



Molecular and Functional Properties of Milk - MFPM

Notes for the course NFOK17002U, at the University of
Copenhagen; February 2025 - April 2025

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Link to Git repo.: https://github.com/DanishUnicorn/mfpm_notes



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Chapter 1

Course Description

1.1 Content

This course has the overall aim of providing students with a detailed knowledge of both theoretical and practical aspects of the molecular, colloidal and functional properties of milk and milk production. In addition, the functionality of food ingredients derived from milk will be covered. The course primarily deals with the following subjects:

Introduction: The chemistry of milk components such as lactose, milk proteins and peptides and lipids.

Milk production and its effects on milk: Milk secretion, the influence of housing and feeding regimes, milking technologies and their influence on raw milk quality, organic production of milk.

The colloidal system of milk: The properties of the casein micelles and the interplay with milk serum (whey) proteins and lipids.

The effects of heat treatment of milk: Denaturation and aggregation reactions, Maillard reactions, milk proteolytic enzymes and analytical separation processes, such as chromatography, used to characterise these. The effects of processes involving mainly heat treatment on the bioactivity of milk ingredients.

Functionality of milk constituents and methods applied for characterization of functionality: Solubility, gelation properties, rheology, microscopy, design of model systems and tests for functional properties. Biological activity of milk components.

1.2 Learning Outcome

After completing the course the student should be able to:

1.2.1 Knowledge

- Describe in detail the composition of milk
- Describe the colloidal interactions governing stabilization and destabilization of milk and dairy products
- State the major factors influencing milk production and raw milk quality, including milk storage
- Outline the factors affecting milk ingredient functionality in food systems and how they can be analysed
- Appreciate the effects of dairy production and processing on milk at a molecular level.

1.2.2 Skills

- Apply the principles of colloid science to milk processing and milk ingredient functionality
- Structure a scientific presentation and produce a condensed synopsis.

1.2.3 Competences

- Plan experiments related to the effect of a culinary technique on the sensory properties of food
- Discuss the factors influencing milk ingredient functionality
- Reflect on the health functionality/bioactivity of milk constituents
- Recognise the importance of working effectively in a group during laboratory practicals.

1.3 Literature

See Absalon for a list of course literature.

1.4 Recommended Academic Qualifications

Knowledge equivalent to a dairy internship is recommended.

Academic qualifications equivalent to a BSc degree is recommended.

1.5 Teaching and Learning Methods

The course consists of lectures and tutorials (4-8 hrs/week) and laboratory practicals (4-8 hrs/week), as well as e-learning, and excursions.

Lectures and tutorials provide an overview of milk quality and thermal processing on the functionality and bioactivity of milk and milk ingredients. Laboratory practicals dealing with aspects of milk as a colloidal system and milk protein functionality are also included as well as excursions.

Minor costs in relation to excursions are paid by the students.

1.6 Remarks

It is recommended to follow the course on the first year of the MSc Programme in Food Innovation and Health.

1.7 Workload

See table 1.1 for an overview of the workload for the course.

1.8 Feedback Form

Continuous feedback during the course of the semester Peer feedback (Students give each other feedback)

Table 1.1: A table with an overview over the workload for the course.

Category	Hours
Lectures	26
Preparation	90
Theory exercises	15
Practical exercises	32
E-Learning	3
Excursions	30
Exam	10
Total	206

1.9 Sign Up

Self Service at KUnet

<http://www.science.ku.dk/english/courses-and-programmes/>

<https://www.science.ku.dk/english/continuing-and-professional-education/single-subject-courses/practical/>

1.10 Exam

Table 1.2: The table shows the details of the course exam, as defined from the website of the University of Copenhagen.

