Acid-induced gelation, where low pH (4.6) reduces repulsive charges between micelles to cause aggregation, used for cottage and cream cheeses.
3. Acid-heat precipitation, which uses both acid and high heat to precipitate both casein and whey proteins for varieties such as Ricotta.

The manufacturing process proceeds through several defined stages to control product consistency. Standardisation of milk composition is necessary to achieve target fat-in-dry matter (FDM) and yield, typically by adjusting the casein-to-fat (C/F) ratio or adding milk solids. After standardisation, milk usually receives a heat treatment, such as pasteurisation at 72°C for 15 s, which eliminates pathogens while generally requiring no modifications to the manufacturing protocol beyond the potential addition of calcium chloride. Starter cultures are then added to convert lactose to lactic acid, which increases the rate of coagulant activity and syneresis while causing CCP dissolution.

The subsequent stages involve structural and chemical manipulation of the curd:

1. Coagulation and cutting: The coagulum is cut into small pieces (grains or curd) to facilitate whey removal. Cutting a "fine" or soft coagulum promotes rapid shrinking and "healing" of the curd surface, whereas a "coarse" or firm coagulum heals more slowly and is more prone to breakage.

2. Stirring, heating, and syneresis: The combination of mechanical stirring, heating, and acid development causes the casein network to tighten and expel moisture.
3. Whey removal and salting: Methods vary by variety, including draining through moulds, "cheddaring" (matting and stacking curd slabs), or the "stirred curd" process. Salt is added via direct addition to the curd, dry surface salting, or brine immersion.
4. Pressing: This operation forms the desired shape, forces out remaining whey, and ensures curd particles knit together into a cohesive mass.

The final ripening or maturation stage involves chemical and enzymatic reactions mediated by the starter culture and adventitious microflora that transform the bland curd into finished cheese. Modern whey technology utilizes membrane filtration—including ultrafiltration (UF), reverse osmosis (RO), nanofiltration (NF), and microfiltration (MF)—to recover functional proteins and lactose.

6 RM 06: Milk Protein Gels; Chapter 16 - Milk Proteins: From Expression to Food

John A. Lucey, Wisconsin Center for Dairy Research, University of Wisconsin-Madison, 2020

Summary

The gelation of milk proteins forms the structural basis for cheese and fermented products, achieved through heat, rennet enzymes, or acidification. Casein micelles, the fundamental building blocks for rennet and yogurt gels, are assembled via hydrophobic interactions and the formation of calcium phosphate nanoclusters across phosphoserine clusters. These micelles are protected by κ-casein, which features a hydrophilic C-terminal "hairy" layer providing steric stabilization and a barrier against association. Gelation methods, including heat treatment, impact the microstructure and digestion rates of the resulting milk gels.

Rennet-induced coagulation proceeds in two overlapping stages. In the primary enzymatic phase, the C-terminal part of the κ-casein molecule is hydrolyzed, diffusing into the serum phase as caseinomacropeptide (CMP) or glycomacropeptide (GMP). This proteolysis typically obeys first-order kinetics and leads to a $\approx 50\%$ reduction in zeta (ζ)potential, which decreases electrostatic repulsion between micelles. Aggregation begins during the secondary phase once the removal of protective κ-casein hairs reaches approximately 70%, though true system-spanning network formation requires at least $\approx 87\%$ hydrolysis. These gels are viscoelastic and are characterized by:

1. The elastic or storage modulus (G'), measuring stored energy per oscillation.
2. The viscous or loss modulus (G"), measuring energy dissipated as heat.
3. The loss tangent, representing the ratio of viscous to elastic properties.

Syneresis in rennet-induced gels is a dehydration process where moisture is lost through the contraction of the casein network. This one-dimensional shrinkage is governed by Darcy's equation, written as equation 2.1:

$$v = \frac{B}{\eta} \times \frac{p}{x} \tag{2.1}$$

where the superficial flow velocity (v) is determined by:

1. B: the permeability coefficient.
2. η: the viscosity of the liquid.

3. p: the pressure acting on the liquid.
4. x: the distance over which the liquid flows.

In commercial practice, cheesemakers enhance syneresis by cutting curd into smaller grains, increasing stirring speeds, or raising the cooking temperature.

Acid-induced gels, such as yogurt, form as the pH of milk is reduced toward the isoelectric point (≈ 4.6), causing the dissolution of colloidal calcium phosphate (CCP). This process involves three distinct pH regions:

1. pH 6.7 to ≈ 6.0: Reduction in net negative charge with minimal CCP dissolution.
2. pH ≈ 6.0 to ≈ 5.0: Shrinkage of κ-casein hairs and complete dissolution of CCP.
3. pH < 5.0: Declining net negative charge and increased hydrophobic interactions.

Factors influencing yogurt texture include fortification levels, fat content, homogenization conditions, and the specific starter culture used.

Whey protein gels are formed by heating protein solutions (usually $\geq 6\%$) to induce irreversible denaturation and unfolding, which exposes hydrophobic residues. The resulting network type depends on the balance of attractive and repulsive forces; fine-stranded transparent gels form at neutral pH and low ionic strength, while opaque particulate gels form at high ionic strength or pH values near 5. Properties are further influenced by the ratio of β-lactoglobulin to α-lactalbumin and the concentration of divalent cations like Ca^{2+}. Mixed gels, combining rennet and acid action, undergo complex rearrangements and demineralization that often result in a higher final storage modulus than acid-only gels.

7 RM 07: Technology of Cheesemaking; Chapter 10.4 - Cheese Structure and Eye Formation

Edited by Barry A. Law and A.Y. Tamime, 2010

Summary

Cheese structure and eye formation in Swiss-type varieties are fundamentally dependent on a close-knit texture and appropriate cheese cohesion. Cohesion is determined by specific mechanical properties, including elongational viscosity and fracture stress, which are influenced by the chemical composition of the matrix and proteolysis changes during ripening. A crucial factor in this development is the pH at one day, which affects the structural state of the protein; eye development is specifically promoted within a pH range of 5.15-5.45. Consistency that is too soft (associated with pH below this range) results in blind cheese, while consistency that is too tough (above this range) increases the risk of fracture and slit formation.

The study of eye formation involves various technological and rheological measurements:

1. X-ray tomography and transmission to monitor the growth of identified holes.
2. Scanning electron microscopy (SEM) to observe the nucleation of eyes and isotropic deformation of curd particles.
3. Nuclear magnetic resonance (NMR) imaging for the high-resolution detection of eye features, air pockets, and micro-bubbles.
4. Compression and relaxation testing to evaluate fracture stress, fracture strain, and elastic properties at ripening temperatures.
5. Ultrasonic techniques for non-destructive monitoring of the gas-solid structure of the cheese.

Proteolysis is essential for texture development, with the presence of native caseins in the matrix contributing to the firmness and deformability necessary for openness. Intensified proteolysis, particularly reflected by higher levels of phosphotungstic-acid soluble nitrogen (PTASN), has been significantly correlated with the intensity of slit defects in varieties like Comté. Furthermore, the fat content affects mechanical resistance; higher fat levels increase elasticity and decrease resistance, though excessive fat may reduce propionic fermentation. During the warm-room ripening stage, the cheese paste reaches its lowest firmness and highest deformability, providing the most appropriate mechanical conditions for eye formation.

Slit development, which occurs primarily during the cold storage stage, is a consequence of increased internal pressure combined with low fracture resistance. A slit forms instead of a round eye if the local overpressure is higher than the local cheese fracture stress. Fracture patterns in slits often exhibit concentric folds and radial tracks, and these defects are typically oriented perpendicular to the pressing axis. Factors driving this late-stage defect include secondary fermentation by specific propionibacteria strains and changes in carbon dioxide solubility and matrix rigidity induced by the temperature decrease. In summary, successful eye formation requires the precise synchronization of gas production with the optimal mechanical and biochemical properties of the cheese matrix.

8 RM 08: Technology of Cheesemaking; Chapter 11.7 - Cheese Defects

Edited by Barry A. Law and A.Y. Tamime, 2010

Summary

Microbiological contamination is a major cause of quality defects in cheese, which may originate from raw milk, other ingredients, or post-heat-process sources within the dairy. Because fresh curd may be matured for several weeks, months, or years, spoilage problems often manifest long after the cheese was produced. Early blowing caused by gas production from coliforms is now rare due to pasteurization and effective post-pasteurization control. While bacteriophage attack has been considered a problem of the past, its reemergence is a concern in artisan and farmhouse environments characterized by poor management of airflow or extended propagation of starter mother cultures. The inhibition of citrate-fermenting starter cultures by bacteriophages or antibiotics allows residual levels of citric acid to remain in the curd; heterofermentative non-starter lactic acid bacteria (NSLAB) are then capable of fermenting citrate to produce carbon dioxide, resulting in gassing, splits, and fissures.

The involvement of yeasts in cheese maturation and spoilage is complex, with dominant species belonging to the genera *Kluyveromyces* and *Debaryomyces*. Excessive growth is implicated in defects such as softening, early blowing of Parmesan, and various forms of discoloration or slime formation. While a level of 10^4 cfu g^{-1} may be considered normal in traditionally produced hard cheeses with a natural rind, an initial level of 10^2 cfu g^{-1} or below in vacuum-packaged blocks can cause loosening of the cheese bags during maturation due to the production of metabolic carbon dioxide.

Mould growth is the primary cause of defects in modern cheese production. Cheese possesses a specific associated mycoflora, and identification of the species present is an essential prerequisite for the control of problems. In a study of hard, semi-hard, and semi-soft cheeses, 91% of fungal isolates were *Penicillium* spp., with *P. commune* occurring in 42% of samples. The specific associated mycoflora and related defects include:

1. *Penicillium commune*: The wild type of the white Camembert mould and the most frequent contaminant.
2. *Penicillium roqueforti*: Used in blue cheese manufacture but undesirable as a cross-contaminant.

3. *Cladosporium* spp.: Common where cheese surfaces have become damp.
4. *Phoma*-type moulds: Associated with 'thread-mould' defect in vacuum-packed block Cheddar.
5. *Aspergillus versicolor*: Found on some Dutch cheeses.
6. *Penicillium discolor*: Significantly resistant to the antifungal agent natamycin.
7. *Penicillium verrucosum*: The only potential ochratoxin A producer found in the cheese environment.

Analysis of cheese has failed to demonstrate significant or persistent quantities of mycotoxins beneath the rind of correctly manufactured cheese without fissures. Spoilage moulds do not produce the antibiotic penicillin; significant quantities are only produced by *Penicillium chrysogenum*, which is rare as a food spoilage agent and is not used in mould fermentations.

9 RM 09: Fundamentals of Cheese Science; Chapter 07

Patrick F. Fox, Timothy P. Guinee, Timothy M. Cogan, Paul L. H. McSweeney, 2017

Summary
The enzymatic (rennet-induced) coagulation of milk is a two-stage process consisting of a primary enzymatic phase and a secondary non-enzymatic phase. The primary phase involves the specific hydrolysis of κ-casein at the $Phe_{105} - Met_{106}$ bond. This action destroys the micelle-stabilizing properties of κ-casein, producing para-κ-casein, which remains with the micelle, and caseinomacropeptide (CMP), which is released into the serum. The secondary phase involves the aggregation of these rennet-altered (para-casein) micelles into a three-dimensional gel network in the presence of Ca^{2+} at temperatures above 20°C.
Summary Hydrolysis of κ-casein is influenced by several environmental factors:
1. pH: The optimum pH for the first stage in milk is $\approx$ 6.0 at 30°C.
2. Temperature: Coagulation has a broad minimum at 40–45°C, but is typically performed at 31°C to optimize starter growth.
3. Heat Treatment: Severe heating (> 72°C) results in denatured β-lactoglobulin complexing with κ-casein, which inhibits both the primary and secondary phases.

The secondary phase occurs when the zeta potential of the micelles is reduced from -10/-20 to -5/-7 mV and approximately 85% of the total κ-casein has been hydrolyzed. Aggregation is driven by calcium bridges and hydrophobic interactions and is highly temperature-dependent, with a Q_{10} of $\approx$ 16. Methods for measuring rennet coagulation properties include:
1. Measurement of rennet coagulation time (RCT) using rotating bottles or tubes to detect the onset of visual coagulation.
2. Dynamic gel firmness testing using the Lattodinamografo to determine parameters such as rennet coagulation time (r), time to reach a firmness of 20 mm (k_{20}), and curd firmness at specific times (a_t).
3. Low-amplitude strain oscillation rheometry to compute storage modulus (G'), loss modulus (G"), and phase angle (δ).
4. On-line sensors, such as hot wire probes or near-infrared (NIR) diffuse reflectance probes, to monitor gel firmness in the cheese vat.

Rennets include traditional animal extracts, primarily containing chymosin, and rennet substitutes. Chymosin is a single-chain polypeptide with a molecular mass of 35,600 Da and two catalytically active aspartyl residues. Acceptable rennet substitutes include:
1. Bovine, porcine, and chicken pepsins.

2. Acid proteinases from *Rhizomucor miehei*, *R. pusillus*, and *Cryphonectria parasitica*.
3. Fermentation-produced chymosin (FPC) cloned in organisms such as *Aspergillus niger* or *Kluyveromyces marxianus* var. *lactis*.

Efficiency is maximized by using rennets with a high ratio of milk-clotting activity to proteolytic activity, such as calf chymosin or FPC, which minimize non-specific proteolysis and loss of fat and protein in the whey.

