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Effect of Dairy Cow Diets on the Composition and Processing Characteristics of Milk

A thesis submitted to National University of Ireland Maynooth in fulfilment of the requirements of the degree of

Doctor of Philosophy

By

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Dedicated to my both set of parents and Atul

Without their love and encouragement none of my success would be possible

Declaration

I hereby certify that this thesis has not been submitted before, in whole or in part, to this or any university for any degree and is, except where otherwise stated, the original work of the author.

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Abstract

Milk composition is affected by many factors including stage of lactation, type and level of feed intake, and environmental conditions. Changes in milk composition affect milk processability and the yield and quality of dairy products. The overall aim of this thesis was to investigate the effect of different herbage allowance and feeding systems on seasonal changes in milk composition, biochemical, processing characteristics, or product manufacture and their quality. Effects of reducing daily herbage allowance (DHA) from 15.0 to 11.8 kg dry matter per cow to a spring-calved herd during early lactation (EL; 29-70 days in milk, DIM) on milk composition and processability (e.g., rennet gelation, heat coagulation time) were examined throughout lactation (up to 267 DIM). Reducing DHA led to reductions in milk yield and concentrations of protein during EL; otherwise, it had little effect on milk composition or on the selected processing characteristics in mid- or late lactation. The comparative effects of three different dairy cow diets or feeding systems on the milk composition and its impacts on the quality of Mozzarella cheese and low heat skim milk powder (LHSMP) were also studied: grazing perennial ryegrass (*Lolium perenne* L.) only (GRO), grazing perennial ryegrass and white clover (*Trifolium repens* L.) (GRC), and housed indoors offered total mixed ration (TMR). Feeding system affected milk composition and processability to an extent dependent on stage of lactation and year of study. Most notably, GRO-based milk had higher concentrations of protein, casein, Ca, lower concentrations of lactose, I, Cu and Se, and stronger rennet gelation characteristics than TMR milk. Milk from grass based feeding system had a higher mozzarella cheese-yielding capacity than milk from TMR based feeding system, and produced cheese which had lower levels of I, Cu and Se, was more yellow and became more fluid and flowable on heating to 95 °C. Moreover, the use of a novel technique called cavitation rheology (CR) to measure the mechanical properties of Mozzarella cheese, produced from GRO milk, during ageing at

4 °C. The linear modulus (*E*) obtained from CR was much lower when compared *G′* (storage modulus) from shear rheology, but correlated significantly with G′ and firmness of the cheese. Finally, composition and functionality of low-heat skim milk powder produced from three different feeding systems was studied. Compared to TMR milk, LHSMP from GRO milk had a higher protein content and a lower lactose, I, Cu and Se content, a more green-yellow colour, and on reconstitution (10%, w/w; RSM) had better rennet coagulability. RSM from all three feeding systems had similar casein micelle size and hydration, ethanol stability and yoghurt making characteristics. The differences in milk and product characteristics obtained from different feeding systems may provide a foundation for product differentiating parameters suited to different consumers, processors and markets.

Abbreviations

$\dot{\gamma}$	Shear rate
%	Percent
α-Lac	α-lactalbumin
AMF	Anhydrous milk fat
ANOVA	Analysis of variance
BCS	Body condition score
β-Lg	β-lactoglobulin
CCP	Colloidal calcium phosphate
cm	Centimetre
CoT_c	Cross-over temperature while cooling
CoT_h	Cross-over temperature while heating
CR	Cavitation rheology
d	Day
Da	Daltons
df	Degrees of freedom
DHA	Daily herbage allowance
DIM	Days in milk
DLS	Dynamic light scattering
DM	Dry matter
DMI	Dry matter intake
EL	Early lactation
EW	Work required for uniaxial extension of molten cheese
FDM	Fat-in-dry-matter
FOF	Follow on formulae
FS	Feeding system
FTIR	Fourier transform infrared spectroscopy
g	Gram
G'_{40}	Gel firmness at 40 min after rennet addition
$G'_{pH4.6}$	Storage modulus at pH 4.6 of acid-gel
GFR_{max}	Maximum gel firming rate
GLM	General linear model
Go_{pH}	Gelation onset pH
GRC	Grass-clover diet
GRO	Grass-only
h	Hour
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HCT	Heat coagulation time
H-DHA	High daily herbage allowance
HNO_3	Nitric acid
Hz	Hertz
IMCU	International milk coagulating units
IMF	Infant milk formulae
K	Consistency index (Pa.s)
kg	Kilogram
L	Litre
L-DHA	Low daily herbage allowance
LHSMP	Low heat skim milk powder
LL	Late lactation
LS	Lactation stage

LT_{max}	Maximum loss tangent
m	Metre
M-DHA	Medium daily herbage allowance
mg	Milligram
min	Minutes
mL	Millilitre
ML	Mid lactation
mM	Milimolar
MNFS	Moisture-in-non-fat substances
M_w	Molecular weight
N	Newton
n	Flow behaviour index
NaCl	Sodium chloride
NAOH	Sodium hydroxide
NCN	Non-casein nitrogen
nm	Nanometre
NPN	Non protein nitrogen
Pa.s	Pascal seconds
pH 4.6-SN	pH 4.6 soluble nitrogen
RCT	Rennet coagulation time
RP-HPLC	Reverse phase high pressure liquid chromatography
rpm	Revolutions per minute
RSM	Reconstituted skim milk
s	Seconds
S/M	Salt-in-moisture
SH	Sulfhydryl
TCA	Trichloroacetic acid
TFA	Trifluroacetic acid
TMAH	Tetra-methyl ammonium hydroxide
TMR	Total mixed ration
TN	Total nitrogen
TS	Total solids
v/v	Volume/volume
vs.	Versus
w/w	Weight/weight
WHC	Water holding capacity
WPR	Water-to-protein-ratio
WSN	Water-soluble nitrogen
Ya	Actual cheese yield
Yn	Normalised cheese yield
μg	Microgram
σ_o	Shear stress (Pa)

List of Publications

Peer-reviewed articles

1. Gulati, A., N. Galvin, E. Lewis, D. Hennessy, M. O'Donovan, J. J. McManus, M. A. Fenelon, T. P. Guinee. 2018. Outdoor grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on gross composition and mineral content of milk during lactation. *Journal of Dairy Science*, 101, 2710 – 2723.
   (Based on Chapter 4)

2. Gulati, A., D. Hennessy, E. Lewis, M. O'Donovan, J. J. McManus, M. A. Fenelon, T. P. Guinee. 2018. Effect of dairy cow feeding system on milk composition and processability. European Grasslands Federation, *Grass Science in Europe*, 709-711.
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3. Gulati, A., N. Galvin, D. Hennessy, S. McAuliffe, M. O'Donovan, J. J. McManus, M. A. Fenelon, T. P. Guinee. 2018. Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid- and late- lactation. *Journal of Dairy Science*, 101, 8737-8756.
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4. Gulati, A., N. Galvin, E. Kennedy, E. Lewis, J. J. McManus, M. A. Fenelon, T. P. Guinee. 2019. Reducing daily herbage allowance during early lactation has little influence on seasonal changes in the composition and processing characteristics of milk from spring-calved herds. International Dairy Journal. International Dairy Journal, 92, 69-76.
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Submitted manuscripts

1. Gulati, A., D. Hennessy, S. McAuliffe, M. O'Donovan, J. J. McManus, M. A. Fenelon, T. P. Guinee. 2018. Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low heat skim milk powder composition, physico-chemical and processing characteristics in mid- and late- lactation. *Journal of Dairy Science*. Resubmitted February 2019.
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Conference Proceedings

1. Oral - Impact of cow diet and season on composition, physico-chemical and processing characteristics of milk. International Dairy Federation Parallel Symposia, Dublin, Ireland, 11-13 April 2016.

2. Poster - Varying daily herbage allowance to cows in early lactation: effects on milk composition, physico-chemical and processing characteristics of milk. IUFoST, 18th World Congress of Food Science and Technology, Dublin, Ireland, 21-25 August 2016.

3. Poster - Effect of dairy cow diet on the milk composition, nitrogen fractions and mineral profile of milks collected from spring calved herds. Society of Dairy Technology Spring Conference, Cork, Ireland, 10-12 April 2017.

4. Oral - Effects of different feeding systems on milk composition and processability: Outdoor grazing on pasture vs. indoors feeding on TMR. European Grassland Federation, 27th general Meeting, Cork, Ireland, 17-21 June 2018.

5. Poster - Effect of dairy cow feeding system on milk composition during mid-to-late lactation: minerals and nitrogen fractions, Grass-Fed Dairy Conference, Dublin, Ireland, 25 October 2018.

6. Short Communication - Effect of dairy cow feeding system on milk composition and processability. Grass-Fed Dairy Conference, Dublin, Ireland, 25 October 2018.

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Chapter 1

Introduction

1.1 Introduction

In the last three decades, world bovine milk production has increased by more than 50%, from 500 million tonnes in 1983 to 769 million tonnes in 2013 (FAO, 2018). Domestic bovine milk intake in Ireland has increased from 4.9 to 6.6 million tonnes from 2008 to 2017 (CSO, 2018). The biological function of milk is to provide energy and nutrition to the neonate. Milk and dairy products are also consumed by children and adults where it contributes a significant proportion of the dietary and energy intake, especially of proteins (Lucey et al., 2017). Milk can be pasteurised, sterilised or ultra-heat treated (UHT) before consumption or can be processed to different dairy products, such as, cheese, milk powder, yoghurt, butter, evaporated milk, cream and alcohol-dairy beverages. These product manufacturing applications utilise functional properties of milk towards rennet, acid, heat or ethanol. These functional or processing characteristics of milk are affected by variations in milk composition due to factors such as breed, diet, stage of lactation, season, physiological and husbandry practices.

1.2 Milk composition

Bovine milk contains lactose (4.8 %, w/w), fat (3.7 %, w/w), protein (3.5 %, w/w), minerals (0.8 %, w/w), vitamins and water (87 %, w/w) (Fox et al., 2015). Milk proteins are heterogeneous with 75-80 % casein, 15-20 % whey protein and the remaining 5 % is non-protein nitrogen. Caseins can be separated by reducing pH of milk to 4.6 where they form coarse precipitates, while other fractions of milk such as whey proteins, NPN, minerals and colloidal calcium phosphate remain soluble at pH 4.6 (Swaisgood, 2003). Casein and whey protein differ significantly in their molecular structure, physicochemical properties and functionality (Fox et al., 2015).

1.2.1 Casein fractions

Caseins are heterogeneous consisting of four major casein types, α_{s1}-, α_{s2}-, β- and κ-casein, in the ratio 4:1:3.5:1.2 (Swaisgood, 2003). They have a molecular mass ranging from 19- 24 kDa and αs_{1}-, αs_{2}-, β- and κ- caseins have 199, 207, 209 and 169 amino acids, respectively (Table 1.1). The four caseins also differ at the structural level due to variations in their residues with respect to their phosphorylation, disulphide linked polymerisation, or glycosylation (Fox & Brodkorb, 2008). All caseins are phosphorylated at serine residues by esterification. α_{s1}-, α_{s2}-, β- and κ- casein contains 8-9, 10-13, 4-5, 1-3 mol phosphate (PO_4) per mole of protein, respectively (Farrel et al., 2004). The relatively high charge and ionisation of caseins at neutral pH is explained by these high levels of phosphorylation. The isoelectric point of casein varies according to the degree of phosphorylation. The isoelectric point of α_{s1}-, α_{s2}-, β- and κ- casein on average is 4.4-4.8, 4.9, 4.7 and 5.5, respectively. The phosphoserine groups also bind strongly to cations such as calcium. Thus caseins with a higher degree of phosphorylation, i.e., α_{s1}-, α_{s2}-, and β-, are more sensitive to calcium ion concentration and precipitate even at low calcium ion activity (>6 mM). Alternatively, κ-casein is less sensitive to calcium ion activity and stabilises the other caseins by the formation of casein micelles. Phosphorylation of caseins thus contributes to higher heat stability, which is technologically important in the processing of milk and manufacture of milk products.

Disulphide linkages: Both α_{s2}- and κ- casein have two cysteine residues in each polypeptide chain which may form intermolecular disulphide bond (-S-S-), thus having limited flexibility in the structure. α_{s2}-casein usually occurs as dimers and κ-casein as multimers (Ramussen et al., 1994). κ-casein can also form quaternary structures through intermolecular disulphide bonds (Swaisgood, 2003). The disulphide linkage in κ-casein

forms a complex with exposed sulfhydryl groups of β-lactoglobulin when it is heat-denatured. It may also participate in sulfhydryl-disulphide interchange reactions with α-lactalbumin and can form disulphide-linked polymers with α_{s2}-casein on heating.

Glycosylation: κ-casein is a highly glycosylated protein containing carbohydrate groups across two-thirds of its structure, and these occur as oligomers containing 5-11 linked monomers. The carbohydrate moieties are composed of galactose, *N*-acetylneuraminic (sialic acid) and *N*-acetylgalactosamine which are esterified to threonine residue (Thr_{131}) in C-terminal of κ-casein. These oligosaccharides are quite hydrophilic thus contributing to the hydrophilicity of the C-terminal of κ-casein.

Structure: The abundance of proline residues in caseins, where 17, 10, 35 and 20 moles of proline per mole of αs_1-, αs_2, β-, κ- casein, respectively, limit the formation of secondary structure including α-helices and β-sheets (Swaisgood, 2003). Thus caseins are considered rheomorphic with an open, flexible, mobile conformation due to the presence of high charge density as a result of phosphoseryl residues, glutamic- and aspartic-acid residues, and high proportion of proline residues (Holt & Sawyer, 1993).

Hydrophobicity: Caseins are highly hydrophobic due to their open structures, where the hydrophobic residues are exposed to solvent at surface of the protein. Hydrophobic and polar residues are not uniformly distributed but occur as clusters or patches throughout the casein sequences (Horne, 2017) thus conferring caseins, their amphipathic properties. The order of hydrophobicity of caseins is β- > κ- > αs_1- > αs_2- casein. The average hydrophobicity per residue for caseins was calculated by Swaisgood (1982) and is given in Table 1.1. The potential of these hydrophobic residues to participate in hydrophobic interactions is not only based on their hydrophobicity values but also

depends on their positioning and clustering in the casein sequence (Lucey & Horne, 2018).

Table 1.1 Characteristics of bovine milk proteins (adapted from Fox et al., 2015 and Farrel et al., 2017)

	Caseins				Whey proteins	
Item	**α_{s1}-casein**	**α_{s2}-casein**	**β-casein**	**κ-casein**	**β-Lg**	**α-Lac**
Proportion	40	10	35	12		
Mw (Da)	23614	25230	23983	19023	18363	14176
Amino acids (no.)	199	207	209	169	162	123
P residues per mole	8-9	10-13	5	1	0	0
SH groups per mole	0	2	0	2	1	0
Disulphide bonds per mole	0	1	0	1	2	4
Free thiol group	0	0	0	0	1	0
Residues						
Glycosylated	0	0	0	yes	no	no
Proline	17	10	35	20	8	2
Cysteine	0	2	0	2	5	8
Glutamic acid	24	25	18	13	16	7
Aspartic acid	8	11	5	1	10	13
Apolar (%)	36	40	33	33	2	8
Isoionic pH	4.96	4.9	4.7	5.5	5.2	4.8
Hydrophobicity (kJ/residue)	4.89	4.64	5.58	5.12	5.03	4.68
Sensitive to Ca	yes	yes	yes	no	no	no
Sensitive to chymosin	no	no	no	yes	no	no

1.2.2 Casein micelles

In bovine milk, 95 % of caseins exist as large spherical colloidal particles with molecular mass of 10^6 to 10^8 Da (Dalgleish, 2011). Casein micelles are composed of proteins and ions including calcium, phosphorous, magnesium and citrate. The size of casein micelles range from 50-600 nm in diameter with average size ranging between 100 to 200 nm (Fox & Brodkorb, 2008). An increase in the size of the casein micelle is correlated with a decrease in the proportion of κ-casein (Dalgleish et al., 1989). Casein micelles are highly hydrated with 3.4 g water/ g casein. The caseins account for ~ 92 % (w/w) of the dry weight of casein micelles, with Ca and inorganic P constituting 7 %

(w/w), and the remainder comprising of Mg, citrate and traces of some other constituents. The primary biological role of casein micelles is transport of nutrients (calcium, phosphate and amino acids) from mother to neonate.

Table 1.2 Characteristics of bovine casein micelles (adapted from Fox et al., 2015)

Characteristic	Value
Diameter	120 nm (range: 50 -500)
Surface area	8 X 10^{-10} cm^2
Molecular mass	1.3 X 10^9 Da
Water content	63 %
Hydration	3.7 g water/ g protein

1.2.2.1 Structure of casein micelles

The structure of casein micelles has been studied for over 50 years and many models and theories have been proposed and reviewed (Schmidt 1982; Holt, 1992; Dalgleish, 1998; de Kruif, 1999; Walstra, 1999; de Kruif & Holt, 2003; Horne, 2002; Horne, 2006; Fox & Brodkorb, 2008; Dalgleish, 2011; de Kruif et al., 2012; McMahon & Oommen, 2013; Huppertz et al., 2017).

The different models proposed over the years include the submicelle model (Schmidt 1982; Walstra 1990), dual binding model (Horne 1998), nanocluster model (Holt et al., 1992; de Kruif & Holt, 2003), and Dalgleish (2011) and are shown in Figure 1.1. All these models agree regarding the presence of κ-casein on the micelle surface and colloidal calcium phosphate (**CCP**) clusters in the micelle core.

The submicelle-model by Schmidt (1982) stated that the casein submicelles were cemented by CCP where κ-casein depleted micelles formed the internal submicelles and κ-casein rich sub-micelles were mainly concentrated at the surface. In the dual binding model (Horne 1998), it was postulated that micellar integrity is not only maintained by CCP but also by electrostatic and hydrophobic effects. The interior of the micelle is composed of α_s- and β-casein in which hydrophobic regions interact

via the hydrophobic effects, and electrostatic interactions between calcium ions and carboxyl groups of acidic amino acids and phosphates on phosphoseryl residues. The N-terminal region of κ-casein located at the surface interacts via hydrophobic effects with α_s- and β-caseins and orient their hydrophilic C-terminal to the solvent/serum and prevent further polymerisation. The nanocluster model (de Kruif & Holt, 2003) is an extension of the tangled cross-linked web model first proposed by Holt (1992), where a tangled mass of open rheomorphic casein chains cross-linked by CCP nanoclusters in the core with similar casein composition and is stabilised by an external region of lower density – a 'hairy layer'. The dual-binding model is analogous to the nanocluster-model for consideration of polymerisation of casein via the hydrophobic effects and electrostatic interactions; however, the dual-binding model does not describe the detail of the interior structure of the micelle. Later, a study by McMahon & McManus (1998) found no clear evidence for casein micelle subunits as studied by electron microscopy.

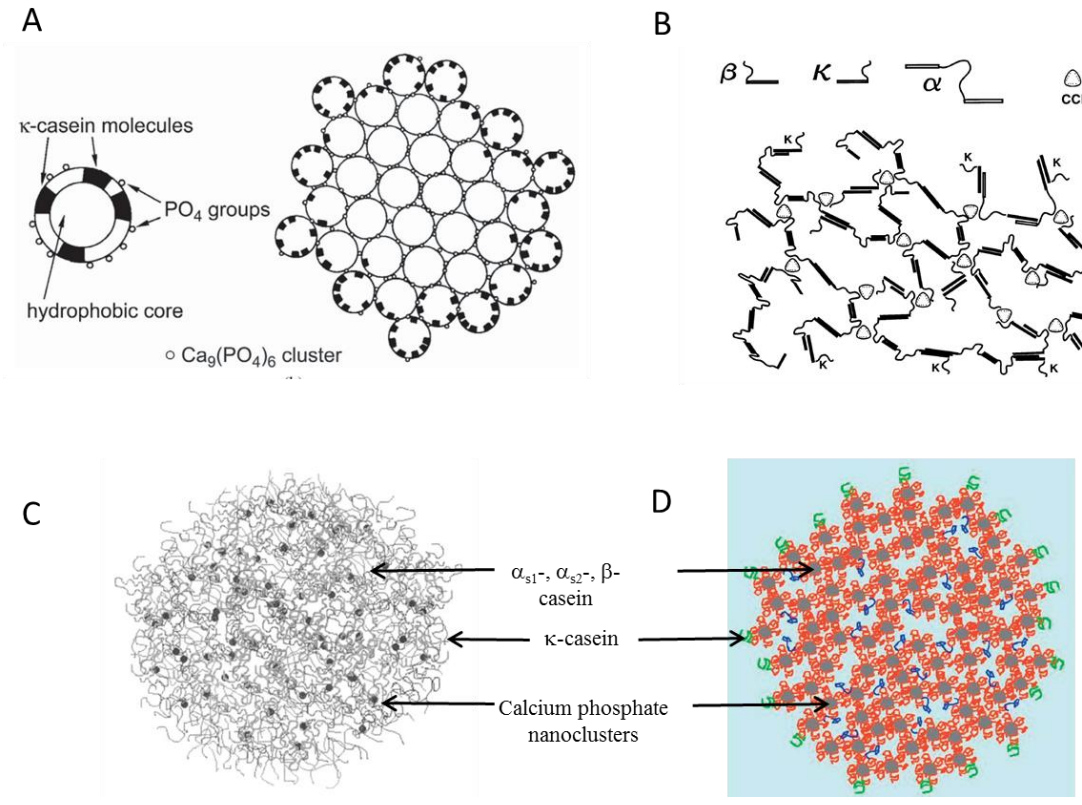

Figure 1.1: Representation of casein micelle models proposed by (A) Schmidt (1982): Submicelle-model; (B) Horne (1998): Dual binding model; (C) de Kruif & Holt (2003): Nanocluster model; (D) Dalgleish (2011).

In one of the latest reviews of casein internal structure, Dalgleish (2011) postulated that the water in the internal structure of casein micelles is not evenly distributed. It was proposed that the hydrophobic character of α_{s-} and β- caseins is incompatible with the highly hydrated structure of casein micelles. Thus the water in casein micelle is likely to be distributed unevenly throughout the interior micelle. Huppertz et al. (2017) found that the 25-30 % of total water associated with casein micelles is found on the surface layer of κ-casein, ~ 15 % is associated with caseins in the form of primary hydration water and the remaining ~ 55-60 % is entrapped within the micelle structure.

1.2.2.2 Interactions among casein micelles

Casein micelle integrity is maintained by a balancing of a number of forces. Colloidal calcium phosphate is vital in maintaining the casein micelle structure. Ionic bridges are formed between calcium ions and phophoseryl residues in caseins to maintain integrity. However, studies have shown that after solubilisation of CCP on acidification to pH 4.9, casein micelles still maintained their integrity (Dalgleish & Law, 1989). This suggests that casein micelles are stabilised by other internal forces including hydrogen bonding and the hydrophobic effects (Horne 1998, 2006). About 20 % of the total β-casein has been reported to leave and re-enter the micelle during temperature fluctuations (Creamer et al., 1977). This indicates that it is associated in the micelle to a large extent by hydrophobic effects and to a lesser extent by ionic bridges (Downey and Murphy, 1970). Another major force stabilising casein micelles against aggregation is a steric stabilising force, provided by a C-terminal hydrophilic protruding hairy layer of κ-casein, which causes micelles to repel each other on close approach (Walstra, 1990). Thus, casein association is a result of number of weak interactions including hydrogen

bonding, the hydrophobic effects, electrostatic interactions and van der Waals attraction (de Kruif & Holt, 2003; Dalgleish, 2011; Horne & Lucey 2017).

1.2.3 Whey proteins

Whey protein in bovine milk is present at a level of ~ 0.6 % (w/w). Whey proteins are heterogeneous and consist of β-lactogloblulin (β-Lg), α-lactalbumin (α-Lac) immunoglobulins and bovine serum albumin in the proportion of 51, 21, 16 and 17 %, respectively of total whey protein. It also contains trace amounts of other proteins, such as lactoferrin, serotransferrin and several enzymes.

β-Lactoglobulin has a molecular weight of 18.3 kDa and has 162 amino acids residues per monomer (Fig. 1.2). It exists as a globular negatively-charged dimer in the pH range 6.0 to 7.5 and at temperature below 40 °C (Cheison et al., 2010). It is rich in sulphur-containing amino acids cystine and cysteine. Whey protein contains two disulphide groups and one free thiol group, which, on exposure to heating (>60 °C), allows β-Lg to react with κ-casein and/or with α-Lac via SH/S-S (sulfhydryl-disulphide) interchange reaction mechanisms (Jang & Swaisgood, 1990). This can lead to aggregation of partially denatured proteins as well. β-Lg exists as 10-15 % α-helix, 43 % β-sheet and 47 % unordered structure, including β-turns. The isoelectric pH of β-Lg is ~ 5.2.

α-Lac has a low molecular mass of approx. 14.2 kDa and comprises of 123 amino acids with four disulphide bonds and no thiol group (Fig. 1.2; Farrel et al., 2017). α-Lac is capable of binding Ca^{2+}, which connects the α-Lac and β-sheet domains in the native α-Lac. It can also bind other mono- and di-valent cations such Zn^{2+}, Mg^{2+}, Mn^{2+}, K^{+} and therefore known as metallo-protein. The isoelectric pH of α-Lac is ~ 4.8.

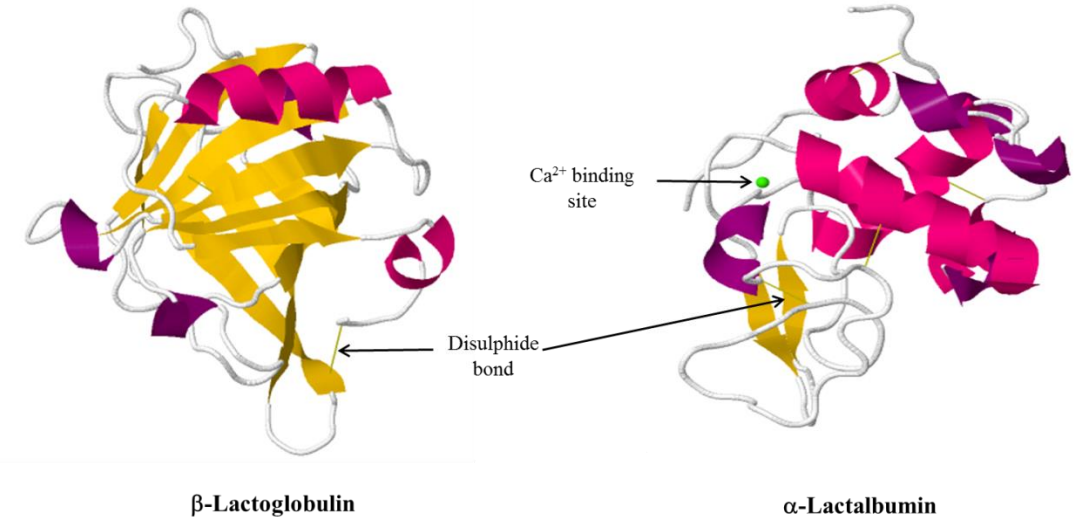

Figure 1.2: Structure of β-lactoglobulin and α-lactalbumin monomer. α-helices are represented in pink, β-sheets are represented in yellow and 3_{10}-helices are represented in purple, disulphide bonds are represented in greenish-yellow. Ca^{2+} binding site on α-Lac is represented in green (Source: PDB)

1.2.4 Non protein nitrogen

NPN comprises of urea, creatine, creatinine, uric acid, opporic acid, hippuric acid, proteose-proteone, ammonia and amino acids (Fox et al., 2015). Urea accounts for 90 % of NPN, thus urea-nitrogen accounting for nearly 50 % NPN. It is soluble in 12 % trichloroacetic acid (TCA) solution, thus also a widely used method for extraction of NPN from milk.

1.2.5 Lipids

Bovine milk typically contains 3.5 % fat. Triacylglycerides represent ~ 97.5 % of total lipids in bovine milk (Figure 1.3). The remaining lipids consist of phospholipids (0.6%) mainly present in milk fat globule membrane (MFGM), diacylglycerides (0.36 %), sterol mainly cholesterol (0.31 %), monoacylglycerols (0.027 %), and trace amounts of carotenoids and free fatty acids. The main fatty acids in milk include saturated (butyric, caprylic, capric, lauric, myristic, palmitic, oleic and stearic) and unsaturated (linoleic, linolenic and arachidonic) fatty acids (Fox et al., 2015). Saturated fatty acids contain an

alkane chain of only single-bonded carbon atoms whereas unsaturated fatty acids contain at least one alkene group of double-bonded carbon atoms in the chain. Milk is an oil-in-water emulsion, in which fat exists as small globules stabilised with protein at the interface. The milk globules range from ~ 0.1-20 μm. The distribution of fat globules in milk can be determined by light microscopy or light scattering.

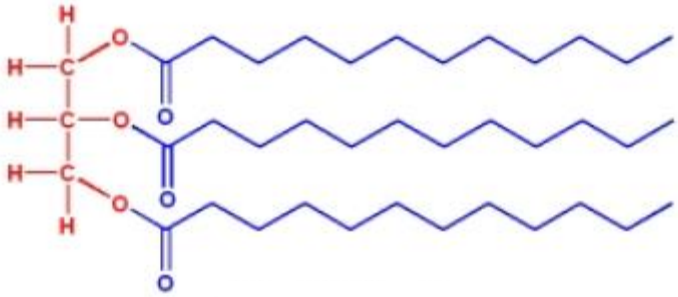

Figure 1.3: Schematic diagram of triacylglyceride.

1.2.6 Lactose

Lactose (Mw 342.3 Da) is disaccharide consisting of glucose and galactose bonded by β-1,4-glycosidic linkage, and is present as α- and β- lactose in milk. It is a major sugar present in bovine milk and the concentration is nearly 4.8 %. Lactose maintains 50 % of the osmotic pressure of milk, which is isotonic with blood. The rest of the osmotic pressure is maintained by inorganic salts. The relationship between the lactose and inorganic salts in milk is expressed as Koestler number (KN):

$$KN = \frac{\%\ chloride}{\%\ lactose} \times 100 \qquad \text{(Eq. 1.1)}$$

During late lactation, there is an influx of blood constituents into milk, thus to maintain osmotic pressure in milk, lactose is reduced. An inverse correlation has also been observed between lactose and casein in milk (Holt & Jenness, 1984). Nutritionally, lactose aids in the absorption of calcium in the intestine, by increasing osmotic pressure. Lactose is an important constituent in cheese ripening and milk powder manufacturing. During cheese making, most of the lactose is lost in whey, but during early stages of

ripening residual lactose is metabolised rapidly to lactate, which is an important precursor for a series of reactions including oxidation and microbial metabolism (McSweeney, 2004). Lactose is a principal constituent of milk powders and being a reducing sugar may participate in Maillard reactions resulting in the production of off-flavours and brown product during storage especially at high temperatures and humidity (Fox et al., 2015).

1.2.7 Minerals

Milk contains both organic and inorganic salts including salts of phosphates, citrates, chlorides, sulphates, carbonates and bicarbonates of calcium, magnesium, sodium, potassium and potassium. Trace-elements are also found in milk, such as, zinc, iron, iodine, copper, molybdenum, manganese, selenium and cobalt.

These salts or elements can be present in association with caseins, whey proteins or fat, or in the free state in the soluble/serum phase of milk (Fransson & Lönnerdal, 1983; Vegarud et al., 2000). The salts or minerals present in association with casein micelles are called micellar or sedimentable salts as they sediment with casein micelles on ultracentrifugation. The soluble salts are the salts in the serum and are referred to the non sedimentable salts on ultracentrifugation. The distribution of salts between micellar and soluble phase has been extensively studied and reviewed (Gaucheron 2005 & 2013; Cashman 2011 a & b).

The most important elements in milk, both nutritionally and technologically, are calcium and phosphorous. In bovine milk Ca and P are present at the levels of 120 and 95 mg/100 g, respectively (Gaucheron, 2005). Calcium in milk is present in sedimentable micellar and serum soluble form in the ratio of 7:3. Micellar Ca is partly associated with colloidal inorganic phosphate, forming CCP, and rest is bound to the organic phosphate, phosphoserine residue of caseins in the micelle. The distribution

between colloidal Ca and caseinate Ca is at a ratio of 6:4. Soluble Ca can be present as unionised Ca (complexed with citrate, phosphate or serum proteins) or ionised Ca, usually in a ratio of 7:3 (White & Davies 1958). Colloidal calcium phosphate in bovine milk is calcium phosphate present in association with magnesium which forms a 61 kDa nanocluster with a hydrodynamic radius of ~ 2.4 nm and contains 500 calcium ions and 450 phosphate ions citrate (de Kruif et al., 2012).

The partitioning of minerals between the micellar and soluble phase, especially calcium and phosphorous, has a large influence on the structure and stability of casein micelles (Lucey & Horne, 2009). The partitioning is influenced by physico-chemical changes in temperature, heat treatment and pH (de la Fuente, 1996; Gaucheron, 2005). Heating milk shifts the calcium phosphate equilibrium between colloidal and serum phases with a concurrent release of H^+ and reduction in milk pH, as shown in below:

$$3Ca^{2+} + 2HPO_4^{2-} \overset{heating}{\underset{cooling}{\longleftrightarrow}} Ca_3PO_4 + 2H^+ \quad \text{(Eq. 1.2)}$$

Partitioning has a major impact on dairy processes where casein micelles are involved such as rennet and acid coagulation in the production of cheese and yoghurts. CCP and free ions play a crucial role during rennet induced gelation and in the cheese-making process (Lucey & Fox, 1993; Choi et al. 2007). Partitioning also affects the stability of milk and concentrated products during heating and evaporation for manufacture of powders or beverages. Preheating of milk before powder manufacture results in the transfer of soluble Ca and P to the colloidal phase which reduces the Ca^{2+} in reconstituted skim milk powder (Oldfield et al., 2005)

1.3 Factors affecting milk composition

The composition of bovine milk is impacted by a number of factors including the nutritional (diet of cow), physiological (stage of lactation), animal dependent (breed, genetics, health status and lactation number) and environmental factors (Auldist &

Hubble 1998; Mackle et al., 1999; O'Brien et al., 1999a, b; Auldist et al., 2000a, b; O'Callaghan et al., 2016b, Lin et al., 2017).

1.3.1 Diet of cows

The effect of the diet of a cow on milk composition generally depends on the type of feed and level of feed intake. To achieve the daily dietary requirement of dairy cows, they can be grazed on fresh pasture or can be fed grass silage, hay or straw which are often supplemented with concentrates such as grains (corn, oats, wheat, barley) or a protein supplement source (soybean, cottonseed, linseed, groundnut). Alternatively, dairy cows can also be fed indoors on total mixed ration (TMR) composed of forages, grains, by-products, minerals and vitamins. The type of feeding system employed in a specific region depends on factors such as climate and its effect on pasture growth, cost efficiency, sustainability, and animal welfare.

Grazed pasture has been traditionally the major source of energy due to their cost-competitiveness (O'Neill et al., 2011) in countries with temperate climate (New Zealand, Australia, Western Europe e.g., France, Ireland and UK) where grass growth is abundant (Roche et al., 2017). Dairy grazing systems are designed to produce high dry matter annually where pasture can be present as grass-legume mix (Roche et al., 2017). Thus pasture composition can vary with region, but generally includes perennial ryegrass (*Lolium perenne* L.) with other species such as leguminous white clover (*Trifolium repens* L.), meadow grass (*Poa trivialis* L.), cocks foot (*Dactylis glomerata* L.) and fescue (*Festuca arundinacea* L.). Research has been carried out on grazing of dairy cows on pure leguminous white clover, which has shown its advantages over grazing on ryegrass, with higher milk yield and similar protein concentration (Thomson et al., 1985), however this approach is unrealistic due to lower dry matter production of white clover (Harris et al., 1997). Thus, incorporation of white clover to a pasture can

be advantageous and also fixes atmospheric N and reduces nitrous oxide emissions and carbon footprint (Ledgard et al., 2009; Yan et al., 2013). The diet of dairy cows is often supplemented with concentrates after parturition and towards end of lactation, to meet the dietary recommendation of dairy cows when grass growth is limited in temperate countries (Dillon et al., 1997; McEvoy et al., 2008).

TMR is used more extensively in the USA, parts of Europe and the southern hemisphere and evolved in last 50 years (Schingoethe et al., 2017). Advantages of TMR include a more consistent feed composition and quality, better regulation of dry matter intake (**DMI**), and higher milk yield (Kolver and Muller, 1998; McAuliffe et al., 2016).

1.3.1.1 Effect of diet on milk composition

Increasing the dietary crude protein intake either by increasing the level of intake or by altering the type of feed by incorporating supplements can affect the milk yield, component (protein/fat/lactose/casein) yield and composition. Increasing the level of feed intake by increasing the pasture allowance by 32% (13-19 kg dry matter per cow) early-or mid-lactation has been found to coincide with increases in milk yield and concentrations of protein and casein but not affecting lactose and fat concentration to an extent dependent on differences in daily herbage allowance, **DHA** (Kennedy et al., 2007; McEvoy et al., 2008 O'Brien et al., 1997; Auldist et al., 2000b). In contrast, altering the type of feed does not always result in the consistent effects on milk composition. The change in milk composition from variation in feed arises from the multiple factors that feed contributes to, such as, total dietary crude protein intake, metabolisable energy, forage:concentrate ratio and the amount of rumen degradable protein. The variation in the type of pasture or supplement (wheat/corn/canola, alfalfa hay/lucerne, partial mixed ration) had no effect on concentrations of protein or casein in milk from pasture-fed cows at 45 DIM (Auldist et al., 2016 & 2017). Although, there

were significant differences in the composition of milk from pasture-fed cows and cows that were fed indoors on TMR. Milk from pasture-fed cows had higher mean concentrations of fat, protein and casein, and a lower concentration of lactose throughout lactation (Auldist et al. 2000a; O'Callaghan et al., 2016). In contrast Dillon et al. (1997) reported that supplementing concentrates (2-4 kg) to dairy cows in early-lactation (April-June) increases the milk yield but had no effect on the concentrations of protein, fat or lactose.

The proportion of nitrogen fractions can also be affected by change in diet or nutrition of cows. Mackle et al (1999) reported that partial substitution of pasture with grain and silage when cows were 203±14 DIM did not affect the concentrations of total protein or casein, but resulted in higher casein number (casein as % of total protein) and lower levels of NPN and urea, as proportions of total N. Although, the proportions of individual caseins do not appear to change with the alteration of dairy cow diet (Thomas, 1983; Auldist et al., 2016). Conversely, in one study where DHA was restricted to ~ 40 % of *ad libitum* intake led to lower proportions of α_s- and β-caseins and a higher proportion of γ-casein in early lactation-milk (60 DIM) but not in mid-lactation milk (180 DIM).

The type and level of supplementation to dairy cows did not result in milk with significantly different casein micelle size being in the range 175-191 nm and 159-172 nm, respectively (Devold et al. 2000; Auldist et al. 2016). There is a paucity of information on the impact of dairy cow diet or feeding system on casein micelle hydration.

1.3.2 Stage of lactation or season

Stage of lactation has a pronounced effect on composition of milk, especially in the bulk milk from compact-calved herd that graze on pasture. Countries such as New Zealand,

Ireland and some parts of UK where the climate is temperate and abundancy of grass in spring-summer-autumn, dairy herds are usually compact calved between late-winter and early spring. In temperate countries, where grass growth is sufficient from spring-autumn, grass is utilised as a feed to be cost-effective. The production of milk is from February to November or December, with increasing milk production until May-June and decreasing steadily thereafter until end of lactation (O'Brien & Guinee, 2011; O'Brien et al., 1999b). Stage of lactation can have a significant impact on the composition of herd bulk milk obtained from compact calved herd containing predominantly either spring or autumn calved milk proportions. Thus large variations in milk composition are expected throughout the year (White & Davies, 1958). On the other hand, the herd management systems that utilise year-round calving, in countries such as Netherlands, USA and most parts of Europe, enable milk production throughout the year and the effect of stage of lactation is diminished. Lactational changes in milk composition occur as a result of physiological changes that occur in mammary glands of healthy cows fed on good quality diets, whereas, seasonal changes in milk are referred as to those arising due to lactation and the overlaid effects of other environmental factors such as climate. The effect of stage of lactation and/or season on milk composition and its physico-chemical and processing characteristics has been widely studied. These studies were conducted in different countries around the world such as Ireland (Kelly et al., 1982; Keogh et al., 1982; O'Keeffe et al., 1982; Phelan et al., 1982; O'Brien et al., 1999 a, b; Mehra et al., 1999), New Zealand (Auldist et al. 2000a), United Kingdom (Chen et al., 2014), Netherlands (Heck et al., 2009), Sweden (Lindmark-Månsson et al., 2003) and Italy (Bernabucci et al., 2015).

1.3.2.1 Effect of stage of lactation or season on milk composition

Total protein content and casein concentration, which are important factors in determining the nutritional value of milk and in evaluating how milk is processed vary throughout lactation. For a spring or an autumn calved herd, total solids, protein, casein and fat concentrations increase while concentration of lactose decreases with lactation up to 280 days in milk; these changes are concurrent with the decrease in milk yield over lactation (O'Brien et al., 1999b; Auldist et al., 1998, Guinee et al., 2006, Hickey et al., 2006, O'Callaghan et al., 2016b). Increase in milk proteins and fat concentrations during lactation can be related to the reduced milk volume in late-lactation and the concentrating effect of milk constituents in the udder (Auldist et al., 1998). In contrast, the reduced concentration of lactose in late-lactation coincides with the increase in the concentrations of sodium and chloride (Auldist et al., 1998), to maintain the osmotic pressure of milk similar to that of blood. Non-protein nitrogen (NPN), as a proportion of total N, and urea are also known in increase during late-lactation (Phelan et al. 1982; Mehra, et al., 1999; Auldist et al., 1998). NPN is particularly important as it does not partake in cheese-making process, thus may reducing the cheese-yielding efficiency. The effect of stage of lactation on major milk component (protein, fat, lactose) produced from spring-calved herd is shown in Fig. 1.4.

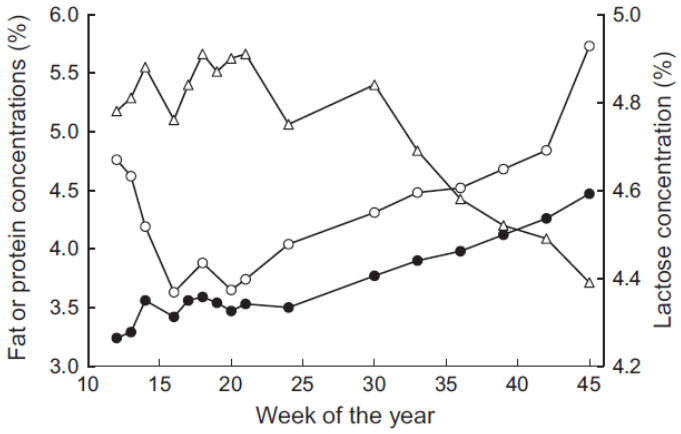

Figure 1.4: Effect of stage of lactation in milk protein (●), fat (○) and lactose (Δ) of Irish whole milk from spring-calved herds during the period mid-March to early November 2015 (Adapted from Soodam & Guinee, 2018).

Comparatively little has been published on variations in serum nitrogen fractions, proportion of individual caseins and casein micelle characteristics (such as casein micelle size and casein hydration) due to lactation. In Irish manufacturing milk (predominantly from bulked spring-calved herd milk), it reported that the proportion of α_s-casein decreased progressively throughout the year, while that of β-casein decreased from July onwards; the concentration of γ-caseins showed an opposite trend to that of β-casein and the levels of κ-casein remained constant throughout the year (Donnelly & Barry 1983). A different trend was observed for milk from herds comprised of spring- and autumn- calving cows which had a higher proportion of α_{s1}-casein than milk from Spring, Summer or Winter, and a lower proportion of β-casein than Spring milk (Lin et al., 2017). Alternatively, Heck et al. (2009) found that season had no effect on Dutch bovine casein proportions over the year.

Mineral concentration in milk have been found to be affected by the stage of lactation and season throughout the year. Elements such as Ca and P which are important nutritionally and functionally range from 111-147 and 55-112.3 mg/100g, respectively, over the lactation stage from spring-calved herd milk (Keogh et al., 1982; Auldist et al., 2004). Seasonal variation in other macro (e.g., Mg, Na) and trace-elements (e.g., Zn, Fe, Cu, Mo, Se, Mn) of bulk herd milk has also been reported (White & Davies, 1958; Moreno-Rojas et al. 1993; Moreno-Rojas et al. 1994; Pechova et al., 2008; Nantapo & Muchenje 2013).

Little or no lactational or seasonal effects have been observed in the milk for casein micelles size, casein hydration or ionic calcium by various studies (White and Davies, 1958; Keogh et al., 1982; Grimely et al., 2009; Glantz et al., 2010; Chen et al., 2014). In contrast, there was a pronounced seasonal trend in casein micelle size of milk

from Ayrshire cows, with smaller micelles in summer as compared to winter (Holt & Baird, 1978).

1.4 Processing characteristics of milk

Milk is processed at an industrial scale for products such as cheeses, yoghurts, milk powders, beverages and other dairy products (Auldist et al., 1996b; Deeth & Lewis, 2017; Fox et al., 2017). The functional properties of milk are majorly influenced by stability of casein micelles. Although casein micelles are very stable under natural conditions, they can be destabilised by several mechanisms including rennet, acid, heat, Ca^{2+} or a combination of these (Guinee et al., 1997; Sievanen et al., 2008; Horne, 2016; Huppertz, 2016). Milk processability can be determined by a nu1qmber of specific tests that include rennet- and acid- induced gelation, heat stability and ethanol stability of milk, which are described in further sections.

1.4.1 Rennet-induced gelation

Rennet gelation of milk is a crucial step in the manufacture of rennet-curd cheeses such as Cheddar, Mozzarella and Gouda (Fox et al., 2017). Chymosin is the specific milk clotting enzyme present in rennet, which is present in abomasum of young ruminants. Rennet induced gelation of milk involves two steps, enzymatic hydrolysis of the polyelectrolyte brush of casein followed by aggregation of the destabilised casein micelles. Chymosin hydrolyses κ-casein specifically at the Phe_{105}-Met_{106} bond producing *para*-κ-casein (f1-105 κ -casein) and caseino-macro-peptide (CMP, f106-169 κ-casein); this is called the primary phase of coagulation and follows first order kinetics where the reaction rate is proportional to enzyme or protein concentration (van Hooydonk et al., 1984). The hydrophilic C-terminus of κ-casein (CMP) is hydrolysed from casein micelles and disperses into the surrounding medium. The loss of CMP

results in the removal of the steric stabilising layer of casein micelles and a decrease in electrostatic repulsion as measured by a reduction in zeta (ζ)-potential, which is the difference of the potential at the interface between the particle and the dispersant in a colloidal system (Anema & Kostermeyer, 1996). The decrease in the ζ-potential at the surface increases the effective net attraction of N-terminal *para*-κ-casein, which is attached to the micelle core. The colloidal stability of micelles decreases when 85-90 % of total κ-casein has been hydrolysed by chymosin and they begin to aggregate to form a viscoelastic gel or coagulum at temperatures greater than 18 °C in the presence of calcium ions (Sandra et al., 2012). This is referred to as secondary phase of coagulation which is marked by rapid increase in viscosity and elastic shear modulus, G′, which is a measure of gel firmness. The hydrophobic effects become dominant after removal of the hydrophilic C-terminal during primary enzymatic phase is likely to be responsible for coagulation, along with decrease in electrostatic repulsion and steric stabilisation. The rennet-gelation of milk can be monitored by low-amplitude strain oscillation rheometry which dynamically records elastic or storage modulus (G′) over time. A rapid increase in the storage modulus ≥0.2 Pa is referred to as coagulation point where 90 % CMP is released (Sandra et al., 2007). Other techniques used to monitor coagulation of milk are reported by Lucey (2002) which includes diffusing wave spectroscopy and dynamic light scattering (Sandra et al., 2007).

