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Ollscoil na hÉireann, Corcaigh
The National University of Ireland, Cork
University College Cork

School of Food and Nutritional Sciences

Head of School: Prof. Alan L. Kelly

Supervisors: Prof. James A. O'Mahony and Dr. Tom F. O'Callaghan



**Influence of post-production storage conditions and
seasonality on techno-functional properties of butter**

Thesis presented by

Órla Dinneen

B.Sc. Food Science, University College Cork

for the degree of

Master of Science

in

Food Science and Technology

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Declaration

I hereby declare that the work submitted is entirely my own and has not been submitted as an exercise for a degree at this or any other university or higher education institute, or for any other academic award in this university.

Name: Órla Dinneen

Órla Dinneen

Date: 11/March/2025

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Dedication

I would like to dedicate this thesis to my parents, siblings and grandparents.

Abstract

Several properties of butter, including colour, texture and meltability are key determinants of the quality and acceptability of the product. Consistency in the delivery of these properties in finished product, across multiple application scenarios, is key to the continued success and consumer acceptance of the product. To achieve this, it is essential to understand how each unit operation and processing step in the overall process impacts the processability and quality attributes of finished product. The aim of this thesis was to investigate how (a) post-production storage conditions, and (b) seasonality impacted these properties. Variation in the quality and chemical properties of milk and cream (e.g., fatty acid profile), as well as post-production storage conditions (i.e., temperature and time), are known to exist within large-scale butter production systems, making the research objectives of this thesis particularly relevant for dairy scientists and industry alike.

In the first experimental chapter, texture analysis, differential scanning calorimetry (DSC) and rheological analyses were employed to understand how post-production storage impacted techno-functional properties of butter in mid-late season (MLS) and late season (LS). Fatty acid profiling was performed to provide a baseline understanding of the dominant chemical constituents of butter. Six different temperature treatments were used to store butter in the days directly following production. Specific storage temperatures used included temperatures between -30°C and +5°C. It was concluded that, although numerical differences existed across all measured parameters, when considering standard deviation, no differences in key functionality parameters were found between the different temperature treatments investigated. These conclusions informed the authors that it would be relevant to focus on the impact of pre-processing factors, and specifically, seasonality, for the second experimental chapter.

In the second experimental part of this study, the focus was on the techno-functional properties of butter across a season, derived from the milk of numerous herds with changing stage of lactation and dietary factors. These techno-functional properties included melt properties, fatty acid profile, textural properties, and colour. There was opportunity from this study to gain a greater understanding of how the specific chemical changes within milk, observed across a season in an industrial milk processing setting, could influence the quality of the resultant butter, while also understanding and identifying how some of these inter-related factors are correlated with one another. It was found that statistically significant differences existed across many fatty acid profiles including C16:0 (palmitic acid) and C18:1 (oleic acid). Statistically significant differences were also found across some measured textural properties, such as peak force, and workability. Both were found to be significantly lower in butter produced in autumn, when compared with butter produced in spring and summer. L* colour chromaticity values remained consistent throughout the year, while a*, and especially b*, colour chromaticity values showed significant differences between seasons. In addition, many thermal properties exhibited significant differences between seasons. First peak crystallisation temperature, and crystallisation onset temperature, were highest in spring, and significantly lower in summer and autumn. Second peak crystallisation temperature was significantly higher in spring and summer when compared with autumn, while melting completion was highest in spring, and significantly lower in the other two seasons. This is valuable new knowledge for the scientific community and industry stakeholders, to inform choices on raw material selection for processing and the setting of production parameters. All results are discussed in detail throughout the chapter and the learnings from this thesis provide beneficial insights for dairy processors and the scientific community.

Chapter 1

Introduction and context for research

Órla Dinneen^{1,2}, Anna G. Behan², James A. O'Mahony¹, Tom F. O'Callaghan¹

¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*

²*Kerry Dairy Ireland, Princes Street, Tralee, Co. Kerry, Ireland*

Declaration

This chapter was written by author Órla Dinneen (Ó.D.) and reviewed by all co-authors.

1.1 Introduction and context for research

Butter is a widely produced and consumed dairy product in Ireland and internationally. In 2023, Ireland produced 273,150 tonnes of butter, representing approximately 13% of all butter produced in the EU, second to only Germany and France (Eurostat, 2024; CLAL.IT, 2025). This was in line with 2021, when Ireland accounted for approximately 14% of all butter produced in the EU, also positioned third after Germany and France (Eurostat 2022). This consistently high volume of production is significant when considering a 2024 report showing Irish-based dairies as processing 6% of the 145 million tonnes of milk processed by dairies within the EU in 2023 (Eurostat, 2024). With approximately 8.5 billion litres of milk collected in Ireland in 2023 (CSO, 2024), the national milk pool is modest compared to other European countries such as Germany and France (Eurostat, 2024).

The efficient production of consistent quality butter and other dairy products is not without its challenges, due to the naturally varying composition and physicochemical properties of the raw material involved (i.e., milk). There are a wide variety of factors which can impact milk chemistry, and the functionality and characteristics of the products derived from it. Many studies have previously been performed on this topic, both nationally and internationally, assessing the impact of factors such as breed (Prendiville *et al.*, 2011; Kostovska *et al.*, 2024), feed (Couvreur *et al.*, 2006; Glover *et al.*, 2012; O’Callaghan *et al.*, 2016; Kostovska *et al.*, 2024) and seasonality (Hayes *et al.*, 2023) on raw milk and selected dairy products (e.g., butter, cheese, powder) made therefrom. Seasonality is a particular challenge in the Irish dairy industry, and methods to overcome and standardise resultant chemical composition, processing performance and finished product functionality and quality are continually being researched and evaluated. Seasonality is attributed to the calving patterns and feeding practices typically used in countries such as Ireland and New Zealand. For example, in Ireland, on the majority of farms, calves are born in the Spring months (February, March and April) while cows graze

predominantly on fresh pasture, with the general exceptions of the very start and end of the year. This results in a herd profile which is synchronously changing throughout the year in terms of its stage of lactation and feed consumed, resulting in a direct impact on milk quality parameters. This is in contrast to non-seasonal farming approaches practiced elsewhere around the world. These farms produce a more consistent milk product throughout the year, in part due to the animals being fed a more consistent diet throughout the year (typically indoor fed with concentrates and silage), but also due to the calving of cows occurring in a staggered pattern throughout the year instead of in a block in Spring. These two factors greatly increase product consistency as the herd is being fed the same diet, while at the same time being broadly in the same stage of lactation, culminating in a more balanced cow, and milk composition/production, profile throughout the season.

Butter is a versatile product, with two main routes of use following production – business to business, where product may be sold to an intermediate company who then incorporate it into their own products, and business to customer where butter is sold directly to the end user from the producing company. Within both of these routes of use, butter has a wide variety of diverse applications and technical requirements worldwide, including for example, commercial bakery, confectionary, food service and home consumption. For example, some applications such as butter used in bakery/pastry may require product with attributes such as high firmness, lower melting point and higher workability/plasticity, while butter intended for home consumption generally requires a more spreadable, softer product, with a slightly lower melting point for ease of use.

Consumer acceptance of butter is critical to the continued success of the product. A 2007 study investigated the key parameters driving purchasing and consumption behaviour of butter by consumers. In the study of Krause, Lopetcharat, and Drake (2007), separate focus groups were conducted for (a) butter consumers and (b) butter and margarine consumers. For the butter only

study, it was found that flavour of the product, health attributes, price, and if the product was salted or unsalted, were four of the main aspects that impacted most strongly on consumer purchasing decisions (Krause, Lopetcharat, and Drake, 2007). When considering products viewed by the consumer as being “natural”, interestingly, the study found that of the 52 consumers who only consumed butter, 89% either agreed, or strongly agreed, that butter fitted within this category (Krause, Lopetcharat, and Drake, 2007).

This research project is designed to evaluate two specific scientific research questions relating to quality and applications of butter; the impact of (a) post-production storage conditions and (b) seasonality, on texture, functionality, and quality of Irish butter. It is important to better understand both of these questions for dairy processors, but also for the scientific community, such that premium quality butter products can be manufactured to consistently meet the ever-increasing demands of consumers for products with tailored composition, texture, functionality, and quality across a variety of applications.

More specifically, the impact of cream tempering on its processability and physicochemical properties of the resultant butter has been widely studied and presented in the scientific literature (Drelon *et al.*, 2006; Buldo *et al.*, 2013; Logan *et al.*, 2021). In this process, melting of some crystals present in the milk fat occurs, before it is cooled again to a reduced temperature (Logan *et al.*, 2021). It involves ageing of the pasteurised cream for at least 4 h, while sometimes, employing the use of temperature cycling, depending on raw material chemistry (O Callaghan *et al.*, 2022), which generally has a significant impact on final quality characteristics of butter such as textural properties (Rønholt *et al.*, 2012; O’Callaghan, 2022). Studies have also been performed to assess the impact of varying post-production storage conditions of butter on its final characteristics such as hardness and flavour; however, such studies tend to largely focus on long term effects of single storage temperatures, e.g., refrigerated vs. frozen storage as opposed to adjusting the temperature of the product in the

days immediately after production (Krause *et al.*, 2008). Given the seasonal nature of milk supply in Ireland, devising methods of producing a consistent product throughout the year for customers is vital. From analysis of the relevant scientific literature, it is well established that the approach of tempering cream can assist greatly in achieving consistent, targeted functionality from a seasonal cream supply, therefore, it was not designed to be within the scope of this study.

Alternatively, to compliment the effectiveness of cream ripening strategies, the aim of this study was to focus on the effects of post-production changes in temperature and time conditions of storage on product functionality and quality. The attributes studied include fatty acid profile, texture, rheology and melt properties. This research is of particular interest to consumers, producers, and the broader research community for two main reasons; (1) in many butter producing dairy companies, post-production storage is already modified (normally in response to specific requirements of individual customers), while the specific scientific rationale for the differences obtained in quality attributes of resultant butter may not be fully elucidated, and (2) it is important to establish systematically the extent of the changes in functional and quality attributes which can be achieved for seasonally produced butter by a targeted modification of the temperature-time storage conditions post-production, without making any modifications to the butter-making procedure (e.g., cream ripening).

No research studies, to the authors knowledge, have directly asked the question if altering post-production storage conditions in the days immediately following production could deliver targeted functional parameters, and as a result, a knowledge gap was identified, and this study was developed with the aim of answering these questions.

While it is important to understand the impact extrinsic, post-production process adjustments can have on milk and butter (such as altering post-production storage conditions), it is equally

important to acknowledge and assess further the impact of pre-processing factors on milk and butter chemistry.

To do this in a scientifically robust and systematic way, it is first necessary to better understand the nature of the raw material throughout an entire season and to assess the inter-relationships between factors such as fatty acid profile, textural properties and melt properties. No studies were found detailing the impact of seasonality, specifically in the Southwest/West region of Ireland on butter properties, and this information is of interest to not only the Irish dairy company involved in this study, but also to the wider scientific community, which formed the aim for the second part of this study.

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Chapter 2

Milk fat: a review

Órla Dinneen^{1,2}, Anna G. Behan², James A. O'Mahony¹, Tom F. O'Callaghan¹

¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*

²*Kerry Dairy Ireland, Princes Street, Tralee, Co. Kerry, Ireland*

Declaration

The relevant literature was critically reviewed by Órla Dinneen (Ó.D.). This chapter was written by author Ó.D. and reviewed by all co-authors.

2.1 Bovine milk

Bovine milk is a complex mammalian fluid with a wide range of applications across the food industry. Understanding the intrinsic composition of milk is of considerable importance when investigating and developing applications associated with its use. Many factors can impact milk composition, including but not limited to, genetics, feed, health and stage of lactation (Hayes *et al.*, 2023). As described by Joubran *et al.* (2021), pasture-based grazing is typical in the Irish production system due to the temperate climate, and the adoption of this system facilitates an environmentally efficient system for producing high quality milk (Bord Bia, 2024). On average, Irish cows across the country spend approximately 240 out of 365 days of the year in the outdoors on pasture (Bord Bia, 2024). Milk production in Ireland has significantly increased in recent years. Between 2013 and 202, milk intake at processing facilities rose by 52.9% (CSO, 2025). Despite the significant increase in milk production, the average herd size remains relatively modest at 93 cows (Bord Bia, 2024). This cost-effective system of feeding is also practiced in New Zealand, due to its similar temperate climate (Joubran *et al.*, 2021; Timlin *et al.*, 2021). Pasture-based grazing has been reported to have profound positive effects on the resulting milk from cows fed this diet (Joubran *et al.*, 2021). As well as having positive effects on the milk itself, a pasture-based feeding regime has been shown to have a lower impact on the environment with regards enteric (methane) emissions than a total mixed ration feeding regime (O'Neill *et al.*, 2011).

2.1.1 Synthesis of milk

The synthesis of bovine milk takes place in the mammary gland (Early, 2012; Chandan *et al.*, 2016). Located in the udder, the first milk secretion from the mammary gland of a cow commences prior to calf birth (Chandan *et al.*, 2016). A cows' udder comprises of two halves,

segregated by ligaments known as suspensory ligaments (Nickerson and Akers, 2011). As shown in Figure 2.1, each half is comprised of two individual quarters, thereby a cow has 4 quarters in total, with each having its own mammary gland for milk synthesis (Chandan *et al.*, 2016).

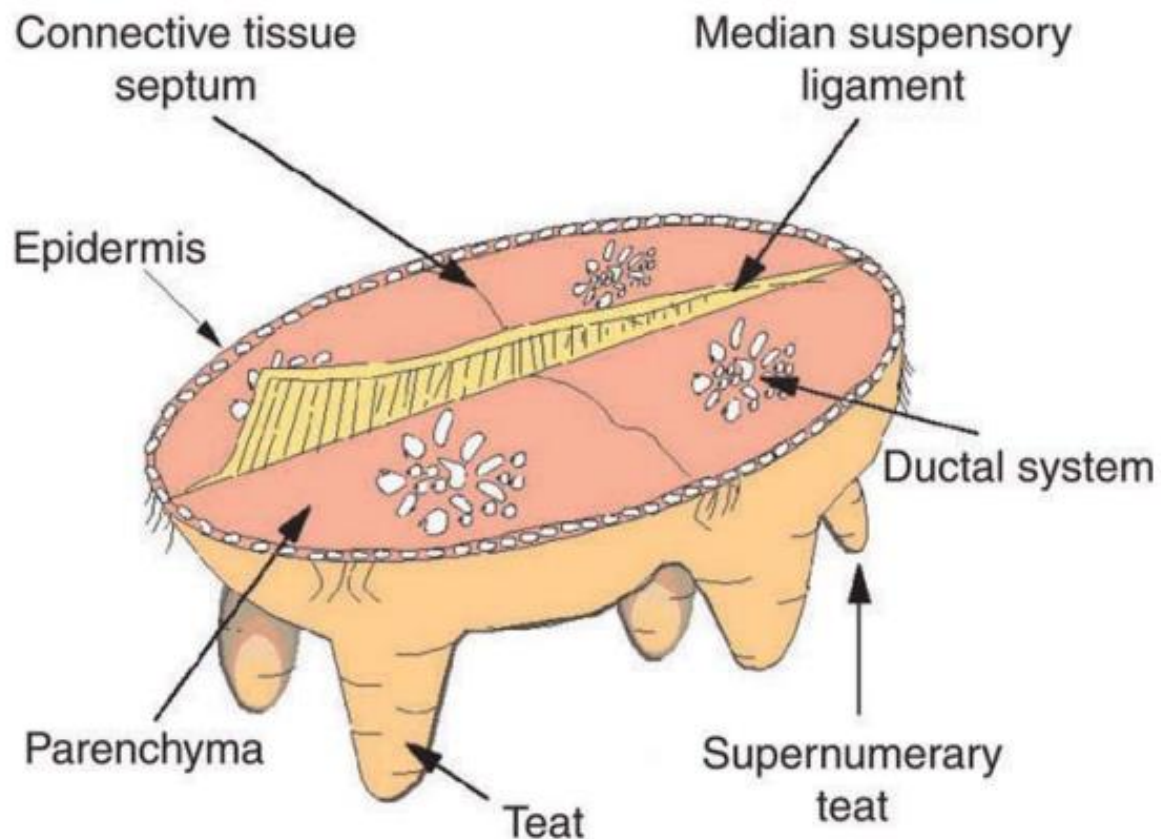


Figure 2.1. Diagrammatic representation of the udder in a cow, showing the two halves separated by the suspensory ligament and each half separated into 2 quarters in each (Nickerson and Akers, 2011)

Epithelial cells are found within the mammary glands of cows, with these cells existing in a grouped arrangement, forming the alveoli, and are responsible for the synthesis of milk constituents using molecules of blood origin (Fox *et al.*, 2015). Milk is released from these cells into the lumen before being transported to the cistern of each individual quarter in the

presence and action of oxytocin (Fox *et al.*, 2015). From the cistern, the milk can be moved into the teat channel prior to milking in the presence of oxytocin (Chandan *et al.*, 2016). A muscle known as a “sphincter muscle” is present at the end of the teat channel to inhibit passage of milk out of the udder when the cow isn’t being milked or feeding their young (Chandan *et al.*, 2016). Importantly, the precursors for the main components of milk solids (fat, lactose and protein) are transported in the blood and act as substrates in the mammary gland to create these constituents. Other components of milk, such as water, hormones and salts, are simply destined for milk because of leakage from the blood (Chandan *et al.*, 2016).

2.1.2 Chemical composition of milk

The composition of bovine milk has been widely studied. Along with physicochemical properties, the chemical composition of milk can have a significant effect on the composition, quality and functionality of dairy products, and therefore, is of significant importance to the industry as a whole (Li, Ye and Singh, 2019). Indeed, bovine milk composition can be affected by a large number of factors (Kailasapathy, 2016). Milk composition varies throughout lactation, but typically contains ~4.6% fat, 3.4% protein, 4.9% lactose, 0.5% ash and 86.6% water (Kailasapathy, 2016). Many minor constituents are also present in bovine milk (Fox *et al.*, 2015).

Protein plays a significant role in the composition, quality and functionality of bovine milk and dairy products. However, like the other main constituents of milk, its innate function is to provide a source of nutrition to the growing calf (Fox *et al.*, 2015). It has become apparent from the outputs of research over many decades that these dairy proteins have some distinct and unique features compared with other proteins, both nutritionally and functionally (Lagrange, Whitsett and Burris, 2015). Milk proteins are present in two main forms, casein and

whey, representing 80 and 20% of total protein respectively (Kailasapathy, 2016). While the whey proteins are soluble, the caseins are colloiddally dispersed in the form of casein micelles(Goulding, Fox and O'Mahony, 2020). A 2016 study on the Irish production system, reported that the average protein concentration of bovine milk across lactation was $3.65 \pm 0.26\%$ (O'Callaghan *et al.*, 2016a). However, large differences in protein content were observed throughout lactation due to seasonality. These values were reported as part of a study in which the chemical composition of milk from ryegrass pasture fed Friesian cows was investigated. The study incorporated a full lactation cycle with the results highlighting the difference in chemical composition of milk, not only across stage of lactation, but also across different feeding conditions. Further details on the milk protein system are available in Goulding, Fox and O'Mahony (2020).

Fat present in bovine milk has several essential functions. Its primary role is to act as a source of energy for the growing neonate (Fox, 2011). However, milk fat does have benefits beyond this. It can be functionally important as an ingredient in many food systems providing, and enhancing, organoleptic and rheological properties of food stuffs (Fox *et al.*, 2015) but also can act as a valuable nutritional inclusion in many human diets around the world, providing benefits such as being a source of essential fatty acids in the diet. A study carried out by O' Callaghan *et al.* (2016a) found that the yearly average fat content of bovine milk from pasture-based systems was approximately 4.65%, while the month-on-month values ranged from 4.4 to 5.12%. This fat in milk is present as globules which range in size from 1 to 20 μm and will be discussed in greater detail in section 2.3.1 (Tunick, 2018).

Lactose is the primary source of carbohydrate in milk and is present in the majority of dried dairy powders (Huppertz and Gazi, 2016). It is comprised of galactose and glucose (Singh *et al.*, 2022), chemically bound together with a β -1,4 glycosidic bond (Fox *et al.*, 2015). Lactose

exists as one of two anomeric forms, α -lactose and β -lactose, and mutarotates between these forms depending on several factors, including temperature and the concentration of lactose in a given system (Huppertz and Gazi, 2016). Lactose can have a significant effect on dairy powders in terms of water removal during drying (e.g., spray drying), storage (e.g., caking) and reconstitution (e.g., insolubility at low temperature) (Huppertz and Gazi, 2016). It is also a reducing sugar, with a strong ability to participate in the Maillard reaction (Huppertz and Gazi, 2016), resulting in browning and flavour formation (Jaeger, Janositz and Knorr, 2010). This reaction takes place when amino acids and reducing sugars react in the presence of heat (Jaeger, Janositz and Knorr, 2010). Lactose can also play a role in fermented products such as yoghurts whereby it is the substrate for fermentation by lactic acid bacteria (Huppertz and Gazi, 2016). The lactose content of cows' milk, and its variation throughout a lactation, was investigated as part of the study carried by O'Callaghan *et al.* (2016a). The authors reported yearly averages for lactose of 4.87%. Like protein, these values also varied greatly throughout the lactation. This value broadly aligned with the reported value of 4.8% for lactose content of bovine milk by Fox *et al.* (2015).

Different values for ash content in bovine milk have been reported by various authors. Kailasapathy. (2016) presented an approximate ash content of 0.5% for cows' milk, while Fox *et al.* (2015) reported it as being typically between 0.7 and 0.8%. This variation is likely due to the fact that the ash content of bovine milk can vary between breeds and across stage of lactation (Chandan, 2016). The salts making up the ash in milk exist in equilibrium between the colloidal and the serum phase, with the split between both impacted by a wide range of factors, one being the pH of milk (Kailasapathy, 2016). In summary, the concentrations at which some of these significant salts are present in bovine milk are as follows: chloride (30.2 mmol/L), calcium (29.4 mmol/L), sodium (24.2 mmol/L), phosphate (20.9 mmol/L), citrate (9.8 mmol/L) and magnesium (5.1 mmol/L) (Fox *et al.*, 2015). Importantly, it has been

highlighted that for a number of reasons, the salt levels in milk are not fully reflected by the ash content; for example, some organic ions may not be retained following the laboratory procedure to determine ash content (Fox *et al.*, 2015).

2.1.3 Variability in chemical composition of bovine milk

Variation in the composition of milk can occur for a number of reasons. One such reason is a consequence of feed, while others include stage of lactation and breed. This can cause processing challenges, but also opportunities throughout any given season. For these reasons, it is important to develop a good understanding of the factors affecting these changes in composition of milk across season in grass-based milk production systems.

2.1.3.1. Impact of breed on the chemical composition of milk

The Friesian breed is the most dominant breed in Ireland, with approximately 40% of the Irish total live herd being made up of Friesian cattle at the end of 2021 (Department of Agriculture, Food and the Marine, 2021). With regards to the Jersey breed, in 2021, the number of calves registered to a Jersey dam was 70,600 (Department of Agriculture, Food and the Marine, 2021). Breed has been demonstrated to have a significant effect on the composition of milk (Hayes *et al.*, 2023). A 2011 study investigated the differences in milk yield and composition from cows of different breeds (Prendiville *et al.*, 2011). Data was presented for Holstein Friesian (H), Jersey (J) and Holstein x Jersey cross breeds (H x J) in this study, with the cows fed on pasture between February and the middle of November, and milked twice per day. It was reported that the highest milk yielding periods for all breeds was at the start of lactation, with volumes decreasing thereafter, until the end of lactation for all three (Prendiville *et al.*, 2011). The J

group yielded the least volume of milk, followed by the H x J group (however, both groups were similar at the end of lactation), with the H group consistently providing the largest volume of milk across lactation (Prendiville *et al.*, 2011). In terms of protein and fat contents of milk from the three groups, the authors reported that the Jersey cows produced milk with the highest concentrations of both, followed by the H x J group, with the Holstein Friesians producing milk with the lowest fat and protein contents throughout the lactation (Prendiville *et al.*, 2011). A similar study was carried out by Prendiville, Pierce and Buckley.(2009) in Ireland, in which the three aforementioned breed types were investigated for a number of factors including milk production and composition. Results were similar in overall trends to the previously mentioned study by Prendiville *et al.* (2011). Milk yield was highest in the H group, followed by the H x J group, with the J group having the lowest milk yield (Prendiville, Pierce and Buckley, 2009). Values reported for milk yield were 18.3, 16.7 and 13.8 kg/d, respectively (Prendiville, Pierce and Buckley, 2009). Trends for fat and protein contents were also in line with results of the above study(Prendiville *et al.*, 2011), with the J group producing the highest levels for both, followed by the H x J group, and finally followed by the H group (Prendiville, Pierce and Buckley, 2009). In the case of protein, it is important to note that breed not only plays a major impact on the protein content of milk, but also the quantities of specific caseins and whey proteins (Penasa *et al.*, 2014; Niero *et al.*, 2021). Similarly, breed has been shown to have an impact on individual fatty acid quantities in cows milk (Gottardo *et al.*, 2017) but also, feed can play a major role in this (Bobe *et al.*, 2003).

2.1.3.2 Impact of seasonality and stage of lactation on the chemical composition of milk

The study of O'Callaghan *et al.* (2016a) investigated milk composition across a complete lactation for Friesian cows on a pasture-based diet. A seasonal change in composition was clearly identified in this study. In early lactation, milk from cows fed ryegrass via grazing, the

protein concentration was initially $3.37 \pm 0.06\%$ in March, with a reduction in April, followed by a steady increase in protein concentration to reach a maximum of $4.02 \pm 0.13\%$ in August. After this point, the authors reported a decrease in protein concentration, with similar extents of variability evident for fat content. In March (early lactation), fat content was reported as $4.48 \pm 0.31\%$, increasing to $4.64 \pm 0.17\%$ in April, before reducing to 4.40-4.56% throughout May to August. As the late lactation period approached, fat content increased sharply to $5.12 \pm 0.18\%$ in September before finishing the lactation cycle at $5.03 \pm 0.11\%$ in October. Lactose concentrations also changed throughout the season, with a lactose content of $5.02 \pm 0.14\%$ at the beginning of lactation in March before decreasing to $4.93 \pm 0.02\%$ in April. Lactose concentrations increased in mid lactation, but steadily declined thereafter to the lowest level measured of $4.67 \pm 0.02\%$ at the end of lactation. This variability observed throughout the study across the three main solids constituents of milk highlights the variability across a season in a Spring calving system fed predominantly on a pasture-based diet.

