University of Reading

PROTEOLYTIC ACTIVITY AND THE CHEMICAL AND MICROBIOLOGICAL QUALITY OF JERSEY RAW MILK AND ITS IMPACT ON QUALITY AND SHELF LIFE OF ULTRA-HIGH-TEMPERATURE (UHT) TREATED MILK

DAgriFood Sustainable Food Quality for Health

The University of Reading, Department of Food and Nutritional Sciences

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This dissertation is dedicated to my beloved son Benjamin Drabble.

“Science knows no country because knowledge belongs to humanity and is the torch which illuminates the world”.

“It is the microbes who will have the last word”.

-Louis Pasteur

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Magdalena Drabble

Jersey, December 2022

DECLARATION OF ORIGINAL AUTHORSHIP

I, Magdalena Drabble, declare that the PhD thesis “Proteolytic activity and the chemical and microbiological quality of Jersey raw milk and its impact on quality and shelf life of Ultra-High-Temperature (UHT) treated milk” is no more than 90,000 words in length, including quotes and exclusive of tables, appendices, bibliography, references, and footnotes.

I confirm that this is my own work and the use of all material from the other sources has been properly and fully acknowledged.

Reading, December 2022

Magdalena Drabble

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LIST OF ABBREVIATIONS

A = Autumn

ANOVA = Analysis of variance

ATP = Adenosine triphosphate

CAGR = Compund annual growth rate

CFU = Colony forming unit

cP = Centipoise

ES = Ethanol stability

EU = European Union

FAN = Free amino nitrogen

Fluorescamine = 4-phenylspiro [furan-2(3H), 1-phthalan]-3, 3-dione

FPD = Freezing point depression

FTIR = Fourier transform Infra-red spectroscopy

GNB = Gram-negative bacilli

GPB = Gram-negative psychrotrophic bacteria

GPB = Gram-positive bacilli

HTST = High-temperature short time pasteurisation

IBC = Individual bacteria count

IR = Infra-red spectroscopy

J = Jersey

JDL = Jersey Dairy Laboratory

JMMB = Jersey Milk Marketing Board

MALDI-TOF = Matrix assisted laser desorption ionisation time-of-flight

MIR = Mid Infra-red spectroscopy

PBC = Psychrotrophic bacteria count

PCA = Principal Component analysis

PL = Proteolysis

PLS = Partial Least Squares

RP-HPLC = Reversed-phase high-performance liquid chromatography

SCC = Somatic cell count

SD-PAGE = Sodium dodecyl sulphate gel electrophoresis

SM = Summer

SNF =Solids Non-fat

SP = Spring

TBC = Thermoduric bacteria count

TCA = Trichloroacetic acid

TNBS = 2, 4, 6- Trinitrobenzenesulfonic acid

TNTC = Too numerous to count

TS = Total solids

QMMS = Quality Milk Management System

UHT = Ultra high temperature

UK = United Kingdom

W = Winter

ABSTRACT

The demand for aseptically packed ultra-heat-treated milk (UHT) with a validated long shelf life has been gradually increasing worldwide. However, extracellular enzymes secreted by psychrothrophic bacteria that are present in raw milk can hydrolyse milk protein and cause quality defects in the final UHT product. Therefore, robust means of controlling the microbiological quality of raw milk are required, to reduce incidences of UHT product defects and extend their shelf-life. The aim of this thesis was to develop a raw milk improvement strategy to enhance UHT milk stability that is transferrable into industry practice. UHT processing and storage stability studies were carried out at pilot and commercial scale with raw milk of varying quality. The importance of establishing control measures over psychrothrophic bacteria counts was confirmed. UHT milk produced from raw milk of low microbiological quality destabilised within five weeks at ambient storage conditions. The UHT milk also developed significant levels of free amino nitrogen concentrations that indicated protein hydrolysis was caused by bacterial proteolytic activity. The study established microbiological quality requirements for raw milk that were key to validate the stability of UHT milk, stored at various temperatures (4, 21 and 30°C) over a period of 360 days. Free amino nitrogen values coupled with viscosity and sensory observations were also used to confirm UHT milk acceptability.

The incorporation of additional psychrothrophic and thermoduric bacteria counts in raw milk producers' payment incentives, resulted in the production of premium microbiological quality raw milk by reference and comparison to various guidelines and published data. Historical analysis of Jersey raw milk quality used for UHT milk production provided a significant benchmark to other dairy processors and expanded on the literature for milk produced by this type of breed.

The findings of this thesis indicated that UHT milk processors need to further evaluate and monitor raw milk microbiological suitability by focusing on specific bacteria counts, as means of improving UHT milk stability and validation of product shelf-life extensions.

Keywords: raw milk, UHT milk, shelf-life, microbiological quality, psychrothrophic bacteria, protein hydrolysis

CHAPTER 1

INTRODUCTION

1.1. BACKGROUND

According to the Food and Agriculture Organisation of the United Nations (OECD/FAO, 2016), the demand for milk and milk products is expected to increase by 20% by 2025, mainly due to rising incomes, population growth, and changes in diets in developing countries (Paludetti et al., 2018). The demand for ultra-heat-treated milk (UHT) that is processed and aseptically packed has been gradually increasing worldwide since the 1960s when the first aseptic packages were developed. According to the IMARC Group (2019), the global UHT milk market has grown at a CAGR of 5.9% during 2011 to 2018, reaching a volume of around 107.4 billion litres in 2018.

The Covid-19 pandemic boosted demand for ultra-high-temperature-treated milk as people tried to reduce the number of visits to the shops by stocking milk with longer shelf life. This indicates a substantial change in consumers' behaviour towards a preference for products with a long shelf life as a defence mechanism for any future eventualities. Additionally, a rising population that requires high availability of hygienic milk and increasing urbanisation has also induced a fast-paced lifestyle making it easier for them to buy packaged food and beverage items. VPA Research (2021) suggests that the Covid-19 pandemic significantly impacted the regional and country level UHT milk markets worldwide. The outlook of economic progress across most countries is optimistic. Additionally, Infinium Global Research Report (2020) suggests a rise in the living standards among the working and middle-class population, growing e-commerce channels, rise in the numbers of product innovations which supports the raising UHT milk consumption trend. The report predicts the growth of an ultra-high temperature milk market with a CAGR of 8% over the forecast period to 2025. The global UHT milk market is expected to reach 124.77 million tons by 2024 (Global UHT milk market, 2020).

Various companies have developed a range of UHT milk products fortified with vitamins and minerals to fulfil nutritional requirements for bodily functions. Additionally, they are also available in a lactose-free variant, which is offered for lactose intolerant population. The innovation activities improved UHT milk quality and the acceptability of the product flavour. This partially resolved previously reported negative consumers perceptions; cooked and stale flavour (Zabbia et al., 2012), rancid and oxidised flavour and development of sedimentation, gelation, and discolouration of the milk products (Richards et al., 2016).

Ultra-high temperature (UHT) processing involves heating milk at a high temperature (130-145°C) for a short time (2 to 45 s) to obtain a product that is microbiologically stable at ambient temperatures with long shelf life, typically between 4-6 months (D'Incecco et al. 2018) or 6-9 months (Bimbo et al. 2016; Richards et al. 2014). During this process, bacteria are destroyed but some heat-stable enzymes of native and bacterial origin such as lipases and proteases survive the sterilisation process and contribute to product physical defects such as gelation and off-flavours (David et al., 1996). Psychrotrophic bacteria are the group of main concerns. They are defined as bacteria that can thrive under refrigerated temperatures (3-7°C) and their extracellular enzymes remain active after heat-treatment processes (Robinson, 2002). Some reviews confirm that *Pseudomonas fluorescens* protease AprX induces compact gels (Zhang et al.2018), hydrolyses casein, decreases its zeta potential, and induces sediment (Matéos et al.2015), *Serratia liquefaciens* Ser2 protease forms aggregates and releases peptides during product storage (Bagliniére et al.2017).

The improvement in raw milk microbiological quality is one of the factors that contribute to improved UHT milk quality and extended shelf life (Deeth and Lewis, 2017). To date, improvements in raw milk suitability have been focused on improving milk hygiene by reducing somatic cell counts and bacterial counts. There are specific legislative requirements for those

two parameters established in many countries. Additionally, the United Kingdom (UK) and European Union (EU) multi-component pricing system includes compositional parameters such as fat and protein percentage and milk hygiene parameters such as Bactoscan count and Somatic cell counts. Some milk producers in the United Kingdom introduced thermoduric bacteria testing to their payments testing suite to improve pasteurised product shelf life and product keeping quality (NML, 2019). The development of knowledge in this area is critical to ensuring mechanisms can be developed so that UHT milk is of consistently high quality and stability. It is essential for the raw milk producers to tackle problems at the farm level and for the milk processors to be familiar with multivariable factors, including the level of bacterial contamination that triggers UHT milk defects and reduces UHT milk shelf life.

In 2012, to drive improvements in UHT milk shelf life, Jersey Dairy Milk Marketing Board (JMMB) integrated thermoduric bacteria counts into the routine testing programme. This was followed by the introduction of psychrotrophic bacteria counts incentive in 2016.

To date, the leading indicators of milk quality and suitability for UHT processing are confirmed to be somatic cell counts, bacterial count, heat coagulation and ethanol stability tests. Other tests can potentially be introduced by processors to improve and extend UHT product shelf life, i.e., psychrotrophic bacteria count test and testing of free amino nitrogen groups (FAN) that could indicate the development of proteolysis in raw milk prior to UHT processing.

This thesis will provide fundamental data regarding Jersey raw milk quality and recommendations for processing mechanisms that could be used to enhance UHT milk product stability and shelf life. The impact of implementing the raw milk payment structure is also discussed. This fundamental knowledge is required as although many factors have been reported at the laboratory scale to reduce UHT milk shelf life, this work is based additionally on industrial scale experiments. It has yet to be established and subsequently validated at an

industrial scale what metrics and limits should be considered embedding into quality systems to ensure the stability of the products and acceptability of the product by the consumer throughout the shelf life.

1.2. OBJECTIVES

The overall aim of this thesis was a critical assessment of the microbiology of raw milk by processors, in relation to final UHT product quality during its shelf life, by determining the role of microbiological quality and proteolytic enzymes activity of raw milk to improve the control of raw milk quality used during UHT processing and thereby maximise product quality and shelf life of UHT milk. The specific objectives set to meet this aim were as follows:

1. To produce a state-of-the-art review on the impact of microbiological and proteolytic quality of raw milk on the overall quality, shelf life and protein stability of UHT processed milk.

2. To assess variations in chemical and microbiological properties of Jersey raw milk produced and the impact of pricing schedules, between 2014 and 2019, sourced from pedigree Jersey cows on the island of Jersey and establish a profile of Jersey raw milk used for UHT milk production.

3. To establish evidence-based thresholds for specific bacterial strains related to the consistency of UHT milk quality, understand microbiological factors affecting UHT milk shelf-life and utilise this knowledge as a tool in improving of raw milk quality at the farm level.

4. To evaluate the impact of bacterial counts on the rate of proteolysis in UHT Jersey milk and determine appropriate mechanisms to prevent or reduce proteolysis during storage of UHT milk.

1.3. STRUCTURE OF THE THESIS

This thesis is divided into 7 Chapters:

Chapter 1- Introduction. Introduced the background of the research and the objectives.

Chapter 2- A literature review. This chapter provides a literature review of chemical, microbiological, and proteolytic variations in the quality of Jersey raw milk and milk sourced from other breeds. It critically reviews published literature regarding the impact of chemical and microbiological properties of raw milk used to produce ultra-heat-treated milk. It also critically examines the current state of the art in enzymatic action in milk and its link to sensory defects in UHT processed milk. A review of raw milk and UHT product microbiological and physicochemical testing methodology is presented.

Chapter 3- Chemical and microbiological quality of Jersey raw milk. This chapter provides an assessment of the variation in chemical and microbiological properties of Jersey raw milk produced between 2014 and 2019, from pedigree Jersey cows on the island of Jersey, Channel Islands. Additionally, this chapter indicates the dominating spoilage bacteria as prevailing species and discusses payment structure and its impact on improvements in Jersey raw milk quality and changes in milk microbiome over the years of study.

Chapter 4 – Impact of raw milk quality on proteolysis and storage stability of Jersey UHT unstandardized milk produced at pilot scale. This chapter provides an initial assessment and effect of raw milk quality (Individual Bacteria Counts, psychrotrophic bacteria and thermoduric bacteria, Somatic cell counts and *Enterobacteriaceae* spp*., Streptococcus* spp*., E. coli,* and *Pseudomonas* spp*.* and microbiome identification) on proteolysis in UHT milk processed by indirect (tubular) pilot plant system (138°C for 4 seconds, 10 ml h^{-1}) during storage of milk at 19°C and 55°C for five weeks.

In this chapter, the effects of psychrotrophic bacteria counts and microbiological activity on the shelf life of UHT unstandardized milk stored for a short period of time are analysed. This evaluates unstandardized samples produced from Jersey raw milk collected from farms and processed at the UHT pilot plant and observation of changes in protein degradation and production of peptides due to protein hydrolysis over five weeks of storage while two different products storage temperatures were trialled. Additionally, this chapter evaluates individual bacteria strains present in raw milk samples to establish evidence-based thresholds for specific bacteria strains to ensure consistency of UHT milk quality that processors could use when bands for payment purposes for quality of milk are introduced. The source analysis was carried out to establish a root cause of raw milk contamination to provide a tool to be able to address contamination issues at the farm level. This chapter provided a good foundation for the following commercial-scale experiment by indicating and confirming that initial high counts of bacteria contribute to the development of UHT milk quality defects.

Chapter 5 – Impact of raw milk quality and storage conditions on proteolysis and storage stability of UHT Jersey standardized milk produced at commercial scale. This chapter provides an assessment and effect of raw milk quality (Individual Bacteria Counts, psychrotrophic bacteria and thermoduric bacteria, Somatic cell counts and *Enterobacteriaceae* spp*.*, *Streptococcus* spp*., E. coli* and, *Pseudomonas* spp*.* and microbiome identification) on proteolysis in UHT whole fat and UHT skimmed milk processed by commercial indirect (tubular) system (138°C for 4 seconds 3800 L h^{-1}) during storage of milk at different temperatures 4°C, 21°C, 30°C for up to 360 days and at 55°C stored for 5 weeks.

In this chapter, the effects of psychrotrophic bacteria counts and microbiological activity on the longer shelf life of UHT skimmed and whole fat milk is analysed. This evaluates samples produced at a commercial plant and observation of sensory defects, compositional changes,

colour and viscosity changes, and changes in protein degradation and production of peptides during protein hydrolysis during product shelf life while different product storage temperatures were trialled. Additionally, this chapter evaluates individual bacteria strains present in raw milk samples to establish evidence-based thresholds for specific bacteria strains to ensure consistency of UHT milk quality that could be used by processors when bands for payment purposes for quality of milk are introduced.

Chapter 6- Overall conclusions and recommendations. This chapter provides a discussion of the overall findings of the thesis and its potential impact on industrial practice.

Chapter 7- Future work recommendations. This chapter highlights recommendations for additional future work.

CHAPTER 2

2. LITERATURE REVIEW

2.1 INTRODUCTION

This chapter reviews past findings regarding raw milk composition and properties and its suitability for UHT processing. It also discusses raw milk physicochemical properties and their relevance with factors affecting the stability and shelf-life of UHT milk during storage. It briefly discusses Jersey raw milk composition compared to milk sourced from different breeds. It reviews current raw milk microbiology and levels of bacteria counts recorded worldwide. It presents the review of legislative microbial criteria and payment initiatives. It discusses the UHT processing methods, current chemical and microbiological testing methodology and quality requirements for UHT milk processing that are detailed in the latest scientific reviews. The main objective of these critical reviews is to determine the role of microbiological quality and proteolytic enzymes activity of raw milk to improve the control of raw milk quality used during UHT processing and thereby maximise product quality and shelf life of UHT milk. This chapter assesses the case and provides the subject knowledge required to establish future improvements in raw milk microbiological quality in relation to UHT milk productions.

2.2 RAW MIK CHEMICAL COMPOSITION

2.2.1 Main components

Bovine milk is a nutritionally rich, chemically complex biofluid consisting of hundreds of different components. The chemical composition of cow's milk sourced from different breeds has been studied for decades, however much of the information according to Foroutan et al. (2019) is very dated. The major component of milk is water, the remainder consists of fat, lactose, and protein (casein and whey protein). Milk also contains smaller amounts of minerals, vitamins and specific blood proteins, enzymes, and small intermediates of mammary synthesis. All these

components have specific properties and influence milk characteristics and have significant consequences for milk processing and different behaviours during product shelf life (Robinson, 2002).

According to the data in **Table 2.1,** the highest nutritional values are recorded for raw milk produced by the Jersey breed. It needs to be noted that within a herd of cows of a single breed, there are considerable variations between different cows, also due to breeding methodology, genetics, location and farming practice; these factors contribute to significant variations in the composition of the milk produced.

Table 2.1 Typical composition of milks of some breeds of cow (g/ 100g)(Robinson, 2002)

Breed	Protein	Fat	Lactose	Ash
Jersey	4.0	5.2	4.9	0.77
Friesian	3.4	4.2	4.7	0.75
Brown Swiss	3.5	4.0	4.9	0.74
Guernsey	3.7	3.7	4.7	0.76
Holstein	3.3	3.5	4.7	0.72
Ayrshire	3.5	3.9	4.6	0.72

2.2.2 Fat composition

The major lipid component of cow's milk is triglycerides, which make up about 98% of milk fat. The other 2% of milk lipids consist of diglycerides, monoglycerides, cholesterol, phospholipids, free fatty acids, cerebrosides, and gangliosides. Only thirteen fatty acids are present in milk at reasonable concentrations and these can be arranged in many ways to give hundreds of different triglycerides. Various triglycerides have a large melting point range (Robinson, 2002).

The highest value of milk fat was reported for the Jersey breed located in New Zealand, equal to 6.23 g/100g by Macle et al. (1996). Free fatty acids elevate in Jersey milk within milking days once they reduce in Holstein milk. There are some differences between the proportions of some individual fatty acids in milk sourced from those breeds (Dong-Hyun et al., 2020). Jersey milk has a higher concentration of short and medium-chain fatty acids and as a result, the concentration of saturated fatty acids is higher. Jersey milk has also a lower level of long-chain trans-fat and beneficial conjugated linoleic acid (Beaulieu & Palmquist, 1995; White et al., 2001; Martini et al., 2003; Soyeurt et al., 2006). The feeding system has a significant impact on the fatty acid composition of raw milk (White et al., 2001).

During storage of UHT milk, fat globules can aggregate and float to the top resulting in fat separation and fat adhesion to the packaging. Fat separation is closely correlated to and will increase with fat content, storage temperature, and fat globule size. The rate of fat separation is affected by the homogenisation efficiency in which, a higher efficiency retards the fat separation by contributing to a larger reduction in fat globule size (Karlssonn et al., 2019). The mean milk fat globule size of Jersey milk is significantly higher than a fat globule of milk sourced from Holstein cow and it has been reported to be 4.5 µm versus 3.5 µm (Singh, 2006) or 5.31 µm versus 4.93 µm (Martini et al., 2003).

2.2.3. Protein composition

Proteins are fractioned into two main groups. On acidification of milk to pH 4.6 at 20°C, about 80% of the total protein precipitates out of the solution; these proteins are called caseins. The proteins that remain soluble under these conditions are referred to as whey proteins or serum proteins. Caseins can be fractioned into four proteins: α_{s1}-, α_{s2}-, β- and κ-caseins. There are also several derived caseins, resulting from the action of indigenous milk proteinases, especially plasmin. These are usually referred to as γ-caseins. Caseins are all phosphoproteins. The phosphate groups bind substantial amounts of calcium and they are

important to the structure of casein micelles. They can bind a considerable amount of calcium ions, leading to strong aggregations. The whey protein fractions are β-lactoglobulin, bovine serum albumin, α-lactalbumin, and immunoglobulins (Robinson, 2002).

Table 2.2 presents protein and nitrogen fractions of the raw cow's milk produced from five dairy breeds in the UK Ayrshire, Brown Swiss, Guernsey, Holstein, and Jersey. The highest reported values for crude protein, true protein, casein, β-lactoglobulin, total nitrogen, protein nitrogen, casein nitrogen, and casein number are reported for milk produced by the Jersey breed. The highest value of protein is reported for the Jersey herd in Poland; 4.15g/100g by Barlowska et al. (2006). The differences in protein composition impact on quality of UHT milk products from different breeds, and variations impact on the rate of development of quality defects that involve proteins. Those defects include sediment formation and age gelation. Sediment formation involves the formation of a compact layering adhering at the bottom of the package and is suggested to consist of aggregates of protein or protein particles of various sizes. Sediment formation has been shown to increase with storage temperature. Age gelation consists of a three-dimensional, voluminous network of proteins, and can occur either through enzymatic or non-enzymatic (i.e., physico-chemical) processes (Karlsson et al., 2019).

Table 2.2 Means of protein and nitrogen fraction (g/100 ml) of raw cow's milk produced from five major dairy breeds in the UK (Chen et al. 2017).

Protein and nitrogen fraction	Ayrshire	Brown Swiss	Guernsey	Holstein	Jersey
Crude protein	3.47	4.05	3.7	3.22	4.22
True protein	3.30	3.84	3.56	3.07	4.07
Casein	2.73	3.14	2.88	2.53	3.39
True whey protein	0.57	0.69	0.68	0.54	0.68
B-lactoglobulin	0.16	0.22	0.22	0.18	0.28
Other whey protein	0.41	0.47	0.46	0.36	0.40
Total nitrogen	0.54	0.63	0.58	0.51	0.66
Nonprotein nitrogen	0.026	0.034	0.023	0.024	0.023
Protein nitrogen	0.52	0.60	0.56	0.48	0.64
Casein nitrogen	0.43	0.49	0.45	0.40	0.53
Total whey protein nitrogen	0.116	0.142	0.129	0.109	0.131
True whey protein nitrogen	0.0896	0.1084	0.1065	0.0849	0.1073
Casein number	78.7	77.4	77.7	78.2	80.2

2.2.4 Lactose composition

Lactose, the major carbohydrate in milk, is found in cow's milk at 4.8%. Lactose is less sweet than most of the other carbohydrates (e.g., sucrose, glucose, or galactose). Lactose is a disaccharide, made up of glucose and galactose units and contributes the majority to the colligative properties of milk (osmotic pressure, freezing point depression, boiling point elevation). It exists in both α- and β- isomeric forms and it is less soluble in water, which causes some manufacturing problems. Lactose crystals are gritty in texture and additionally, like other

reducing sugars, can react with free amino groups of protein to give products that are brown in colour (Robinson, 2002). Intense UHT treatment initiates non-enzymatic browning reactions, Maillard reactions that involve condensation reactions between reducing sugars in milk (mainly lactose) and amino groups (mainly lysine residues) that lead to the formation of the protein bound Amadori product lactulosyllysine. In the initial stage, firstly, the carbonyl compounds react with the amino compounds to form an unstable Schiff base, which is a reversible process. Then, the reduction efficiency can be enhanced during the formation of stable Amadori or Heyns rearrangements via double-bond migration and rearrangement process of the Schiff base. The intermediate stage of the Maillard reaction involved degradation, sugar fermentation, etc. The processes are related to pH value. Amadori products produce furfural or hydoxymethylfurfural (HMF) via the 1,2-enolation reaction pathway at ph ≤ 7. Coloured compounds e.g. melanoidins and volataile compounds are formed in the advanced stage of the process where low molecular weight intermediates undergo a series of reactions, including cyclization, dehydration, rearrangements, post-acetal reaction, isomerization, and other reactions to produce high molecular weight polymers with coloured compounds (Xiang et al., 2021). Alessio et al. (2016) reported that the lactose content of milk is influenced by somatic cell count, and although it varies seasonally, it is not related to breed, milk yield, milk fat content or protein levels.

2.2.5 Minerals and minor components

Calcium is an important mineral for the internal structure and stability of casein micelles. Milk sourced from Jersey cows contains higher levels of total calcium, as well as most of the other mineral components (**Table 2.3**). Most of the minerals are involved in the reactions and development of quality defects.

Table 2.3 Minerals composition of milk from Holstein and Jersey cows (Bland, 2015).

Parameter (mg per 100 g of milk)	Holstein	Jersey	Reference
Total calcium (mg %)	120.57	163.97	Czerniewicz et al. (2006)
	118.0	130.0	McCance and Widdowson (2010)
Colloidal calcium (mg %)	77.1	112.2	Czerniewicz et al. (2006)
Soluble calcium (mg %)	43.4	51.7	Czerniewicz et al. (2006)
Ionic calcium (mg %)	7.0	8.2	Czerniewicz et al. (2006)
Magnesium	11.3	12.7	Harmensen et al. (2005)
	10.9	11.7	Auldist et al. (2004)
	11.0	12.0	McCance and Widdowson (2010)
Phosphorus	102.0	114.0	Hermensen et al. (2005)
Sodium	35.3	28.0	Auldist et al. (2004)
	43.0	54.0	McCance and Widdowson (2010)
Potassium	151.2	141.0	Auldist et al. (2004)
Sulphur	34.0	40.0	Hermensen et al. (2005)

2.2.6 pH

The developed or real acidity of milk is due to lactic acid formed as a result of bacterial action on lactose. The stage of lactation and the health of the animal impact on this process. Milk acidity is the sum of developed and natural acidity. Milk pH is influenced by many factors i.e. microbial activity, and the addition of soluble calcium salts. Milk pH varies with species, breed, individuality, stage of lactation and health of the animal. If the animal is suffering from udder disease like mastitis the pH would be higher than the normal milk (Jenness et al., 1988).

Any addition of substances that rise pH improve milk heat stability (Deeth and Lewis, 2017). The pH of raw milk varies between 6.4 to 6.8 (Robinson, 2002). Schmidt (1996) reported that the factor having the greatest effect on the pH of raw milk is bacterial count; as the bacteria

count increases, the pH decreases. Protein content can influence pH, but not to the same extent. Differences in protein content and chemical composition between milk sourced from different breeds may influence pH (Czerniewicz et al. 2006).

Temperature and pH are the two major parameters that affect acidic milk coagulation (Eleya et al., 1995). Changes in pH impacts on the casein micelle. Increased pH causes casein micelle swelling, micelles decrease on acidification to pH 5.5 and increase when pH is dropped below 5.5, due to shrinkage of casein at lower pH before their aggregation at pH below 5.5 (Sinaga et al., 2016). In the complex non-enzymatic Maillard reactions, that usually take place during milk processing or storage, lactose is subject to isomerisation and degradation, creating significant amounts of formic acid that lowers the pH. As the pH decreases, the negative net charge on proteins is reduced, promoting inter-micellar interactions, and resulting in precipitation and gelation (Xiang et al., 2021).

2.2.7 Viscosity

Consumer acceptance of milk is highly dependent on its consistency, which requires information about its rheological behaviour during milk processing and storage. Viscosity, a rheological property, is considered an important physical characteristic for assessing the quality change of processed milk during storage. Variation of milk viscosity during prolonged storage is dependent on macromolecular substances including casein, whey protein and fat content. The viscosity of processed milk depends on the temperature and pH. The stability of casein micelles plays a significant role in the overall properties of milk. An increase or decrease in the pH of milk causes an increase in casein micelle voluminosity. Cooling temperature increase viscosity due to the increased voluminosity of casein micelle and temperature above 65°C increases viscosity due to the denaturation of whey protein. It is confirmed that during product storage the milk viscosity remains steady, followed by an increase for a short period and then a sharp increase in the temperatures 10-20°C. Milk stored at the temperature of 2-5°C shows a decrease and then a

sharp increase in milk viscosity (Ting et al. 2016). Therefore, the measurement of milk viscosity is a potential method for measuring milk stability during product shelf life. The viscosity of freshly produced bovine milk ranges from 1.52 to 2.36 mPa·s (mean 1.93 ± 0.21) with no seasonal variation observed (Deeth and Lewis, 2017).

2.2.8 Impact of breed, feed, seasonality, and region

The composition and microbiological quality of milk can be affected by many factors, such as seasonal variations (Kazeminia et al. 2019), breed (Nóbrega and Langoni, 2011), stage of lactation and nutritional status (Kalac and Samkova, 2010), age (Haile-Mariam and Pryce, 2015), regional variations (Skeie et al. 2019) and animal health (Gonçalves et al. 2015).

Environmental factors such as temperature, rainfall, and sunshine often impact on the performance of dairy cows (Lambertz et al., 2014) and different management conditions and housing systems have an influence on milk quality (Bradley et al. 2018).

Milk fat and protein percentages are the highest during the autumn and winter and lowest during the spring and summer. This variation is led to changes in both the types of feed available and climatic conditions. Lush spring pastures low in fibre depress milk fat. The weather and high humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fibre intake (Looper M.,1994). The higher environmental temperatures affect milk fatty acid composition. Milk fat in the summer tends to be lower in palmitic acid. Change in milk fat relates to changes in blood plasma lipids (Linn J.G.,1988).

Nóbrega and Langoni reported (2011) that Holstein and Jersey cows kept under the same conditions in the dry season had estimated means of 3.03% and 4.11% fat in milk respectively and in the rainy season increased to 3.25% and 4.50% respectively. In the dry season protein for Holstein and Jersey cows were 2.87% and 3.32% in the milk, respectively. In the rainy season protein and milk urea, nitrogen levels differed between the breeds.

Milk from Jersey cows has excellent heat stability and reported good ethanol stability (77.00 ±5.70%) when compared to Ayrshire, Brown Swiss, Guernsey, and Holstein cows breeds. The reason for this was increased casein content, a higher level of lactose, and higher κ-casein content. McLean et al. (1987) indicated that milk containing a higher level of both β, and κ-casein resulted in better heat stability, which for Jersey milk was reported to be as high as 91%.

2.2.9 Testing methodology overview

Standard methods have been accepted as official reference methods and have been used for years to evaluate the composition of dairy products. These methods are often labour intensive, while automated routine methods have been developed that allow faster, simpler, and sometimes cheaper procedures. Reference methods are used to assess and verify the reliability of other measurement procedures. They are having the highest metrological properties. Therefore, they are used for routine method calibration and estimation of combined enlarged uncertainties of measurement results by routine methods.

In terms of fat determination, the following individual methods are identified; butyrometric, Folch, Bligh and Dyer, dry column, supercritical fluid extraction, and Weibull-Berntrop. The butyrometric method was developed in 1891 by Niklaus Gerber, and involves a separation of protein from milk fat by adding sulfuric acid. The Folch method principle involves a two-phase participation of the lipid fraction in the organic (chloroform) phase. Bligh and Dyer method principle is the same as the Folch method but it has been adjusted addition of an additional filtration stage so it can be used for testing other dairy products as well as milk. The dry column method involves the extraction of lipid by solvent elution using a dry column composed of anhydrous sodium sulphate and diatomaceous earth. Supercritical fluid extraction is an alternative to the solvent-based extraction method and it is based on a separation technology that uses supercritical fluid as the solvent. The Weibull-Berntrop gravimetric method includes hydrolysis by hydrochloric acid and Soxhlet extraction by n-hexane (Kala et al., 2018).

The following two methods are often used as reference methods; Röse-Gottlieb gravimetric method, that involves hydrolysis by ammonia and extraction by diethyl ether and petroleum ether. Secondly, reference Majonnier gravimetric method principle is based on the separation of the fat fraction (limited to the lipophilic ether phase) from the rest of the milk sample. All stated methods are time consuming, they also show other defects and measurement errors that might evolve i.e. type of glassware or purity of chemicals used potentially can impact on the final result (Kleyn et al, 2001). Within years, the need for the development of fast, reliable and cost effective methods to support daily dairy production evolved. Firstly, turbidimetric analysis were developed where the correlation between fat content and the detected elimination of light dispersed by a milk sample as specific wavelengths was analysed. The ultrasound procedure involves measurement of high-frequency ultrasound radiation passing through the sample. NMR (nuclear) method is based on the absorption of radiofrequency electromagnetic radiation of the nuclei of some atoms in the molecules of the analysed samples located in the magnentic field. TD-NMR (time domain nuclear) is a method that consists of a combined relaxation analysis. Development of the optical spectroscopy quickly replaced some of the standard methods. Spectroscopic methods of determination of fat are based on components absorption. Infrared (IR) spectroscopy involves the measurement of infrared irradiation (invisible, short, near, mid, long or thermal spectrum) absorbed or reflected by a sample. There are various methods identified based on the same principles; FTIR (Fourier transform), ATR-FTIR (attenuated total reflectance) and DRIFTS (diffuse reflectance Fourier transform infrared spectroscopy) (Kala et al., 2018).

Similarly to fat determination, protein measurement through the years involved the development of the standard, reference, and routine testing methods. The same principle is applied, the development of faster, cheaper, and more reliable methods that are used as daily routine tests by the laboratories in commercial production.

The Dumas method, described by Jean-Baptiste Dumas in the early 19th century, involved the burning of the sample and the release of oxygen. This procedure leads to the release of carbon dioxide, water, and nitrogen oxides. The gasses pass through a column that absorbs carbon dioxide and water. The nitrogen was measured of the chemiluminescence reaction of nitrogen dioxide or in the elemental form by the thermal conductivity detection (Kala et al., 2019).

The reference standard method for measurement of protein content is based on the determination of the nitrogen content by the Kjeldahl method. The method consists of transforming all nitrogen in a weighed sample into ammonium sulfate by digestion with sulfuric acid, alkalizing the solution and determining the resulting ammonia by distilling it into a measured volume of standard acid, the excess of which is determined by titration. The other methods are also identified; formol titration where free amino acids and protein-bound amino acids and peptides react with formaldehyde, producing methylene amino acid derivatives and changing the acidity of these amino groups. Colorimetry methods involve measurement of the quantity of electricity after previous neutralisation, oxidation, reduction, eventually precipitation and dye-binding methods. Those are based on the formation of an insoluble dye-protein complex. The insoluble complex is separated and the unbound dye can be determined by spectrophotometer. This led to the development of faster routine methods and as fat measurement involves the use of automated flow analysers which are based formerly on the dye-binding methodology and then replaced as an example by the infrared (IR) spectroscopy: mid-infrared (MIR) using optical selective filters with specific wavelength corresponding to the measured component, Fourier transform (MIR-FT). MIR-FT uses the interferometer (in a whole spectrum) (Kala et al., 2018).

Individual proteins can be separated by electrophoresis and chromatography. 1D PAGE, 2D PAGE and Reversed-phase (RP) HPLC is being used for milk protein analyses (Deeth and Lewis, 2017).

Lactose can be determined by volumetric determination using chloramine T. Lactose can be also determined by polarimetry and enzymatically, based on the determination of NADH formed by the oxidation of β-galactose or the determination of NADPH formed by the oxidation of glucose. Gas chromatography and high-performance liquid chromatography (HPLC) are also used for lactose and other carbohydrates measurements.

Normally, as with fat and protein measurements, nowadays typical dairy laboratory uses automated milk analysers based on Infrared (IR), Mid-infrared (MIR) and Fourier Transform Infrared technique FTIR spectroscopy for lactose measurements (Britz and Robinson, 2008).

Freezing point depression (FPD) is a method of raw milk used to detect any accidental or deliberate additions of water. It is measured by cryoscope that is calibrated by accurate standards. Additionally, Deeth and Lewis (2017) reported that a cryoscope potentially also determines the extent of the reaction during lactose hydrolysis and measures the extent of some chemical changes during the storage of UHT milk.

The total titratable acidity is determined by using the titration method where the milk sample is titrated against standard alkali to the phenolphthalein endpoint. The pH of milk does not truly represent the titratable acidity because of the non availability of the ionic groups to titration in normal milk. pH is measured by the most convenient method by using a pH probe which is an example of a selective ion electrode (Jenness et al., 1988).

2.3 RAW MILK MICROBIOLOGY AND PROTEOLYTIC ACTIVITY

2.3.1 Raw milk microbiome

Milk microbiota is quite dynamic and linked to several host-related and environmental factors (**Table 2.4**). Normally the udder of a healthy animal is habitated by bacteria that belong to *Streptococcus, Staphylococcus and Micrococcus* genera which account for > 50% of total raw milk microflora, followed by *Corynebacterium* spp*., Escherichia coli* and others. Microbial counts of aseptically drawn milk are acceptable, less than 100 CFU/ml but in practice, they usually range from 1,000 CFU/ml to 20,000 CFU/ml (Özer and Akdemir-Evrendilek, 2014). It is difficult to assess if this count is acceptable, and this will depend on the microbiological composition of the count.

There are studies linking the variation of the bacterial population in raw milk with the weather conditions (Li et al. 2018), farms and geographical location (Skeie et al. 2019), breed (Cremonesi et al., 2018), as well as housing management practices (Bradley et al. 2018). As an example, there is a significant difference in microbiological counts and bacteria microbiome identified in raw milk, due to the different bedding materials used for the cows at the farm. Bedding material can cause cross-contamination of milk, therefore different bedding materials are trialled and diffirent milking practices have been validated and when implemented have reduced the initial counts. As an example, foremilking is associated with a reduced total bacterial count in milk (Bradley et al., 2018) and dipping teats in disinfectant and drying, prior to milking is associated with lowered numbers of *Streptococcus* spp*.* and *Enterococcus* spp*.* in milk (Gleeson et al., 2009). Disinfecting clusters between milking different cows reduces thermoduric and psychrotrophic bacteria (Bradley et al.2018). Different drying techniques are proved to be better than others in relation to bacteria management. Gleeson et al. (2009) reported use of disinfecting wipes resulted in a significant reduction in the microbiological counts compared to no teat preparation or washing and drying technique that did not involve any chemical.

Table 2.4: Sources of contamination of raw milk linked with the identified problem and typical bacteria species.

Source	Problem	Bacteria	Study
Udder hygiene	Inflammatory disease of udder (mastatis)	*Staphylococcus areaus* *Staphylococcus agalactiae* *Staphylococcus dysagalactiae* *Streptococcus uberis* *E. coli* *C. freundii* *Enterobacter ssp.* *Klebsiella ssp* *Actinomyces pyogenes* *Listeria monocytogenes* *P. aeruginosa* *C. bovis* *M. bovis* *B. cereus* *B. abortus* *C. brunetti* *Serratia liquefaciens*	(Özer and Akdemir-Evrendilek, 2014) Rainard (2017) Griffiths (2010)
Environment	Faeces	*Enterococci* *Lactobacilli* *Coliforms* *Bifidobacterium pseudolongum* *E. coli* *Salmonella* *Shigella* *Yersinia enterocolitica* *Klebsiella aerogenes*	Özer and Akdemir-Evrendilek (2014) Kagkli et al. (2007)
	Weather conditions (summer)	*Bacillus cereus sensu lato* *Bacillus anthracis* *Bacillus thuringiensis* *Bacillus cereus*	Özer and Akdemir-Evrendilek (2014) Buehner et al.(2014)
	Water	*Pseudomonas* *Achromobacter* *Flavobacterium* *Alcaligenes* *E. coli*	Özer and Akdemir-Evrendilek (2014)
	Hay dust	*Bacillus subtilis* Yeast Mould	Özer and Akdemir-Evrendilek (2014) Robinson (2002)
	Feed	*Bacillus cereus* *Bacillus subtilis* *Geobacillys stearothermophilus*	
	Air	*Micrococci* *Corynebacterium* *Bacillus* *Streptococcus*	Robinson (2002) Lei et al. (2019)
	Bedding	*Kocuria rhizophila* *Kocuria salsicia*	Gagnon et al. (2020)

Source	Problem	Bacteria	Study
Milking practices	Poorly cleaned and desinfected milking equipment	*Lactococcus lactis* *Brevibacterium lines* *Leconostoc mesenteroides* *Lactobacillus* *Lactococcus* *Enterococcus ssp.* *Pseudomonas* *Achromobacter* *Aureomonas* *Flavobacterium* *Micrococcus* *Microbacterium*	Özer and Akdemir-Evrendilek (2014) Fitzgerald and Cotter (2013) Gleeson et al. (2015)
Milk handler	Poorly followed hygiene practices	Human pathogenes	Robinson (2002)

Seasonality-related changes in bacterial counts are also documented and Li et al. (2018) reported that in Shanghai (China), the highest diversity of the bacterial population is in June (the warmest month), while the lowest was reported in December during the coldest weather. *Pseudomonas* spp.*, Lactococcus* spp. and *Acinetobacter* spp. were found to be the most prevalant genera (>1%) identified in raw milk. The highest abundance of *Pseudomonas* spp., *Propionibacterium* spp. and *Flavobacterium* spp. were correlated with low temperatures, which might be due to its psychrotrophic features. *Acinetobacter* spp. was abundant in the summer months since most species are widely distributed in nature and can survive at a broad range of temperatures. There was no obvious trend observed in *Lactococcus* ssp. whose presence can be impacted on by other environmental factors than temperature and humidity. Nóbrega and Langoni (2011) reported that environmental pathogens were more frequently isolated from the Jersey breed, regardless of the season and there seem to be differences in the immune response of Jersey and Holstein breeds. Holstein cows with intramammary infections presented a higher increase in somatic cell counts when compared to Jersey cows. Jersey cows have lower chances

of showing intramammary infections signs and symptoms than Holstein cows during the rainy season.

Cremonesi et al. (2018) suggested that further studies need to be carried out on larger groups of animals from different breeds to identify inter-breed microbiological differences. These differences are due to the innate mechanism of host defence, as well as discrimination below the genus level. Additional studies are needed in order to understand the milk microbiome and its associations with microbiological animal health factors in order to be effectively tackled at the farm level.

Routine identification of bacteria in farm bulk milk samples is crucial as it helps to identify the root cause of problems and sources of contamination. Consequently, different techniques of milk testing can be trialled, in order to eliminate high microbial counts and establish correct management practices specific to the individual farm at various locations.

Deeth and Lewis (2017) stated that the milk microbiome, the body of bacteria that inhibit milk should lead to a better understanding of factors affecting dairy product shelf-life. Deeth and Lewis (2017) reported that raw milk quality used for UHT processing is important to the final product and raw milk should have a total count of bacteria of less than 10^6 CFU/ml and preferable less than 10^5 CFU/ml. This should be considered only as a guide as the risk of contamination from extracellular enzymes cannot be associated with those levels as different bacteria have different prosperities to produce those enzymes.

2.3.2 Individual bacteria counts

There are different methods adopted as a standard in different countries to measure the hygienic condition of the milk produced by the herd. Individual Bacteria Count (**IBC**) called "Bactoscan count" is used for regulatory purposes but is also frequently used by milk processors to determine the milk price received by milk producers. The milk Bactoscan count is a comprehensive reflection of the hygienic conditions of the herd and can be influenced by teat

preparation, the prevalence of intramammary infections in the herd, sanitation of milking equipment and milk storage temperature (O'Connell et al., 2015).

Table 2.5 presents maximum bacteria count levels in raw milk supply implemented by legislation in different countries. There is a national conversion formula completed for individual countries to convert colony forming units per milliliter, established by standard plate count methods count identified in the country legislation, into the Bactoscan count which is expressed by individual bacteria count per milliliter of raw milk. However, Britz and Robinson (2008) reported that the relationship between the standard plate count and Bactoscan values at lower plate counts (i.e. <10,000 CFU/ml) is less consistent than at higher CFU levels.

Table 2.5 Legal requirements comparison in bacterial count results in raw milk reported in different world locations.

Area	Standard (maximum)	Method	Study
USA	100,000 cfu/ml	Plate count at 30°C	US Food and Drug Administration (2017)
Canada	50,000 cfu/ml 121,000 IBC/ml	Mesophilic aerobic plate count Flow cell cytometry on a Bactoscan	National Dairy Code (2015)
Europe	100,000 cfu/ml	Plate count at 30°C	Regulation (EC) 853/2004
Australia New Zeland	100,000 cfu/ml	Aerobic plate count at 30°C / 72 hours or Bactoscan	Code of Practice (2010)
Brazil	750,000 cfu/ml	Plate count at 30°C	Technical Regulation on Production (2002)

Payment schemes developed worldwide by various stakeholders show the ranges of raw milk microbiological quality, which helps to maintain acceptable quality and helps milk processors to produce microbiologically safe and high quality dairy products (**Table 2.6**).

