UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

Molecular and Functional Properties of Milk - MFPM

Group 05 project: Milk Enzymes - Membrane Associated Enzymes

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Link to Git repo.: https://github.com/DanishUnicorn/mfpm_group_05

Contents

Chapter 1
Requirements

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

Chapter 2
Notes for the resume

2.1 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.

2.2 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

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.

Biological Role of Flavin-Dependent Sulfhydryl Oxidase and Significance in Milk

- QSOX1 in High Secretory Cells:
  Associated with Golgi membranes, cell surface, and secreted as an enzyme.
  Overexpressed in breast tissue, pancreas, and prostate cancer, suggested as a diagnostic cancer marker.
  Also linked to heart failure diagnosis.
- Function in Milk:
  May contribute to protein folding, but exact substrates remain unidentified.
  "Cooked" and "flat" off-flavou rs in UHT milk are associated with sulfur-containing compounds, methyl ketones, and aldehydes.
  QSOX1 may impact oxidation of these compounds; 60% of its activity remains post-pasteurization.
  QSOX immobilized on glass beads has been used to reduce UHT milk off-flavours.
- Catalytic Properties:
  Activity measured using GSH or reduced RNase at pH 7, producing a yellow product (412 nm absorption).
  Oxidation of thiol substrates generates hydrogen peroxide aerobically; ferricenium ion acts as an alternative electron acceptor anaerobically.
  Reduction with dithionite or dithiothreitol forms a two-electron intermediate (EH2) with a charge transfer band at 560 nm.
  Redox-active disulfide bridge involved in catalysis, with similarities to pyridine nucleotide dependent disulfide oxidoreductases.
- Comparison with Other QSOX1 Enzymes:
  Bovine milk QSOX1 has slightly higher kcat/Km values than the chicken egg white enzyme for

oxidation of DTT, glutathione, and reduced RNase.

Differences may stem from variations in electron flow mechanisms.

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2.3 Catalse

- Catalase (EC 1.11.1.6):
  Catalyzes hydrogen peroxide breakdown into water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$).
  Found in most aerobic organisms, primarily located in peroxisomes.
- Catalase in Milk:
  One of the first enzyme activities detected in milk due to easy peroxide monitoring.
  Historically classified as an indigenous MFGM enzyme, but proteomic data do not confirm its presence in MFGM.
  Mainly derived from non-milk-secreting cells, present in milk primarily during mastitis.
- Reassessment of Catalase in Milk:
  Likely originates from somatic milk cells or microorganisms (added/contaminating).
  Not a true membrane-associated enzyme, but included in discussion due to historical classification.

Structure of Catalase

- Bovine Liver Catalase:
  Reliable amino acid sequence (526 amino acids) published by Schroeder et al. (1982).
  Crystallization studies reported, including a 2.5 Å resolution structure (Murthy et al. 1981).
  Sequence data available in Uniprot entry P00432.
- Catalase in Bovine Milk:
  Purified from bovine milk by Ito and Akuzawa (1983a, b).
  Differences exist between bovine and human catalase, but functionally important amino acid regions are conserved.
  Structurally similar across mammalian species.
- Structural Features:
  Homotetramer of 60-kDa subunits.
  Belongs to the monofunctional catalase group.
  Each subunit contains a heme (ferriprotoporphyrin IX) at the active site.
  Substrate access limited to small molecules.
- Secretion and Localization:
  Encoded as a precursor without a signal sequence.
  Mature protein has a blocked N-terminal, indicating it is not destined for secretion.

Biological Role of Catalase and Significance in Milk

- Catalase as a Mastitis Indicator:
  Catalase activity has been proposed as a marker for mastitis detection (Kitchen 1976, 1981; Fitz-Gerald et al. 1981).
  Correlation between catalase activity and bacterial counts can be used in biosensor scans for microbial-challenged milk (Zhang et al. 2014).

Traditional confirmatory tests are still required for mastitis diagnosis.