Category	Details
Credit	7.5 ECTS
Type of assessment	Oral examination, 20 minutes
Type of assessment details	One week prior to the actual exam, the student draws a subject and prepares an oral presentation in this subject. Assessment will be based on the oral presentation and following questions within the course curriculum. The presentation should take no more than 8 minutes. Following the presentation the student will be asked questions for approx. 7 minutes.
Exam registration requirements	Approval of reports from practicals.
Aid	All aids allowed
Marking scale	7-point grading scale
Censorship form	No external censorship Several internal examiners
Re-exam	Same as ordinary exam. The student draws a new subject a week before the re-exam and a new presentation is prepared and given at the examination. If the student has non-approved reports from the practicals, these must be handed in for approval at least two weeks prior to re-examination.

Criteria for exam assessment See Learning Outcome.

Chapter 2

Basic Knowledge Recap

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 microbiology concepts.

2.1 1st RM

Introduction

Chemistry evolved over a millennia, merging with biology to form diverse fields like **food chemistry**, which focuses on sustenance and nutrition. This chapter outlines chemistry's relation to food sciences and its educational context, addressing challenges like increased teaching loads and exploring new opportunities [2].

On the Need for Chemistry

Food chemistry studies the composition, structure, and properties of food and their transformations. It builds on general chemistry, including **organic**, **physical**, and **inorganic** branches. This chapter surveys these areas and introduces key terms for future topics [2].

Organic Chemistry

Organic chemistry focuses on carbon compounds and **covalent bonds** with hydrogen. Hydrocarbon nomenclature (e.g., **alkanes**, **alkenes**, and **alkynes**) introduces systematic naming. Topics include bonding theories, **valence**, and molecular conformation constraints [2].

Functional Group Chemistry

Organic compounds belong to families like **alkanes**, **alcohols** (R-OH), and **carboxylic acids** (R-COOH). Functional groups have consistent behaviour, e.g., **hydroxyl** groups act similarly across compounds. Their reac-

tivity may vary with the R group, as in alcohol vs. phenol [2].

Aromatic Compounds

Aromaticity in compounds like benzene involves **electron delocalisation** and resonance. Conjugated double bonds in unsaturated lipids and fat-soluble vitamins contribute to their color and oxidative sensitivity. Free radicals drive polymerization of natural monomers like **isoprene** [2].

Organic Reaction Mechanisms

Organic reactions include **addition**, **substitution**, and **rearrangement**, often occurring in raw and processed foods. **Free radical** reactions drive polymerization and impact **lipid oxidation**, photochemical processes, and ageing-related deterioration [2].

Stereochemistry

Stereochemistry studies chiral compounds, often with asymmetric carbons attached to four groups. Molecules with N stereogenic centers yield (2^N) stereoisomers. Chirality includes helices lacking stereogenic centers. Chiral molecules rotate plane-polarized light, but **racemic mixtures** show no net optical activity [2].

Physical Chemistry

Physical chemistry explores material transformations, focusing on three key themes: **thermodynamics**, **chemical kinetics**, and **quantum mechanics** [2].

Thermodynamics

Thermodynamics examines energy forms, transformations, and efficiency. The **first law** states energy is conserved, expressed as ($U = Q + W$). The **second law** highlights entropy increase, with **Gibbs free energy** (ΔG) predicting spontaneity. Applications include **reaction calorimetry** and energy content in food [2].

Chemical Kinetics

Chemical kinetics studies reaction rates, influenced by factors like concentration, temperature, and catalysts. Reaction rates follow Equation 2.1.

$$\text{rate} = k[A]^x[B]^y \quad (2.1)$$

With (k) described by the **Arrhenius equation** which can be seen from Equation 2.2

$$\ln k = \ln k_0 - \frac{\Delta E^\#}{RT} \quad (2.2)$$

The **Eyring theory** relates k to $\Delta G^\#$, $\Delta H^\#$, and $\Delta S^\#$, emphasizing transition states [2].

Inorganic Chemistry

Inorganic chemistry covers non-carbon elements, including metals, nonmetals, and metalloids. Periodic trends, such as **atomic radii**, **ionization energies**, and **electronegativity**, arise from effective nuclear charge and electron shielding [2].

