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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

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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 4ºC 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 4ºC 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.

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LIST OF ABBREVIATIONS

A	=	Hamaker's constant
ALA	=	α-linolenic
ANOVA	=	One-way Analysis of Variance
BC	=	Before Christ
biovar.	=	biovariance
BSG	=	Balangu seed gum
BT	=	Beef tallow
CB	=	Cocoa butter
CLA	=	Conjugated linoleic acid
CLSM	=	Confocal laser scanning microscope
cm	=	Centimetre
CNO	=	Coconut oil
CO_2	=	Carbon dioxide
D	=	Particle diameter
ES	=	Electric sensing
FA	=	Fatty acid
g	=	Gramme
G	=	Shear modulus
G'	=	Elastic modulus
G''	=	Viscous modulus
h	=	Hour
HDPE	=	High density polyethylene
H_o	=	Interparticle distance
HPKO	=	Hardened palm kernel oil
HSBO	=	Hardened soyabean oil
k	=	Boltzmann's constant
kg	=	Kilogram

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

SD	=	Standard deviation
SDL	=	Starter distillate
SFC	=	Solid fat content
SNF	=	Solids-not-fat
SPME	=	Solid phase microextraction
spp.	=	Species
subsp.	=	Subspecies
T	=	Absolute temperature
T.A.	=	Titratable acidity
TPA	=	Textural profile analysis
tr	=	Trace
TVA	=	Trans vaccenic acid
w/w	=	Weight/weight
XG	=	Xanthan gum
μm	=	Micrometre
ϕ	=	Volume fraction of solids

1.0 Introduction

It is well-known that manufacturers of dairy products have a large market share, of which butter is an important product, especially in western countries (Bylund, 1995; White, & White, 1999; Mallia, Escher, & Cerny, 2008; Food Processing Staff, 2015). People have been consuming butter made from bovine milk fat for thousands of years, not only because of its pleasant buttery flavour, but there is also evidence that the dairy product has a variety of positive effects on human health (Oeffner, Qu, Just, Quezada, Ramsing, Keller, & Bobe, 2013). Butter is rich in fat-soluble vitamins, such as vitamins A, E and K2, unsaturated fats, and butyrate (Oeffner, Qu, Just, Quezada, Ramsing, Keller, & Bobe, 2013). Chemically, butter is a water-in-oil emulsion containing not less than 80% fat (White, & White, 1999; & Mallia, Escher, & Cerny, 2008). It is usually classified as cultured (sour cream) butter and sweet cream butter. The dairy spread can also be classified according to salt content (Bylund, 1995; Spreer, 1998). In addition, flavoured butters can be created for using on breads and desserts, by adding spices, herbs and seasonings (Spreer, 1998; Goff, 2015).

The technology of producing butter has evolved from cottage industry to a large-scale highly automated processing sector due to significant advances in science and technology (Huber, 1931; Frede, & Buchheim, 1994). The technology of making butter dates back to thousands of years Before Christ (BC) (Huber, 1931; Frede, & Buchheim, 1994). Archaeologists found some evidence that milk was whipped into butter grains as early as the 4th millennium BC, when a Sumerian frieze in relief showed milk processing from this time (Huber, 1931; Frede, & Buchheim, 1994). Thus, the technique of butter-making was probably already known at this time (Frede, & Buchheim, 1994). The basic principles of butter processing continue to be applied even today. However, the main difference is that milk has been replaced by cream whipped in specialised butter churns (Frede, & Buchheim, 1994; Spreer, 1998). In earlier times, butter was churned by hand after skimming cream from the top of the

milk (Spreer, 1998; Mallia, Escher, Cerny, 2008). There is also evidence that, naturally soured milk was used in butter making in early times due to opportunistic bacteria from the environment, milk handler, equipment and udders of the cow (Spreer, 1998; Goff, 2015). Milk is sterile when it comes from the udder, but it easily becomes contaminated by environment microorganisms as mentioned here, leading to spontaneous or natural fermentation. The bacteria responsible for milk fermentation were mainly dominated by lactic acid bacteria (LAB) (Law, 1997). Natural fermentation of milk gave birth to new fermented products including cultured butter (Lane, 1998; Goff, 2015). Later, addition of previous naturally soured milk or acidified buttermilk (back-slopping) was used to initiate the fermentation of sweet cream (Bylund, 1995). The practice of back-slopping improved reduced fermentation time and improved the quality of cultured products (Goff, 2015). It eventually became possible to make safe and consistent high quality cultured butter under more controlled conditions using pure starter cultures (White, & White, 1999).

With the invention of the butter making separator, it became possible to skim the cream before the milk had become sour (White, & White, 1999). The butter making separator was devised by Nielsen-Petersen in 1878 (Frede, & Buchheim, 1994; Bylund, 1995; Smit, 2003), which was a turning point from small scale to large scale butter making (Mick, Mick, & Schreier, 1982). The quality of the large scale produced butter was further enhanced by the introduction of pasteurisation, coupled by advances in lactic starter technology (Bylund, 1995; Smit, 2003). The knowledge and experience gained over the years led into the development of continuous commercial butter making (Mick, Mick, & Schreier, 1982). Currently, the two major varieties of the dairy spread that can be produced are sweet and cultured (soured) butter which may be salted (Bylund, 1995; Spreer, 1998).

Although the dairy industry has been successful in large scale commercial butter processing, small scale butter still exists in small communities and specialised

products. At present, there is also a market demanding for special or particular products such as organic dairy butter (Frede, & Buchheim, 1994; Bylund, 1995; Spreer, 1998). The consumer market for organic products is growing and it is important for the industry to respond positively for the growth of various sectors (Frede, & Buchheim, 1994; Bylund, 1995; Spreer, 1998). Small scale butter production uses low capacity equipment and in some cases, domestic kitchen type which is labour intensive. This level of production is associated with several challenges such as hygiene and sanitation, churning efficiency and product consistency (Bylund, 1995; Smit, 2003; Goff, 2015). Of particular importance to the safety and keeping quality of butter, is the size and even distribution of water droplets in the butter (Frede, & Buchheim, 1994; Bylund, 1995; Spreer, 1998).

As the technology of butter making becomes more advanced, processors can modify the properties of spreadability to meet consumer demands (Walstra, & Jenness, 1984; Lane, 1998; Goff, 2015). Processors can produce more spreadable butter by either increasing the moisture content during production or incorporating vegetable oil (Bylund, 1995; Lane, 1998; Spreer, 1998). Due to seasonal variations of milk composition, spreadable butter can be also produced by modifying the cream ripening procedure (Bylund, 1995; Goff, 2015). In summer, milk fats contain a large proportion of unsaturated fatty acids (FAs), which usually have low melting point (MP) (Bansal, Habib, Rebmann, & Chen, 2009; Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009). On the contrary, winter milk has a larger proportion of solid fat, as most of fats are saturated with high MP (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). Thus, to produce spreadable butter from the two types of butter fat, the respective cream ripening temperatures are adjusted to produce a spreadable product.

These years, the market share of butter has been continuously increasing, and it overtook margarine to become the most popular spread choice in western countries

(Food Processing Staff, 2015). Hence, studying butter has become more significant. In New Zealand, there is a small but flourishing market for cultured butter made from Hawke's Bay fresh cows' milk, by small scale producers using conventional kitchen/domestic type equipment. Thus, the overall quality of the butter may be variable. Therefore, the aim of this project was to standardise small scale production of cultured butter using kitchen/domestic scale equipment.

The specific objectives of the study were:

To standardise the production of cultured butter using kitchen/domestic scale equipment;

To modify the confocal laser scanning microscope method for measuring the size of water droplets in cultured butter during storage at 4ºC;

To determine the stability of cultured butter during storage for 21 days at 4°C;

To determine moisture content, solids-not-fat content and fat content of cultured butter during storage for 21 days at 4ºC;

To measure pH, colour and texture of cultured butter during storage for 21 days at 4ºC;

To determine titratable acidity, and coliforms of cultured butter during storage for 21 days at 4ºC; and,

To evaluate consumer acceptance of cultured butter using the hedonic scale during storage at 4ºC.

2.0 Literature Review

2.1 Introduction

Fermented milks, including cultured butter, are an essential part of our diet (Bylund, 1995; Rudd, 2013). In ancient times, milking was done by hand with buckets rather than machines (Leaver, 1983; Valenze, 2011). Cows were usually milked beside a restraining wooden post erected near the main kraal or under a tree, twice a day, in the morning and evening (Leaver, 1983; Rudd, 2013). Since these places were not kept clean except for the removal of dung, the milking cows became soiled with dung and urine (Leaver, 1983; Valenze, 2011; Rudd, 2013). It was not a common practice for the ancient people to clean the udder and hindquarters before milking, thus the milk was contaminated by microorganisms (Leaver, 1983; Valenze, 2011). The obtained milk became sour and thick due to opportunistic microorganisms from the handling equipment, the milker and the general environment (IDF 227, 1988; Rudd, 2013). In some instances, the soured milk was consumed after shaking to mix and some households drained off the liquid (whey), leaving a much thicker curd. The sour milk and the thick curd were consumed with various food products including cereals (Leaver, 1983). In some societies, the sour milk was shaken in the fermenting vessel until butter grains formed and the whey was drained off (Leaver, 1983). The butter grains were matted together and using a source of cooking fat (Leaver, 1983). The traditional practice of making butter gave birth to modern day sweet and cultured butter (Robinson, 1986). Although commercial butter is presently produced using modern technology under controlled processing conditions, the traditional practice is still common in small or traditional communities (Valenze, 2011). Also, the traditional practice may be preferred to produce nice products with specific sensory characteristics (Robinson, 1986). However, traditional butter technology suffers from poor yield, inconsistent quality of products and low safety (Robinson, 1986). In the early days, there was less attention on the fermentation process until the beginning of

20th century, when specific microorganisms were used to ferment dairy products (Robinson, 1986; Rudd, 2013). Cultured butter is now commercially being produced use pure cultures with known characteristics (Robinson, 1986; Rudd, 2013).

2.2 Milk

2.2.1 Gross composition of milk

Milk is in general an oil-in-water emulsion, although there are many uncertain factors influencing its composition and structure, such as breed of cow, climate, season, lactation stage and feeding regime (Mulder, & Walstra, 1974; Walstra, & Jenness, 1984). It is mainly composed of fat globules, casein micelles, serum proteins, lipoprotein particles, mineral constituents and somatic cells (Walstra, & Jenness, 1984; Belitz, Grosch, & Schieberle, 2004). Table 2.1 shows the main elements of milk composition.

Table 2.1 Properties of the main structural elements of milk, including approximate numerical values (Walstra, & Jenness, 1984).

	fat globules	plasma		
		casein micelles	serum	
			globular proteins	lipoprotein particles
Main component	Fat	casein, water and salts	serum proteins	lipids and proteins
To be considered as	Emulsion	fine dispersion	colloidal solution	colloidal dispersion
Content (% dry matter)	3.8	2.8	0.6	~0.01
Volume fraction	0.042	0.065	0.006	~0.0001
Particle diameter[1]	0.1-10 µm	10-300 nm	3-6 nm	~10 nm
Number per ml	10^{10}	10^{14}	10^{17}	10^{14}
Surface area (cm^2/ml milk)	700	40 000	50 000	100
Density (~20^0C)(g/ml)	0.92	1.11	1.34	1.1
Visible with	microscope	ultra microscope	(electron microscope)	electron microscope
Light scattering	very turbid, white	turbid, bluish	light haze	negligible
Separable with	dairy separator	high speed centrifuge	gel filtration	gel filtration
Filterable with	Fine sintered glass	Chamberland filter	cellophane	cellophane
Diffusion rate (mm in 1 h)	-	0.1-0.3	0.6	0.4
Flocculation	by 'agglutinin' in the cold	by acid or by rennet	by heat	

[1] 1 mm = 10^3 µm = 10^6 nm = 10^7 Å

Note: milk includes fat globules and plasma; plasma includes casein micelles and serum; serum includes globular proteins and lipoprotein particles.

Milk is valuable due to its rich components. Fat globules contribute to the status of milk; when the inside of globule is not liquid, it is partly crystallised at room temperature, and milk becomes partially solid (Lane, 1998). When milk becomes crystallised, the status of milk changes, including the properties of butter which will be discussed in section 2.2.3. Casein micelles comprise of proteins, minerals and water (Walstra, & Jenness, 1984; Belitz, Grosch, & Schieberle, 2004). Casein is a complex of several different types of molecules with different properties. The proportion of the molecules is constant (Walstra, & Jenness, 1984; McKenna, & Kilcast, 2003). The principal casein fractions are α_s-, κ-, and β-caseins; also, there are genetically determined variants with slightly different composition and properties (McKenna, & Kilcast, 2003; Belitz, Grosch, & Schieberle, 2004). Casein, which is not a true protein, contains residues of phosphate and glucides like hexoses and sialic

acid, besides amino acids (Mulder, & Walstra, 1974; Walstra, & Jenness, 1984). In milk, casein functions like a caseinate, mainly of calcium; casein is combined with a calcium phosphate of varying composition with ion-exchange properties (Mulder, & Walstra, 1974). The micelles are about 10-12 nm in diameter, consisting of almost spherical subunits (Mulder, & Walstra, 1974; Lane, 1998). Compared with casein, serum proteins are true globular proteins, whose particles are considered as macromolecules (Walstra, & Jenness, 1984; Lane, 1998). Also, serum proteins (β-lactoglobulin, β-lactalbumin, blood serum albumin and immunoglobulins) contain proteose-peptone, which are poly-, tri- and di-peptides, and finally amino acids. Lipoprotein particles contain phospholipids and other glycerides, cholesterol, protein, nucleic acids, and several enzymes (Walstra, & Jenness, 1984; Lane, 1998).

2.2.2 Milk fat

The most important component in butter is milk fat, which should not be less than 80% (ANZFS Code, 2010). Therefore, the characteristics of milk fat determine the final quality of butter, which will be discussed in subsequent sections.

According to Logan, Auldist, Greenwood, and Day (2014), milk fat is typically about 3 to 6% by weight of total milk, and dispersed within the serum phase. The average milk fat content varies for different breeds ranging from 3.8% for Friesian to 5.1% for Jersey cattle (Leaver, 1983; Walstra, & Jenness, 1984). Milk fat is made up of FAs derived from two sources. Those containing 4-10 carbon atoms are synthesised in the udder from acetate and *β*-hydroxybutyrate which is mainly derived from acetic and butyric acids produced in the rumen (Leaver, 1983; Damodaran, Parkin, & Fennema, 2008; Boerman, & Lock, 2014). FAs in the milk containing 18 or more carbon atoms are directly transferred from the blood triglycerides within the udder, while those with intermediate chain lengths are derived from the both udder and rumen (Leaver, 1983;

Damodaran, Parkin, & Fennema, 2008; Boerman, & Lock, 2014).

A fat globule is an intimate mixture of liquid and solid phases whose main constituents are triglycerides (shown in Figure 2.1) (Timms, 1994; Dewettinck, Rombaut, Thienpont, Le, Messens, & Van Camp, 2008; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). In milk, milkfat is predominantly present as spherical globules, the bulk of which are 1-8 μm in diameter (Timms, 1994; Logan, Auldist, Greenwood, & Day, 2014). Each globule is surrounded by a membrane which is comprised mainly of protein and phospholipid (Figure 2.1) (Logan, Auldist, Greenwood, & Day, 2014; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). Fat globules have a high interfacial tension with water and occur in the form of small droplets in most occasions, and are soluble in non-polar organic solvents, while insoluble in aqueous liquids (Lane, 1998; Dewettinck, Rombaut, Thienpont, Le, Messens, & Van Camp, 2008). In aqueous phase, lipids associated with other organic materials, such as proteins, and presented in a very finely divided state; thus, lipids are not prone to distinguish these lipoproteins and tiny fat globules (McKenna, & Kilcast, 2003; Logan, Auldist, Greenwood, & Day, 2014). Although the chemical composition and structure of fat is complicated, the physical properties, chemical reactivity and its food values are dependent on FA pattern (McKenna, & Kilcast, 2003).

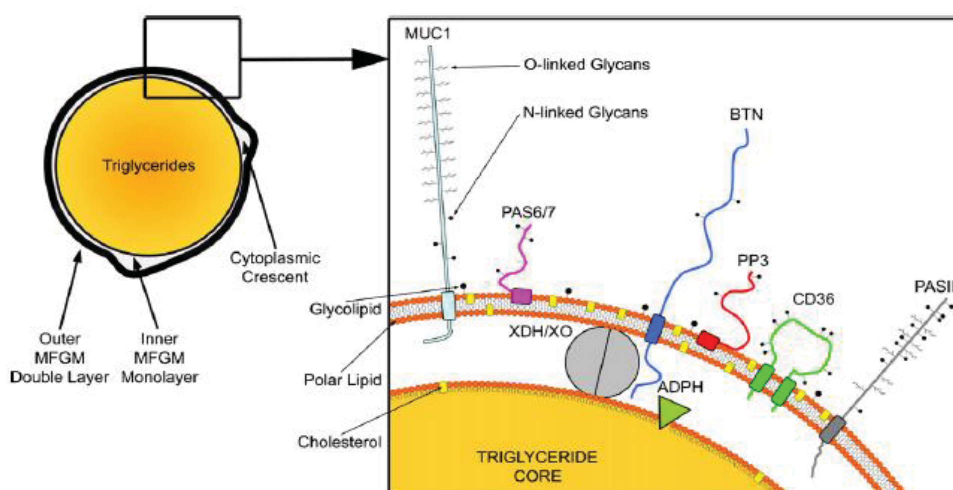


Figure 2.1 Structure of the fat globule of the main milk fat globule membrane (MFGM) (Dewettinck, Rombaut, Thienpont, Le, Messens, & Van Camp, 2008).

The chemical composition of milkfat is very different from other fats with similar MP. The quality of milk fluctuated when FA changed (Chilliard, Ferlay, Rouel, & Lamberet, 2003). Table 2.2 gives the FA composition of milkfat compared with other edible fats (Timms, 1994). The Table shows that milkfat has a higher level of shorter-chain acids, which produces a softer fat (Timms, 1994). However, the physical properties of milkfat are not significantly different from other semi-solid fats of similar MP. Some basic physical properties, namely, density, specific heat, heat of fusion and viscosity are showed in Table 2.3 (Timms, 1994). The percentage of solid fat content (SFC) analysed by nuclear magnetic resonance (NMR) is shown in Table 2.4, which shows that lauric fats, palm kernel and coconut oils are similar to milkfat. Thus these fats are good replacements for milkfat in filled-milk products or so-called non-dairy products (Timms, 1994).

Table 2.2 FAs composition of milkfat compared with other edible fats (Timms, 1994).

	Fatty acid (% wt as methyl ester)										
Fat	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3
Milkfat	4	3	1.5	2.5	3	10	26	13	24	1.5	1.5
MF stearin	3.5	2.5	1	2.5	3.5	12	31	12	20	1.5	1
MF olein	4.5	3	1.5	3	3	10	27	10	24	1.5	1.5
CNO	0	0.5	8.5	6.5	48	16	8.5	2.5	7	2.5	0
PKO	0	0.5	4	3.5	47	15	9	4.5	14	2.5	tr
HPKO	0	0.5	4	3.5	47	15	9	15	5.5	0.5	0
HSBO	0	0	0	0	0	tr	11	10	76	2.5	tr
BT	0	0	0	0	0.5	3.5	25	23	39	2	1
CB	0	0	0	0	0	tr	26	35	34	3	tr
PO	0	0	0	0	tr	1	45	4.5	39	10	0.5

MF = milkfat; CNO = coconut oil; PKO = palm kernel oil; HPKO = hardened PKO (MP = 35ºC); HSBO = hardened soyabean oil (MP = 35ºC); BT = beef tallow; CB = cocoa butter; PO = palm oil; tr = trace.

Table 2.3 Density, specific heat, heat of melting and viscosity of milkfat compared with other edible fats (Timms, 1994).

Fat	Density (kg/m^3 at 50℃)	Specific heat (kcal/kg at 50 ℃)	Heat of melting (kcal/kg)	Viscosity (cP at 50℃)
Milkfat	895	≈0.50	20-24	22
Palm oil	891	0.51	≈23	27
Palm kernel oil	898	≈0.51	≈30	21
Coconut oil	901	≈0.51	≈26	19

Table 2.4 Solid fat content (%) by NMR of milkfat compared with other edible fats (Timms, 1994).

	Temperature (℃)						
Fat	10	15	20	25	30	35	40
Milkfat	50	39	21	13	7	4	0
MF stearin	65	54	37	27	18	9	1
MF olein	42	27	10	2	1	0	0
CNO	80	63	35	2	0	0	0
PKO	67	-	39	15	0	0	0
HPKO	85	-	73	53	21	8	3
HSBO	60	-	40	26	15	4	0
BT	59	55	47	35	25	18	11
CB	82	79	76	69	32	0	0
PO	54	-	26	16	8	5	1

MF = milkfat; CNO = coconut oil; PKO = palm kernel oil; HPKO = hardened PKO (MP = 35ºC); HSBO = hardened soyabean oil (MP = 35ºC); BT = beef tallow; CB = cocoa butter; PO = palm oil; tr = trace.

Although the FAs in milk comes from rumen and udder of the cow, they originally come from the natural forages, which are the grasses and pastures (Chilliard, Ferlay, & Doreau, 2001; Boerman, & Lock, 2014). In temperate countries, fresh grass contains 1-3% FAs, spring and autumn have been observed the highest FAs content (Chilliard, Ferlay, & Doreau, 2001). Among these FAs, 55-65% comprise α-linolenic acid, whereas, tropical pastures present a different FA component, where α-linolenic acid is 15-40 % of total FAs (Chilliard, Ferlay, & Doreau, 2001).

Blaško, Kubinec, Górová, Fábry, Lorenz, and Soják (2010) reported on the

composition of unsaturated FAs, including conjugated linoleic acid (CLA), α-linolenic (ALA), oleic acid (OA), and trans vaccenic acid (trans-11 18:1, TVA), and saturated FAs, including palmitic acid (PA) and myristic acid (MA), in butter produced in summer and winter. Summer butter had higher levels of potentially health-promoting unsaturated FAs compared with winter products. Cow's milk produced in summer had higher average contents of FAs compared with winter milk (Blaško, Kubinec, Górová, Fábry, Lorenz, & Soják, 2010).

The softness of fat is determined by the relative amounts of FAs with low MPs (Bylund, 1995; Spreer, 1998; Smit, 2003). Soft fat generally contains a high content of low-melting FAs, and this fat has a large continuous phase of liquid fat at room temperature (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). On the contrary, high-melting FA contains high levels of hard fat, and the ratio of liquid-to-solid fat is low (Spreer, 1998; Damodaran, Parkin, & Fennema, 2008). Moreover, soft fat in butter results in a softer and greasier butter, whereas, hard fat makes butter harder and stiffer (Walstra, & Jenness, 1984; Bylund, 1995; Spreer, 1998). The unsaturation of FAs in milk controls the texture of milk fat; generally, the more unsaturation FAs, the softer of milk fat (Schingoethe, Brouk, Lightfield, & Baer, 1996). Schingoethe, Brouk, Lightfield, and Baer (1996) studied the response of dairy cows to breeding using extruded soybeans and sunflower seeds compared to normal pasture. Dietary manipulation can be used to alter the FA composition of milk, even the proportion of unsaturated FAs in milk fat can be increased by about 40% (Schingoethe, Brouk, Lightfield, & Baer, 1996). When the FA component of oilseeds varies, sunflower seeds and safflower seeds contain the most unsaturated fat sources (Schingoethe, Brouk, Lightfield, & Baer, 1996; Damodaran, Parkin, & Fennema, 2008). The two latter seeds typically contain 66 and 77% linoleic acid ($C_{18:2}$), respectively, with barely linolenic acid ($C_{18:3}$) (Schingoethe, Brouk, Lightfield, & Baer, 1996; Damodaran, Parkin, & Fennema, 2008). Soybeans also contain highly unsaturated FAs, but less linoleic acid (58% $C_{18:2}$) and measurable amounts (8%) of

$C_{18:3}$ (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). Schingoethe, Brouk, Lightfield, and Baer (1996) found that the $C_{18:3}$ in milk increased when soybean oil was infused into cow feeds. Also, milk fat from cows fed by soybeans or sunflower seeds contained higher concentrations of unsaturated FAs and long-chain FAs than milk fat from cows fed with normal pasture (Schingoethe, Brouk, Lightfield, & Baer, 1996).

2.2.3 Milk fat crystals

Milk fat crystallisation is an important step that controls the texture and viscosity of the dairy products, including cultured butter. For milk fat, crystallisation commences between 40°C and -40°C (Mulder, & Walstra, 1974; Spreer, 1998; Smit, 2003; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). Milk fat is liquid >40°C, and a solid at <-40°C (Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). Thus, milkfat is a mixture of crystals and oil at intermediate temperatures (e.g. room temperature), where the oil is normally continuous (Spreer, 1998; McKenna, & Kilcast, 2003; Smit, 2003). Temperature treatment can therefore contribute to the rheological properties of crystals. At the crystallisation temperature, the concentration of the solute to be crystallised above the concentration of a saturated solution is increased, in order to obtain crystallisation (Timms, 1994; Spreer, 1998; Walstra, 2003). A typical saturation-supersaturation diagram is shown in Figure 2.2. Below the continuous line, crystallisation is not possible because solution is not saturated. The dashed line divides the metastable zone from the unstable zone, namely, crystallisation zone. In the metastable zone, crystallisation is possible, but does not occur spontaneously or immediately without assistance such as seeding (Timms, 1994; Walstra, 2003). Crystallisation can occur spontaneously in the unstable zone (Timms, 1994). From Figure 2.2, the position of the dashed line boundary between unstable and metastable

is variable (area CAE) depending on variables, such as cooling rate, agitation and contamination by non-fat particles. The position of continuous line depends only on the substance being crystallised (Timms, 1994).

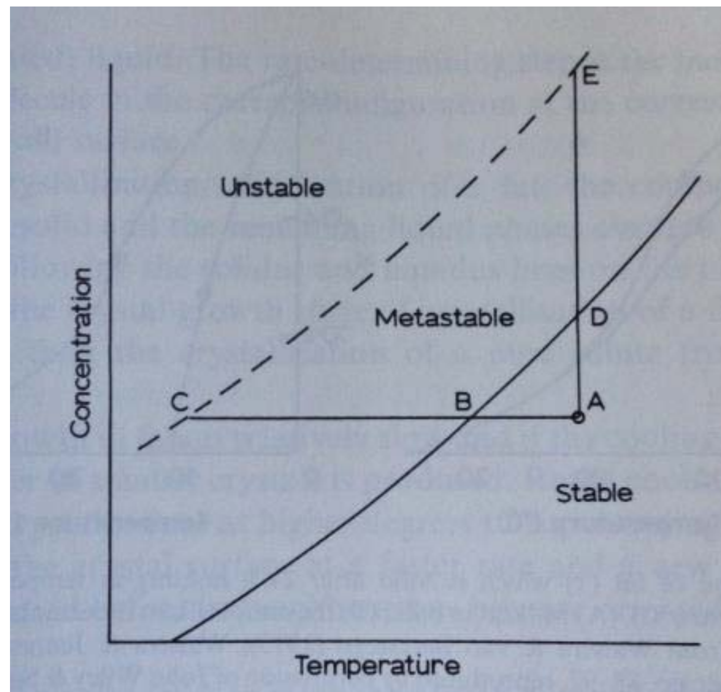


Figure 2.2 Saturation-supersaturation solubility diagram (Timms, 1994).

In all fat crystals, the rate of crystallisation can be affected by several substances such as surfactants, when they are dissolved in small quantities in the fat (Deman, & Beers, 1988; Spreer, 1998; Walstra, 2003). Due to that fat crystals flocculate into a network suspended in liquid oil, the crystals are able to grow together in aggregates, and eventually forming a solid network or structure of considerable mechanical strength (McKenna, & Kilcast, 2003). The fat then has both liquid and solid phases in the continuous network, which is held by van der Waals attraction forces (Lane, 1998; Walstra, 2003). The mass looks completely solid when 80% of fat is solid, while the liquid phase is still exist, but can be hardly expressed, nor spread at the air-water interface (Lane, 1998).

If crystallisation is slow, large crystals form, whereas, rapid cooling to low temperatures produces numerous small crystals of 10^{12}/mL (Walstra, & Jenness, 1984; Lane, 1998). A crystal nucleus is the smallest crystal existing in a solution of a certain concentration and temperature (Timms, 1994). Crystals that are smaller than the nucleus would re-dissolve (Timms, 1994). Once a crystal nucleus has formed, it starts to grow by the incorporation of other molecules. Brownian motion is one of the incorporations. With Brownian motion, small crystals are prone to aggregate to large crystals, which are less soluble in solvents, and tend to grow at the expense of small ones, due to Ostwald ripening (Lane, 1998). Small crystals may be sometimes not strong enough to build a solid network, where recrystallisation has taken place (Lane, 1998; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). Any slow recrystallisation, whether by polymorphic transition or through separation of mixed crystals, increase the size of crystals (Walstra, 2003). In butter production, recrystallisation is always used to improve stronger solid networks (Bylund, 1995). Also, small fat crystals are required to obtain desirable product consistency and physical properties (Lane, 1998).

Fat crystal network is held together by van der Waals forces, where the average bond energy is about 10 kT units (k = Boltzmann's constant and T = absolute temperature), and the interparticle distance about 1 nm (Deman, & Beers, 1988; Walstra, 2003). A linear chain model for the network structure in plastic fats was proposed by Van Den Tempel (1961). In this model, the fat network is made up of straight chains oriented in three mutually perpendicular directions, where each chain consists of a linear array of particles held together by two types of bonds (Van Den Tempel, 1961). The two irreversible bonds lie on secondary bonds or primary bonds, the latter may consist of a relatively strong van der Waals bond between favourably oriented particles and a growing together of crystals due to recrystallisation processes or polymorphic transition, involving formation of new solid phases from solution (Van Den Tempel, 1961; Walstra, 2003). The energy content and contribution to stiffness in these solid

phases is much higher than those of the secondary bonds, which can be disrupted by kneading and do not reform easily, thus causing a high degree of work-softening (Van Den Tempel, 1961). Van Den Tempel (1961) worked out Equation 1 for the value of the shear modulus G, assuming that the interaction in network is caused by van der Waals forces only.

$$G = 5A\phi D^{0.5}/24\pi H_o^{3.5} \quad (1)$$

Where, A = Hamaker's constant (determining the van der Waals attraction energy), ϕ = volume fraction of solids, D = particle diameter, H_o = interparticle distance (Van Den Tempel, 1961; Deman, & Beers, 1988). Equation 1 reveals a continuous drop in stress with increasing strain and the shear modulus is directly proportional to ϕ and D (Van Den Tempel, 1961; Deman, & Beers, 1988).

Fat normally behaves like a rigid solid until the deforming stress exceeds the yield value and fat tends to flow like a viscous liquid (Deman, & Beers, 1988). This theory can be proved by Figure 2.3, which is obtained from a pumpable shortening using a cone and plate viscometer (Davis, 1973). From Figure 2.3, as shear stress increased, there is no significant deformation until it reaches the static yield value (y1). This is followed by a decrease in stress which is attributed to breakdown of the structure in sample (Davis, 1973). Plastic viscosity is the slope of the original down curve, and the deformation which occurs before the yield value is reached due to elastic stretching of bonds, whereas stresses beyond the yield value bonds break and reform (Davis, 1973).

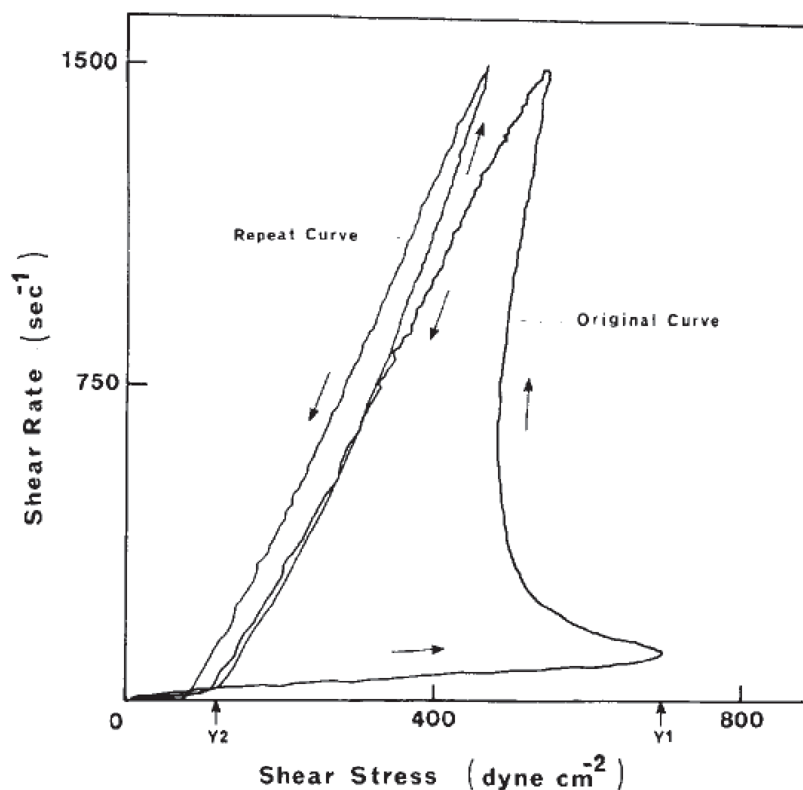


Figure 2.3 Rheogram for pumpable shortening obtained with a rotational viscometer (Davis, 1973).

Crystallisation is especially crucial for butter products to build a good plasticity, which is affected by the ratio of solid to liquid fat (Lane, 1998; Walstra, 2003; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). A plastic fat is usually workable at room temperature when the solid fat content is between 20 and 38% (Deman, & Beers, 1988). A liquid fat forms small crystals of needle to platelet shape through rapid cooling, about 0.1-3 µm long, with a ratio of length : width : thickness of 4:2:1 (Deman, & Beers, 1988). As crystals grow, crystals are aggregated to larger crystals with similar shape. In milk, the large crystals are usually spherulites, as much as 50 µm across (McKenna, & Kilcast, 2003; Walstra, 2003); the clusters of crystals, spherulites or aggregates can reach sizes of over 100 µm, then fat is perceptibly grainy (Deman, & Beers, 1988).

Plastic fat products mainly include shortening, margarine and butter (Deman, & Beers,

1988). Among these products, shortening consists of a dispersion of fat crystals in oil, margarine contains water droplets within the fat crystal network, and butter consists of free oil, fat crystals, water droplets and fat globules (Deman, & Beers, 1988). Small fat crystals can bind in the liquid oil, and give a structure which will be deformed under shear (Lane, 1998). If fat crystals are larger than 20 μm, they will aggregate to cause a gritty texture in the product (Lane, 1998). This phenomenon appears in some margarine when certain triglycerides predominate (Lane, 1998). In butter products, the variety of triglycerides and the short chain FAs to the liquid fat phase contribute to enhance the plasticity (Lane, 1998; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). In butter, the droplets are in the aqueous phase, where emulsifiers are added to play a significant role, tend to be larger and their interface structure also seems to differ from margarines (McKenna, & Kilcast, 2003). Moreover, the plasticity influences the spreadabiltiy of butter, which will be discussed in section 2.6.2.

