Cheddar and Mozzarella cheesemaking characteristics of bovine milks containing κ-casein A and B variants

Thesis

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for Degree of

Doctor of Philosophy

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: Deirdre Walsh **ID No.:** **94970238**

Date: 22/2/'99

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Abbreviations

$A_{60:}$	Curd firmness after 60 minutes of rennet coagulation
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
CN	Casein
FAA	Free amino acids
FDM	Fat in dry matter
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
HPLC	High performance liquid chromatography
IEF	Isoelectric focusing
Ile	Isoleucine
K_{20}	Rate of curd firming
LA	Lactalbumin
LG	Lactoglobulin
Lys	Lysine
MNFS	Moisture non-fat substance
NS	Not significant
PAGE	Polyacrylamide gel electrophoresis
pI	Isoelectric point
Pro	Proline
PTA	Phosphotungstic acid
RCT	Rennet coagulation time
S/M	Salt in moisture
SED	Standard error of difference
Ser	Serine
TBC	Total bacteria count
TCA	Trichloro acetic acid
TEMED	N,N,N',-tetramethylethylenediamine
Thr	Threonine
Val	Valine

Abstract

Cheddar and Mozzarella cheesemaking characteristics of bovine milks containing κ-casein A and B variants

C. Deirdre Walsh

This investigation studied the influence of κ-casein A and B variants on the composition, production and for the first time the pilot-scale Cheddar and low moisture part-skim Mozzarella cheesemaking properties of milks from Irish Holstein Friesians. Analysis of 6,007 individual animal milks showed that only 1.98 % of animals had the κ-casein BB phenotype. No statistically significant associations ($P < 0.05$) were observed between κ-casein variant and milk yield and composition (Chapter 1). Renneting studies showed that rennet coagulation time was related to κ-casein variants as follows: κ-casein AA>AB>BB while the rate of curd firming and curd firmness values were such that κ-casein AA<AB<BB (Chapters 2 & 5). The moisture adjusted Cheddar and Mozzarella cheese yields for AA and BB variant milks were 92.5 and 100.1, and 91.5 and 102.5 kg cheese/1000 kg milk, respectively. The moisture and protein adjusted Mozzarella cheese yields for κ-casein AA, AB and BB variants were 90.2, 93.2 and 95.2 kg cheese/1000 kg milk, respectively (Chapters 2 & 5). Cheddar cheeses made from κ-casein BB variant milk had significantly higher ($P<0.05$) free amino acid levels after 270 days ripening than κ-casein AA cheeses. Rheometric analysis during a 270 day ripening period showed that κ-casein BB Cheddar cheeses were significantly softer ($P<0.05$) at 90 and 180 days and had a lower fracture stress at day 180 than AA variant cheeses. κ-Casein variant had no significant effect on the sensory characteristics of Cheddar cheese (Chapter 3 & 4). Furthermore, κ-casein variant had no significant effect on the proteolysis (nitrogen soluble at pH 4.60 and nitrogen soluble in 5 % phosphotungstic acid), rheological (hardness) or functional characteristics (melt, flow and stretch) of Mozzarella (Chapter 5). Given the above results, it was concluded that κ-casein BB variant milk resulted in improved rennet coagulation properties and higher yields of Cheddar and Mozzarella cheese while having no detrimental effects on cheese quality. Furthermore, increasing the frequency of the B variant of κ-casein in Irish Holstein Friesian dairy herds should be beneficial in the production of milk for the manufacture of Cheddar and Mozzarella cheese.

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Dedicated to my son Seán

Literature Review

1.1 Bovine Milk

Milk is a fluid secreted by lactating females of all mammalian species. There are over 4,000 mammalian species in existence. The main function of milk is to meet the complete nutritional requirements of the neonate of the species. In addition to neonatal nutrition, milk also serves some physiological functions. Many of these functions are carried out by proteins and associated peptide fragments in the milk e.g. immunoglobulins, enzymes and enzyme inhibitors, binding or carrier proteins, growth factors and antibacterial agents.

The milk of domestic cows, *Bos taurus*, has become an important protein source for man. Milk is an aqueous solution of proteins, fat, lactose, minerals, and certain vitamins carried in emulsified fat globules and colloidally dispersed casein micelles. The gross composition of bovine milk is given in Table 1.1.1. The normal levels of fat, protein and lactose in bovine milks are 3.5, 3.2 and 4.5 % respectively. These levels can vary in different breeds and they change during lactation. The fat and protein levels are high initially but fall over the first 3-4 days (Fig. 1.1.1) after which the levels increase steadily into late lactation. The lactose level decreases steadily throughout the lactation period. If the fat is removed from milk, skimmed or skim milk is obtained. If the casein in skimmed milk is precipitated by reducing the pH to 4.60 (at 20°C), the resulting supernatant liquid is known as acid whey or milk serum.

Table 1.1.1 Gross composition of bovine milk

Component	Normal Level	Range
Fat (%)	3.5	2.8-5.5
Protein (%)	3.2	2.8-3.9
Lactose (%)	4.5	3.5-5.0
Ash (%)	0.7	0.65-0.80
Casein (%)	2.5	2.1-3.0
Potassium (mg/100 ml)	150	120-170
Sodium (mg/100 ml)	50	40-80
Chloride (mg/100 ml)	100	80-160
Calcium (mg/100 ml)	90	75-105
Phosphorus (mg/100 ml)	90	75-105
Citrate (mg/100 ml)	160	120-220
NPN/T.P. (%)	6.0	5.0-7.5
Urea/T.P. (%)	3.4	1.8-5.0

NPN: non protein nitrogen; T.P.: Total protein
(taken from Holland *et al.* (1989))

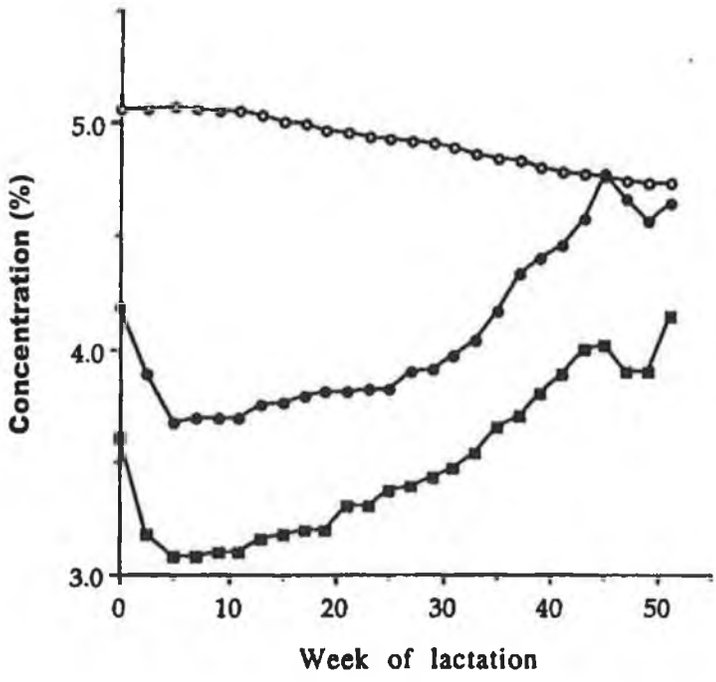

Figure 1.1.1 Typical changes in the concentration of fat (●), protein (■) and lactose (○) in bovine milk during lactation (taken from Fox and McSweeney (1998)).

1.2 Bovine Milk Proteins

The contribution of bovine milk proteins to human nutrition can be appreciated when it is realized that on a *per capita* basis, about 25 % of a persons daily protein intake in the industrial world comes from dairy products. Milk protein's are responsible for the stability of milk fat, which exists as an emulsion in the aqueous phase of milk. The major proteins of milk can be divided into two groups by the nature of their structure and physicochemical behaviour. These groups are the "caseins" and "whey proteins" (Fig. 1.2.1). The ratio of casein : whey in the bovine species is *c.* 80:20. βeta- and α_{s1}-casein make up the bulk of the casein group (>75 %) while β-lactoglobulin is the most abundant (> 50 %) fraction in the whey group. While κ-casein represents only 12.7 % of the casein fraction its role in casein micelle structure (Section 1.2.2.2) makes it an important protein in milk.

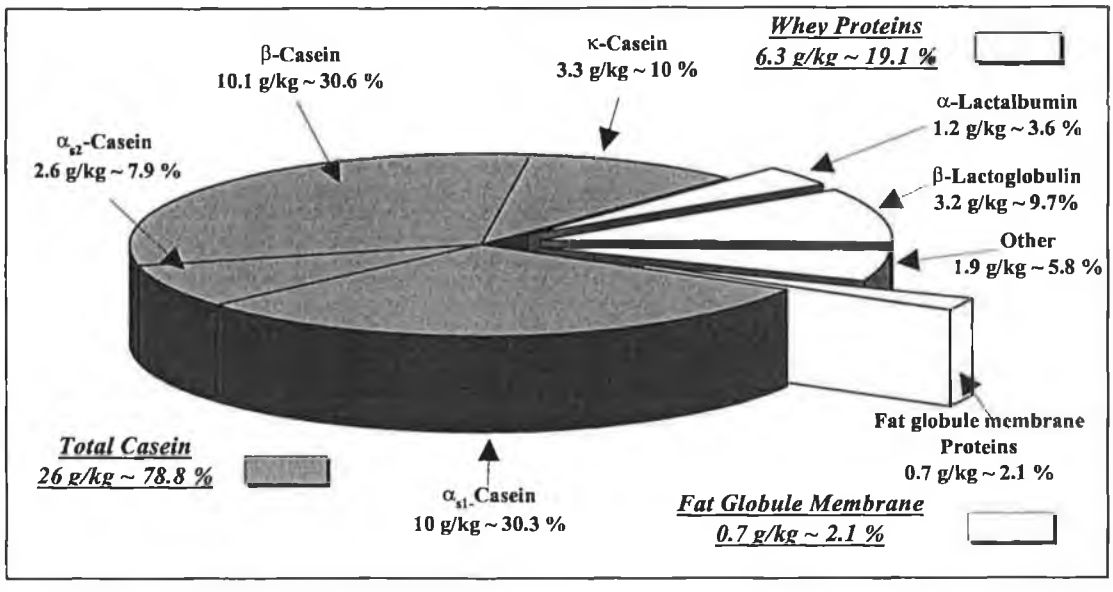

Figure 1.2.1 Approximate concentration of individual proteins in bovine milk (taken from Eigel *et al.* (1984)).

1.2.1 Whey Proteins

About 20 % of the total protein of bovine milk belongs to a group of proteins generally referred to as whey, serum proteins or non-casein nitrogen.

Whey protein can be divided into two main groups by fractionation with saturated $MgSO_4$ or half saturated $(NH_4)_2SO_4$. The resultant precipitate is referred to as lactoglobulin and the soluble fraction is referred to as lactalbumin. The lactoglobulin fraction consists mainly of the immunoglobulins (Ig) while the lactalbumin fraction consists of three main proteins (1) β-lactoglobulin, (2) α-lactalbumin and (3) blood serum albumin. While the whey proteins represent 20 % of the total protein in bovine milk, this study concentrates on κ-casein and therefore more time will be devoted in this review to the casein fraction of milk.

1.2.2 Caseins

"Whole Casein" has been defined as a heterogeneous group of phosphoproteins precipitated from raw skim milk at pH 4.6 and 20°C. Casein comprises about 79 % of total milk protein and bovine milk ranges from 2.1 to 3.0 % casein. The principle casein fractions are α_{s1}-, α_{s2}-, β- and κ–casein. The distinguishing property of all caseins is their low solubility at pH 4.6. The common compositional factor in caseins is that they are conjugated proteins, most with phosphate groups esterified to serine residues. These phosphate groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content (Fox and McSweeney, 1998). About 95 % of the casein in milk exists as large colloidal particles known as micelles

1.2.2.1 Casein micelles

Since micelles are of colloidal dimensions, they are capable of scattering light and the white colour of milk is due largely to light scattering by casein micelles; the white colour is lost if the caseins are disrupted (Fox and McSweeney, 1998). The structure of casein micelles has attracted the attention of scientists for a considerable time. Knowledge of micelle structure is important because the stability and behaviour of the micelles is central to many dairy processing operations e.g. cheese manufacture, stability of sterilised, sweetened-condensed and reconstituted milks and frozen products (Fox and McSweeney, 1998).

1.2.2.2 Casein micelle structure

Various models of casein micelle structure have been proposed and refined over the past 40 years (for review see Fox and McSweeney, 1998). There has been strong support for many years on the view that micelles are composed of sub-micelles of mass ~ 10^6 Da and diameter 10-15 nm. Morr (1967) proposed a model in which sub-micelles are linked together by colloidal calcium phosphate (CCP) which gives the micelle a porous open structure. The micelles disintegrate on removal of CCP by e.g. acidification/dialysis, EDTA, citrate or oxalate. However disintegration may also be achieved by treatment with urea, SDS or at pH values > 9 which does not solubilise CCP, suggesting that other factors contribute to micelle structure e.g. hydrogen and hydrophobic bonds.

The sub-unit model for casein micelle structure has been refined several times. Currently the view is that the content of κ-casein varies from sub-micelle to sub-micelle. κ-Casein rich sub-micelles predominate at the surface of the micelle with some α_{s1}-, α_{s2}- and β-caseins also exposed on the surface but with the κ-casein deficient micelles concentrated in the interior. The C-terminal region of κ-casein is thought to protrude from the surface of the micelle giving a 5-10 nm thick hairy layer (Fig. 1.2.2). The stability of the micelle is due to this hairy layer which contributes to the zeta potential (-20 mV) and steric stabilization.

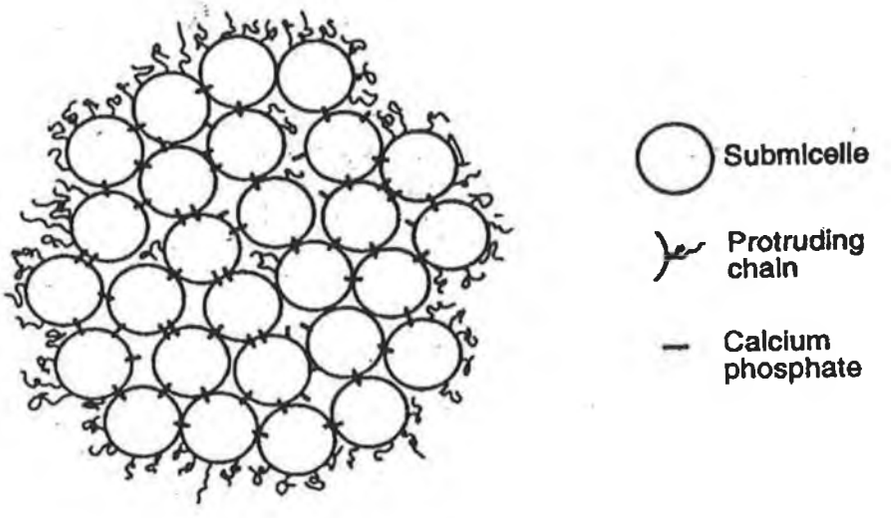

Figure 1.2.2 Sub-micelle model of the casein micelle (taken from Walstra and Jenness (1984))

The colloidal stability of micelles is destroyed and they coagulate or precipitate when this surface hairy layer is removed, e.g. following hydrolysis of κ-casein with rennet, or when the layer is collapsed, e.g. by inclusion of excess ethanol.

While this sub-micellar model explains the principle features and physicochemical reactions undergone by the micelles it has never enjoyed unanimous support. Visser (1992) has proposed an alternative model in which the micelles are spherical conglomerates of individual casein molecules randomly aggregated and held together partly by salt bridges in the form of amorphous calcium phosphate and partly by other forces, e.g. hydrophobic bonds, with a surface layer of κ-casein. A second model has been proposed by Holt (1992, 1994) in which the casein micelle is depicted as a tangled web of flexible casein molecules forming a gel-like structure in which microgranules of colloidal calcium phosphate are an integral feature and from the surface of which the C-terminal region of κ-casein extends, forming a hairy layer (Fig. 1.2.3). The cementing role of CCP is retained in both these models as is the proposed predominant surface location of κ-casein.

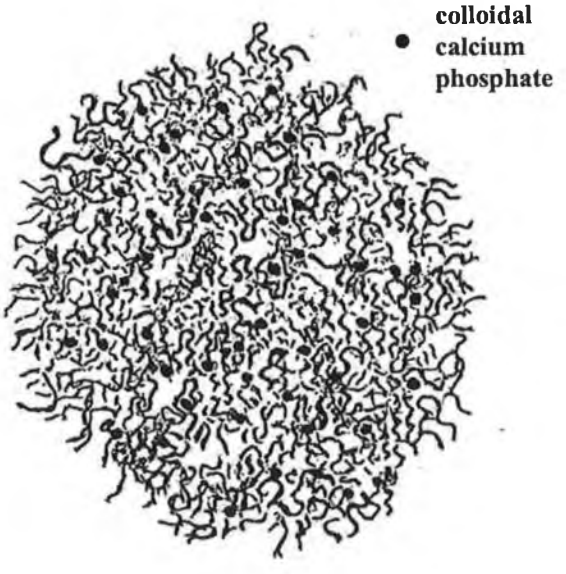

Figure 1.2.3 Model of the casein micelle (taken from Holt (1994)).

Two principle factors stabilize the micelles (1) a surface (zeta) potential of *c* -20 mV at pH 6.7, which alone is probably too small for colloidal stability, and (2) steric stabilization due to the protruding κ-casein hairs (Holt, 1994).

1.3 Milk protein polymorphism

All caseins and major whey proteins have been found to display genetic polymorphism (Fig 1.3.1). Genes are made up of defined sequences of nucleotides which code for the synthesis of specific polypeptide chains. Genes exist as two alleles at a locus in diploid organisms (one on each homologous chromosome). Genetic polymorphism in milk proteins arises from either substitutions of an amino acid or deletion of certain amino acid sequences along the polypeptide backbone. This is brought about by mutations in the sequence of base pairs of the DNA molecule which constitute the protein gene. This leads to a change in the amino acid composition of the protein resulting in a new genetic variant (Eigel *et al.*, 1984). If the frequency of occurrence of the most common allele at a given locus is less than 0.99, this locus is said to be polymorphic. A polymorphic locus produces different protein products termed "genetic variants". Figure 1.3.1 summarizes the genetic variants which have been discovered for the major caseins and whey proteins. The number of variants per protein varies with α-lactalbumin having 3 variants to β-lactoglobulin having 11 different variants. Even though 11 variants have been reported for β-lactoglobulin, Table 1.3.1 shows that only two of these variants are common in Western cattle breeds. More information on the structure of the proteins and the discovery of their variants is given in Sections 1.3.1 and 1.3.2. Many polymorphs have been found over the years using electrophoretic methods and silent variants continue to be found in various cattle breeds.

Table 1.3.1 Genetic variants of the major milk proteins commonly found in Western cattle breeds

Protein	Occurrence		
	Common	Uncommon	Not found
α-Lactalbumin	B	A, C	
β-Lactoglobulin	A, B	C, D, H, I, J, W	E, F, G
α_{s1}-Casein	B, C	A, D, F, G	E
α_{s2}-Casein	A	B, C, D,	
β-Casein	A^1, A^2, B	A^3, A^4, C, E, F,	D
κ-Casein	A, B	C, E, F, G	

(taken from Creamer and Harris (1997)).

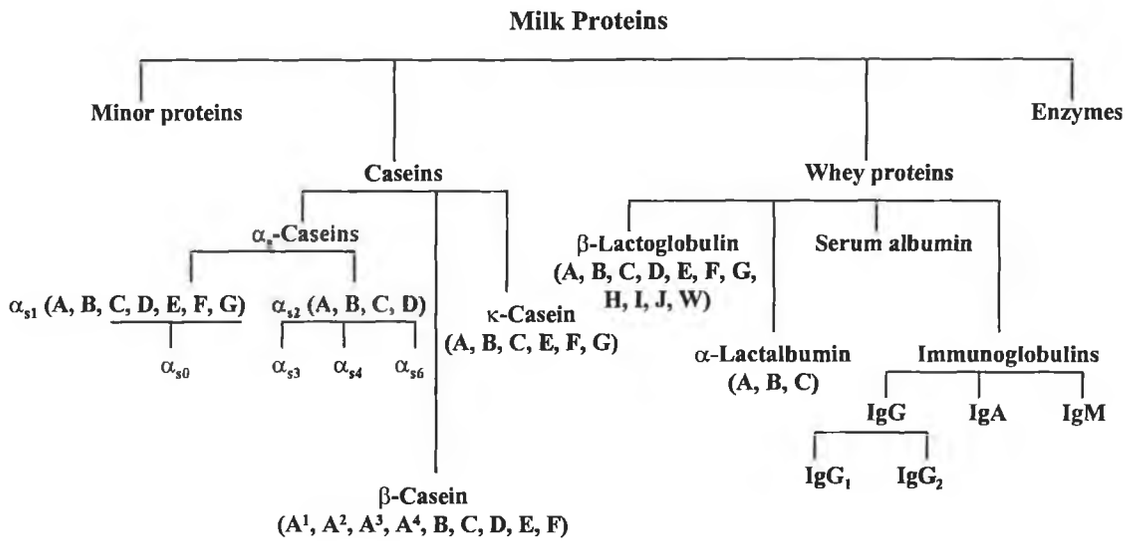

Figure 1.3.1 Genetic variants of the major casein and whey proteins found in bovine milk (adapted from Ng-Kwai-Hang and Grosclaude, 1994; Lodes, 1995; Ikonen *et al.*, 1996 and Creamer and Harris, 1997)

1.3.1 Identification of milk protein genetic variants

The milk protein variants are identifiable by a range of analytical techniques as they differ in their amino acid sequence. Variants differing in charge or size can

generally be distinguished by electrophoretic or ion-exchange chromatographic techniques. Where the difference between the variants is caused by the substitution of a neutral amino acid with a neutral amino acid these variants are said to be "silent" variants. The identification of these variants usually requires peptide mapping protocols.

1.3.1.1 Electrophoresis

Electrophoresis is a process by which molecules of different size or charge can be separated by migration in an electric field. It is over 40 years since the occurrence of milk protein genetic polymorphism was first reported. In 1955 Aschaffenburg and Drewry found two distinct β-lactoglobulin bands when milk samples from individual cows were subjected to electrophoresis on filter paper in barbitone buffer at pH 8.6. These bands were then denoted as β_1- and β_2-lactoglobulin in order of decreasing mobility. On discovery that these two types of β-lactoglobulin were controlled by di allelic autosomal genes, β_1 and β_2 were renamed as the A and B variants of β-lactoglobulin, respectively (Aschaffenburg and Drewry, 1957). The discovery of Aschaffenburg and Drewry was followed by an active search for genetic polymorphisms in other whey proteins and caseins. The predominant methods used to search for these polymorphs were electrophoretic due to its simplicity and ease of application to large sample numbers. Polyacrylamide gel electrophoresis (PAGE) has long been used as a method for fractionating and analysing proteins. Paper electrophoresis was commonly used as a supporting medium for electrophoresis up until 1955 when Smithies introduced starch gels which was then followed by polyacrylamide gels (Smithies, 1955). A chronological summary of the use of electrophoretic techniques in the discovery of milk protein genetic variants is given in Table 1.3.2

1.3.1.2 Iso-electric focussing

Iso-electric focusing (IEF) is a special type of electrophoresis whereby each protein migrates to a specific point across a pH gradient, that is to its isoelectric point (pI). The pH at which any given protein has a net overall charge of zero is termed the

Table 1.3.2 Chronological summary of the use of electrophoretic techniques in the discovery of milk protein variants

Protein	Variant	Author	Year	Methods/Remarks
α-LA		Blumberg and Tombs	1958	Paper electrophoresis (PE)
α-LA		Davoli	1981	Cellulose acetate strip supports with tris-glycine buffer containing 6 M urea at pH 9.0 in presence of 2-mercaptoethanol
β-LG	β_1, β_2	Aschaffenburg and Drewry	1955	PE
β-LG	A, B	Aschaffenburg and Drewry	1957	Nomenclature reassigned
β-LG	A, B, C	Bell	1962	Starch gel electrophoresis (GE) pH 8.5 borate buffer
β-LG	A, B, D	Grosclaude *et al.*	1966*a*	Method of Wake and Baldwin (1961, zone electrophoesis in urea) with 2-mercaptoethanol
β-LG		Aschaffenburg	1965	Agar gel in veronal buffer at pH 8.6
β-LG	A, B	Bell and Stone	1978	Cellulose acetate strips under alkaline conditions
α_{s1}-CN	A, B, C	Thompson *et al.*	1962	Starch gel urea electrophoresis, pH8.6
α_{s1}-CN		Davoli	1981	Cellulose acetate strip supports with tris-glycine buffer containing 6 M urea
α_{s2}-CN	A, B, C	Grosclaude *et al.*	1976*a*	Starch gel slabs, 2-mercaptoethanol, 7 M urea alkaline conditions
β- CN	A, B, C	Aschaffenburg	1961	PE-6 M urea (dissociation agent), pH 7.5 buffer
β- CN	A, B, C	Thompson *et al.*	1964	Starch GE
β- CN	A, B, C	Michalak	1967	Starch
β- CN	A^1, A^2, A^3	Peterson and Kopfler	1966	Polyacrylamide pH 2.8, 4.5 M urea
β- CN	"	Grosclaude *et al.*	1966*b*	Starch GE at low pH
β- CN	"	Arave	1967	" " " " " "
κ–CN	2 bands	Neelin,	1964	Starch GE with 2-mercaptoethanol in sample buffer
κ–CN	"	Schmidt	"	" " " " " " " "
κ–CN	"	Woychik	"	" " " " " " " "
κ–CN	A, B	Grosclaude *et al.*	1965	Concluded 2 bands found by above method were variants of κ–casein
κ-CN		Davoli	1981	Cellulose acetate strip supports with tris-glycine buffer containing 6 M urea at pH 9.0 in presence of 2-mercaptoethanol

LA: lactalbumin, LG: lactoglobulin, CN: casein

pI. Separation is achieved by establishing a pH gradient in a gel, following which proteins move under the influence of an electric field to a position where they have no net charge. The pH gradient is formed by including amphoteric compounds in the gel. As many as twenty bands can be obtained when whole casein is subjected to electrophoresis across a pH gradient between pH 3.0 to 10.0 in 5% polyacrylamide containing urea. This separation method has the ability to resolve proteins differing by only 0.02 of a pH unit in their isoelectric points (Peterson, 1969). Ultra thin (0.5 mm) polyacrylamide gels were first used by Vesterberg (1972) and Görg *et al.* (1978) for protein separation. Thin gels offer many advantages over the conventional thicker gels as follows: lower operating current generating less heat, superior heat dissipation permitting use of higher field strengths, reduced amounts of ampholytes required and more rapid staining and destaining. All these factors lead to improved resolution, reduced running time and diminished cost per sample.

A summary of the milk protein variants resolved using IEF is given in Table 1.3.3. Josephson (1972) reported successful identification of κ-casein A and B through the use of pH gradient electrophoresis of bovine milk caseins. Peterson (1969) and Josephson (1972) were among the first to separate bovine caseins (β-casein A^1, A^2, A^3, B, C) using IEF. Their method was improved upon by Addeo *et al.* (1983) to simultaneously type the four major caseins. The same group (Di Luccia *et al.*, 1988) reported that κ-casein C had an isoelectric pH between the A and B variants. Seibert *et al.* (1985) used ultrathin layer IEF on 100 μm polyacrylamide gels to phenotype all the milk proteins in a single run. The method of Seibert *et al.* (1985) was ideal for large-scale screening studies as it had the added advantages of short separation time, low cost and high resolution. Bech and Munk (1988) investigated the possibility of IEF on 0.5 mm agarose gels using 7 M urea for detection of milk protein polymorphism. However, the casein and whey proteins were run separately to give improved resolution. Bovenhuis and Verstege (1989) and Vegarud *et al.* (1989) again described a method for simultaneously typing all milk protein variants by IEF in a single run using a Phast System® (Pharmacia, Uppsala, Sweden). Table 1.3.4 summarizes the pI values of bovine milk protein genetic variants. It is interesting to note that since milk proteins from different species have different pI values, that Rispoli and Sauques (1989) used IEF to detect the presence of cow's milk in ewe's cheeses.

Table 1.3.3 Chronological summary of the use of isoelectric focusing techniques in the discovery of bovine milk protein variants

Protein	Variant	Author	Year	Method/Remarks
β-CN	A^1, A^2, A^3, B & C	Peterson	1969	pH gradient PAGE electrophoresis
κ-CN	A, B	Josephson	1972	" " " "
β-LG	A & B	Pearce and Zadow	1978	IEF pH 3.5-5.0 in 5% polacrylamide containing 6M urea
α_{s1}-, α_{s2}-,β- & κ-Cn	All	Addeo *et al.*	1983	PAGE IEF (simultaneous phenotying of 4 major caseins)
κ-CN	D	Seibert *et al.*	1987	Ultra-thin 4.5 % PAGE containing 8 M urea, 2-mercaptoethanol
κ-CN	C	Di Luccia *et al.*	1988	C isoelectric pH was found to be between A and B
α_{s1}-, β-	A, B, C/A^1, A^2, B	Bech and Munk	1988	Agarose gel containing 7 M urea (simultaneous phenotying
κ-CN, β-LG	A, B/A, B	" "	"	of 4 major caseins and β-lactoglobulin)
κ-CN	E	Erhardt	1989	0.3 mm PAGE IEF
All milk protein variants		Bovenhuis and Verstege	1989	PhastSystem® (precast PAGE gels)
" " " "		Vegarud *et al.*	"	" " " " "
α_{s2}-CN	D	Erhardt	1993	0.3 mm PAGE IEF

LG: lactoglobulin, CN: casein

TABLE 1.3.4 Summary of isoelectric point (pI) values for various bovine milk protein variants.

Protein	Genetic Variant	pI Range		
		Seibert *et al.* (1985)	Josephson (1972)	Trieu-Cout and Gripon (1981)
α_{s1}-Casein	A	4.16-4.40		
	B	4.23-4.47	4.20-4.60	4.44-4.76
	C	4.27-4.49		
α_{s2}-Casein	A	4.83-5.13		
β-Casein	A^1	4.68-4.96		
	A^2	4.60-4.84		
	A^3	4.50-4.74	4.60-5.10	4.83-5.07
	B	4.78-5.10		
	C	4.97-5.29		
κ-Casein	A	5.43-5.81	5.30-5.80	5.45-5.77
	B	5.54-6.12		
α-Lactalbumin	B	4.66-4.89		
β-Lactoglobulin	A	4.64-4.90		
	B	4.72-4.98		
	C	4.77-5.13		

(taken from Seibert *et al.* (1985))

1.3.1.3 Chromatography

There are four main types of chromatographic separation, (1) separations dependent on molecular size, (2) separations dependent on the overall charge or charge distribution on molecules, (3) reversed-phase separations and (4) affinity separations (in which the chromatographic medium has a particular affinity for a molecule). In the first case, the separations are based on the use of media with different pore sizes, small molecules enter the pores while large molecules do not and so elute from the column first. Intermediate size molecules pass through the column more slowly resulting in different retention times. Separations may be carried out under denaturing or non- denaturing conditions. In the former the separation is based on molecular weight of the molecule; in the later the conformation of the molecule will affect the separation observed. In the second chromatography technique, separation proceeds because ions of opposite charge are retained to different extents. The resolution of the chromatographed sample is influenced by pH and ionic strength of the eluent. The typical conditions for reversed-phase chromatography used are low pH and a gradient to high concentration of organic solvents i.e. which favours denaturation of proteins. Solutes are eluted from reversed-phase matrices in order of decreasing polarity. The fourth type, affinity separations, occur where the molecule to be purified is specifically and reversibly adsorbed by a complementary binding substance (ligand) immobilized on an insoluble support (matrix).

During the past three decades many chromatographic techniques have been developed and adapted for the study of milk proteins variants. A summary of these techniques used is outlined in Table 1.3.5. As can be seen from this Table, the most popular techniques used in the separation of different milk protein variants is anion exchange chromatography.

Table 1.3.5 Chronological summary of the use of chromatographic techniques in the discovery of milk protein variants.

Protein	Variant	Author	Year	Methods/Remarks
Whey proteins	8 fractions	Schober *et al.*	1959	Anion exchange DEAE-cellulose step-wise changes in pH and [NaCl]
β-LG	A & B	Yaguchi *et al.*	1961	Improvement on above method
β-CN	A, B & C	Thompson and Pepper	1964	DEAE-cellulose in presence of urea
α_{s1}-CN	A, B & C	Thompson and Kiddy	1964	" " " " " "
κ-CN	A & B	Thompson	1966	DEAE-cellulose using imidazole-HCL-urea at pH 7.0
α-LA	A & B	Pearce	1983	HPLC system short alkyl chain (C6) reverse phase column
β-LG	A & B	Humphrey and Newsome	1984	Anion exchange HPLC Mono Q® HR5/5, using buffered gradients of
" " "	"	" " "	"	increasing ionic strength
Whey proteins		Andrews *et al.*	1985	Mono Q® and Mono S™ columns
κ-CN	A & B	Dalgleish	1986	Anion exchange FPLC (Pharmacia Mono Q® column)
" "	" "	Visser *et al*	1986	Reversed-phase HPLC (RP-C18)
" "	" "	Guillou *et al.*	1987	Anion exchange FPLC Mono Q®
Milk proteins		Laezza *et al.*	1991	Method of Andrews *et al.* (1985) to detect bovine milk in ovine milk
β-CN	A^1, A^2 & A^3	Hollar *et al.*	1991	Cation exchange FPLC on a Mono S™ column

LA: lactalbumin, LG: lactoglobulin, CN: casein

1.3.1.4 DNA-based techniques

The use of electrophoretic, IEF and chromatographic techniques for the analysis of milk protein polymorphism is limited to lactating animals. Indirect determination of a sires genotype using the above techniques would require at least 5 years before sufficient numbers of lactating daughters become available such that analysis could be carried out on their milks (Lin *et al.*, 1992). Knowledge of a sires genotype is essential in considering a breeding programme targeted to select for cows expressing a specific milk protein variant in their milks. The use of DNA-based techniques i.e. restriction fragment length polymorphism (RFLP), either on genomic DNA (Leveziel *et al.*, 1988) or on DNA amplified by the polymerase chain reaction (PCR) procedure (Medrano and Aguilar-Cordova, 1990; Schlieben, *et al.*, 1991), have made it possible to type animals irregardless of sex or age. PCR was introduced by Saiki *et al.* (1985) and involves enzymatic synthesis in vitro of millions of copies of a specific DNA fragment. PCR can therefore be used to amplify and quantify the DNA associated with a specific milk protein variant gene. This amplified DNA can then be digested with restriction endonuclease enzymes and the resultant products analysed by agarose gel electrophoresis. Nierop-Groot *et al.* (1995) reported on an allele-specific primer approach for the rapid determination of κ-casein genotypes in semen and blood samples from commercial Irish sires.

