Effect of Temperature on Textural and Sensory Properties of Butter

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Texture and sensory studies are important to understand the functionality and improve the quality of butter. Seven commercial butter samples were studied, using traditional and novel parameters. Both instrumental penetration test and sensory analysis methods were valid to study the butter texture. The melting rate was based on the changes in hardness over temperature, where limited literature presents. Contradictory results for sample US1 (unsalted butter sample 1) were identified between instrumental and oral melting rates, requiring further investigation. Temperature and sample type had significant effects on all the instrumental textural parameters, while there were insignificant effects on some sensory attributes. The increase in temperature made the samples soft, spreadable, less adhesive and less cohesive. At the highest temperature, 25°C, all the samples were similar in all instrumental parameters and some sensory attributes. At lower temperatures, US1 was significantly harder and less spreadable. Sample SS (spreadable butter) was the softest and most spreadable, which may be due to the temperature-cycle tempering process on winter butter or anhydrous milk fat (AMF) fractionation. The culturing of cream may contribute to the soft texture of CS (Lightly salted cultured butter). The characteristic buttery flavour was stable in the samples against temperature changes, while highly volatile diacetyl was not detected, or confused with the samples' un-freshness. Creaminess was valid to evaluate the combined flavour and texture properties, which was strongly correlated with buttery flavour, softness and oral melting rate.

Solid fat content (SFC), differential scanning calorimetry (DSC) and fatty acid profile (FA) may require further study to confirm the results and to address the problems in the significant differences of US1 in hardness, spreadability, adhesiveness, cohesiveness and melting rate. In further sensory analysis, diacetyl content may be studied, without confusion with tangy flavour, and saltiness may also be evaluated for unsalted samples, to investigate the salt effects.

Keywords: butter, temperature, texture, instrumental, sensory, melting behaviour, solid fat content, fatty acid, polymorphism

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Table of Contents

Abstract i

Acknowledgements ii

Table of Contents iii

List of Tables iv

List of Figures v

Abbreviations vi

Chapter 1 Introduction 1

1.1 Background 1

1.2 Objective 2

Chapter 2 Literature Review 3

2.1 Milk fat composition 3

2.2 Factors affecting the melting and crystallisation properties of milk fat 4

2.2.1 Fatty acid composition 4

2.2.2 Polymorphism and polytypism of milk fat 6

2.2.3 Temperature effect 7

2.3 Milk fat products and butter manufacturing 8

2.4 Temperature effects on the texture and sensory properties of butter 10

2.4.1 Texture properties 10

2.4.2 Sensory properties 11

Chapter 3 Materials and methods 14

3.1 Experimental design 14

3.2 Materials 14

3.3 Methods 15

3.3.1 Temperature control 15

3.3.2 Texture analysis 15

3.3.3 Consumer panel sensory analysis 15

3.3.4 Data analysis 16

Chapter 4 Results and Discussion 18

4.1 Instrumental texture analysis 18

4.2 Sensory analysis 27

Chapter 5 Conclusion 35

References 36

Appendix A Instrumental texture analysis result 44

A.1 Instrumental texture analysis result 44

A.2 Sensory analysis results 44

List of Tables

Table 2.1.1 Milk fat globule composition. Adapted from (Mohan et al., 2020) 3
Table 2.2.1 Fatty acid composition of milk fat (FA), adapted from Bylund (1995). 5
Table 3.2.1 Sample information factsheet. 14
Table 3.3.1 Sensory terms used for the sensory analysis. 16
Table 4.1-1 Sample melting rate with the increase in temperature from 5 to 25°C. 21
Table 4.1-2 Pearson’s correlation among textural properties[1]...23
Table 4.2-1 Pearson’s correlation among sensory attributes. 29
Table 4.2-2 Pearson's correlation between instrumental and sensory textural attributes.........32

List of Figures

Figure 4.1-1 Sample hardness as a function of temperature (mean±SE, denoted in the graph). 20
Figure 4.1-2 Sample adhesiveness as a function of temperature (mean±SE, denoted in the graph)....22
Figure 4.1-3 Sample cohesiveness as a function of temperature (mean±SE, denoted in the graph)....24
Figure 4.1-4 Work of shear as a function of temperature (mean±SE, denoted in the graph).....25
Figure 4.2-1 Intensity rating for sensory attributes at 5°C, 15°C and 25°C for all samples. 28
Figure 4.2-2 Overall acceptance for flavour, texture, combined flavour and texture, and spreadability at 5°C, 15°C and 25°C for all samples....30

Abbreviations

AMF	Anhydrous milk fat
CS	Slightly Salted Cultured Butter
DSC	Differential scanning calorimetry
FA	Fatty acid composition
SFC	Solid fat content
S1	Salted Butter 1
S3	Salted Butter 3
S2	Salted Butter 2
SS	Spreadable Butter
US1	Unsalted Butter 1
US2	Unsalted Butter 2

Chapter 1

Introduction

1.1 Background

Milk fat is an essential component of cow's milk and mostly made into value-added products, such as cream, butter, anhydrous milk fat (AMF), ice cream, cheese, and milk powder. The Codex Alimentarius requires that butter should consist of minimum 80% dairy fat (FAO/WHO, 1971), and it has been part of the human diet since ancient times. Not until the 19th century, did butter manufacturing advance from handmaking to industrial production, following the development of efficient cream separation machines and facilities. The consumption of fat from dairy products was constant (22% of the total diet) since the 1960s (FAOSTAT, 2016). Saturated fatty acids were perceived to be associated with cardiovascular disease, and the reduction in consumption of dairy fat with higher saturated fatty acids started in the 1980s (Jenkins & McGuire, 2006). Then butter regained its popularity with further health concerns over the hydrogenated vegetable fat in margarine. The EU and New Zealand are the primary sources of butter globally, and New Zealand is expected to keep its position, but a slight decrease till 2026 (FAO, 2017), may due to the soaring price. New Zealand is famous for high-quality dairy products, for the grass feeding and naturally good pasture conditions. Each butter manufacturing company needs to ensure its products are of good quality, in keeping its market share.

Butter has various applications, and it can be directly consumed as a spread at room temperature or melted as a seasoning or for cooking and baking. It is also an essential ingredient in bakery and sauce products. Sound knowledge of the quality of butter at every usage condition, is important for butter manufacturers and marketers, especially in the texture and sensory properties.

Both instrumental and sensory analysis can be used to study the textural and sensory properties of butter. Texture analysis is an important technique to measure hardness, adhesiveness and cohesiveness, and to indicate spreadability using parameters like work of shear (Oeffner et al., 2013; Sert et al., 2020). Traditional sensory analysis with human subjects is the most commonly used to study the sensory properties of food products. Both texture analysis and sensory evaluation can together provide a comprehensive understanding of the texture of the butter on the shelf and in the mouth (Bobe et al., 2003; Brenner & Nishinari, 2014; Wright et al., 2001). This research will apply instrumental texture analysis and sensory analysis by consumers to study the textural and sensory properties of commercial butter as a function of temperature.

1.2 Objective

The objective of the research is to study the texture and sensory properties of commercial butter products locally available at different temperatures, through texture and sensory analysis methods.

Chapter 2

Literature Review

2.1 Milk fat composition

Whole milk and cream are fat-in-water emulsions, where fat droplets are dispersed in the milk serum as little globules of a diameter broadly ranging from 0.2- 15µm (mean 4µm) (Mulder & Walstra, 1974). Fat globules have a triglyceride core and are surrounded by a very thin biological membrane. The membrane consists of proteins and phospholipids and is called "milk fat globule membrane" (MFGM). Fat droplets are stable in the skim milk serum, as MFGM reduces the surface tension (Evers, 2004; Lee & Sherbon, 2002). MFGM of the fat globules protects the triglyceride core from hydrolysis and oxidation (Lopez, 2005).

Milk fat mainly consists of triglycerides. Other components include mono- and diglycerides, fatty acids, sterols, carotenoids and vitamins (A,D,E and K), with minor trace elements. Triglycerides have many types and a broad range of melting points from –50 to 80 °C (Rybak, 2016), contributing to the thermal behaviour of butter. Table 2.1-1 shows the composition of milk fat. During the processing of dairy product, the structure of MFGM is easily broken down, by agitation, pumping, high shear and particle size changes from homogenisation, pasteurisation and crystallisation (Campos et al., 2002; Herrera & Hartel, 2000; Himawan et al., 2006; Alejandro G. Marangoni et al., 2012). These processes are part of butter manufacturing. Upon damages, the fat globules will be immediately covered by the milk serum abundant with surface-active substances, such as proteins and phospholipids, resulting in profound changes in the composition and structure of the globules (Mulder & Walstra, 1974).

Table 2.1.1 Milk fat globule composition. Adapted from Mohan et al. (2020).

Component	Percentage/Composition as main component
Milk fat globule core (94%–98% of globule mass)	
Triglycerides	96%–98%
Diglycerides	0.3%–2.3%
Free fatty acids	0.1%–0.3%
Phospholipids	0.2%–0.4%
Carotenoids	2–13 µg/g fat (95% β-carotene)
Sterols	0.02%–0.5%
Vitamins (fat soluble)	Vitamin A (all-trans and 13-cis retinol): 9–4 µg/g fat; Vitamin E (mostly α and trace amounts of β and γ tocopherols): 18–35 µg/g fat; Vitamin D: 0.01–0.02 µg/g fat; Vitamin K (mainly phylloquinone K1): 0.1–0.2 µg/g fat
Flavour compounds	Lactones (δ-octalactone, δ-decalactone, γ-dodecalactone), Aldehydes, Methyl ketones
Milk fat globule membrane (2%–6% of globule mass)	

Triglycerides	0.73%–2.12%
Diglycerides	0.03%–0.08%
Monoglycerides	0.005%–0.01%
Free fatty acids	0.01%–0.03%
Polar lipids	0.53%–1.54%
	Phosphatidyl choline: 19%–37%; Phosphatidyl ethanolamine: 20%–42%; Sphingomyelin: 18%–34%; Phosphatidyl inositol: 1%–14%; Phosphatidyl serine: 2%–16%
Membrane proteins	0.56%–1.7%
	Xanthine oxidoreductase, butyrophilin, and adipophilin together—70%; Others are Mucin-1, alkaline phosphatase, glycoprotein ecto 5′-nucleotidase, γ-glutamiltranspeptidase, PAS 6/7
Total polar and phospholipid composition of milk fat	
Polar lipids	0.25%–0.96%
	Phosphatidyl ethanolamine: 26%–73%; Phosphatidyl inositol: 3%–14%; Phosphatidyl serine: 2%–16%; Phosphatidyl choline: 8%–45%; Sphingomyelin: 4%–29%

2.2 Factors affecting the melting and crystallisation properties of milk fat

2.2.1 Fatty acid composition

Fatty acid composition and the distribution of the fatty acids in the triglyceride structure influences crystallization behaviour, melting behaviour and nutritional aspects (Gonzalez et al., 2003). Melting and crystallisation properties of milk fat influence the texture and sensory characteristics of butter. Physical and chemical properties of milk fat influenced by fatty acid profile include hardness, melting point, solid fat and liquid fat content, viscosity, oxidative stability, and sensory attributes (Bobe et al., 2003; Chen et al., 2004; Esmaeili fard et al., 2016; Kaylegian & Lindsay, 1995; Mallia et al., 2008; Oeffner et al., 2013; Penedo et al., 2013).

Various fatty acids and a glycerol component constitute triglycerides in milk fat, the predominant components in milk fat (Bylund, 1995). In each tatty acid molecule, there is a hydrocarbon chain and a carboxyl group. Fatty acids can be saturated or unsaturated, depending on the way how the carbon atoms are linked to each other. The main difference between saturated and unsaturated fatty acids is the number of double bonds between carbon atoms in the hydrocarbon chain: saturated fatty acids only have single bonds and no double bonds, while unsaturated fatty acids have at least one double bond.

As in Table 2.2.2, milk fat contains both saturated and unsaturated fatty acids. They have different melting points, ranging from -49.5 to 69.3℃, therefore, whether the fatty acids are liquid or solid at room temperature is determined by their melting points compared to the room temperature. In this sense, the varied status that the contained fatty acids present will result in different melting behaviour of the triglycerides. The milk fat product will be harder if the triglycerides have more high-melting fatty acids, while softer if the triglycerides have more low-melting fatty acids. The major fatty acids in milk fat are palmitic acid, myristic acid, stearic acid in a solid form (high melting), and oleic acid in a liquid form (low melting) at room temperature.

Table 2.2.1 Fatty acid composition of milk fat (FA), adapted from Bylund (1995).

Fatty acid	Content in total FA %	Melting point °C	Number of atoms			Status at room temperature	Saturation degree
			H	C	O		
Saturated							
Butyric acid	3.0-4.5	-7.9	8	4	2	Liquid	0
Caproic acid	1.3-2.2	-1.5	12	6	2	Liquid	0
Caprylic acid	0.8-2.5	16.5	16	8	2	Liquid	0
Capric acid	1.8-3.8	31.4	20	10	2	Solid	0
Lauric acid	2.0-5.0	43.6	24	12	2	Solid	0
Myristic acid	7.0-11.0	53.8	28	14	2	Solid	0
Palmitic acid	25.0-29.0	62.6	32	16	2	Solid	0
Stearic acid	7.0-3.0	69.3	36	18	2	Solid	0
Unsaturated							
Oleic acid	30.0-40.0	14	34	18	2	Liquid	1
Linoleic acid	2.0-3.0	-5	32	18	2	Liquid	2
Linolenic acid	<1.0	-5	30	18	2	Liquid	3
Arachidonic acid	<1.0	-49.5	32	20	2	Liquid	4

Cow's diet affects the fatty acid composition in milk, hence, the fatty acid difference in the milk fat product, as a result of feeding systems, season and cow breed (Capuano et al., 2014; Sébastien Couvreur et al., 2006; Heck et al., 2009; Ineichen et al., 2019). Pasture or more fresh grass feeding increases the contents of unsaturated fatty acids, medium-chain fatty acids, conjugated linoleic acid, and decreases the contents of saturated fatty acids and palmitic acid in the milk (Capuano et al., 2014; S. Couvreur et al., 2006; O'Callaghan et al., 2019; Sant'Ana et al., 2019). Likewise, supplement or concentrate feeding produces more saturated and short-chain fatty acids in the milk (Bargo et al., 2006; Villeneuve et al., 2013). The same trend can be seen with the seasonal effects, as the summer effect can be taken as more fresh grass in the diet, while winter less fresh grass and more supplements. The reason is that the summer grass is usually younger than the cut grass to make silage for winter shortage, making the content of polyunsaturated fatty acids (especially C18:3) content lower in the silage (Chilliard et al., 2001; Ferlay et al., 2006; Heck et al., 2009). The breed variation of dairy cows also has significant effects on the fatty acid composition, due to the differences in the biosynthesis of cows. It is generally agreed that Holstein cows have significantly higher contents of polyunsaturated fatty acids, linoleic acid and lower saturated fatty acids and palmitic acid than Jersey cows (Drackley et al., 2001; Larsen et al., 2012; Palladino et al., 2010). Contradictory results were found for palmitic content, (White et al., 2001) found insignificant difference between Holstein and Jersey, while (Beaulieu & Palmquist, 1995) reported significant content for Holstein.

