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

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Chapter 1
Membrane-Associated Enzymes

1.1 Introduction

Many enzymes have been identified in milk fat globules (MFGs) and extracellular vesicles (EVs), with advancements in proteomic techniques continuously expanding this knowledge. However, many of these enzymes are present in low abundance and often originate from endoplasmic reticulum (ER), Golgi membranes, or cytosolic remnants. A large proportion remains inactive in milk due to the absence of relevant substrates or a suitable environment. This chapter focuses on sulfhydryl oxidase, catalase, lactoperoxidase (LPO), xanthine oxidoreductase (XOR), γ-glutamyltransferase (GGT), and 5′-nucleotidase, discussing their relevance in mammary gland biology, milk integrity, and physiological function upon consumption [2].

1.2 Classification of Mechanisms of Enzymes

1.3 Role of Enzymes in Milk and Dairy Products

1.4 Summary of Recent Scientific Studies

To limit the scope of this report, it was decided to investigate one enzyme from the given literature [2]. Lactoperoxidase was selected due to its antimicrobial properties and a relevant scientific study by Aouadhi et al. (2024) was identified and used in current section.

Lactoperoxidase (LPO) combined with hydrogen peroxide and thiocyanate forms a well described system called the Lactoperoxidase System (LPS), known for its antimicrobial properties through production of hypothiocyanite which impairs protein functions in organisms like E. coli, yeasts, and molds [1]

A recent study by Aouadhi et al. (2024) investigated the impact of combining the LPS system with nisin and/or heat treatment to enhance the microbial safety and shelf life of raw milk products through decrease of microbial load. Nisin was chosen as it increases membrane permeability thereby facilitate transport of hypothiocyanite through membranes to impair cellular proteins. In contrast, heat treatment reduces the initial microbial load, enhancing the system's efficacy.

As noted in [2], the LPS system remains inactive in bovine milk due to low concentrations of hydrogen peroxide and thiocyanate. Aouadhi et al. (2024) confirmed this and activated the system by adding hydrogen

peroxide and thiocyanate to the raw milk. Following activation, nisin was added in different concentrations, and samples were incubated at 25°C for 72 hours. Microbial analysis following the experiment revealed:

- 6-log reduction in total mesophilic bacteria
- 5-log reduction in coliforms
- 4-log reduction in yeasts and molds

These reductions were achieved with 7 mg/L SCN, 15 mg/L hydrogen peroxide, and 50 IU/mL nisin after 72 hours of incubation at 25°C.

With the optimal concentrations an experiment was prepared including pasteurization. The LPS system was activated for 2, 4 and 6 hours before pasteurization at 63°C for 30 minutes and subsequent incubation at 4°C and 25°C for 96 hours. All samples were analyzed for total bacterial count, coliforms, yeasts and mold. Results for coliforms are shown below:
From 1.1, it is evident how the concentration of coliforms steadily increases in the control sample, incubated without any treatment. In contrast, the heat-treated sample show an initial decrease in coliforms, followed by an increase after 24 hours of incubation at both temperatures (4°C and 25°C). Samples where LPS was activated before heat treatment shows the greatest log reduction in coliforms when stored at 4°C for 96 hours. Although a significant reduction is also observed at 25°C, the effect is less pronounced compared to storage at 4°C, highlighting the impact from incubation temperature on microbial growth.

Identical results were obtained for yeasts, and molds with complete destruction at 4°C with 4 and 6-hour LPS activation before heat treatment.

The article concludes that LPS activation cycles of 4 and 6 hours before heat treatment (pasteurization) show promising results in reducing the microbial load of milk and thereby the quality. This while utilizing an already present enzyme in milk, LPO, combined with a food grade approved preservative nisin.