2.1.3.3. Impact of feed on the chemical composition of milk

In Ireland, cows are typically fed on pasture for an average of 240 d of the year (Bord Bia, 2024) with the exact number of days being dependent on the prevailing weather and rate of grass growth. Approximately 95% of a typical dairy cow's feed in Ireland is grass based (Bord Bia, 2024). It is widely known that the feed of a dairy milk producing animal has an impact on the composition of the resulting milk and fatty acid profile (Fox *et al.*, 2015). The study of O'Callaghan *et al.* (2016a) investigated the impact on milk composition of feeding cows' pasture-based perennial ryegrass (RG), pasture-based perennial ryegrass and white clover (RGC) or indoor TMR-based (TMR) diets. The RG diet in this study consisted of feeding 18 Friesian cows approximately 18 kg of dry matter daily via grazing on Perennial Ryegrass, the RGC diet

consisted of feeding 18 different cows the same amount of dry matter through perennial ryegrass and white clover and the TMR diet consisted of feeding 18 further cows 7.15 kg of both grass and maize silage and 8.3 kg of concentrates daily. The study was performed from early March to late October in 2015 in Ireland, and the authors reported significant differences across a number of parameters when comparing the milk composition and yield from both feeding regimes. The differences reported in lactose levels in milk between the three feeding regimes were not deemed significantly different; however, fat, protein and milk yield were different. Averaged across a lactation, milk from cows fed a RG diet had the highest protein and fat contents at $3.65 \pm 0.26\%$ and $4.65 \pm 0.32\%$, respectively, followed by milk from cows fed the RGC diet and TMR diet, respectively (in the case of protein) and the TMR and RGC diet (in the case of fat) (O’Callaghan *et al.*, 2016a). Milk yield, averaged across the experimental duration, was significantly higher for the TMR group (27.71 litres per d) than the PB group (20.98 l per d). Importantly, these trends did change throughout the season; for example, when considering the RG and TMR based diets, while RG-fed cows produced higher levels of fat averaged throughout the season, in August, results showed that the RG-fed herd were producing milk with lower concentrations of fat than those fed a TMR-based diet, whereas in April, the RG-fed cows were producing milk with approximately 0.5% higher fat (O’Callaghan *et al.*, 2016a).

These results are similar in overall trends to those reported by O’Neill *et al.* (2011), which contrasted similar feeding regimes to the study of O’Callaghan *et al.* (2016a). Importantly, the study by O’Neill *et al.* (2011) only incorporated a 10-week study period in early lactation. Cows fed on pasture were fed 14.28 kg of grass dry matter per, day while cows fed a TMR diet consumed 19.69 kg of dry matter daily. The TMR diet in this study consisted of maize and grass silages, a concentrate mix, barley straw and molasses. Similar to the study of O’Callaghan

et al. (2016a), the cows fed a TMR diet produced significantly higher volumes of milk than the cows fed a pasture-based diet with yields of 21.1 kg per cow daily and 29.5 kg per cow daily reported (O'Neill *et al.*, 2011). This study also investigated and reported on fat and protein levels in milk from concentrate-based diets, as well as milk from pasture fed cows (O'Neill *et al.*, 2011) with results reporting higher yields for fat and protein from cows fed a RG-based diet as opposed to a TMR-based diet (O'Neill *et al.*, 2011).

2.2 Milk fat

The majority (approx. 97.5%) of the total lipid present in bovine milk is made up of triacylglycerols (Fox *et al.*, 2015). The remaining 2.5% mainly consists of mono, di and tri glycerides, cholesterol, phospholipids, and free fatty acids, with the phospholipids in milk being of critical importance, even though they are only present at concentrations of approx. 0.6 – 1.0 % (Fox *et al.*, 2015). The structure in which fat exists in milk has been widely reported and is agreed to exist as globules surrounded by a membrane i.e., the milk fat globule membrane(MFGM) (Tunick, 2018). Both the fat globule and its associated membrane will be discussed in further detail in the section 2.

2.2.1 Synthesis of milk fat

Fatty acids in milk originate from one of two sources – plasma lipids and those which are created in the mammary gland of the animal through *de novo* synthesis (Taylor and MacGibbon, 2011). *De novo* synthesis is responsible for the synthesis of fatty acids C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, as well as a fraction of C16:0, while fatty acids in the C18 group and remaining C16:0 are derived from blood plasma (Taylor and MacGibbon, 2011). Taylor and MacGibbon

(2011) note that, due to hydrogenation of polyunsaturated fatty acids, diet mainly affects long chain fatty acids, including C16:0, C18:0 and the monounsaturated fatty acid C18:1.

2.2.2 Milk fat constituents

2.2.2.1 Fatty acids and triacylglycerols

Triacylglycerols make up more than of 95% of the total lipid present in milk fat (Kailasapathy, 2016). These molecules are comprised of a glycerol backbone and three fatty acids (Taylor and MacGibbon, 2011; Logan *et al.*, 2021). Selected chemical and physical properties of milk can be significantly impacted by its fatty acid composition (Chilliard *et al.*, 2001). Different types of fatty acids exist in bovine milk, such as saturated fatty acids and fatty acids with varying degrees of unsaturation (Kailasapathy, 2016). Saturated fatty acids in milk fat are those without any double bond (Huppertz, Kelly and Fox, 2009). The most abundant of these saturated fatty acids tend to have carbon atoms of even number although some odd carbon numbered saturated fatty acids do exist at lower concentrations (Taylor and MacGibbon, 2011). Unsaturated fatty acids differ significantly in that they contain carbon atoms bound by at least one double bond, and often, more (Huppertz, Kelly and Fox, 2009).

Although approximately 400 fatty acids have been identified in milk fat, many of these are only present in small amounts (Jensen, 2002). Fatty acids which are present in amounts greater than 1% of total fat include: C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C18:0, C18:1 and C18:2 with C18:1, C16:0, and C14:0 being among the most abundant (Kailasapathy, 2016). Significant saturated fatty acids in milk are those which are hydrocarbon entities with no branching in their structures (Taylor and MacGibbon, 2011). The most abundant of these fatty acids found in milk fat is palmitic acid (Taylor and MacGibbon, 2011).

2.2.2.1.1 Influence of feed on milk fatty acid composition

It has been reported that the nutrition of a cow impacts the fatty acid profile of the milk (Bobe *et al.*, 2003). This can, in turn, lead to implications for the rheological properties of products made from this milk, such as butter (Bobe *et al.*, 2003). For example, butter made with milk from cows consuming higher amounts of natural grass is said to have favourable properties both rheologically and nutritionally such as lower rancidity, lower firmness in the mouth, achieving a higher score for melting in the mouth when scored in sensory analysis and having a lower atherogenicity index (Couvreur *et al.*, 2006).

O’Callaghan *et al.* (2016a) investigated the impact of diet on the fatty acid composition of milk from Holstein Friesian cows. This study reported lactation-long results for 27 fatty acids and concluded that there were 16 fatty acids which displayed statistically significant variation when comparing three different feeding strategies, namely C:11, C13:0, C15:0, C16:0 C17:0, C18:2 n-6t, C18:2 n-6c, C18:3n-6c, C20:1, Conjugated linoleic acid (CLA; c9t11 & c12t10), C18:3n-3, C22:0, C22:1n-9, C23:0 & C20:4n-6. The three feeding regimes included for analysis in this review were total mixed ration(TMR), ryegrass (RG) and ryegrass with clover (RGC). Upon analysis of the yearly average data, the TMR group produced milk with the highest amount of saturated fatty acids, followed by the RG group, with the RGC group producing milk with the least amount of saturated fatty acids, averaged across a season (O’Callaghan *et al.*, 2016a). When analysing the yearly average data, the TMR and RG groups produced milk with almost identical levels of unsaturated fatty acids (19.06 ± 3.36 g/100 g milk fat and 19.05 ± 3.32 g/100 g milk fat, respectively), followed by milk from the RGC group (18.35 ± 2.65 g/100 g milk fat). Importantly however, within both groups, the concentrations of some specific fatty acids were impacted more significantly by diet than others. Concentrations of butyric (C4:0), caproic (C6:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1n-9c), γ linoleic (C18:3n-6c), CLA (c12t10) and erucic acid (C22:1n-9) were highest in milk from the TMR

group, followed by milk from the RG group, with the RGC group producing milk with the lowest concentrations when considering average values across the year (O'Callaghan *et al.*, 2016a). The only exception to this trend was that the same value was reported for C6:0 in milks from RG and RGC-fed animals as a yearly average; this was also true for C18:2n-6t (O'Callaghan *et al.*, 2016a).

In contrast, concentrations of capric (C10:0), lauric (C12:0), tridecanoic (C13:0), linolelaidic (C18:2n-6t), eicosenoic (C20:1), α -linolenic (C18:3n-3), tricosanoic (C23:0) and arachidonic (C20:4n-6) acids were lowest in milk from the TMR group, followed by milk from the RG group, with the RGC group producing milk with the highest concentrations, again when analysing average values across the year (O'Callaghan *et al.*, 2016a). Eicosatrienoic acid concentration was unaffected by feed, based on yearly average data presented (O'Callaghan *et al.*, 2016). Concentrations of caprylic (C8:0), myristic (C14:0), linoleic (C18:2n-6c) and behenic (C22:0) acids were reported as being highest in milk from TMR-fed cows, and lowest in RG-fed cows, while in contrast, concentrations of pentadecanoic (C15:0) heptadecanoic (C17:0) acids and CLA (c9t11) were reported to be highest in milk from RG-fed cows and lowest in milk from TMR fed cows (O'Callaghan *et al.*, 2016a).

Myristoleic acid (C14:1) was found to be present at the highest concentration in cows fed an RG diet, and at the lowest in milk from cows fed an RGC diet, although values reported across each of the three diets were very similar. When considering yearly average values, TMR-derived milk contained the highest concentration of saturated fatty acids, followed by RG-derived milk, with RGC-derived milk having the lowest concentration of saturated fatty acids. This trend was also true for unsaturated fatty acids, but to a significantly lesser degree, with the authors reporting almost identical values for TMR and RG derived milks averaged over a lactation. Palmitic and oleic acids were found to be the two most abundant fatty acids in the

milks of all three experimental groups (O'Callaghan *et al.*, 2016a). Palmitic acid appeared to have been affected much more by diet than oleic acid, albeit some differences were also present between groups with regards oleic acid concentrations. Palmitic acid was present at concentrations of 24.39 g/100 g milk fat in milk from TMR fed cows, while it was present in significantly lower amounts in RG fed cows (20.73 g/100 g milk fat) and even lower amounts in RGC fed cows (19.98 g/100 g milk fat) (O'Callaghan *et al.*, 2016a). Oleic acid concentrations followed a broadly similar trend, being present at concentrations of 14.59 g/100 g milk fat, 13.99 g/100 g milk fat and 13.23 g/100 g milk fat in milk from TMR, RG and RGC-fed cows, respectively (O'Callaghan *et al.*, 2016a). Separately, Capuano *et al.* (2014) conducted a study to assess if fatty acids could successfully verify feed type. Similar to O'Callaghan *et al.* (2016a), this study concluded that fatty acid profile of milk is affected by different feed type.

2.2.2.1.2 Influence of seasonality and stage of lactation on milk fatty acid composition

Seasonality has been shown to have an effect on fatty acid composition in seasonal calving systems (O'Callaghan *et al.*, 2016a; Li, Ye and Singh, 2022). Li, Ye and Singh (2022) investigated the effect of seasonality in a seasonal calving system in New Zealand on various properties of milk fat, including fatty acid composition. Early-season milk was defined as between days 8 and 100 in milk between August and October, mid-season milk was defined as milk produced between days 101 and 220 in milk in the months of November, December, January and February, while late season milk was defined as milk produced between days 221 to 300 in the months of March, April and May (Li, Ye and Singh, 2022). The levels of C4:0 were highest in early season, while minimal differences in its concentration were observed between mid- and late-season. C6:0 varied to a lesser degree, being present at the highest concentrations in early season, decreasing gradually as the season ensued (Li, Ye and Singh,

2022); variation in concentrations of C8:0, C10:0, C12:0 and C18:3 were not statistically significant throughout the season (Li, Ye and Singh, 2022). Concentrations of C14:0 were lowest in early season, followed by an increase in concentration in mid-lactation before again decreasing to lower concentrations towards the end of the season, although not to the same low levels measured at the start of season (Li, Ye and Singh, 2022). The authors reported little difference in the concentrations of C14:1 and C15:0 between mid- and late-season, but both were present in lower concentrations in early season (Li, Ye and Singh, 2022). Levels of C17:0 were low when compared with other fatty acids; $0.6 \pm 0.0\%$ in early- and mid- and $0.5 \pm 0.0\%$ in late- season, with values expressed as wt. % of fatty acids (Li, Ye and Singh, 2022). These authors reported similar trends for C18:0 and C18:2 throughout the season, with concentrations of both being highest in early season, before decreasing, and then subsequently partially recovering to early season highs in mid- and late-season. The concentrations of CLA were reported as increasing steadily from early- to mid-, and mid- to late- lactations (Li, Ye and Singh, 2022). In this study, C16:0 was found to be the fatty acid present in the highest amounts in early-, mid-, and late-lactation milks, followed by C18:1, which is in agreement with the results of O'Callaghan *et al.* (2016a). Li, Ye and Singh (2022) reported that C16:0 was present in lowest amounts in early season, with a significant increase in mid-season, followed by a subsequent decrease in late season; however, concentration in late season was still higher than that observed in early season. Oleic acid (C18:1) was found to be present in the highest concentration in early season, before decreasing significantly in mid-season, and recovering to a degree in late season (Li, Ye and Singh, 2022).

O'Callaghan *et al.* (2016a) investigated the change in certain milk fatty acids across a lactation of Spring-calving Irish-based cows from March to October fed a grass-based diet. The authors reported similar trends to those reported in the study of Li, Ye and Singh. (2022), with the two most significant exceptions being the two most abundant fatty acids – C16:0 and C18:1 (O'

Callaghan *et al.*, 2016a). O'Callaghan *et al.* (2016a) reported that CLA (c9t11) increased significantly from early-mid-lactation, with a further, less substantial, increase recorded from mid-late-lactation, while it was reported that concentration of C18:1 was highest in early-lactation, followed by a significant decrease in mid-lactation and a further decrease in late-lactation milk.

2.2.3 Structural organisation of fat in milk

2.2.3.1 Milk fat globule

The vast majority (approx. 98%) of triacylglycerols in milk fat are contained within the milk fat globule (Tunick, 2018). Given the range of fatty acids identified in milk, many triacylglycerol configurations are possible. For example, a study by Wang *et al.* (2022) analysed triacylglycerols in milks from a number of species, with the authors reporting 109 triacylglycerols identified in milk from Holstein Friesian cows. The cream layer formed in untreated bovine milk exists as a result of these globules naturally rising to the surface (Tunick, 2018). The stability of globules in bovine milk is aided by the MFGM (Logan *et al.*, 2014), which is a membrane consisting of three layers surrounding the globule itself (Logan *et al.*, 2014; Fleming *et al.*, 2017). The diameter of individual milk fat globules can range from 1 to 20 μm (Tunick, 2018); however, many factors can affect this, such as lactation stage (Fox *et al.*, 2015), daily fat yield (Wiking *et al.*, 2004) and cow breed (Fox *et al.*, 2015; Fleming *et al.*, 2017). Size is very significant when discussing milk fat globules, as this property can influence the stability of milk fat, amongst other attributes (Wiking *et al.*, 2004). A recent study investigated the impact of fat globule size on cream and its churnability in butter making (Panchal *et al.*, 2017). In this study, two additions were used to allow for decreased fat globule size, while retaining stability for the purpose of investigation and comparison (Panchal *et al.*,

2017). Samples of cream with different fat globule sizes in a stable environment were achieved using these two additions paired with homogenization. Various addition combinations of tween 80 and sodium caseinate were utilized and investigated, and it was found that the churnability was not solely dependent on fat globule size but also on the emulsifier type; however, the authors reported trends indicating as the fat globule size decreased, the churning time increased (Panchal *et al.*, 2017). Significantly, there were also differences in churning times depending on the agent used to stabilise the cream (Panchal *et al.*, 2017).

Fox *et al.* (2015) suggested that as lactation progresses, milk fat globule size decreases, with the smallest milk fat globules occurring at the end of lactation. A study by Wiking *et al.* (2004) reported a strong relationship between the daily fat yield of Holstein Friesian cows and milk fat globule size; milks greater fat yield had larger milk fat globule size. The authors concluded from these trends that MFGM production is lower when the amount of fat produced is higher (Wiking *et al.*, 2004), which is relevant when considering the views of Fleming *et al.* (2017) that smaller milk fat globules tend to have a greater proportion of membrane than larger milk fat globules. Wiking *et al.* (2004) also reported that as levels of four fatty acids (C16:0, C18:0, C16:1 and C18:1) increased, so too did the milk fat globule diameter. Fleming *et al.* (2017) found large variations between cow breed and milk fat globule size when comparing Jersey, Brown Swiss, Ayrshire, and Holstein cows/herds, with the Holstein Friesian-derived milk having the smallest sized globules, followed by Brown Swiss, Ayrshire, and Jersey breeds.

2.2.3.2. Milk fat globule membrane

The MFGM, as shown in Figure 2.2, is derived from the secretory cells (Wiking *et al.*, 2004), where the fat globules are surrounded by the layer found at the exterior of these cells before passing towards the lumen (Tunick, 2018). This is of significance in the prevention of globule

aggregation and also helps to inhibit the actions of enzymes (Dewettinck *et al.*, 2008). The main constituent of the approx. 10-20 nm thick MFGM (Dewettinck *et al.*, 2008) is protein, which is said to be present at a concentration of approximately 90% (Tunick, 2018), while sphingolipids (Dewettinck *et al.*, 2008), sterols, phospholipids, and other minor constituents (Tunick, 2018) are also present in the membrane.

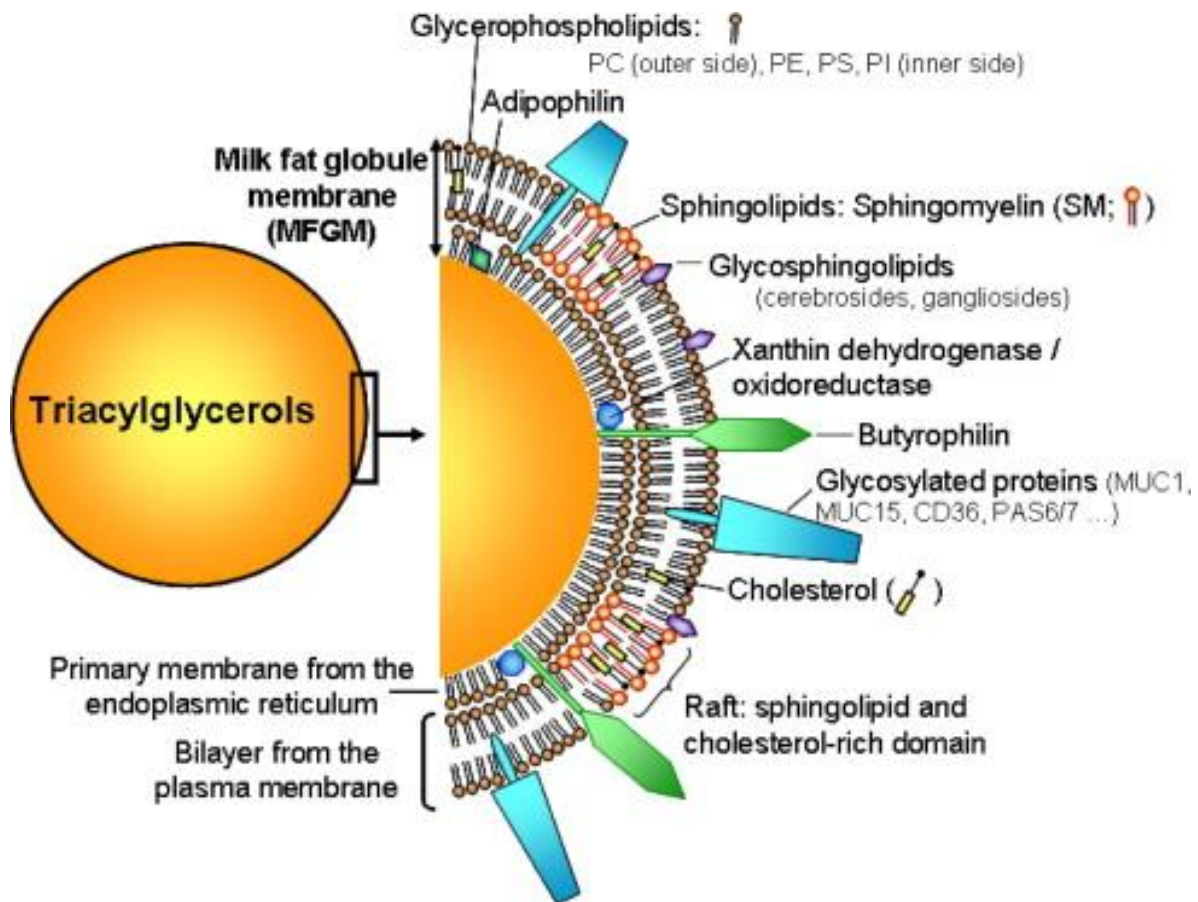


Figure 2.2. Schematic representation of the milk fat globule membrane (Lopez, Madec and Jimenez -Flores, 2010)

The integrity of the MFGM is affected by processing conditions such as thermal treatment (Huang *et al.*, 2023). Churning of cream also greatly affects the MFGM as described by Zheng *et al.* (2020). In this study, double defatted cream (fat content of 30.25 g/100 g) was mixed in a blender to imitate butter churning for 8 min. At 2, 4, 6 and 8 min during churning, the expelled

buttermilk was collected, measured in terms of volume and also for major constituents. The authors reported that, throughout the imitation butter making process, proteins originating from the MFGM passed into the buttermilk and were released with it. The protein concentration in the buttermilk released following freeze drying was 6.56, 60.86, 57.37 and 78.81 g/100 g after 2, 4, 6 and 8 min respectively (Zheng at al., 2020). This study also investigated fat content in the released buttermilk with concentrations of 53.34, 21.75 , 24.58, and 18.68 g/100g reported after 2,4,6 and 8 min respectively (Zheng at al., 2020).

2.3 Butter

Butter consists of three main components – fat, protein and water at a maximum legally permitted level of 16% (Funahashi and Horiuchi, 2008). According to Codex Alimentarius, butter cannot legally contain less than 80% m/m milkfat and no more than 2% m/m milk solids non-fat components (CODEX standard 279-1971). It is a widely renowned dairy product in today's world (Deosarkar, Khedkar and Kalyankar, 2016) with its production dating far back in history as a means of retaining the microbial integrity and extending the shelf life of the highly nutritious fat portion of milk (Mortensen, 2016). Originally produced using more laborious methods, modern butter making practices are significantly more advanced (Deosarkar, Khedkar and Kalyankar, 2016). Equipment and processes involved in this will be discussed in the following sections.

2.3.1 Butter production

The vast majority of butter produced industrially currently is produced using continuous production processes (Deosarkar, Khedkar and Kalyankar, 2016), normally following the Fritz method of production (Wilbey, 2009). Churning of cream, followed by buttermilk drainage,

and subsequent working of the remaining butter, are the main steps involved in modern butter making (Mortensen, 2016). A typical butter making process is depicted in Figure 2.3.

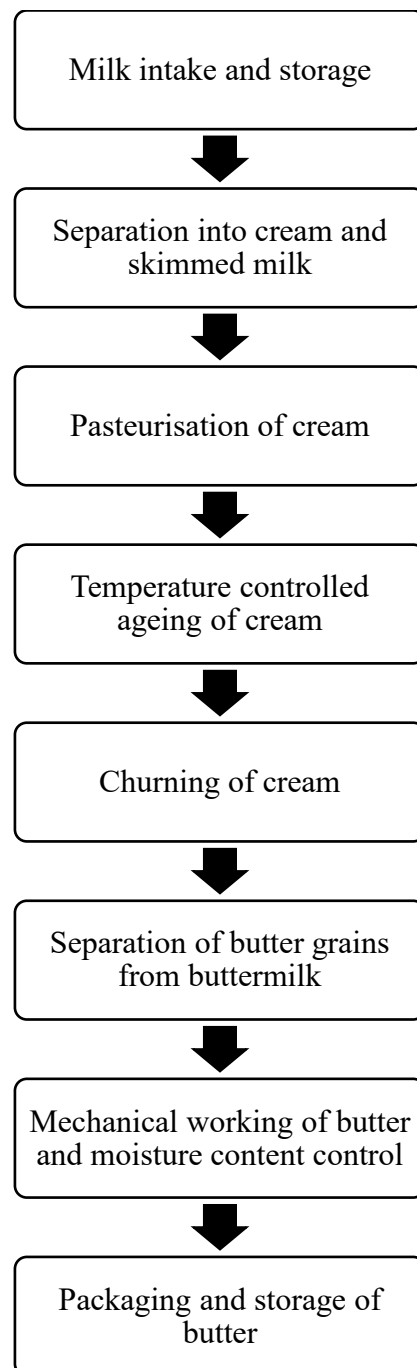


Figure 2.3. Key steps involved in the production of butter

Raw milk is taken into a process facility from dairy farms in a catchment area and stored at refrigeration temperature in clean silos; ideally stored $< 4^{\circ}\text{C}$. It is gently mixed to prevent creaming and to maintain the integrity of the fat component in milk (Frye and Kilara, 2016). It is then separated into skimmed milk and cream using a centrifugal style separator at approximately 50°C , similar to that shown in Figure 2.4. This separation process is carried out at temperatures which allow the fat phase to be fluid, to avoid premature breaking of the globules (Wilbey, 2009).