2.2.2 Polymorphism and polytypism of milk fat

The crystallization process of triacylglycerols is very slow with a constant state flux. Milk fat is initially liquid (globule) at the body temperature, and it crystallizes during cooling or tempering. This thermal behaviour is affected by the triglyceride crystalline structure properties, on the nucleation behaviour and crystal growth (Alejandro G Marangoni et al., 2012). The microstructural changes are expressed in the functional, technological, and sensory properties of milk fat, such as the melting behaviour of butter (Michalski et al., 2004).

Milk fat has different polymorphic forms, exhibiting varied crystal structures and melting points, which are attributed to the various types of fatty acids (chain lengths and degrees of saturation of fatty acids) and the order of arraignments of the fatty acids on the glycerol backbone. The three main polymorphic forms are (i) α (hexagonal, lowest stability and melting point), (ii) β’ (orthorhombic, medium stability and melting point) and (iii) β (triclinic, highest stability and melting point) (Mazzanti et al., 2009; Rønholt et al., 2012). The triacylglycerols also pack into lamellar structures in the longitudinal direction, most often in stacks either two or three fatty acids long (termed 2L or 3L respectively) in different directions (polytypism). The chains are perpendicular to the lamellae planes (possibly tilted) and the in-plane packing of the chains generate various polymorphic forms (Rønholt et al., 2012). Stability is not correlated with the stacking, and 2L is not necessarily stable than 3L (Lopez, Lavigne, Lesieur, Keller, et al., 2001). The state of milk fat (anhydrous or emulsion) is affected by the triglyceride microstructures at a specific temperature. (Lopez et al., 2002; Lopez, Lavigne, Lesieur, Bourgaux, et al., 2001; Lopez, Lavigne, Lesieur, Keller, et al., 2001; Lopez et al., 2005).

Polymorphism and polytypism describe the nano-scale structural characteristics of the elements that form milk fat products. In semisolid milk fat products, such as AMF and butter, 3-dimensional fat crystalline network formed by their solid components (formed by the nano-scale elements) determines the melting behaviour, hence, product texture properties. During crystallisation, these fat crystals aggregate and form clusters, then clusters gather as flocs which subsequently form a floc network, as shown in Figure 2.2.2-2 (Alejandro G Marangoni et al., 2012).

2.2.3 Temperature effect

Melting and crystallisation are two ways in which milk fat dynamically behaves with the change in heat, either melting from solid to liquid or crystalising from liquid to solid (Tomaszewska-Gras, 2013). Affected by temperature, the fatty acid composition of the triglycerides, polymorphism and polytypism, and the interactions among different triglycerides contribute to the solid to liquid ratio, resulting in varied texture and properties. Each triglyceride has its own polymorphic properties and melting behaviour, but the mixture of triglycerides behaviour is an interactive effect and not predictable based on the individual triglycerides (Pattarino et al., 2014). Milk fat has a wide range of triglycerides, and the behaviour of the whole material is complicated and hard to understand (De Graef et al., 2012). Most studies have been on the integrated melting and crystallisation properties of milk fat, for the ease of observation and understanding, and their direct correlations with the macro functionalities of the milk fat products (Minato et al., 1997; Narine & Marangoni, 1999; Vereecken et al., 2010). Minato et al. (1997) observed two polymorphisms with the same fat mixture. Vereecken et al. (2010) reported that different fatty acid compositions with the same saturation had different polymorphic transitions.

Butter is a major milk fat product. Its manufacturing is a complicated process, where the temperature affects the triglycerides mixtures in various ways, via pasteurisation of milk and cream, cooling and ripening of the cream (some cases). Many studies focused on fatty acid composition, polymorphism, and microstructure development. Time-resolved synchrotron X-ray diffraction techniques and differential scanning calorimetry are methods to observe the crystallisation dynamics and polymorphism of the triglycerides in milk fat and AMF (Campos et al., 2002; Lopez et al., 2002; Lopez et al., 2005; Mazzanti et al., 2004). In general, fast cooling promotes the formation of smaller crystals, resulting in a firm crystal structure, while a fewer number of larger crystals are formed and weakly structured after slow cooling (Rønholt et al., 2012; Wiking et al., 2009). In a fast cooling with a low final temperature, the metastable α forms changed to a mixture of more stable β'and β forms. In contrast, a slow cooling with a high final temperature resulted in the β' forms only. The starting amount of α forms affected the formation of β forms (Campos et al., 2002; Lopez et al., 2002; Lopez et al., 2005; Mazzanti et al., 2004). When the cooling is fast at a low crystallisation temperature, polar lipids may have acted together with the high melting fraction in the α phase and initiated the crystallisation (Mazzanti et al., 2004). Wiking et al. (2009) reported similar results that, at the same final temperature, a fast cooling induced the transition from α to β' forms, but no confirmation on the coexistence of β forms. The maturing (setting) of cream could also lead to the transition from stable β' to more stable β, but slow-cooled matured cream can result in a soft product, due to a weak crystal network (Ali et al., 2018; Herrera & Hartel, 2000; Rønholt et al., 2012).

Studies also made efforts to relate polymorphism with polytypism. Lopez et al. (2005) found that the melt of 3L (72 Å) and 2L (46 Å + 38.5 Å) correlated with the decreasing α forms. Two diffraction peaks (about 3.8 and 4.2 Å) in the X-ray diffraction patterns graph indicated newly-formed 2L is characterised as the formation of β′ form. This stacking is the characteristic of the longitudinal arrangement of triglyceride, where short or unsaturated fatty acid chains and long saturated fatty acid chains are linked together. Rønholt et al. (2012) observed that when the cream was being matured, the 3L arrangement changes to a 57 Å stacking, indicating the polymorphic transition from α to β′.

The change in the fraction melting point can also induce microstructural transformation. The incorporation of low-melting cream into traditional or heat-stepped cream lowered the average melting rate, resulting in either cold spreadability or warm spreadability, which indicated that the microstructure changes mainly contributed to the different textures (Schäffer et al., 2001). Melting and crystallisation occur at the same time in the sample when exposed to heat (Ratta et al., 2001), where the stearin and the olein fractions exhibited the formation of a β′ 2L (41.1–42.6 Å) structure and a β′ 3L (66 Å) structure, respectively, by which, determined the solid to liquid ratio (Lopez & Ollivon, 2009). The higher unsaturated fatty acids can also make the sample thick, with the stacking of 3L and β′ 2L (Bugeat et al., 2015).

The temperature effect on the fat crystal network state can be addressed using microscopic imaging (Staniewski et al., 2020). The increase in temperature (8 to 23°C) showed a noticeable liquefaction effect on the microstructure. At a temperature above 20°C, the liquefaction structure was obvious and typical, with more round- and spherical crystals. This phenomenon may result from the incomplete melting of the higher-melting triglycerides or the great level of liquefaction of the lower-melting triglycerides in the samples. The structure at a lower temperature was sharper, with pointed surfaces, caused by the sequential crystallisation of the higher-melting triglycerides in the sample. Therefore, more liquefaction in the sample at a higher temperature will lead to low hardness and higher spreadability, to varying degrees among these butter samples. The crystal network structure at different temperatures can also show the difference in solid to liquid ratio (Bugeat et al., 2015; Lopez et al., 2002; Vithanage et al., 2009; Wiking et al., 2009).

2.3 Milk fat products and butter manufacturing

Milk fat is a high-value milk product and mainly made into cream, butter, AMF, cheese and ice cream, and this paper focuses on butter. Cream is the starting point for other milk fat products. The fat in milk consists of smaller globules dispersed in the milk serum, and if the unprocessed milk is settled without disturbance, milk fat will float onto the milk surface and aggregate as a thick fat layer on the top. This is a slow process naturally, but it is often accelerated in industrial processing, via centrifugation by separators. The cream skimmed directly from milk is called “sweet cream”, while many countries ferment the cream and make “sour cream”.

Butter is formed from the aggregation of fat globules into butter granules in a series of processes. Fat globules have both crystal fat and liquid fat phases. Upon agitation of the cream, large protein bubbles form and become a compact mixture with small bubbles, when the liquid fat is pressed out of the fat globules. At the same time when the crystallised fats aggregate, and liquids cover the surfaces of both the protein bubbles and the fat globules, making the mixture dense (butter granules) and the protein bubbles collapse (Bylund, 1995).

The cream is churned after temperature treatment and after souring, where applicable. In traditional batch production, cylindrical, conical, cubical, or tetrahedral churns are used, with speed ranges suitable for different needs. In recent years, the size of churns increased to 8 000 to 12 000 litres capacity or more. Based on traditional churning, the Fritz method was developed, as a continuous churning process. The following churning methods were more or less based on the Fritz method's continuous churning principle (Bylund, 1995).

Industrial continuous churning generally consists of a series of steps. First, either raw milk is pasteurised and then separated, or raw milk is separated, and the skim and fat streams are pasteurised separately. Vacuum deaeration before pasteurisation is sometimes needed if the cream has undesirable aroma or flavour. Vacuum deaeration is done through preheating and fast cooling. Then culture is added into cream to make sour cream if needed. After transferred to the ripening tank, where the cream is being soured or setting (Lopez et al., 2005), the cream will be under a temperature program to ensure that the fat crystalline structure is up to requirement, subject to the fatty acid composition, and the iodine values. The following steps are done at a low temperature, starting from churning. The cream is agitated to coagulate the fat into butter grains during continuous churning when buttermilk is continuously drained out. Working of the butter is to obtain a fine and consistent water-in-fat emulsion in the butter, when salt is also added and well dispersed in the product (Ali & Fischer, 2005; Fearon, 2011).

Processing conditions of butter making can affect the crystalline kinetics of milk fat, including cooling and agitation rate, crystallisation temperature, and processing of milk. Rapid cooling generally produces well-defined crystals that distinguishable from each other and are easy to be separated from a liquid phase (Campos et al., 2002; Ceylan & Ozcan, 2020; Herrera & Hartel, 2000; Lopez et al., 2002). Fast agitation tends to break the already-aggregated crystal clusters, leading to porosity and diffusion of liquid fat into the crystal network (Alejandro G Marangoni et al., 2012). When shear is applied to the initial crystalline network, the alterations of the polymorphic forms were found (Kaufmann et al., 2013). In this study, a higher shear rate reduced the intensity of α form and accelerated the form transition into β’, although the amounts of β’and β were not affected. High crystallisation temperature increases the formation of larger crystals because of the encouraged coalescence formation (Herrera & Hartel, 2000; Lee & Martini, 2018). Milk processing technologies, including pasteurisation and homogenisation, result in different contents of low-, medium- and high-melting fractions in the crystallisation development (Ali et al., 2018; Ren et al., 2020).

2.4 Temperature effects on the texture and sensory properties of butter

2.4.1 Texture properties

The expression of the milk fat melting and crystallisation is in the textural properties of the products. Creaminess, hardness and spreadability are important textural characteristics of butter. They are also associated with milk fat composition, the crystallisation and polymorphism of fat crystal network (Buldo et al., 2013; Campos et al., 2002; Herrera & Hartel, 2000; Lopez, 2005; Lopez et al., 2002; Tang & Marangoni, 2007; Wright et al., 2008), and processing methods that manipulate the crystal structures, such as cooling, tempering and ripening of cream (Ali et al., 2018; Herrera & Hartel, 2000; Alejandro G Marangoni et al., 2012). Research has been done to study these textural characteristics of butter products (Glibowski et al., 2008; Oeffner et al., 2013; Vithanage et al., 2009). Creaminess is a combined property of flavour and texture and an essential quality factor of butter. The natural milk fat-related creaminess is preferred by consumers (Krause et al., 2007).

While spreadable butter has been developed using tempering (temperature-cycle) to alter the crystal polymorphism or fractionantion of AMF (low melting point fraction) to increase the butter spreadability (Abbas et al., 2006; Queirós et al., 2016), there are still many butter products exhibiting a high hardness and low spreadability at cold storage temperature (5-10°C) (Viriato et al., 2019). With the increase in temperature from storage to consumption conditions (5-25°C), the butter texture properties change (Wright et al., 2008). The increase in temperature induces the decreased solid fat content (SFC), hence, higher spreadability (Glibowski et al., 2008; Vithanage et al., 2009), contributed by the fatty acid composition, polymorphism, and the interactions among triglycerides (De Graef et al., 2012). Decreased saturated fatty acids, increased unsaturated fatty acids, and cow-diet induced changes in fatty acid composition all increase spreadability (Bobe et al., 2003; Sébastien Couvreur et al., 2006; Heck et al., 2009). At a higher temperature, the fat crystal structure becomes round, and the solid crystals are not easy to be distinguished, while the microstructure is sharper at a lower temperature (Herrera & Hartel, 2000; Staniewski et al., 2020). Pasture feeding increases the amounts of C18:0 C18:1 C18:3 and decreases C16:0 (Chilliard et al., 2001; Sébastien Couvreur et al., 2006), thus, decrease the spreadability index (ratio of the major SFA C16:0 on the sum of C18:1 fatty acids), making the products more spreadable (Sébastien Couvreur et al., 2006). The textural parameters are related to each other: samples with a lower adhesiveness, a higher cohesiveness (in TPA) tend to be more spreadable (Glibowski et al., 2008).