- Functional Role of Catalase in Milk:

  Catalyzes a hydrogen peroxide-dependent reaction converting nitrite to nitrate (Silanikove et al. 2014).

  Helps maintain low levels of free radicals and oxidation products in milk.

  Active in the udder, contributing to early milk quality maintenance.

  Prevents accumulation of oxidation products post-milking and during storage.

  Inhibition of catalase increases nitrotyrosine and lipid peroxides.

- Stability and Heat Sensitivity:

  Catalase is highly heat-sensitive, similar to alkaline phosphatase (Hirvi et al. 1996).

  Activity is significantly reduced after heating at 72 °C for 15 s (Griffiths 1986).

  Some evidence suggests catalase activity increases after pasteurization due to release from somatic cells and bacteria.

  Variability in catalase activity may contribute to challenges in raw milk cheese ripening (Gatti et al. 2014; Yoon et al. 2016).

  Due to post-pasteurization variability, catalase is unsuitable as a pasteurization index.

- Catalase Isolation and Structural Properties:

  First isolated from milk by Ito and Akuzawa (1983a), purified 23,000-fold and crystallized.

  Three isozymes were identified in milk catalase (Ito and Akuzawa 1983b).

  SDS dissociation showed subunits ranging from 11 to 55 kDa.

  Similar in structure to bovine liver catalase, a homotetramer of 60–65 kDa (total mass 250 kDa).

  Heterogeneity in milk catalase likely results from proteolysis during isolation.

- Catalase Activity Measurement:

  Traditionally measured by monitoring H_2O_2 disappearance at 240 nm (Beers and Sizer 1952).

  New and more sensitive assay methods continue to be developed (Hadwan 2018).

  Commercially available assays exist for precise catalase activity quantification.

2.4 Lactoperoxidase

- Lactoperoxidase (LPO) (EC 1.11.1.7):

  A glycoprotein found in exocrine secretions such as saliva, tears, and milk.

  Belongs to the family of heme-containing peroxidases, also found in plants and fungi.

  In mammalian peroxidases, the heme group is covalently bound, unlike in fungal and plant peroxidases (Sharma et al. 2013).

- Catalytic Function:

  Catalyzes the breakdown of hydrogen peroxide, generating water (H_2O_2 + reduced acceptor $\rightarrow$ oxidized acceptor + $2H_2O$).

  The acceptor can be a phenolic compound, aromatic amine, aromatic acid, thiocyanate, bromide, or iodine (Kohler and Jenzer 1989).

- Detection and Historical Aspects:

  LPO activity is easily monitored and was one of the first enzymes described in milk (Arnold 1881).

  Many chromogenic substrates have been used, but ABTS is currently the most common.

  Historical aspects of LPO activity in milk have been reviewed (Fox and Kelly 2006).

- Presence in Milk Fractions:

  Traditionally assigned to skim milk and whey.

Small amounts detected in MFGM and EVs, likely due to cytosolic contamination.

More MS-spectral counts for LPO were found in EVs compared to MFGM in an iTRAQ proteomic study (Reinhardt et al. 2013).

- Classification Considerations:

  LPO is not considered a true membrane-associated milk enzyme.

  It is still included in discussions as it may be present in phospholipid-containing milk fractions.

Structure of Lactoperoxidase

- Bovine Lactoperoxidase (LPO) Sequence:

  Complete amino acid sequence published in 1990–1991 (Dull et al. 1990; Cals et al. 1991).

  Mature protein consists of 612 residues after cleavage of signal and propeptides.

  3D structure resolved at 2.3 Å (Singh et al. 2009).

- Structural and Functional Aspects:

  Database entry (UniProt P80025) details disulphide bridges, glycosylations, phosphorylations, and heme-binding.

  Encoded as a secreted protein; no indication of membrane association.

- Purification and Analysis:

  Various methods include salting-out, membrane filtration, chromatography, and immune-affinity techniques.