10 RM 10: Fundamentals of Cheese Science; Chapter 08

Patrick F. Fox, Timothy P. Guinee, Timothy M. Cogan, Paul L. H. McSweeney, 2017

Summary

Following rennet-induced gelation, the coagulum is subjected to a series of treatments designed to encourage syneresis, which is the contraction of the para-casein matrix and the expulsion of the aqueous whey phase. This process effectively concentrates the fat and casein of milk approximately tenfold by removing moisture and soluble milk constituents. Methods used to measure the rate and extent of syneresis include quantifying the volume of expressed whey, monitoring changes in curd mass or density, using tracers to measure whey volume, and observing changes in electrical conductivity.

Syneresis is significantly influenced by compositional and processing variables. Fat tends to reduce syneresis, while casein concentration is directly related to it. The rate of syneresis is inversely related to pH, reaching optimality at the isoelectric point of casein (≈ 4.6), and is promoted by the addition of $CaCl_2$. Major processing factors that regulate dehydration include:

1. Curd Particle Size: Smaller pieces accelerate syneresis due to the increased surface area available for the loss of whey.
2. Cook Temperature: Heating the curd-whey mixture promotes syneresis, with temperatures ranging from 31°C for soft varieties to 52–55°C for extra-hard varieties.
3. Rate of Acid Development: Lower pH values increase the rate and extent of syneresis by reducing inter-protein repulsion.
4. Stirring: Agitation facilitates heat transfer, prevents matting, and promotes syneresis through collisions between curd pieces.
5. Pressing: External pressure applied to the curd mass contributes significantly to the removal of remaining whey.

Specific cheese varieties undergo texturization during manufacture to induce characteristic physical properties. Cheddaring involves piling and turning blocks of curd, causing the curd to flow under its own weight and results in the fusion of curd particles. This is accompanied by the solubilization of micellar calcium and an increase in para-casein hydration. In *pasta filata* varieties, the curd is heated to $\approx$ 58–60°C and mechanically stretched, linearizing the para-casein matrix into fibers with fat trapped in elongated pools.

Final operations include moulding, pressing, and packaging. Moulding allows the curd to form a continuous mass, with high pressure applied to low-moisture curds to ensure adequate fusion. Packaging protects the finished cheese from physical or microbial contamination, reduces moisture loss, and prevents physical deformation. Internal bacterially-ripened cheeses are often vacuum-packed in plastic film or coated with wax to maintain stability during ripening.

Chapter 3
Exam Questions and Answers

This chapter of the course notes compiles the exam questions for the course held in February 2026, along with their respective answers prepared by me. The purpose of this section is twofold: firstly, to provide a reflective exercise that consolidates understanding of the course material; and secondly, to document my comprehension of the course topics as assessed through the exam questions.

To ensure citation accuracy and academic transparency, NotebookLM has been employed as the primary generative AI platform. Its use has focused on verifying that all citations accurately reference the uploaded course materials and lecture slides provided by the professors. Beyond citation control, this section also represents an ongoing exploration of prompt engineering - refining interaction design to optimise AI output quality, precision, and academic reliability. Through this approach, the work aims to maintain a high academic standard while enhancing clarity, structure, and depth in written responses.

There are a total of 10 questions in the exam, each consisting of multiple structured sub-questions. The questions are designed to assess both theoretical understanding and applied knowledge within dairy product technology. The numbering of sections corresponds directly to the numbering of the exam questions, ensuring a clear and consistent structure throughout. Questions 1-8 primarily address cheese manufacture and ripening, while questions 9-10 focus on fermented milk products, including structure formation and production processes. Each question is presented as a comprehensive topic intended for oral presentation, followed by examiner-led follow-up questions and discussion.

Examination Details

Exam is oral (total time 20 minutes)

At the oral exam a question will be randomly drawn by the student.

For this question, all aids are allowed (15 minutes oral exam). You should present your answer within 7-8 min, and you will then be asked some follow-up questions for the remainder of the time.

For the final part of the exam (approx. 5 minutes), topics related to the report from the practical part of the course will be discussed.

It is recommended to bring written answers to the questions in a more or less ready form, which may be used as an aid for answering the drawn question. It may also be recommended to bring a printed copy of the report for the final part of the exam.

1 Controlling the Moisture Content of Cheese

Water is expelled from the milk gel during production of cheese, and this is an important processing step for regulating the moisture content of the final cheese.

1.1

Give the name of this process and describe the mechanism (also on a microstructural level) behind how water is being expelled from the milk gel.

1.2

Explain how cutting the milk gel, stirring of the curd grains and temperature changes in the cheese vat effect the amount of water that is expelled. Elaborate on how pH at whey drainage affects calcium level in the cheese.

2 Influence of Type of Coagulants on Cheese Manufacture and Ripening

2.1

Coagulants from different sources (animals, microorganisms, plants) can be used in the production of cheese. Give examples of different types of commonly used coagulant preparations and describe differences in their primary action on casein in the cheese vat as well as what kind of differences the different coagulants may cause during ripening?

2.2

Describe what is meant by clotting activity versus proteolytic activity of a coagulant. What is the optimal proteolytic activity of a coagulant?

2.3

How could the different activity of coagulants during cheese ripening be investigated?

3 Cheese Yield

The cheese yield varies due to a number of cheese making parameters, and it varies between different cheese types.

3.1

Describe which major milk components of the cheese milk that are retained, and which are not retained in the cheese, and describe why they are retained or not retained. Elaborate on the difference between Cheese Yield and Moisture Adjusted Cheese Yield (MACY).

3.2

Describe how milk quality parameters can affect cheese yield. Describe industrial methods/ingredients which may be used to increase cheese yield.

4 Importance of pH Development in Cheese Ripening

During the first day(s), pH in cheese decreases to a minimum and then it increases at different rates depending on cheese variety.

4.1

Describe how different pH minima (the lowest pH obtained during cheese making) may be obtained, and give examples on cheese varieties with low, medium and high minimum-pH.

4.2

Give examples on cheese varieties with low, medium and high final pH (pH of the ripened cheese), respectively, and describe which microbial/enzymatic activities that are involved.

4.3

Differences in pH between the interior and the surface of cheeses are most pronounced in cheeses with surface ripening. Mention two different cheese varieties with different kind of surface ripening. Explain how pH gradients develop during ripening and how they influence ripening using one of the cheese varieties as an example.

5 Gas Production in Cheese

5.1

Gas may be produced from the metabolism of lactose and citrate. Which are the main microorganisms involved in this metabolism, and in which type of cheeses is this relevant?

5.2

Gas production in cheese is not always desirable and may be considered as a serious defect. Mention three different types of gas producing reactions (substrate and the organisms involved) in which undesired gas production may occur. What measures are available to the cheese producer to control undesired gas production in cheese?

5.3

In Swiss cheese, eye formation is an important cheese quality parameter. What factors (microbial and physio-chemical) are necessary to ensure that satisfactory eye formation develops?

6 Cheese Proteolysis

6.1

Proteolysis is a fundamental process in cheese ripening. List enzymes that hydrolyse peptide bonds in intact casein molecules in rennet coagulated semi-hard cheeses, describe the specificity of these enzymes and give the first peptides that are produced.

6.2

Explain how the first peptide released from α_{S1}-casein by chymosin in cheese (α_{S1}-casein (f1-23)), may be further hydrolysed all the way to amino acids, and mention which enzymes that are involved and what the origin of these enzymes may be.

6.3

Give examples of two cheese types with different primary proteolysis (first attack on intact casein) and explain how the differences are related to cheese manufacture. Elaborate on suitable laboratory methods for measuring proteolysis in cheese.

6.4

What approaches can be used to accelerate proteolysis and what are the advantages/disadvantages of each approach?

7 Amino Acids Catabolism and Cheese Flavour Formation

7.1

Amino acids are released from casein derived peptides in cheese during ripening and used to different extents by different microorganisms. Describe the enzymes that are directly involved in amino acid release incl. their name, specificity and origin.

7.2

Amino acids may be catabolized to aroma compounds in cheese. What are the main pathways (and the enzymes involved) in the catabolism of amino acids?

7.3

Which are the four groups of amino acids, which breakdown products may contribute to the cheese flavours

1. Malty
2. Floral
3. Cooked cabbage/garlic
4. Buttery

respectively?

Answer:

Table 3.1: *A table with an overview over the workload for the course.*

Flavour	Amino Acid group
Malty	X
Floral	XX
Cooked cabbage/garlic	XXX
Buttery	XXXX

7.4

Which analytical method can be used for identification of aroma compounds in cheese?

8 Importance of Milk Fat in Cheese Flavour

Low-fat cheese commonly lacks the complex and balanced flavour of normal-fat cheese.

8.1

Describe three different roles that milk fat may have in cheese flavour and describe how the off-flavour bitterness is related to fat content in cheese.

8.2

During ripening, fat is degraded to different extent in different cheese varieties from less than 1% to about 10% or more. Which lipolytic enzymes are involved in different kinds of cheeses, and where do they origin from? Which kind of aroma compounds may be produced from lipolysis and further catabolism of the fatty acids released, and what flavour notes do each of these aroma compounds introduce.

8.3

Give examples of compounds from milk fat catabolism that contribute to the characteristic flavour of the two cheeses, Parmesan and Blue cheese. How can lipolysis be enhanced in cheese during ripening?

9 Structure Formation in Fermented Milk Products

During acidification of milk, e.g. during fermentation using a standard culture of lactic acid bacteria for yoghurt production, several changes occur in the milk components, leading to formation of the final structure of fermented milk products.

9.1

Describe the changes occurring in the casein micelle as a result of acidification.

9.2

What are the main factors affecting structure formation in fermented milk products, and how can these be used to control the structural properties of the final product? How can you measure the structure formation?

10 Production of Fermented Milk Products

A number of basic processing steps are included in most production processes for fermented milk products.

10.1

Present an outline of a standard production process for yoghurt manufacture (set or stirred), including a short description of the aim and outcome of the selected parameters for each processing step.

10.2

How can concentration for production of fermented milk products like Greek yoghurt, ymer and skyr be obtained? Both traditional methods and modern technologies should be addressed.

10.3

Describe which molecules (apart from lactic acid) that are largely responsible for the flavour of plain yoghurt and describe how they are formed.

Chapter 4
Group Reports

This chapter contains the full group reports for the projects undertaken during this course. Each report is included as a PDF to preserve formatting and layout.

UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

Dairy Product Technology 2 - Practical: Fermented Dairy Products

Manufacture and measurements on yoghurt made with different treatments

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Last compiled: 17-12-2025
Link to Git repository

Preface

This written report has been prepared as part of the course Dairy Product Technology 2- Practical: Fermented Dairy Products at the University of Copenhagen. The practical focuses on the manufacture and evaluation of yoghurt produced under controlled variations in fat content, added milk protein, and mechanical post-treatment. Through experimental work, students explore how processing conditions influence fermentation behaviour, texture formation, syneresis, viscosity, and sensory quality of fermented dairy products.

The present project is based on the two-day practical in which yoghurt is produced from milks differing in composition and treated under varying backpressures after fermentation. The work includes monitoring acidification, performing mechanical smoothing, and conducting analytical measurements such as pH, water binding, flow properties (Posthumus funnel), and viscosity profiling. The collected data form the basis for discussing how fat content, added whey protein, and mechanical treatment affect product characteristics and overall yoghurt quality.

The assignment was carried out by Niclas Hauerberg Hyldahl (JNC117), Simon Riis Kjær (WMF954), and Lucas Daniel Paz Zuleta (TZS159), MSc students at the University of Copenhagen, as group report fulfilling the requirements for the Dairy Product Technology 2 practical on fermented dairy products.

Contents

1 Introduction

The purpose of this laboratory exercise is to analyse how changes in process parameters and yoghurt composition impact the final product. Specifically, the exercise focuses on variations in fat and protein content, as well as the influence of different backpressure levels during post-treatment. The yoghurts were produced on day 1, followed by a sensory evaluation. On day 7, a second sensory evaluation was conducted, along with measurements of viscosity and water-binding capacity.

2 Methods

2.1 1st day of production: Production and sensory evaluation of yoghurt.

Commercially homogenized milk with either 1.5% or 3.5% fat was used for production. Before producing the yoghurt, the milk was pasteurized at 85°C for 15 minutes by the supervisors. After pasteurization, whey protein isolate was added to four of the six batches as described below and in table 1.

- Batches 1 and 2 consisted of milk with 1.5% fat and 1% added protein.
- Batches 3 and 4 consisted of milk with 3.5% fat and 1% added protein.
- Batches 5 and 6 consisted of milk with 3.5% fat with no added protein.

Table 1.1: *A table with an overview of the different yoghurt samples produced.*

Sample	Fat [%]	Added Protein	Back Pressure
1	1.5%	1%	1
2	1.5%	1%	4
3	3.5%	1%	1
4	3.5%	1%	4
5	3.5%	-	1
6	3.5%	-	4

After pasteurization and addition of protein the milk was warmed to 44°C in a water bath. The starter culture "YF-L706" was thawed, and 20 g was mixed with approximately 200 g of cold 1.5% milk before addition in to the yoghurt base.

Each batch received 20 g of the starter culture, after which the pH was measured. pH Measurements were taken every 20 minutes until the pH declined from roughly 6.5 to 4.6 (1.2). Once the desired acidity was reached, the yoghurt was cooled to 20°C.

After reaching the desired pH all batches (1 thorugh 6) underwent additional mechanical treatment, where one sample in each pair (1 & 2, 3 & 4, 5 & 6) was processed at 1 bar backpressure and the other at 4 bar. The finished yoghurts were transferred into containers and stored at 5°C in the refrigerator.