Rennet coagulation of milk is affected by a number of factors including the concentrations of protein, calcium ions, CCP and rennet, pH, temperature and preheat treatment (Corredig & Salvatore, 2016). The effect of protein concentration is mainly on the secondary stage of rennet gelation where aggregation and fusion of particles occur resulting in formation of rennet gel. Several studies have shown a positive relationship between protein concentration (0.3-7 %, w/w) and rennet gel strength and firming rate (Guinee et al., 1996, 1997; Jõudu et al., 2008; Salvatore et al., 2011). Although the

effects of increasing protein concentration on rennet gelation time which marks the primary phase of coagulation are not always consistent. Serum casein and whey proteins do not partake in the rennet gelation process and may inhibit the enzymatic action of chymosin and *para*-casein aggregation by shielding the hydrophobic or calcium-sensitive patches on the surface of the protein particles (Corredig & Salvatore, 2016; Gamleth et al., 2018). Casein micelle size is also known to influence rennet gelation where smaller micelle size results in shorter gelation time and firmer gels due to higher κ-casein proportions (Niki et al., 1994; Glantz et al., 2010).

Addition of calcium ions in the form of calcium chloride reduces the rennet gelation time and increases gel firmness (Udabage et al 2001; Tsioulpas et al 2007). The firmer gels might be due to higher affinity of *para*-κ-casein to Ca^{2+} and further decrease in electrostatic repulsion between rennetted micelle (Udabage et al., 2001). However, excessive levels of added Ca^{2+} (>40 mg/100g) results in adverse effects on rennet gelation (Udabage et al., 2001). Depletion or reduction of colloidal calcium from the micelles results in the formation of weaker gel or no gel formation (Shalabi & Fox, 1982a) due to reduction in forces such as hydrogen bonds, van der Waals interaction and calcium bridges (Choi et al 2007).

At temperatures below 18 °C, the gelation time increases (Raynal & Remeuf, 2000) and no visible aggregation of caseins occurs due to very high temperature coefficient of the secondary phase (Fox et al., 2015) and partly due to solubilisation of β-casein in milk at low temperatures (Dalgleish & Law, 1988). Increasing temperature (25-50 °C) is correlated with decreasing coagulation time and increasing gel firmness, thus suggesting a role of the hydrophobic effects (Panthi et al., 2018).

The pH optimum for the hydrolysis of κ-casein by chymosin is 5.1-5.3 (Shalabi & Fox, 1982a). Reduction of milk pH from 6.8 to 5.6 decreases the steric repulsion, therefore the extent of CMP release necessary to start aggregation decreases (de Kruif,

1997). Reducing pH of milk also causes solubilisation of colloidal calcium phosphate and increasing Ca^{2+} level, both of which are associated with increasing coagulation time (Shalabi & Fox, 1982a).

1.4.2 Acid-induced gelation

Acidification of milk is the basis of dairy products such as yoghurts and acid cheeses. Yoghurts are manufactured by fermentation of lactose in milk, with thermophilic bacterial cultures comprising of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* to lactic acid (Lucey, 2004). To simulate acidification in milk, it has also been acidified with hydrochloric acid (Roefs et al., 1990; Roefs & van Vliet 1990) or with addition of glucono-δ-lactone (GDL) which hydrolyses to gluconic acid (Lucey & Singh, 1997). The manufacture, formation and physical properties of acid gels have previously been reviewed (Tamime & Robinson, 2007; Lucey & Singh 1997, 2003; Horne 1999, Lee & Lucey 2010).

During acidification of milk using bacterial cultures (*Lactobacillus),* there is a slow decline of pH at the start due to buffering capacity of milk and *lactobacillus* itself (Lucey, 2002). On acidification of milk, physicochemical properties of casein micelles are altered, especially in the pH range 5.0-5.5. Decrease in pH results in the following changes in the casein micelles: (1) reduction in surface charge leading to collapse of hairy κ-casein on the surface allowing aggregation of casein micelles via electrostatic interactions (de Kruif, 1999), (2) increased voluminosity and casein solvation (Walstra 1990). (3) solubilisation of colloidal calcium phosphate especially at pH ≤ 6, liberating casein into the soluble phase depending on temperature (Dalgleish & Law, 1988). When the pH of the system reaches 4.6, the isoelectric point of casein, there is charge neutralisation at the surface which facilitates aggregation of casein to form acid gel via electrostatic attraction as well as increased by hydrophobic effects. This leads to the

formation of chains and clusters linked together to form a three-dimensional structure. Some studies suggest that the hydrophobic effects does not play a part in the gel strength of acid gels, as when assay temperature of gels was decreased, G′ (storage modulus) was found to increase (Roefs & van Vliet 1990).

The gel network formation in acid-coagulated milk involves an association-dissociation phenomena of milk caseins and the firmness is influenced by many factors including milk composition, heat treatment, salt system, temperature, starter culture, pre-acidification of milk and the addition of stabilisers (Guinee et al., 1995; Peng et al., 2009; Meletharayil et al., 2016). Acid gels are mostly produced from heated milk (85-140 °C for 4s to 30 min) that reduce unwanted micro-organisms and competition for starter cultures (Lee & Lucey, 2010; Lucey, 2016). High heat treatment of milk also results in whey protein denaturation which then complexes with κ-casein via sulfhydryl linkages. When this heated milk is acidified with bacterial cultures whey proteins, specifically β-Lg, aggregates at pH 5.3, which is their isoelectric point (Farrel et al., 2004), thus increasing the gelation pH of heated milk when compared to unheated milk. Acidification of heated milk also results in a more firm and viscous gel due to the interaction of whey proteins associated with casein micelles and cross-linking casein particles in the gel (Lucey et al., 1997; 1998). This increases the number and strength of bonds between protein particles thus more branching and inter-connectivity of the gel network.

1.4.3 Heat Stability

Heat stability of milk is defined as the ability of milk, to withstand high temperatures without visible flocculation, aggregation or coagulation of milk. Heat stability of milk is an important characteristic in production of evaporated milk, UHT milk, milk beverage, milk powders and yoghurts, during which heat treatment is applied to milk in order to

achieve either a pasteurised, sterilised or dehydrated product (Singh, 2004). Over the years, a number of methods have been developed to assess heat stability of milk including heating milk in sealed tube in thermos-statistically controlled oil bath (Davies & White 1966). Heat coagulation of milk has been extensively reviewed (O'Connell & Fox 2003; Singh, 2004; Huppertz, 2016). Heat coagulation time (HCT) of milk is defined as the length of time which elapses between placing of samples in an oil bath rocking at a definite speed and temperature and onset of coagulation as indicated by flocculation, gelation or changes in protein sediment ability.

Heat induced coagulation is a complex process with contributions from (1) heat induced acidification of milk resulting in collapse of κ-casein from the micelle, (2) heat induced dissociation of κ-casein and its removal from the micelle, (3) heat induced precipitation of calcium phosphate, and (4) heat induced denaturation of whey proteins and their interaction with κ -casein.

Development of acidity in milk during heating is a result of thermal degradation of lactose to organic acids (formic acid being the most prominent), dephosphorylation of casein, and precipitation of tertiary calcium phosphate with a concomitant release of H^+ (O'Connel & Fox, 2003)

HCT-pH profiles of milk can either be Type A or type B. Type A profile is characterised by a maximum (HCT_{max}) around pH 6.7 followed by a minimum (HCT_{min}) at pH 6.9. The stability of milk increases again at pH > 6.9. Conversely in a Type B profile, a minimum is not found, and the HCT increases as a function of pH (O'Connell & Fox, 2003) as shown in Figure 1.5. Type A heat stability profile corresponds to unconcentrated milk and Type B profile to concentrated or evaporated milk.

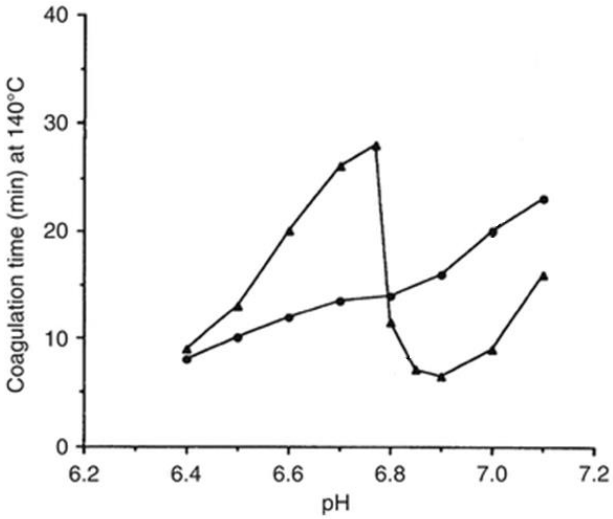

Figure 1.5: Effect of pH on the heat stability of type A milk (filled triangles) and type B milk (filled circles) (adapted from Fox, 1982).

The characteristic feature of the maximum and minimum in a type A profile is attributed to heat induced dissociation of κ-casein and its dependence on the pH. The dissociation of κ-casein increases significantly at pH > 6.7 thus becoming the significant contributor to the process (Singh, 2004). The dissociation of κ-casein from the micelles leads to the decreased electrostatic repulsion and steric stabilisation of the micelle, which reduces the colloidal stability. Also, κ-casein depleted casein micelles are very susceptible to heat due to high sensitivity of α_{s1}, α_{s2} and β-caseins to calcium ion in serum leading to aggregation. At pH > 6.9, HCT increases again as function of pH due to increase in zeta potential, and decrease in Ca^{2+} in the serum as a result of reduced solubility of calcium at higher pH.

Urea at low concentrations enhances heat stability of milk in the regions outside minima region of HCT-pH profile (Muir & Sweetsur 1977, Shalabi & Fox 1982b). It was demonstrated that the addition of 5-20 mM urea to milk, increases the maximum heat stability of milk at 140 °C (Shalabi & Fox, 1982b). A number of studies investigated changes in milk composition either by changes in diet or over a season and heat stability of milk, established a positive correlation between urea and heat stability (Muir & Sweetsur 1976; Holt 1978, Banks et al., 1984). Urea in milk gradually

decomposes on heating to ammonia and carbon dioxide. The ammonia formed stabilises the milk against heat-induced acidification through the neutralisation of acid and reduced Ca^{2+} activity in heated milk due to pH buffering (Singh, 2004).

Lactose is also one of the most important contributors of heat induced acidification of milk, thus reducing heat stability. On heating milk, lactose degrades into galactose, formic acid and C5/C6 compounds (Singh, 2004). Formic acid accounts for 80 % of total acid formed, thus is the major determinant in reducing pH of the heated milk. Shalabi & Fox (1982b) found that increasing the concentration of lactose from 0.9-6.9 % (w/w) in milk caused a decreased in heat stability of milk. Removal of lactose in milk by enzymic hydrolysis with β-galactosidase was also found to increase heat stability (Tan-Kintia & Fox, 1996).

Calcium and phosphate both in colloidal and soluble phase play key roles in heat stability of milk. It has been shown that removal of 40 % of the total CCP increases HCT in the pH range of 6.4-7.4, while removal of 60-100 % of total CCP increases HCT in the pH range of 6.4-7.0 but does not increase stability at pH > 7.0 (Fox & Hoynes, 1975). Increase in soluble calcium in milk, either by addition of calcium chloride or ultrafiltrated permeate, has a negative impact on heat stability (Rattray & Jelen, 1996; Sievanen et al., 2008, Omoarukhe et al., 2010; Kaushik et al., 2015).

1.4.4 Ethanol stability

The stability of milk to ethanol is a prerequisite for food formulations containing both milk and alcohol such as cream liqueurs, egg nogs and other alcohol dairy-based beverages (Abbott & Savage, 1985). ES is defined as the minimum concentration of aqueous ethanol solution required to induce instantaneous visible flocculation or precipitation in milk (Horne & Parker, 1980).

It is postulated that ethanol induced protein aggregation is due to the collapse of the κ-casein 'hairy layer' on the micelle, removal of steric stabilising layer, electrostatic interactions, and precipitation of calcium phosphate (Ye & Harte, 2013; Horne, 2016). The hydrodynamic radius of casein micelles decreases with added ethanol concentration below critical levels. As ethanol concentration exceeds critical concentration, a dramatic increase in hydrodynamic radius occurs due to collapse of 'hairy layer' on the casein micelles, which leads to reduction in steric stabilisation and subsequent protein aggregation (Horne, 1984). A typical ES-pH profile is shown in Figure 1.6, ethanol stability is a sigmoidal function of artificial adjustment of milk pH in the range 6.0 to 7.4. The pH dependent increase of ethanol stability is due to an increased precipitation of colloidal calcium phosphate at higher pH, which draws caseinate-bound calcium reducing soluble calcium [Ca^{2+}] and increases the protein charge. At higher pH, an increase in protein-charge drives the system towards stability thus requiring higher alcohol content for protein precipitation (Horne, 2003).

Ethanol stability is largely affected by serum composition and pH of milk, as suggested by the role of these components in micellar stability against ethanol. Ethanol stability of milk is pH dependent, as can be seen in Figure 1.6. Increasing the pH of milk increased the critical concentration of ethanol required to induce visible flocculation. Higher levels of soluble Ca and Mg in milk reduced ES (Horne & Parker 1981; Horne & Muir, 1990), while retaining the sigmoidal ph/ES profile. Other studies (Chavez et al., 2004; Tsioulpas et al., 2007) also found a significant negative correlation with ES at natural pH of bovine milk either obtained from individual animals or bulk milk over the season/stage of lactation. Addition of phosphate, citrate (up to 5 mM), monovalent cation (Na^{+}), whey proteins, lactose or urea had little, or no, effect on ES (Horne & Parker, 1981).

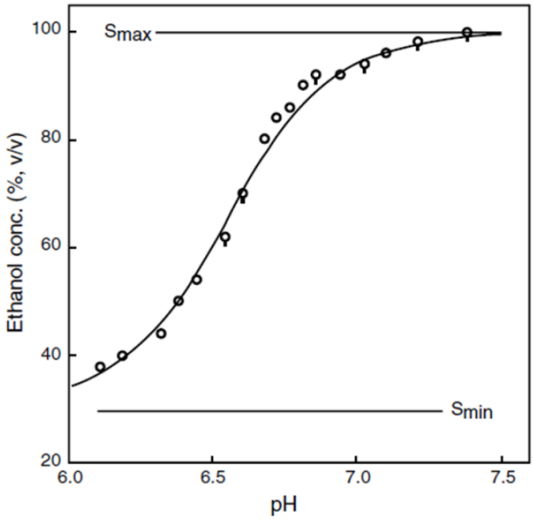

Figure 1.6: Effect of adjusting milk pH on the minimum concentration of ethanol solution required to coagulate the milk. (Adapted from Horne & Parker, 1980)

1.4.5 Effect of diet and stage of lactation on milk processing characteristics

Dairy cow diet (DHA or type and level of supplement) and stage of lactation effect the composition of milk as discussed in Section 1.3. These compositional changes in milk can result in altered functionality of milk. Few studies showed alteration of diet (DHA or type and level of supplement or different pastures) changed the casein content in the range of 0.1-0.3 % (w/w), which were probably insufficient to manifest any change in the rennet gelation properties or ethanol stability (O'Brien et al., 1997; Guinee et al., 1998; Hermanssen et al., 1999; Auldist et al., 2016). Alternatively, Massolini et al. (2005) found the change in diet leads to the variation in protein and casein, which affects the processing characteristics, specifically rennet gelation. Supplementation of hay and protein concentrates to silage diet for dairy cows, resulted in an increase in maximum heat stability of milk, which was correlated positively with urea content in milk (Banks et al., 1984). There is paucity of information on comparative effects of major feeding systems, i.e., grazing on pasture versus feeding indoors on total mixed ration on the processing characteristics.

Rennet gelation properties of milk are greatly influenced by casein concentration in milk, thus increased gel firmness is observed as the lactation progresses where casein

concentration increases (O'Brien et al., 1999). No consistent trends have been observed for the heat stability of manufacturing milk during lactation or across the season. However, ~ 70 % of the changes in heat stability have been correlated with the urea content in milk (Holt et al., 1978; Kelly et al., 1982). Ethanol stability has been found to show some lactational trends, with ES at pH 6.6 higher in early lactation thereafter decreasing throughout lactation (Horne et al., 1986). A high salt balance ratio, Ca and Mg- to-inorganic phosphate and citrate may be a contributor to reduced ethanol stability of milk in late lactation.

1.5 Product Manufacture

1.5.1 Mozzarella

Mozzarella cheese is a 'pasta filata' cheese which means 'stretched curd' where the curd is subjected to a mechanical stretching in hot water before moulding. This step results in the elongation and reordering of cheese structure where protein fibres run in a parallel fashion, thereby imparting these cheeses with the unique functionality of heat induced flow. Mozzarella is one of the world's most widely produced cheeses and is made in the largest quantities in the USA (USDA, 2017). It is an ingredient cheese used in pizza, lasagne and sandwiches, where its heat induced flow properties are utilised. According to the Codex Alimentarius Standard for low-moisture Mozzarella cheese (WHO/FAO, 2011) and the Code of Federal Regulations for low-moisture part-skim (LMPS) Mozzarella (CFR, 2016), low-moisture Mozzarella cheese contains between 30 to 45 % milk fat-in-dry-matter and 45 and 52 % (w/w) moisture.

1.5.1.1 Mozzarella manufacture

The production of Mozzarella involves several steps including, milk standardisation, pasteurisation, acidification, coagulation, gel cutting, dehydration of curd, salting,

stretching and brining. The central step involved in the production of Mozzarella is rennet induced gelation of milk, which is significantly affected by the composition of milk.

The cheese-milk for Mozzarella is standardised to a protein-to-fat ratio of 1.15-1.20. The standardised cheese-milk is pasteurised and pumped into the cheese vats, where it is inoculated with thermophilic cultures (*Streptococcus thermophilus and Lactobacillus delbruckii*). The starter culture is allowed to ripen in the cheese-milk to produce lactic acid. Acidification plays a vital role in determining the enzymatic activity of rennet and solublisation of calcium phosphate during cheesemaking. The inoculated cheese milk is coagulated enzymatically using rennet. The mechanism of rennet action and factors affecting renneting properties are described in Section 1.4.1. The rennet-altered casein micelles with exposed *para*-κ-casein on the micelle surface forms the gel by means of inter-micellar interactions due to increased hydrophobicity, which is called *para*-casein gel. Once the rennet-altered *para*-casein gel has reached the desired firmness, the gel is cut. It is important to cut the gel at certain gel strength as it influences the cheese yield and moisture content of final cheese (Fagan et al., 2007). The cutting of a *para*-casein gel is followed by a cooking procedure where the curd particles are cooked from 36 °C to 42 °C in a defined time, usually at a rate of 1 °C/5 min. This step is called a cooking or dehydration process where the *para*-casein gel contract resulting in expulsion of whey from the curd, also known as curd syneresis. The extent of syneresis affects the moisture content of final cheese and hence the biochemical and flow properties (McMahon & Oberg, 1998; Everard et al., 2008). During cooking, the curd and whey mixture is continuously stirred to ensure the heat is distributed throughout and the sticking of curd particles is avoided. When the pH of the whey inside the curd has dropped to 6.1, the vat is drained into the finishing vat then trenched, slabbed and piled. This process is called Cheddaring, where the slabs of curd

are acidified due to the inoculum activity and high temperature. The reduction in pH solubilizes colloidal calcium phosphate and increases the hydration of *para*-casein gel (Lucey & Fox, 1993). Once the pH has reached the optimum value of 5.3-5.1, the slabs are milled and dry salted. Salt diffuses into curd via osmosis which results in loss of moisture. The milled and salted curd are then plasticised, kneaded and stretched mechanically with hot water (>70 °C) which reorients the amorphous curd structure into an aligned unidirectional fibrous network surrounded by serum channels and fat globules (McMahon et al., 1999). The hot plasticised curd (58 °C) is then moulded into rectangular blocks and cooled in the dilute brine, drip dried, vacuum packaged and stored at 4 °C.

1.5.1.2 Cheese yield

The yield of cheese is affected by a number of factors, including, the composition of raw milk, milk pre-treatments (i.e., milk standardisation of protein-to-fat, homogenisation, pasteurisation temperature, and manufacturing process of cheese (i.e., firmness of gel at cutting, cutting/stirring speed, rate of cooking the curd-whey mixture, temperature to which it is cooked). These factors exert their effects by influencing the composition of rennet milk gel, the extent of moisture expulsion and fat loss from the curd particles formed due to cutting the gel.

Increasing the concentrations of protein and fat in milk generally coincides with linear increase in cheese yield (Fenelon & Guinee, 1999). In contrast, increasing the protein-to-fat ratio in cheese-milk reduces the cheese yield (Guinee et al., 2007).

1.5.1.3 Development of cheese structure

The microstructure of Mozzarella cheese changes during manufacture and ripening as studied by scanning electron microscopy (Oberg et al., 1993) and confocal scanning

laser microscopy have shown (Fig. 1.7; Everett & Auty, 2017). The microstructure of curd shows concentrated gel consisting of a calcium-phosphate para-casein network that encloses fat in the form of globules and varying degrees of coalescence. The gel undergoes further aggregation and concentration, concomitant with expulsion of whey drainage, cheddaring, salting and plasticisation steps (McMahon et al., 1999). During the storage of low-moisture Mozzarella cheese at 4 °C, the *para*-casein gel swells with occluded fat-serum channels due to an increase in proteolysis and solubilisation of calcium bound to casein (McMahon et al., 1999; Lucey et al., 2003). The increase in casein hydration or water holding capacity of casein during ripening influences the melting properties of cheese (McMahon et al., 1999) The fat-serum channels act as lubricant which facilitates the displacement of adjacent *para*-casein on heating (Guinee 2002).

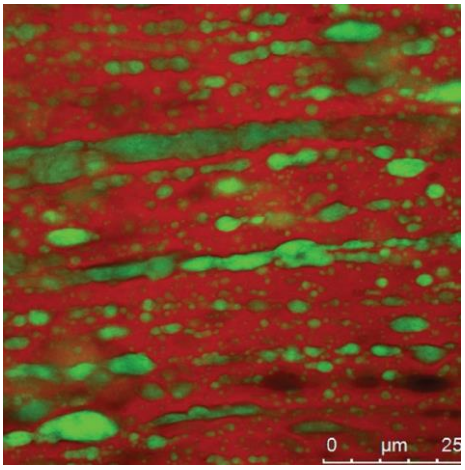

Figure 1.7: Confocal micrograph of Mozzarella cheese showing the protein phase (red), the fat phase (green), and the serum phase (black) (Adapted from Everett & Auty, 2017)

1.5.1.4 Functional Properties of Mozzarella

Mozzarella is viscoelastic in nature, i.e., it has both viscous (or fluid), and elastic (or solid) like characteristics. Rheology is the study of the deformation and flow of matter when exposed to stress or strain (Gunasekaran & Ak, 2003). Rheological properties of cheese relate to its composition, structure and strength of attractions among its structural constituents (O'Callaghan & Guinee, 2004). Low-moisture Mozzarella cheese is mostly

used in pizza and other prepared food that contains melted cheese. Thus apart from texture, its heat induced properties such as flow and melt properties are major determinants of the quality and acceptability of Mozzarella (McMahon et al., 1993).

Large strain deformation tests on cheese are employed, which are a measure of non-linear rheological properties of cheese, to obtain fracture characteristics and firmness of cheese. The firmness of Mozzarella is dependent on cheese composition including protein, fat or moisture content, and the level of proteolysis; and it significantly reduces over storage time due to an increase in the level of proteolysis (Yun et al., 1993a; Kindstedt et al., 1995; Henneberry et al., 2015). Heat induced flow of cheese can be studied either by measuring the increase in cheese disc diameter on heating in an oven for specified time or by low amplitude strain oscillatory rheology (LASOR). The rheological parameter, loss tangent, which is tan of phase angle (δ), is a measure of fluidity of cheese. Phase angle is measure of viscous to elastic characteristic of cheese:

$$\tan \delta = \frac{G''}{G'} \qquad \text{(Eq. 1.3)}$$

where, G″ is loss modulus and G′ is storage modulus. The heat induced flow of cheese increases over storage time (Yun et al., 1993b; Guinee et al., 2002) which is associated with increase in protein hydration and proteolysis over storage. Fat and moisture in Mozzarella cheese act as a lubricator during heating and enables the flow of molten cheese (Rudan et al., 1999).

Variation in milk composition, especially, casein and fat in the range of 3.0 to 4.5% (w/w) and 0.1-3.5 % (w/w), respectively, is highly correlated with the yield of cheese but has little impact on protein recovery or cheese composition (Gille & Lawrence 1985; Guinee et al. 1994, 1996, 2001; Fenelon & Guinee, 1999; Soodam & Guinee, 2018). Reducing protein-to-fat ratio of milk, by changing fat content in the range, has pronounced effects, the most notable being increases in cheese firmness and

fracture stress, impairment of cooking properties, and a deterioration in sensory qualities (e.g., a loss of typical cheese flavour and creaminess). The effects of altering fat content depend on the degree of fat reduction and make procedure (Rudan et al., 1999; Fenelon & Guinee, 2000b; Henneberry et al., 2015; McCarthy et al., 2016).

1.5.2 Skim milk powder

Skim milk powder (SMP), also referred to as non-fat dry milk, is used extensively as an ingredient in the manufacture of dairy-based beverages and formulated food products (e.g., coffee creamers; ice cream, dairy-based desserts, sauces, soups, processed cheese products, bakery products). Depending on the application of SMP and functionality required, different heat treatments to skim milk can be applied prior to evaporation and drying to produce low-, medium- or high-heat powders. Manufacture of SMP involves heat treatment of milk, evaporation to 45-50% (w/w) total solids, and spray drying to ~ 96 % (w/w) total solids. The temperature and time of heat treatment affects the extent of whey protein denaturation, binding of denatured whey protein to casein micelles and portioning of components (minerals, caseins and whey proteins) between serum and micellar phases of milk (Dunato et al., 2009).

Low-heat SMP is used for the preparation of recombined milk for cheese manufacture, or for standardising the content of milk protein or solids in products such as cheese milk, yoghurt and fermented milk products (Kelly & Fox, 2016). The functional or processing characteristics of skim milk powders depend on the original raw milk composition and the drying. Dehydration of skimmed milk to a powder with specified moisture content (~ 4-5 %, w/w) removes the impact of seasonality-associated differences in the total solids content of milk on the total solids content of the powder or the reconstituted skim milk (RSM), prepared by dispersing and dissolving the powder on a given weight basis (e.g., 10 %, w/w). Nevertheless, the variations in the

proportions and ratios of individual constituents (e.g., lactose, protein, calcium, urea) due to diet or seasonality in skimmed milk will still prevail in powders and thus in RSM. Such compositional variation may influence the processing characteristics (stability towards rennet, acid, heat or ethanol) of RSM as they would in raw or skim milk as discussed in Section 1.4. Some studies have shown that increasing protein content (from 3.07 to 3.50 %) and decreasing Ca^{2+} (to 1.14 mM) of RSM from low heat skim milk powders has positive effect on the heat stability of RSM (Faka et al., 2009; Sikand et al., 2010).

1.5.3 Stirred yoghurt

There are two major types of yoghurts, set and stirred yoghurt. Set yoghurts are the gels that are formed undisturbed and stirred type yoghurt are produced by breaking the gel via stirring and storing in cold (Lee & Lucey, 2010). Yoghurt manufacturing involves standardisation of milk, homogenisation, heat treatment and fermentation process by addition of starter culture. The milk solids content for yoghurt manufacture ranges from 9-20 %, but many commercial types of yoghurt have a milk solids content of 14-15 % (Tamime & Robinson, 2007). Homogenisation improves yoghurt quality including consistency and water retention. Following homogenisation, heat treatment of milk is used to destroy unwanted micro-organisms and to improve the gel strength. Milk is then cooled to incubation temperature and inoculated with bacterial cultures and fermented until pH drops to 4.6. For stirred type yoghurt, yoghurts are stirred and cooled immediately to reduce further acid development.

Similar to monitoring gelation for rennet-induced gelation (Section 1.4.1), rheological parameters during acid gelation can also be characterised using low amplitude dynamic oscillatory rheometry (Lucey et al., 1997). During gel formation, storage modulus (G′) increases because of the increase in number and strength of bonds

in protein particles and rearrangement of the protein gel (Lee & Lucey, 2010). A characteristic rheological feature during acid gel formation from heated milk is the initial increase in loss tangent up to a maximum and then a decrease thereafter (Lucey et al., 1998). Loss tangent increases due to loosening of the protein network as a result of colloidal calcium phosphate solubilisation and decreases thereafter indicating strengthening of gel network due to decreased electrostatic interactions between casein particles and increased casein-casein interaction near their isoelectric points (Lucey, 2004; Lin et al., 2018). Flow behaviour of stirred-type yoghurt has been studied by shear rate sweep analysis, which suggests that yoghurt is a non-Newtonian and shear thinning product (Ramaswamy & Basak, 1991).

1.5.4 Effect of diet and stage of lactation or season on product manufacture

The changes in milk composition due to changes in diet or season can result in the altered product manufacturing characteristics, quality and their functionality. The findings from varying the diet of cows and its effect on cheese yield, fat and protein recoveries, cheese composition and functionality are not consistent. It has been reported that increasing the daily DMI of dairy cows in the range ~ 11.3 kg per cow and ~ 21 kg per cow by increasing pasture allowance or by supplementing with concentrates or high protein and energy constituents, increases the cheese yield and may decrease Cheddar cheese moisture, which can be associated with higher casein in milk from cows with higher DMI (Kefford et al., 1995; Christian et al., 1999; Guinee et al., 2001). Cheese moisture in turn can influence the functionality of unheated and heated cheese. The increase in cheese moisture while maintaining the compositional parameters, such as protein and fat, relatively constant can lead to reductions in firmness and fracture stress due to reduction in concentration of volume fraction of para-casein network, making it less elastic and more susceptible to fracture on compression. The increase in cheese

moisture can also lead to increased flowability and fluidity of heated cheese, where moisture acts as a lubricant between the contiguous planes of the para-casein network during heat-induced displacement (Visser 1991; Fox et al., 2017). On the other hand, some studies on effect of varying pasture allowance on Mozzarella cheese-making and supplementation on Cheddar cheese-making have reported no effect on cheese yield, fat or protein recoveries and gross composition of cheese (Guinee et al., 1997; Auldist et al., 2016). More recently, O'Callaghan et al. (2016, 2017) reported the effect of feeding system on Cheddar cheese; cows were grazed on pasture or offered total mixed ration indoors (TMR). Cheese from milk produced by cows on the pasture feeding systems had higher concentrations of β-carotene, being softer at 20°C and more yellow in colour. Jasińska et al. (2010) found that the hardness of set yoghurt made from non-standardised milk from dairy herds fed on grass (supplemented with concentrates) or on total mixed ration varied with month of year, with no consistent effect of season evident.

Advancing stage of lactation has been found to increase the moisture content of Cheddar cheese (O'Keeffe 1984; Hickey et al. 2006). Moisture content of cheese can be correlated positively with moisture in non-fat substances (MNFS), which is known to increase microbial and enzymatic activity in cheese. Stage of lactation has also been found to affect the yield of Cheddar cheese, being higher in late lactation than in mid-lactation (Kefford et al., 1995; Auldist et al., 1996a) but not affecting the cheese composition. The higher cheese yield was correlated with higher protein and fat content of the milk. Seasonal variation has been shown in stirred-yoghurt production, where seasonal milk powders over the year were found to have varying viscosity which was positively correlated with casein content of milk powder (Cheng et al., 2002). The relationship between protein content and yoghurt quality was due to pivotal role of protein in gel formation, building viscosity and water holding capacity.

There is limited information available on effect of diet or season on the processing characteristics of low heat skim milk powders. We are also unaware of any studies done on the comparative effects of two major feeding systems around the world, i.e., grazing on pasture versus feeding indoor on total mixed ration on the product manufacturing characteristics and product composition, such as, Mozzarella and skim milk powder, throughout the lactation.

1.6 Conclusion

This chapter reviews the current understanding of milk composition and the effect on processing properties of milk including rennet-induced gelation, heat stability, ethanol stability and acid-induced gelation. Milk is processed to produce functional dairy products such as cheese, powders, yoghurts and beverages. A thorough review of literature is conducted on the effect of cow diet and season on the processing characteristics of milk and milk product quality. There are limited studies available on the direct comparison of two major feeding systems of dairy cows, i.e, grazing pasture versus feeding indoors on total mixed ration, throughout the season. The variation in milk composition due to feeding of the dairy cows, stage of lactation or season, might influence milk processability which in turn can have a potential implication on manufacture and quality of dairy products.

Chapter 2

Materials and Methods

2.1 Herd treatments, management and milk collection

2.1.1 Herd treatments for reducing DHA to dairy cows in early lactation

This herd treatment corresponds to Chapter 3. Thirty-six spring-calved cows from the Teagasc Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland) were made available for the study. The cows had a mean calving date of February 9 2015 ± 8.4 d. The 36 cows were assigned to three different herds which were placed on different DHA for 6 weeks in early lactation from March 9 to April 19 (29-70 days in milk, **DIM**). The three DHA, expressed as kg dry matter (DM) intake per cow, were 11.8, 14.4 and 15.0, respectively >3.5 cm; these are denoted as low (L-DHA), medium (M-DHA) and high (H-DHA), respectively. The details of the herd management are given in Table 2.1. Paddocks were managed, as described by Kennedy et al. (2007), to ensure the desired DHA for each treatment. The herds were balanced with respect to a number of factors as shown in Table 2.2.

Table 2.1: Experimental and herd management details for DHA experiment.

Period	Dates	DHA (kg DM)	Supplementation	Other details
Mean calving date	Feb 9 (1 DIM)	7	-	-
Partial Turnout	Feb 9- Feb 22	7	ad-libitum access to grass silage by night + 5 kg concentrates	Following calving, the cows had access to pasture during day and housed during night until February 22
Full turnout	Feb 23-Mar 2	13	Concentrates gradually reduced to 0 by March 2	
Experimental period in early lactation	Mar 9-Apr 19 (29 –70 DIM)	36 cows were assigned to 3 herds on different DHA: 11.8, 14.4 and 15.0	None	Herds grazed within the same paddocks divided with temporary fence.
Post experimental period	Apr 20-Nov 23	18	None	all 36 cows grazed once more as a single herd
Drying off	-	-	-	Individual cows were dried off when daily milk yield decreased to 8 kg per cow, BCS dropped to 2.5, or when the interval from next calving date was 8 weeks

Table 2.2: Factors on which three herds were balanced.

Period	Details	SD
Mean calving date	Feb 9, 2015	8.4
Breed	16 Holstein Friesian (HF), 13 HF X Jersey, 7 HF X Norwegian Red	
Lactation number	2.56	1.42
Bodyweight	523	53.2
Body condition score (BCS)	3.17	0.0142
Pre-experimental daily milk yield (kg)	25.3	3.70
Milk solids yield (kg)	2.20	0.33

All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee (TAEC69/2014) and authorised by the Health Products Regulatory Authority (Project licence No.: AE19132/P017), which is the competent authority in Ireland responsible for the implementation of European Union legislation for the protection of animals used for scientific purposes.

2.1.1.1 Milk sampling from DHA experiment

Milk was sampled at 10 day intervals during the period March 9-April 19, 2015 (29-70 DIM), when the herds were on different DHA; this period was denoted early lactation (EL). Thereafter, milk was sampled at 1-3 week intervals for the remainder of the lactation, which was arbitrarily divided into two sub-periods, namely mid lactation (ML, April 27-August31) when cows were 78-183 DIM, and late lactation (LL, September 20-November2) when cows were 205-267 DIM. The number of milk samples in EL, ML and LL were 4, 7 and 4, respectively.

All 36 cows were milked daily at 07:00 and 15:30 and ~250 mL of milk was collected separately from each cow in the evening and morning milkings in sterilised plastic screw cap bottles; evening milk samples in separate 250 mL bottles were held at 4 °C overnight prior to blending with morning milk samples. A composite sample (2 L) for each of the treatment herds was generated by blending the milk samples from

individual cows in the herd, in quantities proportional to the total milk yield of each cow in both evening and morning milkings. The three composite herd milk samples were preserved with sodium azide (0.02 %, w/w) and held at 4 °C until required for further analysis, which was completed within 3-48 h after collection.

2.1.2 Herd treatment for different dairy cow feeding systems

This herd treatment corresponds to Chapters 4, 5, 6 and 8. A separate set of 60 spring-calving dairy cows were allocated to one of the three feeding systems at the Teagasc Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland) both in 2015 and 2016. The feeding systems, imposed from mid-February (1 DIM) to November (300 DIM), were: grazing on perennial ryegrass (*Lolium perenne* L.) pasture (GRO), grazing on perennial ryegrass and white clover (*Trifolium repens* L.) pasture (GRC), or housed indoors and offered total mixed ration (TMR). The herds were balanced with respect to a number of factors as shown in Table 2.3, including 2-week pre-experimental milk yield and milk solids yield, as described by McAuliffe et al. (2016). The details of the herd management and feeding systems are given in Table 2.4.

Table 2.3 Factors on which three herds were balanced.

Period	Details
Mean calving date	Feb 19 (2015 and 2016)
Breed	48 Holstein Friesian + 12 Holstein Friesian × Jersey
Lactation number	12 primiparous + 48 multiparous
Economic Breeding Index (EBI)	€185 ± €43

Table 2.4: Experimental and herd management details for DHA experiment.

Period	Details
Turnout	The cows were placed on the different feeding systems 1 week after calving
Sward Management	GRO and GRC swards were fertilised at 250 kg N/ha/yr. Nitrogen was applied to GRO and GRC swards treatments as urea (46 % N) until the end of April and as calcium ammonium nitrate (27 % N) from early May to mid-September. Grass was allocated to the grazing groups each day to achieve a post grazing sward height of 4 cm. Pasture allocation for the grazing treatments was measured using

	pre-grazing herbage mass (>4 cm) and area (m2). Average sward clover content across the year was 23.8 % of herbage dry matter. The target pre-grazing herbage mass was between 1300 and 1600 kg DM/ha above 4 cm.
Stocking rate	The grazing treatments were stocked at 2.75 livestock units/Ha in a fully closed farm system.
Grazing rotations	Both grazing groups were rotationally grazed, achieving 8.3 grazing rotations in the season.
Grazing cow supplementation	They had a dry matter intake of 18 kg/day per cow. Grazing cows received a mineral supplement in the form of a liquid mineral preparation injected into the water supply (Terra Liquid Minerals, Moone Lodge, Moone, Athy, Co. Kildare, Ireland), giving a mean intake (mg/day per cow) of Na, Mg, Zn, Cu, Se and Co of 5.0, 1.2, 219, 106, 3.8, and 3.0 respectively. The GRO and GRC diets were supplemented with concentrates at a level of 2 kg per cow in November for a period of 25 days, which included the last 2 sampling occasions in 2015 when the cows were 257 and 281 DIM, respectively. During this period, the DMI decreased to 17 kg/day per cow.
TMR cow diet	It consisted of grass silage, maize silage and concentrates, including beet pulp, soybean meal, maize distillers' grains, rolled barley, rapeseed meal, Megalac®, acidbuf and mineral balancer (O'Callaghan et al., 2016a). The dry matter intake of TMR-fed cows was 7.15 kg grass silage, 7.15 kg maize silage, and 8.3 kg concentrate. The concentrates portion of the TMR feed was supplemented with a commercial mineral balancer, Dairy Hi-Phos (McDonnell Bros. Agricultural Suppliers Ltd, Fermoy, Co. Cork, Ireland) to give added Ca, Na, P, Zn, Cu, Mn, I, Co and Se of 3340, 2000, 1200, 140, 100, 70, 10, 2 and 0.8 mg/kg, respectively. Cows on TMR feeding system were housed in cubicles and fed ad-libitum at 0830 h everyday into individual feed bins which were electronically controlled by Griffith Elder Mealmaster (Griffith Elder and Company Ltd., Suffolk, UK).
Drying off	Individual cows were maintained on the treatments until the milk yield dropped to < 8L/d or on November 26 in 2015 and November 29 in 2016.

All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee and authorised by the Health Products Regulatory Authority (HPRA), which is the competent authority in Ireland responsible for the implementation of European Union legislation (Directive 2010/63/EU) for the protection of animals used for scientific purposes.

2.1.2.1 Milk sampling from different feeding system experiment

Milk from each of the three herds on the GRO, GRC or TMR feeding systems was collected separately in the designated tanks, and denoted as GRO, GRC or TMR milk, respectively. Evening (1530 h) and morning (0730 h) milk from each herd was collected separately in designated refrigerated bulk tanks (5000 L) and milk yields were recorded using DairyMaster milk meters (DairyMaster, Causeway, Co. Kerry, Ireland) en-route

to the bulk tank. Following completion of morning milking, the collected pm and am milks were agitated intermittently for ~ 30 min prior to sampling.

For the study in 2015, ~350 L milk from each herd was collected on 10 separate occasions, at two or three weekly intervals, from June 17 (119 DIM) to November 26 (281 DIM). The milk from each herd was collected in a separate refrigerated bulk tank. A representative 2-L herd milk sample was then withdrawn through the sampling port of each bulk tank into clean 2-L glass bottles and taken immediately to the laboratory for analysis. Milk was analysed for gross composition and elements over the period from June 17 to November 26 (ML: June 17-September 9; LL: September 22-November 26). In the 2016 study, bulk herd milks were collected in ML (May 23-July 20, 94-152 DIM) and LL (September 27-November 5, 221-260 DIM), and evaluated for their Mozzarella cheese- and low-heat skim milk powder (LHSMP) making characteristics.

2.2 Milk and milk serum analysis

2.2.1 Preparation of skim milk and skim milk serum

Milk was skimmed to a fat content of <0.1% (w/w) fat using a disc bowl centrifuge (FT15 Disc Bowl Centrifuge, Armfield Limited, Ringwood, UK), preserved using sodium azide (0.02 %, w/w; Sigma-Aldrich, St. Louis, MO, USA), and held at 4 °C prior to analysis (within 24-48 h). Representative sub-samples (~ 10 mL) of the skim milk for analysis of protein profile and concentrations of elements were stored at -20 °C and thawed at 4 °C overnight prior to analysis.

Milk serum was prepared by heating the cold skim milk samples at 40 °C for 30 min (to reverse cold ageing effects, including solubilisation of casein and calcium phosphate; Dalgleish & Law, 1988) and ultracentrifugation at 100,000 *g* at 25 °C for 1 h (Sorvall Discovery 90SE ultracentrifuge, Kendro Laboratory Products, Asheville, North

Carolina, USA). The supernatant was filtered through superfine glass wool (11 μm) (VWR International, Dublin, Ireland), to obtain fat-free serum which was preserved with sodium azide (0.02 %, w/w) and stored at 4 °C for further analysis and at -20 °C until analysed for minerals.

2.2.2 Total solids and fat

Total solids (**TS**) and fat in milk samples or reconstituted milk from LHSMP (RSM) were analysed in triplicate using FOSS Fourier Transformed Infrared (FTIR) analyser MilkoScan^{TM} FT+ analyser (N. Foss Electric A/S, Hillerød, Denmark).

TS in cheese and LHSMP was determined by the oven drying method (ISO, 2004a). 3 g of sample was dried in an oven at 102 ± 2 °C for 5 h and TS or moisture was expressed as percentage by recording weight prior and after drying. Fat in cheese was measured by gravimetric method also called as Röse-Gottlieb method (ISO, 2010). Cheese sample (~ 10 g) was weighed into the extraction tube and extracted with 10 mL of ammonia and 10 mL ethanol, 25 mL diethyl ether and 25 mL light petroleum ether. The mixture was shaken vigorously for 1 min and the extracted supernatant was evaporated to determine the fat content. Fat in LHSMP was analysed by CEM Smart Trac (CEM Corporation, Matthews, NC, USA).

2.2.3 Lactose and urea

Milk samples or RSM were analysed for lactose and urea in triplicate using FOSS Fourier Transformed Infrared (FTIR) analyser MilkoScan^{TM} FT+ analyser (N. Foss Electric A/S, Hillerød, Denmark).

2.2.4 Total protein

Protein content milk (~ 2 g), milk serum (~ 2 g), cheese (~ 0.2 g) and LHSMP (~ 0.1 g) was determined by measurement of nitrogen (**N**) by the Kjeldahl method (ISO, 2014). It involves sample digestion with 20 mL sulfuric acid, 12 g potassium sulfate to elevate boiling point of sulfuric acid and 1 mL copper sulfate (II) as a catalyst, at ~ 430 °C (to convert N to non-volatile ammonium sulphate), neutralisation (liberation of ammonia by addition of 65 mL sodium hydroxide (40 %, w/w) to ammonium sulphate), steam distillation of liberated ammonia into boric acid, and titration of resultant ammonium borate with hydrochloric acid. Typical digestion times can vary from 1-3 h. The N content of milk and milk products was converted to protein by multiplying by 6.38 which is the N-to-protein conversion factor. The conversion factor is the ratio of average molecular weight of amino acids (Mw = 89.32) present in milk protein to the atomic weight of nitrogen.

$$Organic\ N \xrightarrow[H_2SO_4+K_2SO_4+CUSO_4]{Digestion} (NH_4)_2SO_4 \xrightarrow[NaOH]{Neutralization} NH_3 \xrightarrow[H_3BO_3]{Distillation} (NH_4)_3BO_3 \xrightarrow[HCl]{Titration} NH_4^+ + Cl^- + H_3BO_3 \quad \text{(Eq. 2.1)}$$

The Kjeldahl block digesters, distillation and titration unit was run by Technical services lab, Teagasc Food Research Centre Moorepark.