  Recent approach using salting-out and ion-exchange yielded high purity (Li et al. 2019).

  Purity assessed by absorbance at 412 nm (heme content) and A_{280} nm (total protein) (Andersson et al. 1996).

Biological Role of Lactoperoxidase and Significance in Milk

- The LPO System:

  Comprises LPO, hydrogen peroxide, and oxidized products, forming a non-specific humoral immune response (Ihalin et al. 2006).

  Uses H_2O_2 to oxidize thiocyanate, iodides, and bromides into antimicrobial hypothiocyanite, hypoiodides, and hypobromides.

  Inhibits bacterial, fungal, and viral growth by oxidizing thiol groups in proteins (Reiter and Härnulv 1984).

- LPO in Milk:

  Functions in neonatal defence against digestive pathogens (Koksal et al. 2016).

  Activity in milk debated; requires added thiocyanate and H_2O_2 for activation (Silanikove et al. 2006).

  Reacts with nitrite to produce nitric dioxide (NO_2^-), involving xanthine oxidase and catalase.

  Second most abundant enzyme in milk, constituting $\sim$0.5% of whey proteins (30 mg L^{-1}).

  LPO reported absent in human milk (Hamosh 1988), but later studies confirmed its presence (Shin et al. 2001).

- Pasteurization and LPO Testing:

  LPO loses activity upon heat treatment (Arnold 1881).

  Basis for the Storch test, used to verify super-pasteurized milk ($\geq$ 76 °C for 15 s) (Storch 1898).

- Industrial and Food Applications:

  Used in dairy to inhibit microbial growth and preserve raw milk (Seifu et al. 2005).

Expanded applications include oral hygiene, food preservation, fish farming, and carcinogen degradation (Li et al. 2019).

2.5 Xanthine Oxidoreductase

- Xanthine Oxidoreductase (XOR):
  Identified in bovine milk as an aldehyde reductase (Schardinger, 1902).
  Catalyzes oxidation of hypoxanthine to xanthine and xanthine to uric acid (Booth, 1938).
- Structural and Functional Aspects:
  XO (EC 1.17.3.2) and XD (EC 1.17.1.4) are interchangeable enzyme forms (Stirpe & Della Corte, 1968, 1972).
  Widely distributed molybdenum-containing enzyme involved in purine degradation.
- XOR in Milk:
  Found in skim milk and cream (Kitchen, 1974), released from MFGM during phase inversion.
  Classified as a peripherally associated membrane protein (Briley & Eisenthal, 1975).
- Presence in Milk Fractions:
  Detected in EVs and MFGs in both human and bovine milk.
  Proposed as a microRNA marker in commercial milk (Benmoussa et al. 2017).

Structure of Xanthine Oxidoreductase

- Structure and Redox Centers:
  XOR is a dimer of identical monomers, each with 1332 residues and four redox centers: 2Fe/2S clusters, FAD, and molybdopterin (Berglund et al. 1996).
  XO catalyzes xanthine oxidation with O_2 reduction, while XD uses NAD^+ (Hille, 2013).
- Interconversion Between XO and XD:
  XD is the native form; XO results from oxidation or proteolysis (Stirpe & Della Corte, 1968).
  Conversion involves disulfide bond formation between Cys535-Cys992, altering NAD^+ binding (Nishino et al. 2005).
  XO/XD equilibrium is dynamic, with structural flexibility allowing interconversion (Nishino et al. 2015).
- XOR in Milk:
  Early assays suggested XOR absence in human milk, later disproven (Bradley & Günther, 1960).
  Low XOR activity in human milk due to molybdenum deficiency (Godber et al. 1997).
  XOR activity in goat and sheep milk increases with dietary molybdenum supplementation (Atmani et al. 2004).
- Isolation and Purification:
  Early methods used pancreatin, causing irreversible XO conversion (Battelli et al. 1972).
  Alternative isolation methods include butanol partitioning and ion-exchange chromatography (Rajagopalan, 1986; Kristensen et al. 1996).
  Affinity chromatography with XO inhibitors improves purification (Beyaztaş & Arslan, 2015).