Table 2.6 Compared Bacterial counts obtained from Bactoscan for milk quality payment schemes in different countries.

Area	Premium (IBC/ml)	Acceptable Standard 1 (IBC/ml)	Sub-standard Standard 2 (IBC/ml)	Unacceptable Sub-standard (IBC/ml)	Study
Australia	≤71,000 ≤80,000	71,001-100,000 >80,000-≤200,000	100,001-264,000 >200,000-≤400,000	>264,000 >400,000	Dairy Australia (2016)
UK	0-15,000	16,000-50,000	51,000-100,000	>100,000	Glanbia (2016)
Spain	<30,000				Reguillo et al.(2018)
Brazil	≤75,000cfu/ml	75,001-300,000	301,000-750,000	>750,000	Botaro et al. (2013)

2.3.3 Themoduric bacteria

Thermoduric bacteria have significant implications on the quality of products and reduce product shelf life. Thermoduric bacteria are heat resistant bacteria in milk that survive pasteurisation. Their increased stability is due to protein and cell membranes. Archaeal membranes consist of lipid containing ether bonds, whereas most of bacterial and eucary membranes contain lipids with ester bonds. Lipid membranes with majority of ether-containing lipids stay in liquid crystal state at a wider range of temperatures, and that helps them to survive in such conditions. In addition, several strains produce heat-stable enzymes which continue to break down fat and protein during product storage (Tonget al., 2016).

Thermoduric bacteria include the genera of *Microbacterium* spp*., Micrococcus* spp*., Bacillus* spp*., Clostridium* spp*.,* and *Alcaligenes* spp*.* These microorganisms survive heating at 63°C for 30 minutes and as such can survive the pasteurisation process, to varying extents (Robinson, 2002).

Thermoduric bacteria are used as an indicator of milking equipment sanitation. A high thermoduric bacteria count (**TBC**) indicates chronic cleaning failure. A TBC < 200 CFU/ml is considered normal, whereas TBC < 10 CFU/ml indicates excellent equipment hygiene (O'Connell et al., 2015). Control of thermoduric bacteria is necessary in order to achieve excellent product quality and shelf life, in relation to pasteurised products. Few regions and producing facilities decided to introduce thermoduric bacteria counts into their payment schemes (**Table 2.7**). Additionally, data found for Australia and the UK shows huge differences in recommendations.

Table 2.7 Thermoduric bacteria counts for milk quality payment schemes comparison.

Area	**Premium (cfu/ml)**	**Acceptable Standard 1 (cfu/ml)**	**Sub-standard Standard 2 (cfu/ml)**	**Unacceptable Sub-standard (cfu/ml)**	**Study**
Australia	≤2,000 ≤2,000	2,001-5,000 >2,000-≤5,000	5,001-10,000 >5,000-≤10,000	>10,000 >10,000	Dairy Australia (2016)
UK	0-250		250-500	>500	Glanbia (2016)

2.3.4 Psychrotrophic bacteria

Psychrothrophic bacteria are microorganisms that have the ability to grow at low temperatures but have optimal and maximal growth temperatures above 15°C and 20°C, respectively (Oliveira et al., 2014). These microbes are able to produce thermostable extracellular enzymes such as proteases and lipases in raw milk, a fact which has been highlighted as the cause of numerous defects in dairy products (Kazeminia et al.,2019). An initial count of as little as 10^2 CFU/ml can spoil raw milk during cold storage within five days. Kazeminia et al. (2019) reported that psychrotrophic bacteria have a high ability to multiply from the initial count of 2.6×10^2 CFU/ml to 10^6 CFU/ml in 2.9 days at 6°C and 5 days at 2°C.

Psychrothrophic bacteria in raw milk are represented predominantly by gram-negative bacteria such as *Pseudomonas* spp*., Enterobacter* spp*., Klebsiella* spp*., Acinetobacter* spp*., Aeromonas* spp*., Alcaligenes* spp*., Serratia spp., Achromobacter* spp*., Flavobacterium* spp., and *Chromobacterium* spp., and less so, by gram-positive bacteria such as *Lactobacillus* spp*., Bacillus* spp*., Streptococcus* spp*., Clostridium* spp*., Corynebacterium* spp. *and Microbacterium* spp*.*

Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas fragi, Pseudomonas putrefaciens and *Pseudomonas lundensis* are reported as the most isolated species. *Pseudomonas ssp* and *Enterobacteriaceae* spp. are the most abundant microorganisms that account for up to 95% of the isolates. Gram-negative microorganisms are estimated to account for 90% of the psychrotrophic microflora in raw milk (Özer and Akdemir-Evrendilek, 2014).

Lei et al. (2019) reported that the levels of psychrotrophic bacteria differ in raw milk samples collected from different regions and in different seasons (from 10^2 to 10^7 CFU/ml) with animal husbandry factors such as bedding, air, water, feed, and the cow's teat and udder all listed as potential sources.

According to Sørhaug and Stepniak (1997), a psychrotrophic count of 5.9 log CFU/ml in raw milk causes UHT milk gelation after 20 weeks of storage at 4 - 7°C, while populations between 6.9 and 7.2 logs CFU/ml will cause the same effect between 2 and 10 weeks. UHT milk with these bacterial counts showed gradual development of lack of freshness, slightly stale, unclean, bitter flavour. Oliveira et al. (2015) reported an average psychrotrophic bacteria count of 1.3×10^5 CFU/ml in a silo at several dairies in Scotland. *Pseudomonas* spp. counted for 70.2%, *Enterobacteriacea* spp. 7.7% and Gram-positive bacteria 6.9%.

Worldwide there are few instances of payment schemes introduced for psychrotrophic bacterial counts regularly measured in raw milk supply. National Milk Laboratories (NML, 2019) gives a guideline for acceptable quality milk to be for PBC <500 CFU/ml, and milk of poor quality

PBC >5,000 CFU/ml. Reguillo et al. (2018) report that the EU standard also requires a maximum of 5,000 CFU/ml psychrotrophic count.

2.3.5 Bacterial enzymes

Bacteria produce extracellular proteases and lipases, and their biochemical characteristics are variable and strain dependent. Another characteristic is their thermostability and their resistance can vary between strains (Bagliniére et al. 2012). Proteases and lipases produced by psychrotrophic bacteria may retain activity after heat treatment at 70, 80 or 90°C, and proteases appear to be more heat-stable than lipases (Yuan et al. 2018). Many of the produced enzymes retain significant activity after UHT treatment and degrade proteins and fats present in processed products (Oliveira et al. 2015).

Bacterial proteases prefer κ-casein. However, Deeth and Lewis (2017) stated that the reported specificities of bacterial proteases vary, and this is due to different bacterial species and strains tested or to variation in experimental conditions.

Deeth and Lewis (2017) report that *Pseudomonas* spp. enzymes are 20-30% active following heat treatment for 140°C for 5 sec. However, as an example, *Pseudomonas fluorescens* MC60 protease has been reported to remain 10% active following 149°C for 90 sec (Jelen, 1983). Barach et al. (1976) found that the heat-resistant *Pseudomonas fluorescens* MC60 protease underwent a deactivation at 55°C for 10 min and resulted in the loss of enzyme activity.

Figure 2.1 confirms that most proteases produced by psychrotrophs are heat stable but also suggests that there are differences present between enzymes produced by specific genera and specific strains that show varying thermostability. The processing temperature and time impact on the enzyme thermostability. The increase in time and temperature causes the deactivation of enzymes. The process of enzyme denaturation involves the breaking of many the bonds in which case the enzyme loses its activity. Griffiths et al. (2011) suggests that product shelf life can be extended by deactivating enzymes by deploying low-temperature inactivation at 55°C

for 60 min, innovative steam injection heating, membrane processing, and high-pressure treatments.

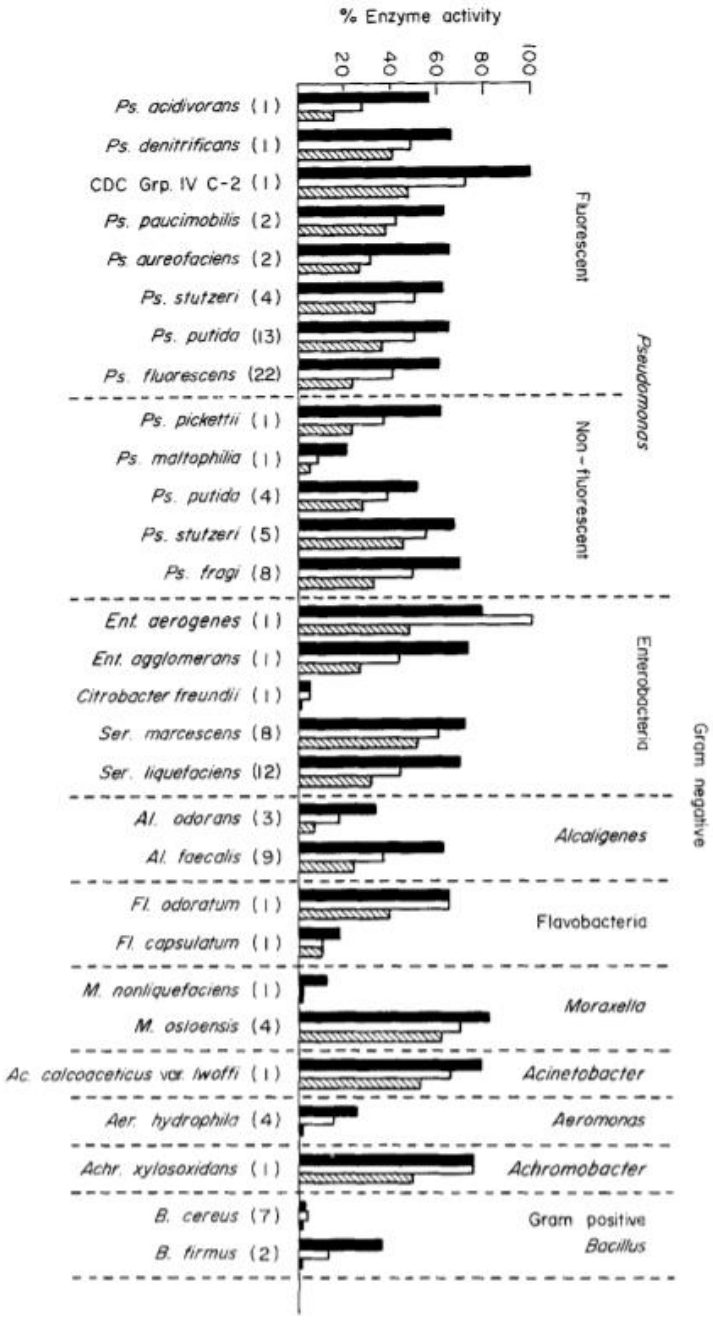

Figure 2.1: Effect of various heat treatments on the protease activity of psychrothrophic bacteria. Cell-free supernatants of bacterial cultures were subjected to heating at 77°C for 17s ■ 77°C for 17s followed by 55°C for 1h □ and 140°C for 5s ▨ The number in parentheses refers to the number of strains of the species for which values have been obtained (Griffiths et al., 1981).

Richardson and Newstead (1979) reported that UHT milk containing as little as 1ng bacterial proteinase/mL may have a shelf-life of only 3 months. Button et al. (2011) reported that as little as 0.0003% of a cell-free supernatant of a *Pseudomonas fluorescens* culture (grown to ~ 10^6 cells/ml in sterile milk), when added aseptically to UHT milk could cause detectable proteolysis during storage at room temperature.

2.3.6 Somatic cells

Milk somatic cell count (**SCC**) is a general indicator of the overall health status of a herd. Somatic cells are part of the innate immune system of the cow and are almost always a reflection of the inflammatory response in dairy cows (Kirkeby et al., 2020). Bulk tank milk from herds with increased SCC has an increased risk of containing antibiotic residues and is an indicator of animal welfare. Barbano et al. (2006) reported that SCC is correlated with increased amounts of heat-stable protease (plasmin) and lipase (lipoprotein lipase) in raw milk. Enzymes associated with high SCC will cause protein and fat degradation during product storage. Both flavour and shelf life of pasteurised dairy products are altered when high SCC milk is used. It is also confirmed that cows with subclinical mastitis produce less milk (O'Connell et al., 2015). SCC is regularly monitored (**Table 2.8 and 2.9**) and used in payment structure by many milk processors. Similarly, to bacterial count, national legal requirements are available for SCC worldwide (**Table 2.10**).

Table 2.8 Somatic cell count averages in different regions of the world.

Area	Year	Count (cells/ml)	Study
Norway	2004	115,000	More (2009)
Sweden	2004	<200,000	More (2009)
Iceland Denmark	2004	<250,000	More (2009)
Ireland	2004	251,000	More (2009)
Brazil	2013	393,000	Botaro et al.(2013)
UK	2018 2019 2020	161,000 165,000 164,000	AHDB (2021)

Table 2.9 Legal requirements comparison in Somatic cell counts in raw milk reported in different world locations.

Area	Standard Maximum legal limit	Source
USA	750,000 cells/ml	US Food and Drug Administration (2017)
Canada	400,000 cells/ml	National Dairy Code (2015)
Europe	400,000 cells/ml	Regulation (EC) 853/2004
Brazil	750,000 cells/ml	Technical Regulation on production (2002)

Table 2.10: Compared Somatic cell counts obtained for raw milk quality payment schemes in different countries.

Area	Premium (cells/ml)	Acceptable Standard 1 (cells/ml)	Sub-standard Standard 2 (cells/ml)	Unacceptable Sub-standard (cells/ml)	Study
Australia	≤250,000 ≤300,000	250,001-400,000 >300,000-≤400,000	400,001-600,000 >400,000-≤600,000	>600,000 >600,000	Dairy Australia (2016)
UK	<150,000	150,000-200,000	200,000-400,000	>400,000	Glanbia (2016)
Brazil	≤300,000	301,000-500,000	501,000-750,000	>750,000	Botaro et al. (2013)

Acceptable levels of SCC counts are achieved in the UK with the average count in 2020 of 164,000 cells/ml (AHDB, 2021).

Nóbrega and Langoni (2011) reported different responses between Holstein and Jersey cows kept under the same conditions. In dry season had estimated SCC means of 282,000 cells/ml and 260 000 cells/ml. In the rainy season this increased to 313,320 cells/ml and 266,070 cells/ml. Jersey cows are reported to have lower SCC levels when compared with SCC levels in milk sourced from other breeds kept under the same conditions. Sabuncu et al. (2013) discussed the effect of cows' age and season on the somatic cell count of dairy cows with subclinical mastitis. When the age of dairy cow increase, SCC increases thus mastitis incidence may rise. It has been reported that the incidence of mastitis can increase in spring and summer, the others reported that the incidence of clinical mastitis is significantly higher in colder months, in winter, and autumn compared to spring and summer (Shathele M.S, 2009). Interestingly, Ivanov et al. (2017) reported no significant seasonal variations of SCC and found that the SCC of milk from the Jersey breed is statistically higher than the other breeds studied, Holstein and Simmental. This is opposite to what has been reported by Nóbrega and Langoni (2011).

2.3.7 Plasmin activity

Plasmin is an endogenous alkaline protease in milk, confirmed to be at higher levels in early and late lactation milk and in milk with increased somatic cell counts. It is an alkaline serine proteinase with an optimum pH of 7.5 at 37°C (Chove et al., 2011). Plasmin is very heat-resistant and survives pasteurisation and even partially higher temperatures such as UHT processing. Deeth and Lewis (2017) suggest that plasmin becomes problematic in products with longer shelf life due to its ability to induce proteolysis-produced peptides which impart a bitter flavour, destabilise the protein and cause age gelation. The preferred substrate for plasmin is β-casein, however, α_{s1}- and α_{s2}-caseins are also hydrolysed, whereas κ-casein is generally considered to be resistant to plasmin activity.

The plasmin system includes the active enzyme plasmin, inactive form plasminogen, plasminogen activator inhibitors, plasmin inhibitors and plasminogen activators.

All components in the plasmin system work together to regulate the proteolytic activity of plasmin. Plasmin activity is determined by the interaction of these components (Deeth and Lewis, 2017). The pH of milk and storage conditions (Ismail and Nielsen, 2010), stage of lactation, feeding methods and time of the year (Nicholas et al. 2002), udder health and increased somatic cells counts (Urech et al., 1999) have the detrimental impact on plasmin system activity.

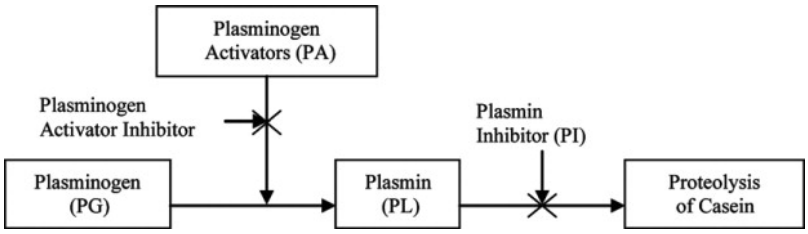

Figure 2.2: Plasmin system in cow milk.

Deeth and Lewis (2017) stated that plasmin activity is also influenced by heat treatment and reported that the higher the heat treatment, the greater inactivation of plasmin. Additionally, an indirect UHT heat treatment process results in less active plasmin in the product than direct UHT processing methods. Ismail and Nielsen (2010) recorded that pre-heating milk at 80-95°C for 30-60 seconds before UHT treatment at 135-150°C for a few seconds inhibited the formation of complexes between whey proteins and caseins. This additional process delays proteolysis but does not interrupt the plasminogen system completely. Plasmin increases during UHT milk storage due to the conversion of plasminogen to plasmin. It is suggested by Ismail and Nielsen (2010) that there is no difference in plasmin activity between milk sourced from Jersey and Hollstein cows.

2.3.8 Spore-forming bacteria

The destruction and removal of spore forming bacteria is the fundamental aim of Ultra-high temperature treatment. A wide variety of bacteria produce highly heat-resistant spores that have emerged as problems over years and are the major cause of UHT milk sterility cases.

G. stearothermophilus, B. licheniformis, B. coagulans, B. macerans and *B. subtilis* are the major cause of the "flat sour" defect. Several other spore formers have been isolated from UHT milk including *B. sporothermodurans, Paenibacillus* spp*., G. stearothermophilus, B. licheniformis, B. coagulans, B. ciculans, B. badius, B. subtilis, B. cereus, B. polymyxa* and *B. spericus*. Some of them can survive the heating process and others would have been present due to post-sterilisation contamination (Deeth and Lewis, 2017). Spores enter the raw milk from a variety of sources. A major source is the environment of a housed cow. Bedding materials, silage and concentrates often carry a high spore load. Bacteria spores are also present in faeces which can be picked up on teats and udders and enter the milk during milking (Schedlman et al., 2005).

2.3.9 Testing methodology review

Microbiological methods

Microbiological methods for raw milk bacteria enumeration include standard plate colony count at 30°C with reference method described by IDF Standard 100B:1991 and ISO Standard 13559:2002. The microbes that are identified are aerobic mesophiles and include lactic acid bacteria, psychrotrophic bacteria, thermoduric bacteria and sporeformes, including pathogenic bacteria. The direct microscopic count is based on the technique developed by Breed (1911). The method does not distinguish between dead and viable cells and, it is time consuming and incorporate significant error due to the sample size. The direct epifluorescent technique (DEFT) is capable of counting individual bacteria counts due to a selective preparation procedure however it remains time consuming method. Spiral plate count (SPC) can be carried out on approximately 50 samples per hour and indicate result in the range of 500 to 500,000 bacteria

per ml. Bactoscan is based on direct microscopic counting of centrifuged and separated bacteria from raw milk and stained with a fluorescent dye. This is an automated method and it has been improved through the years. The newest development from Foss Electrics Bactoscan FC is based on flow cytometry where the DNA / RNA of the bacteria is stained with the fluorescent dye and counted electronically as light impulses in a continuous flow fluorescent microscope. Certain buffers and enzymes are added during sample preparation to reduce the influence of other milk constituents. The sample is treated with a lysing solution and so bacterial clumps are dissolved, a fact that leads to more accurate counts. This is currently the quickest and the most accurate measurement available however involves higher capital expenditure than compared with other methods (Britz and Robinson, 2008).

In addition to the total bacterial counts, the enumeration of contaminating and indicator microbes needs to be examined. For these, reference methods are used. These include methods for thermoduric bacteria and psychrotrophic bacteria enumeration. Those methods are labour intensive, require prolonged incubation periods and rely on the ability of the microorganisms to replicate and their growth rate. During the last years, many rapid microbiological tests have been developed, including antibody and nucleic-acid-based methods that include DNA hybridization, polymerase chain reaction (PCR), miniaturised biochemical kits, modified conventional methods and selective membranes (Britz and Robinson, 2008).

Protein stability

According to Shew (1981), ethanol stability (**ES**) provides a simple way of indicating whether raw milk is suitable for UHT processing, with ethanol stability of 74% being the threshold below which milk is not suitable. Milk with lower ethanol stability is more susceptible to fouling and sedimentation during heat treatment and storage (Chen et al., 2012). Deeth and Lewis (2017) explain that milk may have a low ethanol stability (<74%) due to either poor microbiological quality, which is accompanied by a drop in pH, or salt imbalance. Any factors that reduce the

negative charge of the protein and incur change in the proportions of casein fractions in the micelle may impact ethanol stability.

Ethanol stability (**ES**) is determined by mixing equal volumes of milk and a range of ethanol solutions. The milk-ethanol solution is observed to identify the presence of any milk clots/ coagulation. The highest ethanol concentration at which coagulation does not occur was determined as the ethanol stability of the milk.

Protease activity and proteolysis measurements

Different methods are used to measure protease activity and proteolysis measurement during product storage and shelf life. There are two groups of methods for measuring proteolysis in UHT milk during storage. First group measures aromatic amino acids in trichloroacetic acid (**TCA**)-soluble peptides. These aromatic amino acids are detected by reaction with the Folin-Ciocaltey phenol reagents as in the methods of Hull (1947) and Lowry et al., (1951). The other group of proteolysis detection methods measure free amino groups (**FAG**) in TCA-soluble peptides. These include the trinitrobenzensulfonic (**TNBS**) acid, fluorescamine, O-phthaldialdehyde and ninhydrin methods. In addition to these, gel electrophoresis and reversed-phase high-performance liquid chromatography (**RP-HPLC**) are used. Before performing these methods, the proteolysis product gets extracted with TCA or by lowering of pH to 4.6 by acetic acid (Vaghela et al., 2017).

The oldest Hull method that determined the proteolysis in milk is not sensitive. TCA-soluble peptides are mixed with sodium carbonate and react with phenol reagent. Aromatic hydroxyl groups in tyrosine and tryptophan reduce the phenol reagent and give a blue colour that is measured at 650nm in a spectrophotometer. In this method chemicals interfere with the reaction of the phenol reagent and aromatic amino acids and peptides that do not have aromatic amino acids, are not detected.

Fluorescamine is a sensitive method that measures protease activity that is specific for primary amino groups and free of interference. The method is simple and rapid. It requires expensive equipment, a spectrofluorometer. It requires a small quantity of reagents, and it is more reliable than the traditional Hull method.

O-phthalddialdehyde (**OPA**) is also a rapid, sensitive method. A-amino groups released by hydrolysis react with O-phthaldialdehyde and form an adduct that absorbs strongly a 340nm.

Trinitrobenzenesulfonic (**TNBS**) acid method has the possibility to detect lower levels of proteases. The method is simple and rapid. Method principle is based on the reaction of TNBS with amino acids and changing colour to yellow measured at 420nm. The reaction is carried out in borate buffer at pH 9.5. The reaction is stopped by lowering pH after the amino groups have been trinitrophenylated. Chove et al. (2011) compared the TNBS method with other methods i.e. RP-HPLC, gel electrophoresis and fluorescamine for analysis of proteolysis in milk and recommended TNBS for use in routine laboratory analysis based on its accuracy, reliability, and simplicity. The TNBS method was used as it has been confirmed to be a more sensitive and accurate method to measure the proteolysis level in milk (Skaridis and Lewis, 2016).

In the ninhydrin method, amino acids react with ninhydrin hydrate at pH 5 and 100°C for a standard time, yielding a purple-blue compound (diketohydrindylidene and diketohydrindamine) which absorbance is measured at 570nm. Yellow product (proline and hydroxyeproline) is measured at 440nm. The method requires additional cooling and heating steps and it is labour intensive.

Reversed-phase high-performance liquid chromatography (**RP-HPLC**) analysis is reliable and separates peptides from casein hydrolysates. Molecules are separated based on hydrophobicity. RP-HPLC is used for detecting proteolysis in milk and has been shown to exhibit greater sensitivity and reproducibility than a TNBS (Vaghela et al., 2017) and fluorescamine-

based method (Datta and Deeth, 2006). RP-HPLC is accurate and reported as 600 times lower than those determined by the fluorescamine methods. The method's disadvantage is expensive laboratory equipment to perform the analysis (Vaghela ate al., 2017). The RP-HPLC analysis is labour intensive and involves higher capital expenditure once compared with other methods. The RP-HPLC method is useful for distinguishing between peptides produced by plasmin and bacterial proteases. The peptides released by bacterial proteinases are small and hydrophilic while those released by native protease, plasmin, are large and hydrophobic. The peptides formed from plasmin hydrolysis remain soluble at pH 4.6 but precipitate in 4% TCA extracts, it is possible to analyse all the peptides and determine the type of protease(s) causing the proteolysis. Deeth and Lewis(2017) Suggested that in order to differentiate these peptides products formed by either plasmin or bacterial proteinases, 12% trichloroacetic acid (**TCA**) must be analysed. The TCA filtrate shows substantial peptide peaks only if milk was contaminated by bacterial proteinase, while the pH 4.6 filtrate showed peptide peaks when either or both bacterial and native milk proteinases caused the proteolysis. A procedure based on these analyses is proposed as a diagnostic test for determining which type of proteinase- milk plasmin, bacterial proteinase, or both is responsible for proteolysis in UHT milk.

Polyacrylamide Gel Electrophoresis (**PAGE**) analysis is used to separate milk proteins and peptides derived from them. In peptide analysis, PAGE can be used qualitatively for identifying the presence of peptides or quantitatively with the use of a densinometer. Gels are commonly run with a protein-denaturant sodium dodecyl sulphate (**SDS**) or urea to break non-covalent bonding within and between proteins. They can also be run under non-reducing or reducing conditions. In the latter, disulfide bonds are cleaved to sulfhydryl groups by heating the sample with mercaptoethanol or dithiothreitol before being loaded onto the gel. After the gel is run, the protein bands are visualised by staining the gel in a solution of a protein-binding dye such Comassie Blue and then distaining the background gel with dilute acetic acid. For proteolysis of

milk proteins, the major bands of interest are those of para-κ-casein, which is a fair indication of bacterial proteolysis and γ-caseins, protease-peptones and λ-casein which are a good indication of plasmin-mediated proteolysis of β-, α_{s1}- and α_{s2}-caseins (Deeth and Lewis, 2017).

One of the applied methods is the free amino nitrogen (**FAN**) method which is a ninhydrin calourimetric method used for the calculation of the concentration of amino acids and small peptides that are utilised by microorganisms reported in the European Brewery Convention (Lie, 1973).

2.4 ULTRA HIGH TEMPERATURE PROCESSING

2.4.1 UHT processing

Ultra Heat Treatment (**UHT**) is a thermal process to obtain commercially sterile products and it is designed to result in preferably 12 log reduction of *Clostridium botulinum*. Commercially sterile means that the treated product is free of viable microorganisms and their spores which would be capable of growing in the treated product when kept in a closed container under normal non-refrigerated conditions at which the food is likely to be held during manufacturing, distribution, and storage (Britz and Robinson, 2008). UHT processing of milk combined with aseptic packaging was introduced to produce shelf-stable products with much less chemical change compared to in-container sterilised milk (Deeth and Lewis, 2017). During UHT treatment, the product is exposed to a brief but intense heating, normally temperatures in the range of 130 to 145°C. Common holding times range from 2 to 45 seconds (David et al. 1996). Codex Alimentarius (2004) states that the continuous flow of heat for a short time should be no less than 135°C and in the range of 135 to 150°C in combination with a suitable holding time. The process should be able to secure that product remain microbiologically stable after incubating for 15 days at 30°C in closed containers or for 7 days at 55°C in closed containers or after any other method demonstrating that the appropriate heat treatment has been applied.

2.4.2 UHT milk processing and packing methods

There is a few manufacturers of aseptic equipment. Commercial manufacturers include Tetra-Pak, Scholle, Elopak, SIG Combibloc, and the Dole Aseptic Canning System. Aseptic packaging systems for dairy products include drum and bin systems, heat during blow moulding, carton packaging machines, bag-in-box packaging systems, bulk tanks and containers, plastic cups, pots, cartons, pouches, and sachets. The equipment uses as sterilant either heat, pressurised steam, hydrogen peroxide on its own or with heat, radiation (Holdsworth, 1992).

2.4.3 Process flow and requirements

According to Deeth and Lewis (2017), there are two basic principles of UHT processing that distinguish it from in-container sterilisation. Principle one claims that for the same bacterial destruction, a high-temperature-short-time treatment results in less chemical change than a low-temperature-long time treatment. Principle two establishes that minimum times and temperatures are dictated by the need to inactivate thermophilic bacterial spores while the maximum times and temperatures are determined by the need to minimise undesirable chemical alterations. For this reason B value (9-log reduction of thermophilic spores), C value (3% reduction of thiamine), and F_0 value (lethality index) are calculated to support and establish the correct processing conditions.

There are two main heat exchanger technologies used for heating in UHT processing, direct and indirect systems. Steam, hot water, and electricity are heating methods for UHT equipment. The heat exchangers utilising steam or hot water can be subcategorised as direct or indirect heating systems. In the indirect system, the product and heating medium do not come into contact, as a barrier (stainless steel) is present. Direct heating modes include steam injection (steam into milk), steam infusion (milk into steam), and scraped surface. Indirect heating modes include indirect spiral tubes, indirect tubes, indirect plates, scraped surfaces and electricity. The indirect heating with electricity includes electric elements, conductive heating, and friction.

Documented experience suggests that a direct UHT system results in less fouling, better heat transfer and less heat damage to the final product. Indirect technology requires less capital expenditure than the direct system (Chevan et al., 2011).

2.4.4 Sterility and shelf life testing methods

Product packages are tested by the milk producers for sterility by a selection of incubating temperatures. A standard temperature of 30°C and incubation for up to 15 days is chosen to check for mesophilic bacteria growth. Additionally, the temperature at 55°C for incubating for up to 7 days for testing for spore-forming bacterial growth is carried out.

David et al. (1996) stated that an effective sampling inspection plan will catch all defective units. For example, to verify an overall defect rate of 1:10,000, 22,500, 30,000 and 46,000 units at 90, 95 and 99% confidence limits will need to be tested. Routine inspections of packs as well as strict control over all processing parameters are necessary in order to detect serious errors. Commercial sterility is assured by testing a small but statistically random sample, followed either by visual testing, pH testing or subculturing and bacteriological analysis. The bacterial analysis involves streak standard plating or more innovative methods based on bioluminescence technology. The current testing technologies developed by either Celsis or 3M utilises adenosine triphosphate (ATP) bioluminescence and use the enzyme luciferase to catalyze the consumption of microbial ATP which produces light. Those technologies reduce testing time and give instant results. Manufacturing factories preincubate products at intervals to ensure regulatory compliance and self-imposed internal Food Safety and Quality Assurance programmes.

The shelf-life testing methodology involves regular sampling and testing of products during their shelf life. This involves measurement of the quality parameters to ensure compliance with product specification that is based on customer product acceptability. Currently, standard dairy manufacturers visually assess UHT samples for all quality attributes that include taste, appearance, smell, or viscosity.

2.5 CHEMICAL, MICROBIOLOGICAL AND PROTEOLYTIC MILK REQUIREMENTS FOR UHT PROCESSING

Although UHT milk is commercially sterile, it is not possible to inactivate all heat-resistant enzymes and thereby prevent chemical and physical, and sometimes enzymatic, reactions from taking place and changing the quality attributes of the product and reducing product shelf life (Deeth & Lewis, 2017). Milk deterioration during product shelf life is due to microbial, structural, and chemical degradation (Wilby, 1997).

2.5.1 Shelf life

Shelf life is the storage time before quality drops to an unacceptable level. David et al. (1996) reported that long life product is by definition product that is commercially sterilised, kept in refrigeration and will have a shelf life from 2 to 3 months. Sterile product is shelf-stable with a shelf life of about 1 to 2 years at ambient temperatures. However, storage of products at elevated temperatures may reduce the shelf life. Temperature abuse throughout storage and distribution can result in product quality defects as discolouration, separation, and gelation (Rippen, 1969).

UHT milk is produced either as a stand by-product to be used when pasteurised milk is not available or it is produced as a major type of milk available and is used regularly. In the first case, shelf life is expected to reach a longer period of time 6-12 months and in the second case 3 months shelf life is applicable and there is no need for the extension. According to Deeth and Lewis (2017) UHT milk may have a shelf-life of up to 12 months, although in practice is usually consumed much earlier than this.

The reported validated shelf life of UHT dairy products stored at ambient temperatures is between 4-6 months (D'Incecco et al. 2018), and 6-9 months (Bimbo et al. 2016; Richards et al. 2014).

Export market shelf life expectations can be longer, as long part of the shelf life product spends in the distribution chain before it reaches the final customer.

2.5.2 UHT milks sensory defects

The chemical and physical product changes during storage are the most important factors affecting product shelf-life. Those changes impact the product quality resulting in customer complaints. These changes include i.e., proteolytic, lipolytic, oxidative and Maillard type reactions (Singh et al., 2009).

2.5.2.1 Chemical and physical reactions

Fat separation (creaming), sediment formation, browning (Maillard reactions), age gelation or development of off-flavours during subsequent storage are the most common sensory defects reported in the literature for UHT milk.

Higher storage temperature causes faster loss of stability, with the exemption of solubilisation of caseins and colloidal calcium phosphate, a process that is faster at lower storage temperature (Anema, 2017). The other factors that affect the stability of milk include: heat during UHT processing, dissolved oxygen availability, and milk composition and raw milk microbiological quality (Deeth, 2010).

<u>Creaming / Fat rise in UHT milk</u>

Fat separation is an undesirable UHT milk quality defect. It negatively impacts general product perception by the customer. Creaming or fat rise is the formation of a fat-rich layer at the top of containers of milk products. Obviously, creaming is only an issue in samples that contain significant levels of fat. The excessive fat rise is usually due to inadequate homogenisation and/or flocculation of fat globules to form larger fat particles. Data & Deeth (2003) state that the cause of fat separation is unclear and it can be associated with age gelation due to aggregation of casein associated with homogenised fat globules. Creaming is also reported to be faster at higher storage temperatures than at lower storage temperatures (Anema, 2019).

Sedimentation

The phenomenon can be described as the natural settling of the colloidal particles in milk. The rate of settling can be estimated by Stokes' law. Lewis et al. (2011) confirmed that there is a relationship between the level of sedimentation and the pH and/or ionic calcium levels in milk. Low pH (≤6.5) at processing is a major cause of sedimentation and relates to the heat stability of the caseins. At constant milk pH conditions, the sediment levels increased when ionic calcium levels are increased (by adding soluble calcium), whereas sediment levels decrease when ionic calcium levels are reduced (Anema, 2018).

Gaur et al. (2018) reported that direct UHT processing is more prone to sediment formation than the indirect system because less fouling deposit attaches to walls of heat exchanges and stays in milk. Regardless of the process, sediment from UHT milk is composed of κ-casein-depleted casein micelles and low levels of denatured whey protein. The stability of casein micelles is largely associated with the concentration of K-casein at the surface of the micelles, therefore the casein micelles that are depleted in K-casein are less stable and will be more prone to aggregation via calcium bridging, either when the pH of the milk is lower, or the ionic calcium level is higher than certain critical levels. The aggregated casein micelles settle to form the sediment. In order to minimise sedimentation, control over pH and ionic calcium levels in raw milk supply are necessary. Raising the pH or lowering the ionic calcium levels of the milk reduces or eliminates sedimentation, whereas lowering the pH or adding ionic calcium levels increases the level of sedimentation. Additional reports suggest that downstream post-processing homogenisation reduces sedimentation (Anema, 2018).

Maillard reactions

Those involve the formation of flavour compounds and brown pigments. Maillard reaction additionally impacts pH. As lactose is subject to isomerisation and degradation, significant amounts of formic acid are created that lower the milk pH.

2.5.2.2 Microbiological and enzymatic reactions

In milk, enzymes can be endogenous or exogenous (i.e., of bacterial origin). Endogenous enzymes originate from cows' blood or from the somatic cells (Le at e., 2017).

Proteolysis caused by endogenous enzyme activity

Plasmin activity in relation to proteolysis is important. Plasmin induces hydrolysis of β-casein, resulting in γ-casein, protease-peptone, and smaller peptides, and is strongly correlated with milk storage temperature (Mortensen et al., 2010). Plasmin and plasminogen activators can be active during cold storage of milk resulting in increased proteolysis and levels of plasmin activity. However, storage of milk at 5°C results in decreased levels of plasmin activity caused by autolysis of plasmin compared to milk stored at room temperature. (Ismail and Nielsen, 2010, Somers et al., 2002, Cruddenet al., 2005).

Proteolysis is also caused by bacterial proteases produced by a variety of bacteria that are in raw milk. Those either act in milk prior to heat-treatment or survive heat treatment and induce proteolysis during milk storage. Proteolysis is characterised by the development of bitter flavour and age gelation.

Age gelation and protein cross-linking

According to Malmgren et al. (2017), age gelation is a process that involves proteins. It is a physico-chemical effect involving non-enzymatic rearrangements of proteins during UHT milk processing and enzymatic modification of caseins by proteases. Gonzálwa et al.(2020) suggest that there is no agreement on a single mechanism describing this process, but report that the UHT milk age gelation occurs as a two-step process, where firstly polypeptides dissociate from casein micelles. During UHT processing of milk, β-lactoglobulin (β-Lg), the major whey protein in milk, denaturates, because of the applied heat. It complexes with κ-casein (κ-CN) forming a β-Lg- κ-CN complex on the surface of the casein micelles. In the second stage, a three-dimensional gel network is formed (Raynes et al., 2018). The enzymes that are involved in this

process is mainly of two types: the native milk alkaline proteinase, plasmin and heat-stable extracellular proteinases produced by bacteria in milk, prior to treatment. Plasmin may cause soft gels while bacterial proteases give rise to hard gels (Data & Deeth, 2003). Rauh et al. (2014) demonstrated that increased plasmin activity could be a potential cause of age gelation and bitterness in UHT milk produced. Data & Deeth (2003) confirmed that storage of UHT milk at low (4°C) and high (35-40°C) temperatures delays onset of age gelation. Storage at 25-30°C is optimum for gel formation. **Table 2.11** presents confirmation of example of defects caused by the presence of bacteria and their enzymes in dairy products.

Table 2.11 Overview of defects caused by psychrotrophic bacteria and their enzymes in a range of dairy products.

Bacteria / Enzyme	Criteria	Impact	Study
Psychrothrophic bacteria	Pasteurised milk	Ropy milk Synthesis of polysaccharides	Jay et al. (2005)
High SCC **High psychrothrophic bacteria**	UHT milk Direct steam processing system	Bitterness, gelation, and sedimentation due to bacterial protease and plasmin activity	Topçu et al. (2006)
Gram negative psychrothropic bacteria	Apparent when numbers reach 10^7-10^8 cfu/g	Unpleasant, putrid odour	Bell et al. (2005)
High SCC and high psychrothrophic bacteria count	SCC 621,000 cells/ml PBC 4,872 cfu/ml	Bitterness, gelation, and sedimantation Defects were reduced when milk was processed at a higher temperature (150 rather than 145°C)	Topçu et al. (2006)
***Pseudomnas* spp.**	Pasteurised milk	Detection of fruity, bitter, rancid, and unclean off-flavours Coagulation of milk proteins	Robinson et al. (2002)
Bacillus cereus	Counts >10^6 cfu/ml	Sweet curdling	Özer and Akdemir-Evrendilek (2014)

Bacteria / Enzyme	Criteria	Impact	Study
Psuedomonas weihenstephanensis ***Pseudomonas proteolytica*** ***Psueodomonas* spp.**	Not identified Apparent enzyme activity ≥0.03pkat mL^{-1}	Product defects occurred in order: bitterness-particles-creaming-sediment-gelation A linear correlation found between proteolitic activity and onset of product defects, apart from onset of gelation	Stoeckel et al. (2016)
Psychrothrophic bacterial count	< 8.0 x 10^6 8.0 x 10^6 5.0 x 10^7	Age gelation noticed within, days >140 ~63 ~12	Chavan et al. (2011)
Heat stable proteases	UHT milk	Development of bitter peptides and age-gelation Protease the most active against κ-casein and β-casein with minor activity against α-casein and little /no activity against whey protein	Robinson (2002)
Pseudomonas fluorescens **Protease AprX**	UHT milk	Induced compact gels Almost all κ-casein was hydrolysed (degree of hydrolysis (DH) exceeded 1.3%) Plasmin induced soft gels. Around 60% of both β- and α_{s1}-casein were hydrolysed and DH reached 2.1%	Zhang et al. (2018)
Psuedomonas **LBSA1** **Protease AprX** **Serralysin family**	UHT milk Activity pH 6-10 Optimal temperature activity 40°C	Presence of sediment Zeta potential of casein decreased Content of non-casein nitrogen and non-protein nitrogen increased Hydrolysed casein but not whey protein	Matéos et al. (2015)
Serratia liquefaciens L53 **Ser2**	UHT milk	Presence of sediment Zeta potential increased Formation of aggregates Released peptides were identified during storage Caseins were hydrolysed in the preferential order β->α_{s1}->κ->α_{s2}. No specific peptidic hydrolysed bond was detected.	Baglinière et al.(2017)

SCC- Somatic cell counts

Fernandes et al. (2008) reported that increased somatic cell counts has an impact on UHT milk stability after 120 days of storage. Increased SCC induced lipolysis as measured by free fatty acids increased during storage. The viscosity of UHT milk increased and casein degradation was confirmed. SCC increase the proteolysis, although the correlation coefficient between SCC and casein content was not high, hence indicating that other factors are involved in the process. The other factor impacting on UHT milk stability is plasmin activity. Plasmin activity was confirmed to cause proteolysis, and increased rate of casein hydrolysis. Plasmin caused a decrease in pH and changes in colour. Gelation occurred along with an increase in viscosity and extensive proteolysis of αs- and β-caseins. Plasmin activity was confirmed to be involved in age gelation and bitterness caused by proteolysis (Rauh et al., 2014).

Flavour issues

The are various flavour issues reported in the literature. Cooked flavour normally is caused by heat treatment and it can be reduced by minimisation of UHT process temperature and time. Stale and oxidised flavours are reported due to the development of aldehydes. Rancid and soapy flavour due to free fatty acids caused by residual bacterial lipases. The bitter flavour is developed by the activity of residual bacterial proteases or plasmin and the development of bitter peptides. Additionally, volatile compounds that negatively impact on UHT milk flavour are developed during Maillard reactions.

Lipolysis

Enzymatic hydrolysis of milk lipids to free fatty acids and partial glycerides is caused by endogenous milk enzymes and those of microbial origin (Deeth and Fitz-Gerald, 1983). The endogenous lipoprotein lipase is inactivated by the UHT process; however, bacterial lipases can create increased amounts of free fatty acids, resulting in rancid flavours. Indirect heat treatment is confirmed to be more effective in inactivating lipases than the direct heat treatment process

(Panfil-Kuncewicz et al., 2005). The heat resistant bacterial lipases can survive UHT treatment. *Pseudomonas* spp. MC50 lipase is extremely heat resistant, and it can survive a temperature process of 121 to 149°C for 5 to 8 seconds. The optimum temperature for MC50 lipase activity is 40°C (Adams and Bradley, 1981).