2.2.4 Seasonal variations of milk components

Seasonal variations impact on milk composition which is caused by changes in the stage of lactation and feeding (Auldist, Walsh, & Thomson, 1998; Skeie, 2007; Bansal, Habib, Rebmann, & Chen, 2009). Thus, seasonal variations in milk will influence the texture and characteristics of butter, making it difficult to achieve consistent butter products over time (Auldist, Walsh, & Thomson, 1998; Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003; Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). It is therefore important to know the variations of milk components in different seasons.

The concentrations of protein and fat in milk is higher in winter, and lower in the summer (Bansal, Habib, Rebmann, & Chen, 2009; Heck, van Valenberg, Dijkstra, &

van Hooijdonk, 2009). The composition of raw milk in New Zealand during February and April in 2008 is shown in Table 2.5. In New Zealand, concentrations of protein and fat are higher in April, whereas, the trend is different with lactose as the disaccharide decreases. A similar tendency was reported in The Netherlands as shown in Table 2.6 (Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009). Large seasonal variations of FAs in milk were also reported in The Netherlands. The concentrations of unsaturated FAs were higher in summer milk, while lower in the winter (Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009). The presence of higher unsaturated FAs in milk product result in a softer milk fat, which means milk fat in summer is softer than in winter (described in section 2.2.2) (Schingoethe, Brouk, Lightfield, & Baer, 1996). However in New Zealand, concentrations of unsaturated FAs were reported be to higher in winter than in summer as shown in Table 2.7 (Auldist, Walsh, & Thomson, 1998).

Table 2.5 Composition of raw milk in Palmerston North, New Zealand during February and April in 2008 (Bansal, Habib, Rebmann, & Chen, 2009).

Time	Protein%	Fat%	Lactose%
Early February	<3.50	~4.50	4.60-4.75
Mid-April	3.75-4.00	5.75-6.00	4.30-4.60

Table 2.6 Mean and seasonal variations of components of The Netherlands bovine raw milk in 2005 (Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009).

Component	Mean	Minimum	Maximum
Protein (g/100g)	3.48	3.39 (July)	3.56 (December)
True protein (g/100g)	3.30	3.21 (July)	3.38 (December)
Fat (g/100g)	4.38	4.10 (July)	4.57 (December)
Lactose (g/100g)	4.51	4.46 (October)	4.55 (May)

Table 2.7 Proportions of unsaturated fatty acids at 10°C in cow's milk during lactation in New Zealand (Auldist, Walsh, & Thomson, 1998).

Fatty acid%	Spring			Summer			Autumn			Winter		
Monounsaturates	26.3	25.4	27.8	22.8	20.8	22.3	27.1	22.8	24.5	28.4	24.7	25.0
Polyunsaturates	4.29	4.27	4.70	3.31	3.30	3.52	3.96	3.95	4.44	3.90	4.08	4.08

Solid fat content (SFC) fluctuates the most depending on the seasons as well. In summer, when fresh grass is abundant, the SFC values are lower at about 55% at 5℃ (Lane, 1998). Butter products aim at fat content no less than 80% (ANZFS Code, 2010), thus, Jersey cow milk is popular to producing cream used for butter making. Jersey milk is naturally high in butter fat content, and will yield more cheese (20% to 25%), butter (>30%), non-fat dry milk (~10%) than average milk, and at a lower cost per pound of product (McKenna, & Kilcast, 2003).

2.3 Aroma compounds of butter

The popularity of butter for thousands years is largely attributed to its aromatic flavours (Bylund, 1995; Spreer, 1998; Smit, 2003; Mallia, Escher, & Cerny, 2008). Aroma components in butter depend on animal feeding, season of production, manufacturing temperature and storage conditions of the final products (Vas, & Vekey, 2004). Although more than 230 volatiles have been identified as natural constituents of butter, only a small proportion contributes to the odorants of butter; for example, diacetyl presents buttery-like flavour, butanoic acid shows cheesy aroma, and δ-decalactone reveals a peach flavour (Spreer, 1998; Mallia, Escher, & Cerny, 2008).

The aroma compounds in butter can be obtained in two ways: fermentation of cream and addition of starter distillate (SDL) (Leroy, & De Vuyst, 2004). During fermentation, mixed mesophilic lactic starter cultures are added to the cream to produce aroma compounds. During fermentation, starter culture produces rapid acidification of the raw cream through production of organic acids, mainly lactic acid including aroma compounds, such as diacetyl and acetoin (Leroy, & De Vuyst, 2004). Aromatic butter can be also produced using SDL (Robinson, 1986). SDL is included in the formulation of many dairy products as water- or oil-based liquid or as

encapsulated powder (Robinson, 1986). The aromatic distillates contain high levels of diacetyl (Rincon-Delgadillo, Lopez-Hernandez, Wijaya, & Rankin, 2012). Depending on the types of starter culture used, production of SDL may take several hours to a whole day (Robinson, 1986; Spreer, 1998). Adding SDLs can boost the buttery flavour by changing sweet cream to cultured cream (Robinson, 1986). Since starter cultures can only produce certain aroma compounds, SDLs are added together with starter cultures in butter production to obtain more flavours (Robinson, 1986).

2.3.1 Diacetyl and citrate metabolism

LAB are responsible for the production of diacetyl and other aroma compounds in the butter during cream fermentation (Spreer, 1998; Smit, 2003). During the production of cultured butter, LAB metabolise sugars present in butter, producing lactic acid through homo- or heterofermentative pathways (Spreer, 1998; Smit, 2003; Mallia, Escher, & Cerny, 2008). The efficiency of LAB is principally evaluated on their ability to metabolise some milk components, during specific biochemical process, such as glycolysis, proteolysis, lipolysis and diacetyl production (Macciola, Candela, & Leonardis, 2008). LAB also metabolise other substrates including citrate producing 4-carbon compounds (Spreer, 1998; Damodaran, Parkin, & Fennema, 2008; Macciola, Candela, & Leonardis, 2008). Diacetyl or 2,3-butanedione is one of the most important aroma substances among the 4-carbon compounds produced by citrate metabolism, responsible for the buttery aroma of dairy products, including butter, acid cream and cottage cheese (Spreer, 1998; Macciola, Candela, & Leonardis, 2008; Quintans, Blancato, Repizo, Magni, & López, 2008). A diagrammatic overview of citrate metabolism in LAB is shown in Figure 2.4.

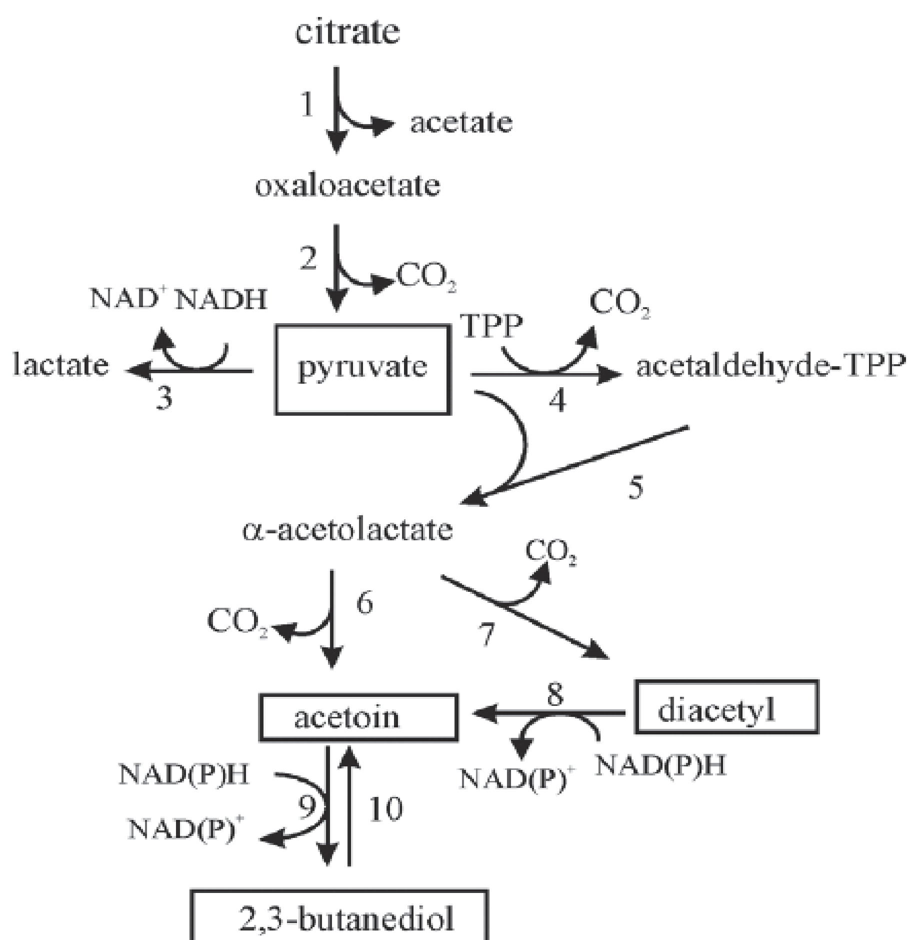


Figure 2.4 C4- compound biosynthetic pathway in LAB (Quintans, Blancato, Repizo, Magni, & López, 2008).

1, citrate lyase; 2, oxaloacetate decarboxylase; 3, lactate dehydrogenase; 4, pyruvate decarboxylase; 5, acetolactate synthase; 6,acetolactate decarboxylase; 7, non-enzymatic oxidative decarboxylation of α-acetolactate; 8 and 9, diacetyl acetoin reductase; 10,2,3-butanediol dehydrogenase; TPP, thiamine pyrophosphate.

Diacetyl is produced from the spontaneous oxidative decarboxylation of α-acetolactate which can also be transformed into acetoin by α-acetolactate decarboxylase or by spontaneous non-oxidative decarboxylation (Figure 2.4) (Macciola, Candela, & Leonardis, 2008; Gokce, Akdogan, Divriklib, & Elci, 2014). Diacetyl is the final product of citrate metabolism by certain LAB such as *Lactococcus* (*L.*) *lactis* subsp. *lactis* biovariance (biovar.) *diacetylactis* and *Leuconostocs* (*Leuc.*) (IDF, 1988; Macciola, Candela, & Leonardis, 2008; Gokce, Akdogan, Divriklib, & Elci, 2014). Diacetyl is the important aroma compound that

characterises the flavour of butter and cheese (Mallia, Escher, & Cerny, 2008). Due to this reason, it is important to increase the quantity and quality of diacetyl in butter and cheese products. Law (2001) reported that mutant strains have been developed through genetic engineering to increase the production of diacetyl in cottage cheese and other soft cheese products. The precursor of diacetyl is citric acid, which is approximately 2000 mg per litre in cow's milk (Fox, Law, McSweeney, & Wallace, 1993). The identification of the volatile compounds butter has been achieved by using static and dynamic headspace methods, such as solid phase microextraction (SPME) and static headspace analysis (Zhang, Zeng, Wang, & Chen, 2013). Head space technique coupled with gas chromatography equipped with flame-ionization or mass spectrometer detectors is used to identify and quantify the volatile compounds (Frank, Owen, & Patterson, 2004; Vas, & Vekey, 2004). The aroma of butter oil has also been widely studied over the years (Mallia, Escher, & Cerny, 2008). Butter oil is produced by removing the aqueous phase from butter (Mallia, Escher, & Cerny, 2008). In the early studies, Siek and Lindsay (1968), found over 100 compounds in the steam distillates from butter-fat, including alkanals, alkanones, alcohols, esters, hydrocarbons and aromatic compounds. Flavour threshold studies showed that decanoic acid, lauric acid, δ-lactones, indole and skatole were the main volatile compounds in butter oil, whereas, the phenolic compounds were on the borderline (Stark, Urbach, Hamilton, & Forss, 1973).

Since butter oil consists of several kinds of flavour compounds, butter as the main product, contain more aroma compounds than butter oil. The flavour compounds in cultured butter are also present in sweet cream butter (Spreer, 1998; Smit, 2003; Mallia, Escher, & Cerny, 2008). Lindsay, Day and Sather (1967) reported that 2,3-butanedione (diacetyl), acetic acid and lactic acid were the most important aroma compounds in cultured products during the metabolism of LAB. This was confirmed by Mick, Mick, and Schreier (1982), with other contributions including 2-methylketones and alcohols. Shooter, Jayatissa and Renner (1999) studied volatile

sulphur compounds in butter. A variety of reduced sulphur compounds have been reported in raw milk, including hydrogen sulphide, methanethiol, dimethylsulphide, and dimethyldisulphide, thus the quality of milk directly influences butter (Shooter, Jayatissa, & Renner, 1999). In their study, the highest concentration of sulphur compounds was reported in spring milk in New Zealand. The sulphur compounds were significantly lower after butter samples were stored for 5 weeks (Shooter, Jayatissa, & Renner, 1999). The water fraction of butter has been reported to contain aroma compounds as well. Adahchour, Vreuls, Van der Heijden and Brinkman (1999), reported 23 compounds in the water fraction of butter including 1-methoxy-2-propanol, 3-hydroxy-2-butanone, 1-ethoxy-2-propanol, 2,3-butanediol, butanoic acid and benzoic acid.

2.3.2 Off-flavours and spoilage of butter

The desirable and unique aroma compounds of butter are a result of delicate balance of concentrations of compounds (Spreer, 1998; Smit, 2003; Mallia, Escher, & Cerny, 2008). The balance of the compounds can be broken by addition or depleted of aroma components, thus, the interaction between volatile and non-volatile compounds and the food matrix are especially important (Adahchour, Vreuls, Van der Heijden & Brinkman, 1999). The presence of off-flavours of butter affect sensory characteristics and aroma compounds, as well as loss of nutritional values, containing vitamins, oxidation of unsaturated lipids, which lead to significant economic losses (Spreer, 1998; Mallia, Escher, & Cerny, 2008). The origins of butter off-flavours in butter such lipid oxidation, lipolysis and microbial growth occur during butter manufacture, packaging and storage (Spreer, 1998; Mallia, Escher, & Cerny, 2008).

The presence of oxygen in butter can lead to autoxidation of milk fat producing off-flavours, which have been described as cardboard-like, metallic, oily, fishy, painty

and tallow (Spreer, 1998; Mallia, Escher, & Cerny, 2008). During autoxidation, unsaturated FAs breakdown to hydroperoxides, which decompose into flavourful compounds (Gonzalez, Duncan, O'Keefe, Sumner, & Herbein, 2003). The rate of autoxidation in butter depends on the composition of FAs, presence of antioxidants and pro-oxidants (Spreer, 1998; Mallia, Escher, & Cerny, 2008). For instance, linoleic acid oxidises ten times faster than oleic acid. Alpha-tocopherol, ascorbic acid and carotenoids are present as antioxidants in butter, whereas, peroxides and heavy metals act as pro-oxidants (Spreer, 1998; Damodaran, Parkin, & Fennema, 2008). The pro-oxidants can be heavy metals, such as iron and copper ions, originating from the metal equipment used in the production of butter (Gonzalez, Duncan, O'Keefe, Sumner, & Herbein, 2003). The oxidation rate of butter is also susceptible to external factors, such as oxygen pressure, temperature, exposure to light and moisture content (Spreer, 1998; Mallia, Escher, & Cerny, 2008).

Oxidised off-flavours formed in butter during prolonged storage have been widely reported (Spreer, 1998; Mallia, Escher, & Cerny, 2008). Van der Waarden (1947) is considered to be the first conclusive report on the oxidative degradation of lipid components during cold-storage of butter. Badings (1970) reported flavour defects in butter during cold storage caused by auto-catalytic oxidation of unsaturated FAs. Hexanal was chosen as an indicator of lipid oxidation in butter during storage, as it originates from autoxidation of linoleic acid present in the volatile fraction of oxidised foods (Belitz, Grosch, & Schieberle, 2004). (E)-2-Nonenal and 1-octen-3-one are easily formed through oxidation of linoleic acid (Badings, 1970), which can explain the metallic off-flavour in butter (Spreer, 1998; Mallia, Escher, & Cerny, 2008).

Heat is another factor that can induce off-flavours in butter, described as cooked, burnt, sulphurous and caramelised (Spreer, 1998; Mallia, Escher, & Cerny, 2008). The off-flavours can be formed during pasteurisation and high temperature treatment above 76.7ºC leading to Maillard reaction (Bodyfelt, Tobias, & Trout, 1988). Lactones

are also generated in butter oil resulting in undesirable coconut-like off-flavour (Spreer, 1998; Mallia, Escher, & Cerny, 2008). Lee, Macku and Shibamoto (1991) studied lipid degradation of sweet cream butter heated at 100, 150 and 200ºC for 5 h, respectively, generating several volatile compounds. Aldehydes and ketones produced at the higher temperature. Lipid degradation is the major reaction occurring in butter during heating (Lee, Macku, & Shibamoto, 1991). Heterocyclic compounds including thiazoles, pyrroles and pyridines were produced at temperatures above 150ºC (Lee, Macku, & Shibamoto, 1991).

Lipoprotein lipases which are naturally present in skim milk can produce lipolysed off-flavours, described as goaty or soapy (Mallia, Escher, & Cerny, 2008). Lipolysis can cause rancid odour when the double layer membrane protecting fat globule is disrupted by agitation or churning, thus, free FAs increased in the disrupted double layer (Allen, 1994). Schieberle, Gassenmeier, Guth, Sen, and Grosch, (1993) worked on traditionally processed sour cream butter. Their study reported a rancid and sweaty odour in the butter is due to high concentrations of butanoic and hexanoic acids formed by lipolysis (Schieberle, Gassenmeier, Guth, Sen, & Grosch, 1993). Lipases present in butter can be caused by external microbial contamination as well. Apart from the unpleasant odours in butter, lipolysis reduces the churning efficiency of cream (Allen, 1994).

Microbial off-flavours in butter are caused by the metabolism of microorganisms in raw milk, which is influenced by the initial microflora, the processing conditions, and post heat-treatment contamination (Mallia, Escher, & Cerny, 2008). Musty and malty are the main odours in microbial off-flavours of butter, which can be prevented by using high microbial quality sweet cream with proper sanitation during storage and processing (Mallia, Escher, & Cerny, 2008).

Microorganisms are easier to grow in raw milk compared with butter, and a variety of

spoilage microorganisms are found in milk, including Gram-negative psychrotrophs, coliforms, LAB, yeasts, and moulds which are killed during heat-treatment (Law, 1997; Pantoja, Reinemann, & Ruegg, 2011). A high quality of butter is produced with high microbiological quality of milk (Law, 1997). Growth of microorganisms in butter can be more strictly controlled when the sizes of water droplets in butter are less than 5 μm (Van Dalen, 2002; Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008).

As a by-product, buttermilk is more susceptible to microbiological spoilage due to its high water content and rich composition (Law, 1997). Buttermilk can be easily spoiled by growth of pseudomonads, psychrotrophs, yeasts and coliforms, which can reduce the diacetyl content of buttermilk and sour cream (Wang, & Frank, 1981). It is therefore important to drain as much buttermilk as possible from butter during production to reduce spoilage.

Another defect associated with butter, is the presence of high quantities of acetaldehyde which produces a green flavour (Keenan, & Lindsay, 1966; Siek, & Lindsay, 1968). The action of *Leuc. mesenteroides* subsp. *cremoris* converts acetaldehyde to alcohol, thus reducing the “green” or yogurt-like flavour to alcohol through alcohol dehydrogenase activity (Keenan, & Lindsay, 1966; Siek, & Lindsay, 1968). A diacetyl to acetaldehyde ratio of 4:1 is considered desirable to impart the buttery flavour, whereas the green flavour is perceived when the ratio is 3:1 or less (Lindsay, & Day, 1965).

2.4 Butter making

2.4.1 Types of butter

According to current manufacturing processing, there are two main types of butter, each with specific flavour, namely, sour cream butter and sweet cream butter (Spreer, 1998; Smit, 2003; Mallia, Escher, & Cerny, 2008). Butter can also be categorized according to salt content; unsalted, salted, and extra salted (Bylund, 1995; Spreer, 1998).

Cultured (sour) butter is also described as lactic butter, which is obtained from bacteriologically soured cream ripened by lactic acid culture (Spreer, 1998; Mallia, Escher, & Cerny, 2008). To produce cultured butter, the cream is inoculated with lactic starter cultures after pasteurisation (Leaver, 1983; Bylund, 1995; Spreer, 1998). Then, the cream is allowed to ferment during which aroma compounds are produced (section 2.3), which contribute to the sensory characteristic taste of butter (Bylund, 1995; Spreer, 1998). Sweet butter is made from the cream standardised to around 40% fat and it is derived from unfermented cream (White, & White, 1999). The procedures of making cultured and sweet butter are similar as discussed in the subsequent sections.

2.4.2 Mesophilic lactic starter cultures

Mesophilic fermented milk products, as well as cultured butter, are usually produced by adding mesophilic bacteria as starter cultures (Cogan, 1983; Johansen, & Kibenich, 1992; Law, 1997). *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *L. lactis* spp. *lactis*, *L. lactis* spp. *cremoris*, *Leuc. mesenteroides* spp. *cremoris*, and *Leuc. lactis* are the main bacteria used in cultured butter (Cogan, 1983; Johansen, & Kibenich, 1992; Law, 1997; Bissonnette, Labrie, Deveau, Lamoureux, & Moineau, 2000). These mesophilic

cultures are more active in the temperature range from 15 to 40°C (Law, 1997). Lactic acid is produced from the metabolism of lactose mainly during fermentation by *L. lactis* spp. *lactis* and *L. lactis* spp. *cremoris* (Cogan, 1983; Johansen, & Kibenich, 1992). *L. lactis* subsp. *lactis* biovar. *diacetylactis* produces aroma compounds, of which diacetyl is the most important for impacting aroma in cultured butter (Cogan, 1983; Johansen, & Kibenich, 1992). The leuconostoc strains produce diacetyl, and reduce the acetaldehyde produced by the lactococci (Keenan, & Lindsay, 1966; Cogan, 1983; Johansen, & Kibenich, 1992; Law, 1997).

The mesophilic starter cultures used in fermented dairy products are divided into two main groups, mixed and defined (Bissonnette, Labrie, Deveau, Lamoureux, & Moineau, 2000). Mixed starters are composed of undefined numbers and ratios of strains which are less predictable and more difficult to control under automated production of dairy products (Bissonnette, Labrie, Deveau, Lamoureux, & Moineau, 2000). Meanwhile, defined starters consist of a mixture of two or three well characterised strains, which are ideally phage-unrelated to prevent possible lysis of the starter and subsequent fermentation failure (Law, 1997; Bissonnette, Labrie, Deveau, Lamoureux, & Moineau, 2000). It is also common practice to rotate defined starters to prevent phage development (Law, 1997; Bissonnette, Labrie, Deveau, Lamoureux, & Moineau, 2000). A heterofermentation including defined commercial mixed starter cultures of *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. cremoris* was used in this study. The utilisation of citrate during fermentation by *L. lactis* and *Leuc.* is dependent on pH of the medium (Starrenberg, & Hugenholtz, 1991). The highest conversion rates of citrate were observed at pH ranging from 5.5 to 6.0, when citrate was added to cultures (Starrenberg, & Hugenholtz, 1991).

2.4.3 Processing of butter

The procedure of butter making has undergone significant changes over the years, whereas, the principles have essentially remained the same, irrespective of the scale of production. (Mulder, & Walstra, 1974; Spreer, 1998). In the early days, butter was made for household use only using a simple process and equipment as shown in Figure 2.5. During churning and discharging of buttermilk in a shallow trough, butter grains were collected manually with replicated squeezing until dry and structured butter was produced (Bylund, 1995). As more advanced and sophisticated equipment was developed, butter manufacturing processes developed into large scale, but still involving several processing stages (Figure 2.6). However, hand-churning still exists in some traditional communities.

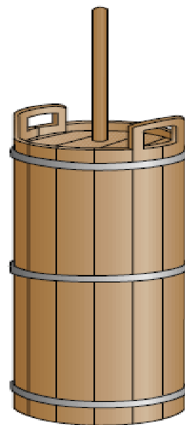


Figure 2.5 Traditional hand-churning, formerly used for domestic butter-making (Bylund, 1995).

In both small scale and large scale butter productions, fresh cream is the main raw material (Spreer, 1998). The cream is usually pasteurised by the supplier before delivery to the plant for butter production. The delivery and storage of cream is strictly controlled to prevent contamination, aeration and foaming (Bylund, 1995). Upon delivery of cream at the plant, the cream is stored in cooled storage tanks or

refrigerator (Bylund, 1995; Spreer, 1998). The key procedures comprise of pasteurisation, bacterial souring, temperature treatment, churning and working (Mulder, & Walstra, 1974; Frede, & Buchheim, 1994; Bylund, 1995; Spreer, 1998).

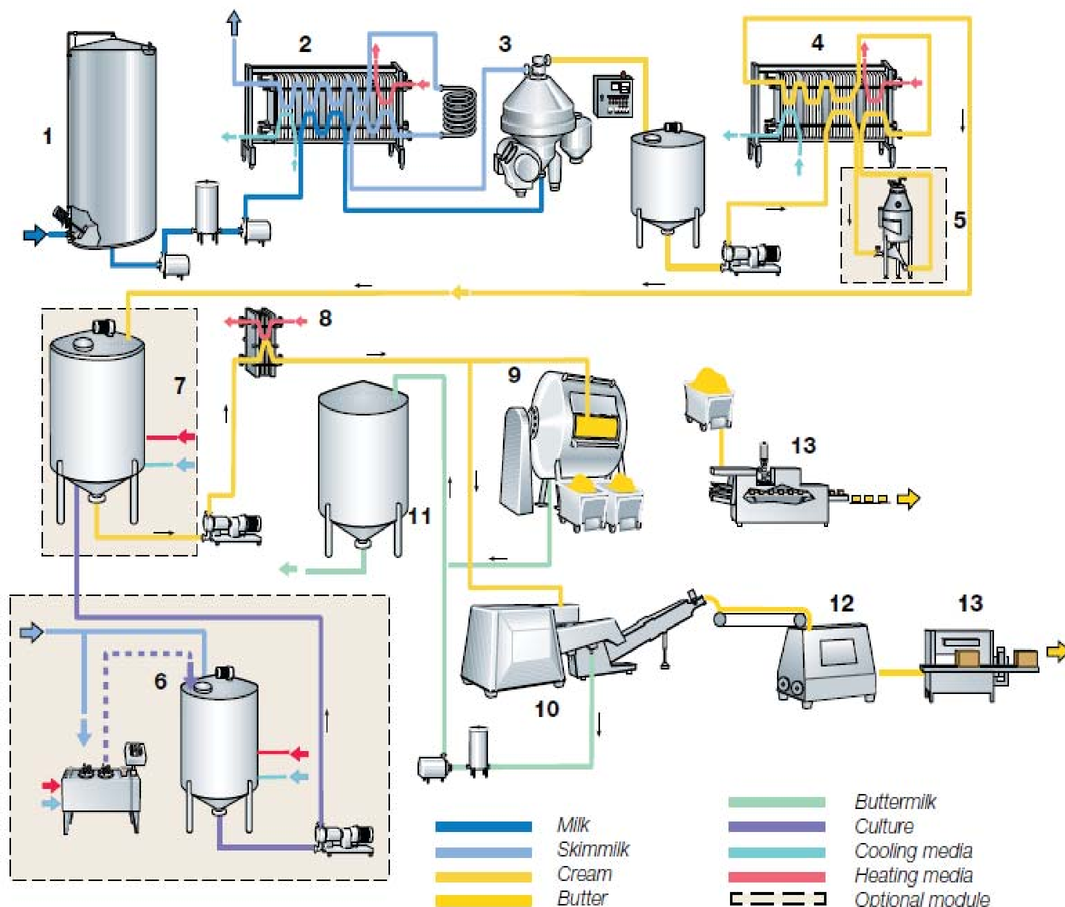


Figure 2.6 General process steps in batch and continuous production of cultured butter (Bylund, 1995).

1 Milk reception; 2 Preheating and pasteurisation of skimmilk; 3 Fat separation; 4 Cream pasteurisation; 5 Vacuum deaeration, when used; 6 Culture preparation, when used; 7 Cream ripening and souring, when used; 8 Temperature treatment; 9 Churning/working, batch; 10 Churning/working, continuous; 11 Buttermilk collection; 12 Butter silo with screw conveyor; 13 Packaging machines.

Unsaturated fatty acids of milk fat

The degree of unsaturated FAs in milk fat (cream) is determined by the iodine value using Wijs' method (Mulder, & Walstra, 1974; Kirk, & Sawyer, 1991). The iodine value of an oil or fat is defined as the weight of iodine absorbed by 100 parts by weight of the sample (Kirk, & Sawyer, 1991). Low iodine values indicate low unsaturation of fats (Bylund, 1995; Damodaran, Parkin, & Fennema, 2008). There is a direct relationship between unsaturation and rancidity in fat, that is, the greater the degree of unsaturation or the higher the iodine value, the greater rancid liability by oxidation of the oil or fat (Kirk, & Sawyer, 1991). In large scale butter processing, the heat treatment and ripening time are based on the iodine value of cream (Table 2.8), which influences the technology of butter making (Bylund, 1995). In this study, unsaturation of FAs was not analysed due to regulations of the analytical laboratory.

Vacuum deaeration

Vacuum deaeration is not usually done in small scale production, as it is difficult to achieve manually. It usually happens before pasteurisation, as the preheating processing (Bylund, 1995; Spreer, 1998). Any undesirable flavouring in the cream is normally bound in fat and transmitted to butter product unless it is removed, thus, vacuum treatment is essential to remove these natural volatile substances, such as onion taste (Bylund, 1995; Mallia, Escher, & Cerny, 2008). Onion off-flavour is a common defect in summer when various onion plants grow in the fields (Bylund, 1995; White, & White, 1999). The cream is first heated to 78℃ and then pumped to a vacuum chamber where the pressure is similar to a boiling temperature of 62℃ (Frede, & Buchheim, 1994; Spreer, 1998). Volatile flavouring and aromatic substances escape in the form of gas due to the reduced pressure. In this way, the cream is flash-cooled to free any entrapped gas and volatile substances (Frede, & Buchheim, 1994). After vacuum treatment, the cream is returned to the pasteuriser for further heat treatment

(Bylund, 1995; White, & White, 1999).

Pasteurisation

In both small scale and large scale butter production, the cream is usually pasteurised to 95℃ or higher without any holding time after being pumped from the intermediate storage tank or refrigerator (Bylund, 1995; Spreer, 1998). This high temperature is essential to kill pathogenic bacteria and enzymes as well as other microorganisms that would affect the keeping quality of the butter (Montville, Matthews, & Kniel, 2012). The destruction of unwanted microorganisms is also crucial as sour cream butter creates pleasant growth conditions for other bacteria cultures (Monu, Blank, Holley, & Zawistowski, 2008). In addition, strong antioxygenic sulfhydryl compounds are released through heat treatment, which reduce the risk of oxidation (Damodaran, Parkin, & Fennema, 2008).

Cream souring

The souring of cream and temperature treatment take place at the same time, and they give fat the necessary crystalline structure for optimum butter consistency (Mulder, & Walstra, 1974; Bylund, 1995; Spreer, 1998; Goff, 2015). Cultured butter is produced by adding starter cultures in both small scale and large scale productions after cooling the cream to inoculation temperatures (Mulder, & Walstra, 1974; Spreer, 1998; Goff, 2015). The mesophilic starter cultures convert more citrate during fermentation between pH 5.5 and 6.0 (Starrenberg, & Hugenholtz, 1991). The amount of starter culture added in cream during temperature treatment programme depends on the iodine value of the cream. As previously mentioned, the iodine value determines the unsaturation of fat (Kirk, & Sawyer, 1991). A higher iodine value indicates higher unsaturation of the fat in cream, resulting in a softer butter (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). Table 2.8 shows the iodine

value, temperature treatment and concentration of starter culture used in temperature programmes during butter making (Bylund, 1995; Spreer, 1998).

Table 2.8 Typical principal temperature programmes adjusted to the iodine value and recommended volumes of starter culture used (Bylund, 1995).

Iodine value	Temperature programme(℃)	% of starter culture in cream
<28	8-21-20	1
28-29	8-21-16	2-3
30-31	8-20-13	5
32-34	6-19-12	5
35-37	6-17-11	6
38-39	6-15-10	7
>40	20-8-11	5

Temperature treatment

In both small scale and large scale butter production, temperature treatment is important for fat crystallisation (Bylund, 1995; Spreer, 1998; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). In small scale production, this step is achieved by thermal transformation between water bath and the cream (Mulder, & Walstra, 1974; Spreer, 1998). The cream is placed in a stainless pot in a water bath to which contains cold or hot water (Mulder, & Walstra, 1974; Spreer, 1998).

The fat in fat globules is present as liquid after pasteurisation, and it starts to crystallise when the cream is cooled to below 40℃ (Bylund, 1995; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). Due to the different MP, fats start to crystallise at diverse temperatures if cooling is gradual (Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). This type of cooling is ideal for soft butter, as it results in a minimum of solid fat (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). Crystal

formation is slow during gradual cooling, thus, crystallisation process may take several days. This is susceptible to microbial contamination as the fat would be sensitive to bacterial infection at the cooling temperature (Montville, Matthews, & Kniel, 2012).

Cooling the cream quickly to low temperature can increase the rate of crystallisation, where the formation of crystals is very rapid (Bylund, 1995; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). The disadvantage of this method is that mixed crystals are formed, due to triglycerides with low MPs being trapped in the same crystals (Damodaran, Parkin, & Fennema, 2008). A higher proportion of fat would be crystallised, which makes the ratio of liquid to solid fat lower, thereby affecting texture of butter (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). The disadvantage could be avoided if the cream is heated carefully to a higher temperature, which can melt the low-melting triglycerides out of the crystals (Bylund, 1995; Spreer, 1998). The melted fat is then recrystallised at a relatively lower temperature, thus, purer crystals form and the mixed crystals are lower, which makes a higher liquid to solids ratio and a softer fat in the butter (Bylund, 1995; Spreer, 1998). Temperatures of heat treatment are selected according to the iodine value of the fat (Table 2.8). Data in second row (winter method) shown in Table 2.8 were used as the temperature programme of the cream samples in this study (Bylund, 1995).

Churning

Churning takes place in both small scale and large scale productions to generate butter grains (Mulder, & Walstra, 1974; Bylund, 1995; Spreer, 1998). Churning in small scale production and large scale production is illustrated in Figure 2.5 and 2.6, respectively. The cream is churned after temperature treatment and ripening (White, & White, 1999). Churns have a working speed range suitable for any set of butter

parameters. The shape, setting and size of the dashers are related to the speed of the churn, and the end product (Spreer, 1998; White, & White, 1999). As technology improved, the size of churns has increased in recent years for large scale production. Typically, a capacity of 8000–12000 L or more are used in large central creameries (Frede, & Buchheim, 1994; Funahashi, & Horiuchi, 2008).

