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

Doctor of Philosophy (PhD)

in

Food Technology

At Massey University, Manawatu, New Zealand

Abbas Khodamoradi Dashtaki

2018

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 (PSLT) 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 PSLT 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 law 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.

Acknowledgments

I would like to give my sincere gratitude to all the people who were directly or indirectly involved and also assisted me in completing this project. The thesis could not have been completed without the help of my supervising team. I would like to especially thank Dr. Alistair Carr for his knowledgeable insights and inspiring thoughts and conversation. Also for allowing me to come to his office whenever I had a question or idea to consult.

I would like to express my sincere gratitude to Dr. James Harnett (Fonterra) who agreed to be my supervisor and helped me greatly to look at things from a microbiologist's point of view, and kindly provided me the bacterial strains and some milk-based powders. I would like also to thank Mr Rod Bennett for being a part of supervisory team at some stage. I also would like to express my gratitude to Professor Steve Flint for his help and support in post-submission, examination and amendments of my thesis.

I am especially thankful to Mr Allan Hardacre for helping me to stop being the "the pH-stat system's alkali addition pump". Without his help, I have no idea how many more nights I had to stay beside the equipment to keep the pH constant by dripping caustic soda in the fermenter tank.

I would like to thank Professor Matt Golding for the discussions which shaped my thesis greatly.

I would like to also thank Professor Julian Haynes for a being a source of continuous inspiration and support.

I am thankful to the staff of IFNHH and Riddet Institute, especially Mr. Garry Radford, Mr. Steve Glasgow, Mr. Warwick Johnson and Mrs. Michelle Tamehana, Janiene Gilliland and Maggi Zou, Mr. Byron McKillop and Mrs. Yvonne Parkes for providing me with the material and equipment I needed for the study and also the right advice and help at the best time.

I would like to especially thank Ann-Marie Jackson for her support in providing me with whatever I needed in the microbiology lab and many helpful advice. I also wish to thank Julia Good for her practical advice during microbiological lab work. I would like to thank Joella Lauridsen for her help during my visit to Fonterra's microbiology unit. I am grateful to the Manawatu Microscopy

and Imaging Centre (MMIC) for their help and advice during my study. I would like to express my gratitude to Mr Trevor Loo from Institute of Fundamental Sciences for his help and practical advice for protein analysis. I wish to thank Matt Levin from Massey IT services for his help with computer and software used in this study and many fun chats.

I am grateful to my friend and colleagues who I have become to know during my studies, shared experiences and knowledge, and will always remember their support and encouragements. I would especially like to thank Miss Jasmine Soukieh for her many advice and intriguing discussions.

Finally, I am greatly thankful to my family, to my parents for supporting me extraordinarily throughout my life. I can't put it in words how blessed I am to have you both as my parents, idols and source of true love. To my brothers and sisters for being a source of inspiration and happiness. I wish to express my sincere gratitude to my father-in-law, Dr Majid Dehghan-Shoar, Zahra Dehghan-Shoar and my brother-in-law Mohammad Hossein Dehghan-Shoar for their support and encouragement throughout my PhD work. This thesis is dedicated to my love, Dr Zeinab Dehghan-Shoar, who has supported me from the beginning of this study till the end. It would have not been possible to finish this piece of work without her being so understanding and patient, especially by looking after the eternal source of happiness in our life, Rose Khodamoradi. And above all, I thank almighty God who is the source of the 'Energy' to me, the casein interactions, the starter bacteria enzymes, and each and every other 'vibrating matter' in the universe.

Table of Contents

Table of Figures

Table of Tables

List of abbreviations and symbols

°C: Celsius degree

AA:Aminoacid

pI: Isoelectric pH

ANOVA: Analysis of variance

a_w: Water activity

CCP: Colloidal calcium phosphate

CFU: Colony forming unit

CLSM: Confocal laser scanning microscopy

CMP: Caseinomacropeptide

Da: Dalton

DLAOR: Dynamic low amplitude oscillatory rheometry

EtOH: Ethanol

FAA: Free amino acid

g: gram

G': Elastic modulus

G": Viscous modulus

GMP: Glycomacropeptide

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-ß-gal: Phosphor-ß-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

η: Complex viscousity*

TPA: Texture profile analysis

UF: Ultrafiltration

ß-gal: ß-galactosidase