It is generally agreed that the microstructure of small uniform crystals is strong and the resultant product is firm, and large and nonhomogeneous crystals formed network is weak, making a soft product (Herrera & Hartel, 2000; Lopez et al., 2002; Lopez et al., 2005; Wiking et al., 2009). However, the interactions among triglycerides may lead to the formation of links among the large crystals and harder products (Herrera & Hartel, 2000). AMF crystallized rapidly was harder than AMF crystallized slowly and had a higher SFC. Moreover, its solid state was in a more metastable polymorphic form. Upon slow crystallization, AMF had a lower SFC and its solid state was in a more stable polymorphic form (Campos et al., 2002). Similar results were reported in other studies (Lopez et al., 2005; Wiking et al., 2009). Sour cream is made by acidification or culturing of the cream, which alters the microstructure of milk fat, due to the destruction of already-formed nucleation sites or minor fatty acids (Ceylan & Ozcan, 2020; Ewe & Loo, 2016; Jinjarak et al., 2006).

2.4.2 Sensory properties

Creaminess, hardness and spreadability are also sensory factors indicating butter quality and consumer acceptability. Sensory attributes, such as flavour, texture, mouthfeel, of butter are associated with the fat crystal structure, and its melting behaviour in the mouth (Engelen et al., 2003; Hyvönen et al., 2003). The temperature effect on butter in the mouth is in two aspects: the temperature of the butter before put in the mouth and the temperature increase to the body temperature that melts the butter. The melting of milk fat in the mouth assists the release of flavour compounds and the texture of milk fat, and the crystalline network can also be sensed by the mouth (Yılmaz & Öğütcü, 2015).

The texture sensed in the mouth can be mimicked by instrumental texture analysis, and hardness and spreadability are the most studied attributes, which are associated with the fatty acid composition, crystal microstructure and the reflected SFC (Brenner & Nishinari, 2014; Krause et al., 2008; Mallia et al., 2008; RadoČAj et al., 2011). With the increase in temperature, hardness and spreadability decreases and increases, respectively, as discussed in the previous section, which was also found in sensory studies. Milk of cows with more monounsaturated fatty acids yielded butter products with more monounsaturated fatty acids that were softer and had a satisfactory flavour

(Bobe et al., 2003; Chen et al., 2004; Mallia et al., 2008). Higher temperature also results in a less brittle structure (Engelen et al., 2003; Staniewski et al., 2020).

Fatty acids have their own notes of flavour. Twenty odour-active compounds were detected for fresh butter: hydrogen sulphide, acetaldehyde, dimethyl sulphide, 2,3-butanedione, hexanal, 2-methylbutanal, 3-methylbutanal, 1-hexen-3-one, butanoic acid, dimethyl trisulphide, 1-octen-3-one, hexanoic acid, δ-hexanolactone, nonanal, (Z)-2-nonenal, (E)-2-nonenal, δ-octanolactone, skatole, δ-decanolactone and γ-dodecanolactone) (Mallia et al., 2008; Peterson & Reineccius, 2003). In general, butyric acid and caproic acid contribute to the characteristic milky flavour of dairy products (Su et al., 2017; Zhao et al., 2018), and more compounds have been analysed and assigned to creamy and fatty notes (Mallia et al., 2008).

Salt is a traditional ingredient in butter to enhance flavour, but its effect on texture was scant for butter and contradictory on cheese (Mistry & Kasperson, 1998; Saint-Eve et al., 2009). Diacetyl is highly volatile and a characteristic flavour compound in culture butter (Schieberle et al., 1993). It can evaporate and disappear under a low-temperature environment, such as a refrigerator and freezer (Chauhan et al., 2010; Park & Drake, 2005). Diacetyl and tangy are similar flavours, as they are both tasted slightly acidic (Rakib et al., 2017). Tangy is a characteristic sensory attribute associated with fermented dairy products, such as yoghurt and acidophilus milk (ÖZer & Kirmaci, 2010; Yildiz, 2016). However, tangy can also be undesirable and associated with off-flavour in dairy products, attributed by the increased short-chain fatty acids, such as butyric acid (C4:0), caproic acid (C6:0) and caprylic acid (C8:0), produced in the hydrolysis of milk triacylglycerols (Chen et al., 2003).

Creaminess is a complex sensory term that describes the overall perception of food flavour and texture (Frøst & Janhøj, 2007). It is easier for human beings to describe the term creaminess than instrumental prediction methods and closer associated with smoothness (Szczesniak, 2002). The relationship between creaminess and the microstructure of the fat crystal microstructure was not fully confirmed, but smaller particles evenly distributed in the structure was found contributing to creaminess (Kilcast & Clegg, 2002). Higher liquid fat content in the crystal network can also improve creaminess through a softer and fast-melting structure (Akhtar et al., 2005; Kilcast & Clegg, 2002). The buttery flavour was also found strongly correlated with creaminess (Hyvönen et al., 2003; Saint-Eve et al., 2004; Tournier et al., 2007). After the food is put in the mouth, it is rubbed between the palate and the tongue, by which a thin film is formed on the oral mucosa. The oral mucosa can sense the friction that is correlated with creaminess, and the less the friction, the more the creaminess (Chojnicka-Paszun et al., 2012). The reason may be that the coalescence on the tongue surface, generated from the emulsion (Dresselhuis et al., 2008).

Literature is rare on the temperature effects on a comprehensive sensory profile for butter. In addition, products from different manufacturers also differ in these characteristics (Vithanage et al., 2009). A comprehensive understanding of the temperature effect and product differences can assist in quality improvement and product innovation.

Chapter 3

Materials and methods

3.1 Experimental design

Commercial butter samples were purchased from the local market at a similar time. Five temperatures (5, 10, 15, 20 and 25°C) were tested for texture analysis, while three (5, 15, and 25° C) for sensory analysis. The samples needed for testing were kept in a temperature-controlled incubator for a minimum of 24 hours to reach the required temperature before the testing of texture or sensory analysis.

3.2 Materials

Pure butter and pure spreadable butter samples used in this research came from commercially available products. They included six types of butter and one type of spreadable butter products. Efforts were made to procure the products from the same batch and milk season; however, there were variations in the products we finally sourced, as shown in Table 3.2. For texture analysis, samples will be used in the original size to void over manipulation, and three replicates (pats) are required for each testing. For sensory analysis, small pieces of 3cm x 1cm x 1cm are cut from the pats using a dough scraper.

Table 3.2.1 Sample information factsheet.

Code	Description	Weight	Estimated production date[1]	Milk season[2]
S1	Salted Butter 1	400g	19 August 2020	Winter
S2	Salted Butter 2	500g	22 November 2020 (texture analysis) 30 July 2020 (sensory analysis)[3]	Summer Winter[3]
S3	Salted Butter 3	500g	4 December 2020	Summer
US1	Unsalted Butter 1	400g	28 October 2020	Summer
US2	Unsalted Butter 2	500g	19 November 2020	Summer
CS	Slightly Salted Cultured Butter	250g	7 June 2020	Summer
SS	Spreadable Butter	375g	19 September 2020	Winter

[1] *The production date was not available for each sample. The production date was estimated as 12 months before the best before date, based on two of the samples having both the production and best before date printed on the packaging.*

[2] *Milk season refers to the season when the milk was collected and used for butter making.*

[3] *Product dates for the S2 in the texture and sensory analysis were different.*

3.3 Methods

3.3.1 Temperature control

All the samples were stored in the refrigerator until required, at 5±2℃. The instrumental texture analysis was conducted on samples at five different temperatures 5℃, 10℃, 15℃, 20℃, 25℃. The sensory analysis was done at three temperatures, 5℃, 15℃, and 25℃. All the temperatures were reached and maintained by keeping the samples in the incubator (stable at the set temperature in advance) for a minimum of 24 hours. The temperature was monitored during the entire time in the incubator, with a probe monitoring the inside temperature of control butter pat or cut samples. Each day of the experiment was for all the samples at the same temperature.

3.3.2 Texture analysis

The texture profile analysis consists of the testing of four parameters: hardness, adhesiveness, cohesiveness, and work of shear to indicate spreadability. The measurements were done on a texture analyser (TA-XT2, Stable Micro Systems, Godalming, UK). A cylindrical probe (6mm) was used on the texture analyser to conduct the testing, with a trigger force of 3g. The speed and depth into the sample surface were 1mm/s and 12mm, respectively (Oeffner et al., 2013). Hardness was defined as the maximum penetration force, cohesiveness the maximum negative force. Adhesiveness referred to the negative area and work of shear the positive area under the penetration curve. The work of shear indicated spreadability: less work of shear means higher spreadability. For each temperature, ten measurements were conducted on each of the three replicates (pats) of each type of the butter samples. The experiment for the same temperature was done on the same day. Melting rate was defined as the absolute value of the slope of the trendline in the hardness scattered plot.

3.3.3 Consumer panel sensory analysis

A consumer panel consisting of 128 males and 172 females participated in the sensory evaluation of the butter samples at three different temperatures of 5°C, 15°C and 25°C. The evaluation was carried out in individual sensory booths, at room temperature, and under a mixture of red and green lights to mask colour differences among samples. Unsalted crackers and distilled water were provided to the panellists for palate rinse. A sensory software (REDJADE, Redjade Sensory Solutions, LLC of Martinez, CA) was used to gather data. Salted or unsalted products at three temperatures were analysed on separate days, to avoid physical and psychological fatigue. In each evaluation session, samples were coded with randomly picked 3-digit numbers in a balanced presentation manner. The panellists were given documents of product information and consent, and tablets for conducting the analysis.

The testing comprised of two parts: oral tasting and non-oral mechanical testing of spreadability. In general, the sensory attributes evaluated by oral tasting are categorised as flavour, texture attributes or combined flavour and texture. The specific terms under each category were assessed by asking the panellists to rate the intensity on an unstructured line scale, marked with proper anchors, and the two extreme ends of the scales were defined with scores as 0 (left end) and 100 (right end). The non-oral mechanical testing involved evaluating the spreadability of the butter samples. Spreadability is expressed as the force necessary to spread the sample on a surface. The panel were required to conduct the mechanical test by spreading the sample for three strokes: backward, forward and backward on an unsalted cracker (1.5cm from the tip of the knife) (Krause et al., 2008).

The overall liking of flavour, texture, combined flavour and texture, and spreadability were evaluated using the 9-point hedonic scale (1=Dislike Extremely, 2=Dislike Very Much, 3=Dislike Moderately, 4=Dislike Slightly, 5=Neither Like Nor Dislike, 6=Like Slightly, 7=Like Moderately, 8=Like Very Much, 9=Like Extremely). Sensory terms used are shown in Table 3.3-1. Salty and tangy flavours were evaluated only for salted butter samples, to understand their possible effects on other sensory attributes and overall acceptance.

Table 3.3.1 Sensory terms used for the sensory analysis.

Category	Term	Instruction given to panel	Applied sample
Flavour	Buttery	N/A	All samples
	Salty	N/A	Salted samples
	Tangy	Tangy was the substitute term of Diacetyl[1] flavour and its meaning is easier to understand for consumer panel	Cultured sample (among salted sample group)
Texture	Softness	Softness of the sample as soon as it is put in the mouth	All samples
	Melting rate	Melting rate in the mouth	All samples
	Spreadability -cutting	The softness detected through the marked tip of the knife (1.5cm from the top)[2] when cutting the sample into two equal pieces	All samples
	Spreadability -spreading	The spreadability detected through the marked tip of the knife (1.5cm from the top)* when spreading one half of the cut sample on a cracker	All samples
Combined flavour and texture	Creaminess	Creaminess is defined as a combination of flavour (e.g. fatty flavour) and texture (e.g. softness and melting rate in the mouth).	All samples

[1] Diacetyl flavour refer to the characteristic flavour of cultured butter (Schieberle et al., 1993)
[2] Refer to (Krause et al., 2008)

3.3.4 Data analysis

Analysis of variance (ANOVA) (participants as the random effect for sensory analysis), with a general linear model (GLM) was conducted. Tukey's test was used to assess significant differences between the means based on samples, temperatures, and sample*temperature models, $\alpha = 0.05$. Pearson's correlation test was conducted to evaluate the correlation among texture or sensor parameters or between the instrumental and sensory attributes. The statistics analysis was conducted using statistics software (Minitab®, MiniTab LLC, 1829 Pine Hall Rd, State College, PA 16801, USA). Scattered plots and spider plots were made for instrumental texture analysis and sensory analysis results, respectively, using Microsoft Excel (Microsoft Corporation, USA). Melting rate based on instrumental texture analysis was defined as the absolute value of the trendline slope in the hardness scattered plot, as a function of increasing temperature.

Chapter 4

Results and Discussion

4.1 Instrumental texture analysis

As shown in Figure 4.1-1,2,3,4, the instrumental texture analysis measured hardness, adhesiveness, cohesiveness, and work of shear. The texture analysis reported significant effects of both temperature and sample type on the results ($P<0.05$). For the same sample, all the texture attribute measurements (hardness, adhesiveness, cohesiveness, and work of shear) significantly decreased as temperature increased ($P<0.05$). This means that as temperature increased, the samples became soft, less adhesive to the probe, and more spreadable. At lower temperatures, different samples exhibited more significant differences for all the texture parameters. However, as the temperature increased the differences among samples decreased, and at the highest temperature of 25°C, all the seven samples exhibited similar texture properties ($P>0.05$). The present study results on the downward trend and the decreasing differences among samples with the increase in temperature were consistent with previous research (Glibowski et al., 2008; Vithanage et al., 2009).

From Figure 4.1-1,2,3,4, US1 exhibited the highest values for hardness, cohesiveness and work of shear, indicating that it was the hardest and least spreadable butter sample, compared to all other salted or unsalted samples. However, it exhibited a lower adhesiveness than S1, S2, S3, CS and US2 at 5°C, and a lower adhesiveness than S2, S3 and US2 at 10°C. SS, the spreadable butter product, was the lowest in all four parameters, indicating its soft and spreadable characteristics. CS was generally much higher than SS and less low than the other samples for the four parameters. As mentioned in the Introduction, US1 may have the highest SFC, making it the hardest and least spreadable, while SS may have the lowest SFC, contributing to its lowest hardness and highest spreadability, at the temperatures below 25°C. The samples may have the same SFC at 25°C. Also, US1 may have a significantly higher content of high-melting fractions, maybe due to polymorphism changes (Glibowski et al., 2008; Vithanage et al., 2009). Based on other studies observing the microstructure changes during melting, β’ 2L and β’ 3L, or 3L stacking with other polymorphic forms may have occurred in the sample, affecting SFC (Bugeat et al., 2015; Lopez & Ollivon, 2009).