Following a brief cooling stage, the yoghurt batches were assessed based on taste and visual appearance 1.2.

2.2 2nd day of production: Measurements and sensory evaluation of yoghurt.

The yoghurt samples from day 1 were removed from the refrigerator, tasted, and compared with the initial sensory evaluation.

After the sensory evalution, the yoghurt was poured into a cloth tea bag, allowing the whey to drain. The difference in weight before and after draining was used to determine the water-binding capacity [%]. The pH was

Table 1.2: *results from day 1, pH and sensory evaluation of yoghurt samples.*

Sample	Fat [%]	Added Protein	Back Pressure	Initial pH	Final pH	Sensory Evaluation
1	1.5%	1%	1	6.51	4.58	Grainy, but primarily in appearance.
2	1.5%	1%	4	6.54	4.55	Fairly fine but somewhat neutral.
3	3.5%	1%	1	6.55	4.57	Very grainy and unpleasant mouthfeel.
4	3.5%	1%	4	6.55	4.57	Very nice and appealing mouthfeel.
5	3.5%	-	1	6.55	4.55	Boring and neutral, fair mouthfeel
6	3.5%	-	4	6.56	4.58	Has a drinking-yoghurt–like consistency.

measured using a calibrated pH meter, and the viscosity was measured at different RPM settings using a Brookfield viscometer. Finally, the post-humus value was determined using the designated small-hole funnel where the time was recorded for yoghurt to pass the funnel.

3 Results

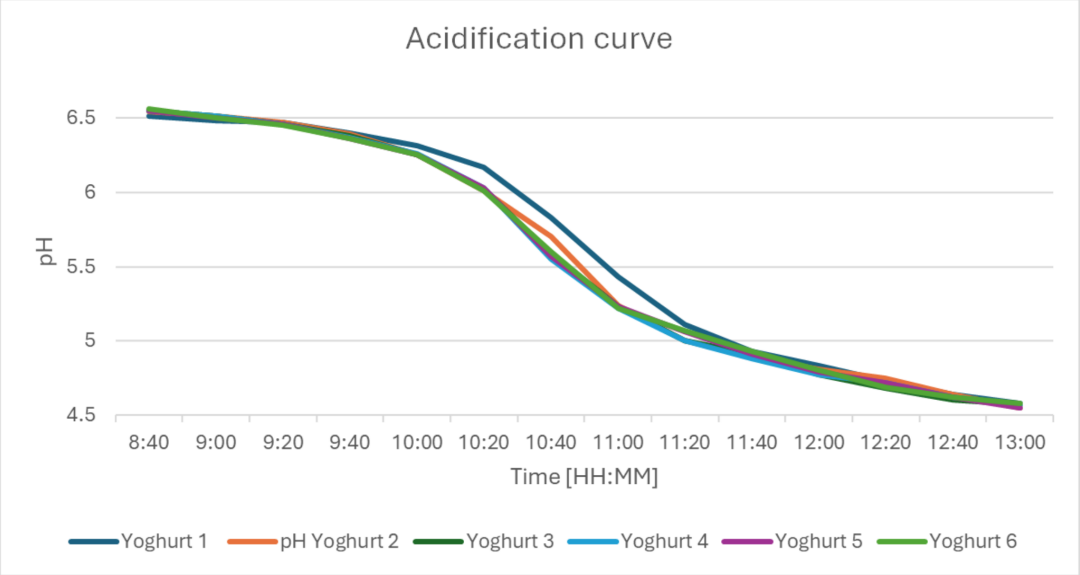

Figure 1.1: *Acidification curve.*

Post hummus and water-binding capacity results:
Measurements of water binding:

$$\text{Water Binding [\%]} = \frac{\text{Weight of whey}}{\text{Weight of yoghurt}} \times 100 \tag{1.1}$$

Equation 1.1 shows how to calculate water binding capacity [%]

$$\frac{38.76}{182.16} \times 100 = 21.68\% \tag{1.2}$$

Table 1.3: *Sensory evaulation for day 7*

Sample	Fat [%]	Added Protein	Back Pressure	Initial pH	Final pH	Sensory Evaluation
1	1.5%	1%	1	6.51	4.58	Grainy, but primarily in appearance. (No significant changes in texture, though a little in taste)
2	1.5%	1%	4	6.54	4.55	Fairly fine but somewhat neutral (No significant changes in texture, though a little in taste)
3	3.5%	1%	1	6.55	4.57	Very grainy and unpleasant mouthfeel (No significant changes in texture, though a little in taste).
4	3.5%	1%	4	6.55	4.57	Very nice and appealing mouthfeel (No significant changes in texture, though a little in taste).
5	3.5%	-	1	6.55	4.55	Boring and neutral, fair mouthfeel (No significant changes in texture, though a little in taste).
6	3.5%	-	4	6.56	4.58	Has a drinking-yoghurt–like consistency (Quite a lot of syneresis).

Table 1.4: *Viscosity results (Day 7) for yoghurt samples.*

Sample	Fat [%]	Added Protein	Back Pressure [bar]	10 rpm	20 rpm	30 rpm	40 rpm	50 rpm
1	1.5%	+	1	9530	4560	3600	2780	2180
2	1.5%	+	4	10500	6000	3840	2670	2130
3	3.5%	+	1	4290	2185	1530	1120	930
4	3.5%	+	4	8630	2115	1377	992	832
5	3.5%	-	1	7340	3535	2357	1685	1370
6	3.5%	-	4	2620	1465	940	707	584

Table 1.5: *pH, Posthumus time, and water binding results for yoghurt samples.*

Sample	Fat [%]	Added Protein	Back Pressure [bar]	pH	Posthumus [s]	Water Binding [%]
1	1.5%	+	1	4.37	83	21.68
2	1.5%	+	4	4.31	28	18.61
3	3.5%	+	1	4.33	270	18.05
4	3.5%	+	4	4.33	73	14
5	3.5%	-	1	4.25	25	26
6	3.5%	-	4	4.24	7	43

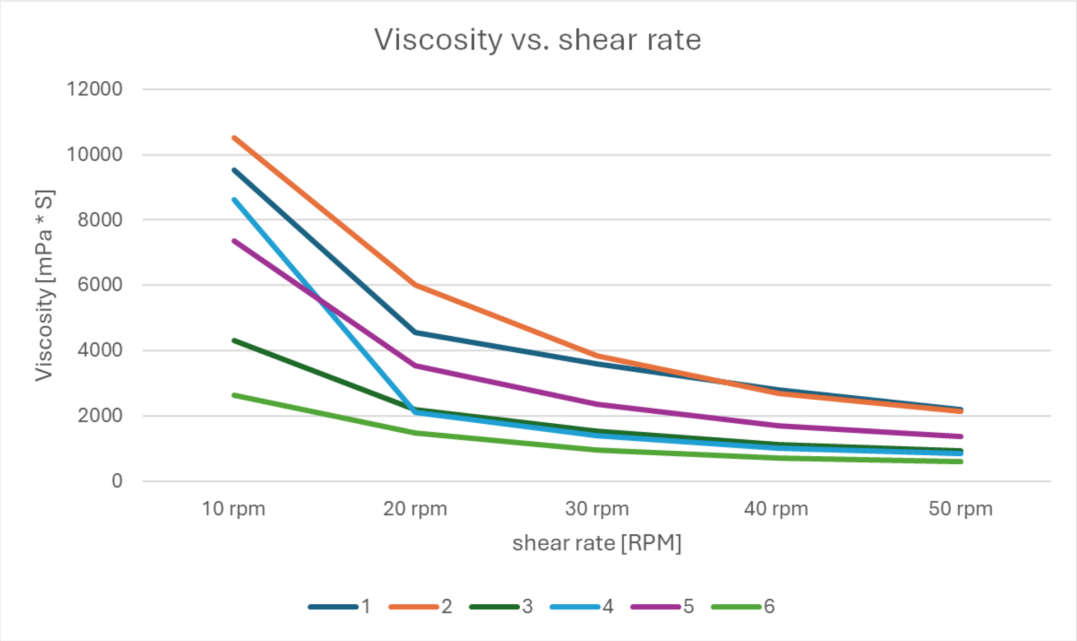

Figure 1.2: *An illustration of the rpm's influence on both viscosity and shear rate.*

Equation 1.2 shows our data integrated, where 38.76 g of whey was drained from 182.16 g of yoghurt, resulting in a water binding capacity of 21.68%.

4 How does the fat content affect yoghurt quality and why?

Reducing fat content in yogurt is associated with poor texture. Compared to full-fat yogurt, reduced-fat yogurt exhibits lower tension, firmness, and adhesiveness. The removal of fat can also lead to quality defects such as a powdery taste, excessive firmness, and higher whey expulsion. Fat globules are crucial as structure promoters because they act as linking protein agents in the network. Protein-coated fat spheres, formed during processing, reinforce the gel by associating with casein micelles. Consequently, the reduced-fat yogurt protein network is less dense, more open, and contains more void spaces than full-fat yogurt. This results from the casein micelles forming chains instead of extensively fused aggregates, due to fewer fat globules being present to link the proteins.

5 How does added milk protein affect yoghurt quality and why?

Added milk proteins, like Whey Protein Concentrate, can improve reduced-fat yogurt texture, sometimes mimicking full-fat quality. It increases strength and firmness because its denatured proteins become completely integrated into the protein network. protein additions (like skim milk powder) in large amounts can lead to quality defects such as excessive firmness, grainy texture, and higher whey expulsion. The protein matrix is primarily responsible for the firmness and springiness of yogurt.

6 How does increased back pressure affect yoghurt quality and why?

Increasing backpressure during yoghurt production can improve texture by strengthening the protein gel, resulting in a thicker, creamier product. Applying too much backpressure may lead to syneresis and can also harm live cultures, compromising flavour.

7 What would you recommend as the optimal processing parameters for the manufactured yoghurt?

Depeding on which type of yoghurt that is desired, we would recommend the processing parameters that were used for the sample 4 for a classical Greek-stylish yoghurt, but if the desired product should be like a drinking yoghurt we would recommend the processing parameters for sample 6. The two types of samples were the same except that sample 4 had 1% extra protein added. They had the same fat content and had the same backpressure.

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Dairy Product Technology 2 - Practical: Fermented Dairy Products

Manufacture and measurements on cheese made with different treatments

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Last compiled: 06-01-2026
Link to Git repository

Contents

1 Introduction

Feta cheese holds a Protected Designation of Origin (PDO) status, meaning that authentic feta may only be produced in specific regions of Greece. As part of the PDO, the used milk must come from sheep or from a mixture of sheep and goat milk, where the amount of goat milk cannot exceed 30% [1].
The production steps follow a general cheesemaking process, but with some difference unique to feta. After coagulation, the curd is transferred into perforated moulds and allowed to drain under its own weight, as feta is not a pressed cheese. Once the moisture level has decreased sufficiently, the cheese is dry salted for several days before placed into a brine solution, where it matures for at least two months [1]. Traditionally, the ripening took place in wooden barrels or stainless-steel containers, although modern production has started to incorporate plastic containers.
Most PDO feta is produced from pasteurized milk and inoculated with standardized starter cultures, typically *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [1]. Although the micro flora can vary among producers, beforementioned species dominate commercial production.
The feta cheese analysed in this course was purchased from Ängelmark and produced in Greece. It contained 23% fat and had a maximum moisture content of 56%. According to the nutrition label, the cheese provides 278 kcal per 100 g, consisting of 23 g fat, 16 g protein, and 5.5 g salt.
Sensory-wise, the cheese had the characteristic salty, tangy and aromatic flavour associated with long brining and a subtle goat-like note was also perceived.

2 Phenotypic Clustering

The heatmap of scaled relative abundance of aroma compounds shows a clear phenotypic separation among the seven cheeses. Feta forms its own distinct cluster, placed on the far left, indicating that its volatile profile differs from all other cheeses (Danbo, Brie, Emmental, Grana, Cheddar, Havarti, and Gouda). This positioning results from a unique set of compounds present at high abundance in Feta cheese, many of which are either absent or present at much lower levels in the other cheeses.
These compounds show high positive scores for Feta but appear blue/neutral for nearly all other cheeses which indicates that Feta has higher levels of these volatiles than the rest. Compared with harder, longer-ripened cheeses like Grana, Cheddar and Emmentale, Feta shows low levels of ketones, lactones and low aldehyde complexity.

2.1 Ethyl acetate

Ethyl acetate is an ester known to contribute pleasant fruity and floral notes to cheese, helping to create a flavour balance by reducing the sharpness originating from free fatty acids.
The formation of esters occurs through esterification reactions between short- to medium-chain fatty acids and alcohols, a process catalyzed by lipases and/or esterases produced by yeasts and bacteria in the cheese. In Feta-type cheese, the formation of esters, including ethyl acetate, has been associated with the use of specific auxiliary cultures such as *Lb. paracasei* subsp. *paracasei* and *D. hansenii*, resulting in high levels of ethyl acetate in the cheeses produced with these cultures [2].
In addition, many types of esters, including ethyl acetate, have been found in sheep's Feta cheese, suggesting that these compounds have a particular importance for the characteristic flavor of Feta made from sheep's milk. It is likely that the high fat content of sheep's milk enhances the activity of certain lipases and/or esterases involved in ester formation [2].