At the beginning of churning, fat globule crystals in the cream can to some extent become structured, for cream contain both crystallised fat and liquid fat (Bylund, 1995; Spreer, 1998; Damodaran, Parkin, & Fennema, 2008). The fat globules form a shell closely to the membrane of the fat globule, and largely protein bubble foams form when cream agitated (Damodaran, Parkin, & Fennema, 2008; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015). Fat globules active on the surface are drawn towards the air/water interface, and then the fat globules are concentrated in the foam (Frede, & Buchheim, 1994). The cream foams rapidly increase in volume until it is about 190% of the original under vigorous agitation (Jebson, 1994). As churning continues, the bubbles become smaller and more numerous, while the protein gives off water to make the foam more compact, thus applying pressure on the fat globules (Bylund, 1995; Spreer, 1998). Continuous agitation induces some of the membranes disintegrate and liquid fat be pressed out of the fat globules (Frede, & Buchheim, 1994; Bylund, 1995). The membrane walls are thinner as the total surface area of the film increases. The fat globules tend to concentrate at the surface of the film (Jebson, 1994; Spreer, 1998). A point is reached where the film is so thin that there is no room for the fat globules to slide past one another, and collisions become frequent (Jebson, 1994). When collisions accelerate at the correct ratio of solid to liquid fat in the globules, they are disrupted in the collisions, liquid fat leaks out, and cement the globules together to form butter granules (Jebson, 1994). The foam bubbles become unstable, as coating the foaming. Butter granules increase in size with continued agitation, until they are suitable for draining off the buttermilk (Jebson, 1994).

The churning step controls the yield of butter. Churning recovery indicates the amount of fat in the cream converted to butter (Bylund, 1995; Spreer, 1998; Goff, 2015), and it is expressed as the ratio of fat remaining in buttermilk to amount of total fat in the cream (Frede, & Buchheim, 1994; Spreer, 1998; Goff, 2015). For instance, a churning yield of 0.50 indicates that 0.5% of the cream fat in buttermilk and, thus 99.5% has been transformed into butter. A good churning recovery must be less than 0.70 (Bylund, 1995).

Working

When buttermilk has been drained off, working process takes place to press and squeeze the moisture in butter grains, while dispersing the water phase into fine droplets (Bylund, 1995; Spreer, 1998). In small scale production, butter grains are washed manually by distilled water (Mulder, & Walstra, 1974). Increasing washing time can decrease the moisture content in butter (Mulder, & Walstra, 1974; Bylund, 1995; Spreer, 1998). The finished butter must be dry, with no visible water droplets (Bylund, 1995; Spreer, 1998).

2.5 Packaging of butter

At the end of the processing, butter is commonly packed in aluminium foil and stored at 5℃ (Bylund, 1995; Spreer, 1998). Retail packaging of butter is commonly done using 0.009 mm thick aluminium foil, laminated to either 40 g/m^2 grease-proof paper or vegetable parchment (Robertson, 2012). Other types of packaging materials, including plastic tubs thermoformed from polystyrene (PS) or polyvinyl chloride (PVC) with a tight-fitting lid, are also used for packaging butter (Robertson, 2012). When using tubs, butter must be packed directly from the churn or reworked immediately prior to packaging to allow it to flow into the package and fill efficiently

(Robertson, 2012).

Butter is susceptible to light-induced or oxygen-induced flavour defects, it is therefore important to select suitable packaging materials (McKenna, & Kilcast, 2003). The degree of deterioration depends on factors such as light source, wavelength of light, exposure time, distance of butter from the light source, and β-carotene content in butter (Robertson, 2012). Figure 2.7 shows the transmission of light by different materials. The transmission of parchment (A) ranges from about 46-64%, whereas material B can markedly reduce the transmission to 1-17% for wavelengths between 300-500 nm of yellow pigments. The reduction of transmission below 500 nm does not appreciably reduce oxidation, indicating that longer wavelengths have a better effect (Robertson, 2012). Metallised paper (material C) transmits less than 10% of light, and foil laminate (material E) does not transmit measurable light (Robertson, 2012).

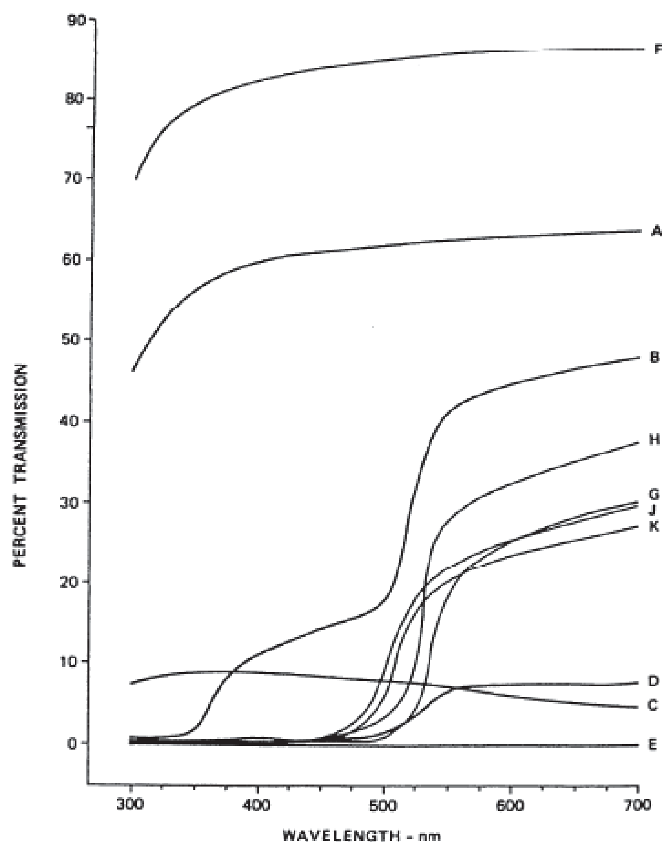


Figure 2.7 Spectra of light transmission: paper-based (A, B, C and D); foil laminate (E); polyethylene-based packaging materials (F, G, H, J and K) (Robertson, 2012).

2.6 Characteristics of butter

2.6.1 Texture and rheology

Butter is highly prised not only for its flavour, but also texture attributes, which is defined as the sensory and functional manifestation of foods, including structural, mechanical and surface properties (Krause, Lopetcharat, & Drake, 2007; Lawless, & Heymann, 2010). The diet stage of lactation, dietary supplementation of the animal and the season of the year are also factors that influence the texture of butter (Krause, Miracle, Sanders, Dean, & Drake, 2008). It was reported that the FA profile affects texture of dairy products (Oeffner, Qu, Just, Quezada, Ramsing, Keller, Cherian, Goddick and Bobe, 2013).Using unsaturated FA, such as C18:3n-3, as an ingredient

of butter processing, is prone to decrease hardness and adhesiveness of refrigerated butter, which can improve spreadability (Oeffner, et al., 2013).

Bobe, Hammond, Freeman, Lindberg and Beitz (2003) used the TA-XT2 Texture Analyser (Stable Micro Systems, London, UK) equipped with a 40º conical probe to analyse the penetration force of butter. Five (5) mm surface layer of butter was removed, and the probe was lowered at 1 mm/s to a depth of 12 mm from the sample (new) surface and then withdrawn at the same speed. Butter samples from cows with a more unsaturated milk FA composition had a lower atherogenic index, and the samples were more spreadable, softer, and less adhesive (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003). Another study (Bobe, Zimmerman, Hammond, Freeman, Porter, Luhman, & Beitz, 2007) showed that feeding supplements to cows influences texture of butter. In their study(Bobe et al., 2007), the TA-XT2 Texture Analyser (Stable Micro Systems, London, UK) with similar settings (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003) was used to determine the texture of butter. The study indicated that milkfat from cows feded with roasted soybean produced a softer butter (Bobe, et al., 2007).

Rheology provides important information about the structural organisation of food, processing, transportation, stability, and mouth feel of the products during development (Damodaran, Parkin, & Fennema, 2008; Emadzadeh, Razavi, & Schleining, 2013). Chemically, butter is a water-in-oil (w/o) emulsion (Belitz, Grosch, & Schieberle, 2004; Wu, 2009). The suspension phase of butter exists as discrete droplets of water, whose diameter is generally between 0.1 and 100 μm (Belitz, Grosch, & Schieberle, 2004; Wu, 2009). Water stays in the continuous phase of oil, and there is an interfacial layer between the two phases, where surfactant materials are occupied (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008; Wu, 2009). The original oil-in-water (o/w) emulsion (e.g. cream) is broken in a temperature range of 10-15ºC, where milk fat is partially liquid. The finished product,

with 16% to 20% aqueous phase, is dispersed in droplets varying between 1 and 30 μm in size (Buchheim, & Dejmek, 1997). The stabilisation of the formulation is dependent on the viscoelasticity of the continuous phase (Emadzadeh, Razavi, & Schleining, 2013). The characteristics of butter can be influenced by external additives such as sugar replacer and fat replacer. The replacers are considered as important dietary ingredients for preventing certain diseases (Emadzadeh, Razavi, & Schleining, 2013). Butters containing replacers are considered to be more glycaemic, low insulinaemic, low in calories, and do not promote teeth cavity (Emadzadeh, Razavi, & Schleining, 2013).

Emadzadeh, Razavi, and Schleining (2013) reported on small and large deformation properties of low-calorie pistachio butter. In their study, different low-calories pistachio butter formulations were treated with different fat replacers and sweeteners, in order to have a better understanding of the changes in viscoelasticity and textural characteristics. All the formulations of butter showed viscoelastic behaviour (Emadzadeh, Razavi, & Schleining, 2013), where elastic modulus (G') were stronger than that of viscous modulus (G") (Belitz, Grosch, & Schieberle, 2004; Damodaran, Parkin, & Fennema, 2008). During the study of the three fat replacers of Reihan seed gum (RSG), Balangu seed gum (BSG) and xanthan gum (XG), a higher amount of isomalt resulted in higher magnitudes of adhesiveness containing BSG as a fat replacer (Emadzadeh, Razavi, & Schleining, 2013). In addition, both textural profile analysis (TPA) and back-extrusion showed that the BSG formulation produced the hardest butter (Emadzadeh, Razavi, & Schleining, 2013).

Different characteristics of shea kernel butter treated with diverse heat treatment have been reported (Honfo, Linnemann, Akissoe, Soumanou, & Boekel, 2013). The kernel was heated with smoking and boiling, respectively. The study showed that neither method influenced the moisture content of kernel, whereas, treatment by boiling resulted in freer FA (Honfo, Linnemann, Akissoe, Soumanou, & Boekel, 2013). The

boiling method produced butter with a softer texture and intense smell, characterised by high moisture content, unsaponifiable matter, tocopherol compounds, peroxide value and iodine value (Honfo, Linnemann, Akissoe, Soumanou, & Boekel, 2013). The report also showed that the lightness values were higher for smoking than heat-treated butter. A yellow colour corresponds to the natural colour of butter, where lighter butter is more attractive (Honfo, Linnemann, Akissoe, Soumanou, & Boekel, 2013).

2.6.2 Spreadability of butter

Although butter is usually spreadable at about 15℃, the methods of increasing spreadabiltiy at low temperature are still being investigated (Lane, 1998). According to the ANZFS Code (2010), moisture content in butter should be no more than 16%. The spreadability of butter can also be improved by increasing aeration. Air or nitrogen is beaten into butter to increase its volume by 50-125%, thereby increasing the spreadability of butter (McKenna, & Kilcast, 2003). During processing, butter is pumped through a mixing device such as a pin worker or similar in-line mixer, into which either air or nitrogen is injected (Lane, 1998). This method physically manipulates the crystalline structure without changing the chemical composition (Bylund, 1995; Lane, 1998). Furthermore, modifying cow's diet is another method that increases spreadability of butter. Polyunsaturated FAs initially present in grass lipids are largely hydrogenated by the action of microorganisms in cow's rumen (Chilliard, Ferlay, & Doreau, 2001). Coating globules of fat with formaldehyde-treated proteins protects the fats from hydrogenation, resulting in the increase of unsaturated FAs in milkfat which is softer (Lane, 1998). More spreadable butters have been made from milk of cows fed with protected oils (Lane, 1998).

Buchheim and Dejmek (1997) reported that a higher amount of undamaged fat

globules in butter results in better spreadability, because of reduced intermeshing of fat crystals. Softer and more spreadable butter can also be achieved by using low MP milk fat in summer, when unsaturated FAs are rich in the milk fat (Damodaran, Parkin, & Fennema, 2008; Goff, 2015). Kaylegian, and Lindsay (1992) reported that butter samples made from low melting liquid fractions or a combination of low melting liquid fractions, produced good spreadability at 4℃, whereas, the samples were almost melted at room temperature at 21℃. More spreadable butters can be made from olein fractions. When fat is cooled very slowly to below its MP, fewer nuclei are formed and can grow into large crystals up to 300 μm of high melting glycerides (Lane, 1998). The nucleuses are made up of many needle-like crystals which can be filtered out from the remaining liquid fat to yield two fractions: a stearin of higher MP than original butterfat, and an olein with MP close to the fractionation temperature (Lane, 1998). This process will continue at increasingly low temperature where olein produces more fractions (Lane, 1998).

2.6.3 Microstructure of butter

The characteristics of texture and rheology previously described are ultimately affected by the microstructure of butter; that is, the spatial arrangement of components including fat globules, the water droplets size distribution, and casein particles (Ong, Dagastine, Kentish, & Gras, 2011). The microstructure of butter is also related to the shelf life, which is the growth of microorganisms. The microorganisms grow rapidly in a coarse water dispersion and low salt environment; whereas, the growth are delayed when water droplets are smaller than 5 μm, due to the insufficient nutrients per droplets (Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). As fat spreads are in general water-in-oil (w/o) emulsions, the matrix of liquid oil and fat consists of a network of fat crystals (Van Dalen, 2002; Wu, 2009). A shell of crystals surrounds the water droplets preventing them from merging

(Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). In low water content fat spreads like butter, spheres are mainly formed, as droplets will tend to minimise the surface-volume ratio. Whereas, in high water content fat spreads, droplets are deformed and have at least a bimodal distribution, as water droplets interact with each other (Van Dalen, 2002).

The commonly used methods to determine water droplet size distribution in fat spreads are microscopy, nuclear magnetic resonance spectroscopy (NMR), laser diffraction (LD) and electric sensing (ES) (Van Dalen, 2002). Fat spreads have to be diluted when using LD, ES and conventional wide-field light microscopy, the dilution may modify the water droplet distribution (Van Dalen, 2002). Although LD and ES are relatively easy and fast to perform, considerable differences were found in inter-laboratory tests between and within the results (Van Dalen, 2002). Pulsed field gradient nuclear magnetic resonance (pfg-NMR) was used to determine water droplet size in butter (Van Duynhoven, Goudappel, Van Dalen, Van Bruggen, Blonk, & Eukelenboom, 2002; Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). The pfg-NMR was less time consuming on sample preparation, measurement and data analysis, however, it gave no visual information, and its analysis is based on a much bigger sample volume (Van Duynhoven, Goudappel, Van Dalen, Van Bruggen, Blonk, & Eukelenboom, 2002; Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). Another widely used benchtop equipment is pulsed field gradient low resolution NMR, which is suitable for undiluted samples (Sørland, 2014). It is easy, fast and relatively cheap compared to high resolution NMR. Nevertheless, NMR has not been validated to analyse fat (W/O) spreads, because NMR requires a priori assumptions about the shape of the droplets and the form of distribution, such as, whether it is unimodal or bimodal (Sørland, 2014).

For these reasons, microscopy has the advantage of giving visual information about

the droplet size distribution of fat spreads. For conventional wide-field light microscopy, the sample has to be diluted and squeezed between glass slides to achieve a thin specimen, which may deform the large droplets (Van Dalen, 2002). Undiluted fat spreads can be observed by confocal laser scanning microscope (CLSM) without the need for thin specimens (Van Dalen, 2002; Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). The sections are observed optically using a focused laser beam across the plane of focus, where the lower limit of droplet size that can be measured is about 0.5 μm (Van Dalen, 2002). The reflected light reaches the detector (usually a photomultiplier tube) through a small pin hole (Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). Laser beam is reflected by a beam splitter (dichroic mirror), which reflects light of short wavelengths and transmits light of longer wavelengths. The laser beam is then focused to a spot by the microscope objective. An illumination spot is formed in the focal plane of the objective (Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). Cryo scanning electron microscopy is also applicable to microstructure; its images have higher resolution without the need for artificial staining (Ong, Dagastine, Kentish, & Gras, 2011). However, fat globules can hardly be identified by cryo scanning electron microscopy, because of the lack of artificial staining. Due to this reason, fat globule size distribution is usually measured by CLSM (Ong, Dagastine, Kentish, & Gras, 2011).

The types of dyes used are also important when observing the microstructure of butter. Van Dalen (2002) used Nile Red, Nile Blue, and Rhodamine, to stain a fat spread sample. Among these dyes, Nile Red (0.01%) in a mixture of polyethylene glycol/glycerol/demineralised (50/45/5) water showed the greatest staining effect (Van Dalen, 2002). Nile red or 9-diethylamino-5*H*-benzo[α]phenoxazine-5-one is a fluorescent dye which has good affinity to both polar and non-polar liquids, and the method depends on the choice of excitation and emission wavelengths (Montalbo-Lomboy, Kantekin, & Wang, 2014). Several studies have shown that Nile

red is an excellent fluorescent dye for lipids (Van Dalen, 2002; Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008; Montalbo-Lomboy, Kantekin, & Wang, 2014). Its fluorescence is quenched in the presence of water, and the fluorescence in aqueous solution decreases over time until it is finally supressed, because of hydrophobic property of Nile red (Montalbo-Lomboy, Kantekin, & Wang, 2014).

For CLSM results, the particle size distributions of water droplets in different types of butter are assumed to be log-normal (Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). A volume-weighted log-normal distribution *F* as a function of particle diameter *d* is described in Equation 2. *F (d)* is characterized by the volume-weighted geometric mean diameter $\bar{D}_{3.3}$ and the geometric standard deviation $\sigma_{g.}$ The number-weighted geometric mean diameter $\bar{D}_{0,0}$ can be calculated using Equation 3.

$$F(d) = \frac{1}{d \ln \sigma_g \sqrt{2\pi}} \exp\left(-\frac{(\ln d - \ln \bar{D}_{3,3})^2}{2 \ln^2 \sigma_g}\right) \quad \text{......(2)}$$

$$\ln(\bar{D}_{0,0}) = \ln(\bar{D}_{3,3}) - 3\ln^2(\sigma_g) \quad \text{......(3)}$$

2.6.4 Sensory evaluation of butter

The sensory evaluation of butter is essential as it gives opportunity to the consumer to evaluate sensory characteristics, and also provides useful information for product developers (Lawless, & Heymann, 2010). Jinjarak, Olabi, Jimenez-Flores and Walker (2006) conducted sensory tests of whey butter, cultured butter and regular sweet butter of their composition, hardness, colour, and MP using descriptive analysis and

hedonic test. The butter was processed by a series of procedures which included preheating cream at 20ºC, inoculation with 0.07% (w/w) commercial mesophilic cultures (*L. lactis*, *L. cremoris*, and *Leuc. cremoris*), incubating samples at 20ºC to pH 4.90±0.05, following by churning (Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). The sensory panellists generated 25 sensory attributes and defined the attributes of the butter together with reference standards shown in Table 2.9. The study by Jinjarak, Olabi, Jimenez-Flores, and Walker (2006) reported that no major differences on textural characteristics among the types of butters. However, one commercial sample of whey butter had a higher level of porosity compared to the rest of the samples (Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006).

Table 2.9 Terms used in descriptive analysis of sweet cream, whey and cultured butters (Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006).

Attribute	**Definition as worded on score sheet**
Shiny	Amount of light reflected from the sample's surface
Smooth	The smoothness of the sample surface area with no bumps/bundles
Porous	Number and size of pores present on sample surface
Hardness	Force necessary to push the spoon into the butter at a 90º angle
Sticky	Extent of adherence to the spoon of sample in the cup when spoon is lifted after cutting sample
Crumbly	Extent to which sample appears dry and readily falls apart when cut with spoon, not waxy or homogeneous
Spreadability	Ease of spreading a 1-cm^3 sample on toast
Melting rate	Rate at which the butter changes from a solid to a liquid while the sample is melting in the mouth
Even melting	Uniformity of melting of sample in mouth
Mouth coating	Degree to which the mouth remains coated after expectoration
Diacetyl odour/flavour	Odour/flavour of fresh whipping cream, not rancid, butyric
Cheesy odour	Odour of yellow ripened cheese, resemblance to odour of Parmesan cheese powder
Acidic odour	Odour of sour cream, over ripened cream
Nutty odour/flavour	Odour/flavour of hazelnut
Rancid odour/flavour	Odour/flavour associated with oxidised oils/old butter
Cardboard odour	Odour of cardboard box packaging
Acidic aftertaste	Flavour of sour cream, over ripened cream
Grassy flavour	Flavour of cut grass
Sweet flavour	Flavour stimulated by milk sugar, lactose

3.0 Materials and Methods

The use of lactic starter cultures and temperature treated cream are vital steps for the production of cultured butter (Bylund, 1995; Spreer, 1998). Fermentation of cream is achieved by controlling the concentration of starter cultures used and adjusting temperature at each step of butter processing to produce cultured butter with good aroma and texture.

3.1 Experiment design

In this study, three batches of fresh cream samples (10 L per batch) were supplied in August (late winter), September (early spring), and October (mid spring) in New Zealand in 2014. Following the standardisation of the process, the samples were used to produce three batches (1, 2, and 3) of cultured butter, respectively. Cultured butter was produced using fresh cream of approximately 40% fat content (Appendix Table VI) and commercial mixed starter cultures, *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. cremoris* (1:1). The cream was pasteurised, cooled, allowed to ferment by adding starter cultures, recrystallised, ripened, churned, and worked to produce final cultured butter, which was stored for 21 days in a refrigerator at 4ºC.

Various characteristics were analysed during the storage period which comprised moisture content, solids-not-fat content, fat content, colour, hardness, springiness, and adhesiveness, titratable acidity, coliforms counts, sensory evaluation (hedonic test) and water droplet size. pH was measured in the final product and also monitored during processing. Churning efficiency was also estimated. All the analyses were conducted in triplicate, with the exception of sensory evaluation and water droplet size which were conducted once. Chemicals used in this study were of reagent grade

or higher. All materials used in the coliform test of butter samples were sterilised.

In this study, moisture content, solids-not-fat content, fat content in butter, colour, texture, pH, titratable acidity, coliform counts, sensory evaluation, water droplet size and churning efficiency were the response variables, whereas cultured butter product was the treatment.

Constraint and limitation

In this study, the unsaturation of fatty acids in the cream was not analysed due to regulations of the analytical laboratory. The chemical analytical laboratory does not allow the use of Wij's reagent because the composition of reagent includes carbon tetrachloride. Sensory evaluation was conducted once in batch 2 due to insufficient samples.

3.2 Manufacture of cultured butter

3.2.1 Cream and starter cultures

In this study, pasteurised fresh cream with fat content of 40% was supplied by Green Valley Dairies, Mangatawhiri, Auckland. The pasteurised cream was packed in high density polyethylene (HDPE) bottles and delivered under cold chain at 4°C to Massey University, Albany Campus, further heat-treated as described in Section 3.2.2 before use. The cream was inoculated with 2% freeze-dried mixed starter cultures (CHOOZIT™ MA 16 LYO 25 DCU, DANISCO) comprising *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. cremoris*.

3.2.2 Methods

In this study, the butter making was produced following the methods described by Frede, and Buchheim (1994), Bylund (1995), and Spreer (1998). The process flow chart used in this study is shown in Figure 3.1.

The equipment and various appliances used in butter making were cleaned and sanitised prior to processing. The cleaned equipment and appliances were allowed to air-dry. The fresh cream was delivered at 08:00 h in the morning. Upon receipt, the cream was immediately transferred into a stainless pot (5 L) and placed on a hot plate (13, Roband, Australia) for pasteurisation. The cream was heated to 95°C, and held at this temperature for 5 minutes to kill pathogenic bacteria and enzymes that could affect keeping quality in the product (Bylund, 1995). Meanwhile, a water bath (GD120, Global Science, England) with frozen ice packs was setup to create a cold water environment below 8°C. After pasteurisation, cream was transferred into a plastic beaker (5 L) and placed in the cold water bath, to rapidly cool the cream to 8°C. During cooling, the melted ice packs were replaced by frozen ice packs to maintain a constant temperature; whilst the cream was being swirled by a spoon to allow even cooling. Once the temperature of the cream had stabilised at 8°C, a 2 % dry mixed starter culture was weighed and added into the cream. Temperature of cream was monitored by a temperature thermocouple (FLUKE 51, Fisher Scientific, New Zealand).

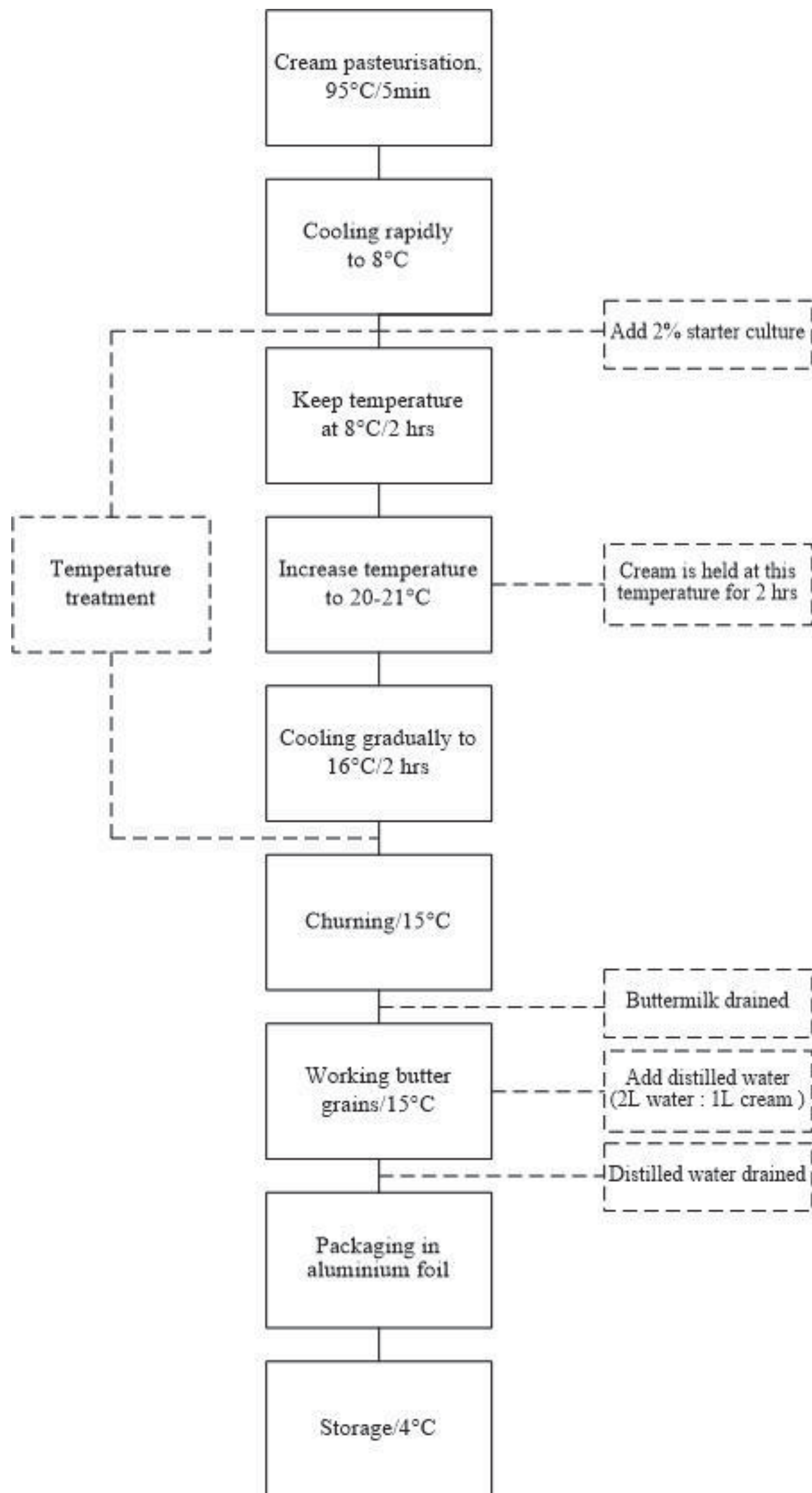


Figure 3.1 Laboratory scale cultured butter processing.

The cream was allowed to ferment at 8°C for 2 h, after which the temperature was increased to 20-21°C and kept at this temperature for 2 h. This was followed by gradual cooling of the cream to 16°C. The cream was kept at this temperature for another 2 h. The temperature of the cream was reduced slightly to churning temperature (15°C) and allowed to stabilise at this temperature before churning (Mulder, & Walstra, 1974; Funahashi, & Horiuchi, 2008). The cream was churned using a churning mixer (KitchenAid Heavy Duty, K5SS, USA) (Figure 3.2). Approximately 1 L of cream was transferred into a churning pot (4 L) for a single production of butter. To avoid spillages, churning of cream (10 L per batch) was done in 1-L lots. The churning speed was set at position number 8 for 4-5 min; churning was terminated when butter grains were separated from the buttermilk (Bylund, 1995; Goff, 2015). Buttermilk was drained and 100 mL were stored in the refrigerator at 4°C for analysis of fat content. The butter grains were washed 5 times using 2 L of distilled water (400 mL portions of water were used at a time). Butter was kneaded by hand to remove moisture content. Butter was then packed in aluminium foil (Glimours Caterers Foil, 440 mm × 90 m) (Robertson, 2012) and stored in the refrigerator at 4°C until required for various analyses.

(a)

(b)

Figure 3.2 (a) Assembled kitchen/domestic scale churning mixer (KitchenAid Heavy Duty, K5SS, USA); (b) Disassembled kitchen/domestic scale churning mixer (KitchenAid Heavy Duty, K5SS, USA).

3.2.3 Butter analysis

3.2.3.1 Water droplet size

The size of water droplets in butter is related to the growth of microorganisms, which can be limited when the droplets are smaller than 5 μm, as the amount of nutrients per droplet is also reduced (Van Dalen, 2002; Van lent, Vanlerberghe, Van Oostveldt, Thas, & Van der Meeren, 2008). The methods of Van Dalen (2002), and Van lent, Vanlerberghe, Van Oostveldt, Thas, and Van der Meeren (2008) were modified to estimate the water droplet size in cultured butter.

Preparation of samples and staining

The butter was transported from Massey University Albany campus to Manawatu Microscopy and Imaging Facility Centre (MMIC) at Massey University's Palmerston North (PN) Campus within 24 h at below 4°C. To maintain the cold-chain, the butter samples were packed with frozen ice packs. Upon delivery of the butter at the MMIC, the samples were stored in a refrigerator at 4°C for four weeks, with weekly analysis of water droplet size. Preparation of each sample involved cutting about 50 mm^3 cube of butter from the original sample using a single sided razor blade (Personna 0.009 regular blade, American Safety Razor) on a precooled (-20°C/24 h) cylindrical stainless steel ingot surface (90 mm diameter x 26 mm deep). The remaining sample was immediately returned to the refrigerator for further storage. To stain the cold butter in further section, a length of 1.8 kg strength standard fishing tippet (Airflo-Hunting and Fishing NZ) was used to shave about 500 μm thick strip from the end of the cube (Figure 3.3).

Figure 3.3 Shaving butter strip from a cube using 1.8 kg strength standard fishing tippet (Airflo-Hunting and Fishing NZ).

The butter strip was recovered and transferred into a microscope concave slide (Citoglas, Interlab Ltd). The butter samples were immersed in equal quantities of Nile Red (Invitrogen; 0.02% in methanol) (Figure 3.4) and 0.05% Acridine Orange (Riedel de Haen-Sigma Aldrich) (Figure 3.5) dissolved in double distilled water. The stain and butter preparation were covered with a No. 1 coverslip (Figure 3.6) and left to incubate at 4°C for about 45 min prior to viewing under a confocal laser scanning microscope (CLSM).

Figure 3.4 Staining sample with Nile Red.

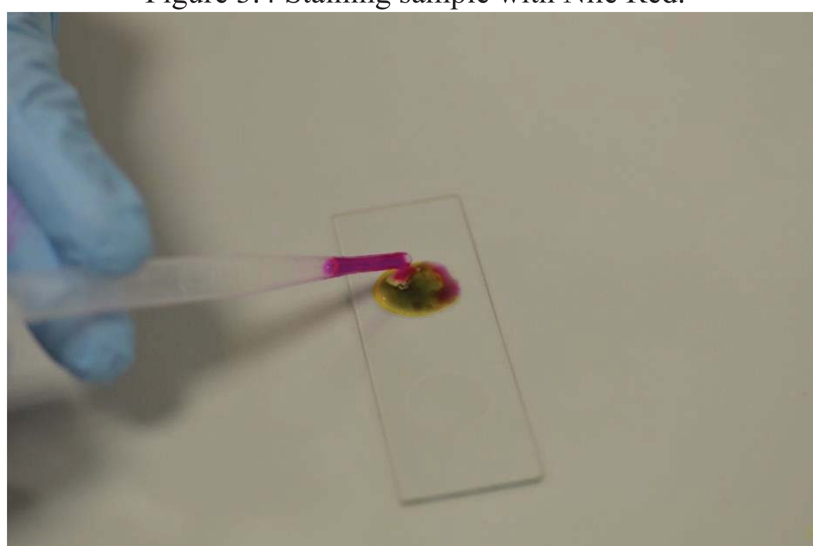


Figure 3.5 Staining sample with Acridine Orange.

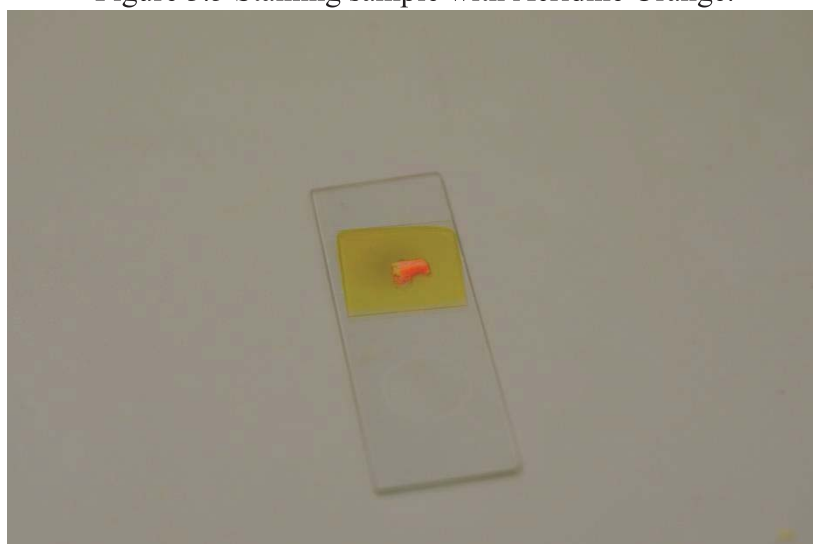


Figure 3.6 Coverslip on the top of sample with Nile Red (Invitrogen; 0.02% in methanol) and Acridine Orange (Riedel de Haen-Sigma Aldrich).