2.2.5 Non protein nitrogen (NPN)

NPN (milk, serum, reconstituted skim milks from LHSMP) was determined by precipitation of protein in ~ 10 g milk sample by addition of ~ 40 g of 15 % (w/w) trichloroacetic acid (TCA, Fisher Scientific, UK) solution such that the final concentration of TCA in the mixture was ~ 12 % (ISO, 2001). TCA precipitates proteins by partial denaturation of protein via resulting in aggregation (Rajalingam et al. 2009).

The precipitated milk protein was removed by filtration using WhatmanTM filter paper No. 1 (GE Healthcare, Life Sciences, UK) and the remaining filtrate contains the NPN components. The nitrogen content of the 20 mL filtrate was determined by the Kjeldahl procedure described in Section 2.2.4.

2.2.6 Casein

Total casein (milk, serum, reconstituted milk from LHSMP) was determined by an indirect method of measuring non-casein nitrogen (NCN) in milk (ISO, 2004b). Milk sample (~ 10 g) was diluted to 50 mL using distilled water at 40 °C and casein in the diluted milk sample was precipitated using 1 M acetic acid (1 mL) reaching its isoelectric pH (pH ~ 4.6), and kept in water bath for 10 min maintained at 40 °C. After precipitation, 1 mL of sodium acetate was added to buffer the mixture to pH 4.5 and diluted further to 100 mL with distilled water. A filtrate containing NCN was obtained by filtering this solution through Whatman^{TM} filter No. 42 (GE Healthcare, Life Sciences, UK) and the N content in the 30 mL filtrate was determined by the Kjeldahl analysis.

Casein N, casein (protein %, w/w) and casein number (casein as a % of total protein) were calculated by the respective formulae:

$$Casein\ N = Total\ N - NCN \quad \text{(Eq. 2.2)}$$

$$Casein = Casein\ N \times 6.38 \quad \text{(Eq. 2.3)}$$

$$Casein\ Number\ (CN) = Casein\ N\ (\%\ total\ N) = 100 - NCN\ (\%\ total\ N) \quad \text{(Eq 2.4)}$$

$$True\ protein\ N\ (\%\ total\ N) = 100 - NPN\ (\%\ total\ N) \quad \text{(Eq. 2.5)}$$

2.2.7 Whey protein

Whey protein in milk, serum or RSM was calculated as follows:

$$Whey\ protein = (Total\ N \times 6.38) - Casein - (NPN \times 6.38) \quad \text{(Eq. 2.6)}$$

$$Whey\ protein\ (\%\ total\ N) = 100 - CN - NPN\ (\%\ total\ N) \quad \text{(Eq. 2.7)}$$

2.2.8 Reverse-phase high pressure liquid chromatography

2.2.8.1 Principle

Chromatography is an analytical technique used to separate, identify and purify components in a mixture. Chromatography techniques are classified based on the nature of the stationary phase and the separation process. Reverse-phase high pressure liquid chromatography (RP-HPLC) is a chromatographic technique that is used in the separation of components in a mixture based on their polarity and hydrophobic interactions of the components with the stationary phase. The mobile phase is polar while the stationary phase is non-polar and usually made of silica gel with long hydrocarbon chains. The polar mobile phase is then pumped under high pressure to separate components through stationary phase. The polar components will move quickly with the polar mobile phase and are eluted first, while less polar components interact with the stationary phase and elute slower.

2.2.8.2 Instrumentation and sample run

The concentrations of individual caseins (κ-casein, α_{s1}-casein, α_{s2}-casein, β-casein) and whey proteins (α-lactalbumin, β-lactoglobulin) were analysed in duplicate using RP-HPLC, according to the method of Visser et al. (1991). The system consisted of an Agilent 1200 separation module (Agilent Technologies, Santa Clara, CA) fitted with a diode array detector (DAD) and Agilent Chemstation Software using a 300 SB-CIS RP poroshell column (Agilent Technologies, Santa Clara, USA). The detector wavelength was 214 nm and the column temperature was 35 °C. The hydrophilic mobile phase (A) consisted of Milli-Q water (18.2 MΩcm), acetonitrile (9:1) containing 0.1 % trifluoroacetic acid (TFA) and the hydrophobic mobile phase (B) of acetonitrile, Milli-Q

(9:1) containing 0.1 % TFA. Mobile phase A and B were filtered through hydrophobic and hydrophilic filters, respectively (0.45μm, Merck Millipore, MA, USA). A stepped gradient of 26-100 % of mobile phase B was used for elution of proteins. The flow rate was 0.5 mL/min and column pressure was 150-200 bar as shown in Table 2.5.

Table 2.5 Stepped gradient of mobile phase B (%) for elution of milk proteins.

Time (min)	% Mobile phase B
0.00	26.0
10.00	37.0
23.00	45.0
26.00	100.0
31.00	100.0
34.00	26.0
36.50	26.0

7 M dissociating buffer was prepared by dissolving 42 g urea and 0.56 g bis-tris propane in 80 ml distilled water. The pH of the solution was adjusted using 0.5 M HCl/NaOH to pH 7.5 and the solution was brought up to 100 mL by addition of distilled water. Prior to sample preparation, 200 μl of 2-mecaptethanol to 40 mL of urea buffer was added and stirred. This was mixed in a 1:20 and 1:10 ratio with skim milk and milk serum, respectively. After 1 h, the sample was filtered through 0.22 μm PES syringe driven filters (Chromacol, Thermo Scientific, Rockwood, USA) into 2 mL glass snap vials (Agilent Technology, Santa Clara, USA) and loaded onto the autosampler. 10 μl volume of sample was injected onto the column.

Protein standards used for calibration of the RP-HPLC assay were κ-casein (0.5-2.5 μg), α_{s1}-casein (0.5-2.5 μg), α_{s2}-casein (0.072 – 0.288 μg), β-casein (0.5-2.5 μg), α-La (0.1-0.5 μg), β-Lg a (0.250-1.250 μg) and β-Lg b (0.250-1.25 μg) (Table 2.6). All proteins were analysed by HPLC individually at five different concentrations to obtain retention time and calibration curve for individual proteins (Fig. 2.1).

The minor whey proteins, including bovine serum albumin (BSA), lactoferrin and immunoglobulins were not detected by the RP-HPLC protocol used. The concentration of individual proteins was calculated using Agilent Chemstation software with the aid of calibration curves for individual milk proteins, by identifying the peaks and drawing the baseline.

Table 2.6: Proteins used for the calibration and their retention time.

Peak No.	Protein	Retention time (min)	Molecular weights (kDa)
1	κ-casein	6.0	19
2	αs_2-casein	7.8	25
3	αs_1-casein	10.6	23.7
4	β-casein	12.2	24
5	α-lactalbumin	13.3	14
6	β-lactoglobulin a	15.6	18.4
7	β-lactoglobulin b	16.8	18.4

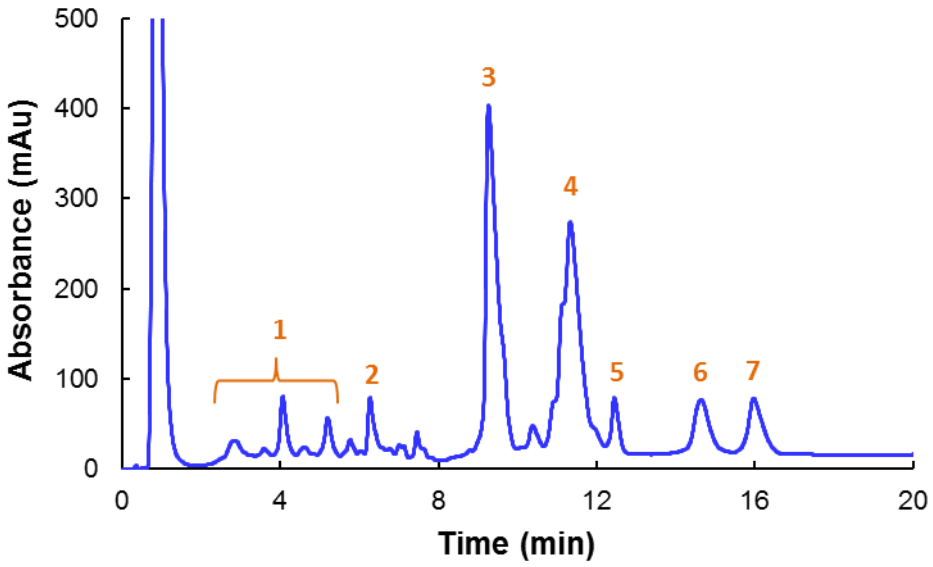

Figure 2.1: Representative RP-HPLC profile of casein and whey proteins in a skim milk sample.

2.2.9 Inductively coupled plasma mass spectrometry (ICPMS)

2.2.9.1 Principle

ICP-MS is an analytical technique used to identify and determine element concentrations. It combines high temperature inductively coupled plasma (ICP) which is

partially ionised gas (argon, helium or air) and mass spectrometry. The technique works on the principle of identification and separation of plasma induced ionised atoms based on their mass and charge ratio (m/z).

2.2.9.2 ICPMS Instrumentation

The inductively coupled plasma mass spectrometry instrument consists of an automated sample introduction, inductively coupled plasma, interface, mass spectrometry and data processing unit. The concentrations of macroelements (Na, Mg, P and Ca) and trace-elements (Mn, Fe, Co, Cu, Zn, Se Mo and I) in samples were analysed using ICP-MS (Agilent ICPMS 7700x, with ASX-500 series autosampler and MassHunter software A.01.02 Patch 4).

Table 2.7: Details of instrumental conditions for determination of elements by ICPMS

ICP-MS Instrument	Agilent ICP-MS 7700x
RF generator	27 MHz oscillators RF unit max power (1.6 kW)
ICP torch	Quartz torch 2.5 mm id
Spray chamber	Quartz double pass spray chamber
Nebuliser type	Quartz concentric micromist nebuliser
Peristaltic pump	ISMATEC MS-4 Reglo/8-100
Sampling cone	Nickel sampling cone 1.00 mm
Skimmer cone	Nickel sampling cone 0.4 mm
Incident power	Fwd power 1500 W
Reflected power	1500 W
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	0 – 1 L/ min
Nebulizer gas flow rate	0.1 to 1.1 L/ min
Solution uptake rate	0.4 mL/ min
Scanning parameters	Amn 0 to 250

2.2.9.3 Sample preparation

Skim milk (1 g), milk serum (2 g), cheese (0.1 g), LHSMP (0.2 g) and Standard reference material (SRM®) 1849a (0.5 g; LGC Standards, London, UK) were each digested with a mixture containing 5mL of concentrated HNO_3 (> 69.0 % TraceSELECT®, for trace analysis), 0.5 mL of concentrated HCl (> 37 %, TraceSELECT®, for trace analysis, Fluka Analytical, Sigma-Aldrich Ireland Ltd., Arklow, Co. Wicklow, Ireland) and 2 mL of H_2O_2 (30 %, AnalaR NORMAPUR for

trace analysis; VWR International BDH chemicals VWR, Dublin, Ireland). Samples were digested in a teflon tube at 1600 W using a microwave digestor (MarsXpress, CEM, North Carolina, USA). Samples were held at 160 °C for 2 min and then at 180 °C for 15 min and, cooled for 30 min thereafter. The acid-digested samples were diluted to 50 g with Milli-Q water and analysed for Cu, Co, Fe, Se, Mn and Mo using ICP-MS. A portion of the diluted digest was further diluted 1:4 with 3.6 % (v/v) nitric acid prior to analysis of Ca, P, Mg, Na and Zn. A Multi-Element Standard solution (Inorganic Ventures, Virginia, USA) with different concentrations of each element, diluted in 3.6 % (v/v) HNO_3 to give a range of concentrations (0, 0.1, 0.5, 1, 2, 5 or 10 ppb) was used for instrument calibration. The wavelength of plasma emission used was 588.9, 279.1, 213.6, 315.6, 257.6, 259.9, 228.6, 324.8, 214.4, 204 and 379.8 nm for Na, Mg, P, Ca, Mn, Fe, Co, Cu, Zn, Se and Mo, respectively.

The measurement of iodine, (I), involved alkaline extraction of a 0.5 g sample using tetramethyl-ammonium hydroxide (**TMAH**; Inorganic Ventures, Christiansburg, VA) and analysis of the extract with ICPMS, as described by BSI (2007). Samples and Standard Reference Material® 1849a were diluted with 5 mL of 5 % (v/v) TMAH, digested by holding at 90˚C for 3 h, cooled to room temperature. Tellurium (1000 μg/mL; Reagecon, Shannon, Ireland) was added to the cooled sample digest at a level 0.5 g and the sample digest was diluted to 50 g with 1 % (v/v) TMAH solution to give a final dilution factor of 100. Iodine standards for calibration were prepared from stock iodide solution (500 μg/L; Inorganic Ventures). Serial dilutions of iodide solution with 1 % (v/v) TMAH solution and Tellurium at a concentration of (10 μg/g) were prepared to give iodine concentrations ranging from 0 to 50 μg/L.

The proportion of each element that sedimented on ultracentrifugation was then calculated as the difference between the concentration of the element in skim milk and that of the serum, expressed as a proportion (%) of that in skim milk.

2.2.10 Ionic calcium

Ionic calcium (Ca^{2+}) in skim milk was measured in triplicate at room temperature using a calcium ion selective electrode (sensION+ 9660C, Hach, Colorado, USA), calibrated each time using calcium chloride solutions (0-5 mM). Potassium chloride (3 M) was added to the standard solution during calibration and in milk at a level of 1 % (v/v) to bring the standards and samples at similar ionic strength. The samples were stirred and the measured immediately for Ca^{2+} concentration while stirring. There was a logarithmic relationship between the electrical output (mV) from the electrode and the concentration of Ca^{2+}.

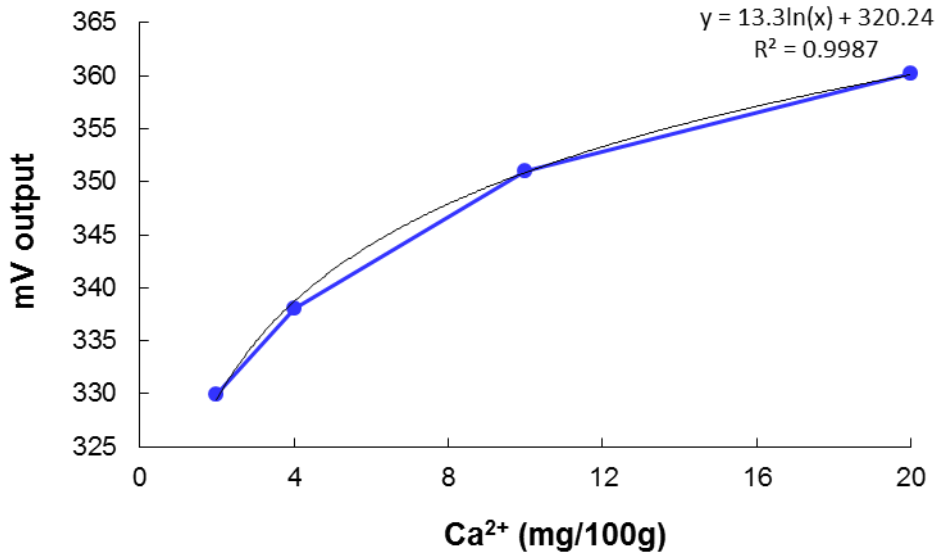

Figure 2.2: Calibration curve of Ca^{2+} using ion selective electrode.

2.2.11 Casein micelle size

Casein micelle size was measured by dynamic light scattering (DLS). DLS is also referred to as quasi-elastic light scattering or photon correlation spectroscopy. It measures the diffusion of particles, i.e., the movement of particles due to their random collision with liquid molecules surrounding them, and relates it to their hydrodynamic diameter. The hydrodynamic diameter and translational diffusion coefficient is defined by the Stokes-Einstein equation:

$$d_h = kT/3\pi\eta D \quad \text{(Eq. 2.8)}$$

where, d_h is the hydrodynamic diameter, D is the translational diffusion coefficient, k is the Boltzmann constant, T is the absolute temperature and η is the viscosity of the medium.

Skim milks were diluted in simulated milk ultrafiltrate (SMUF; Jenness & Koops, 1962) in a ratio of 1:100 (v/v) to give a protein concentration of ~ 0.035-0.040 % (w/w). 3 mL of each diluted milk sample was measured in a clear disposable cuvette using the Malvern Zetasizer Nanoseries Nano-ZS (Malvern Instruments Ltd, Worcestershire, UK) equipped Zetasizer software 7.01. Casein micelle size was measured at 25 °C in triplicate each consisting of 13 sub-measurements with 20 s acquisition time. The particles were illuminated with a Helium-Neon laser (633 nm) and the scattered light was collected at a back-scatter angle of 173°. The Z-average hydrodynamic diameter of casein micelles was measured using an intensity distribution. The refractive indices used for size calculation were 1.38 and 1.33 for casein and dispersant, respectively.

2.2.12 Determination of casein hydration

After ultracentrifugation of the skim milk at 100,000 *g* for 1 h at 25 °C and decantation of the serum, the pellet was weighed, frozen at -80 °C and lyophilised at -46 °C and at a vacuum pressure of <13 Pa in a freeze dry unit (FreeZone Freeze Dry Systems, Labconco, Kansas City, MO, USA). The moisture content of the pellet was calculated from the difference in weight before and after lyophilisation. The analysis was undertaken in triplicate and casein hydration was expressed as grams of water/grams of sedimented casein. The casein content of the sedimented pellet (*g*) was calculated as the difference in casein content (*g*) between the skim milk and the resultant serum.

2.2.13 Rennet gelation

The pH of skim milk was adjusted to 6.55 at room temperature using 0.1M HCl, and then heated to 31 °C, and inoculated with chymosin (Chy-Max® plus, 200 IMCI/mL; Chr. Hansen, Hørsholm, Denmark) at a rate of 10.6 IMCU per g protein. The chymosin was diluted 1:20 in distilled water just prior to inoculation and mixed into the milk for 60 s by stirring on a magnetic stirrer at a speed of 300 rpm. 10 mL of the rennet/chymosin-treated sample was placed in a rheometer cell of a controlled stress rheometer (CSL^2_{500} Carri-Med; TA Instruments, Inc., New Castle, DE) with a concentric cylinder geometry with 15 mm inner radius, a bob radius of 13.83 mm and immersed height of 32 mm. There was a 12 mm gap between the bob and the bottom surface of cylinder. The storage modulus, G', (index of gel firmness or stiffness) was measured dynamically as a function of time over 1 h at a strain of 0.025 and frequency of 1 Hz.

The following parameters were calculated from the resultant G'/time curve: rennet gelation time (RGT), defined as the time required for G' to increase to a value of ≥0.2 Pa; GFR_{max}, maximum gel firming rate, calculated as the maximum slope of the G'/time curve; and G'_{40}, G' at 40 min from rennet addition.

2.2.14 Heat stability

20 mL aliquots of each skim milk at room temperature were adjusted to pH values in the range 6.2 – 7.2, at increments of 0.1 pH unit using 0.1 M HCl/NaOH. 3.4 g of each pH-adjusted samples and the milk sample equilibrated at room temperature (sample at natural pH) were placed in a 4 mL heat-resistant tubes (120 mm tube length, 10 mm outer radius, 7 mm inner radius; Hettich Benelux BV, Geldermalsen, Netherlands), and were capped with a rubber stopper, placed and secured in a metal rack. The loaded rack was placed in the temperature-controlled oil bath (Hettich ESP oilbaths; Hettich

Benelux BV) at 140 °C and rocked gently at a constant frequency (7 oscillations/ min). The heat coagulation time (**HCT**) of samples was measured as the time for visual flocculation of milk at 140 °C (Lin et al., 2016).

The following heat coagulation parameters were obtained from the resultant pH/HCT curves, all of which had a typical type A HCT/pH profile (Huppertz, 2016): HCT_{max}, HCT at the first inflection point; HCT_{min}, HCT at second inflection point and HCT_{npH}, HCT at natural pH.

2.2.15 Ethanol stability

Ethanol stability of pH-adjusted milk samples was measured in the pH range of 6.2-7.2 at 0.2 interval units. The pH of 20 mL aliquots for each milk was adjusted using 0.1 M HCl/NaOH. Ethanol stability is defined as the lowest concentration of ethanol required for visual flocculation on blending the milk with ethanol solutions of varying strengths (Horne and Muir, 1990). ES was assayed by blending 1 mL of sample with absolute ethanol (99.9%, v/v, Scharlau, Barcelona, Spain) at different concentrations prepared by dilution of ethanol with distilled water, ranging from 100 to 30 % (v/v), at an increment of 2 % (v/v), in the ratio of 1:2 while keeping protein-to-diluted ethanol ratio constant at 0.017. The mixture was mixed by vibration (Whirlimixer™, Fisons, Holmes Chapel, UK) for 30 s and checked for visible flocculation.

2.3 Mozzarella cheese manufacture, compositional and functional characteristics

2.3.1 Standardisation and pasteurisation of milks

Milk from each feeding system (GRO, GRC or TMR) was standardised to a protein-to-fat ratio of 1.15 in ML, or 1.20 in LL and held overnight in separate tanks at 4°C. The next day, on the morning of cheese-making, milk (~600 kg) was pasteurised at 72 °C for 15 s using a heat exchanger, cooled to 36 °C, and 460 kg of pasteurised milk was

pumped to the cheese vats. Cheese vats were cylindrical and jacketed stainless steel vats with automated variable speed cutting and stirring (500-L; APV Schweiz AG, CH-3076 Worb 1, Switzerland).

2.3.2 Mozzarella cheese manufacture

Milk (~ 460 kg) was maintained at 36 °C and was inoculated with direct vat cultures TH4, consisting of *Streptococcus thermophilus*, and LHB02, consisting of *Lactobacillus helveticus*, at levels of 10 and 5 g per 100 kg milk with 3.4 % (w/w) protein, respectively, as recommended by the supplier (Chr. Hansen, Little Island, Cork, Ireland). After a 40-min inoculation period when the milk pH was 6.50-6.55, chymosin (single strength Chy-Max® plus, 200 IMCU; Chr. Hansen, Hørsholm, Denmark), diluted 1 in 10 in distilled water, was added at 36 IMCU/kg milk with 3.4 % (w/w) protein. Culture inoculum and rennet dosage were increased pro-rata with milk protein concentration to ensure similar acidification rates and rennet-to-casein ratio, respectively, in ML and LL, despite the difference in milk protein concentration. Added chymosin was thoroughly mixed for 90 s with the milk to ensure uniform distribution, and immediately, a sample (40 ml) of the rennet-treated cheese milk was taken from the cheese vat, and monitored for changes in storage modulus, G', at 36 °C. Rheology was performed at a strain of 0.025 at a frequency of 1 Hz at 36 °C in a controlled stress rheometer (CSL^2_{500} Carri-Med, TA Instruments, Inc., New Castle, DE, USA). The gel strength (G') was measured as a function of time as described in Section 2.2.1.4, and cutting of the gel in the cheese vat was initiated when G' reached 30 Pa as monitored by the controlled stress rheometer. The defined firmness of the coagulum was sufficient to withstand the cutting programme.

Cheese manufacture was as previously described by Guinee et al. (2002). In brief, the rennet-treated milk was cut at a gel strength (G') of 30 Pa, and the curd-whey

mixture was cooked to 42 °C at a rate of 0.2 °C/min. The curd-whey mixture was pumped to a draining vat when the pH of the curd reached a pH value of 6.1, and the resultant curd was cheddared, milled and salted at rate of 4.6 % (w/w) when the pH reached 5.2. The salted curds were held for 20 min and mixed at 5-min intervals (mellowed) to ensure uniform salt distribution. The curd was kneaded in hot water (78-80 °C) and heated to 58-59.5 °C (Automatic Stretching Machine, Model d; CMT, S. Lorenzo di Peveragno CN, Italy), and the plasticised curd was moulded into 2.3 kg rectangular blocks which were cooled in dilute brine (10 %, w/w NaCl, 0.2 %, w/w Ca, pH 5.1, 4-8 °C) for 30 min, allowed to drip-dry for 10 min, vacuum-packed, and stored at 4 °C.

2.3.3 Sampling and mass balance

All inputs (cheese milk, diluted rennet, salt) and outputs (whey, stretch water, curd, cheese) were collected and weighed as described previously (Fenelon & Guinee, 1999). Cheese milk refers to milk from each cheese vat following pasteurisation and cooling; bulk whey to the composite of whey collected during whey drainage and curd cheddaring; salty whey to the whey expressed during salting and mellowing; and stretch water to a mixture of the hot water added during plasticisation and the curd serum released during curd plasticisation.

Raw milk and standardised cheese milk samples were analysed for fat, total N and casein using standard IDF methods as described in Section 2.2.2, 2.2.4 and 2.2.6, respectively. Element analysis on standardised cheese milk was performed using ICPMS (Section 2.2.9). Whey streams (bulk whey, salty whey) and stretch water were analysed for protein by the Kjeldahl method as described in Section 2.2.4, and fat using the Röse-Gottlieb method (ISO, 2010). All samples were heated to 40 °C prior to fat analysis to ensure uniform distribution of fat.

2.3.4 Cheese yield and component losses

Cheese yield was expressed as actual yield, $\mathbf{Y_a}$, defined as kg cheese per 100 kg of cheese milk. Normalised yield, $\mathbf{Y_n}$, defined as kg cheese per 100 kg 'reference' milk with fat and protein of 2.89 % (w/w) and 3.40 % (w/w), respectively. Normalised yield which is milk protein plus fat adjusted yield eliminates the effects of differences in milk composition to yield. Thereby allowing the cheese yield from milk of different compositions to be compared.

The percentages of total milk fat or protein lost in the different whey/stretch water streams were calculated from the percentages of fat and protein in, and weight of, the milk and individual streams, as described previously (Guinee et al. 2006).

2.3.5 Analysis of unheated cheese

2.3.5.1 Composition

Grated cheese samples were analysed in duplicate at 1 day for protein by the Kjeldahl method as described in Section 2.2.4, moisture by oven drying at 102 °C for 5 h (ISO, 2004a), fat by Röse-Gottlieb method (ISO, 2010) and elements using ICPMS, as described in Section 2.2.9, where the sample weight used was ~ 0.2 g). Salt was analysed using a potentiometric method (ISO, 2006) where 3 g cheese was blended with with 30 mL of distilled water (40 °C), 3 mL of nitric acid was added to the slurry and titrated against silver nitrate until the voltage reaches 240 V (pH M210, Meterlab, radiometer Analytical, Hach, CO, USA). A grated cheese slurry was prepared by mixing 20 g of cheese and 12 g heated (40 °C) distilled water. The pH of this slurry was measured at all sampling points using a pH meter (BSI, 1976

2.3.5.2 Proteolysis

A mixture of cheese and water (45 °C), at a weight ratio of 1:2, was homogenised for 5 min (Stomacher, Lab-Blender 400; Seward Medical, London), held at 40 °C for 1 h, and centrifuged at 3000 g for 30 min at 4 °C (Sorvall LYNX 6000 superspeed centrifuge, Thermo Scientific, Dublin, Ireland). The supernatant was filtered through superfine glass wool 11 μm (VWR International, Dublin, Ireland), adjusted to pH 4.6 using 0.1 N HCl and re-centrifuged and filtered. The resultant supernatant was assayed for nitrogen concentration using the Kjeldahl method as described in Section 2.2.4, which was expressed as pH 4.6 soluble N (**pH 4.6-SN**) as a percentage of total N in cheese. The cheese was assessed for proteolysis at days 1, 10, 20, 30 and 50 of storage at 4 °C.

2.3.5.3 Water holding capacity of cheese

The water holding capacity (WHC) of cheese or non-expressible serum is used as an indicator of the water holding capacity of cheese matrix. Grated cheese (120 g) was centrifuged at 12,500 *g* for 75 min at 25 °C (Sorvall LYNX 6000 superspeed centrifuge, Thermo Scientific, Dublin, Ireland) to obtain expressible cheese serum. The centrifugal force, duration and temperature used in this method were optimised by Guo & Kindstedt (1995). Serum and fat were collected in a Startsted bottle (50 mL) and stored at 4 °C until the fat layer was solidified. The solidified fat layer was punctured and the serum was collected and weighed. The WHC was calculated by subtracting the weight of expressible serum per 100 g cheese from the weight of moisture per 100 g, and expressed as g serum/g protein.

2.3.5.4 Texture profile analysis

Texture profile analysis was done to test large strain deformation of cheese which is a measure of non-linear rheological characteristics. This involves the application of strain

which permanently deforms the microstructure. Six cheese cubes (25 mm each side) were obtained from a block of cheese using cheese blocker (Bos Kaasgereedschap, Boven graven, Postbus, the Netherlands). The cube shaped samples were tightly wrapped in a tin foil and equilibrated at 4 °C overnight. Cheese cubes were taken from the fridge and immediately compressed to 70 % of original height in 2 successive strokes (bites) using a TAHDi texture analyser (Stable Micro Systems, Goldalming, UK) equipped with a 5-mm compression plate and a 100-kg load cell at a rate of 1 mm/s (Guinee et al., 2015). A typical TPA (force-time/distance) profile is represented in Figure 2.3 and the parameters obtained from the resultant curve are described in Table 2.4.

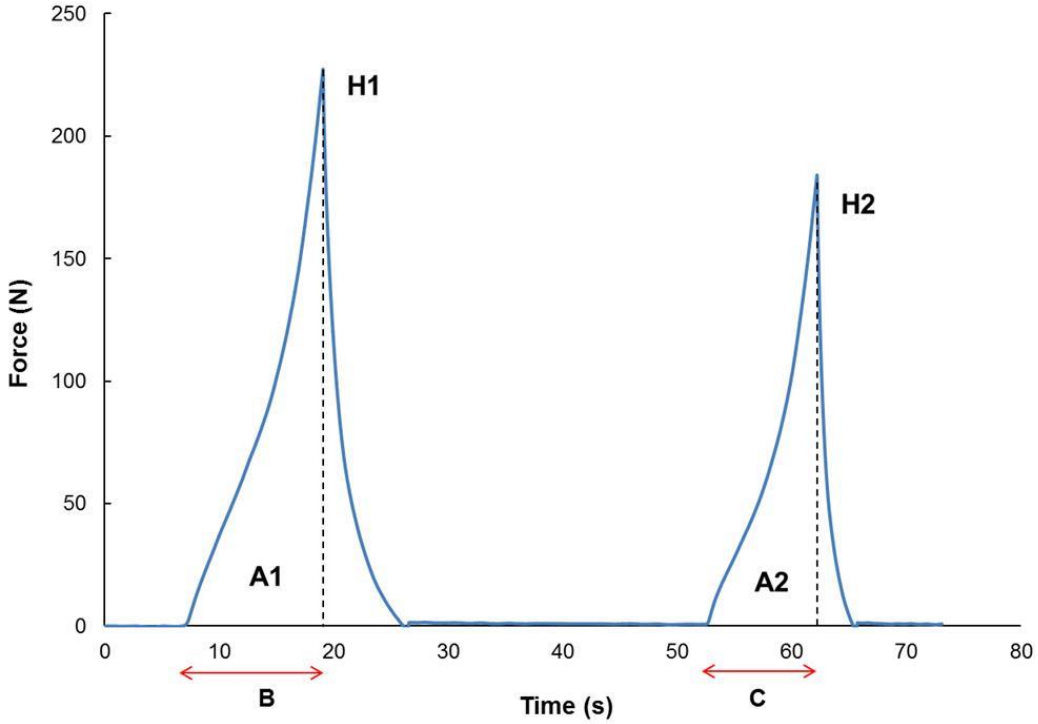

Figure 2.3: A typical force-time (force-distance) profile by TPA on compressing a cube-shaped sample of Mozzarella to 70% of its original height at a rate of 60 mm/min at 4 °C is shown. A1 refers to area under the force-distance curve in bite 1, A2 to the area under the curve in bite 2, B to the compression distance in bite 1, and C to the compression distance in bite 2.

Table 2.8*:* Texture profile analysis (TPA) parameters and definitions.

Parameters	Description	Units
Firmness	Force at full compression in bite 1 (H1)	N
Cohesiveness	Ratio of the compression area (force X distance = work) during bite 2 to that during bite 1 (A2/A1), an index of the work required to chew the sample during 2 compressive bites	-
Springiness	Ratio of sample compression distance in bite 2 to that in bite 1 (B), an index of the recovery of sample height between bite 1 and bite 2	-
Chewiness	Product of firmness by cohesiveness by springiness (H1 X A2/A1 X C/B), an index of the work required to masticate the sample to a state ready for swallowing.	N
In case of fracture		
fracture stress (σ_f)	The force at fracture as determined from the inflection point of the force/time curve.	Pa
fracture strain (ε_f)	The displacement at fracture as a % of original sample height.	-

2.3.5.5 Cavitation rheology

Cavitation rheology (CR) involves quantifying the pressure dynamics of a growing bubble, or, cavity, within the material (Blumlein et al., 2017). Experiments were performed on Mozzarella cheese cubes using a custom-built set-up at 15 °C. Cheese cubes were obtained as described in the Section 2.3.5.4. The instrument consisted of custom setup at Maynooth University, consisting of a piston syringe pump (New Era syringe pump NE1000), stainless steel syringe needles of different internal diameters ranging from (Hamilton, NV, USA) and a pressure sensor (High Accuracy Silicon Ceramic pressure sensor HSCDANT001PG3A3, Honeywell) connected to a computer with custom written programme which recorded pressure at the needle tip during the experiments. A set of four different needles of different gauge and internal radius (i.r.) were used, namely needles 10g (i.r., 1.35 mm), 12g (i.r., 1.08 mm), 14g (i.r., 0.78 mm) and 16g (i.r., 0.58 mm) were used.

On each sampling occasion an entire block of cheese (~ 2.2 kg) was sampled; the outer surfaces (~ 0.5-1.0 cm) were removed and the remainder was cut into cubes

(15.6 cm^3), which were foil-wrapped and refrigerated overnight at 4 °C. On testing, individual cubes were withdrawn and CR measurements were performed immediately. The syringe needle was connected, via rubber tubing, to the piston pump, mounted on a stand, clamped gently, and manually pushed unidirectionally though the top face of the cheese cube to a depth of ~ 10 mm. Using the piston pump, air was compressed through the tubing and the needle at a flow rate of 1.5 mL/min and the pressure was recorded dynamically. The compression was continued until the air pressure, as detected by the sensor and captured on the software, had reached a critical value and then decreased to ~ 25-50 % of the critical value. The critical pressure (P_c) is the pressure at which the compressed air forms into a bubble within the cheese matrix; the formation of the bubble increases the volume of the system and results in a rapid decrease in pressure. The critical pressure (P_c) can be related to the modulus of the material by:

$$P_c = \frac{5}{6}E + \frac{2\gamma}{r} \qquad \text{(Eq. 2.9)}$$

Where, γ is the surface tension between the sample and air, E is the modulus; and r, is the inner radius of the needle used.

2.3.5.6 Cheese elasticity

Cheeses were sliced into thin discs (40 mm in diameter, 2 mm thick) and dynamic strain oscillatory experiments were performed (Anton Paar Rheometer MCR50, Anton Paar GmbH, Graz, Austria). The cheese discs were placed between two parallel, serrated plates (40 mm in diameter) of the rheometer cell, tempered at 13 °C for 5 min, and subjected to a shear strain sweep (0.001% to 10%) at an angular frequency of 6.283 rad/s (1 Hz). Storage modulus (G′), which represents the ratio of shear stress (τ)-to-shear strain (γ̇), was measured dynamically. The region where G′ remains relatively constant with shear rate (γ̇) describes linear viscoelastic deformation (strain), where

τ increases proportionally with (γ̇), and full-recovery of the sample to original dimensions occurs on removal of strain. The critical strain refers to the strain at which G′ decreases on further increasing strain rate. It was calculated from the shear rate (γ̇/G′) curves and defined as the strain at which G′ decreased to a threshold value of ≥ 1 % on further increasing strain. The mean value of G′ in the region of linear deformation was calculated as mean of G′ values up to the threshold G′ value.

2.3.5.7 Colour

The colour space co-ordinates, namely the L*, a* and b* values were measured on cheese discs (47.5 mm diameter) using the CR-400 Chroma Meter (Konica Minolta, Osaka, Japan), which had been calibrated using the Minolta calibration plate. Four discs shaped samples were taken from each cheese, wrapped tightly in tin foil, equilibrated at 4°C overnight, withdrawn and immediately assayed in quadruplicate. The L* value varying from 0 (black) to 100 (white) is an index of lightness, whereas a*- and b*-values represent the variation and intensity in colour from green (– values) to red (+ values), and of blue (– values) to yellow (+ values), respectively.

2.3.6 Thermophysical properties of cheese

2.3.6.1 Flow

The flow or spread of a cheese disc (47.5 mm diameter and 3.5 mm depth), placed on circular glass dish, was measured in quadruplicate on heating in a convection oven at 280 °C for 4 min (Binder FD 35, Binder GmbH, Tuttlingen, Germany). The melted cheese disc was withdrawn, cooled to room temperature, and measured for diameter on four equally-spaced locations (spokes); flow was expressed as the percentage increase in disc diameter. The flow of cheese is an index of melting behaviour of cheese when exposed as a topping (e.g., on pizza) during oven heating (Guinee et al., 2015)

2.3.6.2 Stretchability

Stretchability of grated cheese was analysed on quadruplicate samples on uniaxial extension of the hot molten cheese (95 °C) to a distance of 380 mm at 10 mm/s using a TAHDi Texture Analyser (Guinee et al., 2015). A plastic container (9 cm X 5.5 cm X 4 cm) containing a comb was filled with grated cheese (60 g) and heated in a microwave (Whirlpool MW201, Fonthill Industrial Estate, Dublin, Ireland) set at 750 W for 60 s. The plastic container with molten cheese was placed in the cell of texture analyser with the comb stuck to the crosshead, which was programmed to pull the comb upwards to a maximum distance of 380 mm at a rate of 10 mm/s. The extension work (**E_w**), was calculated as the area of resultant force (F) - time curve at full extension, where E_w = F X extension distance.

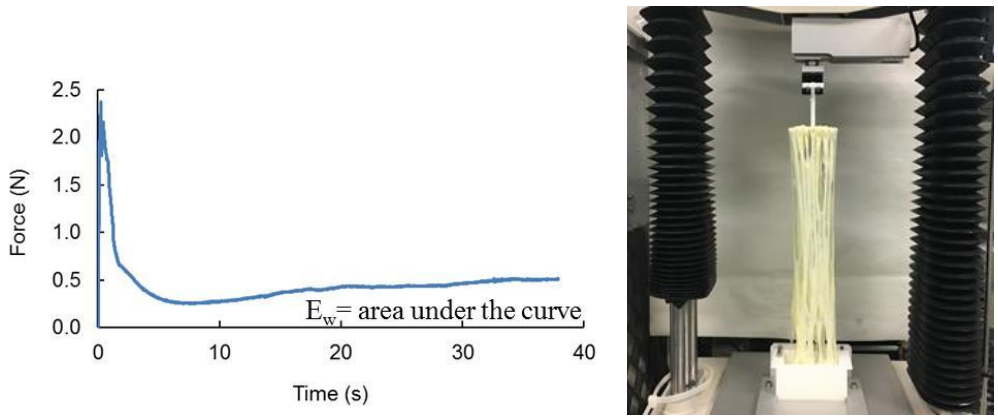

Figure 2.4. Typical force-time curve on extending molten Mozzarella on the texture analyser. Extension work (E_w) is derived by the integral of force by distance curve. Hot molten Mozzarella cheese is also shown being extended up to a distance of 380 mm at a rate of 10 mm/s.

2.3.6.3 Viscoelastic changes during heating and cooling

Dynamic changes in storage modulus (G'), loss modulus (G") and loss tangent (G"/G') on heating of cheese discs (40 mm in diameter, 2 mm thick) at a rate of 3.25 °C/min from 25 to 90 °C and immediately re-cooling at a rate of 3.25 °C/min to 25 °C were

measured using low-amplitude strain oscillation rheometry (Anton Paar Rheometer MCR50, Anton Paar GmbH, Graz, Austria), as described previously (Guinee et al., 2015). The cheese discs were placed between two parallel, serrated plates (40 mm in diameter) of the rheometer cell, tempered at 25 °C for 15 min, and subjected to a low amplitude shear strain (γ) of 0.0063 at an angular frequency of 1 Hz during heating and re-cooling The following parameters can be calculated from the resultant G'-, G"-temperature curves:

Table 2.9: Viscoelastic parameters during heating and cooling and their definitions.

Parameters	Description
During heating	
COTh	Cross-over or melting temperature during heating, the temperature at which G" attains to a value equal to that of G' and the cheese changes from a viscoelastic solid to a viscoelastic fluid, an index of melting point of cheese
LT_{max}	Maximum value of loss tangent, an index of the maximum fluidity attained by the cheese during heating.
TLT_{max}	Maximum value of loss tangent, an index of the maximum fluidity attained by the cheese during heating.
LT_{90}	Loss tangent at 90 °C during heating
$LT_{max} - LT_{90}$	Difference between LT_{max} and LT_{90}, an index of the loss of fluidity on heating to temperatures >TLT_{max}.
G'h90	G' at 90 °C, an index of elasticity of cheese at 90 °C.
During cooling:	
COTc	Cross-over or congealing temperature during cooling, the temperature at which G" and G' become equal and the cheese transitions from a viscoelastic fluid to a viscoelastic solid.
G'c25	G' after cooling to 25 °C, an index of the elasticity of the cooled cheese.

2.4 Manufacture of low-heat skim milk powder (LHSMP)

LHSMP were produced in the Bio-functional Food Engineering pilot plant unit of Moorepark Technology Limited (Teagasc, Moorepark, Fermoy, Co. Cork). Milk was separated at 55 °C (Westfalia model MM1254 separator, Westphalia, Germany),

pasteurised at 72 °C for 15 s using a pilot-scale tubular heat-exchanger (Microthermics UHT/HTSTLab-25 EHVH, Raleigh, NC, USA), cooled to 45 °C, and concentrated to ~ 45-46 % TS in a Falling Film Evaporator (Anhydro, Type F, SPX Flow Technology Denmark A/S, Soeborg, DK-2860, Denmark). The concentrate was spray dried (Anhydro spray dryer, SPX Flow Technology Denmark A/S, Soeborg, Denmark) using centrifugal disc atomisation using inlet and outlet air temperatures of 18 °C and 85 °C, respectively. Powders (~ 4 kg of each type) were packed in silver aluminium bags and stored at 15 °C until they were used for further analysis. LHSMP was produced at 3 separate occasions in both mid and late lactation in 2016. LHSMP produced from GRO, GRC or TMR milks are denoted GRO-, GRC- and TMR- powders, respectively.

2.4.1 Composition analysis of powders

LHSMP was analysed for TS and fat using CEM SMART Trac II (CEM, Matthews, NC, USA), protein by the Kjeldahl method (Section 2.2.4), whey protein nitrogen index (WPNI; ADPI, 2016), and colour using CR-400 Chroma Meter (Konica Minolta, Osaka, Japan) (Section 2.3.5.7). The analyses of lactose, casein and whey protein were measured on reconstituted skim milk prepared by dispersing the LHSMP to 10 % (w/w) in distilled water, as described below. Samples (~ 0.2 g) were assayed for macroelements (Ca, P, K, Na, Mg) and trace-elements (S, Zn, Fe, I, Mn, Cu, Mo, Se and Co) using inductively coupled plasma mass spectrometry (ICPMS), as described in Section 2.2.9.

2.4.2 Preparation of reconstituted skim milks and analysis

Reconstituted skim milk (**RSM**; 10 %, w/w) was prepared by dispersing LHSMP in distilled water at 50 °C and holding in a water bath (50 °C) on stirring at 400 rpm for 2 h; the milk was then dispersed in 1 L glass containers (DURAN, Mainz, Germany) and

stored at 4 °C for 18 h to allow hydration of the proteins. Prior to all analyses, the reconstituted skim milk was heated to 40 °C in a thermostatically controlled water bath (Grant, Cambridgeshire, UK) on stirring (Variomag-USA) and held for 30 min to reverse the cold-ageing, and cooled to 21 °C for analysis. Reconstituted skim milk prepared from GRO, GRC or TMR powders are denoted GRO-, GRC- and TMR- RSM, respectively.

RSM was analysed for TS, lactose and urea using the FOSS MilkoScanTM FT+ analyser (N. Foss Electric A/S, Hillerød, Denmark), for total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN; including whey proteins and non-protein N) to obtain concentrations of total protein, true protein, casein, whey proteins and NPN. RSM was also analysed for individual caseins using RP-HPLC, ionic calcium, casein micelle size, casein hydration, and processing characteristics including rennet-gelation, heat stability and ethanol stability as functions of pH in the range 6.2-7.2, and yoghurt preparation as described in Sections 2.2 and 2.5. Skim milk serum was obtained from RSM as described in Section and assayed for TN and NCN, to obtain serum (soluble) casein as described above.

2.5 Stirred-yoghurt manufacture

Yoghurt was prepared from reconstituted LHSMP which was dispersed to 12.7 % (w/w) TS in distilled water at 50 °C, continually stirring at 5000 rpm (Silverson model L4RT, Silverson, Chesham, UK) for 15 min, stored at 4 °C overnight, and heated to 50 °C. The constituted skim milk (10 L) was then blended with heated-treated (90 °C) anhydrous milk fat (AMF, Glanbia, Ireland), added at a level required to give 2.3 % (w/w), and stirred at 5000 rpm for 2 min (Silverson model L4RT, Silverson, Chesham, UK). The recombined milk was heat treated at 95 °C for 5 min, homogenised at first and second-stage pressures of 15 and 5 MPa, respectively, and cooled to 43 °C (Microtherimics

UHT/HTSTLab-25 EHVH, Raleigh, NC, USA). A portion (2 L) of the homogenised, heated treated milk was inoculated direct-vat starter culture from Chr. Hansen Ireland Ltd (Little Island, Co. Cork, Ireland), i.e., a blend of YC380 YoFlex (*Streptococcus thermophilus*) and CH1 YoFlex (*Lactobacillus delbrueckii* ssp. *bulgaricus*) at a weight ratio of 1:3. The weight of the starter culture inoculum was standardised to a level 0.01 % (w/w) for milk with 5 % protein and was varied accordingly where the protein content of the milk varied with treatment or stage of lactation. The inoculated milk was incubated at 42 °C (Heratherm Advance Protocol Microbiological Incubators, Thermo Scientific, Waltham, MA) until the pH reached 4.6 and gelled. After starter culture inoculation, a well-mixed subsample (10 mL) of the dispersion was immediately withdrawn and monitored for changes in storage modulus (G'), loss modulus (G″) and loss tangent (tan δ = G″/G′) at 42 °C, using low-amplitude strain oscillation rheometry as described for rennet gelation in Section 2.2.14, until pH dropped to 4.6. Moisture evaporation during measurement was prevented by placing a thin layer of tetradecane (Sigma-Aldrich, St. Louis, MO) on the surface and covering the sample with an evaporation blocker.