Chemical Bonding

Chemical bonding can be described with **Lewis structures** which illustrates electron sharing or transfer. The **octet rule** explains covalent bonds, while differences in electronegativity lead to polarized or ionic bonds. **Resonance** applies to compounds like nitrate, where multiple structures describe electron distribution [2].

The Shapes of Molecules

Molecular shapes are predicted using the **VSEPR model**, based on repulsions between electron pairs. Geometries like **linear**, **trigonal planar**, **tetrahedral**, and **octahedral** depend on bonded atoms and lone pairs, as seen in molecules like H_2O and NH_3 [2].

Valence

Valence-bond theory explains covalent bonds through orbital overlap. Some valencies require **hybrid orbitals** (e.g., sp^1 , sp^2 , sp^3), formed via electron promotion and orbital mixing. Orbital shapes are influenced by electron repulsion [2].

Molecular Orbital Theory

Molecular orbital theory explains bonding via atomic orbital combinations forming equal numbers of bonding σ and anti-bonding σ^* orbitals. Molecular orbitals follow **Pauli's exclusion principle** and fill singly before pairing. Diatomic oxygen illustrates these principles [2].

Food Chemistry

Definition and Scope

Food chemistry applies chemistry principles to food systems, studying macroconstituents (e.g., **water**, **carbohydrates**) and microconstituents (e.g., **vitamins**, **additives**). Post-WWII advances improved **shelf-life**, **packaging**, and analysis of toxicants. Food sciences ensure nutritious, safe, and affordable food [2].

Areas of Expertise Required by the Institute of Food Technology

The **Institute of Food Technology** identifies five core competencies for food scientists: **chemistry**, **analysis**, **nutrition**, **microbiology**, and **engineering** [2].

Chemistry and the Food System

Food chemistry encompasses all levels of the food system, from **soil pH** in agronomy to the effects of **pasteurization**. Its scope includes **harvesting**, **processing**, **packaging**, and **distribution**, as well as studying **ingredient behaviour** during manufacture [2].

2.2 2nd RM

Osmosis and Osmotic Pressure

Osmosis involves water moving to solute-rich areas across membranes. **Osmotic pressure** drives this process, dehydrating microbes in salted foods. Preservation methods like salting meats and sugaring jams rely on this principle, as seen in **beef jerky** and jellies [1].

2.2.1 Carbohydrates

Foods High in Carbohydrates

Carbohydrates, including sugars, starches, and fibers, primarily originate from plants. Sources include **grains**, **legumes**, **fruits**, and **vegetables**. Exceptions are **milk**, containing lactose, and animal muscles, storing glycogen. Table sugar comes from **sugar cane** and **sugar beets**, while honey is floral nectar [1].

Composition of Carbohydrates

Carbohydrates consist of **carbon**, **hydrogen**, and **oxygen**, following the formula $C_n(H_2O)_n$. Synthesized via **photosynthesis**, they form saccharides classified as **monosaccharides**, **disaccharides**, **oligosaccharides**, and **polysaccharides** based on saccharide units [1].

Monosaccharides

Monosaccharides are simple sugars; common types include **pentoses** (ribose, arabinose) and **hexoses** (glucose, fructose, galactose). The ending *-ose* indicates that the compound is a sugar [1].

Ribose and Arabinose

Ribose is vital in **DNA**, **RNA**, and **ATP**. It also contributes to **vitamin B₂**. **Arabinose** supports the structure of vegetable **gums** and **fibers** [1].

Glucose

Glucose is the most common **hexose** in foods and blood. It is found in fruits, honey, and corn syrup. Refined glucose (**dextrose**) is used in **candies**, **baked goods**, and **alcoholic beverages**. It is the main component of corn syrup, where it is made by hydrolyzing cornstarch [1].

Fructose

Fructose, or **fruit sugar**, is the sweetest sugar, found in fruits and honey. It causes unwanted properties as **stickiness**, **over-browning**, and lowers **freezing points**. **High-fructose corn syrup** is widely used in soft drinks [1].