1.3.2 Individual milk protein genetic variants

The substitutions or deletions of amino acids which lead to polymorphism and their positions in the primary sequence of bovine milk proteins are shown in Table 1.3.6.

1.3.2.1 α-Lactalbumin

α-Lactalbumin has 123 amino acid residues and there are three known variants (Table 1.3.6). The amino acid sequence of the A and B variants was identified in 1970

TABLE 1.3.6 Summary of the amino acid substitutions or deletions which lead to polymorphism in the major milk proteins obtained from different bovine species.

Protein	Genetic Variant	Amino Acid Position	
α-lactalbumin	A ➪ B	10 Gln ➪ Arg	
	B ➪ C	*Asp ➪ Asn	
β-lactoglobulin	A ➪ B	64 Asp ➪ Gly	118 Val ➪ Ala
	B ➪ C	59 Gln ➪ His	
	B ➪ D	45 Glu ➪ Gln	
	B ➪ E	158 Glu ➪ Gly	
	B ➪ F	50 Pro ➪ Ser	130 Asp ➪ Tyr
		158 Glu ➪ Gly	
	B ➪ G	78 Ile ➪ Met	158 Glu ➪ Gly
	B ➪ I	108 Glu ➪ Gly	
	B ➪ J	126 Pro ➪ Leu	
	B ➪ W	56 Ile ➪ Leu	
α_{s1}-casein	B ➪ A	14-26 deleted	
	B ➪ C	192 Glu ➪ Gly	
	B ➪ D	53 Ala ➪ ThrP	
	B ➪ E	59 Gln ➪ Lys	192 Glu ➪ Gly
α_{s2}-casein	A ➪ C	33 Glu ➪ Gly	47 Ala ➪ Thr
		130 Thr ➪ Ile	
	A ➪ D	50-58 deleted	
β-casein	A^1 ➪ A^2	67 His ➪ Pro	
	A^2 ➪ A^3	106 His ➪ Gln	
	A^1 ➪ B	122 Ser ➪ Arg	
	A^1 ➪ C	35 SerP ➪ Ser	37 Glu ➪ Lys
	A^2 ➪ D	18 SerP ➪ Lys	
	A^2 ➪ E	36 Glu ➪ Lys	
	A^1 ➪ F	152 Pro ➪ Leu	
κ–casein	A ➪ B	136 Thr ➪ Ile	148 Asp ➪ Ala
	B ➪ C	81 Asp ➪ Asn	97 His ➪ Arg
	A ➪ E	155 Ser ➪ Gly	
	A ➪ F	10 Arg ➪ His	
	A ➪ G	97 Arg ➪ Cys	

* exact location not yet clarified. (adapted from Eigel *et al.* (1984); Martin and Grosclaude (1993); and Creamer and Harris (1997))

by Brew *et al.* The exact amino acid substitutions involved in the differentiation of the C variant as observed by Bell *et al.* (1981*a*) has not yet been established. It is believed to be substitution of an aspartic acid residue with asparagine (Bell *et al.*, 1981*a*).

1.3.2.2 β-Lactoglobulin

β-lactoglobulin is composed of 162 amino acid residues. As reported earlier, the A and B variants, were discovered by Aschaffenburg and Drewry in 1955. Bell detected the presence of the C variant in Jersey cows in 1962. A fourth variant, the D variant was detected in the French Montbeliarde breed by Grosclaude *et al.* in 1966a. A β-lactoglobulin variant found in Yak milk (Grosclaude *et al.*, 1976, 1982) which has the same electrophoretic mobility as β-lactoglobulin D has been designated E by Eigel *et al.* (1984). Two additional variants, F and G, have been found in Banteng cattle by Bell *et al.* (1981*b*). Presnell *et al.* (1990), using a rapid micropore HPLC method, detected the W variant of β-lactoglobulin. Baranyi *et al.* (1993) reported an unidentified band near β-lactoglobulin B variant on immobilized pH gradient isoelectric focusing gel containing carrier ampholytes, which they denoted β-lactoglobulin X. Godovac-Zimmermann *et al.* (1996) isolated and sequenced two further novel bovine β-lactoglobulin variants from the previous X band which were denoted I and J.

1.3.2.3 α_{s1}-Casein

There are five known variants of α_{s1}-casein which is composed of 199 amino acid residues (Table 1.3.6). Thompson *et al.* (1962) discovered the first three variants which they denoted A, B and C. The A variant is an example where deletion of certain amino acid sequences along the peptide backbone is involved. It differs from the B variant by a deletion of residues 14-26. The fourth variant of α_{s1}-casein, termed the D variant, was detected by Grosclaude *et al.* (1966*a*) in French Famande cattle. The E variant of α_{s1}-casein was found in the milk of the Nepalese Yak by Grosclaude *et al.* (1976). Erhardt (1993) reported on a new α_{s1}-casein variant, the F variant. The G variant of α_{s1}-casein was reported by Mariani *et al.* (1996) in Italian Brown cattle.

1.3.2.4 α_{s2}-Casein

α_{s2}-Casein has 207 amino acid resisdues. There are four known genetic variants of this milk protein (Table 1.3.6). The variants A, B and C of α_{s2}-casein were detected by Grosclaude *et al.* (1976). A fourth variant termed the D variant was also detected by Grosclaude *et al.* (1979) in the Vosgienne and Montbeliarde breeds.

1.3.2.5 β-Casein

β-Casein is composed of 209 amino acid residues. At present there are nine known genetic variants of β-casein. Aschaffenburg (1961) discovered the A, B and C variants. Peterson and Kopfler (1966) found that the A variant could be resolved into three further variants and these were designated A^1, A^2 and A^3. The E variant was discovered by Voglino in the Piedmont cattle breed in Italy in 1972. Grosclaude *et al.* (1972) reported that replacement of serine at position 18 of A^2 by a lysine gives variant D of β-casein. In 1995, Chung *et al.* reported an A^4 variant of β-casein in Korean native cattle. Visser *et al.* (1995) reported a new genetic variant of β-casein using HPLC and mass spectrometric analysis which was denoted the F variant.

1.3.2.6 κ-Casein

κ-Casein provides colloidal stability to the casein micelle (Walstra and Jenness, 1984; Walstra, 1990; Creamer, 1991) and therefore plays an important role in the physical chemistry of milk. κ-Casein is very soluble unlike α_{s1}- and β-caseins and is insensitive to precipitation by calcium ions. This solubility of κ-casein is attributed to the high negative charge at the C-terminal end, which contains phosphate and carbohydrate groups (Fig. 1.3.2). The N-terminal portion in contrast is hydrophobic (Fig. 1.3.3) and is able to form associations with α_{s1}- and β-casein. κ-Casein can bind about 10 times its own weight of α_{s1}- and β-casein (Zittle and Walter, 1963; Thompson *et al.*, 1969; Walstra and Jenness, 1984; Imafidon and Ng-Kwai-Hang, 1992). As already mentioned, κ-casein is mostly located at the periphery of the micelle, with the hydrophilic C-terminal sequence of κ-casein protruding into the solvent. A combination

of steric and electrostatic effects prevents flocculation of micelles (Walstra, 1990; Creamer, 1991).

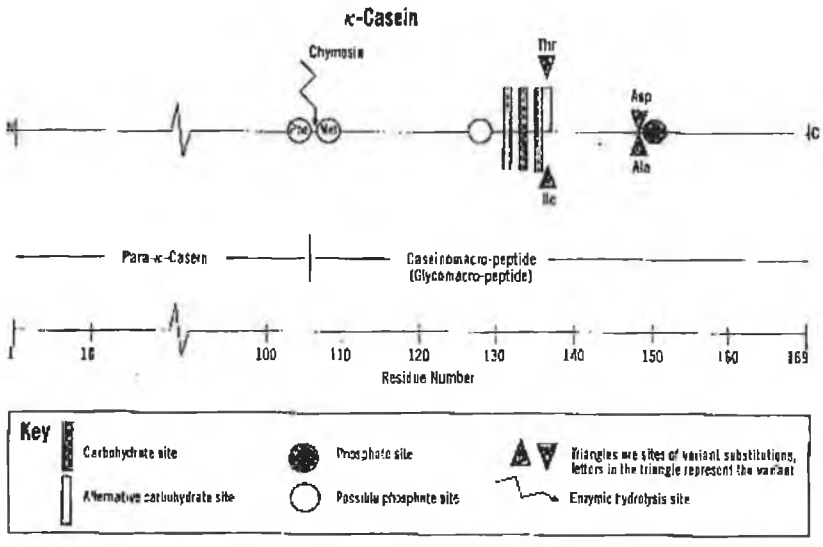

Figure 1.3.2 Diagrammatic representation of the various structural features of the κ-casein molecule (taken from Creamer and Harris (1997)).

When milk is exposed to chymosin, the casein micelles are destabilised and precipitated. It has been demonstrated that chymosin cleaves between Phe_{105}-Met_{106} in the κ–casein molecule (Fig. 1.3.2 and 1.3.4). This results in the liberation of the soluble C-terminal portion of the molecule. This (glyco) macropeptide has a molecular weight of ~6,800 Da and contains the serine phosphate and carbohydrate groups of the molecule. The remainder of the κ–casein molecule is called para-kappa-casein. It has a molecular weight of ~12,000 Da and a net positive charge at pH 6.7. Para-κ-casein is extremely hydrophobic and insoluble

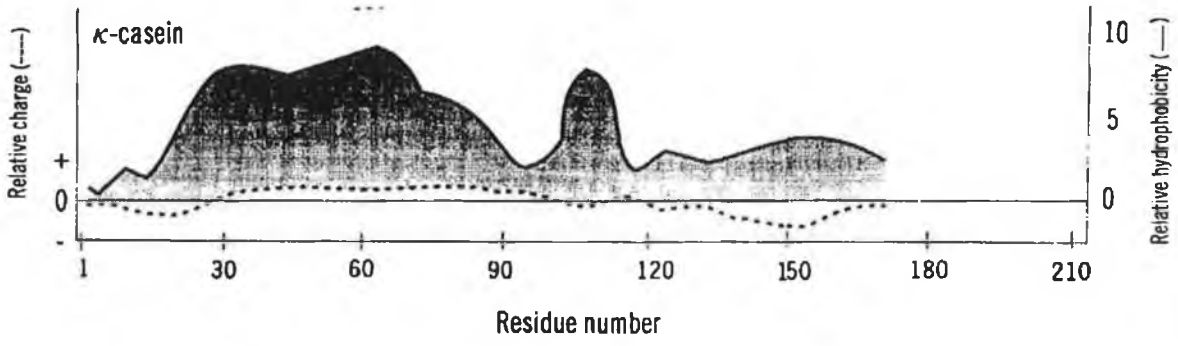

Figure 1.3.3 Diagrammatic representation of the distribution of net charge and hydrophobicity along the sequence of κ-casein. The traces shown are for the aglyco B variant. (taken from Creamer and Harris (1997)).

κ-Casein has 169 amino acid residues (Fig. 1.3.4) and at present there are six known variants of this milk protein. Grosclaude *et al.* (1965) first discovered the existence of the A and B variants. The primary structures of these variants were established by Grosclaude *et al.* (1972) and Mercier *et al.* (1973). Di Stasio and Merlin (1979) discovered the C variant. Siebert *et al.* (1987) and Erhardt (1989) found two additional variants i.e. D and E. More recent results of amino acid sequencing indicate that the C and D variants are similar. Ikonen *et al.* (1996) and Erhardt (1996) have detected two new κ-casein variants which they have termed F and G, respectively.

1
Glu-Glu Gln-Asn-Gln-Glu-Gln-Pro-Ile-Arg-Cys-Glu-Lys-Asp-Glu-Arg-Phe-Phe-Ser-
20
Asp Lys-Ile-Ala-Lys-Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg-Tyr-Pro-Ser-Tyr-Gly-Leu
41
Asn-Tyr-Tyr-Gln-Gln-Lys-Pro-Val-Ala-Leu-Ile-Asn-Asn-Gln-Phe-Leu-Pro-Tyr-Pro-Tyr-
61
Tyr-Ala-Lys-Pro-Ala-Ala-Val-Arg-Ser-Pro-Ala-Gln-Ile-Leu-Gln-Trp-Gln-Val-Leu-Ser-
81
Asp-Thr-Val-Pro-Ala-Lys-Ser-Cys-Gln-Ala-Gln-Pro-Thr-Thr-Met-Ala-Arg-His-Pro-His
101 105 ⇓106
Pro-His-Leu-Ser-Phe-Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys-Thr-Glu-Ile-Pro
121
Thr-Ile-Asn-Thr-Ile-Ala-Ser-Gly-Glu-Pro-Thr-Ser-Thr-Pro-Thr-Thr-Glu-Ala-Val-Glu-
141
Ser-Thr-Val-Ala-Thr-Leu-Glu-Asp-Ser-Pro-Glu-Val-Ile-Glu-Ser-Pro-Pro-Glu-Ile-Asn-
161 169
Thr-Val-Gln-Val-Thr-Ser-Thr-Ala-Val

Figure 1.3.4 Primary structure of bovine κ-casein A genetic variant. The arrow (⇓) indicates the point of attack by chymosin. (taken from Swaisgood (1992)).

1.3.3 Genetic variant frequency distribution

There are numerous reports on the occurrence and frequency distribution of milk protein variants for a wide selection of bovine breeds. The lack of comparability in the results, even within a given breed in a given country or area, can be due to various reasons, including differences in analytical techniques and size and structure of data sets (Ng-Kwai-Hang and Grosclaude, 1994). In Western breeds the most common genetic variants of the different milk protein fractions are α-lactalbumin B, β-lactoglobulin A and B, α_{s1}-casein B and C, α_{s2}-casein A, β-casein A^1, A^2 and B and κ-casein A and B (Li and Gaunt, 1972; Creamer and Harris 1997, Table 1.3.1).

An extensive list for the gene frequency of the most common κ-casein variants is given in Table 1.3.7. The more common breeds used for milk production (Ayrshire,

Holstein-Friesian, Holstein and Jersey) have been studied extensively in various countries. The findings of these studies vary greatly even within a breed. The allelic frequency of the A and B variants of κ-casein in Ayrshire cattle was reported to be 0.59 and 0.41 (Lin *et al.*, 1986) and 0.88 and 0.12 (Ng-Kwai-Hang and Kim, 1994) in Canada and 0.86 and 0.14 (Aaltonen and Antila, 1987) and 0.61 and 0.08 (Ikonen *et al.*, 1996) in Finland (Table 1.3.7). While studies within a particular breed and in the same country vary some studies display good agreement e.g. Zadworny and Kuhnlein in 1990 reported frequencies of 0.87 and 0.13 for κ-casein A and B variants, respectively, for Holstein cattle in Canada and six years later Sabour *et al.* (1996) reported frequencies of 0.86 and 0.14 for κ-casein A and B variants, respectively, for Holstein cattle also in Canada. Similarly, Hines *et al.* in 1976 reported frequencies of 0.80 and 0.20 for κ-casein A and B variants respectively for Holstein cattle in the USA while in 1987 Gonyon *et al.* reported gene frequencies of 0.79 and 0.21 for the κ-casein A and B variants of κ-casein, respectively, for Holstein cattle also in the USA. Medrano and Sharrow (1989) in the USA studying the frequency of κ-casein variants reported gene frequencies of 0.63 and 0.37 for the A and B variants of κ-casein in Holstein cattle. It is mostly in the lesser known breeds that the C, D, E and G variants are reported (Vlasenko and Vyshnyakova, 1986; Seibert *et al.*, 1987; Baranyi *et al.*, 1993; Erhardt, 1996; Mayer *et al.*, 1997*b*). The above therefore shows that it is necessary to actually determine phenotypes in order to estimate gene frequencies for a given cattle breed in a given country.

TABLE 1.3.7 The allelic frequencies for κ–casein variants in different cattle breeds

Breed	Country	No.[#]	Allele			Reference
			A	B	Other	
Ayrshire	Canada	158	0.59	0.41		Lin *et al.* (1986)
Ayrshire	Canada	*	0.88	0.12		Ng-Kwai-Hang & Kim (1994)
Ayrshire	Finland	31	0.86	0.14		Aaltonen & Antila (1987)
Ayrshire	Finland	20990	0.61	0.08	E:0.31	Ikonen *et al.* (1996)
Ayrshire	UK	52	0.80	0.20		Aschaffenburg (1968)
Ayrshire	USA	78	0.70	0.30		Medrano & Sharrow (1989)
Bk Pied	Estonia	140	0.70	0.30		Pupkova (1980)
Bk Pied	Russia	90	0.49	0.46	C:0.05	Vlasenko & Vyshnyakova (1986)
Brown	Italy	98	0.39	0.61		Aleandri *et al.* (1997)
Br Cattle	Austria	1747	0.40	0.59	C:0.01	Mayer *et al.* (1997*b*)
Br Swiss	Canada	*	0.71	0.29		Ng-Kwai-Hang & Kim (1994)
Cnde	Canada	*	0.79	0.21		Ng-Kwai-Hang & Kim (1994)
Finncattle	Finland	7	0.64	0.36		Aaltonen & Antila (1987)
Friesian	Australia	260	0.68	0.32		McLean *et al.* (1984)
Friesian	Finland	30	0.85	0.15		Aaltonen & Antila (1987)
Friesian	N. Z.	2544	0.40	0.60		Hill (1994)
Guernsey	USA	100	0.74	0.26		Woychik (1965)
Guernsey	USA	3888	0.73	0.27		Haenlein *et al.* (1987)
Hg-grey	Hungary	120	0.64	0.36		Baranyi *et al.* (1993)

[#], Number of animals phenotyped; *, Number of animals in study not given; Bk Pied, Black Pied; Br, Brown; Cnde, Canadienne; Hg, Hungarian. contd.....

TABLE 1.3.7 The allelic frequencies for κ–casein variants in different cattle breeds

Breed	Country	No.	Allele			Reference
			A	B	Other	
Holstein	Canada	377	0.69	0.31		Lin *et al.* (1986)
Holstein	Canada	42	0.87	0.13		Zadworny & Kuhnlein (1990)
Holstein	Canada	566	0.86	0.14		Sabour *et al.* (1996)
Holstein	USA	6531	0.80	0.20		Hines *et al* (1976)
Holstein	USA	3571	0.79	0.21		Gonyon *et al.* (1987)
Holstein	USA	783	0.63	0.37		Medrano & Sharrow (1989)
Holstein	Israel	112 (S)	0.89	0.11		Ron *et al.* (1994)
Holstein	Sweden	250	0.80	0.20		Lunden *et al.* (1997)
Hlst.-Fr	Italy	2005	0.75	0.25		Aleandri *et al.* (1990)
Hlst-Fr	Italy	3904	0.85	0.15		Aleandri *et al.* (1997)
Hlst.-Fr	U.K.	189	0.83	0.17		Aschaffenburg (1968)
Hlst.-Fr.	Canada	6509	0.75	0.25		Ng-Kwai-Hang & Monardes (1990)
Hlst.-Fr	USA	1152	0.82	0.18		van Eenennaam & Medrano (1991)
Hlst.-Fr	Holland	6,803	0.80	0.20		Bovenhuis *et al.* (1992)
Hlst.-Fr	Ireland	696	0.75	0.25		O'Hara (1995)
Hlst.-Fr	Austria	185	0.84	0.09	C:0.01 E:0.05	Mayer *et al.* (1997*b*)

[#], Number of animals phenotyped; Hlst-Fr, Holstein-Friesian; (S) sires.

contd....

TABLE 1.3.7 The allelic frequencies for κ–casein variants in different cattle breeds

Breed	Country	No.	Allele			Reference
			A	B	Other	
Jersey	USA	100	0.10	0.90		Woychik (1965)
Jersey	U.K	47	0.09	0.91		Aschaffenburg (1968)
Jersey	Australia	308	0.23	0.77		McLean *et al.* (1984)
Jersey	USA	186	0.11	0.89		Medrano & Sharrow (1989)
Jersey	Denmark	157	0.31	0.69		Bech & Kristiansen (1990)
Jersey	Canada	*	0.26	0.74		Ng-Kwai-Hang & Kim (1994)
Jersey	N. Z.	639	0.70	0.30		Hill (1994)
Jersey	Ireland	116	0.28	0.72		O'Hara (1995)
Jersey	Austria	120	0.34	0.66		Mayer *et al.* (1997*b*)
Kerry	Ireland	123	0.93	0.07		Murphy (1965)
Kerry	Ireland	41	0.90	0.10		O'Hara (1995)
Limpurger	Germany	125	0.72	0.24	C:0.016 E:0.032	Erhardt (1996)
Mtbde	France	74	0.49	0.51		Delacroix-Buchet *et al.* (1993)
Normande	France	155	0.34	0.66		Aschaffenburg (1968)
Normande	France	89	0.34	0.66		Delacroix-Buchet *et al.* (1993)
Pinzgauer	Germany	353	0.78	0.21	E:0.003 G:0.003	Erhardt (1996)
Pinzgauer	Austria	200	0.74	0.24	C:0.002 E:0.018	Mayer *et al.* (1997*b*)

#, Number of animals phenotyped; *, Number of animals in study not given; Mtde, Montbeliarde. contd.....

TABLE 1.3.7 The allelic frequencies for κ–casein variants in different cattle breeds

Breed	Country	No.	Allele			Reference
			A	B	Other	
Rd and Wt	Sweden	394	0.83	0.17		Lunden *et al.* (1997)
Red Danish	Denmark	169	0.81	0.19		Bech & Kristiansen (1990)
Rendena	Italy	306	0.68	0.32		Pagnacco & Caroli (1987)
Simmental	Germany	1557	0.75	0.24	D:0.01	Seibert *et al.* (1987)
Simmental	Italy	1462	0.80	0.20		Aleandri *et al.* (1997)
Simmental	Italy	279	0.77	0.23		Falaki *et al.* (1997)
Simmental	Austria	2078	0.70	0.28	C:0.02	Mayer *et al.* (1997*b*)
Spotted	Hungary	101	0.75	0.22	C:0.03	Baranyi *et al.* (1993)
Tarentaise	France	60	0.57	0.31		Delacroix-Buchet *et al.* (1993)
Tyrn Grey	Austria	197	0.54	0.45	C:0.01	Mayer *et al.* (1997*b*)
Wt Danish	Denmark	223	0.85	0.15		Bech & Kristiansen (1990)

[#], Number of animals phenotyped; Rd, Red; Wt, White; Tyrn, Tyrolean.

1.4 Associations between κ-casein variants with bovine milk composition and production

Specific milk protein variants have been associated with changes in the overall composition and processing parameters of bovine milks. The reports which exist on these relationships are, in many cases, conflicting. The following details some of the relationships reported between κ-casein variants and milk yield, milk composition, dairy production related traits, κ-casein concentration and casein micelle size and other milk protein genetic variants.

1.4.1 Milk yield

Conflicting reports exist on the effect of κ–casein variant on milk yield. Several workers reported no differences in milk yield for the different phenotypes of κ-casein (McLean *et al.*, 1984; Ng-Kwai-Hang *et al.*, 1984; Lin *et al.*, 1986; Haenlein *et al.*, 1987; Ng-Kwai-Hang *et al.*, 1990; O'Hara *et al.* 1994). However, Gonyon *et al.* (1987), Bovenhuis *et al.* (1992) and Chung *et al.* (1991, 1996) reported that κ-casein AA was associated with higher milk yield than the κ-casein BB, with the heterozygous AB being intermediate for the Holstein breed. Ozbeyaz *et al.* (1991) also reported higher milk yields with κ-casein A in Jersey cows. In contrast, Ng-Kwai-Hang *et al.* (1986) and Kim (1994) found the heterozygous κ-casein AB cows were higher producers than either of the homozygous cows.

There are also conflicting reports on the effect of κ-casein genetic variant on protein yield and protein %. Some studies have reported that κ-casein BB variant is associated with an increase in protein yield when compared to the AB and AA variants in Holstein cattle (Ng-Kwai-Hang *et al.*, 1986; Aleandri, 1990; Bovenhuis, 1993). Van den Berg *et al.* (1990) also reported an increase in protein yield associated with the BB variant in Friesian cattle. Pabst, (1992) reported the opposite trend, κ-casein AA variant had a higher protein yield than κ-casein BB for Holstein cattle. Bovenhuis, (1993) and Ron *et al.*, (1994) reported an increase in protein % associated with the BB variant of κ-casein when compared with the AA and AB variant in Holstein cattle. Van den Berg *et*

al., (1992) also reported increased protein % associated with the BB variant compared to the AA variant in Dutch black and white cattle.

Conflicting reports are also found in relation to the effect of κ–casein variant on % milkfat and milkfat yield. Ng-Kwai-Hang *et al.* (1986) and Aleandri *et al.* (1990) reported a positive association between κ–casein BB phenotype and both the fat % (Ng-Kwai-Hang *et al.*, 1986) and fat yield (Aleandri *et al.*, 1990) whereas Bovenhuis *et al.* (1992) and Ron *et al.* (1994) reported decreased fat yields in κ–casein BB phenotype milk. O'Hara *et al.* (1994) reported no significant effect of κ-casein variant on milk protein, milk fat or lactose yield. While there was no consistent trend found in relation to κ–casein E phenotype and milkfat content (Oloffs *et al.*, 1992; Piironen *et al.*, 1992) κ–casein C, when present, was seen to be associated with an increase in milkfat content (Vlasenko and Vyshnyokova, 1986; Macheboeuf *et al.*, 1993).

1.4.2 Milk composition

Higher protein and casein contents and higher casein numbers were reported in the milks from animals homozygous for the B variant of κ–casein when compared to other κ-casein variant milks (Schaar *et al.*, 1985; Lin *et al.*, 1986; Rampilli *et al.*, 1988; Aleandri *et al.*, 1990; Ng-Kwai-Hang *et al.*, 1990; van den Berg *et al.*, 1992; Bovenhuis, 1993). Ng-Kwai-Hang *et al.* (1984) reported that κ-casein BB variant milk had 0.13 % more protein than κ-casein AA milk. Lodes *et al.* (1997) reported no effect of κ-casein variant on the protein concentration of milk. O'Hara *et al.* (1994) and Lodes *et al.* (1997) reported that κ-casein variants did not significantly affect the casein concentration in milk. Schaar *et al.* (1985) reported that κ-casein AA variant milk had a higher milk citrate content than κ-casein BB variant milk. N-acetyl neuraminic acid content is reported to be higher in milk of κ-casein AB phenotype cows than in milk of κ-casein AA cows, thus suggesting that the B variant is more efficiently glycosylated than the A variant (Robitaille *et al.*,1991).

1.4.3 κ-Casein concentration and casein micelle size

An increase in κ-casein concentration is associated with the B variant of κ–casein (McLean *et al.*, 1984; Aaltonen and Antila, 1987; Ng-Kwai-Hang *et al.*, 1987; Hill *et al.*, 1992; van den Berg *et al.*, 1992; Anema and Creamer, 1993; Delacroix-Buchet *et al.*, 1993; Law, 1993; Macheboeuf *et al.*, 1993; Lodes *et al.*, 1996*a*; Mayer *et al.*, 1997*a*). Van Eenennaam and Medrano, (1991) reported approximately 30 % more κ–casein B than κ–casein A in κ–casein AB milk of Holsteins and approximately 16 % more κ-casein B and κ-casein A in κ-casein AB milk of Jerseys, while they found only approximately 5 % more κ-casein B than κ-casein A in κ-casein AB standards (Sigma Chemical Company, St Louis MO).

Casein micelle size is related to the relative amount of κ-casein present in the micelle (Donnelly *et al.*, 1984; Dalgleish *et al.*, 1989; Anema and Creamer, 1993; Delacroix-Buchet *et al.*, 1993; Ng-Kwai-Hang and Grosclaude, 1994; Lodes *et al.*, 1996*b*). Delacroix-Buchet *et al.* (1993) and Lodes *et al.* (1996*b*) reported casein micelle diameters for κ-casein AA and BB of 196 and 145 nm and 224 and 191 nm respectively. Large micelles tend to have less κ–casein than small micelles (Davies and Law, 1983; Ford and Grandison, 1986) and when κ–casein is added to a suspension of micelles the average micelle diameter decreases. This suggests that κ–casein limits the size of casein micelles by interacting at the surface of micelles. In a situation of constant casein concentration and therefore a fixed total summed volume for all casein micelles in a system, increased κ-casein content can only be accommodated by an increase in total surface area which can only be achieved by a decrease in micellar size (Horne *et al.*, 1996).

1.4.4 Dairy production-related traits

Some work has been carried out on milk protein variant effects on production related traits. The reason for doing such work is that milk production is dependent on such factors as growth rate of the heifer, reproductive performance and health status of the cow. Lin *et al.* (1987) examined the relationship between κ-casein locus and body weights. They reported that although calves with κ-casein BB type were found to be 1.7

kg heavier than those with AA type at birth, this difference disappeared when they reached 350 days of age. In a study by Lin *et al.* (1989) on the effect of milk protein genetic variant on herdlife and total yield over fixed parities and to a fixed age it was reported that cows with the BB phenotype of κ-casein outproduced those with AA or AB phenotype. Ng-Kwai-Hang and Monardes, (1990) reported no association between milk protein variant and days to first breeding, days open and number of services per conception during the first, second and third lactation. Jairim and Nair (1983) reported that cows with κ-casein AB, α_{s1}-casein BC, β-casein AA, α-lactalbumin BB, and β-lactoglobulin AB phenotypes had lower age at first calving than other genetic combinations at these loci. Saama *et al.* (1992) reported that there was no evidence to suggest that κ-casein phenotype had an effect on weight gain of bulls.

1.4.5 Other genetic variants

The relative amounts of α_{s1}- and β-casein (McLean *et al.*, 1984) have been reported to vary with κ–casein phenotype in the following order, κ–casein AA> AB>BB. No association between the content of α_{s2}-casein and κ–casein phenotype was reported (Law, 1993; Mariani *et al.*, 1993). McLean *et al.* (1984) reported that the level of β-lactoglobulin in κ–casein BB milk was lower than that of κ–casein AA or AB milks. These workers also reported that κ-casein AA and AB milk had a higher concentration of α-lactalbumin than κ-casein BB milk. Ng-Kwai-Hang *et al.* (1987) also reported an increased concentration of β-lactoglobulin associated with the BB variant of κ-casein.

1.5 Association between κ-casein variants with bovine milk processing properties

1.5.1. Rennet coagulation properties of bovine milk

As outlined in Section 1.3.2.6, κ-casein is the only major casein hydrolysed by chymosin during the primary phase of milk coagulation, which is the first step in the manufacture of most cheese varieties. κ-Casein is hydrolysed specifically at the Phe_{105}-Met_{106} bond, producing para-κ-casein and glycomacropeptide (Fig. 1.3.2 and 1.3.4). The hydrophilic glycomacropeptides diffuse into the surrounding medium and the para-κ-casein remains attached to the micelle core. Removal of the macropeptide from the surface of the casein micelles reduces their zeta potential from about -20 to -10 mV and removes the steric stabilizing layer. The proteolysis of κ-casein is referred to as the primary phase of rennet-coagulation of caseins.

When about 85 % of the total κ-casein in milk has been hydrolysed, the colloidal stability of the micelles is reduced to such an extent that they coagulate at temperatures greater than 20°C, an event referred to as the secondary phase of rennet coagulation. Calcium ions are essential for the coagulation of rennet-altered micelles. Gelation is indicated by a rapid increase in viscosity (η) as shown in Figure 1.5.1. Coagulation commences at a lower degree of hydrolysis of κ-casein if the temperature is increased, the pH reduced or the Ca^{2+} concentration increased (Fox and McSweeney, 1998).

The actual reactions leading to coagulation are not known. Calcium ions are essential but calcium binding by caseins does not change on renneting. Colloidal calcium phosphate is also essential for coagulation to occur. Fox and McSweeney (1998) suggest that perhaps hydrophobic interactions, which become dominant when the surface charge and steric stabilization are reduced on hydrolysis of κ-casein, are responsible for coagulation (the coagulum is soluble in urea). The adverse influence of moderately high ionic strength on casein coagulation suggests that electrostatic interactions are also involved.