Biological Role of Xanthine Oxidoreductase and Significance in Milk

- Biological Role of XOR:

Housekeeping enzyme in purine catabolism; mutations cause xanthinuria (Bray, 1975; Ichida et al. 1997).

Involved in innate immunity, linked to NF-κB signaling (Vorbach et al. 2006).

Generates ROS/RNS with LPO, contributing to bactericidal properties (Björck & Claesson, 1979).

- XOR in Milk:

  Plays a structural role in milk fat globule secretion via butyrophilin interaction (Vorbach et al. 2002).

  Found in a stable complex with butyrophilin and adipophilin (Heid et al. 1996).

  Affects oral microbiome by releasing antibacterial compounds in neonatal saliva (Al-Shehri et al. 2015).
- Heat Stability and Industrial Impact:

  UHT treatment inactivates XOR (Ozturk et al. 2019).

  Not a reliable heat treatment marker, though debated (Griffiths, 1986; Andrews et al. 1987).

  Contributes to oxidative rancidity and aldehyde formation in milk (Aurand et al. 1967).
- XOR in Cheese Production:

  Inhibits *Clostridium tyrobutyricum* spores via ROS/RNS production (Vissers et al. 2007).

  Nitrate addition for spore control is controversial due to nitrosamine risks (Beresford et al. 2001).
- XOR Assays:

  Commonly measured via xanthine-to-urate conversion at 295 nm (Cerbulis & Farrell, 1977).

  Alternative methods include HPLC and fluorometric detection of H_2O_2 (Rashidinejad et al. 2016; Hwang et al. 2016).

2.6 γ-Glutamyltransferase

- γ-Glutamyltransferase (GGT) (E.C. 2.3.2.2):

  Catalyzes transfer of γ-glutamyl groups from glutathione to amino acids, peptides, or water.

  Found in cell membranes and suggested to aid amino acid supply for milk protein synthesis (Calamari et al. 2015).
- Presence in Milk:

  Primarily located in skim milk rather than MFGM (5.8:1 ratio, Kitchen 1974; 8:1 ratio, Reinhardt et al. 2013).

  74.3% of GGT activity detected in skim milk, only 6.6% in MFGM (Baumrucker 1979).
- Detection Methods:

  Traditionally measured by p-nitroanilide release at 410 nm.

  Other methods include HPLC, fluorescence, and chemiluminescent probes for real-time detection (Ziobro & McElroy 2013; An et al. 2019).

Structure of γ-Glutamyltransferase

- Structure of Bovine GGT:

  Sequence deduced from whole genome (UniProt G3N2D8).

  Expressed as a 568-residue precursor, autocleaved into a large (41.0 kDa) and small (19.8 kDa) subunit.

  Anchored to the plasma membrane via a single-pass transmembrane domain (residues 5–26).
- Catalytic Mechanism:

Active site threonine (Thr380) facilitates autocleavage and acts as a catalytic nucleophile.

Hydrolyzes γ-glutamyl amide bonds, releasing glutamate.

Can perform transpeptidation, transferring γ-glutamyl groups to peptide acceptors (West et al. 2013).

- Purification and Structural Insights:

  Few studies on purification from milk; extensive work on rat kidney GGT (Castonguay et al. 2007).

  Human GGT crystal structure resolved at 1.67 Å (West et al. 2013).

Biological Role and Significance of Glutamyltransferase in Milk

- Biological Role of GGT:

  Hydrolyzes extracellular γ -glutamyl compounds, maintaining intracellular glutathione levels.

  Involved in cysteine homeostasis, redox balance, and inflammation.

  Used as a diagnostic marker for liver disease, alcohol consumption, and metabolic disorders (Ndrepepa et al. 2018).
- Purification and Structural Properties:

  Purified from MFGM and skim milk membranes via a seven-step process (Baumrucker 1980).