Galactose

Is not often found in its free form, but is a component of **lactose**. A derivative, **galacturonic acid** is very important in fruits ripening process [1].

Disaccharides

A disaccharide is formed by two monosaccharides linked together. In this next section, sucrose, lactose and maltose will briefly be discussed [1].

Sucrose

Most commonly known as **table sugar**, and is in its chemical form a disaccharide composed of **glucose** and **fructose** [1].

Lactose

Most commonly known as **milk sugar**, and is in its chemical form a disaccharide composed of **glucose** and **galactose**. Many people are unable to produce the enzyme **lactase**, which is responsible for **breaking down** lactose, thus making them **lactose intolerant**. The characteristic symptoms are **bloating** and **abdominal pain**. In some **fermented milk products**, bacteria break down the lactose to **lactic acid**, resulting in a product some lactose intolerant individuals can tolerate [1].

Maltose

Also called **malt sugar**, is a disaccharide composed of **two glucose** units. It is primarily used in the production of **beer** and **breakfast cereals** [1].

Oligosaccharides

Oligo comes from Greek and means **few**. Oligosaccharides are composed of 3-10 monosaccharides. The two most common oligosaccharides are **raffinose** and **stachyose**. These are found in **legumes** and **cruciferous vegetables**. They are not digested in the small intestine, but are fermented by the gut microbiota in the large intestine [1]. Fructo-oligosaccharides (FOS) are also a type of oligosaccharides, and are found in e.g. **onions** and **garlic**. They are used as **pre-biotics** [1].

Food Industry Uses

Oligosaccharides serve as **bulking agents** in diet foods e.g. confections, yogurt, and beverages (also as **fat replacers** in beverages). They are **non-cariogenic** and non **cavity-producing** (they do not promote **tooth decay**), unlike disaccharides. Food-grade oligosaccharides are derived from **soybeans** or via starch processing [1].

Polysaccharides

Polysaccharides are composed of more than 10 monosaccharides. The most common polysaccharides are **starch**, **glycogen**, and **fiber**. They are subdivided into digestible (e.g. starch and glycogen) and indigestible (e.g. fiber) [1].

Starch - Digestible Polysaccharide from Plant Sources

Starch stores energy in **granules** in foods like rice and potatoes. It consists of **amylose** (straight chains) and **amylopectin** (branched chains with **alpha-1,6 bonds**), joined by **alpha-1,4 bonds**. Starches are typically 75%

amylopectin and **25% amylose**. In foods, factors as heat, acids and enzymes are used to break down the starches into **dextrins** (smaller segments of starches) [1].

Glycogen - Digestible Polysaccharide from Animal Sources

Glycogen, a highly **branched** glucose polymer, is found in animal **liver** and **muscles** as an energy reserve. It is, during slaughter, hydrolysed to maintain blood **glucose levels**. Glycogen in meat converts to **lactic acid**. **Oysters and scallops** retain small amounts, adding a sweet taste compared to other types of sea creatures [1].

Fiber - Indigestible Polysaccharide

Measuring Dietary Fiber

Soluble vs. Insoluble Fiber

Common Fibers and Their Food Industry Uses

Cellulose

Hemicellulose

Pectic Substances

vegetable Gums

Inulin

Lignin

Function of Carbohydrates in Foods

2.2.2 Lipids, Or Fats

Foods High in Lipids

Composition of Lipids

Triglycerides

ghhgh

Chapter 3

Lecture Notes

3.1 1st Lecture - Plants and Food Colours

Lecture Goals

After this lecture, the students will be able to:

- Describe the structures of fruits and vegetables
- Identify structural carbohydrates in plants & changes they undergo during ripening and processing
- Describe important factors responsible for texture, colours, flavours, and taste of plants

Plant Organs

Plants have different organs, each serving a specific function. Table 3.1 shows some plant organs and examples of fruits and vegetables which has the following trait.