Figure 2.4. Diagram of a typical modern separator used to separate milk into cream and skimmed milk (Alfalaval, 2025)

Due to milk having the capacity to allow growth of harmful microbes (Frye and Kilara, 2016), it is necessary to pasteurise it or its derivatives before further processing to prevent spoilage, and to ensure the products are safe for the consumer. In this case, pasteurisation of cream before production of butter products takes place, and following pasteurisation, cream is stored in clean silos to allow it to age and crystallise (Wilbey, 2009). After this step, the cream is churned, which leads to breaking of the MFGM (Zheng *et al.*, 2020) and as a result, buttermilk can be drained away (Mortensen, 2016). Buttermilk, which has a somewhat similar chemical composition to skimmed milk, contains high amounts of minerals and protein (Doosh, Alhusyne and Almosawi, 2014). Once drained, it can be then transformed into more usable materials such as into a powder format (Zheng *et al.*, 2020). The butter itself at this point is worked to bring the grains of butter together (Wilbey, 2009) and finally packaged.

Modern butter making equipment offers some distinct advantages when compared with traditional butter making techniques. In past years, wooden barrels or churns would have been the traditional vessel in which butter was produced; however, significant advancements have been made in the areas of process technology in recent decades (Wilbey, 2009). These include continuous butter making options using equipment such as that shown in Figure 2.5. There are many distinct advantages to modern day butter making equipment such as increased control of temperature, feed and moisture, as well as automated cleaning in place facilities (GEA, 2025).

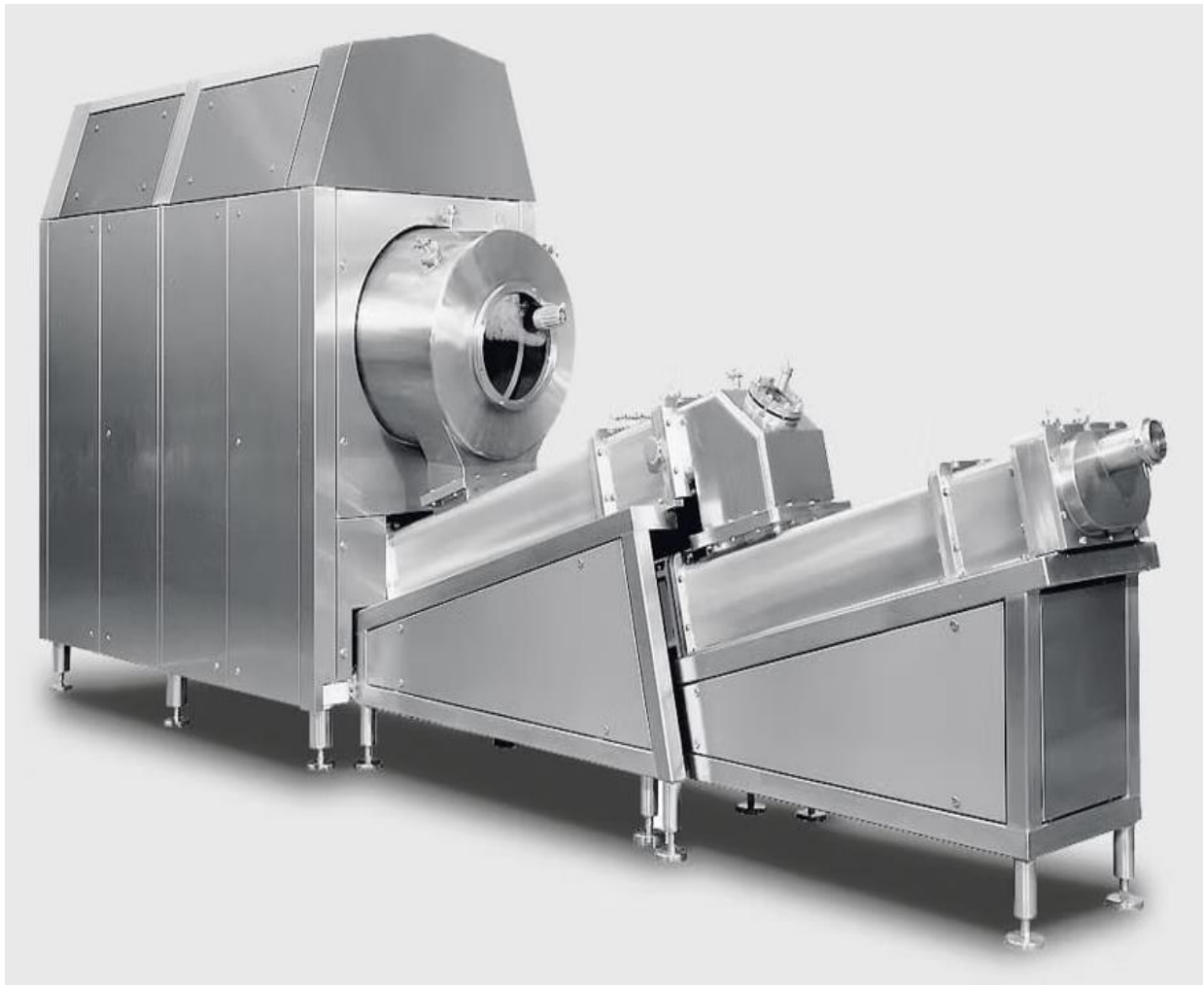


Figure 2.5. Example of a modern-day butter churn suitable for butter making (GEA, 2025)

2.3.2 Butter products

Butter products, produced from cream separated from skim milk, tend to be grouped into four main types, sweet cream, lactic, salted or unsalted butter (Early, 2012). As well as this, cream separated from cheese whey in the production of cheese, can be churned and worked to produce whey butter (Patel, Sharma and Patel, 2016). While salted butter is commonly used for domestic applications, butter typically going forward for further manufacturing and incorporation into a food product as an ingredient, is often unsalted (Patel, Sharma and Patel, 2016). In English-speaking countries, sweet-cream products are typically more popular, while

lactic products are more common in other regions (Fox *et al.*, 2015), highlighting that butter produced for one market may be very different to the butter produced for another.

Salted butter is generally produced by injecting a salt brine into the butter at the first working section of a butter making system, and previous issues with inconsistent combination of this mixture has been overcome by increasing the extent of mixing in the working sections (Wilbey, 2009). Sweet cream is considered by some as being the easiest to produce, in preparation for sweet cream butter. Sweet cream butter is produced from fresh cream with $\leq 0.20\%$ titratable acidity (Patel, Sharma and Patel, 2016). This method may be considered the most straightforward, owing to the fact that it has no additions, and typically has a final pH value of approximately 6.6 (Fox *et al.*, 2015).

Lactic or cultured cream is typically prepared using two different methods – using cultured cream, or making additions to the butter in the working sections of the process. The first method involves addition of cultures to pasteurised cream which has been only allowed to cool to approximately 20 °C (Wilbey, 2009; Patel, Sharma and Patel, 2016). This cream is then held at this temperature and allowed to ferment across 12-18 hours until a pH of 5.3 is reached, following which, the flavour compound diacetyl is produced (Wilbey, 2009). At this point, a mild lactic flavour exists, and the cream can be sent forward for butter making; however, if a stronger lactic flavour is required, fermentation is allowed to continue until the cream reaches approximately pH 4.5, an approach often seen in the northern European countries and Scandinavia (Wilbey, 2009; Fox *et al.*, 2015). There are however some limitations to producing lactic butter in this way, such as producing lactic buttermilk in the butter-making process, and as a result, lactic butter can sometimes be produced by adding cultures and flavour additions in the working section (Wilbey, 2009). Often, concentrated lactic flavours are added to lactic butter to improve the flavour and the consistency of the final product (Fox *et al.*, 2015).

2.3.3 Effect of animal feed on properties of butter

The presence of fresh grass in the feeding practices of cows has been reported to be favourable with regards to butter properties – both nutritionally and rheologically (Couvreux *et al.*, 2006). A study by O’Callaghan *et al.* (2016b) investigated the impact of three different feeding regimes on a variety of butter properties. In this study, three different regimes were investigated for their impact on mid-lactation butter – TMR (indoor), RG (outdoor) and RGC (outdoor) (O’Callaghan *et al.*, 2016b). Many parameters of the butter manufactured from the milk of three groups of 18 cows fed these diets were analysed over a period of 6 months, during which time the butter was stored at 4-6°C (O’Callaghan *et al.*, 2016b). The butter making method used in this study involved pasteurisation and standardisation of the creams to 38-40% fat content using skim milk, storage of this cream for 3 d at 5°C to allow the fat in the cream to crystallise before churning of the cream to produce butter (O’Callaghan *et al.*, 2016b). As expected, variations in fatty acids were observed between the butter samples, with the butter made from the milk of cows fed a TMR diet containing the highest concentrations of saturated fatty acids, while this butter also contained the highest concentrations of palmitic acid (O’Callaghan *et al.*, 2016b) – a fatty acid which is known to be a significant contributor to the firmness of butter (Marangoni *et al.*, 2021). The TMR diet derived butter was found to have the highest spreadability index, which is the ratio of C16:0 to C18:1, followed by the Ryegrass derived butter, with the ryegrass and clover butter having the lowest spreadability index while this trend was also observed for the thrombogenic index of the three butters (O’Callaghan *et al.*, 2016b). The onset temperature of crystallisation was also found to be different when considering the 3 feeding systems, with the TMR derived butter having the highest onset temperature at $17.24 \pm 0.3^{\circ}\text{C}$, followed by the perennial ryegrass derived and the ryegrass and clover derived butters, respectively (O’Callaghan *et al.*, 2016b). It was found that the hardness differences between the three butters were not statistically significant at refrigeration

temperatures; however, at room temperature, the TMR derived butter was hardest across the study duration, while all three butters individually increased in hardness as the study ensued across the 6 months both at room temperature and refrigeration temperature (O'Callaghan *et al.*, 2016b). The butter produced from the milk of perennial ryegrass fed cows scored the highest upon sensory analysis of the three butters with regards liking of flavour and appearance (Hedonic) and colour, diacetyl flavour and aroma , and cream flavour (O'Callaghan *et al.*, 2016b).

2.4 Crystallisation of milk fat

In milk fat, crystallisation, a process which cannot take place before nucleation occurs, is very relevant in terms of impacting functionality of butter (Huppertz, Kelly and Fox, 2009). After this initial step, crystals are allowed to increase in size (Huppertz, Kelly and Fox, 2009)

Crystallisation in an oil in water emulsion such as cream is is greatly dependant on collisions between formed crystals (from within globules) and adjacent fat globules (Logan *et al.*, 2021).

2.4.1 Mechanisms of crystallisation in milk fat

Triacylglycerol crystallisation occurs generally in one of three polymorphic arrangements – α , β' or β (Huppertz, Kelly and Fox, 2009; Taylor and MacGibbon, 2011) and it, along with melting behaviour can be greatly impacted by properties of the individual fatty acids found within TAG structures (Himawan, Starov and Stapley, 2006; Fox *et al.*, 2015). Each crystalline form has different levels of stability with the β form being the most stable and the α form being the least stable (Sato & Ueno, 2011). Figure 2.6 depicts the different forms of crystals which occur in fats (Rye, Litwinenko and Marangoni, 2008). The form in which milk fat crystallises to can be greatly determined by both the TAG composition and what temperatures it has been

exposed to previously (Fox *et al.*, 2015). This in turn has the capacity to impact the functionality of any resulting butter (Rønholt *et al.*, 2012). This is especially significant in cream tempering which can be used to decrease butter hardness(Fox *et al.*, 2015).

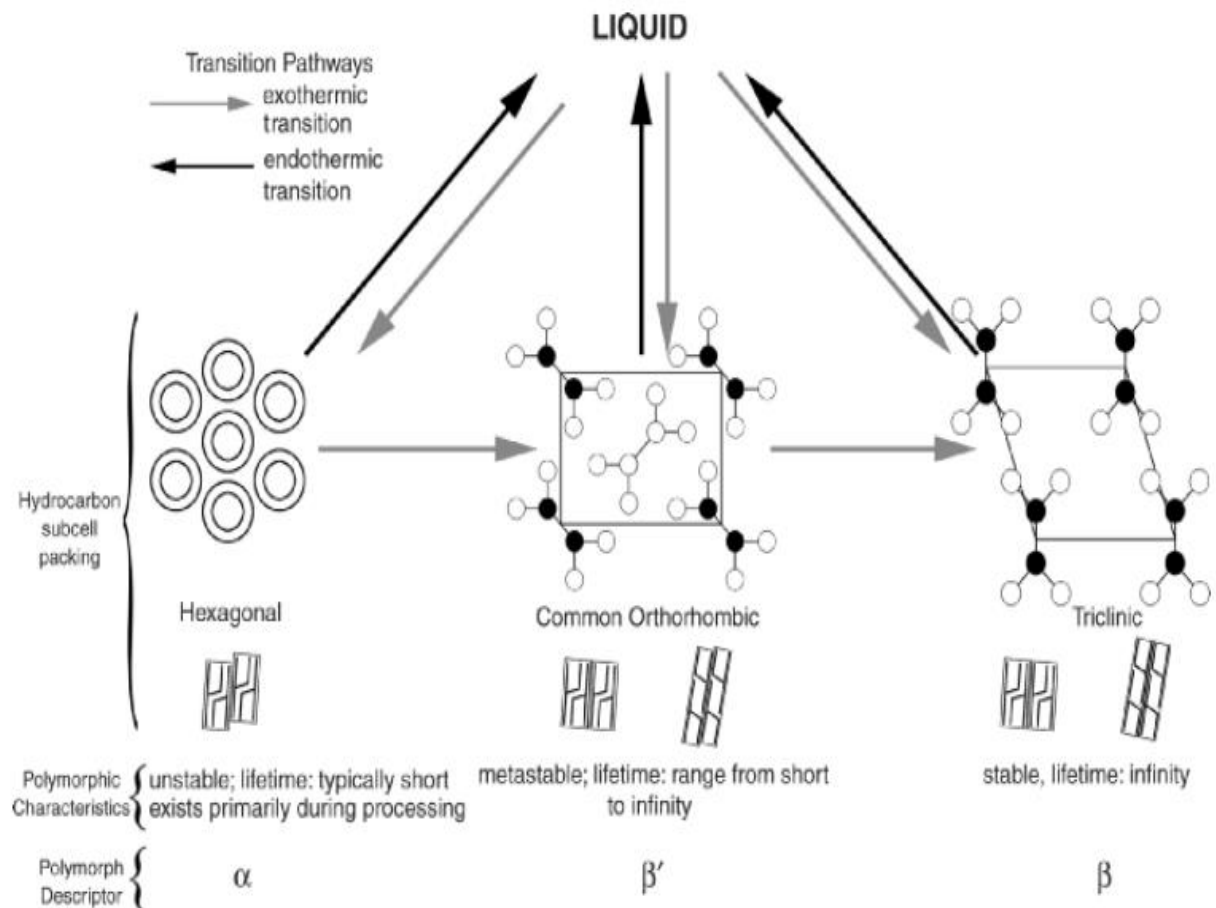


Figure 2.6. Different crystal forms found in fat (Rye, Litwinenko and Marangoni, 2008)

Many factors can affect the final arrangement in which fatty acids crystallise such as chain length and degree of saturation (Himawan, Starov and Stapley, 2006). For example, it has been outlined that triacylglycerols containing fatty acids which are unsaturated will melt at a lower temperature than those which contain lower degrees of unsaturation - this being attributed to double bonds causing interference with efficient packing (Himawan, Starov and Stapley, 2006).

Crystals in milk fat globules in cream begin to form from the inside the globule, thus increases in viscosity of cream in the ripening stage may not be noted as explained by Buldo *et al.* (2013). The β form of polymorphism is considered to be the most stable (Sato and Ueno, 2011), while it is also considered to be the most sensitive to differences in chain length and unsaturation of fatty acids involved (Himawan, Starov and Stapley, 2006). The α polymorphism is considered to be the form with the least stability and can metamorphose easily to other polymorphic forms (Sato and Ueno, 2011). As well as differences in stability, polymorphic forms also differ in their structure, with the α , β and β' polymorphic forms having hexagonal, orthorhombic perpendicular and triclinic parallel subcellular structure respectively (Sato and Ueno, 2011). In many instances in the case of milk fat, the beta prime polymorphic form is the most favoured (Sato and Ueno, 2011). Sato and Ueno, (2011) also outlines that in general, melting temperatures can be significantly related to the polymorphic form in which a triacylglycerol has crystallised – β in general tends to be associated with the highest melting point while α tends to be associated with the lowest melting point.

2.4.2 Cream ripening and storage

Although it takes some time, aging/ripening of cream before use in butter production has a significant impact on the fat crystallisation, and in turn, the final product qualities (Buldo *et al.*, 2013). In addition, varying the storage temperature of cream (tempering) can significantly influence the crystallisation process, as described by Logan *et al.* (2021). In this study, unprocessed cream (20% w/w) and homogenised cream (20% w/w) were both analysed. The unprocessed cream had an average milk fat globule size of 4.43 μm while the homogenised cream had an average milk fat globule size of 1.28 μm (Logan *et al.*, 2021). The authors reported that these values remained constant following retaining of the samples at 4°C x 4 d. Furthermore, following heating of the unprocessed cream to 25°C and holding for 10 min in

the tempering process, and subsequent re-cooling to 4°C and holding for 30 min, average fat globule size remained constant again (Logan *et al.*, 2021), but the authors did report significant changes in the droplets from the homogenised sample exposed to the same temperature treatments, with an increase in droplet size from 1.29 ± 0.13 to 1.56 ± 0.11 µm, with the authors attributing this to coalescence of droplets (Logan *et al.*, 2021).

In the context of butter-making, as cream has a longer length of time to ripen, crystallisation of the fat occurs and can lead to a lower content of moisture in the final product (Funahashi and Horiuchi, 2008).

Buldo *et al.* (2013) investigated the effect of cream ripening time on churning time and reported a clear relationship between these factors. It was found that un-ripened cream had the longest churning time while the cream which had been ripened for the longest in this study (17 h) led to the shortest churning time (Buldo *et al.*, 2013). In addition, the longest aged sample resulted in a higher viscosity measurement at the point of phase inversion compared with lesser aged samples (Buldo *et al.*, 2013). It was observed that the samples which had been aged for longer, resulted in butter grains of larger size – the authors reported that cream which had been aged for 5 h produced butter grains which were 1.75 times the size of those derived from cream which had not gone through an aging process (Buldo *et al.*, 2013).

2.5 Functionality and applications of butter

Butter has a wide range of applications worldwide. These include bakery, food service and confectionary. When considering butter, there are a number of functional parameters which are of particular interest for both business to business and business to consumer applications. Four of these parameters include texture, colour, melt properties and rheological properties. In order to tailor make products to suit these applications, it is necessary to understand some extrinsic

factors of dairy production which impact such attributes. Due to the fact that milk fat is comprised of fatty acids, much of the literature focus is in this area in terms of factors affecting fatty acid profile and its subsequent impact on functionality of butter.

2.5.1 Factors affecting butter functionality and texture

Many factors can affect the fatty acids found in fat component in milk, including diet and stage of lactation (Huppertz, Kelly and Fox, 2009). Both of these factors, as well as phenotypic variation in milk fatty acids will be discussed in greater detail with respect to their impact on butter functionality in the following sections.

2.5.1.1. Feed of the cow

The fatty acids found in milk fat can influence milk fat characteristics (Paduret, 2021; Staniewski *et al.*, 2021). Due to a wide variety of factors impacting the fatty acid content of milk, cream and in turn butter, it is important to know what exact affect this variation can have on butter physicochemical properties. Acknowledging Irelands predominantly grass based model of dairy farming, as well as consumers desire for pasture-based products (Timlin *et al.*, 2024) it is important to understand how pasture inclusion in cows diet can impact end product characteristics and in this instance, butter characteristics. It has been acknowledged that diet can have an impact on the present of these fatty acids as well as impacting butter texture (Bobe *et al.*, 2003; Timlin *et al.*, 2024).

Couvreur *et al.* (2006) investigated the relationship between milk fatty acid composition and resultant butter properties. The mechanism by which the authors aimed to impact the fatty acid profiles was by varying cow diet. This study experimented with 4 different feeding regimes

with varying amount of fresh grass, with yield, chemical composition and resulting butter investigated for impact. It was found that along with inducing an increase in milk yield of a linear nature, butter produced from the milk of the various groups of cows was significantly impacted (Couvreur *et al.*, 2006). It was found that 30% substitution of corn silage with fresh grass induced an increase in butter hardness but thereafter, for the samples produced from the milk of animals fed a 60% and 100% substitution of corn silage with fresh grass, the firmness decreased incrementally. A separate study by Timlin *et al.* (2024) also investigated the impact of a cow's diet on butter textural, colour and rheological properties. In this study, cows were split into 3 groups and fed either (a) a high pasture diet, (b) a high total mixed ration diet or (c) a mix of both. Similarly to the forementioned study by Couvreur *et al.* (2006), in this study it was too found that feed impacted a large number of the fatty acids tested for in this study and in turn, butter functional properties. Oleic acid quantities in butter decreased as the amount of grass in the diet decreased, while palmitic acid quantities were inversely effected as quantities increased with a increase in TMR in the diet. This increased TMR/decreased grass in the cows diet induced an expected increase in butter hardness, while also inducing an increase in both peaks of crystallisation temperatures, enthalpy of crystallisation and enthalpy of melting (using differential scanning calorimetry). The colour of butter was also impacted, with the average L* and a* values being lowest in the grass-fed butter and highest in the TMR fed butters. Conversely, the b* value was highest in the grass-fed butter and lowest in the TMR fed butter (Timlin *et al.*, 2024). These results showed some similarities to the results from O'Callaghan *et al.* (2016b), who found that increasing TMR in the diet induced an increase in palmitic acid levels in butter, butter hardness, both peak crystallisation temperatures and b* values.

2.5.1.2 Phenotypic variation in milk fatty acids and stage of lactation

A 2003 study investigated the impact of phenotypic variation among milk fatty acids from cows fed the exact same diet on the textural properties of butter. This was to assess the direct impact of fatty acid content on butter properties (Bobe *et al.*, 2003). A larger number of samples were tested for fatty acid content and 10 cows were chosen for the study who's fatty acid contents varied significantly in terms of atherogenic index (AI), while being fed the same diet. A number of different breeds were involved in this study and it was found that at refrigeration temperature, butter was more spreadable (46% higher), softer (38% softer) and less adhesive (35% lower) in the butter from cows who produced milk fat with a lower AI than the butter produced from the milk of the cows with a higher AI – with this trend remaining consistent for hardness and adhesiveness when testing temperature was increased (Bobe *et al.*, 2003).

For the most part, how these triglycerides are made up, depends greatly on what fatty acids are present and how much of those fatty acids are present (Jensen, 1991). A 2020 study investigating the impact of both of these factors was conducted by Staniewski *et al.* (2021) in which the impact they had on butter properties was discussed. Over 60 commercial products were included in the study with just over half of these produced from cows fed a pasture-based diet while the remainder of the samples originated from the milk of cows fed an indoor housed diet. The pasture-based butters were Summer produced butters while the remainder were winter butters. A cone penetration test was utilized to determine the butter hardness. The authors found a significant difference in hardness when comparing the Summer (Grass Fed) and winter butters (Stall Fed). It was found that across a range of temperatures, butter hardness was lower in the Summer samples when compared with winter samples. This study found that in order to have the butter at a level of spreadability considered acceptable by consumers, the winter butter required warming to 15.3°C compared with 11.9°C for the Summer butter (Staniewski *et al.*, 2021). This suggests an effect on the melting point of the butter which can have impacts on

consumer acceptance but also processability of butter in business-to-business trading. Timlin *et al.*, (2024) also investigated the impact of stage of lactation on butter textural properties across 2 seasons. It was found that for cows fed a predominantly pasture based diet across the lactation that stage of lactation did have a significant impact on the final butter product (Timlin *et al.*, 2024). Many fatty acids were found to be impacted such as palmitic acid, oleic acid, as well as the total quantities of saturated and unsaturated fatty acids. This, as expected caused variations in butter texture. The C16:0/C18:1 *cis* ratio was lower in early season compared with mid and late season while the peak crystallisation temperatures for both crystallisation peaks were highest in early season and lowest in late season. In terms of butter hardness, trends reported were different for samples tested at room temperature and samples tested at refrigeration temperature – hardest in mid lactation and softest in early lactation for refrigerated samples and hardest in late lactation and softest in early and mid-lactation (2.87 N and 2.86 N respectively) at room temperature (Timlin *et al.*, 2024).

2.5.1.3 Cream ripening

Cream ripening and variations thereof is another factor which can impact the texture of butter. (O’Callaghan, 2022). It is known that seasonal changes in milk fat can cause different textural properties at different times of the year and to overcome this phenomenon, cream ripening/tempering methods have been employed (Wilbey, 2009). The Alnarp method of cream ripening, dating as far back as 1973, is the predominant method employed to achieve this effect, and this process is used to create a more consistent butter product throughout the year regardless of the fatty acid profile of the cream (Wilbey, 2009). In this method, cream is pasteurised, before being cycled through various time - temperature combinations, depending on the iodine value, time of year, and fatty acid profile with a view to reducing the hardness,

and producing a consistent product (Wilbey 2009; O’Callaghan, 2022). Examples of such time - temperature treatments for both Summer and Winter butters can be seen in Wilbey. (2009).

2.5.2 Quality and consumer acceptance of butter

Butter is a widely used product worldwide, and while the foundational principles of butter-making are understood since pre-historic times, it is a product which can encounter quality issues if not produced to a high standard, often leading to consumer disliking. The three main groupings of quality parameters, from a sensory standpoint, to which the quality of a butter product is judged on are: appearance, texture, and taste (Wilbey, 2009). These groupings, and key quality attributes within each, are summarised in Table 2.1.