Oxidation

During lipid oxidation, fatty acids are broken down into oxidation products. Several biochemical changes take place which decrease the nutritional value of the product but also leads to oxidation products. The rate of oxidation depends on the fatty acid composition. Indigenous and bacterial lipases cause hydrolysis of triglyceride molecules resulting in the formation of free fatty acids and glycerol. Free fatty acids caused by residual bacterial lipases lead to the development of objectionable rancid, soapy flavour in UHT milk. Most of the fatty acids in milk fat are esterified in triacylglycerols or phospholipids. Non-esterified fatty acids also called free fatty acids (FFA) are primarily formed in dairy products by the enzymatic breakdown of glycerides by lipase activities. The free fatty acids content in milk is low and has low flavour thresholds, especially short-chain FFA and provides the characteristic flavour and odour of many dairy products, particularly, the flavour of fermented dairy products, and especially cheese. However, elevated levels of short-chain free fatty acids, especially C4:0, are also responsible for rancidity in milk and other dairy products. The rancid flavour becomes unacceptable to the consumer (Amores and Virto, 2019). Bacterial enzymes that survive UHT treatment can cause hydrolytic rancidity of milk during ambient storage conditions (Ajmal et al., 2018).

2.5.2.3 Impact of processing conditions

The UHT processing conditions that have an impact on the quality and presence of defects in UHT milk (**Table 2.12**). It becomes apparent from the review that intense heat treatment and also direct heat treatment method result in more noticeable defects: age gelation, sedimentation.

Heat coagulation depends on the pH, calcium ion concentration and casein micelle size. The reduction of pH significantly decreases the heat coagulation time. This became a key factor to predict milk stability and its suitability for UHT processing. High protein stability minimises fouling during the UHT process and reduces sediment formation in the product.

Table 2.12 Review of defects caused by chosen processing UHT conditions.

Product	Processing condition	Impact	Study
UHT milk	Direct system	Shows more sediment formation than milk from indirect systems	McMahon et al. (1996)
	Increased heat load and increased storage temperature	Sedimentation increased	
UHT skimmed milk	Heat treatment of 75°C/3s +143°C/3s Direct steam injection	Gelled relatively rapidly due to the proteolitic degradation of caseins through the indigenous plasmin enzymes in milk	Anema (2017)
	More severe heating through pre-heating and indirect UHT treatment	Confirmed to destroy the plasmin enzymes Increased UHT milk stability	
UHT milk	Microfiltration	Delayed the formation of gel particles and sediment. Slowed down the proteolysis in terms of accumulation of peptides although no correlation was observed between the two phenomena	D'Incecco et al. (2018)
	Double homogenisation	Narrowed the fat globule distribution and distrupted the fat-protein aggregates The adopted conditions avoided the appearance of the cream layer	

Product	Processing condition	Impact	Study
UHT milk	Direct steam processing Higher temperature (150°C rather than 145°C)	The proteolysis and defects were reduced by processing the milk at a higher temperature This caused a more intense cooked flavour but the overall acceptability was not affected.	Topçu et al. (2006)

2.6 Conclusion

The Jersey breed has been found to produce milk with a higher percentage of fat, protein, and solids than milk sourced from Holstein or Guernsey cows. Somatic cell counts levels were reported to be lower for Jersey cows, however, plasmin activity was reported to be at a similar level. There is limited information available in the literature, especially in relation to herds' size once the values of the components are reported. The development of genetics, technology, and nutritional studies in relation to feeding has the potential to improve those chemical values further.

Raw milk microbiological quality is important worldwide due to its impact on processed milk and other dairy products' shelf life and keeping quality. In many parts of the world, legislation was introduced to control raw milk quality, but the threshold values for legislative and recommended requirements can vary significantly between different countries. Additionally, in some areas, payment initiatives are introduced by processors to control and improve raw milk quality used at processing facilities. It was confirmed that raw milk microbiological microflora is influenced by many aspects including environmental factors, hygiene, husbandry, and management procedures at the farm but also by breed and season. The importance of the control over raw milk microbiological quality i.e. psychrothrophic bacteria counts due to the enzyme's direct impact on milk components and additionally the aspect of enzymes

thermostability on UHT milk stability was confirmed relevant and studied extensively. Solely from raw milk quality, this expanding knowledge requires to be taken into consideration once the processor intends to produce a product with long shelf life and with excellent quality that is valued and accepted by the customer.

From this review, there is a recognised benefit to report the nutritional and microbiological values of Jersey raw milk produced on the island of Jersey to provide a benchmark and expand on the literature for milk produced by this type of breed.

It is proved to be necessary to investigate further region tailored knowledge of the different type of bacteria and heat resistant spoilage enzymes producing microbiota in raw milk that will help to trace the contamination sources in the supply chain as currently there is a lack of expanded knowledge about the impact of many isolates and enzymes produced by raw milk bacteria on the stability of UHT shelf life. The rate of physicochemical changes in UHT milk stability caused by specific factors needs to be better understood and resolutions applied by the industry practice.

CHAPTER 3

3. IMPACT OF SEASON AND PAYMENT STRUCTURE ON CHEMICAL AND MICROBIOLOGICAL QUALITY OF JERSEY RAW MILK PRODUCED BETWEEN 2014 AND 2019

3.1. INTRODUCTION

In this chapter, the impact of season, raw milk quality incentives and payments structure on the chemical and microbiological quality of Jersey raw milk produced by pedigree Jersey cows on the island of Jersey (**Figure 3.1**) between 2014 and 2019 are discussed. The objective of this study was to present the chemical and microbiological profile of Jersey raw milk used in UHT milk processing and the impact of changes in payment structure on the quality of Jersey raw milk from 2014 to 2019.

The chemical and microbiological quality of raw milk is routinely monitored by dairy manufacturers in many countries, to ensure that milk-derived products meet legislative requirements and to minimise the development of quality defects in the final dairy products. There is a growing commercial interest from more dairy manufacturers to produce products with extended shelf life such as UHT milk. These products enable dairy manufacturers to secure additional export contracts. As such, the relationship between raw milk microbiological quality and its impact on UHT shelf life needs to be investigated in more detail.

In Jersey, the quality of raw milk is routinely measured by using Individual Bacteria Count (**IBC**), thermoduric bacteria count (**TBC**), psychrotrophic bacteria count (**PBC**), somatic cell counts (**SCC**), fat, lactose, protein contents, and freezing point depression (**FPD**). In addition, the agreed payment structure specifies bands for bonuses and penalties for all those quality parameters. This structure is reviewed annually and adjusted to ensure continuous improvements in the Jersey raw milk supply. Jersey Dairy produces a wide range of dairy products; pasteurised milk and cream, butter, yogurts, ice cream, UHT milk, and UHT recipe-based products (**Figure 3.2**).

For all those products, control over raw milk quality supply becomes important, as it enables longer shelf life of products and brings company confidence that pasteurised and ultra-high-temperature (UHT) treated products are maintaining their excellent quality until the end of their shelf life.

In this chapter, an assessment of the seasonal variation in chemical and microbiological properties of Jersey raw milk produced between January 2014 and December 2019, from pedigree Jersey cows on the island of Jersey was performed. Additionally, an assessment of silo milk prevalence and variance of bacteria in the years 2014 -2019 was carried out. This knowledge will aid in improving the development and choice of new sanitation procedures and process controls to ensure the consistent production of high-quality raw milk and dairy products.

Figure 3.1: Pedigree Jersey cows grazing on the island of Jersey.

Figure 3.2: Jersey Dairy processing facility.

3.2. MATERIALS AND METHODS

3.2.1. Experimental design

Data collection was conducted on the island of Jersey, Channel Islands, UK. Milk samples were collected as a part of the Rules of Supply sampling routine by the Jersey Dairy laboratory under my supervision from individual Jersey milk producers of all farms located on the island. All herds comprised pedigree Jersey cows. I fully managed this entire project as I was employed by Jersey Dairy as Head of Quality and Senior Manager during years of study.

Samples from 23 farms were tested weekly between January 2014 and December 2019 for pH, composition (fat, protein, lactose, total solids (**TS**) content), and the somatic cells count (**SCC**). Thus, 1,196 samples were analysed each year, giving a total of 7,176 samples analysed during 6

years of study. Individual bacterial count (**IBC**) was carried out two or three times a week and weekly averages were calculated for each of the farms. Thus, 840 samples were collected from each producer, giving a total of 19,320 samples tested for **IBC** during 6 years of study. All assays were performed in duplicates. Bi-monthly samples were collected for thermoduric bacteria count (**TBC**) and psychrotrophic bacteria count (**PBC**). Thus, 24 samples were analysed annually from each producer. This resulted in a total of 3,312 samples of raw milk being collected from the 23 milk producers between January 2014 and December 2019.

Additionally, raw milk samples were taken bi-monthly during 6 years of data collection from January 2014 till December 2019 from two Jersey Dairy silos at the processing plant for analysis of milk prevalence bacteria and reported accordingly, giving a total of 288 observations.

The seasons were defined as spring **SP** (March, April, and May), summer **SM** (June, July, and August), autumn **A** (September, October, and November) and winter **W** (December, January, and February). Jersey's daily temperature (mean air) (°C), monthly rainfall (mm) and monthly sunshine (h) were sourced from Jersey Government Climate statistics (**Appendix 1**).

3.2.2. Milk sampling

Jersey raw milk samples were aseptically collected in sterilised plastic containers at milk delivery to the processing plant and transported at a maximum of 2 hours at 3°C +/-2°C to an accredited Jersey Dairy testing laboratory (**JDL**). The weekly samples were immediately used to carry out the physicochemical analysis. The bi-monthly samples were kept in ice coolers and transported the same day by air to the Quality Milk Management Services in Somerset (**QMMS**) for further analysis to be initiated on the morning of the second day. Delivered samples were checked on arrival for temperature and were accepted for analysis if the temperature was less than 6°C.

3.2.3 Microbiological testing

Individual Bacterial Counts (**IBC**) were measured by flow cytometry method using Foss Bactoscan FC (Foss, Hillerød, Denmark) and Foss Bacsomatic (Foss, Hillerød, Denmark). Somatic cell count (**SCC**) was measured by flow cytometry method using Delta Combiscope, Model FTIR 600 (QCL, Forest Row, UK) and Foss Bacsomatic (Foss, Hillerød, Denmark).

Thermoduric bacterial count and direct plating were carried out in in QMMS (Somerset, UK).

Thermoduric bacterial counts (**TBC**) were determined by using Milk Plate Count Agar (VWR, Lutterworth, UK). Milk (5 ml) was heated to a temperature that stimulates pasteurisation (63.5 +/- 0.5°C for 30 min), followed by immediate refrigeration at 20°C. After treatment, serial dilution of the samples was performed up to 10^{-3} is sterile peptone (VWR, Lutterworth, UK) and spread on Milk Plate Count Agar and incubated at 30°C for 72h. Following this time thermoduric bacteria were enumerated. Psychrotrophic bacteria counts (**PBC**) were carried out in Jersey Dairy Laboratory in Jersey and determined using Milk Plate Count Agar (VWR, Lutterworth, UK) incubated at 3°C for 10 days.

Direct plating was performed using sheep blood agar (Thermoscientific, Basingstoke, UK) and then the identity of isolates to confirm species was further confirmed using Matrix Assisted Laser desorption/ionisation time of flight- mass spectrometry MALDI-TOF MS (MALDI Biotyper Microflex, Bruker Daltonics, Coventry, UK).

3.2.4 Physicochemical testing

All physicochemical analysis was carried out at Jersey Dairy Laboratory. Analysis of fat, protein, lactose, and solids non-fat contents was performed by Fourier transform Infra-Red Spectrophotometry (FTIR) using Foss Milkoscan FT120 (Foss, Hillerød, Denmark). Freezing point

depression (FPD) of milk samples was measured by cryoscopy using 4D3 Cryoscope (Advanced Instruments Inc., Metuchen, USA).

3.2.5 Data sets

Combined data sets represented 100% of the population of Jersey dairy herds (n=23) on the Island. Small farms were classed as those that produce less than 1,000,000 litres of raw milk annually (n=13 herds), whereas large farms were classed as those producing more than 1,000,000 litres of raw milk annually (n=10 herds). The combined data set represents 100% of the entire population of Jersey dairy herds, silo represents the average adjusted by volume of milk delivered from the individual farm (n=23), which represents milk used at the processing facility.

3.2.6 Payment data

Jersey Dairy pricing data is confidential. Bonus and penalty structures are shared (**Table 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6**) and represent quality classification bands and adjustments in pence per litre of milk that were made in payment structure to improve Jersey raw milk quality in years from January 2014 till December 2019.

In April 2016 changes were made in payment structure for Individual bacteria counts and the highest bonus was paid for milk with IBC < 16,000 IBC/ml and penalty was paid for milk with IBC > 31,000 IBC/ml. In April 2017 further changes were made and adjustment was three times higher for milk with IBC < 16,000 IBC/ml and penalty applied for milk IBC > 51,000 (**Table 3.1**). High bonuses were paid for fat content from April 2013 till the end of March 2014. Following discussions about trends on the market, the decision was made to reduce bonuses paid for fat content from April 2014. They remained static till April 2018. Following bonuses were increased and remained at this level till the end of 2019 (**Table 3.2**).

Bonuses were paid for SCC ≤ 200,000 cells/ml with the highest bonus for SCC ≤ 100,000 cells/ml. This was changed to SCC ≤ 120,000 cells/ml in April 2018. Penalties were applied for SCC > 251,000 cells/ml from April 2013 till December 2019 (**Table 3.3**).

The payment structure for thermoduric bacteria counts was introduced in April 2013. Bonuses and bands were adjusted after the first 12 months of running the payment scheme. After 1st April 2014, the highest bonus was paid for milk with TBC ≤ 175 CFU/ml and involved an additional 1ppl for milk. Further adjustments were made from April 2016 where a bonus was increased by another 0.5ppl and the band threshold was changed to ≤ 100 CFU/ml. At the same time penalty was introduced for milk with TBC > 500 CFU/ml which previously was at the same level for milk with TBC > 700 CFU/ml. Following those changes, the payment scheme remained unchanged till the end of 2019 (**Table 3.4**).

The payment structure for psychrotrophic bacterial counts was introduced in April 2016. The highest bonus is paid for milk with PBC ≤ 25 CFU/ml and the penalty were deducted from the base milk price for milk with PBC > 500 CFU/ml. The scheme structure remained unchanged since it was introduced (**Table 3.5**).

The payment structure for protein content was introduced in April 2018 once trends on the milk market were noted in relation to the value of higher protein content in customer diets. The highest bonus was implemented for protein content above 4.5% which accounted for additional 1.5 ppl added to the base milk price (**Table 3.6**).

Table 3.1 Payment structure for Jersey raw milk Individual Bacteria Count (**IBC**, Bactoscan) from January 2014 till December 2019.

Bands	01/04/2013-31/03/2014 (IBC/ml)	adj.[1]	1/04/2014-31/03/2015 (IBC/ml)	adj.[1]	1/04/2015-31/03/2016 (IBC/ml)	adj.[1]	01/04/2016-31/03/2017 (IBC/ml)	adj.[1]	01/04/2017-31/03/2018 (IBC/ml)	adj.[1]	01/04/2018-06/04/2019 (IBC/ml)	adj.[1]	01/04/2019-31/12/2019 (IBC/ml)	adj.[1]
1	0-30,000	0.5	0-30,000	0.5	0-30,000	0.5	**0-15,000**	0.5	0-15,000	1.5	0-15,000	1.5	0-15,000	1.5
2	31,000-50,000	0	31,000-50,000	0	31,000-50,000	0	**16,000-30,000**	0	16,000-30,000	0.75	16,000-30,000	0.75	16,000-30,000	0.75
3	51,000-75,000	-0.5	51,000-75,000	-0.5	51,000-75,000	-0.5	**31,000-50,000**	-0.5	31,000-50,000	0	31,000-50,000	0	31,000-50,000	0
4	76,000-100,000	-1	76,000-100,000	-1	76,000-100,000	-1	**51,000-100,000**	-1	51,000-100,000	-1	51,000-100,000	-1	51,000-100,000	-1
5	101,000-150,000	-2	101,000-150,000	-2	101,000-150,000	-2	**101,000-150,000**	-2	101,000-150,000	-2	101,000-150,000	-2	101,000-150,000	-2
6	>151,000	-5	>151,000	-10	>151,000	-10	**>151,000**	-10	>151,000	-10	>151,000	-10	>151,000	-10

[1] Payment adjustment to the base milk price in ppl.

Table 3.2 Payment structure for Jersey raw milk **fat** results from January 2014 till December 2019.

Bands	01/04/2013-31/03/2014 (%)	adj.[1]	1/04/2014-31/03/2015 (%)	adj.[1]	1/04/2015-31/03/2016 (%)	adj.[1]	01/04/2016-31/03/2017 (%)	adj.[1]	01/04/2017-31/03/2018 (%)	adj.[1]	01/04/2018-06/04/2019 (%)	adj.[1]	01/04/2019-31/12/2019 (%)	adj.[1]
0	<5.40	0												
1	5.40-5.59	0.75	> 6.00	0.3	> 6.00	0.3	> 6.00	0.3	> 6.00	0.3	> 6.00	1.5	> 6.00	1.5
2	5.60-5.79	1.5	5.70-5.99	0.2	5.70-5.99	0.2	5.70-5.99	0.2	5.70-5.99	0.2	5.70-5.99	1	5.70-5.99	1
3	5.80-5.99	2.25	5.40-5.69	0.1	5.40-5.69	0.1	5.40-5.69	0.1	5.40-5.69	0.1	5.40-5.69	0.75	5.40-5.69	0.75
4	6.00-6.19	3	< 5.40	0	< 5.40	0	< 5.40	0	< 5.40	0	< 5.40	0	< 5.40	0
5	6.20-6.39	3.75												
6	6.40-6.59	4.5												

[1] Payment adjustment to the base milk price in ppl.

Table 3.3 Payment structure for Jersey raw milk somatic cell counts (**SCC**) from January 2014 till December 2019.

Bands	01/04/2013-31/03/2014 (cells/ml)	adj.[1]	1/04/2014-31/03/2015 (cells/ml)	adj.[1]	1/04/2015-31/03/2016 (cells/ml)	adj.[1]	01/04/2016-31/03/2017 (cells/ml)	adj.[1]	01/04/2017-31/03/2018 (cells/ml)	adj.[1]	01/04/2018-06/04/2019 (cells/ml)	adj.[1]	01/04/2019-31/12/2019 (cells/ml)	adj.[1]
1	0-100,000	1.5	0-100,000	1.5	0-100,000	1.5	0-100,000	1.5	0-100,000	1.5	0-120,000	1.5	0-120,000	1.5
2	101,000-150,000	1.2	101,000-150,000	1.2	101,000-150,000	1.2	101,000-150,000	1.2	101,000-150,000	1.2	120,000-150,000	1	120,000-150,000	1
3	151,000-200,000	0.4	151,000-200,000	0.4	151,000-200,000	0.4	151,000-200,000	0.4	151,000-200,000	0.4	151,000-200,000	0.4	151,000-200,000	0.4
4	201,000-250,000	0	201,000-250,000	0	201,000-250,000	0	201,000-250,000	0	201,000-250,000	0	201,000-250,000	0	201,000-250,000	0
5	251,000-300,000	-1	251,000-300,000	-1	251,000-300,000	-1	251,000-300,000	-1	251,000-300,000	-1	251,000-300,000	-1	251,000-300,000	-1
6	> 301,000	-2	> 301,000	-2	> 301,000	-2	> 301,000	-2	> 301,000	-2	> 301,000	-2	> 301,000	-2

[1] Payment adjustment to the base milk price in ppl.

Table 3.4 Payment structure for Jersey raw milk thermoduric bacteria count (**TBC**) from January 2014 till December 2019.

Bands	01/04/2013 - 31/03/2014 (cfu/ml)	adj.[1]	1/04/2014- 31/03/2015 (cfu/ml)	adj.[1]	1/04/2015- 31/03/2016 (cfu/ml)	adj.[1]	01/04/2016- 31/03/2017 (cfu/ml)	adj.[1]	01/04/2017- 31/03/2018 (cfu/ml)	adj.[1]	01/04/2018- 06/04/2019 (cfu/ml)	adj.[1]	01/04/2019- 31/12/2019 (cfu/ml)	adj.[1]
1	0-299	0.5	0-175	1	0-175	1	0-100	1.5	0-100	1.5	0-100	1.5	0-100	1.5
2	300-699	0	176-300	0.5	176-300	0.5	101-200	1	101-200	1	101-200	1	101-200	1
3	700-999	-0.5	301-700	0	301-700	0	201-300	0.5	201-300	0.5	201-300	0.5	201-300	0.5
4	> 1000	-1	701-1000	-0.5	701-1000	-0.5	301-500	0	301-500	0	301-500	0	301-500	0
5			> 1000	-1	> 1000	-1	501-1000	-0.75	501-1000	-0.75	501-1000	-0.75	501-1000	-0.75
6							> 1000	-1.5	> 1000	-1.5	> 1000	-1.5	>1000	-1.5

[1] Payment adjustment to the base milk price in ppl.

Table 3.5 Payment structure for Jersey raw milk Psychrothrophic bacteria count (**PBC**) from January 2014 till December 2019.

Bands	01/04/2016-31/03/2017 (cfu/ml)	adj.[1]	01/04/2017-31/03/2018 (cfu/ml)	adj.[1]	01/04/2018-06/04/2019 (cfu/ml)	adj.[1]	01/04/2019-31/12/2019 (cfu/ml)	adj.[1]
1	0-25	1	0-25	1	0-25	1	0-25	1
2	26-50	0.5	26-50	0.5	26-50	0.5	26-50	0.5
3	51-500	0	51-500	0	51-500	0	51-500	0
4	501-750	-1	501-750	-1	501-750	-1	501-750	-1
5	751-1000	-2	751-1000	-2	751-1000	-2	751-1000	-2
6	> 1000	-3	> 1000	-3	> 1000	-3	>1000	-3

[1] Payment adjustment to the base milk price in ppl.

Table 3.6 Payment structure for Jersey milk **protein** results from January 2014 till December 2019.

Bands	01/04/2018-06/04/2019 (%)	adj.[1]	01/04/2019-31/12/2019 (%)	adj.[1]
1	> 4.50	1.5	> 4.50	1.5
2	4.00-4.49	0.75	4.00-4.49	0.75
3	3.50-3.99	0.5	3.50-3.99	0.5
4	< 3.50	0	< 3.50	0

[1] Payment adjustment to the base milk price in ppl.

3.2.7. Statistical Analysis

Statistical analysis was carried out using Minitab Software 2020 (Minitab Ltd, State College, Pennsylvania). Descriptive statistics were used to analyse and present Jersey's raw milk profile. Data were subject to ANOVA to detect any statistical differences between seasonal variations and the impact of month and season on the quality of raw milk. Differences were considered significant at $p < 0.05$. The Pearson correlation method was used to establish whether the correlation coefficients between quality and seasonal parameters were significant. These included Jersey quality milk parameters including fat, protein, lactose and SNF (Solids Non-Fat) content, freezing point depression, somatic cell counts, individual bacteria count, psychrotrophic bacteria count and thermoduric bacteria counts and Jersey island weather parameters including daily temperature, monthly rainfall, daily sunshine and seasonality by season and month. Yearly and monthly means were compared using One-way Anova. The Tuckey test was used to group means variance between months. Grouping Information using the Tukey method and 95% confidence. The effect of Jersey payment adjustments and milk quality variables was assessed using paired t-test and was found significant at $p < 0.05$.

3.3 RESULTS AND DISCUSSION

3.3.1 Physicochemical analysis of Jersey raw milk

Table 3.7 presents the fat, protein, lactose, solids non-fat (SNF), and freezing point (FPD) composition of the samples. The highest recorded fat result was 6.80%, protein 4.70% and lactose 4.87%. The lowest and the highest results recorded for fat, protein, and lactose were recorded from milk produced by the small herds. The mean fat for the silo data set was 5.29%, protein 3.76%, lactose 4.51%, SNF 9.24% and FPD 523 (-m°C).

Table 3.7 Distribution of fat, protein, and lactose, and solids non-fat, freezing point of 23 Jersey dairy farms from 2014 to 2019.

Item	Data set[1]	No. of records	Mean	St Dev	Minimum	Median	Maximum
Fat (%)	Combined	6279	5.28	0.34	4.20	5.30	6.80
	Small	3435	5.26	0.38	4.20	5.20	6.80
	Large	2844	5.31	0.29	4.40	5.30	6.50
	Silo	6279	5.29	0.21	4.82	5.29	5.71
Protein (%)	Combined	6258	3.80	0.17	2.80	3.80	4.70
	Small	3424	3.84	0.19	2.80	3.80	4.70
	Large	2834	3.75	0.14	3.40	3.80	4.40
	Silo	6258	3.76	0.11	3.57	3.76	4.01
Lactose (%)	Combined	6279	4.49	0.09	3.11	4.50	4.87
	Small	3435	4.47	0.10	3.11	4.48	4.80
	Large	2844	4.51	0.08	4.02	4.52	4.87
	Silo	6279	4.51	0.04	4.43	4.51	4.62
SNF (%)	Combined	6279	9.26	0.19	6.20	9.30	10.00
	Small	3435	9.28	0.20	6.90	9.30	10.00
	Large	2844	9.24	0.17	6.20	9.20	9.90
	Silo	6279	9.24	0.13	9.00	9.24	9.62
FPD (-m°C)	Combined	6279	523	5	357	523	544
	Small	3435	522	6	357	522	544
	Large	2844	523	5	486	523	539
	Silo	6279	523	2	520	523	528

[1] Combined data set represents 100% of the entire population of Jersey dairy herds (n=23); small data set represents producers that produce < 1,000,000 litres of milk annually (n=13 herds); large data set represents producers that produce >1,000,000 litres of milk annually (n=10 herds); silo represents an average adjusted by volume of milk delivered from the individual farms (n=23). **SNF-** Solids Non-fat **FPD**- Freezing point depression.

3.3.2 Microbiological analysis of Jersey raw milk

Microbiological results were presented in **Table 3.8**. The mean Bactoscan for the silo data was 17,900 IBC/ml, for SCC 137,569 cells/ml, thermoduric bacteria 242.2 CFU/ml and psychrotrophic bacteria 103.9 CFU/ml.

Table 3.8 Distribution of Bactoscan (**IBC**), Somatic cell count (**SCC**), Thermoduric count (**TBC**), Psychrothrophic count (**PCB**) of 23 Jersey dairy farms from 2014 to 2019.

Item	Data set[1]	No. of records	Mean	SE Mean	St Dev	Minimum	Median	Maximum
Bactoscan (IBC/ml)	Combined	24507	22,000	405	63,460	1,000	18,000	8388,000
	Small	12859	23,780	703	79,718	1,000	19,000	8388,000
	Large	11648	20,780	353	38,114	1,000	17,000	3044,000
	Silo	24507	17,900	540	4,582	8,000	19,000	27,000
Somatic cell count (SCC) (cells/ml)	Combined	6279	157,194	915	72,500	21,000	145,000	1147,000
	Small	3435	174,601	1,355	79,431	21,000	164,000	1147,000
	Large	2844	136,169	1,057	56,378	28,000	124,000	498,000
	Silo	6279	137,569	930	7,890	111,000	139,000	159,000
Thermoduric count (TBC) (cfu/ml)	Combined	2850	395.6	35.9	1,865.7	< 1	175.0	62,600.0
	Small	1559	507.9	63.2	2,496.0	<1	180.0	62,600.0
	Large	1291	259.9	9.7	347.9	<1	170.0	6,000.0
	Silo	2850	242.2	12.4	105.0	79.0	213.0	544.0
Psychrothrophic count (PCB) (cfu/ml)	Combined	2826	552.7	178.3	9,476.7	<1	40.0	492,000.0
	Small	1546	870.0	35.1	12,784.1	<1	50.0	492,000.0
	Large	1280	169.5	22.9	819.2	<1	30.0	18,650.0
	Silo	2826	103.9	13.9	117.5	6.0	70.0	649.0

[1] Combined data set represents 100% of the entire population of Jersey dairy herds (n=23); small data set represents producers that produce < 1,000,000 litres milk annually (n=13 herds); large data set represents producers that produce >1,000,000 litres of milk annually (n=10 herds); silo represents an average adjusted by volume of milk delivered from the individual farms (n=23).

3.3.3. Jersey raw milk seasonality and improvements

The impact of season on the composition of Jersey silo milk is shown in **Table 3.9 and 3.10**.

Table 3.9 Seasonal distribution of fat, protein, lactose, and solids non-fat, the freezing point of milk collected from 23 Jersey dairy farms from January 2014 to December 2019.

Item	Season	Mean	SE Mean	St Dev	Minimum	Median	Maximum
Fat (%)	Autumn	5.35	0.05	0.20	4.99	5.36	5.71
	Spring	5.28	0.03	0.14	4.98	5.28	5.51
	Summer	5.05	0.03	0.12	4.82	5.07	5.28
	Winter	5.47	0.03	0.01	5.25	5.46	5.71
Protein (%)	Autumn	3.82	0.03	0.11	3.65	3.81	4.01
	Spring	3.74	0.01	0.06	3.63	3.74	3.85
	Summer	3.63	0.01	0.05	3.57	3.64	3.74
	Winter	3.83	0.01	0.06	3.76	3.82	3.98
Lactose (%)	Autumn	4.48	0.01	0.02	4.43	4.48	4.52
	Spring	4.54	0.01	0.03	4.50	4.54	4.61
	Summer	4.53	0.01	0.03	4.48	4.54	4.56
	Winter	4.50	0.01	0.05	4.43	4.49	4.62
SNF (%)	Autumn	9.28	0.03	0.14	9.02	9.25	9.62
	Spring	9.22	0.01	0.06	9.12	9.22	9.37
	Summer	9.13	0.02	0.08	9.00	9.12	9.32
	Winter	9.35	0.03	0.13	9.16	9.29	9.58
FPD (-m°C)	Autumn	522	0	2	520	522	526
	Spring	523	0	2	521	523	526
	Summer	522	0	1	520	522	524
	Winter	524	1	2	521	523	528

SNF- Solids Non-fat; **FPD**- Freezing point depression.

Number of records is based on weighted average for season from combined data set.

Table 3.10 Seasonal distribution of Individual bacteria counts (**IBC**), somatic cell counts (**SCC**) thermoduric bacteria counts (**TBC**) and psychrotrophic bacteria counts (**PBC**) of milk collected from 23 Jersey dairy farms from January 2014 to December 2019.

Item	Season	Mean	SE Mean	St Dev	Minimum	Median	Maximum
Bactoscan (IBC/ml)	Autumn	18,111	1,087	4,613	10,000	19,000	27,000
	Spring	17,444	1,097	4,655	8,000	19,500	23,000
	Summer	16,056	1,074	4,556	9,000	16,000	23,000
	Winter	20,000	0,925	3,926	13,000	21,000	25,000
SCC (cells/ml)	Autumn	137,556	1,939	8,226	111,000	139,000	149,000
	Spring	135,222	1,610	6,830	124,000	136,000	146,000
	Summer	140,889	1,802	7,646	128,000	142,500	159,000
	Winter	136,611	1,954	8,290	124,000	139,000	152,000
TBC (cfu/ml)	Autumn	260.5	24.1	102.2	79.0	251.5	422.0
	Spring	182.6	14.7	62.2	81.0	174.0	321.0
	Summer	306.3	28.9	122.5	146.0	283.5	544.0
	Winter	219.6	20.7	87.7	93.0	199.5	427.0
PBC (cfu/ml)	Autumn	90.2	23.9	101.4	7.0	79.5	447.0
	Spring	81.9	18.8	79.7	8.0	63.0	354.0
	Summer	143.2	39.7	168.3	6.0	79.0	447.0
	Winter	100.1	24.0	101.9	16.0	62.0	449.0

SCC- Somatic cell counts; **TBC**- Thermoduric bacteria count; **PBC**- psychrothrophic bacteria count

Number of records is based on weighted average of combined data set.

A seasonal trend was observed for monthly compositional results (**Figure 3.4**). Mean results were statistically different ($p < 0.05$) between months and seasons indicating a seasonality trend in fat, lactose, protein, and SNF (Solids Non-Fat) content. The means between seasons were statistically significant for the freezing point ($p=0.0053$) but they were not between months ($p=0.0590$).

There is no statistical difference recorded for Bactoscan (IBC) ($p>0.05$) and psychrothrophic bacteria counts (PBC) ($p>0.05$) between months and seasons (**Figure 3.5**). Thermoduric bacteria

count means were statistically different between months (p=0.0376) (**Figure 3.5b**) and seasons (p=0.0019), the lowest mean was recorded for spring 183.0 CFU/ml (April 175.0 CFU/ml) and the highest for summer 306.0 CFU/ml (July 346.0 CFU/ml). Somatic cell counts were statistically different between months (p=0.0421) (**Figure 3.5d**) but not between seasons (p=0.1683).

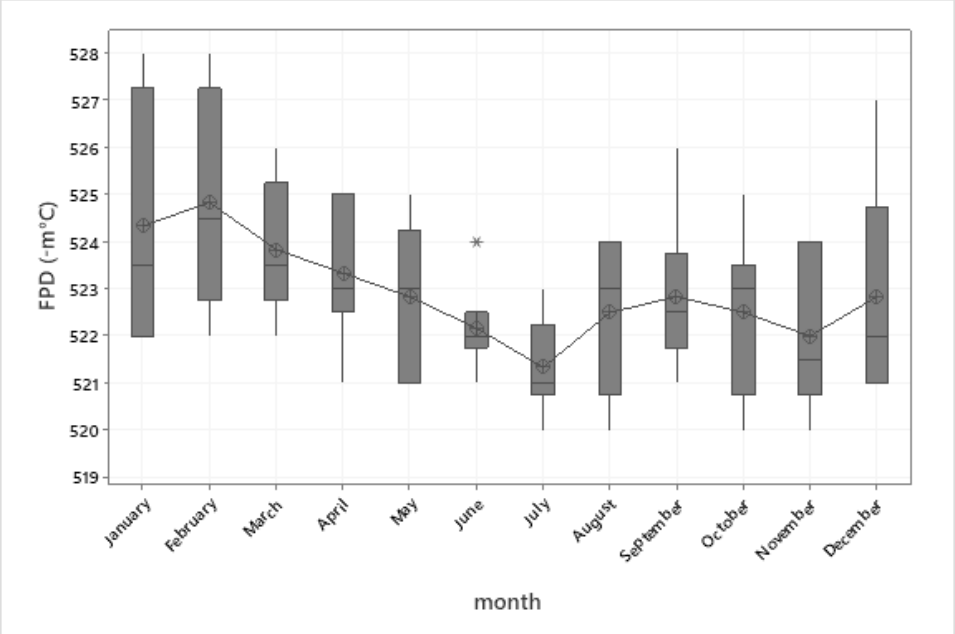

Figure 3.3 Boxplots of freezing point depression (p=NS, R^2=0.2572) versus months for Jersey dairy raw milk from January 2014 to December 2019.

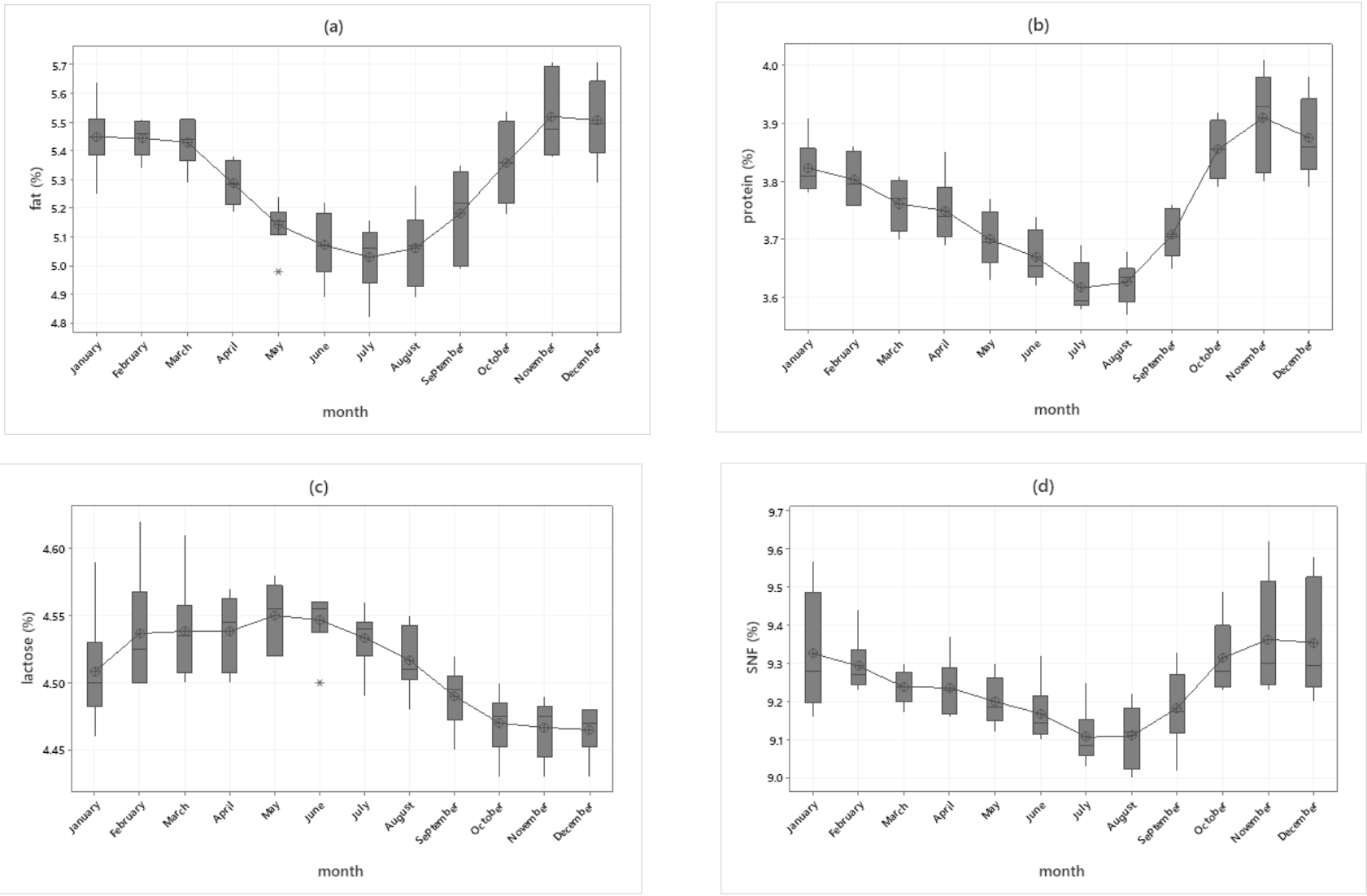

Figure 3.4: Boxplots of (a) fat (p=0.000, R^2=0.7188), (b) protein (p=0.000, R^2=0.7837), and (c) lactose (p=0.000, R^2=0.5679), and (d) solids non-fat (p=0.000, R^2=0.4501), versus months for Jersey dairy raw milk from January 2014 to December 2019.

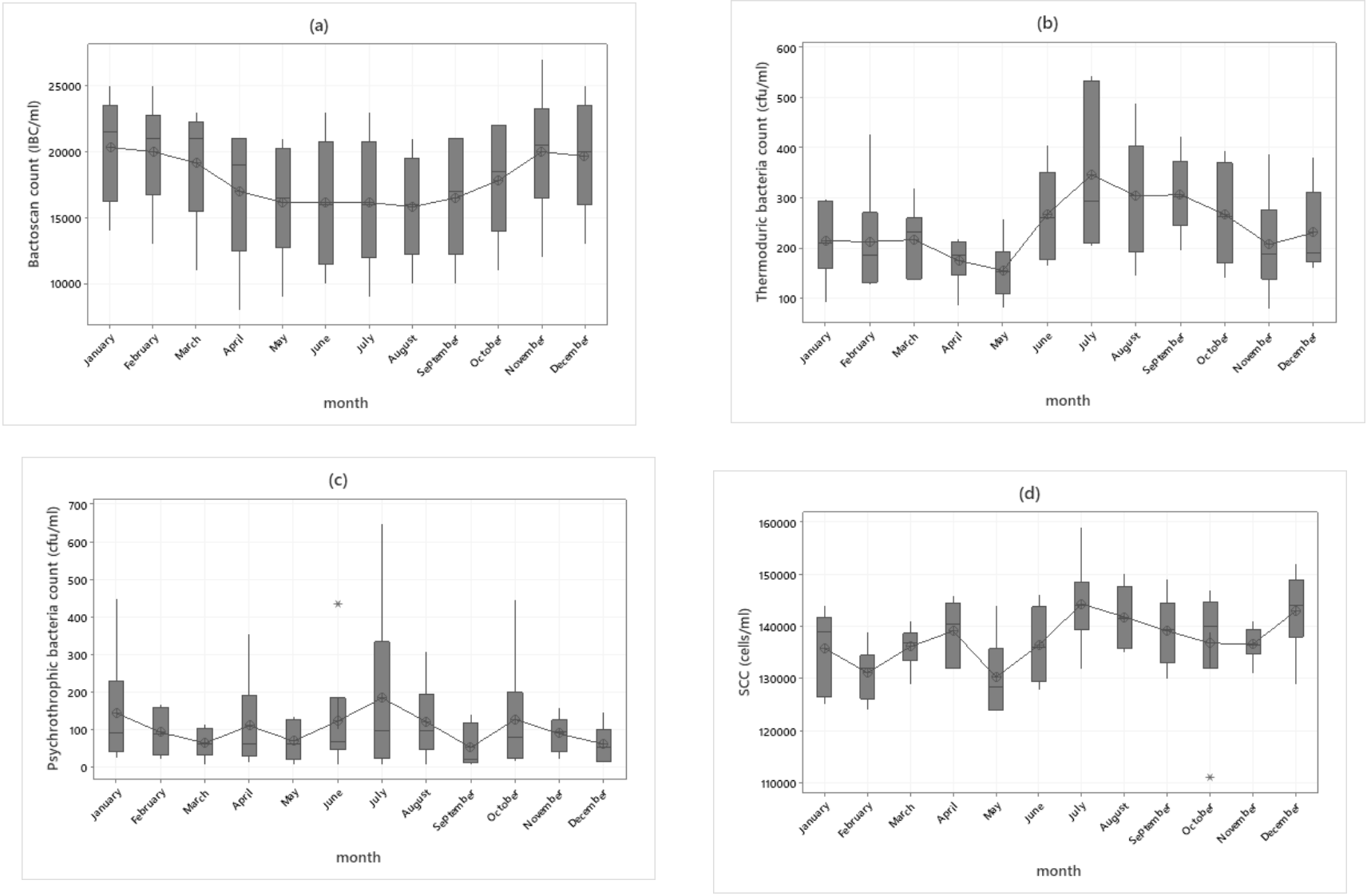

Figure 3.5: Boxplots of (a) Bactoscan (p=NS, R^2=0.1430), (b) thermoduric bacteria count (p=0.038, R^2=0.2743), and (c) psychrothrophic bacteria count (p=NS, R^2=0.1034), and (d) somatic cell counts (p=0.042, R^2=0.2700), versus months for Jersey dairy raw milk from January 2014 to December 2019.

The lowest silo means fat result was reported in 2018 in July (4.8%) and the highest value was reported in November 2019 (5.7%). Bonus for fat was introduced in April 2018 (**Table 3.2**). Paired t-test confirmed that fat content results significantly improved after the bonuses and penalties scheme was adjusted in April 2018 (p=0.0004). Since the introduction of protein bonuses in the payment scheme (**Table 3.6**), an increase in protein content has been observed. Paired t-test confirmed that protein content means increased after the bonuses and penalties scheme was adjusted in April 2018 (p=0.0007). Changes in cow's feed made an improvement in this composition.