The higher hardness of a sample indicates that the sample has more high-melting-point fractions than a softer sample, at the same temperature. This is related to the higher existence of polymorphic β’ forms in the harder sample with a higher melting point, which was confirmed by previous studies (Lopez, Lavigne, Lesieur, Keller, et al., 2001; Lopez et al., 2005; Wiking et al., 2009). It was also reported in another study that cooling rate had significant effects on the melting profile of the samples. Therefore, the significantly harder sample US1, may have more β’ forms transformed from α, resulting from a fast cooling process during manufacturing.

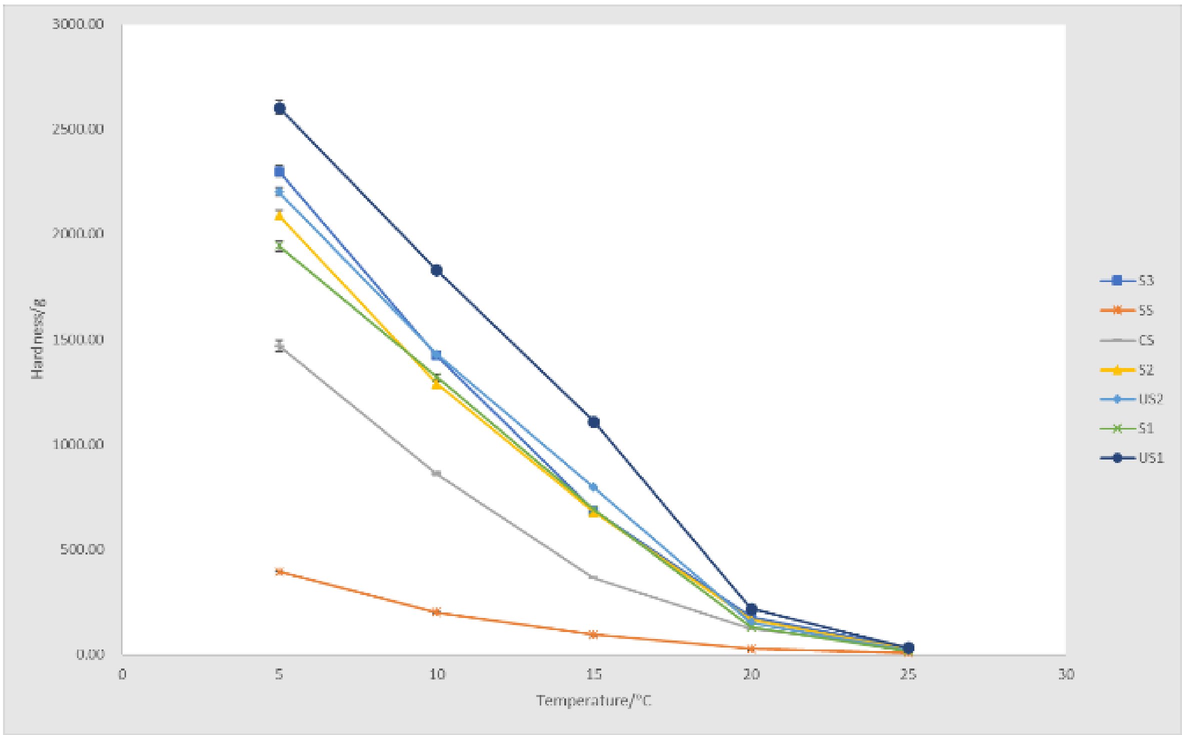
Based on the colour of CS and the manufacturing company's region, CS is likely to be made with milk from cows fed on supplements or concentrate. CS had a light colour, while butter from pasture grazing is well-known for its yellow colour, due to the higher intake of β carotene (Martin et al., 2004). There is also a possibility that the bacteria in the cultured butter have converted the β carotene and made the product colour light, yet the evidence is lacking. CS’s lower hardness and high spreadability may be due to the regional or feeding differences in fatty acid composition. As a lower level of fresh grass in the cow’s diet can decrease the polyunsaturated fatty acids in the milk fat, change the ratio of spreadability index (C16:0/C18:1), thus, increase the final melting rate and SFC in the butter, resulting in a harder product (Bargo et al., 2006; Bobe et al., 2003; Sébastien Couvreur et al., 2006). However, the texture properties of CS were not in line with the literature.

The culture of cream in the production of cultured butter, such as CS, may be an alternative reason for its lower hardness and spreadability. The acidification of the cream can break down the already-formed crystals and the originally-formed nucleation site, resulting in a weaker structure (Ceylan & Ozcan, 2020). Cultured cream butter was found to be softer than sweet cream butter (Ewe & Loo, 2016; Jinjarak et al., 2006), may be due to the higher content of polyunsaturated fatty acids in the cultured one mainly contributed by linoleic acid (C18:2, c9,12) (Ewe & Loo, 2016; Florence et al., 2009). The decreasing effect of polyunsaturated on hardness, and an increasing effect on spreadability has been reported (Bobe et al., 2003).

Hardness Hardness is an important quality factor of butter, affecting its applications in both domestic and commercial uses. Softer products can be used as a spread or sauce, while harder butter can be a good ingredient in biscuits, ice cream, and chocolate (Granger et al., 2005). From Figure 4.1-1, as mentioned above, differences among the samples reduced with the increase in temperature, at below 25℃, which can be attributed to SFC and microstructural changes. US1 was significantly higher than all the other samples, indicating that it may be suitable for biscuit making where a sufficient amount of crystals in butter are required to retain air during mixing and the early state of baking (Shahidi, 2005).

Samples were equally hard at 25°C, but at different rates. As shown in Table 4.1-1, samples had significantly different melting rates, as the temperature increases form from 5 to 25°C ($P<0.05$), indicating that they varied in melting profile. Limited literature focused on melting rate changes depending on temperature and sample type. Melting rate means how fast the sample melts with the increase in temperature. Sample melting fast means that more crystals in the sample are transiting or have transited from solid to liquid state, per degree Celsius of increase in temperature, which also is also expressed as SFC. There is less solid phase than liquid phase if the sample melts faster. US1 had the highest melting rate, while SS the lowest. S3 (similar with US2), S2, S1 and CS are in decreasing order, between the highest and lowest ($P<0.05$). US1 may have the highest SFC, followed by SS and S3, S2, S1 and CS. The total change in SFC of different samples may vary. As per the research by (Vithanage et al., 2009), the SFC of pure butter decreased by 50%, while the percentage for butter-vegetable fat blends was only 25%, due to their varied SFC.

Figure 4.1-1 Sample hardness as a function of temperature (mean±SE, denoted in the graph).



Hardness Significant difference[1]	S3	SS	CS	S2	S1	US2	US1
5°C	B,a	G,a	F,a	D,a	E,a	C,a	A,a
10°C	B,b	E,b	D,b	C,b	C,b	B,b	A,b
15°C	C,c	E,c	D,c	C,c	C,c	B,c	A,c
20°C	AB,d	C,d	B,d	AB,d	B,d	AB,d	A,d
25°C	A,e	A,d	A,e	A,e	A,e	A,e	A,e

[1] *In a row or column, table cells not sharing a letter mean that the corresponding hardness values (mean) (refer to Appendix A-Table A.1) are significantly different. Uppercase letters refer to the (in)significant differences among samples at the same temperature; lowercase letters refer to the (in)significant differences among different temperatures for the same sample. Alphabetical order indicates the decrease in the mean values (mean with the lettering of A/a has the highest value in its category).*

Table 4.1-1 Sample melting rate (mean±SE) with the increase in temperature from 5 to 25°C.

Sample	Melting rate
US1	135.09±1.69 ^{a} *
S3	116.9±1.36 ^{b}
US2	112.74±1.06 ^{b}
S2	105.13±1.15 ^{c}
S1	100.98±1.22 ^{c}
CS	72.11±1.2 ^{d}
SS	18.85±0.1 ^{e}

** Means not sharing a letter are significantly different. Alphabetical order indicates the decrease in the mean values (mean with the lettering of a has the highest value).*

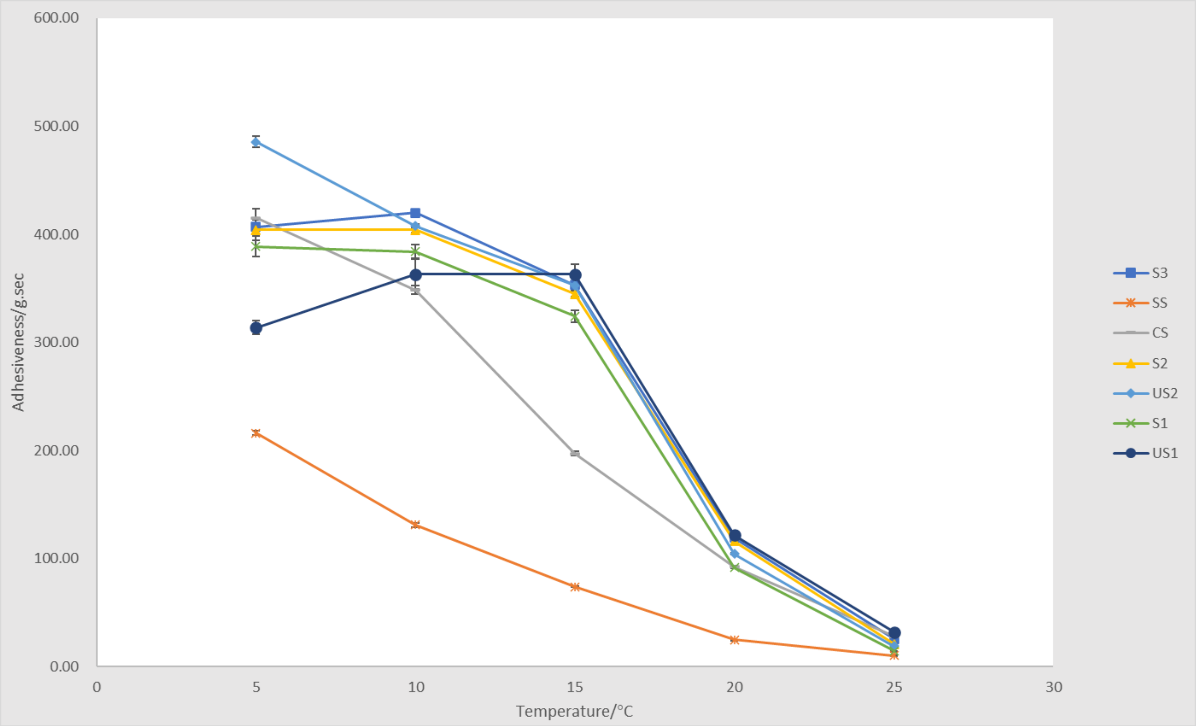
Adhesiveness Adhesiveness indicates the interaction between the sample and the probe or knife in practical use. Adhesiveness is negatively correlated with spreadability, and a less adhesive sample tends to be more spreadable (Glibowski et al., 2008; Oeffner et al., 2013), which was confirmed in the present study, as shown in Table 4.1-2. As mentioned in the method section, work of shear is negatively correlated with spreadability, and lower work of shear means higher spreadability. In Table 4.1-2, there is a strong positive correlation between work of shear and adhesiveness (0.862), meaning a strong negative correlation between the two.

From Figure 4.1-2, as mentioned above, the adhesiveness decreased with the increase in temperature, except that US1 had an increase from 5 to 10°C. Also, the significant differences among samples reduced with the increasing temperature ($P<0.05$), until 25°C when wall the samples were similar ($P>0.05$). The decreasing trend with temperature was reported with previous studies, attributed to the decreased SFC (Bobe et al., 2003; Glibowski et al., 2008; Oeffner et al., 2013), but they did not find the similar adhesiveness at 25°C, as this temperature was not included. Figure 4.1-2 also shows that SS was significantly lower than all the other samples at below 25°C ($P<0.05$). Except for SS, US1 had the lowest adhesiveness at 5°C ($P<0.05$), but the highest adhesiveness at 15 and 20°C, which indicated a significant change over high temperatures for this sample. The different levels of adhesiveness among samples may be due to the different unsaturated fatty acids, which were found in these previous studies. SS may have the highest percentage of unsaturated fatty acids, resulting in the lowest adhesiveness.

Glibowski et al. (2008) also reported a higher SFC resulted in higher adhesiveness, vice versa, thereby, the SFC in US1 at below 10°C could be lower, and that at above 15°C may be lower than other samples except for SS. However, the inference for US1 under 10°C may be contradictory with the results for hardness (Figure 4.1-1) that the highest hardness of US1 may result from the highest SFC. Given that more evidence exists to prove the positive correlation between hardness and SFC

(Campos et al., 2002; Herrera & Hartel, 2000; Narine & Humphrey, 2004; Oeffner et al., 2013; Vithanage et al., 2011), the more possible reason could be that the SFC effect was overridden by the microstructure difference in US1 at below 10°C, as SFC may not reflect the microstructural characteristics which can also alter the melting properties of butter (Vithanage et al., 2009). Since adhesiveness refers to the interaction between the sample and the probe, a higher adhesiveness may be caused by the breakdown of the crystalline structure. At 5°C, US1's lower adhesiveness may be due to its less-disrupted structure, which could be formed mainly by β' forms, and smaller traces of β and α forms. Furthermore, the recrystallisation may have occurred early with newly-formed β' to compensate for the transition to unstable forms (Lopez & Ollivon, 2009).

Figure 4.1-2 Sample adhesiveness as a function of temperature (mean±SE, denoted in the graph).



Adhesiveness Significant difference[1]	S3	SS	CS	S2	S1	US2	US1
5°C	B,a	D,a	B,a	B,a	B,a	A,a	C,b
10°C	A,a	E,b	D,b	A,a	BC,a	AB,b	CD,a
15°C	B,b	D,c	C,c	AB,b	B,b	A,c	A,a
20°C	AB,c	C,d	B,d	AB,c	B,c	AB,d	A,c
25°C	A,d	A,d	A,e	A,d	A,d	A,e	A,d

[1] *In a row or column, table cells not sharing a letter mean that the corresponding adhesiveness values (mean) (refer to Appendix A-Table A.1) are significantly different. Uppercase letters refer to the (in)significant differences among samples at the same temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the mean values (mean with the lettering of A/a has the highest value in its category).*

Table 4.1-2 Pearson's correlation among textural properties[1].