Confocal laser scanning microscopy

Imaging of sample was carried out using the Leica DM6000B SP5 confocal laser scanning microscope system running LAS AF software (version 2.7.3.9723; Leica Microsystems CMS GmbH). To prevent sample distortion due to temperature changes during the acquisition process, samples were maintained at 4°C using a Linkam Thermoplate PE120 cooling stage (Linkam Scientific Equipment, Bio-Strategy). Images were acquired with a HCX PL FLUOTAR 40x (N.A. 0.75) lens with an optical zoom of 3x. Each probe was imaged sequentially using the following settings: Acridine Orange excitation at 488 nm with emission collection at 500-538 nm and Nile Red excitation at 561 nm with emission collection at 571-676 nm. Brightfield data were collected simultaneously using 561 nm light for visualisation and a transmitted light detector. To minimize potential artefacts due to sample cutting, all images were acquired at a depth of 10 µm beneath the sample surface.

Image analysis

Image analysis of CLSM micrographs was performed using ImageJ software (Version 1.47, Java, National Institutes of Health, USA).

3.2.3.2 Texture analysis

Hardness, springiness, and adhesiveness are important factors for analysis of texture of butter (Bourne, 2002). Texture was measured by TA.XT2 Texture Analyser (Model 11390, Stable Micro Systems, UK), equipped with a 5-kg load cell of Texture Profile Analysis (TPA) and a 40º conical probe, at a testing speed of 1 mm/s to a depth of 12 mm from the sample surface, and then withdrawn at the same speed (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003). The texture analyser was first equipped with a

5-kg load cell, calibrated for force and height, and then attached with a 40º conical probe. Measurement parameters of the TA.XT2 Texture Analyser were set as shown in Table 3.1. Butter sample (150 g) with flat surface was retrieved from the refrigerator and placed on the bench of texture analyser. The equipment was operated following the manufacturer's instructions and data were automatically uploaded into the interfaced computer (AUD3470JWG, Dell Incorporated, USA). Figure 3.7 shows a typical force-by-time curve of TPA graph. Values for the attributes were determined or calculated from the graphs generated by the TA.XT2 Texture Analyser. From Figure 3.7, hardness is the distance to positive peak in the first load; springiness is the distance of Length2 (time)/distance of Length1 (time); adhesiveness is the negative area (Area 3) in the first load (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003).

Table 3.1 Set parameters of the Texture Profile Analysis for measuring texture of butter using the TA.XT2 Texture Analyser (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003).

Caption	Value	Units
Pre-Test Speed	1.00	mm/sec
Test Speed	1.00	mm/sec
Post-Test Speed	5.00	mm/sec
Target Mode	Distance	
Distance	12.0	mm
Time	5.0	sec
Trigger Type	Auto (Force)	
Trigger Force	1.0	g
Break Mode	Off	
Tare Mode	Auto	
Advanced Options	On	
Control Oven	Disabled	
Frame Deflection Correction	Off (XT2 compatibility)	

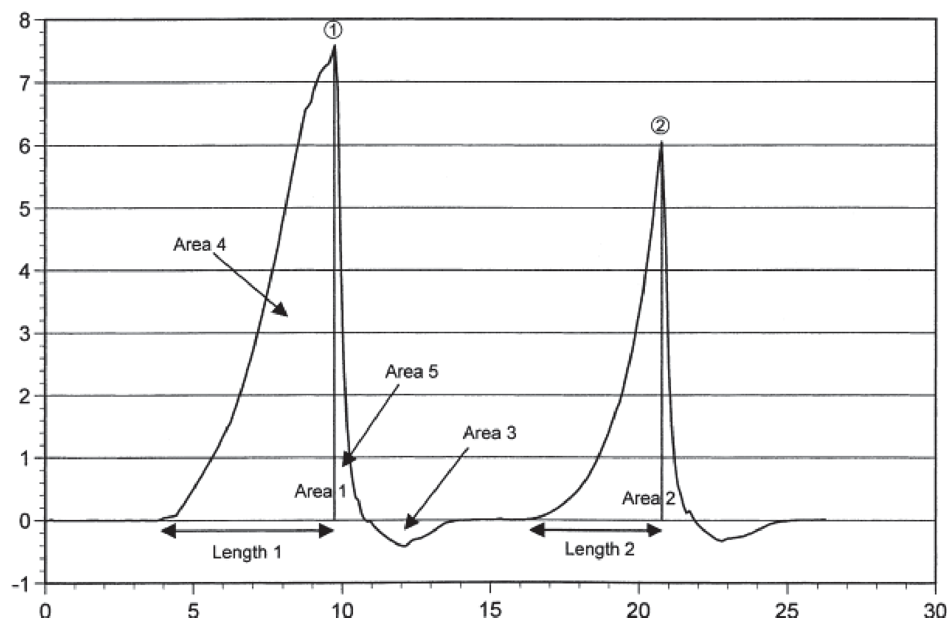


Figure 3.7 TPA force-by-time loading map (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003).

3.2.3.3 Moisture content in butter

The moisture content in butter should be no more than 16% (ANZFS Code, 2010) to reduce microbiological spoilage (Law, 1997). In this study, moisture content in butter was measured according to the method of Evers, Crawford, & Kissling (2003). Cultured butter sample was warmed in the original unopened package to between 24ºC and 28ºC, to facilitate thorough mixing without any rupture of the emulsion. An empty beaker (200 mL) was placed in a drying oven at 102ºC for 1 h, and then cooled to ambient temperature (20°C) in a desiccator. Ten (10) g butter sample were transferred into the beaker and weighed on an analytical balance (New Classic MF, ML 204, Switzerland). The beaker was transferred onto a hot plate (HP130915Q, Thermo Scientific, USA) for heating. The melted butter was continuously agitated and swirled during heating. The heating and agitation was done gently to avoid losses caused by spattering and frothing. To achieve this, the heating was done intermittently while observing frothing. The melted butter was heated to light brown or yellow brown. The beaker was removed from the heater, allowed to cool and weighed.

Moisture content in butter was calculated using equation 4 (Evers, Crawford, & Kissling, 2003):

$w_m = [(m_1 – m_2)/(m_1 – m_0) * 100]$ %..(4)

Where, w_m is the moisture content (g 100 g^{-1} product), m_0 is the mass (g) of the empty beaker, m_1 is the mass (g) of the test portion and beaker before heating and, m_2 is the mass (g) of the test portion and beaker after heating.

3.2.3.4 Solids-not-fat (SNF) content in butter

Solids-not-fat content is defined as the components in milk other than butterfat and water (Walstra, & Jenness, 1984) and was analysed using the method of Evers, Crawford, & Kissling (2003). The residue from moisture test was melted in 60 mL petroleum ether (40-60ºC AR, Scharlau, ACS, USP), and the contents were mixed carefully by rotating the beaker. The beaker was placed on a sloping stand and the SNF was allowed to settle, then the solvent was decanted as waste. The SNF in the beaker was heated gently to 70-80ºC for about 3 min on a hot plate until all solvent had been volatilised and the residue (SNF) was thoroughly dried. A glass rod was rinsed with 20 mL of solvent into the beaker. The content was mixed carefully by rotating the beaker. The SNF was allowed to settle and the solvent was decanted to waste as previously described. The beaker and content were again heated gently to 70–80ºC on a hot plate until all the SNF was thoroughly dried. The beaker was cooled in a desiccator and weighed to the nearest 1 mg. The SNF content was calculated using equation 5 (Evers, Crawford, & Kissling, 2003):

$w_s = [(m_1 – m_0)/(m_2 – m_0) * 100]$ %..(5)

Where, w_s is the SNF content (g 100 g^{-1} product), m_0 is the mass (g) of empty beaker, m_1 is the mass (g) of beaker with dried SNF and, m_2 is the mass (g) of test portion

plus beaker.

3.2.3.5 Fat content in butter

Fat content in butter was calculated using equation 6, based on equations (4) and (5) (Evers, Crawford, & Kissling, 2003):

$w_f = 100\% - (w_m + w_s)$……………………………………………………………(6)

Where, w_f is fat content (g 100 g^{-1} product), w_m, and w_s are moisture content (g 100 g^{-1} product) and SNF content (g 100 g^{-1} product) from equations 4 and 5, respectively.

3.2.3.6 Titratable acidity (lactic acid %)

Eighteen (18) g butter sample were mixed with 90 mL hot boiled water, and titrated against 0.02 M standardised sodium hydroxide solution using 1% phenolphthalein as indicator. Titratable acidity was calculated using equation 7 (Kirk, & Sawyer, 1991; Belitz, Grosch, & Schieberle, 2000):

Titratable acidity (lactic acid %) = [0.02M NaOH requied (mL)/100] %..................(7)

3.2.3.7 Churning efficiency

Churning efficiency estimates the amount of fat in the cream converted to butter (Bylund, 1995). A good churning efficiency should be more than 99.3% (Bylund, 1995; Goff, 2015). It is calculated in terms of fat content in buttermilk as a percentage of the fat content in the cream (Bylund, 1995; Goff, 2015). In this study, the fat content in buttermilk and the cream was measured using the Mojonnier test (AOAC 989.05, 2006) as described in subsequent sections.

Sample preparation

Ten (10) g buttermilk or cream were accurately weighed into a dry Mojonnier tube. Two (2) mL of ammonium hydroxide were added, and mixed well in the lower bulb. The tube was then placed in a 60°C water bath (TC 120, Grant Instruments, Cambridge Ltd.) for 10 min, and then cooled, followed by adding 2-4 drops phenolphthalein. Ten (10) mL of ethanol were added and mixed by allowing the liquid to flow backwards and forwards between the two bulbs. Then 25 mL diethyl ether (Scharlau, AR, ACS, USP) were added to the tube and sealed by a stopper. The tube was shaken gently for about 1 min.

Fat extraction procedure

The stopper on the tube was removed, and 25 mL petroleum ether (40-60ºC boiling point, AR, Scharlau ACS, USP) were added. The first few millilitres of the solvent were allowed to run down the side of the neck of the tube and stopper to rinse any adhering fat particles into the tube. The tube was sealed by the stopper and shaken gently for 30 s and centrifuged (Super Vario N, Funke Gerber, Germany) for 2 min at 44 gravitational force. The stopper was removed again, and the organic solvent layer was decanted carefully into a pre-weighed aluminium dish under the fume hood, and the dish was placed on a hotplate (50°C) for heating. Another 5 mL ethanol were

transferred into the Mojonnier tube, and the extraction was repeated using 25 mL of diethyl ether and 20 mL of petroleum ether. The organic layer was decanted into the same aluminium dish, and placed on the same hotplate for heating until the liquid stopped foaming. The amount of fat content in buttermilk was calculated using equation 8 (AOAC 989.05):

Fat content in buttermilk = $[(w_2 - w_1)/w_3 * 100]$ %..(8)

Where, W_1 is the weight of empty flask (g); W_2 is the weight of flask and fat (g); W_3 is the weight of sample taken (g).

3.2.3.8 Colour

Carotenoid, which is sensitive to light, oxygen and heat, gives butter a yellowy colour (Bylund, 1995; Iglesias, Mayer, Chavez, & Calle, 1997; MacDougall, 2002). In this study, the colour of solid butter was measured by colourimetry (MINOLTA, CR-300, 82981046, Japan). A solid butter, with the flat surface of no less than 10 cm^2, was used in this experiment. The colorimeter was first calibrated following manufacturer's instructions, then the probe was placed on the flat surface of butter, and the colour of butter was measured directly. The results were recorded immediately after measurement. The colour of butter was measured in triplicate on different flat surface areas of each sample.

3.2.3.9 Microbiological analysis

Coliforms are found in the intestine of warm-blooded animals, aquatic environments, soil and vegetation (Pantoja, Reinemann, & Ruegg, 2011). They can easily contaminate dairy products including butter, through milk handling and other processing (Pantoja, Reinemann, & Ruegg, 2011; Sengul, Erkaya, BaAlar, & Ertugay, 2011). The determination of coliforms has been used as an indicator of hygiene and sanitation during food processing as well as possible contamination of products by enteric pathogens. Violet Red Bile Agar was used to determine the presence of coliforms in butter (Adams, and Moss, 2008).

Sample preparation

Fifty (50) g solid cultured butter sample was melted at 37°C in a stomacher bag (LABPLAS, Quebec, Canada), and 50 g peptone water (Merck Darmstadt, Germany) were weighed in the same stomacher bag at room temperature. The stomacher bag was mixed by hand shaking for 30 s to homogenise the sample of butter. Then the stomacher bag was allow to settle for 5 min to allow peptone water and melted butter to form separate layers. Violet Red Bile Agar (OXOID, UK) was prepared following manufacturer's instructions.

Coliform test

One (1) mL sample from the stomacher bag was aseptically withdrawn and added to 9 mL sterile peptone water bottle and mixed to give a 10^{-1} dilution. Subsequent suitable dilutions were made from 10^{-1} dilution. For plating, 1 mL of each dilution was plated in liquid agar using the pour plate method. The solidified agar plates were incubated (Clayson Laboratory Apparatus Pty Ltd, IM 1000, Australia) at 35°C for 48 h.

3.2.3.10 Sensory evaluation

Sensory evaluation of food is defined as a scientific method used to evoke, measure, analyse, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Lawless, & Heymann, 2010). It gives opportunity for consumers to evaluate sensory characteristics of food, and also provides useful information on the attributes of the product for the producer (Lawless, & Heymann, 2010; Stone, Bleibaum, & Thomas, 2012). The method and materials used in this study were based on Jinjarak, Olabi, Jimenez-Flores, and Walker (2006) who reported on the sensory evaluation of butter.

Consumer panellists (30) at Massey University's Albany Campus were randomly recruited by email and word-of-mouth to evaluate cultured butter during storage at 2, 8, 15 and 22 days at 4°C. The panellists comprised academic staff, general staff, under- and post-graduate students, and PhD students. Stainless knives and paper plates were pre-cooled in the refrigerator (4°C) for 30 min prior to sensory evaluation to ensure that the butter sample is not distorted during cutting. Butter samples were cut into 2 cm^2 cubes onto paper plates, and then placed in the refrigerator ready for sensory evaluation. Each panellist was served with a cube of cultured butter, a piece of original cracker (Arnott, New Zealand), a cooled stainless knife or plastic knife (section 4.8), sensory form and a tumbler of water (20°C). Panellists were required to rinse their palate with water first, tasted the sample, and then indicated their degree of liking of the sample by completing a sensory form shown in Appendix 2.0.

3.2.3.11 Data analysis

All the figures of the results were generated using Microsoft Excel 2010 (Microsoft Office, USA), except for the scatter plot and linear regression which were constructed

by SPSS Version 21 (IBM, USA), and interaction plot was done by Minitab Version 16 (Minitab, USA). One-way Analysis of Variance (ANOVA) was used to determine differences of characteristics (moisture content, solids-not-fat content, fat content, colour, hardness, springiness, adhesiveness, pH, titratable acidity, and sensory evaluation) of cultured butter within batches, and storage days at P-value of 0.05.

4.0 Results and Discussion

The data obtained from the three batches were analysed separately, as the compositional quality of the cream was significantly different (Auldist, Walsh, & Thomson, 1998; Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009). According to Auldist, Walsh, and Thomson (1998), components of bovine milk produced in New Zealand are affected by seasonal variations. In their report, there were large monthly variations in fat content, an important component to this study. Heck, van Valenberg, Dijkstra, and van Hooijdonk (2009) also reported large seasonal variations in the concentrations of fat and protein of bovine raw milk produced in The Netherlands.

4.1 pH of cultured butter during processing

The pH of cultured butter during processing is shown in Figure 4.1. Samples of fresh cream were delivered under cold chain (<5°C) and immediately pasteurised as described in section 3.2.2. The average pH of the three batches of the cream used in the experiments ranged from pH 6.55 to 6.58. The pH of fresh cream was within the expected range (Walstra, & Jenness, 1984; Goff, 2015). After pasteurisation, the temperature of cream was rapidly decreased to 8°C and a freeze-dried lactic starter culture was added, followed by immediate measurement of pH. It was observed that the average pH of the cream samples had decreased by less than 0.05 in the three batches when the temperature was cooled from 95°C to 8°C. The cream was held at 8°C for 2 h (to initiate the formation of low MP fat crystals); then the temperature was increased to 21°C (to melt high MP fat crystals). The cream was held for 2 h at the higher temperature (21°C) to recrystallise and form pure crystals, at which point pH was measured again. It was observed that the pH of the three batches had slightly decreased from average 6.53 to 6.49 (Figure 4.1). A further decrease of pH (6.49 to

6.44) of the cream of the three batches was observed after ripening, before churning at 15°C. After churning, pH was measured in the collected buttermilk and the final butter product. There was no marked change in pH between buttermilk and butter.

The pH decreased after adding lactic starter cultures to the cream due to the metabolism of lactose (main carbohydrate) in the cream to produce a range of organic acids, of which the main one is lactic acid (Cogan, 1983; IDF227, 1988; Mallia, Escher, & Cerny, 2008). Also, the lactose in the cream is metabolised by the lactic starter cultures to produce CO_2, which contributes to pH reduction during cream ripening and churning (Cogan, 1983; IDF227, 1988). The average pH of the final cultured butter of about 6.30 was slightly higher than values reported by the previous studies (Hurtaud, Faucon, Couvreur, & Peyraud, 2010; Goff, 2015). The discrepancy between our results and previous studies may be attributed to the point when the pH was measured. In this study, pH was measured after washing butter grains, while in the previous studies, pH was measured before washing (Hurtaud, Faucon, Couvreur, & Peyraud, 2010; Goff, 2015). Before washing, the buttermilk in butter grains contributes towards lower pH due to lactic acid, which have significant impact on the acidity of butter (Walstra, & Jenness, 1984). The pH of unwashed or salted fermented butter can range from 5.2-5.5 (Hurtaud, Faucon, Couvreur, & Peyraud, 2010; Goff, 2015). However, in this study, buttermilk was washed-off from the butter grains to prolong shelf life (Bylund, 1995). Cultured butter is sensitive to oxidation defects, and can give the product a metallic taste if buttermilk is retained in the butter (Gonzalez, Duncan, O'Keefe, Sumner, & Herbein, 2003). The metallic taste is accentuated by presence of traces of copper or other heavy metals, and this may considerably affect the chemical keeping quality of the butter (Gonzalez, Duncan, O'Keefe, Sumner, & Herbein, 2003). Further, the presence of buttermilk in the final cultured butter product can promote the microbial spoilage of the product, as the moisture content would be increased by buttermilk (Bylund, 1995; Spreer, 1998). Thus, it is important to remove buttermilk or serum from the final product for public safety and improved shelf life.

The higher pH of cultured butter observed in this study may also be explained by the compositions of the starter cultures. The starter culture used in this study comprised *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. cremoris.* The main function of the leuconostoc is to produce aroma compounds, since it is known to be a poor acid producer. In the mixed starter culture used in this experiment, acid production was therefore mainly attributed to the lactococci (Cogan, 1983; Law, 1997; Hache, Cachon, Wache, Belguendouz, Riondet, Deraedt, & Divies, 1999).

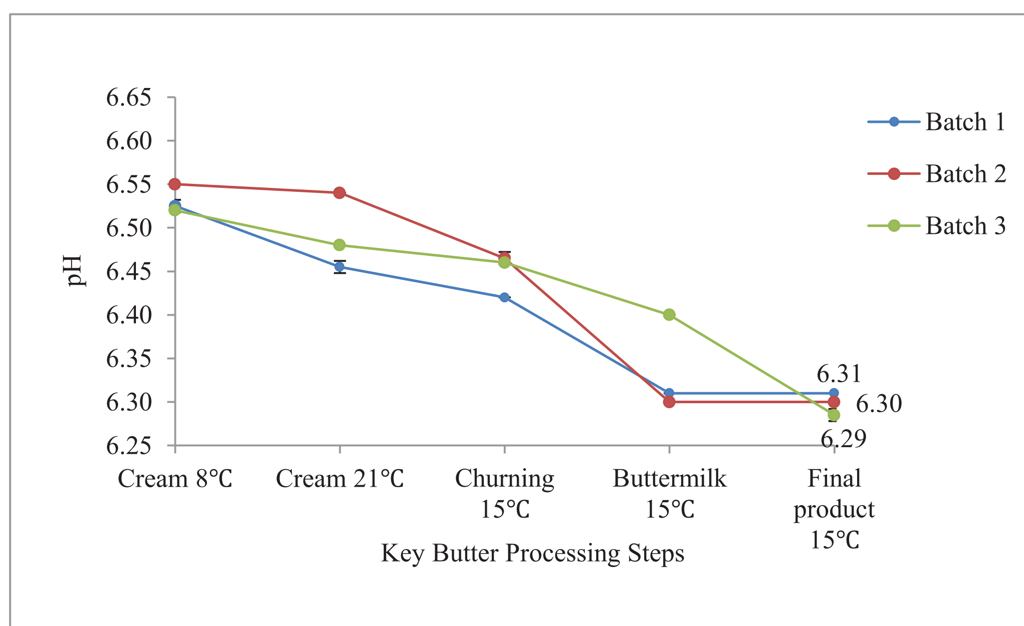
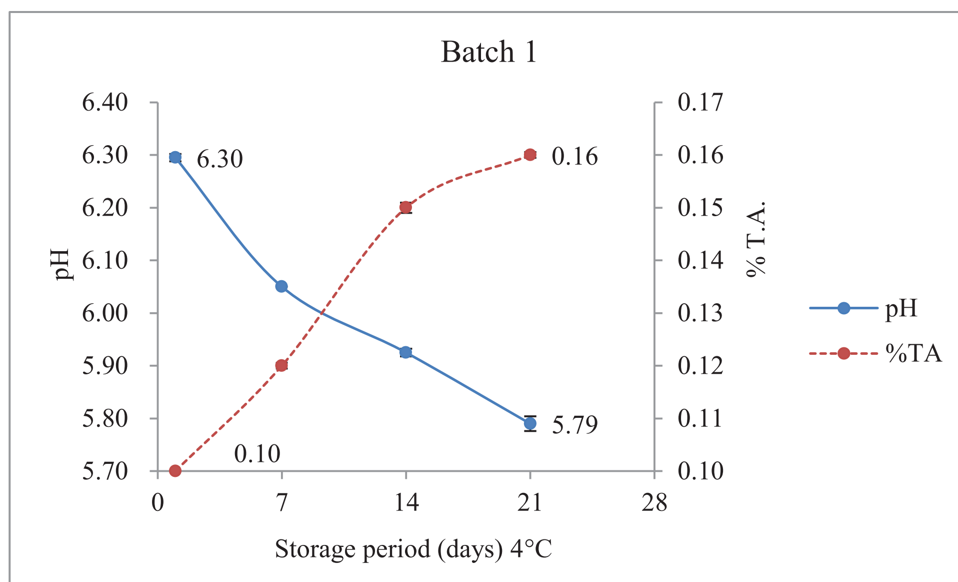


Figure 4.1 Mean pH of the cream during temperature treatment. n=2; error bars = ±SD.

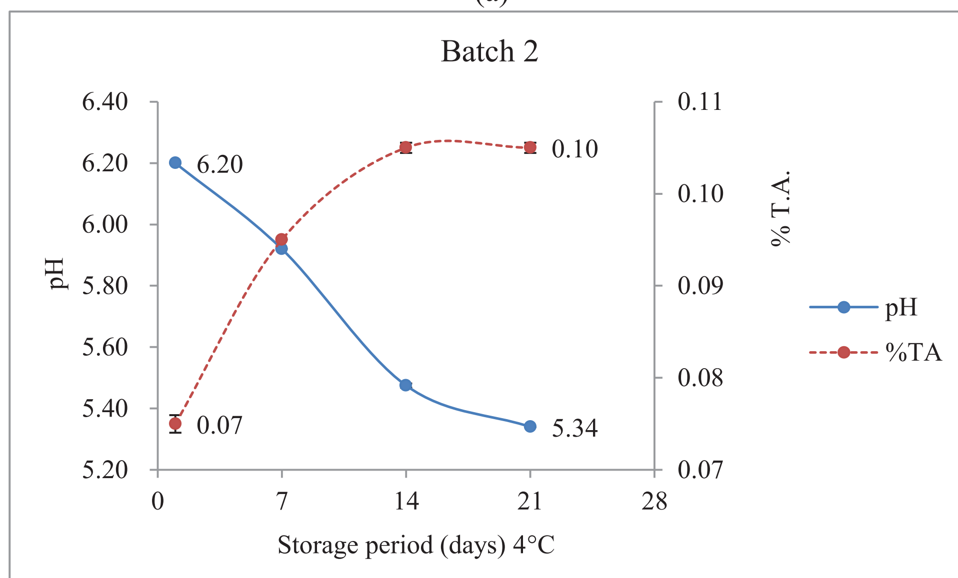
4.2 pH and titratable acidity of cultured butter during storage at 4°C

Figure 4.2 and Figure 4.3 show the pH and titratable acidity (% T.A.) of cultured butter during storage at 4ºC. As expected, the pH of butter decreased with concomitant increase in T.A. in the three batches during processing. In batch 1, the pH decreased from 6.30±0.01 to 5.79±0.01, while T.A. increased from 0.10%±0.00 to 0.16%±0.00. For batch 2, the pH decreased from 6.20±0.00, to 5.34±0.00, and T.A. slightly increased from 0.07%±0.00 to 0.10%±0.00. In batch 3, pH decreased from

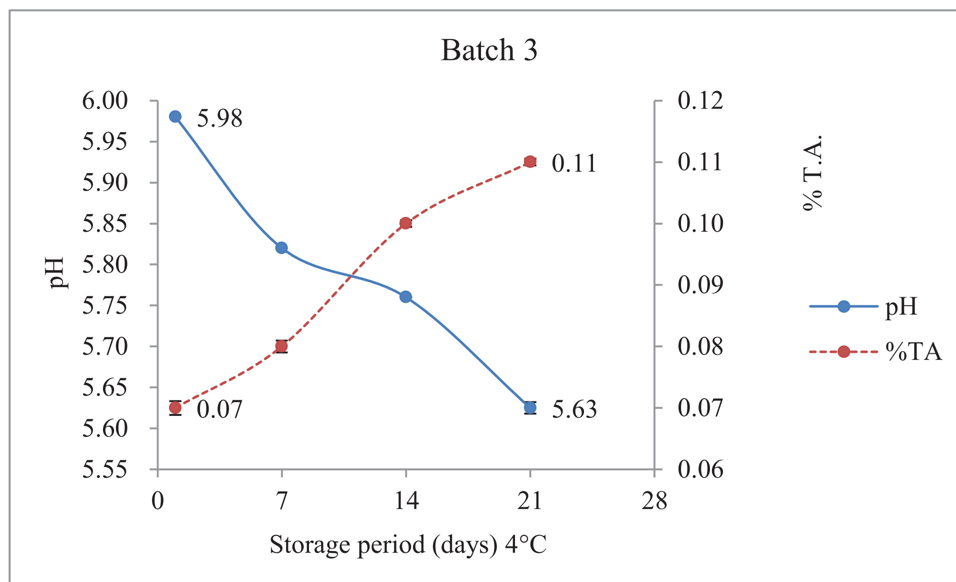
5.98±0.00 to 5.63 ± 0.01 while T.A. increased from 0.07%±0.00 to 0.11%±0.00.



(a)



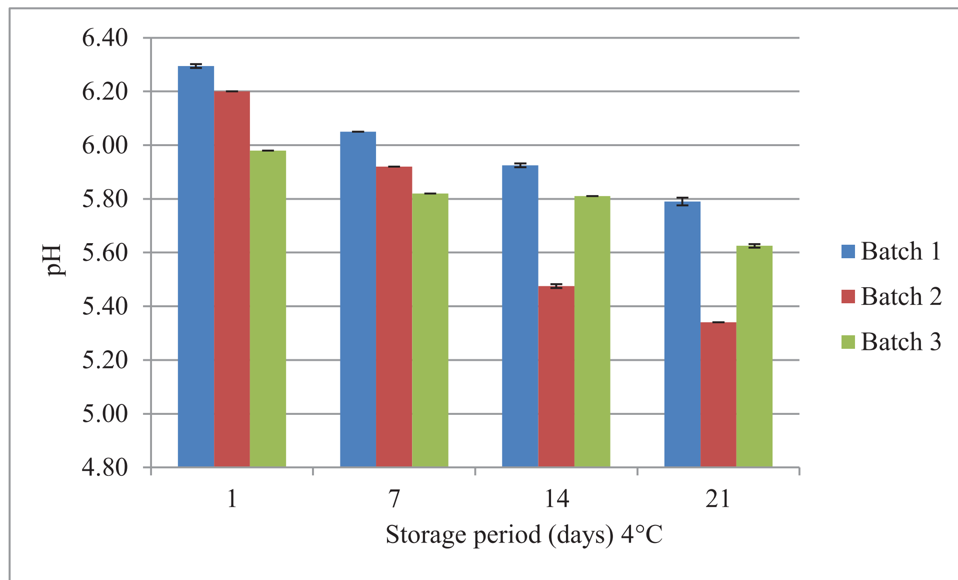
(b)



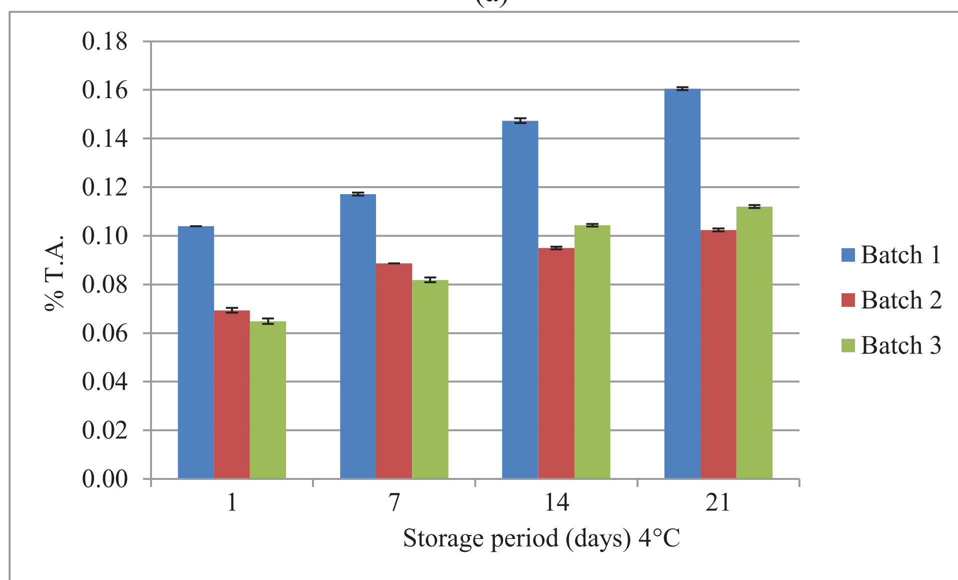
(c)

Figure 4.2 (a), (b), (c) Means of pH (n=2) and titratable acidity (% T.A.) (n=3) of cultured butter during storage at 4ºC; error bars = ±SD.

In Figure 4.3, butter produced in batch 1 had the highest pH during storage for 21 days (4°C) compared with the other two batches. Batch 2 had the second highest pH in the first 7 days of storage, but the pH was lower than batch 3 in the last 14 days. Overall, batch 1 had the highest T.A. during storage. Both pH and T.A. were significantly different ($p<0.05$) in the three batches. The results discussed here are in agreement with Mulder and Walstra (1974) who reported that acidity in milk increases by 1 mmol/L when pH decreases by 0.01. Mulder and Walstra (1974) also reported a direct relationship between T.A. and moisture content. The pH range (5.3-5.8) of cultured butter during storage in this study was slightly higher than values (4.7-5.2) reported in other studies (Mulder, & Walstra, 1974; Hurtaud, Faucon, Couvreur, & Peyraud, 2010). The discrepancy in the pH values obtained in our study and other reports may be attributed to the use of the leuconostoc (weak acid producer) in the mixed starter culture (Cogan, 1983; Law, 1997; Hache, Cachon, Wache, Belguendouz, Riondet, Deraedt, & Divies, 1999). In the previous reports cited here, the researchers used mixed lactic starter cultures which included a leuconostoc.



(a)



(b)

Figure 4.3 (a) (b) Comparison of the means of pH (n=2) and titratable acidity (% T.A.) (n=3) of cultured butter during storage at 4ºC; error bars = ±SD.

4.3 Fat content of buttermilk

Fat content of buttermilk was measured to determine the amount of fat in the cream converted to butter (Bylund, 1995). Buttermilk was drained after churning, and the amount of fat content was determined to estimate churning efficiency, which is shown in Figure 4.4. According to Walstra, and Jenness (1984), the fat content in buttermilk

ranges from 0.3 to 0.7%. The fat content of buttermilk obtained in this study ranged from 2.47 to 4.41% which was higher than the data (1.8%) reported by Dat, Manh, Hamanaka, Hung, Tanaka, and Uchino (2014). The higher fat content of buttermilk recorded in our study may be attributed to the inefficiency of churning equipment. In this study, churning was achieved by using a churning mixer (KitchenAid Heavy Duty, K5SS, USA). Another possible reason for the higher fat content in buttermilk maybe attributed to the loss of liquid fat from milk fat globules. This suggests inadequate crystallisation of butter fat during temperature treatment (Walstra, & Jenness, 1984; Walstra, 2003). During processing, when the temperature of cream is increased from 8ºC to churning temperature (15ºC), some low MP fats convert from solid fat to liquid fat (Mulder, & Walstra, 1974). The fats inside the fat globule are protected by a thin layer fat globule membrane. The membrane is broken during churning and liquid fat oozes out, and flows into buttermilk; it therefore increases the fat content in buttermilk (Walstra, 2003; Staniewski, Smoczyński, Staniewska, Baranowska, Kiełczewska, & Zulewska, 2015).

Churning efficiency is expressed as the ratio of fat remained in buttermilk to total fat in the cream. A good churning efficiency should be more than 99.3% (Bylund, 1995; Goff, 2015). In this study, however, the fat content of buttermilk was higher than expected, which resulted in lower churning efficiency (Figure 4.5). The churning efficiency of batch 1 was 88.91%, which indicates that 88.91% of the cream fat was transferred into butter. For batch 2 and batch 3, 93.82% and 89.68% of cream fat were transferred into butter, respectively.

