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INFLUENCE OF GENETIC VARIANTS ON FUNCTIONAL PROPERTIES OF MILK PROTEINS

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DOCTOR OF PHILOSOPHY

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ABSTRACT

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INFLUENCE OF GENETIC VARIANTS ON FUNCTIONAL PROPERTIES OF MILK PROTEINS

Isolated α_{s1}-casein, α_{s2}-casein, ß-casein, κ-casein of different phenotypes, whole casein of different phenotype combinations of α_{s1}-/ß-/κ-casein, and ß-lactoglobulin of different phenotypes were prepared and used in the study. Surface properties at the air-water interface, voluminosity and hydration, emulsifying capacity and emulsion stability, foaming and gelling properties were investigated to understand the relationship between the structure and functionalities of milk proteins. Phenotype CC of α_{s1}-casein, A^1A^1 and A^1A^2 of ß-casein, AB of κ-casein decreased surface tension at a faster rate than the other phenotypes within the casein system under consideration. ß-Casein, when compared to α_{s1}-casein, α_{s2}-casein and κ-casein, was the most surface active protein. The α_{s1}-/ß-/κ-casein haplotypes of BB/A^2A^2/AA and BB/A^1A^1/BB were found to be associated with higher surface activity than the other ten combinations of whole casein. α_{s1}-Casein BB, ß-casein A^2A^2 and κ-casein AB had higher values for voluminosity and hydration than the other phenotypes of the corresponding individual casein. Within the four caseins, ß-casein had the strongest ability to entrap water and the highest value for voluminosity, followed by α_{s1}-casein, α_{s2}-casein and κ-casein. Whole casein with the combination of BB/A^2B/BB had the highest value for

voluminosity and hydration. Analysis of model emulsions suggested that the emulsifying properties of different sources of protein were dependent on the oil content. α_{s1}-Casein BB in 10% oil emulsion, ß-casein A^2A^2 in 10% and ß-casein A^2B in 40% oil emulsions were the best stabilizers. κ-Casein AB, in both levels of oil emulsions, was superior to either AA or BB phenotypes. More stable emulsions were obtained for the whole casein with the haplotype $BB/A^1A^2/AB$ and $BB/A^1A^1/AB$ in 10% oil emulsions, and $BB/A^2A^2/AA$ in 40% oil emulsions. Characterization of foams formed with ß-lactoglobulin indicated that B variant was responsible for higher foaming capacity and higher stability, however, B variant produced less elastic and less firm foams than A variant. The foaming capacity and stability decreased and the foam firmness and elasticity increased gradually with increasing proportion of A variant in ß-lactoglobulin. The texture analysis of the gels that were produced with ß-lactoglobulin showed that AA phenotype generated the strongest, most elastic gels, with the highest water holding capacity. These levels decreased with the decreasing proportion of A variant in ß-lactoglobulin.

RÉSUMÉ

Hong GAO

Ph.D

Biochimie des produits laitiers
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L' influence des variations génétiques sur les propriétés fonctionnelles des lactoprotéines

Dans cette étude, nous avons préparé et utilisé : la α_{s1}-caséine, la α_{s2}-caséine, la ß-caséine et la κ-caséine de différents phénotypes, la caséine entière de différentes combinaisons de phénotypes de α-/ß-/κ-caséine, ainsi que la ß-lactoglobuline de différents phénotypes. Pour comprendre la relation entre la structure et les fonctions des protéines du lait, plusieurs propriétés ont été investiguées, comme les propriétés tensio-actives à l'interface air-eau, la voluminosité et l'hydratation, la capacité émulsifiante, la stabilité de l'émulsion, la propriété moussante et la propriété gélifiante. Le phénotype CC de la α_{s1}-caséine, le phénotype A^1A^1 et A^1A^2 de la ß-caséine, le phénotype AB de la κ-caséine diminuent les propriétés tensio-actives à un rythme plus élevé que les autres phénotypes du système de la caséine, pris en considération. Comparé à la α_{s1}-caséine, α_{s2}-caséine et à la κ-caséine, la ß-caséine a été la protéine dont les propriétés tensio-active ont été les plus efficaces. L'haplotype α_{s1}-/ß-/κ-caséine de BB/A^2A^2/AA et BB/A^1A^1/BB ont présenté des propriétés tensio-actives plus élevées que les dix autres combinaisons de la caséine entière. La α_{s1}-caséine BB, la ß-caséine A^2A^2 et la κ-caséine AB ont des valeurs de voluminosité et d' hydratation plus élevées que les autres phénotypes de chacune des caséines individuelles. Parmi les quatre caséines , la ß-caséine a eu la plus grande habileté de capter l'eau et les plus