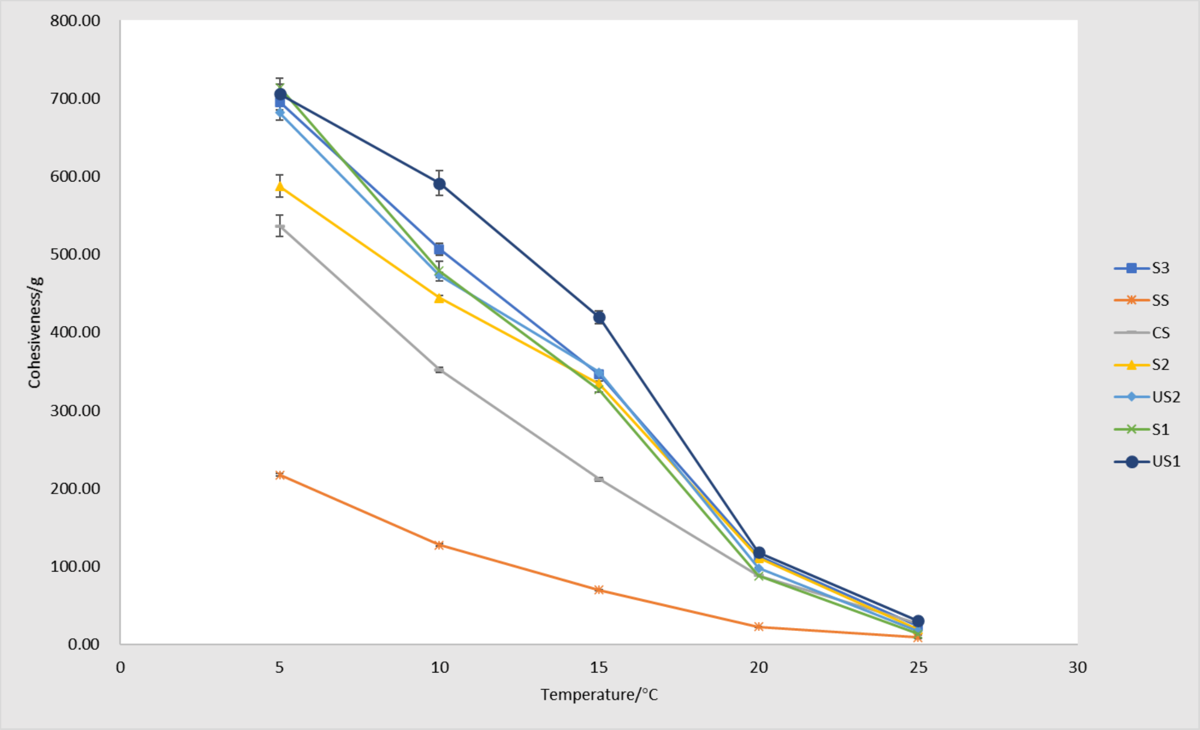
	Hardness	Adhesiveness	Cohesiveness
Adhesiveness	0.85		
Cohesiveness	0.972	0.923	
Work of shear	0.999	0.862	0.974

[1] *A positive value means that the two factors are positively correlated, while a negative value indicates a negative correlation. High degree: a coefficient value between ± 0.50 and ± 1, the correlation is considered as strong. Moderate degree: If the value is between ± 0.30 and ± 0.49, the correlation is a medium correlation. Low degree: a value between ± 0.10 and ± 0.29 refers to a small correlation.*

Cohesiveness Cohesiveness refers to the internal bonds among the fat crystals, and a higher value indicates a stronger interaction among the crystals. Butter with a high cohesiveness was found less spreadable (Glibowski et al., 2008; Oeffner et al., 2013), which was confirmed in the present study, although the literature is scarce in finding this correlation. In Table 4.1-2, cohesiveness and work of shear are in a strong correlation with work of shear (0.974), meaning that cohesiveness and spreadability were negatively correlated. Figure 4.1-3 shows that the samples were significantly different at below 20°C, instead of 25°C, indicating similar inter-crystal bonds among the samples at a lower temperature.

Literature is also limited on the temperature effect on the cohesiveness of butter. (Glibowski et al., 2008) reported that cohesiveness increased with the increase in temperature and that lower SFC contributed to higher cohesiveness. US1 had the significantly highest values at 10 and 15°C, maybe due to its lowest SFC at these temperatures. At 5°C, S1, S2 and S3 were all similar with US1, which may indicate the similar SFC of these four samples. The inferred low SFC of US1 at 5 to 15°C would be contradictory to that in the hardness results. Similar to the scenario for the adhesiveness of US1 at below 10°C, the SFC differences between US1 and S1, and S2 and S3 may be dominated by the variations in the bonding among triglycerides (De Graef et al., 2012; Vithanage et al., 2009). Furthermore, Figure 4.1-3 also shows that the cohesiveness of CS was lower than S1, S2, S3, US1 and US2, which may also indicate the weaker structure and higher polyunsaturated fatty acids in CS, due to the culturing of cream, as discussed earlier in this chapter.

Figure 4.1-3 Sample cohesiveness as a function of temperature (mean±SE, denoted in the graph).



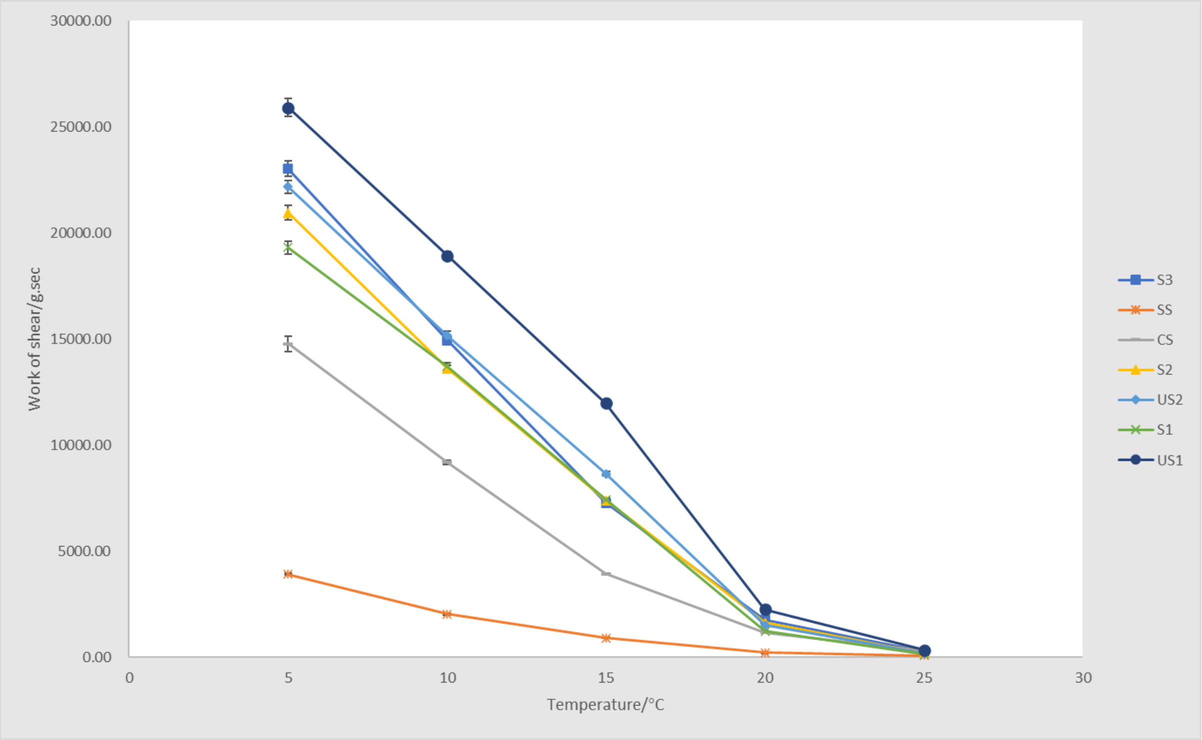
Cohesiveness Significant difference[1]	S3	SS	CS	S2	S1	US2	US1
5°C	A,a	D,a	C,a	B,a	A,a	A,a	A,a
10°C	B,b	E,b	D,b	C,b	BC,b	BC,b	A,b
15°C	B,c	D,c	C,c	B,c	B,c	B,c	A,c
20°C	A,d	B,d	A,d	A,d	A,d	A,d	A,d
25°C	A,e	A,d	A,e	A,e	A,e	A,e	A,e

[1] *In a row or column, table cells not sharing a letter mean that the corresponding cohesiveness values (mean) (refer to Appendix A-Table A.1) are significantly different. Uppercase letters refer to the (in)significant differences among samples at the same temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the mean values (mean with the lettering of A/a has the highest value in its category).*

Work of shear Work of shear is negatively correlated to spreadability, and lower work of shear means higher spreadability. Spreadability is an important functionality for butter, especially when it is used as a table spread at room temperature. Spreadability is also strongly and negatively correlated with hardness (Glibowski et al., 2008; Sert & Mercan, 2020), which was also reported in the present study. Table 4.1-2 shows that hardness had a very strong positive correlation with work of shear (0.999), thus, a very strong negative correlation with spreadability, meaning that the harder samples is less spreadable.

From Figure 4.1-4, work of shear decreased with temperature or spreadability increase with temperature. As mentioned above, the samples were significantly different and the difference reduced with the increase in temperature (P<0.05), until 25°C when there were similar (P>0.05). US1 had the significantly highest value at all temperatures below 25°C, indicating that US1 was the least spreadable sample, due to its highest SFC, as discussed above. In contrast, SS had the lowest value of work of shear, hence, the highest spreadability, due to a much lower SFC. In addition, US1 may have more saturated, or lower unsaturated fatty acids than other samples, as spreadability was found related to the saturation of fatty acids (Bobe et al., 2003; Sébastien Couvreur et al., 2006; Heck et al., 2009).

Figure 4.1-4 **Work of shear as a function of temperature (mean±SE, denoted in the graph).**



Work of Shear Significant difference[1]	S3	SS	CS	S2	S1	US2	US1
5°C	B,a	F,a	E,a	C,a	D,a	B,a	A,a
10°C	B,b	E,b	D,b	C,b	C,b	B,b	A,b
15°C	C,c	E,c	D,c	C,c	C,c	B,c	A,c
20°C	AB,d	C,c	B,d	AB,d	B,d	AB,d	A,d
25°C	A,e	A,c	A,d	A,e	A,e	A,e	A,e

[1] *In a row or column, table cells not sharing a letter mean that the corresponding work of shear values (mean) (refer to Appendix A-Table A.1) are significantly different. Uppercase letters refer to the (in)significant differences among samples at the same temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the mean values (mean with the lettering of A/a has the highest value in its category).*

SS was a spreadable butter made by pure milk fat, and exhibited significant differences for hardness, adhesiveness, cohesiveness and spreadability, to a greater extent than that between other butter samples. The big difference of SS may be the result of special processing methods, such as temperature cycling or AMF fractionation. Temperature cycling refers to the method using fluctuation in temperature to make the butter soft (Bylund, 1995). Cold-warm-cold tempering procedure is likely the processing used on SS, and this method is recognised to improve the spreadability of butter made from winter cream (Fearon, 2011). From Table 3.2-1, SS was likely to be made from winter milk, and it may be suitable for the temperature cycling method. The procedure starts with a low-temperature crystallisation of cream to produce more small crystals, followed by a gentle increase of temperature where partial melting takes place, and then the secondary cold phase induces the recrystallisation of lower melting-point fractions. As a result, the crystal size may be larger, and SFC is reduced, thereby, the spreadable butter can exhibit good spreadability at refrigerator temperature (Fearon, 2011; Kaylegian & Lindsay, 1992). The most used fractionation methods may be the conventional crystallisation method. Low melting point fraction is to keep and make soft products (Abbas et al., 2006). It is a slow cooling process with constant agitation, where AMF is partial crystallised and separated as stearin (more solid with a greater melting point) and olein fractions (more liquid with a lower melting point). The two fractions can both be used for different purposes, or the olein fraction is to be further fractionated at a lower temperature (Queirós et al., 2016).

Figure 4.1-1,2,3,4 shows that the seasonal effect on the butter samples through fatty acid composition may not present. Winter butter is harder than summer butter, for the difference in fatty acid saturation. Milk tends to have higher unsaturated fatty acids in summer, and lower unsaturated fatty acids in winter. In contrast, saturated fatty acids were lower and higher in summer and winter, respectively (Ferlay et al., 2006; Heck et al., 2009). The SFC is also higher in winter, resulting in harder butter (Fearon, 2011). The colour of the samples indicated that CS might be from the milk of cows fed on less fresh grass (as mentioned above), while the equally yellow colour of other samples may indicate that they were from the milk of cows grazing fresh grass at a similar proportion. Among the grass-grazing butter samples except for SS that may be manufactured differently, S1 was made from winter milk, while others from summer milk. As winter butter is harder than summer butter, S1 was expected to be the hardest among the samples, which, however, was contradicted in this study. US1, a summer butter, was the hardest, instead. Cows in winter may not experience less-available fresh grass, as the colour was similar among the grass-grazing butter. An alternative explanation may be the breed variation, and US1 may be made from the milk of Jersey cows that tend to produce milk with higher saturated fatty acids and the butter may be harder (Drackley et al., 2001; Palladino et al., 2010). The causes for the significant highest values of US1 for hardness, adhesiveness, cohesiveness and spreadability may require further investigation on the fatty acid composition of microstructure characteristics.

