STANDARDISATION OF CULTURED BUTTER PROCESSING FOR SMALLSCALE PRODUCTION

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Food Technology

Massey University

Albany, New Zealand

Jia Shi

August 2015
SUMMARY

Butter is one of the most popular dairy products that have been transformed from a cottage industry to successful large scale productions. In western countries, consumption of butter has slowly overtaken margarine as the most popular spread. Different kinds of butter are now available on the markets, of which sweet cream butter and salted butter constitute significant proportions. The popularity of cultured cream butter is mainly attributed to its unique flavour and nutritional properties. Butter contains large amounts of β-carotene (provitamin A carotenoid) and is characterised by the buttery flavour due to the presence of diacetyl as well as other organic aroma compounds. Although butter has been produced successfully in large scale commercial processing, small scale productions still exists in small communities and for use in specialised products. New Zealand, like in many other western countries, is dominated by small to medium scale food processing enterprises which produce speciality foods for discerning markets. The domestic market in New Zealand enjoys a variety of dairy products which includes cultured butter. Some small food processing enterprises in outlying areas of New Zealand produce their own cultured butter to cater for the local businesses and their inhabitants. Thus, the main objective of this project was to standardise small scale production of cultured butter using kitchen/domestic scale equipment.

Fresh cream (40% fat) used to produce cultured butter was fermented by a mixed lactic starter culture (Lactococcus lactis subsp. lactis biovar. diacetylactis and Leuconostoc cremoris) following a modified standard method. The cream was pasteurised at 95°C/5 min, rapidly cooled to 8°C, and then starter culture (2%) was added. The cream was held at 8°C/2 h to initiate the formation of low melting point fat crystals. The temperature of the cream was then increased to 20-21°C, and held at this temperature for further 2 h to melt fat crystals with high melting point and recrystallise the crystals. The temperature was then decreased to 16°C/2 h to form pure fat crystals. This was then followed by slightly decreasing the temperature to 15°C for butter churning in a K5SS KitchenAid Heavy Duty (USA) churning mixer.

Buttermilk (100 mL) was collected and stored at 4°C for analysis and the remained buttermilk in the butter churn was drained. The butter grains were washed with distilled water to remove any residual buttermilk. Final cultured butter (product) was packed in heavy duty aluminium foil and stored at 4°C for 21 days. Cultured butter was produced on three different occasions commencing in August 2014 (batch 1), September 2014 (batch 2), and October 2014 (batch 3). Various analyses and measurements were conducted during processing and storage to monitor the shelf life stability of the butter. Standard methods were used to measure chemical, physical, consumer sensory acceptance of the products and presence of coliforms were enumerated by Violet Red Bile Agar. Thus, fat content in buttermilk was determined by the Mojonnier test to calculate churning efficiency. Colour was measured by colourimetry, while texture analysis was determined by the TA.XT2 Texture Analyser. Water droplet size of butter was examined by confocal laser scanning microscope.
after staining with Nile Red and Acridine Orange. The cultured butter samples were also evaluated by consumer sensory panellists using hedonic scaling of six sensory attributes (smoothness, hardness, spreadability, melting rate, buttery flavour, and overall acceptance). Data were plotted on graphs and also analysed by analysis of variance (P<0.05), linear regression and interaction plot.

There were significant differences (P<0.05) in moisture content of the three batches of butter which ranged from 13.90 to 19.19%. Although the moisture content of two batches (1 and 3) of butter was slightly higher than the standard (16%), it was within expected range. Manual washing butter grains after churning to remove water droplets may be inefficient to remove water droplets on the surface of butter. Most of the water droplets had a diameter of 5 μm which is desirable to inhibit the growth of spoilage microorganisms. No coliforms were detected in the cultured butter, indicating good hygiene standard during production. There were significant differences (P<0.05) in hardness of the three batches of cultured butter. Batch 2 had higher hardness than the other two batches, probably attributed to its low moisture content.

The fat content of cultured butter of the three batches ranged between 75% and 80%, which was slightly lower than the expected 80%. However, the results were reasonable, considering the higher moisture content of the butter. The cultured butter was well accepted by sensory panellists. Linear regression and interaction plot showed that spreadability and buttery flavour had significant effects (P<0.05) on the overall acceptance of the butter. The products were spreadable, presumably due to higher moisture content. The buttery flavour could be attributed to the aroma compounds produced by lactic acid bacteria through citrate metabolism during cream ripening. The dominant hue in the butter was yellowness, which slightly decreased during storage, presumably due to the loss of β-carotene. The pH of butter samples (5.3 to 5.8) during storage was slightly higher than in previous studies (4.7-5.2). The higher pH may be caused by poor acid production of the leuconostoc in the mixed culture.