2.2 3-Methyl-1-butanol

3-Methyl-1-butanol is classified as an alcohol. This compound is responsible for a pleasant alcoholic floral note in some soft cheeses. It is formed from the amino acid leucine via Strecker degradation [2].
High concentrations of 3-methyl-1-butanol have previously been found in Feta cheese. Alcohols such as this can be formed rapidly from aldehydes under the highly reducing conditions present in cheese, or via other metabolic pathways, such as amino acid catabolism [2].

2.3 Hexanoic acid

Hexanoic acid is a volatile fatty acid and is one of the dominant volatile fatty acids in traditional Feta cheese. It is the most abundant fatty acid in traditional Feta cheese made from raw milk. This high concentration is likely attributed to increased hexanoic acid-specific lipase activity from the wild lactic acid bacteria strains found in Feta cheese. Hexanoic acid contributes to a "goat/barn"-like odor but is often considered a desirable characteristic in mature cheese [3].

3 Most Abundant Compounds

The volatile profile of Feta cheese is characterized by its distinct set of aroma compounds that differentiate from other cheese varieties. The most abundant volatiles include free fatty acids, alcohols, and esters, respectively [4]. In this section, these three groups will be described with emphasis on the most abundant individual compounds with respective tables for visualisation. Data has been derived from the study by Kondyli et al. [4].

3.1 Free Fatty Acids

In the study by Kondyli et al., free fatty acids were found to be the most abundant compounds in the volatile fraction of Feta cheese. After 180 days of ripening, the five dominant FFAs were C10:0 (decanoic acid), C2:0 (acetic acid), C16:0 (palmitic acid), C14:0 (myristic acid) and C12:0 (lauric acid), as shown in Table 1.1. These fatty acids contribute to the characteristic flavour profile of Feta, with C10:0 and C2:0 in particular.

It was shown that packaging affected the quantitative distribution of these FFAs. Cheeses that was stored in tin vessels contained significantly ($P < 0.05$) lower levels of C10:0 compared with those ripened in wooden barrels. The levels of C16:0 and C14:0 was slightly lower, and C12:0 showed only a minor decrease in the tin vessels Kondyli et al. [4].

Table 1.1: *Concentrations of the five most abundant free fatty acids (FFAs) in Feta cheese stored for 180 days in wooden barrels and tin vessels. Values are given as* $\mu g\, g^{-1}$ *cheese* $\pm$ *SD.*

FFA	Wood Barrels	Tin Vessels
C10:0	299.03 ± 10.13	253.92 ± 20.17
C2:0	284.53 ± 20.15	184.87 ± 15.12
C16:0	226.27 ± 4.62	207.48 ± 12.72
C14:0	159.78 ± 4.97	129.20 ± 15.85
C12:0	138.74 ± 4.27	134.25 ± 3.57

To get a better understanding of the overall FFA profile, Table 1.2 has been compiled, illustrating the top five most abundant FFAs in the feta cheese with sensoric descriptors.

Table 1.2: *Sensoric descriptors of the five most abundant free fatty acids (FFAs) in Feta cheese. Data has been adapted from The Good Scents Company database at their respective minimum thresholds in the database The Good Scents Company [5].*

FFA	Odour	Flavour
C10:0	Rancid, Sour, fatty, and citrus	Soapy, waxy, and fruity
C2:0	Pungent acidic and dairy-like	Acidic, dairy with a pronounced fruity lift
C16:0	Low heavy waxy, with a creamy, candle waxy nuance	Waxy, creamy fatty, soapy with a crisco like fatty, lard and tallow like mouth feel and a dairy nuanc
C14:0	Faint, waxy and fatty with a hint of pineapple and citrus peel	Waxy, fatty, soapy, creamy, cheesy, with a good mouth feel
C12:0	mild fatty	Fatty, waxy

3.2 Alcohols

The second most abundant group of volatiles in feta cheese is represented by alcohols. At 180 days of ripening the five dominant alcohols were ethanol, butan-2-ol, 3-methylbutan-1-ol, phenylethanol and butan-1-ol [4], as shown in Table 1.3. Ethanol was present at the highest concentration, reflecting the fermentative activity during brining and storage. Butan-2-ol and 3-methylbutan-1-ol were also present at significantly high levels.

Packaging had also an influence on the quantitative profile of the alcohols. Cheeses that were ripened in wooden barrels contained substantially higher levels of ethanol, butan-2-ol, phenylethanol and butan-1-ol compared with cheeses stored in tin vessels, indicating a more active fermentation and microbial pathways in the wooden environment. Conversely, 3-methylbutan-1-ol showed similar concentrations in both packaging types. Despite these differences, the dominant alcohols remained consistent across packaging type Kondyli et al. [4].

Table 1.3: *Concentrations of the five most abundant alcohols in Feta cheese stored for 180 days in wooden barrels and tin vessels. Values are given as peak area in TIC* $\times 10^5 \pm$ *SD.*

Alcohol	Wood Barrels	Tin Vessels
Ethanol	54,634.4 $\pm$ 3512.30	35,458.0 $\pm$ 2160.70
Butan-2-ol	20,990.60 $\pm$ 1549.10	9,004.70 $\pm$ 512.30
3-Methylbutan-1-ol	17,671.2 $\pm$ 2085.0	18,641.4 $\pm$ 1999.8
Phenylethanol	8,560.7 $\pm$ 343.5	1,226.7 $\pm$ 229.7
Butan-1-ol	3,114.5 $\pm$ 48.5	381.1 $\pm$ 103.7

Table 1.4 was compiled to illustrate the top five most abundant alcohols in the feta cheese. Sensoric descriptors have been added for better understanding of their individual contributions to the overall aroma profile of the cheese.

Table 1.4: *Sensoric descriptors of the five most abundant alcohols in Feta cheese. Data has been adapted from The Good Scents Company database at their respective minimum thresholds in the database The Good Scents Company [5].*

Alcohol	Odour	Flavour
Ethanol	Strong alcoholic ethereal, medical	n.d.
Butan-2-ol	Sweet apricot	n.d.
3-Methylbutan-1-ol	Fusel, alcoholic, pungent, etherial, cognac, fruity, banana and molasses	Fusel, fermented, fruity, banana, etherial and cognac
Phenylethanol	Sweet, floral, fresh and bready with a rosey honey nuance	Floral, sweet, rosey and bready
Butan-1-ol	Fusel oil sweet balsam whiskey	Banana fusel

3.3 Esters

Esters formed the third most abundant group of volatile compounds in Feta cheese at 180 days of ripening. The five dominant esters were ethyl hexanoate, ethyl octanoate, ethyl butanoate, ethyl decanoate and 2-phenylethyl acetate, as shown in Table 1.5. These compounds are primarily formed through esterification between free fatty acids and ethanol or other alcohols [4], and contribute with fruity, floral and sweet notes that balance the sharper acidic components of the cheese.

A clear effect of packaging was observed on the quantitative levels of esters. Cheeses ripened in wooden barrels contained significantly higher concentrations of all five esters compared with cheeses stored in tin vessels, indicating that the wooden environment favoured ester synthesis. The differences were especially pronounced for ethyl hexanoate, ethyl octanoate and ethyl butanoate Kondyli et al. [4].

Table 1.5: *Concentrations of the five most abundant esters in Feta cheese stored for 180 days in wooden barrels and tin vessels. Values are given as peak area in TIC* $\times 10^5 \pm$ *SD.*

Ester	Wood Barrels	Tin Vessels
Ethyl hexanoate	17,310.2 $\pm$ 947.1	1,437.4 $\pm$ 157.8
Ethyl octanoate	16,930.3 $\pm$ 1001.3	3,198.7 $\pm$ 394.1
Ethyl butanoate	13,189.6 $\pm$ 844.7	1,561.8 $\pm$ 87.2
Ethyl decanoate	9,861.6 $\pm$ 449.8	733.8 $\pm$ 44.1
2-Phenylethyl acetate	7,862.3 $\pm$ 298.9	821.5 $\pm$ 104.7

A compilation of the top five most abundant esters in Feta cheese is shown in Table 1.6, along with their sensoric descriptors to illustrate their individual contributions to the overall aroma profile of the cheese.

Table 1.6: *Sensoric descriptors of the five most abundant esters in Feta cheese. Data has been adapted from The Good Scents Company database at their respective minimum thresholds in the database The Good Scents Company [5].*

Ester	Odour	Flavour
Ethyl hexanoate	Sweet, fruity, pineapple, waxy, fatty and estry with a green banana nuance	Sweet, pineapple, fruity, waxy and banana with a green, estry nuance
Ethyl octanoate	Waxy, sweet, musty, pineapple and fruity with a creamy, dairy nuance	Sweet, waxy, fruity and pineapple with creamy, fatty, mushroom and cognac notes
Ethyl butanoate	Sweet, fruity, tutti frutti, lifting and diffusive	Fruity, sweet, tutti frutti, apple, fresh and lifting, ethereal
Ethyl decanoate	Sweet, waxy, fruity, apple	Waxy, fruity, sweet apple
2-Phenylethyl acetate	Sweet, honey, floral rosy, with a slight yeasty honey note with a cocoa and balsamic nuance	Sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel

3.4 Semi-Quantitative Concentrations

Although concentrations reported in the literature provide a useful reference for the typical volatile composition of Feta cheese, they do not necessarily reflect the specific product analysed in this exercise. Therefore, semi-quantitative evaluation was performed based on the GC-MS analyses conducted during the practical. Chromatographic data were processed and compiled in an Excel spreadsheet, from which peak areas were extracted and compared. Among the previously identified abundant aroma compounds, only 3-methyl-1-butanol ranked among the four most intense volatiles in the analysed Feta sample. Based on the measured peak areas and the internal standard response, semi-quantitative concentrations were calculated according to Equation 1.1.

$$C_i = 0.5 \times (\frac{A_{peak\_compound}}{A_{peak\_reference}}) \tag{1.1}$$

The sensory detection thresholds used for Table 1.7 were obtained from a reference compilation reporting thresholds defined across multiple studies, serving as a consolidated database for detection limits. The four compounds exhibiting the highest GC-MS peak areas represent the most abundant volatiles in the analysed Feta cheese sample based on semi-quantitative data. The remaining compounds, shown in italics, were included due to their known relevance to cheese aroma as reported by Kondyli et al. [4].

Among the four most abundant volatiles, only hexanoic acid, ethyl ester exhibited a semi-quantitative concentration (2.775 ppm) below its reported sensory detection threshold (3 ppm). All other compounds among the four most abundant volatiles had concentrations above their respective thresholds, indicating a high detection potential in the analysed cheese.

Table 1.7: *Semi-quantitative concentrations of selected aroma compounds in the analysed Feta cheese sample, along with their respective sensory detection thresholds and sensoric descriptors, obtained from Gemert and The Good Scents Company, respectively [6, 5].*

Compound	**Peak Area**	**Chemical Group**	**Semi-Quantitative Concentrations [ppm]**	**Detection Thresholds**	**Sensoric Descriptors**
Ethyl acetate	11,390	Ester	4.982	0.005	Etherial, fruity, sweet, grape and rum-like
1-Butanol, 3-methyl-	10,948	Alcohol	4.790	0.004	Fusel, fermented, fruity, banana, etherial and cognac
Hexanoic acid, ethyl ester	6,343	Ester	2.775	3	Sweet, fruity, pineapple, waxy, fatty and estry with a green banana nuance
Styrene	6,307	Benzenoid	2.759	0.08	Almond
Acetic acid, 2-phenylethyl ester	*1,371*	*Ester*	*0.600*	*0.019*	*Sweet, honey, floral*
Acetic acid	*1,050*	*Acid*	*0.460*	*22*	*Sharp pungent sour vinegar*
Octanoic acid, ethyl ester	*593*	*Ester*	*0.259*	*3*	*Waxy, sweet, musty, pineapple and fruity with a creamy, dairy nuance*
Decanoic acid, ethyl ester	*230*	*Ester*	*0.101*	*0.023*	*Sweet, waxy, fruity, apple*
1-Butanol	*191*	*Alcohol*	*0.083*	*0.5*	*Fusel, oil, sweet, balsam, whiskey*
2-Butanol, (R)-	*105*	*Alcohol*	*0.046*	*3.3*	*Oily, wine*
Decanoic acid	*104*	*Acid*	*0.046*	*10*	*Unpleasant, rancid, sour, fatty, citrus*

Overall, the semi-quantitative data primarily support relative ranking of aroma compounds and their *potential* sensory significance in the analysed Feta cheese.

4 Compare the volatile composition of your cheese with the tasting you did in the beginning of this course.

Based on question 3 & 4 the compounds with the highest concentrations in the feta cheese was ethyl acetate, 1-butanol-3-methyl, and hexanoic acid, all of which contribute to the final flavour in the cheese. In the initial tasting performed earlier in the course, the feta was described as salty, tangy, slightly sweet and sour, with a subtle goaty flavour and an overall mild balanced aroma. These sensory observations align with the identified volatile compounds as described below.

Ethyl acetate is a common ester in fermented dairy products and is typically associated with fruity and sweet flavours. Although feta is not known for complex fruity notes, low to moderate levels of ethyl acetate contribute to the fresh, mildly aromatic flavours in brined cheeses aligned with the observation made during the cheese tasting

1-butanol -3-methyl contributed with malty, fusel-like, tangy and fermented notes that can vary in pungency depending on the concentration. In the feta cheese the compound contributes to the tangy notes and balancing the flavour profile which was also identified during the cheese tasting

Hexanoic acid, ethyl ester is an ester formed from the medium-chain fatty acid hexanoic acid which is associated with the goaty, tangy flavour typical of sheep and goat milk cheeses. Hexanoic acid itself has a this pungent goaty aroma however esterification transforms the compound into a fruitier and more balanced compound. Esterification results in a milder expression of the goaty aroma rather than an intense or pungent flavour from hexanoic acid. The presence of this ester aligns well with the sensory evaluation of the cheese, which was described as having a subtle but noticeable goaty flavour consistent with traditional feta cheese.
Overall, the dominant volatiles detected in the heat map (question 3&4) align with the sensory evaluation and the expected characteristics of authentic Greek feta cheese.