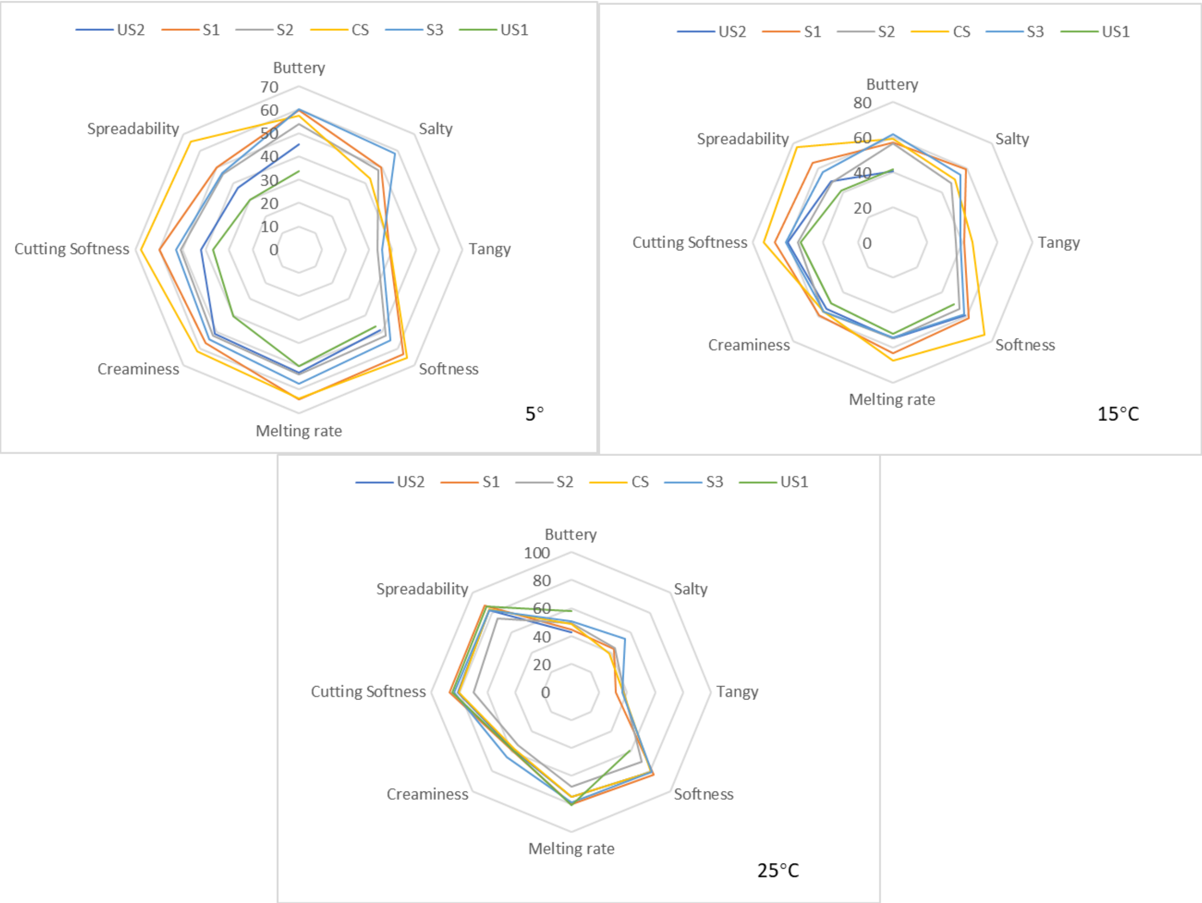
The variation in processing conditions may be another essential factor for the sample differences. Pasteurisation of milk and cream, ripening and cooling of cream, churning and working in butter, making all have effects on the crystallisation of milk fat. In general, fast cooling, high agitation rate and low crystallisation temperature produce smaller crystals and harder products (Campos et al., 2002; Herrera & Hartel, 2000; Lopez et al., 2002; Lopez et al., 2005). The processing history contributes to the product's microstructure nature and its different behaviour by the future heat effects (Lopez, Lavigne, Lesieur, Bourgaux, et al., 2001; Lopez, Lavigne, Lesieur, Keller, et al., 2001). The samples were from different manufacturers where the processing methods may be different, and result in products with different textural properties. US1 may be made with fast cooling, a high agitation rate, with a low crystallisation temperature. Therefore, the textural properties can be different, even though the fatty acid composition may be very similar based on the common regional feeding characteristics.

In summary, temperature and sample type had significant influences on hardness, adhesiveness, cohesiveness and work of shear (spreadability) ($P<0.05$). All the parameters decreased with the increase in temperature, which may be due to the reduction in SFC. US1 was the hardest, adhesive (except at below 10℃), most cohesive and least spreadable sample, while SS the softest and the least adhesive, cohesive and spreadable. CS was in between SS and all the other samples, likely attributed to the culturing of cream giving weak structure. SS may be made with temperature-cycle tempering method to gain a good softness and spreadability. SFC may be tested in future research to confirm the findings. Problems remain unsolved and require further investigation in the significantly different values of US1 in hardness, spreadability, and adhesiveness and cohesiveness (warmer temperature). Microstructure (DSC) and fatty acid composition may be suitable to explain these problems.

4.2 Sensory analysis

Flavour: Figure 4.2-1 shows that temperature and sample type played significant roles in affecting intensities and overall acceptance of flavour. For butter flavour, no significant differences in temperature were found for the same sample ($P>0.05$), except that US1 had significantly lower buttery flavour at 5°C and 15°C than 25°C. At 25°C, all samples had the same intensity of buttery flavour. The insignificant difference in buttery flavour among samples and temperature, except for unsalted samples at 5 and 15 °C, indicated that buttery flavour was outstanding and characteristic for all the samples, and was relatively stable in the changes of temperature. The signature flavours of butter, including buttery, milky and fatty flavours, and the corresponding compounds were found abundant and stable in butter samples over storage time and temperature fluctuation (Mallia et al., 2008). Results from the present and previous studies were consistent. From Table 4.2-1, Buttery flavour had a strong positive correlation with the coverall flavour liking (0.604), indicating that consumer's acceptance for the overall flavour was mainly attributed to buttery flavour, which was previously recognised (Nursten, 1997).

Figure 4.2–1 Intensity rating for sensory attributes at 5°C, 15°C and 25°C for all samples.



Buttery[1]	S1	S2	S3	CS	US1	US2	Melting rate	S1	S2	S3	CS	US1	US2
5°C	A,a	A,a	A,a	A,a	B,b	AB,a	5°C	A,b	AB,b	AB,b	A,a	B,b	AB,b
15°C	AB,a	AB,a	A,a	A,a	BC,b	C,a	15°C	AB,b	AB,ab	AB,b	A,a	B,b	AB,b
25°C	A,a	A,a	A,a	A,a	A,a	A,a	25°C	A,a	A,a	A,a	A,a	A,a	A,a
Salty	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**	**Creaminess**	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	AB,a	AB,a	A,a	B,a	*	*	5°C	A,a	AB,a	AB,a	A,a	A,a	AB,a
15°C	A,a	A,a	A,a	A,a	*	*	15°C	A,a	A,a	A,a	A,a	A,ab	A,a
25°C	AB,a	AB,a	A,a	B,a	*	*	25°C	A,a	A,a	A,a	A,a	A,a	A,a
Tangy	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**	**Cutting Softness**	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	A,a	A,a	A,a	A,a	*	*	5°C	AB,b	BC,b	BC,b	A,b	D,c	CD,c
15°C	A,a	A,a	A,a	A,a	*	*	15°C	A,b	B,b	AB,b	A,ab	B,b	B,b
25°C	A,a	A,a	A,a	A,a	*	*	25°C	A,a	B,a	A,a	AB,a	A,a	AB,a
Softness	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**	**Spreadability**	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	AB,b	BC,b	ABC,b	A,b	C,b	C,b	5°C	B,c	B,b	B,b	A,b	C,b	BC,b
15°C	AB,b	B,b	B,b	A,ab	B,b	B,b	15°C	AB,b	C,b	BC,b	A,ab	C,b	C,b
25°C	AB,a	B,a	AB,a	AB,a	A,a	AB,a	25°C	A,a	A,a	A,a	A,a	A,a	A,a

[1] *In a row or column, table cells not sharing a letter mean that the corresponding values (mean±SE) (refer to Appendix A-Table A.2) are significantly different. Uppercase letters refer to the (in)significant*

differences among samples at the same temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the values of means (mean with the lettering of A/a has the highest value in its category).

Table 4.2-1 Pearson's correlation among sensory attributes.

Correlation[1]	Buttery	Salty	Tangy	Flavour overall	Softness	Melting rate	Texture overall	Creaminess	Combination overall	Cutting softness	Spreadability
Salty	0.301										
Tangy	0.257	0.317									
Flavour overall[2]	0.604	0.144	0.08								
Softness	0.053	-0.09	0.006	0.023							
Melting rate	0.111	0.002	0.019	0.107	0.727						
Texture overall[2]	0.406	0.039	0.024	0.626	0.198	0.254					
Creaminess	0.449	0.164	0.06	0.445	0.31	0.327	0.527				
Combination overall[2,3]	0.514	0.12	0.056	0.779	0.135	0.217	0.755	0.639			
Cutting softness	0.048	-0.088	-0.016	0.027	0.724	0.579	0.16	0.318	0.127		
Spreadability	0.048	-0.086	0.043	0.018	0.666	0.527	0.114	0.271	0.09	0.813	
Spreadability overall[2]	0.211	0.007	0.028	0.33	0.393	0.349	0.516	0.395	0.443	0.505	0.614

[1] *A positive value means that the two factors are positively correlated, while a negative value indicates a negative correlation. High degree: a coefficient value between ± 0.50 and ± 1, the correlation is considered as strong. Moderate degree: If the value is between ± 0.30 and ± 0.49, the correlation is a medium correlation. Low degree: a value between ± 0.10 and ± 0.29 refers to a small correlation.*

[2] *Overall means the overall acceptance.*

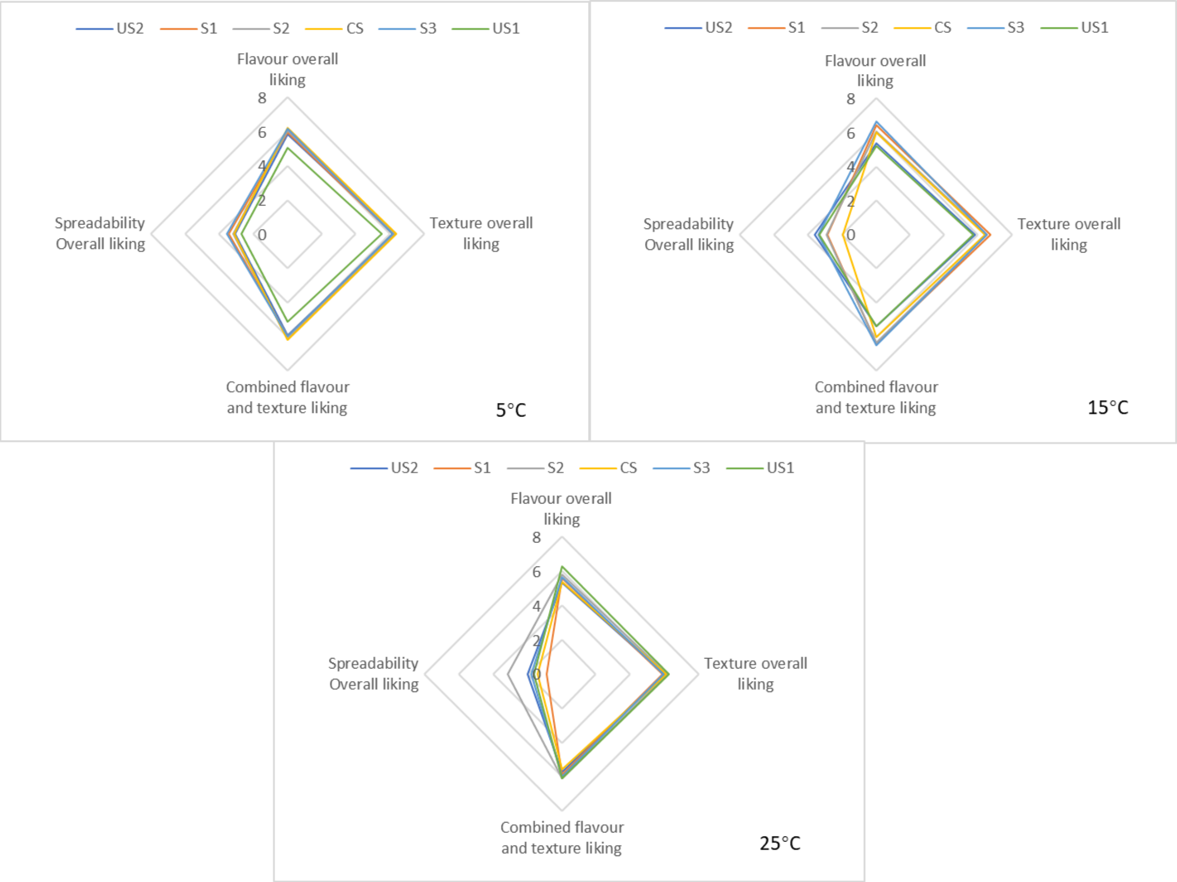
[3] *Combination refers to the overall acceptance for the combined flavour and texture.*

Salty and tangy flavours were only tested for salted samples, to understand their possible impacts on other sensory attributes and overall acceptance. In Figure 4.2-1, CS had lower saltiness at 5 and 25°C (P<0.05), while S1, S2 and S3 were similar in the saltiness intensities across three temperatures (P>0.05). From Table 4.2-1, saltiness was in a moderate positive correlation with buttery flavour (0.301), a small positive correlation with overall flavour liking (0.144), indicating that salt may have played a moderate role in contributing to the overall flavour acceptance. The flavour enhancing effect of salt only on butter has long been reported (Syarifuddin et al., 2016; Tuorila-Ollikainen et al., 1986), but it was insignificant in the present study. It is more likely that the salty and buttery flavours together characterised the overall flavour. The cooperation of these two exhibited a typical "cool-melting" sensation affecting the release of flavour compounds (Saint-Eve et al., 2009).

As mentioned above, US1 had significantly lower buttery flavour at 5°C and 15°C, than 25°C. Figure 4.2-1 also shows significant difference between salted and unsalted samples, at 5 and 15°C (P<0.05). US1 had a significantly less buttery flavour than all the salted samples at 5°C, while US2 was the lowest at 15°C. The fat matrix with reduced salt could hinder the release of characteristic aroma compounds (Boisard et al., 2014; Guichard, 2002). The lower intensities of buttery flavour for the two unsalted samples, US1 and US2, may be explained by the lack of salt that could have encouraged their buttery flavour. However, this blocking effect may be insignificant, as the overall likings for flavour were similar most of the times, with a neutral acceptance (scored 5-6.6) (P>0.05), except that the unsalted samples were the least liked at 15°C, compared to the salted ones, or to themselves

(*P*<0.05), as shown in Figure 4.2-2. Further investigation could be undertaken by evaluating saltiness intensity for unsalted samples too.