Cultured butter was successfully processed using a kitchen/domestic churning mixer. The churning efficiency of the equipment was lower than the expected range. The butter had good keeping quality and was well accepted by sensory panellists. The quality of the butter during storage was probably attributed to the optimal size of water droplets, which were successfully measured by the confocal laser scanning microscope method modified in this study.
ACKNOWLEDGEMENTS

It has been a great experience and great honour for me to pursue my Master of Food Technology at Massey University in New Zealand. There are several people I am grateful to who helped me to accomplish my studies and enjoy my life at Massey University.

First and foremost, I would like to appreciate Dr. Tony Mutukumira, my supervisor, who believed in me and gave me unlimited support and guidance throughout the whole process of my postgraduate study and life. It was my great fortune and honour to be his student and thank you for giving me the opportunity to work with you. I appreciate all his contributions of time, encouragements, expertise, experiences, and patience.

I would like to thank Origin Earth Limited, Hawkes Bay, for financial support, and in particular Joanie Williams for technical support and encouragement.

I would like to thank Corrie den Haring, General Manager of Green Valley Dairies, Mangatawhiri, Auckland, who provided the fresh cream used in this study to produce cultured butter.

I also would like to thank Niki Murray, Laboratory Manager at Manawatu Microscopy and Imaging Centre (MMIC), Massey University, for her assistance with measurement of water droplets size of cultured butter.

I also thank Rachel Liu and Helen Matthews, both Laboratory Managers in the School of Food and Nutrition, at the Albany Campus, for their assistance in various aspects of the project. I also want to thank my friends who shared experiences with me.

Lastly, I would like to acknowledge my parents for all their unconditional love and encouragement, as well as financial support.
# TABLE OF CONTENTS

SUMMARY ................................................................................................................... i  
ACKNOWLEDGEMENTS ........................................................................................... iii 
TABLE OF CONTENTS ............................................................................................. iv 
LIST OF TABLES ....................................................................................................... vi 
LIST OF FIGURES ................................................................................................. viii 
LIST OF ABBREVIATIONS ...................................................................................... x 

1.0 Introduction ....................................................................................................... 1  
2.0 Literature Review ........................................................................................... 5 
   2.1 Introduction ...................................................................................................... 5 
   2.2 Milk .................................................................................................................. 6 
      2.2.1 Gross composition of milk ........................................................................ 6 
      2.2.2 Milk fat ..................................................................................................... 8 
      2.2.3 Milk fat crystals ....................................................................................... 13 
      2.2.4 Seasonal variations of milk components .................................................. 18 
   2.3 Aroma compounds of butter ........................................................................ 20 
      2.3.1 Diacetyl and citrate metabolism .............................................................. 21 
      2.3.2 Off-flavours and spoilage of butter ......................................................... 24 
   2.4 Butter making ................................................................................................ 28 
      2.4.1 Types of butter ....................................................................................... 28 
      2.4.2 Mesophilic lactic starter cultures ......................................................... 28 
      2.4.3 Processing of butter .............................................................................. 30 
   2.5 Packaging of butter ...................................................................................... 37 
   2.6 Characteristics of butter ............................................................................... 39 
      2.6.1 Texture and rheology ............................................................................ 39 
      2.6.2 Spreadability of butter ...................................................................... 42 
      2.6.3 Microstructure of butter ..................................................................... 43 
      2.6.4 Sensory evaluation of butter ............................................................... 46 
3.0 Materials and Methods .................................................................................. 48 
   3.1 Experiment design ....................................................................................... 48
3.2 Manufacture of cultured butter ................................................................. 49
  3.2.1 Cream and starter cultures ................................................................. 49
  3.2.2 Methods .......................................................................................... 50
  3.2.3 Butter analysis .................................................................................. 54
4.0 Results and Discussion ............................................................................ 67
  4.1 pH of cultured butter during processing .............................................. 67
  4.2 pH and titratable acidity of cultured butter during storage at 4°C .......... 69
  4.3 Fat content of buttermilk ....................................................................... 72
  4.4 Moisture, SNF and fat content of cultured butter during storage at 4°C ... 75
  4.5 Colour of cultured butter during storage at 4°C .................................... 78
  4.6 Texture analysis of cultured butter during storage at 4°C ....................... 83
  4.7 Microbiology quality of cultured butter during storage at 4°C ............... 88
  4.8 Consumer sensory evaluation of cultured butter during storage at 4°C .... 89
  4.9 Examination of water droplet size of cultured butter during storage at 4°C .. 99
5.0 Conclusions .............................................................................................. 103
6.0 Recommendations .................................................................................... 104
7.0 References ............................................................................................... 105
Appendix ......................................................................................................... 123
  Appendix 1.0 Characterisation of cream, buttermilk and cultured butter ....... 123
  Appendix 2.0 Sensory evaluation test forms ................................................. 132
  Appendix 3.0 Statistical outputs ................................................................. 135
LIST OF TABLES