The gelation onset-pH (GO_{pH}) was defined as the pH at which tan δ decreases to 1. When the pH decreased to pH 4.6, the yoghurt gel was placed in ice water, cooled to ~ 8 °C and stirred at 70 rpm (model RW16; IKA-Werke GmbH & Co., Staufen im Breisgau, Germany), and stored at 4 °C for 36 h prior to analysis.

2.5.1 Rheological properties of stirred yoghurt

Yoghurt was stirred at 70 rpm for 1 min at room temperature (model RW16; IKA-Werke GmbH & Co.) to ensure sample homogeneity before rheological measurement. A 10 g sample was placed in the measuring cell of a controlled-stress rheometer (CSL^2_{500} Carri-Med; TA Instruments, Inc., New Castle, DE). The sample was

equilibrated at 8 °C for 5 min, and then subjected to a shear rate ($\dot{\gamma}$) sweep, where shear rate was increased from 10 to 120 s^{-1}. Shear stress (σ; Pa) and viscosity (Pa.s) were measured as a function of shear rate. The resultant shear rate versus shear stress data were fitted to the Herschel–Bulkley model using TA Rheology Advance Data Analysis software (version V5.7.0; TA Instruments):

$$\sigma = \sigma_o + K \dot{\gamma}^{n} \qquad \text{(Eq. 2.10)}$$

where, σ_o is the yield stress (Pa), *K* is the consistency coefficient (Pa.s), and *n* represents the flow behaviour index (Ramaswamy & Basak, 1991).

2.5.2 Water holding capacity of yoghurt (WHC)

After cooling the yoghurt samples to 8 °C, 6 aliquots of each yogurt were poured immediately into 50-mL centrifuge tubes, held at 4 °C for 36 h, and centrifuged at 300 *g* at 8 °C for 30 min. The expressed serum was decanted and weighed. The WHC of the yoghurt was calculated as the difference between total serum in yoghurt (moisture, fat, lactose, NPN expressed as protein, undenatured whey protein) and the serum expressed on centrifugation of 100 g of yoghurt.

Chapter 3

Effect of reducing daily herbage allowance during early lactation on composition and processing characteristics of milk from spring-calved herds

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3.1 Abstract

This study investigated the effects of reducing DHA from 15.0 to 11.8 kg dry matter per cow (> 3.5 cm post grazing sward height) to a spring-calved herd during early lactation on the composition, rennet coagulability and heat stability characteristics of milk during early lactation (EL, 29-70 days in milk, DIM), mid- lactation (ML, 78-183 DIM), and late lactation (LL, 205-267 DIM) DHA was varied. Samples of milk were taken at approximate 10 d intervals during EL and at 1-3 week intervals during ML and LL. Reducing DHA led to reductions in milk yield, milk solids yield, and concentrations of protein (~0 .22 %, w/w) and casein (0.13 %, w/w) during EL. Otherwise, it had little effect on milk composition or on the selected processing characteristics in ML, LL or overall lactation. Stage of lactation resulted in comparatively large changes in most compositional parameters, rennet gelation and heat stability.

3.2 Introduction

Grazing of dairy cows on pasture grass features prominently in temperate regions, such as Ireland and New Zealand, where grass growth occurs over most of the year. Grazing with compact calving of cows in early spring is often the most cost-effective approach for milk production, as the maximum milk production volume coincides with maximum grass growth (O'Brien and Hennessy, 2017). The diet of pasture-grazed dairy herds may be supplemented with a cereal grain or pelleted concentrates offered at the extremes of the pasture growing season, i.e., in early and late lactation.

Commercial milk primarily from spring-calved, pasture-grazed dairy herds has a seasonal supply pattern, with peak supply occurring in late spring-early summer when cows are in early lactation and grass growth is high (O'Brien & Hennessy, 2017). Moreover, the milk also displays variation in composition and yield over the year to an extent dependent on stage of lactation (Auldist et al., 2000a), quality/allowance of feed

(Mackle et al.,1999; Auldist et al. 2016), animal health, weather, and husbandry practices (Chen et al., 2017). Variation in the DHA of pasture-based, spring-calved herds can vary according to pedoclimatic conditions and local changes in weather conditions. Data on DHA of cows in Ireland indicates that the DHA varies from ~ 12 to 16 kg dry matter (DM) per cow during the initial 12 weeks of lactation (Lewis et al., 2011). Cold wet weather in spring (March-April) can significantly reduce grass growth and, hence, the DHA available to grazing herds in early lactation especially where stocking rate is high (Kennedy et al., 2015). The following questions arise: 'does a shortage of herbage in early lactation affect composition and processability of milk?', 'at what level of herbage reduction do effects become significant?', and 'are effects of a shortage of herbage in early lactation carried into mid- and late-lactation when herbage again becomes plentiful?'

Lowering DHA in the range 13-19 kg DM per cow in early lactation (15-95 DIM) has been generally found to coincide with reductions in milk yield and protein concentration, but to have no effect on the concentration of fat or lactose (Kennedy et al., 2007; McEvoy et al., 2008). A similar trend for effect of DHA on milk yield and concentrations of protein and casein was reported by Auldist et al. (2000b) when DHA was limited to ~ 40 % of ad-libitum allowance in early lactation (~ 60-68 DIM). Similarly, Bargo et al. (2002) found that a decrease in DHA from 40 to 25 kg DM per cow during four different periods across the year (93-113, 114-134, 209-229, and 230-250 DIM) reduced milk yield, but had no effect on the mean concentrations of milk protein, fat or urea nitrogen.

Variations in the concentrations of the major milk constituents (e.g., casein, whey protein, lactose) are of relevance in dairy processing as they affect the manufacturing efficiency of dairy products such as cheese, casein and milk powder, and processing characteristics such as rennet gelation (Guinee et al., 1997; Lin et al., 2017),

heat stability (Huppertz, 2016) and ethanol stability (Chen et al., 2014; Lin et al., 2017). However, other composition-related factors are also likely to affect the processing behaviour of milk, e.g., concentration of different elements (Tsioulpas et al., 2007), proportions of different caseins (Jõudu et al., 2008), and the partitioning of components (e.g., casein, calcium) between the serum and casein micelle (Lin et al., 2018b). Wedholm et al. (2006) reported that a low concentration of κ-casein and a low proportion of κ-casein (% total casein) in individual cow milk samples collected from different breeds (Swedish Red and White, Swedish Holstein and Danish Holstein-Friesian) correlated with poor rennet-coagulating properties. Little information is available on how variation in DHA in early lactation affects these other compositional parameters and milk processability, or whether such effects are carried over into mid- and late-lactation.

3.3 Aims of the study

The current experiment had the aim of investigating the effect of reducing DHA (15.0 to 11.8 kg DM per cow) in early lactation (29-70 DIM) on the composition, rennet gelation and heat stability of cow's milk during early-, mid-, and late- lactation. The typical DHA required for spring-calved cows in early lactation in Ireland is ~15 kg DM per cow (Lewis et al., 2011).

3.4 Results and discussion

Milk from three herds on different DHA (H-DHA, M-DHA and L-DHA) in early lactation (March 9-April 19, 2015; 29-70 days in milk, DIM) was collected every 10 days during this period. Thereafter, milk was sampled at 1-3 week intervals for the remainder of lactation, which was further divided into two sub-periods, namely mid lactation (ML, April 27–August 31) when cows were 78-183 DIM, and late lactation

(LL, September 20 –November 2) when cows were 205-267 DIM. The data set relating to the bulk milk from each of three DHA treatments (L-DHA, M-DHA and H-DHA) in the individual lactation stages (EL, ML and LL) was analysed using analysis of variance (ANOVA), to determine the effect of DHA in each lactation stage, the effect of DHA in overall lactation, and the effect of lactation stage across all the DHA treatments. The experimental unit was herd milk, while the replication unit was the sampling time. The effects of DHA and lactation stage were determined using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). Tukey's multiple-comparison test was used for paired comparison of means and the level of significance was determined at $p < 0.05$.

R-3.2.2 software (R Core Team, 2014) was used to compute a Pearson correlation between the different compositional variables, where significance was determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$, according to Students t-test.

3.4.1 Gross composition and pH of milk

The yield and composition of milk from the different DHA treatments, applied during early lactation, are shown in Fig. 3.1 and Table 3.1 Milk yield and composition was significantly affected by DHA, the extent of which depended on compositional parameters and lactation stage.

Reducing DHA from 15.0 (H-DHA) to 11.8 (L-DHA) kg DM per cow during the six-week EL period resulted in lower milk yield and concentrations of total protein, true protein and casein in EL, but had no effect on the concentrations of total solids, lactose, fat, whey protein, NPN, urea, casein number (casein as % of total protein), or proportions of individual caseins. The results concur with those of previous studies (O'Brien et al., 1997; Kennedy et al., 2007; McEvoy et al., 2008), which found that reducing DHA in the range 19.0 to 13.0 kg DM per cow in early- (15-95 DIM) or mid-

(88-177 DIM) lactation led to lower milk yield and concentrations of protein and casein, but did not affect the concentrations of fat and lactose. Likewise, the absence of an effect of DHA on the proportions of different caseins (α_{s1}-, α_{s2}-, β- or κ-caseins) concur with the findings of Auldist et al. (2000b) on restricting DHA to ~ 40 % of *ad libitum* intake in early lactation (~ 60-68 DIM).

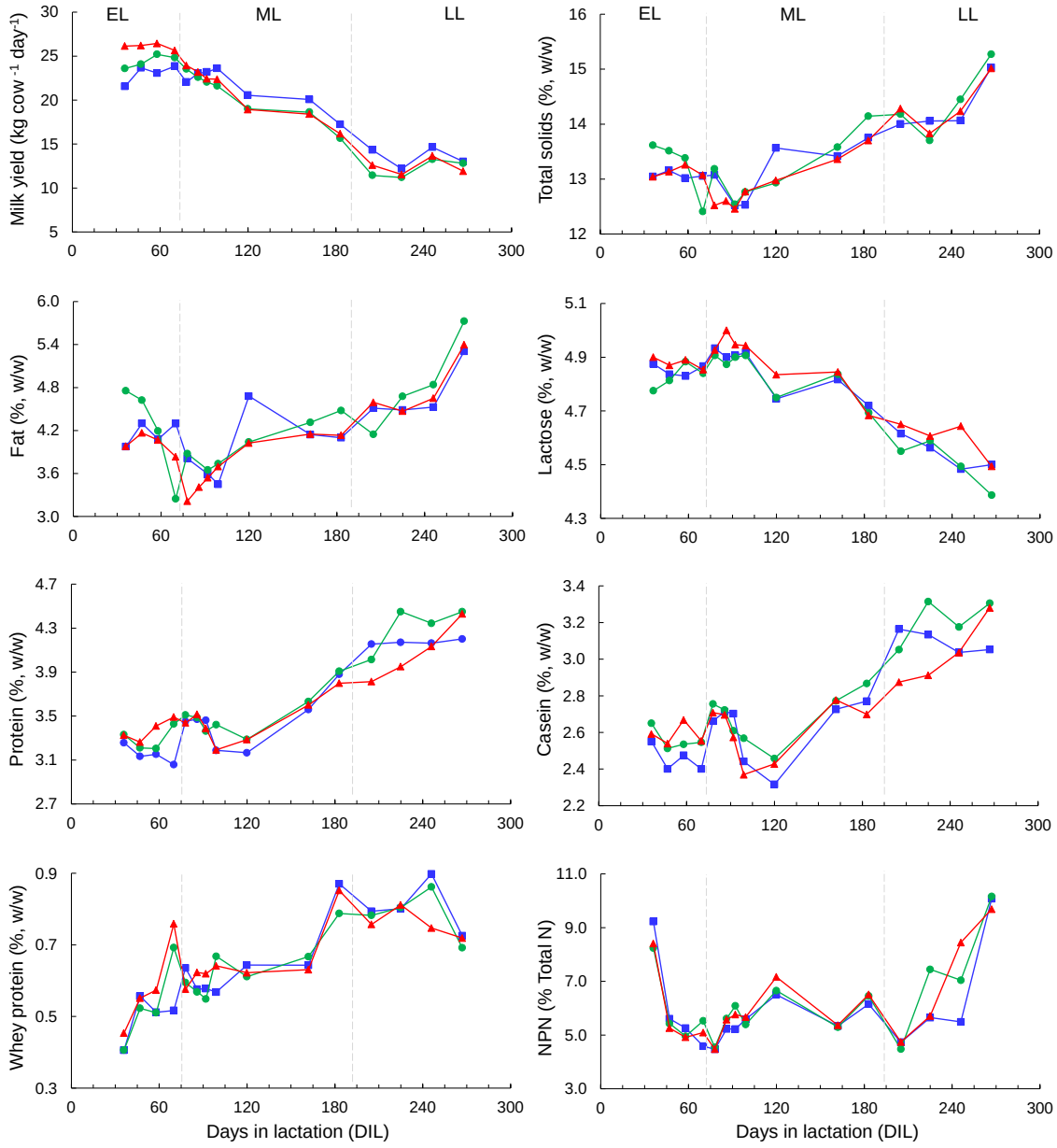

Figure 3.1. Seasonal changes in yield and composition of milk from spring-calved herds on high- (H-DHA: 15.0 kg dry matter per cow, ▲), medium- (M-DHA: 14.4 kg dry matter per cow, ●) or low- (L-DHA: 11.8 kg dry matter per cow, ■) daily herbage allowance during early lactation. Milk from each DHA treatment was analysed in early (EL)-, mid (ML)- and late (LL)- lactation.

Table 3.1: Effect of reducing daily herbage allowance (DHA) in early lactation on the composition of whole milk in early-, mid- and late-lactation.[1]

	Effect of DHA treatment in different lactation stages															Overall effects in lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LS
Item	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	*P*	*P*
Milk yield (kg/cow/day)	26.1^{a}	24.4^{b}	23.0^{b}	0.34	**	20.8	20.4	21.4	0.03	-	12.4^{ab}	12.2^{b}	13.5^{a}	1.14	**	-	***
Milk solids yield (kg/cow/day)	3.43^{a}	3.23^{ab}	3.01^{b}	0.06	**	2.67	2.72	2.82	0.04	-	1.78	1.83	1.91	0.16	-	-	***
Total solids (%, w/w)	13.1	13.2	13.1	0.16	-	12.9	13.3	13.2	0.13	-	14.3	14.6	14.4	0.18	-	-	***
Lactose (%, w/w)	4.88	4.83	4.85	0.02	-	4.88	4.84	4.85	0.01	-	4.6^{a}	4.48^{b}	4.52^{ab}	0.05	*	-	***
Fat (%, w/w)	4.01	4.20	4.16	0.02	-	3.74	4.19	4.05	0.14	-	4.78	4.9	4.77	0.10	-	-	***
Total protein (%, w/w)	3.37^{a}	3.29^{ab}	3.15^{b}	0.05	*	3.46	3.52	3.45	0.02	-	4.08	4.31	4.17	0.11	-	-	***
True protein (%, w/w)	3.17^{a}	3.09^{ab}	2.95^{b}	0.04	*	3.26	3.31	3.26	0.02	-	3.78	4.00	3.90	0.13	-	-	***
Casein (%, w/w)	2.59^{a}	2.56^{a}	2.46^{b}	0.02	*	2.61	2.68	2.62	0.02	-	3.03	3.21	3.10	0.08	-	-	***
Individual caseins (% milk casein)																	
α_{s1}-casein	42.3	42.7	40.3	1.1	-	37.4	37	39.4	2.2	-	39.3	39.1	41.0	0.69	-	-	*
α_{s2}-casein	8.54	8.74	8.51	0.72	-	11.1	9.83	9.58	3.2	-	10.24	11.49	8.56	0.61	-	-	-
β-casein	32.4	32.2	33.9	1.1	-	31.8	33.2	33.8	3.3	-	32.4	28.9	31.2	1.14	-	-	-
κ-casein	16.8	16.3	17.3	1.1	-	19.7	20.0	17.2	2.8	-	18.1	20.5	19.2	1.42	-	-	-
Casein number	76.8	77.8	78.0	0.81	-	75.4	76.2	75.9	0.35	-	74.2	74.5	74.2	0.46	-	-	**
Whey protein (%, w/w)	0.58	0.53	0.50	0.02	-	0.65	0.64	0.65	0.01	-	0.76	0.79	0.80	0.06	-	-	***
α-Lac: β-Lg	0.21	0.22	0.22	0.01	-	0.21	0.22	0.23	0.38	-	0.23	0.20	0.25	0.07	-	-	-
Casein:whey protein	4.47	4.83	4.92	0.20	-	4.02	4.19	4.03	0.17	-	3.99	4.06	3.88	0.14	-	-	**
NPN (% total N)	5.92	6.04	6.17	0.20	-	5.79	5.72	5.50	0.09	-	7.15	7.28	6.49	0.81	-	-	-
Urea (mg/100g)	28.5	27.2	28.2	1.2	-	28.5^{ab}	29.6^{a}	27.8^{b}	0.40	*	32.5	34.5	34.7	2.30	-	-	-
pH	6.70	6.74	6.72	0.02	-	6.69	6.65	6.67	0.01	-	6.64	6.61	6.63	0.01	-	-	***
Soluble protein (%, w/w)	0.91^{ab}	0.97^{a}	0.84^{b}	0.03	*	1.06	1.07	1.01	0.02	-	1.53	1.6	1.49	0.08	-	-	***
Soluble casein (% milk casein)	3.4	7.9	4.6	1.44	-	6.4	7.1	5.1	0.71	-	13.2	13.9	11.7	1.58	-	-	***

Table 3.2: Effect of reducing daily herbage allowance (DHA) during early lactation on the elemental composition of milk in early-, mid- and late-lactation.[1]

	Effect of DHA treatment in different lactation stages															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LS
Item	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	*P*	*P*
Ca (mg/100g)	121	128	122	3.1	-	126	130	124	1.7	-	143	142	144	2.1	-	-	***
P (mg/100g)	92.7	98.3	92.7	3.0	-	91.6	92.6	91.4	1.1	-	98.7	102	101.7	1.7	-	-	*
Na (mg/100g)	36.6	39.5	36.8	1.1	-	41.3	41.6	40.5	0.36	-	55.0	58.2	56.9	2.2	-	-	***
Mg (mg/100g)	10.5^{b}	11.4^{a}	11.4^{a}	0.11	**	10.8	11.2	11.0	0.15	-	13.4	13.6	14.0	0.18	-	-	***
Zn (µg/kg)	4086	4212	4467	171	-	3957	4066	3859	93.9	-	4300^{b}	4521^{ab}	4603^{a}	119	*	-	*
Fe (µg/kg)	332^{ab}	371^{a}	276^{b}	19.1	*	304	375	323	50.7	-	295	262	274	24.9	-	-	-
Cu (µg/kg)	98.3	117.9	101	7.6	-	72.1	78.6	81.3	4.9	-	42.3	48.9	48.7	7.2	-	-	***
Mo (µg/kg)	29.6	31.2	33.9	2.67	-	45.4	46.2	45.0	1.4	-	44.5	44.8	45.8	3.5	-	-	**
Mn (µg/kg)	30.9	33.9	29.1	2.1	-	34.5	37.3	35.9	3.2	-	33.8	34.3	32.0	1.5	-	-	-
Se (µg/kg)	8.9	9.2	9.2	0.29	-	14.7	15.7	15.1	0.39	-	16.2	17.8	17.4	1.2	-	-	***
Co (µg/kg)	0.75	0.71	0.83	0.03	-	0.52	0.71	0.60	0.01	-	0.88	0.74	0.80	0.02	-	-	*

[1] H-DHA, M-DHA and L-DHA denote high- , medium- and low- DHA, i.e., 15.0, 14.4 and 11.8 kg dry matter/cow, respectively. Early (EL)-, mid (ML)- and late (LL)-lactation correspond to March 16-April 19, April 27-August 10, and September 1-November 2, when cows were 29-70, 78-183, and 205-267 days in milk, respectively. Values within a row relating to effect of DHA treatment in EL, ML or LL and not sharing a common lower-case superscripted letter differ significantly for effect of DHA; values within a row without a superscript do not differ for effect of DHA ($p > 0.05$). SED = standard error of difference between means; *P* values denote statistical significance, where ***, **, * and - denote P < 0.001, < 0.01, < 0.05 and > 0.5, respectively. The statistical significance (*P*) for the effects of DHA in overall lactation, and lactation stage (LS) across all DHA treatments are also shown.

Apart from a quite small, but significant, change in urea in ML, reducing DHA in EL had no carry-over effect on milk composition into ML or LL (Table 3.1), as evidenced by the similar values for the latter variables in ML and LL. This trend confirms the results of Kennedy et al. (2007), which showed that differences in milk yield and composition as a result of DHA variation in EL (15-91 DIM) disappeared on normalisation of DHA to 20 kg DM per cow in mid-lactation (92-119). Conversely, Roche (2007) found that restricting DHA from 13.5 to 8.6 kg DM per cow in early lactation (1-35 DIM) coincided with lower yields of milk, fat and protein, and concentrations of fat and protein during later lactation (36-105 DIM). McEvoy et al. (2008) also found that lowering DHA (from 17 to 13 kg DM per cow) in early lactation (19-95 DIM) resulted in a significant reduction in milk protein (0.13%, w/w) in mid-lactation (96-181 DIM). Roche (2007) concluded that the most plausible reason for this carryover effect is a negative effect of energy restriction on mammary secretory cell number and activity, and potentially a reduced uptake of nutrients by the mammary gland. The inter-study discrepancy (Kennedy et al., 2007, Roche, 2007; McEvoy et al., 2008) on the effects of DHA restriction in early lactation on the composition of milk as lactation advances may relate to factors such as extent and duration of feed restriction, and level of nutrient intake in the pre-calving and post-restriction periods. Despite its effects in EL, reducing DHA in EL had no effect on the mean values for milk yield or different compositional parameters over the entire lactation.

The mean daily milk yield decreased progressively with stage of lactation, from ~ 24.5 kg in EL per cow to 12.7 kg per cow in LL. Lactation stage also had a significant effect on milk composition (Table 3.1; Fig. 3.1), with LL milk having higher mean concentrations of fat, protein, casein, whey protein and NPN, a lower concentration of lactose, and a lower pH value. However, casein number (casein as a % of total casein) decreased, while whey protein as a proportion of total protein increased with advance in

lactation. It has been suggested that the reduction in the casein:whey protein ratio in LL milk is due to an influx of blood components (including albumin and immunoglobulins) into the milk, concomitant with an increase in the permeability of the alveolar epithelium as involution approaches (Auldist & Hubble, 1998; Bobbo et al., 2017). The mean proportion of α_{s1}-casein in ML milk (37.9 %) was lower than that in EL milk (41.8 %); otherwise lactation stage did not affect the proportions of κ-, β- and α_{s2}-caseins, or the ratio of α-lactalbumin-to-β-lactoglobulin. The trends in protein and lactose with lactation stage are similar to those reported previously for milk from spring-calved herds (Mehra et al., 1999; Auldist et. al. 2000a; O'Callaghan et. al. 2016b).

3.4.2 Macro- and trace-elements

The elemental content of milk affects its processing behaviour, and the nutritional value and stability of dairy products (Gaucheron, 2013). The mean concentrations of macroelements (Ca, P, Na and Mg) and trace elements (Zn, Fe, Cu, Mo, Mn, Se and Co) are shown in Table 3.2. Overall, reducing DHA in EL had little influence on the concentrations of most elements, apart from giving higher and lower concentrations of Mg and Fe, respectively, in EL and a higher concentration of Zn in LL. The results concur with O'Brien et al. (1997) who reported that on alteration of DHA in mid-lactation (88-177 DIM) did not significantly alter the concentrations of Ca or P. The absence of an effect of DHA on the concentrations of Ca and P might be explained on the basis that the animal skeleton acts as a reservoir for these minerals where mineral intake in the diet is deficient (Fox et al., 2015).

Lactation stage significantly affected the mean concentration of most elements, apart from Fe, Mn and Co (Table 3.2). Late-lactation milk had higher mean concentrations of Ca, Mg, P, Na, Se, Zn and Mo, and a lower concentration of Cu, than

EL or ML milk. The trend aligns with the lactational increase in casein, with which a relatively high proportion of many of the latter elements (Ca, P, Mg, Zn) associate (Vegarud, Langsrud, & Svenning, 2000) in the formation of the casein micelles (Lucey & Horne, 2018). Hence, the concentrations of Ca, P and Mg correlated positively with casein content (Fig. 3.2a). Nevertheless, the ratio of total Ca or P to casein decreased slightly, but significantly, as casein increased (Fig. 3.2b) from 120 DIM onwards.

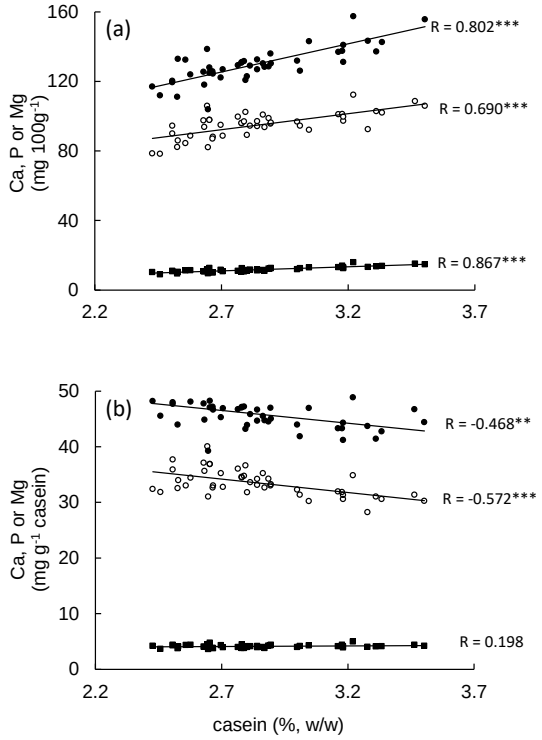

Figure 3.2. Concentrations of: Ca (●), P (○) and Mg (■) (a), and the ratio of Ca- (●), P- (○) and Mg- (■) to-casein (b), as a function of casein content of milk. The data are from 45 milk samples collected on 15 different occasions throughout the year from three spring-calved herds on different daily herbage allowance in early lactation. Linear regression lines (-) were fitted to the experimental data points. The regression coefficient (R) and significance of correlations are shown, where statistical probability is denoted by: ***, $p < 0.001$; **, $p < 0.01$.

3.4.3 Composition of milk serum

Reducing DHA from 14.4 (M-DHA) to 11.8 (L-DHA) kg DM per cow in EL led to a significant reduction in serum protein (0.13 %, w/w) in EL milk, but otherwise had no effect on serum composition (soluble casein, casein profile) in EL, ML or LL milks (Table 3.1).

The concentration of protein and soluble casein in serum increased over lactation, concomitant with the increase in the concentrations of total protein, casein and whey protein in milk. Casein in serum, as a proportion of total casein in milk, increased significantly from a mean value of 5.3 % in EL to 12.9 % in EL; the range of values (3.4- 13.9 %) over lactation was broader than that (3·6–10·5 %) reported by Lin et al. (2017b) for a mixed mixed-herd of spring- and autumn-calving cows over the year, but narrower than that (7-25 % of total casein) found by Rose (1968) for fresh milk from individual cows and equilibrated at 35 °C prior to ultracentrifugation. The overall mean proportions of α_s-, β and κ-caseins, as percentages of casein in serum, were ~ 26, 41 and 33, respectively, and were not influenced by DHA or lactation stage (data not shown); the values are of similar magnitude to those reported by Lin et al. (2017b). A tentative explanation for the significantly higher proportion of soluble casein in the LL milk is the reduction in ratio of Ca and P-to-casein (Fig. 3.2b). Rose (1968) investigated the effects of an incremental reduction in the colloidal calcium content of milk from 18.6 to 0.6 mM, and concluded that the calcium phosphate content of micelles and the polymerisation of temperature-sensitive caseins (Dalgleish & Law, 1988), especially β-casein, is the major factor controlling the level of intact casein in serum. High levels of casein in serum (>> 15% of total casein) are undesirable as they impair rennet gelation and curd syneresis, and reduce cheese yield (Ali et al., 1980; O'Keeffe, 1984).

3.4.4 Rennet gelation

Rennet gelation is a key functional parameter of milk used for cheesemaking as it determines the rate at which the milk sets and the changes in gel strength (storage modulus, G') and gel firming rate as a function of time from rennet addition. It influences the ability of the gel to withstand fracture during cutting and to synerese, and consequently the moisture content and quality of the cheese, the recovery of fat, and

cheesemaking efficiency (Fox et al., 2017). Alteration of DHA in EL had no effect on rennet coagulation time, (RCT), maximum gel firming rate (GFR_{max}) or gel firmness at 40 min (G'_{40}) during EL, ML or LL (Fig. 3.3, Table 3.3). The lack of an effect of DHA on rennet coagulation characteristics is consistent with the results of O'Brien et al. (1997) and is scarcely surprising based on the relatively small, or lack of, difference between the DHA treatments with respect to casein concentration in EL (maximum difference of 0.13 %, w/w, in EL) (O'Brien et al., 1997; Auldist et al., 2004), individual caseins, soluble casein, and ratios of Ca- and P- to casein (Guinee et al., 1997; Horne & Lucey, 2017).

Late-lactation milk had enhanced coagulability, as evidenced by the lower value of RCT and higher values of GFR_{max} and G'_{40} relative to EL or ML milks (Table 3.3; Fig. 3.3). The improved gelation characteristics of LL milk were most likely associated with the higher casein concentration, which correlated positively with GFR_{max} and G'_{40} (Table 3.4). Hence, the increase in casein concentration over lactation was sufficiently large to outweigh the slight, but significant, reductions in the ratios of Ca- and P- to casein and increase in the proportion of soluble casein, which are expected to impair rennet gelation (Ali et al., 1980; Fox et al., 2017). The levels of α_{s1}-,α_{s2}-, β-, and κ-caseins (as proportions of total casein) had no effect on rennet gelation properties. Conversely, Jõudu, et al. (2008) found significant effects of the proportions of α_{s1}-,α_{s2}-, β-, and κ-caseins on the rennet gelation characteristics of individual milk samples from different breeds (Estonian Red, Red-and-White Holstein, Estonian Holstein) over 1.5-year period. The inter-study discrepancy may relate to the magnitude of the changes in the proportions of individual caseins over the investigation periods, which were relatively small in the current study (Table 3.1); these data were not presented by Jõudu et al. (2008).

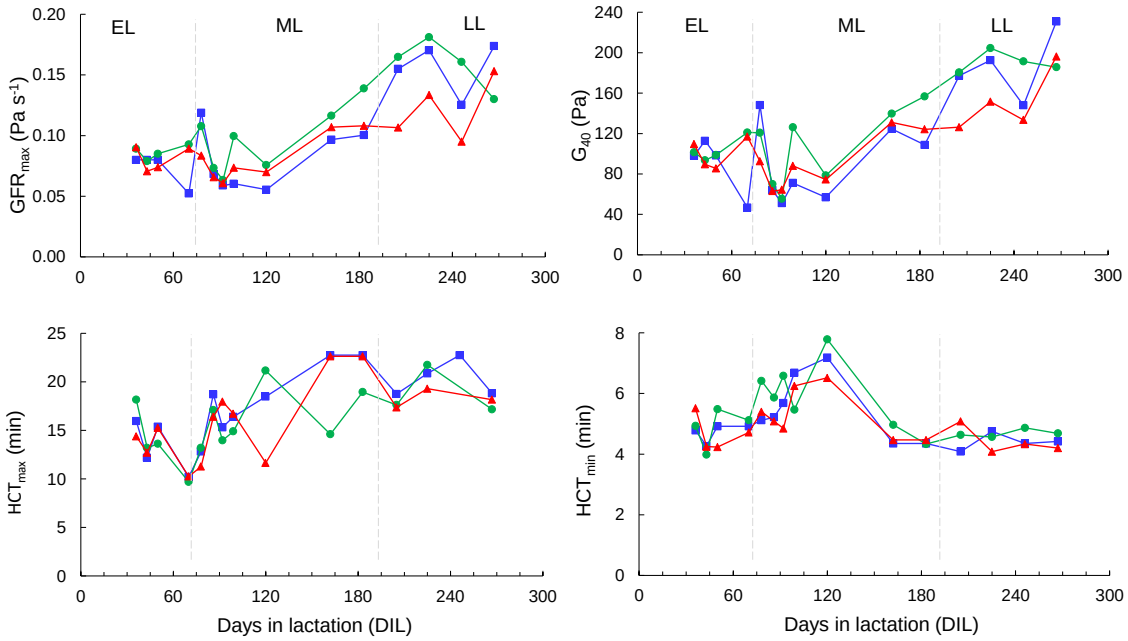

Figure 3.3. Seasonal changes in rennet gelation (maximum gel firming rate, GFR_{max}; gel firmness at 40 min, GF_{40}) and heat stability characteristics (maximum heat coagulation time, HCT_{max}; minimum heat coagulation time, HCT_{min}) of milk from spring-calved herds on high- (H-DHA: 15.0 kg dry matter per cow, ▲), medium- (M-DHA: 14.4 kg dry matter per cow, ●) or low- (L-DHA: 11.8 kg dry matter per cow, ■) daily herbage allowance during early lactation. Milk from each DHA treatment was analysed in early (EL)-, mid (ML)- and late (LL)- lactation.

3.4.5 Heat coagulation time

Heat coagulation time determines the stability of milk to high temperature treatment, as applied for example during the preparation of milk-based beverages (e.g., UHT milk, infant milk formula, and nutritional drinks), condensed milk and recombined milks (Sharma et al., 2012). Commercially, beverages are frequently prepared from skim milk concentrates that have a lower pH than native milk, which increases the susceptibility to heat-induced aggregation and destabilisation (Lin et al., 2018b). All milks exhibited a type A HCT *versus* pH profile with a maximum at pH 6.6 to 6.7 and a minimum at pH 6.8 to 7.0 (Fig. 3.3). The mean values for HCT_{npH}, HCT_{max} and HCT_{min} at 140 °C over the lactation stage (Table 3.4) are comparable to those reported by Lin et al. (2017b) for mixed herd milk. Reducing DHA in EL had no effect on the heat stability characteristics at 140 °C (HCT_{min}, HCT_{max} and HCT_{npH}) of milk in EL, ML or LL. The absence of an effect of DHA is consistent with the similar values of lactose,

Table 3.3: Effect of reducing daily herbage allowance (DHA) during early lactation on rennet gelation and heat stability of skim milk in early-, mid- and late-lactation.[1]

	Effect of DHA treatment in different lactation stages															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LS
Item	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*p*	*P*	*P*
Rennet gelation																	
RCT (min)	13.0	13.3	14.0	1.5	-	15.3	15.5	15.4	0.63	-	12.4	11.7	12.3	0.10	-	-	*
GFR_{max} (Pa/s)	0.08	0.09	0.07	0.01	-	0.08	0.10	0.08	0.01	-	0.12	0.16	0.16	0.01	-	-	***
G'_{40} (Pa)	100.4	103.7	88.9	12.1	-	91.1	106.8	89.3	6.49	-	151.9	190.5	187.1	13.7	-	-	***
Heat coagulation time (HCT)																	
HCT_{npH}	13.1	13.7	13.4	0.54	-	17.0	16.3	18.2	1.57	-	14.0	15.2	20.3	1.4	-	-	-
HCT_{max}	13.8	14.9	13.5	0.22	-	14.0	15.4	18.1	0.46		14.6	16.2	17.0	0.50	-	-	-
HCT_{min}	4.7	4.9	4.7	0.62	-	5.3	6.6	5.5	1.1	-	5.2	5.7	4.4	0.79	-	-	**

[1]H-DHA, M-DHA and L-DHA denote high- , medium- and low- DHA, i.e., 15.0, 14.4 and 11.8 kg dry matter/cow, respectively. Early (EL)-, mid (ML)- and late (LL)-lactation correspond to March 16-April 19, April 27-August 10, and September 1-November 2, when cows were 29-70, 78-183, and 205-267 days in milk, respectively. Values within a row relating to effect of DHA treatment in EL, ML or LL and not sharing a common lower-case superscripted letter differ significantly for effect of DHA; values within a row without a superscript do not differ for effect of DHA ($p > 0.05$). SED = standard error of difference between means; *P* values denote statistical significance, where ***, **, * and - denote $p <$ 0.001, < 0.01, < 0.05 and > 0.5, respectively. The statistical significance (p) for the effects of DHA in overall lactation, and lactation stage (LS) across all DHA treatments are also shown. Abbreviations: RCT, rennet coagulation time; GFR_{max}, maximum gel firming rate; G'_{40}, gel firmness at 40 min; HCT_{npH}, HCT at natural pH; and HCT_{max} and HCT_{min} are the maximum and minimum heat coagulation times, respectively, of the HCT/pH (6·2–7·2) curve.

Table 3.4: Significant relationships between milk composition and rennet gelation or heat stability characteristics.[1]

Processing characteristic	Compositional parameter	Correlation coefficient (r)
Rennet gelation		
RCT, rennet coagulation time	casein (%, w/w)	-0.41**
GFR_{max}, maximum gel firming rate	casein (%, w/w)	+0.84***
	Ca (mg/100g)	+0.70***
	P(mg/100g)	+0.62***
G'_{40}, gel firmness at 40 min	casein (%, w/w)	+0.82***
	Ca (mg/100g)	+0.72***
	P(mg/100g)	+0.65***
Heat stability		
Maximum heat coagulation time, HCT_{max}	lactose (%, w/w)	-0.44**
	protein (%, w/w)	+0.55***
	NPN (%, w/w)	+0.35*
	urea (mg/100g)	+0.50***
	Soluble casein (%, w/w)	+0.40**
	Soluble casein (% total casein)	+0.37*
Heat coagulation time at natural pH, HCT_{npH}	lactose (%, w/w)	-0.31*
	protein (%, w/w)	+0.35*
	NPN (%, w/w)	+0.38*
	urea (mg/100g)	+0.42**
	Soluble casein (%, w/w)	+0.36*
	Soluble casein (% total casein)	+0.33*

[1]The data set comprised 45 milk samples collected on 15 different occasions throughout the year from three spring-calved herds on different daily herbage allowance in early lactation. Correlations were obtained using simple linear regression analysis; only relationships found to be statistically significant are shown: ***, $p < 0.001$; **, $p < 0{\cdot}01$; *, $p < 0{\cdot}05$. Positive and negative correlations between two parameters are indicated by a positive sign (+) and a negative sign (-), respectively.

urea, Ca, P and pH for each of the DHA treatments in EL, ML and LL (Holt et al., 1978; Huppertz, 2016); and the relatively small difference in protein content (0.22 %, w/w) (Rattray & Jelen, 1996) between the treatments in EL.

While stage of lactation also had no effect on HCT_{min} or HCT_{npH}, the mean HCT_{max} across the three DHA treatments in EL was lower than that in ML or LL (Table 3.4). The results suggest that changes in the various compositional parameters during lactation have interactive effects on HCT, to an extent dependent on the magnitude of the change (Huppertz, 2016). Hence, while the relatively low concentration of lactose

and high concentration of urea might be expected to enhance the HCT_{npH} of LL milk compared to ML- or EL- milk, such an increase may be offset by the higher protein concentration and lower pH of LL milk (Sikand et al., 2010; Meena et al., 2016). Regression analysis indicated that HCT_{max} and HCT_{npH} correlated positively with concentrations of urea, NPN, total protein and soluble casein, and proportions of κ- and α_{s1}- or α_{s2}-caseins, and negatively with lactose (Table 3.4). The positive effect of higher soluble casein on HCT_{max} and HCT_{npH} is analogous to the increase observed on increasing the proportion of soluble casein by addition of NaCl (1.2 %, w/w) or addition of sodium caseinate (≥ 0.3 %, w/w) (Tessier & Rose, 1964; Lin et al., 2017a).

3.5 Conclusion

Reducing DHA of a spring-calved herd from 15 to 11.8 kg DM per cow in EL (9 March – 19 April; 29-70 DIM) led to lower milk yield and concentrations of total protein and casein, but had little, or no, effect on other aspects of composition (e.g., concentrations of fat, lactose, non-protein N, urea, elements or proportions of individual caseins), rennet gelation or heat stability at pH values 6.2-7.2. Moreover, there was little, or no, impact of reducing DHA in EL on milk composition, rennet gelation or heat stability in ML (27 April - 31 August; 85-190 DIM), LL (20 September - 2 November; 210-275 DIM) or overall lactation (EL + ML + LL). The absence of an effect of lowering DHA in EL on most compositional parameters and processability characteristics (rennet gelation and heat stability) in EL, ML or LL suggests that restricted grazing without concentrate supplementation can, within limits, be applied in early lactation with little consequence apart from the lower yields of milk and milk solids during that period. This is of relevance to farm management where adverse weather in spring can reduce grass availability to cows, especially where stocking rate is high.

Chapter 4

Grazing of dairy cows on pasture *versus* indoor feeding on total mixed ration: Effects on gross composition and mineral content of milk during lactation

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4.1 Abstract

The influence of feeding system and stage of lactation on the gross composition, macro-elements (Ca, P, Mg, and Na) and trace-elements (Zn, Fe, Cu, Mo, Mn, Se and Co) of bovine milk was investigated. Three feeding systems were compared: cows grazing on perennial ryegrass pasture (GRO), cows grazing on perennial ryegrass and white clover pasture (GRC), and cows housed indoors offered total mixed ration (TMR). Sixty spring-calving Holstein Friesian dairy cows were assigned to three herds, each consisting of 20 cows, and balanced with respect to parity, calving date and pre-experimental milk yield and milk solids yield. The herds were allocated to one of the three feeding systems from February to November. Milk samples were collected on 10 occasions over the period June 17 to November 26, at two or three weekly intervals, when cows were on average 119 to 281 days in milk (DIM). The total lactation period was arbitrarily sub-divided into two lactation stages based on DIM, namely mid lactation (ML), June 17 to September 9 when cows were 119-203 DIM, and late-lactation (LL), September 22 to November 26 when cows were 216-281 DIM. With the exception of Mg, Na, Fe, Mo and Co, all other variables were affected by feeding system. Milk from GRO feeding system had highest mean concentrations of total solids, total protein, casein, Ca and P. Milk from TMR feeding system had the highest concentrations of lactose, Cu and Se, and lowest level of total protein. Milk from GRC feeding system had levels of lactose, Zn and Cu similar to those of GRO milk, and concentrations of TS, Ca and P similar to those of TMR milk. Stage of lactation affected all variables, apart from the concentrations of Fe, Cu, Mn and Se. On average, the proportion (%) of total Ca, P, Zn, Mn or Se that sedimented with the casein on high speed ultracentrifugation at 100,000 x g, was ≥ 60 %, whereas that of Na, Mg or Mo was ≤ 45 % total. The results demonstrated how the gross composition and elemental composition of milk can be affected by different feeding systems.

4.2 Introduction

The most widely used feeding methods for dairy cows globally include, grazing on pasture, usually with a low quantity of concentrate supplementation offered only at the extremes of the pasture-growing season, or indoors offered total mixed ration (TMR) comprised mainly of silage, grain, protein and added vitamins and minerals. Pasture-based feeding is common in temperate regions where sufficient pasture growth is possible, including Ireland, New Zealand and parts of Australia, while TMR is used more extensively in the USA, parts of Europe and the southern hemisphere.

Advantages of pasture-based feeding include its cost competitiveness, its lower contribution to enteric methane emissions (O'Neill et al., 2011), and provision of a 'more-natural' environment for animal welfare (Verkerk, 2003). Additional advantages of pasture incorporating clover include atmospheric N fixation and reduction in nitrous oxide emissions (Ledgard et al., 2009). Advantages of TMR include a more consistent feed composition and quality, better regulation of DMI, less reliance on climatic conditions, and higher milk yield (Kolver & Muller, 1998; McAuliffe et al., 2016).

Despite the widespread use of pasture and TMR feeding systems, there is a paucity of published information on the comparative effects of these feeding systems on milk characteristics other than gross composition, such as the profile of proteins and minerals. Minerals such as calcium (Ca) and phosphorus (P) are important modulators of protein-protein interactions, casein micelle structure, and the susceptibility of the protein to aggregation during dairy processing (Holt & Jenness, 1984). Consequently, Ca and P have a major influence on processing characteristics, such as rennet coagulation, heat stability and ethanol stability (Tsioulpas et al., 2007; Sandra et al., 2012; Horne 2016). Apart from their effects on protein aggregation (Sievanen et al., 2008; Sandra et al., 2012), variation in mineral content can also alter the nutritional value of milk and milk products (Cashman, 2011a). The role of trace elements in human

nutrition has been extensively reviewed (Cashman, 2011b; Gaucheron, 2013); they contribute biological and physiological functions such as the role of iron (Fe) in haemoglobin production, copper (Cu) and selenium (Se) in enzyme functioning, cobalt (Co) as a constituent of vitamin B_{12}, and Se as a component of glutathione peroxidase, an antioxidant. Further, little is known about how trace elements partition between the casein and serum phases of milk, even though this is likely to influence their bioavailability (Cashman, 2011b). Protein and minerals (elements) in first stage (0 – 6 months) infant milk formulae (IMF) and follow-on formulae (FOF) are provided by the skim milk powder and whey ingredients used in formulation. The total concentration of elements in the latter ingredients, determines the level of element fortification required to meet the target label claim for IMF and FOF (European Commission, 2006; McSweeney et al., 2013; McCarthy et al., 2016b). Hence, any changes in the concentrations of protein and elements owing to the feeding system of the dairy cow and stage of lactation could necessitate reformulation of the IMF and affect 'in-process' thermal stability.

4.3 Aims of study

The current study investigated the effect of the following feeding systems on the gross composition and concentrations of macro-elements and trace elements in milk over the period June to November 2015: grazing on perennial ryegrass pasture (GRO), grazing on perennial ryegrass and white clover pasture (GRC), and housed indoors and offered total mixed ration (TMR).

4.4 Results and discussion

Milk from three herds on different feeding systems (GRO, GRC, TMR) was collected on 10 occasions, at two or three weekly intervals, from June 17, 2015 (119

DIM) to November 26, 2015 (281 DIM), and analysed for gross composition. The data were classified by three feeding system (GRO, GRC, and TMR) and two lactation stages based on DIM, namely mid-lactation from June 17 to September 9 when cows were 119-203 DIM, and late-lactation from September 22 to November 26 when cows were 216-281 DIM. The data were analysed by one-way analysis of variance (ANOVA) as a factorial design looking at the main effects of feeding system, lactation stage and their interaction using general linear model (GLM) procedure of SAS 9.3 (SAS Institute, 2011). Tukey's multiple-comparison test was used for paired comparison of means and the level of significance was determined at $p < 0.05$.

Mid-lactation milk, denoted ML milk, refers to the composite of the milk samples collected from the herds on the GRO, GRC and TMR feeding systems in mid-lactation; the composite of the late lactation milk samples was similarly denoted as LL milk. Milk from GRO, GRC or TMR feeding systems in mid-lactation are coded as GRO-ML, GRC-ML and TMR-ML, respectively; similarly, milk samples from GRO, GRC or TMR feeding systems in late lactation are GRO-LL, GRC-LL, TMR-LL) were, similarly, denoted. R-3.2.2 software (R Core Team, 2014) was used to compute a Pearson correlation between the different compositional variables, where significance was determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$, according to Students t-test.