5 Conclusion

The Three dominant aroma compounds identified in the heat map & aroma sheet (Ethyl acetate, 3-methyl-1-butanol and Hexanoic acid) has been further investigated and are considered classical flavour compounds in feta cheese. The three compounds contribute to the fruity sweetness, acidity earthy and goaty flavour that aligns with cheese tasting in the beginning of the course.

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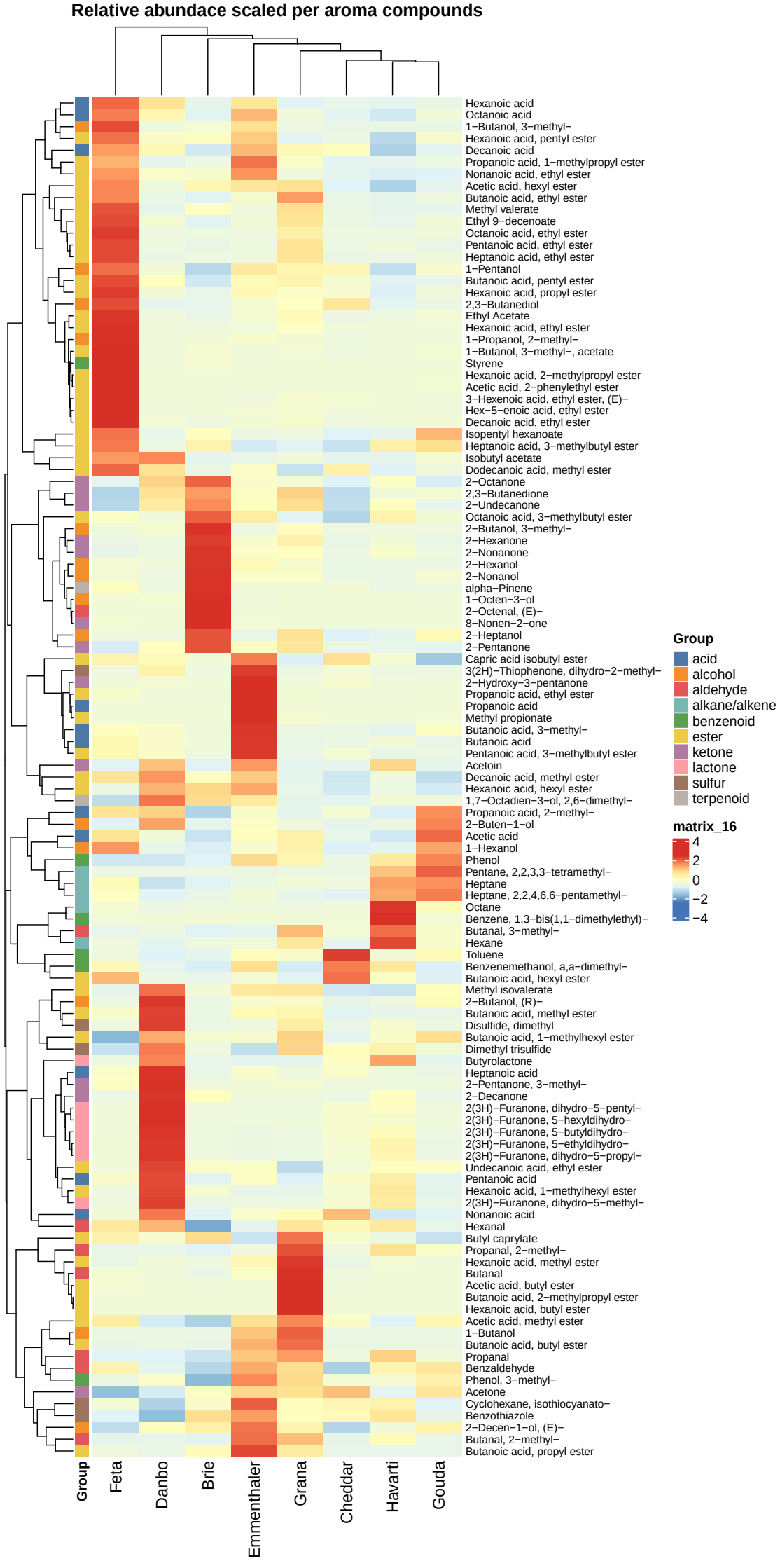
Appendices

1 Appendix 1 - GAI declaration

The heatmap is shown on the next page, since the size is too large to fit in the normal text area.


Relative abundace scaled per aroma compounds
Hexanoic acid
Octanoic acid
1−Butanol, 3−methyl−
Hexanoic acid, pentyl ester
Decanoic acid
Propanoic acid, 1−methylpropyl ester
Nonanoic acid, ethyl ester
Acetic acid, hexyl ester
Butanoic acid, ethyl ester
Methyl valerate
Ethyl 9−decenoate
Octanoic acid, ethyl ester
Pentanoic acid, ethyl ester
Heptanoic acid, ethyl ester
1−Pentanol
Butanoic acid, pentyl ester
Hexanoic acid, propyl ester
2,3−Butanediol
Ethyl Acetate
Hexanoic acid, ethyl ester
1−Propanol, 2−methyl−
1−Butanol, 3−methyl−, acetate
Styrene
Hexanoic acid, 2−methylpropyl ester
Acetic acid, 2−phenylethyl ester
3−Hexenoic acid, ethyl ester, (E)−
Hex−5−enoic acid, ethyl ester
Decanoic acid, ethyl ester
Isopentyl hexanoate
Heptanoic acid, 3−methylbutyl ester
Isobutyl acetate
Dodecanoic acid, methyl ester
2−Octanone
2,3−Butanedione
2−Undecanone
Octanoic acid, 3−methylbutyl ester
2−Butanol, 3−methyl−
2−Hexanone
2−Nonanone
2−Hexanol
2−Nonanol
alpha−Pinene
1−Octen−3−ol
2−Octenal, (E)−
8−Nonen−2−one
2−Heptanol
2−Pentanone
Capric acid isobutyl ester
3(2H)−Thiophenone, dihydro−2−methyl−
2−Hydroxy−3−pentanone
Propanoic acid, ethyl ester
Propanoic acid
Methyl propionate
Butanoic acid, 3−methyl−
Butanoic acid
Pentanoic acid, 3−methylbutyl ester
Acetoin
Decanoic acid, methyl ester
Hexanoic acid, hexyl ester
1,7−Octadien−3−ol, 2,6−dimethyl−
Propanoic acid, 2−methyl−
2−Buten−1−ol
Acetic acid
1−Hexanol
Phenol
Pentane, 2,2,3,3−tetramethyl−
Heptane
Heptane, 2,2,4,6,6−pentamethyl−
Octane
Benzene, 1,3−bis(1,1−dimethylethyl)−
Butanal, 3−methyl−
Hexane
Toluene
Benzenemethanol, a,a−dimethyl−
Butanoic acid, hexyl ester
Methyl isovalerate
2−Butanol, (R)−
Butanoic acid, methyl ester
Disulfide, dimethyl
Butanoic acid, 1−methylhexyl ester
Dimethyl trisulfide
Butyrolactone
Heptanoic acid
2−Pentanone, 3−methyl−
2−Decanone
2(3H)−Furanone, dihydro−5−pentyl−
2(3H)−Furanone, 5−hexyldihydro−
2(3H)−Furanone, 5−butyldihydro−
2(3H)−Furanone, 5−ethyldihydro−
2(3H)−Furanone, dihydro−5−propyl−
Undecanoic acid, ethyl ester
Pentanoic acid
Hexanoic acid, 1−methylhexyl ester
2(3H)−Furanone, dihydro−5−methyl−
Nonanoic acid
Hexanal
Butyl caprylate
Propanal, 2−methyl−
Hexanoic acid, methyl ester
Butanal
Acetic acid, butyl ester
Butanoic acid, 2−methylpropyl ester
Hexanoic acid, butyl ester
Acetic acid, methyl ester
1−Butanol
Butanoic acid, butyl ester
Propanal
Benzaldehyde
Phenol, 3−methyl−
Acetone
Cyclohexane, isothiocyanato−
Benzothiazole
2−Decen−1−ol, (E)−
Butanal, 2−methyl−
Butanoic acid, propyl ester
Group
Feta
Danbo
Brie
Emmenthaler
Grana
Cheddar
Havarti
Gouda
Group
acid
alcohol
aldehyde
alkane/alkene
benzenoid
ester
ketone
lactone
sulfur
terpenoid
matrix_16
4
2
0
−2
−4

UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

Dairy Product Technology 2 - Practical: Chemical Analysis of Cheese

A laboratory report describing standardised chemical methods for the characterisation of cheese composition and ripening, based on IDF starndards and widely used dairy industry and research practices.

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Link to Git repository

Contents

1 Introduction

Feta cheese is a traditional Greek cheese that is protected under the Protected Designation of Origin (PDO) scheme, ensuring that authentic feta is produced in specific regions of Greece and from specific milk types. Feta must be produced from sheep's milk or from a blend of sheep's and goat's milk, with goat's milk comprising no more than 30% of the total milk content [1].

Most of the PDO-certified feta is produced from pasteurized milk and relies on defined starter cultures to ensure consistent fermentation. Commonly used bacterial strains include, but are not limited to, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which dominate commercial feta production however minor variation in microflora between producers can be observed [1].

The feta cheese analysed in this course was sourced from the brand Änglamark and manufactured in Greece. According to the product information the feta cheese has a energy value of 278 kcal per 100 g, with 23 g fat, 16 g protein, and 5.5 g salt per 100 g.

2 Manufacturing

The manufacturing process of Greek feta cheese differs from other semi-hard cheeses such as Gouda, Danbo and is illustrated in figure 1.1. Feta is a brined, non-pressed cheese, and its production is characterized by high moisture content, acidic fermentation, and maturation in brine.

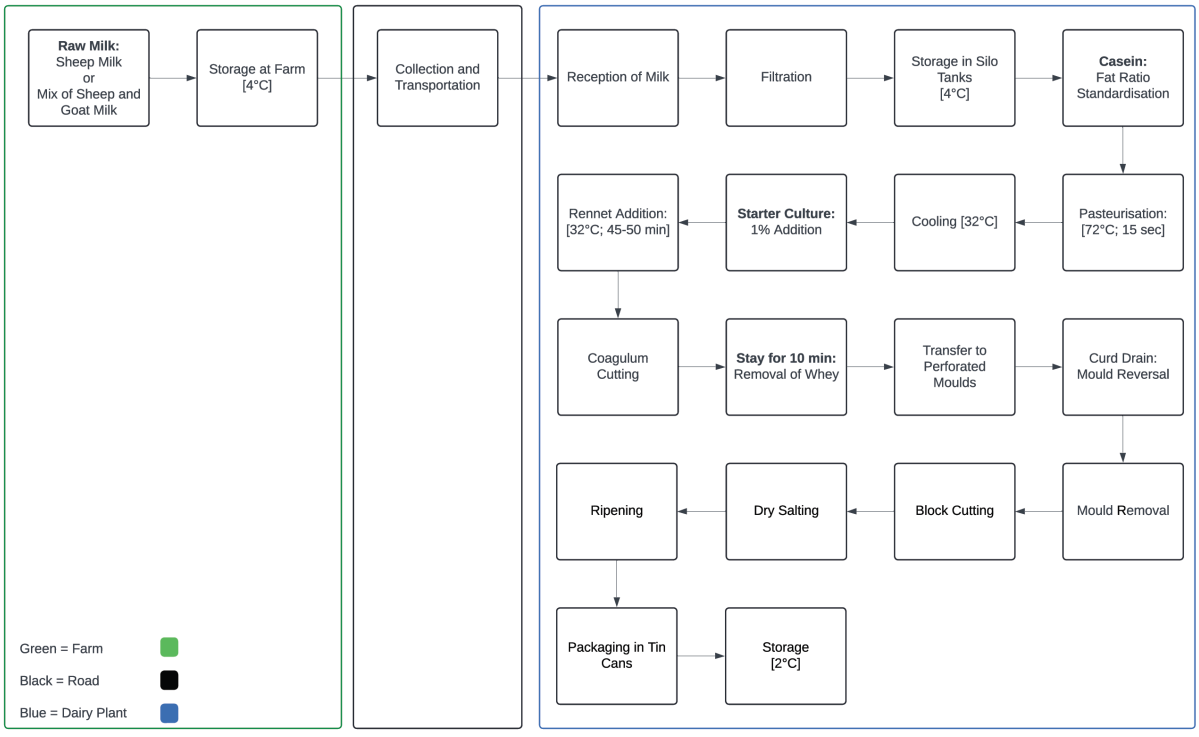

Figure 1.1: *Schematic overview of the feta cheese manufacturing process.*

Milk intended for feta production must comply with PDO regulations and consist of 100% sheep's milk or a mixture of sheep's and goat's milk, with the total amount of goat's milk not exceeding 30% [1].