Table 2.1 Properties of the main structural elements of milk, including approximate numerical values…………………………………………………………………………………7

Table 2.2 FAs composition of milkfat compared with other edible fats…………………10

Table 2.3 Density, specific heat, heat of melting and viscosity of milkfat compared with other edible fats……………………………………………………………………11

Table 2.4 Solid fat content (%) by NMR of milkfat compared with other edible fats.11

Table 2.5 Composition of raw milk in Palmerston North, New Zealand during February and April in 2008………………………………………………………………..19

Table 2.6 Mean and seasonal variations of components of The Netherlands bovine raw milk in 2005…………………………………………………………………….19

Table 2.7 Proportions of unsaturated fatty acids at 10°C in cow’s milk during lactation in New Zealand………………………………………………………………………19

Table 2.8 Typical principal temperature programmes adjusted to the iodine value and recommended volumes of starter culture used……………………………………………………………………34

Table 2.9 Terms used in descriptive analysis of sweet cream, whey and cultured butters………………………………………………………………………………………47

Table 3.1 Set parameters of the Texture Profile Analysis for measuring texture of butter using the TA.XT2 Texture Analyser …………………………………………………58

Appendix Table I pH of cream, buttermilk, and cultured butter at different temperature treatment during butter processing…………………………………………………………123

Appendix Table II Mean±SD pH of cream, buttermilk, and cultured butter at different temperature treatment during butter processing………………………………………123

Appendix Table III pH and titratable acidity (%T.A.) of cultured butter stored at 4°C
for 21 days ........................................................................................................123

Appendix Table IV Mean±SD pH and titratable acidity (%T.A.) of cultured butter stored at 4°C for 21 days ........................................................................................................124

Appendix Table V %Fat in buttermilk ................................................................124

Appendix Table VI %Fat in cream ......................................................................124

Appendix Table VII Hunter Lab values of cultured butter during storage days at 4°C ................................................................................................................125

Appendix Table VIII Mean±SD Hunter Lab values of cultured butter during storage days at 4°C ........................................................................................................126

Appendix Table IX Moisture content, SNF content, and fat content (%) of three batches of cultured butter during storage days at 4°C ........................................126

Appendix Table X Mean±SD moisture content, SNF content, and fat content (%) of three batches of cultured butter during storage days at 4°C ..........................127

Appendix Table XI Six sensory attributes of batch 2 cultured butter during storage days at 4°C ........................................................................................................127

Appendix Table XII Mean±SD of six sensory attributes of batch 2 cultured butter during storage days at 4°C ............................................................................130

Appendix Table XIII Hardness, springiness and adhesiveness of cultured butter stored at 4°C for 21 days ..................................................................................130

Appendix Table XIV Mean±SD hardness, springiness, and adhesiveness of cultured butter stored at 4°C for 21 days .........................................................................131
LIST OF FIGURES

Figure 2.1 Structure of the fat globule of the main milk fat globule membrane........9
Figure 2.2 Saturation-supersaturation solubility diagram.................................14
Figure 2.3 Rheogram for pumpable shortening obtained with a rotational viscometer.................................................................17
Figure 2.4 C4- compound biosynthetic pathway in LAB................................22
Figure 2.5 Traditional hand-churning, formerly used for domestic butter-making....30
Figure 2.6 General process steps in batch and continuous production of cultured butter........................................................................31
Figure 2.7 Spectra of light transmission............................................................39
Figure 3.1 Laboratory scale cultured butter processing.................................51
Figure 3.2 (a) Assembled kitchen/domestic scale churning mixer; (b) Disassembled kitchen/domestic scale churning mixer.......................................................53
Figure 3.3 Shaving butter strip from a cube using 1.8 kg strength standard fishing tippet...............................................................................55
Figure 3.4 Staining sample with Nile Red........................................................56
Figure 3.5 Staining sample with Acridine Orange...........................................56
Figure 3.6 Coverslip on the top of sample with Nile Red and Acridine Orange.....56
Figure 3.7 TPA force-by-time loading map.......................................................59
Figure 4.1 Mean pH of the cream during temperature treatment......................69
Figure 4.2 Means of pH and titratable acidity (% T.A.) of cultured butter during storage at 4°C.................................................................70
Figure 4.3 Comparison of the means of pH and titratable acidity (% T.A.) of cultured
butter during storage at 4ºC……………………………………………………………………72

Figure 4.4 Mean fat content in buttermilk……………………………………………………74

Figure 4.5 Calculated churning efficiency of cultured butter products………………74

Figure 4.6 Mean moisture content of cultured butter during storage at 4ºC…………76

Figure 4.7 Mean SNF content of butter during storage at 4ºC………………………77

Figure 4.8 Mean fat content of butter during storage at 4ºC…………………..78

Figure 4.9 Mean Hunter Lab values of cultured butter during storage at 4ºC……79

Figure 4.10 Comparison of the mean Hunter values of ‘L’, ‘a’, and ‘b’ of cultured butter during storage at 4ºC…………………………………………………………………..82