4.4.1 Gross composition: fat, protein and lactose

The composition of milk resulting from the different feeding systems are presented in Table 4.1. The concentrations of fat, protein, casein and lactose were affected by feeding system, lactation stage, and the interaction of feeding system and lactation stage. Most notably, the GRO milk had highest mean concentrations of TS, total protein and casein, and TMR milk had the highest concentration of lactose ($p < 0.01$). Conversely, TMR milk had the lowest mean level of total protein ($p < 0.01$). The mean

protein content of the GRO milk (3.98 %) was similar to that reported by O'Callaghan et al. (2016b) but markedly higher than that reported by Mehra et al. (1999) for bulk silo milk collected from pasture-fed spring-calved herds (3.20-3.75 %) over a similar part of the season (June 2 - November 19). The mean fat content of the GRO milk was significantly higher than that of the GRC milk but not differ from that of the TMR milk.

Milk composition changed significantly with the lactation stage. Late-lactation milk had higher mean concentrations of total solids, fat, total protein and casein than ML milk; conversely, the lactose content of ML milk was higher than that of LL milk. The increase in levels of total protein and casein over the experimental period for milk from all feeding systems is typical of that reported for the effect of stage of lactation in spring-calved herds (Mehra et al., 1999; Auldist et. al. 2000a; O'Callaghan et. al. 2016b).

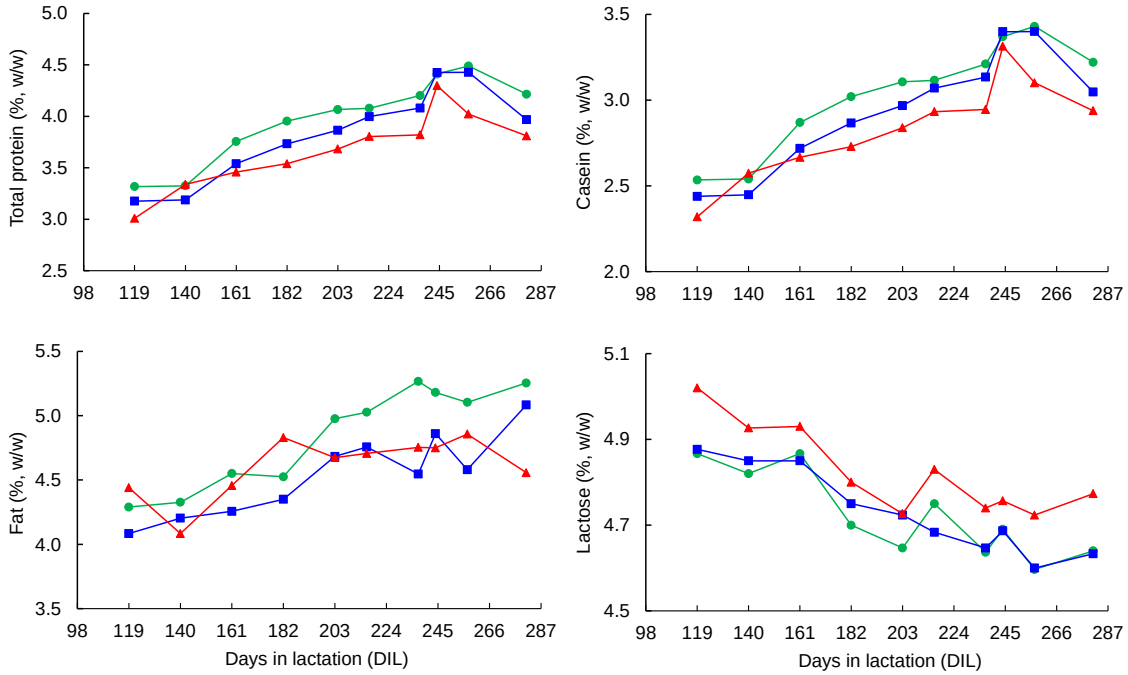

Figure 4.1. Seasonal variation in the concentration of total protein, casein, fat and lactose in skim milk from spring-calved herds fed using three different feeding systems: grazing perennial ryegrass pasture, GRO (●), grazing perennial ryegrass and white clover pasture, GRC (■), or housed indoors and offered total mixed ration, TMR (▲). Milk from each of the feeding systems was analysed over the period June 17 to November 26, 2015.

Table 4.1: Seasonal variation in the compositional characteristics of milk from cows on different feeding systems.[1]

	Effect of feeding system				Effect of lactation stage		Effect of interaction Feeding system x lactation stage			
Item	Feeding system	Mean	SED	Range	Lactation stage	Mean	Feeding system in mid lactation (ML)	Mean	Feeding system in late lactation (LL)	Mean
Composition (%, w/w)										
Total solids	GRO	14.3^{a}	0.087	13.4 - 14.8	ML	13.7^{b}	GRO-ML	14.0^{ab}	GRO-LL	14.6^{a}
	GRC	13.9^{b}		13.3 – 14.4	LL	14.2^{a}	GRC-ML	13.6^{b}	GRC-LL	14.1^{ab}
	TMR	13.8^{b}		12.9 - 14.3			TMR-ML	13.7^{b}	TMR-LL	14.0^{ab}
Fat	GRO	4.85^{a}	0.061	4.29 – 5.27	ML	4.45^{b}	GRO-ML	4.54^{bc}	GRO-LL	5.17^{a}
	GRC	4.54^{b}		4.08 – 5.08	LL	4.89^{a}	GRC-ML	4.31^{c}	GRC-LL	4.77^{ab}
	TMR	4.61^{ab}		4.08 – 4.86			TMR-ML	4.50^{bc}	TMR-LL	4.73^{bc}
Lactose	GRO	4.72^{b}	0.020	4.60 – 4.87	ML	4.82^{a}	GRO-ML	4.78^{abc}	GRO-LL	4.66^{bc}
	GRC	4.73^{b}		4.60 – 4.88	LL	4.69^{b}	GRC-ML	4.81^{ab}	GRC-LL	4.65^{c}
	TMR	4.82^{a}		4.72 – 5.02			TMR-ML	4.88^{a}	TMR-LL	4.76^{abc}
Total protein	GRO	3.97^{a}	0.046	3.32 – 4.49	ML	3.53^{b}	GRO-ML	3.79^{bcd}	GRO-LL	4.16^{a}
	GRC	3.84^{b}		3.18 – 4.43	LL	4.14^{a}	GRC-ML	3.65^{cd}	GRC-LL	4.03^{ab}
	TMR	3.66^{c}		3.01 – 4.30			TMR-ML	3.53^{d}	TMR-LL	3.80^{abc}
Casein	GRO	3.05^{a}	0.072	2.53 – 3.43	ML	2.71^{b}	GRO-ML	2.81^{bc}	GRO-LL	3.27^{a}
	GRC	2.95^{b}		2.44- 3.40	LL	3.17^{a}	GRC-ML	2.69^{bc}	GRC-LL	3.21^{a}
	TMR	2.84^{b}		2.32 – 3.31			TMR-ML	2.63^{c}	TMR-LL	3.05^{ab}
Yield of milk and components (kg/cow/day)										
Milk	GRO	17.0^{c}	0.893	12.3 – 22.6	ML	23.5^{a}	GRO-ML	19.9^{b}	GRO-LL	14.0^{c}
	GRC	19.7^{b}		15.0 – 26.7	LL	17.5^{b}	GRC-ML	23.3^{b}	GRC-LL	16.1^{c}
	TMR	24.9^{a}		20.6 – 29.1			TMR-ML	27.4^{a}	TMR-LL	22.3^{b}
Total solids	GRO	2.4^{c}	0.114	1.8 – 3.1	ML	3.3^{a}	GRO-ML	2.8^{b}	GRO-LL	2.1^{c}
	GRC	2.7^{b}		2.1 – 3.6	LL	2.5^{b}	GRC-ML	3.2^{b}	GRC-LL	2.3^{c}
	TMR	3.4^{a}		2.8 – 4.0			TMR-ML	3.8^{a}	TMR-LL	3.1^{b}

Fat	GRO	0.81[b]	0.034	0.63 – 0.97	ML	1.04[a]	GRO-ML	0.90[cd]	GRO-LL	0.73[a]
	GRC	0.88[b]		0.72 – 1.10	LL	0.85[b]	GRC-ML	1.00[bc]	GRC-LL	0.77[de]
	TMR	1.14[a]		0.94 – 1.30			TMR-ML	1.23[a]	TMR-LL	1.05[b]
Lactose	GRO	0.81[c]	0.046	0.56 – 1.10	ML	1.14[a]	GRO-ML	0.95[bc]	GRO-LL	0.66[d]
	GRC	0.94[b]		0.70 – 1.30	LL	0.82[b]	GRC-ML	1.12[b]	GRC-LL	0.75[cd]
	TMR	1.20[a]		0.98 – 1.43			TMR-ML	1.34[a]	TMR-LL	1.06[b]
Total protein	GRO	0.66[c]	0.023	0.55 – 0.77	ML	0.82[a]	GRO-ML	0.73[cd]	GRO-LL	0.60[e]
	GRC	0.74[b]		0.60 – 0.85	LL	0.72[b]	GRC-ML	0.81[bc]	GRC-LL	0.67[de]
	TMR	0.91[a]		0.78 – 1.00			TMR-ML	0.93[a]	TMR-LL	0.88[ab]
Casein	GRO	0.51[c]	0.018	0.42 – 0.59	ML	0.63[a]	GRO-ML	0.56[cd]	GRO-LL	0.46[e]
	GRC	0.57[b]		0.46 – 0.65	LL	0.55[b]	GRC-ML	0.62[bc]	GRC-LL	0.52[de]
	TMR	0.70[a]		0.60 – 0.77			TMR-ML	0.72[a]	TMR-LL	0.68[ab]

[1]Values within a column relating to effect of feeding system, lactation stage or interaction and not sharing a common superscripted letter a-c, a-b or a-e, respectively differ significantly ($p < 0.05$). Feeding system: GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): Mid lactation (June 17- September 9, 2015; 119-203 DIM) and late lactation (September 22 – November 26, 2015; 217-281 DIM).

4.4.2 Component Yields

The yields of milk, total solids, fat, protein, casein and lactose are shown in Table 4.1. Yields were significantly affected by feeding system, lactation stage, and their interaction which means the response of different feeding systems on compositional parameters depended on the lactation stage.

Total mixed ration feeding gave a significantly higher yield of milk and all milk components (total solids, fat, protein, casein and lactose) than the GRO or GRC feeding systems. The higher milk yield with TMR, therefore, more than compensated for its relatively lower concentrations of TS, protein, casein and fat. The difference between the feeding systems with respect to milk yield and component yields is consistent with the findings of previous studies (Kolver & Muller, 1998; McAuliffe et al., 2016). The higher yield of milk from TMR feeding is attributed to a higher DMI (Kolver & Muller, 1998). The yield of milk and milk solids (lactose, fat, total protein and casein) were significantly higher in ML milk than in LL milk.

4.4.3 Macroelements in skim milk

Changes over the experimental period in the concentration of macro-elements and the proportions of each that sedimented with the casein on high speed ultracentrifugation are shown in Fig. 4.2. The range and mean for total concentration of macro-elements (Ca, P, Na and Mg) are shown in Table 4.2 and are similar to those reported previously (White & Davies, 1958; Keogh et al., 1982; O'Brien et al., 1999b; Auldist et al., 2004; Sola-Larrañaga et al., 2009; Bijl et al., 2013).

Calcium (Ca)

Calcium content was significantly affected by feeding system, lactation stage and their interaction (Table 4.2; Fig. 4.2). The mean Ca content of GRO milk was higher ($p <$

0.05) than that of GRC or TMR milk. The high Ca content of the GRO milk reflects its higher casein concentration, as evidenced by the similar mean values for Ca-to-protein ratio for GRO, GRC and TMR milk (33.6 – 34.4 mg/g protein; data not shown). Most of the Ca in milk exists in association with P as an insoluble calcium phosphate hydroxyapatite attached to the caseins where it contributes to the structural integrity of the casein micelle (de Kruif et al., 2012). The mean concentration of Ca in LL milk

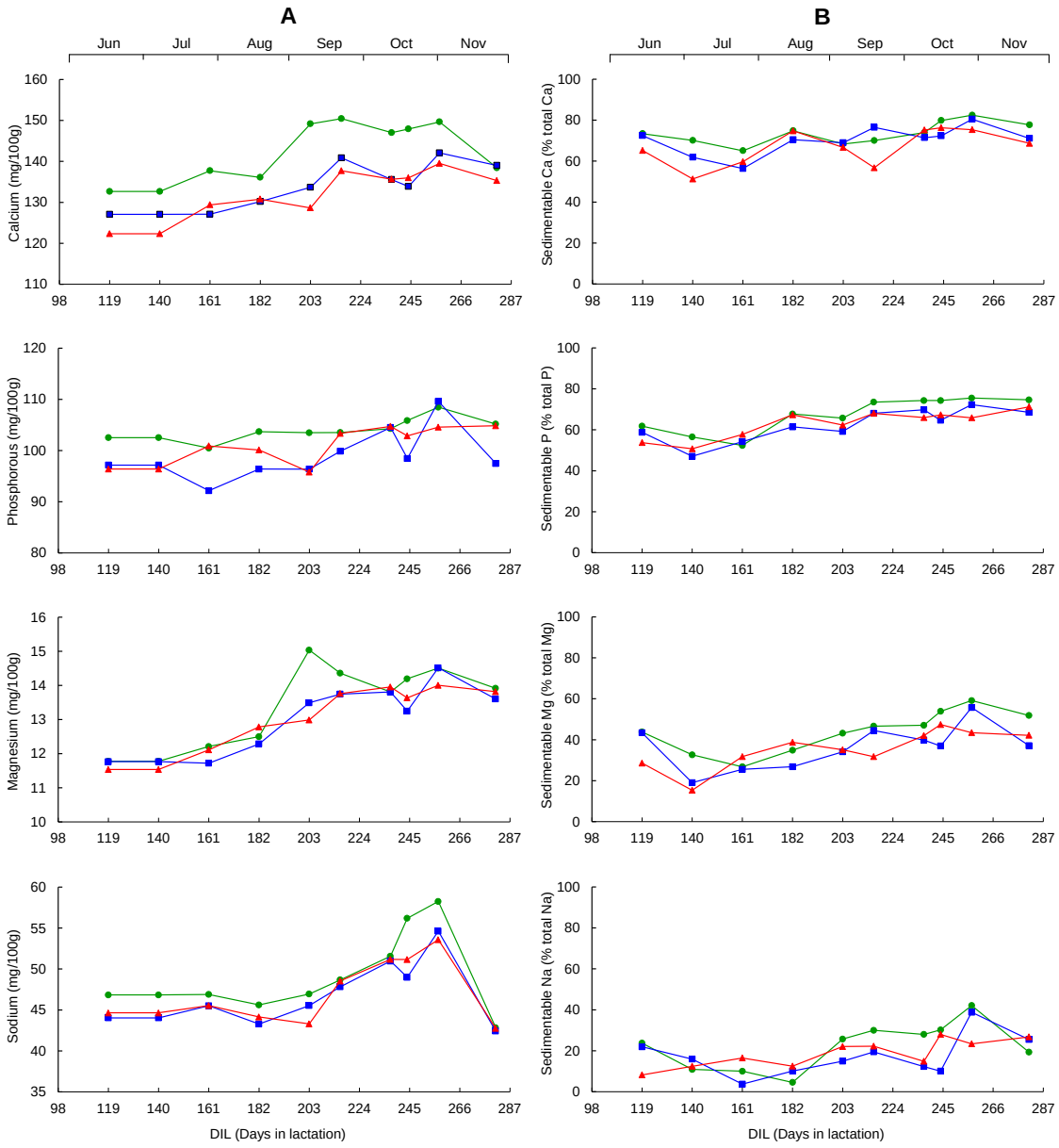

Figure 4.2. Seasonal variation in the concentration of macro-elements in skim milk from spring-calved herds fed using three different feeding systems: total concentration (A) and the proportion of each element that sedimented with the casein on ultracentrifugation at 100,000 g for 1 h at 25 °C (B). The different feeding systems were grazing perennial ryegrass pasture, GRO (●),grazing perennial ryegrass and white clover pasture, GRC (■), or housed indoors and offered total mixed ration, TMR (▲). Milk from each of the feeding systems was analysed over the period June 17 to November 26, 2015.

was significantly higher than that of ML milk (Table 4.2). This trend is consistent with the relatively high concentration of casein in LL milk.

Sedimentable, or micellar, Ca represents the calcium associated with the casein micelle as colloidal calcium phosphate and as calcium that interacts electrostatically with the side chain carboxyl groups of acidic amino acids (Holt & Jenness, 1984). It contributes to casein interactions within the micelle and is important in the stability of milk to chymosin, heat and ethanol (Horne, 2016). The mean level of sedimentable Ca, as a percentage of total Ca, varied from 67 to 74 % over the experimental period (Fig. 4.2). The proportion of sedimentable Ca was significantly affected by lactation stage but not by feeding system. Overall, the proportion of sedimentable Ca was highest in GRO-LL milk, lowest in TMR-ML milk and intermediate in the GRO-ML, TMR-LL, GRC-ML and GRC-LL milk.

Phosphorus (P)

Phosphorus content of milk from each of the feeding systems is shown in Table 4.2. The mean P concentration over the experimental period was relatively high when compared to the studies reported previously (65-78.5 mg/100g) (Keogh et al., 1982; Sola-Larrañaga et al., 2009). The high P content of the current milk samples was consistent with their relatively high casein content (~ 2.9-3.2 %, w/w) compared to that (~ 2.4-2.6 %, w/w) in milk from the foregoing studies. Hence, the mean P-to-casein ratio (~ 33-35 mg/g casein) for milk from each of the different feeding systems (data not shown) was comparable to that (25.9 mg/g casein) reported by Keogh et al. (1982). Moreover, the molar ratio of Ca-to-P (1.01-1.06) of milk from the three feeding systems was comparable to that (0.96) reported by Sola-Larrañaga et al. (2009), but lower than that (1.44) found by Keogh et al. (1982).

The concentration of P was affected by feeding system, lactation stage and their interaction. The mean P content of the GRO milk over the experimental period was ~ 3-5 mg/100g higher than that of the corresponding GRC or TMR milk. The mean P content of LL milk was significantly higher than that of ML milk. Owing to the interactive effect of feeding system and lactation stage, the increase in P content of milk on advancing from ML to LL was significant in GRC and TMR milk but not in GRO milk.

The mean level of sedimentable P ranged from 62 % to 68 % of total P. Pearson correlation analysis of the entire data set indicated a significant positive correlation between P and casein ($p < 0.001$) and between P and Ca ($p < 0.001$). The level of sedimentable P was affected by feeding system, lactation stage and their interaction (data not shown), with the mean values of sedimental P over the lactation being higher in GRO milk (67.6 %) than in GRC milk (62.4 %); and with LL milk (70.3 %) being higher than ML milk (58.4 %).

Magnesium (Mg)

Magnesium partitions between the casein micelle and the serum phase of milk. In the micelle, Mg complexes with Ca and phosphate (PO_4^{3-}) to form small inclusions or nanoclusters (~ 2 nm diameter) that attach to the micellar matrix of caseins via serine phosphate groups, and Mg in the serum phase occurs as a co-ion to citrate and inorganic phosphate (de Kruif et al., 2012). The range of Mg across all milks was of similar magnitude to that previously reported in literature (White and Davies, 1958; Keogh et al., 1982;

O'Brien et al., 1999b; Rodríguez-Rodríguez et al., 2001). The mean Mg content was

Table 4.2: Seasonal variation in the concentration of macro-elements, and the proportions that sediment with casein on ultracentrifugation, in skim milk from cows on different feeding systems.[1]

	Effect of feeding system				Effect of lactation stage		Effect of interaction Feeding system x lactation stage			
Item	Feeding system	Mean	SED	Range	Lactation stage	Mean	Feeding system in mid lactation (ML)	Mean	Feeding system in late lactation (LL)	Mean
Macro elements total concentration in milk (mg/100 g)										
Calcium	GRO	142.2^{a}	1.4	132.7 – 150.4	ML	131.1^{b}	GRO-ML	137.7^{b}	GRO-LL	146.7^{a}
	GRC	133.7^{b}		127.1 – 142.1	LL	140.6^{a}	GRC-ML	129.0^{cd}	GRC-LL	138.3^{b}
	TMR	131.8^{b}		122.3 – 139.5			TMR-ML	126.7^{d}	TMR-LL	136.8^{cb}
Phosphorous	GRO	104.0^{a}	0.763	100.5 – 108.5	ML	98.8^{b}	GRO-ML	102.5^{ab}	GRO-LL	105.5^{a}
	GRC	98.9^{b}		92.2 – 109.6	LL	103.8^{a}	GRC-ML	97.9^{c}	GRC-LL	102.0^{ab}
	TMR	101.0^{b}		95.8 – 104.8			TMR-ML	95.9^{bc}	TMR-LL	104.1^{a}
Sodium	GRO	49.1^{a}	0.767	42.8 – 58.2	ML	45.2^{b}	GRO-ML	46.6^{ab}	GRO-LL	51.5^{a}
	GRC	47.0^{b}		42.4 – 54.6	LL	50.0^{a}	GRC-ML	44.5^{b}	GRC-LL	49.0^{ab}
	TMR	46.7^{b}		42.8 – 53.6			TMR-ML	44.5^{b}	TMR-LL	49.4^{ab}
Magnesium	GRO	13.4^{a}	0.193	11.8 – 15.0	ML	12.4^{b}	GRO-ML	12.7^{bc}	GRO-LL	14.2^{a}
	GRC	13.0^{a}		11.7 – 14.5	LL	13.9^{a}	GRC-ML	12.2^{c}	GRC-LL	13.8^{ab}
	TMR	13.0^{a}		11.5 – 14.0			TMR-ML	12.2^{c}	TMR-LL	13.8^{ab}
Trace-elements total concentration in milk (μg/kg)										
Zinc	GRO	4589^{ab}	600	4080 – 5320	ML	4467^{b}	GRO-ML	4480^{ab}	GRO-LL	4698^{ab}
	GRC	4417^{b}		4151 – 4770	LL	4753^{a}	GRC-ML	4307^{b}	GRC-LL	4526^{ab}
	TMR	4822^{a}		4357 - 5171			TMR-ML	4611^{ab}	TMR-LL	5034^{a}
Iron	GRO	542^{a}	66	263 – 1282	ML	472^{a}	GRO-ML	480^{a}	GRO-LL	603^{a}
	GRC	504^{a}		235 – 1663	LL	445^{a}	GRC-ML	597^{a}	GRC-LL	410^{a}
	TMR	331^{a}		215 – 575			TMR-ML	339^{a}	TMR-LL	322^{a}
Copper	GRO	60.3^{b}	3.4	47.9 – 117.0	ML	64.8^{a}	GRO-ML	70.9^{ab}	GRO-LL	49.8^{ab}
	GRC	47.2^{b}		33.8 – 77.9	LL	58.1^{a}	GRC-ML	45.7^{b}	GRC-LL	48.7^{b}
	TMR	76.9^{a}		71.9 – 82.6			TMR-ML	77.8^{a}	TMR-LL	75.9^{a}

Molybdenum	GRO	45.9[a]	1.28	39.5 – 57.6	ML	42.3[b]	GRO-ML	47.1[ab]	GRO-LL	44.6[ab]
	GRC	43.4[a]		36.4 – 55.4	LL	48.2[a]	GRC-ML	39.1[b]	GRC-LL	47.8[ab]
	TMR	46.4[a]		36.8 – 59.1			TMR-ML	40.7[ab]	TMR-LL	52.1[a]
Manganese	GRO	42.5[a]	2.16	33.7 – 71.2	ML	39.1[a]	GRO-ML	43.2[a]	GRO-LL	41.8[a]
	GRC	40.7[ab]		27.1 – 62.1		35.8[a]	GRC-ML	45.0[a]	GRC-LL	36.3[a]
	TMR	29.2[b]		22.6 – 37.3			TMR-ML	29.0[a]	TMR-LL	29.3[a]
Selenium	GRO	15.7[b]	1.15	13.5 – 18.4	ML	18.4[a]	GRO-ML	16.1[b]	GRO-LL	15.2[b]
	GRC	13.9[b]		10.3 – 17.1	LL	19.4[a]	GRC-ML	13.6[b]	GRC-LL	14.2[b]
	TMR	27.1[a]		23.7 – 30.0			TMR-ML	25.6[a]	TMR-LL	28.7[a]
Cobalt	GRO	0.80[a]	0.08	0.26 – 2.34	ML	0.62[b]	GRO-ML	0.54[a]	GRO-LL	1.06[a]
	GRC	0.75[a]		0.44 – 1.47	LL	0.96[a]	GRC-ML	0.66[a]	GRC-LL	0.83[a]
	TMR	0.82[a]		0.55 – 1.50			TMR-ML	0.66[a]	TMR-LL	0.98[a]

[1]Values within a column relating to effect of feeding system, lactation stage or interaction and not sharing a common superscripted letter a-c, a-b or a-e, respectively differ significantly ($p < 0.05$). Feeding system: GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): Mid lactation (June 17- September 9, 2015; 119-203 DIM) and late lactation (September 22 – November 26, 2015; 217-281 DIM).

unaffected by feeding system. For all feeding systems, Mg in LL milk was significantly higher than that of the corresponding ML milk. Similar seasonal trends were noted by Keogh et al. (1982) and O'Brien et al. (1999b). The higher content of Mg in LL milk reflects its higher casein content; hence, Pearson correlation analysis indicated significant positive correlations between Mg and total casein ($p < 0.001$), and Ca and P ($p < 0.001$).

The proportion of sedimentable Mg ranged from 36 to 44 % of total. Analogously, Fransson & Lönnerdal (1983) and Mekmene et al. (2009) found that most of the Mg (> 62 %) in cow's milk was serum soluble. Sedimentable Mg was positively correlated with sedimentable Ca and P ($p < 0.001$), supporting its association with Ca and P in the casein micelle. The proportion of sedimentable Mg was unaffected by feeding system, but was significantly affected by lactation stage and the interaction of lactation stage and feeding system (Table 4.2). The concentration of Mg in LL milk was higher than that in ML milk; this trend concurs with the higher casein content in LL milk.

Sodium (Na)

The spread of Na concentration across the milks from the different feeding systems was relatively narrow compared to that reported previously, i.e., 23-87 mg/100 g (Keogh et al., 1982; Rodríguez-Rodríguez et al., 2001; Sola-Larrañaga et al., 2009). The mean Na content was affected significantly by lactation stage and the interaction of lactation stage and feeding system, but was unaffected by feeding system (Table 4.2). Overall the concentration of Na in LL milk was significantly higher than that of ML milk. The higher concentration of Na in LL milk is consistent with the previous study (Keogh et al., 1982) and its lower lactose content (Fox et al., 2015); as the lactose content decreases in late lactation, the concentrations of Na^+ and Cl^- increase to maintain the osmotic pressure of milk in the mammary gland isotonic with that of the blood in the lactating cow (Fox, 2011).

Regression analysis of the current data indicated that Na was inversely correlated with lactose content ($p < 0.05$).

The current results showed that the mean concentration of sedimentable Na was 17.3-22.5 % of total. It is generally assumed that all Na is soluble or non-sedimentable (Holt & Jenness, 1984; Gaucheron et al., 2013). The proportion of sedimentable Na was unaffected by feeding system but increased significantly with lactation, as indicated by the higher in levels in LL milk than in ML milk (Fig. 4.2).

4.4.4 Trace-elements in skim milk

The mean and range of concentrations of trace elements are shown in Table 4.2. Seasonal variation in the concentration of trace-elements and the proportion of each element that sedimented on ultracentrifugation (100,000 X *g* for 1 h) at 25 °C from three different feeding systems are shown in Fig. 4.3. For all feeding systems, the mean concentration decreased in the following order: Zn > Fe > Cu > Mo > Mn > Se > Co. The range of concentration (μg/kg) for each of these elements was comparable with that reported in the literature: Zn, 2300-6600; Fe, 200-1500; Cu, 34-220; Mo, 27-52; Mn, 10-299; Se, 6.8-29; and Co, 0.2-2.0 (Moreno-Rojas et al., 1993; O'Brien et al., 1999b; Cashman, 2011b; Rodríguez-Rodríguez et al., 2001; Pechová et al., 2008; Sola-Larrañaga et al., 2009; Nantapo & Muchenje, 2013)

Zinc (Zn)

The concentration of Zn was significantly affected by feeding system, lactation stage, and their interaction (Table 4.2; Fig. 4.3). The mean level in TMR milk over the experimental

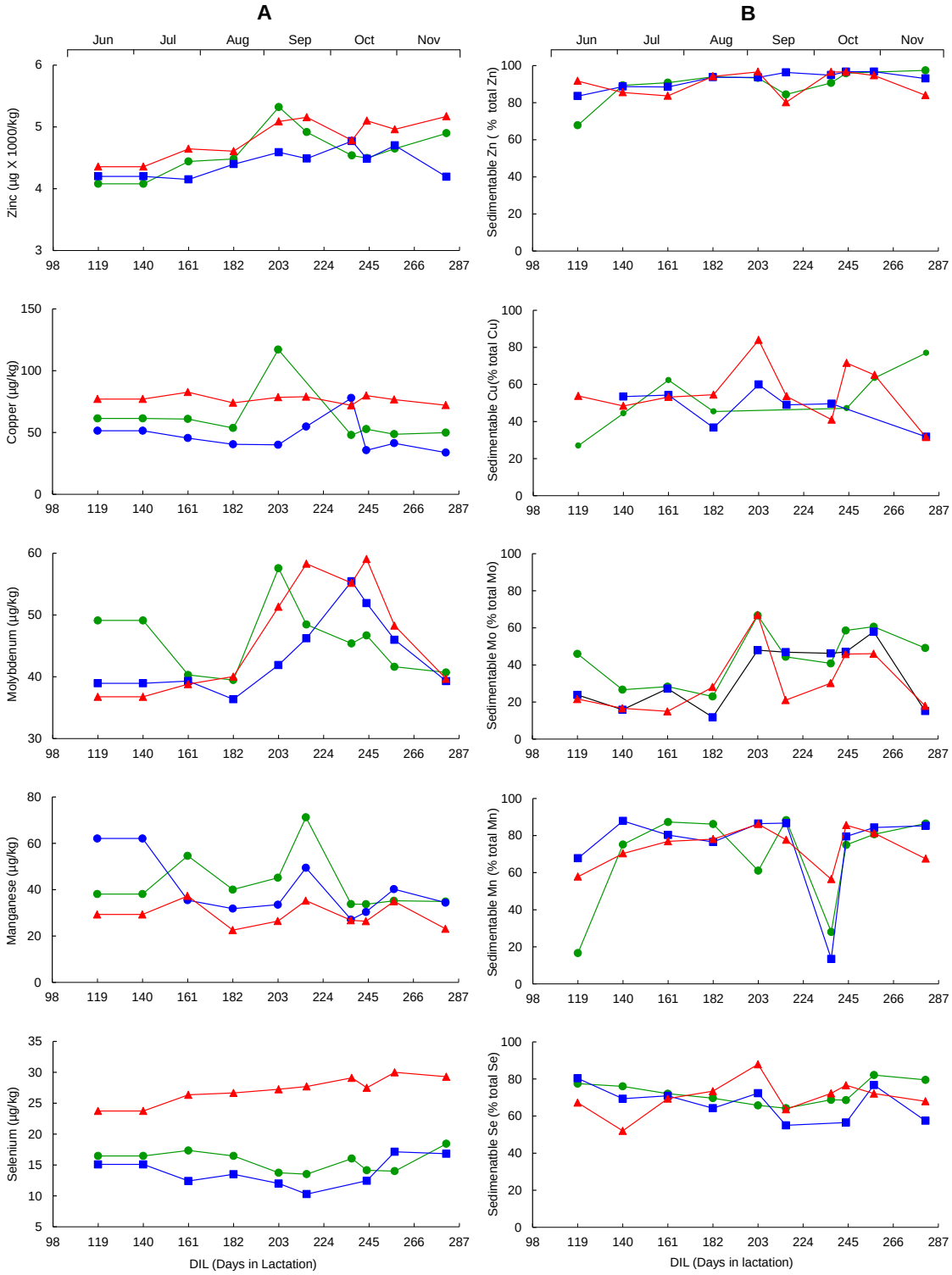

Figure 4.3. Seasonal variation in the concentration of trace elements in skim milk from spring-calved herds fed using different feeding systems: total concentration (A) and the proportion of each element that sedimented with the casein on ultracentrifugation at 100,000 g for 1 h at 25 °C (B). The different feeding systems were grazing perennial ryegrass, GRO (●), grazing perennial ryegrass and white clover pasture, GRC (■), or housed indoors and offered total mixed ration, TMR (▲). Milk from each of the feeding systems was analysed over the period June 17 to November 26, 2015.

period was significantly higher than that in GRC; however, TMR milk had the highest ($p < 0.05$) mean ratio of Zn-to-protein, i.e., 126 μg/g compared to ~ 110 μg/g in GRO or GRC milk. Late-lactation milk had significantly higher (by ~ 300 μg/kg) Zn than in ML milk, the current results concurred with those of Nantapo & Muchenje (2013) who found the Zn content of South African milk from cows fed on pasture, containing *Lolium multiflorum* L. and *Trifolium repens* L., was higher in Winter (4560 μg/kg) than in Spring (4250 μg/kg).

In agreement with other studies (de la Fuente et al., 1996), Zn was predominantly (~ 90 %) sedimentable. The high proportion of sedimentable Zn in milk has been attributed to its association with the casein micelle, most likely by interactions with phosphoseryl residues on the caseins, and calcium phosphate (Gaucheron, 2013). The mean proportion of sedimentable Zn was unaffected by feeding system or lactation stage.

Iron (Fe)

Fe concentrations showed large variation, ranging from 215 to 1663 μg/kg (Table 4.2). Other studies have also reported large seasonal variation in Fe content, for example, 460-1490 μg/kg (O'Brien et al., 1999b), 190-1000 μg/kg (Rodríguez-Rodríguez et al., 2001), and 780-1560 μg/kg (Nantapo & Muchenje, 2013). The mean concentration was comparable to that reported by Moreno-Rojas et al. (1993), i.e., 440 μg/kg. Feeding system or lactation stage did not affect the concentration in milk.

Copper (Cu)

Cu in milk is of relevance as it can induce lipid oxidation in dairy products such as liquid milk and butter (Wedding & Deeth, 2009); Cu can also promote oxidation of proteins (Ramirez et al., 2005) which can impair the nutritional status of the protein (Meyer et al.,

2012). The concentration of Cu was affected by feeding system and the interaction of feeding system and lactation stage, but not by lactation stage (Table 4.2). Milk from TMR feeding system had the highest ($p < 0.05$) mean concentration of Cu over the experimental period, and Cu-to-protein ratio, i.e., 2.01 μg/g compared 1.46 μg/g in GRO milk or 1.20 μg/g in GRC milk. Analogously, other studies have also found that the Cu content of milk increases when cows were brought indoors from pasture in late autumn and switched to a diet that relies more on concentrates (Ford et al., 1986; O'Brien et al., 1999b).

The mean proportion of sedimentable Cu varied from 35-56 % of total (Fig. 4.3), and was not affected by feeding system or lactation stage. The results were in alignment with the findings of previous studies which found that Cu partitions with fat globules, sedimentable casein and serum (specifically with whey proteins and low molecular weight protein fractions with molecular mass <10kDa) at levels of ~ 2, 44 and 55 % of total, respectively (Fransson & Lönnerdal, 1983; Al-Awadi & Srikumar, 2001; Gaucheron, 2013).

Molybdenum (Mo)

Milk may contribute as much as ~ 36 % of the recommended daily allowance for Mo. It is an essential component of several enzymes, including xanthine oxidase, which is associated mainly with the fat globule membrane (Cashman, 2011b). The range and mean of Mo concentration for the GRO, GRC and TMR milk samples are shown in Table 4.2. Mo concentration was not affected by lactation stage ($p < 0.05$), but not by feeding system. The mean concentration of Mo in LL milk was higher than that in ML milk. The mean proportion of sedimentable Mo ranged from 31-44 % of total and was not affected by

feeding system or lactation stage. The authors are unaware of published data on the partitioning of Mo between sedimentable and serum phases in milk.

Manganese (Mn)

The mean concentration in the GRO milk was significantly higher than that in the TMR milk (Table 4.2) and ~ 69-75 % of total Mn was sedimentable (Fig. 4.3), which was similar to that reported previously. Mn content was unaffected by lactation stage or the interaction of feeding system and lactation stage, and sedimentable Mn was unaffected by feeding system or lactation stage.

Selenium (Se)

The concentration of Se was affected by feeding system but not by lactation stage. The mean concentration of Se in TMR milk was significantly higher than that of GRO or GRC milk (Table 4.2; Fig. 4.3); the Se-to-protein ratio of TMR milk (0.67 μg/g) was also higher ($p < 0.05$) than that of GRO or GRC milk (0.38 μg/g) or GRC milk (0.25 μg/g). Considering that the Se content in milk increases with the level in the diet (Givens et al., 2004), the higher Se in TMR milk may reflect a higher Se content in TMR compared to pasture, owing to its inclusion of molasses, a relatively rich source of Se (Givens et al., 2004).

The level of sedimentable Se was unaffected by feeding system or lactation stage. The high proportion of sedimentable Se, which has also been reported in previous studies (~ 55-75 %, Knowles et al., 1999), reflects its binding to milk protein, especially casein (van Dael et al., 1991). In contrast, Al-Awadi and Srikumar (2001) found that ~ 80 % of Se

in skim milk was found in the serum where it was associated with whey proteins and low molecular weight protein fractions.

Cobalt (Co)

The mean concentration was unaffected by feeding system but was affected by lactation stage, with the level in LL milk being higher than that of ML milk (Table 4.2). The range and mean of Co concentration are comparable to those given by Cashman (2011b), i.e., range, 0.4-1.1 μg/kg, with a mean 0.5 μg/kg. The data of O'Brien (1999b) showed a wider range (0.2-2.7 μg/kg) and higher mean concentration (1.0-1.3 μg/kg).

4.4.5 Correlation of variables

The data for all milk samples collected over the experimental period were analysed by linear regression to establish potential inter-relationships. The analysis indicated significant relationships, denoted by S1, S2 and S3, at $p < 0.001$, 0.01 and 0.05, respectively. The analysis indicated significant positive correlation between the concentration of protein or casein and the total, and sedimentable, concentrations of Ca, P, Mg Na and Zn ($p < 0.01$) and Mo ($p < 0.05$). There were also significant inter-relationships between the concentrations of the macro-elements, Ca, P, Mg and Na ($p < 0.01$). Positive correlations between macro-elements and trace elements included: Ca or Mg with Zn, Fe, Mo and Co; P with Zn and Mo, and Na with Mo ($p < 0.05$). The strong correlations between Ca, P and Mg are consistent with the pivotal role played by these elements in casein micelle formation and stability (Holt & Jenness, 1984; de Kruif et al., 2012). The concentrations of Cu and Se, both of which were high in TMR milk, were positively correlated ($p < 0.001$).

4.5 Conclusion

This study investigated the effect of three different feeding systems (perennial ryegrass, GRO; perennial ryegrass and white clover, GRC; or total mixed ration, TMR) on composition and yield of spring-calved herd milk. Feeding system influenced the concentration and yield of TS, fat, lactose, protein, casein, macroelements, and trace-elements. The changes in milk composition with feeding system have potential implications for product manufacture and quality. The significantly higher protein and casein content of GRO milk, despite the lower milk yield, would be advantageous in terms of reducing the plant capacity required for the manufacture of dairy products and ingredients. Differences in the concentration of milk protein and the ratio of individual elements-to-protein are of particular relevance when formulating products with target levels of protein and minerals from dairy ingredients, for example, beverages such as IMF, and medical or therapeutic applications. Nevertheless, the consistency of any feeding system is likely to vary due to differences in grazing strategies, type and level of fertilisation, soil type and weather in the case of pasture, and to the proportions and consistency of ingredients used in TMR formulation.

Chapter 5

Grazing dairy cows on pasture versus indoor feeding on total mixed ration: effects on compositional and processing characteristics of milk in mid-lactation

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27th European Grassland Federation (EGF) General Meeting on, Sustainable meat and milk production from grasslands

5.1 Abstract

This study investigated the effect of feeding system on the compositional, physico-chemical and processing characteristics of bovine milk in mid-lactation. Three feeding systems were compared: cows grazing on perennial ryegrass pasture (GRO), cows grazing on perennial ryegrass and white clover pasture (GRC), and cows housed indoors offered total mixed ration (TMR). Milk was collected on 6 occasions in mid-lactation, over the period June 17 to September 22, when the cows were 119 to 216 days in milk. Relative to TMR milk, GRO milk had higher mean concentrations of total protein (~ 0.26 %, w/w) and casein (~ 0.24 %, w/w), a higher proportion of α_{s2}-casein, and enhanced rennet gelation, as evidenced by higher gel-firming rate and gel firmness at 40 min. The GRC milk was intermediate between GRO and TMR milk for most parameters. Feeding system did not affect the mean levels of non-protein nitrogen (NPN), urea N, ionic calcium or heat stability at 140 °C in the pH range 6.2 – 7.2.

5.2 Introduction

Milk composition is of relevance in dairy processing as it affects processing characteristics such as rennet gelation (Guinee et al., 1997), heat stability (Huppertz, 2016) and ethanol stability (Horne, 2016), and the manufacturing efficiency, composition, and quality of dairy products, such as milk powders (Auldist et al., 1996b), cheese (Fox et al., 2017) and beverages (Deeth & Lewis, 2017). Composition, including the concentrations of fat, protein and lactose, of cow's milk are affected by several factors including stage of lactation, diet/nutrient intake, breed, genetic merit, health, calving pattern (i.e., year-round calving or compact calving over a condensed period in Spring or Autumn) and environmental conditions (Auldist & Hubble 1998; Mackle et al., 1999; Lin et al., 2017b).

In general, the effect of diet on milk composition depends on the type of feed offered and the level of intake. Several studies reported that an increase in the DHA (e.g. from ~ 60 to 120 % of normal) coincides with an increase in casein and a reduction in lactose, to an extent dependent on the difference in DHA and cow phenotype (O'Brien et al., 1997; Auldist et al., 2000b). However, the increase in casein concentration is relatively small (< 0.2 %) with little effect on rennet gelation or cheese-yielding properties of the milk (O'Brien et al., 1997; Guinee et al., 1998). Auldist et al. (2000b) found that restriction of DHA to ~ 40 % of *ad libitum* intake led to lower proportions of α_s- and β-caseins and a higher proportion of γ-casein in early lactation-milk (60 DIM) but not in mid-lactation milk (180 DIM). Mackle et al. (1999) reported that partial substitution of pasture with grain and silage when cows were 203±14 DIM did not affect the concentrations of total protein or casein, but resulted in higher casein number (casein as a % of total protein) and lower levels of NPN and urea, as proportions of total N. A more recent study (Auldist et al., 2016) found that varying the type or level of supplement (wheat/corn/canola, alfalfa hay, partial mixed ration) did not significantly affect the concentrations of protein and casein, or the rennet gelation properties of milk from pasture-fed cows at 45 DIM.

A number of studies have compared grazing on pasture to feeding total mixed ration to cows housed indoors for their effects on milk yield, gross composition and fat composition (McAuliffe et al., 2016; O'Callaghan et al., 2016b). Auldist et al. (2000a) found that milk from pasture-fed cows had significantly higher mean levels of fat, protein and casein, a lower concentration of lactose, and a similar concentration of urea to that of milk from TMR-fed cows over the early- to mid- lactation stage (September - April). The authors are unaware of previous studies on the comparative effects of pasture grazing and TMR on casein profile or processing characteristics.

5.3 Aims of study

The current study investigated the effect of different feeding systems on biochemical characteristics, rennet gelation properties and heat stability of mid-lactation milk (June 17 to September 22, 119 – 216 DIM), when ~ 40-50 % of the total milk for processing is produced in Ireland (Central Statistics Office, 2017). The feeding systems investigated were: cows grazing on perennial ryegrass pasture (GRO), cows grazing on perennial ryegrass and white clover pasture (GRC), and cows housed indoors offered TMR.

5.4 Results and discussion

Milk was collected from herds on three different feeding systems in mid-lactation from June 17 (119 DIM) to September 22 (216 DIM) at 2-3 weekly intervals on 6 occasions and was analysed for nitrogen fractions, individual caseins and whey proteins (RP-HLPC), rennet-coagulability and heat stability at 140 °C and pH values 6.2-7.2. The data from the three feeding systems (GRO, GRC, TMR) over the total period were analysed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute, 2011). Tukey's multiple-comparison test was used for paired comparison of treatment means and the level of significance was determined at $p < 0.05$. R-3.2.2 software (R Core Team, 2014) was used to compute a Pearson correlation between the different compositional variables, where significance was determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$, according to Students t-test.

5.4.1 Nitrogen fractions: Casein, Whey protein, non-protein—nitrogen (NPN, urea and serum casein)

The mean and range of nitrogen fractions in skim milk over the experimental period in mid lactation are shown in Table 5.1. Similar to the trends found in previous studies for spring-calving herds over the same period (June - September), the concentrations of protein and casein increased progressively over the period (Mehra et al., 1999; Auldist et al., 2000a), by ~ 0.83 % (w/w) and ~ 0.60 % (w/w), respectively. The relatively large seasonal change in concentrations of protein and casein during this period necessitated that the quantity of added coagulant or starter culture added to the milk is increased *pro-rata* with the content of protein, or more specifically casein, to optimise yield and ensure consistent composition and quality of products such as cheese, rennet casein or lactic-acid casein (Fox et al., 2017).

Feeding system affected the mean concentrations of protein and casein, but not that of serum casein, whey protein, NPN or urea. In agreement with the results of Auldist et al. (2000a), the mean concentrations of true protein and casein in the GRO milk were significantly higher than those of the GRC or TMR milks. The range of NPN (4.4 to 7.9% TN), urea (~ 17-45 mg/100 mL), and Urea N (27-48 % NPN) in the current study were similar to those reported previously for spring-calved herds or manufacturing milks (Kelly et al., 1982; Mackle et al., 1999; Mehra et al., 1999; Heck et al., 2009).

