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**The effects of pH-stat long-term lactic acid bacterial  
activity prior to curd formation on the development of  
cheese structure in a fat free model cheese**

**A thesis presented in partial fulfilment of the requirements for the degree of**

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## Abstract

Cheese ripening is an important step in most cheese production practices during which a tasteless fresh cheese is converted to a tasty and flavourful product with specific textural attributes. However, the complexity of its composition (pH, solubilisation of calcium from the colloidal casein proteins, salt concentration, acid production rate, indigenous and added enzymes, residual activity of the enzymes etc.) coupled with the length of time associated with manufacturing which in some cases can be in excess of two years has made it a complicated area of study. The influences of the composition and process contributors are confounded and it is impossible to connect the impact of one particular parameter on cheese making steps and quality attributes. pH has proven to be an important influential factor to influence the extent of effects of other parameters with significant influence on other ruling parameters in milk, curd and cheese. Proteolysis during ripening is the most important physicochemical pathway to define the quality of cheese. One of the major factors governing cheese ripening reactions is the starter bacteria.

This study has aimed to characterise the effects of starter bacteria activity on curd formation and resultant cheese textural attributes of the long fermented cheesemilk. By developing a pH-stat system, long fermentations carried out to assess the proteolytic activity of selected starter lactic acid bacteria (LAB) on a milk based medium before rennet addition. It was attempted to assess the degree of hydrolysis of cheesemilk through extended bacterial fermentation, conducted under pH-stat conditions, prior to curd formation. The effects of the bacterial activity on casein proteins during pH-stat long term (PLST) fermentations were evaluated by assessing proteolysis index from pH4.6 soluble nitrogen as a fraction of total nitrogen (pH4.6SN/TN). The proteolysis of proteins during PLST was further assessed by doing reverse-phase high performance liquid chromatography (RP-HPLC) on 70%Ethanol soluble (70%EtOHS) and insoluble (70%EtOHI) fractions of pH4.6 soluble fraction of the samples. The effects of PLST fermentation on formation of small-size peptides were assessed by quadrupole time-of-flight mass spectrometry (Q-ToF MS) on the 70%EtOHS fraction. The effect of PLST fermentation on 'depth of proteolysis' during

cheese ripening were assessed by analysing the quantity of free amino acid (FAA) formed in resultant cheese after 12 months storage at 4°C. The impact of PLST fermentation on gel formation attributes were assessed by doing dynamic low amplitude oscillatory rheometry (DLAOR). The consequent effects on resultant cheese texture were evaluated using texture profile analysis (TPA). The impact of PSLT on microstructure were assessed by confocal laser scanning microscopy (CLSM).

The results provided evidence for the adequacy of developed fermentation to conduct PSLT with reproducible results. High correlation between the parameters of the PSLT fermentation system were obtained. The proteolysis index measured from the PSLT fermentations with different durations showed evidences on the significance of LAB proteolytic system on cheese milk prior to curd formation. The proteolysis index for the longest fermentation prior to curd formation was 5% which was comparable to day one cheese proteolysis index, in presence of rennet, in most cheeses varieties. Peptide profiling of the 70%EtOHS and 70%EtOHI sub-fractions of pH4.6S showed significant ( $p < 0.05$ ) effects arising from PSLT fermentations. Analysis of FAA of ripened cheese also showed a significant increase ( $p < 0.05$ ) in the samples with longer PSLT (20 times increase in total free amino acids compared to non-fermented treatment) fermentations. The differences in gelation behaviour of the sample and textural attributes of cheese and microstructure of final cheese were connected to the extent of proteolytic activity of LAB during PSLT fermentations. The hardness of cheese significantly ( $p < 0.05$ ) decreased (up to ~60%) by increasing fermentation duration over the studied timescale.

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## List of abbreviations and symbols

*°C: Celsius degree*

*AA: Amino acid*

*pI: Isoelectric pH*

*ANOVA: Analysis of variance*

*a<sub>w</sub>: Water activity*

*CCP: Colloidal calcium phosphate*

*CFU: Colony forming unit*

*CLSM: Confocal laser scanning microscopy*

*CMP: Caseinomacropptide*

*Da: Dalton*

*DLAOR: Dynamic low amplitude oscillatory rheometry*

*EtOH: Ethanol*

*FAA: Free amino acid*

*g: gram*

*G': Elastic modulus*

*G'': Viscous modulus*

*GMP: Glycomacropptide*

*h: Hour*

*HPLC: High performance liquid chromatography*

*LAB: Lactic acid bacteria*

*Lc.: Lactococcus*

*LEP: Lactose elimination point*

*LTLT: Low temperature long time*

*MALDI-ToF: Matrix-assisted laser desorption/ionization time of flight*

*min: Minute*

*mL: Millilitre*

*MM: Molecular Mass*

*MPC: Milk protein concentrate*

*NSLAB: Non-starter lactic acid bacteria*

*PAGE: Polyacrylamide gel electrophoresis*

*PEP-PTS: Phosphoenol pyruvate phosphotransferase system*

*PSLT: pH-stat long-term*

*PTA: Phosphotungestic acid*

*P- $\beta$ -gal: Phosphor- $\beta$ -galactosidase*

*Q-ToF: Quadrupole time of flight*

*RCT: Rennet coagulation time*

*RP-HPLC: Reverse phase high performance liquid chromatography*

*RSM: Reconstituted skim milk*

*s: Second*

*TCA: Trichloroacetic*

*$t_g$ : Gelation time*

*$\eta^*$ : Complex viscosity*

*TPA: Texture profile analysis*

*UF: Ultrafiltration*

*$\beta$ -gal:  $\beta$ -galactosidase*