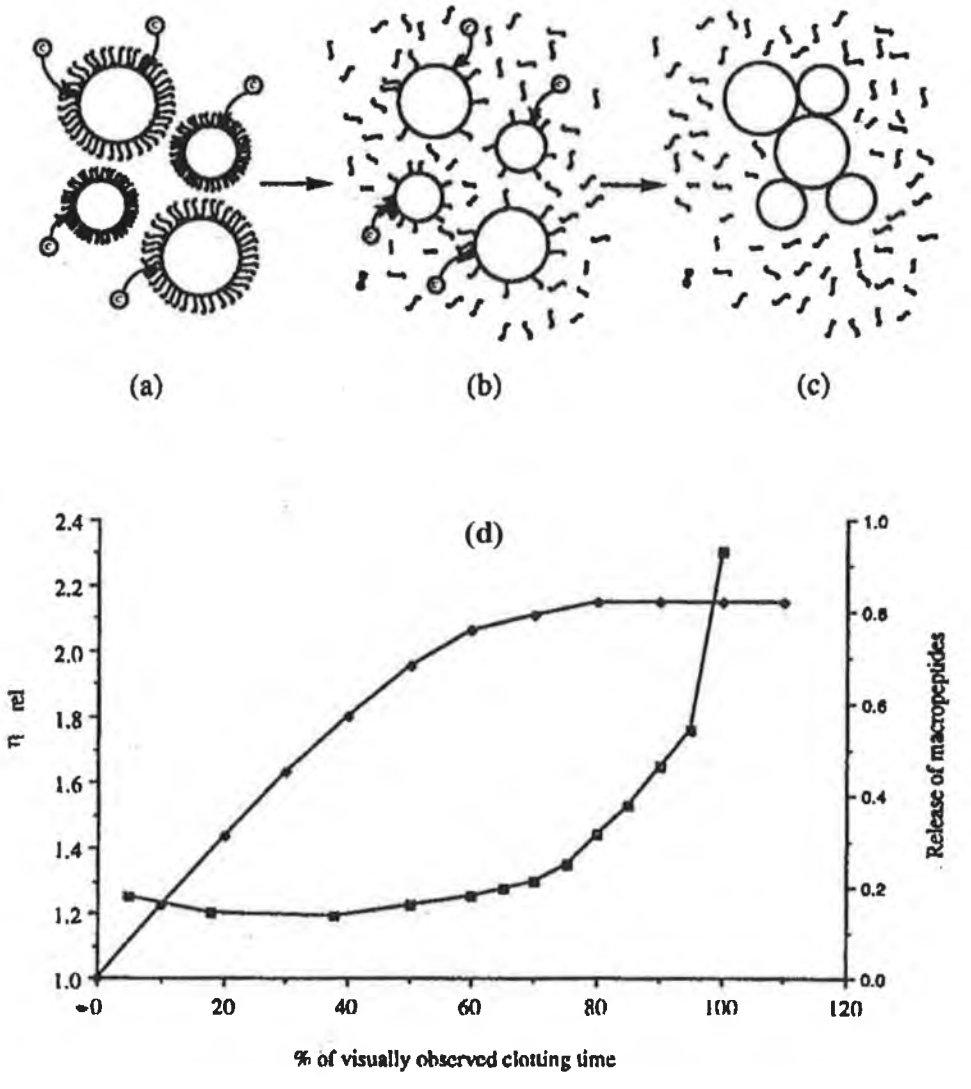

Figure 1.5.1 Schematic representation of the rennet coagulation of milk. (a) Casein micelles with intact κ-casein layer being attacked by chymosin ©; (b) micelles partially denuded of κ-casein; (c) extensively denuded micelles in the process of aggregation; (d) release of macropeptides (◆) and changes in relative viscosity (❑) during the course of rennet coagulation (taken from Fox and McSweeney (1998)).

1.5.1.1 Factors affecting rennet coagulation

A summary of the various compositional and environmental factors which effect the primary and secondary phases of rennet coagulation and the overall coagulation process is shown in Figure 1.5.2. Coagulation does not occur below 20°C, due mainly to the very high temperature coefficient of the secondary phase. At temperatures above 55-60°C the rennet is denatured. Rennet coagulation time increases with an increase of the reaction pH. RCT is reduced with increased calcium concentration. Preheating milk at temperatures above 70°C will prolong or prevent rennet coagulation. This effect is due to the interaction of β-lactoglobulin with κ-casein via sulphydryl-disulphide interchange reactions; both the primary and the secondary phase of coagulum are adversely affected. Rennet concentration is inversely proportional to rennet coagulation time. An increase in protein concentration gives a lower rennet coagulation time (Fig. 1.5.2.)

Factor	First phase	Second phase	Overall effect see panel
Temperature	+	++	a
pH	+++	-	b
[Ca]	-	+++	c
Pre-heating	++	++++	d
Rennet concentration	++++	-	e
Protein concentration	+	++++	f

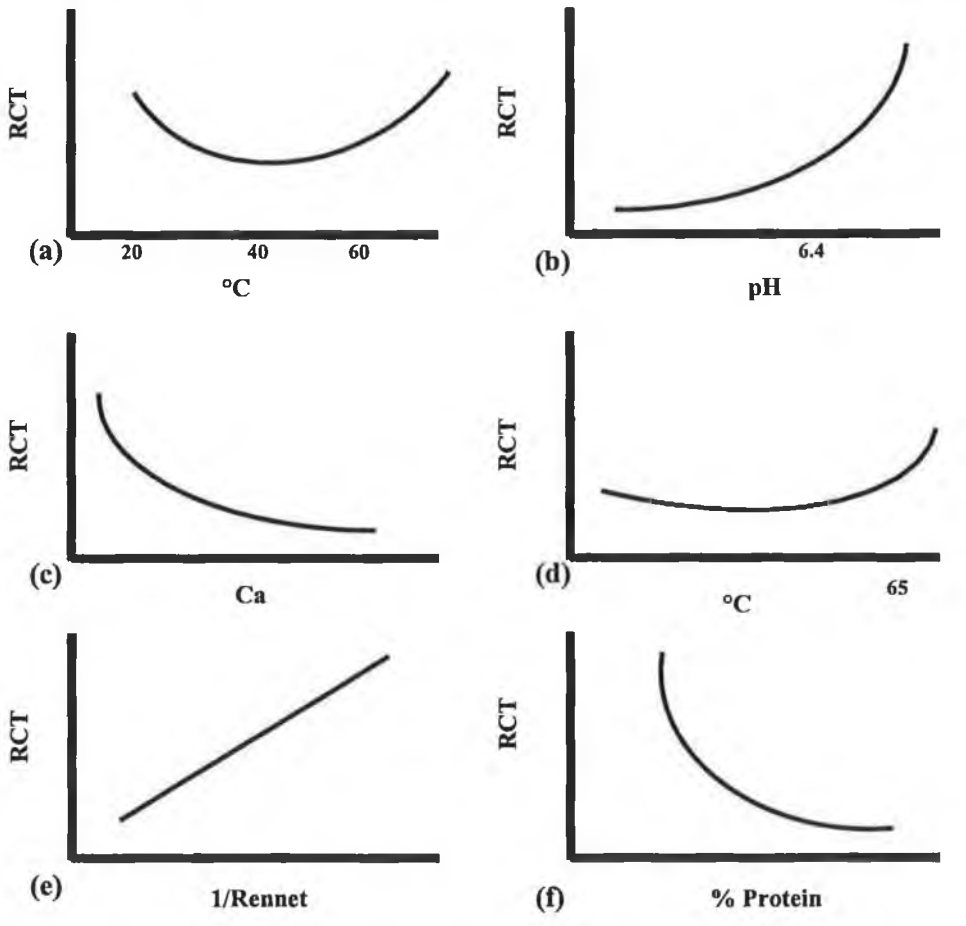

Figure. 1.5.2 Principal factors affecting the rennet coagulation time (RCT) of milk. Effect of (a) temperature of reaction, (b) pH of reaction, (c) [Ca] of reaction, (d) preheat temperature of the milk, (e) rennet concentration, (f) protein concentration on rennet coagulation of milk (taken from Fox and McSweeney (1998)).

1.5.1.2 Measurement of rennet coagulation properties

As shown in Figure 1.5.1 the viscosity of milk increases sharply when milk coagulates and this property may be used to determine the coagulation point. While there are many ways of studying rennet induced coagulation of milk, the Formagraph® instrument (Foss Electric, Hillerod, Denmark) is a viscometer specifically designed to assess the rennet coagulation properties of milk. Samples of the milk to be analysed are placed in cavities in an electrically heated metal block. Rennet is added and the loop-shaped pendulum of the instrument placed in the renneted milk. The metal block is moved back and forth, creating a "drag" on the pendulum in the milk. The arm to which the pendulum is attached contains a mirror from which a flashing light is reflected on to photosensitive paper, creating a trace. While the milk is fluid, the viscosity is low and the drag on the pendulum is slight and it scarcely moves from its normal position; hence a single straight line appears on the paper. As the milk coagulates, the viscosity increases and the pendulum is dragged out of position, resulting in bifurcation of the trace. The rate and extent to which the arms of the trace move apart is an indication of the strength of the coagulum. The time required from the start of coagulation for the arms of the formagram to bifurcate by 20 mm, an indication of the rate of curd firming is termed K_{20} and the extent of bifurcation 60 min after rennet addition, an indication of curd firmness, is termed A_{60}. As can be seen from Figure 1.5.3 rennet coagulation time (RCT), K_{20}, and A60 can be assessed using the Formagraph® instrument.

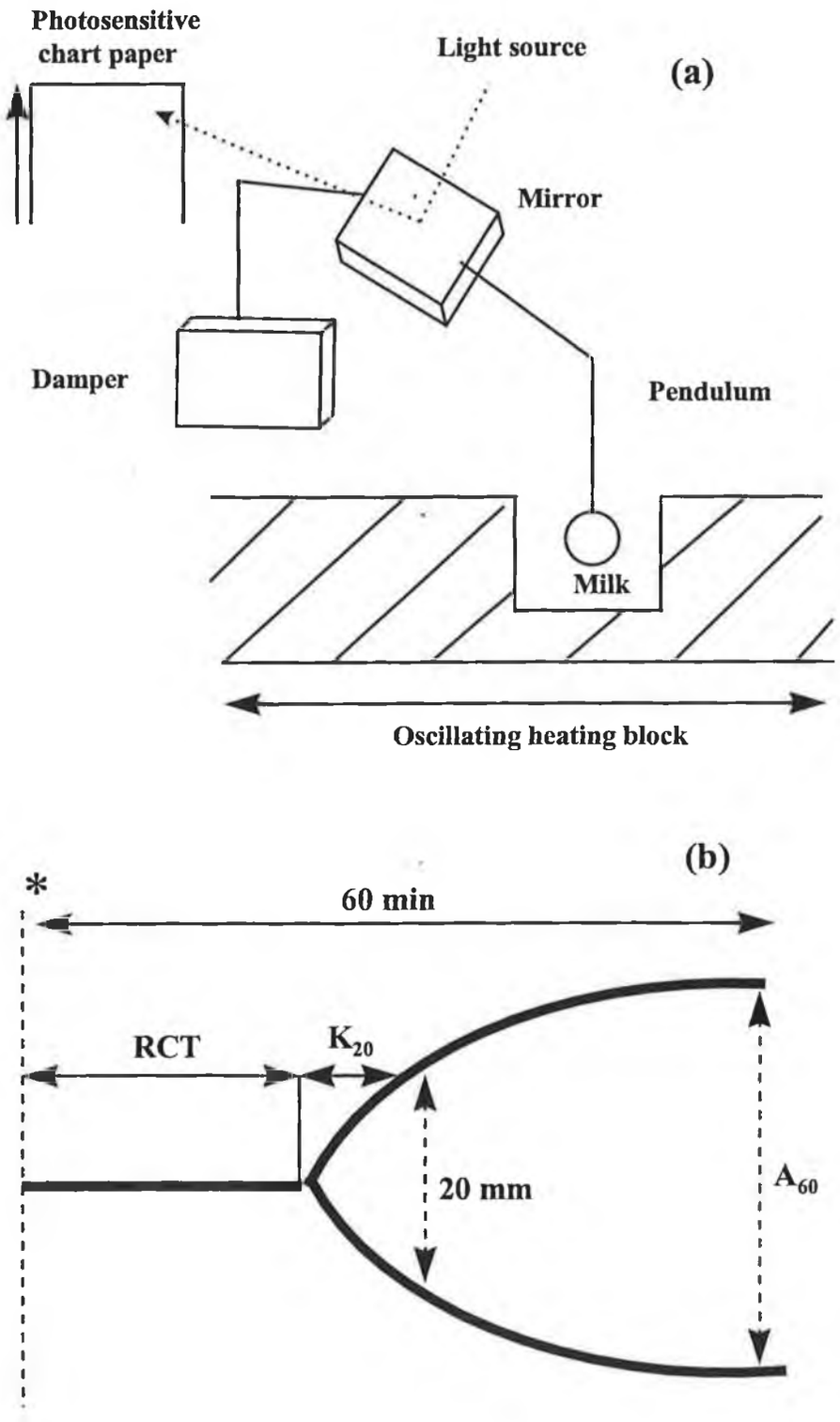

Figure 1.5.3 Schematic representation of (a) the Formagraph® apparatus for determining the rennet coagulation of milk (b) typical formagram. Point of rennet addition, RCT is rennet coagulation time; K_{20} is the time required from the start of coagulation for the arms of the formagram to bifurcate by 20 mm; A_{60} is the extent of bifurcation in mm 60 min after rennet addition (taken from Fox and McSweeney (1998)).

1.5.1.3 Effect of κ-casein variant on rennet coagulation properties of bovine milk

κ-Casein BB variant milk has been shown to have superior renneting properties with shorter rennet coagulation time, faster rates of curd firming and producing a firmer curd in comparison to the other κ-casein variant milks (Schaar, 1984; Schaar *et al.*, 1985; Aaltonen and Antila, 1987; Pagnacco and Caroli, 1987; Jakob and Puhan, 1992; Delacroix-Buchet, 1993; Macheboeuf *et al.*, 1993; Horne and Muir, 1994*a*; O'Hara *et al.*, 1994; Lodes *et al.*, 1996*b*; FitzGerald, 1997). While the differences observed in rennet coagulation properties between κ-casein phenotypes can be influenced by soluble calcium and pH, they are not due to variations in mineral content of these milks (Horne and Muir, 1994*b*). Marziali and Ng-Kwai-Hang (1986*b*) reported that κ-casein genetic variant *per se* did not effect the renneting properties of milk but that it was the amount of κ-casein in the milk which was the most significant factor in the rate of curd firming. Horne and Muir (1994*a*) and O'Hara *et al.* (1994) reported, that κ-casein BB milks at natural pH had significantly shorter rennet coagulation times than κ-casein AA but that this difference was eliminated by adjusting the pH to 6.40. Generally the effect of κ-casein variant on renneting time was shown to be less pronounced than the effect on curd firmness and rate of curd firming (O'Hara, 1995). The better curd firmness obtained with κ-casein AB and BB type milk when compared with κ-casein AA type milk could be explained, partly, by their higher casein contents (Mariani *et al*, 1976; Rampilli *et al.*, 1988; Rahali and Menard, 1990; van den Berg *et al.*, 1990). Rahali and Menard (1991) demonstrated that milk containing κ-casein B variant has superior rennet coagulation properties when milks of equal casein content but different κ-casein variants were contrasted. κ-Casein E variant milks have been reported to have similar renneting properties to that of κ-casein A milks (Gravert *et al.*, 1991).

1.5.2 Cheese

This thesis is concerned with κ-casein genetic variant effects on Cheddar and Mozzarella cheese manufacture, yield, composition, functional and ripening characteristics. As the literature on cheese *per se* is vast, this review, therefore, will

only concentrate on Cheddar and Mozzarella cheese manufacture and some associated compositional, functional and ripening characteristics

Cheese is a very varied group of dairy products, produced mainly in Europe, North and South America, Australia and New Zealand and to lesser extent in North Africa and the Middle East, where it originated during the Agricultural Revolution, 6,000-8,000 years ago. There are at least 1,000 named cheese varieties. The principal families are Cheddar, Dutch, Swiss and Pasta filata (e.g. Mozzarella), which together account for about 80 % of total global cheese production. Production of cheese is essentially a concentration process in which the milkfat and casein are concentrated about tenfold while the whey proteins, lactose and soluble salts are removed in the whey (Fox and McSweeney, 1998). Table 1.5.1 shows typical composition of Cheddar and Mozzarella cheese varieties.

Table 1.5.1 Typical composition of Cheddar and Mozzarella cheeses

Parameter	Cheddar	Mozzarella
Moisture %	36.7	54.1
Protein %	24.9	19.4
Total Fat	33.1	21.6
Total Carbohydrate %	1.3	2.2
Fat in Dry Matter %	52.4	47.1
Ash %	3.9	2.6
Calcium %	0.72	0.52
Phosphorus %	0.51	0.37
Sodium %	0.62	0.37
Potassium	0.09	0.07

(adapted from Holland *et al.* (1989))

1.5.2.1 Manufacture of Cheddar and low moisture part-skim Mozzarella cheese

Cheese is formed from the coagulation of the casein in milk, followed by the removal of the whey from the curd. The curd is then generally textured, salted and pressed in a mould prior to storage under conditions that will allow the cheese to ripen.

Milk for cheesemaking should be normal in composition and of good hygienic quality. It should be free of residual antibiotics, preservatives, neutralisers and traces of detergents and sterilisers.

A block diagram of the cheesemaking processes used for Cheddar and low moisture part-skim Mozzarella cheese is given in Figure 1.5.4. The first step in the manufacture of cheese is standardisation. Cheese type is often characterized according to its "fat-in-total-solids" content. Therefore the fat content of the cheesemilk must be adjusted to the required fat content of the cheese. It must always have a relationship with the casein content, and hence protein content, of the whole milk. For this reason the content of fat and casein in milk is monitored throughout the year and the ratio between the fat and casein standardised to the required value. The protein:fat ratios for Cheddar and Mozzarella are 0.96:1 and 1.2:1 respectively.

Pasteurisation is used to eliminate pathogens and spoilage organisms which may exist in raw milk. Pasteurisation destroys not only pathogens but also denatures many endogenous milk enzymes which may be detrimental during ripening. Cheeses from pasteurised milk ripen at a slower rate than those made from raw milk and may fail to develop the full-bodied well-aged flavour of raw milk cheeses. On the other hand spoilage organisms present in raw milk may produce defects in flavour and body. In the interests of safety, pasteurisation should always be employed during cheesemaking. Minimum pasteurisation treatments of milk (72 °C x 15 sec) are desirable for cheese. Temperature treatments above this cause chemical and physical changes in milk (Guinee *et al.*, 1998*a*).

The purpose of adding a starter to milk for cheesemaking is to establish a predominance of a desirable bacterial flora i.e. lactic acid producing bacteria. As well as producing acid which performs many important functions during cheese manufacture i.e. curd dehydration, development of curd texture, control of harmful disease-causing and spoilage of micro-organisms, starter bacteria determine to a large extent the flavour,

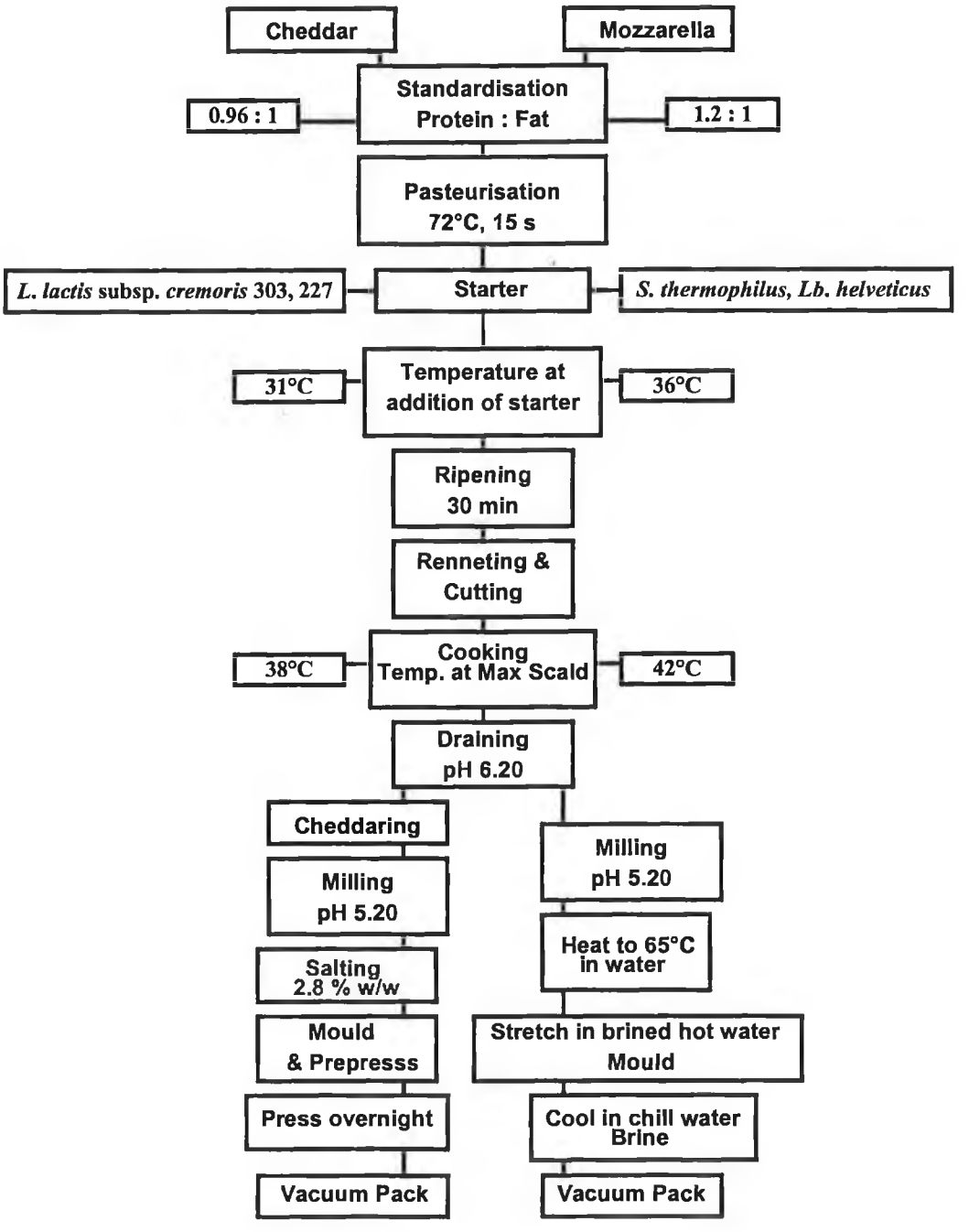

Figure 1.5.4 Diagrammatic representation of the Cheddar and low moisture part-skim Mozzarella cheesemaking processes.

body and texture of the final cheese. The starter bacteria used in most varieties of cheese belong to the Streptococcus genus; Lactobacilli and Propionbacteria are used for a limited range of cheese varieties. Typical starters used in Cheddar and low moisture Mozzarella cheese manufacture are shown in Figure 1.5.4. The temperature at which milk is held prior to starter addition is dependent on the optimum temperature of the starter. After the addition of starter, the cheesemilk can be allowed to stand for 30 minutes to ripen (an increase in titratable acidity of 0.01 % to 0.02 %).

The renneting process has been outlined in Section 1.5.1. The quantity of rennet added to the cheese vat is dependent on the strength (milk clotting units per mL) of the rennet.

The readiness of the curd for cutting can be assessed by pressing the back of the hand on to the curd surface and observing if the curd comes away cleanly from the side of the vat or by cutting a slit in the curd with a knife, the cut should be clean and the whey issuing should be clear. When the coagulum has reached the required degree of firmness it is cut into cubes with knife blades or wire. The object of cutting is to facilitate whey drainage during cooking. The finer the cut the greater the total surface area and hence the greater the amount of whey released. The size of the cut curd particles will depend on the type of cheese to be manufactured. The curd is cut relatively finely for cheeses which require a low moisture content (e.g. Swiss, Cheddar). Cheeses which require a high moisture content are cut more coarsely (e.g. Camembert, Irish Blue). After cutting, the curd is allowed to stand for 5 min to allow contraction of the curd particles and allow whey expulsion to get under way. The free whey then permits the curd to move freely when agitation is applied. Agitation is applied which initially should be just sufficient to keep the curd particles suspended in the whey. The rate of agitation may be increased when the curd particles become less fragile during subsequent curd heating or cooking steps. Stirring prevents the curd from settling in the cheese vat, prevents matting of the curd particles, facilitates heat conduction and gives uniform cooking and promotes contraction and firming of the curd particles.

Heat is applied to the cheese vat during the so-called cooking step of cheesemaking. The objective of heating is to cause the curd particles to contract thus promoting further whey expulsion from the curd particles. Acid production by lactic acid bacteria continues during heating. The rate of heating/cooking in cheesemaking

should be carefully controlled. If the heating rate is too rapid the surface of the curd particles will become dehydrated and free diffusion of the whey through the particle will be restricted. If the temperature is allowed to exceed the required temperature, heat shocking of the lactic bacteria will occur. Heat shocking of the lactic acid bacteria will cause a reduction or a cessation of acid production. Cutting, stirring, heating and development of acidity during cooking bring about whey expulsion and consequently a change in curd consistency. The duration of cooking and the temperature used vary with the variety of cheese to be manufactured, and has a significant bearing on the final moisture content.

After cooking, the curd is pitched. Pitching refers to the point when stirring is stopped and the curd is allowed to settle in the cheese vat. The whey can be drained from the vat when the correct acidity has been developed. The final pH of the cheese will depend primarily on the pH or acidity of the whey in the curd at this stage and to a lesser extent on the whey (lactose) content of the curd. The lower the acidity when draining off the whey, the higher the pH of cheese of any given moisture content. The procedures for handling curd immediately after pitching vary for different cheese varieties. In Cheddar and low moisture Mozzarella cheese the curd with the whey is allowed to run into a shallow cheddaring vat. The whey is then drained from the curd with special strainers used to retain curd particles. The curd particles form a bed at the end of the cheddaring vat. When whey drainage is complete the curd is cut into blocks. After drainage a process called cheddaring is carried out.

The objectives of cheddaring are to allow acidity development to continue, to permit drainage of whey from the curd and to develop the proper consistency or texture in the curd. During manual cheddaring the blocks are inverted every 10-15 minutes and the whey allowed to drain freely from the curd. As the acidity of the whey develops the curd blocks are piled 2 to 3 high and later piled higher. The bottom of the cheese vat is kept warm to facilitate the further growth of the starter bacteria. During the cheddaring operation, the physical condition of the curd changes from a tough rubber like consistency with a high water content to a mass having a smooth appearance and a texture which is likened to chicken breast.

Cheddar cheese curd is milled when cheddaring is complete (curd pH 5.3-5.4). The purpose of milling is to reduce the curd slabs to small pieces to permit proper

distribution of salt throughout the cheese. Salting is carried out immediately after milling. In Cheddar cheese, salting is usually carried out by dry-salting (applying dry salt to the cheese surface). The function of salt in cheesemaking is to control bacterial activity and regulate the rate of ripening, to dissolve some of the protein and so assist matting of the curd, to contribute to flavour, to aid in the further removal of whey and to harden and shrink the curd and to curb undesirable forms of fermentation.

After salting, the curd for Cheddar cheese is mellowed and stirred, it is then placed in moulds and pressed overnight. The cheese is then vacuum packed and stored for the required ripening period.

Low moisture Mozzarella differs from Cheddar cheese in that it is milled into brined water at 65°C. It is then stretched in the 65°C water. This stretching or kneading in hot water gives the cheese a characteristic fibrous structure and melting and stretching properties. The molten mass of curd is filled into moulds. It is then cooled in chilled water. After cooling the moulded cheese is brine salted. The brined cheese is then vacuum packed and stored over its ripening period.

Cheddar cheese is ripened at about 7 °C. A temperature of 4 °C is used in New Zealand. The higher the temperature the more rapid the flavour development. Cheese is more likely to develop off-flavours when it is ripened at temperatures above 10°C.

Mozzarella cheese has a short ripening period and it is ready for consumption at about one week after manufacture. It has a short period of consumer acceptability (3-4 weeks) as it becomes excessively soft on storage after this time period.

1.5.2.2 Specific characteristics of Cheddar cheese

Cheddar cheese received its name from a village in Somerset. The technique of piling the curds in heaps was first used in this village around the middle of the 19th century. The purpose of piling the curds in heaps was to prevent the temperature from falling which would reduce acid production. The first Cheddar cheese factory, as opposed to farmhouse cheesemaking began operation in the United States in 1861 in New York State.

The typical composition of a full fat Cheddar cheese is summarized in Table 1.5.1. The range of acceptable values for salt-in-moisture (S/M), moisture in non-fat substance

(MNFS), fat-in-dry matter (FDM) and pH required for the cheese to be considered either first or a second grade cheese are depicted in Figure 1.5.5.

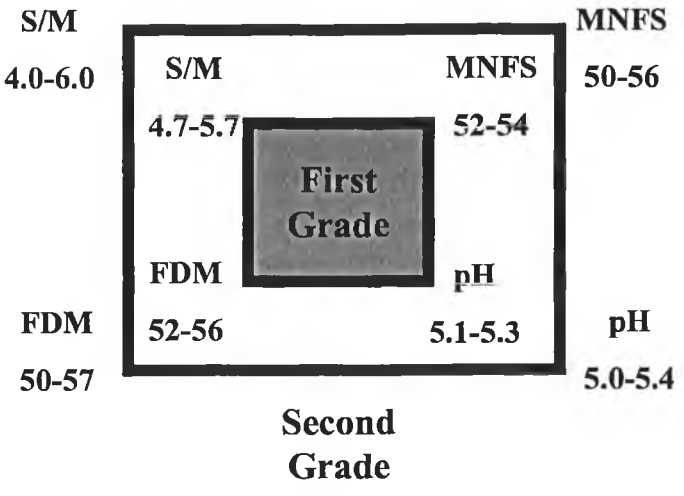

Figure 1.5.5 Suggested ranges of salt-in-moisture, (S/M) moisture in non-fat substance (MNFS), fat-in-dry matter (FDM) and pH for first and second grade Cheddar cheese following analyses 14 days after manufacture (taken from Gilles and Lawrence (1973)).

The ratios of moisture to casein and of salt to moisture are critical factors in cheese quality (Gilles and Lawrence, 1973; Lawrence and Gilles, 1986) since both parameters effect the rate of proteolysis in cheese. Proteolysis in cheese during ripening contributes to the characteristic flavour, aroma, texture and appearance of individual cheese varieties. Since it is difficult to measure the casein content in cheese, the moisture non-fat substance has been determined rather than the casein to moisture ratio. Casein generally represents 85 % of the solids-not-fat in the cheese and therefore changes in MNFS correlate well with changes in the ratio of moisture to casein. The MNFS gives a much better indication of potential final cheese quality than does moisture content of the cheese. The higher the MNFS percentage the faster the rate of proteolytic breakdown in cheese ripening at a given temperature (Lawrence *et al.*, 1993)

The quality of a cheese is dependent upon its characteristic pH range (Lawrence *et al.*, 1984) which results from both its composition and the manner in which it is manufactured (Lawrence *et al.*, 1983). Cheese curd pH is important in that it provides an indication of the extent of acid production throughout the cheesemaking process.

The main factors effecting the pH of Cheddar cheese during manufacture are summarized in Figure 1.5.6. As can be seen from this Figure, the pH of the cheese is dependent on many factors, e.g. milk composition, starter numbers, casein and mineral content of the curd, time between cutting and draining and residual lactose in the salted curd.

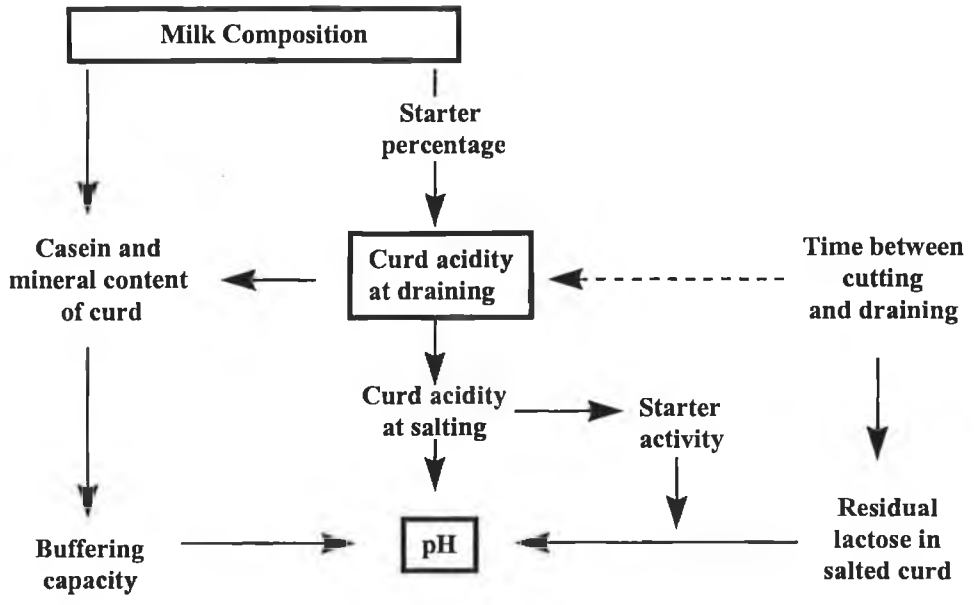

Figure 1.5.6 Main factors determining the pH of Cheddar cheese (taken from Lawrence *et al.* (1993)).

The main factors determining the percentage S/M in Cheddar cheese are outlined in Figure 1.5.7. In young Cheddar cheese the S/M ratio is the main factor controlling water activity which in turn controls the rate of bacterial growth and enzyme activity. A low S/M value (< 4.5 %) can lead to high starter numbers reaching levels which could result in off-flavours (Lowrie and Lawrence, 1972; Breheny *et al.*, 1975). For this reason cheesemakers normally try to maintain the S/M value within the range 4.5 and 5.5 % (Lawrence and Gilles, 1982). Within this S/M range, the rate of metabolism of the lactose is controlled by a second factor, the temperature of the cheese during the first few days of ripening, since this controls the rate of growth of non-starter bacteria such as Lactobacilli and Pediococci.