Hemicelluloses

- Hemicelluloses contain a variety of sugars in their long chains—unlike starch and cellulose
They contain both pentoses and hexoses
- Hemicelluloses, matted with pectic substances, serve as a connection between fibrillar cellulose
- Alkaline medium has a strong effect—vegetables cooked with baking soda added become flaccid & mushy
Baking soda also has a destructive effect on thiamine

Xylan and arabinan are two particularly common hemicelluloses with glucuronic acid attached, which is a common feature of pectic. Figure 3.1 shows the structure of these.

Pectic Substances

- Pectic substances is a general term for member of this family of polygalacturonic acid compounds
Protopectin, pectin and pectic acid
- Contained in the primary cell wall and the middle lamella (the outer region of the cell wall)
- Pectic substances in the middle lamella change form during the maturation process

Table 3.1: A table showing plant organs and examples

Organ	Function	Plant material and cell type	Example
Roots	Anchor plants into ground, absorb nutrients	Tough fibrous material – cells have thick, cellulose-rich cell walls.	Inedible
		Some roots swell up with storage cells full of <i>amyloplasts</i>	Carrots, parsnips, radishes, sweet potatoes
Stems, stalks	Conduct nutrients to roots and leaves, gives structural support	Fibrous material (stems, stalks) - cells have thick, cellulose-rich cell walls	Asparagus stems, cellery stalks
Tubers, rhizomes		Some stems swell up with storage tissue (tubers rhizomes) -cells are full with <i>amyloplasts</i>	Potato, turnip, ginger
Leaves	Produce sugar molecules by photosynthesis	Plant material is thin so gases can penetrate/escape. Almost no structural support—cell walls are thin and flexible. Cells have many chloroplasts and large air pockets between them for gases.	Spinach leaves, lettuce leaves
Flowers	Reproduction	Contain reproductive organs, Often colorful to attract pollinators	Cauliflower, broccoli
Fruits	Seed dispersal	Fleshy or dry structures, Contain seeds	Apples, oranges
Seeds	Germination	Contain embryo and nutrients, Protected by seed coat	Peas, beans

- Combine with hemicellulose in the primary cell wall to form the "cement" surrounding the cellulose fibers

Protopectin is water-insoluble, and occurs in immature fruits and, to a lesser extent, in vegetables. Pectin is water-soluble, and is found in ripe fruits and vegetables. Pectic acid is formed when pectin is heated in an acid medium. Figure 3.2 shows the structure of these.

Starchy Vegetables and Texture

When vegetables are raw, their starch granules are hard and give a chalky feeling when chewed. When cooked, the starch granules begin to soften around 60°C, when cell membranes are also affected. Starch granules absorb water which disrupt their structure and they swell, forming a gel. Vegetables becomes tender but dry. When cell walls are weak, the gel-filled cells pull away from each other as separate particles giving a mealy impression.