grandes valeurs de voluminosité, suivie par la α_{s1}-caséine, α_{s2}-caséine et κ-caséine. La caséine entière, avec les combinaisons de BB/A^2B/BB, a présenté les plus grandes valeurs de voluminosité et le plus haut degré d'hydratation. L'analyse des modèles d'émulsion suggère que les propriétés émulsifiantes de différentes sources de protéines dépendent de leur contenu en huile. L'émulsion de la α_{s1}-caséine BB dans 10% d'huile, l'émulsion de la ß-caséine A^2A^2 dans 10% d'huile ainsi que l'émulsion de la ß-caséine A^2B dans 40% d'huile se sont avérées être les émulsions les plus stables. La κ-caséine AB, dans les deux concentrations d'huile, a été supérieure aux phénotypes AA et BB dans la formulation des émulsions. Des émulsions plus stables ont été obtenues pour la caséine entière avec les haplotypes BB/A^1A^2/AB et BB/A^1A^1/AB émulsionnés dans 10% d'huile et l'haplotype BB/A^2A^2/AA émulsionné dans 40% d'huile. La caractérisation de la mousse formée avec la ß-lactoglobuline a indiqué que la variant B était responsable d'une capacité moussante plus grande ainsi que d'une plus grande stabilité. Cependant, la variant B a produit une mousse moins ferme et moins élastique que la variant A. La capacité moussante et la stabilité de la mousse ont diminué et sa fermeté et son élasticité ont augmenté graduellement avec l'augmentation de la concentration de A. L'analyse de la texture des gels produits avec de la ß-lactoglobuline purifiée, a montré que le phénotype AA génère les gels les plus solides, les plus élastiques et ayant la plus grande capacité de rétention d'eau. Ces propriétés diminuent avec la diminution de la concentration de la variant A dans la ß-lactoglobuline.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

The original discovery of two types of genetic variants of ß-lactoglobulin (ß-Lg) by Aschaffenburg and Drewry (1955) has initiated considerable research activities on milk protein genetic polymophism. Genetic polymorphism of milk proteins arises from substitutions or deletions of some amino acids in the polypeptide chains. Variants A, B, C, D and E of α_{s1}-casein (α_{s1}-Cn); A, B, C and D of α_{s2}-casein (α_{s2}-Cn); A^1, A^2, A^3, B, C, D and E of ß-casein (ß-Cn); A, B, C and E of κ-casein (κ-Cn); A, B, C, D, E, F and G of ß-Lg; A , B and C of α-lactalbumin (α-La) in bovine milk constitutes the genetic polymorphism of bovine milk proteins (Eigel *et al.*, 1984; Ng-Kwai-Hang and Grosclaude, 1992). Three decades of studies have shown the existence of relationships between milk protein polymorphism and milk production traits, milk composition and technological properties of milk.

With regard to considering coagulation and cheesemaking properties of milk, it was found that variant B of κ-Cn has more favorable renneting properties with shorter coagulation time and firmer curd (Graham *et al.*, 1984; Maziali and Ng-Kwai-Hang, 1986a; McLean, 1986; Schaar, 1985). At the ß-Cn locus, the B variant is associated with shorter time of coagulating than the other variants (Graham *et al.*, 1984; McLean, 1986). The curd was found to be firmer for ß-Lg AA than for AB and BB phenotypes (Marziali and Ng-Kwai-Hang, 1986a). Cheese yield has been associated with the phenotypes of the milk proteins by various workers (Graham *et al.*, 1984; McLean, 1986, 1987; Marziali and Ng-Kwai-Hang, 1986b). Higher cheese yield was obtained with ß-Cn A^1A^1, κ-Cn BB and ß-Lg BB than from A^1A^2 of ß-Cn, AA and AB of κ-Cn and ß-Lg, respectively. The different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn were also found to affect cheesemaking properties and it was demonstrated that α_{s1}-Cn BB/ß-Cn BB/κ-Cn BB was associated with highest cheese dry matter yield, cheese solids, protein and fat recoveries than other phenotype combinations (Graham *et al.*, 1984; McLean, 1986). Heat stability of milk is another important technological property. In a large experiment including 4853 Angler and Friesian cows, Schulte-Coerne *et al.* (1992) confirmed earlier studies which related κ-Cn B variant with better heat stability. Similar differences were found for ß-Lg phenotypes. The observation showed that the heat coagulating time was in the order of

BB>AB>AA (Schulte-Coerne *et al.*, 1992). With studies on thermal stability of ß-Lg and a mixture of ß-Lg and κ-Cn, Imafidon *et al.* (1991a) reported that different genetic variants of ß-Lg have different onset temperatures, denaturation temperatures and width at half-peak height on differential scanning thermograms. They indicated that BB phenotype of ß-Lg is more stable than the other phenotypes at various of ß-Lg:κ-Cn ratios in phosphate buffer.

Physicochemical properties and functionalities of milk protein are also important characteristics that are affected by genetic variants even though not many reports have been published in that area. Recently, caseinates and whey protein concentrates have been increasingly used in the food industry because they are easy to prepae with high level of purity at low cost (Lorient *et al.*, 1989; Patel and Kilara, 1990). As food ingredients, their use relies upon their specific functional characteristics, such as surface activity, water holding capacity, emulsion and foaming properties, gelation, and heat stability. Milk protein functionalities extensively depend on their molecular structure, the association with other components (protein, fat, carbohydrates, ions), the temperature, the pH and the ionic strength of the medium in which they exist. The functionality will change more or less depending on the influence of the environmental conditions on the protein structure.