  Consists of two glycosylated subunits (57 kDa and 25 kDa) with a total molecular mass of 80 kDa.

  Optimal activity at pH 8.5–9.0 and 45°C; inhibited by diisopropylfluorophosphate, iodoacetamide, Cu^{2+}, and Fe^{3+} (Farkye 2003).
- Heat Stability and Industrial Relevance:

  More heat-resistant than alkaline phosphatase, but less than LPO.

  50% inactivation at 69°C after 3 min; residual activity found in raw milk cheese (Blel et al. 2002).

  Suggested as a marker for milk pasteurization (Vetsika et al. 2014).

2.7 5'-Nucleotidase

- 5'-Nucleotidase (EC 3.1.3.5):

  Glycoprotein enzyme involved in nucleotide catabolism, hydrolyzing 5'-mononucleotides to nucleosides and phosphate (Reis 1934).

  Found in various tissues, bacteria, and plants with substrate specificity and pH variability (Zimmermann 1992).
- Structural and Functional Diversity:

  Exists in membrane-bound (ecto-5'-nucleotidase) and cytosolic forms, including mitochondrial variants (Misumi et al. 1990).

  Mammalian forms require Mg^{2+} or Mn^{2+}, while bacterial forms are activated by Co^{2+} and Ca^{2+} (Neu 1967).
- Detection and Assays:

  Activity measured spectrophotometrically or via radioactive labeling (Edelson & Duncan 1981).

  Commercial kits available using colorimetric detection of ammonia production.

  Specific inhibitors aid in distinguishing nucleotidase variants in lysates (Bianchi & Spychala 2003).

Structure of 5'-Nucleotidase

- Structural Variants of 5'-Nucleotidase:

Exists in multiple forms with distinct substrate affinities across organisms.

Ecto-5′-nucleotidase forms homodimers, while cytosolic variants exist as monomers, dimers, or tetramers (Bailyes et al. 1984; Bianchi & Spychala 2003).

- Structural Insights:

  Crystal structures determined for eukaryotic and bacterial 5′-nucleotidases (Walldén et al. 2007; Knöfel & Sträter 1999).

  Human ecto-5′-nucleotidase structure resolved at 1.55–2.00 Å in open/closed conformations.

  Bovine ecto-5′-nucleotidase has 574 residues, 62,966 Da mass, and four disulfide bonds (Fini et al. 2000).
- Functional Features:

  Glycosylation patterns vary by species and tissue; contains four or five N-linked sites (Misumi et al. 1990).

  Active site located at the interface of N- and C-terminal domains, inaccessible to solvents.

  C-terminal contains substrate binding site and GPI anchor attachment (Knapp et al. 2012).

Biological Role and Significance of 5'-Nucleotidase in Milk

- 5′-Nucleotidase as a Marker:

  Used to study plasma membrane and milk secretion mechanisms.

  Inhibition of milk flow after concanavalin A injection suggests a role in exocytosis (Snow et al. 1980).
- Isolation and Properties:

  First isolated from bovine MFGM using detergents, precipitation, heat treatment, and chromatography (Huang & Keenan, 1972).

  Exists in two isoforms with differing substrate affinities (K_M values: 0.94 and 5 mM).

  Maximum activity at 69°C; loses 50% activity after 60 min at 60°C.

  Extractable using n-butanol, with higher efficiency at lower temperatures (Ahn & Snow, 1993).
- Detergent Sensitivity:

  Mild detergents (Triton X-100, Tween 20) enhance activity, while SDS inhibits it (Bhavadasan & Ganguli, 1978).

  Higher concentrations of nonionic detergents inhibit bovine MFGM 5′-nucleotidase (Kanno & Yamauchi, 1979).
- Potential Role in Milk:

  May aid nucleotide digestion in neonates by generating absorbable nucleosides (Janas & Picciano, 1982).

  Nucleotide content is higher in human milk than bovine milk.