Table 2.1 Key quality attributes associated with butter products to ensure consumer acceptance and product quality (Wilbey, 2009)

Parameter	Key quality attributes
Appearance	<ul style="list-style-type: none"> • Surface should be free from oil and evidence of moisture. • Colour will vary, depending on region and product; however, should be consistent throughout the product.
Texture	<ul style="list-style-type: none"> • A smooth texture should be present, free from air gaps, gritty mouthfeel, and inconsistencies. • The product should melt and disperse evenly in the mouth, while not being overly crumbly nor over-worked and gummy
Taste	<ul style="list-style-type: none"> • Product flavour should be consistent with the product type being produced, and the market and application it is being produced for. • Flavour and taste will vary considerably between different products but should be consistent, free from off-flavours and clean

Butter is a product which by some may be considered as not being healthy, especially among older consumers; however, it is known for its taste and flavour attributes (Krause, Lopetcharat, and Drake, 2007; Cheng *et al.*, 2023). A proportion of this perception is based on the fact that butter is considered as being unhealthy due to its contents of saturated fat (Cheng *et al.*, 2023). This is one of the reasons that Irish butter has distinct advantages over butter produced in many countries worldwide where cows are fed a higher proportion of TMR feed and as a result, butter contains more saturated fatty acids. A 2007 study evaluated what consumers consider as important, and factors which drive liking when purchasing butter and margarine. It was found through the use of focus groups that two of the most important factors to consumers, when purchasing butter were good flavour and a natural image (Krause, Lopetcharat, and Drake, 2007). It is important to note that quality parameters which are considered favourable in one region, may be perceived differently in another. Consumer perception remains critical when considering butter, not least from a nutritional perspective, as individuals become more health cognisant, and as a result, studies into its benefits (both nutritional and functional) are important going forward.

2.6 Butter storage

For reasons of seasonality, and suitability for processing of milk, butter products are often produced in large volumes at specific time intervals of the year and stored for sale, and ultimately, consumption at a later date (Krause et al., 2008). With this in mind, the appropriate storage of butter post-production is important to maintain functionality, as well as microbiological and chemical integrity of the product during this time (Laikoja, Teder and Joudu, 2017). Butter, following its production, is typically packaged and moved directly to low-temperature storage (Tetra Pak, 2025), where it is either refrigerated (typically at 4°C) or frozen (typically at -18°C), for use at a later stage. Consequently, it is critical for both dairy

processors and the scientific community alike, to understand the effects, if any, various time-temperature combinations can have on the storage stability and end-quality of butter.

In recent years, temperature and length of storage of butter have been shown to not significantly impact key quality attributes such as acid and peroxide values (both indices of lipid oxidation; Laikoja, Teder and Joudu, 2017). Conversely, it has been shown that differences in texture and off-flavour development can be linked to post-production temperature and duration of storage (Krause et al., 2008). In addition, Krause et al. (2008) demonstrated that refrigerated storage of bulk and smaller pack sized butter induced earlier oxidation, and resulting off-flavour development when compared with frozen storage where these effects were delayed (Krause et al., 2008). As mentioned, some studies have been carried out which discuss the impact of various single storage temperatures on chemical properties of butter (e.g., oxidation and off-flavour development). However, few, if any, studies have focussed on the impact of storage temperature on melt properties of butter, while even more importantly, to the authors knowledge, no studies have assessed the effects of strategically changing storage temperature of butter following production on its techno-functional properties. This study was designed with the aim of addressing this scientific knowledge gap.

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Chapter 3

Impact of post-production storage conditions on techno-functional properties of butter

Órla Dinneen^{1,2}, Anna G. Behan², James A. O'Mahony¹, Tom F. O'Callaghan¹

¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*

²*Kerry Dairy Ireland, Princes Street, Tralee, Co. Kerry, Ireland*

Declaration

This chapter was written by author Órla Dinneen (Ó.D.) and reviewed by all co-authors. Ó.D. co-designed the study with co-authors and performed all of the experimental work.

Abstract

The aim of this study was to assess the impact of post-production storage temperature on the quality of butter. This was achieved by applying six different temperature treatments to butter directly after production for a period of 20 d and examining their impact on its techno-functional properties. The study was conducted using a standard, lactic unsalted butter produced at two different times of the year in 2023; Mid-Late season (MLS; September) and Late season (LS; November). Butter was produced in an industrial setting from the milk pool of a large dairy processor in Ireland and stored in 454 g containers. Different storage treatments were applied to the butter samples through the use of various fridges and freezers. Parameters measured in this study included texture, thermal melt properties, rheological properties and fatty acid quantification. Textural properties were measured through the use of texture profile analysis, rheological properties were measured by observing the storage and loss modulus throughout a temperature ramp between 4 and 40°C and noting their crossover point, while thermal melt properties were quantified by running a differential scanning calorimetry ramp between -15 and 50°C and analysing the resulting thermograms. No significant impacts of changing the post-production storage conditions of butter were found across all of these parameters, although there were significant changes between the trend of results measured in the MLS trial and the LS trial, which was to be expected. These results include higher hardness levels, higher concentration of saturated fatty acids, lower unsaturated fatty acids, and higher melting points in the LS trial. Some minor differences were observed in terms of melt properties of butter between the different temperature treatments; however further research is required to understand the robustness of these results, given the nature of the product and the high standard deviation observed in the analysis.

3.1. Introduction

Butter is a widely produced and consumed dairy product in Ireland and indeed internationally. In Ireland alone, butter production volumes have consistently remained above 260,000 Tonnes per annum over the past five years, making Ireland the third largest butter producer within the European Union (EU) in this time period (CLAL.it, 2025). While a portion of this produce has been consumed and utilised at a domestic level, the majority of Irish butter is exported to 140 markets globally (Bord Bia, 2024). In 2024, Ireland reached record heights for agri-food exports, and butter exports were particularly high in value in Ireland over the last twelve months (Bord Bia, 2025). This has in part helped to compensate for the recent reduction in value of other dairy commodities such as skim milk powder (Bord Bia, 2025).

From production to consumption, butter is subjected to several different stages of storage, including storage immediately post-production, in transit storage, warehouse storage and end use storage (e.g., at domestic and food service levels). Butter is commonly produced in bulk (normally in 25 kg blocks) and is often stored for months at a time (Krause *et al.*, 2008). Some research has been conducted on the impact of long-term storage, at either frozen or refrigerated temperatures, on the physicochemical changes in butter over time (Krause *et al.*, 2008; Mendéz-Cid *et al.*, 2017). However, to the authors knowledge, no studies have been identified which specifically investigate changing the storage temperature of butter immediately after production, to assess the impact, if any, this has on physicochemical and key quality attributes of butter. This was identified as a gap in knowledge in the field of butter storage.

Post-production storage, and any subsequent implications for product quality and functionality, is of particular interest to industry producers and end-users alike. In some instances, butter may be produced, refrigerated, and remain refrigerated up to the point of end use, while in other cases, it may be frozen for the first number of days/weeks, prior to refrigeration for tempering before shipment to a customer.

It is important to understand if the differences in such post-production storage protocols impact the end product, across a number of functionality categories, such as texture, rheological and thermal melt properties. These functional properties are relevant, individually, and collectively, both in business-to-consumer and business-to-business contexts, across application areas such as food service, bakery and confectionary. It is important to answer this research question to assess if a particular cooling regime could potentially be more suitable for a given application over another, as butter texture and melting point requirements can be different depending on the intended application. An example of where this is the case is in bakery applications, more specifically in the production of puff pastry, croissants, and other pastry-based products, where, if butter is too soft, it can have an adverse effect on the processing and sheeting of butter layers between dough laminate layers. Implications of this include breaking of the fat layer in the pastry and difficulty producing sheets of fat which are workable. This can lead to processing and product functionality issues. It has been widely reported that the milk fatty acid profile, as well as cream treatment pre butter production (e.g., tempering of cream) can impact the functionality of butter (Bobe *et al.*, 2003; Drelon *et al.*, 2006; Buldo *et al.*, 2013; Logan *et al.*, 2021; Timlin *et al.*, 2024); however, it is not as widely understood if the temperature treatment post butter production in the warehouse etc. can impact this too.

The objective of this study was multifaceted. Primarily, it was to assess the impact, if any, of utilising various storage treatments of butter directly after its production on the rheological, melt and textural properties of butter. It was important to understand if factors such as harsh temperatures (e.g., -30°C), rate of cooling post-production, and stability of temperature throughout storage could impose physicochemical changes on butter. This is relevant for food processors and shipping companies, in addition to the receiving partner in business-to-business scenarios. It is important for all food producers to understand the impact that changing any factors in their supply chain from intake to customer delivery can have on the end product.

Secondly, and stemming from the primary objective, was to identify the storage treatments, if any, which may have the potential to be used in certain applications over others, to tailor the techno-functional requirements of butter products.

3.2. Materials and methods

3.2.1. Butter samples

A standard unsalted lactic butter with a fat content of 82% and a moisture content of no greater than 16% was used to perform this study. The butter was produced and supplied by Kerry Ingredients, Islandmacloughry, Tralee Road, Listowel, Co. Kerry. It was produced using a Simon Frères butter churn (Simon Frères, Cherbourg-en-Contentin, France). Butter was taken directly from the processing plant at the time of production and stored in standard ~454 g butter containers for the duration of the study.

3.2.2. Experimental design

The butter was taken directly from the butter churn (Kerry Ingredients, Islandmacloughry, Tralee Road, Co. Kerry, Ireland) and stored at its respective temperature over a period of 10 d. Six different temperature treatments were trialled in this study. Each treatment involved different time - temperature storage combinations of the butter directly after production as described in Table 3.1 and shown visually in Figure 3.2. Trials were performed at two different times of the year in 2023; September (Mid-Late season; MLS) and November (Late season; LS). Four of the treatments involved two different storage temperatures, both 5 d in duration. The final two temperature treatments involved the use of one temperature held isothermally over 10 d. All butter samples were stored at $9 \pm 3^{\circ}\text{C}$ for 10 d after being in their respective storage, to equilibrate all samples to the same temperature.

Temperature loggers, supplied by Sensor-Tech Limited, Dunleer, Co. Louth, Ireland were utilised to measure and record the temperature history of the products throughout storage. The temperature logger probe was inserted into the side of each reference tub to reach the core of the sample as shown in Figure 3.1.



Figure 3.1. Photographic image of a temperature probe inserted in the side of temperature reference tub for one temperature treatment.

An example of the output data from these loggers is presented in Appendix 3.1. Each storage treatment had a corresponding temperature reference sample which was stored in combination with the samples to achieve this tracking.

Table 3.1. Summary of the six different temperature treatments involved in this study performed on butter manufactured in Mid-Late season (MLS) and Late Season (LS).

Sample Code	Storage temperature (°C)		
	1–5 d	6–10 d	11–20 d
MLS -30/-30 & LS -30/-30	- 30	- 30	+9
MLS -20/-20 & LS -20/-20	-20	-20	+9
MLS -30/-20 & LS -30/-20	- 30	-20	+9
MLS -20/-30 & LS -20/-30	-20	- 30	+9
MLS +5/-20 & LS +5/-20	+5	-20	+9
MLS -20/+5 & LS -20/+5	-20	+5	+9

The different storage temperature treatments examined in this study were achieved through the use of various fridges and freezers in Kerry Ingredients, Islandmacloughry, Tralee Road, Listowel, Co. Kerry., operating at each of the respective target temperatures.

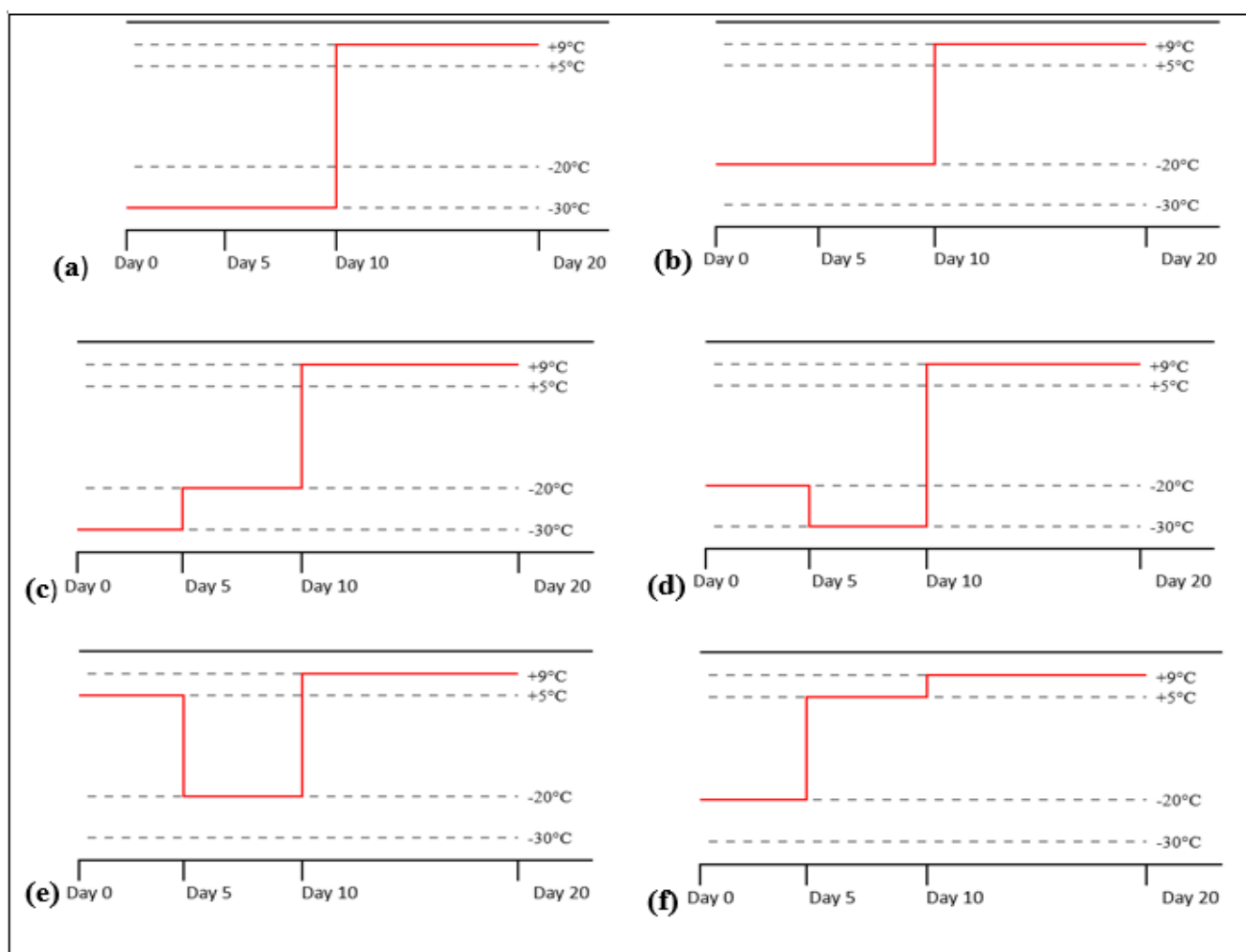


Figure 3.2. Temperatures (°C) used for Mid-Late season (MLS) and Late season (LS) trials throughout a post-production storage period of 20 days: **(a)** MLS -30/-30 & LS -30/-30, **(b)** MLS -20/-20 & LS -20/-20, **(c)** MLS -30/-20 & LS -30/-20, **(d)** MLS -20/-30 & LS -20/-30, **(e)** MLS +5/-20 & LS +5/-20, **(f)** MLS -20/+5 & LS -20/+5

3.2.3. Analytical methods

3.2.3.1. Fatty acid profile

Analysis of fatty acids was performed using gas chromatography-flame ionisation detection (GC-FID) based on BS EN ISO 12966. Samples were saponified and methylated with the resulting fatty acid methyl esters extracted, and their profile analysed via gas chromatography, with an internal standard used for identification and quantification. The analysis was conducted by an external analytical service provider (Eurofins Food Testing Ireland Limited, Finglas, Co. Dublin, Ireland). Analysis of butter from both times of the year was carried out in triplicate.

3.2.3.2. *Differential scanning calorimetry*

Differential scanning calorimetry (DSC) analysis was performed on frozen butter samples, using a TA DSC 2500 calorimeter (TA Instruments, Delaware, United States). Each storage treatment was imitated using the DSC method before bringing the samples to -15°C and undertaking a melt analysis to 50°C to assess if varying storage temperatures influenced the melting properties of the butter. To choose the precise temperatures used on the DSC instrument for each treatment, temperature logger data was used, to determine the average temperature the butter samples reached throughout storage. This was completed to take into account any potential temperature fluctuations from fridges and freezers. The temperature - time steps involved for each treatment are summarised in Table. 3.2.

The butter was pre-melted to 60°C in the DSC to erase crystal memory before each independent temperature treatment was applied to the samples in the DSC instrument and a subsequent melting ramp completed. Initially, the pan weight and sample weight were recorded on the software, and a reference pan of known empty weight was included as a reference in all analysis. The same reference pan of equal weight was used for the MLS and LS trials. Frozen butter (3-8 mg) was carefully weighed into aluminium pans, with care taken to not get any butter on the lip of the pan as this can interfere with sealing and affect the results. A seal press was used to seal the edges of the pans before loading samples into the DSC instrument, and the photographic images of the sample preparation for this method are presented in Appendix 3.2. Analysis of resulting DSC thermograms was carried out using TRIOS DSC software.

Table 3.2. Temperature-time combinations involved in the Differential scanning calorimetry(DSC) treatment of butter samples from Mid-Late season (MLS) and Late season (LS) trials. The exact temperatures used were chosen from the temperature the butter had reached during storage. This information was available from the temperature logger data.

STEP	MLS -30/-30	MLS -20/-20	MLS -30/-20	MLS -20/-30	MLS +5/-20	MLS -20/+5
	LS -30/-30	LS -20/-20	LS -30/-20	LS -20/-30	LS +5/-20	LS -20/+5
1	Melt to 60°C and hold for 10 min	Melt to 60°C and hold for 10 min	Melt to 60°C and hold for 10 min	Melt to 60°C and hold for 10 min	Melt to 60°C and hold for 10 min	Melt to 60°C and hold for 10 min
2	Ramp to -30°C and hold for 20 min	Ramp -20°C and hold for 20 min	Ramp to -30°C and hold for 10 min	Ramp to -20°C and hold for 10 min	Ramp to 5°C and hold for 10 min	Ramp to -20°C and hold for 10 min
3	Ramp to -15°C and hold for 15 min	Ramp to -15°C and hold for 15 min	Ramp to -20°C and hold for 10 min	Ramp to -30°C and hold for 10 min	Ramp to -20°C and hold for 10 min	Ramp to 5°C and hold for 10 min
4	Melt to 50°C @ 5°C/min	Melt to 50°C@ 5°C/min	Ramp to -15°C and hold for 15 min	Ramp to -15°C and hold for 15 min	Ramp to -15°C and hold for 15 min	Ramp to -15°C and hold for 15 min
5	-	-	Melt to 50°C@ 5°C/min	Melt to 50°C@ 5°C/min	Melt to 50°C@ 5°C/min	Melt to 50°C@ 5°C/min

-: Temperature treatments which did not require a fifth step, due to being held isothermally for 10 d as opposed to changing temperature at day 5

3.2.3.3. Texture analysis

A Stable Micro Systems TA.XTplusC texture analyser (Stable Micro Systems, Surrey, United Kingdom) was used to perform a double compression texture profile analysis (TPA) cycle on all samples which were prepared as follows: butter samples (25 mm cubed) were prepared using a custom-made metal cuber of dimensions 25 mm x 25 mm x 25 mm (W x D x H) (Kerry Ingredients, Listowel County Kerry) which can be seen in Figure 3.3. The butter block was cored using this cuber before being pushed out of the metal cuber using a custom-made tool to suit this application (Kerry Ingredients, Listowel, County Kerry). Butter was stored at 4°C prior to cutting, following cutting, and up to the point of analysis (in aluminium foil).



Figure 3.3. Custom-made metal cuber of dimensions 25mm x 25mm x 25mm (W x D x H) (Kerry Ingredients, Listowel County Kerry)

The butter cubes were taken directly from the fridge and placed on the texture analyser platform for testing. A test speed of 1 mm/s was utilized, and the head of the texture analyser was lowered by 10 mm onto the sample, using a P50 Stable Micro Systems probe. A short recovery time of 15 s between compressions was used as the sample was not expected to recover. The area under the curve of the first TPA peak was calculated using a formula, based on the trapezoidal rule, custom developed in the Food Ingredients Research Group at University College Cork, Ireland. Peak force was extrapolated from each curve and was defined as the highest force reached throughout the first compression. The work of compression was defined as the area under the curve of the first compression.

3.2.3.4. Rheological analysis

Discs of butter were prepared for rheological analysis using a number of steps. The refrigerated butter blocks were removed from their tubs and sliced into 2.25 mm thick slices using a Scharfen meat slicer (Model G330F, Scharfen Slicing Machines GmbH, Witten, Germany). Circular discs were punched from these slices using a 4 mm high, 58.7 mm diameter steel pipe ring (custom made in University College Cork). This diameter was chosen to ensure the discs were larger than the 50 mm smooth parallel plates used for analysis. Discs were stacked using parchment paper placed between the discs and stored at 4°C. Butter discs were stored at this temperature, both prior, and following cutting, after which, samples were ready to be loaded onto the rheometer for analysis, where each was compressed to 2.25mm and trimmed to fit the parallel plates. Sample preparation for this method is presented in more detail in Appendix 3.3.

A butter melt curve was obtained between 4 and 40°C under oscillatory conditions. This was achieved through melting of butter discs from different treatments under controlled conditions between two flat parallel plates of 50mm diameter (Anton Paar, Graz, Austria). Butter was held for two min pre and post temperature ramping to ensure the analysis started at a temperature of 4°C and to assess if results had plateaued at the end of the test. Measured parameters included storage and loss moduli as a function of temperature and time. An Anton Paar modular compact MCR 102e rheometer was used for this study, with cooling controlled using a Julabo 200 I waterbath.

3.2.4. Statistical data analysis

Trials were carried out twice within the same year at different times - MLS and LS. Within each of these trials, each temperature treatment was performed in triplicate, with analysis of each performed in duplicate, giving six results per treatment for each of the trials. A two sided

T-Test in Microsoft excel was used to analyse differences in fatty acid profile between the MLS and LS trials.

3.3. Results and discussion

3.3.1. Fatty acid profile

Fatty acid analysis was undertaken to serve as a baseline to distinguish between the butter at the two different times of the year in terms of fat chemistry. The fatty acids in this study which were present at levels of $> 0.1\%$ of total fatty acids are shown in Table 3.3., with data presented as % of total fatty acids present in the sample. Some fatty acids were found to exist in similar concentrations in both trial butters, such as C6:0(Caproic acid) and C8:0(Caprylic acid) while the levels of other fatty acids were very different between trials. These trends are in line with previous studies where differences have been observed in the concentration of some fatty acids, with small or no changes being observed in the concentration of other fatty acids when comparing mid and late lactation fatty acid profiles of milk and butter (Li, Ye and Singh, 2022; Timlin *et al.*, 2024). The interim time period between conducted trials in this study coincided with a time typically associated with a diet change for cows in a pasture-based system such as Ireland. Fatty acid profile can be influenced by the feed a cow consumes (Bobe *et al.*, 2003).

Palmitic and oleic acids (C16:0 and C18:1, respectively) are the two most commonly found fatty acids in milk fat as shown and documented widely in literature (Jensen, 2002; Bobe *et al.*, 2003; Staniewski *et al.*, 2021; Li, Ye and Singh, 2022; Timlin *et al.*, 2024). The concentration of these two fatty acids varied significantly between the MLS and LS trials; for example, concentrations of palmitic acid were $27.51 \pm 0.07\%$ in the MLS trial, compared with $34.54 \pm 0.01\%$ in the LS trial, while oleic acid concentrations ranged from $17.69 \pm 0.01\%$ in the LS trial to $20.06 \pm 0.09\%$ in the MLS trial, as expected. These results are broadly in agreement with the results of Staniewski *et al.* (2021), who found that commercially produced butter in

winter contained $25.12 \pm 2.72\%$ oleic and $28.06 \pm 4.16\%$ palmitic acid, while Summer butter contained $20.22 \pm 1.55\%$ oleic and $33.69 \pm 2.82\%$ palmitic acid.

Conjugated linoleic and Alpha linolenic acid concentrations were also significantly lower ($p < 0.05$) in the LS samples when compared with the MLS samples. Saturated fatty acid concentrations differed between trials from $65.12 \pm 0.15\%$ in the MLS trial to $71.06 \pm 0.03\%$ in the LS trial, marking a significant increase. This contradicts the results found in the study of Li, Ye and Singh (2022), but when considering the results of Couvreur *et al.*, (2006), which reported that higher quantities of fresh grass in the diet of cows resulted in milk with lower saturated fatty acid quantities, they are in line. This may be explained because it is expected that given the calving and grazing systems utilised in the region within which the butter was produced, butter in MLS came from cows being fed a higher portion of fresh grass than the butter from the LS trial.

The fatty acid profile as a whole was expected to impact melt, DSC and texture results, given that different fatty acids have different melting points (Huppertz, Kelly and Fox, 2009, but the aim of this study was to investigate if the post-production storage could alter these results further.

Table 3.3. Fatty acid profile of butter used in Mid-Late season trial (MLS) and Late season trial (LS).