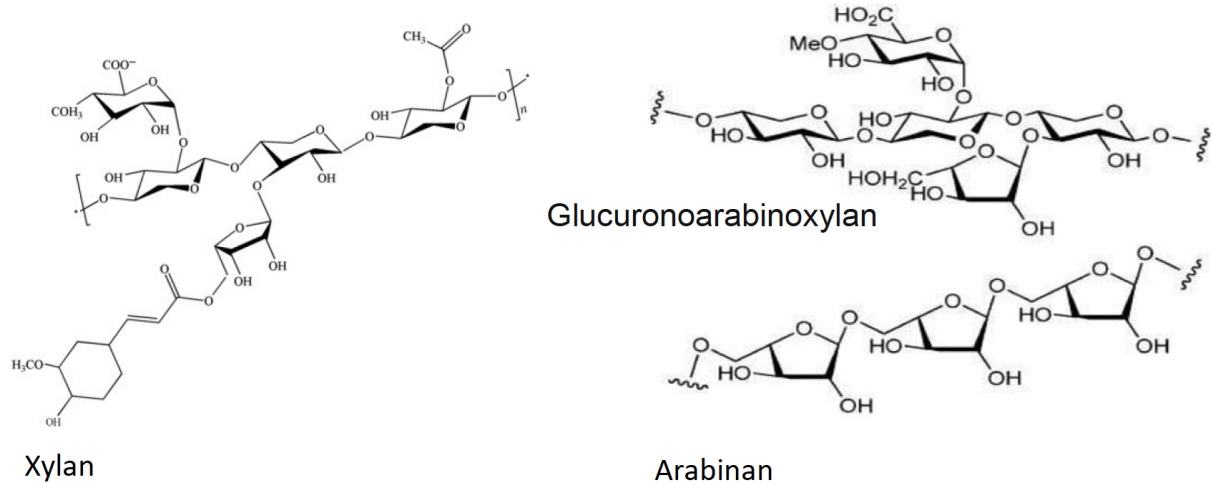


Figure 3.1: Structure of hemicelluloses

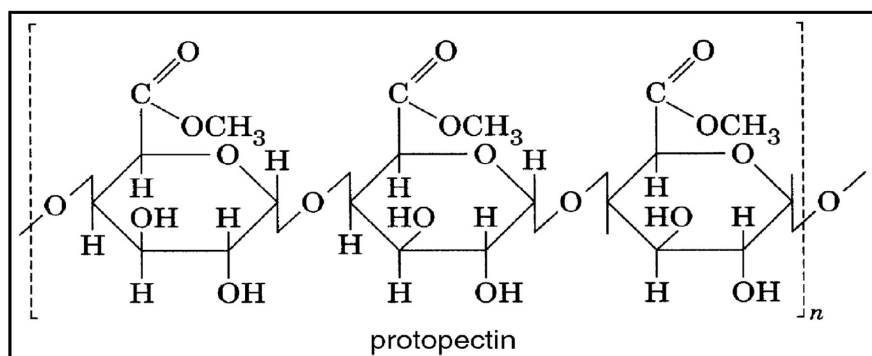


Figure 3.2: This form is a methylated (methyl ester groups), very long polymer of galacturonic acid

Important Factors During Precessing of Potatoes

- Boiling - Pectin crosslinking promoted by enzyme activity at 55-60°C for 20-30 min
Firm potato. According to McGee, enzyme in the cell wall alters cell well pectins - more easily cross-linked by calcium ions (activated around 50°C and inactivated around 70°C)
- Frying - Starch leaks out of granules and glues outer cell walls together when initial frying temperature is kept low (120-160°C) - crisp crust
- Grayish discolouration: caused by a pigmented complex formed by chlorogenic acid, oxygen and iron ions
Minimized at low pH
- Leftover potatoes get a stale, cardboard flavour, due to membrane lipids being oxidized
- Salt speeds up softening (Sodium ions destabilize cell wall)
- Ca²⁺ slows down softening (stabilizes cell wall)
- High pH soften hemicellulose and open starch structure

3.2 2nd Lecture - Carbohydrates I

Carbohydrates in food

In foods various carbohydrates are present. Some examples are listed in Table 3.2.

Table 3.2: Carbohydrates in food with classification based on their molecular size

Monosaccharides	Di-	Oligo-	Poly-
Glucose	Sucrose	Digestible:	Digestible:
Fructose	Lactose	Maltotriose	Starch
Galactose	Maltose	Maltotetrose	(Amylose, amylopectin)
Mannose	Cellobiose	Maltopentose	
Ribose	Trehalose	Non-digestible	Non-digestible
Xylose	...	Rafinose	<u>Soluble:</u> agar, gum arabic, carrageenan, pectin, ...
...		Stachyose	<u>Insoluble:</u> cellulose, protopectin, chitin, ...

Anomeric Carbon and Reducing Sugar

The anomeric carbon originates from the carbonyl group in the open-chain form of a sugar and becomes a stereocenter in the cyclic form, capable of forming alpha and beta isomers. Reducing sugars can switch back to the open-chain form, exposing the reactive carbonyl group, key in reactions like the Maillard reaction. Non-reducing sugars, like sucrose, have their anomeric carbon locked, preventing such reactivity. An example of an open-chain and cyclic form of a monosaccharide is shown in Figure 3.3 where the anomeric carbon is highlighted in red. The term "masked oxo-group" refers to the carbonyl group (C=O) in the open-chain form being hidden in the ring structure.