To ensure microbiological safety and consistent quality, the milk is pasteurized most often using high temperature short time (HTST) pasteurization of 72°C for 15 seconds. Pasteurization decreases the microbial load (pathogenic and spoilage microorganisms) in the milk while preserving the functional properties of proteins necessary for cheesemaking [1].

Following pasteurization, the milk is cooled to inoculation temperature, and thermophilic starter cultures are added. These typically include *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which contribute with a rapid lactose fermentation and lactic acid production. The resulting decrease in pH is essential for curd formation and contributes to the characteristic acidic flavour of feta cheese. After reaching the targeted pH rennet is introduced, leading to enzymatic cleavage of κ-casein and the aggregation of casein micelles into a gel.

Once coagulated, the curd is cut into large cubes to facilitate whey expulsion while retaining a high moisture level. Unlike Gouda and Danbo, feta curds are not washed or scalded, as more lactose is needed during early fermentation and ripening. The cut cheese curd is transferred into perforated moulds, where whey drainage occurs under gravity without mechanically added pressure.

After drainage, the curd is cut into blocks and dry salted for several days. This step increases whey removal, regulates microbial activity and initiates rind formation. After dry salting the blocks are submerged in a salted brine solution and ripening occurs over a minimum of two months as required to be PDO certified. During ripening acidification, proteolysis and salt diffusion continues and contribute to the final texture and flavour of the feta cheese.

3 Materials and Methods

An Änglamark feta cheese was used for all experiments described in this report. The cheese is organic and certified with PDO. It is produced in Hellas, Greece, and made from 70% sheep's milk and 30% goat's milk.

The nutritional information provided on the packaging is shown below and will be references when discussing the laboratory results.

Table 1.1: *Nutritional information of Änglamark feta cheese per 100 g. Data was obtained from the product's webpage [2].*

Nutritional Value	Per 100 g
Content	100 g
Energy	278 kcal
Fat	23 g
Of which saturated fat	16 g
Carbohydrates	0.7 g
Of which sugars	0.7 g
Protein	17 g
Salt	2.2 g

3.1 Preparing the Cheese for the Lab

We were given two 150g feta cheeses. Before preparing the cheese sample, a sensory analysis was done outside the laboratory. The feta was crumbled and divided into two bags with approximately equal amounts in each, with

one part being used on the first day in the laboratory and the other part being put in the freezer for day two in the laboratory for later analysis.

Analysis of the cheese was decided into four areas:

- Compositional analysis
- Proteolysis
- Lipolysis
- Glycolysis

3.2 Compositional Analysis

The compositional analysis included determination of the total solid content (see table 1.2), fat content using the Gerber method, and total nitrogen and protein levels using the DUMAS method. Additionally, the pH, salt, and ash content were measured.

For the total solids content about 5 g of feta was added to a beaker containing pumice and mixed with a spatula. Afterwards it was dried for 16-18 hours at 104°C. The sample was then cooled to room temperature in the desiccator and weighed before it was put back in the oven for 2 hours more. Then it was cooled in the desiccator again and weighed once again to secure that the constant weight was obtained.

3.3 Gerber method for fat content

For measurement of fat content with the Gerber method we added 3 g of feta to a rubber stopper, which was fitted with a glass tube and connected to the bottom of a butyrometer. 10 ml of sulfuric acid was added, after which it was heated in a water bath at a temperature of 65-70°C for 10 minutes. Every 10 minutes, a stopper was inserted into the butyrometer, and it was subsequently inverted ten times to ensure thorough mixing, after which the butyrometer was returned to the water bath. This process continued until the cheese was completely dissolved, at ten-minute intervals. 1 ml of amyl alcohol was added to the butyrometer and mixed with the dissolved cheese to release the fat. Sulfuric acid was added until the liquid surface reached the 30 percent mark, after which the butyrometer was inverted 10 times. The butyrometer was left in the water bath for a period of 5 minutes, after which it was transferred to the Gerber centrifuge. It ran at 65 °C, at 1000-1200 RPM for 5 minutes. After the centrifuge, the butyrometer was put back in the water bath for another 5 minutes. The percentage value could then be read from the butyrometer.

For the determination of total nitrogen and protein in feta using DUMAS, 0.5 grams of feta was added to a crucible and covered with parafilm. The crucible was placed in the autosampler on top of the Rapid Max N Exceed and subsequently analysed using Dumas. The results were subsequently provided to us.

For the NaCl content, we used a Hach TitraLab AT1000 Series to calculate the percentage. 2 grams of feta were weighed and transferred into a 100 mL beaker and mixed with 10 mL of a 0.5M sodium-tri-citrate solution and 40 mL heated deionized water at 50°C. Then placed on an external stirring plate at 50°C for a duration of one hour until the cheese was fully suspended. The Hach TitraLab AT1000 Series calculates the results directly in percentage of NaCl.

To measure the pH of feta, 2-4 g of feta were weighed into a plastic shot glass and mixed with water in a 1:1 ratio to form a paste, after which the pH was measured with a pH meter.

For the ash content 2 g feta was weighed and placed in a pre-weighed crucible, which was then placed in a desiccator and transferred to an oven by the laboratory assistants, where it underwent a dehydration process overnight at a temperature of 104°C. It was then transferred to the muffle furnace at a temperature of about 525°C for 20 hours. The crucible was subsequently weighed to determine its ash content.

For proteolysis, the amount of pH 4.6-soluble nitrogen was determined using DUMAS, along with formol-titratable nitrogen in the pH 4.6 fraction and ammonium nitrogen content.

For pH 4.6-SN - soluble nitrogen, 12.5 grams of feta are placed in a 400 ml beaker and 50 ml of neutralized 0.5 M trisodium citrate solution is added, cover with foil and place in a water bath at 55°C for 30 minutes. Transfer the sample to a 250 ml volumetric flask, cool to room temperature and add deionized water to the 250 ml mark. Return the sample to the beaker and add 28 ml of 1.0 M HCl. The pH is measured and should be between 4.3-4.6.

For the formalin titratable nitrogen, two samples were prepared. 1 cheese sample and 1 blank. For the cheese sample, 20 ml cheese filtrate from the previous experiment is pipetted into a 50 ml beaker. Place on a stirring plate with a magnet in the beaker. Lower the pH electrode into the liquid and add 1 ml 1.0 M NaOH. The titration is carried out with a digital burette. 0.1 M NaOH is added to the desired pH of 8.3. 10 ml neutralized formalin is added, and a new titration is carried out again with 0.1 M NaOH, pH 8.3 again. The amount of 0.1 M NaOH, at each titration is noted as well as the final pH value. For the blank, add 50 ml 0.5 M trisodium citrate and dilute in a 250 ml volumetric flask. The solution is transferred to a 400 ml beaker, and 28 ml 1.0 M HCl is added. 20 ml of the sample is transferred to a 50 ml beaker. Same procedures with magnet, stirring plate and pH electrode. Finally, 1 ml 1.0 M NaOH is added. Same process as before and the amount of 1.0 M NaOH and final pH value are noted. The pH was then adjusted with 1.0 M HCl and the solution filtered into a new 400 ml beaker. The filtrate is then used to determine total nitrogen using the Dumas method and the Formol titratable nitrogen method.

For ammonium N, 2.5 g of feta is weighed into a Kjeldahl digestion tube and mixed with 2 g of barium carbonate and 2-3 drops of anti-foam. A receiving flask is prepared with 50 ml of 1.0% (w/w) boric acid solution and 4 drops of Kjeldahl indicator. The receiving flask is placed in the Kjeldahl instrument and the digestion tubes containing the cheese samples are fixed in a position so that they are in contact with the rubber stopper. The samples are distilled and the beaker containing the distilled sample is removed from the Kjeldahl instrument. A titration with 0.1 M HCl is performed. A colour change from green to grey is observed and the exact amount of HCl is documented. However, this analysis is not done by us, but by the technicians and the results are uploaded to us for further analysis.

Lipolysis was assessed by titrating the acidity of the fat in the cheese. Glycolysis was measured by determining the concentrations of D- and L-lactic acid.

For the acidity of fat in cheese, 30 g of feta were mixed with 1.2 g of sodium polyphosphate, 3.0 ml of 1 M NaOH and 50 ml of deionized water. The mixture was stirred as much as possible until a smooth, thin paste is obtained. The cheese paste formed is transferred to a volumetric flask using 50 ml of 40-50°C warm deionized water. The cheese paste is then heated in a water bath at 100 °C for 15-20 minutes until the fat is clearly separated. 15 ml of 1.0 M HCl is added to the volumetric flask and swirled gently and placed on the table for 15 minutes. 50 ml of BDI reagent is added to the volumetric flask, swirled gently and placed in the water bath for 10 minutes further, until the fat phase is clearly visible. When the fat phase was clearly visible, the volumetric flask was filled with deionized water for marking and left on the table for 5 minutes until the fat appeared as a distinct clear, clean fat phase at the top of the volumetric flask. An amount of 0.5-0.8 g of the clear fat phase was transferred to a 25 ml beaker, after which 15 ml of fat-dissolving mixture was added. Subsequently, titrated with KOH until a clear turn

to pink was observed. The pH and amount of KOH were noted.

For the enzymatic determination of L- and D-lactic acid, 1 g of feta was transferred to 100 ml of cold measuring flask with 80 ml of deionized water and placed in a 60°C water bath for 15 minutes. The sample was then cooled to room temperature and adjusted to the 100 ml mark in the measuring flask. The sample was transferred to a new beaker via a Whatman 1 filter. Four cuvettes were used for each analysis: a blank, a standard and two samples. Subsequently, two different procedures had to be followed in the exercise manual for D-Lactic Acid and L-Lactic Acid, respectively.

Details regarding the equipment, materials, and methods used are provided in the Manual for Chemical Analysis of Cheese (Hougaard and Danielsen, 2025)

4 Results and Calculations

4.1 Compositional Analysis Results

Table 1.2 summarizes the weights recorded during the total solids determination for the two feta cheese samples.

Table 1.2: *Total solids data for feta cheese samples.*

Replicate	**Weigh of beaker + pumice + spatula: m_0 [g]**	**Weigh of grated sample: m_1 [g]**	**Weigh after drying (beaker + pumice + spatula + sample): m_2 [g]**
Sample 1	65.6194	4.9960	67.8421
Sample 2	65.1652	4.9935	68.4598

Total solids were calculated using the formula in equation 1.1.

$$\text{Total Solids } [\%] = \frac{(m_2 - m_0)}{m_1} \times 100 \tag{1.1}$$

Where:

- m_0 = weight of beaker + pumice + spatula [g]
- m_1 = weight of grated sample before drying [g]
- m_2 = weight of the beaker, pumice, spatula, and sample after drying [g]

After determination of total solids (TS), the moisture content was calculated as:

$$\text{Moisture } [\%] = 100 - \text{Total Solids} \tag{1.2}$$

The results from the total solids content calculations are summarized below in equations 1.3 and 1.4:

$$\text{Sample 1: } TS_1 = \frac{67.8421 - 65.6194}{4.9960} \times 100 = 44.49\% \tag{1.3}$$

And,

$$\text{Sample 1: } TS_2 = \frac{68.4598 - 65.1652}{4.9935} \times 100 = 65.98\% \tag{1.4}$$

The difference between the two samples has been calculated as seen in equation 1.5:

$$\Delta \text{TS}\,[\%] = |TS_1 - TS_2| = |44.49 - 65.98| = 21.49\% \tag{1.5}$$

The moisture content for each sample was calculated using equation 1.6 and 1.7:

$$\text{Sample 1: Moisture}_1 = 100 - 44.49 = 55.51\% \tag{1.6}$$

And,

$$\text{Sample 2: Moisture}_2 = 100 - 65.98 = 34.02\% \tag{1.7}$$

4.2 Fat Content - Gerber Method

The fat content of the cheese was determined using the Gerber method. The fat percentage was determined by reading the butyrometer after completion of the analysis. The results are summarised in Table 1.3.

Table 1.3: *Fat content results from Gerber method for feta cheese samples.*

	[g]	**% fat**
Sample 1	3.05	24
Sample 2	3.04	22.5

The difference between the duplicates was calculated as seen in equation 1.8:

$$\Delta \text{Fat}[\%] = |\text{Fat}_1 - \text{Fat}_2| = |24 - 22.5| = 1.5\% \tag{1.8}$$

Lastly the average fat content was calculated using equation 1.9:

$$\text{Average Fat}\,[\%] = \frac{\text{Fat}_1 + \text{Fat}_2}{2} = \frac{24 + 22.5}{2} = 23.25\% \tag{1.9}$$

4.3 Total Nitrogen and Protein Content - DUMAS

The total protein content of the feta cheese was calculated from the nitrogen content using a conversion factor of 6.38, which is commonly used for milk proteins. The results from the DUMAS analysis are summarized in Table 1.4.

Table 1.4: *Total nitrogen and protein content results from DUMAS analysis for feta cheese samples.*

	[mg]	**[N%]**	**g protein/100g**
Sample 1	512.1	2.34	14.92
Sample 2	516.3	2.43	15.50

The protein content was calculated using the formula in equation 1.10:

$$\text{Protein}\,[\%] = \text{Nitrogen}\,[\%] \times 6.38 \tag{1.10}$$

The calculations for the protein % for each sample are shown in equations 1.11 and 1.12:

$$\text{Sample 1: Protein}_1 = 2.34 \times 6.38 = 14.92\% \tag{1.11}$$

And,

$$\text{Sample 2: Protein}_2 = 2.43 \times 6.38 = 15.50\% \tag{1.12}$$

The average protein content was calculated using equation 1.13:

$$\text{Average Protein [\%]} = \frac{\text{Protein}_1 + \text{Protein}_2}{2} = \frac{14.92 + 15.50}{2} = 15.21\% \tag{1.13}$$

4.4 NaCl Content

The salt (NaCl) content of the feta cheese was determined using the method described in the laboratory manual. The salt concentration was given by the analytical equipment. The results are summarised in Table 1.5.