Fatty Acid	MLS	LS	P value
	Mean $\pm$ SD as a % of total fatty acids		
C4:0 Butyric acid	3.52 $\pm$ 0.01	3.17 $\pm$ 0.00	0.000
C6:0 Caproic acid	2.18 $\pm$ 0.01	2.17 $\pm$ 0.00	0.251
C8:0 Caprylic acid	1.27 $\pm$ 0.00	1.26 $\pm$ 0.00	0.116
C10:0 Capric acid	2.68 $\pm$ 0.00	2.66 $\pm$ 0.00	0.005
C10:1(n-1)cis cis-9-Decenoic acid	0.40 $\pm$ 0.00	0.42 $\pm$ 0.00	0.000
C12:0 Lauric acid	3.25 $\pm$ 0.01	3.35 $\pm$ 0.00	0.000
C12:1(n-1)cis cis-11-Dodecenoic acid	0.12 $\pm$ 0.00	0.14 $\pm$ 0.00	0.002
C13:0 Tridecanoic acid	0.18 $\pm$ 0.00	0.21 $\pm$ 0.00	0.000
C14:0 iso 12-Methyltridecanoic acid	0.12 $\pm$ 0.00	0.11 $\pm$ 0.00	N/D
C14:0 Myristic acid	10.39 $\pm$ 0.02	10.81 $\pm$ 0.00	0.000
C14:1(n-5)cis Myristoleic acid	1.17 $\pm$ 0.00	1.37 $\pm$ 0.00	0.000
C15:0 ante-iso 12-Methyltetradecanoic	0.60 $\pm$ 0.00	0.52 $\pm$ 0.00	N/D
C15:0 iso 13-Methylmyristic acid	0.32 $\pm$ 0.00	0.29 $\pm$ 0.00	N/D
C15:0 Pentadecanoic acid	1.20 $\pm$ 0.00	1.45 $\pm$ 0.00	0.000
C16:0 iso 14-Methylpentadecanoic acid	0.30 $\pm$ 0.00	0.28 $\pm$ 0.00	0.007
C16:0 Palmitic acid	27.51 $\pm$ 0.07	34.54 $\pm$ 0.01	0.000
C16:1(n-7)cis Palmitoleic acid	1.83 $\pm$ 0.01	2.32 $\pm$ 0.00	0.000
C16:1(n-9)cis cis-5-Hexadecenoic acid	0.21 $\pm$ 0.00	0.15 $\pm$ 0.00	0.000
C17:0 ante-iso 14-Methylhexadecanoic	0.54 $\pm$ 0.00	0.55 $\pm$ 0.00	N/D
C17:0 Heptadecanoic acid	0.53 $\pm$ 0.00	0.78 $\pm$ 0.00	0.000
C17:0 iso 15-Methylpalmitic acid	0.46 $\pm$ 0.00	0.43 $\pm$ 0.02	0.094
C18:0 ante-iso 15-Methylheptadecanoic	0.23 $\pm$ 0.00	0.30 $\pm$ 0.00	0.000
C18:0 Stearic acid	9.45 $\pm$ 0.02	7.76 $\pm$ 0.01	0.000
C18:1(n-11)trans trans-7-Octadecenoic acid	0.13 $\pm$ 0.01	0.10 $\pm$ 0.01	0.029
C18:1(n-6)cis cis-12-Octadecenoic acid	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	1.000
C18:1(n-6)trans trans-12-Octadecenoic acid	0.20 $\pm$ 0.00	0.16 $\pm$ 0.01	0.003
C18:1(n-7)cis cis-Vaccenic acid	0.32 $\pm$ 0.00	0.31 $\pm$ 0.00	0.016
C18:1(n-7)trans trans-Vaccenic acid	2.78 $\pm$ 0.01	1.32 $\pm$ 0.00	0.000
C18:1(n-9)cis Oleic acid	20.06 $\pm$ 0.09	17.69 $\pm$ 0.01	0.000

C18:1(n-9)trans Elaidic acid	0.27 ± 0.01	0.24 ± 0.01	0.034
C18:2(n-6)cis Linoleic acid	0.99 ± 0.00	1.08 ± 0.00	0.000
C18:2conj Total Conjugated Linoleic acid(CLA)	1.53 ± 0.05	0.69 ± 0.00	0.000
C18:3(n-3)cis Alpha-Linolenic acid (ALA)	0.62 ± 0.02	0.36 ± 0.00	0.000
C20:0 Arachidic acid	0.14 ± 0.00	0.16 ± 0.00	0.002
C20:1(n-11) Gadoleic acid	0.16 ± 0.02	0.16 ± 0.00	0.795
C20:4(n-6)cis Arachidonic acid	0.10 ± 0.00	0.10 ± 0.00	0.230
C22:2(n-6)cis Docosadienoic acid	0.09 ± 0.01	0.10 ± 0.01	0.435
C22:5(n-3)cis Docosapentaenoic (DPA)	0.10 ± 0.00	0.11 ± 0.00	0.000
Monounsaturated fatty acids	24.54 ± 0.09	22.78 ± 0.01	0.000
Polyunsaturated fatty acids	2.25 ± 0.07	1.97 ± 0.00	0.005
Saturated fatty acids	65.12 ± 0.15	71.06 ± 0.03	0.000
Total Omega-3 fatty acids	0.95 ± 0.05	0.59 ± 0.01	0.000
Total Omega-6 fatty acids	1.30 ± 0.00	1.40 ± 0.00	0.000
Total Omega-9 fatty acids	20.30 ± 0.08	17.90 ± 0.00	0.000
Omega-3 : Omega-6 Ratio	0.73 ± 0.03	0.43 ± 0.01	0.000
Total Trans Fatty Acids	3.41 ± 0.00	1.81 ± 0.02	0.000
Unidentified fatty acids	3.14 ± 0.1	1.69 ± 0.04	0.000

Figures shown are mean ± standard deviation of triplicate analysis as a % of total fatty acids.

N/D: Fatty acids which had no standard deviation, therefore statistical analysis could not be carried out and a p-value was not determined

3.3.2 Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) analysis was carried out to assess if the six different temperature treatments impacted the melting profile of butter and especially the final melt temperature of the butter which can have a significant impact on butter functionality in application. When analysing the data, each thermogram was firstly observed for endothermic peaks, which signified a period of melting of fatty acids as temperature increased. When these peaks had been identified, they were then analysed in isolation, with three parameters being focused on within each peak – E_{on} , E_{off} and area. Figure 3.4 shows how this analysis was carried out for MLS -20/-20 for explanatory purposes only. E_{on} is defined as the lower limit of each peak (temperature at which the endothermic peak begins), E_{off} is defined as the upper limit of each peak (temperature at which the endothermic peak ends) and area is defined as the total area of the endothermic peak. The E_{off} value of the final peak in each thermogram was of particular significance when analysing the data in this study, as it signified the temperature at which the butter had melted. Some thermograms produced one peak while others produced more.

While DSC melt profiles were found to be broadly similar for most of the treatments, in terms of the range of temperatures within which endothermic peaks occurred, there were some small variations found in relation to the overall shapes of the curves, especially for treatments MLS and LS -20/+5. These variations will be discussed in greater detail in the following sections.

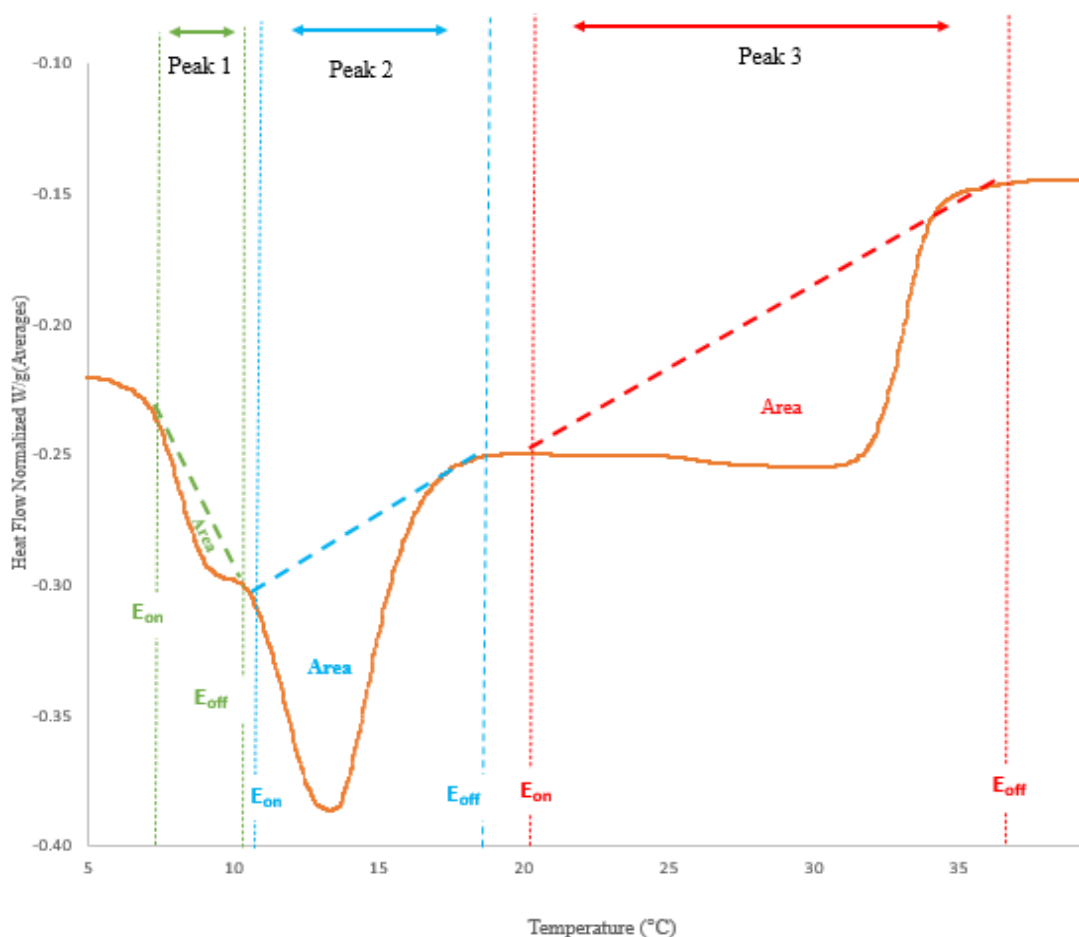


Figure 3.4. Differential scanning calorimetry (DSC) curve obtained for MLS -20/-20 trial (Final melt step) showing how analysis of thermograms was carried out. Markings are shown for explanatory purposes to outline where the lower and upper temperature limits for the peaks were identified (E_{on} and E_{off} respectively). The areas calculated are also outlined for each graph.

Figures 3.5 and 3.6 show the DSC curves obtained for the final melt step between 5°C and 50°C for the MLS and LS trials, respectively. In all curves, the main area of analysis was between approximately 7.5 and 37.5°C, as this is where peaks associated with fatty acid melting were identified. Overall trends and results were broadly in line with previous studies for the E_{off} values (melting complete temperature) such as that of O’Callaghan *et al.*, (2016). In their study, it was found that final melt temperature was $35.27 \pm 0.48^\circ\text{C}$. Given the nature of butter, and its high sensitivity to temperature, some variation is expected, but final melt temperature were in the same area of the thermograms as those seen previously (O’Callaghan *et al.*, 2016).

Results for melting completion temperatures are also in line with values obtained by Timlin *et al.*(2024), Tomaszewska-Gras. (2013), and Lokuge *et al.* (2024) and who found this temperature to be at 34.83°C. This study also found that the first E_{on} was at approximately 3-5°C, which again is broadly in line with the results found for this parameter in the current study.

Melting complete temperatures were overall higher in the LS trial than in the MLS trial – most likely due to increased quantities of saturated fatty acids, leading to higher melting temperatures.

It is evident in the MLS curves that while all other treatments displayed similar curves, albeit with some minor differences, treatment MLS -20/+5 yielded a significantly different shaped curve and associated endothermic peaks. Similarly, for the LS trial, all treatments yielded broadly similarly shaped curves, with the exception of the LS -20/+5 curve which was inherently different.

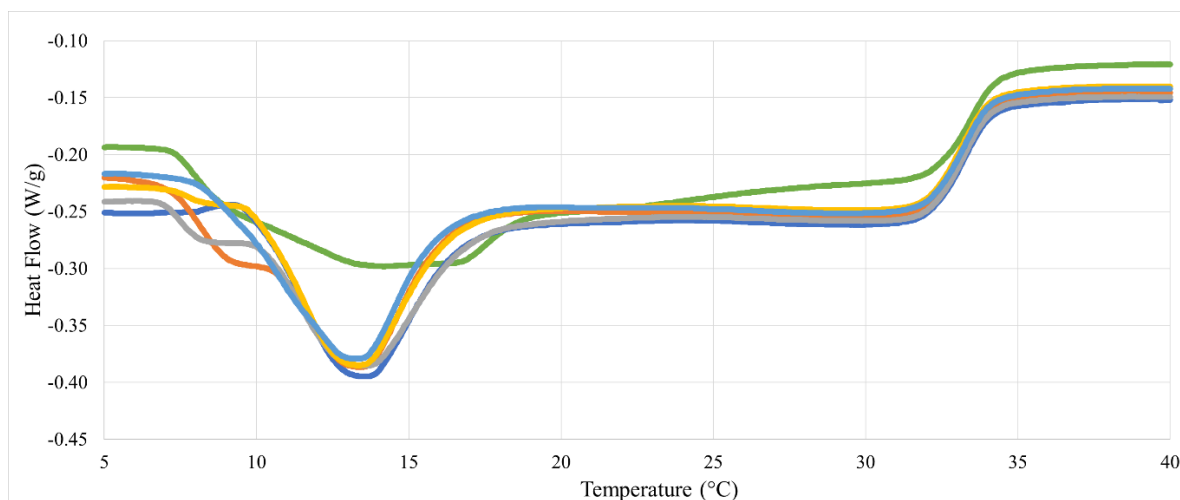


Figure 3.5. Differential scanning calorimetry thermograms obtained for the Mid-Late season (MLS) trial. Figures shown are averages for the final melt step following temperature treatment. - MLS -30/-30, - MLS -20/-20, - MLS -30/-20, - MLS -20/-30, - MLS +5/-20, - MLS -20/+5

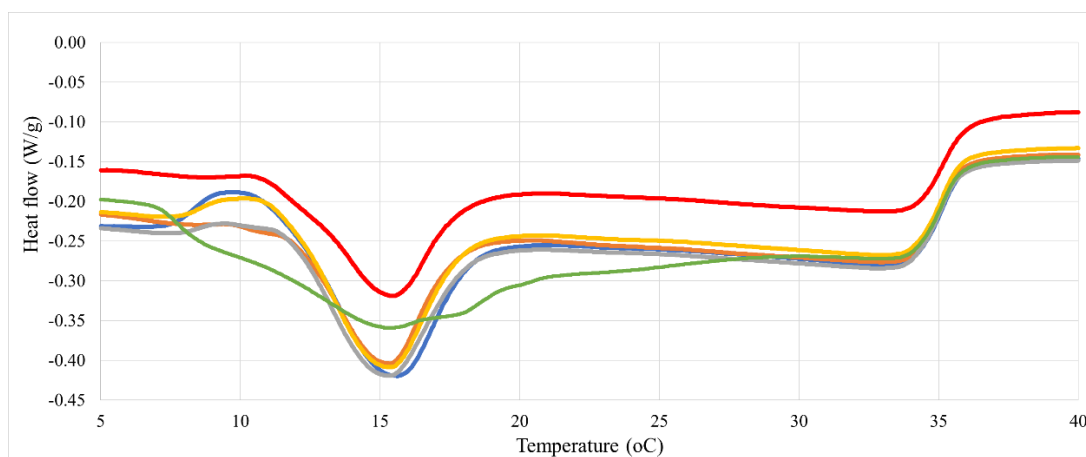


Figure 3.6. Differential scanning calorimetry thermograms obtained for the Late season (LS) trial. Figures shown are averages for the final melt step following temperature treatment. - LS -30/-30, - LS -20/-20, - LS -30/-20, - LS -20/-30, - LS +5/-20, - LS -20/+5

Analysis of each individual replicate within each treatment was carried out to calculate standard deviation. For treatment MLS -30/-30, two endothermic peaks of areas $0.59\text{W/g} \cdot ^\circ\text{C} \pm 0.01$ and $0.49\text{W/g} \cdot ^\circ\text{C} \pm 0.04$ were observed between $9.30^\circ\text{C} \pm 0.10$ and $18.42^\circ\text{C} \pm 0.56$ and $21.54^\circ\text{C} \pm 0.73$ and $35.25^\circ\text{C} \pm 0.41$, respectively.

Two endothermic peaks of varying areas and left and right limits were also found for MLS -20/-30 and MLS +5/-20 while MLS -20/+5 only showed one larger endothermic peak between $6.53^\circ\text{C} \pm 0.31$ and $36.94^\circ\text{C} \pm 1.07$ respectively. Results are summarized in Table 3.4.

For MLS -20/-20, two main endothermic peaks were observed, however, a smaller endothermic peak was also observed between approximately $7-9.9^\circ\text{C}$. The main two peaks were found to be $0.34\text{W/g} \cdot ^\circ\text{C} \pm 0.01$ and $0.61\text{W/g} \cdot ^\circ\text{C} \pm 0.03$ in area and were observed between $10.30^\circ\text{C} \pm 0.12$ and $18.48^\circ\text{C} \pm 0.24$ and $19.43^\circ\text{C} \pm 0.43$ and $35.41^\circ\text{C} \pm 0.43$ respectively while the smaller peak was visible between $7.05^\circ\text{C} \pm 0.36$ and $9.91^\circ\text{C} \pm 0.10$ with an area of $0.02\text{W/g} \cdot ^\circ\text{C}$. MLS -30/-20 was also found to have this smaller peak before two larger peaks.

The trends between all six treatments within the LS trial were similar to the MLS trial. For treatment LS -30/-30, two endothermic peaks of areas $0.82\text{W/g.}^{\circ}\text{C} \pm 0.05$ and $0.82\text{W/g.}^{\circ}\text{C} \pm 0.06$ were observed between $10.23^{\circ}\text{C} \pm 0.09$ and $19.73^{\circ}\text{C} \pm 0.24$ and $20.73^{\circ}\text{C} \pm 0.31$ and $37.03^{\circ}\text{C} \pm 0.49$, respectively.

Two endothermic peaks of varying areas and left and right limits were also found for the remainder of the LS treatments. Results are summarized in Table 3.4. Of note is that for both the MLS and LS trials, the -20/+5 treatment produced results which were in contrast with the other treatments in terms of the overall trend of the graph. Peaks were much less distinguishable, while the E_{on} for the first peak in both were lower than the other treatments. This may have been impacted by the fact that it was the only treatment which had a temperature of above 0°C directly prior to ramping to -15°C and melting out of the sample. For the remaining treatments, there were some small inter treatment differences observed also, both in terms of enthalpy of melting and also onsets of melting. Further work is required to assess if these results are true differences across a number of different samples or simply due to the nature of the butter.

There are indications from the study that storage treatments may have a modest impact on melt properties, however; without statistical analysis across a number of different independent studies, it cannot be stated if these are true statistically significant differences or not. Also, if it was confirmed that small differences are induced by post-production storage of butter, application work would need to be carried out to assess if these differences were tangible in practice or not.

Table 3.4. Summary of differential scanning calorimetry results for the Mid-late season (MLS) trial and the Late-season (LS) trials showing onset temperature (E_{on}), offset temperature (E_{off}) and area of each endothermic peak observed.

Temperature Treatment	Peak 1			Peak 2			Peak 3		
	Mean $\pm$ SD			Mean $\pm$ SD			Mean $\pm$ SD		
	Area (W/g.°C)	E_{on} (°C)	E_{off} (°C)	Area 2 (W/g.°C)	E_{on} (°C)	E_{off} (°C)	Area 3 (W/g.°C)	E_{on} (°C)	E_{off} (°C)
MLS -30/-30 °C	0.59 $\pm$ 0.01	9.30 $\pm$ 0.10	18.42 $\pm$ 0.56	0.49 $\pm$ 0.04	21.54 $\pm$ 0.73	35.25 $\pm$ 0.41	N/A	N/A	N/A
MLS -20/-20 °C	0.02 $\pm$ 0.00	7.05 $\pm$ 0.36	9.91 $\pm$ 0.10	0.34 $\pm$ 0.01	10.30 $\pm$ 0.12	18.48 $\pm$ 0.24	0.61 $\pm$ 0.03	19.43 $\pm$ 0.43	35.41 $\pm$ 0.43
MLS -30/-20 °C	0.01 $\pm$ 0.00	6.68 $\pm$ 0.13	9.23 $\pm$ 0.12	0.47 $\pm$ 0.02	9.69 $\pm$ 0.14	19.03 $\pm$ 0.49	0.49 $\pm$ 0.02	21.69 $\pm$ 0.52	34.86 $\pm$ 0.25
MLS -20/-30 °C	0.56 $\pm$ 0.02	9.37 $\pm$ 0.19	18.94 $\pm$ 0.67	0.51 $\pm$ 0.05	21.17 $\pm$ 1.08	35.04 $\pm$ 0.09	N/A	N/A	N/A
MLS +5/-20 °C	0.65 $\pm$ 0.02	7.63 $\pm$ 0.25	17.91 $\pm$ 0.46	0.65 $\pm$ 0.02	18.45 $\pm$ 0.46	35.05 $\pm$ 0.24	N/A	N/A	N/A
MLS -20/+5 °C	2.33 $\pm$ 0.35	6.53 $\pm$ 0.31	36.94 $\pm$ 1.07	N/A	N/A	N/A	N/A	N/A	N/A
LS -30/-30°C	0.82 $\pm$ 0.05	10.23 $\pm$ 0.09	19.73 $\pm$ 0.24	0.82 $\pm$ 0.06	20.73 $\pm$ 0.31	37.03 $\pm$ 0.49	N/A	N/A	N/A
LS -20/-20°C	0.59 $\pm$ 0.04	10.18 $\pm$ 0.29	19.50 $\pm$ 0.18	0.87 $\pm$ 0.04	20.50 $\pm$ 0.20	37.19 $\pm$ 0.39	N/A	N/A	N/A
LS -30/-20°C	0.66 $\pm$ 0.03	10.31 $\pm$ 0.20	19.39 $\pm$ 0.26	0.83 $\pm$ 0.04	20.50 $\pm$ 0.48	37.18 $\pm$ 0.40	N/A	N/A	N/A
LS -20/-30°C	0.75 $\pm$ 0.04	10.29 $\pm$ 0.39	19.81 $\pm$ 0.33	0.84 $\pm$ 0.05	20.50 $\pm$ 0.35	37.13 $\pm$ 0.37	N/A	N/A	N/A
LS +5/-20°C	0.55 $\pm$ 0.05	10.61 $\pm$ 0.40	19.82 $\pm$ 0.66	0.72 $\pm$ 0.07	21.43 $\pm$ 0.73	38.39 $\pm$ 0.45	N/A	N/A	N/A
LS -20/+5°C	1.32 $\pm$ 0.05	6.32 $\pm$ 0.26	29.10 $\pm$ 0.52	0.21 $\pm$ 0.01	30.05 $\pm$ 0.13	36.50 $\pm$ 0.27	N/A	N/A	N/A

N/A: Not applicable, as no second or third peak observed

3.3.3 Texture analysis

To assess the textural properties of the butter, a double compression TPA cycle was carried out on cubed butter, prepared as explained in section. 3.2.3.3. Two areas of results analysis focused on the work of compression (area under the first compression curve) and the peak force reached throughout the first compression. The texture profiles are shown in Figures 3.7 and 3.8. Results are shown in Table 3.6 detailing the peak force (g) and work of compression (g.s) reached for both the MLS and LS trials. For both parameters, when the standard deviation of repeat samples was taken into consideration, while numerical differences were observed, it was found that no

significant difference was present between all 6 treatments both with respect to the hardness (Peak force of compression 1) and the work of compression (Area under the curve of compression 1).

While there were no intra study differences identified, inter study differences between the MLS and LS trials were present, which was to be expected (Paduret, 2021). Peak forces reached for the MLS trials ranged between 5147.12 ± 647.70 g and 6165.82 ± 502.73 g, while peak forces reached in the LS trial ranged from 7280.12 ± 550.10 g to 8013.27 ± 490.61 g. As expected, this trend was also seen in the work of compression, with obtained values ranging from 43302.41 ± 4043.13 g.s and 50270.58 ± 2773.72 g.s for the MLS trial while they were between 63089.60 ± 3808.4 g.s and 70258.73 ± 4181.29 g.s for the LS trial. These inter study trends can be attributed to the increased level of saturated fatty acids and decreased levels of unsaturated fatty acids such as C18:1 in the LS trial owing from changes in diet and stage of lactation. The impact of increased quantities of saturated fatty acids is known to induce higher butter hardness (O'Callaghan *et al.*, 2016), which too was observed in the texture analysis of this study.