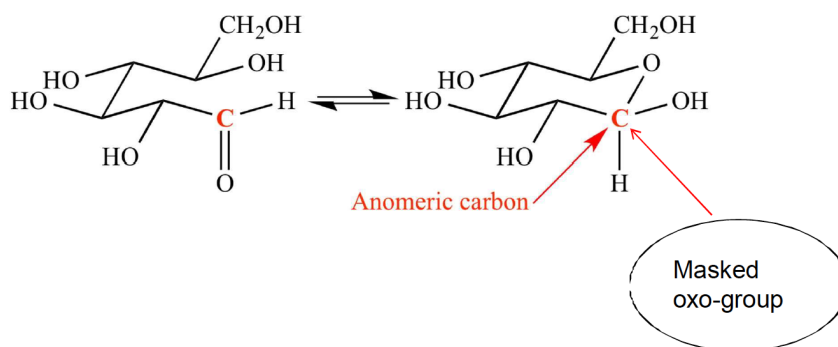


Figure 3.3: An example of an open-chain and cyclic form of a monosaccharide

3.3 12th Lecture - Stocks and Sauces

Chapter 4

Lecture Exercises

4.1 19.11.24 - Exercise 1 - Carrot Soup

You are working in a company that produces frozen soups, ready to heat and eat. You are in the process of developing a new product, a carrot soup with an orange flavour. The basic recipe looks like this:

a.

Describe the structure of the cell wall of the carrot.

b.

Name the three major polysaccharides in the cell wall of the carrot and describe what happens with these polysaccharides during cooking.

c.

The orange you have used for the soup is fairly ripe, what kind of pectic substances do you think predominate in the orange? Explain also briefly the changes that the pectic substances undergo during ripening. Explain also whether you think the pectic substances extracted from the orange will contribute to gel formation in the soup, and why /why not.

Table 4.1: Basic Recipe for Carrot Soup

Ingredient	Preparation
Oil	<ul style="list-style-type: none">• Heat a large saucepan over medium heat. Add oil. Cook the onion, stirring occasionally, for 3 minutes or until soft. Add carrot and cook, stirring often, for 5 minutes or until just soft.• Add stock. Bring to boil. Peel 2 strips of rind from the orange. Add to pan. Reduce heat to medium-low. Simmer for 20 min. or until carrot is tender. Remove and discard orange rind.• Set aside for 5 minutes to cool. Use a blender to puree until smooth. Juice orange. Add to soup. Place over medium heat and cook for 2 minutes or until heated through. Top with parsley.
1 Onion, finely chopped	
1 kg carrots, finely chopped	
1L chicken stock	
1 Orange	
1 tbsp parsley	

d.

Name the pigment that gives a nice orange colour to the soup and explain where it is found in the plant cell and describe its solubility properties.

e.

You consider improving flavour by adding lemon juice to the soup as well as orange juice. To make it a little simpler you want to add all ingredients at the onset of cooking. How do you think that addition of citrus juice might affect the softening of the carrots, if the juice is added at the onset of cooking? Explain why.

Chapter 5

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 microbiology concepts.

5.1 1st lecture

5.1.1 Article 1 - Fermented Foods as Experimentally Tractable Microbial Ecosystems

Introduction

Chapter 6

Group Project - Enzymes - Membrane Associated Enzymes

Formal requirements for the summary

- The summary should not be longer than 3-4 pages excluding references
- The summary should be written as continuous text (a small number of bullet points may be used)
- All references must be cited with full bibliographic information. Citation style can be chosen individually, but should be consistent throughout the summary
- Please address the following aspects:
 - An appropriate title and suitable headings
 - Classification and modus operandi of the enzyme(s)
 - The role of the enzyme(s) in milk and/or dairy products
 - A short summary of 1-2 recent scientific studies related to the enzyme(s)
 - References
- The deadline for submission is February 28