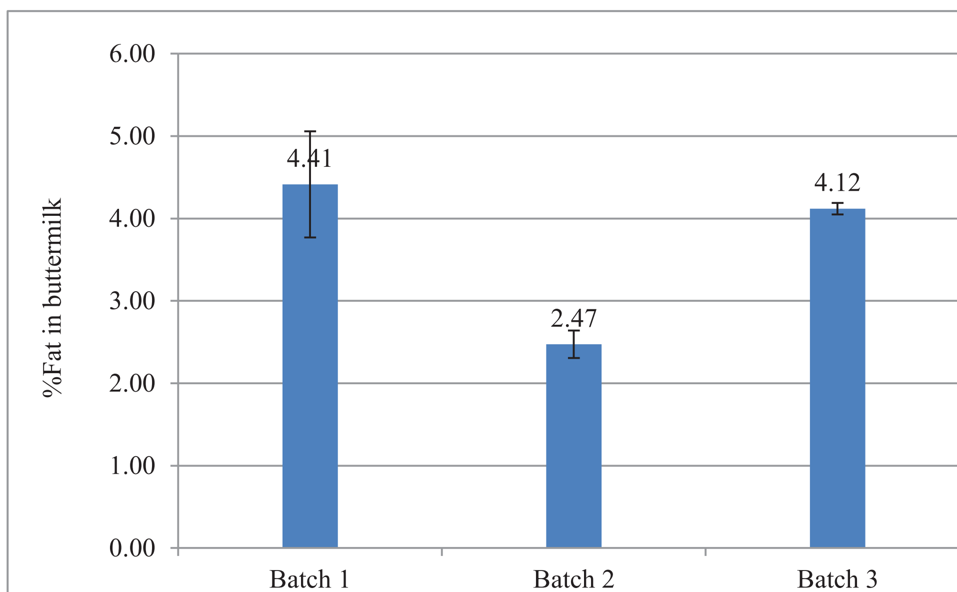


Figure 4.4 Mean fat content in buttermilk. n=3; error bars = ±SD.

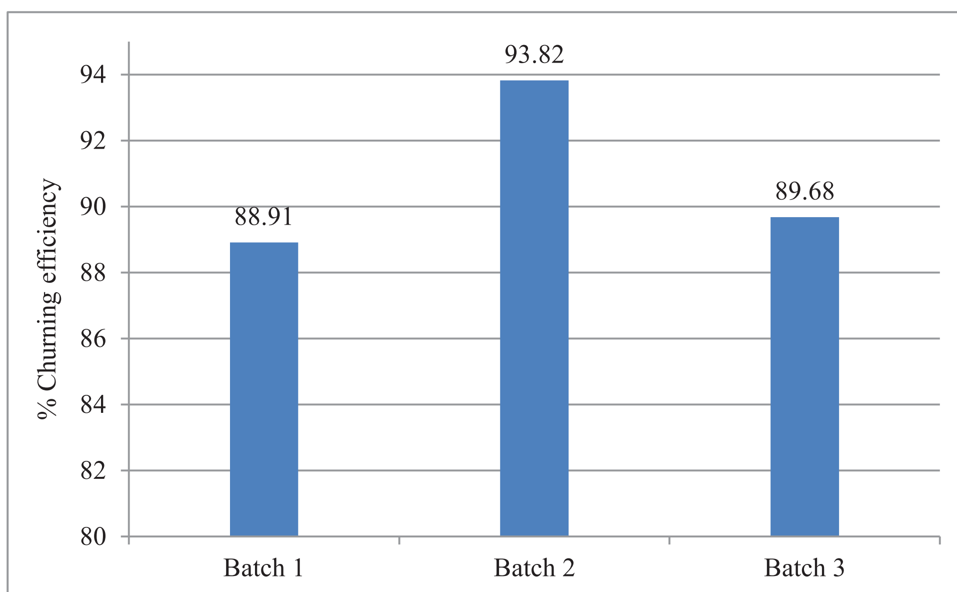


Figure 4.5 Calculated churning efficiency of cultured butter products. n=3.

4.4 Moisture, SNF and fat content of cultured butter during storage at 4°C

Figure 4.6, Figure 4.7 and Figure 4.8 show moisture content, SNF content, and fat content of butter samples during storage for 21 days at 4ºC. Moisture content (Figure 4.6) was stable in the three batches of cultured butter during storage. However, there were significant differences ($P<0.05$) in moisture content among the three batches of the butter during storage. On days 1, 7, and 14, there were significant differences ($P<0.05$) between batch 1 and batch 2, batch 2 and batch 3; while batch 1 and batch 3 were not significantly different ($P>0.05$). On day 21, there were significant differences ($P<0.05$) among the three batches of products.

The average moisture content during storage of batch 1 was 19.19%; for batch 2 it was 13.90%, while batch 3 contained 18.69% (Figure 4.6). The moisture content in batches 1 and 3 were slightly higher than the expected lever of no more than 16% (ANZFS Code, 2010). However, moisture level in butter can be raised to about 25-30% to increase the spreadability (Lane, 1998). The butter of batch 2 had the lowest moisture content, while batch 1 had the highest (Figure 4.6). As previously mentioned in section 4.2, batch 1 also had the highest T.A. among three batches, which was in agreement with Mulder and Walstra (1974), who reported a direct relationship between T.A. and moisture content. It was also observed that the butter in batch 2 was the hardest among three batches (Figure 4.11), and batch 2 had the lowest moisture content (Figure 4.6). Moisture content influences the hardness of butter; higher moisture content in butter results in a softer product (Mulder, & Walstra, 1974).

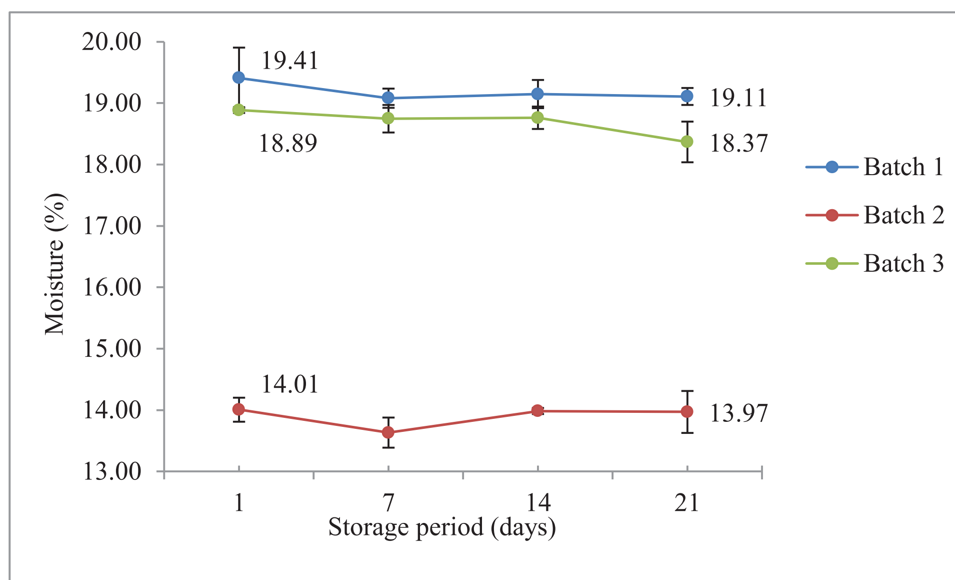


Figure 4.6 Mean moisture content of cultured butter during storage at 4ºC. n=3; error bars = ±SD.

With respect to SNF content of butter samples (Figure 4.7), there were no significant differences (P>0.05) among the three batches on day 1, whereas, significant differences (P<0.05) between batch 1 and batch 2, batch 2 and batch 3 were observed on day 7. Significant differences (P<0.05) in the SNF content were also observed between batch 1 and batch 2, batch 1 and batch 3 on days 14 and 21. With the exception of batch 2 which contained 8.88% SNF on day 7 (Figure 4.7), the rest of the samples contained between 5.19-6.90%. The SNF content reached 8.88%, which is much higher than the recommended level of no more than 2% (ANZFS Code, 2010). A simple kitchen equipment was used to manually produce butter in this study, and this could have resulted in a non-homogeneous product. Further, samples of butter used in the analysis of SNF were obtained from various parts of the block of butter which was uneven due to manual production. The SNF in batch 1 was the lowest among three batches, and batch 2 and batch 3 were higher than batch 1. The content of solids-not-fat of bovine milk is low in winter, which means the content of solid fats is high in winter, as total solids comprise of solids-not-fat and solid fats (Damodaran, Parkin, & Fennema, 2008). The SNF obtained in this study was in agreement with the

report by Auldist, Walsh, and Thomson (1998), who reported 53.2 g/kg fat in late winter, 42.2 g/kg fat in early spring, and 45.3 g/kg fat in mid spring of New Zealand bovine milk.

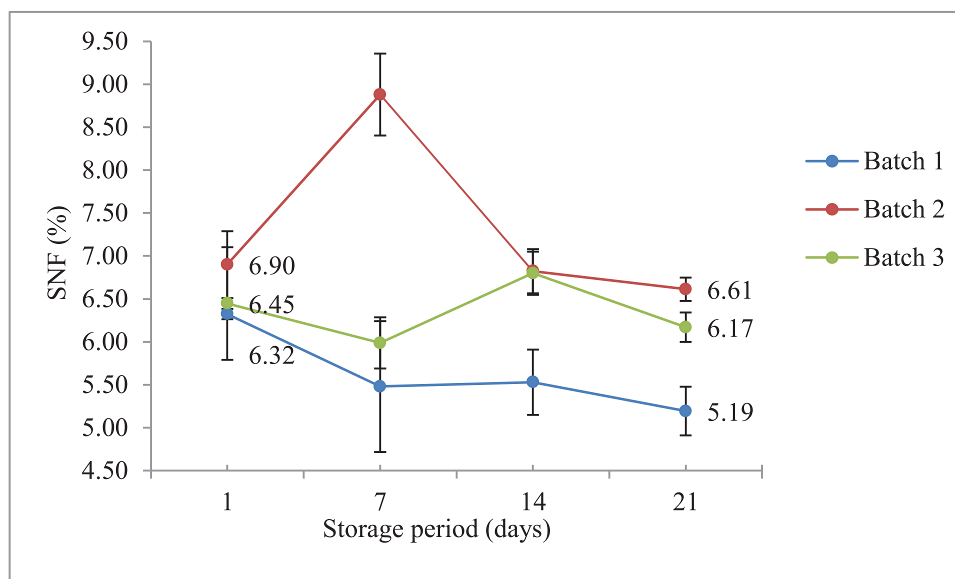


Figure 4.7 Mean SNF content of butter during storage at 4ºC. n=3; error bars = ±SD.

For fat content in cultured butter (Figure 4.8), there were significant differences (P<0.05) between batch 1 and batch 2, batch 2 and batch 3 during storage for 21 days (4°C). No significant differences (P>0.05) were obtained between batch 1 and batch 3 during storage for 21 days. As expected, the fat content in the three batches was slightly lower than the recommended level (80%) (ANZFS Code, 2010), since the moisture and SNF were high. According to equation 6 in section 3.2.3.5, %fat = 100 – (%moisture + %SNF), thus, fat content is expected to be lower if moisture and SNF are higher. Fat content increased from batch 1 (late winter) to batch 2 (early spring), and decreased from batch 2 to batch 3 (mid spring). This tendency is in accordance with Auldist, Walsh, and Thomson (1998), who studied seasonal variations of milk compositions in New Zealand. The latter study reported that fat yield of bovine milk increased from about 0.47 kg/day in late winter to about 0.77 kg/day in early spring, and then decreased to about 0.64 kg/day in mid spring (Auldist, Walsh, & Thomson,

1998).

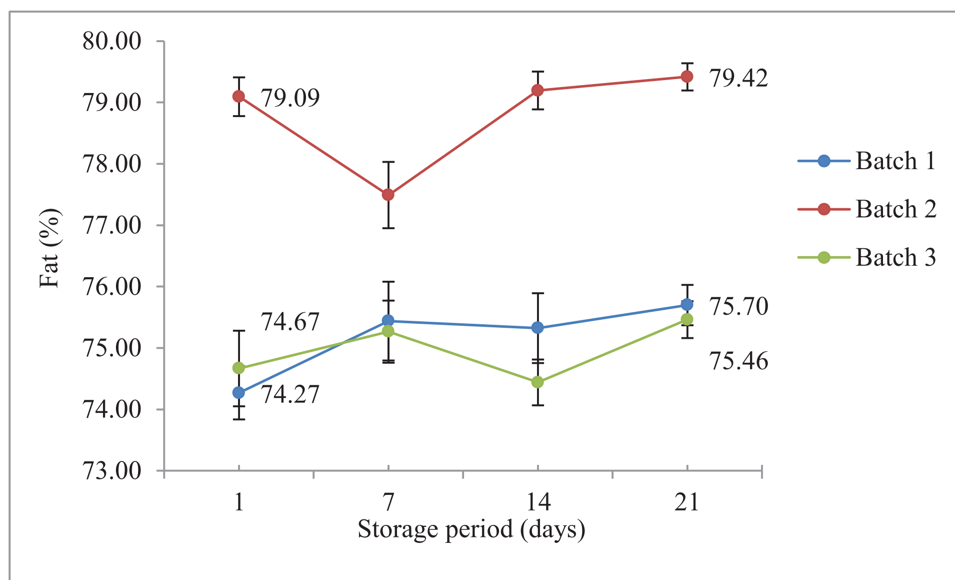
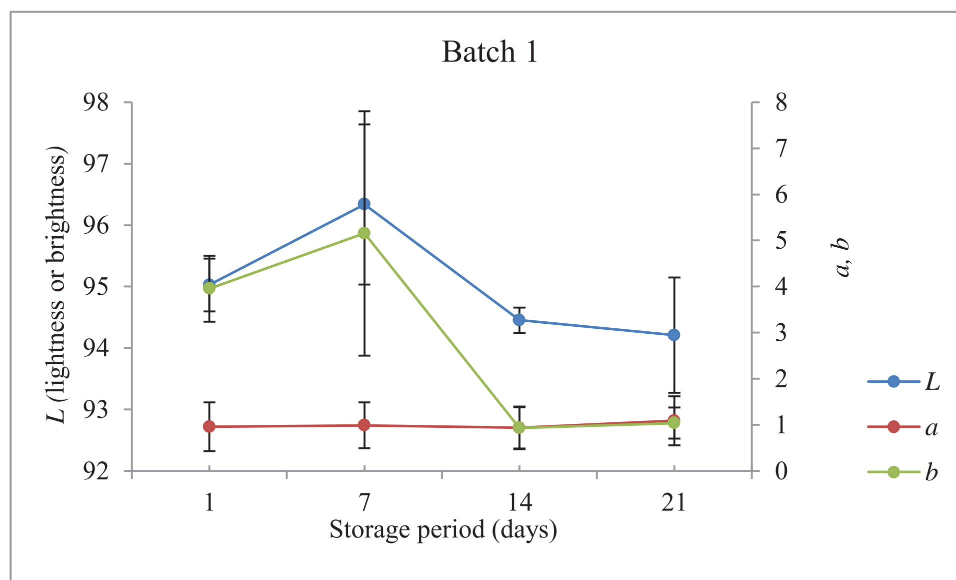


Figure 4.8 Mean fat content of butter during storage at 4ºC. n=3; error bars = ±SD.

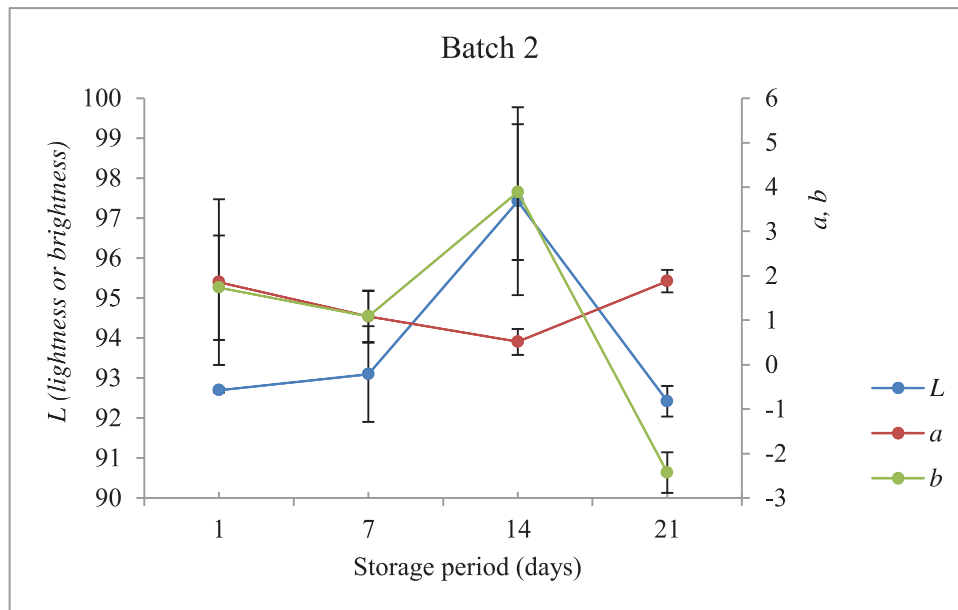
4.5 Colour of cultured butter during storage at 4°C

Colour of food can be defined by several systems, such as Munsell colour system, XYZ tristimulus values, Yxy colour space, L*a*b* colour space, L*c*h colour space, and Hunter Lab colour space (MacDougall, 2002). Among these definitions, the Hunter Lab colour space gives the most pronounced appearance of the yellowness of butter, thus, it was selected to be the colour analysis system in this study (MacDougall, 2002; Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). The Hunter Lab colour space has three values: '*L*' scale showing lightness (or brightness) function; '*a*' scale representing redness-greenness; '*b*' scale expressing yellowness-blueness. The scale of lightness function ranges from 0-100, where 0 is black, and 100 is white (MacDougall, 2002; Damodaran, Parkin, & Fennema, 2008). A positive '*a*' scale, recorded as +*a*, which shows redness, a negative '*a*', recorded as –*a*, which shows greenness, 0 is neutral; similarly, +*b* shows yellowness, and –*b* shows blueness, 0 is neutral (MacDougall, 2002; Damodaran, Parkin, & Fennema, 2008).

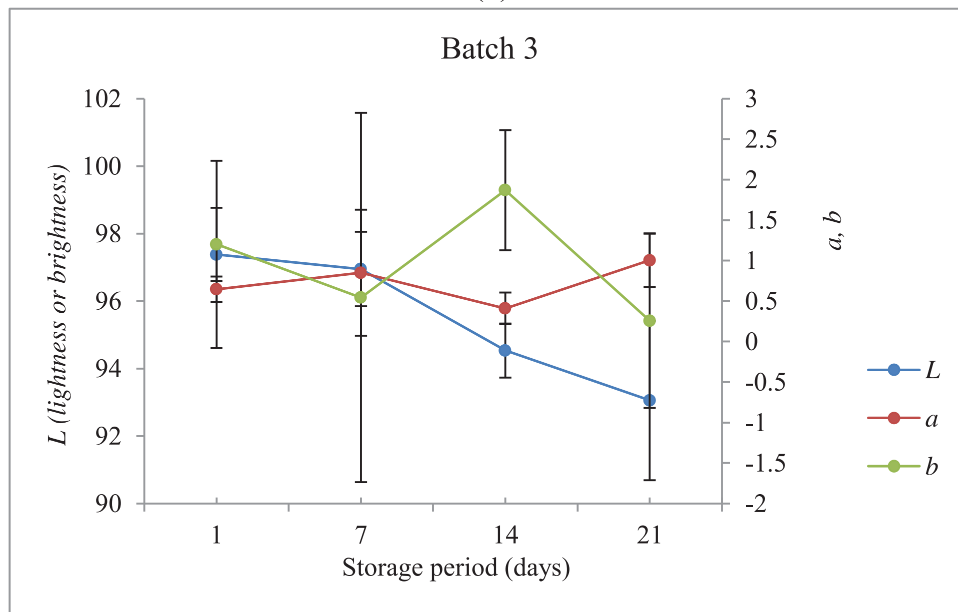
From Figure 4.9, all the data of the '*L*' value were more than 92 in the three batches of cultured butter, which suggested that the butter products were very bright, since the highest brightness scale is 100 (MacDougall, 2002). The redness-greenness values were all between 0 and 2, which indicated that there was almost no redness or greenness in the butter products. For the '*b*' value, (with the exception of the data obtained at 21 days in batch 2), the rest of '*b*' values were between 0 and 5.20, which indicated that the yellow hue was the dominant colour in the butter, which gives butter a pale yellow colour. Butter is naturally pale yellow, because of the natural pigment carotene from the cow's diet (McDowall, 1953; MacDougall, 2002; Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006; Damodaran, Parkin, & Fennema, 2008). During storage of the three batches products, the general tendencies of '*L*' and '*b*' values were decreasing, which suggested that the butter became less bright and less yellow with increased storage. Meanwhile, the '*a*' value was relatively flat, indicating that there was almost no red or green hue in the butter during the storage period.



(a)



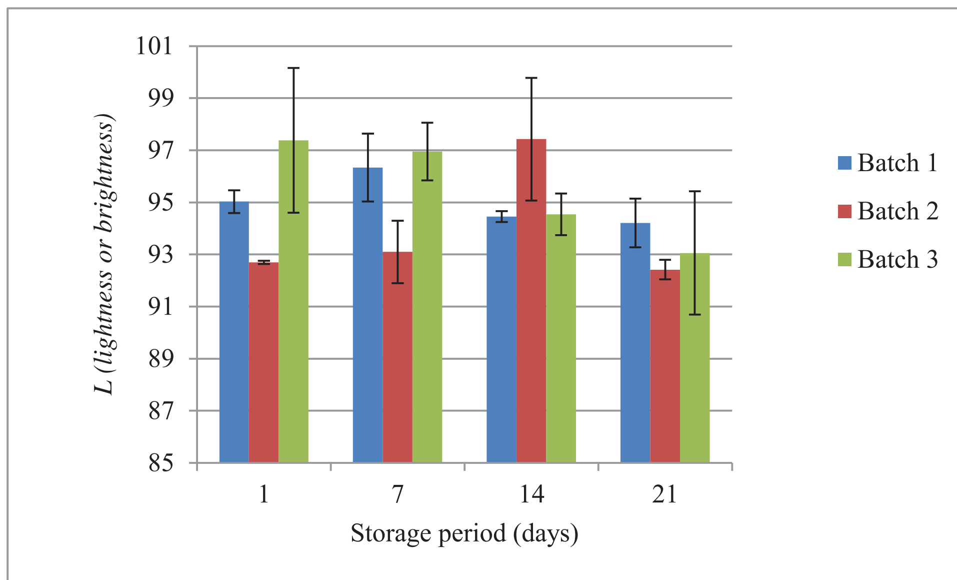
(b)



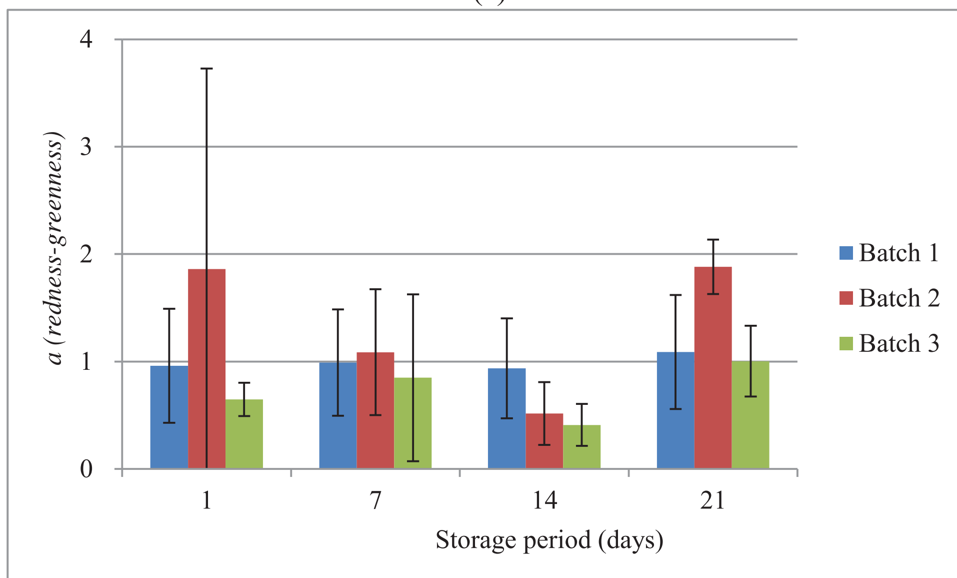
(c)

Figure 4.9 (a), (b), (c) Mean Hunter Lab values of cultured butter during storage at 4ºC. *L* = lightness or brightness, *a* = redness-greenness, *b* = yellowness-blueness. n=3; error bars = ±SD.

Figure 4.10 shows changes of '*L*', '*a*', and '*b*' values of the three batches of cultured butter during storage at 4ºC. For the '*L*' value, there were significant differences ($P<0.05$) among the three batches on day 1 and day 7. For the '*a*' value, there was no significant difference ($P>0.05$) among three batches, although batch 2 was higher than that of batch 1 and batch 3. For the '*b*' value, there were significant differences ($P<0.05$) among three batches of butter on days 1, 14, and 21 during storage. The changes of yellowness (*b*) of butter among the three batches during storage suggested the changes of related pigments (McDowall, 1953; MacDougall, 2002). Meanwhile, carotene in the butter was decreasing. The yellowness colour in butter is attributed to the presence of carotene, which is a group of lipid soluble compounds formed by joining eight isoprenoid units (MacDougall, 2002). Since carotene contains unsaturated double bonds, it is sensitive to light, oxygen and heat (Iglesias, Mayer, Chavez, & Calle, 1997; MacDougall, 2002). The average amount of β-carotene lost in 24 h is less than 4%, and less than 15% in 48 h, when micellar solutions in aqueous medium of β-carotene incubated at 37ºC (Scita, 1992). The results of previous studies discussed here were similar to the observations made in our results, which showed a decreasing tendency of '*b*' value. The loss of moisture of butter during storage (Figure 4.6) can lead to the exposure of deepening colour (McDowall, 1953). Some evaporation of moisture takes place from the surface of butter when it is exposed to the air, thus, the deepening of the colour, called primrose colour, becomes more apparent (McDowall, 1953). β-carotene and primrose colour were however not analysed in the present study.



(a)



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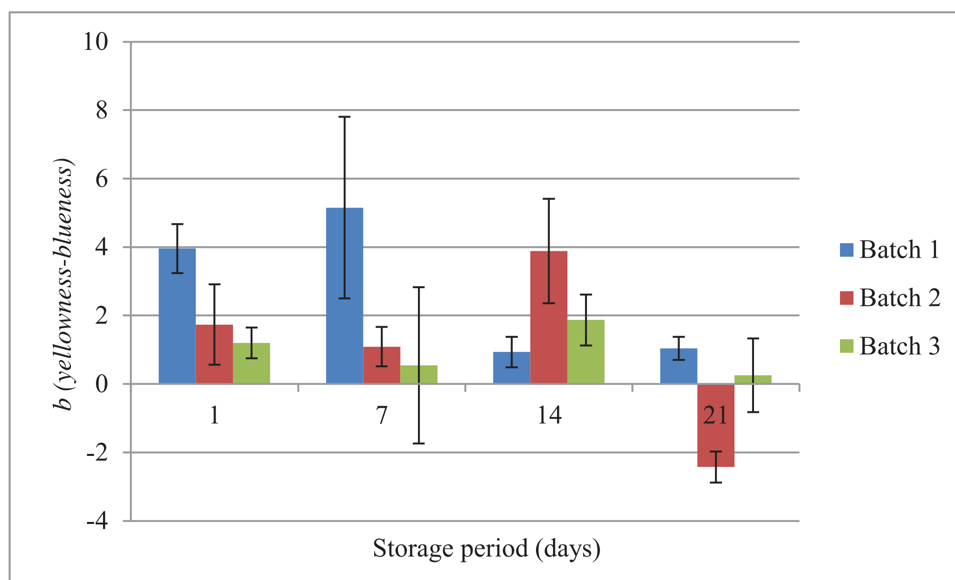


(c)

Figure 4.10 (a), (b), (c) Comparison of the mean Hunter values of '*L*', '*a*', and '*b*' of cultured butter during storage at 4ºC. *L* = lightness or brightness; *a* = redness-greenness, *b* = yellowness-blueness. n=3; error bars = ±SD.

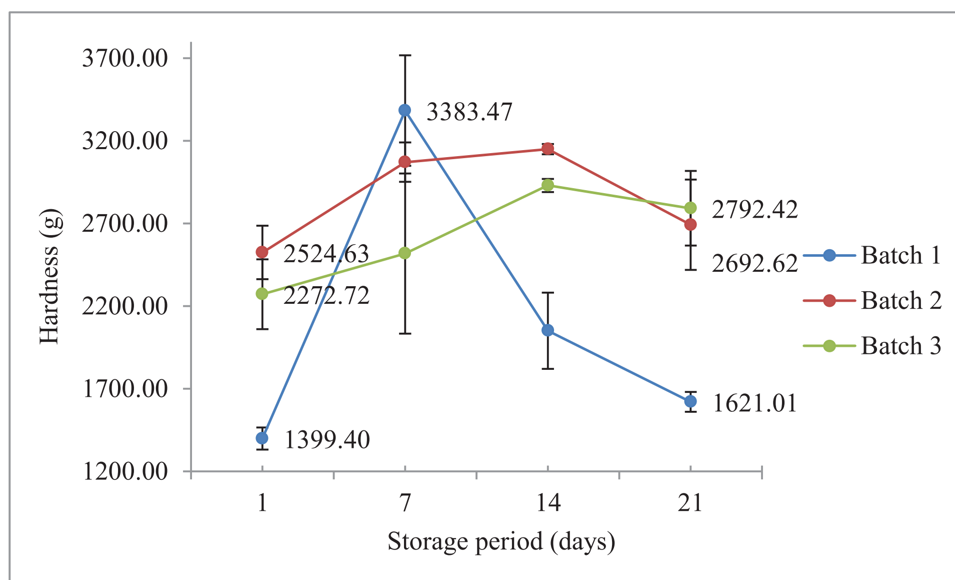
4.6 Texture analysis of cultured butter during storage at 4°C

Figure 4.11, Figure 4.12, and Figure 4.13 show three texture attributes comprising hardness, springiness, and adhesiveness, of cultured butter during storage for 21 days at 4ºC. All the attributes were measured at 4ºC, using the same instrumental settings described in Table 3.1. The standard deviation (SD) of hardness and adhesiveness data were large (Appendix Table XIV), indicating large differences between the two parameters of the butter samples. These results were not unexpected as the samples were uneven due to manual manipulation during production which has mentioned in previous sections.

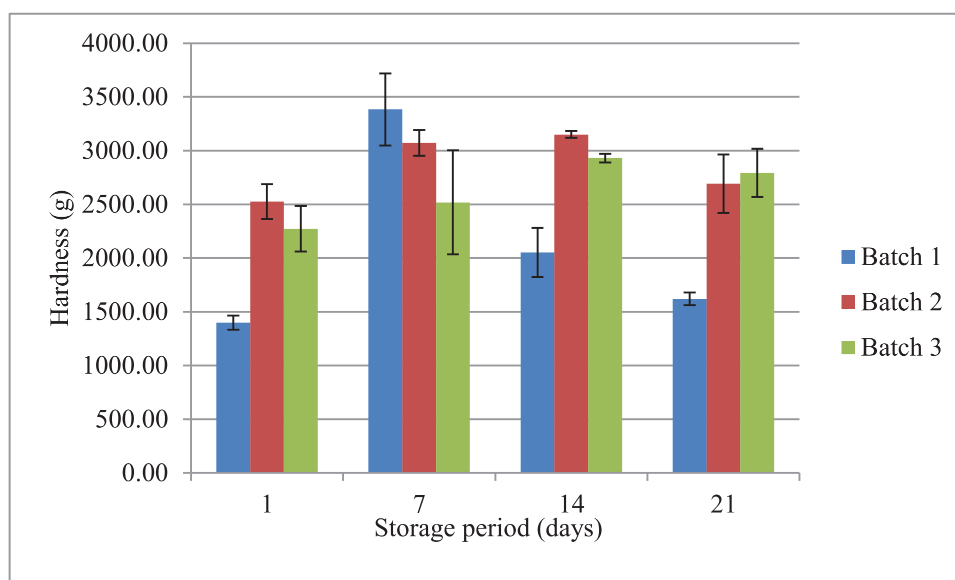
Hardness or firmness is the force required to change the permanent shape of a solid matter between the molars (Bourne, 2002; McKenna, & Kilcast, 2003). It is the peak force of the first compression of the product (Bourne, 2002; McKenna, & Kilcast, 2003). In Figure 4.11, the hardness of butter of batch 1 increased from 1399.40±66.43

g to a peak of 3383.47±335.03 g on day 7, and then decreased to 1621.01±59.51 g by the end of the storage period. The hardness of batch 2 and batch 3 butters increased from 2524.63±161.60 g and 2272.72±210.96 g respectively, and reached a peak on day 14, and then decreased to 2692.62±273.17 g and 2792.42±224.98 g by the end of storage. Figure 4.11 shows that the cultured butter samples were harder on day 7 and day 14, whereas, less force was required to cut the samples on day 1 and day 21. There were significant differences ($P<0.05$) in the hardness of butter on days 1, 14, and 21 during storage at 4ºC.

In this study, the overall hardness of butter samples was about 3000 g after 10 days of storage. The results were similar to De Man and Wood (1958), who reported the hardness of butter made by rapidly cooled cream at around 2000 g in the first 10 days of storage, and then increased to about 3000 g from 10 to 50 days of storage (5ºC). Figure 4.11 shows that the hardness of butter produced in early spring (batch 2) and mid spring (batch 3) were higher than products produced in late winter (batch 1), except for day 7. This observation suggests that softer butter fat was produced in colder weather in New Zealand. It is well known that the texture of butter changes with the unsaturation of FAs of milk (Auldist, Walsh, & Thomson, 1998; Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003; Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). In New Zealand, the unsaturated FAs of summer milk are lower than the winter milk, thus summer butter would be harder (Auldist, Walsh, & Thomson, 1998). The fatty acid composition of the milk used in this study was not analysed.