Figure 4.2–2 Overall acceptance for flavour, texture, combined flavour and texture, and spreadability at 5°C, 15°C and 25°C for all samples.



Flavour[1]	S1	S2	S3	CS	US1	US2
5°C	A,a	A,a	A,a	A,a	A,b	A,a
15°C	AB,a	AB,a	A,a	AB,a	B,b	B,a
25°C	A,a	A,a	A,a	A,a	A,a	A,a
Texture	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	A,a	A,a	A,a	A,a	A,a	A,a
15°C	A,a	A,a	A,a	A,a	A,a	A,a
25°C	A,a	A,a	A,a	A,a	A,a	A,a
Combination	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	A,a	A,a	A,a	A,a	A,a	A,a
15°C	A,a	A,a	A,a	A,a	A,a	A,a
25°C	A,a	A,a	A,a	A,a	A,a	A,a
Overall Spreadability	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	AB,a	AB,a	ABC,a	A,a	C,b	BC,b
15°C	AB,a	BC,a	ABC,a	A,a	C,ab	BC,ab
25°C	A,a	A,a	A,a	A,a	A,a	A,a

[1] *In a row or column, table cells not sharing a letter mean that the corresponding values (mean±SE) (refer to Appendix A-Table A.2) are significantly different. Uppercase letters refer to the (in)significant differences among samples at the same temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the mean values (mean with the lettering of A/a has the highest value in its category).*

In Figure 4.2-1, no significant difference was found for tangy flavour based on temperature or sample. Interestingly, a certain level of tanginess was picked up for all the samples at all temperatures, while tanginess should only be the character of CS, the slightly salted cultured butter. The reason could be that the panellists failed to detect diacetyl and confused it with other flavour attributes that were not included in the questionnaire. As diacetyl is highly volatile even at a cold temperature (Chauhan et al., 2010; Park & Drake, 2005), its limited amount in a small piece of the sample may have all evaporated in the incubator. The minor amount may be nearly impossible for the untrained panel to identify, as their detection ability is quite limited, even in discrimination testing of two samples (Chauhan et al., 2010). Diacetyl may also have been confused with tangy for their similarity as slightly acidic (Rakib et al., 2017). Tangy is characteristic in fermented dairy products, and can also be undesirable and associated with off-flavour in dairy products (Chen et al., 2003). In the present study, the panellists might consider the butter samples to be less fresh, with temperature involvement. Most dairy products require storage at a low temperature, and consumer perception of freshness was found correlated with a decrease in mouth temperature (Jansson et al., 2020). The samples might have a closer-to-mouth temperature and induce a perception of less freshness and the identification of tanginess as an off-flavour. Fortunately, this effect may be insignificant, as the correlation between tanginess and other sensory attributes were very small, less than 0.056 (Table 4.2-2).

Texture Figure 4.2-1 shows significant differences for softness intensity, based on temperature or sample ($P<0.05$). With the increase in temperature, all temperatures exhibited increasing softness or decreasing hardness. Table 4.2-2 shows a strong negative correlation between sensory softness and hardness in the instrumental analysis (-0.746), indicating that sensory softness is equivalent to hardness in the instrumental analysis. In this sense, the increasing softness in the mouth can also be attributed to the decreasing SFC. However, softness values were not the same at 25°C, as in the instrumental test (Figure 4.1-1). Compared to the instrumental test, US1 along was not the significantly lowest at the lower temperatures but companied by other samples, and CS was not always significantly higher, indicating that untrained human panellists may be less sensitive to the textural changes across temperature or samples. However, human perception is different from instrumental measurement, and samples with different qualities measured by the instrument may have the same sensory score (Drake, 2004). In addition, the absence of salt in US1 and US2 may be another reason for their lower softness and melting rate for the two unsalted samples, as salt is a good texture enhancer (Syarifuddin et al., 2016). However, the mechanism of salt influences on texture is unclear.

Table 4.2-2 Pearson's correlation between instrumental and sensory textural attributes.

Correlation[1]	Hardness	Sensory softness	Work of shear	Sensory melting rate	Creaminess	Sensory spreadability
Sensory softness	-0.746					
Work of shear	0.999	-0.76				
Sensory melting	-0.744	0.852	-0.762			
Creaminess	-0.62	0.687	-0.625	0.712		
Sensory spreadability	-0.865	0.889	-0.878	0.948	0.749	
Cutting softness	-0.868	0.88	-0.879	0.951	0.79	0.984

[1] *A positive value means that the two factors are positively correlated, while a negative value indicates a negative correlation. High degree: a coefficient value between ± 0.50 and ± 1, the correlation is considered as strong. Moderate degree: If the value is between ± 0.30 and ± 0.49, the correlation is a medium correlation. Low degree: a value between ± 0.10 and ± 0.29 refers to a small correlation.*

In Table 4.2-2, CS had the same melting rate in the mouth at all three temperatures. As mentioned in the previous section, melting rate describes the amount of solid-to-liquid transition of crystals. The same melting rate of CS indicated the consistent transition per unit temperature, maybe due to that the fatty acids of CS had continuous melting rates, and distributed at equal proportions. In other words, melting rate is an indicator of the SFC at per unit temperature. In contrast, melting rates in the mouth of other samples significantly increased when the samples were at 25°C and reached to the same level, which indicated that from 25°C, the SFC values were likely the same for all the samples, at per unit temperature. As the melting rate in the instrumental analysis was calculated based on the temperature change from 5 to 25°C (Table 4.1-1), the instrumental melting rate is similar to the melting rate in the mouth when the sample was at 5°C. More significant differences were in the samples' instrumental melting rate than the oral melting rate. US1 melted the most slowly in the mouth among all, while exhibiting the highest melting rate based on instrumental hardness. The reason could be that the melting in the mouth is a continuous process, evaluated by human perceptions, so melting rate in the mouth may be more suitable to reflect the melting behaviour. Furthermore, US1 was the hardest, likely with more SFC and melted slower, which were agreed by the sensory melting rate.

From Figure 4.2-1,2, one of the significant differences were found in the overall acceptance of texture, despite of temperature or sample ($P>0.05$). The Pearson's correlation results only showed small correlations between softness or melting rate and the overall texture liking (Table 4.2-1). This may indicate that products with differently scored sensory attributes may have the same overall acceptance (Drake, 2004; Krause et al., 2007). A strong correlation was found between softness and melting rate in the mouth, meaning that softer samples melted faster, or faster-melting samples were softer, which may be attributed to a lower SFC of the sample, or less sharp crystal structure (Staniewski et al., 2020; Vithanage et al., 2009). The strong and positive correlation between softness and melting rate in the mouth may also indicate that they were similar in describing the texture sensation in the mouth, correlation between which two was previously reported (Pascua et al., 2013).

Creaminess and combined flavour and texture No significant difference was found from temperature or sample for creaminess or the overall acceptance for the combination of flavour and texture. This could be due to the integrated effects of differences in flavour and texture attributes, as creaminess is a combined sensory attribute of flavour and texture (Frøst & Janhøj, 2007). In the Pearson's correlation test (Table 4.2-1), creaminess had a strong positive correlation (0.639) with the combined overall liking for flavour and texture, indicating that creaminess was the main attribute for the overall flavour and texture profile. The combined acceptance is also in stronger positive correlations with the overall likings for flavour and texture (0.779 and 0.755, respectively), also indicating the nature of creaminess as a combined flavour and texture attribute. Creaminess had strong negative correlations with instrumental hardness (-0.62) and work of shear (strong positive correlation with instrumental spreadability). Higher creaminess may result from lower SFC in the sample and a fast-melting structure (Akhtar et al., 2005; Kilcast & Clegg, 2002), which could also explain the moderate positive correlations between creaminess and softness in mouth and oral melting rate. Creaminess had a moderate and positive correlation with buttery flavour, consistent with previous research (Hyvönen et al., 2003; Saint-Eve et al., 2004; Tournier et al., 2007). (Saint-Eve et al., 2004) found that samples evaluated as buttery were considered as soft and smooth texture, together correlating creaminess, buttery flavour, softness and fast melting in the mouth.

Mechanical spreadability The mechanical testing of butter samples was to evaluate the texture attributes sensed through the knife, using butter as a table spread. As shown in Figure 4.2-1, all three attributes, softness through cutting, spreadability on a cracker and the overall perception on spreadability, showed significant differences based on temperature and sample type ($P<0.05$). The cutting softness was significantly higher when the samples were at 25°C ($P<0.05$). Table 4.2-2 shows a strong negative correlation between cutting softness and instrumental hardness (Table 4.2-2), which was previously reported (Rousseau & Marangoni, 1998). SFC difference may be the cause as it is a possible effect on SFC. US1 was harder to cut at 5°C, which is in with the instrumental analysis results (Figure 4.1-1).

In Figure 4.2-1, the spreadability on a cracker was significantly higher at 25°C (P<0.05), and all the samples were at the same level (P>0.05), which was in line with the instrumental results where the spreadability (work of shear) values were similar at this temperature (Figure 4.1-1). Table 4.2-2 also shows a strong negative correlation between spreadability and work of shear (strong positive correlation with spreadability on the instrument). Also similar to instrumental analysis, US1 was among the lower group in spreadability, but to a less extent. The consistency between instrumental and sensory analysis indicated that the instrumental texture analysis method well mimicked the way that consumers use the butter products as a spread, and the consistency between the two methods was also reported previously (Estanqueiro et al., 2016; Rohm et al., 1997; Rousseau & Marangoni, 1998). The softness and spreadability detected through the knife were strongly correlated (0.813) (Table 4.2-1), indicating that the consumers perceived these two attributes similarly.

In Figure 4.2-2, the overall acceptance ratings for spreadability were the same at all temperatures ($P>0.05$), except that US1 and US2 scored significantly lower at 5°C than 25°C ($P<0.05$). All the samples were the same at 25°C ($P>0.05$), while at the lower temperature, US1 had the lowest spreadability, at 5 and 15°C. These similarities and differences are were the results from the intensity variations of cutting softness and spreadability on a cracker, as Table 4.2-1 shows that the overall spreadability liking was strongly and positively correlated with spreadability on a cracker and cutting softness. Interestingly, the liking scores were all under 3.7, down to less than 1 (Appendix A-Table A.2), for all temperatures, indicating a low acceptance on the overall spreadability (extremely to moderate dislike), compared to the overall acceptance for other attributes. The reason could be that the way of spreading the butter during the sensory evaluation was different from the way the consumers used butter in their daily life, such as spreading on toast.

In summary, temperature and sample type had significant differences on sensory attributes, but to a less extent than instrumental analysis results, likely due to that instrument measured different products may have the same sensory scores. The characteristic buttery flavour was stable in the sample against temperature changes, while diacetyl flavour was highly volatile and not detected, or correlated with the samples' un-freshness. The effects of salt in enhancing flavour and texture were moderate, maybe due to the fat coating effect. Creaminess was a valid attribute to evaluate the combined flavour and texture, which was strongly correlated with buttery, softness and melting rate. Oral melting rate and the melting rate based on instrumental analysis were contradictory for US1, while the oral melting rate was positively correlated with the instrumental hardness which can be explained by the changes in SFC. Instrumental and consumer sensory texture attributes were highly correlated, indicating their equal validation in measuring the texture of butter.

Chapter 5

Conclusion

The textural properties of seven and sensory properties of six commercial butter samples were studied and compared. Instrumental and sensory analyses were used to study the traditional parameters, and both proved valid. Melting rate in the instrumental analysis was based on the changes in hardness over temperature, where limited literature presents. The oral melting rate was also studied and compared with instrumental melting rate, and they showed contradicting results for US1.

Temperature and sample type had significant differences on all the instrumental textural parameters, while insignificant effects on some sensory attributes. In general, the increase in temperature made the samples soft, spreadable, less adhesive and cohesive. The highest temperature, 25°C, made all the samples similar in all instrumental parameters and some sensory attributes. At lower temperatures, US1 was significantly harder and less spreadable, may be due to the high SFC or varied polymorphism. SS was the softest and most spreadable, may be due to the temperature-cycle tempering process on winter butter or AMF fractionation. The culturing of cream may contribute to the soft texture of CS, due to a weak structure. The characteristic buttery flavour was stable in the sample against temperature changes, while highly volatile diacetyl was not detected, or confused with un-freshness of the samples. Creaminess was valid to evaluate the combined flavour and texture properties, which was strongly correlated with buttery, softness and oral melting rate.

SFC, DSC and fatty acid profile may require further study to confirm the results, and to address the problems in the significant differences of US1 in hardness, spredability, adhesiveness, cohesiveness and melting rate. In further sensory analysis, diacetyl content may be studied, without confusion with tangy flavour, and saltiness may be also evaluated for unsalted samples, to investigate the salt effects.

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Appendix A

Instrumental texture analysis result

A.1 Instrumental texture analysis result

Table A. 1 Instrumental texture analysis results (mean±SE) for hardness(a), Adhesiveness(b), Cohesiveness(c) and work of shear (d) for all seven samples. Means that do not share a letter are significantly different. Uppercase letters refer to the (in)significant differences among samples at the sample temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the values of means (mean with the lettering of A/a has the highest value in its category).