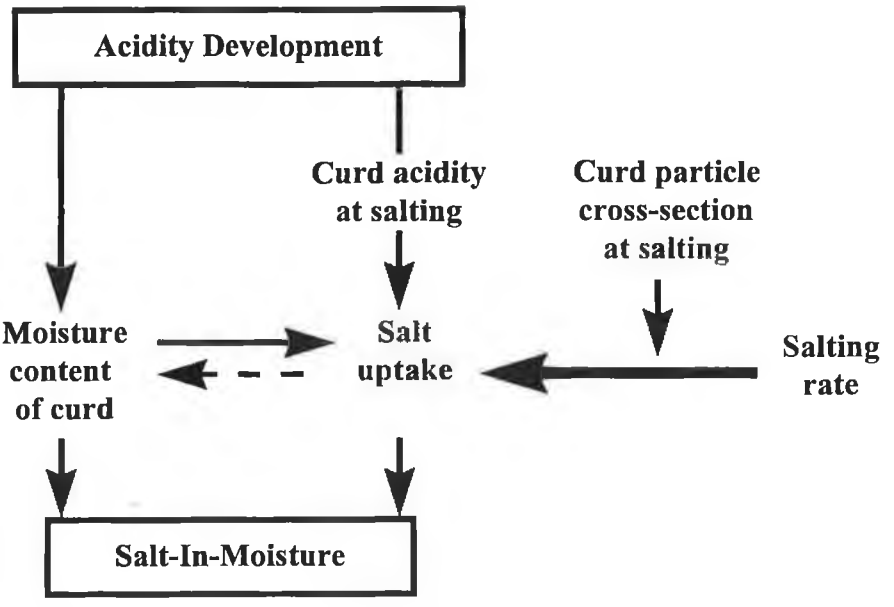

Figure 1.5.7 Main factors which affect salt in moisture level in Cheddar cheese (taken from Lawrence *et al.* (1993)).

The FDM is not as important as MNFS, S/M and pH in determining cheese quality. It only effects cheese quality through its effect on MNFS (Dolby and Harkness, 1955). The percentage FDM has more relevance to the cheesemaker than fat content *per se* since moisture is volatile and legal limits for fat are usually specified as FDM. The FDM can be controlled directly by altering the casein to fat ratio of the cheese milk. An increase of about 0.02-0.04 in the casein/fat ratio will generally cause a decrease of 1 % in the FDM (Dolby and Harkness, 1955) depending on the manufacturing process within a specific cheesemaking plant.

The importance of introducing compositional ranges into a grading system is illustrated by the fact that it is not yet possible to produce a uniform line of cheese within a given day's manufacture. This is particularly the case with respect to S/M values. The quality of some cheeses will not necessarily be poorer but the rate of ripening will differ. The cheese is likely to be acceptable for consumption grade (Fig. 1.5.5) as long as MNFS, S/M, pH and, to a lesser extent, FDM values are within the required range (Lawrence and Gilles, 1980).

Research has shown that changes in cheese flavour during ripening are mainly related to breakdown of the proteins (Aston *et al.*, 1983; Edwards and Kosikowski, 1983). Studies have also correlated protein breakdown with textural changes in cheese

(de Jong, 1977; Creamer *et al.*, 1985). Ripening of the vast majority of cheeses produced by rennet coagulation involves three primary biochemical events, glycolysis, proteolysis and lipolysis. All three reactions are responsible for the desired textural changes and production of the characteristic flavours. Many researchers have found that proteolysis is the single most important event during the ripening of most cheese varieties (Fox, 1989) having a major impact on flavour and texture. The process of proteolysis can be summarized as follows: initial hydrolysis of casein is caused primarily by residual coagulant and to a lesser extent by plasmin and possibly cathepsin D, with the production of large and intermediate sized peptides which are in turn degraded by the coagulant and enzymes from the starter and non-starter flora. The production of small peptides and free amino acids results from the action of bacterial proteinases and peptidases (Fox *et al.*, 1994).

There are five main systems which contribute to the hydrolysis of the caseins; (1) the rennet coagulant, (2) indigenous or native milk proteinase, especially plasmin, (3) proteinases and peptidases produced by the starter cultures once the cells have lysed, (4) enzymes of the adjunct starter which are of major importance and finally (5) enzymes of the non-starter bacteria.

Cheddar cheese ripening is associated with an appreciable extent of proteolysis that involves all of the major caseins to different extents (Fox, 1989) and can be divided into three phases; (1) proteolysis in milk before manufacture, (2) enzymatically-induced coagulation of the milk and (3) proteolysis during cheese ripening (Rosenberg *et al.*, 1995). The products of proteolysis range in size from large polypeptides, comparable in size to intact caseins, through a range of medium and small peptides to free amino acids (Fox *et al.*, 1994).

The pH of vacuum-packed Cheddar cheese does not change significantly during ripening (McSweeney and Fox, 1997). Figure 1.5.8 shows the changes that occur in Cheddar cheese during ripening. Both the nitrogen soluble at pH 4.60, an indication of the degree of primary proteolysis (Fig. 1.5.8 (a)), and the nitrogen soluble in 5 % phosphotungstic acid (a measure of the small peptides and free amino acids produced by the action of bacterial peptidases on the larger peptides produced by rennet (Jarret *et al.*, 1982; Fig. 1.5.8 (b))) increase during the course of Cheddar cheese

ripening. Nitrogen soluble at pH 4.60 increases throughout the ripening period as does nitrogen soluble in 5 % phosphotungstic acid (Fig. 1.5.8 a, b).

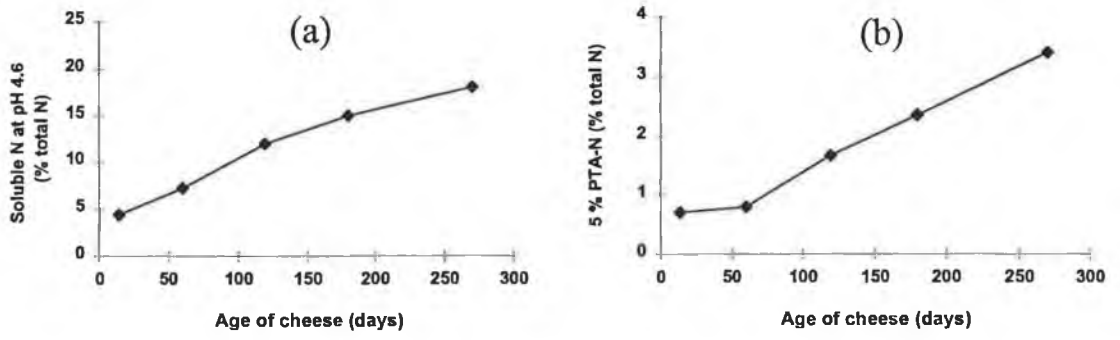

Figure 1.5.8 Changes in Cheddar cheese during ripening (a) formation of nitrogen soluble in water at pH 4.6 and (b) formation of nitrogen soluble in 5 % phosphotungstic acid (adapted from Fenelon and Guinee, (1997)).

Many factors influence the texture of Cheddar cheese. These include cheese pH, protein and fat content and proteolysis during ripening. Figure 1.5.9 outlines the contribution of pH to cheese texture. The Figure shows how the size of the sub-micelles will vary in the cheeses over a range of pH (pH 4.6 - 5.5) it also shows the typical pH's for some cheese varieties and their associated texture. The texture of Cheddar cheese is intermediate (Fig. 1.5.9) between those cheeses having relatively high pH's (Swiss and Gouda), which flow readily when force is applied and to those of a low pH (Cheshire and Feta) which deform by shattering only at their yield point (the point at which the rate of collapse of the stress supporting structure overtakes the build-up of stress due to compression). Scanning electron microscopy has established that cheese consists of a continuous protein matrix and that this matrix is clearly different in the various cheese types (Hall and Creamer, 1972). Figure 1.5.10 is a scanning electron micrograph of Cheddar cheese. The structural units in the protein matrix are essentially in the same globular form as in the original milk. The protein matrix of Cheddar is intermediate between Gouda and Cheshire (Fig. 1.5.9) much of the protein in Cheddar is in the form of smaller particles than in Gouda. As the pH decreases towards that of the iso-electric point of paracasein (pH 4.6), the protein assumes an increasingly more compact conformation and the cheese becomes shorter in texture and fractures under a smaller applied force (Creamer and Olson, 1982; Walstra and van Vliet, 1982). The intermediate position of Cheddar cheese in the cheese spectrum allows it a wider range

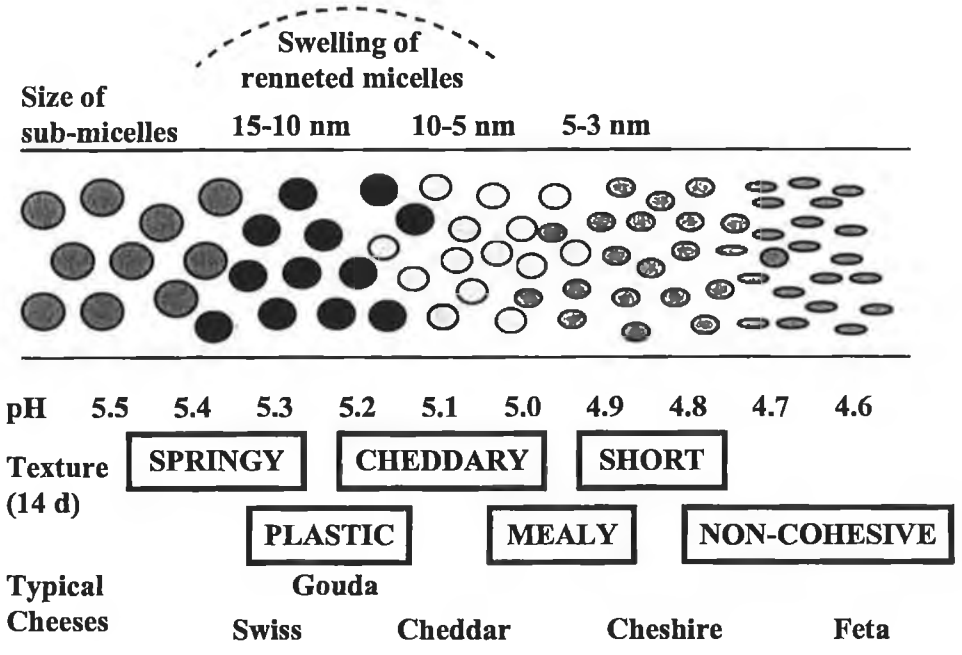

Figure 1.5.9 The contribution of pH to cheese texture (taken from Lawrence *et al.* (1993)).

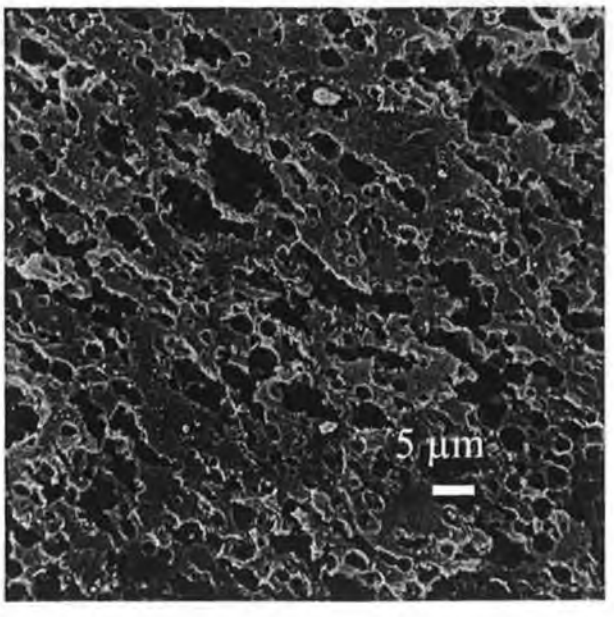

Figure 1.5.10 Scanning electron micrograph of a commercial full-fat Cheddar cheese showing a continuous protein matrix at a magnification of 1800x (taken from Guinee *et al.* (1998*a*))

of consumer acceptability with regard to its texture than for other cheese varieties (Fig. 1.5.9).

Any modification in the amount or nature of the protein present in the cheese will modify its texture, as it is largely the protein matrix which gives rise to the rigid form of cheese. Reduced fat cheese is considerably firmer and more elastic than full fat Cheddar cheese due to a greater percentage of protein matrix (Emmons *et al.*, 1980).

Fat in Cheddar cheese exists as physically distinct globules, dispersed in the aqueous protein matrix (Kimber *et al.*, 1974). Increasing the fat content in cheese leads to a slightly softer cheese. This also occurs on increasing the moisture content as the protein matrix becomes weakened as the protein molecule volume fraction decreases. A large variation in fat content is required before a significant change in texture is seen (Lawrence and Gilles, 1980). An inverse relationship exists between fat content and cheese hardness (Baron, 1949).

Proteolysis during ripening leads to considerable changes in cheese texture. As each peptide bond is cleaved, two new ionic groups are generated which compete for the existing available water in the system. As the available water becomes bound the cheese becomes firmer and less easily deformed. This leads to increased hardness and decreased elasticity over the ripening period (Baron, 1949). The degree of change in texture is dependent on the duration and temperature of maturation and on the concentration of residual rennet, plasmin, and starter and non-starter proteinases all of which affect the rate of proteolysis.

Numerous factors contribute to the development of flavour and aroma in a specific cheese variety. Cheddar cheese flavour may result from the presence of diacetyl (Lawrence and Thomas, 1979), -SH compounds (Lindsay and Rippe, 1986), acetic acid (Forss and Patton, 1966; Morris, 1978; Lawrence and Thomas, 1979), carbon dioxide (Morris, 1978), lactic acid (Morris, 1978) and NaCl together with low levels of specific amino acids and peptides (McGugan *et al.*, 1979; Aston *et al.*, 1983; Aston and Creamer, 1986).

During ripening in Cheddar cheese, as the original casein network is broken down, the desired balance of flavour and aroma compounds is formed. The precise nature of the reactions which produce this balance is poorly understood due to lack of knowledge of the compounds that impart typical Cheddar flavour and the complexity of

the cheese microflora involved. As can be seen from Figure 1.5.11, the flavour of Cheddar cheese can be affected by starter and non-starter bacteria in addition to the presence of chymosin and plasmin activity.

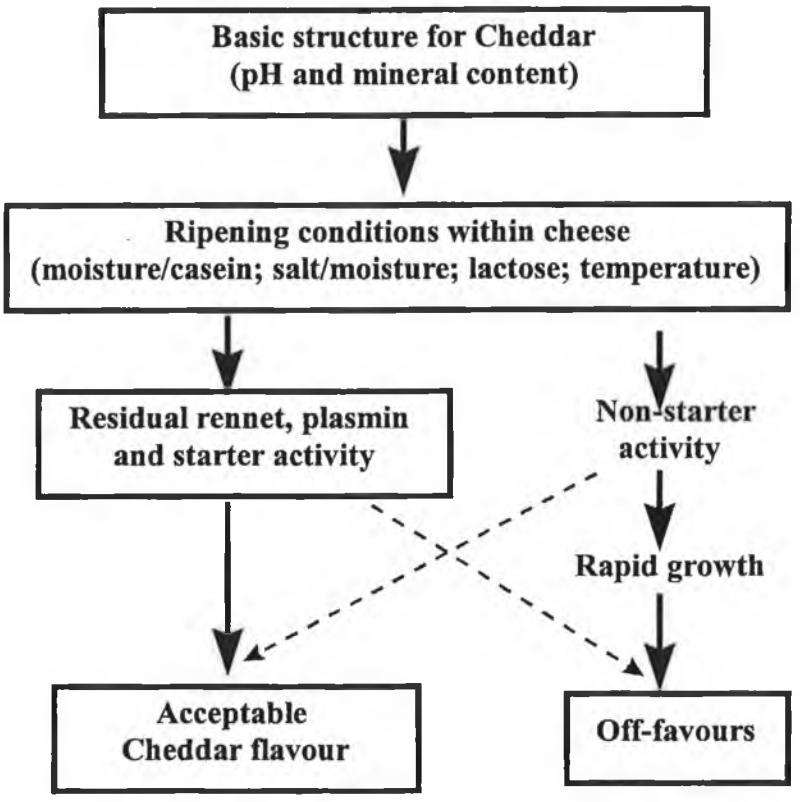

Figure 1.5.11 Main factors which determine the development of acceptable/unacceptable flavour in Cheddar cheese (taken from Lawrence *et al.* (1993)).

Although milk proteins and lactose are the most likely sources of the flavour precursors in Cheddar cheese, fat also plays an important role. It is well known that Cheddar cheese made from skim milk will not give a characteristic Cheddar flavour. The substitution of vegetable or mineral oil for milkfat results in some degree of Cheddar flavour (Foda *et al.*, 1974) suggesting that the water-fat interface is important and that some of the flavour components are retained in the fat.

Lactic acid starter are essential for the development of typical balanced Cheddar flavour while milk fat plays an important role in the perception of Cheddar flavour (Ohren and Tuckey, 1969). The mechanism by which the fat and starter perform

their functions is not totally understood and many contradictory theories have been suggested (Kristofferson, 1973; Law, 1981; Aston and Dulley, 1982; Law, 1984).

During proteolysis a release of flavour compounds, previously bound to protein takes place (McGugan *et al.*, 1979). While a great deal of research has concentrated on the aroma aspect of Cheddar flavour, it is now known that the non-volatile water soluble fraction contributions to flavour intensity. A significant relationship has been found between the level of proteolysis and the extent of flavour development (Aston *et al.*, 1983). While volatile components of Cheddar cheese do not make a measurable contribution to the intensity of Cheddar flavour they are still an important factor in the quality of the flavour (McGugan *et al.*, 1979).

The role of the starter in flavour production can be summarized as follows: starter activity sets up the required redox potential, pH and moisture content in the cheese to allow enzyme activity to proceed at a favourable rate. Other factors such as the temperature during manufacture and the S/M level must be regulated to ensure that the net metabolic activity rate of the starter is low (Lawrence *et al.*, 1972; Lowrie *et al.*, 1974) but adequate to allow it to achieve the required pH at day 1.

1.5.2.3 Specific characteristics of Mozzarella cheese

Mozzarella is a prominent member of the pasta filata, or stretched curd, cheese variety which originated in Italy. Pasta filata cheeses are distinguished by the unique plasticizing and kneading treatment of fresh curd in hot water which imparts to the finished cheese its characteristic fibrous structure, melting and stretching properties. Although Mozzarella cheese originated in Italy, the United States of America has now become the principal producer due to the meteoric rise in popularity of the pizza pie. In the USA, Mozzarella cheese is divided into four separate categories defined by standards of identity on the basis of moisture content and fat-in-dry-matter, as indicated in Table 1.5.2 (Kindstedt, 1993). Low-moisture part-skim is the most common variety of Mozzarella cheese made in Ireland. Mozzarella and part-skim Mozzarella are high in moisture (> 52 %), soft bodied and are often consumed fresh as table cheeses. They are rarely used as an ingredient for pizza due to poor shredding and clumping properties, and limited shelf-life. Low moisture and low moisture part-skim Mozzarella in contrast

have much lower water content (typically 47-48 %), longer shelf-life, firmer body, good shredding properties and are used primarily as ingredients for pizza and related foods (Kindstedt, 1993).

Although most Mozzarella is used for melting purposes, in pizza pie and in toasted sandwiches, the physical properties of the unmelted cheese are also very important. When used for pizza and most other applications, the cheese must first be cut, diced or shredded into discrete particles of uniform size to facilitate even distribution and melting. Therefore shreddability and resistance to clumping after shredding are major determinants of overall cheese quality. Research which specifically addresses these functional properties is scarce.

Table 1.5.2 Compositional standards for Mozzarella cheese

Type	Moisture (%)	FDM* (%)
Mozzarella	> 52 but ≤ 60	≥ 45
Low-moisture	> 45 but ≤ 52	≥ 45
Low-moisture part-skim	> 45 but ≤ 52	≥ 30 but < 45
Part-skim	> 52 but ≤ 60	≥ 30 but < 45

* Fat-in-dry-matter, (taken from Kindstedt (1993)).

One of the most popular methods of testing the rheological properties of cheese is by using the Instron Universal Testing Machine (Chen *et al.*, 1979). Mozzarella consistently shows a high degree of elasticity and a low level of hardness. Chen *et al.* (1979) also reported that Mozzarella, Parmesan and Swiss were among the top three of 11 cheese varieties tested for chewiness, gumminess and adhesiveness. Processed Cheddar and Muenster had a higher cohesiveness than Mozzarella.

The effects of various aspects of composition on the texture of Mozzarella cheese have been studied. Taranto *et al.* (1979) related the rheological properties of Mozzarella and Cheddar cheeses to differences in microstructure and composition. Mozzarella has a lower value for hardness and higher values for work ratio (cohesiveness) and

springiness (elasticity) than Cheddar (Lee *et al.*, 1978; Chen *et al.*, 1979; Imoto *et al.*, 1979). It was reported that these rheological differences could be due to the higher water content and greater compactness of the protein matrix in Mozzarella. The moisture and FDM contents in low-moisture Mozzarella cheese were inversely related to hardness, gumminess and chewiness, while springiness showed an inverse relationship with moisture only and cohesiveness showed an inverse relationship with FDM only (Tunick *et al.*, 1990).

The physical properties of melted Mozzarella cheese are highly complex and give rise to several important functional attributes such as meltability, stretchability, elasticity, flow, free oil formation and browning. Precise definitions for these properties and standard objective methods for their measurement are generally lacking in the literature. In general terms, meltability refers to the capacity of cheese particles to form a uniform continuous melt; stretchability is the ability of the melted cheese to form fibrous strands which elongate without breaking under tension; flow is the ability of melted cheese to spread over an area and elasticity, or "strength of stretch", is the capacity of the fibrous strands to resist permanent deformation. Free oil formation, also called "oiling off" or "fat leakage", is the separation of liquid fat from the melted cheese body into oil pockets, particularly at the cheese surface. Browning which occurs at the cheese surface during high temperature baking is characterized by the formation of a skin-like layer containing coloured patches that may range from light or golden brown to black in extreme cases (Kindstedt, 1993).

Simple tests for analysing some of these attributes, i.e. melt, flow and stretch are outlined in Figure 1.5.12 (a, b and c). Meltability in Figure 1.5.12 (a) is the time in seconds required for a fixed loading (0.173 g/cm^2) of shredded Mozzarella to form a uniform mass on heating at 280°C i.e. no evidence of shredded pieces remaining. Flowability in Figure 1.5.12 (b) is a measure of the percentage increase in diameter of a disc of Mozzarella (44.5 mm diameter x 4 mm height) on heating at 280°C for 4 min (based on the Schreiber test (Park *et al.*, (1984))). Stretchability in Figure 1.5.12 (c) is a measure of the distance (mm) to which two halves of a melted pizza (280°C, 4 min) having a fixed loading (0.242 g/cm^2) of shredded Mozzarella need to be parted in order

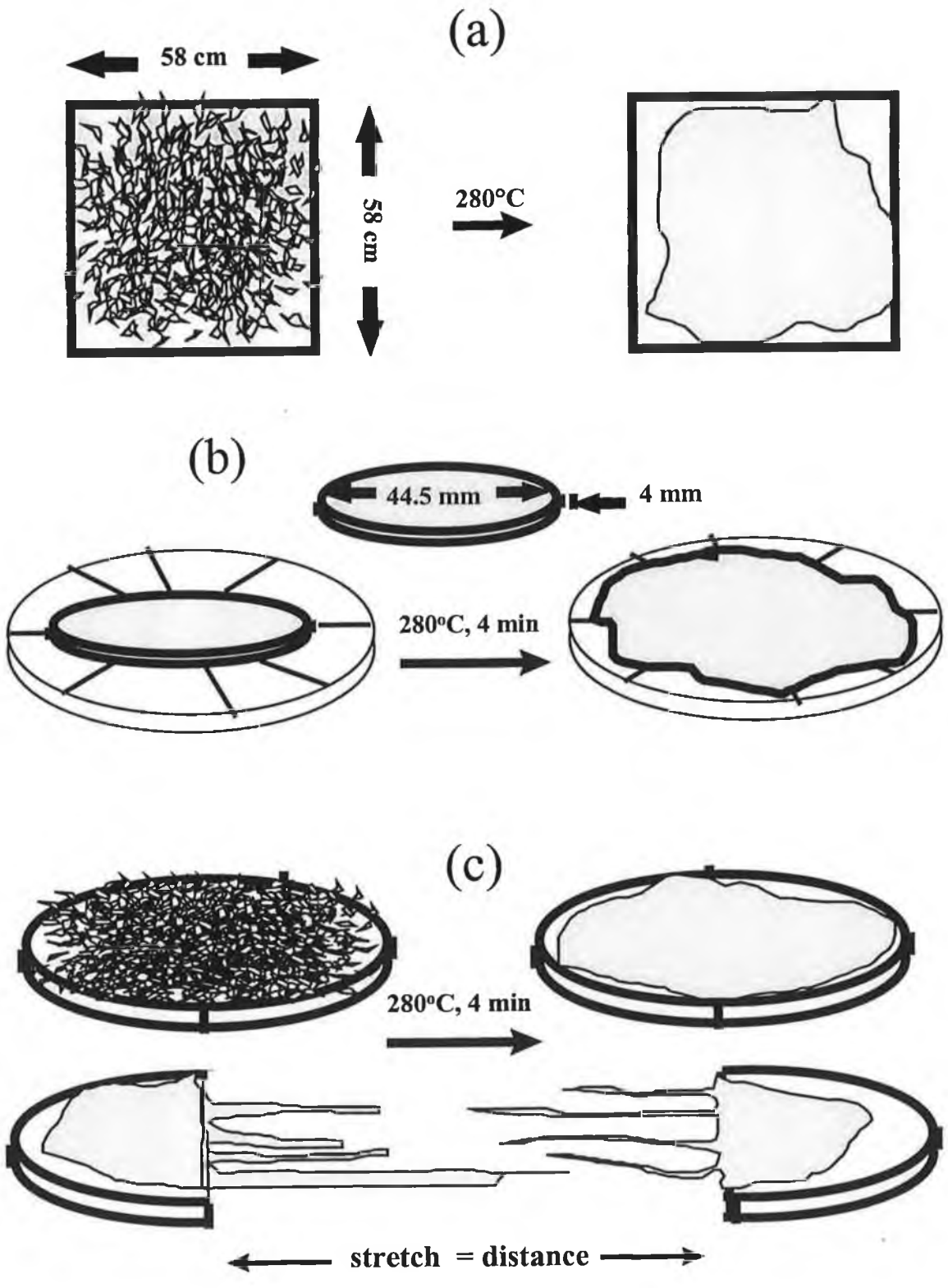

Figure 1.5.12 Diagrammatic representation of (a) meltability, (b) flowability and (c) stretchability tests for Mozzarella.

to achieve complete strand breakage. Free oil formation and browning can also be assessed simply by visual observation during the course of performing these tests.

Mozzarella cheese, directly after manufacture, melts to a tough elastic consistency which is unacceptable for use in pizza pies. In the first week of ageing this consistency mellows to one which is acceptable. As the cheese continues to age it then becomes excessively soft and again unacceptable for use on pizzas. There is, therefore, a relatively short period of acceptability i.e. ~ 3-4 weeks (Kinstedt, 1993).

A graphic representation of the changes occurring during ripening of low moisture, part-skim Mozzarella cheese (brine/dry salted) can be seen in Figure 1.5.13. Ageing of low moisture, part-skim Mozzarella cheese is accompanied by increases in the levels of N soluble at pH 4.6 and in 5 % phosphotungstic acid (Fig. 1.5.13 (a, b)). The pH of low moisture part-skim Mozzarella remains relatively constant (Fig. 1.5.13 (c)) during the ripening period of low moisture part-skim Mozzarella. An increase in cheese softness, in fat expressed on hydraulic pressing and a decrease in the level of free moisture (expressed on hydraulic pressing) during ageing of low moisture part skim Mozzarella cheese (Guinee *et al.*, 1997*b*). Simultaneously, decreases in melt time (Fig. 1.5.13 (e)) and viscosity of the melted cheese while an increase in flowability and stretchability of the melted cheese are observed during ripening (Fig. 1.5.13 (d, f)). Therefore the functionality of the melted cheese shows a marked improvement in the first 1-3 weeks of ripening. The time taken to attain optimum functionality depends on milk composition, make procedure and cheese composition. Further changes in functionality occur more slowly (Guinee *et al.*, 1997*b*). Prolonged ripening (e.g. > 60 days) leads to a loss in the characteristic "chewiness" and promotes excessive softening, and associated problems of shred aggregation and stickiness.

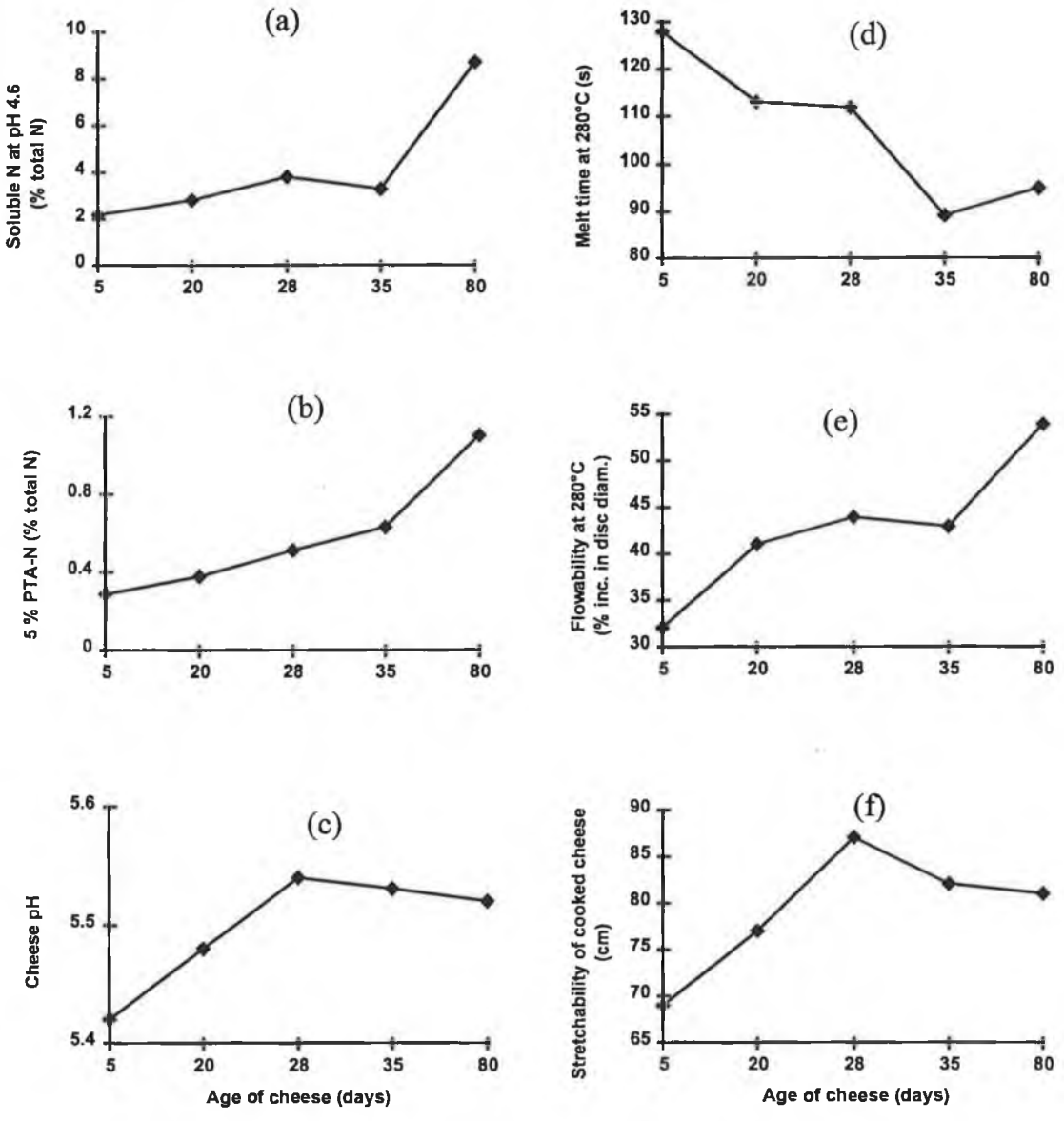

Figure 1.5.13 Changes in (a) nitrogen soluble in water at pH 4.6, (b) nitrogen soluble in 5 % phosphotungstic acid, (c) pH, (d) melt time, (e) flowability, and (f) stretchability characteristics of low moisture part-skim Mozzarella cheese during storage at 4 °C (taken from Guinee *et al.* (1997*b*)).

1.5.2.4 Effect of κ-casein variants on cheesemaking

Given that several research groups report an association between the B variant of κ-casein and a higher casein concentration in milk, it is not surprising that cheeses manufactured from milks containing the BB variant of κ-casein give higher yields than from milks containing the AA or AB variants. A summary of the reports in the literature on the association between κ-casein phenotype with cheese yield is given in Table 1.5.3. It is worth noting that most studies to date on the κ-casein variant effect on cheese yield have been performed at laboratory scale. Mariani *et al.* (1976) and Morini *et al.* (1979) obtained higher yields (8 and 10 %, respectively) in the production of Parmesan cheese from κ-casein BB milks as compared to κ-casein AA milks. Graham *et al.* (1986), Marziali and Ng-Kwai-Hang, (1986*a*), Vink *et al.* (1993) and Banks *et al.* (1994) also reported an increased yield of Cheddar to be associated with the κ-casein B variant. The increase in Cheddar cheese yield (3-5 %) was not as large as that found for Parmesan cheese, possibly due to differences in the cheesemaking process. Schaar *et al.* (1985), using pasteurised milk, reported no effect of κ-casein variant on the yield of Svecia cheese. Aleandri *et al.* (1990) reported that the difference in Parmesan cheese yield between κ-casein AA and BB genetic variants was greater than that expected due to differences in milk composition. Horne *et al.* (1996) reported, that irrespective of milk composition, κ-casein BB milks resulted in a higher moisture adjusted Cheddar cheese yield than κ-casein AA milks. Aleandri *et al.* (1997) recently studied the effect of κ-casein and β-lactoglobulin variants on the yield of three Italian cheese varieties, i.e. Grana (a very hard cheese), Montasio (a semi-hard cheese) and Provalone (a cooked curd cheese). They reported an increase in Grana cheese yield to be associated with the κ-casein BB variant, an increase in Montasio cheese yield to be associated with the BB variant of β-lactoglobulin and no effect on Provalone cheese yield by either κ-casein or β-lactoglobulin variants.