The mean Bactoscan count for silo average which is indicative of the milk quality at the processing facility for the years 2014 – 2019 was 17,900 IBC/ml and for combined data 22,000 IBC/ml (**Table 3.8**). The maximum recorded value was 8,388,000 IBC/ml recorded for milk produced by small producer. Bactoscan counts year on year dropped from 21,750 IBC/ml in 2014 to 10,833 IBC/ml in 2019 due to the work carried out at farms and incentives in the payment structure (**Table 3.11**). There was a significant difference between annual means. There was no significant difference between monthly means (p >0.05), indicating no seasonality impact (**Table 3.10**). The improvement work involved change to the farm plant cleaning regimes. Those included alternative acid and caustic cleans, chemicals strengths review and cleaning validations. Additionally, improvements in efficiency of cooling systems and cow udder pre-milking preparations that involved the use of disinfecting sprays, paper towels, sterilant wipes and wet clean cloths. The equipment preventative maintenance regime for milking equipment at the farm was reviewed. Those alteration had a positive impact on all microbiological raw milk attributes measured during the years of study. Paired t-test shows that Bactoscan results improved following the bonuses and penalties scheme adjustment in April 2016 (**Table 3.11**). Bactoscan results year over year p= 0.2112 indicate no significant change, however long-term effect has been observed as comparison of mean results for two years before the introduction

of adjustment and two years after shows p-value of < 0.0001, which indicate a significant impact of adjustment of payment structure on improvement in milk hygiene (**Figure 3.6**)

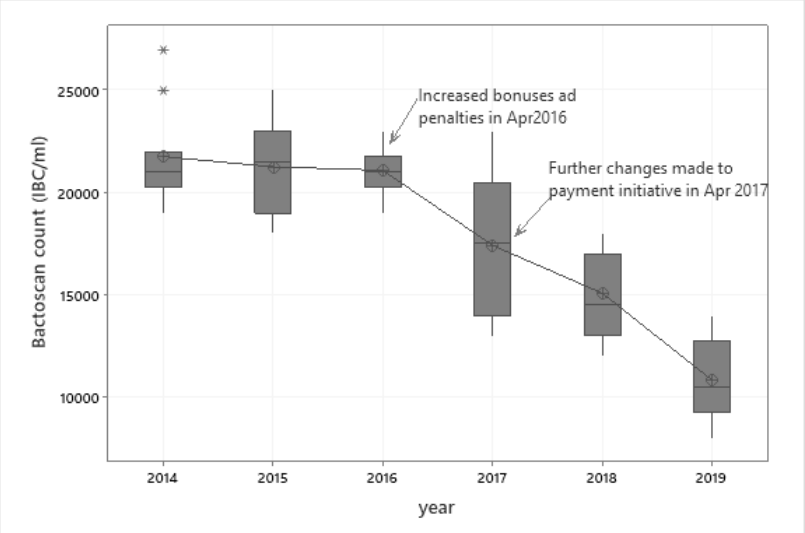

Figure 3.6 Boxplot of monthly means for Bactoscan count (IBC/ml) for the combined data set (23 Jersey dairy herds, 100% of the entire population of dairy herds in Jersey, bulk data) from 2014 to 2019.

Table 3.11 Distribution of Bactoscan (**IBC**) for Jersey dairy farms (silo data set) from 2014 to 2019.

year	Mean (IBC/ml)	St Dev	Minimum (IBC/ml)	Maximum (IBC/ml)
2014	21,750[a]	2,221	19,000	27,000
2015	21,255[a]	2,461	18,000	25,000
2016	21,083[a]	1,165	19,000	23,000
2017	17,417[b]	3,450	13,000	23,000
2018	15,083[b]	2,193	12,000	18,000
2019	10,833[c]	1,850	8,000	14,000

Means within a row with different superscript are significantly different at p < 0.05.

The mean thermoduric bacteria count for bulk data for the years 2014 – 2019 was 242 cfu/ml which classifies the milk as premium, in relation to TBC count (**Table 3.8**). The maximum recorded value was 62,600 CFU/ml recorded for milk produced by the small producer. The lowest recorded mean for silo was 79 CFU/ml and the highest 544 CFU/ml. There is a decreasing trend in TBC year over year with TBC mean of 354 CFU/ml recorded in 2014 and 162 cfu/ml recorded in 2019 (**Table 3.12**). There was a significant difference in annual means ($p<0.05$) indicating improvement in terms of reduction of thermoduric bacteria counts in raw milk supply. Paired t-test shows that thermoduric bacteria results improved after the bonuses and penalties scheme was adjusted in April 2016. Comparison of mean results two years before the introduction of adjustment and two years after confirming the significant impact of payment structure on the thermoduric count p value < 0.0001. Results year over year shows the significant impact of adjustment of payment structure on improvement in milk quality with immediate effect (**Figure 3.7**).

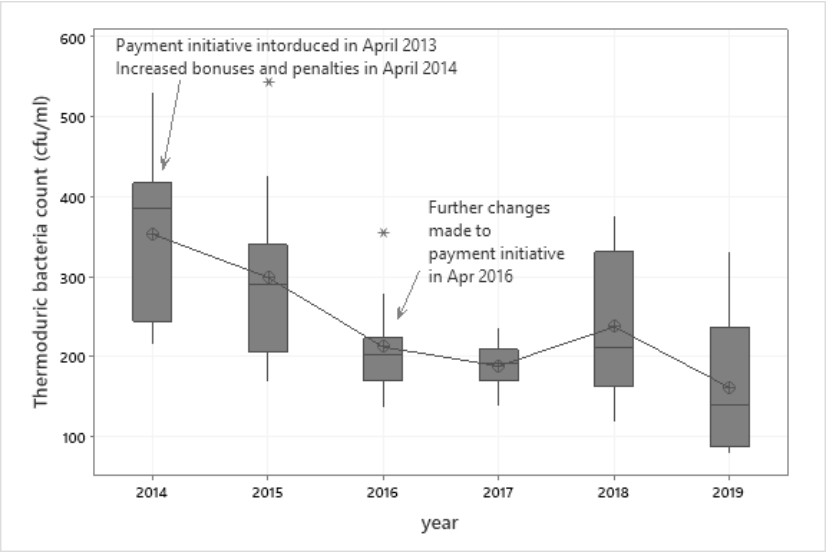

Figure 3.7 Boxplot of monthly means for thermoduric bacteria counts for the combined data set (23 Jersey dairy herds, 100% of the entire population of dairy herds in Jersey, bulk data) from 2014 to 2019.

Table 3.12 Distribution of thermoduric bacteria counts (**TBC**) for Jersey dairy farms (bulk data) from 2014 to 2019.

year	Mean (CFU/ml)	St Dev	Minimum (CFU/ml)	Maximum (CFU/ml)
2014	354[a]	105.9	215	531
2015	300[ab]	107.1	170	544
2016	212[bc]	58.03	137	356
2017	189[c]	26.86	138	236
2018	238[bc]	93.06	118	376
2019	162[c]	85.67	79	331

Means within a row with different superscript are significantly different at $p < 0.05$.

The mean psychrotrophic bacteria count for bulk data for the years 2014 – 2019 was 104 CFU/ml (**Table 3.8**). The maximum recorded value was 492 000 CFU/ml recorded for the milk produced by the small producer. The lowest recorded mean for bulk samples was 6 CFU/ml and the highest 649 CFU/ml. There is a decreasing trend in PBC year on year with PBC mean of 195 CFU/ml recorded in 2014 and 39 CFU/ml recorded in 2019 (**Table 3.13**). The results indicate premium milk quality.

Paired t-test shows that results improved after the bonuses and penalties scheme was introduced. Comparison of mean results for two years before the introduction of the payment structure and two years after shows p-value of 0.0093. Results from one year compared to two years after p= 0.1041 show no significant immediate impact but year after the introduction and three years after showing the significant impact of p=0.0429. Long term effect in improvement was noted (**Figure 3.8**).

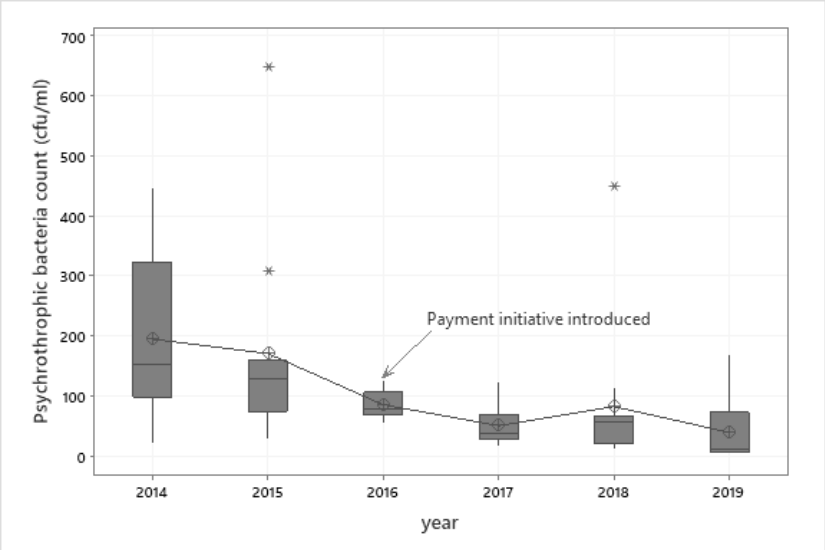

Figure 3.8 Boxplot of monthly geometric means for psychrotrophic bacteria counts for the combined data set (23 Jersey dairy herds, 100% of the entire population of dairy herds in Jersey, bulk data) from 2014 to 2019.

Table 3.13 Distribution of psychrotrophic bacteria counts (**PBC**) for Jersey dairy farms (bulk data) from 2014 to 2019.

year	Mean (CFU/ml)	St Dev	Minimum (CFU/ml)	Maximum (CFU/ml)
2014	195[a]	143.3	21	447
2015	171[ab]	166.2	28	649
2016	86[abc]	22.28	56	126
2017	51[bc]	32.89	16	123
2018	83[abc]	118.9	13	449
2019	39[c]	54.45	6	169

Means within a row with different superscript are significantly different at $p < 0.05$.

Larger producers on all parameters had significantly lower means than smaller producers indicating more focus on hygiene practices and benefits of automation systems introduced at the farms and elimination of manual controls over the milking practices (**Table 3.8**). Standard deviations for all parameters for larger producers are much lower than for small producers

indicating a small variation within the processes for large producers and larger variation in smaller operations.

The mean somatic cell counts for bulk data for the years 2014 – 2019 was 137,569 cells/ml (**Table 3.8**) which is lower than the UK SCC milk recording showing value of 161,000 cells/ml for 2018 and 165,000 cells/ml (AHDB, 2021). The maximum recorded value was 1,147,000 cells/ml recorded for milk produced by the small producer. There was no significant difference between annual means (p=0.232) and counts remained steady and varied from the lowest mean in 2018 134,417 cells/ml and the highest recorded in 2019 142,167 cells/ml (**Table 3.14**). There was a significant difference between monthly means p=0.0442 ($p < 0.05$) indicating a seasonality trend (**Table 3.9**). There were no significant adjustments in payment structure carried out during the time of the study (**Table 3.3**).

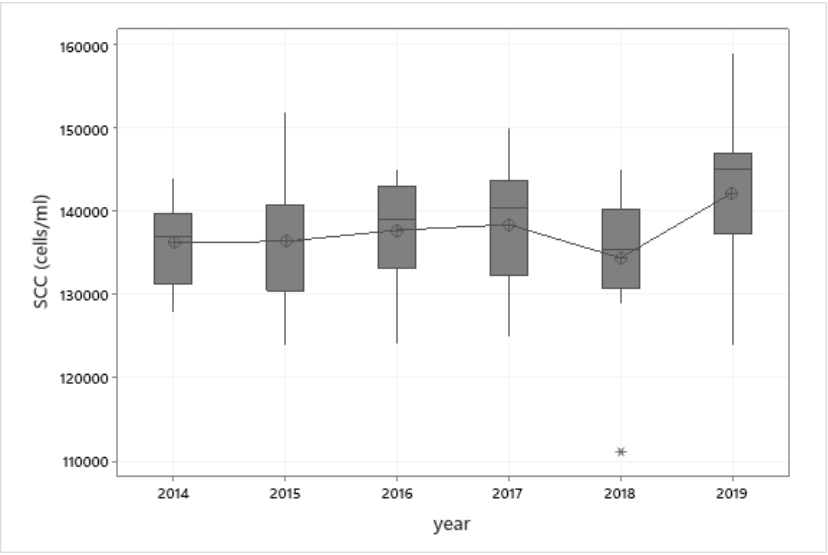

Figure 3.9 Boxplot of monthly means for somatic cell counts for the combined data set (23 Jersey dairy herds, 100% of the entire population of dairy herds in Jersey, bulk data) from 2014 to 2019.

Table 3.14 Distribution of Somatic cell counts (**SCC**) for Jersey dairy farms (bulk data) from 2014 to 2019.

year	Mean (cells/ml)	St Dev	Minimum (cells/ml)	Maximum (cells/ml)
2014	136,250[a]	5,379	128,000	144,000
2015	136,417[a]	7,681	124,000	152,000
2016	137,750[a]	6,524	124,000	145,000
2017	138,417[a]	7,902	125,000	150,000
2018	134,417[a]	8,867	111,000	145,000
2019	142,167[a]	9,600	124,000	159,000

Means within a row with different superscript are significantly different at $p < 0.05$.

The comparison between Jersey and UK raw milk Bactoscan counts (**Figure 3.10**) presents that Jersey raw milk hygiene average results are lower than the UK average for all the years of study. Additionally, there is a trend showing that significant improvement in Jersey milk Individual Bacteria Counts in the years 2014-2019 is present following the introduction of higher bonuses in April 2016 and April 2017. UK Bactoscan count means for the years 2014 - 2019 was 26,514 IBC/ml and Jersey in comparison 17,903 IBC/ml. Tuckey test shows Jersey raw milk Bactoscan count is significantly lower than UK raw milk Bactoscan count (p=0.000).

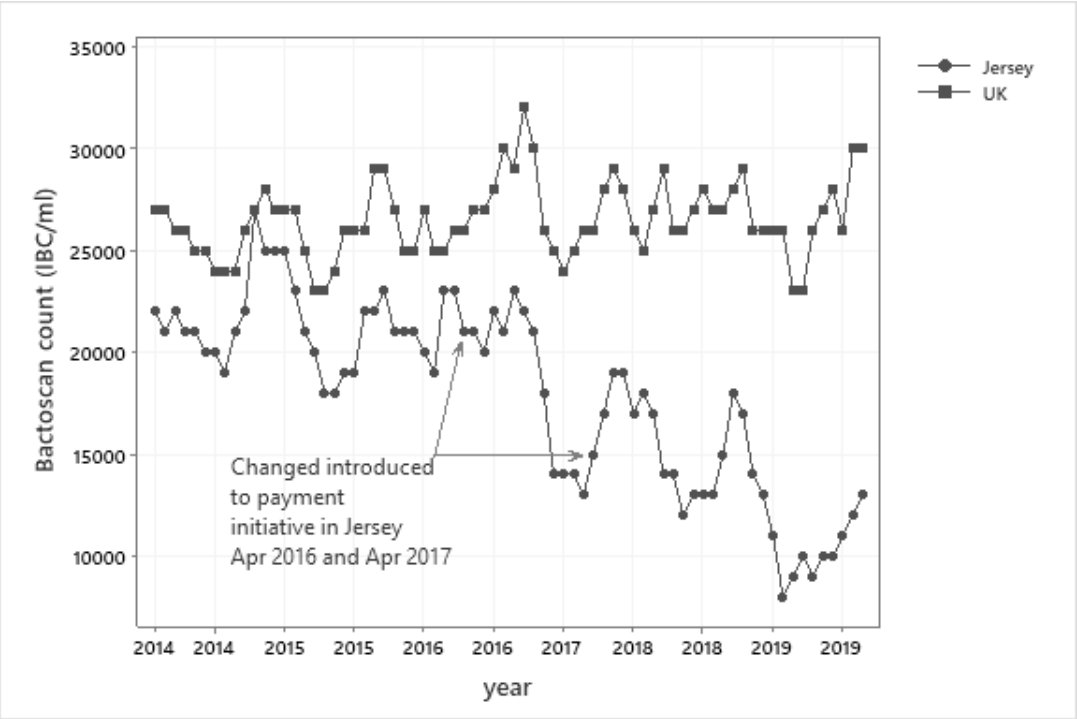

Figure 3.10 Bactoscan Jersey vs the UK. UK data (ref. AHDB.org.UK).

There is no available UK data to present thermoduric or psychrotrophic bacteria counts on any of the raw milk supplies to compare statistical values. However, looking at the recommended reported values, UK premium milk is classified when TBC < 250 CFU/ml. In this case, Jersey raw milk since 2016 would be classified as premium with results below that recommended value. Silo mean is 242.2 CFU/ml for milk collected from 2014 to 2019. National Milk Laboratories guide on psychrotrophic count requires the high-quality milk PBC < 500 CFU/ml and milk of unacceptable quality with PBC > 5,000 CFU/ml. Jersey milk silo means for all years of study was 103.9 CFU/ml. In 2019 mean was reported as low as 39 CFU/ml. This indicates excellent Jersey raw milk quality in relation to psychrotrophic bacteria control.

3.3.4. Impact of Jersey weather conditions on raw milk quality

The objective is to determine the influence of Jersey environmental conditions on Jersey milk quality attributes; fat, protein, and somatic cell counts (**SCC**), individual bacteria count (**IBC**), psychrotrophic and thermoduric bacteria counts which could be contributing to the achievements of premium quality milk. Kazeminia et al. (2019) reported that the composition and microbiological quality of milk can be affected by many factors, such as seasonal variations. Environmental factors such as temperature, rainfall, and sunshine often impact the performance of dairy cows (Lambertz et al., 2014).

Jersey daily temperature (mean air) (°C), monthly rainfall (mm) and monthly sunshine (h) were sourced from Jersey Government Climate statistics (**Appendix 1**).

Correlations among the milk quality data and the environmental data were performed using the Pearson correlation coefficients. Regression coefficients were determined using multiple regression with backward elimination for those correlations that were found to be highly significant ($p < 0.05$).

The Pearson correlations coefficient between various parameters is presented in **Table 3.15**. The correlations coefficient between fat and daily temperature, monthly rainfall and daily sunshine are significant with ($p < 0.05$). There is a negative correlation between daily sunshine and daily temperatures, once those values drop, fat results increase due to the cows grazing and changed feeding regimes at Jersey dairy farms. There is a correlation between those parameters and protein and SNF (Solids Non-Fat) contents.

The Pearson correlation coefficient daily temperature and thermoduric content are 0.4012 ($p = 0.0005$) indicating that the correlation is significant but very moderate. There is a positive correlation which indicates that as daily temperature increases, thermoduric bacteria count also increases which was also confirmed by the seasonality impact reported above. Therefore, it could be stated that control over thermoduric counts is much more challenging in warmer

months. Environmental conditions do not impact somatic cells counts values or psychrotrophic bacteria counts which are confirmed to be purely related to udder and milking hygiene and farm management practices as confirmed by the literature.

Table 3.15: Pearson–correlation (Pearson correlation and p-value) between temperature, sunshine, rainfall, and fat, protein, SCC, IBC, PBC and IBC of Jersey milk collected from January 2014 till December 2019. P-value *< 0.05 ** <0.01 *** <0.001 NS=non-significant.

Correlations	Daily temp (Mean air)	p-value	Monthly rainfall	p-value	Daily sunshine	p-value
Bactoscan	**-0.3267**	**	0.1166	NS	-0.3643	**
SCC	0.2715	NS	0.1088	NS	0.0836	NS
Lactose	0.1046	NS	-0.2364	*	0.4335	**
Fat	**-0.7950**	***	**0.5519**	***	**-0.7723**	***
FPD	**-0.4109**	***	0.2265	NS	-0.2867	*
Protein	**-0.6977**	***	**0.4468**	***	**-0.7244**	***
TBC	**0.4012**	***	-0.1321	NS	0.1971	NS
PBC	0.1449	NS	0.0804	NS	0.0416	NS
SNF	**-0.5504**	***	0.2745	*	**-0.5323**	***

SCC- Somatic cell count; **FPD**- Freezing point depression; **TBC**- Thermoduric bacteria count; **PBC**- Psychrothrophic bacteria count; **SNF**- Solids Non-fat.

Seasonality changes in relation to compositional parameters are purely related with changed in feeding regimes and grazing of cows. Thermoduric bacteria counts are linked with changes in housing practices, and bacteria contamination sources present in the environment at different season temperatures.

3.3.4. Microbiological profile of Jersey raw milk

Altogether, 778 isolates have been identified and assigned to 108 species and 24 genera. *Streptococcus ssp*., *Staphylococcus ssp*., *Pseudomonas ssp*., and *Escherichia coli* were the most abundant genera making up 64.3% of all isolates.

Those isolates are linked with udder inflammatory disease, udder hygiene, water contamination and milking practices that include disinfection of milking equipment (Özer and Akdemir-Evrendilek,2014).

Figure 3.11 shows the biodiversity of **genera** and relative abundance of at least 1% of the number of isolates in Jersey milk produced from January 2014 till December 2019 (**Appendix 2**). **Figure 3.12** presents a relative abundance of the 108 **species** with the abundance of at least 1% of the 778 of the isolates identified in Jersey milk in the year 2014 to 2019 (**Appendix 3**). The microbiome of Jersey milk became more diversified over the year of this study. This included 15 additional species detected in raw milk profile in 2014, gradually increasing and in the end reaching 36 additional species detected in milk in 2019. The Jersey milk microbiome includes a mix of both thermoduric and psychrotrophic bacteria and once the counts of those are reduced over the years, there is no significant changes in the main bacteria species detected in the milk microbiome. The prevalent species for Jersey milk included: *Streptococcus uberis, Pseudomonas fluorescens, Staphylococcus chromogenes, E. coli, Corynebacterium ssp., Bacillus ssp., Staphylococcus aureus, Staphylococcus hyicus, Pseudomonas aeruginosa, Pseudomonas ssp., Enterococcus faecalis, Aerococcus viridans, Serratia liquefaciens, Raoultella terrigena*. Even though, there is a significant drop in Bactoscan individual bacteria count, thermoduric bacteria count and psychrotrophic bacteria count during the years of the study, the same bacteria strains were detected in the milk microbiome, but interestingly the milk microbiome became more diversified. The specific bacteria species identified in the study are representative of Jersey herds raised on the island of Jersey.

Diversification of raw milk could be linked with low counts of the main species and those not dominating the milk microbiome by multiplying to high numbers which allow other bacteria to show their presence. The presence of *Streptococcus uberis, Staphylococcus chromogenes, Staphylococcus aureus, and Staphylococcus hyicus* is linked with udder hygiene, and inflammatory disease of the udder (mastitis) (Rainard, 2017). The presence of *Serratia liquefaciens* is commonly encountered in the environment but also in raw milk samples. This species is known to produce heat-stable extracellular proteases in raw milk Ser1 and Ser2 belonging to the serralysis family. Ser2 hydrolyses sodium-caseinate is confirmed to be very thermoresistant and could be one of the main causes of UHT milk destabilisation during storage (Bagliniére et al., 2017). The presence of *Pseudomonas* species indicates the use of contaminated water sources and the presence of biofilms in milking equipment. *Pseudomonas* species are also known to produce many enzymes that impact on milk stability (Özer and Akdemit - Evrendilek, 2014). *E. coli, Enterococcus faecalis, Serratia liquefaciens* and *Acinetobacter ssp*. indicate environmental factors could be a significant contribution to contamination i.e., faeces (Kagkli et al., 2007). The analysis of direct plating results led to the contamination root causes and therefore corrective actions could have been established. Those corrective actions' impact was monitored directly by the raw milk quality. The direct results coupled with bacteria enumeration are required to establish milk quality and the necessary milk management practices to ensure the improvements in raw milk quality are progressed at farms.

Similarly, Cremonesi et al. (2018) demonstrated that Rendena cows are showing a significantly lower microbial biodiversity than Holstein Friesian breed. Rendena cows showed more stable microbiota. Relative abundance for *Streptococcus* ssp. was reported for Jersey as 16.4%, H-R breed 28% and Rendena cows 74%. Jersey cow's milk currently showed a more diversified milk microbiome. Jersey milk microbiome became much more diversified over the years once

general results were lowered down and this could be the impacting factor on the other reported data.

Metzger et al., (2018) indicated that milk microbiota research is at initial stages, and it is complex, and the methodology can be improved. There is not enough known about the milk microbiota to base treatment decisions on microbiota results. Once again that proves the point that developments in this area are necessary and could be a key to the improvements in relation to milk hygiene results in the future.

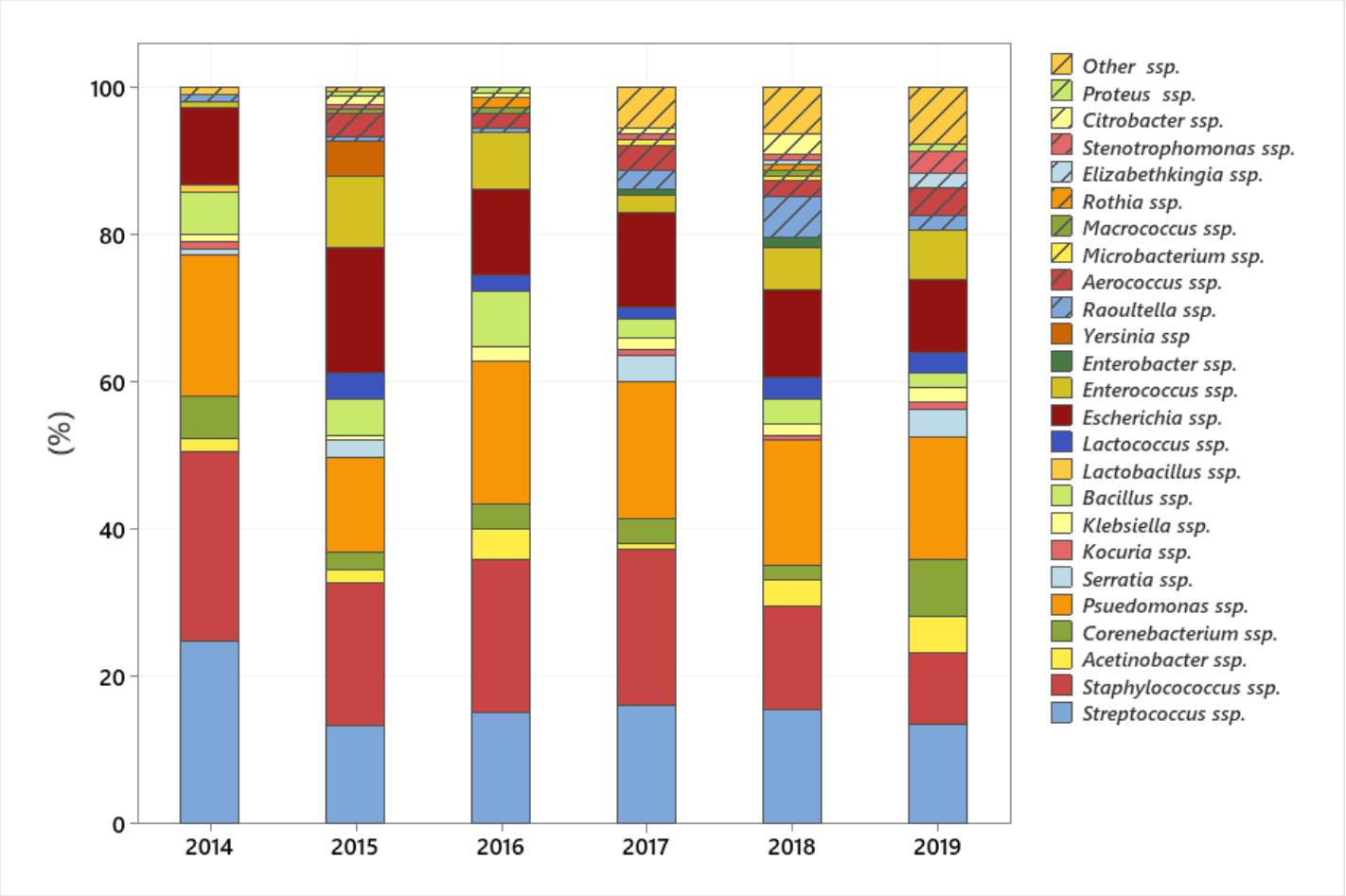

Figure 3.11 Biodiversity of **genera** and relative abundance of at least 1% of the 778 of the isolates in Jersey milk produced from January 2014 till December 2019.

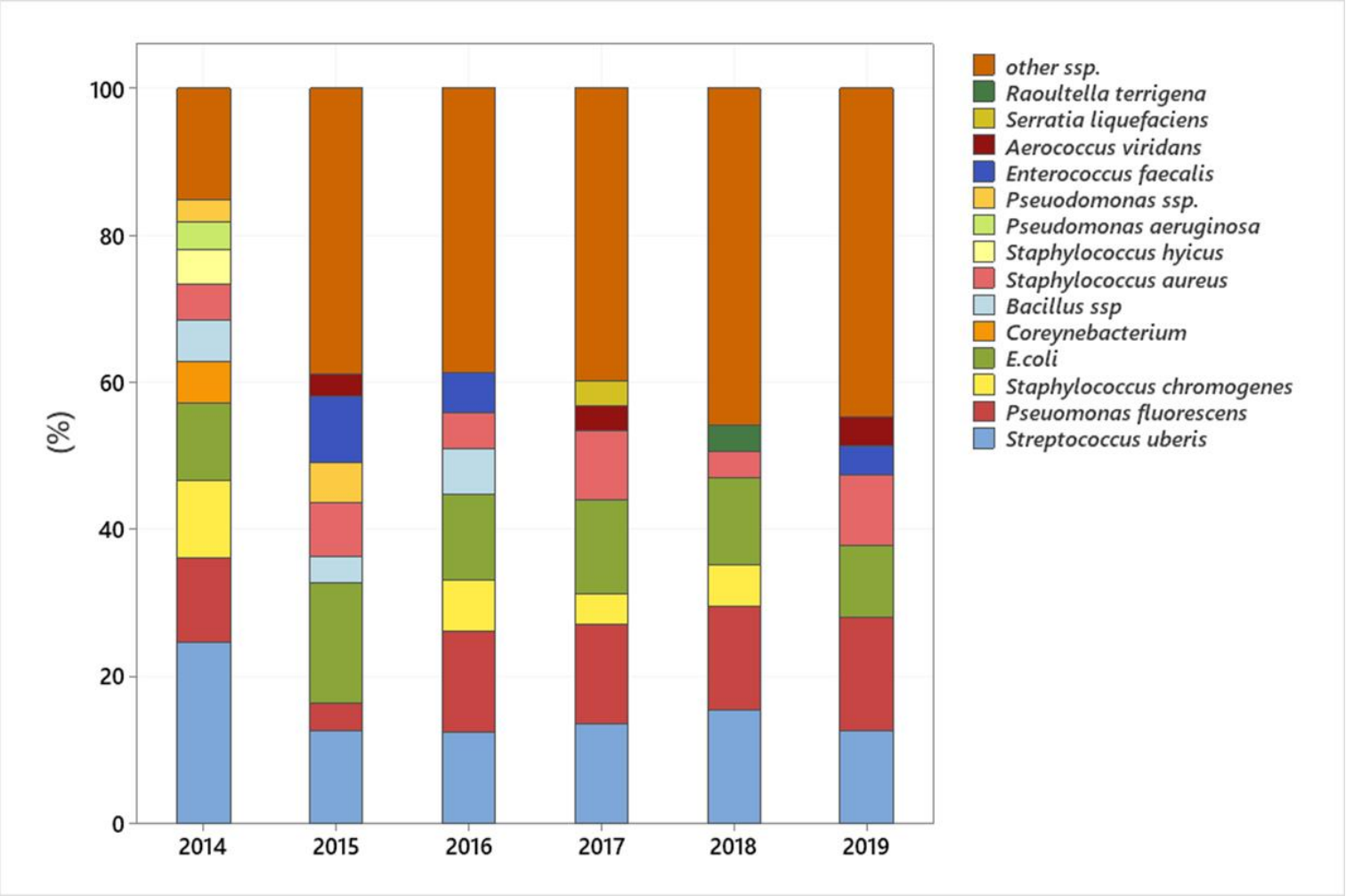

Figure 3.12: A summary of the percentage of isolates belonging to the **species** from 2014 till 2019 with abundance of at least 2%.

3.4. Conclusion

Jersey milk has a specific composition, it differs from milk sourced from different cow's breeds. Due to the incorporation of genetics, and additional payment initiatives focused on hygienic and nutritional feeding programmes in Jersey over the years, Jersey raw milk composition is much improved and consistent overall in compositional values, especially in fat and protein contents. Not surprisingly, compositional results observed seasonality trends that included fat, protein, lactose, and total solids content. Interestingly, the seasonality of microbiological thermoduric bacteria counts was also confirmed. Jersey milk production on the island of Jersey has been consistent with a high premium microbiological quality from January 2014 till December 2019 and at significantly lower levels of bacterial counts than milk produced and reported in the UK. It is worth noting that UK values in legislation and guidelines compared to the other areas of the world are much more demanding, which strengthens the fact that Jersey milk quality presented in this study is of a high-quality standard. No published studies have reported better raw milk microbiological quality than those values observed in this study for the Jersey cattle on the island of Jersey.

The introduction of stringent payment schemes in Jersey has proved to make a significant improvement in the physicochemical and microbiological quality of raw milk. The introduction of additional parameters for testing raw milk microbiological quality, i.e., thermoduric and psychrotrophic bacteria, and a monitoring programme of the prevalence of bacteria presents in raw milk supply allowed for further improvements in Bactoscan counts over those years. It was noted that improvements in those counts did not impact on the change of the main bacteria present in raw milk supply as the same bacteria species remained present in the milk body over the years of study. This study demonstrated that the raw milk microbiome became more diversified over time as other species were detected once some specific bacteria counts were

lowered down. The counts of main bacteria species were not overtaking the milk microbiome and allowing other species to be present and multiply to detectable levels during the analysis.

CHAPTER 4

4. IMPACT OF RAW MILK QUALITY ON PROTEOLYSIS AND STORAGE STABILITY OF JERSEY UHT UNSTANDARDIZED MILK PRODUCED AT PILOT SCALE

4.1. INTRODUCTION

The composition and microbiological properties of raw milk are essential for the stability of UHT dairy milk. Enzymes produced by bacteria in raw milk can significantly impact on UHT milk stability. Proteolysis caused by bacterial proteinases contributes to the development of sensory defects and reduces UHT milk shelf-life. Chemical and physical changes in the milk can lead to off-flavours, browning, fat separation, sediment formation or gelation during the product storage (Deeth, 2010). The enzymatic breakdown of proteins causes age gelation, a significant cause of UHT milk product loss during storage (Datta and Deeth, 2003). UHT processing aims to produce high quality liquid products of extended shelf-life, that remain stable during transportation and storage. This aim is challenged when temperature fluctuations are inevitable in supply chains. For example, UHT milk exported from Europe to Asia may experience temperature fluctuations from 0°C to 50°C as the product is transported across climatic zones and stored in uninsulated warehouses where temperature can reach above 50°C. Despite this, there is still a lack of industry-validated, evidence-based strategies that milk processors can implement to successfully minimise the development of such faults in UHT milk products.

This chapter investigates the effect of raw milk quality on proteolysis in UHT Jersey milk during storage at 19°C and 55°C for a period of five weeks. Samples produced from Jersey raw milk were collected from eight Jersey dairy farms, with variable microbiological quality, and were subjected to UHT processing at a pilot scale. Individual bacterial strains present in raw milk samples were assessed, aiming to establish sources of contamination at the farm level.

4.2. MATERIALS AND METHODS

I fully managed this project as I was employed by Jersey Dairy as Head of Quality and Senior Manager during the time. I arranged and supervised the specific tasks and analysis with the third parties involved in this project.

4.2.1 Raw milk collection

Raw milk samples were collected from eight Jersey farms (Jersey, Channel Islands). The farms were selected based on preceding microbiological analysis such that samples with a range of microbiological quality would be obtained. Hence raw milk was sourced from four farms that were known to produce milk of lower microbiological quality and four farms that consistently produce milk of excellent microbiological quality. Herd size ranged from 50 to 200 Jersey cows. 15 litres of raw milk, cooled below 8°C, was obtained from each farm directly from the bulk tank which contained milk from an evening and a morning milking. Milk samples were collected in January 2020, in sterile bags and were transported on the same day via air to the pilot plant at the University of Reading (Reading, UK). Milk temperature was recorded upon receipt and it was below 5°C.

4.2.2 Raw milk testing

Jersey raw milk from each farm was tested in duplicate for physicochemical, microbiological, and in triplicate for proteolytic quality.

4.2.2.1 Physicochemical analysis

Analysis for fat, protein, lactose, and total solids contents was measured by Fourier transform Infra-Red Spectrophotometry (**FTIR**) using Foss Milkoscan FT120 (Foss, Hillerød, Denmark) in Jersey Dairy Laboratory (Jersey, Channel Islands).

Freezing point depression (**FPD**) of milk samples was measured by cryoscopy using 4D3 Cryoscope (Advanced Instruments Inc., Metuchen, USA). Ethanol stability (**ES**) was determined by mixing equal volumes of 5 ml of milk and a range of ethanol solutions (30% to 80%, v/v) . The test tubes were then left for 1 minute under static conditions. The test tubes were then tilted horizontally and macroscopically observed to identify coagulation along the sides of the tube. The highest ethanol concentration at which coagulation did not occur was identified as the ethanol stability of the milk. Ethanol testing was carried out at Reading University (Reading, UK).

The pH of the milk was tested at 4°C on the day of UHT processing using a pH probe (Thermo Scientific Orion 3-Star pH meter and Sure-Flow Ion-Selective Electrode).

4.2.2.2 Microbiological and proteolytic analysis

Microbiological quality was measured on the day of UHT milk processing. Analysis for Individual Bacteria Count (**IBC**) and Somatic Cell Counts (**SCC**) was performed by flow cytometry method using Foss Bacsomatic (Foss, Hillerød, Denmark) in Jersey Dairy Laboratory (**JDL**) (Jersey, Channel Islands).

Pseudomonas spp. count was determined using Pseudomonas agar base (VWR, Lutterworth, UK) following incubation at 25°C for 48 hours and *Enterobacteriaceae* spp. count was determined by 3M Petrifilm Enterobacteriaceae count plate (VWR, Lutterworth, UK) following incubation at 37°C for 24 hours in **JDL** (Jersey, Channel Islands).

Psychrotrophic bacteria count (**PBC**) was determined using MPC media (VWR, Lutterworth, UK) incubated at 3°C for 10 days. Analysis of thermoduric bacteria count (**TBC**), *E. coli* enumeration, *Streptococcus* spp. enumeration and direct plating were performed by Quality Milk Management Services Ltd. (**QMMS**) (Somerset, UK). Thermoduric bacteria count (**TBC**) were enumerated after heating 5 ml of milk to a temperature that simulates pasteurisation (63.5 +/-

0.5°C for 30 min), followed by immediate refrigeration at 20°C. After treatment, serial dilution of the samples was performed up to 10^{-3} in sterile peptone which were plated using Milk Plate Count agar (Thermoscientific, Basingstoke, UK) and incubated at 30°C for 72 h.

Qualitative direct plating assessment was performed by using sheep blood agar (Thermoscientific, Basingstoke, UK) incubated at 37°C for 72 h and then the identity of isolates was further confirmed using Maldi-Tof MS (Matrix Assisted Laser desorption/ionisation time of flight mass spectrometry,) (Bruker Daltonics, Coventry, UK) performed by Quality Milk Management Services Ltd. (**QMMS**) (Somerset, UK).

4.2.3 UHT milk processing

Jersey raw milk was processed at the University of Reading UHT pilot plant within 24 hours of receipt and 48 hours from milking.

Jersey raw milk was preheated to 65°C in a steam jacketed vessel and then homogenised in a two-stage homogeniser (200 L/T APV Rannie Homogeniser, Winkworth Machinery Ltd., Basingstoke, UK). The milk was then homogenised upstream using a two-stage aseptic homogeniser operating at 22 MPa and 5 MPa in the first and second stage, respectively.

Homogenised milk was then sterilised in a UHT pilot plant system using indirect heating at 138°C for 4 seconds (FT74XTS HTST/UHT System, Armfield, Hampshire, UK). Milk exiting the unit was manually filled under ultra-clean fill conditions using a laminar flow cabinet (Winkworth, model No 38250) into sterile plastic containers (250 ml) and sealed.

Processed milk was stored for 5 weeks at 19°C and 55°C. Three replicates of freshly processed milk samples were tested on the first day and then weekly from each temperature point, giving a total of 30 observations for two temperature points for each of the farms.

4.2.4 Processed milk analysis

Free amino nitrogen content (**FAN**) was carried out at the University of Reading (Reading, UK). All the reagents used to perform **FAN** analysis were prepared as per the ninhydrin colorimetric method reported by the European Brewery Convention (Lie, 1973) and adjusted as described by Patel et al. (2004). Unless otherwise stated, all materials were obtained from Sigma Aldrich (Gillingham, UK). A calibration curve was created using standard glycine solution at varying concentrations of 0.5-2.0 mg/l. The calibration curve was used to determine the **FAN** concentration in the stored UHT milk samples. A sample of milk (1 ml) was transferred into an Eppendorf tube and centrifuged at 21,420 x g for 15 min at 15°C (Multifuge X3R, Fisher Scientific, UK) to separate the fat from the serum. The fat was scraped off, and the solution was vortexed. Trichloroacetic acid (**TCA**) solution at concentration 10% (w/v) (0.5 ml) was added to the sample and then vortexed and centrifuged at 21,420 x g for 10 min at 4°C. TCA (5%)- soluble extracts of milks were prepared by adding 10% (w/v) TCA. This was done to precipitate the whey proteins and solids present in the solution. A sample of the clear serum (1 ml) was transferred into a separate Eppendorf tube, and a ten times dilution was carried out. The diluted solution (0.2 ml) was pipetted into another Eppendorf (in duplicates) to which 0.1 ml of colour reagent (49.71 g $Na_2HPO_4 \cdot 2H_2O$, 5 g ninhydrin, 3 g fructose, 60 g KH_2PO_4, made up to 1 l with distilled water at pH 6.6 to 6.8) was added and vortexed. The eppendorf tubes were heated in a thermoblock (Eppendorf) for 16 min at 100°C and then immediately cooled down using ice. Once cooled, 0.5 ml of dilution reagent (2 g KIO_3, 384 ml absolute ethanol made up to 1 l with distilled water) were transferred into the eppendorf tubes and samples were vortexed. The absorbance of each sample and a blank that used distilled water instead of milk was measured within 30 min of cooling at 570 nm in a Biomate 3 UV/VIS Spectrophotomer (Thermo Spectronic, NY) equipment. The calibration curve (**Appendix 3**) was used to determine the **FAN** concentration in the stored

UHT milk samples as an indication of the concentration of free amino nitrogen and small peptides that are available in the sample due to protein hydrolysis.

4.2.5 Statistical analysis

Statistical analysis was carried out using Minitab Software (Minitab Ltd, State College, Pennsylvania) and SIMCA 17 software (Umetrics, Umea, Sweden).

The variation of microbiological and physicochemical quality of raw milk sourced from different farms was analysed. Principal Component Analysis (**PCA**) was used to provide a map of how the variables related to each other and to identify differences between milk from the farms. Principal component analysis (**PCA**) is defined as an orthogonal linear transformation that transforms the data to a coordinate system such the greatest variance by any projection of the data comes to lie on the first coordinate i.e., principal component (PC1), the second greatest variance on the second coordinate principal component 2 (PC2) etc., (Jolliffee, 2002). The variation of free amino nitrogen (mg/l) between UHT milk produced from raw milk with different quality was assessed using one-way ANOVA. The significance level was established at $p < 0.05$.