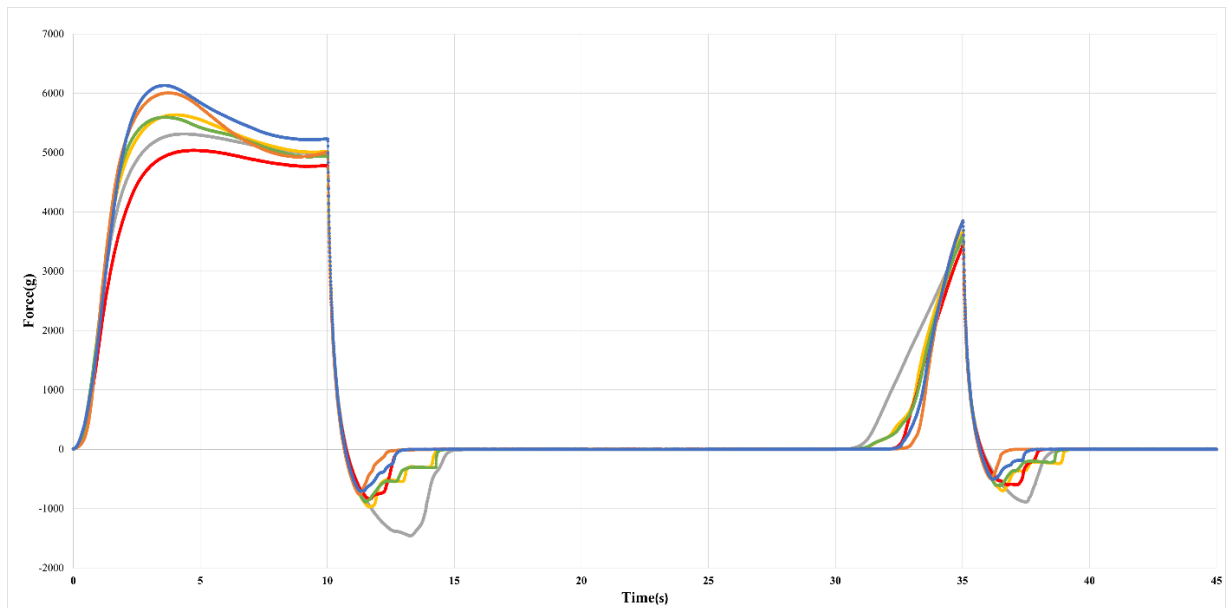


Figure 3.7. Texture profile analysis (TPA) of Mid-Late season(MLS) trial. Figures shown are averages. - MLS -30/-30, - MLS -20/-20, - MLS -30/-20, - MLS -20/-30, - MLS +5/-20, -MLS -20/+5

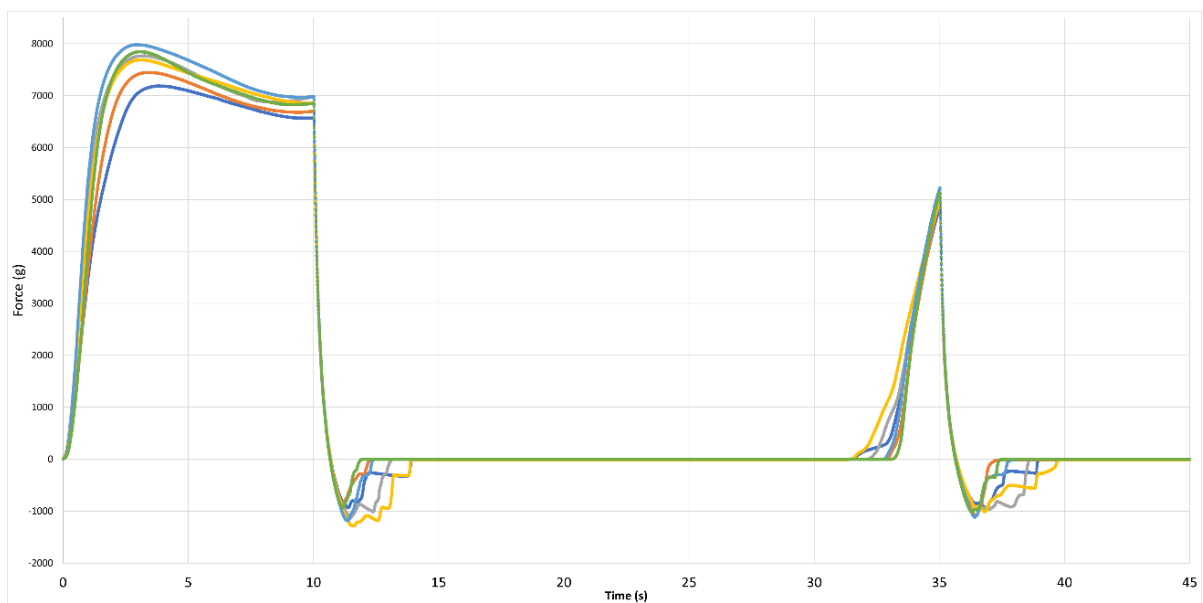


Figure 3.8. Texture profile analysis (TPA) of Late season (LS) Trial. Figures shown are averages - LS -30/-30, - LS -20/-20, - LS -30/-20, - LS -20/-30, - LS +5/-20, - LS -20/+5

It has previously been shown by Timlin *et al.* (2024) that both diet and stage of lactation can impact butter hardness, albeit a different texture analysis method and geometry were used. In their study, both factors were studied in isolation. Mid lactation butter hardness at refrigeration temperature was found to be 21.22 ± 4.40 N, and late lactation butter was found to be 19.07 ± 2.26 N. Butter from cows fed a predominantly grass based diet throughout a season was found to have an average hardness value of 16.74 ± 3.50 N, while butter from cows fed a predominantly total mixed ration diet had an average hardness of 21.50 ± 3.24 N. This shows that the interstudy results seen in the current study are expected. Similarly, in a separate study by Lokuge *et al.* (2024), again using a different method and geometry, butter hardness refrigeration temperature has been shown to be broadly the same as those referenced by Timlin *et al.* (2024)

Table 3.4. Work of compression (g.s) and Peak force (g) for all treatments in Mid-Late season (MLS) and Late season (LS) trials.

Treatment	Work of compression (g.s)	Peak Force (g)
	Mean $\pm$ Standard deviation	
MLS -30/-30 °C	46200 $\pm$ 6330	5360 $\pm$ 731
MLS -20/-20 °C	47700 $\pm$ 2430	5640 $\pm$ 266
MLS -30/-20 °C	43300 $\pm$ 4040	5150 $\pm$ 648
MLS -20/-30 °C	47500 $\pm$ 4460	5650 $\pm$ 520
MLS +5/-20°C	48800 $\pm$ 4090	6030 $\pm$ 618
MLS -20/+5°C	50300 $\pm$ 2770	6170 $\pm$ 503
LS -30/-30°C	63100 $\pm$ 3810	7280 $\pm$ 550
LS -20/-20°C	64600 $\pm$ 3320	7500 $\pm$ 425
LS -30/-20°C	68400 $\pm$ 4600	7790 $\pm$ 544
LS -20/-30°C	69200 $\pm$ 5880	7960 $\pm$ 601
LS +5/-20°C	70300 $\pm$ 4180	8010 $\pm$ 491
LS -20/+5°C	67400 $\pm$ 5100	7880 $\pm$ 596

3.3.4 Rheological analysis

Rheological analysis was carried out to assess the melt profile of the various butter samples on heating from 4 to 40°C at 5°C/min. Profiles for each of the six treatments in the two separate trials (MLS and LS), can be seen in Figures 3.9 and 3.10 respectively. The profile of the plots and overall trends were similar across all samples, however, to draw detailed conclusions from the ramps, raw data was analysed to achieve the key objective of carrying out this analysis - to assess at which point in the melting ramp, the samples would become more liquid than solid. To ascertain the point at which this occurred, it was defined as the first temperature at which the loss modulus value (G'') was higher than the Storage modulus value (G'), indicating the sample was more liquid than solid.

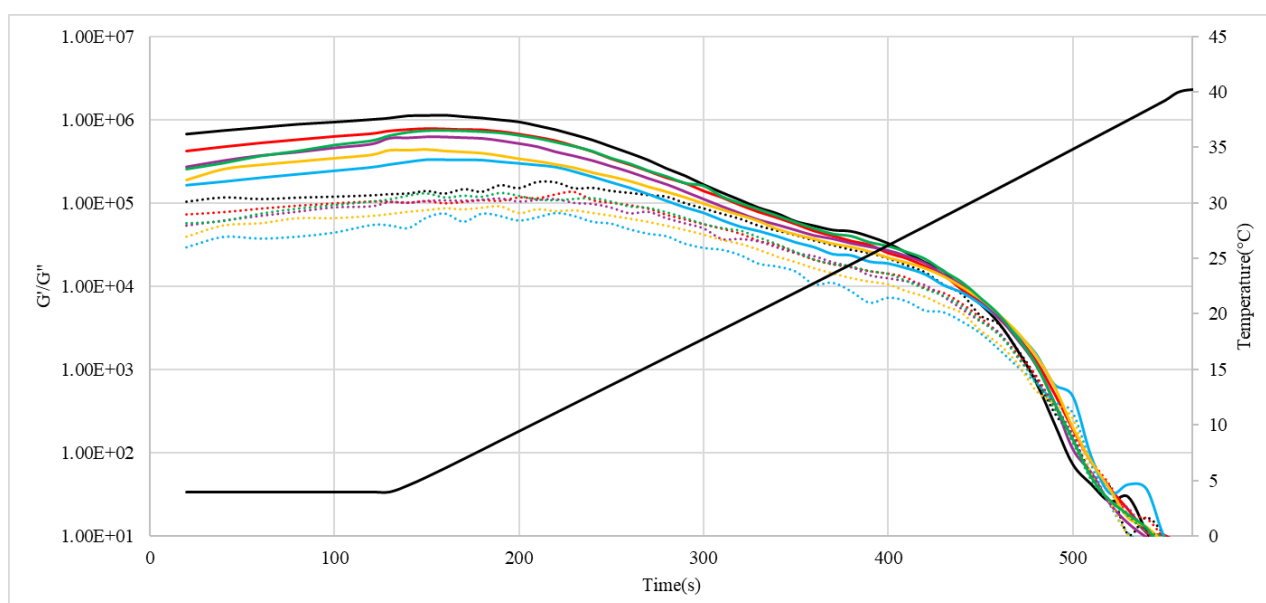


Figure 3.9. Butter melt curve for Mid-Late season (MLS) trial. Figures shown are averages with temperature (-) shown on the right y axis. G' profiles are denoted by a continuous line, G'' profiles are denoted by dotted lines. - MLS -30/-30, - MLS -20/-20, - MLS -30/-20, - MLS -20/-30, - MLS +5/-20, - MLS -20/+5

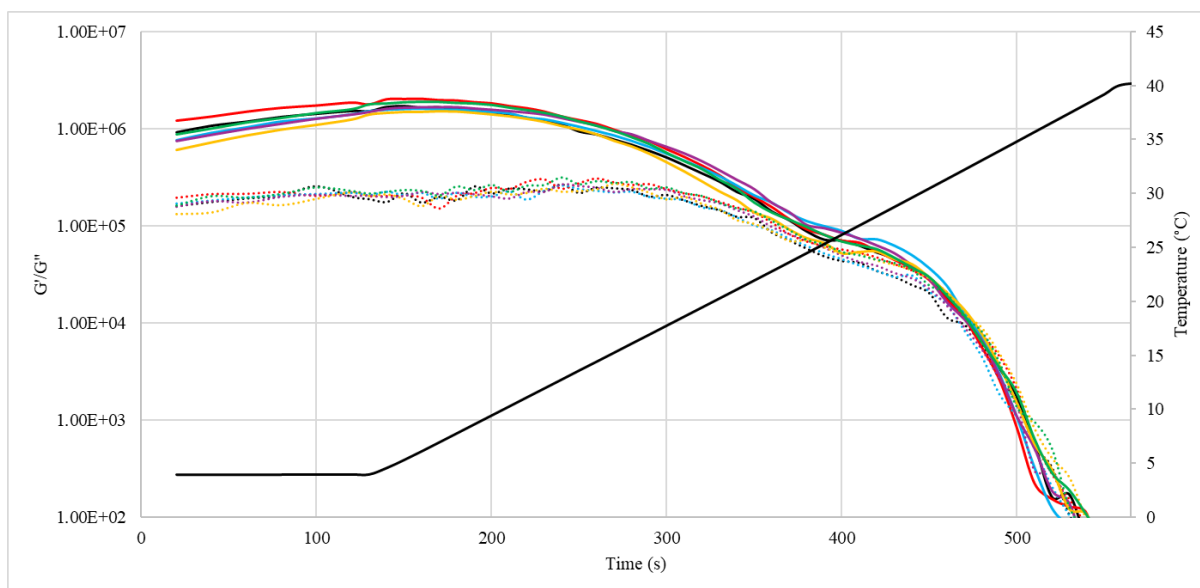


Figure 3.10. Butter melt curve for Late season (LS) trial. Figures shown are averages with temperature (-) shown on the right y axis. G' profiles are denoted by a continuous line, G'' profiles are denoted by dotted lines. - LS -30/-30, - LS -20/-20, - LS -30/-20, - LS -20/-30, - LS +5/-20, - LS -20/+5.

It was expected that the crossover temperatures would coincide with DSC values of melting completion, and they broadly did so in the sense that melting completion was typically observed between 30 and 40°C for DSC thermograms, which coincided with the range of temperatures where G'' was higher than G' for rheometer plots.

These temperatures for MLS and LS trials can be seen in Figures 3.11 and 3.12, respectively. From these results, of note is the high standard deviation in both trials for the -30/-30 treatment, which received the harshest temperature treatment for the longest length of time. For both trials, the standard deviations overlap for each treatment when compared with the others, with the only exception of this being between LS -30/-20 and LS -20/-20. In this instance, while error bars do not overlap, there is significant error observed within treatments and more work would need to be carried out to assess if this is a statistically significant difference. No other instance of this method is documented in the literature to the authors' knowledge, however, melt temperatures of butter have been ascertained previously through other methods such as nuclear

magnetic resonance (NMR), where solid fat content of butter is measured, and DSC. Lokuge *et al.* (2024) found the solid fat content of butter to be close to 0% at 35°C and the same at 40°C, signalling complete melting of the product. These temperatures are also in line with melting completion temperatures extrapolated from DSC thermograms in previous studies (Tomaszewska-Gras, 2013; O’Callaghan *et al.*, 2016; Lokuge *et al.*, 2024). In terms of differences between the overall trends of two different trials (MLS and LS), crossover points were higher for 4 of the treatments and lower for the remaining treatments in the MLS trial when compared with the LS trial, however, when standard deviation was taken into account, this effect was largely negated. When taking all factors into account, while there are some numerical differences between treatments within each individual trial, it cannot be concluded that post-production storage conditions significantly impact the rheological melt properties of butter. A wider ranging study would need to be carried out to assess whether or not this is due to the nature of the product and high standard deviations as a result. Butter is an unstable product when speaking in the context of temperature exposure and it is possible that this is the reason behind the high degree of standard deviation.

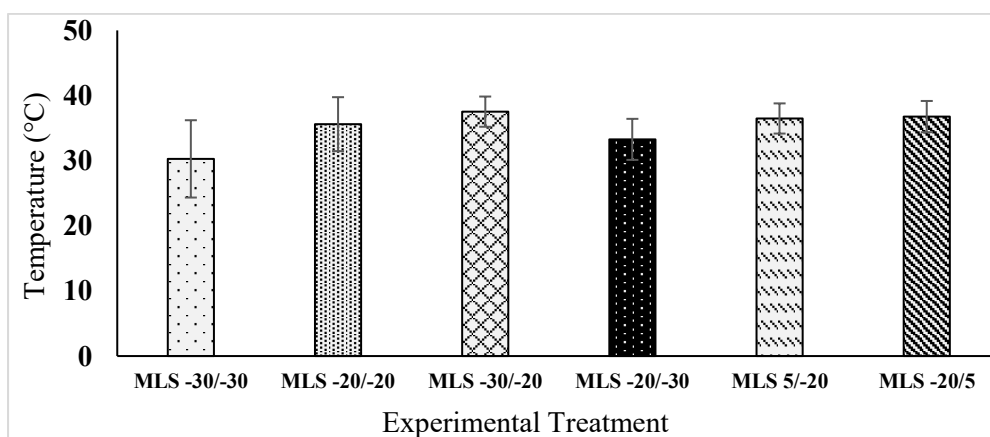


Figure 3.11. Temperature at which the first crossover point of storage modulus (G') and loss modulus (G'') occurs in the Mid-late season (MLS) trials. Values shown are mean $\pm$ standard deviation.

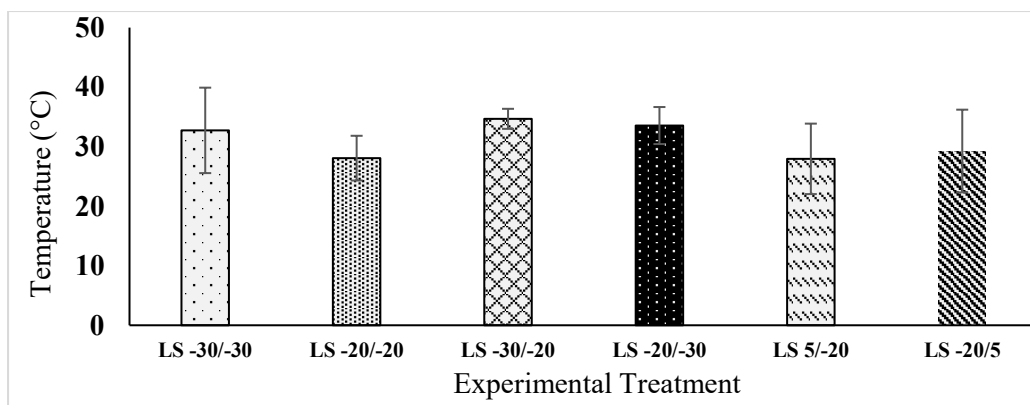


Figure 3.12. Temperature at which the first crossover point of storage modulus (G') and loss modulus (G'') occurs in Late season (LS) trials. Values shown are mean $\pm$ standard deviation.

3.4. Conclusion

While there were numerical differences in the texture, melt and rheological properties of butter made using mid-late and late season trials, changing the post-production storage treatment applied to butter has been shown to not significantly alter these properties. This is important new information to have established in supporting future studies on targeted modification of the key quality attributes of butter.

3.5. References

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Chapter 4

Assessing the impact of seasonality on the techno-functional properties of Irish butter

Órla Dinneen^{1,2}, Anna G. Behan², James A. O'Mahony¹, Tom F. O'Callaghan¹

¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*

²*Kerry Dairy Ireland, Princes Street, Tralee, Co. Kerry, Ireland*

Declaration

This chapter was written by author Órla Dinneen (Ó.D.) and reviewed by all co-authors. (Ó.D.) co-designed the study with co-authors and performed all of the experimental work.

Abstract

The primary aim of this study was to investigate the effects of seasonality on the fatty acid profile and techno-functional properties of Irish butter. These properties included texture, melt and colour. The second aim was to understand if correlations existed between any of these properties. This study was carried out across three seasons in 2024 - Spring (March, April and May), Summer (June, July and August), and Autumn (September, October and November). A total of 28 samples, across 26 weeks from this period were analysed individually, for a variety of compositional and physicochemical parameters. These included colour analysis, texture analysis, fatty acid profile analysis and thermal melt property analysis using differential scanning calorimetry (DSC). Colour analysis was carried out to assess L^* , a^* and b^* values using colorimetry; texture analysis was performed using two different methods – spreadability and texture profile analysis (TPA); parameters such as crystallisation peak temperatures, completion of melting temperatures and enthalpies of crystallisation were quantified using DSC. Significant differences between seasons were observed in the results of all four of these analytical approaches, while correlations existed between a number of the parameters. Of the 38 fatty acids profiled in this study, only 8 showed no statistically significant inter-seasonal differences. Using colorimetry, it was concluded that while L^* values remained constant throughout the year, a^* and b^* values showed significant differences at certain times. Many thermal melt properties showed significant differences also, such as crystallisation onset temperature, crystallisation peak temperatures and temperatures at which melting had completed, while enthalpy of crystallisation showed no significant changes across the year. TPA analysis showed Autumn values to be significantly different to the other two seasons, while spreadability values at room and refrigeration temperatures remained steady throughout.

4.1. Introduction

Butter is an important and valuable commodity to the Irish dairy industry. Exports of butter from Ireland increased to €1.7 billion in 2024, marking a growth of €400 million on the previous year. Of the €6.3 billion in dairy exports from Ireland in 2024, butter accounted for 26% on a value basis, highlighting the value of butter to the sector (Bord Bia, 2025). This is in part due to the increased value per tonne of butter seen over the last twelve months, but also due to increasing demand from Europe and North America (Bord Bia, 2025). Irish butter is renowned worldwide for traits such as higher spreadability, lower quantities of saturated fats and increased yellow colour when compared with butter produced in other countries. This distinctive yellow colour, for example, has been shown to be favourable with both Irish and German consumers when compared with butter produced from cows fed a total mixed ration (TMR) diet (Garvey *et al.*, 2020). The presence of these favourable attributes is one of the factors underpinning the strong record of butter production observed in Ireland in recent years and may be explained by feed and calving pattern choices. These two factors combined give rise to the seasonal effect experienced in Ireland.

Seasonal calving patterns exist in countries with temperate climates such as New Zealand and Ireland, in an effort to maximise the use of grass as a primary feed source. This, in turn, results in seasonal changes to both milk production, milk composition and quality. Seasonal dairy systems are however far less common across mainland Europe and many other milk producing countries, which typically adopt a continuous, year-round calving system. In such systems, farming practices are focused on indoor feeding of cows, with concentrates, silage, and supplemental ration. In these systems, given that cows tend to calve all year around, the seasonality factor is negligible due to consistent feeding practices, and a balance of cows at different stages of lactation existing in a herd throughout the year. This in turn results in more

chemically consistent milk and milk products throughout the year, which can make processing much more streamlined, less varied, and overall, less complex.

Diet and stage of lactation have been shown to significantly impact milk chemistry (Hayes *et al.*, 2023). Against this backdrop, countries like Ireland face the multifaceted factor of seasonality, which occurs due to the effects of both of these factors combined. In order to combat the intricacies involved with this type of system, it is imperative to understand how the raw material varies across the season, and the impact this has on the products produced, in this instance, butter.

The chemical composition of milk has been shown to be significantly impacted by seasonality, both in pasture-based systems and non-pasture-based systems (Gulati *et al.*, 2018; Li, Ye and Singh, 2019; Hayes *et al.*, 2023). Studies have also been conducted which directly assess the impact of seasonality on butter in Spring calving systems (Timlin *et al.*, 2024). These studies have largely tended to focus on trial herds, in a very controlled environment and on small-scale butter production methods. The current study differs significantly from these in that it aims to assess if the trends seen at small scale milk collection and butter production, transfer to large scale production, within one specific dairy processor region. This is important because, within the catchment area of any given processors milk pool, quite a lot of variation can exist in terms of farming practices, days on grass, Spring calving commencement dates, intensity of calving blocks and weather. Seasonality encapsulates all of these factors to culminate in continuous changes to the chemistry of the milk arriving at the factory gate, and in this study, it was important to assess, if the effects of seasonal changes observed previously would be diluted in such a large-scale production or remain evident.

4.2. Materials and methods

4.2.1. Butter collection

A standard unsalted lactic butter product with a fat content of 82% and a moisture content of no greater than 16% was used to perform this study. The butter was produced and supplied by Kerry Ingredients, Islandmacloughry, Tralee Road, Listowel, Co. Kerry, Ireland, using a Simon Frères butter churn (Simon Frères, Cherbourg-en-Contentin, France). All butter samples were produced using a typical production procedure, which is proprietary and confidential to the manufacturer. Twenty-eight samples were taken from the churn across twenty-six weeks over the course of three seasons between the 1st of March and the 13th of November 2024. The seasons were defined as: Spring (March, April, May), Summer (June, July, August) and Autumn (September, October, November). Samples were taken manually from the churn, placed into 454 g containers, and refrigerated at $5 \pm 3^{\circ}\text{C}$ post-production to allow setting for 10 ± 5 d. Samples were then transferred to a freezer which was set to -20°C , until approximately 10 d prior to analysis, to preserve the product, and to closely simulate the typical commercial supply chain temperature profile used by the company. At this point, samples were moved to refrigerated (4°C) conditions, to thaw and equilibrate in the days prior to analysis.

4.2.2 Experimental design

A total of twenty-eight samples from twenty-six different weeks across 2024 were used in this study. Ten of these samples were from Spring months, eleven of these samples were from Summer months and seven of these samples were from Autumn months. A detailed list of sample dates, weeks and seasons can be found in Appendix 4.2. Texture profile analysis and colorimetry was carried out in triplicate on each sample, Differential scanning calorimetry and spreadability tests were carried out in duplicate, while fatty acid profile analysis was carried out in singlicate on all samples.

4.2.3. Analytical methods

4.2.3.1. Fatty acid profile

Analysis of fatty acids was performed using a gas chromatography, with flame ionisation detection (GC-FID) method based on BS EN ISO 12966. Samples were saponified and methylated with the resulting fatty acid methyl esters extracted, and their profile analysed via gas chromatography, with an internal standard used for identification and quantification. The analysis was conducted by an external analytical service provider (Eurofins Food Testing Ireland Limited, Finglas, Co. Dublin, Ireland). Analysis of each sample was performed in singlicate.

4.2.3.2. Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was carried out on butter from refrigerated (4°C) storage. Analysis was carried out using a TA DSC 2500 calorimeter (TA Instruments, Delaware, United States). The pan weight and sample weight were initially recorded on the software. An empty sealed aluminium pan of known weight was included in all analysis performed and was placed in the instrument for each analysis to act as a reference sample. Refrigerated butter (3-8 mg) was weighed into aluminium pans with care taken to not get any butter on the lip of the pan, as this can interfere with sealing, and in turn, the reproducibility of the results. A seal press was used to seal the edges of the pans before loading samples into the DSC instrument. The test method used was similar to that used in the study of O'Callaghan *et al.* (2016). When loaded onto the instrument for analysis, the butter was pre-melted to 60°C, at a rate of 10°C/min, in the DSC instrument to erase crystal memory. The butter was then cooled to -15°C at a rate of 5°C/min to identify crystallisation peaks, before melting to 60°C again at a rate of 2°C/min to identify melting peaks. Analysis of each independent sample was carried out in duplicate. Analysis of DSC thermograms was carried out using TRIOS DSC software.

4.2.3.3. Texture analysis

4.2.3.3.1. Texture Profile Analysis

A Stable Micro System (TA.XTplus) texture analyser (Stable Micro Systems, Surrey, United Kingdom) was used to complete a double compression texture profile analysis (TPA) cycle on all samples, which were prepared as follows: butter samples (25 mm cubed) were cut using a custom-made metal cuber of dimensions 25 mm x 25 mm x 25 mm (W x D x H) (Kerry Ingredients, Listowel County Kerry), with a photograph of this cuber presented in Figure 4.1. The butter block was cored using this cuber, before being pushed out of the metal cuber using a custom-made tool to suit this application (Kerry Ingredients, Listowel, County Kerry, Ireland). Butter was stored at 4°C prior to cutting, following cutting (in aluminium foil) and up to the point of analysis. The butter cubes were taken directly from the fridge and placed on the platform of the texture analyser for testing. A test speed of 1 mm/s was utilised, and the head of the texture analyser was lowered 10 mm into the sample, using a P50 Stable Micro Systems probe. A short recovery time of 25 s between compressions was used as the sample was not expected to recover.



Figure 4.1. Custom-made metal cuber of dimensions 25 mm x 25 mm x 25 mm (W x D x H) (Kerry Ingredients, Listowel County Kerry)

4.2.3.3.2 Spreadability analysis

A Stable Micro System (TA.XTplus) texture analyser (Stable Micro Systems, Surrey, United Kingdom) was used to measure spreadability on all samples in duplicate. A stable micro systems spreadability rig and heavy-duty platform were used to perform this test. Sample preparation is shown in greater detail in Appendix 4.1. Samples were prepared as follows:

Refrigeration temperature analysis:

Butter samples were taken from a 4°C fridge and carefully filled, using a spoon, into empty spreadability conical cups as show in Figure 4.2. Care was taken to avoid as many air pockets as possible, as their presence could impact on results. The prepared samples were then returned to 4°C storage for 1 h prior to analysis.