Pabst *et al.* (1991) reported a 2.7 % higher recovery of milk protein in Edam cheese manufactured from κ-casein BB milk when compared with κ-casein AA variant. Van den Berg *et al.* (1990) reported higher protein recovery in Gouda cheese associated with the κ-casein B allele. Marziali and Ng-Kwai-Hang (1986*a*) reported higher protein and fat contents in Cheddar cheese made with κ-casein BB variant milks. Graham *et al.*

(1986) and Morini *et al.* (1979) also reported an increase in fat content in the cheese made from κ-casein BB milk for Cheddar and Parmesan cheese, respectively. Fat retention efficiency values of 86.4 ± 0.5, 90.7 ± 0.9 and 91.8 ± 0.5 % were reported for κ-casein AA, AB and BB, respectively, by Horne *et al.* (1996). Schaar *et al.* (1985) for Svecia cheese reported no κ-casein variant related effects associated with recovery of milk constituents and cheese composition but they did report increased fat content in the κ-casein BB variant cheese after 3.5 months ripening. Morini *et al.* (1979), showed for Parmesan cheese, Marziali and Ng-Kwai-Hang (1986*a*) for Cheddar, van den Berg *et al.* (1990) for Gouda and Rahali and Menard (1991) for Camembert cheese that κ-casein BB variant milk results in a cheese of higher dry matter content than cheese made from κ-casein AA or AB milk. Graham *et al.* (1986) reported no increase in dry matter content associated with the κ-casein BB variant for Cheddar cheese.

Increases in fat and protein content of cheese associated with the κ-casein BB variant are reflected in a reduced loss of protein and fat in whey (Marziali and Ng-Kwai-Hang (1986*a*, *c*)). Van den Berg *et al.* (1992) reported that whey obtained from κ-casein AA milk had significantly higher amounts of fat and about 30 % more cheese fines than with κ-casein AB milk.

Table 1.5.3 Summary of the literature reports on the association between κ-casein phenotype and cheese yield.

Cheese Variety	Approx. yield increase κ-casein BB > AA (%)	Reference
Parmesan	8	Mariani *et al.*, 1976
Parmesan	10	Morini *et al.*, 1979
Cheddar	5	Graham *et al.*, 1986
Cheddar	> 3	Marziali and Ng-Kwai-Hang, 1986*a*
Camembert	3	Rahili and Menard, 1991
Cheddar	3	Banks *et al.*, 1994
Grana	7	Aleandri *et al.*, 1997

(adapted from FitzGerald and Hill (1997))

Little information exists in the literature regarding the effect of κ-casein variant on cheese ripening and proteolysis a summary of which is given in Table 1.5.4. Morini *et al.* (1979) reported a retardation in proteolysis during ripening of Parmesan cheeses made from κ-casein BB-milk. It was suggested that this observation might be attributed to the lower moisture content in the BB cheese. Schaar *et al*, (1985) reported no differences between the κ-casein variants in the quality of Svecia cheese. He also reported no significant difference in the chemical analysis at maturity, although κ-casein B cheeses appeared to have lower levels of proteolysis. Van den Berg *et al.* (1992), reported no differences in the rate of proteolysis of Gouda cheese made from milks of different κ-casein variants.

Table 1.5.4 Summary of the literature reports on the association between κ-casein phenotype with rate of cheese ripening.

Cheese Variety	Difference in rate of proteolysis between κ-casein variant cheeses	Reference
Parmesan	BB < AA	Morini *et al.*, 1979
Svecia	no difference	Schaar *et al.*, 1985
Gouda	no difference	Van den Berg *et al.*, 1992
Cheddar	no difference	Imafidon *et al.*, 1995
Edam	BB < AA	Mayer *et al.*, 1997*a*

(adapted from FitzGerald and Hill (1997))

While Imafidon *et al.* (1995) reported differences in electrophoretic patterns and reversed-phase HPLC profiles in Cheddar cheeses made from κ-casein AA or AB milks, they reported no differences in the rate of proteolysis of these cheeses as reflected in water soluble nitrogen levels. Mayer *et al.* (1997*a*) reported that 8 week old Edam cheese made with κ-casein AA variant milk had higher levels of water soluble nitrogen than Edam made from κ-casein BB variant milk.

It is clear, therefore, that laboratory-scale studies demonstrate that higher cheese yields are associated with cheese manufactured from milks containing the B variant of κ-casein. However, little or no information is available on the pilot-scale

manufacturing characteristics of these cheeses. Such information is required by commercial cheese manufacturers in order to assess the benefits of selecting for animals displaying specific κ-casein phenotypes. Furthermore, while some information exists on the proteolysis and ripening characteristics of cheese manufactured from milks containing different κ-casein variants, no available detailed systematic study appears to have studied κ-casein variant effects on cheese ripening and proteolysis for cheeses made at pilot-scale. Finally, no information appears to be available on the resultant functional characteristics of cheeses manufactured from milks containing the A or B variants of κ-casein.

Proposed Investigation

The objectives of this thesis were as follows:

- To screen individual milk samples from a large population of Irish Holstein Friesian cows for the A and B variant of κ-casein and to use this information to determine associations, if any, between κ-casein variant and milk production and compositional properties.

- To determine the influence of κ-casein variant on the rennet coagulation properties of pooled milk samples obtained from groups of Holstein Friesians segregated according to κ-casein AA, AB and BB variants.

- To produce Cheddar and Mozzarella cheeses at pilot-scale from bulked Holstein Friesian milk samples obtained from animals which had been segregated into groups containing either κ-casein AA, AB or BB phenotypes.

- To quantify the effect of κ-casein variant on the manufacturing, rheological, sensory and ripening properties of Cheddar cheeses produced at pilot-plant scale.

- Finally, to quantify the influence of κ-casein variant on the manufacturing, functional, rheological and ripening properties of Mozzarella cheeses.

Chapter 1

Effect of κ-casein genetic variant on milk yield and composition in Irish Holstein Friesians

[1]This chapter has been submitted for publication to the *Irish Journal of Agriculture and Food Research*

1.1 Summary

Individual milk samples from 6,007 Irish Holstein Friesian cows were typed for κ-casein phenotype using iso-electric focussing on ultra-thin polyacrylamide gels. The phenotype distribution of the κ-casein variants were as follows: κ-casein AA, 53.07 %; κ-casein AB, 44.95 % and κ-casein BB 1.98 %. Individual cow phenotypes were merged with milk recording data. The resulting data was statistically analysed (using the GENSTAT statistical package) to determine associations, if any, between κ-casein variant and milk yield and composition. While no statistically significant associations ($P<0.05$) were found, data trends indicated that κ-casein BB variant cows had higher milk yield, protein yield and number of days in milk than κ-casein AA or AB variant cows. This data base of information is available at Teagasc, Dairy Products Research Centre, Moorepark, Fermoy, Co. Cork.

1.2 Introduction

Bovine milks containing the BB variant of κ-casein are associated with increased cheese yield (Rahili and Menard, 1991; Banks *et al.*, 1994; Walsh *et al.*, 1995; FitzGerald, 1997; Walsh *et al.*, 1998). Herds having a high frequency of the B variant of κ-casein are therefore expected to be more economically favourable for the production of milk for cheesemaking. However, Holstein-Friesians display a very low frequency of occurrence of the κ-casein BB phenotype (Ng-Kwai-Hang and Monardes, 1990; van Eenennaam and Medrano, 1991; Bovenhuis *et al.*, 1992; O'Hara 1995; Aleandri *et al.*, 1997) and this fact has led to many studies seeking associations between κ-casein variant and milk yield and composition. The results from these studies, however, are conflicting. Some studies report no association between κ-casein variant and milk yield (Ng-Kwai-Hang *et al.*, 1984; Ng-Kwai-Hang *et al.*, 1990; Bovenhuis *et al.*, 1992; O'Hara, 1995). Some studies report κ-casein BB to be associated with an increased milk yield when compared to κ-casein AA (Lin *et al.*, 1986; van Eenennaam and Medrano, 1991) while others report the opposite trend (Gonyon *et al.*, 1987; Bovenhuis *et al.*, 1992). Similarly, conflicting results have been reported in studies

seeking associations between κ-casein phenotype and milk composition parameters (Ng-Kwai-Hang *et al.*, 1986; Aleandri *et al.*, 1990; Bovenhuis *et al.*, 1992). As outlined by Ng-Kwai-Hang and Grosclaude (1994), some of the reasons for the lack of compatability in the results may be due to differences in population size, breed of animal, frequency of genetic variants under consideration, methods of measuring production traits and most importantly the rigor of statistical analysis used to adjust for other important factors contributing to milk production such as age of cow, season, stage of lactation, health status and effects of other genetic variants.

The low frequency of occurrence of the κ-casein BB phenotype in Holstein-Friesians makes it difficult to obtain large groups of κ-casein BB variant animals in order to develop statistically reliable comparisons between milk composition and yield for κ-casein AA and BB variant animals. Therefore, the objective of this study was to firstly determine the frequency distribution of κ-casein variants in a large population of Irish Holstein-Friesians and secondly to determine if there was an association between κ-casein variant and milk yield and composition in this group of animals.

1.3 Materials and Methods

1.3.1 Materials

Bind-silane™, Repel-silane™, Pharmalytes™ in the pH ranges of 5-6, 6.7-7.7 and 4.2-4.9, Ampholine™ pH 5-7 and iso-electric focusing (IEF) electrode strips were obtained from Pharmacia LKB Biotechnology (Uppsala, Sweden). Servalyte™ pH 3-5 was from Serva GmbH (Heidelberg, Germany). Spacer tape (100 μm) was from Desaphor HF (Heidelberg, Germany). Acrylamide, N,N' methylene-bis-acrylamide and Amberlite were from Sigma Chemical Co. (Poole, Dorset, U.K.). Filter paper, (Whatman No. 1) was from Whatman (Maidstone, Kent, U.K.).

Lyophilised defatted whole milk samples of known phenotype used as standards were a kind gift from Prof. Dr. G. Erhardt, Institute for Animal Science, Justus-Liebig-University, Giessen, Germany.

1.3.2 Milk Supply

Individual animal milk samples were collected from 138 herds registered in the DairyGold Co-operative Society Ltd. (Mallow, Co. Cork. Ireland) milk recording service in the North Munster region of Ireland from October, 1993 to March, 1994. The Supplier Numbers and Animal Numbers were recorded and each assigned a code. Sub-samples (1 ml) of individual milks were taken and labeled using this code. These samples were then skimmed by centrifugation at 13,000 g for 10 min at 4°C using a Micro Centaur microfuge (MSE, Sussex, U.K.). Samples were prepared for IEF by mixing 200 µl of skimmed milk with 800 µl of IEF sample buffer (8 M urea, 2.7 % (v/v) β-mercaptoethanol). IEF samples were stored at -20°C for up to one week.

1.3.3 IEF

Iso-electric focussing was carried out using an IEF FBE-3000 Flat Bed Apparatus (Pharmacia Fine Chemicals, Uppsala, Sweden) essentially as described by Erhardt (1989) and O'Hara (1995). Glass plates (230 mm x 115 mm x 2 mm) were used as physical supports for ultrathin gels (300 µm). Three layers of spacer tape was applied to 3 sides of one glass plate. The pH of 500 ml of distilled water was adjusted to 3.5 with 10 % (v/v) acetic acid. Two ml of Bind Silane™ was then added and allowed to mix for 15 min. The plate with the spacer tape was placed in this solution for 1 h. A glass plate without spacer tape was wiped with a tissue soaked in Repel-silane™ and left to dry in a fume hood. Both plates were subsequently rinsed with distilled water and left to air dry.

A stock acrylamide gel solution was prepared containing 8.66 % (w/v) acrylamide, 1.0 % (w/v) amberlite and 0.34 % (w/v) N,N' methylene-bis-acrylamide in distilled water. This was mixed for one hour, filtered through Whatman No. 1 filter paper and stored at 4°C for up to one week in a dark glass bottle. The gel (T = 4.5 %, C = 3.75 %) used to resolve κ-casein genetic variants contained 8 M urea, 50 % (v/v) of the above stock solution, 6.6 % (v/v) glycerol, 0.32 % (w/v) of the Pharmalytes™ pH 5-6 and pH 6.7-7.7 and the Servalyte™ pH 3-5, 0.64 % (w/v) of the Ampholine™ pH 5-7 and 0.8 % (w/v) of the Pharmalyte™ pH 4.2-4.9.

The gel solution was degassed and 1.5 % (w/v) of ammonium persulphate was added immediately prior to pouring the gel between the two treated plates which were clamped and taped together. The gel was allowed to polymerize overnight at room temperature and the plate treated with Repel-silane™ was removed from the gel immediately prior to IEF. IEF electrode strips one soaked in the anode electrode buffer (0.2 M acetic acid, 5 % (v/v) glycerol) and the other soaked in cathode buffer (0.2 M L-lysine, 5 % (v/v) glycerol) were placed on the IEF gel approximately 2 mm from the appropriate edges of the gel.

The gel was prefocused at 650 V, 23 mA and 11 W for 150 volthours (ECPS 3000/150 Power Supply Pharmacia Fine Chemicals. Uppsala, Sweden). Squares of Whatman No. 1 filter paper, approximately 2 mm by 7 mm in size, were placed 3 mm in front of the anodic electrode strip. Samples (2.5 μl) to be electrofocussed were then applied to these squares. The gel was then focussed at 650 V, 23 mA, 11 W for 3000 volthours.

The gels were stained using the one step staining technique of Blakesley and Boezi, (1977).

1.3.4 Statistical Analysis

The milk records were available for the years 1985-1997 and contained production data for each animal i.e. lactation length, lactation number and total and percent yield for protein and fat. While some animals were present in the herds for several years, others were present for a lesser number. The most recent four years data were taken for each animal. While there were initially 6,007 animals typed for κ-casein variant giving 21,078 records this reduced to 4,092 records after the following restrictions were applied: (1) the 1997 data were omitted as records were not complete at the time of statistical analysis, (2) records without lactation length details were omitted, (3) records with missing lactation numbers were omitted, (4) only records with lactation lengths between 230 and 330 days inclusive were used (5) animals calving between 1 January and 4 May for a particular year were designated as Spring calvers and animals calving between 1 September and 31 December were designated as Autumn calvers, animals which calved outside of these dates were omitted (6) only herds containing at least one

κ-casein BB variant cow were included. The data was divided into four sets, such that each animal occurred at most once in each set and the sets in turn were balanced for lactation number. The entire data was divided in this manner in order to ensure the statistical independence of the individual values in each set. The analysis which was performed using GENSTAT version 5 release 3 (Payne *et al.*, 1994), estimated the effects of the κ-casein phenotypes adjusted for the covariates, lactation number, herd, lactation length and calving date. Each of the four data sets was analysed separately, the model used in each case was

$$Y_{ijk} = \mu + X_i + Z_j + (XZ)_{ij} + H_k + \beta_1\gamma_{ijk} + \beta_2\delta_{ijk} + \varepsilon_{ijk}$$

where

Y_{ijk} = The response to the i^{th} κ-casein phenotype for the j^{th} lactation, the k^{th} herd, with lactation length γ_{ijk} and calving date δ_{ijk}

μ = Constant

X_i = Effect of the i^{th} κ-casein phenotype (i = AA, AB, BB)

Z_j = Effect of the j^{th} lactation (j = 1, 2-4, >4)

$(XZ)_{ij}$ = Effect of the interaction of the i^{th} κ-casein phenotype and the j^{th} lactation

H_k = Effect of the k^{th} herd (k = 1, 2, -------, 30)

β_1 = Effect of lactation length

β_2 = Effect of calving date

ε_{ijk} = Random error corresponding to Y_{ijk}

The statistical analyses were performed by Dr. D. Harrington, Statistics Department, Teagasc Headquarters, Dublin 4, Ireland.

1.4 Results

1.4.1 κ-Casein frequency distribution

In total, 6,007 animals from 138 herds were typed for κ-casein genetic variant using the IEF technique. Figure 1.1 shows a typical IEF gel run to resolve the A and B variants of κ-casein. The A and B alleles have isoelectric points between pH 5.43-5.81

and 5.54-6.12, respectively (Seibert *et al.*, 1985). Therefore, the B variant runs nearer the cathode. It is seen from Figure 1.1 that milk samples in lanes 26 and 30 have κ-casein AA phenotypes while samples in lanes 27, 29, 31, 32 and 33 have κ-casein AB phenotypes and the sample in lane 28 has a κ-casein BB phenotype. The phenotype distribution of cows typed was as follows: κ-casein AA, 53.07 %; κ-casein AB, 44.95 % and κ-casein BB, 1.98 %. No κ-casein C, D or E alleles were visualised by the IEF method used in the present study.

1.4.2 Statistical analysis

The κ-casein genetic variant frequency distribution and the lactation category are outlined in Table 1.1 for the Spring only (calving dates between 1st January-4th May) data and the combined Spring and Autumn (calving dates between 1st January-4th May and 1st September-31st December) data. In the Spring data there were 36 BB cows in each of the four data sets. The reason for the apparent discrepancies in the totals (i.e. Data set 1. 899; Data set 2. 903; Data set 3. 898; Data set 4. 904) is that some herds had cows which were Autumn calvers in some lactations and Spring calvers in other lactations. Therefore in restricting the Autumn and Spring data to Spring only calvers, some data sets lost cows whilst others did not. If the omission of cows with Autumn calving dates left a herd with no κ-casein BB cow then the κ-casein AA and AB records for cows in that herd were also omitted.

1.4.3 *Milk yield and compositional analysis*

1.4.3.1 Spring data

Table 1.2 summarizes the mean values for milk yield, milk constituent yields, milk composition and days in milk for the different κ-casein phenotypes in the four data sets of spring calving cows. No statistically significant differences ($P<0.05$) were found between κ-casein variant and milk yield and composition parameters in this data. However, a trend between κ-casein BB variant and greater milk yield was evident. In each of the data sets, κ-casein BB variant animals had numerically higher milk yield than κ-casein AA variant animals. Fat yield and fat concentration showed no distinct

trends between the different κ-casein variant phenotypes in the Spring data. κ-Casein BB variant was consistently numerically higher, in all four data sets, for protein yield compared to κ-casein AA or AB variant animals. κ-Casein BB variant animals had numerically longer days in milk than the κ-casein AA or AB animals in all four data sets.

1.4.3.2 Spring and Autumn data

Table 1.3 summarizes the mean values for milk yield, milk constituent yields, milk composition and days in milk for the different κ-casein phenotypes in the four data sets of Spring and Autumn calving cows. No statistically significant associations ($P<0.05$) were found between κ-casein variant and milk yield and composition parameters in the combined Spring and Autumn data. Furthermore, no distinctive numerical trends were seen in the four data sets for milk yield, fat yield, fat concentration, protein yield, protein concentration or days in milk with κ-casein variants.

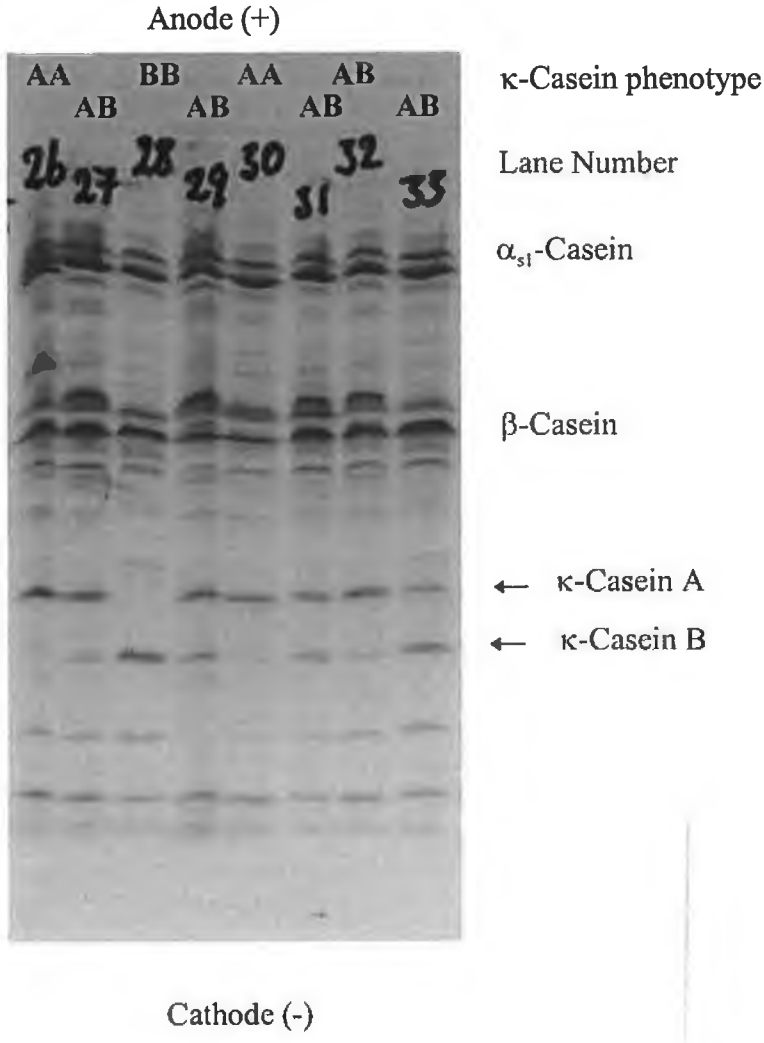

Figure 1.1 Example of a section of an IEF gel used to type κ-casein phenotypes from individual cow milk samples.

Lane No.	κ-Casein phenotype
26	AA
27	AB
28	BB
29	AB
30	AA
31	AB
32	AB
33	AB

Table 1.1 Summary of lactation category and κ-casein variant frequency distribution for four data sets of Spring and Spring and Autumn calving cows.

	Lactation category*	Spring κ-casein phenotype				Spring and Autumn κ-casein phenotype			
		AA	AB	BB	Total	AA	AB	BB	Total
Data set 1	1	81	81	6	168	86	90	6	182
	2-4	267	235	20	522	309	270	21	600
	>4	104	95	10	209	118	110	13	241
	Total	452	411	36	899	513	470	40	1023
Data set 2	1	67	61	2	130	81	73	2	156
	2-4	270	254	22	546	303	288	25	616
	>4	114	101	12	227	129	109	13	251
	Total	451	416	36	903	513	470	40	1023
Data set 3	1	75	65	8	148	88	75	8	171
	2-4	268	250	17	535	300	287	19	606
	>4	112	92	11	215	125	108	13	246
	Total	455	407	36	898	513	470	40	1023
Data set 4	1	73	63	5	141	85	76	6	167
	2-4	268	258	20	546	302	291	21	614
	>4	112	94	11	217	126	103	13	242
	Total	453	415	36	904	513	470	40	1023

*Lactation category: 1 = first lactation animals; 2-4 = animals within their second to fourth lactation inclusive; >4 = animals having a lactation number >4.

Table 1.2 Association between κ-casein genetic variant and milk production properties for Spring calving Holstein-Friesians

Data set		Phenotype AA	AB	BB
1	No. of animals	452	411	36
	Milk yield (kg)	5330 ± 36	5272 ± 38	5455 ± 127
	Fat yield (kg)	191 ± 1.45	188 ± 1.52	198 ± 5.07
	Fat (g/kg)	35.9 ± 0.2	35.8 ± 0.2	36.2 ± 0.5
	Protein yield (kg)	171 ± 1.18	170 ± 1.24	176 ± 4.12
	Protein (g/kg)	32.2 ± 0.1	32.3 ± 0.1	32.3 ± 0.3
	Days in Milk	280 ± 0.88	278 ± 0.92	285 ± 3.07
2	No. of animals	451	416	36
	Milk yield (kg)	5326 ± 37	5388 ± 38	5429 ±137
	Fat yield (kg)	190 ± 1.36	193 ± 1.42	195 ± 5.11
	Fat (g/kg)	35.9 ±0.2	35.9 ± 0.2	35.9 ±0.6
	Protein yield (kg)	171 ± 1.15	173 ±1.20	176 ± 4.31
	Protein (g/kg)	32.4 ± 0.1	32.3 ± 0.1	32.3 ± 0.3
	Days in Milk	279 ± 0.89	280 ± 0.93	281 ± 3.32
3	No. of animals	455	407	36
	Milk yield (kg)	5298 ± 38	5272 ± 40	5327 ± 137
	Fat yield (kg)	189 ± 1.44	188 ± 1.53	188 ± 5.24
	Fat (g/kg)	35.9 ± 0.2	35.7 ± 0.2	35.4 ± 0.5
	Protein yield (kg)	171 ± 1.22	170 ± 1.29	173 ± 4.42
	Protein (g/kg)	32.3 ±0.1	32.2 ± 0.1	32.5 ± 0.3
	Days in Milk	280 ± 0.87	280 ±0.92	285 ± 3.16
4	No. of animals	453	415	36
	Milk yield (kg)	5291 ± 37	5349 ± 39	5333 ± 130
	Fat yield (kg)	188 ± 1.46	190 ± 1.51	191 ± 5.10
	Fat (g/kg)	35.8 ± 0.2	35.7 ± 0.2	35.8 ± 0.6
	Protein yield (kg)	171 ± 1.19	171 ± 1.25	172 ± 4.20
	Protein (g/kg)	32.3 ± 0.1	32.1 ± 0.1	32.4 ± 0.3
	Days in Milk	281 ± 0.89	280 ± 0.93	284 ± 3.13

Data expressed as lactation means ± SEM

Table 1.3 Association between κ-casein genetic variant and milk production properties for combined Spring and Autumn calving Holstein Friesians

Data set		Phenotype AA	AB	BB
1	No. of animals	513	470	40
	Milk yield (kg)	5348±34	5306±36	5439±124
	Fat yield (kg)	191±1.40	190±1.46	195±5.03
	Fat (g/kg)	35.9±0.1	35.9±0.2	35.8±0.5
	Protein yield (kg)	171±1.12	171±1.17	175±4.04
	Protein (g/kg)	32.1±0.1	32.3±0.1	32.2±0.3
	Days in Milk	282±0.84	280±0.88	284±3.02
2	No. of animals	513	470	40
	Milk yield (kg)	5346±35	5383±37	5317±138
	Fat yield (kg)	191±1.33	193±1.40	189±5.23
	Fat (g/kg)	35.9±0.1	36.0±0.2	35.6±0.6
	Protein yield (kg)	172±1.11	173±1.16	171±4.34
	Protein (g/kg)	32.3±0.1	32.3±0.1	32.1±0.3
	Days in Milk	280±0.85	281±0.89	281±3.35
3	No. of animals	513	470	40
	Milk yield (kg)	5350±37	5296±38	5280±134
	Fat yield (kg)	191±1.40	189±1.46	187±5.12
	Fat (g/kg)	35.9±0.1	35.9±0.1	35.5±0.5
	Protein yield (kg)	172±1.18	170±1.23	171±4.32
	Protein (g/kg)	32.2±0.1	32.2±0.1	32.4±0.3
	Days in Milk	282±0.84	281±0.88	286±3.07
4	No. of animals	513	470	40
	Milk yield (kg)	5307±34	5345±36	5328±124
	Fat yield (kg)	189±1.35	191±1.41	192±4.87
	Fat (g/kg)	35.8±0.1	35.9±0.2	35.9±0.5
	Protein yield (kg)	171±1.11	171±1.16	171±4.01
	Protein (g/kg)	32.3±0.1	32.1±0.1	32.3±0.3
	Days in Milk	282±0.83	281±0.87	285±2.99

Data expressed as lactation means ± SEM

1.5 Discussion

1.5.1 κ-Casein frequency distribution

To date studies on the allelic frequency distribution of κ-casein variants in Holstein Friesians have been carried out in the U.K., Canada, Italy, USA, Holland, Ireland and Austria. The κ-casein allelic frequency distribution found in this study (A, 0.755; B, 0.245) is in excellent agreement with that found by Aleandri *et al.* (1990), (A, 0.75; B, 0.25); Ng-Kwai-Hang and Monardes (1990), (A, 0.75; B, 0.25) and O'Hara (1995), (A, 0.75; B, 0.25) for Holstein Friesians. The previous study on Irish Holstein-Friesians (O'Hara, 1995) was performed on 696 cows in the Teagasc, Moorepark herds. Other studies reported an even lower frequency distribution associated with the B allele. The B allele, was reported to have an allelic frequency distribution of 0.2 by Bovenhuis *et al.* (1992) while Aschaffenberg (1968), van Eenennaam and Medrano (1991) and Aleandri *et al.* (1997), found allelic distributions of 0.17, 0.18 and 0.15, respectively in Holstein-Friesians. Mayer *et al.* (1997*a*), in a study carried out in Austria reported the lowest frequency distribution of the B allele for Holstein-Friesians to date with a distribution of 0.09. These researchers also reported an allelic distribution of 0.054 for the E and 0.011 for the C allele of κ-casein. The reasons for the low frequency of occurrence of the B allele in Holstein-Friesians is still unclear. This low frequency of occurrence of the κ-casein BB phenotype may suggest a possible negative effect of the B allele on milk yield or milk composition. The present study, however shows that there were no such negative associations with this allele for Irish Holstein Friesians. It is worth noting that other breeds such as Jersey and Normandes have a high frequency of occurrence of the κ-casein BB phenotype (Woychik, 1965; Aschaffenburg, 1968; McLean *et al.*, 1984; Medrano and Sharrow, 1989; Bech and Kristiansen, 1990; Delacroix-Buchet *et al.*, 1993, Ng-Kwai-Hang and Kim, 1994; O'Hara, 1995; Mayer *et al.*, 1997*b*). It has been suggested that the κ-casein B allele in Holstein-Friesians may be linked to an undesirable locus (Lin *et al.*, 1992) and thereby in some way explain the low frequency of κ-casein BB phenotype in this breed. Further work is required in Holstein Friesians in order to elucidate the reason for the low frequency of occurrence of κ-casein BB phenotype.

1.5.2 Milk yield and composition

The results for the Spring data in this study are in agreement with O'Hara *et al.* (1994) who reported no statistically significant differences between the κ-casein variants (AA and AB) and milk yield and composition in Holstein Friesians. The trend in the present study of the association between κ-casein BB variant and higher milk yield is in agreement with the results found in the studies on Holsteins by Lin *et al.* (1986) and Aleandri *et al.* (1990). However, other Holstein studies (Bovenhuis *et al.*, 1992, Ron *et al.*, 1994), report κ-casein BB variants to be associated with a reduced milk yield when compared to κ-casein AA and AB variants. The trend found in all four groups in the present study, between κ-casein BB variant and higher protein yield for the Spring data, while not statistically significant, is in agreement with the findings of Ng-Kwai-Hang *et al.* (1984) and Aleandri *et al.* (1990). The combined Spring and Autumn data showed no such trends. κ-Casein BB variant was not included as a group in the data set in the study of O'Hara (1995) due to the lower number of animals phenotyped in that study. Therefore, it was not possible to compare the results from this study directly with those of O'Hara (1995) for κ-casein BB variant animals. However the lack of an association between κ-casein AA and AB variants and milk yield and composition for the Spring and Autumn data is in agreement with the results of O'Hara (1995).

The results in this study indicate that there are no detrimental effects, at least in respect to milk yield and composition, in increasing the frequency of the κ-casein B allele in the dairy herd as part of a targeted breeding programme.

CHAPTER 2

Cheddar cheesemaking and rennet coagulation characteristics of bovine milks containing κ–casein AA or BB genetic variants[1]

[1]This chapter was published in *Milchwissenschaft* (1995) **50**(9) 492-496

2.1 Summary

The rennet coagulation and pilot-scale Cheddar cheesemaking properties of Holstein-Friesian milks, having κ–casein AA\β-lactoglobulin AB or κ–casein BB\β-lactoglobulin AB variants, were investigated. The mean protein and fat levels in the κ–casein AA and κ–casein BB milks were 3.38% and 3.47%, and 3.96% and 3.96%, respectively. The κ–casein BB milks had shorter rennet coagulation times, faster curd firming rates and higher curd firmness (at 60 min) at both natural and adjusted (pH 6.60) pH values, than the κ–casein AA milks. Wheys from κ–casein AA milks had higher levels of fat and curd fines. There was a 12% increase in the percentage of fat recovered in the cheese made from κ–casein BB milk. The mean moisture adjusted cheese yields from κ–casein AA and κ–casein BB milks were 9.08 kg and 9.64 kg per 100 kg of protein-adjusted milk, respectively. The significantly increased cheese yield associated with κ–casein BB milk was predominately due to increased recovery of fat in the cheese.