2.8 Conclusion

- True Membrane-Associated Enzymes:

  Four enzymes are strongly linked to milk phospholipid structures: sulfhydryl oxidase, xanthine oxidoreductase, γ-glutamyltransferase, and 5′-nucleotidase.
- Enzymes Not Considered True Membrane Components:

  Catalase likely originates from somatic milk cells or microbial contamination.

LPO lacks membrane-associated structural features; its presence in MFGM and EVs is likely due to fraction contamination.

- Other Enzymes:

  Alkaline phosphatase and lipoprotein lipase are membrane-bound but discussed elsewhere in the book.

Chapter 3
Bullet points & arrangement of the summary

- **Title:**
  Membrane-Associated Enzymes in Milk: Classification, Function, and Recent Insights
- **1. Introduction**
  Overview of membrane-associated enzymes in milk.
  Importance in dairy science and milk composition.
  Scope of the summary (focus on sulfhydryl oxidase, xanthine oxidoreductase, γ-glutamyltransferase, and 5′-nucleotidase).
- **2. Classification and Mechanisms of Enzymes**
  Sulfhydryl Oxidase – Catalyzes oxidation of protein thiols, disulfide bond formation, role in protein folding.
  Xanthine Oxidoreductase (XOR) – Converts hypoxanthine to xanthine and uric acid, exists as xanthine dehydrogenase or oxidase.
  γ-Glutamyltransferase (GGT) – Transfers γ-glutamyl groups, involved in glutathione metabolism and amino acid transport.
  5′-Nucleotidase – Hydrolyzes 5′-mononucleotides, contributes to nucleotide metabolism.
- **3. Role of Enzymes in Milk and Dairy Products**
  Sulfhydryl Oxidase – Stabilization of milk proteins, impact on UHT milk flavor.
  XOR – Antimicrobial properties, role in milk fat globule secretion.
  GGT – Supports amino acid availability for milk protein synthesis, possible thermal processing marker.
  5′-Nucleotidase – Potential role in neonatal digestion and milk secretion studies.
- **4. Summary of Recent Scientific Studies**
  Sulfhydryl Oxidase – Research on enzymatic activity and protein cross-linking effects.
  XOR – Studies on antimicrobial activity and oxidative rancidity.
  GGT – Investigations into its use as a marker for milk pasteurization.
  5′-Nucleotidase – Research on its extraction and enzymatic function in dairy processing.
- **5. Conclusion**
  Key takeaways on membrane-associated enzymes in milk.
  Significance for dairy processing, product quality, and nutrition.
  Future research directions.

- **6. References**

  Full bibliographic information in Harvard (author-year) style, formatted using `natbib`.

Chapter 4
Group Project - Enzymes - Membrane Associated Enzymes

4.1 Introduction

4.2 Sulfhydryl Oxidase

Structure of Flavin-Dependent Sulfhydryl Oxidase

Biological Role of Flavin-Dependent Sulfhydryl Oxidase and Significance in Milk

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4.3 Catalse

Structure of Catalase

Biological Role of Catalase and Significance in Milk

4.4 Lactoperoxidase

Structure of Lactoperoxidase

Biological Role of Lactoperoxidase and Significance in Milk

4.5 Xanthine Oxidoreductase

Structure of Xanthine Oxidoreductase

Biological Role of Xanthine Oxidoreductase and Significance in Milk

Bibliography

[1] Maria Stenum Hansen and Jan Trige Rasmussen. "Enzymes Associated with Milk Phospholipid Membrane Structures: Milk Fat Globule Membranes and Extracellular Vesicles". In: *Agents of Change: Enzymes in Milk and Dairy Products*. Ed. by Alan L. Kelly and Lotte Bach Larsen. Cham: Springer International Publishing, 2021, pp. 127–161. ISBN: 978-3-030-55482-8. DOI: 10.1007/978-3-030-55482-8_6. URL: https://doi.org/10.1007/978-3-030-55482-8_6.