The samples were taken directly from the fridge and placed on the texture analyser platform for testing. A test speed of 3 mm/s was utilized, and the head of the texture analyser was lowered to 2 mm from the bottom of the sample, using a conical spreadability probe as shown in Figure 4.3.



Figure 4.3. Steps involved in the preparation of samples for spreadability analysis.

Room temperature analysis:

Butter samples were taken from a 4°C fridge and equilibrated at room temperature, for 18 h overnight. The following day, analysis was performed on all samples. The temperature equilibrated butter was carefully filled, using a spoon, into empty spreadability conical cups (see Figure 4.3). Care was taken to avoid as many air pockets as possible, as their presence

could impact on results. The prepared samples were then allowed to rest on the bench at room temperature for a further 10 min prior to analysis.

Samples were then placed immediately on the texture analyser platform for testing. A test speed of 3 mm/s was utilised, and the head of the texture analyser was lowered to 2 mm from the bottom of the sample, using a conical spreadability probe as shown in Figure 4.2.



Figure 4.3. Conical spreadability probe used for spreadability analysis (Stable Micro Systems, 2025).

4.2.3.4. Colour analysis

Colour analysis was carried out in triplicate on each individual sample using a Konica Minolta CM-5 spectrophotometer (Konica Minolta, Chiyoda City, Tokyo, Japan). Samples were placed over the top-facing measuring port on a plastic film, and each sample was analysed at three different points, achieved by moving the sample each time. L*, a* and b* values were reported for each sample where L* measured how light or dark each butter was (0-100, light-dark), a* indicated how green or red each butter was (negative indicated more green while positive indicated more red) and b* values were indicative of how much yellow or blue was present in the butters (negative indicated more blue while positive indicated more yellow) (Paduret, 2021; Edward and Buono, 2022).

4.2.3.5. Statistical data analysis

Statistical analysis and graphical figures were created using R version 4.3.3. Unless otherwise stated in the text, all analysis was carried out in duplicate. The impact of seasonality (Spring, Summer and Autumn) on composition and properties of butter was assed using a one-way ANOVA. Post hoc analysis was carried out using Tukey. $p < 0.05$ was considered statistically significant. Pearsons correlation analysis was carried out using the “dlookr” package and “correlate” function whereby subsequent strength of correlations were characterised as follows: 0.00 - 0.19: Very weak or no correlation, 0.20 - 0.39: Weak correlation, 0.40 - 0.59: Moderate correlation, 0.60 - 0.79: Strong correlation, 0.80 - 1.00: Very strong or perfect correlation.

4.3. Results and discussion

4.3.1. Fatty acid profile

The fatty acid profile of butter was measured in this study to evaluate how it changed as a function of season throughout the year. A total of 38 fatty acids were reported on in this study with only 8 of these showing no significant inter-seasonal differences in concentration (% of total fatty acids). These results are summarised for all fatty acids present in amounts greater than 0.1% of total fatty acids in Table 4.1.

In all three seasons, C16:0 (Palmitic acid) and C18:1 (Oleic acid) were the fatty acids present in the greatest abundance. Of the short chain fatty acids reported on, C4:0 (Butyric acid) concentrations were significantly different between Spring-Summer and Spring-Autumn, while C6:0 (Caproic acid) concentrations varied significantly between Spring-Autumn and Summer-Autumn. C4:0 quantities decreased as the season progressed while C6:0 remained relatively consistent through Spring and Summer before declining in Autumn. These results are broadly in line with the findings from other similar studies (O Callaghan *et al.*, 2016; Li, Ye and Singh, 2022).

The three most abundant medium chain fatty acids (MCFA's) in all three seasons were C8:0 (Caprylic acid), C10:0 (Capric acid) and C12:0 (Lauric acid). These MCFA's all followed a similar trend in that their concentrations were highest in Summer and lower in Spring and Autumn. The concentrations of all 3 fatty acids were significantly higher ($p < 0.05$) in Summer when compared to Spring and Autumn which were lower and had no inter-seasonal difference of statistical relevance. The trend of results for all 3 fatty acids were in line with those found by O'Callaghan *et al.* (2016) while they were mostly in line with the work of Li, Ye and Singh. (2022) with the main difference in trend being that when considering C8:0;- their study found no difference between early and mid-season concentrations (Lie *et al.*, 2022).

In terms of long chain fatty acids, the two most abundant of all fatty acids – C16:0 and C18:1, along with C14:0 (Myristic acid) C18:0 (Stearic acid) are of most relevance given their abundance, considering these four fatty acids alone make up approximately 68% of the total fatty acids present in the butter samples. C14:0 and C18:1 concentrations varied significantly between all 3 seasons.

Table 4.1. Fatty acid content of butter across three seasons (Spring, Summer and Autumn) in 2024. Figures presented are Mean $\pm$ standard deviation as a % of total fatty acids present for those present in amounts greater than 0.1%.

Fatty Acid	Spring	Summer	Autumn	Yearly Average
C4.0.Butyric.acid	3.67 $\pm$ 0.16 ^a	3.43 $\pm$ 0.08 ^b	3.37 $\pm$ 0.08 ^b	3.49 $\pm$ 0.16
C6.0.Caproic.acid	2.32 $\pm$ 0.05 ^a	2.34 $\pm$ 0.06 ^a	2.22 $\pm$ 0.03 ^b	2.29 $\pm$ 0.06
C8.0.Caprylic.acid	1.33 $\pm$ 0.08 ^a	1.43 $\pm$ 0.07 ^b	1.31 $\pm$ 0.01 ^a	1.36 $\pm$ 0.06
C10.0.Capric.acid	2.87 $\pm$ 0.28 ^a	3.26 $\pm$ 0.2 ^b	2.9 $\pm$ 0.06 ^a	3.01 $\pm$ 0.22
C10.1.n.1.cis.cis.9.Decenoic.acid	0.31 $\pm$ 0.05 ^a	0.43 $\pm$ 0.02 ^b	0.43 $\pm$ 0.01 ^b	0.39 $\pm$ 0.07
C12.0.Lauric.acid	3.3 $\pm$ 0.33 ^a	3.9 $\pm$ 0.21 ^b	3.54 $\pm$ 0.1 ^a	3.58 $\pm$ 0.3
C12.1.n.1.cis.cis.11.Dodecenoic.acid	0.08 $\pm$ 0.01 ^a	0.12 $\pm$ 0.01 ^b	0.13 $\pm$ 0 ^c	0.11 $\pm$ 0.03
C13.0.Tridecanoic.acid	0.16 $\pm$ 0.03 ^a	0.22 $\pm$ 0.02 ^b	0.2 $\pm$ 0.01 ^b	0.19 $\pm$ 0.03
C14.0.iso.12.Methyltridecanoic.acid	0.09 $\pm$ 0 ^a	0.11 $\pm$ 0.01 ^b	0.12 $\pm$ 0 ^b	0.11 $\pm$ 0.02
C14.0.Myristic.acid	10.08 $\pm$ 0.44 ^a	11.41 $\pm$ 0.23 ^b	10.92 $\pm$ 0.13 ^c	10.8 $\pm$ 0.67
C14.1.n.5.cis.Myristoleic.Acids	0.74 $\pm$ 0.09 ^a	1.07 $\pm$ 0.09 ^b	1.24 $\pm$ 0.02 ^c	1.02 $\pm$ 0.25
C15.0.ante.iso.12.Methyltetradecanoic.acid	0.53 $\pm$ 0.08 ^a	0.64 $\pm$ 0.02 ^b	0.58 $\pm$ 0.04 ^{ab}	0.58 $\pm$ 0.06
C15.0.iso.13.Methylmyristic.acid	0.28 $\pm$ 0.02 ^a	0.31 $\pm$ 0.01 ^b	0.31 $\pm$ 0.01 ^b	0.3 $\pm$ 0.02
C15.0.Pentadecanoic.acid	1.04 $\pm$ 0.04 ^a	1.23 $\pm$ 0.04 ^b	1.22 $\pm$ 0.02 ^b	1.16 $\pm$ 0.11
C16.0.iso.14.Methylpentadecanoic.acid	0.26 $\pm$ 0.02 ^a	0.28 $\pm$ 0.01 ^b	0.31 $\pm$ 0.02 ^c	0.28 $\pm$ 0.03
C16.0.Palmitic.acid	27.15 $\pm$ 1.2 ^a	28.21 $\pm$ 0.9 ^b	28.41 $\pm$ 0.54 ^b	27.92 $\pm$ 0.68
C16.1.n.7.cis.Palmitoleic.acid	1.55 $\pm$ 0.12 ^a	1.59 $\pm$ 0.14 ^a	1.94 $\pm$ 0.12 ^b	1.69 $\pm$ 0.21
C16.1.n.9.cis.cis.5.Hexadecenoic.acid	0.21 $\pm$ 0.02 ^a	0.21 $\pm$ 0.01 ^a	0.2 $\pm$ 0.01 ^a	0.21 $\pm$ 0.01
C17.0.ante.iso.14.Methylhexadecanoic.acid	0.59 $\pm$ 0.09 ^a	0.54 $\pm$ 0.07 ^{ab}	0.46 $\pm$ 0.08 ^b	0.53 $\pm$ 0.07
C17.0.Heptadecanoic.acid	0.73 $\pm$ 0.06 ^a	0.66 $\pm$ 0.08 ^a	0.65 $\pm$ 0.08 ^a	0.68 $\pm$ 0.04
C17.0.iso.15.Methylpalmitic.acid	0.44 $\pm$ 0.04 ^a	0.48 $\pm$ 0.05 ^{ab}	0.5 $\pm$ 0.06 ^b	0.47 $\pm$ 0.03
C17.1.n.7.cis.cis.10.Heptadecenoic.Acids	0.32 $\pm$ 0.03 ^a	0.24 $\pm$ 0.01 ^b	0.22 $\pm$ 0.01 ^b	0.26 $\pm$ 0.05
C18.0.ante.iso.15.Methylheptadecanoic.acid	0.16 $\pm$ 0.1 ^a	0.16 $\pm$ 0.08 ^a	0.19 $\pm$ 0.07 ^a	0.17 $\pm$ 0.02
C18.0.Stearic.acid	11.04 $\pm$ 0.55 ^a	9.18 $\pm$ 0.49 ^b	9.05 $\pm$ 0.28 ^b	9.76 $\pm$ 1.11
C18.1.n.11.trans.trans.7.Octadecenoic.acid	0.13 $\pm$ 0.03 ^a	0.14 $\pm$ 0.02 ^a	0.14 $\pm$ 0.02 ^a	0.14 $\pm$ 0.01
C18.1.n.6.cis.cis.12.Octadecenoic.acid	0.14 $\pm$ 0.01 ^a	0.12 $\pm$ 0.01 ^b	0.11 $\pm$ 0 ^b	0.12 $\pm$ 0.02
C18.1.n.6.trans.trans.12.Octadecenoic.Acids	0.21 $\pm$ 0.01 ^a	0.21 $\pm$ 0.02 ^a	0.21 $\pm$ 0.02 ^a	0.21 $\pm$ 0
C18.1.n.7.cis.cis.Vaccenic.Acids	0.54 $\pm$ 0.04 ^a	0.4 $\pm$ 0.03 ^b	0.33 $\pm$ 0.01 ^c	0.42 $\pm$ 0.11
C18.1.n.7.trans.trans.Vaccenic.acid	2.4 $\pm$ 0.59 ^a	2.79 $\pm$ 0.09 ^a	2.54 $\pm$ 0.34 ^a	2.58 $\pm$ 0.2
C18.1.n.9.cis.Oleic.acid	20.68 $\pm$ 0.8 ^a	17.85 $\pm$ 0.53 ^b	19.32 $\pm$ 0.31 ^c	19.28 $\pm$ 1.42
C18.1.n.9.trans.Elaidic.acid	0.29 $\pm$ 0.03 ^a	0.24 $\pm$ 0.02 ^b	0.25 $\pm$ 0.02 ^b	0.26 $\pm$ 0.03
C18.2.n.6.cis.Linoleic.acid	1.53 $\pm$ 0.06 ^a	1.29 $\pm$ 0.13 ^b	1.18 $\pm$ 0.03 ^c	1.33 $\pm$ 0.18
C18.2conj.Total.Conjugated.Linoleic.acid..CLA.	0.86 $\pm$ 0.23 ^a	1.23 $\pm$ 0.14 ^b	1.34 $\pm$ 0.2 ^b	1.14 $\pm$ 0.25

C18.3.n.3.cis.Alpha.Linolenic.acid..ALA.	0.58 ± 0.1 ^a	0.61 ± 0.03 ^a	0.6 ± 0.04 ^a	0.6 ± 0.02
C20.0.Arachidic.acid	0.16 ± 0.02 ^a	0.14 ± 0.01 ^b	0.14 ± 0.01 ^b	0.15 ± 0.01
C20.1.n.11..Gadoleic.Acids	0.12 ± 0.01 ^a	0.12 ± 0.01 ^a	0.14 ± 0.01 ^b	0.13 ± 0.01
C22.0.Behenic.acid	0.07 ± 0.02 ^a	0.09 ± 0.03 ^a	0.11 ± 0.03 ^a	0.09 ± 0.02
C22.5.n.3.cis.Docosapentaenoic..DPA.	0.09 ± 0.01 ^a	0.1 ± 0.01 ^b	0.11 ± 0 ^c	0.1 ± 0.01
Monounsaturated.fatty.acids	24.76 ± 0.76 ^a	22.18 ± 0.56 ^b	24.05 ± 0.3 ^a	23.66 ± 1.33
Omega.3...Omega.6.Ratio....	0.47 ± 0.05 ^a	0.58 ± 0.04 ^b	0.63 ± 0.04 ^c	0.56 ± 0.08
Polyunsaturated.fatty.acids	2.62 ± 0.18 ^a	2.47 ± 0.16 ^{ab}	2.38 ± 0.03 ^b	2.49 ± 0.12
Saturated.fatty.acids	66.51 ± 0.72 ^a	68.22 ± 0.79 ^b	66.71 ± 0.86 ^a	67.15 ± 0.93
Total.Omega.3.fatty.acids	0.84 ± 0.12 ^a	0.91 ± 0.03 ^a	0.92 ± 0.04 ^a	0.89 ± 0.04
Total.Omega.6.fatty.acids	1.8 ± 0.08 ^a	1.55 ± 0.14 ^b	1.47 ± 0.05 ^b	1.61 ± 0.17
Total.Omega.9.fatty.acids	20.97 ± 0.8 ^a	18.12 ± 0.53 ^b	19.59 ± 0.29 ^c	19.56 ± 1.43
Total.Trans.Fatty.Acids.	3.04 ± 0.61 ^a	3.4 ± 0.12 ^a	3.15 ± 0.36 ^a	3.2 ± 0.18
Unidentified.fatty.acids	2.21 ± 0.27 ^a	2.5 ± 0.18 ^a	2.38 ± 0.38 ^a	2.36 ± 0.15
Spreadability.C16.C18_1	1.31 ± 0.04 ^a	1.58 ± 0.08 ^b	1.47 ± 0.03 ^c	1.45 ± 0.14

Different letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify statistical difference between seasons (P < 0.05 ANOVA)

Matching letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify non statistical differences between seasons (P > 0.05 ANOVA)

The concentrations of C18:1 were lowest in Autumn, which was anticipated given the expected change in the dairy cow's diet in Ireland as they transition into an indoor setting for the winter months. C18:1 is classified as a fatty acid affected by animal feed (Taylor and MacGibbon, 2011) and its concentration has previously been shown to be higher in the milk of cows whose diet included fresh grass compared to the milk of cows whose diet has an absence of this (Capuano *et al.*, 2014).

Conversely, but also expectedly, concentrations of C16:0 were highest in Autumn butter when, animal feed contained more concentrates. Importantly, while its concentration did differ significantly between Spring and Autumn, it only varied numerically between Summer and Autumn. This may be explained by the fact that C16:0 is one such fatty acid which can be

affected by diet but also can be de-novo synthesised in the mammary gland of the animal – a process independent of the diet (Taylor and MacGibbon, 2011). The Omega 3:Omega 6 ratio, Omega 9 contents and spreadability index (C16:0 : C18:1) varied significantly between all 3 seasons as expected, while the total number of trans fatty acids did not. The extent of variation across all fatty acids reported in this study was expected to induce some techno-functional disparity across seasons in terms of texture, melt and colour, based on previous studies (O’Callaghan *et al.*, 2016; Paduret., 2021; Marangoni *et al.*, 2022; Timlin *et al.*, 2024).

The variability seen in the current study is expected due to the calving pattern and feeding practices employed by the majority of milk suppliers of the dairy processing company involved in the study. Variability in milk composition can be expected to be much higher in systems with a cyclical calving pattern compared to the more globally popular system where cows are indoor fed, and an all year around calving approach is practiced. (Timlin *et al.*, 2021). A high degree of variability was found when considering the fatty acid profiles across the three seasons incorporated in this study – Spring (March to May), Summer (June to August) and Autumn (September to November). Principle Component Analysis (PCA) of the fatty acids is depicted in Figure 4.4 and shows a clear difference between the three seasons, with some overlap observed for summer.

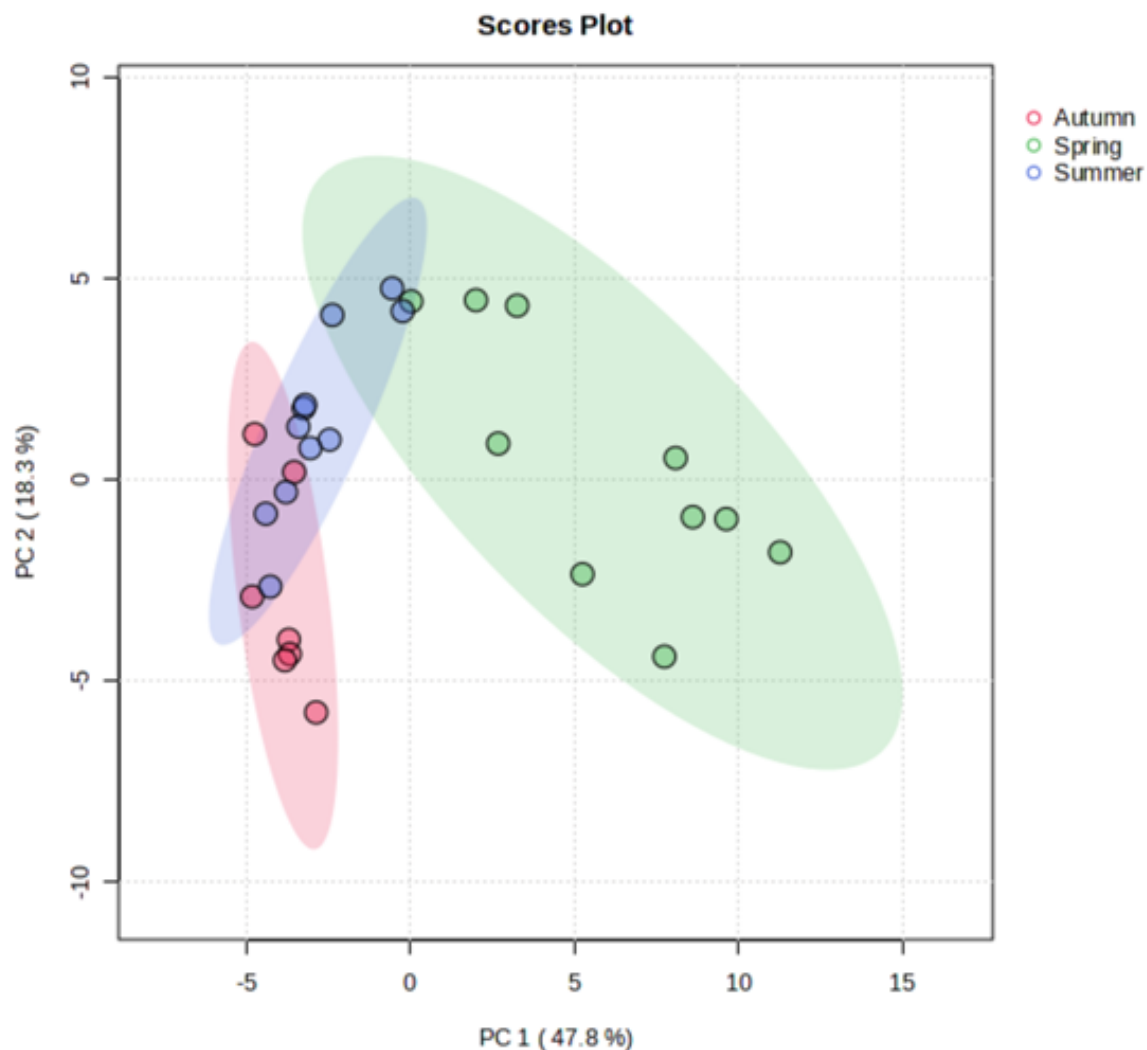


Figure 4.4. Principal component analysis plot of fatty acids across three seasons in 2024 – Spring (●), Summer (●) and Autumn (●).

4.3.2. Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) thermograms showed significant differences across many measured parameters between seasons in this study (Table 4.2). Of the five measured metrics, four of these varied significantly over the year with enthalpy of crystallisation being the only metric which did not. Crystallisation onset temperature was $16.46 \pm 0.74^{\circ}\text{C}$ in Spring, while it was lower at $15.8 \pm 0.11^{\circ}\text{C}$ and $15.51 \pm 0.1^{\circ}\text{C}$ in Summer and Autumn respectively.

The crystallisation onset temperature in Spring was significantly higher when compared with both Summer and Autumn ($P < 0.05$) and exhibited a strong positive correlation with oleic acid content, while displaying a very strong correlation with first and second peak crystallisation temperature and also with melting completion temperatures. The first and second peak crystallisation temperatures exhibited strong correlations with each other while also correlating very strongly and moderately strongly respectively with some long chain and especially saturated long chain fatty acids, such as C18:0. This is to be expected given that melting point of fats tends to increase as saturation levels increase and also when carbon chain length increases (Devi and Khatkar, 2016). C4:0 also was very strongly and moderately strongly correlated with the first and second peak crystallisation temperatures respectively, again, this is to be expected given that it is a saturated fatty acid with a 3-4% presence in butter throughout the year. Finally, the first and second crystallisation peak temperatures both showed very strong and strong correlations with the temperature at completion of melting. The significant results from this study are broadly in line with figures previously reported by O’Callaghan *et al.*

(2016) , Timlin *et al.*, (2024), Tomaszewska-Gras. (2013), and Lokuge *et al.* (2024).

Table 4.2. Differential Scanning Calorimetry (DSC) parameters of butter across the three seasons. Figures presented are Mean $\pm$ standard deviation

Parameter	Spring	Summer	Autumn	Yearly Average
Crystallisation onset temperature (°C)	16.46 $\pm$ 0.74 ^a	15.8 $\pm$ 0.11 ^b	15.51 $\pm$ 0.1 ^b	15.92 $\pm$ 0.49
First peak crystallisation temperature (°C)	14.28 $\pm$ 1.35 ^a	12.65 $\pm$ 0.26 ^b	12.42 $\pm$ 0.09 ^b	13.12 $\pm$ 1.01
Second peak crystallisation temperature (°C)	7.91 $\pm$ 1.05 ^a	7.73 $\pm$ 0.19 ^a	6.95 $\pm$ 0.04 ^b	7.53 $\pm$ 0.51
Enthalpy of crystallisation (J/g)	34.36 $\pm$ 2.59 ^a	35.46 $\pm$ 1.05 ^a	33.73 $\pm$ 0.2 ^a	34.52 $\pm$ 0.88
Melting completion temperature (°C)	36.75 $\pm$ 0.6 ^a	36.13 $\pm$ 0.16 ^b	36.06 $\pm$ 0.13 ^b	36.31 $\pm$ 0.38

Different letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify statistical difference between seasons ($P < 0.05$ ANOVA)

Matching letters (a.b.c) superscripted next to values for Spring, Summer and Autumn signify non statistical differences between seasons ($P > 0.05$ ANOVA)

4.3.3. Texture Analysis

Texture profile analysis peak force and workability results were found to be significantly different between Spring–Autumn and Summer–Autumn. These results are summarised in Table 4.3. Contrastingly, spreadability results, as shown in Table 4.4 and described through a combination of firmness and work of shear in this study were found to not be significantly different between seasons at refrigeration and at room temperature. However, significant differences were observed between the spreadability values at room temperature and refrigeration temperature.

Table 4.3. Texture Profile Analysis parameters of butter across the three seasons. Figures presented are Mean $\pm$ standard deviation

Parameter	Spring	Summer	Autumn	Yearly Average
TPA Workability (g.sec)	48235 $\pm$ 6158 ^a	43635 $\pm$ 7235 ^a	32364 $\pm$ 5564 ^b	41411 $\pm$ 846
TPA Peak force (g)	8814 $\pm$ 1139 ^a	7778 $\pm$ 1828 ^a	5593 $\pm$ 599 ^b	7395 $\pm$ 615

Different letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify statistical difference between seasons ($P < 0.05$ ANOVA)

Matching letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify non statistical differences between seasons ($P > 0.05$ ANOVA)

When considering TPA, the peak force values, which were defined as the maximum force reached in the first TPA compression, ranged from 8814 $\pm$ 1139 g in Spring to 7778 $\pm$ 1828 g and 5593 $\pm$ 599 g in Summer and Autumn respectively, while the workability was positively correlated with these trends. It would have been expected that the peak force would have been higher than it was in Autumn, as previous studies have shown butter hardness at this time of the year to be lower than mid-season butter but not to the same degree as was observed in this study (Timlin *et al.*, 2024). It is possible that the uncharacteristic weather trends observed in Ireland in 2024, and resulting changes in cow diet, have impacted these results, which highlights the effect on product seen in an industrial setting when compared with smaller scale studies. It is possible that the workability trend can be explained by two reasons – area under

the curve is typically expected to be smaller when the peak force reached is less but also, this workability value gives an insight into the workability of the butter and how likely it was to shear upon compression.