2.2 Introduction

The association between genetic variants of milk protein and milk production and processing properties has been extensively reviewed (Jakob and Puhan, 1992; Lin *et al.*, 1992; Jakob, 1994; Ng-Kwai-Hang and Grosclaude, 1994). Cheesemaking involving Parmesan, Svecia, Edam and Gouda and rennet coagulation studies have reported higher cheese yields, shorter renneting times, faster rates of curd formation and higher curd firmness with the κ–casein BB variant (Mariani *et al.*, 1976; Morini *et al.*, 1979; Schaar, 1984; Pabst *et al.*, 1991; Van den Berg *et al.*, 1992; Delacroix-Buchet *et al.*, 1993; Macheboeuf *et al.*, 1993; Horne and Muir, 1994*a*)

Little information is available on the effect of κ–casein variants on Cheddar cheesemaking, apart from three reports on small-scale laboratory Cheddar cheesemaking experiments (Graham *et al.*, 1986; Marziali and Ng-Kwai-Hang, 1986*b*; Banks *et al.*, 1994). Much attention is currently focussed on the genetic selection of cows for the production of milks suited to the manufacture of specific products e.g.

cheese (Jakob, 1994). The objective of the current study was to investigate the rennet coagulation and Cheddar cheesemaking characteristics of bovine milks containing either κ–casein AA or BB variants.

2.3 Materials and Methods

2.3.1 Milk supply

Milk was collected from 14 mid-lactation, spring-calving, Holstein Friesian cows from the Moorepark herds. Seven cows had genotype AA for κ–casein and seven had genotype BB. Cows were divided into seven pairs matching each AA genotype cow with a corresponding BB genotype cow with respect to calving date, lactation number and milk yield. All were genotype AB for β-lactoglobulin. Milks from κ–casein AA or BB cows were separately collected over a 3 day period (given the low frequency occurrence of κ–casein BB phenotypes (Jakob and Puhan, 1992; Lin *et al.*, 1992; Jakob, 1994; Ng-Kwai-Hang and Grosclaude, 1994)), bulked and stored at 4°C. Aseptically taken milk samples from the bulked milks were tested twice daily during the collection period for total bacterial count (TBC) (International Dairy Federation Standard 100B, 1991*a*).

2.3.2 Manufacture of Cheddar cheese

Cheddar cheeses were made, in parallel, from bulked κ–casein AA and κ–casein BB milks on four separate occasions between the 24th of June and the 7th of July 1994. A total of 8 vats of Cheddar cheese were made (4 each for κ–casein AA and BB containing milks). Milks were standardised to a protein to fat ratio of 0.96:1.00. Milks (320-400 kg) were weighed to the nearest 0.01 kg into 500 l cheese vats and inoculated with 1.4% (v/v) of *Lactococcus lactis* subsp. *cremoris* 227 and *Lc. lactis* subsp. *cremoris* 303 (Chr. Hansen's Laboratory (Ireland) Ltd.). After a ripening period of thirty minutes, chymosin (Double Strength Chy-max, 50,000 MCU/ml, Pfizer Inc.,

Milwaukee, WI, USA) was added at a level of 0.07 ml/kg milk. Cheddar cheeses were made according to Guinee *et al.* (1994).

2.3.3 Sampling and mass balance

Representative samples (IDF Standard 50 B, 1985) from the milk in the vats were taken immediately prior to the addition of starter and analysed for composition and rennet coagulation properties (within 2 h).

Representative samples of the bulked pitching and cheddaring wheys from each vat were taken, preserved using sodium azide 0.02 % (w/v) and stored at 4°C prior to compositional analysis. Representative samples from 'White' whey expressed, during overnight pressing, from κ–casein AA and BB curds were collected, pooled, preserved (as above) and stored for subsequent analysis.

Following overnight pressing the cheeses were removed from the moulds, weighed, vacuum packed and stored at 4°C for 14 days at which time they were sampled (IDF Standard 50 B, 1985) for compositional analysis.

All milks, wheys (pitching, cheddaring and 'white' whey) and cheeses were weighed and used for the determination of moisture-adjusted cheese yield and recoveries of fat and protein.

2.3.4 Milk analyses

Fat, protein and lactose were determined by the Infra-red Milk Analyser (Milkoscan, Foss Electric, Hillerød, Denmark) which was calibrated by the IDF method (IDF Standard 29 1990).

A Formagraph instrument (Model 11700, Foss Electric, Hillerød, Denmark) (McMahon and Brown, 1982) was used to assess the rennet coagulation properties of standardised pasteurised (72°C, 15 s) milks with the different genetic variant milks. Milk (10 ml) was tempered for 30 min at 31°C and 66 μl of chymosin was added (1:100 dilution of double strength Chy-max; Pfizer Inc., Milwaukee, WI, USA). The renneted milk was incubated at 31°C and the coagulation properties monitored over a 60 min period. Milks were also adjusted to pH 6.60 before measurement in order to eliminate

the potential effects of different natural pH values on rennet coagulation properties (Schaar, 1984). The pH values were adjusted using lactic acid (0.5 N) except in the case of trial 3 where the κ–casein AA milk was adjusted using NaOH (0.1N).

2.3.5 Whey analyses

Whey samples were analysed for fat by the Röse-Gottlieb method (IDF Standard 22B, 1987), protein by the Kjeldahl method (IDF Standard 20 A, 1986) and fines by a modification of the NIZO method (Van der Berg *et al.*, 1973). In this modification the sediment was poured on to predried and weighed Whatman GF/F filter paper which was then dried and reweighed.

2.3.6 Cheese analyses

Cheese was analysed for pH (British Standard, 1976) moisture (IDF Standard 4A 1982), fat (IDF Standard 152 1991*b*), salt (IDF Standard 88A 1979) and protein (IDF Standard 20B 1993) at 14 days.

2.3.7 Statistical methods

Apart from the K_{20} and A_{60} values at natural pH (Table 2.2) which were analysed using Friedman's test, the rennet coagulation properties, composition, yield and recovery data (Tables 2.1, 2.2, 2.3, 2.4) were examined using the two-tailed paired t-test. The statistical analyses were performed by Dr. D. Harrington, Statistics Department, Teagasc Headquarters, Dublin 4, Ireland.

2.4 Results and Discussion

2.4.1 Microbiological analyses

The TBC's for all bulked milks following 3 days storage at 4°C were <80,000 cfu/ml except for cheese trial 3 (κ–casein AA) which had 342,000 cfu/ml prior to pasteurisation.

2.4.2 Milk composition

The composition of the raw milks is given in Table 2.1. No significant difference (P = 0.98) was observed in the fat content between κ–casein AA and κ–casein BB milks. These results are in general agreement with those of Jakob (1994). Similar to the findings of others (Van den Berg *et al.*, 1992; Jakob, 1994; Ng-Kwai-Hang and Grosclaude, 1994), the protein content of the κ–casein AA and BB milks were numerically different, with the latter displaying a higher protein percentage (Table 2.1). However the difference was not significant (P = 0.07).

2.4.3 Rennet coagulation properties

The effects of the κ–casein variants on the rennet coagulation properties of standardised, pasteurised milks at both natural and adjusted pH (i.e. pH 6.60) values are shown in Table 2.2. The rennet coagulation times (RCTs) were longer for κ–casein AA than κ–casein BB at both natural (P = 0.05) and adjusted (P = 0.004) pH. These observations are in agreement with those of others for raw milk at natural (Jakob and Puhan, 1992) and adjusted (O'Hara *et al.*, 1994) pH values. Moreover, the RCT values for pH adjusted raw (data not shown) and pasteurised milks were generally shorter than those of the corresponding non-pH adjusted milks. When studying the effect of κ–casein variant on the RCT, it is important to eliminate the effect of differences in natural pH on chymosin activity (Imafidon and Farkye, 1994) by adjusting to a common pH value (e.g. pH 6.60, as in this study). Otherwise the effect of genetic variant *per se* on RCT may be obscured.

At both natural and adjusted pH (Table 2.2), the rate of curd firming (K_{20}) of milks with κ–casein AA was slower than in milks with the κ–casein BB variant (i.e. $P<0.05$ and $P=0.002$ respectively). Reflecting the trends in the K_{20} values, the curd firmness values (i.e. A_{60}) were lower for κ–casein AA than κ–casein BB milks at both natural ($P<0.05$) and adjusted pH ($P=0.001$) values. In agreement with the Formagraph results, it was noted that the κ–casein AA milks required a longer time than the corresponding κ–casein BB milks to reach a similar curd cutting firmness (i.e. ~30 min vs. 60 min) during pilot-scale Cheddar cheesemaking trials. The faster curd firming rates and higher curd firmness values of the κ–casein BB milks, also observed by others (Jakob and Puhan, 1992), may be associated with improved cheesemaking (Chapman and Burnett, 1972; Banks and Muir, 1984).

Table 2.1 Composition of bovine milks containing κ–casein AA or BB genetic variants

Composition	Genetic Variant			
	AA	BB	SED	Significance
Fat %				
Trial 1	3.79	4.02		
Trial 2	4.13	4.07		
Trial 3	3.88	3.99		
Trial 4	4.03	3.79		
Mean	3.96	3.96	0.11	NS
Protein %				
Trial 1	3.41	3.47		
Trial 2	3.37	3.47		
Trial 3	3.31	3.48		
Trial 4	3.42	3.44		
Mean	3.38	3.47	0.03	NS

SED, Standard error of difference; NS, not significant ($P > 0.05$)

Table 2.2 Rennet coagulation properties of milk with κ–casein AA or BB genetic variants at natural and adjusted (pH = 6.60) pH value

pH	Genetic Variant	RCT (min)	K_{20} (min)	A_{60} (mm)
		(Mean)	(Median)	(Median)
Natural	AA	42.10	>60	5.25
	BB	22.07	8.87	48.50
SED		6.59	-	-
Significance		*	*	*
		(Mean)	(Mean)	(Mean)
Adjusted	AA	20.25	13.25	38.33
(pH 6.60)	BB	16.71	6.00	54.50
SED		0.23	0.36	0.55
Significance		**	**	***

The K_{20} and A_{60} values at natural pH were analysed using Friedman's test, medians are presented for this data as an indicator of the central location.
Significance levels: ***, P<0.001; **, P<0.01; *, P<0.05;

2.4.4 Whey composition

The mean levels of fat and fines in the κ–casein BB wheys were lower (P=0.001 and 0.003 respectively) than those in the κ–casein AA wheys (Table 2.3); this trend was observed for all trials. The increased levels of fines in the Cheddar cheese wheys form κ–casein AA milks is in agreement with results found for other cheese varieties, such as Gouda (Van den Berg *et al.,* 1992). The κ–casein BB wheys had higher (P=0.009) protein concentrations than the κ–casein AA wheys. However, the level of milk protein in the whey, expressed as a percentage of the milk protein available for incorporation into cheese, was not significantly influenced (P=0.427) by the κ–casein variant. This suggests that the higher protein levels in the cheese wheys from κ–casein BB milks are due to the numerically higher protein levels of the κ–casein BB milk (Table 2.1). The level of fat in the whey, expressed as a percentage of the weight of milk fat, was lower (P=0.004) in the κ–casein BB whey.

2.4.5 Cheese composition and yield

The composition and yields for all cheeses are shown in Table 2.4. There were no significant differences between the cheeses made from κ–casein AA or κ–casein BB milks for pH, moisture, protein, salt in moisture (S/M) and moisture in non fat substances (MNFS). These results agree with those of Graham *et al.* (1986), who found no significant differences in the moisture content between Cheddar cheeses made from κ–casein AA or κ–casein BB milks. However the mean fat in dry matter (FDM) value for cheeses made from κ–casein BB milks was higher than those made from the κ–casein AA milks - a trend observed in each trial. κ–Casein BB milk gave a higher (P=0.002) moisture adjusted Cheddar cheese yield than the corresponding κ–casein AA milk in each trial with a mean values of 9.64 vs. 9.08 kg per 100kg of κ–casein BB and κ–casein AA milks, respectively. The mean level of milk protein recovered in cheese was not (P=0.401) influenced by the κ–casein variant. However, the mean level of fat recovered in the cheese from κ–casein BB milks was higher (P=0.003) than that in the cheeses from κ–casein AA milks (Table 2.4). The composition of all cheeses from κ–casein AA or κ–casein BB milks was within the range for good quality Cheddar

Table 2.3 Composition of Cheddar cheese whey from milks with κ-casein AA and BB genetic variants

Parameter	Genetic Variant AA	BB	SED	Significance
Fat level (% w/w)	0.53	0.26	0.02	***
Protein level (% w/w)	0.88	0.93	0.01	**
Fines (mg/kg)	218.30	100.60	12.60	**
Fat (% of milk fat)	13.69	7.34	0.76	**
Protein (% of milk protein)	25.98	26.16	0.20	NS

Cheese whey consists of pitching whey and whey expressed during cheddaring prior to milling.
Values given are means for four separate cheesemaking trials.
Significance levels: ***, P<0.001; **, P<0.01; NS, not significant
SED, standard error of difference

Table 2.4 Composition, yield and recoveries of fat and protein in Cheddar cheese made from κ–casein AA or BB genetic variant milks

	Genetic Variant				
	AA	BB	SED	CV	Significance
A. Composition					
pH	5.18	5.22	0.03	0.9	NS
Moisture %	39.23	38.12	0.55	2.0	NS
Protein %	24.91	24.39	0.50	2.8	NS
FDM %	50.68	52.61	0.45	1.2	*
S/M %	4.86	5.01	0.20	5.8	NS
MNFS %	56.68	56.50	0.80	2.0	NS
B. Moisture adjusted cheese yield (kg/100 kg milk)	9.08	9.64	0.05	0.8	**
C. Recoveries of milk fat and milk protein in cheese (% of level in milk)					
Fat	77.94	89.93	1.34	2.3	**
Protein	76.01	77.51	1.57	2.9	NS

Values given are means for four separate cheesemaking trials.
SED, standard error of difference; CV, coefficient of variation; FDM; fat in dry matter; S/M; salt in moisture; MNFS; moisture non fat substances.
Moisture adjusted cheese yield expressed as kg of cheese (moisture adjusted to 38% in all cases for comparison purposes) per 100 kg of milk (protein adjusted to 3.12% in all cases for comparison purposes).
Significance levels: **, $P<0.01$; *, $P<0.05$; NS, not significant($P>0.05$)

(Lawrence *et al.*, 1993). However, the FDM level was higher in the cheeses from the κ–casein BB milk, a result which may be attributed partly to the lower fat losses in the κ–casein BB cheese wheys. The absence of significant differences in most composition parameters suggest that Cheddar cheeses of similar quality may be obtained from κ–casein AA or κ–casein BB milks. Further investigations are currently being undertaken to establish the effect of κ–casein variant on the quality and maturation of Cheddar cheese during ripening.

The present pilot-scale study found an increase in moisture adjusted Cheddar cheese yield of 6.2% associated with the κ–casein BB variant compared to the κ–casein AA variant. In line with the reported cheese yield increases (3.0 - 5.0 %) in laboratory scale Cheddar cheesemaking studies (Marziali and Ng-Kwai-Hang, 1986*b*; Graham *et al.*, 1986; Banks *et al.*, 1994), the significantly increased Cheddar cheese yield (i.e. 6.2 %) associated with κ–casein BB milk, as reported in this study, can be at least partly attributed to the increased fat recovery in the cheese and the reduced loss of fines in the whey.

Given that the percentage increase in cheese yield found in this pilot-scale study can be carried through to commercial scale Cheddar production, it is estimated that a Cheddar plant with an annual output of 20,000 tonnes could produce up to 1,240 tonnes more cheese from κ–casein BB variant milk than from κ–casein AA variant milk. Furthermore the reduced curd setting time required for κ–casein BB variant milk (i.e. up to 50% reduction) could potentially result in a higher plant output or reduce the cheese vat capacity requirements for a given milk throughput.

Owing to the marked influence of κ–casein variant on cheese yield as demonstrated in this study and others (Schaar, 1984; Pabst *et al.*, 1991; Jakob and Puhan, 1992; Van den Berg *et al.*, 1992; Horne and Muir, 1994*a*; Ng-Kwai-Hang and Grosclaude, 1994), further work will focus on the potential of varying the processing conditions in order to alter the differences in coagulation properties and cheese yield of milks with different κ–casein variants (including κ–casein AB variant milks).

Chapter 3

Ripening characteristics of Cheddar cheese made from bovine milks containing κ–casein AA or BB genetic variants[1]

[1]This chapter was accepted for publication (19th August 1998) in *Milchwissenschaft*.

3.1 Summary

The ripening characteristics of Cheddar cheeses made from κ-casein AA/β-lactoglobulin AB and κ-casein BB/β-lactoglobulin AB genetic variant milks were investigated over a 274 day period. κ-Casein variant had no significant effect ($P>0.05$) on primary and secondary proteolysis, as monitored by nitrogen solubility at pH 4.6 or in 5 % phosphotungstic acid, and total levels of free amino acids. Moreover, the profile of proteolysis products was not influenced by κ–casein variant, as indicated by the generally similar peptide molecular weight distribution profiles in cheeses from the κ–casein AA or BB milks throughout the 274 day ripening period. Some amino acids, however, (i.e. methionine, leucine, phenylalanine, histidine and lysine) were significantly ($P<0.05$) higher in κ-casein BB cheese compared to κ-casein AA cheese at 274 d ripening. κ–Casein variant had no significant effect on the rheological (fracture stress and firmness) characteristics or the grading scores awarded for flavour/aroma and body/texture.

3.2. Introduction

Extensive research has been undertaken on the effect of κ–casein variant on the suitability of milk for cheesemaking (Mariani *et al.*, 1976; Morini *et al.*, 1979; Schaar *et al.*, 1985; Aleandri *et al.*, 1990; Jakob and Puhan, 1992; Van den Berg *et al.*, 1992; Delacroix-Buchet *et al.*, 1993; Vink *et al.*, 1993; Banks *et al.*, 1994; Ng-Kwai-Hang and Grosclaude 1994; Walsh *et al.*, 1995; Horne *et al.*, 1996; Lodes *et al.*, 1996*b*; Walsh *et al.*, 1998). It is generally agreed that κ–casein BB variant milk is associated with enhanced rennet coagulation properties and increased cheese yields for a range of cheese varieties including Cheddar, Gouda, Parmesan and Mozzarella. These characteristics have been attributed, in part, to the generally higher overall casein level in κ–casein BB milks (Jakob and Puhan, 1992; Ng-Kwai-Hang and Grosclaude, 1994; Walsh *et al.*, 1995) and/or to other factors including differences in κ–casein concentration, and micelle size and structure. Flavour development in Cheddar cheese is affected by the rate and extent of proteolysis during ripening. It is therefore important to ascertain what

effects, if any, the variants may have on the ripening process. While Imafidon *et al.* (1993) reported differences in electrophoretic patterns and reversed-phase HPLC profiles in Cheddar cheeses made from κ–casein AA or AB milks, relatively little information is available on the comparative effects of different κ–casein variants on the changes in cheese during ripening.

The objective of the current study was to compare the proteolysis, rheology and sensory characteristics of Cheddar cheeses made from κ–casein AA or BB milks. The effects of κ–casein variant on rennet coagulation and Cheddar cheesemaking characteristics have been reported earlier (Walsh *et al.*, 1995).

3.3. Materials and Methods

3.3.1 Cheddar cheese manufacture/ripening conditions

The Cheddar cheeses used in this study were those which were described previously by Walsh *et al.* (1995); the cheeses were produced at pilot scale from bulked κ–casein AA and κ–casein BB milks, in parallel, on four separate occasions over a 17 d period in mid-lactation. All milks used for cheese manufacture were phenotype AB for β-lactoglobulin. Cheeses were ripened at 4°C for 30 d and then at 8°C for a further 240 d.

3.3.2 Cheese proteolysis

Proteolysis during ripening was monitored by measuring the percentage of total nitrogen (TN) soluble in water at pH 4.6 (pH4.6SN) and in phosphotungstic acid (50 g/l, PTA-N) as described by Walsh *et al.* (1998).

Individual free amino acids (FAA) in cheeses ripened for 91, 183 and 274 d were determined in 12% TCA filtrates prepared from the pH4.6SN as described by Wilkinson *et al.* (1992). Filtrates were analysed using a Beckman 6300 Analyser (Beckman, High Wycombe, Bucks, UK). FAA concentrations were expressed as mg/kg of cheese.

The pH4.6SN extracts of the cheeses ripened for 91, 183 and 274 d were analysed by gel permeation fast protein liquid chromatography (FPLC) as described by Wilkinson *et al.* (1992). Samples of the pH4.6SN extract (1.5 ml) were centrifuged in Eppendorf tubes at 13,000g for 5 min (Micro Centaur, MSE, Sussex, U.K.), diluted (1:10) with 0.1 mol/l Tris-HCl pH 7.0 containing 0.1 mol/l NaCl and 10 mmol/l $NaNO_3$, filtered through a 0.45-μm Millipore filter (Millipore, Bedford, MA, USA) and applied to a Superose-12 gel permeation column (Pharmacia LKB Biotechnology Ltd., Uppsala, Sweden).

3.3.3 Rheological measurements

Cylindrical samples (diam, 30 mm; height, 29 mm) tempered at 8°C for 4 h prior to testing, were compressed on a Model 112 Universal Testing Machine (Instron LTD, High Wycombe, U.K.), at a cross head speed of 50 mm min^{-1}, using a 50 kgf load cell. The forces required to fracture the cheese cylinders (fracture stress) and compress to 30 % of their original height (firmness) were measured as described by Guinee *et al.* (1996*a*). Six replicate measurements were made. The coefficients of variation for measurements on firmness and fracture stress were 6.8 and 8.1 %, respectively.

3.3.4 Sensory analysis

Cheeses were graded by:

(a) a 10-member in-house Cheddar panel after 91, 183, and 274 days storage at 8°C. Panelists were selected on their abilities to (i) discriminate between the four taste sensations (sweet, sour, salty and bitter) and different intensities of each of these taste sensations and (ii) to discriminate between cheeses with different flavour and texture characteristics (as identified during training of panelists using the descriptive analysis sensory method (Stone *et al.*, 1974)). The cheeses were presented to the panelists at room temperature in individual tasting booths. Samples were presented in a random fashion to avoid consistently high scoring for any one sample. The cheese samples were of a consistent size and no more than four samples were tasted at any one sitting. Non-sparkling mineral water was provided and panelists were instructed to rinse their mouths

between samples. Panelists were asked to score the cheeses for "acceptability". Each panelist was given a sheet of paper on which were drawn a series of 8 cm lines anchored at the extremes by the values 0 (reject) and 8 (excellent). For each cheese the panellist placed a vertical stroke on the line; the distances (from zero) were then measured to determine the acceptability score.

(b) a commercial grader from a local Cheddar factory after 6 and 9 months ripening. Maximum scores for flavour/aroma and body/texture were 45 and 40, respectively. For cheeses to be described as commercial grade, minimum scores of 38 and 32, respectively, are required.

3.3.5 Statistical Analysis

Results of the analyses from the 4 replicate trials for the two κ–casein variants were compared using the two-tailed paired t-test. In the results and discussion that follow effects described as significant were at least $P < 0.05$. The statistical analyses were performed by Dr. D. Harrington, Statistics Department, Teagasc Headquarters, Dublin 4, Ireland.

3.4 Results and Discussion

The compositions of Cheddar cheeses made from κ-casein AA or BB variant milks have been previously reported by Walsh *et al.* (1995). While cheese composition from either κ-casein variant were generally similar, κ-casein AA variant cheese was shown to have a significantly lower level of fat in dry matter and a numerically higher moisture level.

3.4.1 Cheese ripening

The formation of pH4.6SN or PTA-N was not significantly influenced by κ-casein variant throughout the 274 d ripening period (Fig. 3.1). This trend is in agreement with the observations of Imafidon *et al.* (1993), for Cheddar cheese, and Van den Berg *et al.*

(1992), for Gouda cheese. However Mayer *et al.* (1997*a*) found that 8 wk old Edam cheese made with κ–casein variant AA milk had higher levels of water soluble N than Edam made from κ–casein BB milk.

The total concentration of FAA increased significantly with ripening time (Table 3.1), with the levels at 180 d being typical for Cheddar cheese (Wilkinson *et al.*, 1992). κ-Casein variant had no significant effect on the total FAA levels at ripening times ≤ 183 d. This result is in agreement with that for PTA-N, a trend expected as the PTA-N fraction is comprised mainly of very low molecular weight peptides (i.e. < 0.64 kDa) and FAA (Jarrett *et al.*, 1982). However, at Day 274 the total FAA level in κ–casein BB cheese was significantly higher ($P<0.05$) than in the κ-casein AA cheese. κ–Casein variant did not significantly influence the concentration of individual amino acids except at Day 274 when methionine, leucine, phenylalanine, histidine and lysine were significantly higher ($P<0.05$) in the κ-casein BB variant cheese (Fig. 3.2). At each ripening period tested the principle free amino acids in order of descending concentration were glutamic acid ~ leucine > phenylalanine > valine > serine ~ lysine.

The molecular weight (MW) distribution of peptides in pH4.6SN extracts from κ–casein AA and BB cheeses are shown in Fig. 3.3. Ripening was accompanied by a decrease in the concentration of high MW (> 25 kDa), and an increase in the concentration of lower MW (10-25 kDa, 5-10 kDa and <1 kDa) peptides. The concentration of peptide material between 1-5 kDa changed little over the 274 d ripening period (data not shown). The decrease in the concentrations of high MW and the increase in lower MW material during proteolysis in Cheddar cheese reflects the sequential hydrolysis of large MW peptides to peptides of lower MW and FAA's (Altemueller and Rosenberg, 1996; Fox *et al.*, 1996). As seen in the case of pH4.6SN and PTA-N, κ–casein variant also had no significant effect on the MW distribution of components in the pH4.6SN extracts.

Table 3.1 Total free amino acid (FAA) concentrations in Cheddar cheese made from κ-casein AA or BB genetic variant milks during ripening at 8°C.

Ripening time (d)	Genetic Variant AA	BB	SED	Significance
	Total [FAA] (mg/kg)			
91	1985	2400	257	NS
183	4856	5133	298	NS
274	7403	8629	344	*

Values used are means for four separate cheesemaking trials.

SED: Standard error of difference

Significance: NS, not significant ($P > 0.05$); * significant ($P < 0.05$)

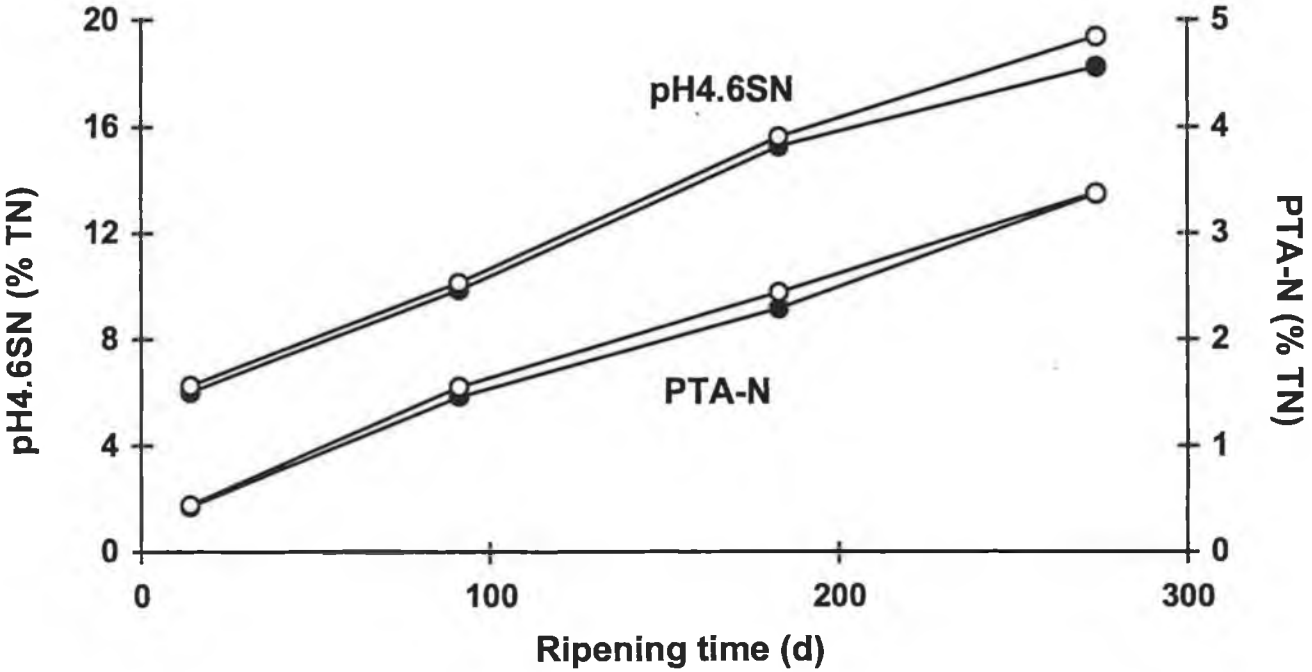

Figure 3.1 Nitrogen soluble in water at pH 4.6 (pH4.6SN) and phosphotungstic acid-soluble nitrogen (PTA-N) levels as a percentage of total nitrogen (% TN) in κ–casein AA and BB genetic variant Cheddar cheeses during ripening (κ–casein AA, ●; κ–casein BB, ○).

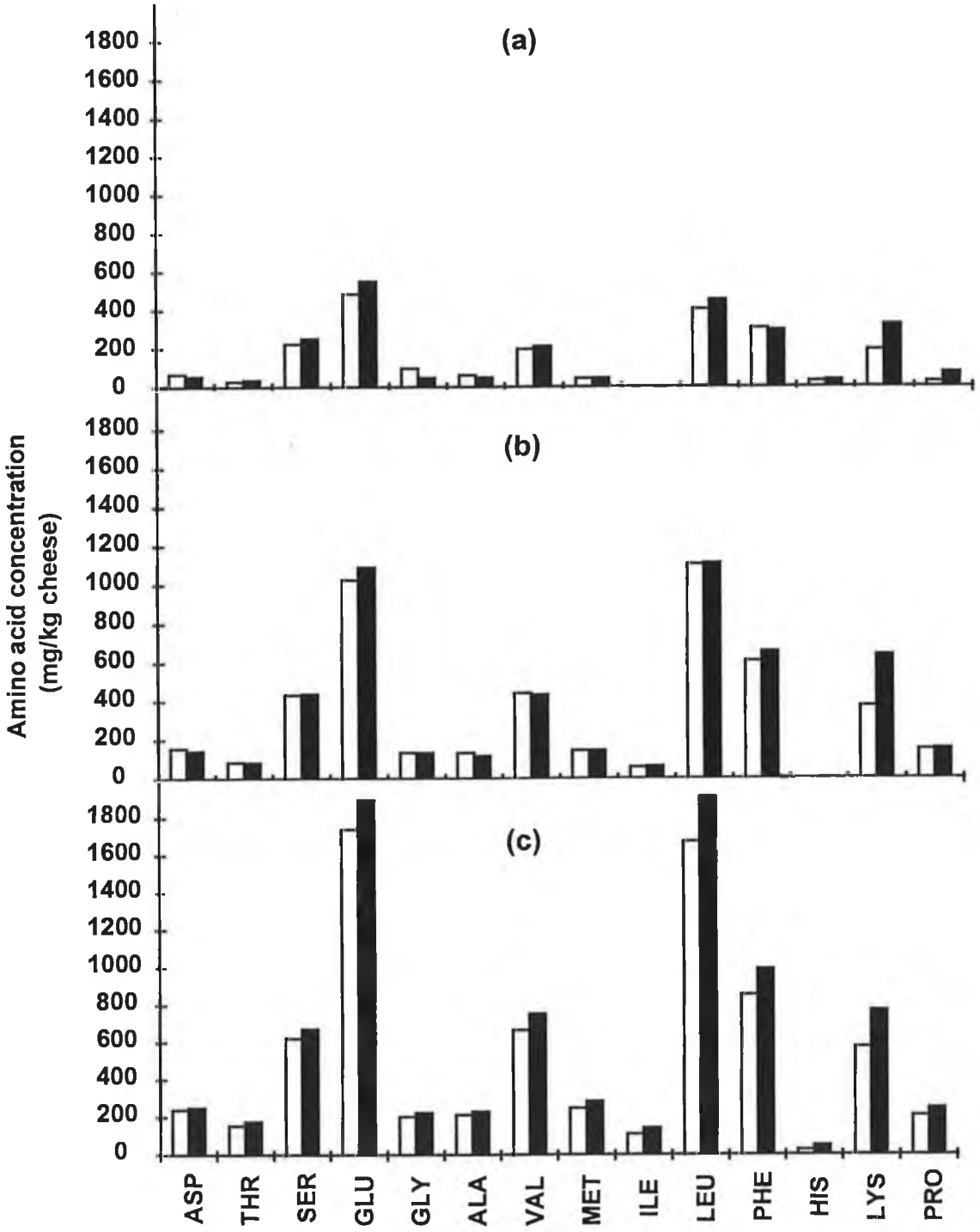

Figure 3.2 Concentration of individual free amino acids in κ–casein AA (□) and BB (■) variant Cheddar cheeses at (a) 91, (b) 183 and (c) 274 d ripening at 8°C.