The correlation between **FAN** concentration (mg/l) and the level of physicochemical and microbiological variables was assessed using Pearson correlation. The significance level was established at $p < 0.05$.

4.3. RESULTS AND DISCUSSIONS

4.3.1 Raw milk quality

The mean values of the microbiological and physicochemical components of the raw milk are shown in **Table 4.1.**

The farms were confirmed to produce milk with a wide range of microbiological quality. Bactoscan individual bacteria counts (**IBC**) varied from 8,000 IBC/ml for farm five to 47,033,000

IBC/ml for farm one. According to the quality classification scheme (Glanbia, 2016) and legal requirements for bacteria counts (Regulation (EC), 853/2004), milk from farms one, two, three and four were classed as sub-standard and legally classed as unacceptable milk for further processing. Milk from farms five, six and eight were classed as premium quality milk (Bactoscan < 15,000 IBC/ml) and farm seven as acceptable standard with Bactoscan count of 19,000 IBC/ml. Somatic cell counts varied between farms. According to the UK quality payment, SCC schemes (Glanbia, 2016) milk from farms two, three and farm eight were below 150,000 cells/ml and classed as premium quality; farms five and six were in acceptable standard (between 150,000 and 200,000 cells/ml) and farms one, four and seven were classed as sub-standard milk, with count between 200,000 – 400,000 cells/ml. There were all below 400,000 cells/ml as per legal requirements. *Enterobacteriaceae* spp. varied from 0 CFU/ml for farm five to outside of countable range (TNTC >100,000 CFU/ml) in the case of milk from farm one. Similarly, psychrotrophic bacteria counts were the highest for farm one (PBC = TNTC >3,000,000 CFU/ml) and the lowest for farm five (15 CFU/ml). Counts for *Streptococcus* spp. and *Pseudomonas* spp. showed a similar pattern, the highest for farm one and the lowest for farm five. The pH of raw milk measured at 4°C from farm one was low 6.57 and other farms' milk pH was consistent and varied between 6.66 - 6.75. The results of compositional analysis, including fat, lactose, protein, and total solids contents, were in line with standard Jersey milk composition and consistent with published data (Robinson, 2002).

Ethanol stability was as low as 30% for milk sourced from farm one, 50% for farm three, and 60% for farms two, seven and eight. These outcomes indicate poor milk processing stability. Low ethanol stability is associated with increased likelihood of experiencing milk processing difficulties, as fouling during UHT treatment may occur. The milk protein gets unstable as a result of disturbance in the mineral balance of milk. Milk with high developed acidity or having calcium and magnesium compounds in greater than normal amounts, will coagulate when

alcohol is added. Reduction of protein stability and higher mineral concentrations increase a heat induced destabilisation of proteins and heat induced precipitation of calcium phosphate are the main drivers for competent fouling during thermal processing (Huppertz and Nieuwenhuijse, 2022). Farm six showed excellent milk stability for UHT milk processing (ES equals to 80%).

Table 4.1 Physicochemical and microbiological quality of Jersey raw milk used for production of Jersey UHT unstandardized milk. Values presented are average counts.

Variable	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8
Bactoscan (IBC/ml)	47,033,000	44,000	434,000	13,882,500	11,000	11,000	19,000	9,000
Somatic Cell Count (cells/ml)	229,500	136,500	143,500	201,500	158,500	158,500	214,500	129,500
Enterobacteriaceae spp. (CFU/ml)	TNTC[1]	10	150	95	15	15	85	5
Psychrothrophic spp. (CFU/ml)	TNTC[2]	10,400	135,000	3,000,000	15	280	190	125
Thermoduric spp. (CFU/ml)	65	505	5,650	55	70	135	235	295
*E. coli (*CFU/ml)	0.0	0.5	2.5	0.0	0.0	0.5	0.5	48.5
Streptococcus spp. (CFU/ml)	6,100	295	385	138	290	230	595	510
Psuedomonas spp. (CFU/ml)	TNTC[2]	14,700	175,500	3,000,000	45	50	20	30
pH	6.57	6.68	6.69	6.75	6.66	6.66	6.70	6.68
Fat (g/100g)	4.98	5.62	5.57	5.13	5.33	5.69	5.18	5.76
Protein (g/100g)	3.73	3.91	3.92	3.70	4.03	3.97	3.87	3.87
Lactose (g/100g)	4.57	4.51	4.51	4.55	4.54	4.42	4.53	4.49
Total solids (g/100g)	14.32	15.10	15.06	14.41	14.97	15.16	14.63	15.18
Freezing point depression (-m°C)	528	523	522	514.5	520.5	520.5	521.5	522.5
Ethanol stability (ES)	30	60	50	70	70	80	60	60

[1] too numerous to count (>100,000 cfu/ml) [2] too numerous to count (>3,000,000 cfu/ml).

The farm milk microbiological direct plating results are shown in **Table 4.2.**

The direct plating results were used to indicate sources of contamination at the farm level. Raw milk bacteria contamination sources as described in **Chapter 2**, **Table 2.3** were used in the investigation and analysis of raw milk direct plating results. In summary, milk from farms 1, 2, 3 and 4 indicated mixed heavy growth of bacteria while milk from farms 5 and 6 scant mixed growth of bacteria and farms 7 and 8 moderate mixed growth with enumeration of bacteria presented below.

Whilst the thermoduric count for milk from farm one was under good control (65 CFU/ml), the psychrotrophic count had risen significantly (>3,000,000 CFU/ml). When coupled with the findings of the direct plating, these results suggest poor milking hygiene, and poorly cleaned and disinfected milking equipment (Gleeson et al., 2015). Increased *Streptococcus* spp. count and increased somatic cell count indicates udder hygiene and a high prevalence of intramammary infection in the herd (Rainard, 2010). The markedly high counts and heavy mixed growth on the direct plating suggest significant problems with milk quality and milking procedures. Both *Pseudomonas* spp*.,* and *Enterobacteriaceae s*pp. are elevated. It is noted that there is a predominance of *Pseudomonas* spp. in this raw milk sample, which is known to produce several proteolytic enzymes. *Pseudomonas* spp*.* could indicate the use of contaminated water sources and the presence of biofilms in milking equipment (Özer and Akdemit - Evrendilek, 2014). Milk from farm 2 showed elevated counts for psychrotrophic (10,400 CFU/ml) and a slightly elevated count of thermoduric bacteria (505 CFU/ml) suggesting the plant cleaning could be a contributing factor. Similarly, the presence of *Pseudomonas* spp., as in milk from farm 1, indicates the use of contaminated water sources or the presence of bacteria biofilm in milking equipment. *Kocuria salsicia* indicates contamination from bedding sources (Gagnon et al., 2020). Milk sourced from farm 3 had elevated psychrotrophic,

thermoduric and *Pseudomonas* spp. counts indicating also poor quality milk. The direct plating has revealed a mixed growth of environmental pathogens suggesting several sources of contamination, such as water, udder hygiene and milking practices (Özer and Akdemit - Evrendilek, 2014). All these findings suggest that the plant cleaning regime and udder cleaning at this farm should be reviewed as a first instance (Griffiths, 2010; Gleeson et al., 2015). All counts were also significantly elevated for farm four; the direct plating indicated mixed heavy growth of bacteria species when coupled with increased bacterial counts of thermoduric and psychrothrophic counts suggests poor milking hygiene and contaminated water source (Fitzgerald and Cotter, 2013; Özer and Akdemit - Evrendilek, 2014), whereas increased SCC and the presence of *Streptococcus uberis* and *Staphylococcus chromogenes* suggest a high prevalence of intramammary infection in the herd (Rainard, 2017). Results for farms 5 and 6 showed an excellent sample, representing milk of high quality. Milk from farm 7 showed slightly elevated counts of *Streptococcus* spp*.* and thermoduric bacteria counts. The isolation of *Staphylococcus aureus* and increased SCC suggested an increased prevalence of intramammary infection in the herd, though effective treatment of mastitis outbreaks could be a key to resolving the problem (Özer and Akdemit - Evrendilek, 2014). Increased thermoduric bacteria count and the presence of *E. coli, Candida utilis,* indicate environmental sources of contamination, i.e., faeces (Kagkli et al., 2007), feed, dust, or air (Robinson, 2002). The presence of *Bacillus oleronius* and *Corynebacterium xerosis* indicate possibilities of contamination from cow's teat skin or presents source of biodiversity of milk sourced from this particular farm (Verdier-Metx et al., 2012). The psychrotrophic bacteria counts remained under good control for milk sourced from farm eight. However, slightly elevated thermoduric bacteria count, and *Streptococcus* spp*.* count joined with findings from the direct plating indicated contaminations related to water sources, faeces, and udder hygiene (Özer and Akdemit - Evrendilek, 2014).

All farms that showed a rise in psychrotrophic counts also showed increased Bactoscan counts and reflected those results in direct plating.

Table 4.2 Jersey raw milk qualitative microbiological direct plating assessment for raw milk used for the production of Jersey UHT unstandardized milk.

Farm	Direct plating
1	Mixed, heavy growth of *Pseudomonas ssp., Aeromonas salmonicida*, *Aeromonas eucrenophila*, *Enterococcus durans*, *Buttiauxella gaviniae* and *Carnobacterium maltaromaticum*.
2	Mixed, heavy growth of *Pseudomonas fluorescens* and *Kocuria salsicia* and scant growth of *E. coli*.
3	Mixed, heavy growth *Psuedomonas fluorescens* and *Citrobacter braaki*, and scant, mixed growth of *E. coli* and *Streptococcus uberis.*
4	Mixed, heavy growth of *Pseudomonas fluorescens*, and scant, mixed growth of *Streptococcus uberis*, *Aerococcus viridans* and *Staphylococcus chromogenes.*
5	Scant, mixed growth of *Aerococcus viridans*, *Staphylococcus chromogenes* and *Kocuria kristinae.*
6	Scant, mixed growth *of E. coli, Aerococcus viridans* and *Staphylococcus epidermidis.*
7	Moderate, mixed growth of *E. coli, Staphylococcus aureus, Aerococcus viridans, Candida utilis, Bacillus oleronius* and *Corynebacterium xerosis.*
8	Moderate, mixed growth of *Raoultella terrigena, Psuedomonas fluorescens, Aerococcus viridans* and *Staphylococcus epidermidis.*

The principal components analysis was used to determine the number of principal components that account for most of the variation in the data. PCA analysis was used to identify the major sources of variance and to highlight any unusual or outlying samples.

In this case, these results show that PCA was used to identify samples and detect outliers within a data set. Principal component analysis is a quick method that was used to identify raw milk quality and suitability for UHT processing. It provides a rapid screen methodology to alert processor to divert milk to other short shelf-life product processing rather than UHT processing.

The contribution of the raw milk quality attributes to the principal components can be evaluated by their correlations with the two components extracted (**Figure 4.1**). A total of 75% of the variability in the 14 quality attributes was jointly explained by the two principal components. The first component (PC1) explained 59.2% of the total variability and was highly and inversely correlated with Bactoscan (r=-0.95), psychrotrophic spp. (r=-0.89), *Streptococcus* spp. (r=-0.86), *Pseudomonas* spp. (r=-0.89), Somatic cell counts (r=-0.85) and *Enterobacteriaceae* spp. (r=-0.87) counts. Thus, PC1 can be labelled as microbiological and proteolytic activity. According to the quality map shown in **Figure 4.1**, milk from farm one (r=-0.96) followed by milk from farm four (r=-0.33) would be characterised as the milk with the lowest microbiological quality, whereas raw milk from farms six (r=0.37) and eight (r=0.32) were classed as of excellent quality.

The second component (PC2) explained 15.8% of the total variability and was highly and inversely correlated with pH (r=- 0.81) and ethanol stability (r=-0.54) which links with raw milk chemical and processing properties. Again, raw milk from farm one, was characterised by the lowest pH and ES. Milk from other farms was stable in terms of those attributes. As illustrated by the loadings, the major quality traits contributing to differences between farms were in relation to microbiological and proteolytic activity.

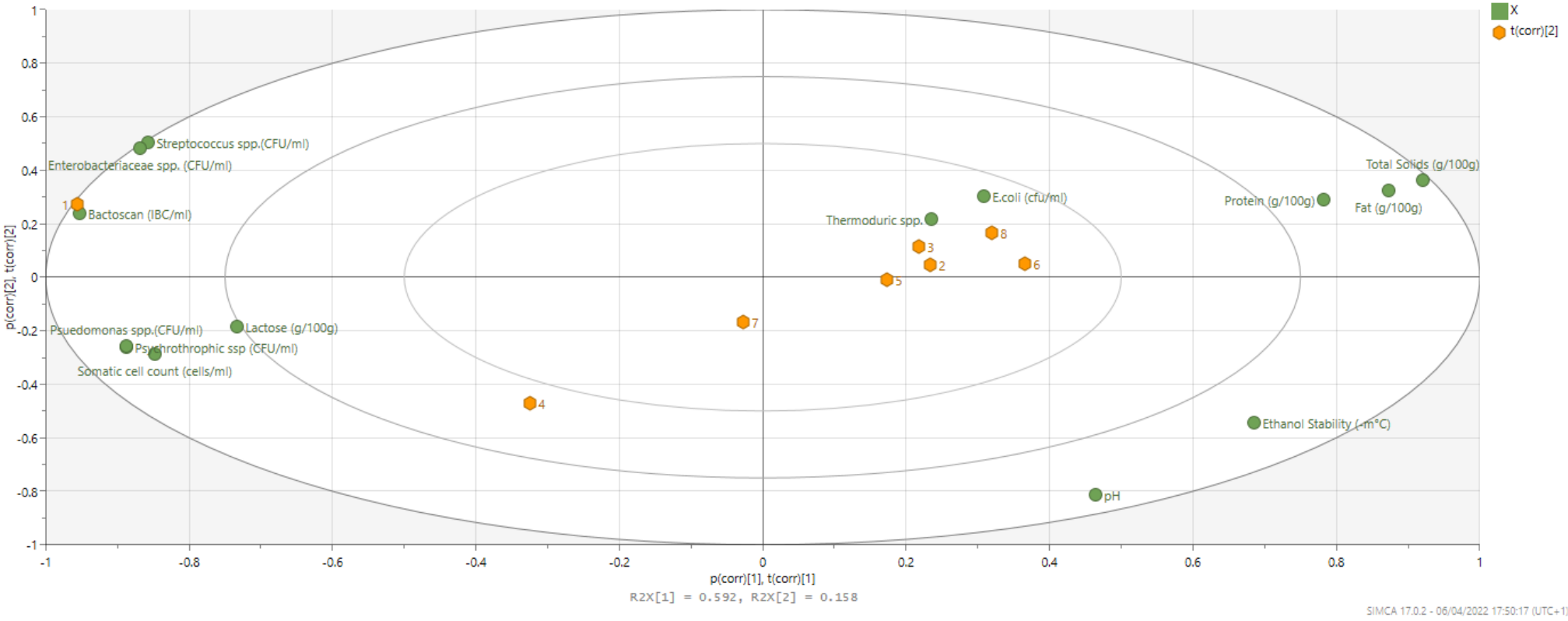

Figure 4.1: Principal component analysis of quality attributes for Jersey raw milk collected from eight Jersey farms. Map of the first and second principal components of the microbiological, proteolytic, and physicochemical characteristics of Jersey raw milk sourced from eight Jersey farms used for further UHT processing.

4.3.2 Protein hydrolysis

Free amino nitrogen (**FAN**) results are presented in **Table 4.3**.

Free amino nitrogen concentration was measured in all samples on day one after UHT processing. The highest value of FAN corresponded to milk from farm five (29.08 mg/l) and the lowest to milk from farm two 23.98 mg/l. A baseline level of proteolytic activity could be expected in all farm milk irrelevant of initial microbial count and enzymatic activity due to the presence of heat resistant enzymes such as plasmin, which is native to milk and has been acting on milk proteins. No currently available data suggests what is the baseline level for milk sourced from different cow breeds and its acceptability levels for further processing. There is no data indicating what value represents a good milk standard and what value triggers the impact on the UHT milk shelf-life stability problems. Therefore, expanded knowledge in this area is valuable.

It was concluded that there was no significant difference between free amino nitrogen concentrations between batches on day 1 after processing suggesting similar level of proteolytic quality for all milk tested regardless of raw milk microbiological quality.

The choice of temperature was related to UHT milk ambient storage at 19°C and the development of an accelerated shelf-life test at an elevated temperature as well as possibility of a high transportation temperatures at 55°C.

Analysis of data (**Table 4.3**) confirmed that in processed milk from farm 1, free amino nitrogen levels were significantly increased during storage at 19°C and 55°C. During storage of samples at 19°C, FAN concentration increased from 29.00 mg/l to 101.87 mg/l on week four. The other milk samples produced from milk of poor microbiological quality sourced from farms 2 and 3, similarly, to farm one also showed FAN results significantly increased once compared to milk

sourced from farms produced from milk of excellent quality. However, the increase of FAN concentrations for farms 2 and 3 was not as substantial as from farm one. The remaining farms showed similar FAN concentration levels (**Figure 4.3a**) and those levels were not increased during the whole storage of five weeks at 19°C. The farms that produced milk of good quality showed the same level of FAN and even slightly decreased values when compared results with results from samples tested on the first day straight after processing. FAN concentrations at storage at 55°C were also increased but not as substantially as at 19°C. At 55°C, the highest value was recorded for milk processed from farm one, however, there was no significant difference in FAN concentrations between all farm samples regardless of the microbiological quality at this temperature point (**Figure 4.3b**). Interestingly, samples produced from milk of good microbiological quality showed an increase in FAN concentrations at 55°C, while there was no noticeable increase in FAN when those samples were stored at 19°C. This could be related to plasmin activity at 55°C present in all farm milk samples.

Table 4.3 Free amino nitrogen concentration (mg/l) of UHT unstandardized milk produced from Jersey raw milk of variable quality on day 1, week 4 and week 5 stored at 19°C and 55°C. Data presented as mean value ± standard deviation of two independent measurements.

	Free amino nitrogen concentration (mg/l)				
Farm	**Day 1**	**Week 4 19°C**	**Week 5 19°C**	**Week 4 55°C**	**Week 5 55°C**
1	29.00 ±0.91^{a}	101.87 ±10.83^{a}	93.87 ±3.63^{a}	56.35 ±8.63^{a}	51.90 ±3.62^{a}
2	23.982 ±1.36^{d}	38.30 ±1.03^{b}	23.70 ±1.29^{c}	54.75 ±1.79^{a}	45.19 ±2.66^{bc}
3	24.20 ±0.97^{cd}	39.12 ±1.84^{b}	23.67 ±0.74^{c}	53.75 ±1.75^{a}	43.95 ±2.37^{cd}
4	25.17 ±1.16^{abcd}	22.88 ±0.82^{c}	23.22 ±1.16^{c}	33.80 ±1.63^{b}	37.22 ±1.19^{e}
5	29.08 ±2.13^{ab}	22.73 ±0.34^{c}	28.69 ±1.83^{b}	35.04 ±7.20^{b}	50.57 ±1.54^{ab}
6	24.37 ±2.91^{bcd}	20.53 ±0.52^{c}	23.55 ±1.43^{c}	32.47 ±1.51^{b}	39.42 ±3.38^{cde}
7	28.78 ±4.49$_{ab}$	21.92 ±1.10^{c}	26.06 ±1.15^{bc}	34.25 ±1.17^{b}	42.93 ±2.91^{cde}
8	28.51 ±2.23^{abc}	20.38 ±0.78^{c}	25.64 ±2.68^{bc}	36.97 ±2.03^{b}	38.85 ±2.12^{de}

Means within a row with different superscript are significantly different at $p < 0.05$.

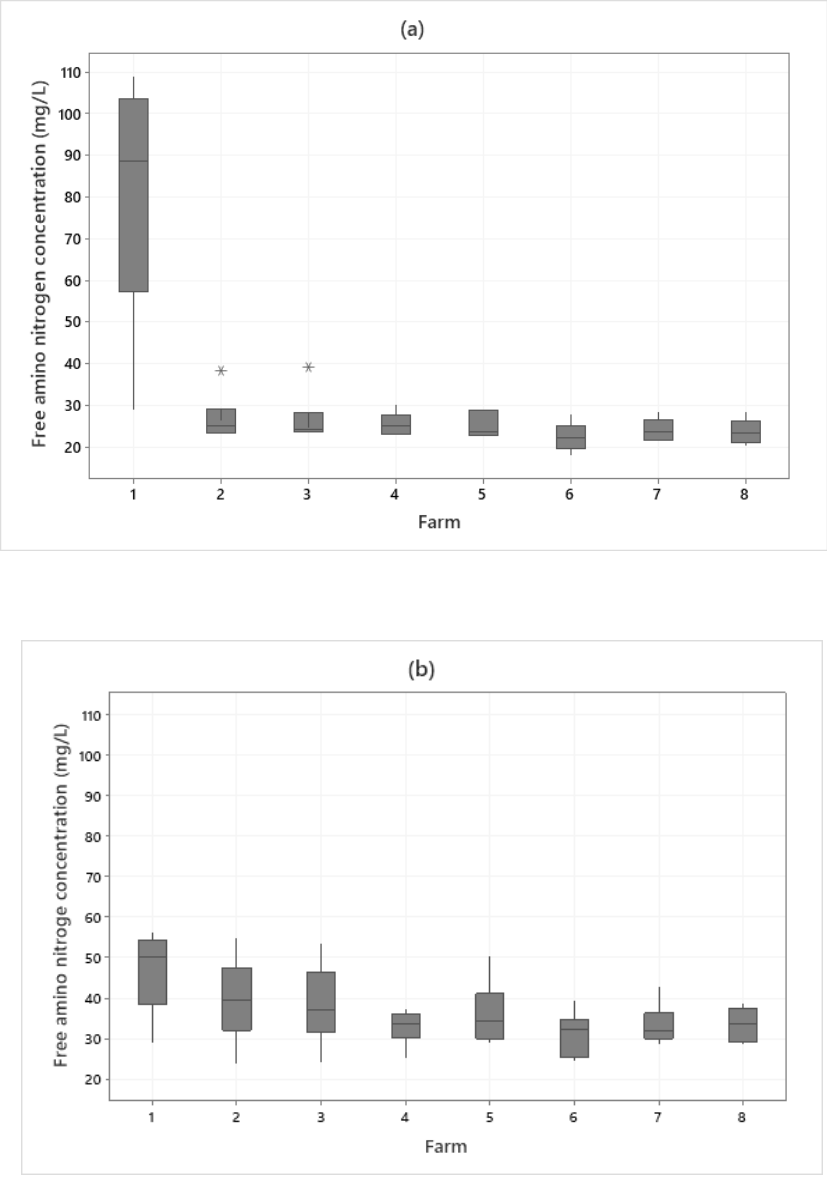

Figure 4.3 Boxplot of Free amino nitrogen concentration (mg/L) for Jersey unstandardized UHT milk samples stored at (a) 19°C (p=0.000) and (b) 55°C (p=0.024).

Interestingly, for storage at 55°C a moderate increase in FAN concentration was noted across all UHT milk samples, which indicates that increased storage temperature promotes proteolysis or different mechanism contributes to the development of proteolysis which can be triggered by temperature. Specifically, a temperature of 55°C could contribute to the prediction of the shelf

life of UHT products by promoting proteolysis reaction, as most proteolytic enzymes are known to demonstrate optimum activity between 40 – 60 °C (Sharma et al., 2019). However, a storage temperature of 55°C for a prolonged time can partially deactivate some native and bacterial enzymes present in milk. Poffé and Mertens (1988) reported that the best inactivation of heat-stable proteases of psychrotrophic origin was after heat treatment of 1 hour at 55°C or 2 min at 140°C. At higher temperatures, increased heat resistance is often observed for bacterial proteinases. The plasmin is more thermoresistant than some bacterial proteinases (Van Asselt et al., 2008). It is likely that storage at 55°C for a longer time inactivated some of the bacterial proteinases present in raw milk (**Figure 2.2**). Though, only an increase related to some bacterial activity might be observed at 55°C and most of the FAN increase is related to plasmin activity in the UHT milk. The highly active bacterial proteinase and native plasmin activity show its presence at 19°C, especially in relation to milk in poor microbiological quality from farm one.

4.3.3 Impact of raw milk attributes on UHT milk stability

The initial free amino nitrogen concentration recorded on day one was similar for all UHT milk samples produced from all farms. Even though, the microbiological quality of the raw milk samples varied significantly on the day of processing, the baseline of detected peptides and progressed protein hydrolysis did not change the initial free amino nitrogen concentration. This could be potentially related to the fact that milk was processed within 48 hours from milking and on day one following processing, there was no evidence of any further enzymatic activity and protein hydrolysis in raw milk samples. However, the level of proteinases due to milk microbiological activity was suspected to be at different levels in different farm milk samples and within the storage time initiated proteolytic activity. This observation confirms that time is required for enzymes to complete some changes during product shelf life and those changes are most likely not detectable on the day of processing. The impacting factor is that milk was

processed within a maximum of 48 hours from milking which is well known a good practice used to minimise the development of bacterial enzymes and limit their activity during UHT milk storage.

Moreover, it is worth to note that the initial levels of free amino nitrogen were established for Jersey milk samples for the first time in literature. FAN levels potentially could vary during a year as plasmin activity is linked with various seasonal factors and the somatic cell count level. The impact of seasonality and breed on FAN levels is an additional study to consider in the future research. There is limited literature knowledge to confirm the levels and differences between FAN concentrations in milk sourced from different breeds at different seasons.

The pilot plant trial suffered from limitations due to a lack of dilutions in raw milk testing, where some counts of enumerations were not obtained and were recorded as TNTC. Most importantly, UHT milk samples were stored for a short period that was restricted to assess the impact due the limited increase of free amino nitrogen concentrations between farms. This study, as expected, highlighted that more trials, ideally commercial trials, are required to build a database with results and variations of raw milk attributes to assess the impact of raw milk microbiological quality on UHT milk quality during longer storage times. The next steps are to consider other attributes measuring the impact of raw milk quality on shelf-life stability are required, i.e., viscosity and measurement and expanded observations of quality defects.

The five weeks of storage were not sufficiently long to confirm and fully support the hypothesis that the microbiological quality of raw milk can impact on the level of milk proteolysis. Raw milk from farm one was confirmed by PCA analysis as considerably of the lowest microbiological quality when compared to milk sourced from other farms 5, 6, 7 and 8, however, was classed as similar milk from farms 2, 3 and 4. However, due to the lack of bacteria enumeration for PBC is believed to have been significantly different from all other milks and with a much-progressed level on bacterial enumeration and enzyme activity initially. Raw milk sourced from farm one

was confirmed as of the lowest microbiological quality, with pH (6.57) and ES (30%). Raw milk from farm one had the highest Bactoscan count and the highest psychrotrophic bacteria level (>3,000,000 CFU/ml), *Pseudomonas* spp. counts (>3,000,000 CFU/ml), and *Streptococcus* spp. (6,100 CFU/ml) enumeration. The concentration results aligned with the raw milk results and supported the findings of higher FAN levels in stored milk samples produced from milk from farm one. Samples for that farm aged during storage within 4 weeks and showed a significantly increased level of protein hydrolysis at 19°C, as indicated by FAN measurement. Additionally, milk from that farm caused processing difficulties and increased fouling at the UHT heat exchanger. This links with pH and ethanol stability results that suggest destabilised proteins or increased mineral content confirming that UHT milk sourced from farm one was not suitable for UHT processing. High stability minimises fouling during the UHT process and reduces sediment formation in the product. It can be confidently said that milk sourced from farm one has already destabilised during the initial 5 weeks of storage and this links with raw milk quality and it is visible by increased FAN values at samples stored at 19°C.

No significant correlations were found between FAN and any other microbiological parameters due to one different result giving a bi-modal distribution of data which invalidated the correlations. The weakness of the experiment was that samples storage time was short and the FAN increase was not significant for the other milk samples collected from farms producing milk of deteriorated quality to be able to fully support the study objective so that valid correlations could be produced, and predictive models developed to establish age gelation capacity.

Milk samples were stored at those two temperatures for initial 5 weeks which is a noticeably short period of time for storage of UHT milk. UHT heat treatment deactivates some percentage of proteinases, so the remaining quantity of enzymes is lower following the heat treatment, therefore the time of measurement of changes would need to be extended to measure the impact of the remaining enzymes on the UHT milk stability.

As noted previously, the values of free amino nitrogen increased at 55°C and did not vary between milk sourced from different farms. The method that includes 55°C storage in protocol could be used to build predictive models in shelf-life estimation but more testing is required to support this evidence and to be able to explain what other processes are happening at this temperature point to confirm if they relate to raw milk microbiological and proteolytic activity rather than other milk chemical changes. As noticed the increase pattern of FAN at 55°C was similar for all farm milk regardless of milk microbiological and physicochemical quality, which could be presenting promoted increase only related to plasmin activity in Jersey milk. This also indicates that other mechanisms could be contributing to the increase of free amino nitrogen at this temperature, or the enzyme activity is suppressed by other chemical reactions developing at this storage temperature. More analysis is required to establish protein degradation at this temperature point in order to confirm its suitability for future indication of protein hydrolysis testing.

As this experiment was carried out on unstandardized milk, it is beneficial to carry out testing on standardised milk, skimmed and whole fat milk, as they might be presenting different behaviours due to their compositional changes and additional processing homogenisation steps making an impact on the development or reduction of quality defects. It is necessary to review other methods to assess protein hydrolysis and changes in product shelf life.

The experiment confirmed that the ninhydrin method could be considered as a good method for detecting the general level of protein hydrolysis in UHT milk.

Additionally, it is beneficial to develop a method that identifies hydrolysis produced from bacterial enzymatic breakdown and eliminates the breakdown caused by plasmin activity that could be activated during UHT milk shelf life and contribute to the formation of UHT milk quality defects. The ninhydrin method used in this study for measurement of free amino nitrogen

concentration identifies plasmin and bacterial enzymatic peptides released during protein breakdown. It would be beneficial to select and amend the testing methodology to identify and measure small peptides from bacterial breakdown and eliminate peptides caused by plasmin activity. That will support the hypothesis of the impact of bacterial quality on the development of quality defects and support thesis objectives to link the microbiological quality of raw milk with the stability of UHT milk during shelf life. In future studies, it would be beneficial to increase the length of storage of milk samples and trial different storage temperatures to observe any different patterns and contributions of other reactions to the development of defects and peptides release.

4.4. Conclusion

This study demonstrated an association between microbiological and chemical quality of raw milk and UHT milk stability. It gave a good base for further studies to establish predictive models and rapid methods that can be used in shelf-life estimation following the analysis of raw milk quality used for UHT milk processing.

This is key knowledge for raw milk producers and dairy manufacturers to use as an indication and guideline to achieve longer UHT shelf life and milk stability. This study confirmed the necessary focus on raw milk microbiological quality in relation to psychrothrophic bacteria counts, that are responsible for producing extracellular thermostable enzymes which negatively impact on UHT milk stability during shelf life.

Qualitative bacteria identification coupled with bacterial enumeration in raw milk was proved as an adequate measurement, aiming to improving raw milk quality by tackling the sources of contamination at the farm level.

Principal component analysis (PCA) revealed differences in microbiological and chemical quality of raw milk between farms in that corresponded with the development of protein hydrolysis during UHT milk storage. The principal analysis could be potentially used to gather multiple quality attributes of raw milk and assess raw milk quality and its suitability for UHT milk processing.

This study confirmed that storage temperature and time had a significant impact on the stability and shelf-life of UHT milk due to the progress of protein hydrolysis. Within time, free amino nitrogen concentrations increased, and FAN concentration varied at different storage temperatures. The latter was associated with the impact of psychrothrophic bacteria and their enzymes on quick protein hydrolysis that trigger UHT milk quality defects. At 19°C within 5

weeks, free amino nitrogen concentration significantly increased for UHT milk produced from raw milk of poor microbiological quality. The FAN concentration at 55°C increased but did not reach the same levels as at 19°C for farms of poor microbiological quality. At 55°C, possible changes in the thermostability and activity of endogenous and exogenous enzymes, as well as the contribution of other physicochemical reactions, may impact on the process of protein hydrolysis; however, further research is required to fully understand the mechanisms of these changes.

CHAPTER 5

5. IMPACT OF RAW MILK QUALITY AND STORAGE CONDITIONS ON PROTEOLYSIS AND STORAGE STABILITY OF UHT JERSEY STANDARISED MILK PRODUCED AT COMMERCIAL SCALE

5.1. INTRODUCTION

Proteolysis of UHT milk causes the development of bitter flavours and leads to an increase in viscosity, with the eventual formation of a gel. Age gelation of UHT milk is a major concern of the dairy industry since it limits the shelf-life and market potential of the milk. Proteolysis of UHT milk is caused by natural milk alkaline serine proteinase, known as plasmin, and proteinases produced by psychrotrophic bacterial contaminants of raw milk (Datta and Deeth, 2003). Bacterial extracellular proteinases produced by psychrotrophic bacteria are problematic as they have high heat stability and may retain some activity even after sterilisation treatments (Griffiths et al., 1981). Plasmin causes soft gels, while bacteria proteases give rise to hard gels (Malmgren et al, 2017).

The development of knowledge in this area is essential to ensure that UHT milk is produced of consistently high quality. Processors must be familiar with multivariable factors, including the level of bacterial contamination that triggers UHT milk defects and reduces UHT milk shelf life. This study aims to provide an analysis of Jersey raw milk quality and its suitability to produce UHT standardised milk. It provides an assessment of Jersey raw milk quality and validates Jersey UHT skimmed and UHT whole fat milk shelf-life. The knowledge gained from this study may be used for developing diagnostic tests to determine the acceptable level of peptides to produce milk with desired shelf-life length and stability.

This study evaluates samples produced from Jersey comingled raw milk collected from 14 Jersey dairy farms and processed at the UHT industrial plant (Jersey Dairy, Jersey) and observation of sensory defects, compositional changes, colour and viscosity changes and changes in protein degradation, during product shelf life up to 360 days of storage while different product storage temperatures were trialled.

Additionally, this chapter evaluates individual bacteria strains present in raw milk supply to establish evidence-based thresholds for specific bacteria strains to ensure consistency of the UHT milk quality. The raw milk direct plating bacteria identification method was used to identify the potential causes of raw milk contamination at the farm level to highlight the requirement of continuous focus on raw milk quality and to provide tool for the necessary improvements.

5.2. MATERIALS AND METHODS

I fully managed this project as I worked at Jersey Dairy as Head of Quality and Senior Manager during the time. I arranged and supervised the specific tasks and analysis with the third parties involved in this project.

5.2.1. Sample collection and experimental design

Bulk milk from 14 Jersey herds on the island of Jersey was transported via milk collection tankers to Jersey Dairy processing facility as part of its standard operations (Jersey, Channel Islands).

Raw milk was classed into three groups according to its psychrotrophic bacteria count as presented in **Table 5.1** and further processed into either UHT skimmed milk or UHT whole fat milk according to the production schedules. For each band intention was to select a minimum of two batches of UHT skimmed milk and two batches of UHT whole fat milk.

Table 5.1 Psychrothrophic bacteria counts bands raw milk classification.

Bands	Psychrothrophic bacteria count (CFU/ml)	UHT skimmed milk batches	UHT whole fat milk batches
1	0-500	2	3
2	501-1,000	5	1
3	1,000-3,500	2	2

In total, 15 UHT milk batches were produced for this experiment between April and September 2019. There were nine batches of UHT whole fat milk and six batches of UHT skimmed milk produced, with milk packed in standard 1 litre Aseptic Tetra Pak cartons. After processing and packing 42 cartons from each batch were then stored at four different temperatures (4°C, 21°C, 30°C, 55°C). Samples where then removed and 2 replicates analysed on day one and at 5 time points over storage, i.e.: day 30, 90, 180, 270, 360 for samples stored at 4°C, 21°C and 30°C and day 7, 14, 21, 28 and 35 for samples stored at 55°C. In total, 630 individual samples were analysed. All assays were performed in duplicates, and free amino nitrogen analysis was performed in triplicates.

5.2.2 Raw milk testing

Jersey raw milk used to produce UHT milk was tested for microbiological and physicochemical quality.

5.2.2.1 Microbiological and proteolytic analysis

Raw milk samples were tested for Bactoscan individual bacteria count (**IBC**), somatic cell count (**SCC**), *Pseudomonas* spp. enumeration, *Enterobacteriaceae* spp., psychrotrophic bacteria count, thermoduric bacteria count, *E. coli*, *Streptococcus* spp. enumeration. Qualitative direct plating

assessment was performed to establish predominant microbiota in raw milk used to produce milk batches.

Individual Bacteria Counts were measured by flow cytometry method using Foss Bactoscan FC and Foss Bacsomatic (Foss, Hillerød, Denmark). Somatic cell count (**SCC**) was measured by flow cytometry method using Delta Combiscope, Model FTIR 400, Model FTIR 600 and Foss Bacsomatic by Jersey Dairy Laboratory (Jersey, Channel Islands).

Pseudomonas spp. count was determined using Pseudomonas agar base (VWR) at 25°C for 48 hours and *Enterobacteriaceae* spp. count was determined by the Enterobacteriaceae count plate (Petrifilm, 3M) at 37°C for 24 hours by Jersey Dairy Laboratory (Jersey, Channel Islands).

Psychrotrophic bacteria count (**PBC**) was determined using MPC media (VWR, Lutterworth, UK) incubated at 3°C for 10 days by Jersey Dairy Laboratory (Jersey, Channel Islands).

Raw milk samples were aliquoted and in refrigerated containers transported at below 5°C to Quality Milk Management Laboratories (**QMMS**) (Somerset, UK) for analysis of thermoduric bacteria count and direct plating and bacteria identification analysis on the day of raw milk collection. Analysis was performed on the second day following the transportation and analysed on the day the raw milk was used for UHT milk production.

Analysis of thermoduric bacteria count (**TBC**), *E. coli* enumeration, *Streptococcus* spp. enumeration and direct plating were performed by Quality Milk Management Services Ltd. (Somerset, UK). Thermoduric bacteria count (**TBC**) was determined using Milk Plate Count agar (Thermoscientific, Basingstoke, UK) incubated at 30°C for 72h. Thermoduric bacteria were enumerated after heating 5ml of milk to a temperature that stimulates pasteurisation (63.5 +/- 0.5°C for 30 min), followed by immediate refrigeration at 20°C. After treatment, serial dilution of the samples was performed up to 10^{-3} is sterile peptone.

Qualitative direct plating assessment was performed by using sheep blood agar (Thermoscientific, Basingstoke, UK) incubated at 37°C for 72 h and then the identity of isolates was further confirmed using Maldi-Tof MS (Matrix Assisted Laser desorption/ionisation time of flight mass spectrometry, Bruker Daltonics, Coventry, UK) by Quality Milk Management Services Ltd. (Somerset, UK).

5.2.2.2 Physicochemical analysis

Representative raw milk samples were tested on the day of raw milk collection and upon delivery to the dairy. Composition tests, ethanol and pH testing were carried out at Jersey Dairy Laboratory (Jersey Dairy, Jersey).

Analysis for fat, protein, lactose, and total solids contents was measured by Fourier transform Infra-Red Spectrophotometry (**FTIR**) using Foss Milkoscan FT120 (Foss, Hillerød, Denmark).

Freezing point depression (**FPD**) of milk samples was measured by cryoscopy using 4D3 Cryoscope (Advanced Instruments Inc., Metuchen, NJ, USA).

Ethanol stability (**ES**) was determined by mixing equal volumes of 2 ml of milk and a range of ethanol solutions (70-80 % v/v at 1% interval resolutions). The milk-ethanol solution was poured into a petri dish and mixed. The Petri dishes were then left to rest for 1 minute and then tilted slightly and observed to identify the presence of any milk clots/coagulation on the concentration at which coagulation did not occur was determined as the ethanol stability of the milk.

The pH of raw milk was analysed at 4°C on the day of processing using a pH probe (Thermo Scientific Orion 3-Star pH meter and Sure-Flow Ion-Selective Electrode).

5.2.3 UHT milk processing

Jersey raw milk was processed within a maximum of 48 hours from milking. The average time from collection to processing was 24 hours. Raw milk was stored between 2°C and 5°C prior to

being standardised to 4.3 % w/w fat content for whole UHT milk, pasteurised and then subsequently UHT processed. All processing took place as part of standard production runs and according to the standard operating procedures of Jersey Dairy.

Standardised raw milk was preheated to 63°C and homogenised upstream using a 2-stage homogeniser operating at 22 MPa and 5 MPa in the first and second stage, respectively. The milk was then pasteurised (74°C for 30 s) and cooled to below 5°C using a plate/tubular heat exchanger. Cold milk was transferred to storage tank and used immediately for further UHT treatment. UHT processing took place on a tubular indirect UHT plant at (138°C for 4 s), followed by downstream homogenisation using a 2-stage aseptic homogeniser operating at 22 MPa and 5 MPa in the first and second stage respectively. The milk was then cooled to 20°C and packed aseptically in 1 litre Tetra Brick cartons.

For the production of UHT skimmed milk raw milk was standardised to maximum of 0.1% fat and thermally processed and packed under the same conditions as whole milk excluding both homogenisation steps.

After processing, UHT milk cartons were randomly selected throughout the run at regular intervals and were removed from the production line and stored in a temperature-controlled incubators for testing over storage as detailed in the experimental design (section 5.2.1).

5.2.3.1 Sample storage

On the day of analysis, two cartons of each batch were opened and analysed in duplicate on each occasion. Results were recorded as means of duplicate analysis and two replications. Free amino nitrogen test was carried out in triplicate on each occasion, and results were recorded as means of triplicate analysis and two replications.

5.2.4 Processed milk analysis

5.2.4.1 Visual and Sterility assessment

Samples were assessed during shelf life for a range of standard visual quality defects: cream layering, age gelation or gel particles and sedimentation. Visual observation of unusual observations and photos of milk samples were taken on each occasion. The presence of individual instability signs was recorded as positive when observed. Colour changes were measured using RHS colour charts supplied by the Royal Horticultural Society (5th edition, UK).

The Celsis Assay was used to determine sterility by checking the presence or absence of microbiological contamination using an amplified ATP-based bioluminescence reaction (Charles River, Denmark).

5.2.4.2 Physicochemical analysis

Physicochemical analysis was carried out in Jersey Dairy Laboratory (Jersey).

Total solids, fat, lactose, and proteins contents were measured by Fourier transform Infra-Red Spectrophotometry (FTIR) using Foss Milkoscan FT120 (Foss, Hillerød, Denmark).

Freezing point depression (**FPD**) of milk samples was measured by cryoscopy using Cryoscope 4D3 Cryoscope (Advanced Instruments Inc., Metuchen, NJ, USA).

Viscosity was measured by Fungilab Rotational Viscometer at temperature 21°C ±1°C. A Ultra-low adapter was used for the determination of the viscosity of milk and spindle no.1 (LCP) was used. Readings were taken after the spindle had been rotating in the milk sample (volume 20ml) for 120s at 0.9 x g, shear rate 220 (1/s), and the mean of two readings was recorded for the analysed sample. The viscosity was recorded as mPa ·s.

Milk pH was measured using a digital pH meter with Automatic Temperature Compensation (Thermo Scientific Orion 3-Star pH meter and Sure-Flow Ion-Selective Electrode) calibrated with standard buffer solutions of pH 4.0 and 7.0.

5.2.4.3 Analysis of proteolysis

UHT milk samples were tested for proteolytic activity by the trinitrobenzenesulfonic acid (**TNBS**) method adapted from Skaridis and Lewis (2016). Unless otherwise stated, all materials were from VWR (Lutterworth, UK).

A calibration curve was created using standard glycine solution at concentrations between 0.5 and 10.0 mg/l. The calibration curve was used to determine the free amino groups concentration in the stored UHT milk samples to indicate the concentration of amino acids and small peptides.