Hardness/g	S3	SS	CS	S2	S1	US2	US1
5°C	2299.89±25.34 ^{B,a}	394.88±1.48 ^{G,a}	1469.97±26.99 ^{F,a}	2090.34±24.13 ^{D,a}	1946.88±25.44 ^{E,a}	2202.73±21.4 ^{C,a}	2604.31±34.46 ^{A,a}
10°C	1422.9±9.71 ^{B,b}	200.41±2.32 ^{E,b}	860.62±8.2 ^{D,b}	1289.95±12.16 ^{C,b}	1321.26±11.8 ^{C,b}	1428.76±12.4 ^{B,b}	1829.8±18.9 ^{A,b}
15°C	685.99±5.27 ^{C,c}	94.27±0.57 ^{E,c}	364.99±2.45 ^{D,c}	679.15±6.01 ^{C,c}	690.08±6.25 ^{C,c}	795.19±6.1 ^{B,c}	1109.22±6.37 ^{A,c}
20°C	181.49±1.36 ^{AB,d}	26.56±0.26 ^{C,d}	125.35±1.14 ^{B,d}	167.63±0.71 ^{AB,d}	130.68±0.7 ^{B,d}	153.11±1.86 ^{AB,d}	218.46±2.2 ^{A,d}
25°C	30.45±0.32 ^{A,e}	9.48±0.08 ^{A,d}	35±0.29 ^{A,e}	24.19±0.3 ^{A,e}	17.46±0.15 ^{A,e}	22.03±0.29 ^{A,e}	33.68±0.24 ^{A,e}

Adhesiveness/g.sec	S3	SS	CS	S2	S1	US2	US1
5°C	406.76±7.01 ^{B,a}	215.97±2.09 ^{D,a}	415.24±8.07 ^{B,a}	404.21±9.85 ^{B,a}	388.89±9.71 ^{B,a}	485.46±5.24 ^{A,a}	313.76±6.41 ^{C,b}
10°C	419.76±3.27 ^{A,a}	130.98±1.96 ^{E,b}	348.52±4.23 ^{D,b}	403.98±2.63 ^{A,a}	383.95±6.29 ^{BC,a}	407.64±1.44 ^{AB,b}	363.17±13.67 ^{CD,a}
15°C	353.13±2.37 ^{B,b}	73.81±0.45 ^{D,c}	197.21±2.16 ^{C,c}	344.83±2.64 ^{AB,b}	324.22±5.45 ^{B,b}	352.69±1.82 ^{A,c}	362.97±9.31 ^{A,a}
20°C	119.29±0.82 ^{AB,c}	24.6±0.2 ^{C,d}	92.18±0.8 ^{B,d}	115.96±0.6 ^{AB,c}	91.32±0.54 ^{B,c}	104.2±0.93 ^{AB,d}	121.36±1.3 ^{A,c}
25°C	25.34±0.24 ^{A,d}	9.97±0.06 ^{A,d}	28.05±0.2 ^{A,e}	20.65±0.19 ^{A,d}	14.49±0.1 ^{A,d}	18.62±0.19 ^{A,e}	31.97±0.24 ^{A,d}

Cohesiveness/g	S3	SS	CS	S2	S1	US2	US1
5°C	695.48±10.57 ^{A,a}	217.51±1.26 ^{D,a}	536.44±13.71 ^{C,a}	587.65±14.61 ^{B,a}	713.96±11.67 ^{A,a}	682.07±9.85 ^{A,a}	705.82±12.4 ^{A,a}
10°C	506.64±7.74 ^{B,b}	127.67±1.48 ^{E,b}	352.06±3.29 ^{D,b}	444.64±2.32 ^{C,b}	478.67±12.39 ^{BC,b}	473.6±3.63 ^{BC,b}	591.6±16.17 ^{A,b}
15°C	346.06±2.59 ^{B,c}	69.95±0.35 ^{D,c}	211.68±2.34 ^{C,c}	333.91±3.38 ^{B,c}	326.45±2.48 ^{B,c}	349.12±2.01 ^{B,c}	419.45±8.56 ^{A,c}
20°C	112.94±0.62 ^{A,d}	22.89±0.18 ^{B,d}	88.53±0.87 ^{A,d}	110.86±0.4 ^{A,d}	88.11±0.42 ^{A,d}	97.97±0.7 ^{A,d}	118.28±1.59 ^{A,d}
25°C	23.95±0.16 ^{A,e}	8.76±0.05 ^{A,d}	26.35±0.18 ^{A,e}	19.26±0.16 ^{A,e}	13.38±0.09 ^{A,e}	17.29±0.15 ^{A,e}	30.4±0.21 ^{A,e}

Work of Shear/g.sec	S3	SS	CS	S2	S1	US2	US1
5°C	23028.69±372.67 ^{B,a}	3925.89±39.87 ^{F,a}	14788.52±363.8 ^{E,a}	20950.98±335.59 ^{C,a}	19305.37±293.46 ^{D,a}	22177.04±292.52 ^{B,a}	25904.47±430.9 ^{A,a}
10°C	14944.28±159.52 ^{B,b}	2036.73±32.22 ^{E,b}	9191.48±108.85 ^{D,b}	13610.14±172.01 ^{C,b}	13712.72±184.45 ^{C,b}	15160.92±195.55 ^{B,b}	18934.72±173.86 ^{A,b}
15°C	7275.55±79.26 ^{C,c}	921.18±6.35 ^{E,c}	3933.76±29.85 ^{D,c}	7406.75±86.13 ^{C,c}	7439.53±80.15 ^{C,c}	8658.32±96.48 ^{B,c}	11976.26±98.79 ^{A,c}
20°C	1790.59±15.88 ^{AB,d}	229.78±2.32 ^{C,c}	1179.45±10.72 ^{B,d}	1635.11±10.58 ^{AB,d}	1242.81±8.17 ^{B,d}	1523.75±22.37 ^{AB,d}	2267.9±22.49 ^{A,d}
25°C	271.09±2.99 ^{A,e}	83.22±0.89 ^{A,c}	312.58±2.91 ^{A,d}	213.1±3.27 ^{A,e}	152.04±1.68 ^{A,e}	195.36±2.9 ^{A,e}	341.02±3.21 ^{A,e}

A.2 Sensory analysis results

Table A.2 Sensory analysis results (mean±SE) for six samples. Means that do not share a letter are significantly different. Uppercase letters refer to the (in)significant differences among samples at the sample temperature; and lowercase letters refer to the (in)significant differences among different temperature for the same sample. Alphabetical order indicates the decrease in the values of means (mean with the lettering of A/a has the highest value in its category).

Buttery	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	59.64±2.83 ^{A,a}	53.9±2.98 ^{A,a}	60.22±2.53 ^{A,a}	57.34±3.06 ^{A,a}	33.74±2.73 ^{B,b}	44.9±3.34 ^{AB,a}
15°C	56.88±3.44 ^{AB,a}	56.38±3.05 ^{AB,a}	61.64±2.83 ^{A,a}	59±3.17 ^{A,a}	41.42±3.48 ^{BC,b}	40.7±3.21 ^{C,a}
25°C	44.4±3.46 ^{A,a}	49.22±3.43 ^{A,a}	50.74±3.55 ^{A,a}	48.78±3.38 ^{A,a}	57.64±2.84 ^{A,a}	42.56±3.16 ^{A,a}
Salty	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	49.74±3.18 ^{AB,a}	47.94±2.8 ^{AB,a}	58.34±3.15 ^{A,a}	43.16±2.68 ^{B,a}	*	*
15°C	58.98±2.66 ^{A,a}	47.42±2.82 ^{A,a}	38.9±2.8 ^{A,a}	50.46±2.92 ^{A,a}	*	*
25°C	43.06±3.32 ^{AB,a}	44.08±3.32 ^{AB,a}	54.04±3.49 ^{A,a}	38.92±3.08 ^{B,a}	*	*
Tangy	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	38.56±3.18 ^{A,a}	33.5±2.66 ^{A,a}	35.5±2.89 ^{A,a}	39.16±3.31 ^{A,a}	*	*
15°C	41.04±3.24 ^{A,a}	36.18±2.65 ^{A,a}	38.9±2.8 ^{A,a}	45.5±2.96 ^{A,a}	*	*
25°C	32.12±2.83 ^{A,a}	38.12±3.32 ^{A,a}	36.7±3.09 ^{A,a}	37.32±3.24 ^{A,a}	*	*
Softness	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	63.24±2.86 ^{AB,b}	52.38±3.08 ^{BC,b}	55.08±2.82 ^{ABC,b}	65.42±2.69 ^{A,b}	46.28±3.39 ^{C,b}	49±3.47 ^{C,b}
15°C	61.54±2.44 ^{AB,b}	53.9±2.77 ^{B,b}	58.1±2.77 ^{B,b}	74.38±1.8 ^{A,ab}	49.78±2.97 ^{B,b}	58.66±3.06 ^{B,b}
25°C	83.42±1.44 ^{AB,a}	70.88±3.3 ^{B,a}	81.1±1.5 ^{AB,a}	80.84±1.57 ^{AB,a}	59.02±1.45 ^{A,a}	80.5±2.43 ^{AB,a}
Melting rate	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	64.2±2.54 ^{A,b}	53.56±3 ^{AB,b}	57.6±2.66 ^{AB,b}	63.74±2.97 ^{A,a}	49.94±3.55 ^{B,b}	52.52±3.07 ^{AB,b}
15°C	63.32±2.59 ^{AB,b}	54.96±2.69 ^{AB,ab}	54.34±2.78 ^{AB,b}	67.78±2.65 ^{A,a}	52.46±3.1 ^{B,b}	54.72±3.21 ^{AB,b}
25°C	80.18±2.08 ^{A,a}	67.64±3.16 ^{A,a}	78.72±1.75 ^{A,a}	74.82±2.33 ^{A,a}	81.02±1.72 ^{A,a}	75.2±2.67 ^{A,a}
Creaminess	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	56.5±2.67 ^{A,a}	52.28±2.46 ^{AB,a}	54.56±2.86 ^{AB,a}	61.72±2.38 ^{A,a}	40.14±2.69 ^{A,a}	51.14±3.01 ^{AB,a}
15°C	59.28±2.96 ^{A,a}	56.24±2.63 ^{A,a}	56.04±2.37 ^{A,a}	55.9±3.34 ^{A,a}	49.34±3.18 ^{A,ab}	53.64±3.4 ^{A,a}
25°C	59.26±3.05 ^{A,a}	53.54±3.16 ^{A,a}	65.56±2.77 ^{A,a}	57.76±3.59 ^{A,a}	59.02±3.04 ^{A,a}	58.76±3.11 ^{A,a}
Cutting Softness	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	59.78±49.76 ^{AB,b}	50.58±45.8 ^{BC,b}	52.76±46.4 ^{BC,b}	67.78±65.66 ^{A,b}	36.86±29.98 ^{D,c}	41.92±37.3 ^{CD,c}
15°C	67.2±64.3 ^{A,b}	54.38±48.74 ^{B,b}	60.92±56.48 ^{AB,b}	73.46±77.02 ^{A,ab}	52.48±41.76 ^{B,b}	60.02±49.4 ^{B,b}
25°C	87.16±87.34 ^{A,a}	70.14±74.14 ^{B,a}	83.74±82.86 ^{A,a}	80.64±83.24 ^{AB,a}	85.26±86 ^{A,a}	80.94±82.5 ^{AB,a}
Spreadability	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	3.45±0.29 ^{AB,a}	3.17±0.23 ^{AB,a}	3.54±0.25 ^{ABC,a}	3.12±0.23 ^{A,a}	2.68±0.25 ^{C,b}	3.07±0.26 ^{BC,b}
15°C	2.84±0.2 ^{AB,a}	2.92±0.23 ^{BC,a}	3.38±0.23 ^{ABC,a}	1.94±0.21 ^{A,a}	3.32±0.25 ^{C,ab}	3.61±0.26 ^{BC,ab}
25°C	0.88±0.25 ^{A,a}	3.13±0.28 ^{A,a}	1.76±0.22 ^{A,a}	1.4±0.22 ^{A,a}	1.59±0.24 ^{A,a}	1.96±0.23 ^{A,a}
Flavour	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	5.94±0.2 ^{A,a}	6.08±0.19 ^{A,a}	6.18±0.2 ^{A,a}	6.24±0.21 ^{A,a}	5.06±0.23 ^{A,b}	5.84±0.24 ^{A,a}
15°C	6.42±0.24 ^{AB,a}	6.06±0.26 ^{AB,a}	6.62±0.21 ^{A,a}	6.02±0.26 ^{AB,a}	5.22±0.26 ^{B,b}	5.38±0.26 ^{B,a}
25°C	5.58±0.28 ^{A,a}	5.8±0.26 ^{A,a}	5.66±0.26 ^{A,a}	5.38±0.29 ^{A,a}	6.26±0.24 ^{A,a}	5.3±0.26 ^{A,a}
Texture	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	6.16±0.19 ^{A,a}	6.26±0.17 ^{A,a}	6.16±0.15 ^{A,a}	6.38±0.19 ^{A,a}	5.52±0.22 ^{A,a}	6.24±0.21 ^{A,a}
15°C	6.74±0.18 ^{A,a}	6.52±0.19 ^{A,a}	6.52±0.18 ^{A,a}	6.42±0.24 ^{A,a}	5.74±0.22 ^{A,a}	5.82±0.25 ^{A,a}
25°C	5.9±0.25 ^{A,a}	6.16±0.24 ^{A,a}	5.94±0.25 ^{A,a}	6.08±0.26 ^{A,a}	6.26±0.25 ^{A,a}	6.02±0.25 ^{A,a}
Combination	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	5.96±0.19 ^{A,a}	6.14±0.16 ^{A,a}	5.96±0.16 ^{A,a}	6.2±0.17 ^{A,a}	5.1±0.23 ^{A,a}	5.94±0.24 ^{A,a}
15°C	6.38±0.23 ^{A,a}	6.34±0.2 ^{A,a}	6.48±0.21 ^{A,a}	6.04±0.27 ^{A,a}	5.4±0.25 ^{A,a}	5.36±0.28 ^{A,a}
25°C	5.86±0.24 ^{A,a}	6.06±0.25 ^{A,a}	6±0.24 ^{A,a}	5.54±0.28 ^{A,a}	6.10±0.24 ^{A,a}	5.74±0.24 ^{A,a}
Overall Spreadability	**S1**	**S2**	**S3**	**CS**	**US1**	**US2**
5°C	3.45±0.29 ^{AB,a}	3.17±0.23 ^{AB,a}	3.54±0.25 ^{ABC,a}	3.12±0.23 ^{A,a}	2.68±0.25 ^{C,b}	3.07±0.26 ^{BC,b}
15°C	2.84±0.2 ^{AB,a}	2.92±0.23 ^{BC,a}	3.38±0.23 ^{ABC,a}	1.94±0.21 ^{A,a}	3.32±0.25 ^{C,ab}	3.61±0.26 ^{BC,ab}
25°C	0.88±0.25 ^{A,a}	3.13±0.28 ^{A,a}	1.76±0.22 ^{A,a}	1.4±0.22 ^{A,a}	1.59±0.24 ^{A,a}	1.96±0.23 ^{A,a}