Table 4.4. Spreadability parameters of butter across the three seasons. Figures presented are Mean $\pm$ standard deviation

Parameter	Spring	Summer	Autumn	Yearly Average
Firmness fridge (g)	27271 $\pm$ 2868 ^a	26991 $\pm$ 1637 ^a	26700 $\pm$ 6252 ^a	26987 $\pm$ 285
Work of shear fridge (g.sec)	40568 $\pm$ 5394 ^a	37216 $\pm$ 3955 ^a	35171 $\pm$ 8807 ^a	37652 $\pm$ 2725
Firmness Room temperature (g)	5454 $\pm$ 2027 ^a	4475 $\pm$ 361 ^a	5099 $\pm$ 1225 ^a	5010 $\pm$ 495
Work of shear room temperature (g.sec)	5524 $\pm$ 2184 ^a	4633 $\pm$ 401 ^a	5549 $\pm$ 1349 ^a	5235 $\pm$ 521

Different letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify statistical difference between seasons ($P < 0.05$ ANOVA)

Matching letters (a.b.c) superscripted next to values for Spring, Summer and Autumn signify non statistical differences between seasons ($P > 0.05$ ANOVA)

4.3.4. Colorimetry

The L* values for butter across the three seasons in 2024 remained consistent, ranging from 91.5 $\pm$ 0.51 in Spring, 91.3 $\pm$ 0.84 in Summer and 90.8 $\pm$ 0.35 in Autumn. Conversely, the a* values differed significantly between Spring – Summer and Spring-Autumn as shown in Table 4.5. The L* values are broadly similar to those previously reported by Lokuge *et al.* (2024), who found L* of 90.50, however, a* in the current study were found to be slightly lower than previously reported (O’Callaghan *et al.*, 2016; Lokuge *et al.*, 2024; Timlin *et al.*, 2024). These studies have focused primarily on butter produced at small scale from research herds and, as a result, it is possible that factors such as limited moisture control has impacted the colour of the resulting butter. As anticipated, the greatest colour change in the current study noted was with respect to the b* value, relating to how blue-yellow the samples were. b* values differed significantly between all 3 seasons with a p value of <0.001 . The samples involved in the study

across all three seasons in 2024 are shown in Figure 4.5 where a clear upward trend is observed in b^* values as the seasons progressed. Yellowness of the samples increased incrementally as the year progressed with values ranging from 33.2 ± 0.95 in Spring to a high of 35.9 ± 1.11 in Autumn. While the overall b^* values are in the same range as previously observed by other authors (O’Callaghan *et al.*, 2016; Lokuge *et al.*, 2024), the overall trend of results was not as expected and differs to what other authors have found (O’Callaghan *et al.*, 2016; Timlin *et al.*, 2024). For example, it has been observed that b^* value can be higher in early lactation, lowest in mid-lactation and recovering to some extent in late lactation (Timlin *et al.* 2024).

Table 4.5. Colour co-ordinates ($L^*a^*b^*$) of butter across the three seasons. Figures presented are Mean $\pm$ standard deviation

Colour co-ordinate	Spring	Summer	Autumn	Yearly Average
L^*	91.5 ± 0.51^a	91.3 ± 0.84^a	90.8 ± 0.35^a	91.2 ± 0.33
a^*	-5.97 ± 0.36^a	-6.33 ± 0.37^b	-6.43 ± 0.18^b	-6.24 ± 0.24
b^*	33.2 ± 0.95^a	34.4 ± 1.2^b	35.9 ± 1.11^c	34.5 ± 1.37

Different letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify statistical difference between seasons ($P < 0.05$ ANOVA)

Matching letters (a,b,c) superscripted next to values for Spring, Summer and Autumn signify non statistical differences between seasons ($P > 0.05$ ANOVA)

The year 2024 was an unusual year of weather throughout Spring and Summer, with large volumes of rain, but a recovery was observed in the final few months of the year, and with this came more grass grazing which may explain why the b^* value was higher than expected in the Autumn months and lower than expected in the Summer months. It is also possible that the large-scale production of butter involved in the current study has had an impact on butter colour in terms of moisture control and a more controlled production environment and processing conditions.

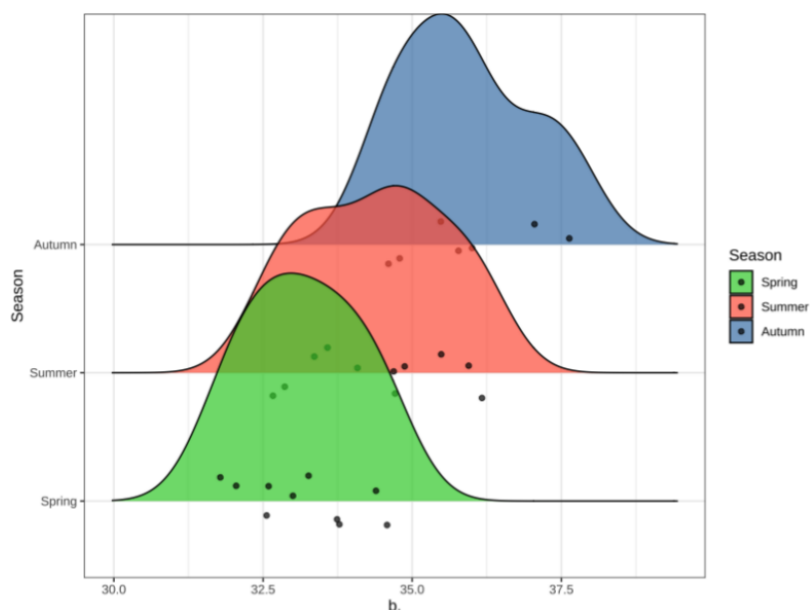


Figure 4.5. Plot of b^* values across three seasons – Spring (-), Summer (-) and Autumn (-).

4.4. Conclusions

The fatty acid profile of butter varied significantly across all three seasons in this study. Conversely, no variation between seasons was seen in spreadability at both room and refrigeration temperature; however, texture profile analysis results were significantly different in Autumn than they were in the previous two seasons. Significant differences were also observed across a range of thermal melt properties. When considering colour analysis, while L^* values remained consistent throughout the seasons, significant differences were observed across all three seasons in b^* values, and between Spring and the remaining two seasons in the case of a^* values.

It is clear from this study that, in the absence of any targeted modifications to cream ripening conditions, milk fat chemistry, and subsequent butter functionality are not consistent throughout the year in Ireland. When considering large-scale production of butter, consistency

in functional parameters is essential to the success of the product. This is largely because the secondary user of these products (e.g., other companies, food service, retail, domestic consumers) expect a certain consistent standard from these products. This is particularly relevant when butter is being sold for further processing into smaller pack sizes, bakery applications etc. To remain consistent with the manner in which their products are produced and how they subsequently perform, controlling the functionality of the raw materials they use (such as butter in this instance) is imperative. This study highlights the need and provides a baseline for, the introductions of strategies (e.g., cream ripening) to enable the industrial-scale production of consistent and predictable quality butter from a seasonally varying milk/cream raw material.

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Chapter 5

General discussion and suggestions for future research

Órla Dinneen^{1,2}, Anna G. Behan², James A. O'Mahony¹, Tom F. O'Callaghan¹

¹*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland*

²*Kerry Dairy Ireland, Princes Street, Tralee, Co. Kerry, Ireland*

Declaration

This chapter was written by author Órla Dinneen (Ó.D.) and reviewed by all co-authors

5.1 General discussion

Butter is a nutritionally and economically important product in the portfolio of dairy products manufactured in Ireland (Bord Bia, 2025). Sales of butter, especially that destined for export, contributed strongly to the upward trend in the value of dairy products to the Irish food and beverage industry in 2024 (Bord Bia, 2025). There is an ongoing need for the development of, not only new, innovative products in this space, but also the requirement to comprehensively and scientifically understand the raw material chemistry of milk and subsequent cream. It also highlights the need to ensure that the best available technology for producing a standardised product, of consistent and predictable functionality throughout the year is identified, especially against the backdrop of unique seasonality challenges in the Irish dairy system. This thesis was designed to address both of these points, encompassing a review of the state of the art, and two independent original experimental chapters. Given the amount of butter exported annually from Ireland (€1.7 billion in 2024), much of which requires intercontinental transport, it was important to understand how altering the post-production environmental storage conditions of the product would impact on its final techno-functional properties. Secondly, given the typical seasonal system of farming adopted in Ireland, and the effects this has been previously shown to infer on milk (Timlin *et al.*, 2021), it was critical to understand the impact of this on the techno-functional properties of butter, in a large-scale production facility, using the pooled milk from cows across a large catchment area. This is to compliment studies conducted in recent years (e.g., Timlin *et al.*, 2024 and O’Callaghan *et al.*, 2016) on research herds, under very controlled conditions.

The aim of the first experimental chapter was multifaceted, and was constructed around a singular research question – “are the techno-functional properties of butter impacted by its post-production storage time-temperature treatment?”. In answering this question, the findings could inform both the scientific community and industrial producers on best practices for post-

production handling of butter. The question was investigated by assessing if different time-temperature treatments, applied directly after commercial production of butter, could influence the techno-functional properties of the product. This is critical in the landscape of aiming to produce the most consistent product. In the case of this study, it was concluded that the techno-functional properties of butter are not significantly impacted by the post-production storage treatments applied in this chapter. Statistically significant differences in fatty acid profiles were observed between the butter produced and used to perform this study in mid-late season (MLS) and late season (LS). However, within each trial, no notable differences in techno-functional properties, such as melting properties, textural properties and rheological properties were observed between the six different temperature treatments.

Two independent trials were conducted at different times of the year in this chapter. As expected, there were significant differences between the fatty acid profiles of the butter from these two time periods. Palmitic (C16:0) and oleic (C18:1) acids were two such fatty acids which varied significantly, which is particularly relevant as these are the two most abundant fatty acids found in milk fat (Bobe *et al.*, 2003). C16:0 was present at concentrations of 27.51 and 34.54% of total fatty acids in the MLS and LS trials respectively, while C18:1 was present at concentrations of 20.06 and 17.69% in the MLS and LS trials, respectively. The concentrations of saturated fatty acids were significantly higher in the LS trial, while conjugated linoleic acid (CLA), alpha-linolenic acid (ALA), unsaturated fatty acids and Omega-3:Omega-6 ratio were all significantly lower in the LS trial when compared with the MLS trial. These differences in fatty acids contributed to differences in measured techno-functional properties between the LS and MLS trials, due to higher amounts of saturated, and lower amounts of unsaturated fatty acids in the MLS butter. Importantly, within each trial (MLS and LS), the post-production handling of butter did not infer changes to these properties. The peak force and work of compression throughout texture profile analysis (TPA) in the MLS

trial for all six temperature treatments were lower than those in the LS trial; however, within each trial, no differences were observed. The temperature at which melting was complete was found to be lower in the MLS trial than the LS trial. However, similar to the texture analysis, no major differences were observed in this value when comparing the different temperature treatments applied. The shape of each individual thermogram was similar for all temperature treatments, except for MLS and LS -20/+5, which showed different shaped profiles, although critically, the melting completion temperature remained in line with that of the other treatments. Rheological analysis also demonstrated no major differences in the crossover point between G' (Storage modulus) and G'' (Loss modulus) between any of the six temperature treatments applied in either trial.

The findings from this chapter highlight that butter cannot be physicochemically altered using different post-production combinations of time and temperature. This limits its potential as a tool to tailor products for specific end-user functionality requirements. Even more importantly, it informs the scientific community and dairy processors that such variation, if required to fulfil supply chain requirements, will not negatively impact the final physicochemical properties of the butter. This is particularly relevant when considering the ever-existing need for delivery of consistent product with regards functionality and the implications of post-production storage not affecting butter quality are significant. It is common industrial practice to store butter in a variety of different ways post-production, and up to the point of sale to a customer/consumer, depending on warehouse capacity, transport distance, transport times and customer requirements. For example, butter may be held in frozen storage for a number of days after production, before being transferred to refrigerated storage in the days prior to shipment, thereby ensuring it is at the desired temperature by the customer at the time of delivery. Alternatively, product may be stored under refrigerated conditions, immediately post-production, and shipped to a customer at that same temperature, thereby avoiding any frozen

storage. These are just two examples of different storage treatments post-production, and many others exist, depending on the company practices and requirements. Critically, when product has been shipped to a customer or consumer, the manufacturer, to an extent, no longer has control of what temperature the product is exposed to. For example, the product can go through varying storage temperatures as it passes through food service, retail, and consumer environments. This makes the findings from this chapter particularly relevant and valuable to all stakeholders. This novel work has shown that butter, to an extent, is robust against such temperature fluctuations, while still delivering the desired techno-functional properties. This chapter has helped to fill an important gap in knowledge in the existing scientific literature. From the results, it was apparent that the main influencers of butter functionality are raw material composition, and processing conditions, as opposed to post-production storage. Therefore, it was important that the second experimental chapter in this study focused on addressing specific scientific research questions arising from this.

The principle aim of Chapter 4 was to better understand the impact of seasonality on the techno-functional properties of butter. In Chapter 3, it had been established that post-production storage did not significantly contribute to differences in functionality often seen in butter, and as a result, it was important to take a step back in the production process to assess how seasonality impacted this functionality. Seasonality is a challenge in countries such as Ireland which adopts a seasonal, pasture-based calving system which can lead to changes in product composition and quality attributes throughout the year (Couvreur *et al.*, 2006; Li, Ye and Singh, 2019). In Chapter 4, the objective was to establish if variation in product characteristics, such as textural properties, colour and thermal properties existed in a large-scale butter production facility across three seasons of Spring (March-May), Summer (June-August), and Autumn (September-November). A secondary objective of this chapter was to understand how these functional properties correlated to one another (e.g., did a specific fatty acid content infer

specific functional attributes on butter). Across the majority of measured parameters in this study, significant variation was found between seasons, with detailed analysis of this discussed in Chapter 4. Such differences include variation in crystallisation peak temperatures, crystallisation onset temperatures, melting completion temperatures, a^* and b^* colour chromaticity values, the quantity/profile of key fatty acids present and textural properties.

Of the thirty-eight fatty acids profiled in this study, thirty of these showed significant differences between at least two seasons. Among these were C16:0 and C18:1, the two most abundant fatty acids in all three seasons in this study, and the two most commonly found fatty acids in milk fat (Li, Ye and Singh, 2022). C16:0 was present at the lowest concentration in spring and was significantly higher in summer and autumn butters. C18:1 concentrations varied significantly between all three seasons and it was present at the lowest concentration in summer butter, with concentrations highest in spring. The concentrations of the three most abundant medium chain fatty acids, C8:0 (caprylic acid), C10:0 (capric acid) and C12:0 (lauric acid), were highest in summer butter, and significantly lower in spring and autumn butter. Many of the measured thermal properties showed significant differences across seasons. Crystallisation onset and first peak crystallisation temperatures were significantly higher in spring when compared with summer and autumn, while second peak crystallisation temperature was lowest in autumn, and significantly higher in spring and summer. The temperature at which melting was complete was highest in spring, and significantly lower in summer and autumn, while no significant inter-seasonal differences were observed for enthalpy of crystallisation. Texture profile analysis results found workability and peak force reached in the first compression to be significantly lower in autumn butter when compared with spring and summer butter. L^* values remained consistent throughout the year, while a^* values were significantly different in spring when compared with summer and autumn, with a smaller negative value reported.

Many of the results in this chapter, such as crystallisation temperatures, were in line with previous studies carried out on this topic at small-scale, using milk from research herds. Conversely, other results were found to be different in magnitude and overall trends, such as b^* values and hardness/peak force, as discussed in detail in Chapter 4. This highlights the value in carrying out further work in this area, to assess if the results seen in this study are repeatable across a number of years, thereby signifying some difference in trends between small- and large-scale production, or if the uncharacteristic weather experienced in 2024, impacted the results obtained. In any case, it may be concluded from this study that seasonality does exist in large-scale processing of butter, highlighting the need for continued research in the area. This is with the aim of being able to (a) when desired, choose certain times of the year to produce a specific butter product with targeted product characteristics, and (b) when necessary, produce a butter which is consistent in functionality all year around, notwithstanding the evolving milk raw material throughout the year. In this context, and for companies aiming to apply tempering methods (e.g., the Alnarp method), or indeed other methods of producing a consistent product throughout the year, such as fatty acid fractionation (Wilbey, 2009), this research, and in particular, the work conducted on fatty acid profiling will be useful. More detail around the Alnarp methodology can be found in Chapter 2 of this thesis.

Overall, the experimental chapters in this thesis have helped to highlight some of the driving factors behind functionality of butter. Chapter 3 has shown that, within the confines of the parameters and ranges studied in the context of butter storage in the days directly after production, the end product remains relatively consistent with regards its textural and thermal melt properties. Conversely, Chapter 4 highlighted that seasonality throughout the year does impact such properties, as well as colour chromaticity values, most significantly b^* values. The conclusions from this thesis provide valuable knowledge for butter producers going forward, but also fulfils the overall research objective of this thesis, which was to add to the scientific

knowledge base through new, original studies. The work carried out helps to highlight that factors early on in the butter production process have a greater impact on end product functionality and characteristics, compared with post-production storage. It also helps to inform producers that a certain level of flexibility in their post-production storage treatments should not negatively impact, nor functionally change, the final product. While producers try to keep processes as streamlined and reproducible as possible, sometimes, different variations of post-production time-temperature treatments are applied, generally to meet consumer demands for a product to be at a specific temperature by the time it has reached their facility. One factor which must also be carefully considered when making warehousing and transport decisions around time-temperature combinations is microbiological quality. It is critical that both the microbial and chemical shelf life of the product is not compromised throughout supply chain. Butter can typically have a shelf life of months, and in some cases, years, and not only should it maintain textural, thermal and rheological performance, but also characteristics such as colour, pH, free fatty acids, among others, which are detailed in Chapter 2.

The conclusions from Chapter 4 are beneficial, as an in-depth understanding of how milk and cream changes throughout the year, and in turn, how techno-functional properties of butter evolve, is essential. While some methods have been identified in literature which aim to increase consistency in functionality throughout the year, without a baseline understanding of the existing variations, it is difficult to apply this knowledge. Chapter 4 has helped to increase the understanding of this essential baseline knowledge.

Overall, the outputs from this thesis have added to the extensive existing research and knowledge in the area of butter functionality, and the factors which affect this. It has helped to fill a gap in existing scientific literature in the areas of post-production storage of butter, and the impact of seasonality in a pasture-based system, on the techno-functional properties of butter produced at industrial scale.

5.2 Suggestions for future research

The outputs from this study, and their consideration, have raised some more possible areas of research going forward, in the area of both post-production modifications, but more so in the area of butter seasonality. Some of the suggestions for future research, arising from this study are outlined as follows:

- 1.** Conducting shelf-life analysis on the various temperature treatments applied in Chapter 3 would be an important next step when considering post-production storage. It is certainly important to understand the physico-chemical implications of post-production storage of butter, but it is even more critical that it is concluded how the microbial integrity of the product withstands these storage treatments, and further studies are required to achieve this.
- 2.** Due to time constraints, the post-production storage element of this study was carried out at only two points in time across a single year, thus it was difficult to conduct robust statistical analysis on the results. While it can be concluded from the current study that the butter was not severely impacted by post-production storage, there were some results and wide standard deviations to indicate that a study which incorporates other times of the year also, would be beneficial to draw more conclusions. These results were predominantly found in DSC and butter melt profile data, and further studies are required to understand if there is a small effect on butter characteristics, or if the temperature-sensitive nature of the product induced the relatively high standard deviations seen across some measured parameters.
- 3.** Large scale post-production storage trials, using 20-25 kg blocks of butter in an industrial warehouse setting would be beneficial to conduct in the future. In the current study, small-scale butter containers were used, meaning product reached its storage

temperature more rapidly than it would in a large, forty-block, pallet of butter, where each block weighs 20-25 kg. This would be beneficial as it would more accurately reflect modern day butter packaging and warehousing practices.

4. Repeating the seasonality study across a number of different years would be beneficial to determine if results seen across 2024 are consistent across other years, or if the weather/grass growth patterns etc. can significantly impact butter produced at an industrial scale.

5. Conducting some application work with butter from across a full year would be a valuable future research concept. It is clear from the current study that variation across seasons in one year exist, using instrumental analysis; however, to evaluate the impact of this at a practical level, application testing is required. This is mainly because different butter applications require different functional properties; for example, harder, more plastic butter is required for bakery applications, such as when making layered pastry.

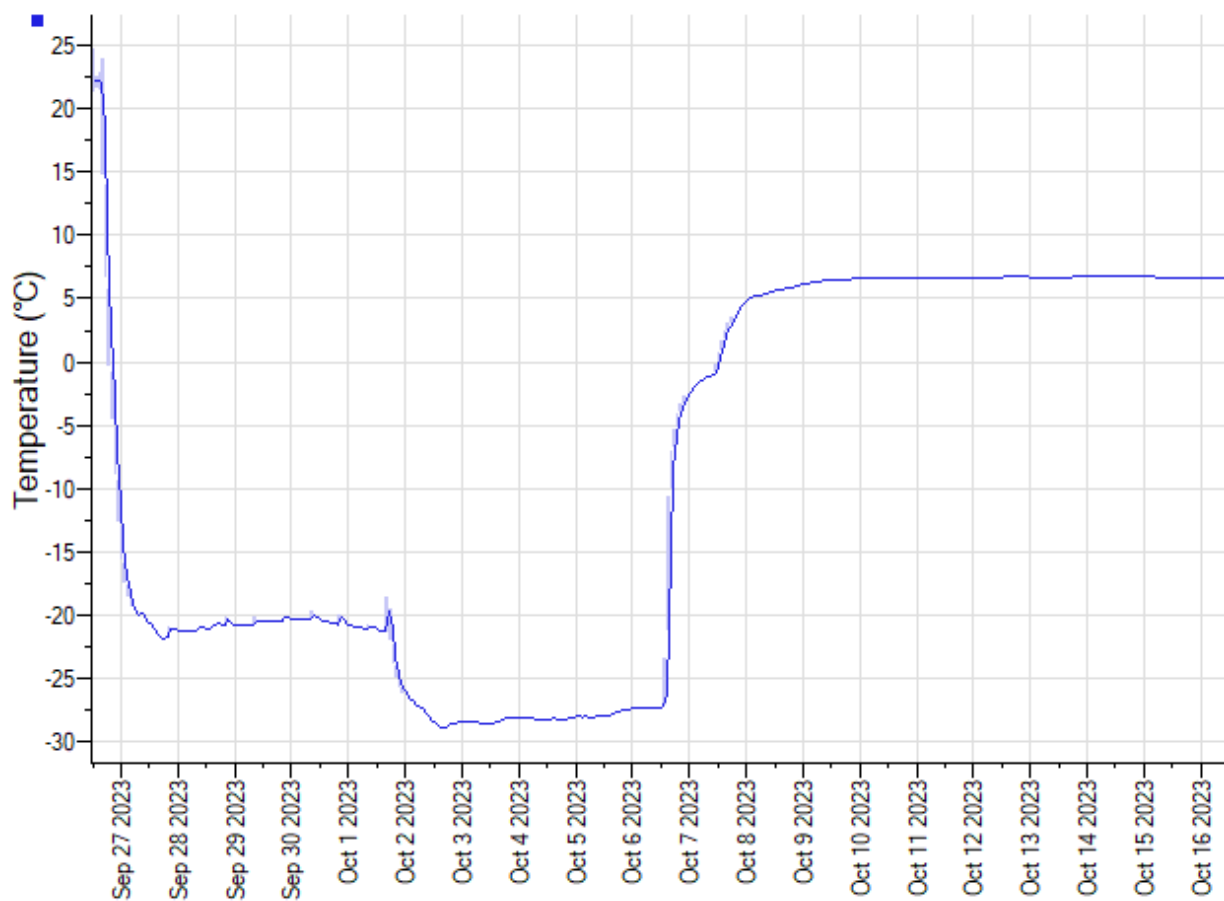
6. Conducting studies/trials on cream tempering methods, where the information found in the current study is used to set the relevant processing parameters. This would be beneficial to assess how consistent a product could be produced, given that this study has shown, that without process intervention, the product is not consistent.

5.3 References

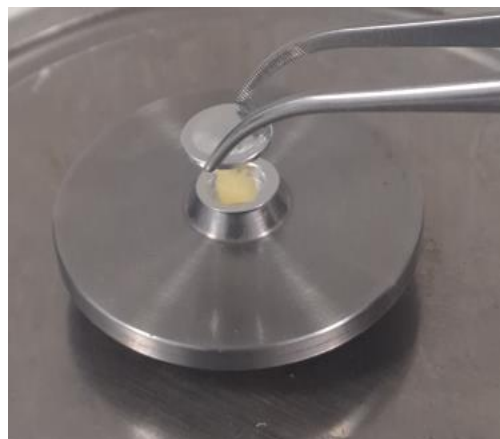
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Appendix

3.1. Example of the graphical output from temperature data loggers. Example shown is MLS -20/-30



3.2. Sample preparation for Differential Scanning Colorimetry (DSC) analysis



3.3. Sample preparation for rheological analysis





4.1. Sample preparation for spreadability analysis





4.2. List of sample dates, months seasons and week numbers used to conduct Chapter 2

Date	Month	Season	Week No.
01-Mar-24	March	Spring	9
18-Mar-24	March	Spring	12
25-Mar-24	March	Spring	13
01-Apr-24	April	Spring	14
08-Apr-24	April	Spring	15
23-Apr-24	April	Spring	17
30-Apr-24	April	Spring	18
07-May-24	May	Spring	19
15-May-24	May	Spring	20
26-May-24	May	Spring	21
02-Jun-24	June	Summer	22
06-Jun-24	June	Summer	23
16-Jun-24	June	Summer	24
27-Jun-24	June	Summer	26
09-Jul-24	July	Summer	28
24-Jul-24	July	Summer	30
02-Aug-24	August	Summer	31
08-Aug-24	August	Summer	32
16-Aug-24	August	Summer	33
22-Aug-24	August	Summer	34
26-Aug-24	August	Summer	35
20-Sep-24	September	Autumn	38
30-Sep-24	September	Autumn	40
03-Oct-24	October	Autumn	40
25-Oct-24	October	Autumn	43
29-Oct-24	October	Autumn	44
01-Nov-24	November	Autumn	44
13-Nov-24	November	Autumn	46