The first step of this TNBS method is a trichloroacetic acid (**TCA**) precipitation procedure. The whole fat milk was first centrifuged at 21,420 x g at 15°C for 15 minutes to remove fat. A sample of skimmed milk (0.7 ml) was mixed with cold 24% (**TCA**) to precipitate proteins and obtain a clear supernatant. In this method, 24% TCA to obtain 12% TCA soluble extracts were obtained. Peptides soluble at pH 4.6 provide a measure of proteolysis from both plasmin and bacterial proteinases, while the peptides soluble in 12% TCA represent proteolysis caused only by bacterial proteinases. Therefore, in this study 12% TCA soluble extracts were tested to measure peptides from proteolysis caused only by bacterial enzymes eliminating the presence of peptides derived from native plasmin activity (Datta and Deeth, 2003).

The TCA soluble extracts were mixed and left at 4°C for 20 minutes, then centrifuged at 21,420 x g at 4°C for 15 minutes. The supernatant was collected and diluted with a dilution reagent (di-

sodium hydrogen phosphate dodehydrate 0.2M pH 9.1). The dilution reagent was warmed up to a stable temperature of 38°C ±1°C and used immediately.

The diluted sample (0.4 ml) was mixed with 0.7ml of 5% v/v TNBS (diluted with 0.1M sodium bicarbonate) and placed in an incubator in the dark at 38°C ±/- 1°C for 60 minutes.

After incubation, the absorbance of this mixture was measured immediately within 2 minutes at 340 nm with Lambda 250 Spectrophotometer using acryl-cuvettes (PerkinElmer, Nantwich, UK). Distilled water was used for blanks, and concentrations were established against the glycine calibration curve (**Appendix 4**). The result was presented as the free amino nitrogen concentrations in mg/l of milk.

5.2.5 Statistical analysis

Statistical analysis was carried out using Minitab Software (Minitab Ltd, State College, Pennsylvania) and SIMCA 17 software (Umetrics, Umea, Sweden).

An approach by means of mathematical models was used in this chapter to analyse the relationships between the **FAN,** viscosity, and the microbiological and physicochemical raw milk parameters from milk stored at different temperatures.

The microbiological and physicochemical variables and impact of time and temperature on UHT samples parameters were assessed using one-way ANOVA, and the Tuckey test was found significant at $p < 0.05$. Tukey's test (at 5% error probability) was used to compare the means for all variables.

The correlations between raw milk attributes were assessed by applying Pearson's correlation coefficient by looking at samples stored at different temperatures.

Minitab was used to calculate a one-way analysis of variance (ANOVA) and Pearson correlation. The correlation between FAN concentration (mg/l) and the level of physicochemical and

microbiological variables was assessed using Pearson correlation. The significance level was established at $p < 0.05$.

5.3. RESULTS AND DISCUSSIONS

5.3.1 Raw milk quality

The average properties of Jersey raw milk used in the experiment are presented in **Table 5.2**. The results show no variation in a data set as far as raw milk physicochemical parameters were analysed for this study that confirms stability of raw milk supply in this respect.

According to Shew (1981), ethanol stability (**ES**) provides a simple way of indicating whether raw milk is suitable for UHT processing, with ethanol stability of 74% being the threshold below which milk is not suitable. Milk with lower ethanol stability is more susceptible to fouling and sedimentation during heat treatment (Chen et al., 2012). Deeth and Lewis (2017) explain that milk has low ethanol stability (< 74%) due to either poor microbiological quality which is accompanied by a fall in pH or salt imbalance. The ethanol stability of raw milk varied from 75% to 78% in this study. Therefore, all milk selected in this trial was suitable for UHT milk processing.

Viscosity varied from 1.78 to 1.84 mPa·s. The pH varied from 6.70 – 6.76.

Table 5.2 Physicochemical quality of raw milk used for the production of Jersey UHT whole and skimmed milk.

Item	No. of samples	Mean	SE Mean	St Dev	Minimum	Median	Maximum
Fat (%)	30	5.20	0.02	0.06	5.10	5.20	5.38
Protein (%)	30	3.73	0.01	0.04	3.64	3.74	3.79
Lactose (%)	30	4.50	0.01	0.03	4.46	4.49	4.59
TS (%)	30	14.40	0.02	0.09	14.29	14.36	14.64
FPD (-m°C)	30	520	0	1	517	520	522
ES (%)	30	76	0	1	75	77	78
pH	30	6.74	0.00	0.02	6.70	6.74	6.76
Viscosity (mPa·s)	30	1.82	0.01	0.02	1.78	1.83	1.84

TS- Total solids; **FPD**- Freezing point depression; **ES**- Ethanol stability.

The microbiological quality of raw milk used to produce UHT skimmed and UHT whole fat milk is presented in **Table 5.3.**

Bactoscan bacteria count varied from 7,000 IBC/ml to 34,000 IBC/ml with mean 13,600 IBC/ml.

Somatic cell count varied from 140,000 cell/ml to 160,500 cell/ml.

According to the current European Union (**EU**) regulation, raw milk should not exceed total bacteria counts of 100,000 CFU/ml and 400,000 cells/ml somatic cell counts. Those legislation criteria were met. EU and UK premium quality milk is described for milk when Bactoscan is below 30,000 IBC/ml and 15,000 IBC/ml, respectively. In this case, all raw milk used in the experiment was classed as premium except one batch number 2 which had a Bactoscan count of 34,000 IBC/ml (**Table 5.3**).

There were variations in other microbiological parameters i.e., *Enterobacteriaceae* spp. and *E. coli* were noted. Thermoduric bacteria count did not vary between batches and raw milk was

classed as premium in relation to this parameter. The UK system classes raw milk as premium if TBC does not exceed 250 CFU/ml. The maximum TBC in this data was 263 CFU/ml but average 169 CFU/ml.

PBC varied, as planned, from the value of 75 CFU/ml up to 3,500 CFU/ml. **Table 5.4** shows variation in raw milk in relation to PBC. National Milk Laboratories (UK) classifies raw milk as poor quality if PBC > 5,000 CFU/ml. Milk is of excellent quality when PBC is less than 500 CFU/ml. *Pseudomonas* spp. being a psychrotrophic bacteria varied from 12 CFU/ml to 3,200 CFU/ml. *Streptococcus* spp. from 15 CFU/ml to 5,900 CFU/ml.

Table 5.3 Microbiological quality of raw milk used to produce Jersey UHT skimmed and UHT whole fat milk.

Item	No. of samples	Mean	SE Mean	St Dev	Minimum	Median	Maximum
Bactoscan (IBC/ml)	30	13,630	178	689	7,000	11,500	34,000
PBC (CFU/ml)	30	941	235	912	75	735	3,500
TBC (CFU/ml)	30	169	13	51	110	150	263
Pseudomonas spp. (CFU/ml)	30	917	241	932	12	750	3,200
E. coli (CFU/ml)	30	7	3	13	0	3	51
Streptococcus spp. (CFU/ml)	30	947	405	1,569	15	303	5,900
Enterobacteriaceae spp. (CFU/ml)	30	61	23	89	0	15	325
Somatic cell count (cells/ml)	30	157,170	170,000	659,000	140,000	152,500	160,500

PBC- Psychrothrophic bacteria count; **TBC**- Thermoduric bacteria count.

Table 5.4 and **Table 5.5** illustrate the microbiological averages of the PBC bands of Jersey raw milk used in this study. The lowest band PBC < 500 CFU/ml was used for batches 4, 7, 8, 10 and 12 (**Figure 5.1**). The highest above > 1,000 CFU/ml for batches 2, 3, 9 and 15. A high number of UHT batches were produced for PBC in the second band for UHT whole fat milk analysis, but only one batch of UHT skimmed milk was produced from raw milk with PBC in the range between 500 and 1,000 CFU/ml. PBC and TBC results were only available after the milk is processed due to the time required to complete microbiological analyses hence there is an imbalance in the number of replicate batches in each PBC class. This was a challenge intrinsic in work on commercial plant and within the timeframe available for this study.

Figure 5.1 shows the variability of the microbiological results used to produce batches of UHT skimmed milk and UHT whole fat milk. The highest Bactoscan count occurred in raw milk used to produce batch 2 (34,000 IBC/ml). The Bactoscan below 10,000 IBC/ml was noted for raw milk used to produce batches 1, 4, 8 and 14. The *Pseudomonas* spp. bacteria count varied with the highest counts of 3,200 CFU/ml and 2,850 CFU/ml for batches 2 and 3, and the lowest counts 12 CFU/ml and 105 CFU/ml for batches 12 and 4.

The increase of *Streptococcus* spp. for raw milk used for batch 2 with a value of 3,150 CFU/ml and 7 of value 5,900 CFU/ml was noted.

Table 5.4 Microbiological quality of Jersey raw milk classified to PBC bands used to produce Jersey UHT whole fat milk. Values presented are mean (StDev).

Items	Band 1	Band 2	Band 3
Bactoscan (IBC/ml)	15,250 ± 3,750	12,200 ± 4,200	23,250 ± 10,750
Enterobacteriaceae spp. (CFU/ml)	88 ± 64	29 ± 40	170 ± 155
Psychrothrophic spp. (CFU/ml)	343 ± 113	742 ± 202	2,525 ± 975
Thermoduric spp. (CFU/ml)	202 ± 52	144 ± 34	143 ± 0
E. coli (CFU/ml)	9 ± 7	3 ± 5	28 ± 24
Streptococcus ssp. (CFU/ml)	3,092 ± 2,523	381 ± 264	2,188 ± 962
Psuedomonas ssp. (CFU/ml)	306 ± 294	738 ± 463	1,805 ± 1,395

Table 5.5 Microbiological quality of Jersey raw milk classified to PBC bands used to produce Jersey UHT skimmed milk. Values presented are mean (StDev).

Items	Band 1	Band 2	Band 3
Bactoscan (IBC/ml)	8,500 ± 2,000	10,500 ± 707	15,250 ± 4,250
Enterobacteriaceae spp. (CFU/ml)	7 ± 8	60 ± 57	90 ± 75
Psychrothrophic spp. (CFU/ml)	168 ± 161	800 ± 42	1,758 ± 452
Thermoduric spp. (CFU/ml)	203 ± 58	153 ± 46	185 ±75
E. coli (CFU/ml)	2 ± 1	5 ± 1	2 ± 1
Streptococcus ssp. (CFU/ml)	348 ± 272	395 ± 78	137 ± 121
Psuedomonas ssp. (CFU/ml)	550 ± 800	450 ± 71	1,875 ± 975

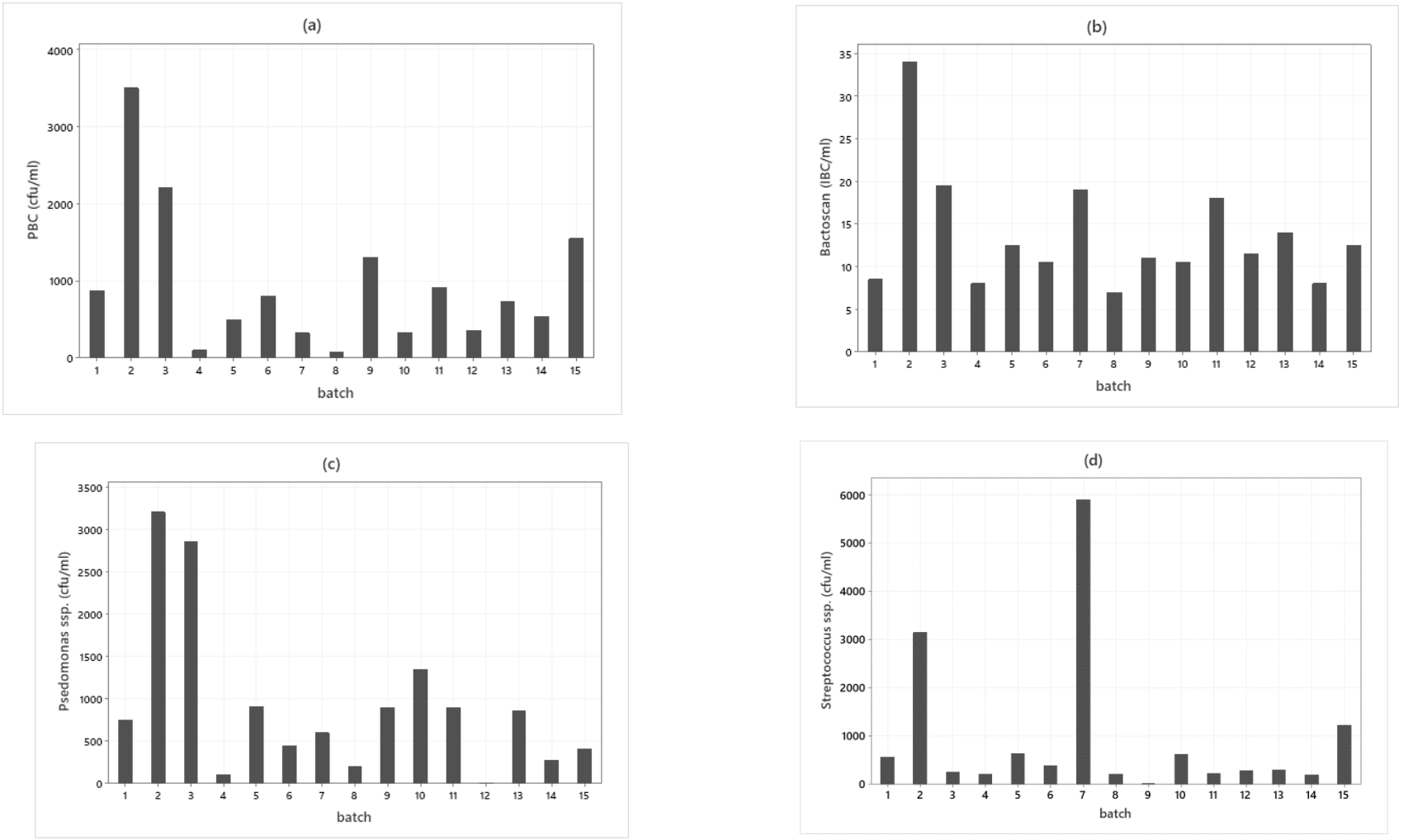

Figure 5.1 Variability of the (a) PBC (b) Bactoscan (c) *Pseudomonas* spp. (d) *Streptococcus* spp. count of raw milk used to produce Jersey UHT skimmed and UHT whole fat milk batches.

The raw milk direct plating results bacteria identification were used to indicate sources of contamination at the farm level (**Table 5.6**). Raw milk bacteria contamination sources as described in **Chapter 2**, **Table 2.2** were used in the investigation and analysis of raw milk direct plating results. The challenging aspect of the analysis in relation to raw milk suitability for UHT milk processing is that all diverse types of psychrotrophic bacteria produce extracellular enzymes with different thermostability. The highest resistance is observed for enzymes produced by *Pseudomonas* spp. and *Enterobacteriaceae* spp. group, *Acetinobacter*, *Achromobacter* and some strains of *Moraxella* could be showing high-temperature resistance. Lower resistance is presented by strains of *Bacillus* spp. or *Aeromonas* spp. (Griffiths et al., 1980).

Joined analysis of direct plating bacteria identification results and other microbiological parameters i.e., psychrothrophic bacteria count, directs the raw milk producer into the potential sources of contamination, helps to establish the root cause and therefore apply the corrective actions. The majority of milk samples analysed and presented in **Table 5.6** indicate that in order to improve raw milk quality the following actions need to be taken; review the plant cleaning regime (Griffiths, 2010; Gleeson et al., 2015); review udder cleaning (Griffiths, 2010; Gleeson et al., 2015); review of water treatment and quality (Özer and Akdemit - Evrendilek, 2014) and improvement of milking regime and udder cleaning procedures (Griffiths, 2010; Gleeson et al., 2015).

The direct plating results (**Table 5.6**) confirmed that raw milk samples identified all bacteria species consistent with findings of typical for Jersey raw milk microbiota (**Figure 3.11 and 3.12**). Predominant microflora consisted of psychrothrophic bacteria, and the most prevalent species were *Streptococcus uberis, Pseudomonas fluorescens, Staphylococcus chromogenes, E. coli, Staphylococcus hyicus, Serratia liquefaciens,* and *Pseudomonas* spp.

Table 5.6 Jersey raw milk microbiological qualitative direct plating assessment used for the production of Jersey UHT whole fat and skimmed milk.

Batch	PBC (CFU/ml)	Band	Direct plating bacteria identification	Potential source of contamination
1	875 ± 78	2	Moderate mixed growth of *Staphylococcus aureus, Bacillus cereus, Serratia* spp*., Streptococcus uberis, Streptococcus dysgalactiae, Enterococcus faecalis, Raoultella ornithinolytica.*	- Udder hygiene (Griffiths, 2010) - A high prevalence of intramammary infection in the herd (Rainard, 2010) - Water quality (Özer and Akdemit - Evrendilek, 2014) - Milking practices (Özer and Akdemit - Evrendilek, 2014)
2	3,500 ± 0	3	Heavy mixed growth of *Streptococcus uberis, Pseudomonas* spp*., E. coli* and *Staphylococcus chromogenes*.	- Plant cleaning (Fitzgerald and Cotter, 2013) - Contaminated water sources (Robinson, 2002) - Bacteria biofilm in milking equipment (Robinson, 2002) - A high prevalence of intramammary infection in the herd (Rainard, 2017) - Udder hygiene (Griffiths, 2010)
3	2,210 ± 226	3	Moderate mixed growth *off E. coli, Pseudomonas fluorescens, Aerococcus viridans* and *Streptococcus uberis.*	- Contaminated water (Özer and Akdemit - Evrendilek, 2014) - Udder hygiene (Griffiths, 2010) - Milking practises (Özer and Akdemit - Evrendilek, 2014)
4	100 ± 0	1	Scant mixed growth of *E. coli, Streptococcus uberis, Aerococcus viridans* and *Staphylococcus* spp*.*	- Udder hygiene (Griffiths, 2010)

Batch	PBC (CFU/ml)	Band	Direct plating bacteria identification	Potential source of contamination
5	650 ± 42	2	Mixed heavy growth of *Serratia liquefaciens* and *Streptococcus uberis*.	- Udder hygiene (Griffiths, 2010) - The presence of inflammatory disease of the udder (Özer and Akdemit - Evendilek, 2014)
6	800 ± 42	2	Moderate, mixed growth of *Pseudomonas fluorescens, Streptococcus uberis, Aerococcus viridans* and *Acinetobacter johnsonii.*	- Contaminated water sources (Robinson, 2002) - Presence of biofilms in milking equipment (Özer and Akdemit - Evrendilek, 2014)
7	325 ± 64	1	Mixed heavy growth of *Streptococcus uberis, Acinetobacter radioresistens* and *Staphylococcus chromogenes Pseudomonas fluorescens, E. coli.*	- The presence of inflammatory disease of the udder (Özer and Akdemit - Evendilek, 2014)
8	75 ± 7	1	Scant, mixed growth of *Acinetobacter baumanii, Moraxella osloensis, Staphylococcus chromogenes* and *Staphylococcus*.	- Milking practices (Özer and Akdemit - Evrendilek, 2014). - Udder hygiene (Griffiths, 2010)
9	1,305 ± 205	3	Heavy, virtually pure growth of *Pseudomonas fluorescens*.	- Milking procedures (Fitzgerald and Cotter, 2013) - Contaminated waste sources (Özer and Akdemit - Evrendilek, 2014) - The presence of biofilms in milking equipment (Özer and Akdemit - Evrendilek, 2014)

Batch	PBC (CFU/ml)	Band	Direct plating bacteria identification	Potential source of contamination
10	330 ± 99	1	Moderate, mixed growth of *Streptococcus uberis, Pseudomonas fluorescens* and *Staphylococcus hyicus*.	- Udder hygiene (Griffiths, 2010) - Water quality (Özer and Akdemit - Evrendilek, 2014)
11	910 ± 85	2	Mixed heavy growth of predominately *Pseudomonas fluorescens* and *Aeromonas salmonicida, Acinetobacter* spp*. and Staphylococcus xylosus*.	- Udder hygiene (Rainard, 2017) - Water quality (Özer and Akdemit - Evrendilek, 2014) - poorly cleaned and disinfected milking equipment (Gleeson et al., 2015)
12	360 ± 113	1	Moderate, mixed growth of *E. coli, Enterococcus faecalis, Serratia liquefaciens* and *Acinetobacter proteolyticus.*	- environmental factors could be a significant contribution i.e., faeces (Kagkli et al., 2007) - water quality (Özer and Akdemit - Evrendilek, 2014) - udder hygiene could be a contributing factor (Griffiths, 2010)
13	735 ± 35	2	Scant, mixed growth of *E. coli, Streptococcus uberis, Corynobacterium xerosis* and *Staphylococcus chromogenes.*	- Milking practises (Fitzgerald and Cotter, 2013) - Udder hygiene (Rainard, 2017)
14	540 ± 42	2	Scant, mixed growth of *E. coli, Streptococcus uberis, Acinetobacter baumanii, Acinetobacter proteolyticus, Aerococcus viridans* and *Corynobacterium xerosis.*	- Udder hygiene (Griffiths, 2010)

Batch	PBC (CFU/ml)	Band	Direct plating bacteria identification	Potential source of contamination
15	1,550 ± 636	3	Mixed, heavy growth of *Klebsiella oxytoca, Pseudomonas fluorescens* and *Staphylococcus hyicus*.	- udder hygiene and inflammatory disease of the udder (Rainard, 2017)

PBC- Psychrothrophic bacteria count.

Pearson correlation was carried out for physicochemical and microbiological raw milk attributes. There was a strong correlation between fat (%) and total solids (%) (r=0.865), that is in line with the literature findings. In relation to microbiological quality as expected (**Table 5.7**), there was a positive relationship between Bactoscan count and other microbiological parameters; PBC (r=0.779), *Pseudomonas* spp. (r=0.706), *E. coli* (r=0.737), *Enterobacteriaceae* spp. (r=0.759). Additionally, PBC and *Pseudomonas* spp. (r=0.796), PBC and *E. coli* (r=0.690) and PBC and *Enterobacteriaceae* spp. (r=0.763). A positive correlation was identified between *E. coli* and *Enterobacteriaceae* spp. (r=0.862) not surprisingly as they belong to the same genera. A negative correlation was identified between PBC and viscosity (r=-0.448) and viscosity and *E. coli* (r=-0.535) and viscosity and *Enterobacteriaceae ssp.* (r=-0.492).

Table 5.7 Pearson-correlation (Pearson correlation and p-value) between raw milk microbiological characteristics*p < 0.05 **< 0.01 ***< 0.001 NS: Non-significant.

Item	Bactoscan	PBC	TBC	*Psuedomonas* spp.	E. coli	*Streptococcus* spp.	*Enterobacteriaceae* spp.
Bactoscan							
PBC	0.779 ***						
TBC	NS	NS					
Pseudomonas spp.	0.706 ***	0.796 ***	NS				
E. coli	0.737 ***	0.690 ***	NS	0.502 **			
Streptococcus spp.	0.556 ***	NS	NS	NS	NS		
Enterobacteriaceae spp.	0.759 ***	0.763 ***	NS	0.651 ***	0.862 ***	NS	
Viscosity	NS	-0.448 **	NS	-0.365 *	-0.535 **	NS	-0.492 **
pH	NS	NS	NS	NS	NS	NS	NS
ES	NS	NS	-0.364 *	NS	NS	NS	NS
SCC	NS	NS	NS	NS	NS	0.408 *	NS

PBC- Psychrothrophic bacteria count; **TBC**- Thermoduric bacteria count; **ES**- Ethanol stability; **SCC**- Somatic cell count.

5.3.2 Analysis of UHT milk

All UHT milk cartons used in the study were subjected to and passed sterility tests. This was to ensure that all samples had been appropriately processed and no post process microbiological contamination had occurred which could result in quality defects.

5.3.2.1 UHT milk on day of processing

Table 5.8 summarises the physicochemical properties of the UHT samples on the day of processing (day 1). There was a minimal variance between processed individual batches in relation to physicochemical parameters. Free amino nitrogen concentration on day 1 varied significantly between skimmed milk and whole fat milk with values of 42.28 mg/L and 35.29 mg/L, respectively as expected due to the compositional differences in UHT milk. Viscosity ranged from 1.86 to 1.91 mPa·s for UHT skimmed milk and 1.76 to 1.87 mPa·S for UHT whole fat milk. There was no difference in pH measurements between UHT skimmed and UHT whole fat milk.

Table 5.8: Physicochemical quality of UHT skimmed and whole fat milk on the day of processing (day 1).

Item	Milk	n	Mean ± Std dev	Minimum	Median	Maximum
FAN concentration (mg/L)	skimmed whole	6 9	42.28^{a} ± 0.68 35.29^{b} ± 3.25	41.28 35.29	42.28 31.59	43.35 41.91
Fat (%)	skimmed whole	6 9	0.05^{a} ± 0.01 4.29^{b}± 0.05	0.04 4.23	0.05 4.34	0.06 4.36
Protein (%)	skimmed whole	6 9	3.92^{a}± 0.03 3.74^{b}± 0.05	3.87 3.67	3.93 3.72	3.96 3.80
Lactose (%)	skimmed whole	6 9	4.73^{a}± 0.04 4.51^{b}± 0.04	4.70 4.47	4.73 4.52	4.80 4.57
TS (%)	skimmed whole	6 9	9.73^{a} ± 0.02 13.54^{b}± 0.03	9.67 13.44	9.71 13.55	9.84 13.69
FPD (-m°C)	skimmed whole	6 9	515^{a} ± 2 518^{b}± 3	512 516	515 517	517 522
pH	skimmed whole	6 9	6.58^{a}± 0.02 6.59^{a} ± 0.03	6.56 6.53	6.59 6.59	6.61 6.63
Viscosity (mPa·s)	skimmed whole	6 9	1.89^{a} ± 0.02 1.80^{b} ± 0.04	1.86 1.76	1.89 1.81	1.91 1.87

Means within a row with different superscript are significantly different at $p < 0.05$. **FAN**- Free amino nitrogen; **TS**- Total solids; **FPD**- Freezing point depression.

5.3.2.4 Analysis of quality defects

The sensory attributes of UHT treated skimmed and UHT whole fat milk produced at the commercial dairy plant and measured over 360 days (at 4°C, 21°C, 30°C) and 35 days (at 55°C) of storage are presented in **Table 5.9**. Quality defects classification was adapted from Karlsson et al. (2019). All batches produced and tested in this timeframe showed no significant defects in relation to cream layering, age gelation and sedimentation above the thresholds of consumer acceptability. The sample was considered unacceptable when large fat lumps were observed on the surface, a large layer of gel and particles was visible on the bottom and ≥25% of the bottom and a large layer of sediment was visible on the bottom and ≥25% of the bottom. Batch 12 displayed low levels of visible cream separation and adhesion on day 360 for samples stored at 21°C and 30°C (**Figure 5.2**) Raw milk used to process batch 12 had moderate, mixed growth of *E. coli, Enterococcus faecalis, Serratia liquefaciens* and *Acinetobacter proteolyticu.* Bactoscan count 11,500 IBC/ml, SCC 160,000 cells/ml, PBC 360 CFU/ml (band 1 classification), TBC 128 CFU/ml, pH 6.75, viscosity 1.80 mPa·s and ES 78%. This indicates good quality raw milk. Two other major contributing factors to fat rise are inadequate homogenisation conditions and temperature of storage. An inadequate homogenisation was a potential cause of fat rise for batch 12 in this study. Slight sedimentation, i.e., a thin sediment layer visible < 25% of the bottom of the package was also noted in batch 12 after 270 days of storage at 4°C and 21°C. The complex reaction of sedimentation during product storage involves the formation of a (usually dense) layer of protein-rich material at the base of the pack of UHT milk. When the sediment layer is voluminous, soft, and gelatinous, it is likely to be the initial stage of age gelation (Anema, 2019). In the case of batch 12, sediment was brown and not gel-like, and it did not look like typical sediment composed of k-casein depleted casein micelles, with low levels of denatured whey protein. Sedimentation similarly to creaming in relation to batch 12 was either

caused by variation in processing conditions i.e., increase in temperature, ineffective homogenisation, or even the possibility of dislodged deposits from the heating surface of the UHT plant and sedimentation of particles over time in UHT milk packages. While the exact cause of the sediment cannot be identified in this case, it was concluded that it was not caused by any of the measured raw milk microbiological quality parameters used for processing of batch 12. However, although the milk was classed in band 1 in relation to PBC (< 500 CFU/ml), the presence of *Serratia liquefaciens* was reported in the bacteria direct plating analysis, and it is known that protease Ser2 increases the sedimentation during shelf life according to Bagliniére et al. (2017).

In batch 6 the presence of gel particles was noted on the bottom of the 1 litre package for samples stored at 21°C after 360 days of storage. Once the milk was poured out, a thin layer was visible covering less than 25% of the bottom of the package. Raw milk used to produce batch 6 had moderate, mixed growth of *Pseudomonas fluorescens, Streptococcus uberis, Aerococcus viridans* and *Acinetobacter johnsonii.* Bactoscan count was 10,500 IBC/ml, SCC 153,000 cells/ml, PBC 800 CFU/ml (classified to band 2), pH 6.75, ES 77 % and viscosity 1.83 mPa·s. Milk was of moderate quality in comparison to milk used for the production of other batches. Anema (2019) reported that there are two mechanisms causing age gelation in the storage of UHT milk, first one involves heat-stable proteases that survive the heat treatment involved in UHT processing. These proteases of native (endogenous naturally found in the milk) and bacterial origin (exogenous enzymes) hydrolyse the milk proteins system, leading to the gelation of the milk. Gelation via proteolytic degradation of the proteins usually occurs within the shelf life of UHT milk, and often relatively quickly after manufacture. The second mechanism is often called “physicochemical” or “non-enzymatic” age gelation. In this age gelation, the proteins are not degraded or hydrolysed during storage even in samples that have gelled. This gelation tends to occur after several months of storage and often beyond the expected shelf life

of the UHT milk, although it may occur sooner in milk at higher total solids or higher protein concentrations. It is also more common in UHT skimmed milk and caused by the polymerisation of caseins and whey proteins by Maillard reactions. FAN concentration for this batch was low and dropped from the baseline figure of 47.05 mg/L to 14.17 mg/L. The reason for this drop in free amino nitrogen concentration is unknown so further studies would need to focus on this finding. The cause age gelation of batch 6 at 21°C could be potentially related to the enzymatic reaction caused by native plasmin that was not tested in this study as only peptides from bacteria reactions were quantified. Another possibility was non-enzymatic age gelation that involved different changes in casein and peptides. However, it is not believed to be caused by raw milk microbiological quality and the level of psychrotrophic bacteria and the impact of their enzymes on UHT milk stability in this case.

Changes in colour followed the same pattern for UHT milk, all samples were the same in colour at the same frequencies of checks and temperatures points. There was no difference in colour noted between milk produced from different raw milk quality. There was as expected a difference in colour between UHT skimmed milk and UHT whole fat milk. Temperature and time had the highest impact on the colour changes observed in the samples. **Figure 5.3** presents colour changes in UHT whole fat milk and comparison with samples on day 1, day 35 stored at 55°C and day 360 stored at 4°C, 21°C and 30°C. Changes in colour were caused by brown pigments formed during Maillard reactions. The higher the storage temperature, the higher the rate of pigment formation, which is widely noted in the literature. It was agreed that the colour of samples stored at 30°C after 360 days of storage would still be classed as acceptable by consumers. However, the colour of samples stored at 55°C for 35 days would be classed as unacceptable as the high rate of pigmentation was observed in a relatively short period of time (35 days).

Table 5.9 Evaluation of the sensory attributes measured for 360 days storage of UHT treated skimmed and whole fat milk produced at a commercial dairy plant and stored at 4, 21, 30 for 360 days and 55°C stored for 35 days. Values represent detection of the quality defect (milk ID) and correspond to storage time in days at which the UHT milk was considered no longer acceptable to consumers.

Sensory attribute	Definition	Scale	Threshold for no longer acceptable for consumption	Storage			
				4°C	21°C	30°C	55°C
Cream layering	Perceived thickness of the fat layer on the surface	(-) = no fat separation (+) = some slight visible cream separation / adhesion (++) = large lumps of fat visible on the surface	≥ (++)	>360d	> 360 d (+)270d (12)	>360 d (+) 270d (12)	>35d
Age gelation	Presence and thickness of the size of gel particles on the bottom of the 1 litre package, after the milk was poured out	(-) = no gel particles (+) = small thin layer visible < 25% of the bottom (++) = large layer of gel and particles visible on the bottom ≥25% of the bottom	≥ (++)	>360d	>360d (+) 360d (6)	>360d	>35d
Sedimentation	Size of the sediment layer on the bottom of the package after the milk was poured out	(-) = no sediment (+) = small thin sediment layer visible < 25% of the bottom (++) = large layer of sediment visible on the bottom ≥25% of the bottom	≥ (++)	>360 days (+)270d (12)	>360 days (+) 270d (12)	>360 days	>35d

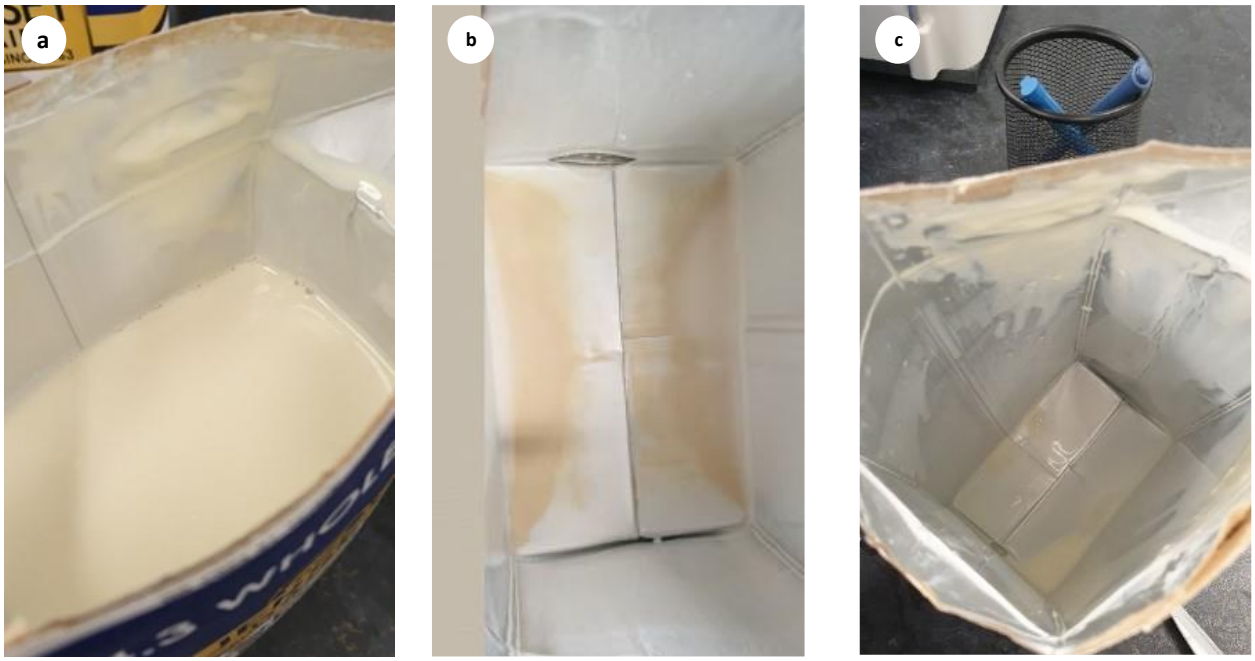

Figure (a) *Figure (b)* *Figure (c)*

Figure 5.2: Presentation of sensory defects captured during samples visual assessments (a) cream separation UHT whole fat milk batch 12 (360 days at 21°C and 30°C) (b) sedimentation-UHT whole fat milk, (c) age gelation-UHT skimmed milk batch 6 (360 days at 21°C).

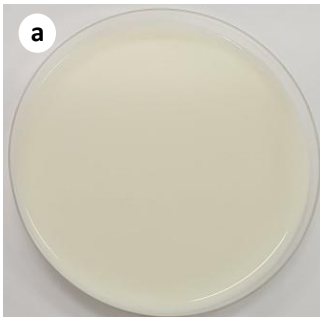

Figure (a)

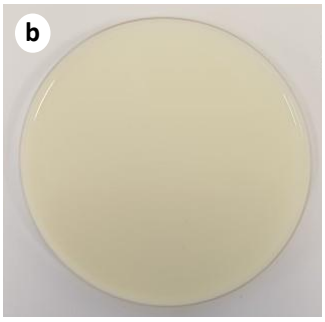

Figure (b)

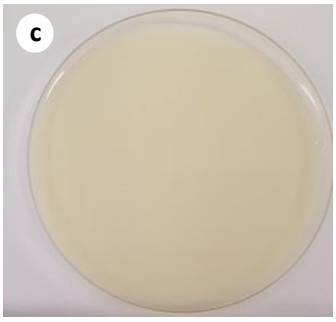

Figure (c)

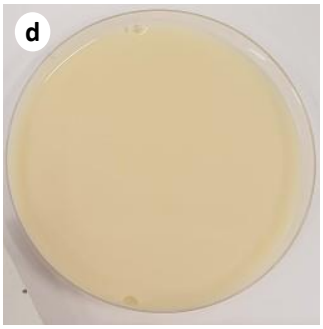

Figure (d)

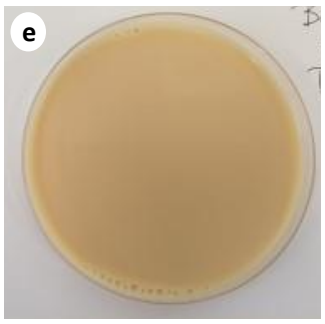

Figure (e)

Figure 5.3: UHT whole fat milk (a) day 1 (155A- white group) (b) day 360 at 4°C (155A- white group) (c) day 360 at 21°C (155B- white group) (d) day 360 at 30°C (159C-orange-white group) (e) day 35 at 55°C (164D- grey-orange group).

5.3.2.2 Analysis of UHT skimmed milk with different PBC levels

The physicochemical and proteolytic activity variance between UHT skimmed milk batches with different PBC levels is reported in **Table 5.10**, separately for different storage temperatures. It was noted that on day 1, there is no difference indicated in relation to composition. There was also no variance in free amino nitrogen concentrations or viscosity of products due to the impact of PBC concentrations on raw milk supply. There was no significant difference in FAN concentration at day 360 for samples with different PBC enumerations stored at either 4°C, 21°C, or 30°C and for samples stored for 35 days at 55°C. The lowest level of free amino nitrogen was observed for samples stored for 35 days at 55°C with PBC < 500 CFU/ml that recorded a concentration of 33.16 mg/L and the highest level was 45.13 mg/L for samples stored at 30°C

for 360 days with PBC > 1,000 CFU/ml. For the same storage time and temperature, but PBC < 500 CFU/ml, FAN concentration was recorded at 44.20 mg/L and confirmed as not significantly different. Storage stability in relation to protein breakdown is expected to be affected by temperature which indicates that higher temperatures promote protein hydrolysis due to increased enzyme activity that proves to reduce UHT stability by triggering protein hydrolysis. The optimum temperature for plasmin activity is 37°C (Crudden et al., 2005). Bacterial enzymes' optimum activity is reported to be the same but there are some studies reporting optimum activity to be usually around 30°C depending on the individual enzyme (Robinson, 2002). This can potentially support the findings that the highest increase of free amino nitrogen was established at 30°C (**Table 5.11**). There was no significant change in free amino nitrogen concentrations for all stored temperatures between all three bands of psychrotrophic bacteria in raw milk used for this study. However, it was noticeable that levels of FAN on day 360 were higher at 30°C, than 21°C and then 4°C (R^2=0.581, $p < 0.05$) (**Table 5.11**).

Free amino nitrogen was measured for peptides derived only from bacterial enzyme breakdown of proteins, eliminating plasmin activity in this study, as opposed to the pilot plant study in **Chapter 4** where ninhydrin measurement indicated peptides derived from potentially both native plasmin and bacterial enzyme activity. In the pilot study, a considerable FAN increase was noted at 55°C with the ninhydrin method as opposed to no increase at 55°C where only bacterial peptides were measured with the TNBS method in the commercial trial study, which indicates plasmin activity was more likely impacting on this increase at 55°C and bacterial enzymes were potentially deactivated at this temperature point when samples were stored for a prolonged time.

There was no significant change in viscosity noted between different PBC bands and they all remained at an acceptable level during the UHT milk storage. However, the highest viscosity was recorded for samples stored at 4°C and 21°C, higher than the viscosity of samples stored at 30°C (R^2=0.720, $p < 0.05$). Colder storage temperature increases viscosity due to increased voluminosity of casein micelle. Ranvir et al. (2020) indicated that an increase in viscosity is related to proteolysis which results in gelation during the UHT milk storage. Higher viscosity changes are observed in samples stored at different temperatures. In this case, no significant change was noted that could be linked with proteolysis caused by bacterial enzyme activity impacting on samples, but the only minor change was noted only in relation to change in casein properties that showed its presence at lower temperatures.

The specified PBC levels did not impact on pH change or any other physicochemical parameters within specified storage time. Storage temperature significantly impacted on pH, freezing point, and lactose content, which according to the literature, with an increase in the temperature, the highest degree of change is noted. Karlsson et al. (2019) reported that storage at a higher temperature considerably decreases the shelf life, and increases changes in sediment formation, taste, and colour which is explained by known mechanisms.

Table 5.10 The impact of storage time and temperature on the physico-chemical properties of UHT skimmed milk produced from Jersey raw milk with varying psychrotrophic bacteria counts (PBC) Data is presented as mean concentrations ± standard deviation.