Table 1.5: *Salt content results for feta cheese samples.*

	g	**% salt**
Sample 1	2.030	3.00
Sample 2	2.002	2.95

The difference between the duplicates was calculated as seen in equation 1.14:

$$\Delta\text{Salt}[\%] = |\text{Salt}_1 - \text{Salt}_2| = |3.00 - 2.95| = 0.05\% \tag{1.14}$$

The salt content of the feta cheese samples was measured as 3.00% and 2.95%. The difference between duplicate measurements (0.05%) is below the maximum allowed difference of 0.06% specified in the laboratory manual and is therefore considered accurate and reliable.

To calculate the salt in moisture, the following formula was used in equation 1.15:

$$\text{Salt in moisture [\%]} = \frac{\text{Salt [\%]}}{\text{Moisture [\%]}} \times 100 \tag{1.15}$$

The calculations for the two samples are shown in equations 1.16 and 1.17:

$$\text{Sample 1: Salt in moisture}_1 = \frac{3.00}{55.51} \times 100 = 5.40\% \tag{1.16}$$

And,

$$\text{Sample 2: Salt in moisture}_2 = \frac{2.95}{34.02} \times 100 = 8.67\% \tag{1.17}$$

Lastly, the average salt in moisture was calculated using equation 1.18:

$$\text{Average Salt in moisture [\%]} = \frac{\text{Salt in moisture}_1 + \text{Salt in moisture}_2}{2} = \frac{5.40 + 8.67}{2} = 7.04\% \tag{1.18}$$

4.5 pH in Cheese

The pH was measured for the two samples and the results are shown in table 1.6.

Table 1.6: *pH results for feta cheese samples.*

	pH
Sample 1	4.28
Sample 2	4.27

The measured pH values of the two feta cheese samples were 4.28 and 4.27. A difference of 0,01 in pH value indicates reliable results.

4.6 Ash Content

Ash contents were calculated using the formula in equation 1.19.

$$\text{Ash Content } [\%] = \frac{(m_2 - m_0)}{m_1} \times 100 \tag{1.19}$$

Where:

- m_0 = the mass of the crucible without ash [g]
- m_1 = the mass of the cheese sample before ashing [g]
- m_2 = the mass of the crucible and ash after ashing [g]

The measured masses used in calculations are summarised in Table 1.7.

Table 1.7: *Masses used for ash content calculations of feta cheese samples.*

	m_0 **[g]**	m_1 **[g]**	m_2 **[g]**
Sample 1	12.8282	1.9745	12.8947
Sample 2	12.4736	2.2283	12.5468

The results from the ash content calculations are summarized below in equations 1.20 and 1.21:

$$\text{Sample 1: Ash Content}_1 = \frac{12.8947 - 12.8282}{1.9745} \times 100 = 3.36\% \tag{1.20}$$

And,

$$\text{Sample 2: Ash Content}_2 = \frac{12.5468 - 12.4736}{2.2283} \times 100 = 3.28\% \tag{1.21}$$

5 Proteolysis Assessment Results

5.1 pH 4.6-Soluble Nitrogen

Soluble nitrogen content at pH 4.6 was determined according to the method described in the laboratory manual. Soluble nitrogen is expressed as grams of nitrogen per 100 g of product (% w/w). The nitrogen content of the soluble fraction was measured using DUMAS, see Table 1.8.

Table 1.8: *pH 4.6-soluble nitrogen results for feta cheese samples.*

	[mg]	**[N%]**
Sample 1	512.1	2.344
Sample 2	516.3	2.43

The pH 4.6-soluble nitrogen content was calculated using the formula in equation 1.22:

$$\text{pH 4.6-SN [\%]} = \frac{m_2}{m_1 \times \frac{2[\text{mL}]}{278\text{mL}}} \times 100 \tag{1.22}$$

Where:

- m_1 = the mass of the cheese sample [g]
- m_2 = the mass of the 2 mL sample used for DUMAS analysis [g]
- $N\%$ = nitrogen content in the filtrate determined by DUMAS [N%]

The calculations for the pH 4.6-soluble nitrogen % for each sample are shown in equations 1.23 and 1.24:

$$\text{Sample 1: pH 4.6-SN}_1 = \frac{0.5121}{12.5 \times \frac{2[\text{mL}]}{278[\text{mL}]}} \times 100 = 13.35\% \tag{1.23}$$

And,

$$\text{Sample 2: pH 4.6-SN}_2 = \frac{0.5163}{12.5 \times \frac{2[\text{mL}]}{278[\text{mL}]}} \times 100 = 13.95\% \tag{1.24}$$

5.2 Formol-Titratable Nitrogen

Table 1.9 summarizes the results from the formol-titratable nitrogen analysis.

Table 1.9: *Formol-titratable nitrogen results for feta cheese samples.*

	0.1 M NaOH [mL]	**pH**
Sample 1	0.21	8.34
Sample 2	0.22	8.29
Blank 1	0.00	8.30
Blank 2	0.00	8.30

The formol-titratable nitrogen content was calculated using the formula in equation 1.25:

$$\text{Ammonium} = \frac{1.40 \times C \times (V_1 - V_2)}{m \times \frac{20}{278}} \tag{1.25}$$

Where:

- C = concentration of NaOH [mol/L]
- V_1 = volume of NaOH used for sample titration [mL]
- V_2 = volume of NaOH used for blank titration [mL]
- m = mass of cheese sample [g]

The calculations for the formol-titratable nitrogen [mg] for each sample are shown in equations 1.26 and 1.27:

$$\text{Sample 1:} Ammonium_1 = \frac{1.40 \times 0.10 \times (0.21 - 0.00)}{12.5} \times \frac{20}{278} = 0.032 \tag{1.26}$$

And,

$$\text{Sample 2:} Ammonium_2 = \frac{1.40 \times 0.10 \times (0.22 - 0.00)}{12.5} \times \frac{20}{278} = 0.034 \tag{1.27}$$

5.3 Ammonium - Nitrate

Table 1.10 summarizes the results from the ammonium nitrogen analysis.

Table 1.10: *Data of the analysed feta cheese and blanks from the exercise, made by Tania, Oliver and Bente.*

	g cheese	mL 0.1 M HCl
Sample 1	2.5740	4.13
Sample 2	2.5782	5.2
Blank 1	-	0.11
Blank 2	-	0.04

The equation from 1.25. is used to calculate ammonium-nitrogen content with the fraction removed, as seen in equation 1.28:

$$\text{Ammonium}_1 = \frac{1.40 \times C \times (V_1 - V_2)}{m} \tag{1.28}$$

The calculations for the ammonium-nitrogen % for each sample are shown in equations 1.29 and 1.30:

$$\text{Ammonium}_1 = \frac{1.40 \times 0.10 \times (4.13 - 0.11)}{2.5740} = 0.2186N\% \tag{1.29}$$

And,

$$\text{Ammonium}_2 = \frac{1.40 \times 0.10 \times (5.2 - 0.04)}{2.5782} = 0.2802N\% \tag{1.30}$$

The ammonium percentage for sample 1 is 0.2186 N% and for sample 2 is 0.2802 N%.

6 Assessment of Lipolysis

6.1 Acidity of Fat in Cheese

Table 1.11 summarizes the results from the acidity of fat in cheese analysis.

Table 1.11: *Acidity of fat in cheese results for feta cheese samples.*

	Fat [g]	KOH [mL]	Degree of acidity [%]
Sample 1	0.55885	2.57	4.59
Sample 2	0.55380	2.18	3.94

The degree of acidity was calculated using the formula in equation 1.31:

$$\text{Degree of Acidity } [\%] = \frac{C \times V}{m} \times 100 \tag{1.31}$$

Where:

- C = concentration of KOH [mol/L]
- V = volume of KOH used for titration [mL]
- m = mass of fat in the sample [g]

The calculations for the degree of acidity % for each sample are shown in equations 1.32 and 1.33:

$$\text{Sample 1: Degree of Acidity}_1 = \frac{0.1 \times 2.57}{0.55885} \times 100 = 4.59\% \tag{1.32}$$

And,

$$\text{Sample 2: Degree of Acidity}_2 = \frac{0.1 \times 2.18}{0.55380} \times 100 = 3.94\% \tag{1.33}$$

7 Glycolysis Analysis

7.1 Enzymatic Determination of L- and D-Lactic Acids

7.1.1 D-Lactic Acid Results

The table 1.12 summarizes the results from the D-lactic acid analysis.

Table 1.12: *D-lactic acid results for feta cheese samples.*

	Before D-LDH	**After D-LDH**					
	A1 (3 min)	A2 (5 min)	A3 (6 min)	A4 (7 min)	A5 (8 min)	A6 (9 min)	A7 (10 min)
Blank	0.450	0.450	0.450	0.462	0.471	0.474	0.468
Standard	0.525	0.761	0.902	0.952	0.994	0.990	1.024
Sample 1	0.540	0.595	0.654	0.673	0.642	0.710	0.719
Sample 2	0.499	0.584	0.632	0.645	0.671	0.701	0.702

Results

	$\Delta A_{D-lactic\ acid}$	**Conc. D-lactic acid [g/L]**	**Content D-lactic acid [g/100 g]**
Blank	0	N/A	N/A
Standard	0.236	0.756	0.00783
Sample 1	0.055	0.176	0.00182
Sample 2	0.085	0.272	0.00282

The concentration and content of D-lactic acid were calculated for all samples. The results are presented in table 1.12, and an example of the calculation for Sample 2 is shown below.

The concentration of D-lactic acid were calculated using the formula in equation 1.34:

$$C = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{D-lactic\ acid} \tag{1.34}$$

Where:

- V = final reaction volume [mL]
- MW = molecular weight of D-lactic acid [g/mol]
- ε = extinction coefficient of NADH at 340 nm (6300 L·mol^{-1}·cm^{-1})
- d = light path length through the cuvette [cm]
- v = sample volume [mL]
- $\Delta A_{D-lactic\ acid}$ = absorbance difference between measurements A1 and A2

Determination of $\Delta A_{D-lactic\ acid}$ is done by subtracting the absorbance of the blank from the absorbance of the sample:

$$\Delta A_{blank} = A_{blank\ 2} - A_{blank\ 1} = 0.450 - 0.450 = 0.000 \tag{1.35}$$

And,

$$\Delta A_{sample} = A_{sample\ 2.2} - A_{sample\ 2.1} = 0.584 - 0.499 = 0.085 \tag{1.36}$$

Now the total ΔA can be calculated:

$$\Delta A_{D-lactic\ acid} = \Delta A_{sample} - \Delta A_{blank} = 0.085 - 0.000 = 0.085 \tag{1.37}$$

Now that the $\Delta A_{D-lactic\ acid}$ is known, the concentration of D-lactic acid can be calculated using equation 1.38:

$$C = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{D-lactic\ acid} = \frac{2.24 \times 90.10}{6300 \times 1.0 \times 0.1} \times 0.085 = 0.272\ g/L \tag{1.38}$$

As the sample was diluted, the content of D-lactic acid was calculated relative to the sample weight using equation 1.39:

$$c_{D-lactic\ acid} = \frac{c_{D-lactic\ acid}}{m_{sample}} = \frac{0.0272}{\frac{0.9653}{100} \times 1000} = 0.00282 \tag{1.39}$$

With an average D-lactic acid content of 0.00232 g/100 g for the two samples, as it can be seen in equation 1.40.

$$Average_{D-lactic\ acid\ content} = \frac{Sample_1 + Sample_2}{2} = \frac{0.00282 + 0.00182}{2} = 0.00232 \tag{1.40}$$

7.1.2 L-Lactic Acid Results

The table 1.13 summarizes the results from the L-lactic acid analysis.

Table 1.13: *L-lactic acid results for feta cheese samples.*

	Before L-LDH	**After L-LDH**		
	A1 (3 min)	A2 (10 min)	A3 (15 min)	A4 (20 min)
Blank	0.540	0.731	0.772	0.798
Standard	0.493	1.400	1.853	2.048
Sample 1	0.517	0.649	0.681	0.698
Sample 2	0.518	0.740	0.939	1.015
Results				
	$\Delta A_{L-lactic\ acid}$	**Conc. L-lactic acid [g/L]**	**Content L-lactic acid [g/100 g]**	
Blank	0.191	N/A	N/A	
Standard	0.907	0.229	0.237	
Sample 1	0.132	0.0189	0.0196	
Sample 2	0.222	0.00993	0.0103	

The concentration and content of L-lactic acid were calculated for all samples. The results are presented in Table 1.13, and the calculations for the concentration of L-lactic acid follows the same principle as for D-lactic acid, see equation 1.34.