Figure 4.11 Mean hardness of cultured butter during storage at 4ºC………………85

Figure 4.12 Mean springiness of cultured butter during storage at 4ºC……………..86

Figure 4.13 Mean adhesiveness of cultured butter during storage at 4ºC…………87

Figure 4.14 Mean sensory scores for overall acceptance, smoothness, hardness, spreadability, melting rate, and buttery flavour of cultured butter during storage at 4ºC……………………………………………………………………………………90

Figure 4.15 Mean intensity scores of six attributes of cultured butter during storage 4ºC…………………………………………………………………………………………94

Figure 4.16 Scatter plot of mean consumer sensory preferences scores of overall acceptance response to the other five attributes (buttery flavour, hardness, melting rate, smoothness and spreadability)………………………………………..97

Figure 4.17 Interaction plot of six attributes of cultured butter during storage at 4ºC…………………………………………………………………………………………………98

Figure 4.18 CLSM images of cultured butter stained with Nile Red and Acridine Orange during storage at 4ºC………………………………………………………………………………101
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Hamaker’s constant</td>
</tr>
<tr>
<td>ALA</td>
<td>(\alpha)-linolenic</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-way Analysis of Variance</td>
</tr>
<tr>
<td>BC</td>
<td>Before Christ</td>
</tr>
<tr>
<td>biovar.</td>
<td>Biovariance</td>
</tr>
<tr>
<td>BSG</td>
<td>Balangu seed gum</td>
</tr>
<tr>
<td>BT</td>
<td>Beef tallow</td>
</tr>
<tr>
<td>CB</td>
<td>Cocoa butter</td>
</tr>
<tr>
<td>CLA</td>
<td>Conjugated linoleic acid</td>
</tr>
<tr>
<td>CLSM</td>
<td>Confocal laser scanning microscope</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CNO</td>
<td>Coconut oil</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>D</td>
<td>Particle diameter</td>
</tr>
<tr>
<td>ES</td>
<td>Electric sensing</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>g</td>
<td>Gramme</td>
</tr>
<tr>
<td>G</td>
<td>Shear modulus</td>
</tr>
<tr>
<td>G’</td>
<td>Elastic modulus</td>
</tr>
<tr>
<td>G”</td>
<td>Viscous modulus</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HDPE</td>
<td>High density polyethylene</td>
</tr>
<tr>
<td>(H_o)</td>
<td>Interparticle distance</td>
</tr>
<tr>
<td>HPKO</td>
<td>Hardened palm kernel oil</td>
</tr>
<tr>
<td>HSBO</td>
<td>Hardened soyabean oil</td>
</tr>
<tr>
<td>k</td>
<td>Boltzmann's constant</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
</tbody>
</table>
L = Litre
L. = Lactococcus
LAB = Lactic acid bacteria
LD = Laser diffraction
Leuc. = Leuconostoc
m = Metre
M = Moles per litre
MA = Myristic acid
MF = Milkfat
MFGM = Milk fat globule membrane
min = Minute
mL = Millilitre
mm = Millimetre
MMIC = Microscopy and Imaging Facility Centre
mmol/L = Millimole per litre
MP = Melting point
n = Number
NMR = Nuclear magnetic resonance
OA = Oleic acid
P = P-value
PA = Palmitic acid
pfg-NMR = Pulsed field gradient nuclear magnetic resonance
PKO = Palm kernel oil
PN = Palmerston North
PO = Palm oil
PS = Polystyrene
PVC = Polyvinyl chloride
RSG = Reihan seed gum
s = Second
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDL</td>
<td>Starter distillate</td>
</tr>
<tr>
<td>SFC</td>
<td>Solid fat content</td>
</tr>
<tr>
<td>SNF</td>
<td>Solids-not-fat</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid phase microextraction</td>
</tr>
<tr>
<td>spp.</td>
<td>Species</td>
</tr>
<tr>
<td>subsp.</td>
<td>Subspecies</td>
</tr>
<tr>
<td>T</td>
<td>Absolute temperature</td>
</tr>
<tr>
<td>T.A.</td>
<td>Titratable acidity</td>
</tr>
<tr>
<td>TPA</td>
<td>Textural profile analysis</td>
</tr>
<tr>
<td>tr</td>
<td>Trace</td>
</tr>
<tr>
<td>TVA</td>
<td>Trans vaccenic acid</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight/weight</td>
</tr>
<tr>
<td>XG</td>
<td>Xanthan gum</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometre</td>
</tr>
<tr>
<td>φ</td>
<td>Volume fraction of solids</td>
</tr>
</tbody>
</table>