Day	Storage Temperature	Parameter	1[1]	2[2]	3[3]
Day 1		FAN (mg/L)	41.83 ± 0.50[a]	42.54 ± 0.00[a]	42.83 ± 0.73[a]
		Viscosity (mPa·s)	1.88 ± 0.00[a]	1.89 ± 0.00[a]	1.89 ± 0.04[a]
		pH	6.59 ± 0.02[a]	6.59 ± 0.00[a]	6.58 ± 0.03[a]
		Fat (%)	0.04± 0.00[a]	0.05±0.00[a]	0.05 ± 0.05[a]
		FPD (-m°C)	514 ± 2[a]	516 ± 0[a]	516 ± 1[a]
		Protein (%)	3.91 ± 0.03[a]	3.93 ± 0.00[a]	3.92 ± 0.06[a]
		Lactose (%)	4.73 ± 0.03[a]	4.74 ± 0.00[a]	4.75 ± 0.07[a]
		TS (%)	9.71 ± 0.01[a]	9.74 ± 0.00[a]	9.75 ± 0.12[a]
Day 35	55°C	FAN (mg/L)	33.16 ± 2.09[a]	36.97 ± 0.00[a]	40.36 ± 3.13[a]
		Viscosity (mPa·s)	1.87 ± 0.02[a]	1.84 ± 0.00[a]	1.87 ± 0.03[a]
		pH	6.13 ± 0.03[a]	6.17 ± 0.00[a]	6.13 ± 0.03[a]
		Fat (%)	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		FPD (-m°C)	537 ± 3[a]	538 ± 0[a]	534 ± 6[a]
		Protein (%)	3.86 ± 0.05[a]	3.93 ± 0.00[a]	3.93 ± 0.04[a]
		Lactose (%)	4.56 ± 0.01[a]	4.61 ± 0.00[a]	4.63 ± 0.04[a]
		TS (%)	9.51 ± 0.08[a]	9.59 ± 0.00[a]	9.63 ± 0.02[a]
Day 360	4°	FAN (mg/L)	37.10 ± 1.16[a]	41.73 ± 0.00[a]	40.41 ± 0.46[a]
		Viscosity (mPa·s)	2.27 ± 0.10[a]	2.33 ± 0.00[a]	2.44 ± 0.02[a]
		pH	6.57 ± 0.01[a]	6.57 ± 0.00[a]	6.58 ± 0.01[a]
		Fat (%)	0.01 ± 0.01[a]	0.04 ± 0.00[a]	0.03 ± 0.00[a]
		FPD (-m°C)	515 ± 2[a]	520 ± 0[a]	517 ± 0[a]
		Protein (%)	3.90 ± 0.05[a]	3.93 ± 0.00[a]	3.91 ± 0.05[a]
		Lactose (%)	4.70 ± 0.01[a]	4.71 ± 0.00[a]	4.72 ± 0.02[a]
		TS (%)	9.62 ± 0.01[a]	9.67 ± 0.00[a]	9.66 ± 0.09[a]
Day 360	21°C	FAN (mg/L)	39.35 ± 4.52 [a]	37.84 ± 0.00[a]	39.02 ± 2.23[a]
		Viscosity (mPa·s)	2.29 ± 0.42[a]	2.83 ± 0.00[a]	2.45 ± 0.03[a]
		pH	6.48 ± 0.00[a]	6.49 ± 0.00[b]	6.50 ± 0.00[ab]
		Fat (%)	0.02 ± 0.01[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		FPD (-m°C)	517 ± 2[a]	522 ± 0[a]	517 ± 0[a]
		Protein (%)	3.81 ± 0.20[a]	3.87 ± 0.00[a]	3.88 ± 0.01[a]
		Lactose (%)	4.71 ± 0.02[a]	4.72 ± 0.00[a]	4.71 ± 0.03[a]
		TS (%)	9.50 ± 0.20[a]	9.57 ± 0.00[a]	9.60 ± 0.04[a]

Day	Storage Temperature	Parameter	1[1]	2[2]	3[3]
Day 360	30°C	FAN (mg/L)	44.20 ± 0.56[a]	44.03 ± 0.00[a]	45.13 ± 0.27[a]
		Viscosity (mPa·s)	1.85 ± 0.01[a]	1.80 ± 0.00[a]	1.84 ± 0.04[a]
		pH	6.32 ± 0.03[a]	6.37 ± 0.00[a]	6.34 ± 0.04[a]
		Fat (%)	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
		FPD (-m°C)	521 ± 3[a]	525 ± 0[a]	523 ±3[a]
		Protein (%)	3.87 ± 0.08[a]	3.90 ± 0.00[a]	3.88 ± 0.03[a]
		Lactose (%)	4.63 ± 0.02[a]	4.65 ± 0.00[a]	4.65 ± 0.00[a]
		TS (%)	9.47 ± 0.01[a]	9.53 ± 0.00[a]	9.51 ± 0.04[a]

[1]**PCB band 1** = 0 – 500 CFU/ml; [2]**PCB band 2** = 500 – 1000 CFU/ml; [3]**PCB band 3** = 1000 – 3500 CFU/ml; **FAN**- Free amino nitrogen concentrations, **FPD**- Freezing point depression; **TS-** Total solids. Means within a row with different superscript are significantly different at $p < 0.05$.

The impact of storage temperature on values of all parameters on day 360 for samples stored at 4°C, 21°C, 30°C and at 55°C for 35 days is presented in **Table 5.11**. Storage temperature has been reported in the literature to have a significant effect on UHT milk stability and contribution to the development of quality defects (Karlsson et al., 2019). The physicochemical changes in milk stability were temperature dependent and those were not caused by the microbiological quality of Jersey raw milk. The results confirmed that the pH value decreased with raised storage temperature which is in line with the literature findings (R^2=0.985, $p < 0.05$). The highest value for samples stored at 4°C of pH 6.57 and 21°C of pH 6.49 and 6.33 at 30°C after 360 days of storage and a value of 6.14 after 5 weeks of storage at 55°C. Additionally, the freezing point (R^2=0.883, $p < 0.05$) and lactose content (R^2=0.827, $p < 0.05$) increased with increased temperature. Changes in pH and freezing point are associated with Maillard reactions, especially at 30°C and 55°C. In this reaction, besides the formation of brown pigments, lactose is subject to isomerisation and degradation, creating significant amounts of formic acid, being responsible for the storage-induced decline in milk pH. The rate of the Maillard reaction increases with temperature, explaining the difference in pH between storage temperatures (Karlsson et al., 2019).

Table 5.11: The impact of storage temperature for UHT skimmed milk on day 360 at temperatures 4°C, 21°C and 30°C and 35 days at 55°C means (St dev.).

Item	4°C	21°C	30°C	55°C
Free amino nitrogen concentration (mg/L)	38.98 ± 2.24^{b}	38.99 ± 3.08^{b}	44.48 ± 0.63^{a}	36.19 ± 4.04^{b}
Viscosity (mPa·s)	2.34 ± 0.14^{a}	2.43 ± 0.34^{a}	1.84 ± 0.03^{b}	1.87 ± 0.03^{b}
pH	6.57 ± 0.01^{a}	6.49 ±0.01^{b}	6.33 ±0.03^{c}	6.14 ± 0.03^{d}
Fat (%)	0.03 ± 0.01^{a}	0.01 ± 0.01^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
FPD (-m°C)	517 ± 2^{c}	518 ± 2^{bc}	522 ± 3^{b}	536 ± 5^{a}
Protein (%)	3.91 ± 0.04^{a}	3.84 ± 0.12^{a}	3.88 ± 0.06^{a}	3.90 ± 0.05^{a}
Lactose (%)	4.71 ±0.02^{a}	4.71 ± 0.02^{a}	4.64 ± 0.01^{b}	4.59 ± 0.04^{c}
TS (%)	9.64 ± 0.06^{a}	9.55 ± 0.14^{a}	9.50 ± 0.06^{a}	9.56 ± 0.08^{a}

Means within a row with different superscript are significantly different at $p < 0.05$. **FPD**- Freezing point depression; **TS**- Total solids.

The parameters trends in UHT skimmed milk were analysed. As far as free amino nitrogen was analysed, there was an unusual trend observed (**Figure 5.4**). In all tested batches, the initial drop in FAN was observed after day 1, which then remained stable and started to increase on day 360. As previously stated, the highest increase was noted for storage of samples at 30°C (R^2=0.773) and the lowest for 55°C as there were only stored for 35 days. Time had a significant impact on viscosity changes (**Figure 5.5**) with a significant increase on day 360 for samples stored at temperatures of 4°C (R^2=0.909), 21°C (R^2=0.660). Storage at 30°C for 360 days and 55°C storage for 35 days was not sufficient to cause viscosity changes. The pH changes also developed during the time at 21°C and 30°C with a sharp decrease on day 360 (**Figure 5.6**) and R^2, values of 0.632 and 0.871, respectively. The impact of time on pH was greatest in samples

stored at 55°C (R^2=0.939). **Figure 5.7** demonstrates changes in relation to FPD at 30°C (R^2=0.703) and 55°C (R^2=0.861).

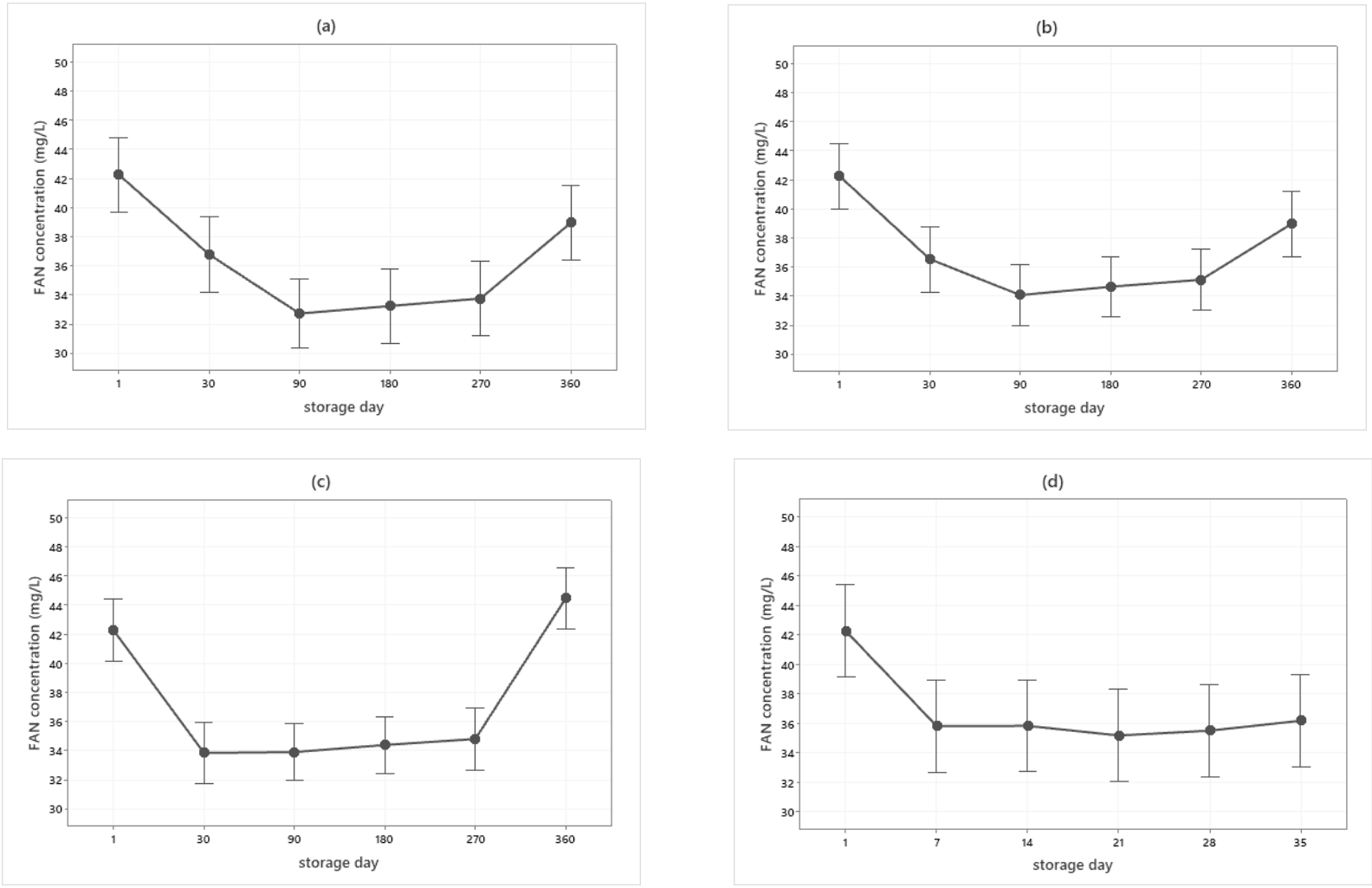

Figure 5.4 Changes in free amino nitrogen concentration of UHT skimmed milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The FAN values shown are mean values for nine UHT skimmed milk batches. The pooled standard deviation is used to calculate the intervals.

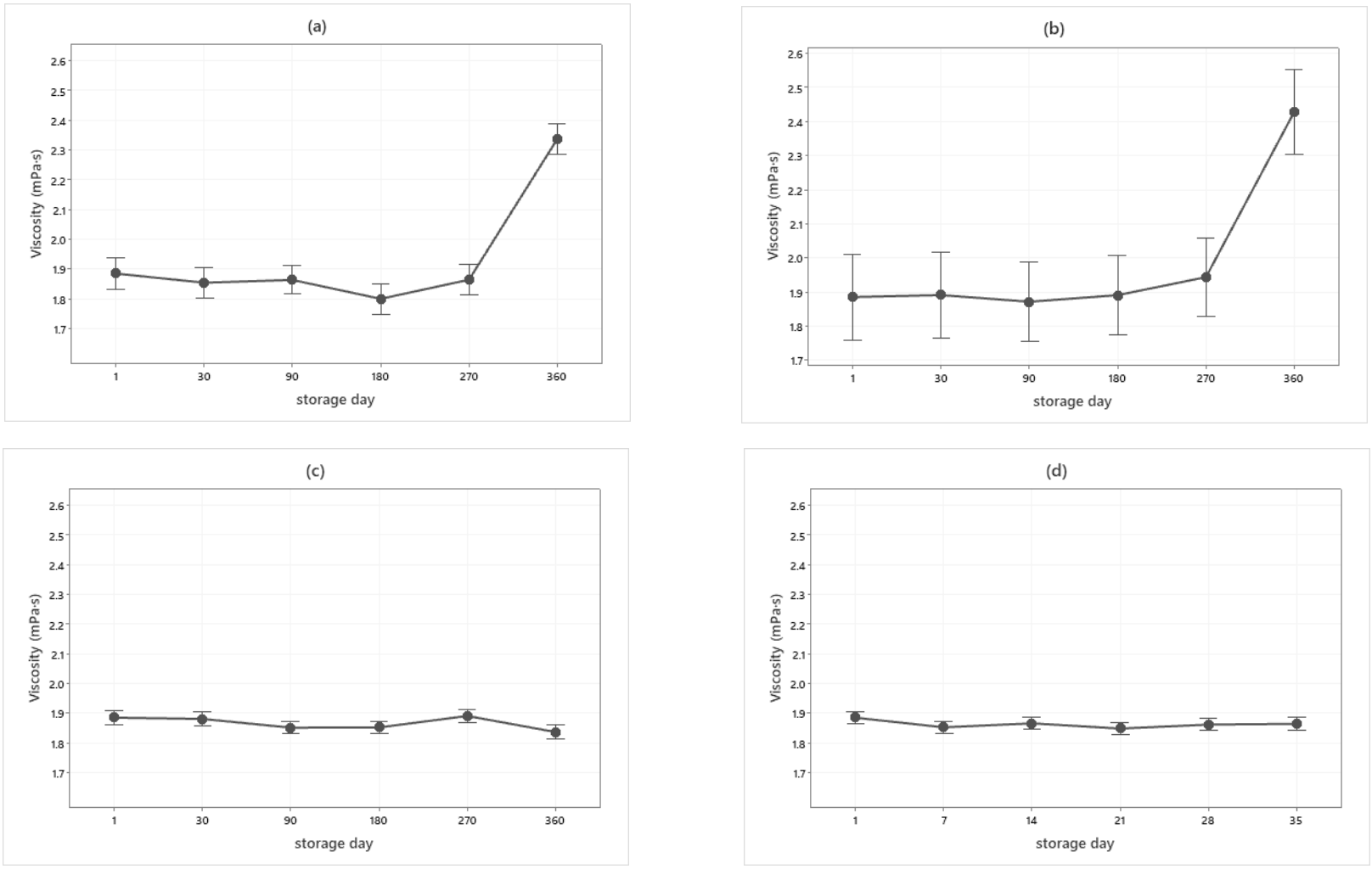

Figure 5.5 Changes in the viscosity of UHT skimmed milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The viscosity values shown are mean values for nine UHT skimmed milk batches. The pooled standard deviation is used to calculate the intervals.

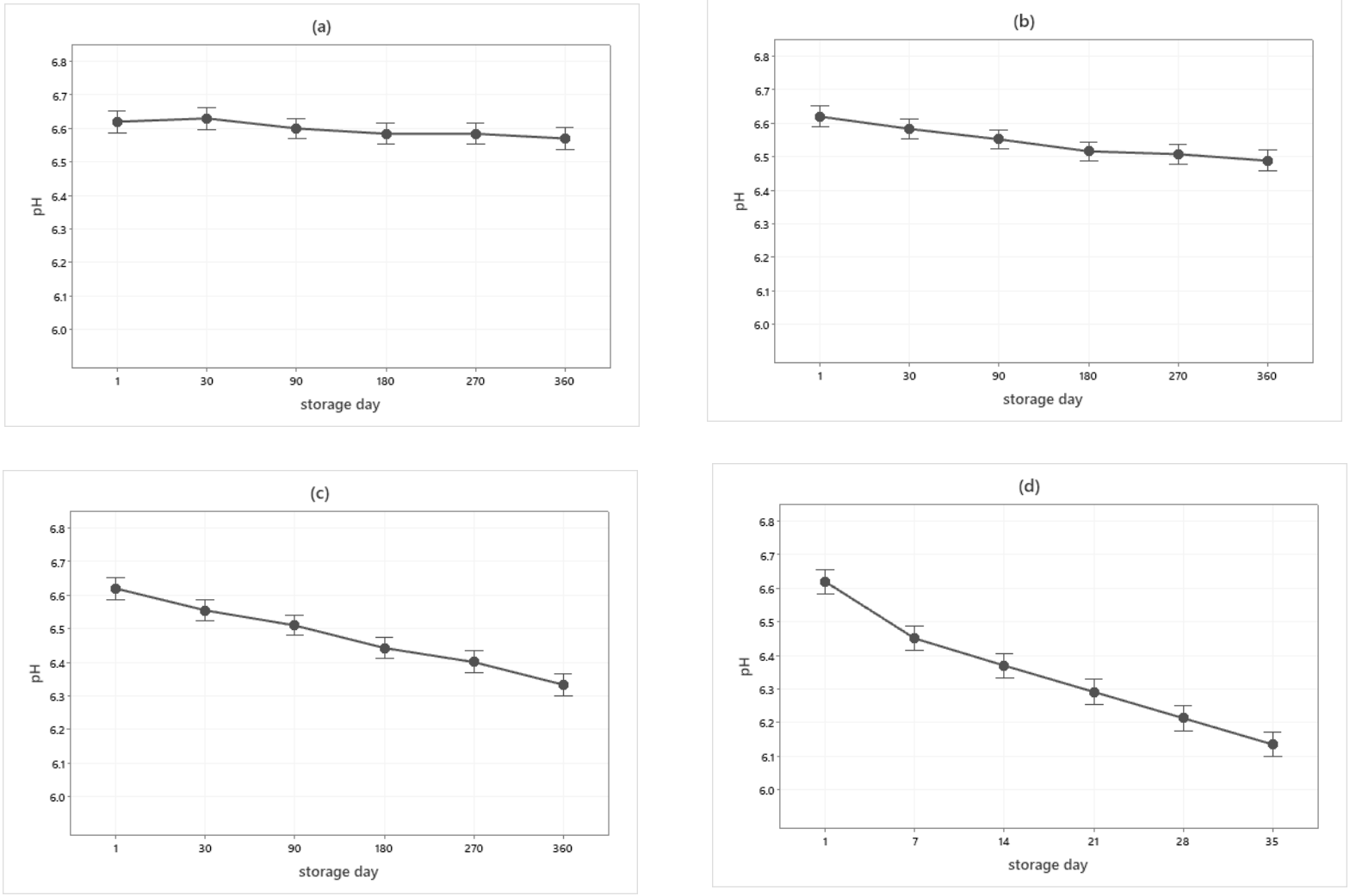

Figure 5.6 Changes in pH of UHT skimmed milk stored at(a) 4°C (b) 21°C (c) 30°C (d) 55°C. The pH values shown are mean values for nine UHT skimmed milk batches. The pooled standard deviation is used to calculate the intervals.

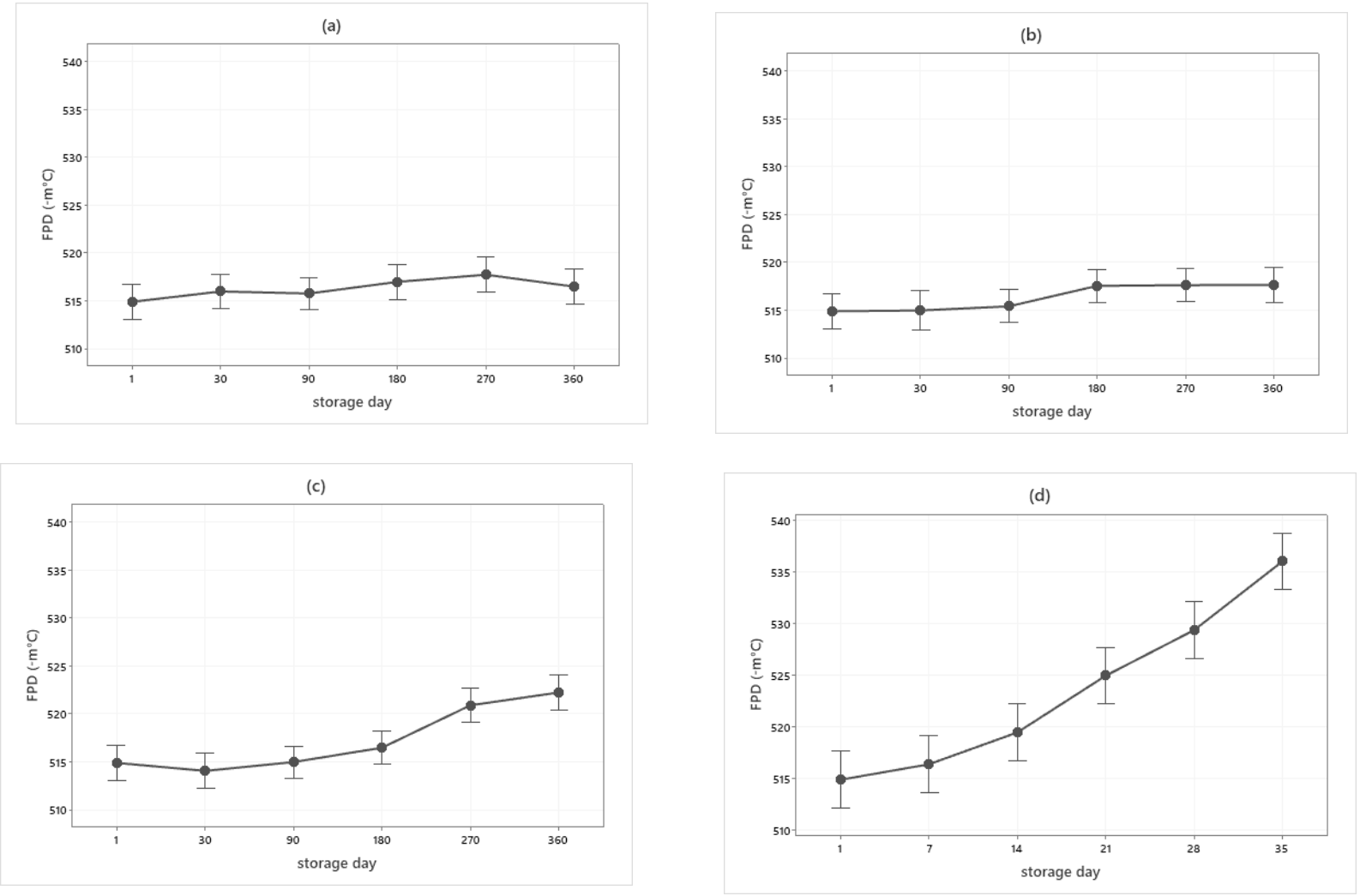

Figure 5.7 Changes in Freezing point depression of UHT skimmed milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The Freezing point depression values shown are mean values for nine UHT skimmed milk batches. The pooled standard deviation is used to calculate the intervals.

5.3.2.4 Analysis of UHT whole fat milk with different PBC levels

The variance of physicochemical and proteolytic activity between UHT whole fat milk produced with PBC classed into different bands is reported in **Table 5.12.** On day 1, there was no difference noted in relation to composition. Average free amino nitrogen concentration values on day 1 were slightly lower in UHT whole fat milk than in UHT skimmed milk with an average of 34.41 mg/L in comparison to 42.40 mg/L in UHT skimmed milk. This variance was linked with changed compositional parameters i.e., the presence of higher protein levels in UHT skimmed milk. There was no impact of PBC count on the free amino nitrogen concentration or viscosity of milk. There was no significant difference in FAN concentration at day 360 for samples with different PBC enumeration stored at either 4, 21 or 30°C and for five weeks for samples stored for 35 days. There was no impact on any physicochemical characteristics of milk caused by the different levels of psychrothrophic bacteria in specified counts in this study.

Similarly, as to UHT skimmed milk, the lowest level of free amino nitrogen was observed for samples stored at 55°C for 35 days with PBC < 500 CFU/ml that recorded a concentration of 33.88mg/L and the highest level of 41.82mg/L for samples stored at 30°C for 360 days with PBC > 1,000 CFU/ml. For the same storage time and temperature, but PBC < 500 CFU/ml, FAN concentration was recorded at 38.44 mg/L and confirmed as not significantly different. There was no significant change in free amino nitrogen concentration for all stored temperatures between all three bands of ranges of psychrotrophic bacteria in raw milk used for this study. It was noticeable that levels of FAN were higher at 30°C, then 21°C and then 4°C, but the degree of change due to different PBC counts in the levels indicated was not statistically significant. The highest viscosity was recorded for samples stored at 4°C (2.45 mPa·s), which was higher than the viscosity of samples stored at 30°C (1.88 mPa·s).

Table 5.12 The impact of storage time and temperature on the physico-chemical properties of UHT whole fat milk produced from Jersey raw milk with varying psychrotrophic bacteria counts (PBC) Data is presented as mean concentrations ± standard deviation.

Day	Storage temperature	Parameter	1[1]	2[2]	3[3]
Day 1		FAN (mg/L) Viscosity (mPa·s) pH Fat (%) FPD (-m°C) Protein (%) Lactose (%) TS (%)	33.51 ± 2.72[a] 1.81 ± 0.05[a] 6.61 ± 0.03[a] 4.33 ± 0.01[a] 518 ± 4[a] 3.75 ± 0.04[a] 4.53 ± 0.02[a] 13.58 ± 0.04[a]	37.04 ± 3.14[a] 1.81 ± 0.04[a] 6.57 ± 0.03[a] 4.28 ± 0.05[a] 517 ± 2[a] 3.73 ± 0.05[a] 4.51 ± 0.03[a] 13.52 ± 0.07[a]	32.68 ± 1.53[a] 1.78 ± 0.04[a] 6.59 ± 0.00[a] 4.30 ±0.09[a] 520 ± 2[a] 3.74 ± 0.07[a] 4.52 ± 0.07[a] 13.56 ± 0.18[a]
Day 35	55°C	FAN (mg/L) Viscosity (mPa·s) pH Fat (%) FPD (-m°C) Protein (%) Lactose (%) TS (%)	33.88 ± 3.84[a] 1.83 ± 0.01[a] 6.15 ± 0.03[a] 4.25 ± 0.03[a] 535 ± 3[a] 3.68 ± 0.09[a] 4.33 ± 0.00[a] 13.28 ± 0.07[a]	35.53 ± 6.45[a] 1.82 ± 0.02[a] 6.14 ± 0.04[a] 4.24± 0.04[a] 536 ± 3[a] 3.69 ± 0.04[a] 4.33 ± 0.03[a] 13.31 ± 0.05[a]	36.64 ± 4.75[a] 1.84 ± 0.01[a] 6.15 ± 0.00[a] 4.23 ± 0.08[a] 535 ± 4[a] 3.72 ± 0.00[a] 4.35 ± 0.03[a] 13.32 ± 0.05[a]
Day 360	4°	FAN (mg/L) Viscosity (mPa·s) pH Fat (%) FPD (-m°C) Protein (%) Lactose (%) TS (%)	36.49 ± 5.65[a] 2.43 ± 0.06[a] 6.57 ± 0.01[a] 4.33 ± 0.03[a] 520 ± 2[a] 3.74 ± 0.05[a] 4.74 ± 0.05[a] 13.63 ± 0.02[a]	40.03 ± 1.84[a] 2.45 ± 0.07[a] 6.57 ± 0.01[a] 4.36 ± 0.12[a] 520 ± 2[a] 3.73 ± 0.07[a] 4.51 ± 0.03[a] 13.64 ± 0.14[a]	40.22 ± 0.89[a] 2.49 ± 0.14[a] 6.56 ± 0.01[a] 4.29 ± 0.12[a] 522 ± 2[a] 3.78 ± 0.02[a] 4.52 ± 0.09[a] 13.64 ± 0.22[a]
Day 360	21°C	FAN (mg/L) Viscosity (mPa·s) pH Fat (%) FPD (-m°C) Protein (%) Lactose (%) TS (%)	36.62 ± 3.37[a] 2.20 ± 0.01[a] 6.48 ± 0.00[a] 4.29 ± 0.01[a] 518 ± 3[a] 3.72 ± 0.04[a] 4.48 ± 0.01[a] 13.53 ± 0.04[a]	40.46 ± 0.94[a] 2.16 ± 0.07[a] 6.48 ± 0.02[a] 4.29 ± 0.03[a] 520 ± 3[a] 3.73 ± 0.06[a] 4.46 ± 0.08[a] 13.55 ± 0.10[a]	40.62 ± 0.57[a] 2.22 ± 0.02[a] 6.47 ± 0.02[a] 4.24 ± 0.07[a] 520 ± 3[a] 3.79 ± 0.02[a] 4.52 ± 0.01[a] 13.59 ± 0.16[a]

Day	Storage temperature	Parameter	1[1]	2[2]	3[3]
Day 360	30°C	FAN (mg/L)	38.44 ± 4.84[a]	40.98 ± 0.69[a]	41.82 ± 0.15[a]
		Viscosity (mPa·s)	1.88 ± 0.00[a]	1.88 ± 0.39[a]	1.88 ± 0.02[a]
		pH	6.32 ± 0.03[a]	6.33 ± 0.02[a]	6.31 ± 0.05[a]
		Fat (%)	4.27 ± 0.00[a]	4.27 ± 0.05[a]	4.27 ± 0.13[a]
		FPD (-m°C)	525 ± 2[a]	525 ± 3[a]	527 ± 4.60[a]
		Protein (%)	3.70 ± 0.05[a]	3.72 ± 0.06[a]	3.78 ± 0.01[a]
		Lactose (%)	4.33 ± 0.10[a]	4.43 ± 0.02[a]	4.44 ± 0.06[a]
		TS (%)	13.42 ± 0.05[a]	13.46 ± 0.13[a]	13.54 ± 0.21[a]

[1]**PCB band 1** = 0 – 500 CFU/ml; [2]**PCB band 2** = 500 – 1000 CFU/ml; [3]**PCB band 3** = 1000 – 3500 CFU/ml; **FAN**- Free amino nitrogen concentrations, **FPD**- Freezing point depression; **TS-** Total solids. Means within a row with different superscripts are significantly different at $p < 0.05$.

The impact of storage temperature on the physicochemical characteristics of UHT whole fat milk is presented in **Table 5.13**. Storage temperature made a significant impact on various parameters; viscosity (R^2=0.966, $p < 0.05$), pH (R^2=0.980, $p < 0.05$), freezing point depression (R^2=0.857, $p < 0.05$) and lactose content (R^2=0.658, $p < 0.05$) which according to literature, with an increase in the temperature, the highest degree of physicochemical change is noted (Karlsson et al.,2019).

Additionally, the rate of changes between UHT skimmed milk and UHT whole fat milk was identical and explained by the same reactions i.e., Maillard reactions.

Table 5.13 The impact of storage temperature for UHT whole fat milk on day 360 at temperatures 4°C, 21°C and 30°C and 35 days at 55°C means (St dev.).

Item	4°C	21°C	30°C	55°C
Free amino nitrogen concentration (mg/L)	39.29 ± 2.88^{ab}	39.64 ± 2.20^{ab}	40.60 ± 2.19^{a}	35.41 ± 5.14^{b}
Viscosity (mPa·s)	2.45 ± 0.08^{a}	2.18 ± 0.06^{b}	1.88 ± 0.03^{c}	1.83 ± 0.02^{c}
pH	6.56 ± 0.02^{a}	6.48 ±0.01^{b}	6.32 ±0.03^{c}	6.14 ± 0.03^{d}
Fat (%)	4.34 ± 0.10^{a}	4.28 ± 0.04^{ab}	4.27 ± 0.06^{ab}	4.24 ± 0.04^{b}
FPD (-m°C)	520 ± 2^{c}	520 ± 3^{c}	525 ± 3^{b}	536 ± 3^{a}
Protein (%)	3.75 ± 0.05^{a}	3.74 ± 0.05^{a}	3.73 ± 0.05^{a}	3.70 ± 0.04^{a}
Lactose (%)	4.51 ±0.04^{a}	4.48 ± 0.07^{a}	4.41 ± 0.06^{b}	4.33 ± 0.02^{c}
TS (%)	13.64 ± 0.01^{a}	13.56 ± 0.09^{ab}	13.47 ± 0.13^{b}	13.30 ± 0.05^{c}

Means within a row with different superscript are significantly different at $p < 0.05$. **FPD**- Freezing point depression; **TS**- Total solids.

The changes in physicochemical trends in UHT whole fat milk were analysed. Similarly, to the UHT skimmed milk, there was an unusual trend observed in free amino nitrogen concentrations (**Figure 5.8**). In all tested batches, the initial drop in FAN was observed after day 30, which then remained stable and started to increase on day 360. As previously stated, the highest increase was noted for storage of samples at 30°C and there was no increase at 55°C after 35 days. Within time a significant change in viscosity was noted (**Figure 5.9**) with a significant increase on day 360 for samples stored at temperatures of 4°C, 21°C and 30°C. Storage at 55°C storage for 35 days did not cause viscosity changes. The pH changes also developed during the time for 21°C

and 30°C with a sharp decrease on day 360 (**Figure 5.10**). The impact of time on pH was the greatest in samples stored at 55°C. **Figure 5.11** demonstrates changes in relation to FPD at 30°C.

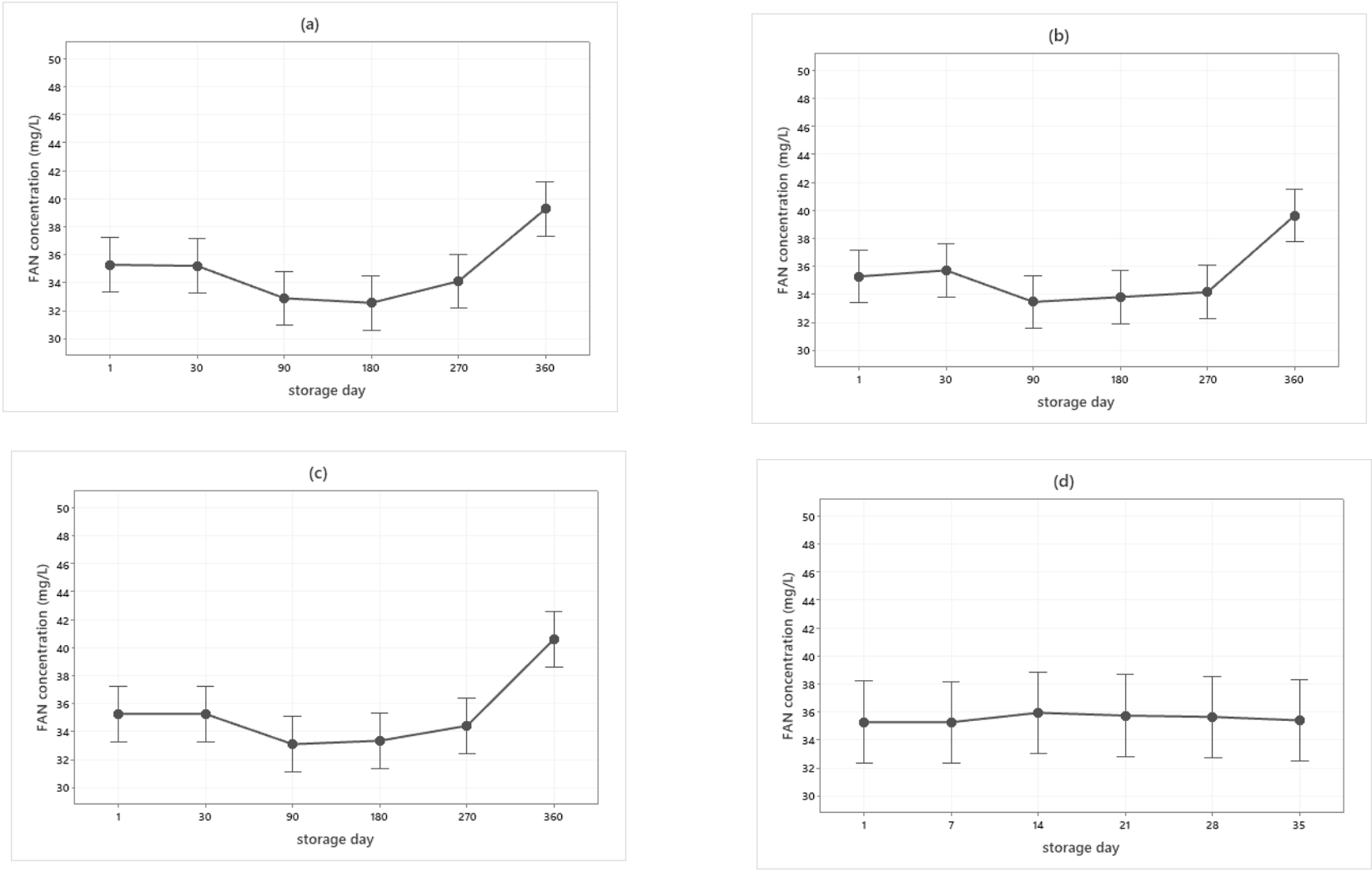

Figure 5.8 Changes in free amino nitrogen concentration of UHT whole fat milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The FAN values shown are mean values for six UHT whole fat milk batches. The pooled standard deviation is used to calculate the intervals.

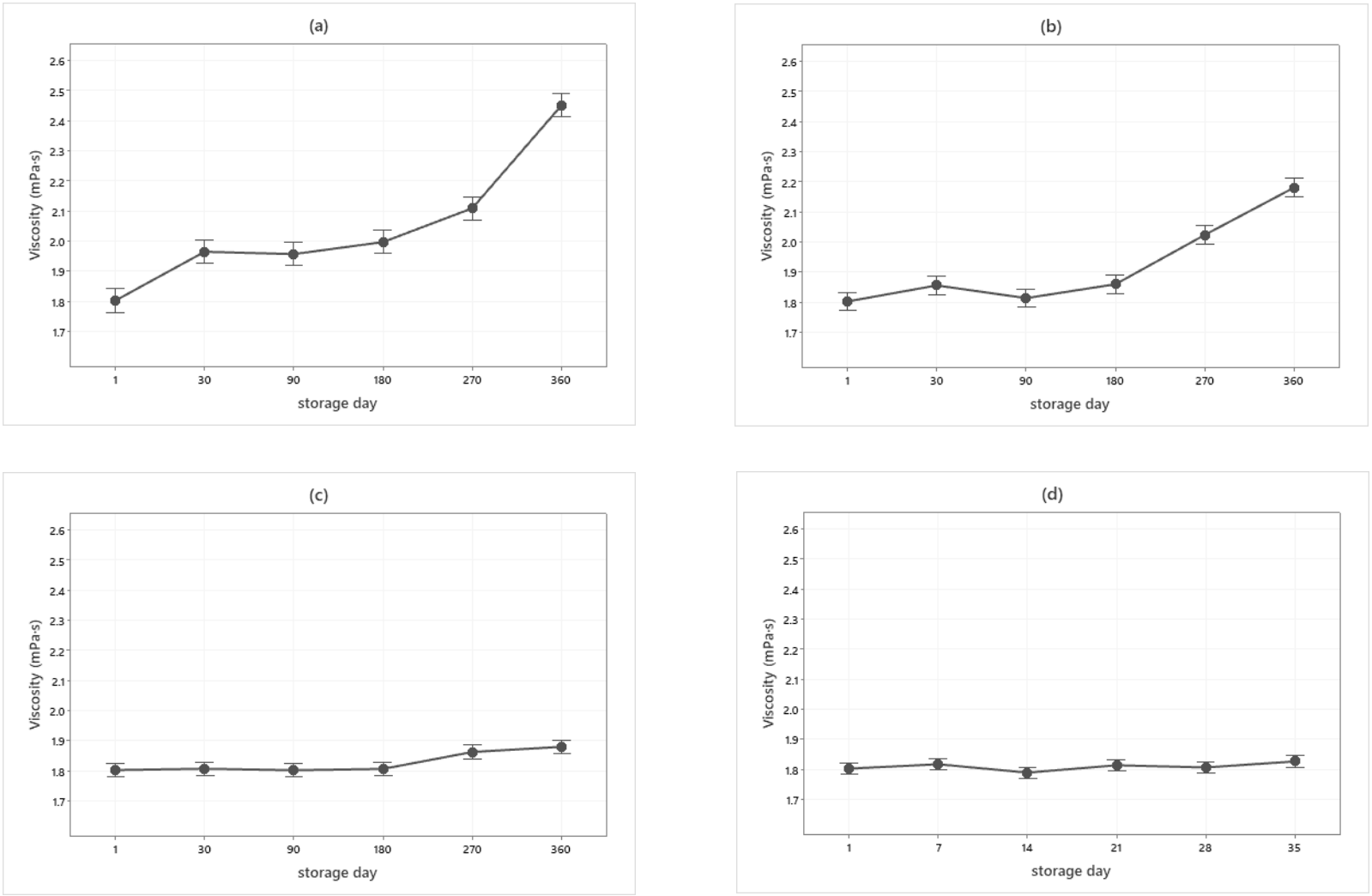

Figure 5.9 Changes in the viscosity of UHT whole fat milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The viscosity values shown are mean values for six UHT whole fat milk batches. The pooled standard deviation is used to calculate the intervals.

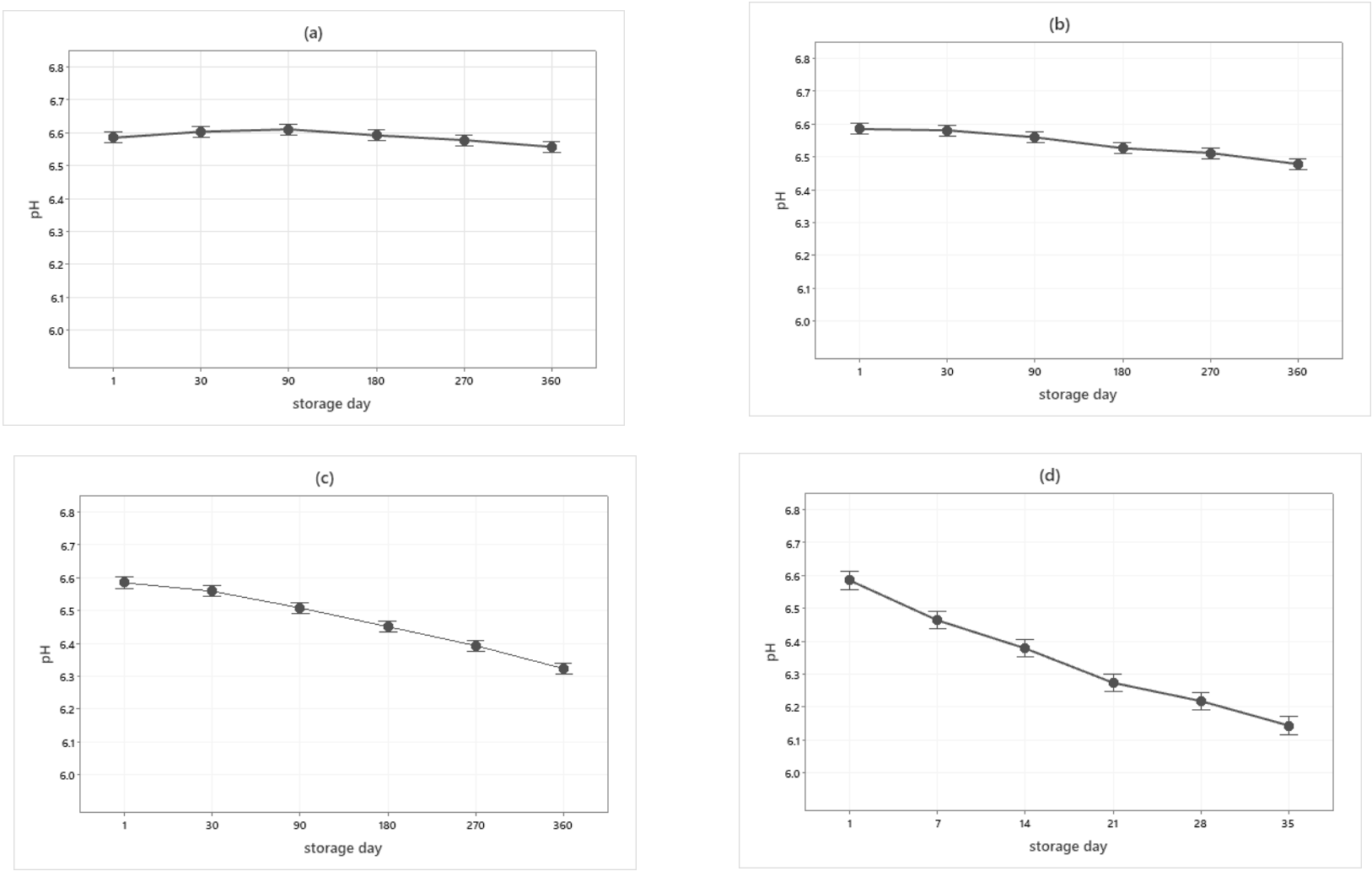

Figure 5.10 Changes in pH of UHT whole fat milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The pH values shown are mean values for six UHT whole fat milk batches. The pooled standard deviation is used to calculate the intervals.