The mean content of serum casein ranged from 0.11-0.18 % (w/w) or 3.7 to 6.2 % total casein (Table 5.1); the level is comparable to that (5-6 % total casein) reported previously (Dalgleish & law, 1988; Lin et al., 2017b). High serum casein content (e.g., 0.76 %, w/w) is indicative of casein hydrolysis (e.g., by plasmin or somatic cell proteinases) or casein dissociation from the micelle on cold storage of milk for extended

Table 5.1: Nitrogen fractions, protein profile, rennet gelation and heat stability of milk from spring-calved herds on different feeding systems in mid-lactation.[1]

	GRO		GRC		TMR	
Item	Mean	Range	Mean	Range	Mean	Range
Total protein	3.82 [a]	3.57-4.02	3.70 [b]	3.55-3.93	3.56 [b]	3.29-3.81
Lactose	4.78 [b]	4.65-4.87	4.79 [b]	4.68-4.88	4.87 [a]	4.73-5.02
True protein (%, w/w)	3.51[a]	3.08-3.89	3.33 [b]	2.93-3.70	3.25[b]	2.80-3.56
Casein (%, w/w)	3.04[a]	2.65-3.42	2.88[b]	2.54-3.22	2.80[b]	2.43-3.07
Serum casein (% total serum casein)	3.71[a]	0-7.69	6.20[a]	0-9.83	3.66[a]	0.00-6.69
Whey protein (%, w/w)	0.67[a]	0.56-0.82	0.61[a]	0.51-0.71	0.60[a]	0.50-0.68
NPN (% TN)	6.52[a]	4.39-7.43	7.05[a]	5.67-7.88	6.38[a]	5.21-7.10
Urea (mg/100 mL)	29.4[a]	17.2-41.0	33.0[a]	22.8-45.2	29.5[a]	21.6-35.0
Urea N (% NPN)	34.1[a]	27.2-42.9	37.3[a]	26.0-43.8	38.1[a]	32.0-48.0
α_{s1}-casein (% total casein)	38.9[a]	36.1-42.7	37.8[a]	35.2-40.0	36.1[a]	38.3-42.0
α_{s2}-casein (% total casein)	12.2[b]	11.0-13.1	13.1[a]	10.8-14.8	11.3[c]	9.6-12.5
β-casein (% total casein)	34.2[a]	32.9-36.5	34.4[a]	33.1-37.0	35.5[a]	30.8-38.8
κ-casein (% total casein)	14.8[a]	12.1-18.1	14.8[a]	11.3-16.8	13.7[a]	10.3-16.0
α-Lac:β–Lg (-)	0.20[a]	0.16-0.23	0.23[a]	0.16-0.32	0.23[a]	0.21-0.26
Rennet gelation						
RCT (min)	13.3[a]	12.0-14.5	13.6[a]	12.1-14.9	14.1[a]	12.5-14.8
GFR_{max} (Pa/s)	0.093[a]	0.067-0.119	0.081[ab]	0.064-0.102	0.079[b]	0.062-0.101
G'_{40} (Pa)	117[a]	84-144	99[ab]	78-125	95[b]	75-122
Heat coagulation time (min)						
HCT_{npH} (min)	11.9[a]	4.0-21.5	13.0[a]	7.2-25.0	13.1[a]	7.7-23.0
HCT_{max} (min)	12.6[a]	8.7-18.3	14.2[a]	10.0-23.0	13.7[a]	8.9-21.3
HCT_{min}(min)	5.7[a]	4.6-8.7	5.4[a]	4.7-6.6	5.3[a]	4.1-6.3

[1] Values within a column relating to GRO, GRC or TMR and not sharing a common lower-case superscripted letter (a, b) differ significantly ($p < 0.05$) for the effect of feeding system.

periods; high levels (e.g., >15 % of total casein) have been associated with poor coagulability and high-moisture cheeses (Ali et al., 1980; O'Keeffe, 1984).

5.4.2 Protein profile

In contrast to the trend for casein concentration, the proportions of individual caseins did not change consistently over the experimental period (Fig. 5.1). The mean proportions of α_{s1}, α_{s2}, β and κ-caseins (as a % of total casein), and mean ratio of α-lactalbumin to β-lactoglobulin (β-LgA +β-LgB) are within the range of values reported previously, i.e., α_s-casein, 42.0-56.0 %; β-casein, 26.2-42.2%; and κ-casein, 8.9-19.8% (Hermansen et al., 1999; Mackle et al., 1999; Auldist et al., 2004; Heck et al., 2009; Lin et al., 2017b).

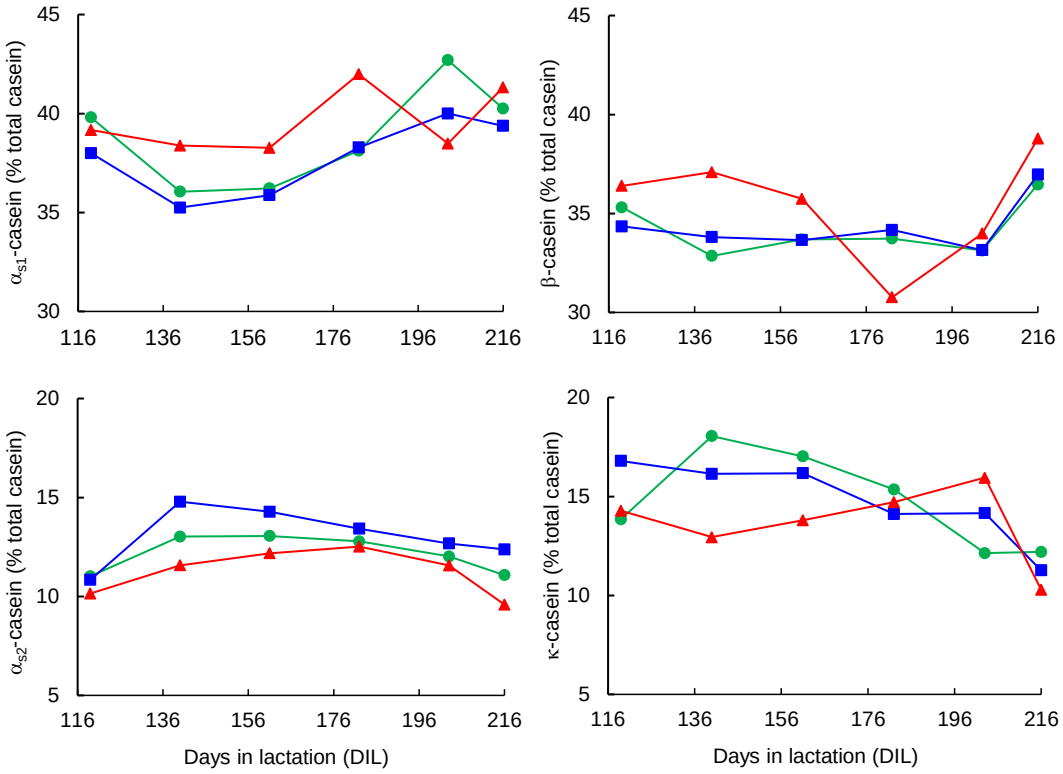

Figure 5.1: Changes in proportions of individual caseins in skim milk from spring-calved herds on different feeding systems during mid-lactation: grazed outdoors on perennial ryegrass pasture, GRO (●); grazed outdoors on perennial ryegrass and white clover pasture, GRC (■); or housed indoors and offered total mixed ration, TMR (▲). Milk from each of the feeding systems was analysed over the period from June 17 to September 22, 2015 (119-216 DIM).

Feeding system did not affect the mean proportions of the different caseins or α-La to β-Lg ratio, apart from α_{s2}-casein, the proportion of which was slightly, but significantly, higher in GRC milk than in TMR milk (Table 5.1). Yousef et al. (1970) reported that increasing the level of grain in the diet, by supplementing hay or silage with grain, led to significant increases in the concentrations of protein and casein, and α_s-casein as a proportion of total casein (from ~ 44.6 to 50.5 %). In contrast, Auldist et al. (2016) reported that supplementation of pasture ryegrass with corn grain and canola meal had no effect on the proportions of different caseins. The absence of an effect of feeding system on the ratio α-La to β-Lg ratio concurs with the results of Mackle et al. (1999), who found that supplementation of a pasture-diet with rolled maize grain or rolled maize + pasture silage did not affect the ratio α-La to β-Lg. The lack of a significant effect of feeding system on the protein profile in the current study is consistent with the conclusion of Thomas (1983), i.e., the changes in nutrition do not appear to have any impact on all of the major milk protein components.

5.4.3 Ionic Calcium and pH

The concentration of ionic calcium, [Ca^{2+}], affects the processability of milk, including its heat coagulation time, ethanol stability and rennet coagulability (Tsioulpas et al., 2007; Omoarukhe et al., 2010; Sandra et al., 2012). There was an upward trend in [Ca^{2+}] for each milk over the experimental period (Fig. 5.2); a similar trend was observed earlier for the concentrations of total calcium (Ca) and phosphorous (P) for the same milk samples (cf. Chapter 4). Nevertheless, the ratio of [Ca^{2+}]-to-casein remained relatively constant over the same period, while that of Ca and P-to-casein showed decreased (Fig. 5.2), indicating that the [Ca^{2+}] increased pro-rata with casein content, while that of Ca or P and did not.

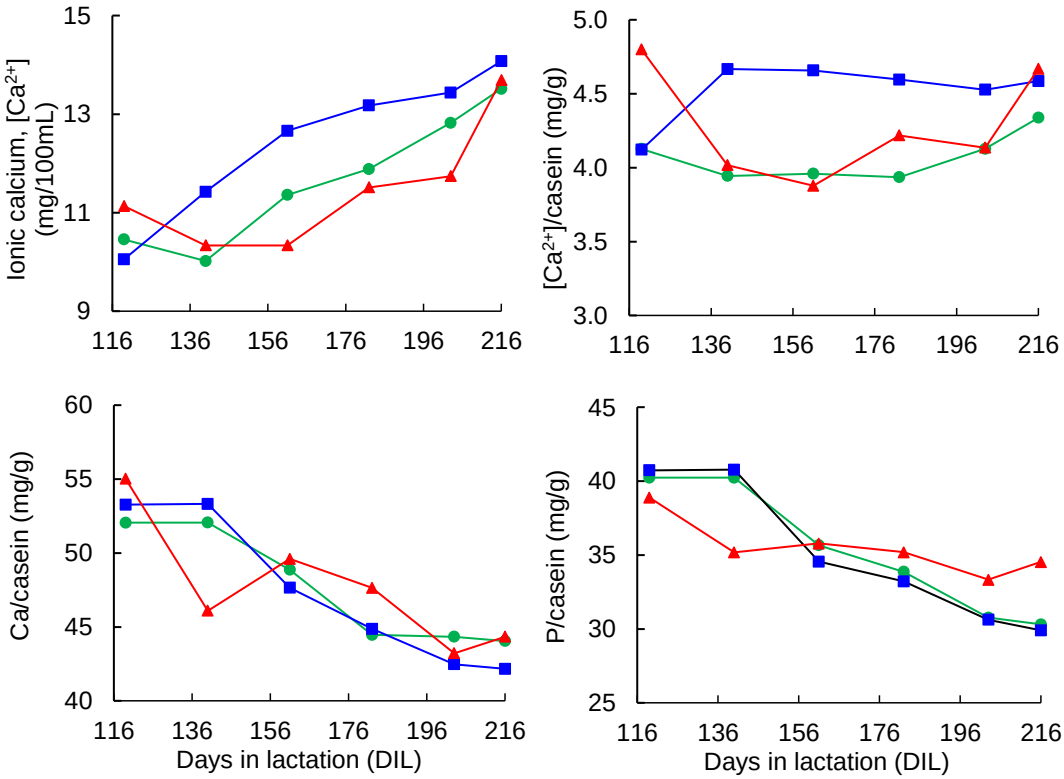

Figure 5.2: Changes in concentration of ionic calcium, [Ca^{2+}], and ratio of [Ca^{2+}]-, Ca-, or P-to-casein in skim milk from spring-calved herds on different feeding systems during mid-lactation: grazed outdoors on perennial ryegrass pasture, GRO (●); grazed outdoors on perennial ryegrass and white clover pasture, GRC (■); or housed indoors and offered total mixed ration, TMR (▲). Milk from each of the feeding systems was analysed over the period from June 17 to September 22, 2015 (119-216 DIM).

The range of [Ca^{2+}] for all milk samples over the experimental period, 10.0-14.1 mg/100mL, or 2.5-3.5 mM, was comparable to that (7.0-14.0 mg 100 mL^{-1}) reported in earlier studies for mid-lactation milk (Lewis, 2011; Bijl et al., 2013; Chen et al., 2014). The mean [Ca^{2+}] was not affected by feeding system. The pH of milk ranged from 6.60 to 6.78 (data not shown); the mean pH value, ~ 6.67, was not affected by feeding system and was similar in magnitude to that reported in previous studies for the same period of the season (Phelan et al., 1982; Mackle et al., 1999).

5.4.5 Rennet gelation

Changes in rennet gelation parameters over the experimental period are shown in Fig. 5.3. The rennet gelation of the GRO milk was stronger that of the TMR, as evidenced by its

higher values of GFR_{max} and G'_{40} (Table 5.1); the gelation behaviour of GRC milk did not differ from that of the GRO or TMR milk. The stronger gelation characteristics of the GRO milk are consistent with its higher content of casein (Guinee et al., 1997), which constitutes the para-casein gel network.

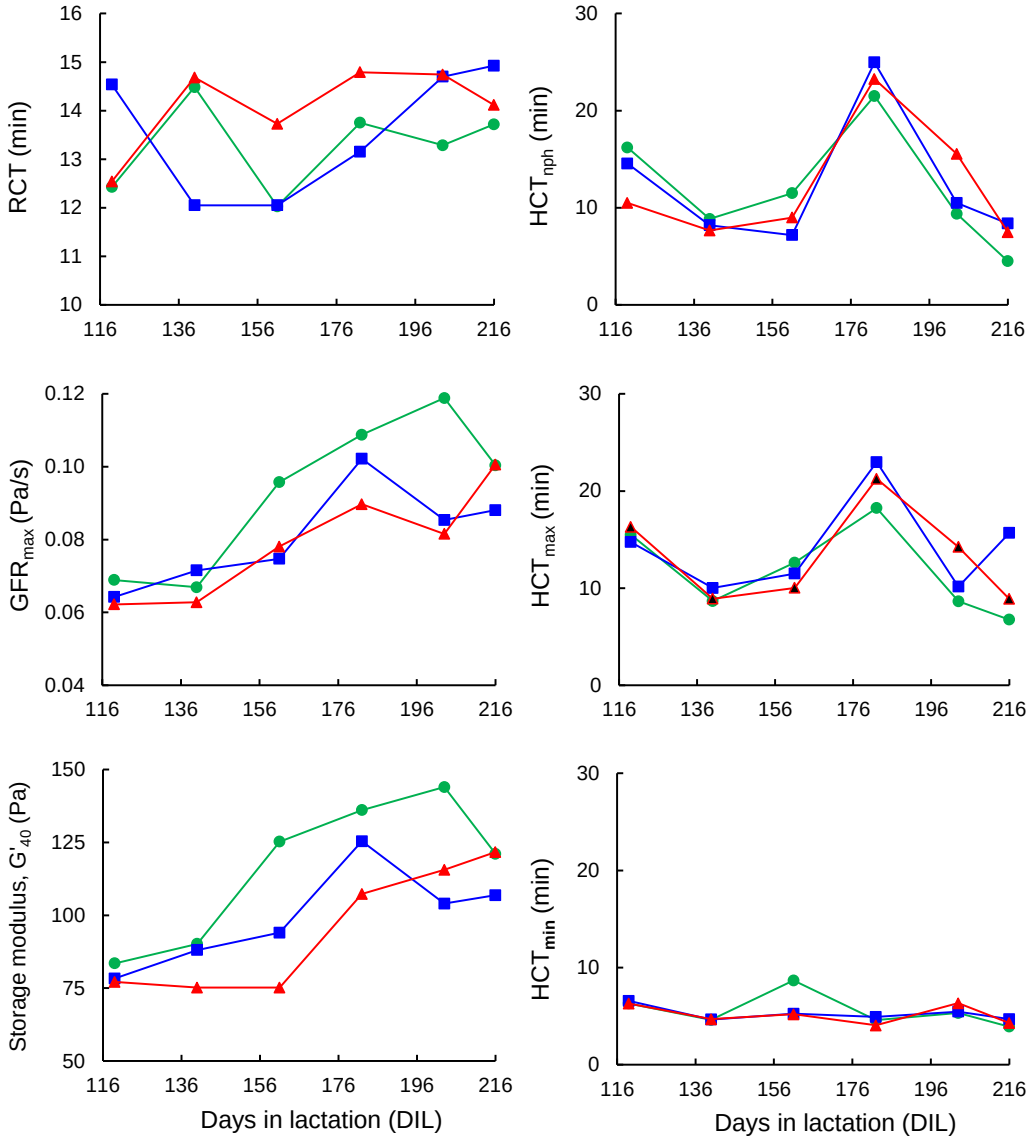

Figure 5.3: Changes in rennet gelation and heat coagulation time (HCT) of milk from spring-calved herds on different feeding systems during mid-lactation: grazed outdoors on perennial ryegrass pasture, GRO (●); grazed outdoors on perennial ryegrass and white clover pasture, GRC (■); or housed indoors and offered total mixed ration, TMR (▲). Milk from each of the feeding systems was analysed over the period from June 17 to September 22, 2015 (119-216 DIM). Rennet gelation parameters: RCT, rennet coagulation time; maximum gel firming rate, GFR_{max}; storage modulus at 40 min, G'_{40}. HCT parameters: HCT_{npH}, HCT at natural pH; HCT_{max}, maximum HCT; HCT_{min}, minimum heat coagulation time.

For all milk samples, GFR_{max} and G'_{40} tended to increase over the experimental period, a trend consistent with the associated increase in concentration of casein and [Ca^{2+}] (Guinee et al., 1997; Tsioulpas et al., 2007; Sandra et al., 2012). Linear regression analysis showed significant positive Pearson's correlation coefficient between the rennet gelation parameters, G'_{40} and GFR_{max}, and concentrations of casein and [Ca^{2+}] ($p < 0.01$). The importance of casein content in modulating the rennet coagulability of milk is further emphasised by seasonal increase in GFR_{max} and G'_{40} despite the simultaneous reduction in the ratio of Ca- and P-to- casein (Fig. 5.3). Several studies (Udabage et al., 2001; Choi et al., 2007) have shown that depletion of insoluble (micellar Ca) leads to a marked deterioration in the rennet-induced gelation of milk

5.4.6 Heat coagulation time

All milk samples displayed a typical type A pH/HCT profile with a HCT_{max} at pH 6.6–6.7 and HCT_{min} at pH 6.8-7.0. Changes in HCT parameters (HCT_{npH}, HCT_{max} and HCT_{min}) over the experimental period are shown in Fig. 5.3. The mean values for different HCT parameters were unaffected by feeding system (Table 5.1). There was no definite trend in HCT_{npH}, HCT_{max} or HCT_{min} over the experimental period. Similarly, Holt et al. (1978) and Kelly et al. (1982) found no systematic variation in HCT parameters of bulk spring-calved manufacturing milk over the period June to September. This trend probably reflects the interactive contributions of different compositional parameters on heat stability of milk; hence, the relatively high concentration of protein in the GRO milk might be expected to reduce HCT (Rattray & Jelen, 1996), the relatively low concentration of lactose (cf. Chapter 4) would likely enhance HCT (Huppertz, 2016).

5.5 Conclusion

This study investigated the effect of three different feeding systems in mid-lactation (June 17 to September 22, 2015; 119-216 DIM) on the composition and processing characteristics of milk from spring-calved Holstein Friesian herds. The feeding systems included cows grazing on perennial ryegrass pasture (GRO), cows grazing on perennial ryegrass and white clover pasture (GRC), or cows housed indoors offered total mixed ration (TMR). Feeding system significantly affected the concentrations of protein and casein and proportion of α_{s2}-casein, but not the mean concentrations of ionic calcium or urea, NPN as a proportion of total N, the proportions of α_{s1}-, β- or κ-casein, or the ratio of α-Lac-to-β-Lg. The changes in milk composition had a significant effect on rennet gelation, but not on heat stability over the pH range 6.2-7.2. Milk from GRO feeding system had higher mean values for casein content, gel firmness and gel firming rate compared to milk from TMR feeding system. The higher protein content of GRO milk may be of interest to the consumer and to cheese manufacturers; higher casein content would favour higher cheese yield and, thereby, reduce the plant capacity required for a given cheese output relative to TMR milk.

Chapter 6

Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid- and late- lactation

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6.1 Abstract

This study investigated the effects of three dairy cow feeding systems on the composition, yield, biochemical and physical properties of low-moisture part-skim Mozzarella cheese in mid- (ML, May-June) and late- (LL, October-November) lactation: grazing on perennial ryegrass (*Lolium perenne L.*) pasture (GRO), grazing on perennial ryegrass and white clover (*Trifolium repens L.*) pasture (GRC), or housed indoors and offered total mixed ration (TMR). Feeding system had significant effects on milk composition, cheese yield, the elemental composition of cheese, cheese colour (a*, b* colour coordinates), the extent of flow on heating, and the fluidity of the melted cheese (LT_{max}). Compared to TMR milk, GRO and GRC milks had higher concentrations of protein and casein, and lower concentrations of I, Cu and Se, higher cheese-yielding capacity, and produced cheese which had lower concentrations of the trace elements I, Cu and Se, and higher b* value and which were visually more yellow. Cheese from GRO milk had higher heat-induced flow and LT_{max} than cheese from TMR milk. These effects were observed over the entire lactation (ML+LL), but varied somewhat in ML and LL. Feeding system had little, or no, effect on gross composition of the cheese, the proportions of milk protein or fat lost to cheese whey, the texture of the unheated cheese, or the energy required to extend the molten cheese. Thus, the differences in colour and melt characteristics of cheeses obtained from milks with the different feeding systems may be provide a basis for creating points of differentiation, suited to different markets

6.2 Introduction

Milk composition is a key factor affecting cheese yield, the recoveries of fat and protein from milk to cheese, and, hence, the profitability of manufacturing plants (Fox et al., 2017).

Consequently, the effects of differences in the concentration of milk constituents, especially fat and protein, on cheese yield and component recoveries have been investigated extensively (Fox et al., 2017). Increasing milk protein in the range 3.0 to 4.5 % when maintaining a standard protein-to-fat ratio, generally results in higher cheese yield, but has little impact on protein recovery or cheese composition (Soodam & Guinee, 2018). The magnitude of the effect depends on the degree to which the protein concentration is increased and cheese-making conditions (Soodam & Guinee, 2018). Hence, the yield of cheese from late-lactation milk is higher than that of mid-lactation milk, because of the increases in the concentrations of total protein during lactation (Govindasamy-Lucey et al., 2005; Guinee et al., 2007).

Auldist et al. (2016) investigated the effect of varying the type and quantity of supplement (wheat grain, corn grain, canola meal, alfalfa hay) to cows grazed on perennial ryegrass. Altering the diet affected milk fat content, fatty acid profile and cheese yield, but not milk protein concentration, protein profile, or rennet gelation properties. More recently, O'Callaghan et al. (2016b, 2017) reported on the effect of feeding system on composition and quality of milk and Cheddar cheese; cows were grazed on pasture, either perennial ryegrass, or perennial ryegrass with white clover, or offered total mixed ration indoors (**TMR**). Significant effects of feeding system were observed for milk composition, and the fatty acid profile, colour, hardness, and sensory characteristics of the cheese. Cheese from milk produced by the pasture feeding systems had higher concentrations of β-carotene, lower weight proportions (g/100 g milk fat) of palmitic acid (C16:0) and linoleic acid (C18:2c) and a higher proportion of linolelaidic acid (C18:2t); being less-hard (softer) at 20 °C and more yellow in colour (O'Callaghan et al., 2017). We are unaware of any studies on the comparative effects of TMR and pasture-based feeding systems on Mozzarella cheese.

6.3 Aims of the study

The current study compared pasture- and TMR-based feeding systems for their effects on composition, yield, colour, texture and thermos-physical properties of low-moisture part-skim Mozzarella (**LMPS**) cheese, manufactured in mid lactation or late lactation. Milk was obtained from three spring-calving herds, each of which assigned to one of the following feeding systems: grazing on perennial ryegrass pasture (GRO), grazing on perennial ryegrass and white clover pasture (GRC), and housed indoors and offered total mixed ration (TMR).

6.4 Results and discussion

Cheese was made from milk from each feeding system (GRO, GRC and TMR) on three separate occasions in ML (94-110 DIM) and four in LL (234-260 DIM). The data were classified according to feeding system and lactation stage, and analysed using analysis of variance, (ANOVA) as a factorial design. The effects of lactation stage, feeding system, and their interaction were determined using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). Tukey's multiple-comparison test was used for paired comparison of means and the level of significance was determined at $p < 0.05$. Mid-lactation milk, denoted ML milk, refers to the composite of the milk samples collected from the herds on the GRO, GRC, and TMR feeding systems in mid lactation; the composite of the late-lactation milk samples was similarly denoted as LL milk. Whey, stretch water and cheese from milks in ML and LL were similarly denoted.

A split-plot design was used to evaluate the effects of feeding system, storage time, and their interaction on the biochemical and physical characteristics of cheese measured during storage. The data were analysed using the PROC MIXED procedure of SAS (SAS

Institute, 2011) with Tukey's multiple-comparison test for paired comparison of means at a significance level of $p < 0.05$. Similarly, the overall effects of feeding system and lactation stage were determined using the PROC MIXED procedure of SAS.

6.4.1 Standardised cheese milk composition

Gross composition

The composition of the cheese milk was affected by feeding system, lactation stage and their interaction to an extent depending on compositional parameter (Table 6.1). The concentration of protein in GRO and GRC milk was higher than that in TMR milk in ML, LL and ML+LL. Feeding system did not affect the mean lactose content of milk in ML or ML+LL, but did in LL when the lactose content in TMR milk was higher than in GRO milk. lactation stage significantly affected the composition, with LL milk having higher protein and fat and a lower concentration of lactose than ML milk. The overall effects of feeding system and lactation stage on gross composition of raw milk are similar to those reported previously (Auldist et al. 2000a; O'Callaghan et al., 2016b; cf. Chapter 4).

Elements

The concentrations of individual elements in the cheese milk from all feeding systems were within the ranges previously reported in bovine milk (O'Brien et al., 1999b; Rodríguez Rodríguez et al., 2001; Bijl et al, 2013; cf. Chapter 4) and are shown in Table 6.1. The concentration of I was high in TMR milk and low in the pasture-based milks compared to that reported in other studies, e.g., ~ 217 or 450 μg/kg in milk from cows with no dietary iodine supplement (O'Brien et al., 2013), or 200 to 530 μg/kg in creamery milk over the year (O'Brien et al., 1999b; O'Kane et al., 2016).

Table 6.1: Composition of standardised pasteurised cheese milk, from cows on different feeding systems in mid- and late-lactation.[1]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (*p*-values) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Fat (%, w/w)	3.00^{b}	3.01^{b}	2.71^{c}	0.036	3.39^{a}	3.29^{a}	3.02^{b}	0.031	<0.001	<0.001	0.297
Protein (%, w/w)	3.46^{c}	3.46^{c}	3.12^{d}	0.031	4.07^{a}	3.95^{a}	3.64^{b}	0.026	<0.001	<0.001	0.141
Lactose (%, w/w)	4.70^{a}	4.70^{a}	4.74^{a}	0.067	4.51^{b}	4.54^{b}	4.61^{a}	0.058	0.476	0.010	0.925
Macroelements (mg/100g)											
Ca	108.3^{bc}	114.3^{abc}	101.8^{c}	4.90	136.4^{a}	132.8^{a}	130.2^{ab}	4.25	0.254	<0.001	0.339
P	82.2^{a}	93.3^{a}	90.1^{a}	4.69	99.3^{a}	96.3^{a}	93.1^{a}	4.06	0.640	0.053	0.211
Na	39.5^{a}	44.1^{a}	46.9^{a}	7.56	51.7^{a}	51.9^{a}	51.3^{a}	6.54	0.877	0.190	0.866
Mg	9.2^{c}	9.9^{bc}	9.8^{bc}	0.61	13.5^{a}	12.7^{a}	12.2^{ab}	0.53	0.815	<0.001	0.266
Trace elements (μg/kg)											
Zn	3,796^{b}	3,855^{ab}	3,540^{ab}	248	4,257^{a}	4,114^{ab}	4,302^{a}	214	0.600	<0.001	0.303
I	177^{c}	67.1^{c}	640^{b}	133	190^{c}	133^{c}	1,245^{a}	115	<0.001	0.041	0.059
Cu	59.1^{bc}	54.2^{c}	94.1^{a}	5.34	42.7^{c}	39.3^{c}	72.0^{ab}	4.62	<0.001	0.001	0.767
Mo	33.3^{a}	34.9^{a}	45.7^{a}	3.57	47.3^{a}	42.0^{a}	47.4^{a}	3.09	0.068	0.013	0.215
Se	17.4^{c}	18.2^{c}	32.8^{a}	1.32	25.1^{b}	22.3^{bc}	32.1^{a}	1.14	<0.001	0.002	0.015

[1]Values within a row relating to mid lactation and late lactation and not sharing a common lowercase superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Presented data are the mean values of 3 replicate trials in mid lactation and 4 in late lactation. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): Mid lactation (May 23-June 8, 2016; 94-110 days in milk, DIM) and late lactation (October 10-November 5, 2016; 234-260 DIM). SED = standard error of difference between means.

The concentrations of macroelements (Ca, P, Mg and Na) were not affected by feeding system in ML, LL or ML+LL. Milk from TMR feeding system had higher concentrations of I, Cu and Se than GRO or GRC milks in ML, LL and ML+LL. The higher concentrations of I and Se in TMR milk is likely to reflect higher concentrations in the total mixed ration compared to pasture (Givens et al., 2004; Borucki Castro et al., 2012; O'Brien et al., 2013). Feeding system did not affect the concentrations of Zn and Mo.

Lactation stage had a significant effect on the concentrations of most elements, with LL milk having higher concentrations of Ca, Mg, Zn, I, Mo and Se than ML milk. In contrast, the concentration of Cu in ML was higher than in LL. Otherwise, lactation stage did not influence the concentrations of P or Na. The generally higher concentrations of Ca, Mg, Zn and Se in LL milks are consistent with the increase in protein, and hence, casein concentration in the milk; these elements are predominantly associated with the casein, as evidenced by their sedimentation with the casein during ultracentrifugation (Vegarud et al., 2000; cf. Chapter 4).

6.4.2 Cheese composition

Gross composition and pH

The compositions of the GRO, GRC and TMR cheeses in ML and LL (Table 6.2) were within the range previously reported for LMPS Mozzarella cheese (Kindstedt et al., 1995; Guinee et al., 2000; Feeney et al., 2001) and comply with the Codex Alimentarius Standard for low-moisture Mozzarella cheese (FAO/WHO, 2011) and the Code of Federal Regulations for LMPS Mozzarella (CFR, 2016).

All compositional parameters and pH at 1 day were unaffected by feeding system in ML, LL or ML+LL. The absence of an effect of feeding system on cheese composition is

consistent with the results of previous studies showing little impact of plane of cow nutrition or breed on LMPS Mozzarella (Guinee et al., 1998) or Cheddar cheese (Auldist et al., 2004; Auldist et al., 2016; O'Callaghan et al., 2017). Lactation stage had a significant effect on composition, with cheeses from LL milk having significantly higher content of moisture, salt-in-moisture and moisture-in-non-fat substances, and lower contents of protein, fat and fat-in-dry matter (FDM).

Elements

The contents of Ca, P and Na in the cheese were similar to those reported previously for low-moisture Mozzarella (USDA, 1976; Guinee et al., 2000; Feeney et al., 2001; Govindasamy-Lucey et al., 2007). The contents of Mg, Zn and Se are comparable to those reported previously for LMPS Mozzarella (USDA, 1976) or Mozzarella (Gaucheron, 2013), i.e., ~ 21 to 26 mg/100 g, 24600 to 31300 μg/kg, and 161 μg/kg respectively. In contrast, the Cu content was generally higher than that (~ 220 μg/kg) given by Gaucheron (2013) for Mozzarella. Inter-study differences in mineral content may relate to differences in: milk as influenced by diet and season (Nantapo & Muchenje, 2013; cf. Chapter 4), cheese-making conditions that alter the extent of moisture loss and mineral solubilisation at whey drainage, and elements present in the dry salt added to the curd or in the water used for curd plasticisation. We are unaware of any previous studies on the concentrations of I and Mo in LMPS Mozzarella cheese.

TMR cheese had higher mean concentrations of I, Cu and Se than the corresponding GRC or GRO cheeses in the overall lactation stage, ML+LL; however, the specific effect of feeding system on the concentrations of these elements in ML cheese or LL cheese depended on the element (Table 6.2). The high concentrations of I, Cu and Se in

TMR cheese are consistent with their significant higher concentration in TMR milk (Table 6.1). No significant differences were observed between the cheeses for concentrations of Ca, P, Na, Mg, Zn and Mo. Lactation stage did not affect the concentration of elements in cheese, apart from Zn, Cu and Mo, the contents of which were higher in ML cheeses than LL cheeses.

The recovery of elements, expressed as the weight of element in 100 g cheese as a percentage of the weight of element in milk required to produce 100 g cheese, were 58-77 % Ca, 55-67 % P, 25-37 % Mg, 96-109 % Zn, 74-93 % Cu, 19-33 % Mo, 46-63 % Se, and 11-29 % I. The significantly higher recovery value for Zn, and low values for Mg and Mo are consistent with the high proportion of sedimentable Zn, and low proportions of sedimentable Mg and Mo, in milk (Gaucheron, 2013; cf. Chapter 4). There was no effect of feeding system or lactation stage on the recoveries of different elements.

6.4.3 Component losses and cheese yield

The percentage of milk protein and fat lost in the combined whey and stretch water streams (Table 6.3), i.e., ~ 23.0-24.4 % (w/w) and ~ 26.0-28.2 % (w/w) respectively, are of similar magnitude to those reported previously by Guinee et al. (2000), i.e., ~ 24-27.0 % (w/w) protein and ~ 20-24 % (w/w) fat. The proportion of fat lost is markedly higher compared to that (~ 10-13 %, w/w of total fat) for cheeses such as Cheddar, Edam and Emmental (Antila et al., 1982; Fenelon & Guinee, 1999). The higher fat loss during the manufacture of LMPS Mozzarella has been attributed to the kneading and stretching of the curd in hot (~ 80 °C) water (Fox et al., 2017), which is conducive to shearing of the fat globule membrane, coalescence of fat into large pools (McMahon & Oberg, 2017) and leaching of free fat into

the stretch water. Hence, the current results show that ~ 13-15 % (w/w) of the total milk fat was lost in the stretch water (data not shown).

The percentage of total milk protein lost in the whey plus stretch water from TMR milk was 1-2 % lower than that of the corresponding whey from the GRO milk in LL and in ML +LL; such an effect was not observed in ML. The percentage of total milk fat lost in the combined whey and stretch water streams was unaffected by feeding system in ML, LL or ML+LL. lactation stage had no effect on the overall losses of fat or protein from milk to whey and stretch water.

Actual cheese yield (Y_a) varied from 8.7 to 11.5 kg/100 kg milk, which is typical of that reported for LMPS Mozzarella cheese (Guinee et al., 1998, 2000; Lilbæk et al., 2006). Y_a from GRO or GRC milk were higher than that from TMR milk ($p < 0.05$) in LL and ML+LL; however, Y_a from GRO or TMR were similar in ML. The generally lower Y_a of TMR cheese milk coincides with its lower protein concentration and fat content (Table 6.1), which are major determinants of cheese yield (Fox et al., 2017). The effect of differences in protein concentration and fat content of milk on cheese yield was confirmed by the absence of a significant effect of feeding system on normalised cheese yield (Y_n) in ML, LL or ML+LL, and by the similar normalised yields for corresponding GRC and TMR milks in ML and LL. Normalising yield to a reference milk with defined percentages of fat and protein and standardised to a fixed protein-to-fat ratio mitigates the effects of differences in milk composition on cheese yield.

6.4.4 Proteolysis

pH 4.6 soluble nitrogen (pH 4.6-SN), as a percentage of total N, increased in all cheeses during storage ($p < 0.05$), from ~ 2-3 % at 1 d to ~ 5 % at 50 d. The range for pH 4.6-SN

Table 6.2: Composition of low-moisture part-skim Mozzarella cheese from milk obtained using different feeding systems mid- and late-lactation.[1]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (*p*-values) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Moisture (%, w/w)	46.4^{b}	46.4^{b}	46.3^{b}	0.481	48.4^{a}	47.8^{ab}	47.1^{ab}	0.416	0.302	0.001	0.408
Protein (%, w/w)	30.4^{a}	29.0^{ab}	29.2^{ab}	0.574	26.6^{b}	26.5^{b}	27.0^{b}	0.497	0.257	<0.001	0.104
Fat (%, w/w)	21.4^{abc}	21.6^{a}	21.5^{ab}	0.293	20.1^{c}	20.3^{bc}	20.5^{abc}	0.253	0.686	<0.001	0.793
SM (%, w/w)	3.47^{a}	3.50^{a}	3.60^{a}	0.099	3.64^{a}	3.87^{a}	3.73^{a}	0.086	0.372	0.010	0.413
MNFS (%, w/w)	59.0^{a}	59.2^{a}	58.9^{a}	0.518	60.6^{a}	60.0^{a}	59.2^{a}	0.449	0.322	0.029	0.399
FDM (%, w/w)[5]	39.9^{a}	40.3^{a}	39.9^{a}	0.472	39.0^{a}	38.9^{a}	38.7^{a}	0.409	0.815	0.006	0.779
pH at day 1	5.36^{a}	5.40^{a}	5.39^{a}	0.022	5.42^{a}	5.42^{a}	5.39^{a}	0.019	0.136	0.241	0.826
Macroelements (mg/100g)											
Ca	899^{a}	845^{a}	838^{a}	51.2	810^{a}	726^{a}	816^{a}	36.2	0.343	0.053	0.529
P	578^{a}	557^{a}	561^{a}	24.6	549^{a}	489^{a}	542^{a}	21.3	0.125	0.476	0.812
Na	689^{a}	706^{a}	744^{a}	40.5	773^{a}	688^{a}	747^{a}	35.1	0.435	0.469	0.388
Mg	36^{a}	34^{a}	38^{a}	4.26	32^{a}	28^{a}	30^{a}	3.69	0.625	0.074	0.839
Trace elements (μg/kg)											
Zn (x 10^{3})	40.7^{a}	43.4^{a}	43.0^{a}	3.20	40.2^{a}	35.9^{a}	42.7^{a}	2.8	0.080	0.005	0.991
I	537^{bc}	123^{c}	1840^{ab}	325	175^{c}	150^{c}	2665^{a}	281	<0.001	0.521	0.173
Cu	580^{ab}	502^{ab}	914^{a}	108.6	338^{b}	298^{b}	517^{ab}	94.1	0.002	0.015	0.890
Mo	108^{ab}	95^{ab}	129^{a}	13.4	84^{ab}	71^{b}	91^{ab}	11.6	0.138	0.013	0.809
Se	114^{bc}	88^{c}	237^{a}	18.3	114^{bc}	93^{c}	171^{ab}	15.9	<0.001	0.170	0.107

[1]Values within a row relating to mid lactation and late lactation and not sharing a common lowercase superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Presented data are the mean values of 3 replicate trials in mid lactation and 4 in late lactation. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): Mid lactation (May 23-June 8, 2016; 94-110 days in milk, DIM) and late lactation (October 10-November 5, 2016; 234-260 DIM). SED = standard error of difference between means. SM = salt-in-moisture; MNFS = moisture-in-non-fat-substances; FDM = fat-in-dry-matter.

Table 6.3: Effect of different feeding systems and lactation stage on losses of total milk fat and protein to cheese whey and stretch water, and cheese yield during manufacture of low-moisture part-skim Mozzarella cheese in mid- and late-lactation.[1]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (*p*-values) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Losses (% of total)											
Protein	23.1^{ab}	24.2^{ab}	23.1^{ab}	0.370	24.4^{a}	23.9^{ab}	22.6^{b}	0.324	0.012	0.576	0.037
Fat	27.1^{a}	25.8^{a}	24.9^{a}	1.41	28.4^{a}	29.1^{a}	26.2^{a}	1.22	0.525	0.112	0.360
Cheese yield (kg/100kg milk)[5]											
Y_a (actual cheese yield)	9.30^{cd}	9.72^{bc}	8.68^{d}	0.166	11.48^{a}	10.96^{a}	10.11^{b}	0.144	<0.001	<0.001	0.020
Y_n (Normalised cheese yield)	9.14^{b}	9.54^{ab}	9.46^{ab}	0.124	9.59^{a}	9.44^{ab}	9.46^{ab}	0.107	0.574	0.007	0.071

[1]Values within a row relating to mid lactation and late lactation and not sharing a common lowercase superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Presented data are the mean values of 3 replicate trials in mid lactation and 4 in late lactation. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): Mid lactation (May 23-June 8, 2016; 94-110 days in milk, DIM) and late lactation (October 10-November 5, 2016; 234-260 DIM). SED = standard error of difference between means. Y_n = normalised cheese yield per 100 kg of milk normalised to reference fat (2.89 %, w/w) and protein (3.40 %, w/w) levels.

Table 6.4: Statistical significances (*p*-values) for changes in physicochemical properties, colour coordinates, texture and thermophysical properties of low-moisture part-skim Mozzarella cheeses from mid- and late-lactation milks from cows on different feeding systems.[1]

	Effects in mid lactation			Effects in late lactation			Overall effects	
Item	Feeding system (FS)	Storage time (ST)	Interaction FS*ST	Feeding system (FS)	Storage time (ST)	Interaction FS*ST	Feeding system (FS)	Lactation stage (LS)
Physico-chemical properties								
pH	-	***	-	-	***	-	-	-
WHC (g water/g protein)	-	***	-	*	***	-	-	***
pH4.6 SN/TN (g/100g)	-	***	-	-	***	-	-	***
Colour coordinates								
L* value	-	***	-	-	**	-	-	-
a* value	*	-	-	**	-	-	*	-
b* value	***	*	-	***	***	-	***	-
Texture								
Firmness	-	***	-	-	-	-	-	*
Cohesiveness (-)	-	-	-	-	***	-	-	-
Springiness (-)	-	-	-	-	-	-	-	-
Thermophysical properties								
Flowability (%)	***	***	-	**	***	-	***	-
Extension work, E_W (mJ)	-	***	-	-	***	-	-	***
CoTh (°C)	-	***	-	**	***	***	**	*
LT_{max} (-)	**	***	**	*	***	-	***	***
COTc (°C)	-	***	-	-	***	*	-	***

[1]Degree of freedom (df): 2 for feeding system, 4 for 50 d storage time of cheese, 8 for interaction of feeding system and storage time in mid- or late- lactation; 2 for effect of feeding system during overall lactation (ML+LL), and 1 for the effect of lactation stage across the different feeding systems. Significance levels: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; -, denotes $p > 0.05$. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): Mid lactation (May 23-June 8; 94-110 days in milk, DIM) and late lactation (October 10-November 5; 234-260 DIM). Abbreviation: WHC, water holding capacity.

was relatively low as compared to hard cheeses such as Cheddar (McCarthy et al., 2016a) but is typical for low-moisture part-skim Mozzarella (Yun et al., 1993a). It reflects the heat-induced denaturation of the coagulant at the high temperature (58-62 °C) during the plasticisation stage of manufacture (Feeney et al., 2001).

Feeding system had no effect on proteolysis in ML, LL or in overall lactation (ML+LL). Such a trend is consistent with the similar composition of cheeses from all three feeding systems (Table 6.2), and concurs with the findings of O'Callaghan et al. (2017) for Cheddar cheese. In contrast, lactation stage had an effect, with the overall mean percentage of pH 4.6-SN in LL cheeses being significantly higher than that of the corresponding ML cheeses.

6.4.5 Water holding capacity (WHC)

The WHC has been used as an index of the serum immobilised by the calcium phosphate *para*-casein network (Guinee et al., 2000). It increased progressively from ~ 1.4 to 1.6-1.8 g/g protein between 1 and 20 d, after which it remained constant (as no further serum was expressed). The increase during early storage reflects the hydration and swelling of the *para*-casein network (McMahon & Oberg, 2017), concomitant with proteolysis and calcium solubilisation (Guo & Kindstedt, 1995; O'Mahony et al., 2005).

The mean WHC of GRO cheese during storage was higher than that in TMR cheese in LL, but not in ML; otherwise, feeding system had no effect in ML, LL or ML+LL. The mean WHC of LL cheeses from the GRO, GRC, and TMR milks was higher than that of the corresponding ML cheeses (Table 6.4).

6.4.6 Texture profile analysis

The range of firmness (250-450 N) was comparable to that (i.e., ~ 320-420 N) previously reported for LMPS Mozzarella cheese, compressed under similar conditions (Guinee et al., 2001a). The values of cohesiveness (~ 0.27-0.52) and springiness (0.64-0.78), which are indices of the resistance of the cheese to fracture and size reduction, were within the range previously reported for Mozzarella and Kachkaval-type cheeses (Guinee et al., 2015; Henneberry et al., 2016); none of the cheeses fractured on compression by 75 % (data not shown).

The firmness of all cheeses from ML milk decreased significantly during storage from ~ 430 N at 1 d to ~ 230-320 N after 50 d ($p < 0.05$; Table 6.4). Similar trends have been previously reported for LMPS Mozzarella (Yun et al., 1993b; Moynihan et al., 2016) and have been attributed to the increases in proteolysis and protein hydration. Nevertheless, storage time did not affect the firmness of LL cheeses. Storage resulted in an increase in the cohesiveness of all LL cheeses ($p < 0.05$) but not in ML cheeses.

The mean values of firmness, cohesiveness, or springiness during storage were not significantly affected by feeding system in ML, LL or ML+LL. The current results differ from those of Combs et al. (2007), who stated that Cheddar cheese made from the milk of cows grazed on pasture (low-endophyte tall fescue with kura clover) was consistently softer than that from milk of cows fed on TMR (grain-based feed with alfalfa silage as the sole forage); no details were given on the conditions of ripening or texture measurement. O'Callaghan et al. (2017) found no difference in the mean firmness or cohesiveness of Cheddar cheeses, made from pasture- or TMR-based milk, when the cheese was tempered to 4 °C prior to rheological evaluation. Nevertheless, Cheddar cheese from TMR milk was significantly firmer than that from GRO or GRC milk, when the cheeses were tempered to

20 °C. The latter trend was attributed to the higher proportion of palmitic acid in fat from TMR milk (O'Callaghan et al., 2016b). Palmitic acid is the major fatty acid in milk fat and has a relatively high melting point (~ 63 °C) compared to oleic acid (~ 14 °C), the second most abundant fatty acid in milk fat (Huppertz et al., 2009; Knothe & Dunn, 2009).

Lactation stage had a significant effect on firmness (Table 6.4), the mean value for the ML cheeses being higher than that of the LL cheeses. Such an effect is consistent with the lower moisture content and pH 4.6-SN as a percentage of total N in the former (Visser, 1991; Watkinson et al., 2001).