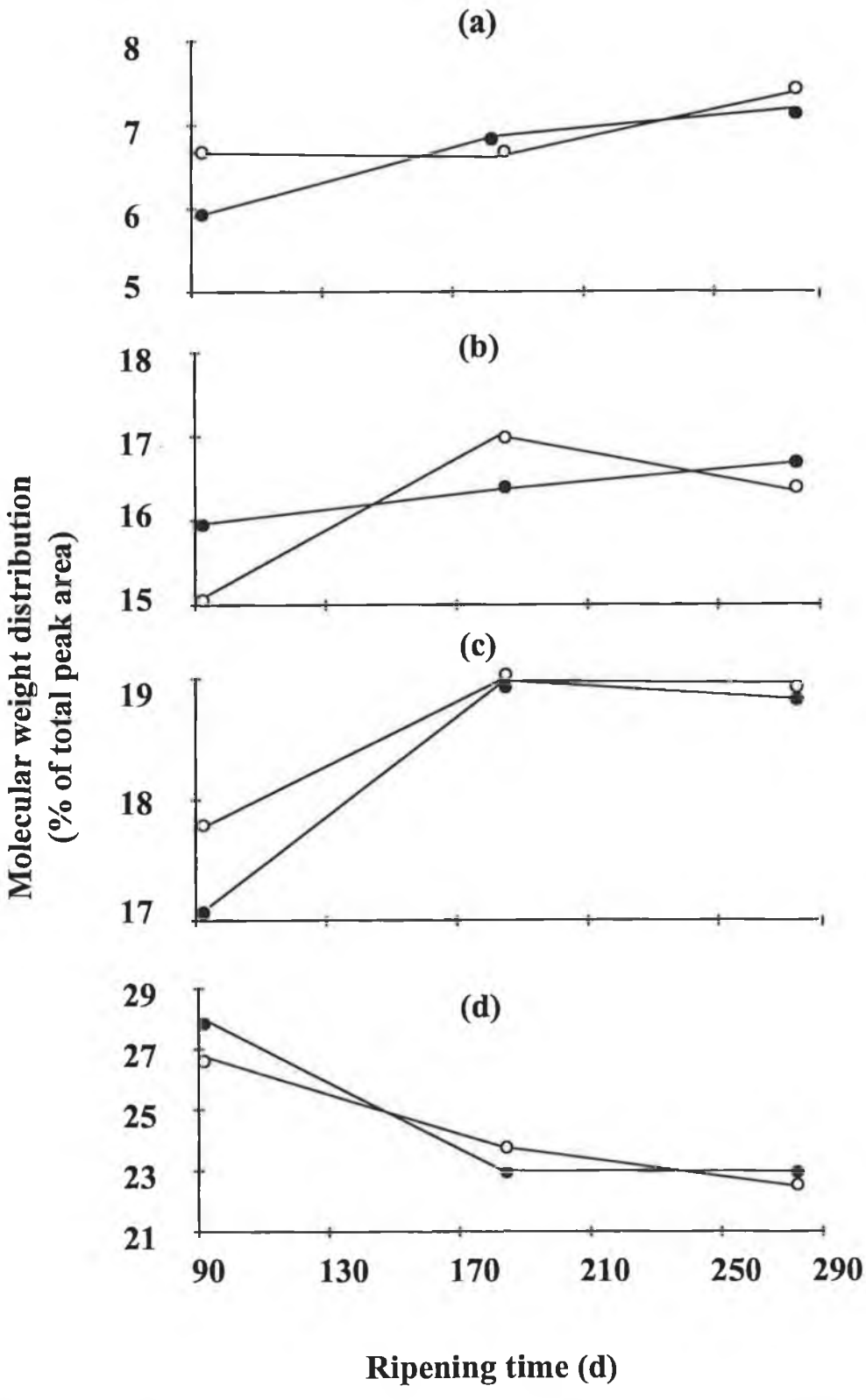

Figure 3.3 Summary of molecular weight distribution of peptides, (a) <1 kDa, (b) 5-10 kDa, (c) 10-25 kDa, (d) > 25 kDa, as obtained at 214 nm for water soluble extracts of κ–casein AA (●) and BB (○) Cheddar cheeses during ripening at 8°C.

3.4.2 Rheological Analysis

Firmness and fracture stress in all cheeses decreased dramatically between 91 and 183 d and thereafter changed little (Fig. 3.4). This trend correlated well with the increase in primary proteolysis as reflected by the levels of pH4.6SN (Fig. 3.1). Firmness and fracture stress were not significantly influenced by κ–casein variant, a trend expected because of the general absence of significant differences in composition (Walsh *et al.*, 1995) and primary proteolysis between the cheeses made from the κ–casein AA or BB milks (FitzGerald, 1997).

3.4.3 Sensory Analysis

The scores awarded, by a commercial grader, for flavour/aroma and body/texture indicated no significant differences between the cheeses made from κ–casein AA or BB milk after 183 and 274 d ripening (Table 3.2). These trends agree with those showing an absence of significant differences in proteolysis and rheological characteristics between the different κ-casein variant cheeses. Likewise, no acceptability differences were observed using an in-house grading of the cheeses during ripening (Table 3.2).

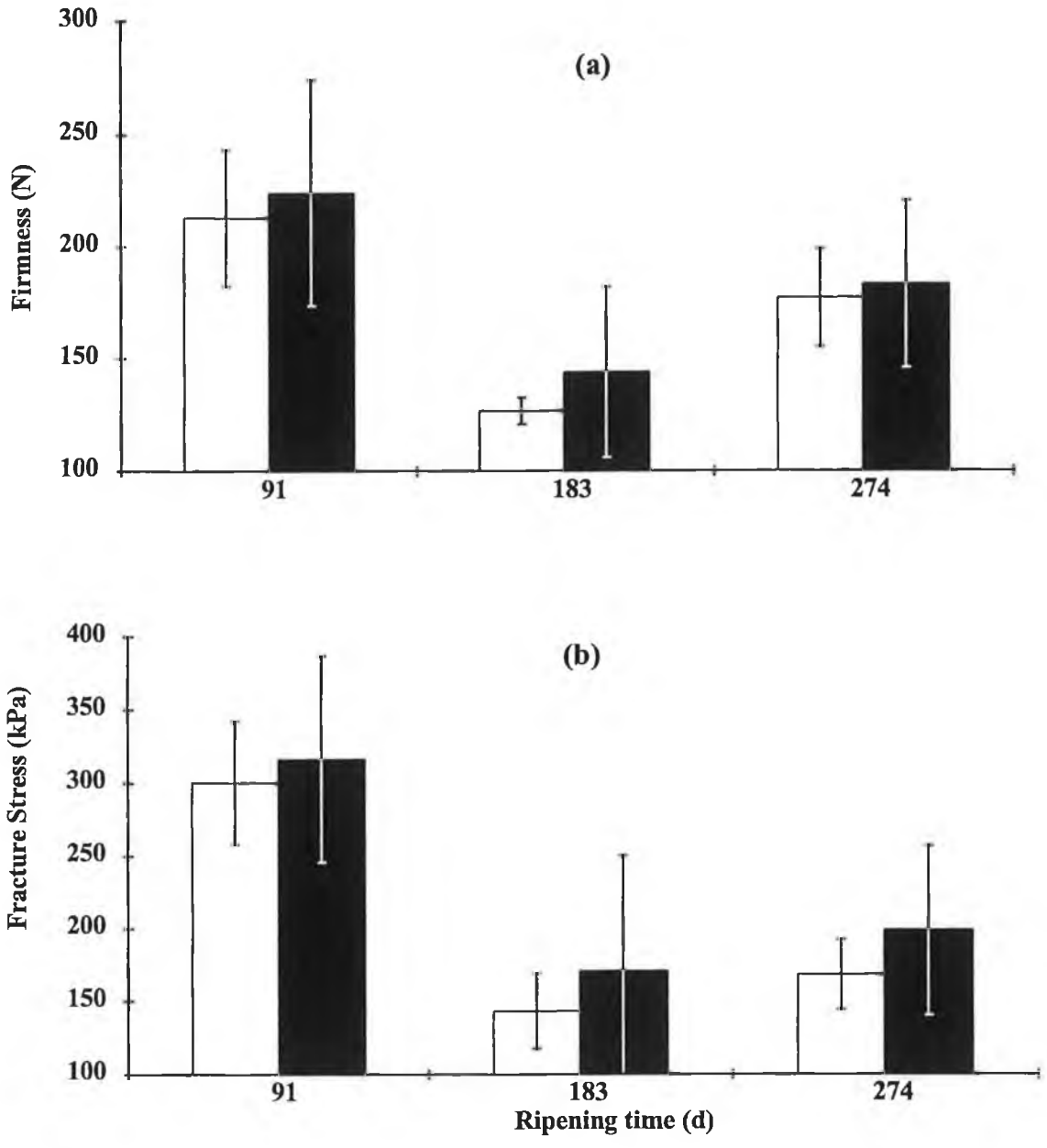

Figure 3.4 Influence of κ-casein AA and BB variant on the force required to fracture Cheddar cheese during ripening at 8°C (κ-Casein AA, ❑; κ-Casein BB, ■).

Table 3.2 Commercial and in-house grading scores for Cheddar cheeses made from κ-casein AA and BB variant milks

Characteristic	Age (d)	κ-Casein Variant AA	κ-Casein Variant BB	SED	Significance
Commercial Grading					
Flavour/Aroma	183	39.00	39.00	0	NS
	274	39.00	38.00	0.41	NS
Body/Texture	183	33.00	32.50	0.65	NS
	274	32.50	32.25	0.63	NS
In-house Grading					
Acceptability	91	4.77	4.63	0.12	NS
	183	4.88	4.65	0.15	NS
	274	5.39	5.18	0.23	NS

Values used are means for four separate cheesemaking trials.

SED: Standard error of difference

NS: not significant ($P>0.05$)

3.5 Conclusions

Cheddar cheeses made from κ–casein AA or κ–casein BB milks resulted in no significant differences in proteolysis (pH4.6SN and total [FAA]), rheology and sensory characteristics over a 274 day ripening period. The absence of κ–casein variant-related differences in maturation characteristics for Cheddar cheese indicates that selective breeding to increase the proportion of κ–casein BB variant may prove worthwhile for increasing cheese yield (Walsh *et al.*, 1995; FitzGerald, 1997).

Chapter 4

Influence of κ–casein genetic variant on rennet gel microstructure, Cheddar cheesemaking properties and casein micelle size[1]

[1] This chapter was published in the *International Dairy Journal* (1998) **8** 707-714.

4.1 Summary

Cheddar cheese was manufactured on three separate occasions over a two week period from milk collected from two mid-lactation, spring-calving, Holstein-Friesian herds (n=11) containing similar casein levels, having phenotype AA or BB for κ–casein genetic variant. κ–Casein variant did not significantly ($P > 0.05$) influence the casein content or gross composition of milk. κ–Casein BB milk had significantly smaller average casein micelle diameter and superior rennet coagulation properties than that of the AA milk. Pilot-scale Cheddar cheesemaking studies showed that the κ–casein BB milk resulted in significantly higher fat recoveries into cheese and higher actual and moisture-adjusted cheese yields. Cheese produced from κ–casein BB variant milk had higher concentrations of fat and lower protein levels than that produced from the AA variant. κ–Casein variant had no significant effect on proteolysis or on the acceptability scores awarded to the cheeses.

4.2 Introduction

Bovine milks containing the BB variant of κ–casein have been associated with improved rennet coagulation properties (Van den Berg *et al.*, 1992; Ng-Kwai-Hang and Grosclaude, 1994; Walsh *et al.*, 1995; Lodes *et al.*, 1996*b*) and increased yields for a range of cheese varieties (Mariani *et al.*, 1976; Morini *et al.*, 1979; Banks *et al.*, 1994; Walsh *et al.*, 1995; Walsh *et al.*, 1998) when compared to milks containing the AA variant. These characteristics have, in part, been attributed to the generally higher overall casein levels associated with κ–casein BB milks (Jakob and Puhan, 1992; Ng-Kwai-Hang and Grosclaude, 1994; Walsh *et al.*, 1995). However, other factors such as differences in κ–casein concentration, variations in micelle size/structure (van Eenennaam and Medrano, 1991; Lodes *et al.*, 1996*a*) and the ensuing differences in the structure and/or molecular properties of the curd may also contribute to the observed variations in cheese yield and rennet coagulation characteristics with the different κ–casein variants.

However to our knowledge there is little documented evidence, apart from that of Schaar *et al.* (1985), on the rennet coagulation and cheesemaking characteristics of κ–casein AA and BB milks containing similar casein levels. The objective of the current study was therefore to compare the rennet gelation and Cheddar cheesemaking properties of κ–casein AA and BB milks containing similar casein levels and cut at similar curd strengths .

4.3 Materials and Methods

4.3.1 Milk supply

Milk was collected from 22 mid-lactation, spring-calving, Holstein Friesian cows from the Moorepark herds. Eleven cows had κ–casein genotype AA and eleven had genotype BB. Cows were matched between the herds with respect to calving date, lactation number, milk yield and β–lactoglobulin variant. Milks from the κ–casein AA or BB herds were separately collected over a 3 day period (given the low frequency of occurrence of κ–casein BB phenotypes), on three separate occasions, when the cows were between 110 and 130 d in lactation. The bulked milks were stored at 4°C over the 3 day collection period and tested daily for total bacterial count (IDF, 1991*a*).

Three replicate trials were undertaken over an 8-day period in mid-lactation (between 10th and 20th August, 1996) in order to minimize the potential effects of stage of lactation and/or changes in diet quality on milk composition and cheese characteristics (Lucey *et al.*, 1992; Auldist *et al.*, 1996; O'Brien *et al.*, 1997; Guinee *et al.*, 1998*b*). In each trial the κ–casein AA and BB milks were analyzed for composition, casein micelle size, rennet gel microstructure and Cheddar cheesemaking characteristics.

4.3.2 Manufacture of Cheddar cheese

Cheddar cheeses were made, in parallel, from bulked κ–casein AA and κ–casein BB milks on three separate occasions between the 13th and 20th of August, 1996. Milks

(~360 kg) were standardised to a casein to fat ratio of 0.73:1.00, pasteurised (72°C x 15 sec), cooled to 31°C and inoculated with bulk starter culture consisting of *Lactococcus lactis* subsp *cremoris* 227 and *Lc. lactis* subsp. *cremoris* 303 at a rate of 1.5 kg/100 kg milk. After a ripening period of thirty minutes, chymosin (double strength Chy-max, 50,000 MCU/ml, Pfizer Inc., Milwaukee, WI, USA) was added at a level of 0.07 mL kg^{-1} milk and the curd was cut at a firmness of ~ 60 Pa as determined using dynamic low-amplitude oscillation rheometry (Guinee *et al.*, 1994). The remainder of the Cheddar cheesemaking process was as described by Guinee *et al.* (1994).

4.3.3 Sampling and mass balance

Pasteurised, cooled milks were weighed to the nearest 0.1 kg into 500 L cheese vats each mounted on three load cells (Type 500 kgf IP 68; Tedea Huntleigh Ltd., Cardiff CF2 2HB, UK). Representative samples were taken immediately before adding starter, preserved using sodium azide (0.2 g L^{-1}) and analysed within 72 h.

All wheys expressed at whey drainage and during cheddaring were returned to the cheese vat, weighed and representatively sampled as described by Guinee *et al.* (1996*a*). Samples were preserved using sodium azide (0.2 g L^{-1}), stored at 4°C and analysed within 72 h.

The curd was weighed at milling and moulded into blocks (23 kg). Following overnight pressing the cheese was weighed to the nearest 0.01 kg and the final cheese weight was determined by adjusting *pro rata* to account for unmoulded curd.

4.3.4 Dynamic measurement of the rennet coagulation properties of the cheese milk

The rennet coagulation properties of vat cheesemilks containing added starter and rennet were determined using dynamic low-amplitude oscillation in a controlled strain rheometer (Bohlin VOR, Bohlin Reologi, Lund, Sweden) essentially as described by Guinee *et al.* (1996*b*). The rheometer measuring cell consisted of two coaxial cylinders, i.e. an outer cup (internal diameter, 27.5 mm), into which the sample was placed, and an inner bob (diameter, 25 mm). An oscillatory motion was applied to the cup, subjecting

the sample to an harmonic, low amplitude shear strain, γ, with respect to time, t, given by:

$$\gamma = \gamma_0 \cos\omega t$$

where: γ_0 = shear strain amplitude, and ω = angular frequency (i.e. $2\pi\nu$). The shear strain imparts a torque to the bob which in turn is used to calculate the shear stress on the sample. Assuming linear viscoelastic behaviour, the resulting shear stress, δ, has the form:

$$\delta = \delta_0 \cos(\omega t + \delta),$$

where: δ_0 = stress amplitude and δ = the phase angle between shear stress and shear strain oscillations which depends on the viscoelasticity (ratio of viscous to elastic properties) of the gelling system. The storage modulus, G′, of the gelling milk system, is given by the equation:

$$G' = (\delta_0 / \gamma_0)\cos\delta$$

G′, which represents curd elasticity or stiffness, was recorded continually from the time of rennet addition. To ensure that measurements were in the linear viscoelastic region, a low amplitude shear strain of 0.025 was applied at a strain frequency of 1 Hz.

4.3.5 Samples for scanning electron microscopy

Immediately after stirring the added rennet into the cheesemilk, a specifically designed curd sampler was placed in the cheese vat to remove a sample of curd without altering the gel structure. The latter consisted of a cylindrical tube, 50 mm in diameter and 10 mm in height one end of which was sealed, which was clamped to the wall of the cheese vat. The sampler was positioned in the cheese vat so that the cylinder was filled with the renneted cheesemilk. When the desired curd strength (~60 Pa) was reached, as determined rheometrically, the sampler containing a representative sample of clotted milk in its cylinder was removed. The ensuing curd disc was placed on a grided plate and sliced using a scalpel blade into cubes (10 mm x 3 mm x 3 mm) which were immediately immersed in 2% (v/v) glutaraldehyde in 0.01 M sodium phosphate buffer (pH 6.55) and stored at 5°C. The samples were dehydrated in graded acetone solutions to 100% acetone and critical point dried from liquid CO_2. The samples were sputter coated with gold and examined in a scanning electron microscope (JOEL JSM-35, Joel

(UK) Ltd., Hertfordshire, U.K.) operating at an accelerating voltage of 20 kV (Harwalker and Kalab, 1981).

4.3.6 Casein micelle size determination

Prior to analysis, samples (~ 300 ml) of raw milk were separated and the resulting skims were ultrafiltered at 21°C through a stirred cell ultrafiltration unit (Model 202, Amicon Ltd. Gloucestershire. U.K.) fitted with a YM10 (10 kDa nominal molecular mass cut-off) Diaflo™ ultrafiltration membrane (Amicon Ltd., Gloucestershire, U.K.). The permeates obtained were used to dilute the corresponding skim milk samples (1:200). The mean casein micelle size was determined on the diluted skim milk samples by photon correlation spectroscopy with a Zetamaster (Model ZEM, Malvern Instruments Ltd., Worcestershire, U.K.). The Z-Average mean size was determined by cumulant analysis at a scattering angle of 90°. Each measurement was an average of ten determinations. The viscosity of the diluent was taken to be 1.14 mPas at 22°C. All samples were pre-equilibrated in the permeate at 22°C prior to measurement. Temperature was maintained at 22°C $\pm$ 0.05°C using an external water bath fitted with a low pressure circulation pump.

4.3.7 Analysis of milk and whey

Cheesemilk was analysed for fat, protein and lactose as described by Walsh *et al.* (1995). Bulk cheesewhey was analysed for fat, protein and fines as described by Walsh *et al.* (1995).

4.3.8 Cheese analyses

Cheese was analysed for pH, moisture, fat, salt and protein at 14 days as previously described by Walsh *et al.* (1995).

Proteolysis in all cheeses at 14 days, 3 months, 6 months and 9 months was monitored by measuring the levels of total N soluble in water at pH 4.6 (pH 4.6SN) and in phospho-tungstic acid (50 g L^{-1}), (PTA-N), as described by Walsh *et al.* (1998)

4.3.9 Cheese rheology

Cylindrical samples (diam, 30 mm; height, 29 mm) tempered at 8°C for 4 h prior to testing, were compressed on a Model 112 Universal Testing Machine (Instron LTD, High Wycombe, U.K.), at a cross head speed of 50 mm min^{-1}, using a 50 kgf load cell. The forces required to fracture the cheese cylinders (fracture stress) and compress to 30 % of their original height (firmness) were measured as described by Guinee *et al.* (1996*a*). Six replicate measurements were made. The coefficients of variation for measurements on firmness and fracture stress were 6.8 and 8.1 %, respectively.

4.3.10 Sensory Analysis

Cheeses were graded by a trained 10-member in-house Cheddar panel after 3, 6, and 9 months storage at 8°C. Panelists were selected on their abilities to (i) discriminate between the four taste sensations (sweet, sour, salty and bitter) and different intensities of each of these taste sensations and (ii) to discriminate between cheeses with different flavour and texture characteristics (as identified during training of panelists using the descriptive analysis sensory method (Stone *et al.*, 1974)). The cheeses were presented to the panelists at room temperature in individual tasting booths. Samples were presented in a random fashion to avoid consistently high scoring for any one sample. The cheese samples were of a consistent size and no more than four samples were tasted at any one sitting. Non-sparkling mineral water was provided and panelists were instructed to rinse their mouths between samples. Panelists were asked to score the cheeses for "acceptability". Each panelist was given a sheet of paper on which were drawn a series of 8 cm lines anchored at the extremes by the values 0 (reject) and 8 (excellent). For each cheese the panellist placed a vertical stroke on the line; the distances (from zero) were then measured to determine the acceptability score.

4.3.11 Statistical Analyses

Experimental data was examined for statistical significance using the two-tailed paired t-test. Statistically significant effects were at the $P < 0.05$ level unless otherwise

stated. The statistical analyses were performed by Dr. D. Harrington, Statistics Department, Teagasc Headquarters, Dublin 4, Ireland.

4.4 Results and Discussion

4.4.1 Microbiological analyses

The total bacterial counts for all bulked milks following 3 d storage at 4°C were <65,000 cfu mL^{-1} except for trial 3 where κ–casein BB milk had 105,000 cfu mL^{-1} prior to pasteurisation.

4.4.2 Milk composition

κ–Casein genetic variant had no significant effect on the gross composition of the cheesemilk, even though the concentrations of fat, protein, casein and whey protein were numerically higher in the κ–casein BB milk (Table 4.1). These trends are in agreement with previous findings (McLean *et al.,* 1984; van den Berg *et al.,* 1992; Ng-Kwai-Hang and Grosclaude, 1994; Walsh *et al.*, 1995; Walsh *et al.*, 1998).

4.4.3 Average micelle size

Consistent with the findings of earlier studies on individual animal milks (Delacroix-Buchet *et al.*, 1993; Horne *et al.*, 1996; Lodes *et al.*, 1996*a*), the average micelle size of the κ–casein BB variant bulk milk was significantly smaller than that of the κ–casein AA variant milk (Table 4.1). The micelle sizes reported in this study are similar in magnitude to the values found by Lodes *et al.* (1996*a*) but higher than those (i.e. 196 and 145 nm for the κ–casein AA and BB milks, respectively) reported by Delacroix-Buchet *et al.* (1993). The observed differences may result from differences in methodology used. The smaller average micelle sizes associated with the κ-casein BB milks than the AA milks has been attributed to the higher κ-casein contents associated

Table 4.1 Effect of the κ-casein variant on the composition of cheesemilk and its suitability for the production of Cheddar cheese.

	Genetic Variant		
	AA	BB	SED
Milk Composition			
pH	6.72	6.77	0.04
Fat, g kg^{-1}	34.7	36.2	1.2
Protein, g kg^{-1}	34.4	35.3	1.0
Lactose, g kg^{-1}	45.8	45.2	0.4
Casein, g kg^{-1}	26.3	27.0	0.9
Whey Protein, g kg^{-1}	5.6	5.9	0.2
Casein/fat	0.757	0.747	0.03
Casein No.	76.47	76.66	0.52
Non protein nitrogen, g kg^{-1}	0.4	0.4	0.0
Average casein micelle size			
Micelle size, nm	266.3^{a}	183.5^{b}	5.84
Whey composition			
Fat, g kg^{-1}	7.5^{a}	3.8^{b}	0.8
Protein, g kg^{-1}	9.6	10.0	0.20
Fines, mg kg^{-1}	544.0	324.0	193.0
Cheese composition			
pH at 14 d	5.15^{a}	5.24^{b}	0.02
Protein, g kg^{-1}	267.1^{a}	255.3^{b}	2.5
Fat, g kg^{-1}	309.6^{a}	345.4^{b}	12.1
Moisture, g kg^{-1}	387.0	373.9	11.8
Moisture in non-fat substances, g kg^{-1}	560.3	571.5	14.6
Fat in dry matter, g kg^{-1}	504.9^{a}	551.7^{b}	16.3
Salt, g kg^{-1}	20.1	20.6	0.6
Salt in moisture, g kg^{-1}	52.0	55.2	2.0
Calcium, mg kg^{-1} protein	20.5	21.9	1.44
Ash, g kg^{-1}	40.1	39.6	0.5
Cheese yield, kg cheese/1000 kg milk			
Actual	93.6^{a}	99.1^{b}	0.6
MACY[1]	92.5^{a}	100.1^{b}	1.8
MACYPFAM[2]	94.3	99.5	3.5
Recovery in cheese			
Milk fat, g kg^{-1}total	80.9^{a}	94.5^{b}	4.34
Milk protein, g kg^{-1} total	69.9	72.9	3.56

[1]MACY, moisture adjusted (to 380 g kg^{-1}) cheese yield. [2]MACYPFAM, moisture adjusted (to 380 g kg^{-1}) cheese yield (kg/1000 kg milk adjusted to 31.2 g protein/kg and 32.5 g fat kg^{-1}). Values are means from triplicate cheesemaking trials; residual df, 4, SED standard error of difference. Values within rows not sharing a common superscript were significantly different, ($P<0.05$).

with the B variant of κ-casein (Lodes *et al.*, 1996*a*). While the overall casein content did not differ significantly between the different κ-casein variant cheesemilks (Table 4.1), the concentrations of κ-casein in the cheesemilks were not quantified as part of the present study.

4.4.4 Rennet coagulation properties

In comparison with κ–casein AA variant milks, κ–casein BB milks had shorter rennet coagulation times, higher curd firming rates (as reflected by the higher slopes of the elastic shear modulus versus time curves) and higher curd firmness (G') values at any given time after rennet addition (Fig. 4.1). This effect was also observed during the cheesemaking process where the set to cut time, at ~60 Pa, for the κ–casein AA milk (i.e. 46 min) was approximately twice that for the κ–casein BB milk. The enhanced rennet coagulation properties of the κ–casein BB milk, has been consistently reported (Aaltonen and Antila, 1987; Pagnacco and Caroli, 1987; Jakob and Puhan, 1992; Horne and Muir, 1994*a*; Walsh *et al.*, 1995; Walsh *et al.*, 1998). The shorter coagulation time of the κ–casein BB milk probably results from its lower critical level of κ–casein hydrolysis required for the onset of gelation (Horne *et al.*, 1996). The slightly higher casein content may also contribute somewhat to the shorter coagulation time (Guinee *et al.*, 1997*a*). The higher curd firming rate in the κ–casein BB milk probably arises from the smaller size of the rennet altered micelles which allows for a more compact arrangement of the sensitised para-casein micelles, and hence more numerous inter-micellar bonds, when forming into a network (Walstra and van Vliet 1986; Horne *et al.*, 1996). Indeed it has been shown by Horne *et al.* (1996) in rennet coagulation studies from modeled milks prepared by re-suspension, in milk serum, of casein micelles, prepared from the same milks using differential ultra-centrifugation, that the curd firming rate for a given casein concentration was inversely proportional to the cube of the diameter. The smaller micelles as in the κ–casein BB milk consequently allow for a greater number of micelles and hence a greater number of inter-micellar bonds per unit surface area.

4.4.5 Scanning electron microscopy (SEM)

Typical SEM's of the rennet gels (which were obtained at similar gel strengths i.e. ~60 Pa) from the κ–casein AA and BB milks at cutting are shown in Fig. 4.2. In each case the protein matrix (grey area) was permeated by a well-distributed system of holes (black areas), which correspond to the spaces originally occupied by the fat globules. The gels from the κ–casein BB milk were consistently found to display finer gel networks, more uniform distribution of the para-casein and smaller pore sizes. This may arise from the smaller casein micelles in the κ–casein BB milk which allows for a more compact arrangement of, and more uniform distribution of, aggregates formed from rennet altered micelles, within the gel network (Horne *et al.*, 1996).

4.4.6 Whey composition

The bulk cheesewhey from the κ–casein AA milk had significantly higher levels of fat and numerically higher levels of fines than that from κ–casein BB milk (Table.4.1). Consistent with these results is the significantly higher fat retention in κ–casein BB cheese. The higher levels of fat in the κ–casein AA cheese wheys is in agreement with results found for Cheddar (Graham *et al.*, 1986; Walsh *et al.*, 1995) and other cheese varieties, such as Gouda (van den Berg *et al.*, 1992) and Mozzarella (Walsh *et al.*, 1998). This trend may be attributed to the finer gel networks (Fig 4.2, plate 2 and 4), and the ensuing lower porosity, of the κ–casein BB gels resulting in improved fat retention. Conversely it is envisaged that the coarser gel network of the κ–casein AA milk with its relatively large pores (Fig 4.2, plate 1 and 3) is more conducive to the leakage of fat to the cheese whey.

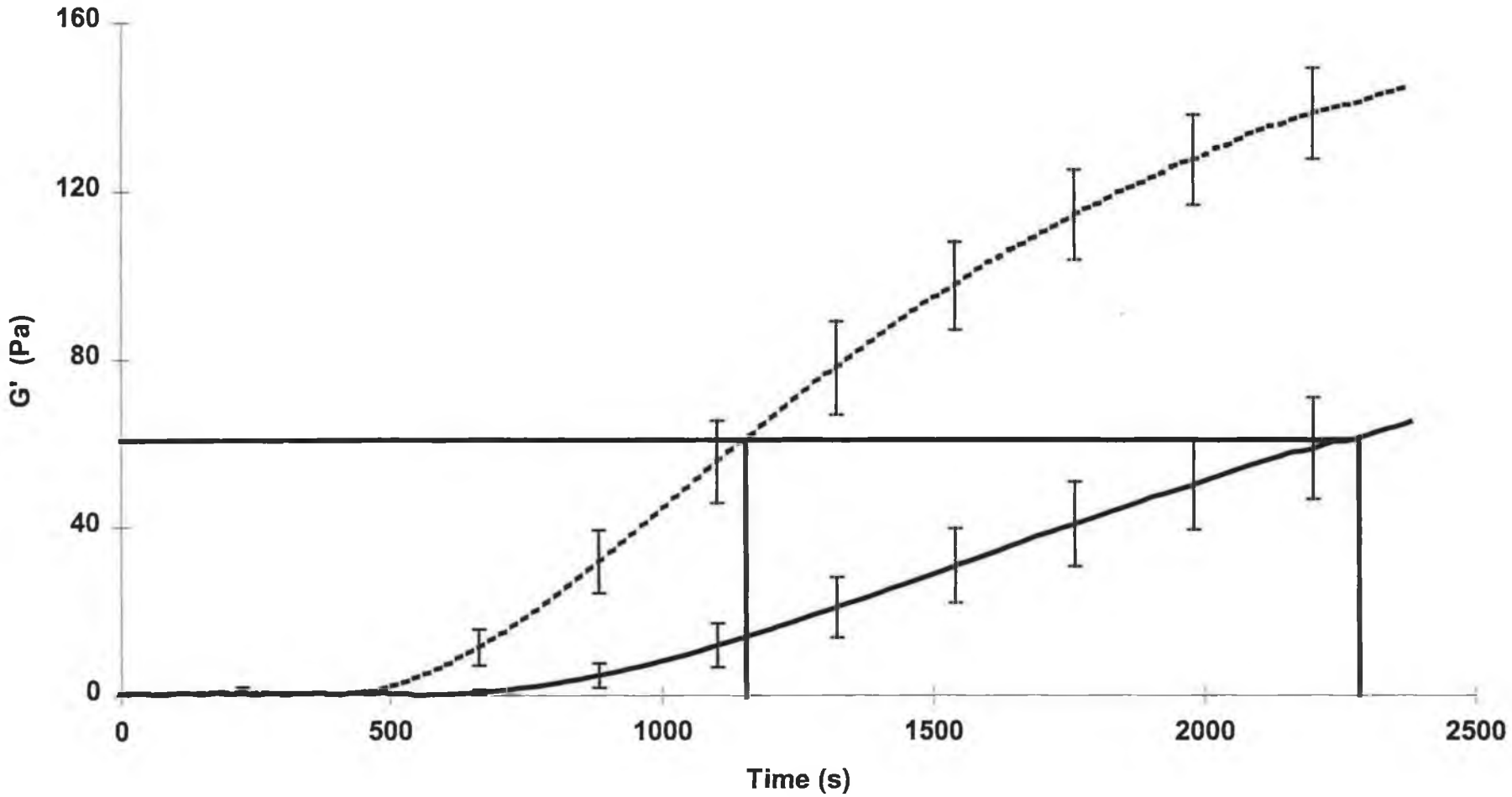

Figure 4.1 Effect of the κ–casein AA and BB genetic variants on the development of the elastic shear modulus, G', of renneted milk. Data shown is the mean from three cheesemilks. Error bars represent standard deviation. κ–Casein variant AA (—), BB (- - -).