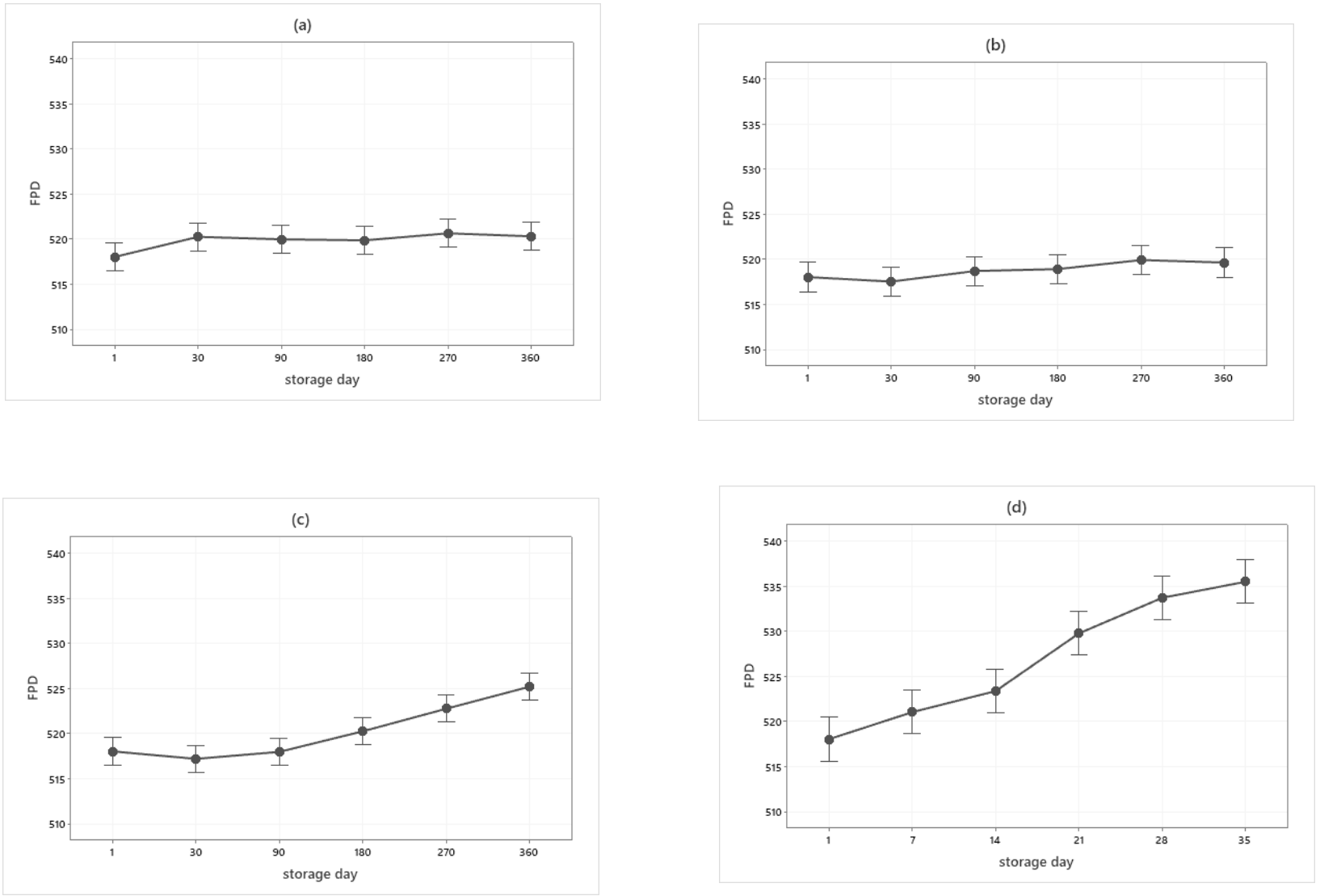

Figure 5.11 Changes in freezing point depression of UHT whole fat milk stored at (a) 4°C (b) 21°C (c) 30°C (d) 55°C. The Freezing point depression values shown are mean values for six UHT whole fat milk batches. The pooled standard deviation is used to calculate the intervals.

5.3.3 Discussion

The analysis of raw milk used for the production of commercial trials of UHT skimmed milk and UHT whole fat milk indicated that premium quality raw milk was used in relation to physicochemical parameters. According to the legislation and industry guidelines, premium microbiological quality milk was used for this study in relation to Bactoscan and SCC counts. The low and steady level of somatic cell counts ensured a minimum level of plasmin activity in the milk, which could potentially trigger any of the quality defects during product shelf life. Jersey raw milk bacteria identification analysis showed bacteria strains that were found and reported in Jersey raw milk in the years of study (2014-2019) and are widely reported in the literature. The direct plating analysis revealed some additional information gathered with bacteria enumeration results that once analysed were beneficial in improving milk quality by tackling the sources of contamination at the farm level.

PBC counts in Jersey raw milk used in the study varied between batches with the highest recorded value of 3,500 CFU/ml.

Raw milk used for the production of all batches was stored below 5°C for a maximum time of 48 hours, minimising the growth of psychrotrophic bacteria prior to processing and consequently, the amount of extracellular bacterial proteinases produced in the milk before heat treatment. The temperature control and processing milk as soon as possible after milking is a good and widely reported procedure used by processors to minimise the impact of raw milk bacterial enzymes on UHT shelf-life stability. Addition of a pasteurisation step before UHT treatment is a good procedure that contributes to the deactivation of some bacterial enzymes present in raw milk that helps to reduce further their impact on UHT milk stability.

The physicochemical analysis of processed milk indicated that there was no variation in any tested physicochemical parameters, and it confirmed good standardisation procedures at the processing facility.

Baseline FAN figure on day 1 straight after processing was established for Jersey UHT skimmed milk and Jersey UHT whole fat milk. FAN figure was only related to bacterial enzyme activity and eliminated plasmin activity, in accordance with the TNBS method. The baseline FAN figure based on the TNBS method was reported to be 42.40 mg/L for UHT skimmed milk and 34.41 mg/L for UHT whole fat milk. The baseline figure established with the ninhydrin method in **Chapter 4** in relation to both bacterial and plasmin activity for Jersey UHT unstandardized milk samples was 26.64 mg/L. The calculated value of the initial baseline FAN level potentially links with compositional parameters of Jersey milk i.e., fat and protein contents. Within time, free amino nitrogen concentration values increased for all standardised and unstandardized milk samples. However, the initial level of free amino nitrogen on day 1 after processing was at the same level regardless of the microbiological quality of raw milk. This confirms that time is required for enzymes to complete some protein changes during product shelf life and the changes are not detectable on the day of processing even though milk varied in microbiological quality in relation to PBC and other microbiological parameters.

The analysis confirmed that the different levels of psychrothrophic bacteria described in specified three bands (0-3,500 CFU/ml) did not impact on the free amino nitrogen concentrations, which confirms no impact of those specific psychrothrophic bands on protein hydrolysis in UHT milk stored for 360 days at a specified temperature.

The analysis of free amino nitrogen, viscosity, compositional results, pH and FPD coupled with sensory analysis confirmed the acceptability of UHT skimmed milk and UHT whole fat milk stored for 360 days at temperatures 4, 21 and 30°C. UHT skimmed milk and UHT whole fat milk were found unacceptable when stored at 55°C for 35 days. Shelf life at 55°C was limited by

deviating colour, attributable to the Maillard reactions. It was confirmed that the microbiology of raw milk samples used in this study was satisfactory to produce milk that lasted 360 days in storage at 4, 21 and 30°C with no impact and changes observed on protein hydrolysis that will trigger viscosity changes and quality defects described by cream layering, age gelation and sedimentation.

Storage time and the temperature had a significant impact on the stability and shelf-life of UHT milks and protein hydrolysation. Within time, free amino nitrogen concentrations increased, and the temperature had an impact on the concentrations once the patterns when analysed. A similar trend of change was noticed for UHT skimmed and UHT whole fat milk, free amino nitrogen dropped from the baseline figure and sharply increased on day 360. However, the drop was observed straight after day 1 for UHT skimmed milk samples and on day 30 analysis for UHT whole fat milk. The reason for this drop is unknown and more analysis would have to be completed to identify this phenomenon. It is suspected that shortly after 360 days of storage FAN values would increase to higher levels triggering the UHT milk quality defects. The highest value of free amino nitrogen concentration in both UHT skimmed and UHT whole fat milk was observed for storage of samples at 30°C after 360 days of storage, 41.82 mg/L and 45.13 mg/L, respectively. The highest values were recorded for storage at 30°C for 360 days, for batches produced with PBC in band 3 > 1,000 CFU/ml in both UHT skimmed and UHT whole fat milk, and the lowest for PBC band 1 < 500 CFU/ml but were confirmed not to be statistically different. Analysis of variance between batches indicated the highest FAN concentration was recorded for UHT whole fat milk produced from raw milk (batch 2) for samples stored at 30°C for 360 days (41.92 mg/L) and the lowest FAN concentration for UHT whole fat milk batches stored at the same time and temperature produced from raw milk 7 (35.02 mg/L). This makes a 6.9 mg FAN difference for products stored under the same conditions. Those findings linked with confirmation of raw milk quality used for the processing. Raw milk used for batch 2 was of the

worst quality in the data set and raw milk used for processing batch 7 was of the best microbiological quality. The degree of change was not significant to cause any viscosity changes or trigger any quality defects observed in the study. UHT skimmed milk and UHT whole fat milk had observed an increase in viscosity and FAN concentration after 360 days of storage.

The highest viscosity change was observed in the Jersey milk stored at 4°C.

The change in rate of pH and FPD was observed and confirmed to be the same for UHT skimmed and UHT whole fat milk for all tested temperatures and all batches in the analysis.

UHT whole fat batch 12 developed slightly increased cream layering adhesion and sedimentation. Batch 12 was classed in band 1 in relation to PBC count. Following this analysis, it was agreed that those defects were potentially linked to malfunction of processing equipment. Alternatively, even though the milk was classed in band 1 in relation to PBC < 500 CFU/ml, it also reported the presence of a strain of *Serratia liquefaciens* in the direct plating analysis which is known to produce protease Ser2 that increase the sedimentation during shelf life according to Bagliniére et al. (2017). This possible cause could not have been excluded as a potential reason of increased sedimentation in this batch.

TNBS method showed a baseline level of peptides in raw milk and FAN trends during the UHT skimmed milk and UHT whole fat milk during storage. Free amino nitrogen TNBS analysis was focused on the measurement of peptides derived only from bacterial hydrolysation and the study eliminated plasmin activity measurements. Optimum temperature activity for bacterial enzymes was confirmed to be a cause of a slight increased free amino nitrogen concentration at 30°C during 360 days of storage.

This method once compared with the ninhydrin method used in **Chapter 4**, showed differences in 55°C storage temperature and as it captured stable level of FAN concentrations. Samples measured in the ninhydrin method showed an increase of FAN in milk produced from milk at the excellent quality (band 1) measured at 38 mg/L after 35 days of storage increased from the

level of 26.64 mg/L. The samples measured with the TNBS method showed no increase for raw milk from PBC band 1 which indicates that plasmin activity was still active at 55°C and measured in ninhydrin method as opposed to no measurement in TNBS method. The plasmin activity is higher than bacterial activity (Crudden et al., 2005), and it has generally higher thermostability (Van Asselt et al., 2008) (Deeth & Lewis, 2017).

The pilot plant experiment presented in **Chapter 4**, showed progress of protein hydrolysis in UHT milk samples in relatively short time that were UHT milk was produced from raw milk of good and low microbiological quality. Commercial experiment in **Chapter 5**, presented development of protein hydrolysis and the development of sensory changes during long period of time. Raw milk used in the study was of good microbiological quality and presented low variation in microbiological and physicochemical characteristics as opposed to raw milk used in **Chapter 4**.

The commercial experiment was lacking flexibility, as there was no control over the PBC in raw milk used in the UHT milk processing. In the given timeframe, the highest PBC level recorded was 3,500 CFU/ml. The highest bacterial activity was noted for samples stored at 30°C, therefore this temperature would be recommended for testing in development in any rapid tests. The weakness of the experiment was that time of storage of samples was still relatively short and the level of free amino nitrogen did not capture an increase in the time frame for the other milk samples collected from farms producing milk from batches classified as band 3 in relation to PBC (1,000 - 3,500 CFU/ml) to be able to fully support study objective so the valid correlations could be produced. Those findings would have been supported by the observation of the sensory defects. It would be recommended to increase the range of PBC in the experiment as well as extend sample storage time.

Additionally, those changes to the project design might have helped to catch the rate of sensory changes to support the study objective and to produce valid correlations.

In future studies, it would be beneficial to add other analyses i.e., RP-HPLC that could capture protein hydrolysis contributing to milk shelf-life assessments to support the main study objectives.

5.4 Conclusion

The chemical composition and microbiological properties of raw milk are essential to ensure the stability of UHT dairy milk. The destabilisation of UHT milk during shelf life is caused by the psychrotrophic bacteria proteases and native milk proteases. Proteolysis caused by bacterial proteases contributes to the development of sensory defects i.e., age gelation that reduces UHT milk shelf-life.

In this study, Jersey raw milk stored below 5°C for a maximum time of 48 hours with Bactoscan below 34,000 IBC/ml and psychrothrophic bacteria count below 3,500 CFU/ml produced UHT whole fat milk and UHT skimmed milk with 360 days shelf life stored at temperatures 4°C, 21°C and 30°C. The free amino nitrogen values coupled with viscosity and sensory observations confirmed UHT milk acceptability.

The highest FAN values were recorded for storage at 30°C for 360 days, for UHT skimmed and whole fat milk batches produced with PBC in range 1,000 – 3,500 CFU/ml and the lowest for PBC band below 500 CFU/ml but confirmed not to be statistically different and more testing is required to confirm psychrotrophic bacteria and protein hydrolysis correlations in these bacteria count range. Storage temperature and time made a significant impact on the stability and shelf-life of UHT milk and protein hydrolysation. Within time and at higher temperatures, FAN concentrations increased. The highest value of free amino nitrogen in both UHT skimmed and UHT whole fat milk was observed for storage of samples at 30°C after 360 days of storage which linked with optimum temperatures for enzyme activity. This initial assessment confirmed that 30°C temperature point is the most acceptable choice to promote protein hydrolysis caused by psychrothrophic bacteria, but further testing is required to support this finding.

No hydrolysis development caused by bacterial proteinases was observed at a storage temperature at 55°C for 35 days which indicates this temperature is potentially not suitable for shelf-life testing in relation to observation of protein hydrolysis caused by bacterial enzyme activity.

The baseline value of FAN was established for UHT Jersey milk produced from milk with known microbiological quality and chemical composition which provides benchmark and valuable data. The qualitative direct plating assessment of raw milk indicating bacteria microbiome coupled with bacterial enumeration revealed additional information of sources of contamination and presented a good methodology in improving raw milk quality by tackling the sources of contamination at the farm level.

This study proves to provide a guideline to processors and validates shelf life for commercial production. Overall, it shows thresholds for raw milk microbiological and chemical quality necessary to produce UHT milk with an identified shelf life of 360 days.

CHAPTER 6

6. OVERALL CONLCUSIONS AND RECOMMENDATIONS

6.1. Overall conclusions

The demand for aseptically packed ultra-high-temperature (UHT) treated milk has been gradually increasing worldwide which highlights the significance of this study. Raw milk physicochemical and microbiological properties are crucial for ensuring stable UHT milk products with a long shelf-life (several months) at ambient temperatures.

The present thesis provided fundamental knowledge with regards to Jersey raw milk quality and provided recommendations for raw milk chemical and microbiological quality parameters that could be used by milk processors to enhance UHT milk product stability and extend its shelf-life. This study confirmed that it is essential for raw milk producers to tackle problems at the farm level in order to improve raw milk microbiological quality. Bacteria identification in raw milk supply is a potential tool to resolve hygiene problems at dairy farm level, by linking identified bacteria with potential contamination sources. Additionally, the introduction of additional payment initiatives in Jersey in 2014, which focused on specific hygienic parameters such as thermoduric and psychrothrophic bacteria counts, Jersey raw milk composition is more consistent and raw milk microbiological quality is significantly improved. The premium quality Jersey raw milk was produced as a result of incorporated incentives. The nutritional and microbiological attributes of Jersey raw milk produced on the island of Jersey provided a significant benchmark to other dairy producers and expanded on the literature for milk produced by this type of breed.

In order to support milk processors to identify the multiple factors, including the level of specific bacterial contamination that triggers UHT milk defects and reduces UHT milk shelf life, a set of specific recommendations were generated based on a commercial study. The study confirmed

that Jersey milk with specified chemical and microbiological quality produced a stable UHT product that lasted 360 days at 4°C, 21°C and 30°C. Free amino nitrogen concentrations coupled with viscosity measurements and observation of UHT milk quality attributes confirmed product acceptability.

Overall, this study initiated specific work to establish thresholds for raw milk microbiological parameters and chemical quality required to produce UHT milk. These can be embedded into quality control systems at the processing facility in order to produce UHT product with an extended shelf-life.

6.2 Future work recommendations

Future research in this field would need to continue to monitor and report raw milk microbiological quality values. Reporting of achievements in the development of tools required to resolve raw milk bacterial contaminations at the farm level, are essential to help producers choose the best standard techniques for consistent production of raw milk of excellent microbiological quality.

Further tailored knowledge should be investigated on different types of bacteria present in raw milk and their heat-resistant spoilage enzymes; it is envisaged that this approach will help to trace the contamination sources in the milk supply chain. This research gave a good base for further studies to initiate the creation of predictive models to measure the impact of psychrothrophic bacteria on UHT milk protein hydrolysis. The establishment of raw milk microbiological quality guidelines including threshold values (i.e., psychrothrophic bacteria) is strongly required by UHT milk processors. In this context, predictive models would also assist in the improvement of other dairy products' shelf life, by offering rapid evaluation of raw milk quality. Currently, processor guidelines for raw milk acceptability for UHT processing are very vague and it has yet to be established and subsequently validated what limits should be

embedded into quality systems to ensure stability and validate product shelf life at a commercial scale.

However, as commercial trials are expensive, pilot trials could be more appropriate to process milk of a wider range of microbiological quality and produce valid correlations. A longer storage trial of processed milk at 30°C to assess quality changes is recommended, complemented by analyses on protein degradation, assessment of enzymatic activity, quantifiable organoleptic checks and product colour measurements.

The study also highlighted the importance of developing rapid methods that can be used in UHT milk shelf-life validation by the dairy industry. Examples of such tools are free amino nitrogen and psychrothrophic bacteria counts; however, to date, no quick method has been developed to support the processors to be able to assess raw milk microbiological and proteolytic suitability for UHT productions. Faster and cheaper methods of analysing raw milk for microbiological quality and proteolytic potential should be developed as those will support manufacturers to produce the UHT milk with long shelf life and of an excellent quality. The microbial quality of raw milk stored under normal, or abuse conditions can be predicted. Further study could look to potentially of employing use of advanced FTIR spectroscopy and chemometrics tools to predict the quality characteristics in raw milk and the possibilities of applying these approaches based on the findings of my thesis. Chemometrics models based on the PCA and PLS regression can be developed to classify raw milk samples and to predict product milk shelf life. Further developments of flow cytometry technology in rapid bacteria or enzyme enumeration and bacteria identification methodology could improve raw milk screening. Additionally, those methods could include amendments to Bactoscan methodology to identify instantly psychrothrophic bacteria count or the development of a rapid FAN test to measure progression of proteolytic reactions. There is a potential for real-time monitoring of microbial raw milk

quality to enable processors to segregate raw milk for different products and markets and real-time shelf-life establishment.

Overall, the rate of physicochemical changes in UHT milk stability caused by specific microbiological factors needs to be better understood and this further research in this field will enable commercial processors to improve UHT milk quality and extend and validate UHT milk shelf life.

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APPENDICES

Appendix 1

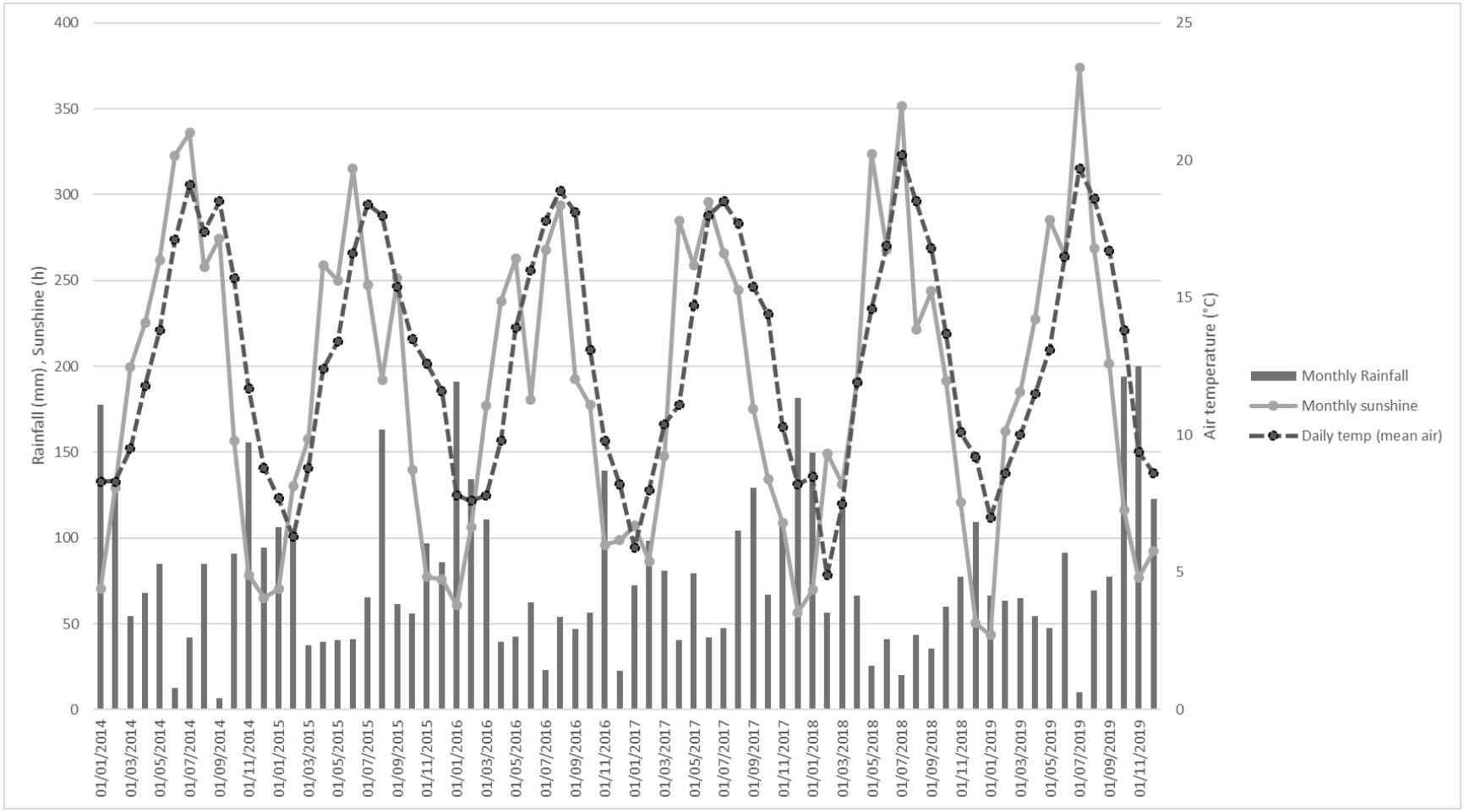


Figure 1 Jersey temperature (°C), monthly rainfall (mm) and monthly sunshine (h) during 2014-2019 (Jersey Gov.,2020).

Appendix 2

Table 1 Biodiversity of **genera** and relative abundance of at least 1% of the number of the isolates in Jersey milk produced from January 2014 till December 2019.

Year	No. samples	No. of isolates	*Streptococcus* (%)[1]	*Aerococcus* (%)[1]	*Lactococcus* (%)[1]	*Kocuria* (%)[1]	*Rothia* (%)[1]	*Macrococcus* (%)[1]	*Microbacterium* (%)[1]	*Lactobacillus* (%)[1]	*Staphylococcus* (%)[2]	*Pseudomonas* (%)[2]	*Raoultella* (%)[2]	*Acetinobacter* (%)[2]	*Serratia* (%)[2]	*Klebsiella* (%)[2]	*Citrobacter* (%)[2]	*Stenotrophomonas* (%)[2]	*Yersinia* (%)[2]	*Elizabethkingia* (%)[2]	*Enterobacter* (%)[2]	*Proteus* (%)[2]	*Escherichia* (%)[3]	*Enterococcus* (%)[3]	*Bacillus* (%)[3]	*Corynebacterium* (%)[3]	Other
2014	47	105	24.8	0.0	0.0	0.95	0.0	0.0	0.0	0.95	25.7	19.1	1.0	1.9	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	10.5	1.0	5.7	5.7	1.0
2015	49	165	13.3	3.1	3.6	0.0	0.0	0.6	0.0	0.0	19.4	12.7	0.6	1.8	2.4	0.6	1.2	0.6	4.9	0.0	0.0	0.6	17.0	9.7	4.9	2.4	0.6
2016	48	145	15.2	2.1	2.1	0.0	1.4	0.7	0.0	0.0	20.7	19.3	0.7	4.1	0.0	2.1	0.7	0.0	0.0	0.0	0.0	0.7	11.7	7.6	7.6	3.4	0.1
2017	44	118	16.1	3.4	1.7	0.8	0.0	0.0	0.8	0.0	21.2	18.6	2.5	0.8	3.4	1.7	0.8	0.8	0.0	0.0	0.8	0.0	12.7	2.5	2.5	3.4	5.5
2018	45	142	15.5	2.1	2.8	0.7	0.7	0.7	0.70	0.0	14.1	16.9	5.6	3.5	0.0	1.4	2.8	0.7	0.0	0.7	1.4	0.0	12.0	5.6	3.5	2.1	6.4
2019	34	103	13.6	3.9	2.9	1.0	0.0	0.0	0.0	0.0	9.7	16.5	1.9	4.9	3.9	1.9	0.0	2.9	0.0	1.9	0.0	1.0	9.7	6.8	1.9	7.8	7.8
mean			16.4	2.4	2.2	0.6	0.4	0.3	0.25	0.16	18.5	17.1	2.1	1.8	1.8	1.5	0.9	0.8	0.8	0.4	0.4	0.4	12.3	5.5	4.4	4.1	3.5

[1] thermoduric bacteria [2]psychrothrophic bacteria [3]some strains are psychrothrophic or thermoduric.

Appendix 3

Table 2 Relative abundance of the 108 **species** with the abundance of at least 1% of the 778 of the isolates identified in Jersey milk in year 2014 till 2019.

Species	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)
Streptococcus uberis	24.76	12.73	12.41	13.56	15.49	12.62
Streptococcus parauberis						0.97
Streptococcus galloticus			1.38	1.69		
Streptococcus dysgalactiae		0.61	1.38	0.85		
Staphylococcus ssp.	1.90		0.69			
Staphylococcus fleuretti				0.85		
Staphylococcus succinus	0.95					
Staphylococcus sciuri		0.61				
Staphylococcus aureus	4.76	7.27	4.83	9.32	3.52	4.85
Staphylococcus chromogenes	42.31	2.42	6.90	4.24	5.63	1.94
Staphylococcus capitis		1.82				
Staphylococcus equorum		2.42		2.54	1.41	0.97
Staphylococcus epidermidis	0.95		0.69	0.85	0.70	
Staphylococcus xylosus	0.95	1.82	1.38			
Staphylococcus hyicus	4.76	1.21	2.07	0.85	0.70	0.97
Staphylococcus simulans		0.61			1.41	0.97
Staphylococcus saphroticus			0.69			
Staphylococcus arlettae			0.69			
Staphylococcus haemolyticus	0.95	1.21	2.76	2.54	0.70	
Aspergillus ssp.	0.95					
Acinetobacter spp.	1.90	1.21				0.97
Acetinobacter beijerinecki						0.97
Acetinbacter baumanii		0.61				0.97
Acetinobacter junii				0.85		
Acinetobacter guillouiae			1.38		2.11	
Acetinobacter gerneri			0.69			
Acetinobacter bereziniae						0.97
Acetinobacter iwofii			0.69			0.97
Acinetobacter johnsonii			1.38		1.41	
Coreynebacterium spp.	5.71	0.61				0.97

Species	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)
Corynebacterium casei		0.61		0.85	1.41	
Corynebacterium xerosis		1.21	1.38			1.94
Corynebacterium variabile						
Corynebacterium glutamicum						0.97
Corynebacterium faecium				1.69		
Corynebacterium stationis			0.69	0.85	0.70	1.94
Corynebacterium frankenforstense						1.94
Pseuodomonas spp.	2.86	5.45	2.76		0.70	
Psuedomonas koreensis		0.61				
Psuedomonas lundenis		1.82	0.69	2.54	0.70	0.97
Pseudomonas fluorescens	11.43	3.64	13.79	13.56	14.08	15.53
Psuedomonas fragi		0.61		0.85		
Pseudomonas aeruginosa	3.81		2.07	0.85		
Pseudomonas putida		0.61				
Psuedomonas cedrina					0.70	
Pseudomonas tolassii					0.70	
Pseudomonas koreensis	0.95			0.85		
Serratia liquefaciens	0.95	2.42		3.39		1.94
Serratia marcescens						0.97
Serratia grimessi						0.97
Kocuria rhixophila	0.95				0.70	
Kocuria salsicia				0.85		0.97
Klebsiella oxytoca	0.95	0.61	1.38	0.85	0.70	1.94
Klebsiella pneumoniae			0.69	0.85	0.70	
Bacillus spp.	5.71	3.64	6.21	2.54	2.82	1.94
Bacillus licheniformis		1.21	0.69		0.70	
Aneurinibacillus aneurinilyticus			0.69			
Lactobacillus spp.	0.95					
Lactococcus garvieae		1.21				
Lactococcus lactis		2.42	2.07	1.69	2.82	2.91
E. coli	10.48	16.36	11.72	12.71	11.97	9.71
Escherichia vulneris		0.61				
Enterococcus faecium	0.95	0.61	1.38	0.85	2.82	2.91
Enterococcus cecorum				0.85		

Species	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)
Enterococcus faecalis		9.09	5.52	0.85	2.11	3.88
Enterococcus devriesei			0.69			
Enterococcus italicus					0.70	
Enterobacter clocae				0.85	0.70	
Enterobacter asburiae					0.70	
Yersinia enterocolitica		2.42				
Yersinia intermedia		2.42				
Raoultella ornitholytica	0.95		0.69	0.85	2.11	1.94
Raoultella terrigena		0.61		1.69	3.52	
Psychrobacter spp.					0.70	0.97
Weissella confusa						0.97
Rhodococcus erythropolis						0.97
Aerococcus viridans		3.03	2.07	3.39	2.11	3.88
Aeromonas ssp.		0.61	0.69		0.70	
Aeromonas bestiarum					1.41	
Micrococcus luteus					1.41	1.94
Microbacterium maritypicum				0.85		
Microbacterium liquefacies						
Microbacterium oxydans					0.70	
Macrococcus caseolyticus		0.61	0.69		0.70	
Rothia spp.			0.69			
Rothia nasimurium			0.69			
Rothia endophytica					0.70	
Elizabethkingia miricola						1.94
Elizabethkingia meningoseptica					0.70	
Stenotrophomonas ssp.						0.97
Stenotrophomonas maltophilia		0.61		0.85		1.94
Stenotrophomonas rhizophila					0.70	
Sphingobacterium ssp.				0.85		
Chryseobacterium bovis						0.97
Lelliottoa amnigena						0.97
Candida lusitaniae						0.97
Candida krusei					0.70	
Truepererella pyogenes				0.85	0.70	

Species	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)
Kluyvera intermedia				0.85		
Hafnia alvei			0.69	0.85		
Achromobacter xylosoxidans				0.85		
Brevibacterium iodum					0.70	
Mucor spp.				0.85		
Citrobacter freundii		0.61				
Citrobacter gillenii		0.61			1.41	
Citrobacter braakii			0.69	0.85	1.41	
Proteus vulgaris						0.97
Proteus spp.		0.61	0.69			

Appendix 4

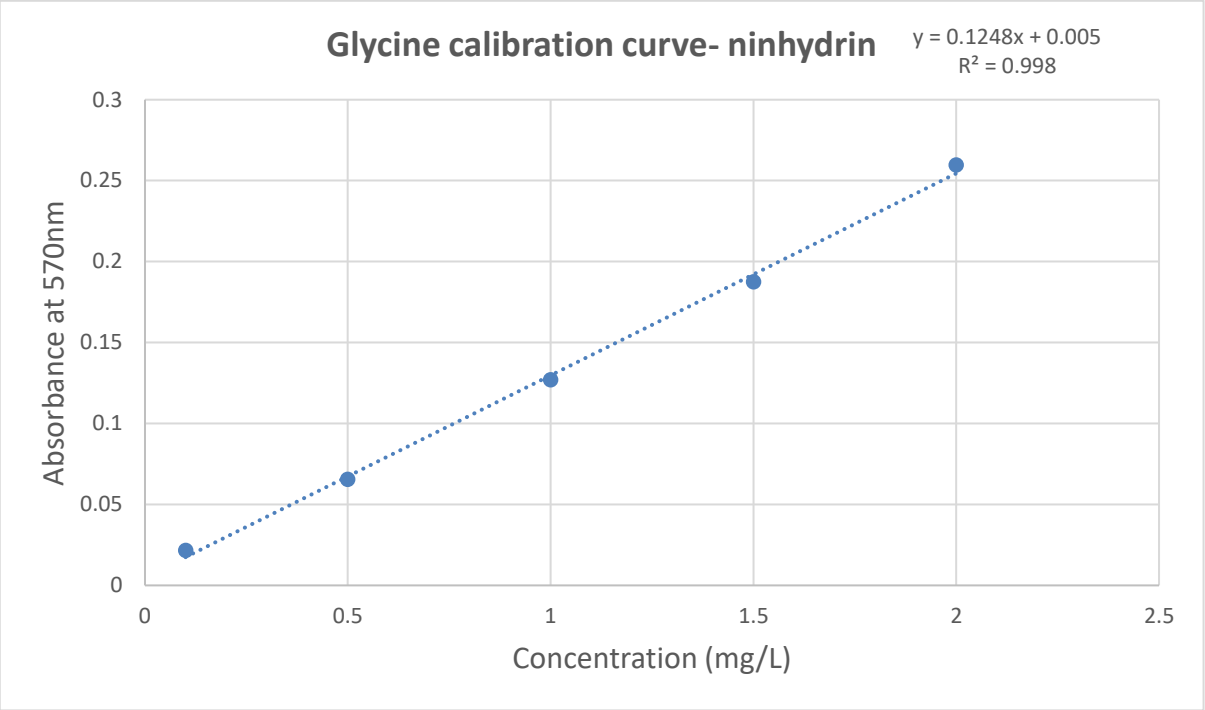


Figure 2 Glycine curve used in ninhydrin method.

Appendix 5

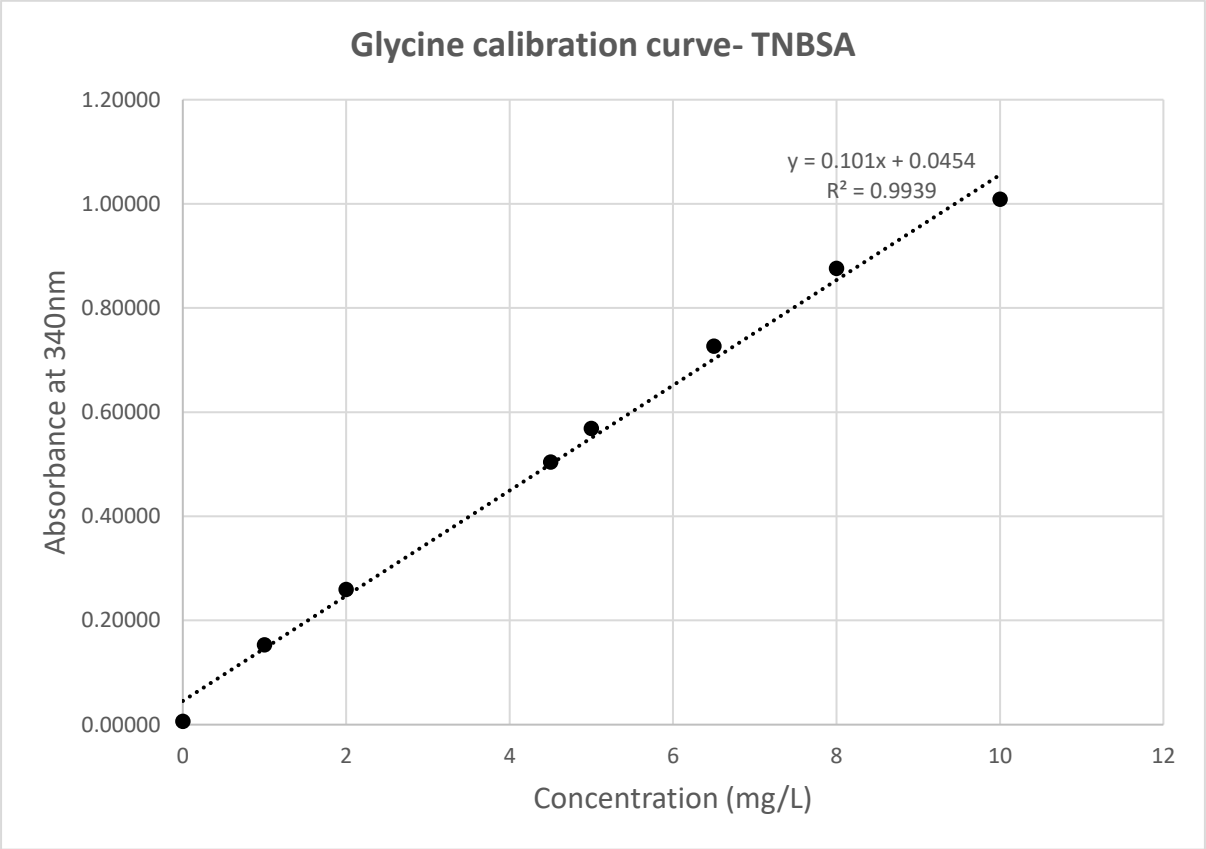


Figure 3: Glycine curve used in TNBS method.

Appendix 6

Poster Presentation

Ohnstadt I., Drabble M., Robine T., Bradley A. 2018. Milk quality improvements initiative on Jersey. Presented at the British Mastitis Conference, Worcester, UK

MILK QUALITY IMPROVEMENT INITIATIVE ON JERSEY

Ian C Ohnstad[1], Magdalena Drabble[2], Thierry Robine[2], and Andrew Bradley[3]
[1] The Dairy Group, Taunton, Somerset; [2] Jersey Dairy, Trinity, Jersey; [3] QMMS Easton, Wells, Somerset

INTRODUCTION

Scientific evidence suggests that defects in dairy products can arise due to the presence of thermoduric and psychrotrophic bacteria in raw milk.
Changes in product distribution patterns, growing export market and greater consumer expectations have resulted in a greater demand for high quality dairy products with a longer shelf life.
The Jersey Dairy quality team has always focused on improving product quality. In 2014, Jersey Dairy took the decision to further improve the raw milk quality from their 20 supplying farms and thermoduric and psychrotrophic tests were introduced. Technical support for producers was organized incorporating a combination of producer meetings with monitoring and improving many husbandry practices at individual farm level. Twice monthly bulk milk tank analysis was initiated with advice and commentary provided after direct plating and identification of predominant bacteria in bulk milk samples. A bonus scheme to reward low levels of thermoduric and psychrotrophic bacteria was also introduced.

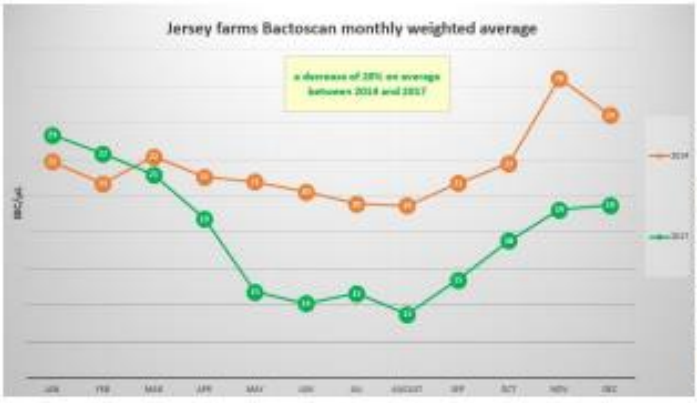
MILK QUALITY INITIATIVE

The initiative followed a number of clearly laid out stages;

- Technical producer meetings held at the dairy where broad concepts and principles were discussed.
- Investment in Bactoscan machine at the dairy.
- Fortnightly bulk milk testing and technical support with QMMS.
- Twice yearly individual farm visits by The Dairy Group to identify and address husbandry issues.
- Bonus scheme introduced. The maximum bonus was achieved with thermoduric counts <100,000cfu/ml and psychrotrophic counts <25,000 cfu/ml.

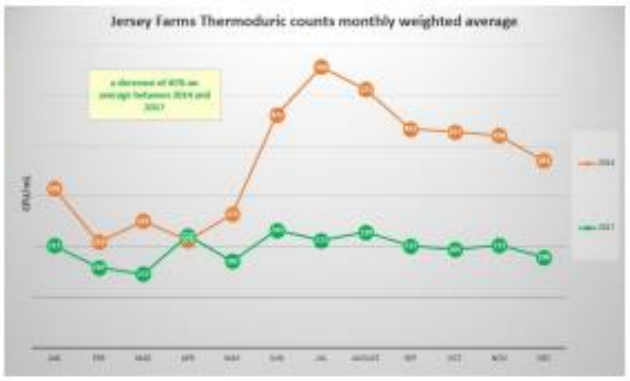
RESULT

Graph1. Bactoscan results



During the same period of time, average bactoscans in England and Wales increased from 26,000cfu/ml to 27,000 cfu/ml (AHDB Dairy 2018)

Graph 3. Thermoduric results



Graph 4. Psychrotrophic results

CONCLUSION

Since the launch of the milk quality initiative, the quality of the raw milk has improved, final product quality was improved while shelf life of UHT, pasteurized milk, butter, cream and recipe based products has been significantly extended. Jersey offers a unique opportunity to assess the effectiveness of a milk quality initiative as all milk is collected, delivered and processed by a single source. This means the benefit can be quantified through the whole chain of production.

This initiative could potentially form a template for other milk quality programs, combining technical support at a group and individual farm level, comprehensive milk quality testing and a focused milk processor committed to improving milk quality.