THESIS

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Thesis title:	**Reduction of Milk Allergenicity Through Enzymatic and Fermentation Hydrolysis**
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Cow's milk is a crucial component of the diets of infants and children worldwide, offering a range of nutrients that contribute to the development of a robust immune system and overall health. However, as we all know, cow's milk is a leading cause of food allergies in children, generating abnormal immunological reactions. While complete milk avoidance is a common approach for preventing milk allergy, these proteins are included in a variety of processed foods and avoiding them may result in nutritional deficits. Heating, enzymatic, and microbial hydrolysis are all food processing procedures that can modify protein structures by removing or degrading epitopes.

In our study, we used papain enzymatic hydrolysis and LAB fermentation. Fermented foods not only improve nutritional value but also include bioactive peptides produced by bacteria during the fermentation process. Yogurt bioactive peptides have functional features such as ACE inhibition and antithrombotic action. This has piqued the food industry's interest in developing novel food supplements and functional goods based on these bioactive peptides.

We examined the texture, molecular weight, antigenicity, ACE inhibitory concentration, and antioxidant capacity of yogurt samples derived from UHT and ESL milk. This investigation involved the application of papain enzymatic hydrolysis and LAB fermentation. Two concentrations of papain, namely 0.008 g/L (UHT-P1, ESL-P1) and 0.012 g/L (UHT-P2, ESL-P2), were prepared. Additionally, we investigated the impact of glucose on lactic acid fermentation by introducing β-galactosidase into individual milk portions. For microbial hydrolysis, LAB strains Streptococcus thermophilus and Lactobacillus bulgaricus were utilized, and fermentation at 45 °C for 6 hours occurred. The resulting fermented samples were designated as UHT-P1-Y, UHT-P2-Y, UHT-P1-Y-NL, UHT-P2-Y-NL, ESL-P1-Y, ESL-P2-Y, ESL-P1-Y-NL, and ESL-P2-Y-NL

The texture analysis of yogurt samples revealed that the hardness of both UHT and ESL milk-derived yogurts decreased with papain and microbial hydrolysis, particularly at higher papain concentrations. Interestingly, UHT milk-based yogurts exhibited a slightly firmer texture,

attributed to increased lactosylation inhibiting papain's proteolytic activity.

To understand the molecular weight and distribution of proteins and peptides, SDS-PAGE gel electrophoresis was conducted. The results showed that higher enzyme-to-substrate ratios led to the conversion of large molecular weight protein subunits into low molecular weight peptides. UHT-P1, UHT-P1-Y, and UHT-P1-Y-NL exhibited a wide range of molecular weights due to partial hydrolysis induced by papain treatment. The combination of papain and LAB hydrolysis further broke down proteins into peptides, with UHT-P2, UHT-P2-Y, and UHT-P2-Y-NL showing more profound protein hydrolysis and lower peptide molecular weights. Notably, yogurt from ESL milk displayed a broader spectrum of lower molecular weight proteins compared to UHT milk, attributed to lower lactosylation in ESL milk, making proteins more susceptible to papain and microbial hydrolysis.

The antigenicity of yogurt samples from UHT and ESL milk using a rabbit polyclonal antibody against cow's milk casein was studied. Papain hydrolysis demonstrated a dose-dependent reduction in allergenicity, with UHT-Y, ESL-Y, UHT-Y-NL, and ESL-Y-NL showing decreased casein antigenicity, likely due to LAB proteolysis. However, microbial hydrolysis alone was insufficient in eliminating casein antigenicity. The sequential application of papain and microbial hydrolysis in UHT-P1, ESL-P1, UHT-P1-Y, ESL-P1-Y, UHT-P1-Y-NL, and ESL-P1-Y-NL further reduced casein antigenicity. Remarkably, UHT-P2, ESL-P2, UHT-P2-Y, ESL-P2-Y, UHT-P2-Y-NL, and ESL-P2-Y-NL nearly eliminated casein antigenicity, attributed to increased papain concentration promoting extensive hydrolysis. Moreover, samples from ESL milk showed greater reduction in antigenicity.

The IC50 values, measuring ACE inhibitory concentration, significantly decreased in a concentration-dependent manner following papain and LAB hydrolysis. The lower molecular weight peptides resulting from reduced steric hindrance had a higher likelihood of interacting with enzymes. Higher papain concentration with LAB hydrolysis further reduced IC50 values, indicating the release of ACE-inhibitory peptides by LAB. Yogurt from ESL milk exhibited lower IC50 values than UHT milk, attributed to ESL milk undergoing more extensive hydrolysis, producing smaller peptides with enhanced enzyme interaction. The addition of glucose further decreased IC50 values, as it facilitated microbial growth and influenced milk protein proteolysis.

The antioxidant capacity was measured by using FRAP and DPPH tests, which revealed dose-dependent antioxidant activity. Because of reduced steric hindrance, peptides with lower molecular weight and higher charge density displayed improved scavenging efficacy against free radicals. Yogurt samples from ESL milk had better antioxidant capacity than UHT milk, which was attributed to the more extensive hydrolysis in ESL milk, which broke down proteins into smaller peptides.

In conclusion, dual hydrolysis with papain and LAB lowered allergenicity in yogurt. Increased papain concentration increased proteolysis while decreasing allergenicity. ESL milk-derived yogurts regularly outperformed, with lesser antigenicity and increased antioxidant capacity due to their non-lactosylated nature, which allows for more thorough hydrolysis. The findings offer suggestions for improving yogurt production in terms of texture, molecular properties, and health-related aspects. This study advances our understanding of enzymatic and microbial hydrolysis in the development of sensitive dairy products.