6.4.7 Colour

The colour co-ordinates (L*, a*, b*) are shown in Fig. 6.1. On storage, a* did not change, b* increased in GRO and GRC cheeses, and L* decreased in all cheeses. Visually, the GRO and GRC cheeses became yellower, and the TMR cheese less white, during storage. The reduction in L* during aging, which has also been observed in other studies (Rudan et al., 1998; Sheehan et al., 2005), may be attributed partly to the reduction in free moisture pockets (droplets), and light scattering, as the water binding of the casein increases (Paulson et al., 1998). In contrast to the current results, O'Callaghan et al. (2017) found that the L*- and a*- values of Cheddar cheese increased, and b* values decreased, during maturation. It is suggested that the reduction in L* in the current study may have been due to the light-induced degradation of carotenoids and riboflavin (Juric et al., 2003). However, β-Carotene has been found to be quite stable during cheese maturation (Nozière et al., 2006). Cheese variety is also likely to affect the colour because of differences in fat content

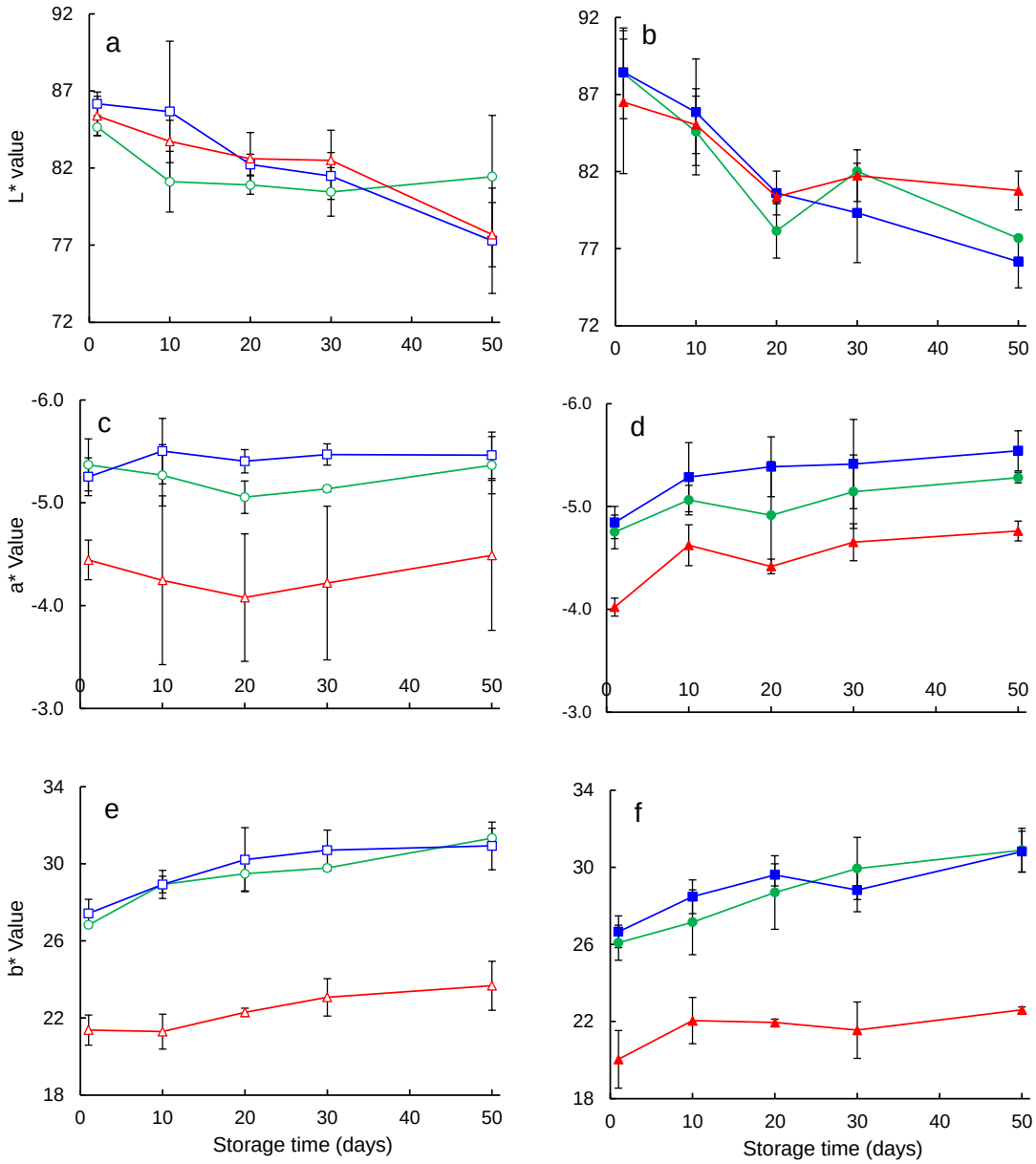

Figure 6.1. Storage-related changes in the colour coordinates, L* (a, b), a* (c, d) and b* (e, f), of low-moisture part-skim Mozzarella cheese in mid lactation (open symbols) and late lactation (closed symbols) from milk produced using different dairy cow feeding systems: grazing on perennial ryegrass pasture, GRO (○, ●), grazing on perennial ryegrass and white clover pasture, GRC (□, ■), or housed indoors and offered total mixed ration, TMR (△, ▲). Presented values are the means of 3 replicate trials in mid-lactation and 4 in late lactation; error bars represent standard deviations of the mean.

and age-related transitions in the distributions of moisture, fat and protein (Auty et al., 2001; McMahon & Oberg, 2017). The GRO and GRC cheeses had a significantly lower mean a*-values and higher mean b*-values compared to TMR cheeses in ML, LL and ML+LL. The higher b*-value in the GRO and GRC cheeses agrees with the general observation that milk from pasture-fed cows is yellower than milk from cows fed indoor on

concentrates, owing to its higher concentration of β-carotene (Nozière et al., 2006). The L-value was unaffected by feeding system. Visually, the TMR cheese was notably whiter than the GRO or GRC cheeses at all storage times; the latter typically had a pale 'butter-yellow' colour. The current results concur with those of O'Callaghan et al. (2016b) showing that Cheddar cheese from GRO or GRC milk had higher b* values and were more yellow than cheese from TMR milk. Lactation stage had no effect on the mean values of L*, a* or b*.

6.4.8 Thermophysical properties of cheese

Flow and extension work

The heat-induced flowability increased in all cheeses during maturation (Fig. 6.2 e, f); simultaneously, the work required to extend the hot molten cheese (E_w) decreased ($p < 0.05$). These changes concur with the increases in proteolysis and WHC. The ensuing increases in hydrolysis of the calcium phosphate *para*-casein network and moisture retention are expected to facilitate the relative displacement of adjoining planes of the cheese mass during heating and subsequent extension (Guinee, 2016).

Feeding system had a significant effect on flow of the heated cheese but not E_w (Table 6.4). The mean flow of the GRO cheese during storage was significantly higher than that of the GRC or TMR cheese in ML, LL and ML+LL. The higher flow of the GRO may be due to its lower content of palmitic acid (O'Callaghan et al., 2017) which would be conducive to the liquefaction and coalescence of fat at lower temperature, and overall less dehydration of the melting cheese mass.

In contrast to feeding system, lactation stage influenced E_w but not flow. The higher mean E_w of the ML cheeses, relative to LL cheeses, accords with their lower moisture content and degree of proteolysis.

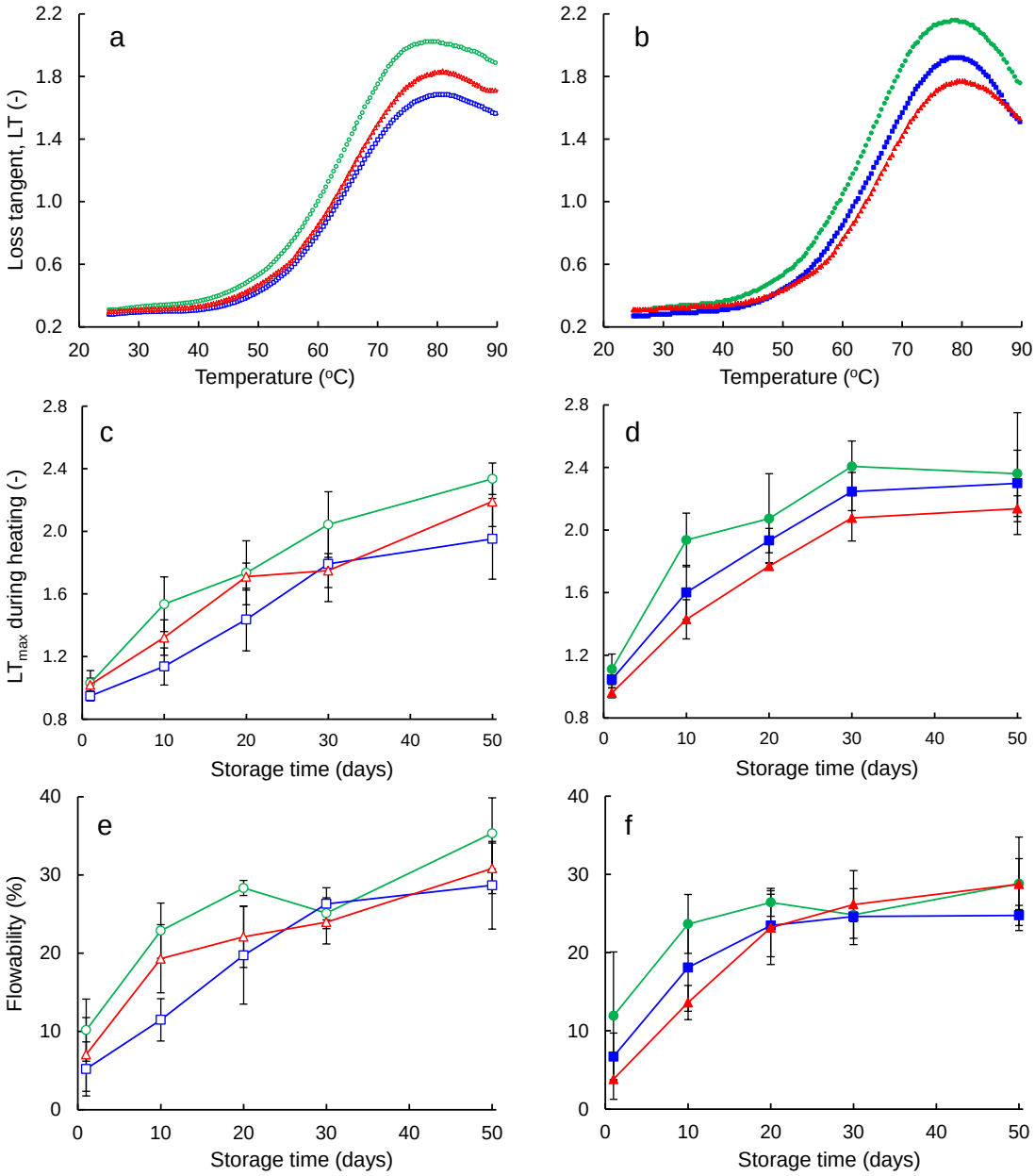

Figure 6.2. Changes in loss tangent during heating (a, b) and storage-related changes in maximum loss tangent, LT_{max} (c, d), and flowability (e, f) of low-moisture part-skim Mozzarella cheese in mid lactation (a,c, e; open symbols) and late lactation (b, d, f; closed symbols) from milk produced using different dairy cow feeding systems: grazing on perennial ryegrass pasture, GRO (○, ●), grazing on perennial ryegrass and white clover pasture, GRC (□, ■), or housed indoors and offered total mixed ration, TMR (△, ▲). Presented data: (a) and (b) are for 20 day-old cheeses from one of the replicate trials in mid- and late- lactation, respectively; c-f are the means of 3 replicate trials in mid-lactation and 4 in late lactation; error bars represent standard deviations of the mean.

Viscoelastic changes during heating and cooling

On heating from 25 to 90 °C, G′ and G″, decreased curvilinearly, and the loss tangent (LT), representing the ratio G″/ G′, increased simultaneously (Fig. 6.2 a, b). The changes in LT,

which represent a transition from a largely elastic cheese at 25 °C (LT < < 1) to a more viscous molten cheese mass at 70-90 °C (LT >> 1), are typical of those reported for various cheese types including LMPS Mozzarella (Guinee et al., 2015; Moynihan et al., 2016). On re-cooling, the molten cheese congealed and LT decreased to < 1 (data not shown). The cooling-induced solidification of the molten cheese has been ascribed to reabsorption of free serum and rehydration of the para-casein network and solidification of fat (Dave et al., 2001; Pastorino et al., 2002; Guinee et al., 2015).

Most of the 1 d old cheeses scarcely melted, with LT_{max} remaining ≤1.0. Loss tangent maximum (LT_{max}) increased significantly in all cheeses during storage (Fig. 6.2 c, d), indicating that the cheeses became more fluid on heating. This trend was consistent with that of flowability; hence, linear regression of the data for all ML and LL cheeses during storage indicated a significant linear correlation between the latter parameters, where LT_{max} = 0.0541flow + 0.64 (R^2 = 0.82). In contrast, the congealing temperature decreased significantly during storage from ~ 68 °C at 10 d to 61 °C at 50 d.

The mean LT_{max} for the GRO cheese over the 50 d storage period was slightly, but significantly, higher than that of TMR cheese in ML, LL and ML+LL (Fig. 6.2 c, d). Cheese from GRC feeding system had the lowest LT_{max} in ML, and a value intermediate between that of GRO and TMR cheeses in LL. The significantly higher LT_{max} of GRO cheese was consistent with its higher flowability, as a more-fluid cheese is expected to flow and spread to a higher degree. Cheese from GRO feeding system also had a slightly lower mean cross-over temperature (COTh) than cheese from TMR feeding system in LL and ML+LL ($p < 0.05$), indicating that it melted more quickly. The congealing temperature on re-cooling (COTc) was not influenced by feeding system in ML, LL or ML+LL. Lactation

stage had a significant effect LT_{max}, COTh and COTc, with the former higher in LL cheese than ML cheese, whereas COTh and COTc were higher in ML cheese.

From a practical viewpoint, the results indicate that the GRO cheese melts at a lower temperature and becomes more fluid and flowable than the TMR cheese, but that feeding system does not affect the temperature or time at which the molten cheese congeals on cooling.

6.5 Conclusion

This study investigated the effect of three different feeding systems (perennial ryegrass, GRO; perennial ryegrass and white clover, GRC; or total mixed ration, TMR) on the properties of milk and LMPS Mozzarella cheese in mid- and late- lactation. Milk from pasture-based systems had a higher concentration of protein, a higher cheese-yielding capacity, and cheese that had a more yellow colour. Moreover, cheese from GRO milk was more flowable and fluid on heating to 90-95 °C. Milk and cheese from TMR feeding system had higher concentrations of I, Cu and Se. Otherwise, feeding systems had little, or no, effect on losses of milk fat and protein to whey, cheese composition or texture. From a manufacturer's perspective, the higher yield, and greater heat-induced flow and fluidity of cheese from GRO milk may prove attractive; varied thermos-physical properties are a means of providing customised cheese ingredient solutions. Nevertheless, the more-yellow colour of LMPS Mozzarella cheese from pasture-based milk may be less acceptable in some markets, more accustomed to eating white-coloured cheese varieties. But they could potentially be marketed to certain regions building a label claim around the cheese, being organic or from the herds grazing pasture.

Chapter 7

Application of cavitation rheology for evaluation of changes in the mechanical properties of low-moisture Mozzarella cheese during ageing

7.1 Abstract

The mechanical properties of low-moisture Mozzarella cheese during ageing at 4 °C was evaluated using cavitation rheology (CR) and standard shear rheology. The critical pressure measured using cavitation rheology was determined at a range of needle radii, which monitors the pressure at which bubble formation and localised collapse of the cheese occurs. While both *E* (linear modulus obtained from CR) and *G′* (shear modulus) decreased with ageing time (25 to 60 d), *E* was found to be significantly lower than G′ at all times. Linear regression showed that *E* correlated significantly with G′ and cheese firmness.

7.2 Introduction

The rheological properties of food materials, including cheese, are of considerable importance as they effect its texture, tendency to fracture or bend, and behaviour while consumption or melting. There are a range of tests used to determine rheological and fracture properties of cheese. Low amplitude strain (< 0.1) oscillation rheometry (LASOR) is used to measure intrinsic rheological properties, such as elastic or storage modulus, *G′*, within the linear viscoelastic region where strain is directly proportional to the applied stress. In this region, the sample behaves like an elastic solid and recovers to its original shape after the strain is removed in a short time scale. Conversely, more empirical measures of rheological behaviour, such as fracture properties (firmness, fracture stress and strain), are measured by subjecting the cheese to relatively large strains (>> 0.1) using a texture analyser (O'Callaghan & Guinee, 2004). The strain applied during these tests mimic to those applied during consumption or cutting processes such as slicing, grating, or shredding.

Cavitation rheology is a recently developed technique which has been used to measure rheological properties of gels and soft tissues, including hydrogels from polyacrylamide, polyethylene oxide, and polyvinyl-alcohol (Zimberlin et al. 2007), vitreous humor in bovine eyes (Zimberlin et al., 2010), bovine eye lens (Jui et al., 2011), rat skin (Chin et al., 2013), protein gels (Blumlein & McManus, 2015), and cell spheroids (Blumlein et al., 2017). Cavitation rheology involves inserting a needle into a sample and inducing cavitation by slow pressurisation. It measures the pressure required to blow a bubble or other defect in the material being measured. The pressure at which the bubble suddenly expands is denoted the critical pressure (P_c), after which the pressure drops. The pressure required for bubble formation is governed by the mechanical properties of the material, surface tension between the air (or water, depending on the medium used for cavitation) and the surrounding material. Thus, cavitation rheology quantifies the pressure dynamics of a growing bubble, or, cavity, within the material (Zimberlin et al., 2007).

To date, cavitation rheology has not been applied in cheese. It has the potential to measure the mechanical properties of cheese as it ripens after manufacture. It is minimally invasive and as such, does not require the cheese to be cut or sampled for measurements, as these can be performed *in-situ*. It may also find application in cheeses such as Mozzarella to gain insight into the effects of different parameters on cheese elasticity, and susceptibility of the shredded cheese to recover following the applications of strain during the retailing/distribution of shredded cheese or during pizza manufacture.

7.3 Aims of the study

As cavitation rheology is a recently developed technique, it has not been used to evaluate mechanical properties of cheese. The aim of this study is to evaluate the potential of

cavitation rheology to measure changes in the mechanical properties of low-moisture Mozzarella cheese during ageing at 4 °C, and to correlate the resultant elasticity values with the age-related changes determined by shear rheology, as measured using low-strain and large strain deformation, and casein hydrolysis (proteolysis).

7.4 Results and discussion

7.4.1 Composition and proteolysis

The composition of the cheeses is shown in Table 7.1; the values of moisture, fat, protein, Ca and P were typical of those previously reported for low-moisture Mozzarella cheese (Yun et al., 1993a; Feeney et al., 2001; cf. Chapter 6).

Table 7.1: Composition of low-moisture Mozzarella cheese.

Compositional parameter	Value
Moisture (%, w/w)	45.8
Protein (%, w/w)	27.76
Fat (%, w/w)	20.2
Salt (%, w/w)	1.59
pH at day 1	5.38
Ca (mg/100g)	785
P (mg/100g)	687

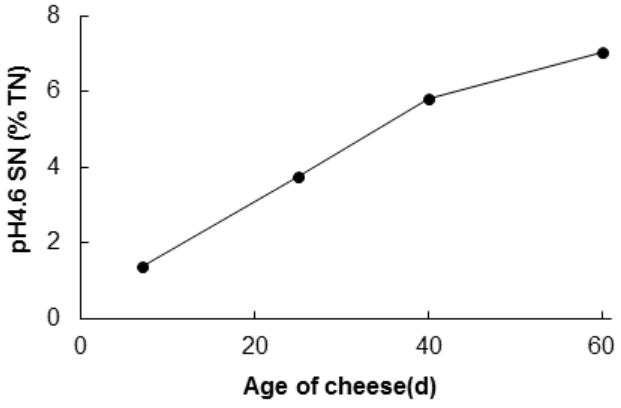

Figure 7.1: pH 4.6 soluble nitrogen (% total N) during ageing of low-moisture mozzarella cheese.

The level of pH 4.6 SN, an index of the degree of primary hydrolysis (proteolysis) of casein, increases during ageing of Mozzarella due to residual chymosin and plasmin activity (McSweeney, 2017; McMahon and Oberg, 2017). It increased from ~ 1.4 % at 7 d to ~ 7.0 % at 60 d (Fig. 7.1), which is typical of that reported in LM Mozzarella, i.e., ~ 7-10 % depending on factors such as ripening time and temperature (Feeney et al., 2001).

7.4.2 Large strain deformation behaviour

The changes in firmness (σ_{max}) and fracture stress (σ_f) during maturation are shown in Table 7.2. The most distinguishing feature of the compression analysis was the shape of the stress–time (displacement) curves, revealing the presence of a distinctive inflection point, representing a fracture peak, in the 7 day-old cheese, but not in the older cheeses (Fig. 7.2).

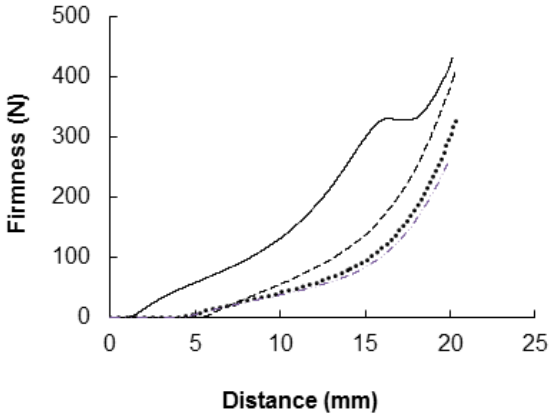

Figure 7.2: Firmness of low-moisture Mozzarella cheese during ageing at day 7 (solid line), day 25 (short-dashed line), day 40 (dotted line) and day 60 (long-dashed line).

Table 7.2: Changes in the rheological characteristics of low moisture Mozzarella cheese during ageing, as measured using cavitation rheology (CR), low amplitude strain oscillation rheometry (LASOR), and large strain deformation (LSD).

	LSD	LASOR		CR
Cheese Age	Firmness, σ_{max} (N)	Critical strain (%)	Shear modulus, G' (kPa)	Linear modulus, E (kPa)
Day 1	488.8	0.130	76.7	-
Day 25	408.1	0.112	56.2	10.6
Day 40	339.3	0.086	22.4	8.2
Day 60	259.1	0.083	14.9	6.5

The occurrence of fracture in the 7 d cheese at a strain of 0.61 and its absence at later times is indicative of a transition from a relatively brittle ('short') matrix to a more plastic pliable one. The value of σ_{max} decreased progressively during ageing of cheese at 4°C, from ~ 489 N at 7 d to 259 N at 60 d. The reductions in firmness are indicative of an attenuation of the stress-bearing capacity of the calcium phosphate *para*-casein network, which is consistent with the increase in primary proteolysis (Guinee, 2016). Ageing of Mozzarella results from various biochemical and enzymatic changes to the casein, which occurs in the form of elongated fibres separated by serum-fat channels (McMahon and Oberg, 2017). These changes are conducive to the swelling of the protein fibres as they absorb water from the serum-fat channels, and thereby become more viscous in nature.

7.4.3 Shear Rheology

Changes in *G′* as a function of shear rate for cheeses aged for 7 to 60 d are shown in Fig. 7.3.

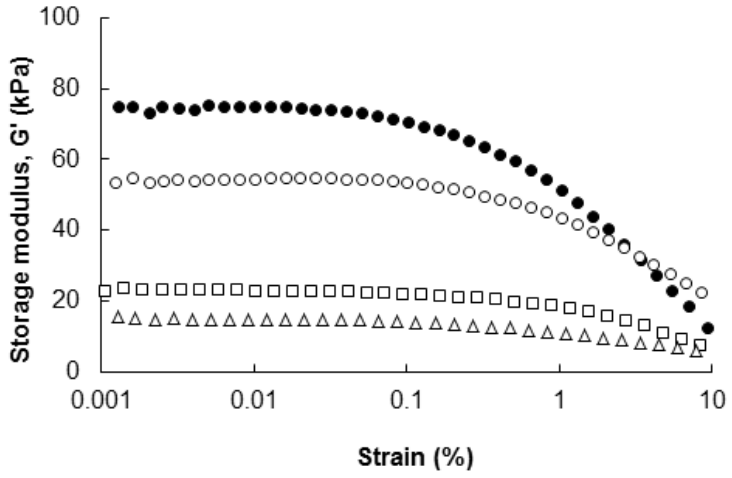

Figure 7.3: Shear strain sweep of low-moisture Mozzarella cheese at day 7 (●), day 25 (○), day 40 (□) and day 60 (△)

Both the critical strain and G' at the critical strain decreased during ageing of the cheese (Table 7.2). The reduction in shear strain on ageing confirms the results of Subramanian & Gunasekaran (1997) who showed that increasing the storage time of LMMC at 8 °C from 7 to 84 d coincided with reductions in critical strain and G' at 1.5 Hz and 40 °C, from ~ 0.21 to 0.17 % and ~ 35 to 18 kPa respectively. It has been previously reported that critical strain for Mozzarella cheese can range from 0.05 to 0.5 % depending upon the temperature and age of cheese (Ak & Gunasekaran, 1996; Subramanian & Gunasekaran, 1997). The decrease in elastic modulus over 60 d storage of Mozzarella cheese is consistent with the increase in proteolysis during ageing.

7.4.4 Cavitation Rheology

Cavitation rheology data were obtained after 25, 40 and 60 d of ageing. It was not feasible to collect data at 7 d, owing to air leakage during measurement, as observed visually by the bubbling of an oil film spread around the point of needle insertion (after the needle had already been inserted) on the cheese surface. Air leakage was most likely due to some fracture along the path of the needle, which would lead to the partial development of space between the inserted needle and the bulk cheese. This suggestion is supported by large strain deformation behaviour which showed that the fracture occurred during compression of the cheese at 7 d, but not at 25, 40 or 60 d. Representative pressure-time plots from Mozzarella cheese obtained at day 40 with 10 g (ir., 1.35 mm) needle are shown in Fig. 7.4.

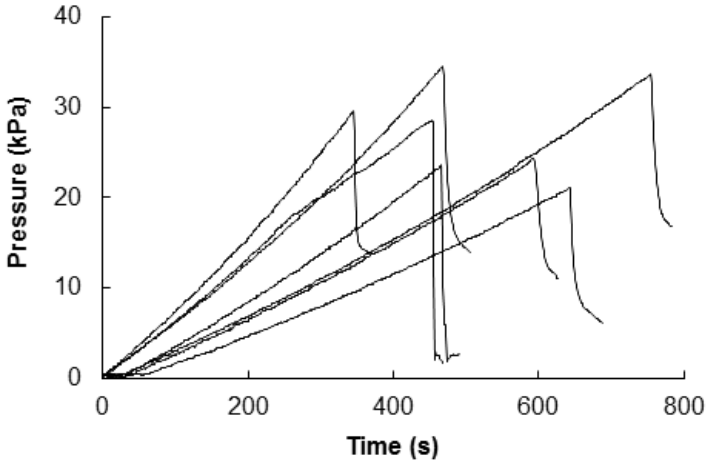

Figure 7.4: Pressure-time plots for low-moisture Mozzarella cheese at 40 d with 10g (ir, 1.35 mm) needle size. Different curves represent replicates of same sample.

At each ripening time, a strong linear relationship was obtained between P_c and the inverse of needle radius, 1/r (Fig. 7.5). Linear regression was used to obtain the slope of the line (2γ) and the intercept (5E/6) according to the following equation (Zimberlin et al., 2007):

$$P_c = \frac{2\gamma}{r} + \frac{5}{6}E$$

where, γ is the surface tension between the sample and air, E is the modulus; and r, is the inner radius of the needle used.

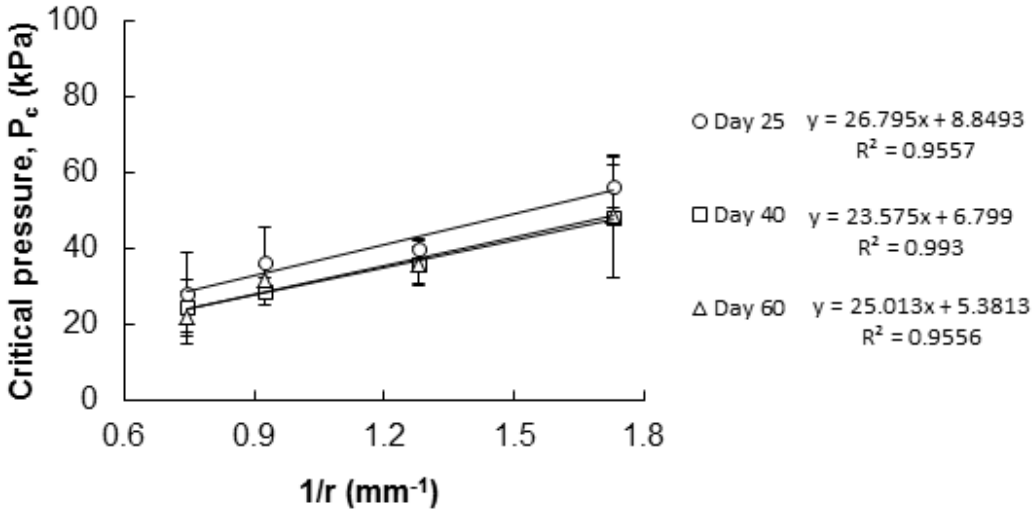

Figure 7.5: Changes in critical pressure as a function of needle radius for low-moisture Mozzarella cheese

The calculated mean values of the modulus (E) at the different ripening times (6.5 - 10.2 kPa, Table 7.2) were significantly lower than the corresponding values of storage modulus, G' (14.9-56.2 kPa) within the linear viscoelastic region (< 0.5 % strain). The lower values for E obtained suggest that more than one mechanism for deformation may contribute to the critical pressure, P_c. One possibility is that the deformation of cheese may have occurred by irreversible fracture before elastic cavitation could take place (Hutchens et al., 2016). Such localised micro-fracture would be conducive to a lower elastic 'counteractive' force by the cheese matrix against the compressed air. Moreover, the structure of cheese is quite heterogeneous on the meso-scale, containing air pockets, curd particle junctions, and curd chip junctions (Kalab, 1977; Lowrie et al., 1982; Fox et al., 2017), which could provide a pathway for material fracture at these length-scales when probed with CR. The heterogeneous structure of cheese would also alter the susceptibility to manual-induced fracture using the needles. Ideally, the insertion procedure would be automated to ensure fixed values of needle velocity and angle of insertion. To further probe if deformation proceeded by irreversible fracture, we plotted P_c against $1/\sqrt{r}$ (data not shown). A better fit to this relationship is obtained (compared to P_c against $1/r$) when irreversible fracture occurs following bubble formation in gels (Kundu & Crosby, 2009). However, for the Mozzarella cheese samples, this is not the case suggesting that P_c contains contributions from both elastic cavitation and irreversible fracture. In a model 60 d old Mozzarella cheese, we investigated this by looking at the reversibility of bubble formation (Fig. 7.6).

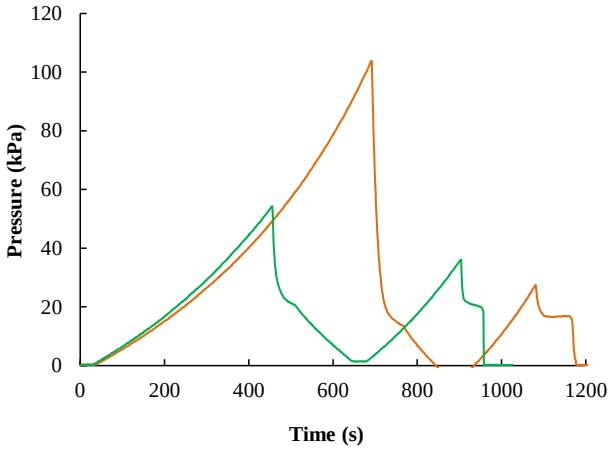

Figure 7.6: Pressure-time plots to monitor reversibility of bubble formation for model low-moisture Mozzarella cheese at 60 d with 16 g (ir., 0.58 mm) needle size. Red and green lines represent the replicates of same sample.

If deformation is purely by irreversible fracture, it is not possible to re-pressurise the system after bubble formation and subsequent removal of the air in the bubble. If the process is fully elastic, bubble formation in a second or subsequent pressurisation cycle occurs at bubble at the same P_c as the initial bubble formation. Fig. 7.6 indicates that while some elasticity remains in the system, a significant fracture event occurs during the initial bubble formation. Therefore, P_c contains information about both the elastic deformation and irreversible fracture. It is very likely that deformation will be length-scale dependent at these smaller length-scales, since heterogeneities at this length-scale will change throughout the ageing process, but a wider range of needle radii are needed to probe for confirmation. Currently, the instrument (which is not commercially available and is custom-made) is not capable of reaching sufficiently high pressures to measure P_c at smaller r values, and a new instrument would need to be built to facilitate these measurements.

Since the contribution of surface tension to the slope is negligible compared to the contribution from the needle radius, deriving values for the surface tension is not possible and gives physically unrealistic values at these needle radii (i.e., values in the range 11-13

N/m compared with literature values of 0.051N/m for fresh cheese curd (Tambat & Srinivasan, 1979). Again, once measurements at smaller needle radii are possible, surface tension will contribute more to the slope relative to the needle radius and it will be possible to determine a value for γ.

The decrease in elastic modulus of cheese obtained from cavitation rheology over ageing (Table 7.2) was consistent with the decrease in G' obtained by the conventional strain-sweep method; hence, regression analysis indicated a linear relationship between P_c and G' for the Mozzarella cheeses of different ages (r= 0.97; df, 2) indicating that relative changes in the mechanical properties of cheese during ageing are obtained using both methods.

7.5 Conclusion

Cavitation rheology was used to evaluate the changes in elasticity in low-moisture Mozzarella cheese during ageing at 4 °C. The results showed that the modulus, E, obtained using cavitation measurement was significantly lower than the elastic shear modulus, G', within the viscoelastic region of the cheese, obtained using low amplitude strain oscillation rheometry. Nevertheless, the reduction in E concomitant with the ageing of the cheese suggests that cavitation rheology could potentially be used as an alternative to low shear strain deformation to monitor changes in elasticity in Mozzarella cheese after ageing for >7 d, when the levels of pH 4.6 SN are ≥ 4 % total N. It was suggested that the discrepancy between values of E and G' may have due to of occurrence of irreversible fractures rather than elastic cavitation while measurement. However, a wider range of needles would need to be employed to confirm if the predominant contribution to P_c is elastic cavitation or irreversible fracture.

Chapter 8

Dairy cow feeding system alters the characteristics of low-heat skim milk powder and processability of reconstituted skim milk

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8.1 Abstract

Low-heat skim milk powder (LHSMP) was manufactured on 3 separate occasions in both mid- (ML, July 4-20) and late- (LL, September 27-October 7) lactation using three different dairy cow feeding systems: grazing on perennial ryegrass (*Lolium perenne* L.) pasture (GRO), grazing on perennial ryegrass and white clover (*Trifolium repens* L.) pasture (GRC), and housed indoors offered total mixed ration (TMR). The resultant powders (GRO-SMP, GRC-SMP and TMR-SMP) were evaluated for composition and colour, and for the compositional, physicochemical and processing characteristics of the reconstituted skim milk (RSM) prepared by dispersing the powders to 10 % (w/w) in water. Feeding system significantly affected the contents of protein and lactose, elemental composition and colour of the LHSMP, and the rennet gel properties of the RSM. Powders from GRO and GRC feeding systems had a higher protein content, lower levels of lactose, Iodine (I) and Selenium (Se), and a more yellow-green colour (lower a*- and higher b*-colour coordinates) than TMR-powder. On reconstitution, the GRO-RSM had higher concentrations of protein, casein and ionic Ca, and lower concentrations of lactose and non-protein nitrogen (NPN, % of total N), and produced rennet gels with a higher storage modulus (G′) than the corresponding TMR-RSM. These effects were observed over the combined mid- and late-lactation (ML+LL) period but varied somewhat during the separate ML and LL periods. Otherwise, feeding system had little, or no, effect on proportions of individual caseins, concentration of serum casein, casein micelle size, casein hydration, heat coagulation time or ethanol stability of the RSM at pH 6.2-7.2, or on the water holding capacity, viscosity and flow behaviour of stirred yoghurt prepared by starter-induced acidification of RSM. The differences in the functionality of the LHSMP may be of greater

or lesser importance depending on the application and the conditions applied during the processing of the RSM.

8.2 Introduction

Skim milk powder (**SMP**), also referred to as nonfat dry milk, is used extensively as an ingredient in the manufacture of dairy-based beverages and formulated food products (e.g., coffee creamers; ice cream, dairy-based desserts, sauces, soups, processed cheese products, bakery products). Depending on the application and functionalities required, the SMP may be low-, medium- or high-heat based on the heat treatment of the skim milk prior to evaporation and drying. Typical heat treatments for low-, medium and high-heat SMPs, are 70-72 °C for 15 s, 85 °C for 60 s, and 120 °C for 60-120 s, or 90 °C for 100-300 s, respectively (Patel et al., 2007). Low-heat SMP (**LHSMP**) is preferably used for the preparation of recombined milk for cheese manufacture, or for standardising the content of milk protein or solids in products such as cheese milk, yoghurt and fermented milk products (Kelly & Fox, 2016).

Owing to seasonal changes in milk composition (Auldist et al., 2000a; Mehra et al., 1999; O'Brien et al., 1999a), the composition and functionality (e.g., rennet gelation, heat stability) of LHSMP is likely to vary across the production season. Dehydration of skimmed milk to a powder with specified moisture content (i.e., 5 %, w/w; FAO/WHO, 1999; FDA, 2018) removes the impact of seasonality-associated differences in the total solids (**TS**) content of milk on the TS content of the powder or the reconstituted skim milk (**RSM**). Nevertheless, seasonal variations in the ratios of individual constituents (e.g., lactose, protein, calcium phosphate, urea, Ca^{2+}) in skimmed milk are not affected by drying, and, hence, occur also in the RSM. Such variations are likely to impact the processing

behaviour of RSM owing to their effects on buffering capacity, degree of heat-induced acidity, or susceptibility of protein to aggregation (Pouliot & Boulet, 1991; Rattray & Jelen, 1996; Sikand et al., 2010). While much information is available on the effects of season and cow diet on the processing characteristics of milk, e.g., rennet coagulability (O'Brien et al., 1999a; Guinee et al., 2001b), heat stability (Holt et al., 1978; Kelly, 1982; Banks, 1984) and ethanol stability (O'Brien et al., 1997; Horne et al., 1986; Chen et al., 2014), relatively few studies have investigated seasonal changes in the composition of SMP or its functionality or processing behaviour on reconstitution to RSM. Most studies on SMP have focused on the heat stability of RSM concentrates (which are standardised to a fixed TS content).

Kelly (1982) found that the maximum heat coagulation time (HCT_{max}) of RSM concentrate (20% TS) at 120 °C increased from ~ 22 min in February to ~ 70-80 min in May, remained relatively constant between May and October, and thereafter decreased to ~ 5-10 min in December. It was suggested that the low HCT at the extremes of lactation (November-February) may be due the incidence of sub-clinical mastitis in the dairy herds and prolonged storage at low temperature. In contrast, Pouliot & Boulet (1991) observed that the HCT of RSM concentrates (~ 31 % TS) at native pH and 121 °C varied little (7-10 min) over the year, and no distinct period of instability was evident. Cheng et al. (2002) reported significant seasonal variations in the consistency and syneresis of set- and stirred-yoghurts made from RSM (10-14%, w/w, TS), prepared by dissolving SMP to a constant TS content. Viscosity and gel strength correlatively positively with protein concentration (4.1-4.9 %, w/w, for RSM with 12 % TS) and negatively with the concentration of inorganic phosphate in the RSM; syneresis decreased with protein content of the RSM. We

are unaware of any studies on the effect of diet/feeding system or lactation on the rennet coagulability or ethanol stability of LHSMP.

8.3 Aims of the study

The objective of the current study was to compare pasture- and TMR- based feeding systems for their effects on composition of low-heat skim milk powder manufactured in mid lactation or late lactation, and its HCT, ethanol stability and rennet coagulability on reconstitution to 10 % (w/w), and properties of reduced-fat (2.3 %, w/w) stirred yoghurt on reconstitution to 12.7 % (w/w).

8.4 Results and discussion

LHSMP was manufactured on 3 separate occasions in both ML (July 4-20, 2016; 137-153 DIM) and LL (Sept 27-Oct 27, 2016; 222-232 DIM) from milk obtained from each of the feeding systems (GRO, GRC and TMR). The data were classified according to feeding system and lactation stage, as a factorial design, and analysed using analysis of variance (ANOVA) for the effects of feeding system, lactation stage, and their interaction. The data for ML and LL were also analysed using ANOVA. ANOVA was performed using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute Inc., Cary, NC), and Tukey's multiple-comparison test was used for paired comparison of treatment means; the level of significance was determined at $p < 0.05$.

When analysing for effect of lactation stage, ML milk refers to the composite of the milks from the herds on the GRO, GRC, and TMR feeding systems in mid lactation, and LL milk to the composite of the corresponding milks in LL milk.

The R-3.2.2 software (R Core Team, 2014) was used to compute Pearson correlation between different compositional parameters, where significance difference was determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$ according to Student's t-test.

8.4.1 Composition of Skim Milk

The gross composition of the milk used for LHSMP manufacture was affected by feeding system and lactation stage, to a degree influenced lactation stage (Table 8.1). Compared to GRO and GRC milks, TMR milk had a significantly lower mean concentration of protein and higher concentration of lactose during overall lactation (ML+LL) ($p < 0.01$).

Lactation stage had a significant effect on skim milk composition, with LL milk having higher protein and lower lactose concentrations than ML milk. The overall effects of feeding system and lactation stage on gross composition of raw milk are similar to those reported previously for milk from spring-calved herds (Auldist et al., 2000a; O'Callaghan et al., 2016b; cf. Chapter 4).

8.4.2 Composition of low-heat skim milk powder

The contents of total protein and lactose were affected by feeding system to an extent dependent on lactation stage (Table 8.2). The protein content in ML, LL and overall lactation (ML+LL) decreased in the following order, GRO > GRC > TMR. The lactose content of TMR powder was higher than that of the GRO- or GRC- powder in LL and ML+LL. Linear regression analysis of the data for all powders in ML and LL indicated a significant inverse relationship between lactose and protein content (df, 16; r = 0.93), with lactose decreasing by ~ 1 % (w/w) for every increase in ~ 0.6 % (w/w) protein content. Otherwise, feeding system did not affect the contents of TS or fat, or the level of

undenatured whey protein, as evidenced by the similar values of WPNI. Apart from increasing the content of non-fat substances, the evaporation and drying processes during manufacture of LHSMP have little, or no, effect on the relative proportions of the different components of milk or denaturation of whey protein (Lin et al., 2018a).

Lactation stage had a significant effect on powder composition, with LL powders having a higher content of protein, and lower content of lactose. The trends for protein and lactose are consistent with those of previous studies for the effects of lactation stage on the composition of milk from pasture-fed, spring-calved herds over the period ~ 15-250 DIM (Auldist et al., 2000a; O'Callaghan et al., 2016b; cf. Chapter 4).

8.4.3 Elemental composition of low-heat skim milk powder

Noting that the solids in skim milk underwent a 10.3 fold concentration during the manufacture of LHSMP, the content of individual elements in all powders (Table 8.2) were within the range previously reported for milk or skim milk from pasture-fed spring-calved herds (O'Brien et al., 2013; cf. Chapter 4, 6). The contents of macroelements (K, Na, S) and trace-elements (Zn, Fe, I, Cu, Mo and Se) were affected to a degree dependent on feeding system and lactation stage. Most notably, TMR powder had significantly higher quantities of I, Cu and Se than GRO or GRC powders in ML and ML+LL. The results concur with earlier work (cf. Chapter 4, 6) that showed that milk from the TMR feeding system had higher concentrations of I and Se than that from pasture-feeding systems. Powder from TMR feeding system also had higher quantities of the trace-elements, Zn and Mo, and a lower quantity of Fe than GRC powder in overall lactation (ML+LL).

Lactation stage had a significant effect on elemental composition, with LL powders having higher mean quantities of Na, Mg and Fe, and a lower quantity of K than ML

powders. Surprisingly, the quantities of Ca and P in powders were not significantly affected by lactation stage despite the slightly higher mean casein content (~ 1.8-2.7 %) of late lactation powder. Bijl et al. (2013) found that the concentrations of Ca and P in milk increased linearly with concentrations of protein and casein. The absence of an effect of feeding system on Ca and P in LHSMP may reflect the diminution of differences in the levels of compositional components (e.g., protein, Ca) between GRO, GRC and TMR milks during evaporation and drying, which involve dehydration to a standard TS content of ~ 96-97 % (w/w), irrespective of the TS content of the milk.

8.4.4 Colour of low-heat skim milk powder

The colour co-ordinates (L*, a*, b*) of the LHSMP are shown in Table 8.2; the values are of similar magnitude to those previously reported for a range of commercial range of skim milk powders on the US market, i.e., L* = 94.0-96.3; a* = -3.4 to -2.1; b* = 12.4-17.9 (Abdalla et al., 2017).

TMR powders had significantly higher mean a*-values and lower b*-values than the corresponding GRO- and GRC- powders in ML, LL and ML+LL. Hence, on visual observation, the colour/hue of the pasture-based powders (GRO, GRC) was more ‘greenish-yellow’ than that the TMR powder. The higher intensities of green (a*) and yellow (b*) colour of the GRO- and GRC-powders may reflect higher contents of riboflavin (Dufossé & Galaup 2009; Božanić et al., 2014) and β-carotene (Nozière et al., 2006), respectively. Analogously, Cheddar and Mozzarella cheeses from pasture milks have been found to have higher b* values and were more ‘straw-yellow’ coloured than cheeses from TMR milk (O’Callaghan et al., 2017; cf. Chapter 6).