6.1 Introduction

- Phospholipids (PL) in Milk: Estimates of PL content in bovine milk vary (0.9–2.3% of total lipids) depending on extraction methods (Röse Gottlieb vs. Folch extraction).
- Milk Fat Globule Membrane (MFGM): Contains 60–65% of milk PL, with the remaining 35–40% found in the skim milk phase.
- Extracellular Vesicles (EVs): Recently recognized nano-sized phospholipid structures in skim milk, distinct from milk fat globules, but involved in intercellular communication.
- Membrane-Associated Enzymes: Identified in both MFGs and EVs, but their abundance is generally low. Many originate from ER, Golgi, or cytosolic crescents.
- Activity Considerations: Many of these enzymes remain inactive in milk due to the absence of substrates or unsuitable environmental conditions.
- Scope of Discussion: The summary will focus on enzymes relevant to mammary gland biology, milk integrity, and physiological functions upon consumption, excluding those related to lipid synthesis.

6.2 Sulfhydryl Oxidase

- Sulfhydryl Oxidase (EC 1.8.3.2): Catalyzes oxidation of protein thiols (cysteine residues) to form disulfide bonds, reducing oxygen to hydrogen peroxide.
- Types in Milk: Exists in metal-dependent and flavin-dependent forms.
- Early Studies:

Iron-dependent sulfhydryl oxidase (89-kDa, contains iron) was initially reported (Janolino & Swaisgood, 1975).

Later studies failed to confirm its presence (Jaje et al., 2007).
- Current Understanding:

Flavin-dependent sulfhydryl oxidase (QSOX1) is well-documented in bovine milk.

Sequence analysis confirmed it as part of the Quiescin-sulfhydryl oxidase family.
- Membrane Association:

Initially believed to be strongly associated with phospholipid membranes (Kitchen, 1974).

Later studies suggest a looser association, making it a more soluble protein (Jaje et al., 2007).
- Proteomic Evidence: QSOX1 has been identified in membrane fractions of both human and bovine milk (Liao et al., 2011; Reinhardt et al., 2013).

Structure of Flavin-Dependent Sulfhydryl Oxidase

- QSOX1 Splice Variants:

QSOX1-L (Long form, 79.6 kDa): Contains a transmembrane region.

QSOX1-S (Short form, 63.8 kDa): Lacks most of exon 12 and is more soluble.

QSOX1-S is more prevalent than QSOX1-L, including in mammary-derived cell lines.
- Structural Features:

Multi-domain enzyme derived from fusion of two ancient genes.

Contains thioredoxin (Trx) domains, FAD-binding module, CxxC motifs (common in redox reactions).

QSOX1-L has a membrane-spanning region, while QSOX1-S does not.
- QSOX1 in Bovine Milk:

Jaje et al. (2007) isolated sulfhydryl oxidase from bovine skim milk, identifying it as QSOX1-L, though later studies suggest it was likely QSOX1-S.

The enzyme migrated as a 62 kDa band in SDS-PAGE.
- Biological Significance:

QSOX1 expression is linked to tumorigenesis (Antwi et al. 2009; Katchman et al. 2013).

QSOX1-S has been isolated from mammalian blood serum (Israel et al. 2014).

QSOX1-L can be proteolytically modified and secreted into the extracellular matrix, possibly after removal of its transmembrane domain (Rudolf et al. 2013).

Dimerization of QSOX1 has been demonstrated in the same study.

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Chapter 7

Abbreviations and Explanations

Topic	Abb.	Description
16S ribosomal RNA	16S rRNA	<i>A component of the 30S subunit of prokaryotic ribosomes, commonly used in phylogenetic studies to identify bacteria and archaea.</i>

Bibliography

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Chapter A

Appendix

A.1 Appendix 1 - Principles for isolation of microorganisms from fermented food and beverages