(a)

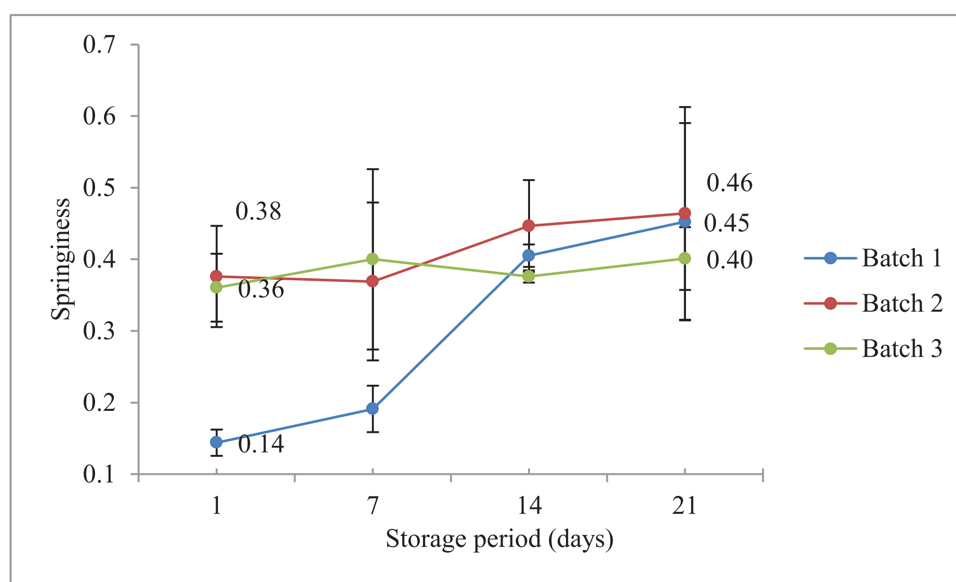


(b)

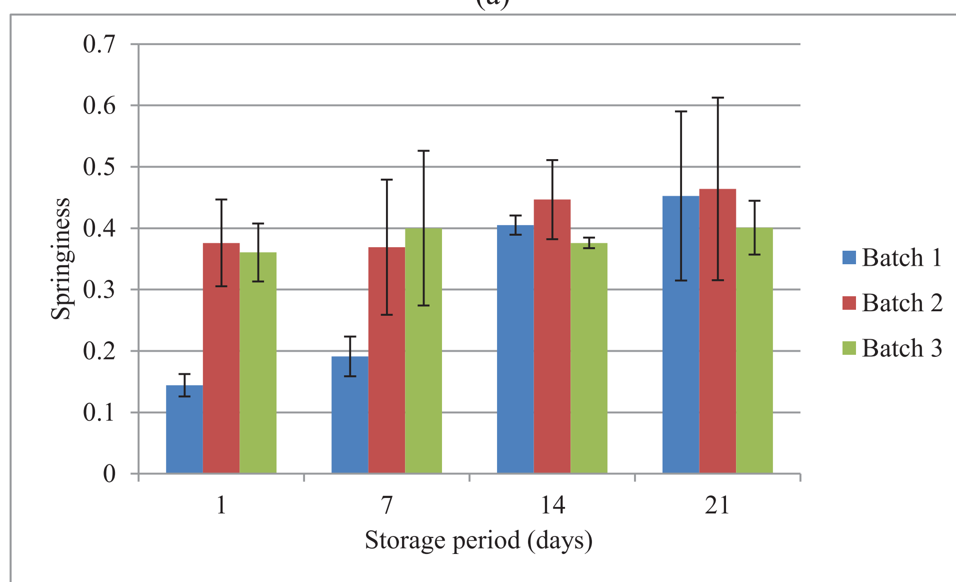
Figure 4.11 (a) (b) Mean hardness of cultured butter during storage at 4ºC. n=2; error bars = ±SD.

Springiness or elastic recovery is the ability of a deformed material to return to its un-deformed state after the deforming force is removed (Bourne, 2002; McKenna, & Kilcast, 2003). The springiness of butter in the three batches increased during storage for 21 days at 4ºC. Springiness of butter samples of batch 1 markedly increased, from 0.14±0.02 to 0.45±0.14 (Figures 4.12). Whereas, the springiness of butter samples of batch 2 and batch 3 slightly increased from 0.38±0.07 to 0.46±0.15, and 0.36±0.05 to

0.40±0.04, respectively. The highest increase in the springiness of butter was observed in the first 14 days of storage (4ºC), while lower increases were noted from day 14 to day 21. The cultured butter became more elastic by the end of storage, that is, it was easier to return to its original size when the load was removed during texture analysis. There were significant differences ($P<0.05$) in the springiness of butter among three batches on day 1. No significant differences ($P>0.05$) were observed among the three batches of butter on the days 7, 14, and 21 during storage.



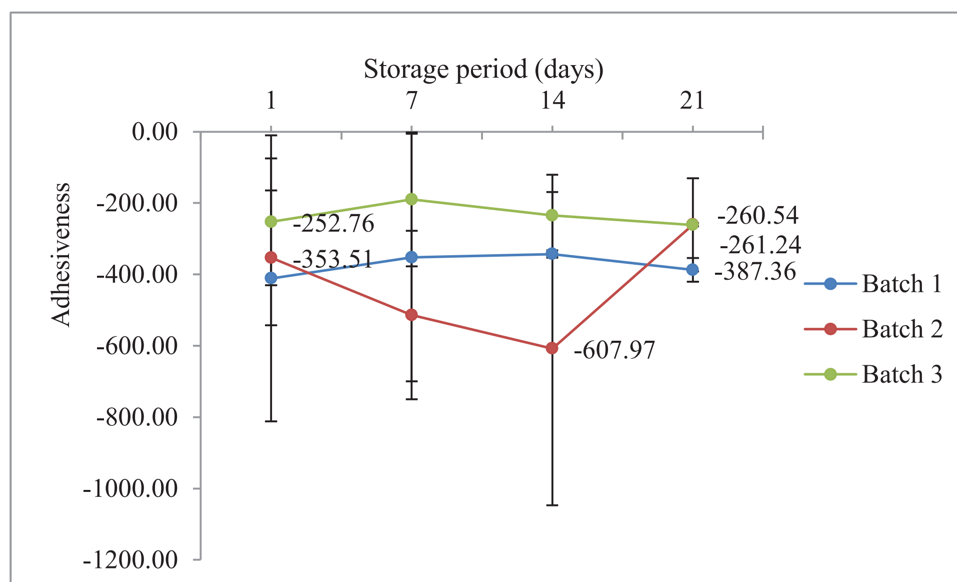
(a)



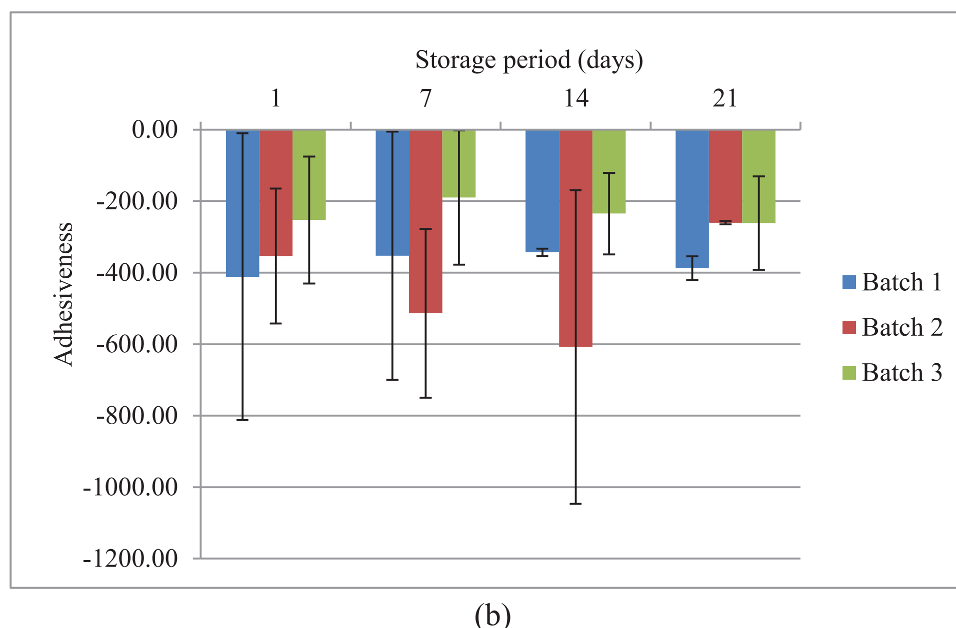
(b)

Figure 4.12 (a) (b) Mean springiness of cultured butter during storage at 4ºC. n=2; error bars = ±SD.

Adhesiveness or stickiness is the force necessary to overcome the attractive forces between the surface of a product and the surface of the probe which is contact with the product (Bourne, 2002; McKenna, & Kilcast, 2003). The data of adhesiveness of cultured butter were all negative, thus showing the work required to pull the plunger away from the sample; the force direction of adhesiveness is opposite to that of hardness and springiness (Bourne, 2002; McKenna, & Kilcast, 2003). Overall, the adhesiveness of cultured butter in the three batches increased, with the exception of batch 2 (14 to 21 days). There were however no significant differences (P>0.05) among the three batches of butter during storage. Figure 4.13 shows that batch 1 and batch 3 had lower adhesiveness than batch 2, which means that batch 2 required more force from the probe when it was withdrawn from the sample. The highest adhesiveness was observed on the 14th day of batch 2, which reached -607.97±438.85. This result indicates that withdrawing the probe was the most difficult on that spot. With the exception of this result, the rest of the data ranged from -250 to -350, which remained relatively stable during storage of butter for 21 days. The results of this study were similar to Oeffner, Qu, Just, Quezada, Ramsing, Keller, and Bobe (2013), who reported the adhesiveness of butter at -302 on the first day of storage at 4°C.



(a)



(b)

Figure 4.13 (a) (b) Mean adhesiveness of cultured butter during storage at 4ºC. n=2; error bars = ±SD.

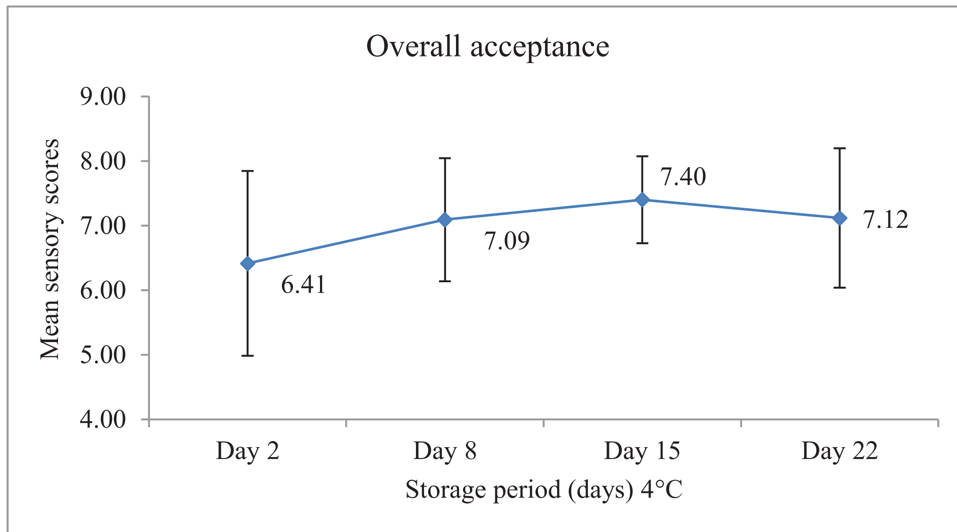
4.7 Microbiology quality of cultured butter during storage at 4°C

Dairy products can be spoiled by undesirable microorganisms such as coliform bacteria present in the initial flora of raw milk, processing environmental conditions, and post-heat treatment contamination (Law, 1997). Coliform bacteria can be found in animal faeces, aquatic environments, soil, and on vegetation (Pantoja, Reinemann, & Ruegg, 2011). Although coliforms can be destroyed by normal pasteurisation temperatures, there is still potential spoilage of the dairy products if pasteurisation fails or by contamination during handling and processing (Sengul, Erkaya, BaAlar, & Ertugay, 2011; Pantoja, Reinemann, & Ruegg, 2011). The coliform test was used to examine the level of hygiene/sanitation of the process and raw materials used to make cultured butter (Mhone, Matope, & Saidi, 2011). No coliform was present in the butter samples. The result indicated that there was no contamination of products during processing, handling and storage.

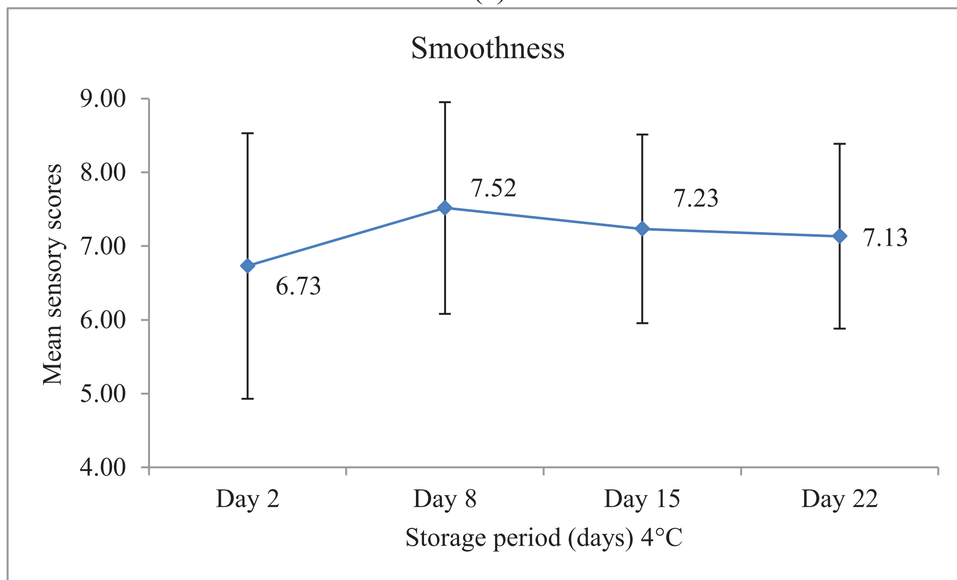
4.8 Consumer sensory evaluation of cultured butter during storage at 4ºC

Sensory evaluation is a vital part of product development, as it provides useful information for product developers to obtain sensory characteristics of their food products (Lawless, & Heymann, 2010; Stone, Bleibaum, & Thomas, 2012). In this study, cultured butter samples of batch 2 were evaluated by consumer sensory panellists to obtain the degree of liking of six attributes (smoothness, hardness, spreadability, melting rate, buttery flavour, and overall acceptance). The samples were evaluated on different occasions by thirty consumer panellists during storage (4ºC) on 2, 8, 15, and 22 days using the method described in section 3.2.3.10.

Figure 4.14 shows the mean sensory scores from the consumer panellists for six attributes of cultured butter evaluated during storage at 4ºC. Overall, the mean sensory scores were higher than 5.0 on the 1-9 hedonic scale, indicating that the products were well accepted by the sensory panellists. The mean overall acceptance score of the samples of cultured butter was 6.41±1.43 on day 2, which peaked to 7.40±0.67 on day 15, and then decreased to 7.12±1.08 at the end of storage. The mean overall acceptance score for smoothness was 6.73±1.80 on day 2, peaking to 7.52±1.43 on day 8, and decreased to 7.13±1.25 at the end of storage. According to consumer sensory evaluation, the average hardness of the butter samples commenced at 6.57±1.83 on day 2, peaked to 7.33±1.09 on day 15, and then decreased to 6.37±1.54 at the end of storage. The mean spreadability score of samples of butter was 5.20±2.59 on day 2, peaked to 6.53±1.41 on day 15, and decreased to 6.03±1.65 at the end of storage. The average sensory score for the melting rate was 7.40±1.59 on day 2, peaked to 7.58±1.15 on day 8, and decreased to 7.20±1.27 at the end of storage. Mean buttery flavour score was 6.17±1.82 on day 2, peaked to 7.27±1.05 on day 15, and decreased to 6.80±1.86 at the end of storage.



(a)



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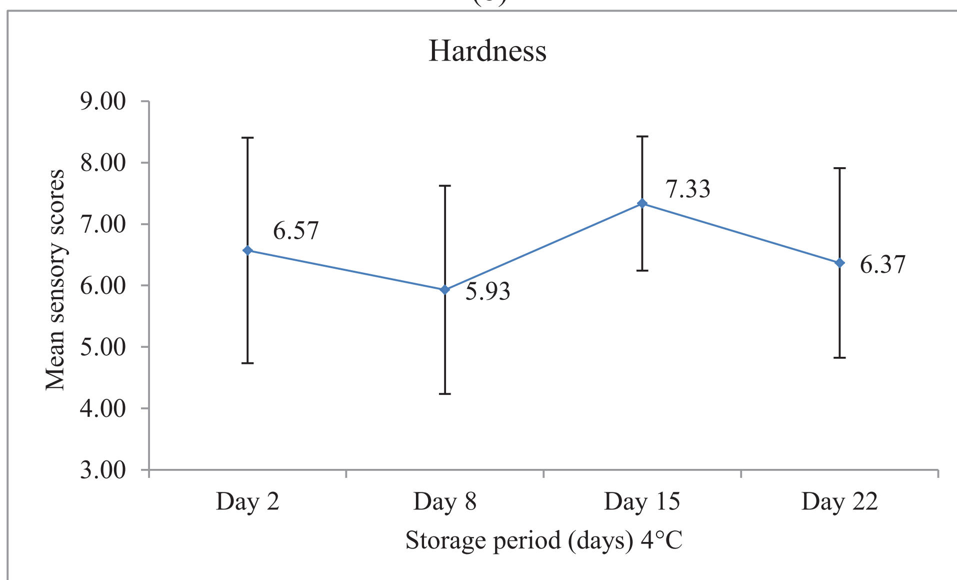

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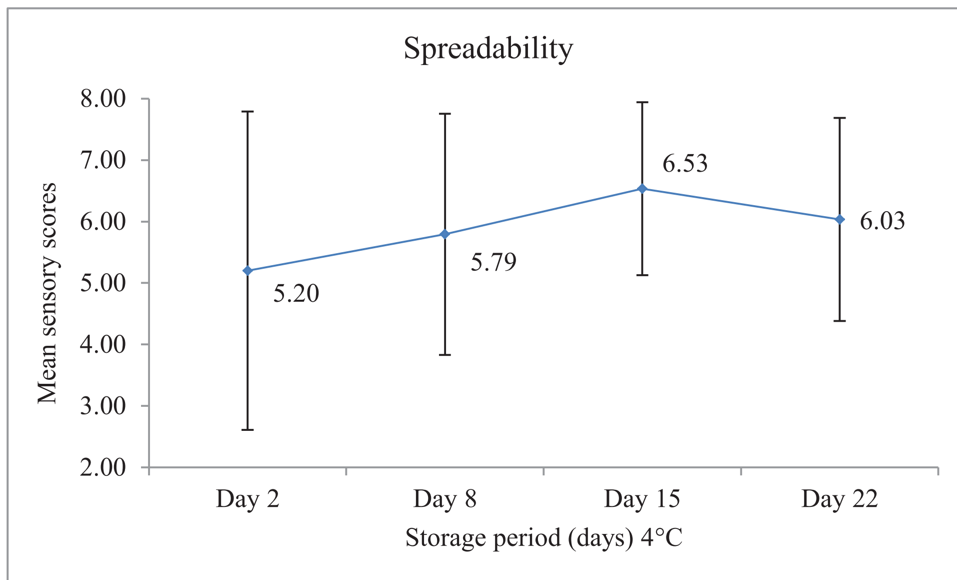

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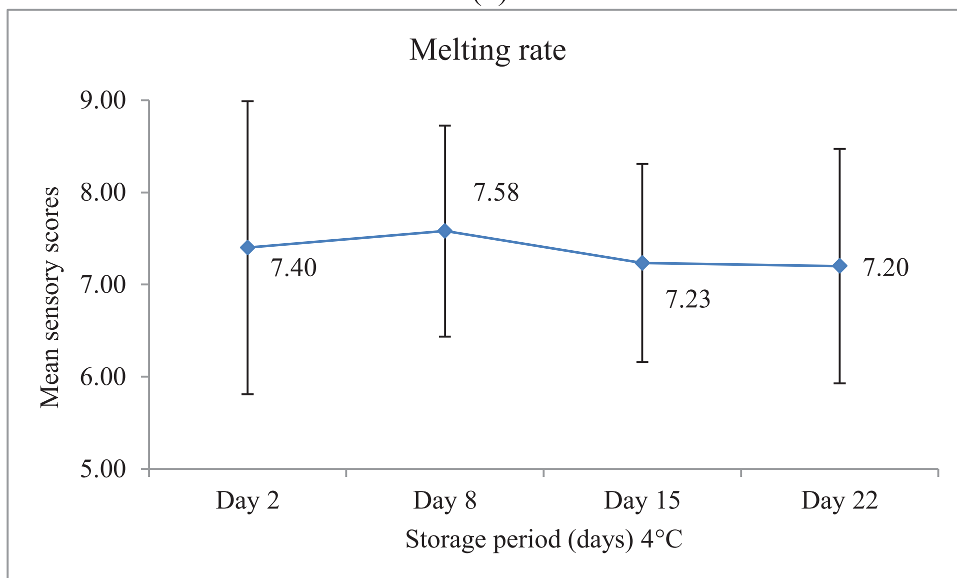
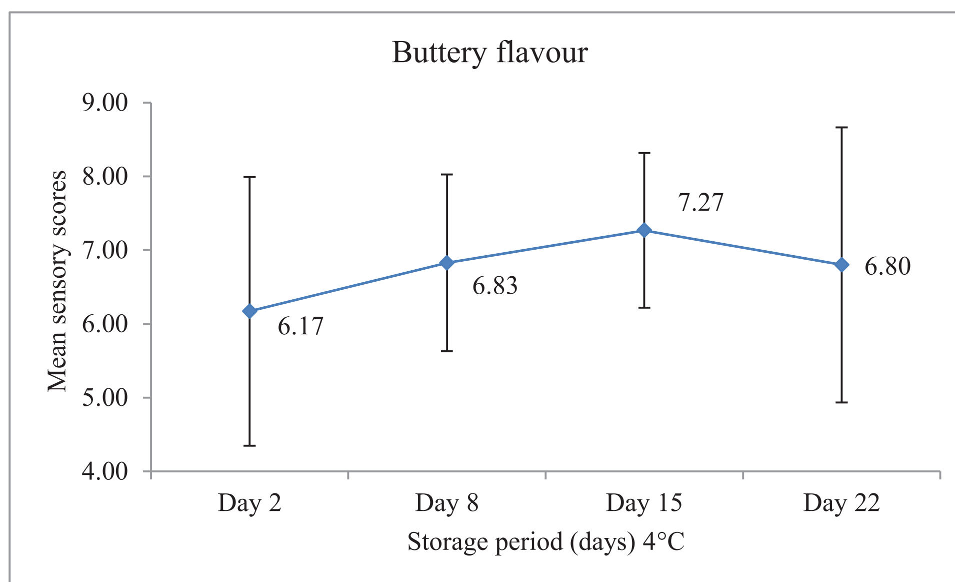


(e)



(f)

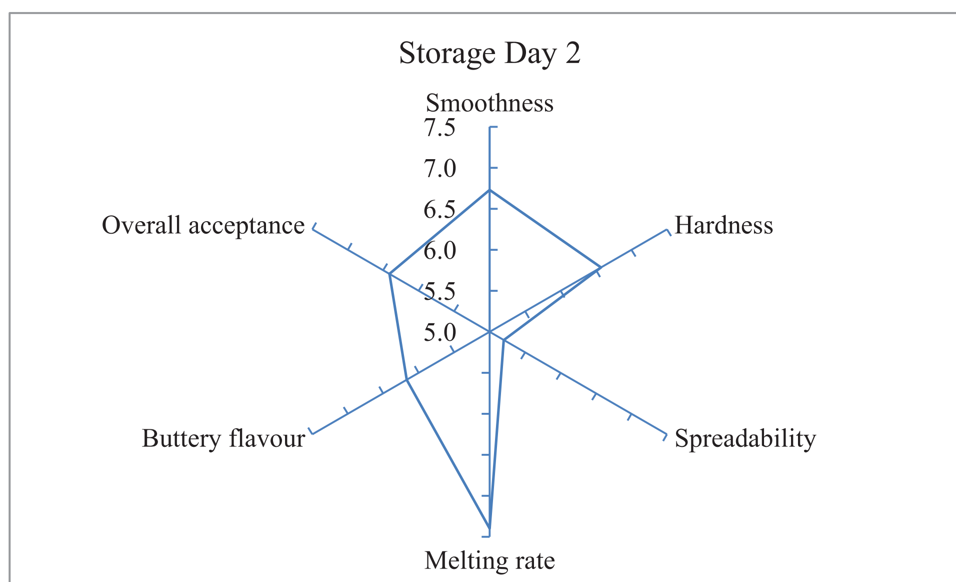
Figure 4.14 (a), (b), (c), (d), (e), (f) Mean sensory scores for overall acceptance, smoothness, hardness, spreadability, melting rate, and buttery flavour of cultured butter during storage at 4°C. n=30; error bars = ±SD. Hedonic sensory scale of 1-9 was used, with 1 as the lowest and 9 the highest.

The intensity sensory scores of cultured butter samples of six attributes during storage at 4ºC are shown in Figure 4.15. Overall, the six attributes (properties) of the cultured butter samples were highly accepted by consumer sensory panellists. It is however apparent that hardness and spreadability had lower scores compared with the other four attributes. The possible reason for the low scores for hardness and spreadability could be due to the storage of samples at 4ºC.It therefore seemed that the butter stored at 4ºC was hard and had poor spreadability. This result agrees with previous studies who also stored butter at low temperatures (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003; Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). Figure 4.15 shows that the sensory scores for hardness and spreadability on days 2 and 8 were lower than the scores on days 15 and 22. A possible explanation of this discrepancy is that plastic knives were used to serve samples on days 2 and 8, while on days 15 and 22, metallic (stainless steel) knives were used. The changeover of utensils was done in response to consumer panellists who requested metallic knives. The supplied plastic knives used

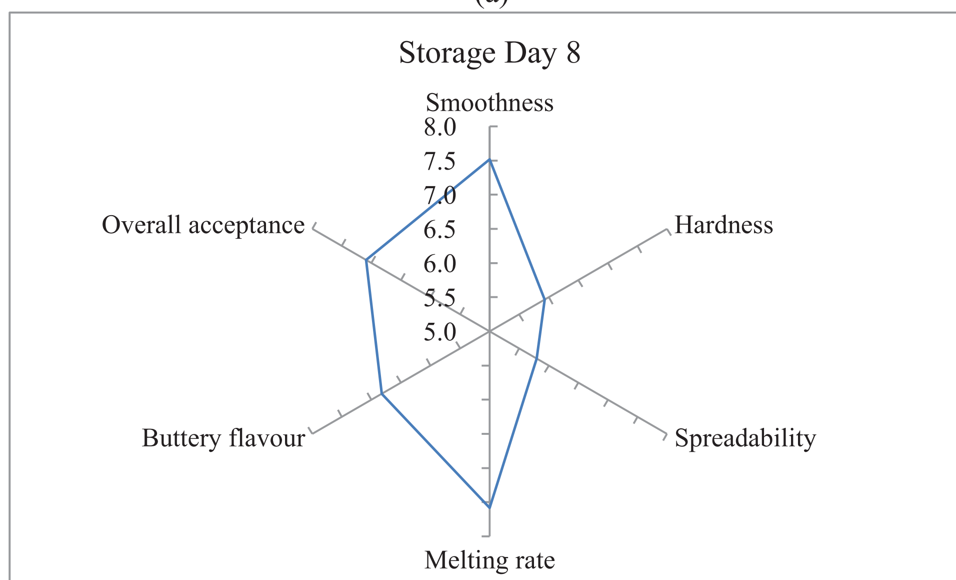
to cut and spread the butter were not rigid enough for the test samples. Thus, the use of flexible plastic would not provide objective responses on the hardness and spreadability of the cultured butter samples. It noted by the panellists that the cultured butter was easier to cut with stainless steel knives, reflected by higher intensity scores on day 15 and day 22 than those of day 2 and day 8 (Figure 4.15).

The buttery flavour (Figure 4.15) of the sample was well-liked by sensory panellists, indicating the optimal presence of aroma compounds in the product (Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006; Lawless, & Heymann, 2010). Aroma compounds were produced by LAB (Cogan, 1983; Law, 1997; Quintans, Blancato, Repizo, Magni, & López, 2008). In this study, a mixed lactic starter was used comprising *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. cremoris*. During fermentation, LAB metabolised sugars, and also a number of non-carbohydrate compounds such as citrate (Hugenholtz, 1993; Quintans, Blancato, Repizo, Magni, & López, 2008). Diacetyl and acetoin, which are responsible for the buttery flavour, are by-products from spontaneous oxidative decarboxylation of α-acetolactate during citrate metabolism (Cogan, 1983; Macciola, Candela, & Leonardis, 2008; Gokce, Akdogan, Divriklib, & Elci, 2014). Diacetyl is the main flavor compound in cultured butter. Other compounds such as γ-decalactone, σ-decalactone, and σ-dodecalactone also contribute to a buttery flavour (Nursten, 1997). During citrate metabolism, acetaldehyde is produced in butter which may result in a green flavour or yogurt-like flavour defect (Keenan, & Lindsay, 1966). The lactococci is responsible for the production of acetaldehyde. However, in a mixed starter culture of the lactococci and leuconostoc, the latter reduces the acetaldehyde to ethanol, which is a neutral compound (Mutukumira, Narvhus, Marzin, Feresu, & Abrahamsen, 2008). It is apparent from Figure 4.15 that smoothness and melting rates maintained relatively high scores during storage. The butter samples were smooth as there were no visible bumps or bundles on the surfaces of the products (Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). Melting rate showed the rate at which a butter sample changed from solid to

liquid, while the sample was melting in the mouth (Jinjarak, Olabi, Jimenez-Flores, & Walker, 2006). The melting rate depends on the proportion of low MP fat and high MP fat (Walstra, 2003). Considering that the butter samples used for sensory evaluation were produced in the early Spring, a large proportion of unsaturated FAs (low MP fat) in the butter was expected compared to the winter sample (Walstra, & Jenness, 1984; Walstra, 2003; Goff, 2015); thus, butter melted relatively quickly in the mouth.



(a)



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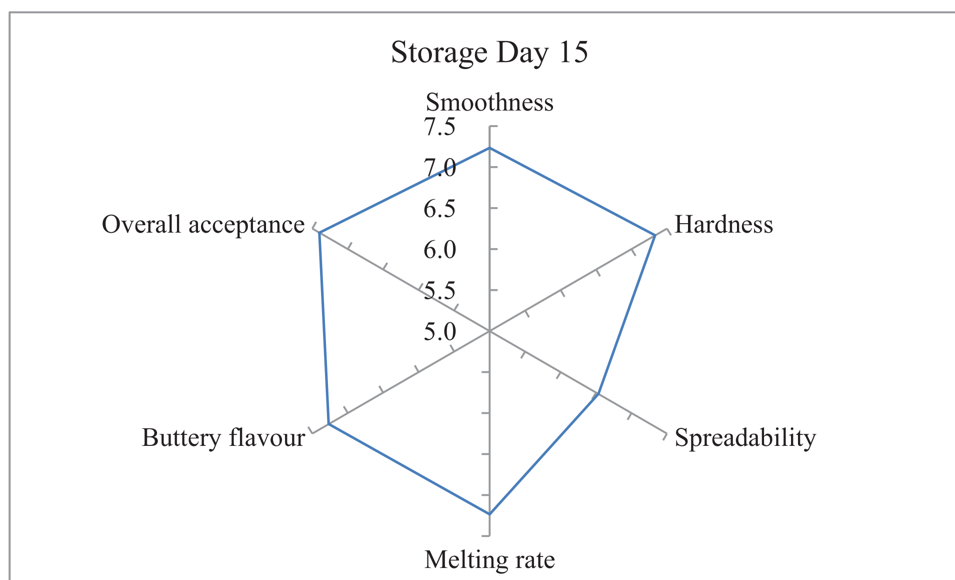

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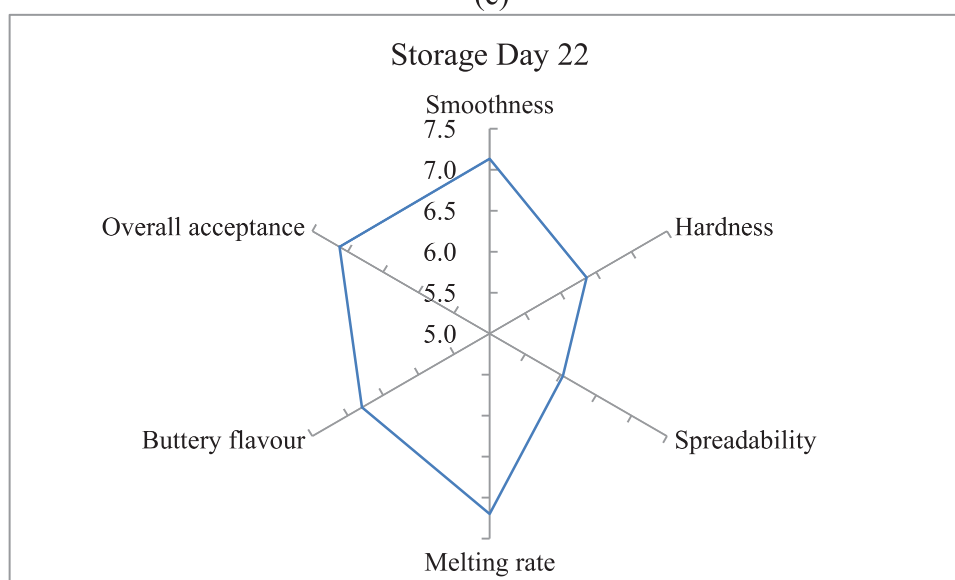


(d)

Figure 4.15 (a), (b), (c), (d) Mean intensity scores of six attributes of cultured butter during storage 4°C. n=30; hedonic scale of 1-9 was used with 1 as the lowest and 9 the highest.

Figure 4.16 shows the response of the overall acceptance of butter samples to the other five sensory attributes (buttery flavour, hardness, melting rate, smoothness and spreadability). The parameters ‘A’, ‘B’, ‘C’, and ‘D’ in Figure 4.16 represent storage days of 2, 8, 15, and 22 at 4ºC, respectively. The coloured circles represent the five attributes. The sensory scores of overall acceptance of the butter samples changed

depending on the changing of the other five attributes. The overall acceptance of butter on day 15 had the highest score, indicating that the samples were most liked by the sensory panellists on this day. However, the relationship between the overall acceptance and the other five attributes could not be observed from the plot easily. Thus, more precise analysis was done using linear regression test of SPSS. The results (Appendix 3.0) indicated that the attributes of spreadability and buttery flavour had significant effects ($P<0.05$) on the overall acceptance of the samples. In this study, the attributes of smoothness and buttery flavour had a positive relationship to the overall acceptance, thus the two attributes impact on the overall acceptance. On the contrary, the effects of melting rate and spreadability were negative to the overall acceptance of cultured butter samples. The model of the relationship between the overall acceptance and the other five sensory attributes can be represented by 'overall acceptance = 0.827 + 0.913*buttery flavour + 0.115*smoothness + 0*melting rate - 0.138*hardness - 0.027*spreadability'. The adjusted R square (Appendix 3.0) illustrated that this model could account for 96.2% of the variance in the sensory scores of the overall acceptance.