1.5 Conclusion

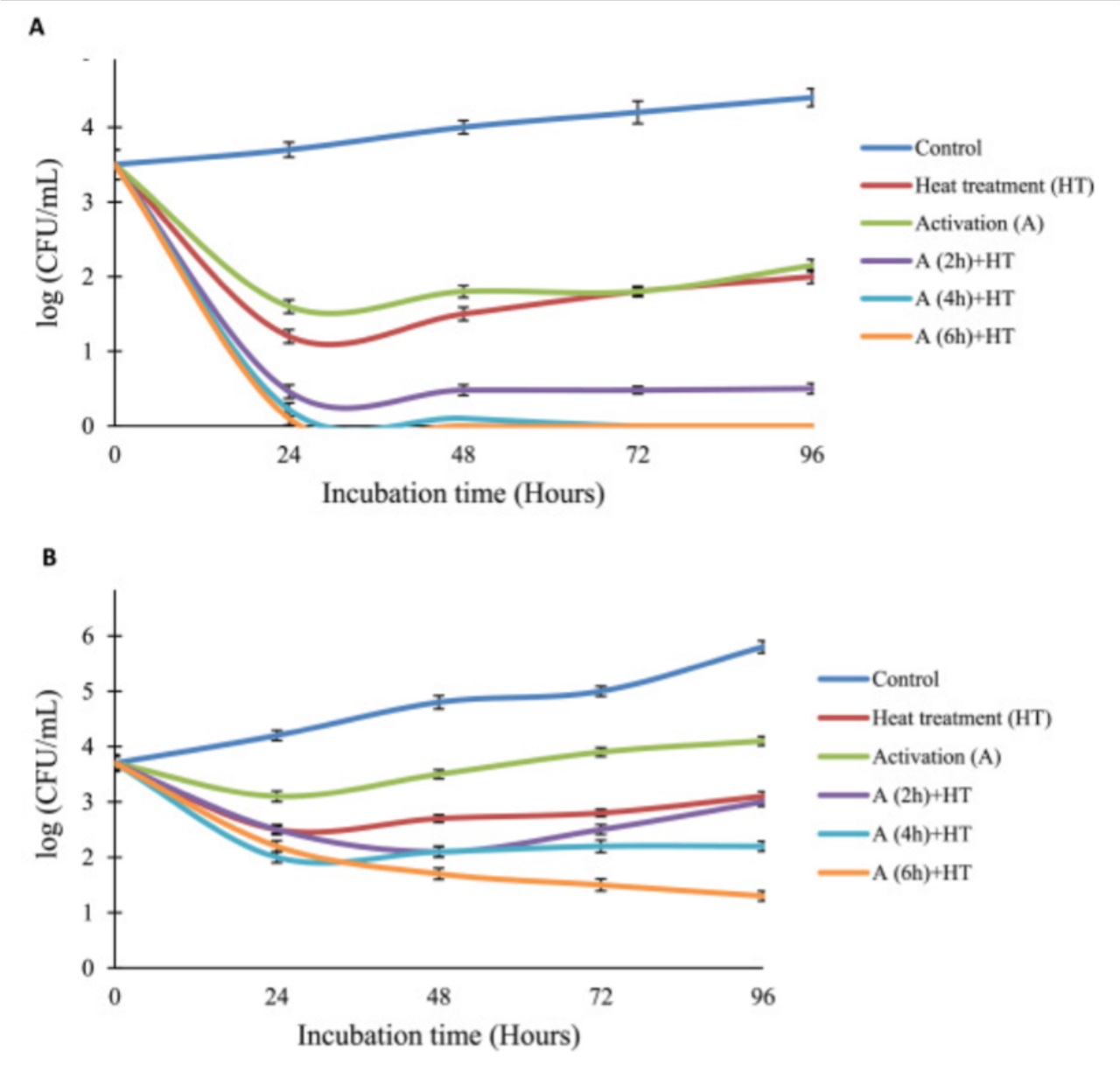

Figure 1.1: Evolution of total coliforms in raw cow milk according to different treatments at 4°C (A) and 25°C (B) for 96 h [1]

Bibliography

[1] Chedia Aouadhi et al. “Study the effect of lactoperoxidase activation in combination with heat treatment or nisin on the microbiological quality of raw milk”. In: *Applied Food Research* 4.2 (2024), p. 100521. ISSN: 2772-5022. DOI: https://doi.org/10.1016/j.afres.2024.100521. URL: https://www.sciencedirect.com/science/article/pii/S2772502224001318.

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