Table 8.1: Composition of skim milk from dairy herds on different feeding systems in mid- and late-lactation.[1]

	Feeding systems								Probability values (*p*) for overall effects		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Total solids (%, w/w)	9.43[a]	9.35[a]	9.17[b]	0.017	9.47	9.35	9.34	0.048	0.022	0.205	0.375
Fat (%, w/w)	0.36	0.36	0.37	0.023	0.32	0.31	0.32	0.019	0.922	0.020	0.951
Lactose (%, w/w)	5.02[b]	5.07[b]	5.11[a]	0.016	4.72[b]	4.77[ab]	4.91[a]	0.027	0.040	<0.0001	0.540
Protein (%, w/w)	3.66[a]	3.66[a]	3.40[b]	0.022	4.05[a]	3.94[b]	3.78[c]	0.016	<0.0001	<0.0001	0.125

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid- and late-lactation; SED = standard error of difference between means. Values within a row relating to mid- or late-lactation and not sharing a common lower-case superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Probability (*P*) values for the effects of feeding system in overall lactation (ML+LL), lactation stage (ML or LL) across the different feeding systems, and their interaction. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): mid lactation [July 4-20; 137-153 d in lactation (DIM)] and late lactation (September 27-October 7; 222-232 DIM).

Table 8.2: Composition of low-heat skim milk powders from milks of dairy herds on different feeding systems in mid- and late-lactation.[1]

	Feeding systems								Probability values (*p*) for overall effects		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Gross composition											
Total solids (%, w/w)	95.9	95.9	95.9	0.026	96.3	96.6	96.5	0.253	0.921	0.138	0.950
Fat (%, w/w)	0.93	0.88	0.85	0.034	1.01	0.95	0.89	0.030	0.112	0.068	0.859
Lactose (%, w/w)	52.3	53.0	53.6	0.040	45.9[b]	47.4[b]	50.4[a]	0.579	<0.001	<0.0001	0.020
Protein (%, w/w)	38.9[a]	38.1[b]	37.0[c]	0.075	42.1[a]	41.0[b]	39.2[c]	0.144	<0.0001	<0.0001	0.408
WPNI (mg N/g)	8.81	8.99	8.46	0.194	9.94	9.82	8.91	0.328	0.067	0.008	0.562
Elements											
Ca (mg/100g)	1253	1213	1281	22.21	1297	1269	1265	12.59	0.526	0.314	0.520
P (mg/100g)	1006	1106	1123	39.5	1048	1101	1122	54.47	0.752	0.635	0.729
K (mg/100 g)	1595	1512	1652	55.1	1329[c]	1396[b]	1470[a]	4.92	0.026	<0.0001	0.178

Na (mg/100g)	359.3	333.0	360.1	16.1	425.5^{a}	381.8^{b}	374.5^{b}	7.57	0.075	0.003	0.219
Mg (mg/100g)	116	110	113	4.01	121	121	117	1.29	0.538	0.043	0.599
S (mg/100g)	293	275	285	15.5	304^{a}	300^{a}	278^{b}	3.81	0.345	0.315	0.405
Zn (μg/kg)	45333	41667	51000	4528	43000^{b}	43667^{b}	51000^{a}	1387	0.032	0.964	0.763
Fe (μg/kg)	2777	2000	2097	267.1	2793^{b}	3923^{a}	2777^{b}	217.4	0.029	0.014	0.008
I (μg/kg)	376.7^{b}	230.0^{b}	6390.0^{a}	1016	616.7^{b}	383.3^{b}	4746.7^{a}	451.7	<0.0001	0.537	0.441
Mn (μg/kg)	6420	7273	4870	2371	2950	4063	2963	646.6	0.593	0.059	0.884
Cu (μg/kg)	750.0^{ab}	660.0^{b}	960.0^{a}	78.1	696.7	546.7	913.3	104.1	<0.01	0.657	0.982
Mo (μg/kg)	360.0	216.7	366.7	39.9	286.7	280.0	313.3	12.47	0.024	0.412	0.091
Se (μg/kg)	203.3^{b}	173.3^{b}	383.3^{a}	11.54	223.3^{b}	210.0^{b}	363.3^{a}	14.27	<0.0001	0.234	0.089
Colour											
L*	92.3^{b}	93.0^{a}	92.6^{b}	0.067	92.4	92.8	92.9	0.214	0.116	0.784	0.609
a*	-3.55^{b}	-3.60^{b}	-2.95^{a}	0.104	-3.73^{b}	-3.61^{b}	-2.93^{a}	0.127	<0.0001	0.580	0.651
b*	13.7^{a}	13.2^{ab}	11.4^{b}	0.376	15.0^{a}	13.8^{ab}	12.3^{b}	0.483	0.0001	0.031	0.728

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid- and late-lactation; SED = standard error of difference between means. Values within a row relating to mid- or late-lactation and not sharing a common lower-case superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Probability (*P*) values for the effects of feeding system in overall lactation (ML+LL), lactation stage (ML or LL) across the different feeding systems, and their interaction. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): mid lactation [July 4-20; 137-153 d in lactation (DIM)] and late lactation (September 27-October 7; 222-232 DIM).

While lactation stage did not affect the colour (L, a* and b* values) of the pasture-based powders, TMR powder from LL milk had a slightly, but significantly ($p < 0.05$), higher b*value than that from ML milk.

8.4.5 Composition of reconstituted skim milk

LHSMP was reconstituted to 10 % (w/w). Due to the similar TS content in all powders (~ 96.2 %, w/w), the TS of all reconstituted skim milks were similar. As expected, the effects of feeding system and lactation stage on concentrations of lactose and protein in the RSM were similar to those for the LHSMP. Similar to the trend for protein concentration, the mean casein content of the GRO-RSM was higher than that of TMR-RSM in ML and ML+LL, but not in LL. Otherwise, feeding system did not influence the mean concentrations of whey protein (~ 0.65 %, w/w) or urea in the RSM.

Non-protein N (NPN), as a proportion of total N, was slightly, but significantly influenced by feeding system, as indicated by the higher value in the TMR-RSM relative to GRO-RSM in LL and ML+LL (Table 8.3). Feeding system did not significantly affect the mean proportions of casein or whey protein (as a % of total protein), soluble casein (% of total casein) or individual caseins (as a % of total casein), the mean values of which were typical of those previously reported for bovine milk (Bernabucci et al., 2015; Auldist et al., 2016; Lin et al., 2017b). The overall trends for effect of feeding system on the composition of RSM are similar to those reported previously for the comparative effects of indoor feeding of TMR versus pasture grazing on the composition of milk during lactation, i.e., from ~ 15-240 DIM (Auldist et al., 2000a; O'Callaghan et al., 2016b; cf. Chapter 4).

Lactation stage had a significant effect on composition, with LL milk having higher mean concentrations of protein, casein, soluble casein (% of total casein), NPN and urea,

Table 8.3: Composition of reconstituted skim milk (10 %, w/w) powders, from milks of dairy herds on different feeding systems in mid- and late-lactation.[1]

	Feeding systems								Probability values (*p*) for overall effects		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Composition											
Total solids (%, w/w)	9.59	9.59	9.59	0.002	9.63	9.66	9.65	0.025	0.921	0.138	0.950
Lactose (%, w/w)	5.23	5.30	5.36	0.004	4.59^{c}	4.74^{c}	5.04^{b}	0.057	0.000	<0.0001	0.020
Total protein (%, w/w)	3.90^{a}	3.81^{b}	3.70^{c}	0.007	4.21^{a}	4.10^{a}	3.92^{b}	0.014	<0.0001	<0.0001	0.410
Casein (%, w/w)	3.09^{a}	2.98^{b}	2.94^{b}	0.016	3.27	3.25	3.12	0.0379	0.023	<0.0001	0.516
Casein number	79.2	78.1	79.5	0.622	77.8	79.3	79.6	0.852	0.409	0.927	0.308
Soluble casein (% total milk casein)	5.91	6.72	4.77	1.37	8.91	9.53	12.39	2.20	0.900	0.048	0.580
Individual caseins (% milk casein)											
α_{s1}+ α_{s2}-casein	47.8	47.5	47.6	0.247	48.8	49.7	48.7	0.481	0.116	0.462	0.975
β-casein	42.0	43.5	42.5	0.398	41.4	40.7	41.4	0.577	0.796	0.004	0.132
κ-casein	10.2	9.0	9.9	0.692	9.8	9.7	9.9	0.335	0.914	0.575	0.976
Whey protein (%, w/w)	0.62	0.62	0.57	0.015	0.69	0.56	0.59	0.029	0.030	0.721	0.089
α-Lac:β-Lg	0.192	0.196	0.195	0.003	0.188	0.190	0.193	0.005	0.914	0.575	0.976
NPN (% total N)	4.96^{b}	5.54^{a}	5.23^{ab}	0.073	4.96^{b}	5.13^{ab}	5.45^{a}	0.076	0.036	0.601	0.138
Urea (mg/100g)	34.7	40.3	35.6	2.00	58.4	54.6	53.3	9.70	0.899	0.005	0.775
Physicochemical characteristics											
pH	6.70	6.69	6.72	0.011	6.71	6.70	6.68	0..012	0.843	0.578	0.230
Ionic calcium (mg/100g)	5.08	4.94	4.65	0.131	5.84^{a}	4.55^{b}	4.85^{b}	0.097	0.001	0.180	0.015
Ionic calcium-to-casein	1.65	1.66	1.58	0.042	1.79^{a}	1.40^{b}	1.55^{ab}	0.034	0.044	0.410	0.051
Casein micelle size (nm)	181	187	172	3.58	182	182	180	4.68	0.235	0.719	0.359
Casein hydration (g water/g casein)	3.20	3.04	3.05	0.087	2.97	3.01	3.16	0.161	0.588	0.261	0.140

Table 8.4: Processing characteristics of reconstituted skim milk (10%, w/w) powders, from milks of dairy herds on different feeding systems in mid- and late-lactation.[1]

	Feeding systems								Probability values (p) for overall effects		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Heat coagulation time (HCT)											
HCT_{npH}	17.1	16.5	16.8	1.14	23.3	23.6	24.1	1.04	0.774	<0.0001	0.925
HCT_{max}	15.9	16.8	15.7	0.946	23.0	22.6	24.6	0.650	0.929	<0.0001	0.285
HCT_{min}	5.3	5.3	5.6	0.304	9.0	8.9	9.8	0.560	0.394	<0.0001	0.961
Ethanol Stability											
$ES_{6.6}$	71.3	72.0	83.3	3.32	56.0	68.0	58.7	3.54	0.240	0.003	0.115
$ES_{6.8}$	86.0^{b}	90.0^{a}	90.0^{a}	0.666	80.7	85.3	86.0	1.24	0.006	0.001	0.968
$ES_{7.0}$	90.0	92.7	94.0	1.21	86.0^{b}	89.3^{a}	89.3^{a}	0.384	0.001	<0.0001	0.204
ES_{npH}	82.0	84.0	84.7	0.902	77.3^{b}	84.0^{a}	80.7^{ab}	0.942	0.026	0.027	0.225
Rennet induced gelation											
Rennet coagulation time (RCT, min)	18.7	18.6	17.1	0.536	14.3	16.0	14.7	1.44	0.128	0.009	0.354
Gel firming rate (GFR_{max}, Pa/s)	0.057	0.055	0.053	0.001	0.090	0.069	0.060	0.008	0.172	0.021	0.286
Gel firmness at 40 min (G'_{40},Pa)	56.6	54.0	55.3	1.63	109.7^{a}	79.5^{ab}	71.1^{b}	8.89	0.033	0.023	0.592

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid- and late-lactation; SED = standard error of difference between means. Values within a row relating to mid- or late-lactation and not sharing a common lower-case superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Probability (P) values for the effects of feeding system in overall lactation (ML+LL), lactation stage (ML or LL) across the different feeding systems, and their interaction. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): mid lactation [July 4-20; 137-153 d in lactation (DIM)] and late lactation (September 27-October 7; 222-232 DIM).

and a lower lactose content. Nevertheless, casein, whey protein, NPN and urea as proportions of total N, or individual caseins as a proportion of total casein, were unaffected by lactation stage.

8.4.6 Physicochemical characteristics of reconstituted skim milk

The values for pH, casein micelle size (**CMS**) and casein micelle hydration (**CMH**) for RSM are typical of those previously reported in the literature for bovine milk (Table 8.3), i.e., pH, 6.73-6.87 (Grimley et al., 2009; Chen et al., 2014); CMS, ~ 160-210 nm

(Glantz et al., 2010; Bijl et al., 2014; Chen et al., 2014); and CMH, ~ 2.8-3.4 g water/g casein at 20-25 °C (Lin et al., 2017b; Huppertz et al., 2017). The [Ca^{2+}], i.e., 1.13-1.45 mM, was relatively low compared to the published values for unheated milk, i.e., ~ 1.7-3.5 mM (Tsioulpas et al., 2007; Bijl et al., 2013; Chen et al., 2014).

The mean [Ca^{2+}] of GRO-RSM was higher than that of GRC- or TMR- RSM in LL and ML+LL. Otherwise, feeding system had no effect on the mean pH, CMS or CMH. The absence of an effect of feeding system on CMS corresponds with the results of Auldist et al. (2016), which showed no effect of the type and level of feed supplement (milled wheat grain, crushed corn grain or canola meal) on the CMS (159-172) of bulk herd milk from Holstein Friesian cows. Analogously, Grimely et al. (2009) found no significant difference in the CMS of bulk herd milks before, during or after the turn out of commercial dairy herds to pasture, when the supply and composition of pasture are likely to vary significantly (McCarthy et al., 2013). However, Devold et al. (2000) found that the type of supplement offered to grazing dairy herds during mid-lactation affected the CMS of milk, as shown by the different values in milk from herds fed rolled barley supplement (191 nm) or commercial concentrate (175 nm). Inter-study differences on the response of CMS to diet or feeding system may relate to a number of factors, including differences in the response of herds with cows of different breed and genetic merit, milk protein polymorphism, and degree of glycosylation (Glantz et al., 2010; Bijl et al., 2014). We are unaware of any published studies on the impact of diet or feeding system on CMH.

Lactation stage did not affect CMS, CMH or [Ca^{2+}]. Other studies (Grimely et al., 2009; Glantz et al., 2010; Chen et al., 2014) have also reported little, or no, effect of season or lactation stage on CMS, CMH or [Ca^{2+}] of herd milks; however, it is difficult to

distinguish between lactation stage and season in the latter studies owing to the lack of detail on calving pattern.

8.4.7 Processing characteristics of reconstituted skim milk

Data on the processing characteristics of the RSM are shown in Fig. 8.1 and Table 8.4. The HCT/pH and ES/pH profiles were typical of those reported for milk, i.e., a type A HCT/pH profile with a maximum HCT (HCT_{max}).

Heat coagulation time

Overall, feeding system had little, or no, effect on HCT in the pH range 6.2-7.2. The absence of an effect of feeding system on the HCT profile of the RSM is consistent with the relatively small differences in compositional parameters (Table 8.4) identified as having a strong influence, e.g., concentrations of lactose, protein, urea, and [Ca^{2+}] (Huppertz, 2016). The current results suggest that the potential HCT-enhancing effect of the slightly lower lactose content in the GRO-RSM relative to TMR-RSM was most likely off-set by its higher contents of protein and casein (Shalabi & Fox, 1982b).

Lactation stage significantly affected HCT (Fig. 8.1; Table 8.4). Most notably, LL RSM had higher mean values of HCT than ML RSM at all pH values, apart from pH 6.2 and 6.3. The higher HCT of LL RSM, suggests that the negative impact of higher concentrations of protein and casein on HCT (Rattray & Jelen, 1996; Meena et al., 2016) is mitigated by the lower and higher concentrations of lactose and urea, respectively (Singh,

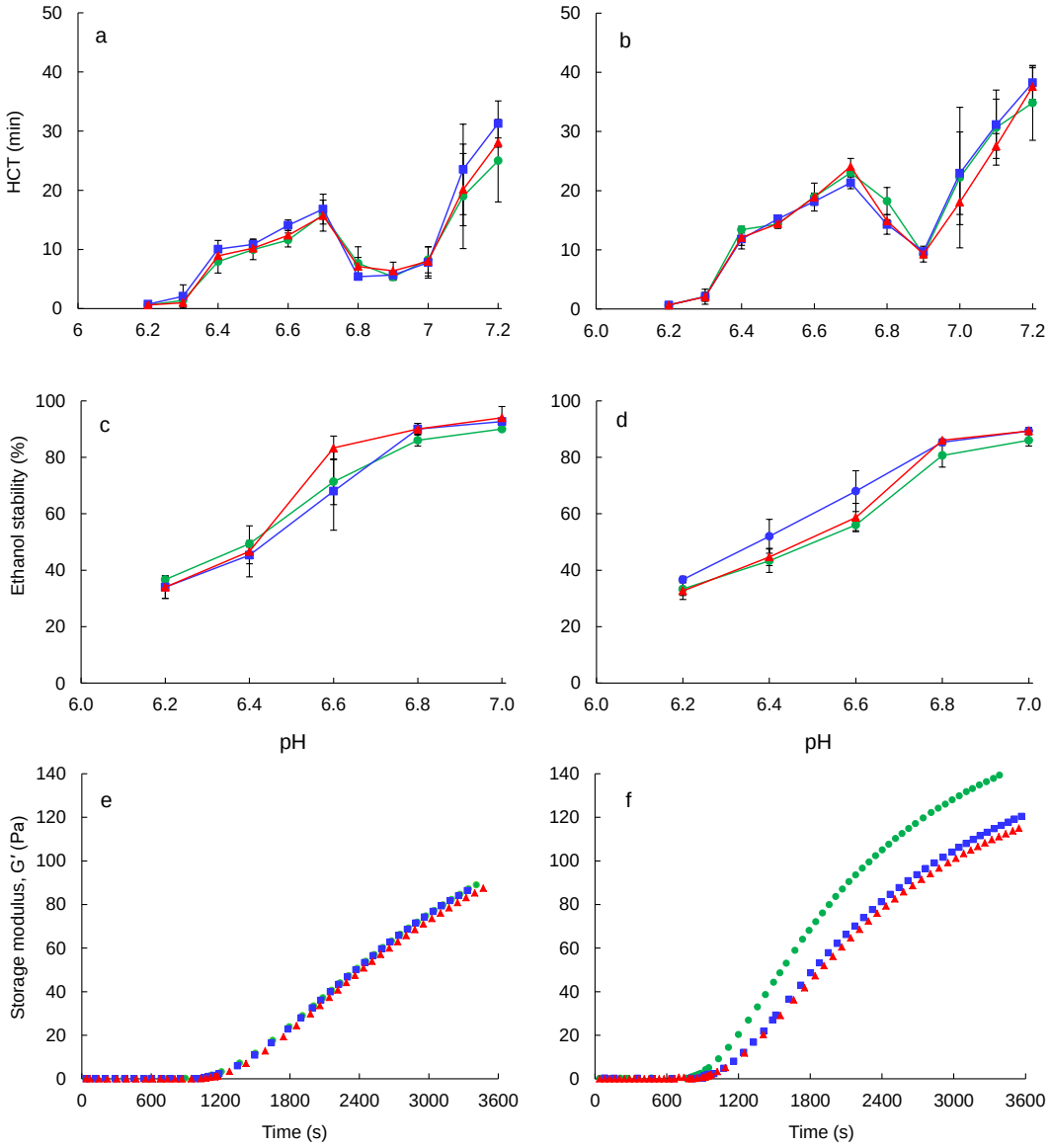

Figure 8.1. Processing characteristics of reconstituted skim milk (RSM, 10%, w/w), prepared from low-heat skim milk powder produced in mid lactation (open symbols; a, c, e) and late lactation (closed symbols; b, d, f) from the milks of dairy herds on different feeding systems: grazing on perennial ryegrass pasture (GRO; ○, ●), grazing on perennial ryegrass and white clover pasture (GRC; □, ■), or housed indoors and offered total mixed ration (TMR; △, ▲). Presented values for heat and ethanol stability over pH range 6.2-7.2 are the means of 3 replicate trials in both mid- and late-lactation; error bars represent standard deviation of the mean. A representative rennet gelation profile (G' vs. time) for one trial in mid- and late-lactation milks is shown in e and f, respectively.

2004; Sikand et al., 2010). Hence, regression analysis of the entire set showed that HCT_{npH} and HCT_{max} correlated negatively with lactose content and lactose-to-protein ratio ($p < 0.01$) and positively with urea content ($p < 0.05$) (Table 8.6). Other studies have also observed a positive correlation between the HCT_{max} and urea concentration of seasonal

milks (Holt et al., 1978; Kelly et al., 1982; Banks et al., 1994). Lactose undergoes thermal-induced degradation to organic acids (e.g., formic) on heating at temperatures of 140 °C, and thereby reduces the pH of the milk during the HCT assay, whereas heat-induced degradation of urea results in the production of ammonia which buffers the pH decrease associated with thermal decomposition of lactose and precipitation of calcium phosphate, and thereby enhances HCT (Singh, 2004). Natural pH and concentration of salts (Ca, Mg, P, Na, K, Ca^{2+}) have also been identified as important factors affecting the HCT of milk and RSM (Newstead, 1977; Faka et al., 2009; Sikand et al., 2010); however, these parameters were scarcely affected by lactation stage (Tables 8.2 and 8.3).

Ethanol stability

As for HCT, feeding system had little impact or ES of the RSM in the pH range 6.2-7.0. Though ES at pH 6.8 and 7.0 differed somewhat with feeding system (Table 8.3), the magnitude of the differences (4-5 %, v/v) was relatively small and unlikely to be of practical significance (Fig. 8.1). Analogously, O'Brien et al. (1997) reported no effect of altering the DHA from 16 to 24 kg dry matter per cow on ES at natural pH, despite an increase in casein content of 0.2 % (w/w). The lack of an effect of feeding system on ES may be attributed to the similarity in composition and casein profile of the GRO-, GRC- and TMR-RSM. Nevertheless, the slightly lower ES of GRO-RSM compared to the GRC or TMR-RSM at pH 6.8 or 7.0 (Table 8.4) is compatible with its higher ratio of Ca^{2+} to casein (Table 8.3; Tsioulpas et al., 2007).

Lactation stage had a significant effect on ES at pH 6.8 and 7.0, with the mean values of the ML milk (across all feeding treatments) being higher than that the corresponding LL milk. However, the magnitude of the difference in ES at these values (4-

5 % ethanol) was also relatively small. A similar trend was noted at pH 6.6, but the magnitude of the difference between the ES between the ML milk (75 %, v/v) and LL milk (61 %, v/v) was much larger. The results concur with those of Horne et al. (1986) who reported that the asymptotic maximum ES (in pH range ~ 6.7-7.5) increased rapidly during the first 5-100 d of lactation and thereafter showed no further lactational trend.

Rennet gelation

Rennet gelation was significantly affected by feeding system and lactation stage.

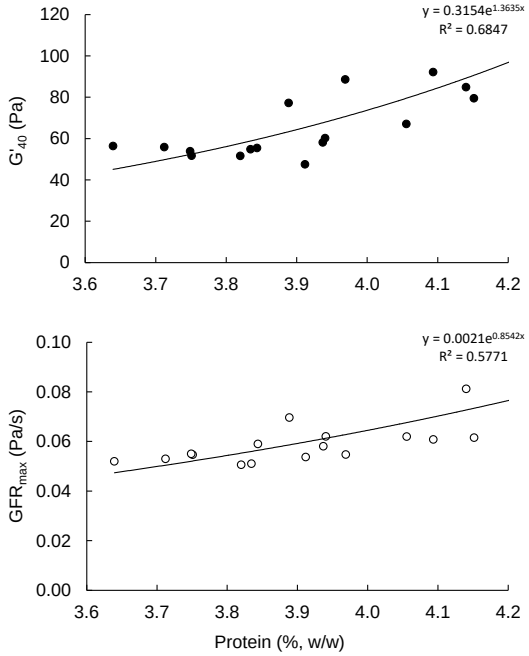

Figure 8.2. Relationship between gel firmness at 40 min, G′40, (●), or maximum gel firming rate, GFRmax, (○) and the protein content of reconstituted skim milk (10 %, w/w) prepared from low-heat skim milk powder made from the milks of dairy herds on different feeding systems in mid- and late-lactation. Regression lines (-) were fitted to the entire data set, comprising three replicate trials for three feeding systems in mid- and late-lactation. Both relationships were statistically significant ($p < 0.001$).

Milk from GRO feeding system had higher gel strength, G'_{40}, than milk from TMR feeding system in LL and ML+LL, but not in ML; G'_{40} for GRC RSM was intermediate between that of GRO- or TMR-RSM. The relatively high G'_{40} of GRO-RSM is most likely due to its protein content (Guinee et al., 1996), as supported by the exponential increase in G'_{40} with protein and casein (Fig. 8.2). Other studies have found similar relationships between milk

protein content and gel strength (Guinee et al., 1996) in the range 3.0-7.5 % (w/w). Late lactation RSM had a lower mean RGT and higher values of GFR_{max} and G'_{40} than the corresponding ML milk. The stronger rennet coagulability of LL is consistent with its higher mean concentration of protein (0.27 %, w/w) casein (0.21 %, w/w) (Table 8.4 or Fig. 8.2).

Stirred-yoghurt forming properties

The changes in pH and G′ during acidification are shown in Fig. 8.3 for one of the trials in ML and LL; similar changes were observed for the replicate trials (data not shown). G′ remained relatively constant until the gelation-onset pH (GO_{pH}; 5.56-5.38, Table 8.5) and then increased sigmoidally; simultaneously, tan δ, the ratio of the viscous or loss modulus (G″) to storage modulus (G′), decreased. The changes mark the gradual aggregation of the dispersed particles (casein micelles, casein micelle-denatured whey protein complexes, protein-covered fat globules) into a gel network as the pH decreased further towards the casein isoelectric point, pH 4.6 (Lucey, 2016). On shearing the resultant yoghurt, shear stress decreased less than proportionally with shear rate. The shear stress versus shear rate data for all yogurts fitted to the Herschel–Bulkley model ($R > 0.99$). All yogurts exhibited a yield stress, σ_o (4-10 Pa) at low shear rate, and thereafter shear thinned on increasing shear rate to 120 s^{-1} (Fig. 8.3). The trend reflects the presence of a particulate protein network which was disrupted during shearing. The viscosity at 120 s^{-1} for all yoghurts, 200-220 $mPas^{-1}$, was of similar magnitude to that previously reported for yogurt with similar protein content and made under similar conditions (Guinee et al., 1995; Lin et al., 2018b).

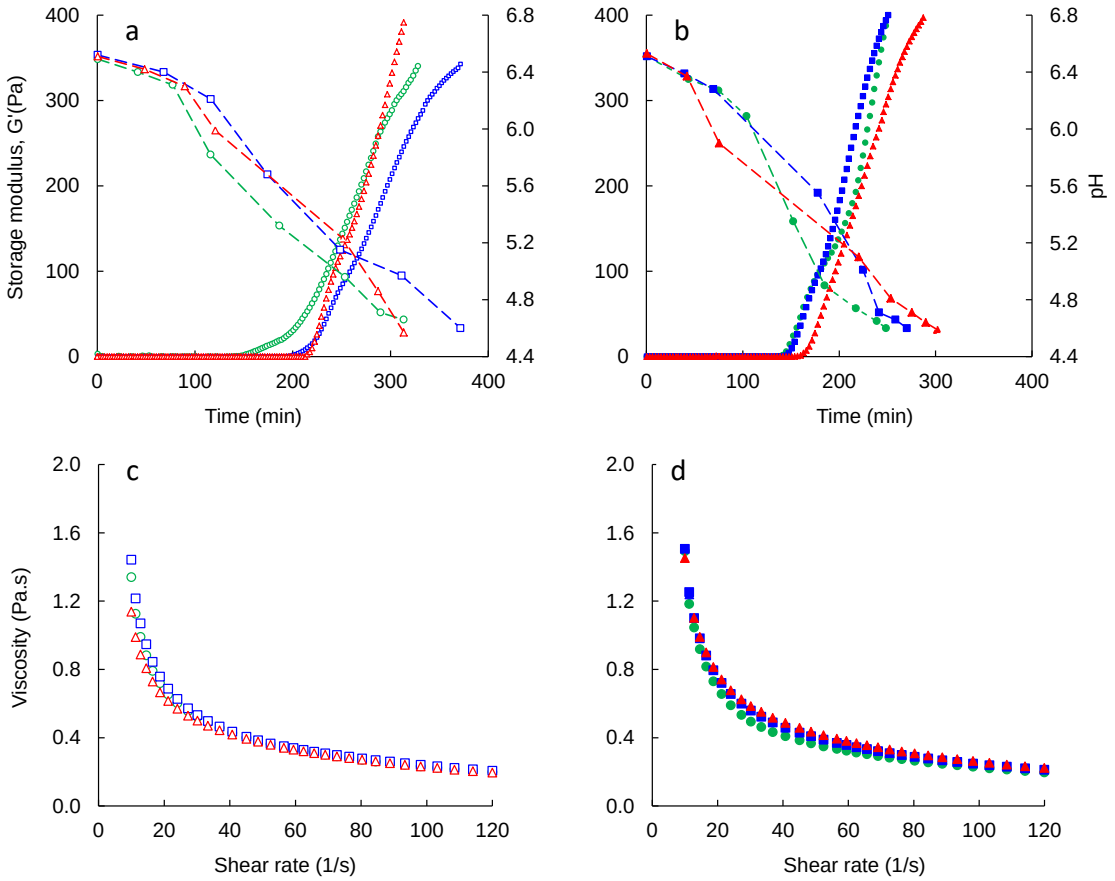

Figure 8.3. Effect of dairy cow feeding system on the properties of model yoghurt prepared in mid lactation (a, c) and late lactation (b, d) using skim milk powder from the milks of dairy herds on different feeding systems: grazing on perennial ryegrass pasture (GRO; ○, ●), perennial ryegrass and white clover pasture (GRC; □, ■), or housed indoors and offered total mixed ration (TMR; △, ▲). Changes in storage modulus, G′ (no line) and pH (broken line) during fermentation of milk at 42 °C (a, b); viscosity of final yoghurt on shearing at 8 °C (c, d) are shown. The presented data and trends shown for one trial in mid- and late-lactation are representative of those in replicate trials.

Feeding system did not affect GO_{pH}, fermentation time (to pH 4.6), G′ at pH 4.6 ($G'_{pH4.6}$), consistency properties (σ_o, K, n or $\eta 120s^{-1}$) or WHC of the final yoghurt (Table 8.5). The absence of an effect on feeding system on GO_{pH} and fermentation time, despite the difference in the protein content between the different feeding systems (e.g., 0.19 % protein in ML and 0.39 % in LL; Table 8.5), most likely reflects the standardisation of the starter culture inoculum *pro rata* with milk protein content. The rate of pH reduction during bacterial-induced lactic fermentation of milk is controlled primarily by the buffering capacity of the milk, which is determined by its concentrations of casein and colloidal

calcium phosphate (Lucey et al., 1993). Previous studies have shown an increase in the G′ of model acid-induced milk gels as a function of casein concentration in the range of 1-5 % (w/w) (Roefs, 1986) and the viscosity of yoghurt on fortification of milk with skim milk powder (1.8 %, w/w; ~ 0.63 %, w/w, protein) or sodium caseinate (1.8 %, w/w; ~ 1.6 % protein) (Tamime & Deeth, 1980). The absence of an effect of feeding system on $G'_{pH4.6}$, σ_o, K or $\eta 120s^{-1}$ suggests that the difference in the mean protein concentration of the yoghurt milk (0.2- 0.4%, w/w; Table 8.5) between the feeding systems was insufficient to override the inter-trial variation associated with factors such as starter culture activity and rate of fermentation. Likewise, Jasińska et al. (2010) found that the hardness of set yoghurt made from non-standardised milk from dairy herds fed on grass (supplemented with concentrates) or on total mixed ration varied with month of year, with no evidence of a consistent effect of feeding system.

Lactation stage had no effect on yoghurt properties (GO_{pH}, $G'_{pH4.6}$, σ_o, K or $\eta 120s^{-1}$), apart from fermentation time which was on average ~ 70 min longer for ML milk than LL milk (Table 8.5). Considering that the starter inoculum was standardised relative to the casein content of the milk, the trend may reflect the slightly higher mean phosphorous-to-casein ratio in ML (36 mg/g casein) milk compared to LL milk (34 mg/g casein) (data not shown), which in turn would favour a higher buffering capacity and resistance to pH decrease (Lucey et al., 1993). In contrast, Muir & Tamime (1993) found a significant effect

Table 8.5: Characteristics of reduced-fat yoghurt prepared using anhydrous milk fat (2.3 %, w/w) and reconstituted skim milk powder (12.7 %, w/w), from milks of dairy herds on different feeding systems in mid- and late-lactation.[1]

	Feeding systems								Probability values (p) for overall effects		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation stage (LS)	Interaction FS*LP
Yoghurt milk composition											
Total solids (%, w/w)	14.5	14.3	14.6	0.267	14.5	14.4	14.5	0.233	0.479	0.297	0.561
Fat (%, w/w)	2.27	2.27	2.21	0.042	2.33	2.32	2.29	0.037	0.546	0.121	0.944
Lactose (%, w/w)	6.66	6.68	6.83	0.056	5.64^{b}	5.99^{b}	6.54^{a}	0.159	0.018	0.001	0.082
Protein (%, w/w)	5.17^{a}	5.08^{ab}	4.98^{b}	0.020	5.60^{a}	5.38^{b}	5.21^{b}	0.023	0.019	0.021	0.349
Denatured whey protein (% of total)	78.9	79.4	79.0	0.401	78.3	80.4	80.1	0.741	0.561	0.434	0.556
Gelation during yogurt manufacture											
Gelation onset pH (GO_{pH})	5.56	5.40	5.40	0.100	5.47	5.55	5.38	0.111	0.597	0.906	0.620
Storage modulus at pH 4.6 ($G'_{pH4.6,}$ Pa)	330.1	367.8	383.0	23.27	372.1	395.2	340.1	27.99	0.683	0.762	0.454
Fermentation time (min)	337.8	326.1	310.1	35.1	235.1	280.0	256.1	16.5	0.676	0.007	0.462
Yogurt properties											
Yield stress ($\sigma_{o,}$ Pa)	7.92	10.40	11.08	0.794	8.18	8.14	4.95	1.93	0.701	0.076	0.208
Consistency coefficient (K, $Pa.s^{n}$)	1.16	0.82	2.87	1.003	1.49	1.76	3.11	1.109	0.196	0.742	0.857
Flow behaviour index (n, -)	0.66	0.62	0.45	0.122	0.69	0.53	0.48	0.093	0.194	0.927	0.817
Viscosity of sample at shear rate of 10 (1/s) (mPa.s)	1317	1538	1469	180.6	1282	1530	1354	128.2	0.363	0.683	0.932
Viscosity of sample at shear rate of 120 (1/s) (mPa.s)	202	209	206	4.16	216	222	210	8.45	0.754	0.261	0.887
WHC at 300 *g* (g of serum retained/100 g yogurt)	71.2	83.0	78.9	2.35	76.8	80.9	80.0	2.42	0.103	0.591	0.528

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid- and late-lactation; SED = standard error of difference between means. Values within a row relating to mid- or late-lactation and not sharing a common lower-case superscripted letter differ significantly ($p < 0.05$) for the effect of feeding system. Probability (P) values for the effects of feeding system in overall lactation (ML+LL), lactation stage (ML or LL) across the different feeding systems, and their interaction. Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration. Lactation stage (LS): mid lactation [July 4-20; 137-153 d in lactation (DIM)] and late lactation (September 27-October 7; 222-232 DIM). WHC = water holding capacity of yoghurt.

Table 8.6: Significant relationships between composition and processing characteristics of reconstituted skim milk (10%, w/w) powders.[1]

Processing characteristics	Compositional parameters	Correlation coefficient (r)
Heat stability		
HCT_{max}, HCT_{npH}	lactose (%, w/w)	-0.753***, - 0.797***
	lactose-to-protein	-0.724***, -0.788***
	protein (%, w/w)	+0.636**, +0.723***
	casein (%, w/w)	+0.553*, +0.660**
	NPN (%, w/w)	+0.549*, +0.659**
	urea (mg/100g)	+0.563*, +0.635**
Ethanol stability		
$ES_{6.8}$, $ES_{7.0}$	$[Ca^{2+}]$	-0.571*, -0.568*
Rennet gelation		
RCT	protein (%, w/w)	-0.525*
	casein (%, w/w)	-0.597**
GFR_{max}, G'_{40}	protein (%, w/w)	+0.718***, +0.803***
	casein (%, w/w)	+0.658**, +0.789**

[1]The data set comprised 18 reconstituted skim milks from low heat skim milk powders obtained using from different feeding systems (GRO, GRC and TMR) in mid and late lactation in triplicates. Correlations were obtained using simple linear regression analysis; only relationships found to be statistically significant are shown: ***, $p < 0.001$; **, $p < 0{\cdot}01$; *, $p < 0{\cdot}05$. Positive and negative correlations between two parameters are indicated by a positive sign (+) and a negative sign (-), respectively. HCTmax = maximum heat coagulation time; HCT_{npH} = heat coagulation time at natural pH; $ES_{6.8}$ = ethanol stability at pH 6.8; $ES_{7.0}$ = ethanol stability at pH 7.0; RCT = rennet coagulation time; GFR_{max} = maximum gel firming rate; G'_{40} = gel firmness at 40 min.

of season on the viscosity of stirred yoghurt from homogenised ovine milk which varied in concentrations of protein (~ 5.0-7.8 %, w/w), fat (~ 5.6-9.5 %, w/w) and Ca (~ 37-53 mM) over the period March to September. Similarly, Cheng et al. (2002) reported that the viscosity of stirred-yoghurt correlated positively with protein concentration (4.1-4.9 %, w/w, for RSM with 12 % TS). The inter-study discrepancy on the effect of seasonality may reflect many factors, including differences in duration of lactation stage and the protein content between treatment yoghurts (i.e., a mean protein of ~5.08 vs. 5.4 %, w/w, in ML and LL, respectively, in the current study) compared to 0.9 or 2.8 % (w/w) in the latter studies.

8.5 Conclusion

This study investigated three different dairy-cow feeding systems (perennial ryegrass, GRO; perennial ryegrass and white clover, GRC; or total mixed ration, TMR) in mid (ML)- and late (LL)-lactation for their effects on composition and colour of low heat skim milk powder, and the biochemical and processing characteristics on reconstituted skim milk prepared by dispersing the powder to 10% (w/w). Powder from the GRO or GRC feeding systems had a higher mean content of protein (~ 2.5 %, w/w), lower contents of lactose (~ 3.5 %, w/w), I, Cu and Se, and a more ‘green-yellow’ colour than the corresponding powder from TMR milk. The GRO-RSM had higher mean concentrations of protein (0.27 %, w/w) and casein, lower concentrations of lactose (~ 0.4 %, w/w) and NPN (% total N), and higher rennet-gel strength than TMR-RSM. These effects were observed for the combined ML+ LL period, but varied in the separate ML and LL periods, depending on the parameter. The levels of protein and NPN (% TN) and rennet gel strength of GRC-RSM were intermediate between those of the corresponding GRO-RSM and TMR-RSM. Feeding

system had little, or no, effect on the physicochemical characteristics, heat coagulation time or ethanol stability of the RSM, or the consistency characteristics of stirred yoghurt prepared from the RSM. The lower lactose-to-protein ratio of the GRO and GRC powders may be more desirable from a nutritional and functional perspective in many applications, for example in recombined milks that are used for cheese manufacture or subjected to high heat treatment. The difference in the elemental composition of the powders from the different feeding systems is of relevance when formulating dairy-based nutritional beverages, e.g., infant milk formula, with target levels of minerals.

Chapter 9

General Discussion

Summary

The composition of milk is a key factor affecting its processability (e.g., rennet- and acid-coagulability, and heat and ethanol stability), product-yielding capacity, and dairy products quality. Milk composition is affected by many factors including breed of cow, stage of lactation of the cow, calving pattern, and plane of nutrition. Both, quantity of feed and type of feed fed to dairy cows can affect composition of milk. Globally, dairy cows are predominantly fed by housing them indoors all year and offering a total mixed ration (TMR), and to a lesser extent by grazing on pasture such as perennial ryegrass (*Lolium perenne* L.) (GRO) and perennial ryegrass + white clover mixed sward (*Trifolium repens* L.) (GRC). Most of the milk supply from Ireland derives from spring-calved herds grazing on pasture. Moreover, for spring calved herds, herbage supply in early spring or early lactation (EL) of cows is of utmost importance, when demand of cows often exceeds grass growth and supply (Kennedy et al., 2007). While there are few studies on the different herbage allowance to cows in EL, and on the separate effects of each of the feeding systems on milk composition; there is very little information on reducing the herbage allowance in EL and comparative effects of feeding systems on milk composition and processability throughout lactation. The effects of reducing DHA in EL on milk composition and processability throughout lactation were demonstrated in this thesis. Three major feeding systems GRO, GRC and TMR were also compared for milk composition, processing characteristics and Mozzarella cheese and LHSMP quality throughout lactation. Additionally, a novel technique called cavitation rheology was utilised to derive mechanical properties of Mozzarella cheese and compared with conventional large strain deformation and low-amplitude strain oscillatory rheometry.

Chapter 3 examined the effects of reducing daily herbage allowance (DHA) in early lactation to dairy herds, resulted in lowered milk yield, milk solids yield, concentrations of protein and casein during EL. Nonetheless, it had little or, no effect on gross milk composition, nitrogen fractions, element profile, rennet gelation and heat stability in mid- or late- lactation. Conversely, stage of lactation resulted in relatively larger effects on milk composition and processing characteristics, when compared to the effects from reduced DHA in EL.

Chapter 4 examined the effects of feeding system on gross composition and mineral composition in mid (ML) - and late-lactation (LL). Milk from GRO feeding system had the highest mean concentrations of total protein, casein, Ca, and P, while TMR milk had the highest concentrations of lactose, Cu, and Se, and lowest level of total protein. The GRC milk was intermediate between GRO and TMR milks for most compositional parameters. However, the exact effects of feeding system on individual variables depended on the lactation stage, with the effects being most pronounced in late lactation, when inter-feeding system differences in the contents of casein, fat and lactose were larger. The mean levels of protein, casein, fat, Ca, P, Na, Mg, Zn, Mo and Co) in late lactation milk were higher than those in mid lactation.

Chapter 5 examined the effect of feeding system on nitrogen fractions, rennet coagulation and heat stability in ML. Compared to TMR milk, GRO milk had higher mean concentrations of total protein, true protein and casein, a higher proportion of αs_2-casein, and enhanced rennet coagulability. The superior rennet coagulability of GRO milk was consistent with its higher concentrations of casein and Ca. Feeding system had little, or no, effect on the proportions of individual caseins or whey proteins, NPN, urea, concentrations of lactose, ionic calcium (Ca^{2+}) and serum casein, or heat stability at 140 °C. The absence

of an effect of feeding system on heat stability in ML was consistent with the similar concentrations of urea and Ca^{2+}.

Chapter 6 examined the effect of feeding system on Mozzarella cheese-making characteristics in ML and LL. Compared to TMR milk, GRO milk had higher concentrations of protein and casein, and lower concentrations of I, Cu and Se, higher cheese-yielding capacity and produced cheese which had: lower concentrations of the trace elements I, Cu and Se; a more yellow colour, higher heat-induced flow and greater fluidity when melted. These effects were observed over the entire lactation (ML+LL), but varied somewhat in ML and LL. Feeding system had little, or no, effect on gross composition of the cheese, the proportions of milk protein, fat or minerals lost to cheese whey, the texture of the unheated cheese, or the energy required to extend the molten cheese. Lactation stage had a significant effect on the elemental composition of milk (Ca, Mg, Zn, I, Cu, Mo, Se) and cheese (Zn, Cu, Mo), cheese yield and cooking properties.

Chapter 7 demonstrates the use of cavitation rheology (CR) to evaluate the changes in mechanical properties of low-moisture Mozzarella cheese during ageing at 4 °C. The results showed that the linear modulus, *E*, obtained using cavitation measurement was significantly lower than the elastic shear modulus (*G′*). The change in E over ageing of cheese was positively correlated with *G′*. Thus, cavitation rheology could potentially be used as an alternative to low shear strain deformation to monitor changes in elasticity in Mozzarella cheese after ageing for >7 d. It was suggested that the discrepancy between values of *E* and *G′* may have due to of occurrence of irreversible fracture rather than elastic cavitation during measurement. However, a wider range of experiments would need to be employed to confirm if mechanism for deformation is fracture or elastic cavitation.

Chapter 8 examined the effect of feeding system on composition and processing characteristics of LHSMP in ML and LL. Over the entire lactation stage (ML+LL), LHSMP from GRO milk had higher contents of total protein, casein and whey protein, and lower contents of lactose and iodine than the corresponding powder from TMR milk. The colour of LHSMP from pasture-based milks (GRO, GRC) was more green-yellow than that from TMR-based milk. Otherwise, feeding system did not affect the levels total solids, NPN (% total N), urea (% total N), or protein profile of the powder. Reconstituted milk from GRO powder (10%, w/w) had higher Ca^{2+}, stronger rennet coagulability, and slightly, but significantly, lower ethanol stability at pH 6.8 and 7.0 than the equivalent reconstituted milk from TMR powder. Otherwise, reconstituted milks from GRO, GRC or TMR powders had similar values for casein micelle size, casein hydration and heat stability. Feeding system did not affect the rheological properties of stirred-yoghurt. Late-lactation RSM had higher concentrations of protein, NPN and urea, serum casein (as a % of total casein), stronger rennet coagulability and heat coagulation time.

Conclusions

The level and type of dairy cow diets had an effect on composition and of milk and some of its processing behaviour. The absence of an effect of lowering DHA in EL on most compositional parameters and processability characteristics (rennet gelation and heat stability) in EL, ML or LL suggests that restricted grazing without concentrate supplementation can, within limit, be applied in early lactation with little consequence apart from the lower yields of milk and milk solids during that period. This of relevance to farm management where adverse weather in spring can further reduce grass availability to cows, especially where stocking rate is high. Feeding system effects on protein, casein, lactose,

Ca, P, I, Cu, Se are important nutritionally. The effect of feeding system on the processability parameters, including, rennet gelation and actual cheese yield can be explained by a higher concentration of protein and Ca in GRO milk relative to TMR milk. The absence of effect of feedings system on heat stability, ethanol stability, or yoghurt-making properties of milk or reconstituted skim milk power can be explained by interactive effects of higher protein and lower lactose concentration in GRO milk relative to TMR milk, which counteract each other and similar ratio of macroelements-to-casein in all milks. Milk from GRO feeding system results in higher cheese yielding capacity of the plant thus contributing towards profitability; although the effects on rennet gelation and actual cheese-yielding capacity could probably be eliminated by milk protein standardisation, e.g., by ultrafiltration to maximize cheese plant capacity. On the other hand, colour effects for products such as cheese, and different ratio of trace elements (I, Cu, Se)-to-protein associated with milk feeding system are more difficult to mask. Product colour is important when manufacturing for a particular market or consumer group, thus Mozzarella from TMR which is whiter could be suitable for some markets. Alternatively trace elements-to-protein ratios are crucial for processors in the formulation of nutritional beverages where target ratios of elements-to-protein are defined and regulated. The differences in elements-to-protein ratio either in milk or powder will necessitate the alteration in mineral mixes added while manufacturing infant milk formula, formulated foods or reformulated milks from powders.

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