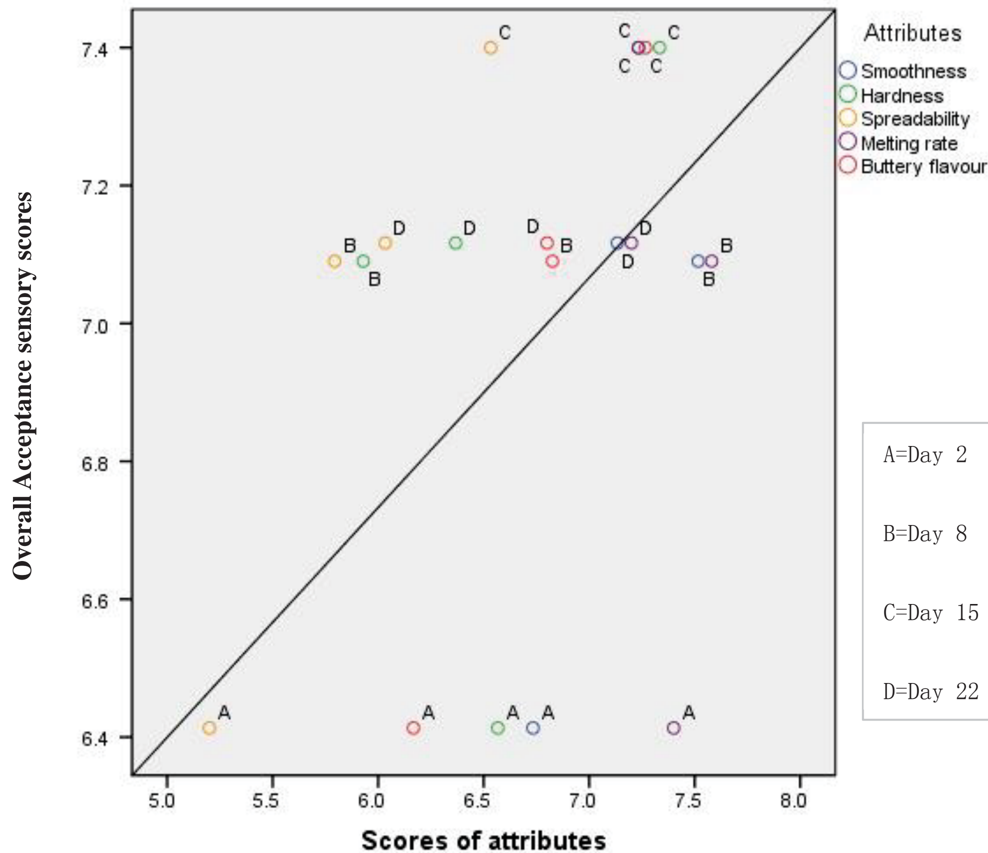


Figure 4.16 Scatter plot of mean consumer sensory preferences scores of overall acceptance response to the other five attributes (buttery flavour, hardness, melting rate, smoothness and spreadability). n=30; hedonic scale of 1-9 was used with 1 as the lowest and 9 the highest.

Figure 4.17 shows the interaction plot of sensory evaluation on six attributes of cultured butter during storage at 4ºC. The data used in the Figure 4.17 are the means of 30 panellists. Statistically, an interaction occurs when the effect of one independent variable on the dependent variable changes depending on the level of another independent variable (Ryan, Joiner, & Cryer, 2013). In this study, the interaction plot was used to determine whether five attributes, comprising buttery flavour, hardness, melting rate, smoothness and spreadability, had effect on the scores of the overall acceptance during storage. In an interaction plot graph, parallel lines indicate no interaction. The greater the difference in slope between the interaction lines, the

higher the degree of interaction (Ryan, Joiner, & Cryer, 2013). However, the absence of interaction between different independent variables does not necessarily mean there is no main effect on the dependent variable. A 'main effect' is the effect of one of the independent variables on the dependent variable, excluding the effects of all the other independent variables (Ryan, Joiner, & Cryer, 2013).

From Figure 4.17, it was apparent that the lines of spreadability and buttery flavour were parallel with the line of overall acceptance, which suggested that there was no interaction among the three attributes. The average scores of the four points (days 2, 8, 15, and 22) of the three lines (overall acceptance, spreadability, and buttery flavour) were different, thus, there were main effects among the three attributes. This phenomenon was in agreement with the result illustrated by linear regression in the previous paragraph, which indicated that spreadability and buttery flavour had significant effects on the sensory scores of overall acceptance.

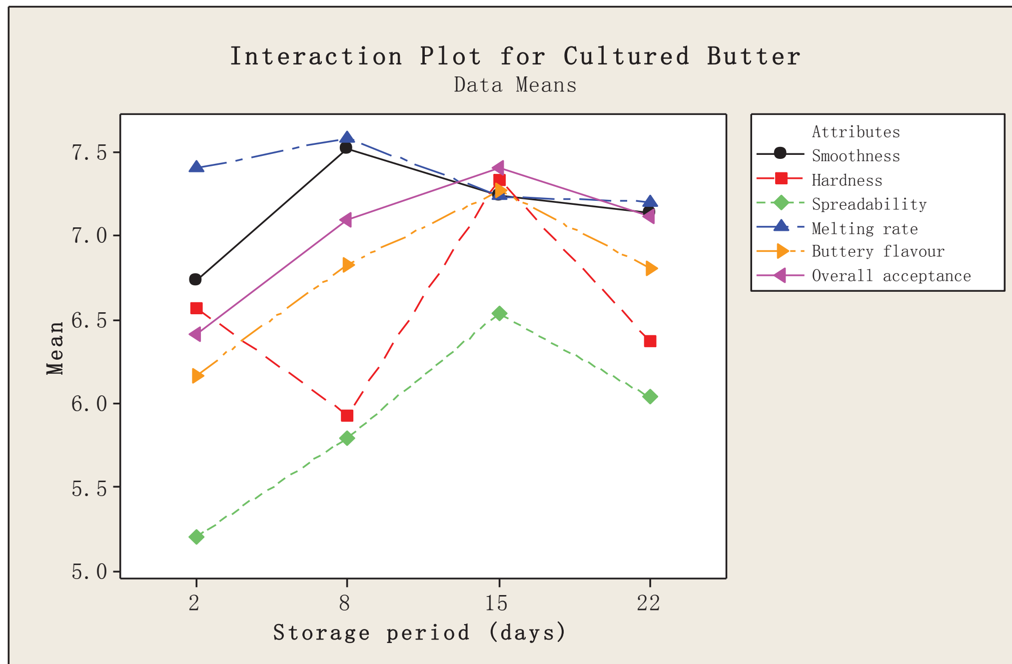


Figure 4.17 Interaction plot of six attributes of cultured butter during storage at 4°C. n=30; hedonic sensory scale of 1-9 was used, with 1 as the lowest and 9 the highest.

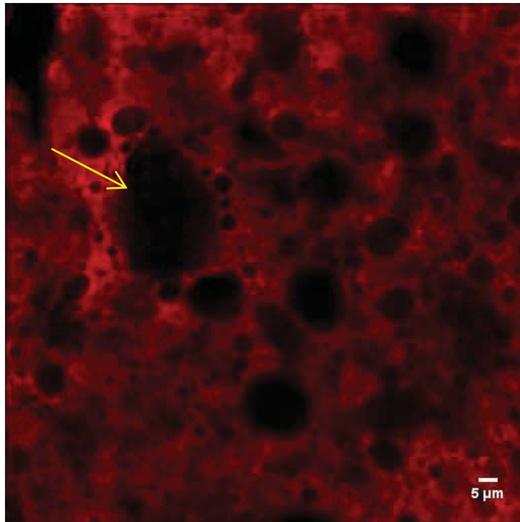
4.9 Examination of water droplet size of cultured butter during storage at 4ºC

Water droplet size is an important parameter in butter as it impacts on the microbiological safety (Van Dalen, 2002). In previous studies (section 2.6.3), the size of water droplet in butter were determined by several specialised equipment, of which confocal laser scanning microscope (CLSM) produced good microstructure of butter (Van Dalen, 2002; Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). Thus, CLSM method was modified to examine the water droplet size in butter samples produced in this study.

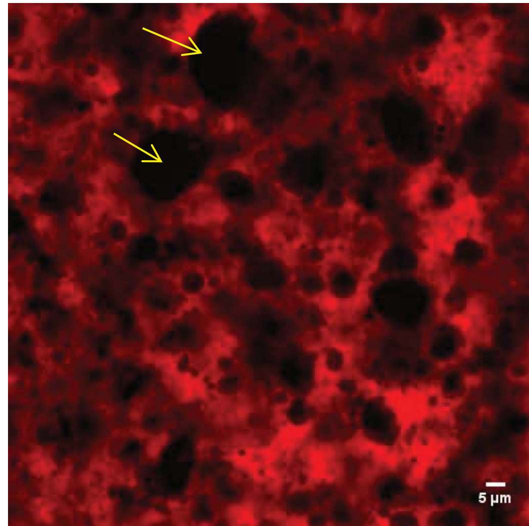
The water droplet size of butter samples produced in October (batch 3) was examined by CLSM during storage (4ºC) on days 5, 12, 19, and 26. In this experiment, different dyes were tested to stain the butter samples in the trials. Images of butter samples stained by Acridine Orange had poor resolution due to poor contact of the sample surface and the well of the cover glass, however, the surface water droplets of the butter could be observed (Van Dalen, 2002). The deeper microstructure of butter samples were observed with Nile Red staining for 45 or 60 min, and all images were acquired at a depth of 10 μm beneath the sample surface (Van Dalen, 2002). Thus, staining butter samples with equal quantities of hydrophobic Nile Red and hydrophilic Acridine Orange produced images with good resolutions and therefore, the method was used to examine the water droplet size of the products.

Figure 4.18 shows the CLSM images of butter stained with Nile Red and Acridine Orange in equal quantities for 45 and 60 min. Fresh butter can contain up to 5% air cells, mostly visible microscopically as air cells (Van lent, Vanlerberghe, Van Oostveldt, Thas, & Van der Meeren, 2008). Air cells are shown by arrows in Figure 4.18. Other small spherical particles in the Figure 4.18 are water droplets in the butter (Van Dalen, 2002); no water channels connecting water droplets in the butter could be

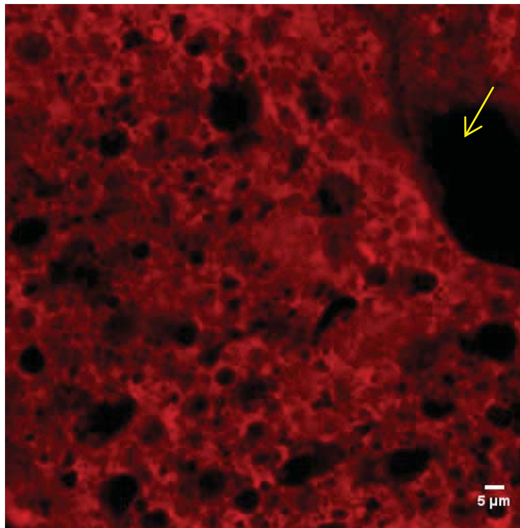
detected. From Figure 4.18, most of the water droplets are smaller than 5 µm in diameter, except for a few water droplets larger than 5 µm. Microorganisms can grow rapidly in a coarse water dispersion and low salt environment, whereas their growth can be delayed when water droplets are smaller than 5 µm, due to insufficient nutrients per droplet (Law, 1997; Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). Results showed uneven distribution of water droplets inside the water phase which had also been previously reported (Van lent, Vanlerverghe, Van Oostweldt, Thas, & Van der Meeren, 2008). This uneven distribution was expected considering that the cultured butter was worked manually after churning. The butter samples stained for 60 min did not show better visual images compared with samples stained for 45 min.



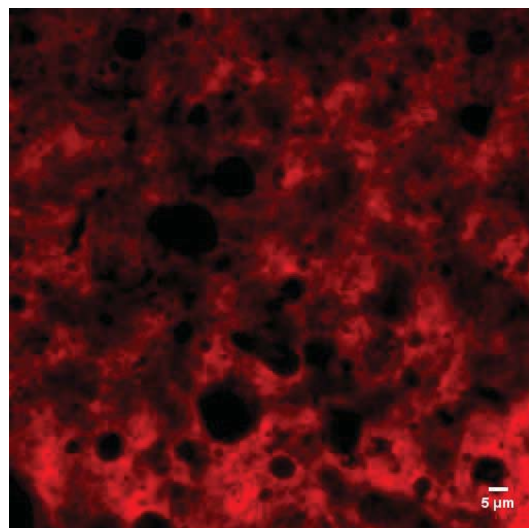
Day 5, stained for 45 min



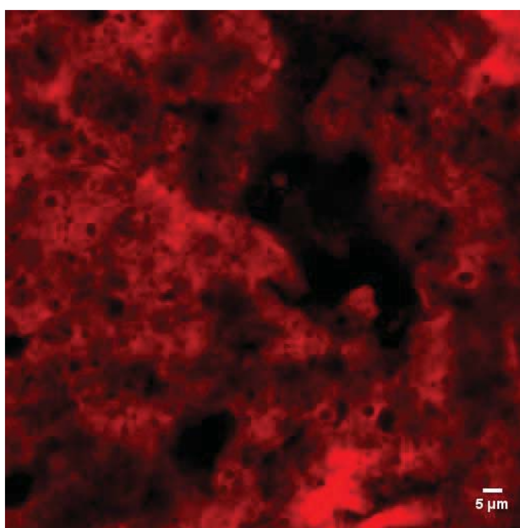
Day 5, stained for 60 min



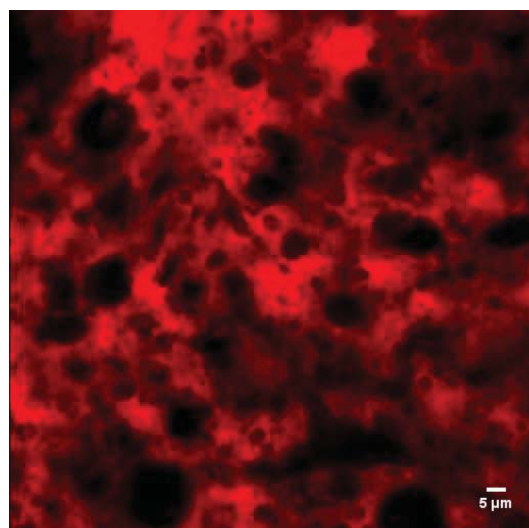
Day 12, stained for 45 min



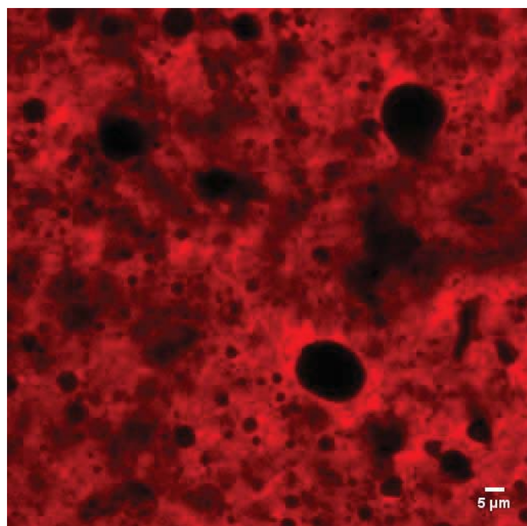
Day 12, stained for 60 min



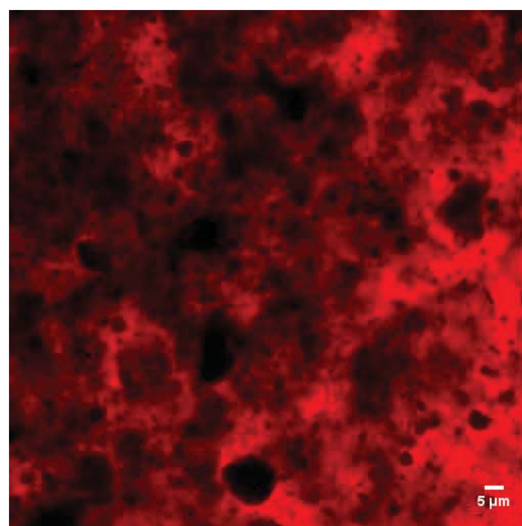
Day 19, stained for 45 min



Day 19, stained for 60 min



Day 26, stained for 45 min



Day 26, stained for 60 min

Figure 4.18 CLSM images of cultured butter stained with Nile Red and Acridine Orange during storage at 4ºC. Samples were stained for 45 min and 60 min; arrows are pointing to air cells.

5.0 Conclusions

The characteristics of cultured butter processed using a small kitchen-scale churning equipment was well accepted by consumer sensory panellists. The dominant colour (yellowness) of the butter samples decreased ($P<0.05$) during storage due to possible reduction of β-carotene. The texture of the butter was slightly harder and more elastic by the end of storage. Overall, the moisture content of the butter samples was higher than expected, presumably due to the efficiency of equipment and manual production. The average size of water droplets (~5 μm), which were successfully measured by the confocal laser scanning microscope method modified in this study, was within the expected range. The droplets of water were however unevenly distributed in the butter. Butter was worked manually and this could have resulted in the poor distribution of water droplets.

6.0 Recommendations

The following recommendations are suggested for future work:

1. Small-scale prototypes were made manually in the present study by culinary churning machine, which may produce uneven products. Information about the iodine value of cream would aid the production of better quality butter. In this study, iodine value of the cream was not measured due to laboratory restrictions, it is however recommended in future experiments.

2. In this study, cream was cooled by replacing frozen ice packs in a water bath. More advanced cooling system is recommended to decrease the temperature rapidly.

3. In this study, the cultured butter was produced only in late winter, early spring, and mid spring, further studies can prolong the production period to the summer and autumn.

4. More analyses, such as components of fatty acids in butter, components of aroma compounds in butter, fat crystals in butter, could be included in further studies, to determine the microstructure and properties of butter.

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Appendix

Appendix 1.0 Characterisation of cream, buttermilk and cultured butter

Table I pH of cream, buttermilk, and cultured butter at different temperature treatment during butter processing (n=2).

Batch	Fresh Cream 4℃	Cream 8℃	Cream 21℃	Cream 15℃	Buttermilk 15℃	Final product 15℃
1	6.58	6.52	6.46	6.42	6.31	6.31
1	6.58	6.53	6.45	6.42	6.31	6.31
2	6.55	6.55	6.54	6.47	6.30	6.30
2	6.55	6.55	6.54	6.46	6.30	6.30
3	6.55	6.52	6.48	6.46	6.40	6.29
3	6.55	6.52	6.48	6.46	6.40	6.28

Table II Mean±SD pH of cream, buttermilk, and cultured butter at different temperature treatment during butter processing (n=2).

Batch	Fresh Cream 4℃	Cream 8℃	Cream 21℃	Cream 15℃	Buttermilk 15℃	Final product 15℃
1	6.580±0	6.525±0.007	6.455±0.007	6.420±0	6.310±0	6.310±0
2	6.550±0	6.550±0	6.540±0	6.465±0.007	6.300±0	6.300±0
3	6.550±0	6.520±0	6.480±0	6.460±0	6.400±0	6.285±0.007

Table III pH (n=2) and titratable acidity (%T.A.) (n=3) of cultured butter stored at 4℃ for 21 days.

	Batch	Day 1	Day 7	Day 14	Day 21
pH	1	6.29	6.05	5.93	5.78
pH	1	6.30	6.05	5.92	5.80
pH	2	6.20	5.92	5.47	5.34
pH	2	6.20	5.92	5.48	5.34
pH	3	5.98	5.82	5.81	5.62
pH	3	5.98	5.82	5.81	5.63
T.A.%	1	0.104	0.117	0.146	0.160

T.A.%	1	0.104	0.117	0.148	0.161
T.A.%	1	0.104	0.117	0.147	0.161
T.A.%	2	0.069	0.089	0.094	0.102
T.A.%	2	0.068	0.089	0.095	0.103
T.A.%	2	0.070	0.089	0.095	0.102
T.A.%	3	0.064	0.082	0.104	0.112
T.A.%	3	0.065	0.081	0.104	0.113
T.A.%	3	0.065	0.083	0.105	0.112

Table IV Mean±SD pH (n=2) and titratable acidity (%T.A.) (n=3) of cultured butter stored at 4℃ for 21 days.

	Batch	Day 1	Day 7	Day 14	Day 21
pH	1	6.30±0.007	6.05±0	5.93±0.007	5.79±0.014
	2	6.20±0	5.92±0	5.48±0.007	5.34±0
	3	5.98±0	5.82±0	5.81±0	5.63±0.007
T.A.%	1	0.104±0	0.117±0.001	0.147±0.001	0.161±0.001
	2	0.069±0.001	0.089±0	0.095±0.001	0.102±0.001
	3	0.065±0.001	0.082±0.001	0.104±0.001	0.112±0.001

Table V %Fat in buttermilk (n=3).

Batch	% Fat in buttermilk	Mean±SD
1	5.14	
1	3.91	4.41±0.64
1	4.19	
2	2.45	
2	2.65	2.47±0.17
2	2.32	
3	4.20	
3	4.08	4.12±0.07
3	4.08	

Table VI %Fat in cream (n=3).

Batch	% Fat in cream	Mean±SD
1	39.47	
1	39.37	39.75±0.57
1	40.40	
2	40.06	

2	40.14	39.96±0.25
2	39.67	
3	39.81	
3	39.74	39.94±0.28
3	40.26	

Table VII Hunter Lab values of cultured butter during storage days at 4ºC (n=3).

Batch	Storage days	L	a	b
1	1	95.41	0.39	3.24
1	1	94.56	1.44	3.96
1	1	95.11	1.05	4.67
2	1	92.71	0.36	0.57
2	1	92.75	1.27	1.72
2	1	92.63	3.95	2.92
3	1	94.18	0.80	0.85
3	1	98.83	0.65	1.04
3	1	99.13	0.49	1.71
1	7	97.82	0.66	2.26
1	7	95.39	0.75	5.74
1	7	95.80	1.56	7.46
2	7	93.99	0.46	1.61
2	7	91.74	1.18	0.47
2	7	93.56	1.62	1.19
3	7	96.82	0.50	1.35
3	7	95.91	0.31	2.31
3	7	98.11	1.74	-2.03
1	14	94.69	0.42	1.43
1	14	94.33	1.07	0.81
1	14	94.34	1.32	0.56
2	14	95.31	0.18	2.14
2	14	99.96	0.68	4.55
2	14	97.00	0.69	4.97
3	14	95.42	0.34	1.95
3	14	94.33	0.63	1.09
3	14	93.86	0.26	2.57
1	21	94.96	0.73	1.07
1	21	93.16	0.84	0.69
1	21	94.51	1.70	1.36
2	21	92.14	2.11	-2.71
2	21	92.27	1.61	-1.90
2	21	92.85	1.93	-2.67
3	21	93.14	1.13	-0.07

3	21	90.65	1.25	-0.62
3	21	95.38	0.63	1.46

Table VIII Mean±SD Hunter Lab values of cultured butter during storage days at 4ºC (n=3).

Batch	Storage days	L	a	b
1	1	95.03±0.43	0.96±0.53	3.96±0.72
1	7	96.34±1.30	0.99±0.50	5.15±2.65
1	14	94.45±0.21	0.94±0.46	0.93±0.45
1	21	94.21±0.94	1.09±0.53	1.04±0.34
2	1	92.70±0.06	1.86±1.87	1.74±1.18
2	7	93.10±1.19	1.09±0.59	1.09±0.58
2	14	97.42±2.35	0.52±0.29	3.89±1.53
2	21	92.42±0.38	1.88±0.25	-2.43±0.46
3	1	97.38±2.78	0.65±0.16	1.20±0.45
3	7	96.95±1.11	0.85±0.78	0.54±2.28
3	14	94.54±0.80	0.41±0.19	1.87±0.74
3	21	93.06±2.37	1.00±0.33	0.26±1.08

Table IX Moisture content, SNF content, and fat content (%) of three batches of cultured butter during storage days at 4ºC (n=3).

%	Batch	Day 1	Day 7	Day 14	Day 21
Moisture	1	19.26	18.92	18.91	19.26
Moisture	1	19.01	19.23	19.37	18.99
Moisture	1	19.96	19.09	19.16	19.07
Moisture	2	13.80	13.88	13.99	14.01
Moisture	2	14.03	13.39	13.93	13.61
Moisture	2	14.19	13.62	14.03	14.29
Moisture	3	18.86	18.80	18.55	17.99
Moisture	3	18.86	18.50	18.87	18.62
Moisture	3	18.94	18.94	18.86	18.49
SNF	1	6.33	6.35	5.11	5.32
SNF	1	6.38	5.16	5.63	5.39
SNF	1	6.26	4.93	5.85	4.87
SNF	2	7.00	9.15	7.06	6.66
SNF	2	7.23	9.16	6.55	6.72
SNF	2	6.47	8.33	6.86	6.46
SNF	3	7.04	6.22	6.67	6.22
SNF	3	6.56	5.65	7.09	5.98

SNF	3	5.74	6.09	6.64	6.31
Fat	1	74.41	74.73	75.98	76.06
Fat	1	74.61	75.98	75.00	75.62
Fat	1	73.78	75.61	74.99	75.42
Fat	2	79.20	78.05	79.15	79.33
Fat	2	78.74	77.45	79.52	79.67
Fat	2	79.34	76.97	78.91	79.25
Fat	3	74.10	74.98	74.78	75.79
Fat	3	74.58	75.85	74.04	75.40
Fat	3	75.32	74.97	74.50	75.20

Table X Mean±SD moisture content, SNF content, and fat content (%) of three batches of cultured butter during storage days at 4ºC (n=3).

%	Batch	Day 1	Day 7	Day 14	Day 21
Moisture	1	19.41±0.49	19.08±0.16	19.15±0.23	19.11±0.14
	2	14.01±0.20	13.63±0.25	13.98±0.05	13.97±0.34
	3	18.89±0.05	18.75±0.22	18.76±0.18	18.37±0.33
SNF	1	6.32±0.06	5.48±0.76	5.53±0.38	5.19±0.28
	2	6.90±0.39	8.88±0.48	6.82±0.26	6.61±0.14
	3	6.45±0.66	5.99±0.30	6.80±0.25	6.17±0.17
Fat	1	74.27±0.43	75.44±0.64	75.32±0.57	75.70±0.33
	2	79.09±0.31	77.49±0.54	79.19±0.31	79.42±0.22
	3	74.67±0.61	75.27±0.51	74.44±0.37	75.46±0.30

Table XI Six sensory attributes of batch 2 cultured butter during storage days at 4ºC (n=30).

Storage days	Smoothness	Hardness	Spreadability	Melting rate	Buttery flavour	Overall acceptance
2	4	7	3	4	3	4
2	6	4	2	3	1	3
2	9	9	7	9	7	8
2	7	8	8	8	8	8
2	9	9	8	9	8	9
2	7	7	8	8	5	7
2	9	7	7	8	8	8
2	7	7	4	6	6	6
2	8	8	4	8	3	6
2	6	4	2	7	5	5
2	7	8	7	9	6	7
2	6	7	6	7	7	7
2	7	6	7	8	7	7

2	8	7	3	9	7	7
2	7	3	1	7	8	5
2	8	9	8	6	6	7
2	4	3	7	8	6	6
2	9	8	9	9	7	8
2	5	6	3	7	2	5
2	7	6	5	9	8	7
2	7	8	5	7	7	7
2	3	8	1	4	5	4
2	5	4	3	6	6	5
2	7	8	9	9	7	8
2	3	4	2	7	7	5
2	4	7	7	8	8	7
2	9	5	2	9	7	6
2	8	5	4	8	6	6
2	8	6	5	7	7	7
2	8	9	9	8	7	8
8	9	8	7	8	9	8
8	8	7	6	7	8	7
8	7	8	8	6	5	7
8	9	7	6	9	8	8
8	8	9	7	9	7	8
8	7	4	3	7	7	6
8	3	4	6	8	8	6
8	4	7	4	8	8	6
8	8	8	8	8	8	8
8	7	6	8	7	7	7
8	7	8	9	7	8	8
8	8	4	8	7	8	7
8	9	9	3	5	7	7
8	8	8	7	8	8	8
8	8	8	8	9	9	8
8	8	6	6	5	7	6
8	8	9	6	8	8	8
8	7	6	4	8	8	7
8	9	8	3	9	8	7
8	9	7	7	9	4	7
8	8	7	6	8	8	7
8	9	7	9	8	9	8
8	9	9	9	9	6	8
8	8	8	8	8	8	8
8	7	7	4	9	8	7
8	9	7	9	8	7	8
8	6	4	4	6	5	5

8	7	6	6	7	6	6
8	6	3	3	8	8	6
8	7	5	5	7	7	6
8	6	5	6	6	6	6
15	6	5	4	6	8	7
15	8	8	6	8	7	8
15	7	7	6	8	8	7
15	9	8	9	9	8	8
15	7	8	5	7	8	7
15	8	6	6	8	9	7
15	8	9	8	8	7	8
15	5	6	4	7	7	7
15	7	8	7	7	8	8
15	7	6	7	7	7	8
15	8	9	5	8	9	8
15	7	7	8	8	7	8
15	8	8	8	5	6	7
15	7	9	6	6	8	7
15	7	8	7	7	8	8
15	5	6	6	7	4	6
15	7	7	8	6	7	8
15	6	6	6	7	7	7
15	9	7	6	9	8	8
15	7	9	9	9	8	8
15	8	8	8	8	7	7
15	4	8	6	5	8	6
15	8	8	8	8	6	7
15	9	7	7	6	7	8
15	8	8	8	7	7	8
15	5	6	4	6	5	6
15	7	6	6	7	7	7
15	8	7	5	8	7	7
15	9	7	6	8	7	8
15	8	8	7	7	8	8
22	7	6	6	6	6	6
22	8	7	4	6	8	7
22	4	7	3	8	3	5
22	4	5	4	8	3	5
22	8	8	9	9	5	7
22	9	8	7	9	9	9
22	6	6	4	4	6	6
22	6	7	6	7	7	7
22	8	6	4	6	9	7
22	8	5	5	9	9	7

22	7	7	6	6	6	6
22	7	7	8	8	7	8
22	7	5	6	7	8	8.5
22	8	5	6	5	7	7
22	7	6	5	6	2	5
22	7	8	6	7	7	7
22	8	6	7	8	8	8
22	7	6	6	7	7	7
22	8	2	5	8	7	8
22	7	3	6	6	8	7
22	5	6	4	7	4	7
22	9	8	8	9	9	8
22	8	7	6	9	8	8
22	8	9	7	8	6	8
22	8	7	9	7	8	8
22	8	9	9	8	8	8
22	7	7	7	7	7	7
22	6	6	4	6	6	6
22	8	7	8	8	9	9
22	6	5	6	7	7	7

Table XII Mean±SD six sensory attributes of batch 2 cultured butter during storage days at 4°C (n=30).

Storage days	Smoothness	Hardness	Spreadability	Melting rate	Buttery flavour	Overall acceptance
2	6.73±1.80	6.57±1.83	5.20±2.59	7.40±1.59	6.17±1.82	6.41±1.43
8	7.52±1.43	5.93±1.69	5.79±1.96	7.58±1.15	6.83±1.20	7.09±0.95
15	7.23±1.28	7.33±1.09	6.53±1.41	7.23±1.07	7.27±1.05	7.40±0.67
22	7.13±1.25	6.37±1.54	6.03±1.65	7.20±1.27	6.80±1.86	7.12±1.08

Table XIII Hardness, springiness and adhesiveness of cultured butter stored at 4ºC for 21 days (n=3).

Attributes	Batch	Day 1	Day 7	Day 14	Day 21
Hardness	1	1352.43	3146.57	1889.01	1663.09
Hardness	1	1446.37	3620.37	2214.95	1578.93
Hardness	2	2638.90	3156.17	3127.99	2499.46
Hardness	2	2410.36	2987.02	3172.34	2885.78
Hardness	3	2421.89	2861.33	2902.05	2951.51
Hardness	3	2123.54	2175.34	2958.72	2633.33
Adhesiveness	1	-694.69	-598.01	-350.27	-364.02
Adhesiveness	1	-127.78	-107.42	-335.64	-410.70

Adhesiveness	2	-486.99	-681.01	-297.65	-263.76
Adhesiveness	2	-220.04	-346.96	-918.28	-257.33
Adhesiveness	3	-127.12	-322.55	-154.06	-168.95
Adhesiveness	3	-378.40	-57.25	-315.87	-353.52
Springiness	1	0.131	0.214	0.416	0.355
Springiness	1	0.157	0.168	0.394	0.550
Springiness	2	0.426	0.447	0.492	0.569
Springiness	2	0.326	0.291	0.401	0.359
Springiness	3	0.327	0.489	0.382	0.370
Springiness	3	0.394	0.311	0.370	0.432

Table XIV Mean±SD hardness, springiness, and adhesiveness of cultured butter stored at 4ºC for 21 days (n=3).

Attributes	Batch	Day 1	Day 7	Day 14	Day 21
Hardness	1	1399.40±66.43	3383.47±335.03	2051.98±230.48	1621.01±59.51
	2	2524.63±161.60	3071.59±119.60	3150.16±31.36	2692.62±273.17
	3	2272.72±210.96	2518.34±485.07	2930.39±40.07	2792.42±224.98
Adhesiveness	1	-411.23±400.87	-352.72±346.90	-342.96±10.34	-387.36±33.00
	2	-353.51±188.76	-513.99±236.21	-607.97±438.85	-260.54±4.55
	3	-252.76±177.68	-189.90±187.60	-234.97±114.41	-261.24±130.51
Springiness	1	0.144±0.02	0.191±0.03	0.405±0.02	0.4525±0.14
	2	0.376±0.07	0.369±0.11	0.4465±0.06	0.464±0.15
	3	0.3605±0.05	0.4±0.13	0.376±0.01	0.401±0.04

Appendix 2.0 Sensory evaluation test forms

SENSORY EVALUATION INFORMATION SHEET

Induction

My name is Jia Shi and I am a Master of Food Technology student at the Institute of Food, Nutrition and Human Health (IFNHH), Massey University, Albany Campus. This study is part of my research project and may contribute to the development of a commercial product. You are invited to take part in a study that assesses some characteristics of cultured butter. The aim of this consumer study is to evaluate the acceptance of the products.

Participant involvement

The trial involves tasting and evaluating unsalted cultured butter. The cultured butter you will taste will contain all of the following ingredients: full fat cream and a commercial butter culture. Your participation will take from 3 to 5 minutes.

SENSORY EVALUATION PARTICIPANT CONSENT FORM – INDIVIDUAL

Project title: Technology of cultured butter production

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further question at any time.

I understand that I have the right to withdraw from the study at any time and to decline to answer any questions.

I agree to voluntarily participate in this study under the conditions set out in the Information Sheet.

Full name: ………………………………………………………………..

Signature………………………………………………………………..