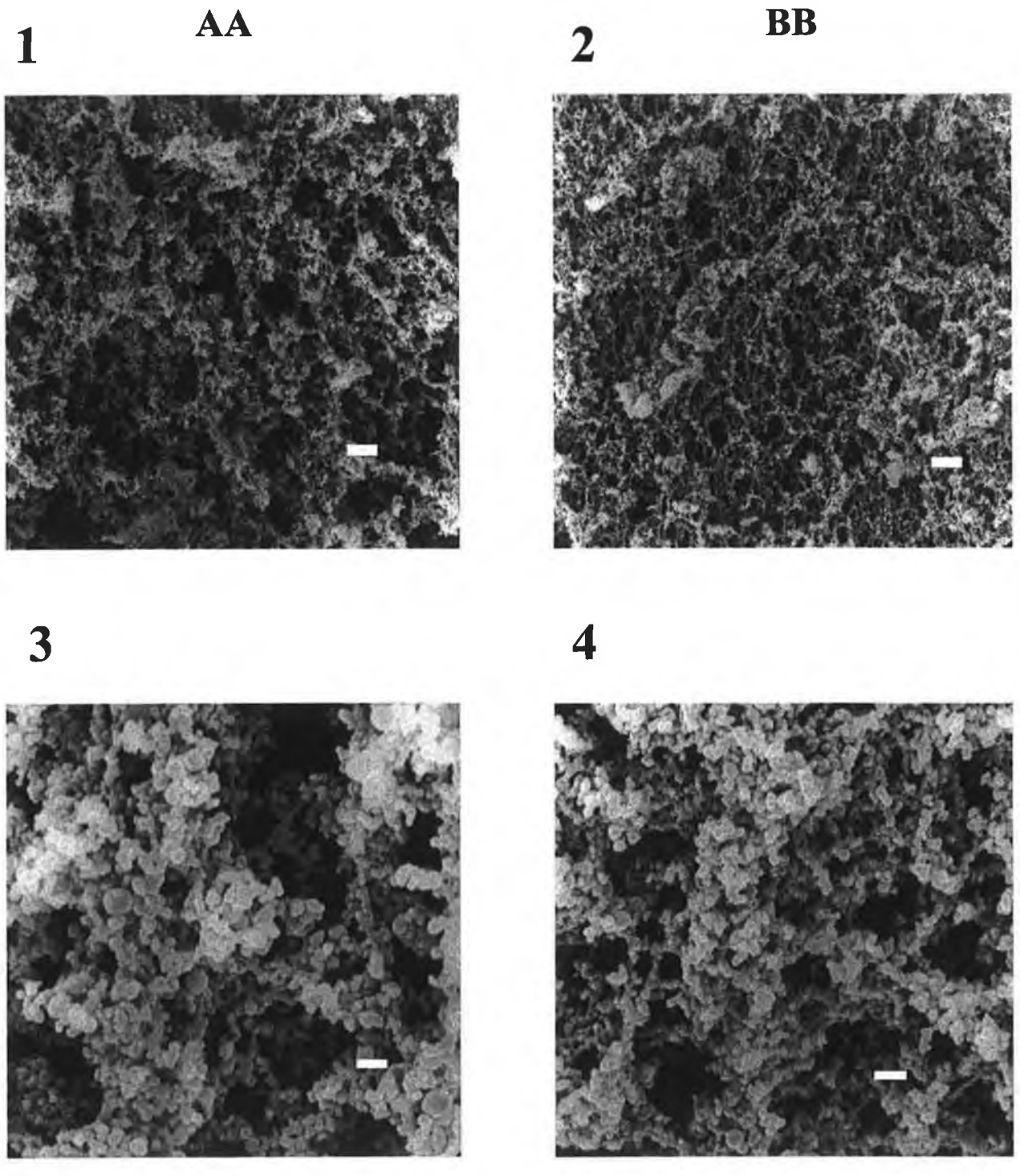

Figure 4.2 Electron micrographs of rennet gels made from κ–casein AA and BB genetic variant milks. Scale bar: 10μm (plates 1,2), 0.5 μm (plates 3,4).

4.4.7 Cheese composition

The compositions of the cheeses, analysed at day 14, are given in Table 4.1. In concurrence with the observations of others, cheese from κ–casein BB milk had significantly higher levels of fat and fat in dry matter (FDM) (Morini *et al.*, 1979; Schaar *et al.*, 1985; Marziali and Ng-Kwai-Hang, 1986*a*; Graham *et al.*, 1986; Walsh *et al.*, 1995; Walsh *et al.*, 1998), and lower levels of protein. Otherwise κ–casein variant had no significant effect on gross composition, even though the level of moisture in the κ–casein BB cheese was numerically lower than that of the κ–casein AA cheese. The higher levels of fat and FDM in the BB cheeses is consistent with the greater fat recovery in the cheese. The lower protein levels in the κ–casein BB cheeses probably ensue from the dilution effect of the higher fat levels. The observed higher pH at day 14 for κ–casein BB cheese may be due to the numerically lower lactose levels in the milk and the lower cheese moisture contents, both factors which would ultimately contribute to lower lactic acid content in the κ–casein BB cheese (Walstra *et al.*, 1993; Guinee *et al.*, 1996*a*).

4.4.8 Cheese yield

The actual yield and moisture adjusted cheese yield (MACY) from the κ–casein BB milk were significantly higher than those from the κ–casein AA milk (Table 4.1). This trend is attributed to the greater fat retention in cheese from κ–casein BB milk. The higher cheese yields (both actual and MACY) associated with the κ–casein BB milks have also been reported for other varieties, including Cheddar (Graham *et al.*, 1986; Vink *et al.*, 1993; Walsh *et al.*, 1995; Horne *et al.*, 1996) and Parmesan (Mariani *et al.*, 1976; Morini *et al.*, 1979). Expressing cheese yield as MACYPFAM (moisture adjusted cheese yield/100 kg milk adjusted to equal levels for protein and fat) eliminates the influence of variations in fat and protein concentrations, associated with the different κ–casein variant milks, on cheese yield. No significant difference was observed between the MACYPFAM from the different κ–casein variant milks. However κ–casein BB milks resulted in numerically higher MACYPFAM in accordance with the trend noted for actual and moisture adjusted yield. In a comparable study on low

moisture Mozzarella cheese (Walsh *et al.*, 1998), trends similar to those in the current study were reported for the effect of κ–casein variant on cheese yield. However, in contrast to the current study, the MACYPFAM from κ–casein BB milk was significantly higher than that from κ–casein AA milk (Walsh *et al.*, 1998), even though the numerical difference between the variants with respect to MACYPFAM in both studies was similar at ~ 5 kg/1000 kg milk. The numerically higher MACYPFAM's in the κ–casein BB milk is consistent with its higher fat retention, an attribute undoubtedly associated with its finer gel structure and its higher fat-retaining ability (Fig. 4.2, plate 2 and 4).

4.4.9 Cheese maturation

The levels of pH4.6SN and PTA-N increased gradually during ripening attaining values at 270 d which are typical for Cheddar (Wilkinson *et al.*, 1992, Chapter 3). κ-Casein variant did not significantly effect the rate of primary or secondary proteolysis throughout the 270 d ripening period as reflected by the similar levels of pH4.6SN and PTA-N respectively in cheeses made from κ–casein AA or BB milks (Fig. 4.3.). These trends are similar to those reported for Gouda (van den Berg *et al.*, 1992), Cheddar (Imafidon *et al.*, 1995) and Mozzarella (Walsh *et al.*, 1998). However Imafidon *et al.* (1995) reported quantitative differences between the gel electrophoretic patterns and reversed-phase HPLC peptide profiles of pH4.6SN extracts of Cheddar cheese made from κ-casein AA or BB milks.

All cheeses became progressively softer and more crumbly with ripening time as reflected by the decreases in hardness and yield strength (Table 4.2). These trends which may be attributed to the reduction in the levels of intact casein (Creamer and Olson, 1982) are consistent with the increases in primary proteolysis. The κ–casein BB cheese were significantly softer at 90 and 180 days, and had a lower yield stress at day 180, than the κ–casein AA cheese (Table 4.2); this trend may ensue from the lower protein levels in the κ–casein BB cheese (Guinee *et al.*, 1996*a*).

Grading of cheeses during maturation by an in-house taste panel indicated no significant differences in the acceptability scores awarded to the cheeses made from different κ–casein variants (Table 4.3).

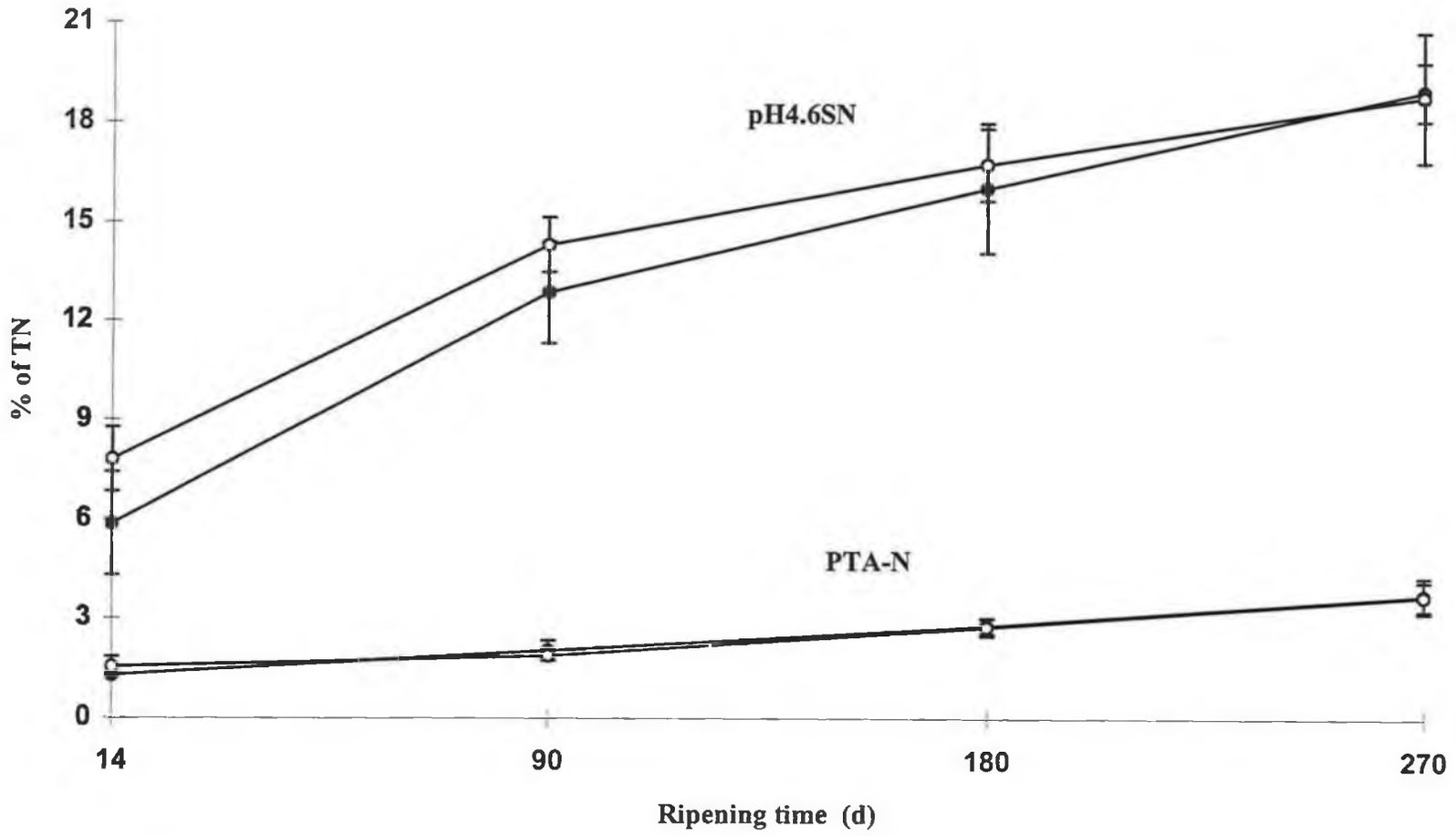

Figure 4.3 Effect of κ–casein variants on the formation of N soluble at pH 4.6 (pH4.6SN) and in phosphotungstic acid (50 g l^{-1}), (PTA-N). Error bars represent standard deviation. κ–Casein variants AA, ●. BB, ○.

Table 4.2 The rheological changes during ripening in Cheddar cheeses made using κ–casein AA or BB variant milks

		Genetic Variant		
	Age, d	AA	BB	SED
Firmness, N	14	291	286	10.4
	90	257[a]	235[b]	5.0
	180	217[a]	191[b]	4.5
	270	199	200	6.7
Fracture stress, kPa	14[1]	-	-	-
	90[1]	-	-	-
	180	237[a]	203[b]	8.6
	270	203	202	11.8

[1]At <180 d, cheese did not fracture during compression.

Values are means from triplicate cheesemaking trials; residual df, 2. SED Standard error of difference. Values within rows not sharing a common superscript were significantly different, ($P<0.05$).

Table 4.3 Effect of the κ-casein variant on the acceptability of Cheddar cheese during ripening.

		Genetic Variant		
	Age, d	AA	BB	SED
In-house grade[1]	90	4.15	4.63	0.39
	180	4.36	4.29	0.23
	270	4.26	3.97	0.23

[1]Cheeses were graded for "acceptability" and were scored between the values 0 and 8, value 0 denoting a rejection rating and 8 an excellent rating.

Values are means from triplicate cheesemaking trials; residual df, 2. SED Standard error of difference.

4.5 Conclusions

For milks with similar casein levels, the κ–casein genetic variant, i.e. AA or BB had a marked influence on the actual and moisture-adjusted yield of Cheddar cheese. This yield increase associated with the κ–casein BB variant is probably attributable to its superior curd forming properties which leads to greater fat recoveries in the cheese. The higher fat recovery with the κ–casein BB milk resulted in cheese with significantly higher fat levels and lower protein levels. κ–Casein variant had little effect on primary and secondary proteolysis during ripening or on the grading scores awarded for acceptability. The increases in the yield of cheese solids per kg of milk associated with the κ-casein BB variant, reported in this study, suggests that selective breeding to increase the proportion of the BB variant may prove advantageous to the dairy industry.

CHAPTER 5

Cheesemaking, compositional and functional characteristics of low moisture part-skim Mozzarella cheese from bovine milks containing κ–casein AA, AB or BB genetic variants[1]

[1]This chapter was published in the *Journal of Dairy Research* (1998) 65 307-315

5.1 Summary

Low moisture part-skim Mozzarella cheeses were made, at pilot scale (450 kg), on five occasions at weekly intervals from milks containing κ–casein AA, AB or BB genetic variants. Compared with κ–casein A variant milks, the κ–casein B variant milks were associated with higher concentrations of casein ($P<0.001$), whey protein ($P<0.02$) and total protein ($P<0.001$), superior curd-forming properties ($P<0.05$) and increased cheese yields ($P<0.05$). The moisture adjusted (to 465 g/kg) cheese yields for the κ–casein AA, AB and BB cheeses were 9.15, 10.06, and 10.25 kg/100kg milk respectively. κ–Casein variant had no significant effect on the proteolysis and ripening of uncooked cheese or on the functionality (melt time, flowability and stretchability) of the cooked cheese during the course of a 90 day ripening period

5.2 Introduction

The composition and cheesemaking properties of milks are affected by milk protein polymorphism. The effect of κ–casein genotype has been studied in a range of cheese varieties including Cheddar, Parmesan, Svecia, Edam and Gouda. In particular it is known to affect rennet coagulation properties, cheese composition and cheese yield (Schaar *et al.*, 1985; Graham *et al.,* 1986; Marziali and Ng-Kwai-Hang, 1986*a*; Jakob and Puhan, 1992; Van den Berg, 1992; Horne and Muir, 1994*a*; Jakob, 1994; Walsh *et al.,* 1995). The different effects on composition and yield obtained in the various cheesemaking studies suggest that the effect of κ–casein genotype is to some extent dependent on cheese variety (Banks *et al.,* 1994; Walsh *et al,.* 1995).

Production of Mozzarella cheese, which belongs to the pasta filata (stretched curd) group of cheeses, has grown considerably in recent years. This is due to the increased popularity of pizza pie, especially in the USA where current annual production of Mozzarella cheese is approximately 938,000 tonnes (International Dairy Federation Group D31, personal communication). Mozzarella is particularly suited for use in pizza pie owing to its unique functional properties, i.e. ability to melt, flow and stretch on cooking (Kindstedt, 1995). To our knowledge, there is little, or no, published

information on the influence of κ–casein genetic variant on Mozzarella cheesemaking and quality.

The objective of this study was to investigate the effect of κ–casein genetic variants (i.e. AA, AB or BB) on rennet coagulation characteristics of bovine milk and on the yield, proteolysis and functionality of low moisture part-skim Mozzarella (LMPM) cheese.

5.3 Materials and Methods

5.3.1 Milk supply

Milk was collected from 42 mid-to-late lactation, spring-calving, Holstein Friesian cows from the Moorepark herds. There were three groups, that is κ-casein AA, AB and BB with 14 cows for each genotype. Each cow within a group was matched with a cow in the corresponding groups for calving date, lactation number, milk yield and β-lactoglobulin genotype. Animals were also typed for polymorphism at the β-casein locus. While it was not possible to match animals with respect to β-casein phenotypes, similar distributions of β-casein variants were observed within the three groups. The distribution of β-casein phenotypes for the κ-casein AA animals was A1A1 x 9, A1A2 x 3 and A2A2 x 2, for the AB variant animals was A1A1 x 7, A1A2 x 3, A2A2 x 1, A1B x 2 and A2B x 1 and for κ-casein BB animals was A1A1 x 8 and A1A2 x 6. Milks from each group were collected separately over a 3-day period, bulked and stored at 4°C. Samples from the bulked milks were tested twice daily during the collection period for total bacterial count (TBC) (International Dairy Federation, 1991*a*).

Milks from individual animals within the Moorepark herds were tested monthly for somatic cell count (SCC) using a Bentley Somacount 300 somatic cell counter (AgriYork 400 Ltd., York YO4 2QW, UK), after calibration and standardization as set out in the International Dairy Federation Standard methods (1984). During the milk collection period in this study, the monthly SCC was determined between 6-9 Sep 1995. The SCC of the bulk milks were estimated from those of the individual animals and the

contribution of these milks to the final volume. Animals were continually monitored for symptoms of clinical mastitis.

5.3.2 Manufacture of low moisture part-skim Mozzarella cheese

Three vats of cheese were made, in parallel, from bulked κ–casein AA, AB or BB milks on five separate occasions between 25 Aug and 22 Sep, 1995.

Milks (~450 kg) were standardized to a protein to fat ratio of 1.2:1, pasteurized (72°C, 15s), cooled to 36°C, and inoculated with *Streptococcus thermophillus* and *Lactobacillus helveticus* (Chr. Hansen's Laboratory (Ireland) Ltd., Little Island, Cork). The starters were grown separately in 100 g/l reconstituted low heat skim milk powder and added at 10 and 5 g/kg, respectively. After 30 min, chymosin (Double Strength Chy-max, 50,000 milk clotting units (MCU)/ml; Pfizer Inc., Milwaukee, WI 53214-4298, USA) was added at 0.077 ml/kg milk. The curd/whey mixture was cooked at 42°C and pitched at pH 6.1. The curd was cheddared, milled at pH~5.15, heated to 60°C and stretched (Automatic Stretching Machine, Mod f.; CMT Construzioni Meccaniche e Technologia S.p.a., Peveragno, Cuneo, Italy). The plasticized molten curd was dry-salted (10 g/kg), moulded into 2.3 kg blocks (Automatic Moulding Machine Mod H2MS; CMT Construzioni Meccaniche e Technologia S.p.a.) cooled to 23°C, brine salted (210 g/l NaCl) for 2 h at 10°C, vacuum wrapped and stored at 4°C.

5.3.3 Sampling and mass balance

Pasteurized, cooled milks were weighed to the nearest 0.1 kg into 500 l cheese vats each mounted on three load cells (Type 500 kgf IP 68; Tedea Huntleigh Ltd., Cardiff CF2 2HB, UK). Representative samples were taken immediately before adding starter, preserved using sodium azide (0.2 g/l) and analysed within 72 h.

All wheys expressed at whey drainage and during cheddaring were returned to the cheese vat, weighed and representatively sampled as described by Guinee *et al.* (1996 *a*). Samples were preserved using sodium azide (0.2 g/l), stored at 4°C and analysed within 72 h.

The curd was weighed at milling and 45 kg were heated, stretched and kneaded. The moulded cheese was weighed to the nearest 0.01 kg before cooling and brining. The weights of the residual unstretched cheese (if any) and unmoulded cheese in the moulding unit were recorded and the weight of the final cheese was adjusted to account for these.

5.3.4 Milk and whey analyses

Milk fat, protein and lactose were determined by the Infra-red Milk Analyser (Milkoscan 605, Foss Electric) which was calibrated according to International Dairy Federation, (1990).

A Formagraph instrument (Model 11700, Foss Electric) was used to assess the rennet coagulation properties (McMahon and Brown, 1982) of raw milks and standardized pasteurized milks (ex vat) from trials 4 and 5. Milks were adjusted to pH 6.60 (using 0.5 M HCl or 0.5 M NaOH), tempered at 36°C, renneted with chymosin (1:100 dilution of double strength Chy-max, 50,000 MCU/ml) at a rate of 77 μl/10 ml, incubated at 36°C and the coagulation properties monitored over a 60 min period. The parameters obtained were rennet coagulation time (RCT), curd firming rate over 20 min (K_{20}) and curd firmness at 60 min (A_{60}).

Whey samples were analysed for fat by the Röse-Gottlieb method and protein and fines as described by Walsh *et al.* (1995).

5.3.5 Cheese sampling

Each rectangular block (~ 230 x 100 x 95 mm) was cut into 4 symmetrical quarters. Cylindrical samples for rheological analysis were cut from one, with a second being used to cut disc-shaped samples for determination of flowability of the melted cheese. Another sub-block was finely grated (Disc 1 Kenwood Euro System 900; Kenwood Ltd., New Lane, PO92NH, UK.) and used for compositional analysis. The remaining sub-block was cut into 24 mm cubes (Cheese Blocker; Bos Kaasgereedschap, NL 2410, Bodegraven, The Netherlands), shredded (length ~24 mm; Hallde RG-3500

Food preparing machine; AB Hallde Maskiner, S 164 22 Kista, Sweden) and used for functional evaluation of cheese on cooking.

Cheeses were sampled for composition at day 10 and for all other characteristics at 10, 20, 30, 45 and 90 d.

5.3.6 Analysis of uncooked cheese

Cheese was analysed for pH, moisture, fat, protein, salt, ash and calcium as described by Guinee *et al.* (1996*a*).

Proteolysis was monitored by measuring the % total N (TN) soluble in water at pH 4.6 (WSN) and in 50 g/l phospho-tungstic acid (PTA-N) as described by Guinee *et al.* (1996*a*).

The hardness was determined by measuring the force required to compress a cylindrical sample by 75 % using the Universal Instron Testing Machine (Model 112 Instron Ltd., High Wycombe HP12 35Y, UK) as described by Guinee *et al.* (1996*a*). For each sample, 4-6 measurements were made and a mean value obtained.

5.3.7 Evaluation of cheese functionality on cooking

Melt time: The melt time was analysed in triplicate as described by Guinee *et al.* (1997*b*).

Flowability: The flowability of the cheese on cooking was determined by the Schreiber test as described by Guinee *et al.* (1997*b*).

Stretch: Prior to loading the cheese, the pizza base (Canadian Pizza (UK) Ltd., Salford M5 2NP, UK.) was cut cleanly in half and the halves were aligned in their original position to form a flush interface. Shredded cheese, stored at 4° C, was distributed evenly over the base (2.42 kg/m^2) which was then placed in an thermostatically-controlled electric fan oven at 280°C for 4 min. The melted pizza pie was then placed on a circular wooden board cut in half and of similar dimensions and the same orientation as the pizza. One half of the pizza was clamped in position. One min after removal from the oven the unclamped section was manually pulled until the extended string(s)/sheet of the molten cheese mass connecting both halves of the pizza base completely broke. Under these test conditions, the average extension time for a 15 day

old LMPM cheese was 2.1 s; the coefficients of variation for extension time and stretch length were 29.5 and 23.8 %, respectively (n=10). Stretch was defined as the distance between the pizza halves at the point of complete strand breakage and extension time as the time taken to complete the stretch i.e from the beginning of pull to the point of string breakage.

5.3.8 Statistical Analysis

The results for the three κ-casein variant groups were compared using analysis of variance. Statistically significant effects were at the $P<0.05$ level unless otherwise stated. The statistical analyses were performed by Dr. D. Harrington, Statistics Department, Teagasc Headquarters, Dublin 4, Ireland.

5.4 Results

5.4.1 Microbiological analyses

The TBC's for all bulked milks following 3 days storage at 4°C were <65,000 colony forming units (cfu)/ml except for trial 1, where κ-casein AA and BB milks, had 260,000 and 305,000 cfu/ml, respectively, prior to pasteurization. The SCC's for the AA, AB and BB milks were 181 X 10^3, 544 X 10^3 and 282 X 10^3 per ml, respectively. No clinical symptoms of mastitis were observed in any of the cows during the course of this study, indicating overall acceptable quality of the bulk milks.

5.4.2 Milk composition and rennet coagulation characteristics

The compositions of the different κ–casein variant milks used in this study are shown in Table 5.1. Significant differences were observed in the fat, protein, whey protein and the total casein contents, with κ–casein AB and BB milks having higher levels. There were no significant differences between the milks in lactose concentration and casein number.

While there were no significant differences in rennet coagulation times between the milks, the K_{20} and A_{60} for κ–casein AB and BB milks were significantly lower and higher, respectively, than those for the κ–casein AA milks (Table 5.1).

5.4.3 Composition of wheys

Cheese wheys from κ–casein AB and BB milks had lower fat and higher protein levels than that from κ–casein AA milk (Table 5.2). No significant differences were observed in the fines levels in the different wheys, however, κ–casein BB wheys had numerically lower fines levels ($P = 0.13$).

5.4.4 Cheese composition

The compositions of all cheeses were typical for LMPM (United States Department of Agriculture, 1976; Guinee and O'Callaghan, 1997). The levels of fat and fat in dry matter in cheeses from κ–casein BB milks were significantly higher than those from κ–casein AB milks which were also significantly higher than those from κ-casein AA milks (Table 5.2).

5.4.5 Cheese yield

The actual, and moisture adjusted (to 465 g/kg) cheese yields per 100 kg milk (MACY) and per 100 kg of milk adjusted for protein (34 g/kg) and fat (28.3 g/kg), (MACYPFAM), were significantly higher for κ–casein BB than for κ–casein AA milk. The increases in actual yield for κ–casein AB and BB milks, expressed as a percentage of the yields obtained for κ–casein AA milk, were 7.9 and 8.8 %, the corresponding increases in MACY were 9.9 and 12.0 % and in MACYPFAM were 3.3 and 5.5 %, respectively. Milk fat recovery in the cheeses was in the following order: κ-casein AA < κ-casein AB< κ-casein BB. Milk protein recovery was not significantly influenced by κ-casein variant (Table 5.2).

Table 5.1 Composition and rennet coagulation properties of standardized pasteurized bovine milks containing κ–casein AA, AB or BB genetic variants

	Genetic Variant			
	AA	AB	BB	SED
Composition				
Fat (g/kg)	29.2[a]	31.3[b]	30.9[b]	0.40
Protein (g/kg)	35.4[a]	37.6[b]	37.5[b]	0.30
Lactose (g/kg)	46.1	45.8	46.2	0.30
Casein No.	74.6	75.2	74.7	0.39
Casein (g/kg)	26.4[a]	28.3[b]	28.0[b]	0.03
Whey protein (g/kg)	7.0[a]	7.4[b]	7.6[b]	0.10
Rennet coagulation property				
RCT (min)	16.66	15.45	14.04	0.65
K_{20} (min)	8.16[a]	5.25[b]	4.21[b]	0.41
A_{60} (mm)	48.75[a]	57.63[b]	58.48[b]	1.01

SED, Standard error of difference. Values within rows not sharing a common superscript are significantly different ($P<0.05$).
Values given for composition are means for five replicate cheesemaking trials. Residual degrees of freedom, 8.
Milk rennet coagulation property values given are from trials 4 and 5 adjusted to pH 6.60. Residual degrees of freedom, 2. RCT, rennet coagulation time. K_{20}, rate of curd firming. A_{60}, curd firmness at 60 min.

Table 5.2 Effect of the κ-casein variant on whey and cheese composition, cheese yield and recoveries in cheese from standardized pasteurized milks

	Genetic Variant			
	AA	AB	BB	SED
Whey composition				
Fat (g/kg)	5.9[a]	4.7[b]	3.5[c]	0.30
Protein (g/kg)	10.3[a]	10.6[b]	10.6[b]	0.10
Fines (mg/kg)	284.0	251.0	64.0	101.3
Cheese Composition				
pH at 10 d	5.51	5.52	5.55	0.02
Protein (g/kg)	285.3	282.0	278.3	6.2
Fat (g/kg)	210.3[a]	220.9[b]	231.8[c]	4.2
Moisture (g/kg)	469.1	459.3	454.3	7.4
MNFS (g/kg)	593.9	589.5	591.3	7.3
FDM (g/kg)	395.9[a]	408.7[b]	424.7[c]	5.0
Salt (g/kg)	13.6	12.6	12.5	0.5
S/M (g/kg)	28.9	27.3	27.6	0.8
Calcium (mg/g protein)	30.44	31.43	30.24	1.17
Ash (g/kg)	34.0	33.5	33.7	0.7
Cheese Yield (kg cheese/100 kg milk)				
Actual	9.23[a]	9.96[b]	10.05[b]	0.18
MACY	9.15[a]	10.06[b]	10.25[b]	0.13
MACYPFAM	9.02[a]	9.32[ab]	9.52[b]	0.15
Recovery in cheese				
Milk fat (g/kg total)	67.4[a]	71.2[b]	76.7[c]	0.96
Milk protein (g/kg total)	77.4	78.0	77.5	1.14

SED, Standard error of difference. Values within rows not sharing a common superscript are significantly different ($P<0.05$).
Values given are means from five replicate cheesemaking trials. Residual degrees of freedom, 8. MNFS, moisture non fat substances; FDM, fat in dry matter; S/M, salt in moisture.
MACY, moisture adjusted (to 465 g/kg) cheese yield. MACYPFAM, moisture adjusted cheese yield per 100 kg of protein (to 34 g/kg) and fat (28.3 g/kg) adjusted milk.

5.4.6 *Proteolysis*

The WSN (as % of TN) increased slowly with ripening time from ~ 1.5 at day 10 to ~ 4.0 % at day 90. The PTA-N levels increased from ~ 0.3 to 1.1 % of TN during the same ripening period. Proteolysis was not significantly influenced by κ–casein variant at any stage of the ripening period.

5.4.7 Changes in rheology during ripening

All cheeses became progressively softer with ripening time with hardness decreasing from ~ 450 N at day 10 to ~ 200 N at day 90. Hardness was not significantly influenced by κ–casein variant at any stage of the ripening period.

5.4.8 Changes in functionality of cooked cheese during ripening

Apart from flowability at Day 20, κ–casein variant had no significant influence on the functional characteristics i.e. melt time, flowability and stretchability of LMPM over the 90 d ripening period (Table 5.3).

Table 5.3 Effect of κ–casein AA, AB and BB genetic variants on the melt time, flowability and stretchability of low-moisture part-skim Mozzarella cheese, during ripening at 4°C.

	Genetic Variant			
	AA	AB	BB	SED
Melt time (sec)				
10 d	103	104	102	2.64
20 d	108	103	107	3.99
45 d	105	103	108	3.17
Flowability (%)				
10 d	43.60	38.00	38.60	5.12
20 d	49.20[a]	40.70[b]	47.20[ab]	2.60
45 d	51.80	50.90	48.00	3.34
Stretchability (mm)				
10 d	812	835	814	98.9
20 d	967	835	836	149.9
45 d	890	966	1029	98.9

SED, Standard error of difference. Values within rows not sharing a common superscript are significantly different ($P<0.05$).
Values given are means from five replicate cheesemaking trials. Residual degrees of freedom, 8.

5.5 Discussion

The higher levels of fat, casein and total protein in the κ–casein AB and BB milks, compared to the κ–casein AA milks, are in agreement with the findings of others (van den Berg *et al.,* 1992; Jakob, 1994; Ng-Kwai-Hang and Grosclaude, 1994; Walsh *et al.,* 1995).

The superior curd-forming properties of the κ–casein AB and BB milks, as previously reported (Jakob and Puhan, 1992; Walsh *et al.,* 1995), may in part be attributed to their higher casein concentrations (Guinee *et al.,* 1996*b*). However, factors other than overall casein level may also contribute to differences in the curd forming properties e.g. differences in κ–casein concentration, variations in micelle size/structure (van Eenennaam and Medrano, 1991; Lodes *et al.,* 1996*a*) and the ensuing differences in the structure and/or molecular properties of the curd. In support of the above Walsh *et al.* (1997) found that milks of similar casein levels but with different κ–casein variants exhibited marked differences in curd forming properties. Moreover, the different fat recoveries found in the current study, suggest differences in the fat-retaining abilities of, and protein matrix structures of, curds from milks of different κ-casein variants. The higher levels of fat in the κ–casein AA cheese wheys is in agreement with results found for other cheese varieties, such as Gouda (van den Berg *et al.,* 1992) and Cheddar (Graham *et al.,* 1986; Walsh *et al.,* 1995). Furthermore, the changes in MACYPFAM between milks containing different κ-casein variants also suggest that variations in gel matrix properties and associated fat-retaining abilities of the curds contribute to the observed yield differences. Expressing cheese yield as MACYPFAM eliminates the influence of variations in fat and protein concentrations, in the different κ-casein variant milks, on cheese yield. The higher cheese yields (both actual and MACY) associated with the κ–casein AB and BB milks have also been reported for other varieties, including Cheddar (Graham *et al.,* 1986; Vink *et al.,* 1993; Walsh *et al.,* 1995) and Parmesan (Mariani *et al.,* 1976; Morini *et al.,* 1979).

Generally κ-casein variant had no significant effect on the rheology of the uncooked cheese or on functionality of the cooked cheese. Such a trend might be expected due to the general absence of significant differences between the cheeses from the different κ-casein variants *vis a vis* pH, composition and proteolysis (Guinee *et al.,*

1997*b*). The trends in the latter parameters with ripening time are typical of those reported by others for LMPM cheese (Yun *et al.,* 1993; Kindstedt, 1995; Guinee *et al.,* 1997*b*).

In conclusion, the absence of κ-casein variant related differences in the functional, rheological and ripening characteristics of LMPM indicate that selective breeding to increase the proportion of the B variant of κ–casein may prove advantageous for increasing cheese yield.

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