The absorbance difference for L-Lactic acid follows equation 1.37 but with values from the L-lactic acid analysis:

$$\Delta A_{L-lactic\ acid\ Blank} = A_2 - A_1 = 0.731 - 0.540 = 0.191 \tag{1.41}$$

And,

$$\Delta A_{L-lactic\ acid\ Sample} = A_2 - A_1 = 0.649 - 0.517 = 0.1325 \tag{1.42}$$

Now the total ΔA can be calculated:

$$\Delta A_{L-lactic\ acid} = \Delta A_{blank} - \Delta A_{sample} = 0.191 - 0.1325 = 0.0585 \tag{1.43}$$

The concentration of L-lactic acid was calculated following equation 1.44:

$$C = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{L-lactic\ acid} = \frac{2.24 \times 90.10}{6300 \times 1.0 \times 0.1} \times 0.059 = 0.0272 \tag{1.44}$$

As the sample was diluted, the content of L-lactic acid was calculated relative to the sample weight, using equation 1.45:

$$c_{L-lactic\ acid} = \frac{c_{L-lactic\ acid}}{m_{sample}} = \frac{0.0189}{\frac{0.9653}{100} \times 1000} = 0.0196 \tag{1.45}$$

The average was calculated using equation 1.46.

$$Average_{L-lactic\ acid\ content} = \frac{Sample_1 + Sample_2}{2} = \frac{0.0196 + 0.0103}{2} = 0.01495 \tag{1.46}$$

8 Discussion

8.1 Composition of the Cheese

8.1.1 Total Solids/Moisture and Composition

First, we will look at the total solids/moisture and the composition of feta cheese. We calculated the moisture to be around 55% and 34%, after we found the total solids from the two samples. Compared to literature [3], we would expect a moisture percentage between 45 and 60. Therefore we think that something went wrong in the second sample, since it so far from the first sample. And as mentioned we should have made a new sample as our duplicates exceeded the 0.20% maximum described in the laboratory manual. But we accept the first sample as usable.

As mentioned in the introduction we would expect a content of 23% fat, 17% protein, and 2.2% salt. The Gerber method gave us an average fat content of 23.25%. Which is considered close enough to be accepted. For the protein content we used the DUMAS, which gave us an average protein content of 15.21%. A bit off, compared to the fat content, but still in an acceptable range. The salt content shows a bigger deviation between the declared amount and what we measured. We would expect a salt content around 2.2%, but measured an average content of 2.975%, which is a bit higher than expected.

Overall to get a more accurate content of the compositions, we could have made more samples and not just two. But we accept the results we got. A summary of the composition results can be seen in table 1.14.

Table 1.14: *Summary of the composition results for feta cheese samples.*

Component	Declaration [%]	Results [%]	Deviation [%]
Fat	23	23.25	1.09
Protein	17	15.21	10.53
Salt	2.2	2.975	35.23

So overall, we find the results we got for total solids and composition acceptable.

8.1.2 pH

Feta cheese typically has a pH of 4.4-4.6 [3]. Our measurements gave pH 4.28 and 4.27, which is a bit lower, but we consider it acceptable. Another possibility could be that the pH-meter was not calibrated correctly, as it was already sat up for the lab. We could except a smaller variability here, since we used to pack feta, so parameters such as starter culture, fermentation, brining and storage could have an impact on our measurements compared to the values from literature.

8.1.3 Ash Content

Ash content is a great indicator for minerals such as Calcium, Magnesium and Sodium. From other results reported in literature, we found that the ash content should be somewhere between 4.10 to 4.60 [4]. For our first sample we had a content of around 3.37% and for the second sample we had 3.28%. So we are a bit lower than what we expected.

8.2 Proteolysis Assessment

Proteolysis is a key biochemical process during cheese ripening. The degree of proteolysis in cheese is strongly dependent on milk composition, the type and activity of proteolytic enzymes present, and processing factors such as salt content and ripening conditions. The feta cheese analysed in this exercise was matured and stored in brine, resulting in a high NaCl concentration, which is known to inhibit enzymatic activity and microbial growth. The proteolysis potential in brined cheeses, such as feta, is generally less expressed than in hard, long-ripened cheeses, i.e. parmesan, where lower salt-in-moisture ratios and prolonged ripening favour protein degradation.

The proteolytic development was evaluated using the ratio between pH 4.6-soluble nitrogen (pH 4.6-SN) and total nitrogen. At pH 4.6, intact caseins precipitate while smaller peptides remain soluble. Therefore, a low ratio reflects limited secondary proteolysis, whereas a high ratio indicates extensive degradation of caseins. The low pH 4.6-SN to total nitrogen ratio observed for the analysed feta cheese is consistent with the cheeses production and ripening conditions.

The nitrogen fractionation further supported this interpretation. The low pH 4.6-SN relative to total nitrogen indicates that most nitrogen remains associated with intact or weakly degraded caseins. Formol-titratable nitrogen was low (0.032-0.034%), indicating a limited accumulation of free amino acids. Ammonium nitrogen was also low, suggesting that deamination was low and extensive proteolysis. As a result, the SN-fraction is dominated by peptide nitrogen.

8.3 Lipolysis

In this report, the fat acidity of feta cheese was determined by measuring the content of free fatty acids (FFA) through titration. The concentration provides an indication of amount of lipolysis, as FFAs are released through the enzymatic degradation of triglycerides in the cheese fat.

As presented in subsection 6.1, the degree of acidity was determined to be 3.94% and 4.59% for the duplicate samples, indicating a relatively high level of lipolysis in the analysed feta cheese. In comparison, similar studies

have reported fat acidity values ranging between 0.51% and 2.07% in white cheeses stored in brine, suggesting that the values obtained in this experiment are high [5].

The observed difference between the duplicate measurements may indicate experimental errors, particularly related to sample handling during the experiment. Determination of FFA involved several handling and transfer steps, and incomplete phase separation during fat extraction could have resulted in partial fat loss. Such losses would typically lead to an underestimation of fat acidity in one or both samples. However, given the relatively high acidity values presented in subsection 6.1, this is not considered to be the primary cause of the high values.

The high level of free fatty acids may instead be influenced by the storage conditions of the cheese. The feta cheese was stored in a freezer for seven days prior to the experiment. During storage, oxidative reactions may occur, contributing to an increase in fat acidity. Oxidation products, such as low-molecular-weight organic acids, can react with the titrant during analysis, thereby increasing the measured degree of acidity. Consequently, the measured acidity percent likely show a combination of lipolytic release of free fatty acids and oxidative degradation of lipids.

Finally, the titration endpoint was determined by visual observation of a colour change, which introduces a degree of subjectivity. Variations in interpretation of the endpoint may have resulted in the addition of excess potassium hydroxide (KOH) before the titration was stopped leading to an overestimation of the fat acidity.

8.4 Glycolysis

Lactic acid is formed under fermentation of lactose by lactic acid bacteria (LAB) typically added as starter cultures for feta production. Lactose is metabolised through glycolysis, where glucose is converted into pyruvate. Pyruvate is then reduced to lactic acid to regenerate NAD^+ allowing glycolysis to continue for multiple cycles.

Lactic acid exists as two stereoisomers, L-lactic acid and D-lactic acid, and the proportion of each depends on the type of lactate dehydrogenase (LDH) present in the LAB. LAB containing L-lactate dehydrogenase (L-LDH) produce L-lactic acid, whereas LAB containing D-lactate dehydrogenase (D-LDH) produce D-lactic acid. Some LAB species have both enzymes and may therefore produce both isomers of lactic acid [6].

As described in the introduction of this report, *S. thermophilus* and *L. delbrueckii* are commonly used in the production of feta cheese. *S. thermophilus* primarily produces the L-isomer of lactic acid, while *L. delbrueckii* mainly produces the D-isomer [7, 8].

The contents of both L- and D-lactic acid were analysed in the feta cheese given in this course, and the average concentrations were determined to be 0.0149 and 0.00232, respectively, as presented in subsection 7.1. The concentration of L-lactic acid were considerably higher than D-lactic acid, which is consistent with expectations for feta cheese and can be explained by differences in the growth behaviour of the used LAB. *S. thermophilus* has a higher growth rate than *L. delbrueckii* and is therefore able to convert a large proportion of the available lactose into lactic acid at an early stage of cheese production [9]. As a fast-growing LAB, *S. thermophilus* dominates the microflora during the initial phase of fermentation, which partly explains the higher concentration of L-lactic acid compared to D-lactic acid.

Additionally, as the pH decreases and salt is added during cheese manufacture, *L. delbruckii* is inhibited more rapidly than *S. thermophilus* [10]. As a result, the formation of D-lactic acid decreases faster than production of L-lactic acid, further contributing to the observed difference in lactic acid concentrations.

Great differences were observed between the individual samples in the determination of L- and D-lactic acid, see subsection 7.1. These variations are likely attributable to experimental inaccuracies during sample preparation, including weighing and pipetting.

8.5 Comparison Across Analysed Cheeses

During the laboratory exercises for this course, multiple types of cheese were analysed using similar methodologies. Comparing the results obtained for feta cheese with those from other cheeses, such as Grana Padano, Brie, and Havarti, provides insights into how cheese type and processing conditions influence biochemical properties.

Two tables were compiled using data from each group attending the course. For this report table 1.15 and 1.16 has been compiled to focus on comparison of our feta cheese, with group 4's Grana Padano, group 12's Brie, and group 15's Havarti.

Table 1.15: *Comparative compositional parameters of the analysed Feta cheese and selected reference cheeses from other groups: Grana Padano (Group 4), Brie (Group 12), and Havarti (Group 15).*

Gr.	Cheese	Total Solids	Ash	pH	NaCl	Salt-In-Moisture	D-Lactate	L-Lactate
1	Feta	55.24	3.33	4.28	2.98	7.035	0.00232	0.01495
4	Grana Padano	65.60	3.91	5.56	1.065	N/A	0.93	0.73
12	Brie	48.45	2.47	7.17	1.21	2.37	-0.0091	0.0022
15	Havarti	65.06	3.40	5.40	1.66	4.72	N/A	0.0547

A lower pH and a higher salt-in-moisture content were observed in the analysed feta cheese compared to the other cheeses, as shown in table 1.15. This reflects the brined manufacture and limited ripening of feta, which contrasts with the longer ripening times and lower salt levels in Grana Padano, Brie, and Havarti. The total solids content of feta was intermediate between the high-moisture Brie and the low-moisture, long-ripened Grana Padano. The elevated salt level in feta is consistent with reduced lactate accumulation, as indicated by comparatively low D- and L-lactate concentrations. In contrast, Grana Padano cheese, which undergoes a prolonged fermentation and maturation, showed higher lactate levels and total solids.

Table 1.16: *Comparative proteolysis- and lipolysis-related parameters of the analysed Feta cheese and selected reference cheeses from other groups: Grana Padano (Group 4), Brie (Group 12), and Havarti (Group 15).*

Gr.	Cheese	N_{Total}	Protein	pH 4.6-SN	N_{Formol}	$N_{Ammonium}$	Fat	Fat acidity
1	Feta	2.38	15.21	0.1365	0.033	0.2494	23.25	4.26
4	Grana Padano	4.838	30.86	1.4089	0.898	0.603	28.4	3.628
12	Brie	4.89	31.2	0.09	0.825	N/A	34.6	4.58
15	Havarti	3.72	23.7	0.214	0.67	N/A	36.2	2.21

The results showed significantly lower total nitrogen and protein content in the analysed feta cheese compared to the other cheeses, as presented in table 1.16. This reflects the limited proteolysis occurring during the short ripening time and high salt conditions of feta production. The pH 4.6-soluble nitrogen fraction was significantly lower in feta compared to Grana Padano, however, it was higher than in Brie, indicating some degree of protein degradation. Formol-titratable nitrogen and ammonium nitrogen were also low in feta, consistent with limited amino acid release and deamination.

9 Conclusion

Based on the analytical data obtain in this report (sections 4, 5, 6, and 7), minor discrepancies were observed between the declared nutritional values and the experimentally determined composition of the feta cheese. These differences are likely attributable to sampling variability, storage conditions prior to analysis, and minor experimental errors during analysis.

The measured pH values of the cheese (4.28 and 4.27) were slightly lower than the typical pH range reported for traditional feta cheese, which is generally between 4.4 and 4.6. Furthermore, the ash content was found to be lower than the value stated on the product label. This deviation may be related to variations in milk composition or differences in brining and mineral uptake.

The distribution of nitrogen in the analysed cheese confirmed that proteolysis was limited. This was reflected in the combined results showing a low pH 4.6-SN/TN ratio, low formol-titratable nitrogen, and a low ammonium nitrogen. These results indicates that the proteolytic potential was dominated by the primary events and less pronounced during the secondary, and that a minimal amino acid release was minimal. These results are consistent with feta cheeses, which are brine-ripened and stored, at high NaCl concentrations. The short ripening time and environment, constrains the enzymatic and microbial activity resulting in the low proteolytic potential.

Most of the nitrogen remained associated with intact or weakly degraded caseins, which contributes to the feta's characteristic firm and crumbly texture with a mild flavour profile.

Lipolysis analysis showed relatively high levels of FFA when compared to values reported in the literature. This is likely influenced by oxidative reactions occurring during storage, which may contribute to an overestimation of FFA originating from lipolysis.

Glycolysis analysis demonstrated a higher concentration of L-lactic acid compared to D-lactic acid, which is consistent with the starter cultures used in feta cheese production and aligns well with existing literature.

Overall, the analyses performed as part of this course, illustrates how manufacturing practices, brining, and ripening conditions influence the properties of feta cheese. Despite minor and major variation, the experimentally determined values generally correspond well with the declared values on the label.

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Chapter 5
Abbreviations and Explanations

Topic	Abb.	Description
Leaching	**n.a.**	*leaching refers to the process by which substances, such as ions, minerals, or nutrients, are removed or lost from the soil. This often occurs due to water penetrating the soil and displacing these substances*