Date: …………………………………………………………………

Consumer Acceptance Test of Cultured Butter

Instructions

- You will be provided with a cultured sample of butter.
- Please rinse your mouth before tasting using the water provided.
- Please evaluate the attributes of the sample using the 1 – 9-point scale and place a tick (√) in the appropriate box of each characteristic:

Please use the following rating scale to express your degree of liking of the butter that you are tasting:

9- like extremely
8-like very much
7-like moderately
6-like slightly
5-neither like nor dislike
4-dislike slightly
3-dislike moderately
2-dislike very much
1- dislike extremely

Sensory Evaluation of Cultured Butter

***Smoothness*: How do you like the smoothness of the butter?**

☐	☐	☐	☐	☐	☐	☐	☐	☐
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

***Hardness*: How do you like the hardness of the butter when cutting with knife?**

☐	☐	☐	☐	☐	☐	☐	☐	☐
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

***Spreadability*: How do you like the spreadability of the butter on the cracker?**

☐	☐	☐	☐	☐	☐	☐	☐	☐
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

***Melting rate*: How do you like the melting of the butter from solid to liquid in your mouth?**

☐	☐	☐	☐	☐	☐	☐	☐	☐
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

***Buttery flavour*: How do you like the flavour of the butter?**

☐	☐	☐	☐	☐	☐	☐	☐	☐
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Have you detected any off-flavours (unpleasant flavour) in the butter: Yes ☐ No ☐

Overall acceptance (use scale 1-9): ______________________________________

Thank you for your help

Appendix 3.0 Statistical outputs

pH

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cream_8_degree_Celsius	Between Groups	.001	2	.001	31.000	.010
	Within Groups	.000	3	.000		
	Total	.001	5			
Cream_21_degree_Celsius	Between Groups	.008	2	.004	229.000	.001
	Within Groups	.000	3	.000		
	Total	.008	5			
Cream_15_degree_Celsius	Between Groups	.002	2	.001	73.000	.003
	Within Groups	.000	3	.000		
	Total	.002	5			
Buttermilk_15_degree_Celsius	Between Groups	.012	2	.006	.	.
	Within Groups	.000	3	.000		
	Total	.012	5			
Final product_15_degree_Celsius	Between Groups	.001	2	.000	19.000	.020
	Within Groups	.000	3	.000		
	Total	.001	5			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cream_8_degree_Celsius	1	2	-.02500*	.00408	.018	-.0421	-.0079
		3	.00500	.00408	.518	-.0121	.0221
	2	1	.02500*	.00408	.018	.0079	.0421
		3	.03000*	.00408	.011	.0129	.0471
	3	1	-.00500	.00408	.518	-.0221	.0121
		2	-.03000*	.00408	.011	-.0471	-.0129
Cream_21_degree_Celsius	1	2	-.08500*	.00408	.000	-.1021	-.0679
		3	-.02500*	.00408	.018	-.0421	-.0079
	2	1	.08500*	.00408	.000	.0679	.1021
		3	.06000*	.00408	.001	.0429	.0771
	3	1	.02500*	.00408	.018	.0079	.0421
		2	-.06000*	.00408	.001	-.0771	-.0429
Cream_15_degree_Celsius	1	2	-.04500*	.00408	.003	-.0621	-.0279
		3	-.04000*	.00408	.005	-.0571	-.0229
	2	1	.04500*	.00408	.003	.0279	.0621
		3	.00500	.00408	.518	-.0121	.0221
	3	1	.04000*	.00408	.005	.0229	.0571
		2	-.00500	.00408	.518	-.0221	.0121
Final product_15_degree_Celsius	1	2	.01000	.00408	.175	-.0071	.0271
		3	.02500*	.00408	.018	.0079	.0421
	2	1	-.01000	.00408	.175	-.0271	.0071
		3	.01500	.00408	.069	-.0021	.0321
	3	1	-.02500*	.00408	.018	-.0421	-.0079
		2	-.01500	.00408	.069	-.0321	.0021

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	.104	2	.052	3133.000	.000
	Within Groups	.000	3	.000		
	Total	.104	5			
D7	Between Groups	.053	2	.027	.	.
	Within Groups	.000	3	.000		
	Total	.053	5			
D14	Between Groups	.219	2	.109	3279.500	.000
	Within Groups	.000	3	.000		
	Total	.219	5			
D21	Between Groups	.207	2	.104	1243.800	.000
	Within Groups	.000	3	.000		
	Total	.208	5			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	.09500*	.00408	.000	.0779	.1121
		3	.31500*	.00408	.000	.2979	.3321
	2	1	-.09500*	.00408	.000	-.1121	-.0779
		3	.22000*	.00408	.000	.2029	.2371
	3	1	-.31500*	.00408	.000	-.3321	-.2979
		2	-.22000*	.00408	.000	-.2371	-.2029
D14	1	2	.45000*	.00577	.000	.4259	.4741
		3	.11500*	.00577	.001	.0909	.1391
	2	1	-.45000*	.00577	.000	-.4741	-.4259
		3	-.33500*	.00577	.000	-.3591	-.3109
	3	1	-.11500*	.00577	.001	-.1391	-.0909
		2	.33500*	.00577	.000	.3109	.3591
D21	1	2	.45000*	.00913	.000	.4119	.4881
		3	.16500*	.00913	.001	.1269	.2031
	2	1	-.45000*	.00913	.000	-.4881	-.4119
		3	-.28500*	.00913	.000	-.3231	-.2469
	3	1	-.16500*	.00913	.001	-.2031	-.1269
		2	.28500*	.00913	.000	.2469	.3231

*. The mean difference is significant at the 0.05 level.

Titratable acidity

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	.003	2	.001	1910.286	.000
	Within Groups	.000	6	.000		
	Total	.003	8			
D7	Between Groups	.002	2	.001	2557.750	.000
	Within Groups	.000	6	.000		
	Total	.002	8			
D14	Between Groups	.005	2	.002	4536.600	.000
	Within Groups	.000	6	.000		
	Total	.005	8			
D21	Between Groups	.006	2	.003	9410.333	.000
	Within Groups	.000	6	.000		
	Total	.006	8			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	.034668*	.000693	.000	.03254	.03680
		3	.039162*	.000693	.000	.03703	.04129
	2	1	-.034668*	.000693	.000	-.03680	-.03254
		3	.004494*	.000693	.002	.00237	.00662
	3	1	-.039162*	.000693	.000	-.04129	-.03703
		2	-.004494*	.000693	.002	-.00662	-.00237
D7	1	2	.028569*	.000524	.000	.02696	.03018
		3	.035310*	.000524	.000	.03370	.03692
	2	1	-.028569*	.000524	.000	-.03018	-.02696
		3	.006741*	.000524	.000	.00513	.00835
	3	1	-.035310*	.000524	.000	-.03692	-.03370
		2	-.006741*	.000524	.000	-.00835	-.00513
D14	1	2	.052323*	.000586	.000	.05052	.05412
		3	.043014*	.000586	.000	.04122	.04481
	2	1	-.052323*	.000586	.000	-.05412	-.05052
		3	-.009309*	.000586	.000	-.01111	-.00751
	3	1	-.043014*	.000586	.000	-.04481	-.04122
		2	.009309*	.000586	.000	.00751	.01111
D21	1	2	.058101*	.000454	.000	.05671	.05949
		3	.048471*	.000454	.000	.04708	.04986
	2	1	-.058101*	.000454	.000	-.05949	-.05671
		3	-.009630*	.000454	.000	-.01102	-.00824
	3	1	-.048471*	.000454	.000	-.04986	-.04708
		2	.009630*	.000454	.000	.00824	.01102

*. The mean difference is significant at the 0.05 level.

Moisture content

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	53.284	2	26.642	282.359	.000
	Within Groups	.566	6	.094		
	Total	53.850	8			
D7	Between Groups	55.994	2	27.997	623.386	.000
	Within Groups	.269	6	.045		
	Total	56.263	8			
D14	Between Groups	49.626	2	24.813	839.539	.000
	Within Groups	.177	6	.030		
	Total	49.803	8			
D21	Between Groups	46.264	2	23.132	281.333	.000
	Within Groups	.493	6	.082		
	Total	46.757	8			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	5.40333*	.25081	.000	4.6338	6.1729
		3	.52333	.25081	.173	-.2462	1.2929
	2	1	-5.40333*	.25081	.000	-6.1729	-4.6338
		3	-4.88000*	.25081	.000	-5.6495	-4.1105
	3	1	-.52333	.25081	.173	-1.2929	.2462
		2	4.88000*	.25081	.000	4.1105	5.6495
D7	1	2	5.45000*	.17303	.000	4.9191	5.9809
		3	.33333	.17303	.212	-.1976	.8642
	2	1	-5.45000*	.17303	.000	-5.9809	-4.9191
		3	-5.11667*	.17303	.000	-5.6476	-4.5858
	3	1	-.33333	.17303	.212	-.8642	.1976
		2	5.11667*	.17303	.000	4.5858	5.6476
D14	1	2	5.16333*	.14037	.000	4.7326	5.5940
		3	.38667	.14037	.074	-.0440	.8174
	2	1	-5.16333*	.14037	.000	-5.5940	-4.7326
		3	-4.77667*	.14037	.000	-5.2074	-4.3460
	3	1	-.38667	.14037	.074	-.8174	.0440
		2	4.77667*	.14037	.000	4.3460	5.2074
D21	1	2	5.13667*	.23413	.000	4.4183	5.8550
		3	.74000*	.23413	.045	.0216	1.4584
	2	1	-5.13667*	.23413	.000	-5.8550	-4.4183
		3	-4.39667*	.23413	.000	-5.1150	-3.6783
	3	1	-.74000*	.23413	.045	-1.4584	-.0216
		2	4.39667*	.23413	.000	3.6783	5.1150

*. The mean difference is significant at the 0.05 level.

Solids-not-fat content

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	.553	2	.277	1.412	.314
	Within Groups	1.175	6	.196		
	Total	1.729	8			
D7	Between Groups	20.188	2	10.094	33.758	.001
	Within Groups	1.794	6	.299		
	Total	21.982	8			
D14	Between Groups	3.286	2	1.643	18.007	.003
	Within Groups	.547	6	.091		
	Total	3.834	8			
D21	Between Groups	3.167	2	1.583	37.325	.000
	Within Groups	.255	6	.042		
	Total	3.421	8			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	-.57667	.36138	.318	-1.6855	.5321
		3	-.12333	.36138	.938	-1.2321	.9855
	2	1	.57667	.36138	.318	-.5321	1.6855
		3	.45333	.36138	.468	-.6555	1.5621
	3	1	.12333	.36138	.938	-.9855	1.2321
		2	-.45333	.36138	.468	-1.5621	.6555
D7	1	2	-3.40000*	.44648	.001	-4.7699	-2.0301
		3	-.50667	.44648	.530	-1.8766	.8632
	2	1	3.40000*	.44648	.001	2.0301	4.7699
		3	2.89333*	.44648	.002	1.5234	4.2632
	3	1	.50667	.44648	.530	-.8632	1.8766
		2	-2.89333*	.44648	.002	-4.2632	-1.5234
D14	1	2	-1.29333*	.24664	.005	-2.0501	-.5366
		3	-1.27000*	.24664	.005	-2.0267	-.5133
	2	1	1.29333*	.24664	.005	.5366	2.0501
		3	.02333	.24664	.995	-.7334	.7801
	3	1	1.27000*	.24664	.005	.5133	2.0267
		2	-.02333	.24664	.995	-.7801	.7334
D21	1	2	-1.42000*	.16817	.000	-1.9360	-.9040
		3	-.97667*	.16817	.003	-1.4927	-.4607
	2	1	1.42000*	.16817	.000	.9040	1.9360
		3	.44333	.16817	.086	-.0727	.9593
	3	1	.97667*	.16817	.003	.4607	1.4927
		2	-.44333	.16817	.086	-.9593	.0727

*. The mean difference is significant at the 0.05 level.

Fat content

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	43.052	2	21.526	97.271	.000
	Within Groups	1.328	6	.221		
	Total	44.380	8			
D7	Between Groups	9.176	2	4.588	14.332	.005
	Within Groups	1.921	6	.320		
	Total	11.096	8			
D14	Between Groups	38.351	2	19.176	103.194	.000
	Within Groups	1.115	6	.186		
	Total	39.466	8			
D21	Between Groups	29.498	2	14.749	179.165	.000
	Within Groups	.494	6	.082		
	Total	29.992	8			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	-4.82667*	.38410	.000	-6.0052	-3.6481
		3	-.40000	.38410	.580	-1.5785	.7785
	2	1	4.82667*	.38410	.000	3.6481	6.0052
		3	4.42667*	.38410	.000	3.2481	5.6052
	3	1	.40000	.38410	.580	-.7785	1.5785
		2	-4.42667*	.38410	.000	-5.6052	-3.2481
D7	1	2	-2.05000*	.46196	.010	-3.4674	-.6326
		3	.17333	.46196	.926	-1.2441	1.5908
	2	1	2.05000*	.46196	.010	.6326	3.4674
		3	2.22333*	.46196	.007	.8059	3.6408
	3	1	-.17333	.46196	.926	-1.5908	1.2441
		2	-2.22333*	.46196	.007	-3.6408	-.8059
D14	1	2	-3.87000*	.35197	.000	-4.9499	-2.7901
		3	.88333	.35197	.101	-.1966	1.9633
	2	1	3.87000*	.35197	.000	2.7901	4.9499
		3	4.75333*	.35197	.000	3.6734	5.8333
	3	1	-.88333	.35197	.101	-1.9633	.1966
		2	-4.75333*	.35197	.000	-5.8333	-3.6734
D21	1	2	-3.71667*	.23427	.000	-4.4355	-2.9979
		3	.23667	.23427	.598	-.4821	.9555
	2	1	3.71667*	.23427	.000	2.9979	4.4355
		3	3.95333*	.23427	.000	3.2345	4.6721
	3	1	-.23667	.23427	.598	-.9555	.4821
		2	-3.95333*	.23427	.000	-4.6721	-3.2345

*. The mean difference is significant at the 0.05 level.

Colour

ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
HunterL	Between Groups	32.901	2	16.450	6.253	.034
	Within Groups	15.784	6	2.631		
	Total	48.685	8			
a	Between Groups	2.380	2	1.190	.942	.441
	Within Groups	7.578	6	1.263		
	Total	9.958	8			
b	Between Groups	12.816	2	6.408	9.171	.015
	Within Groups	4.192	6	.699		
	Total	17.008	8			

a. Days = 1

Multiple Comparisons[a]

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HunterL	1	2	2.33000	1.32431	.260	-1.7333	6.3933
		3	-2.35333	1.32431	.255	-6.4167	1.7100
	2	1	-2.33000	1.32431	.260	-6.3933	1.7333
		3	-4.68333*	1.32431	.028	-8.7467	-.6200
	3	1	2.35333	1.32431	.255	-1.7100	6.4167
		2	4.68333*	1.32431	.028	.6200	8.7467
a	1	2	-.90000	.91759	.614	-3.7154	1.9154
		3	.31333	.91759	.938	-2.5021	3.1287
	2	1	.90000	.91759	.614	-1.9154	3.7154
		3	1.21333	.91759	.435	-1.6021	4.0287
	3	1	-.31333	.91759	.938	-3.1287	2.5021
		2	-1.21333	.91759	.435	-4.0287	1.6021
b	1	2	2.22000*	.68251	.040	.1259	4.3141
		3	2.75667*	.68251	.016	.6625	4.8508
	2	1	-2.22000*	.68251	.040	-4.3141	-.1259
		3	.53667	.68251	.724	-1.5575	2.6308
	3	1	-2.75667*	.68251	.016	-4.8508	-.6625
		2	-.53667	.68251	.724	-2.6308	1.5575

*. The mean difference is significant at the 0.05 level.

a. Days = 1

ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
HunterL	Between Groups	25.692	2	12.846	8.878	.016
	Within Groups	8.682	6	1.447		
	Total	34.374	8			
a	Between Groups	.085	2	.042	.107	.900
	Within Groups	2.383	6	.397		
	Total	2.468	8			
b	Between Groups	38.062	2	19.031	4.550	.063
	Within Groups	25.095	6	4.182		
	Total	63.157	8			

a. Days = 7

Multiple Comparisons[a]

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HunterL	1	2	3.24000*	.98216	.038	.2265	6.2535
		3	-.61000	.98216	.814	-3.6235	2.4035
	2	1	-3.24000*	.98216	.038	-6.2535	-.2265
		3	-3.85000*	.98216	.018	-6.8635	-.8365
	3	1	.61000	.98216	.814	-2.4035	3.6235
		2	3.85000*	.98216	.018	.8365	6.8635
a	1	2	-.09667	.51462	.981	-1.6756	1.4823
		3	.14000	.51462	.960	-1.4390	1.7190
	2	1	.09667	.51462	.981	-1.4823	1.6756
		3	.23667	.51462	.892	-1.3423	1.8156
	3	1	-.14000	.51462	.960	-1.7190	1.4390
		2	-.23667	.51462	.892	-1.8156	1.3423
b	1	2	4.06333	1.66983	.111	-1.0602	9.1868
		3	4.61000	1.66983	.073	-.5135	9.7335
	2	1	-4.06333	1.66983	.111	-9.1868	1.0602
		3	.54667	1.66983	.943	-4.5768	5.6702
	3	1	-4.61000	1.66983	.073	-9.7335	.5135
		2	-.54667	1.66983	.943	-5.6702	4.5768

*. The mean difference is significant at the 0.05 level.

a. Days = 7

ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
HunterL	Between Groups	17.161	2	8.580	4.137	.074
	Within Groups	12.445	6	2.074		
	Total	29.606	8			
a	Between Groups	.465	2	.233	2.060	.208
	Within Groups	.678	6	.113		
	Total	1.143	8			
b	Between Groups	13.666	2	6.833	6.644	.030
	Within Groups	6.171	6	1.028		
	Total	19.837	8			

a. Days = 14

Multiple Comparisons[a]

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HunterL	1	2	-2.97000	1.17592	.099	-6.5780	.6380
		3	-.08333	1.17592	.997	-3.6914	3.5247
	2	1	2.97000	1.17592	.099	-.6380	6.5780
		3	2.88667	1.17592	.108	-.7214	6.4947
	3	1	.08333	1.17592	.997	-3.5247	3.6914
		2	-2.88667	1.17592	.108	-6.4947	.7214
a	1	2	.42000	.27437	.343	-.4219	1.2619
		3	.52667	.27437	.214	-.3152	1.3685
	2	1	-.42000	.27437	.343	-1.2619	.4219
		3	.10667	.27437	.921	-.7352	.9485
	3	1	-.52667	.27437	.214	-1.3685	.3152
		2	-.10667	.27437	.921	-.9485	.7352
b	1	2	-2.95333*	.82802	.027	-5.4939	-.4127
		3	-.93667	.82802	.532	-3.4773	1.6039
	2	1	2.95333*	.82802	.027	.4127	5.4939
		3	2.01667	.82802	.111	-.5239	4.5573
	3	1	.93667	.82802	.532	-1.6039	3.4773
		2	-2.01667	.82802	.111	-4.5573	.5239

*. The mean difference is significant at the 0.05 level.

a. Days = 14

ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
HunterL	Between Groups	4.940	2	2.470	1.119	.386
	Within Groups	13.238	6	2.206		
	Total	18.177	8			
a	Between Groups	1.411	2	.706	4.659	.060
	Within Groups	.909	6	.151		
	Total	2.320	8			
b	Between Groups	19.832	2	9.916	20.059	.002
	Within Groups	2.966	6	.494		
	Total	22.798	8			

a. Days = 21

Multiple Comparisons[a]

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
HunterL	1	2	1.79000	1.21279	.365	-1.9312	5.5112
		3	1.15333	1.21279	.631	-2.5678	4.8745
	2	1	-1.79000	1.21279	.365	-5.5112	1.9312
		3	-.63667	1.21279	.862	-4.3578	3.0845
	3	1	-1.15333	1.21279	.631	-4.8745	2.5678
		2	.63667	1.21279	.862	-3.0845	4.3578
a	1	2	-.79333	.31776	.103	-1.7683	.1816
		3	.08667	.31776	.960	-.8883	1.0616
	2	1	.79333	.31776	.103	-.1816	1.7683
		3	.88000	.31776	.073	-.0950	1.8550
	3	1	-.08667	.31776	.960	-1.0616	.8883
		2	-.88000	.31776	.073	-1.8550	.0950
b	1	2	3.46667*	.57406	.002	1.7053	5.2280
		3	.78333	.57406	.415	-.9780	2.5447
	2	1	-3.46667*	.57406	.002	-5.2280	-1.7053
		3	-2.68333*	.57406	.008	-4.4447	-.9220
	3	1	-.78333	.57406	.415	-2.5447	.9780
		2	2.68333*	.57406	.008	.9220	4.4447

*. The mean difference is significant at the 0.05 level.

a. Days = 21

Hardness

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	1394865.361	2	697432.681	27.886	.012
	Within Groups	75030.921	3	25010.307		
	Total	1469896.283	5			
D7	Between Groups	767875.758	2	383937.879	3.183	.181
	Within Groups	361842.550	3	120614.183		
	Total	1129718.308	5			
D14	Between Groups	1350596.261	2	675298.130	36.366	.008
	Within Groups	55708.275	3	18569.425		
	Total	1406304.536	5			
D21	Between Groups	1687009.421	2	843504.711	19.650	.019
	Within Groups	128780.405	3	42926.802		
	Total	1815789.826	5			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	-1125.23350*	158.146474	.012	-1786.08300	-464.38400
		3	-873.319500*	158.146474	.024	-1534.16900	-212.47000
	2	1	1125.233500*	158.146474	.012	464.38400	1786.08300
		3	251.914000	158.146474	.373	-408.93550	912.76350
	3	1	873.319500*	158.146474	.024	212.47000	1534.16900
		2	-251.914000	158.146474	.373	-912.76350	408.93550
D7	1	2	311.876000	347.295528	.678	-1139.37404	1763.12604
		3	865.132500	347.295528	.169	-586.11754	2316.38254
	2	1	-311.876000	347.295528	.678	-1763.12604	1139.37404
		3	553.256500	347.295528	.373	-897.99354	2004.50654
	3	1	-865.132500	347.295528	.169	-2316.38254	586.11754
		2	-553.256500	347.295528	.373	-2004.50654	897.99354
D14	1	2	-1098.18100*	136.269677	.008	-1667.61354	-528.74846
		3	-878.402000*	136.269677	.015	-1447.83454	-308.96946
	2	1	1098.181000*	136.269677	.008	528.74846	1667.61354
		3	219.779000	136.269677	.367	-349.65354	789.21154
	3	1	878.402000*	136.269677	.015	308.96946	1447.83454
		2	-219.779000	136.269677	.367	-789.21154	349.65354
D21	1	2	-1071.60850*	207.187841	.028	-1937.38804	-205.82896
		3	-1171.41300*	207.187841	.022	-2037.19254	-305.63346
	2	1	1071.608500*	207.187841	.028	205.82896	1937.38804
		3	-99.804500	207.187841	.885	-965.58404	765.97504
	3	1	1171.413000*	207.187841	.022	305.63346	2037.19254
		2	99.804500	207.187841	.885	-765.97504	965.58404

*. The mean difference is significant at the 0.05 level.

Springiness

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	.067	2	.034	13.312	.032
	Within Groups	.008	3	.003		
	Total	.075	5			
D7	Between Groups	.051	2	.025	2.626	.219
	Within Groups	.029	3	.010		
	Total	.080	5			
D14	Between Groups	.005	2	.003	1.691	.322
	Within Groups	.004	3	.001		
	Total	.009	5			
D21	Between Groups	.005	2	.002	.157	.861
	Within Groups	.043	3	.014		
	Total	.047	5			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1	2	-.232000*	.050274	.039	-.44208	-.02192
		3	-.216500*	.050274	.046	-.42658	-.00642
	2	1	.232000*	.050274	.039	.02192	.44208
		3	.015500	.050274	.950	-.19458	.22558
	3	1	.216500*	.050274	.046	.00642	.42658
		2	-.015500	.050274	.950	-.22558	.19458
D7	1	2	-.178000	.098434	.307	-.58933	.23333
		3	-.209000	.098434	.232	-.62033	.20233
	2	1	.178000	.098434	.307	-.23333	.58933
		3	-.031000	.098434	.948	-.44233	.38033
	3	1	.209000	.098434	.232	-.20233	.62033
		2	.031000	.098434	.948	-.38033	.44233
D14	1	2	-.041500	.038534	.588	-.20252	.11952
		3	.029000	.038534	.753	-.13202	.19002
	2	1	.041500	.038534	.588	-.11952	.20252
		3	.070500	.038534	.301	-.09052	.23152
	3	1	-.029000	.038534	.753	-.19002	.13202
		2	-.070500	.038534	.301	-.23152	.09052
D21	1	2	-.011500	.119700	.995	-.51169	.48869
		3	.051500	.119700	.906	-.44869	.55169
	2	1	.011500	.119700	.995	-.48869	.51169
		3	.063000	.119700	.865	-.43719	.56319
	3	1	-.051500	.119700	.906	-.55169	.44869
		2	-.063000	.119700	.865	-.56319	.43719

*. The mean difference is significant at the 0.05 level.

Adhesiveness

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
D1	Between Groups	25730.770	2	12865.385	.169	.852
	Within Groups	227894.001	3	75964.667		
	Total	253624.771	5			
D7	Between Groups	105033.180	2	52516.590	.746	.546
	Within Groups	211326.345	3	70442.115		
	Total	316359.525	5			
D14	Between Groups	147347.001	2	73673.501	1.074	.445
	Within Groups	205790.270	3	68596.757		
	Total	353137.271	5			
D21	Between Groups	21326.440	2	10663.220	1.763	.312
	Within Groups	18144.052	3	6048.017		
	Total	39470.492	5			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Batch	(J) Batch	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
D1	1.0	2.0	-57.720500	275.616885	.976	-1209.44571	1094.00471
		3.0	-158.472500	275.616885	.842	-1310.19771	993.25271
	2.0	1.0	57.720500	275.616885	.976	-1094.00471	1209.44571
		3.0	-100.752000	275.616885	.931	-1252.47721	1050.97321
	3.0	1.0	158.472500	275.616885	.842	-993.25271	1310.19771
		2.0	100.752000	275.616885	.931	-1050.97321	1252.47721
D7	1.0	2.0	161.270500	265.409335	.826	-947.80024	1270.34124
		3.0	-162.816500	265.409335	.824	-1271.88724	946.25424
	2.0	1.0	-161.270500	265.409335	.826	-1270.34124	947.80024
		3.0	-324.087000	265.409335	.520	-1433.15774	784.98374
	3.0	1.0	162.816500	265.409335	.824	-946.25424	1271.88724
		2.0	324.087000	265.409335	.520	-784.98374	1433.15774
D14	1.0	2.0	265.009500	261.909825	.620	-829.43777	1359.45677
		3.0	-107.990000	261.909825	.913	-1202.43727	986.45727
	2.0	1.0	-265.009500	261.909825	.620	-1359.45677	829.43777
		3.0	-372.999500	261.909825	.435	-1467.44677	721.44777
	3.0	1.0	107.990000	261.909825	.913	-986.45727	1202.43727
		2.0	372.999500	261.909825	.435	-721.44777	1467.44677
D21	1.0	2.0	-126.815000	77.768999	.361	-451.78971	198.15971
		3.0	-126.123500	77.768999	.364	-451.09821	198.85121
	2.0	1.0	126.815000	77.768999	.361	-198.15971	451.78971
		3.0	.691500	77.768999	1.000	-324.28321	325.66621
	3.0	1.0	126.123500	77.768999	.364	-198.85121	451.09821
		2.0	-.691500	77.768999	1.000	-325.66621	324.28321

Sensory evaluation

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Smoothness	Between Groups	9.575	3	3.192	1.503	.217
	Within Groups	248.442	117	2.123		
	Total	258.017	120			
Hardness	Between Groups	15.626	3	5.209	2.124	.101
	Within Groups	286.935	117	2.452		
	Total	302.562	120			
Spreadability	Between Groups	29.347	3	9.782	2.562	.058
	Within Groups	446.653	117	3.818		
	Total	476.000	120			
Meltingrate	Between Groups	3.278	3	1.093	.663	.576
	Within Groups	192.722	117	1.647		
	Total	196.000	120			
Butteryflavour	Between Groups	26.880	3	8.960	3.855	.011
	Within Groups	271.930	117	2.324		
	Total	298.810	120			
Overallacceptance	Between Groups	15.783	3	5.261	4.611	.004
	Within Groups	133.503	117	1.141		
	Total	149.286	120			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Storagedays	(J) Storagedays	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Smoothness	2	8	-.7828	.3732	.160	-1.755	.190
		15	-.5000	.3762	.546	-1.481	.481
		22	-.4000	.3762	.713	-1.381	.581
	8	2	.7828	.3732	.160	-.190	1.755
		15	.2828	.3732	.873	-.690	1.255
		22	.3828	.3732	.735	-.590	1.355
	15	2	.5000	.3762	.546	-.481	1.481
		8	-.2828	.3732	.873	-1.255	.690
		22	.1000	.3762	.993	-.881	1.081
	22	2	.4000	.3762	.713	-.581	1.381
		8	-.3828	.3732	.735	-1.355	.590
		15	-.1000	.3762	.993	-1.081	.881
Hardness	2	8	-.1753	.4011	.972	-1.221	.870
		15	-.7667	.4043	.235	-1.821	.287
		22	.2000	.4043	.960	-.854	1.254
	8	2	.1753	.4011	.972	-.870	1.221
		15	-.5914	.4011	.456	-1.637	.454
		22	.3753	.4011	.786	-.670	1.421
	15	2	.7667	.4043	.235	-.287	1.821
		8	.5914	.4011	.456	-.454	1.637
		22	.9667	.4043	.084	-.087	2.021
	22	2	-.2000	.4043	.960	-1.254	.854
		8	-.3753	.4011	.786	-1.421	.670
		15	-.9667	.4043	.084	-2.021	.087
Spreadability	2	8	-1.0258	.5004	.176	-2.330	.278
		15	-1.3333*	.5045	.046	-2.648	-.018
		22	-.8333	.5045	.354	-2.148	.482
	8	2	1.0258	.5004	.176	-.278	2.330
		15	-.3075	.5004	.927	-1.612	.997
		22	.1925	.5004	.981	-1.112	1.497
	15	2	1.3333*	.5045	.046	.018	2.648
		8	.3075	.5004	.927	-.997	1.612
		22	.5000	.5045	.755	-.815	1.815
	22	2	.8333	.5045	.354	-.482	2.148
		8	-.1925	.5004	.981	-1.497	1.112
		15	-.5000	.5045	.755	-1.815	.815
Meltingrate	2	8	-.2129	.3287	.916	-1.070	.644
		15	.1667	.3314	.958	-.697	1.030
		22	.2000	.3314	.931	-.664	1.064
	8	2	.2129	.3287	.916	-.644	1.070
		15	.3796	.3287	.656	-.477	1.236
		22	.4129	.3287	.593	-.444	1.270
	15	2	-.1667	.3314	.958	-1.030	.697
		8	-.3796	.3287	.656	-1.236	.477
		22	.0333	.3314	1.000	-.830	.897
	22	2	-.2000	.3314	.931	-1.064	.664
		8	-.4129	.3287	.593	-1.270	.444
		15	-.0333	.3314	1.000	-.897	.830
Butteryflavour	2	8	-1.1882*	.3904	.015	-2.206	-.171
		15	-1.1000*	.3936	.031	-2.126	-.074
		22	-.6333	.3936	.378	-1.659	.393
	8	2	1.1882*	.3904	.015	.171	2.206
		15	.0882	.3904	.996	-.929	1.106
		22	.5548	.3904	.489	-.463	1.572
	15	2	1.1000*	.3936	.031	.074	2.126
		8	-.0882	.3904	.996	-1.106	.929
		22	.4667	.3936	.637	-.559	1.493
	22	2	.6333	.3936	.378	-.393	1.659
		8	-.5548	.3904	.489	-1.572	.463
		15	-.4667	.3936	.637	-1.493	.559
Overallacceptance	2	8	-.6770	.2736	.069	-1.390	.036
		15	-.9867*	.2758	.003	-1.706	-.268
		22	-.7033	.2758	.058	-1.422	.016
	8	2	.6770	.2736	.069	-.036	1.390
		15	-.3097	.2736	.671	-1.023	.403
		22	-.0263	.2736	1.000	-.739	.687
	15	2	.9867*	.2758	.003	.268	1.706
		8	.3097	.2736	.671	-.403	1.023
		22	.2833	.2758	.734	-.436	1.002
	22	2	.7033	.2758	.058	-.016	1.422
		8	.0263	.2736	1.000	-.687	.739
		15	-.2833	.2758	.734	-1.002	.436

*. The mean difference is significant at the 0.05 level.

Linear regression

Correlations

		Overallacceptance	Smoothness	Hardness	Meltingrate	Spreadability	Butteryflavour
Pearson Correlation	Overallacceptance	1.000	.759	.307	-.331	.964	.987
	Smoothness	.759	1.000	-.291	.331	.564	.711
	Hardness	.307	-.291	1.000	-.670	.540	.425
	Meltingrate	-.331	.331	-.670	1.000	-.528	-.335
	Spreadability	.964	.564	.540	-.528	1.000	.977
	Butteryflavour	.987	.711	.425	-.335	.977	1.000
Sig. (1-tailed)	Overallacceptance	.	.120	.347	.334	.018	.006
	Smoothness	.120	.	.355	.334	.218	.144
	Hardness	.347	.355	.	.165	.230	.287
	Meltingrate	.334	.334	.165	.	.236	.332
	Spreadability	.018	.218	.230	.236	.	.011
	Butteryflavour	.006	.144	.287	.332	.011	.
N	Overallacceptance	4	4	4	4	4	4
	Smoothness	4	4	4	4	4	4
	Hardness	4	4	4	4	4	4
	Meltingrate	4	4	4	4	4	4
	Spreadability	4	4	4	4	4	4
	Butteryflavour	4	4	4	4	4	4

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.987[a]	.975	.962	.08147	2.612

a. Predictors: (Constant), Butteryflavour

b. Dependent Variable: Overallacceptance

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.513	1	.513	77.230	.013[b]
	Residual	.013	2	.007		
	Total	.526	3			

a. Dependent Variable: Overallacceptance

b. Predictors: (Constant), Butteryflavour

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	Collinearity Statistics Tolerance	Collinearity Statistics VIF
1	(Constant)	.827	.704		1.175	.361		
	Butteryflavour	.913	.104	.987	8.788	.013	1.000	1.000

a. Dependent Variable: Overallacceptance

Excluded Variables[a]

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics Tolerance	Collinearity Statistics VIF	Collinearity Statistics Minimum Tolerance
1	Smoothness	.115[b]	.592	.660	.510	.494	2.023	.494
	Hardness	-.138[b]	-1.272	.424	-.786	.819	1.221	.819
	Meltingrate	.000[b]	.000	1.000	.000	.887	1.127	.887
	Spreadability	-.027[b]	-.036	.977	-.036	.045	22.191	.045

a. Dependent Variable: Overallacceptance

b. Predictors in the Model: (Constant), Butteryflavour