Although numerous studies have been performed on the physicochemical properties and functionalities on caseins and whey proteins, only a few (Feagan *et al.*, 1972; Graham *et al.*, 1984; Imafidon *et al.*, 1991a, b, 1992; Jenness and Parkash, 1967; Losi and Mariani, 1985; Marziali and Ng-Kwai-Hang, 1986a, b, c; McLean, 1986, 1987; McLean *et al.*, 1987; Schaar, 1985; Schmidt and Koops, 1965) have investigated the effects of the genetic variants of these proteins. Milk protein polymorphism that could be detected by electrophoresis is due to the differences in net charges of the genetic variants. These variants in the proteins may have profound effects on their particular functionalities. Polymorphic forms of caseins and whey protein have enough differences in their pattern of interactions to influence micelle stability, heat stability and coagulating properties (Imafidon *et al.*, 1991a, b). It is quite conceivable that functionalities such as interfacial activities, gelling, emulsifying and foaming properties may be influenced by genetic variants.

2. REVIEW OF LITERATURE

2.1 Milk and milk proteins

Milk is a complex fluid containing many components in several states of dispersion, which determine its nutritive quality, its values as a raw material for making food products, and many of its physicochemical properties. On the average, milk contains: 87.3% water, 3.6% fat, 3.3% protein, 4.8% lactose, 0.8% minerals, 0.18% organic acids and 0.14% miscellaneous compounds (Fox, 1989). Although milk is fairly constant in composition and properties, there is considerable quantitative variation. Chemical composition, size and stability of structure elements, and physical properties may all differ among batches of milk (Walstra and Jenness, 1984).

Caseins, a group of phosphoproteins, accounting for 76-86% of the proteins (Swaisgood, 1982) precipitate at pH 4.6. The main components of casein are made up of 40% α_{s1}-Cn, 10% α_{s2}-Cn, 38% ß-Cn and 12% κ-Cn (Fox, 1989).

Whey proteins, the other fraction of the milk protein are soluble at pH 4.6, and contain 60% ß-Lg, 20% α-La, 10% bovine serum albumin (BSA) and 10% of other minor proteins, including immunoglobulins and some proteolytic protein products (Harwalkar, 1985).

In addition to being the main nutritive component in the milk and dairy products, milk proteins are very important ingredients used in the food industry and non-food products manufacturing (de Wit, 1989).

2.2 Genetic polymorphism of milk proteins

Aschaffenburg and Drewry (1955, 1957a, b, c) were the first to demonstrate that ß-Lg exhibited genetic polymorphism. This phenomenon which also occurs in other milk proteins is due to substitution of one or more amino acids or deletion of several amino acids in the peptide chain (Fox, 1989). All the genetic variants so far described have been detected by electrophoretic techniques because the mutations involve changes in net charges of the proteins. Table 1 presents the locations of amino acid substitutions or deletions in genetic variants of proteins identified in bovine milk.

2.2.1 Occurrence and molecular basis of genetic polymorphism

Five genetic variants of α_{s1}-Cn, designated as A, D, B, C and E in order of decreasing electrophoretic mobility in alkaline gels containing urea and 2-mercaptoethanol, have been reported. The variants A, B, and C were discovered by Thompson *et al.* (1962) while D was first found in French Flamande cattle by Grosclaude *et al.* (1966a). The fifth variant, E, was reported in Nepalese Yak's milk ten years after the discovery of D variant (Grosclaude *et al.*, 1976b). α_{s1}-Casein is the major milk protein component and its amino acids residues were sequenced by Grosclaude *et al.* (1973) and Mercier *et al.* (1971). The primary structure of α_{s1}-Cn B contains 199 amino acid residues. Thirteen amino acid residues absence at position 14-26 of α_{s1}-Cn B yielded variant A. Variant C and D differ from B by having a glycine instead of a glutamic acid at position 192, and a threonine instead of alanine at position 53, respectively. The substitution of glutamine for lysine at position 59 and glutamic acid for glycine at position at 192 of α_{s1}-Cn B gives α_{s1}-Cn E variant. Figure 1 illustrates the primary structure of one known genetic variant of α_{s1}-Cn, the enclosed amino acid residues are sites corresponding to mutational differences in the genetic variants A, B, C and D.

Grosclaude *et al.* (1976a) first reported the genetic polymorphism of α_{s2}-Cn. They used the designation A for the already known variant common in European breeds and assigned the letter B to a newly discovered variant in cattle (*Bos taurus*) and Zebus (*Bos indicus*), and the letter C to another variant observed in yaks (*Bos gruniens*). Later, Grosclaude *et al.* (1979) reported a fourth variant, D, among the European breeds of *Bos taurus* in France in the Vosgienne and Montbéliarde breeds. The primary structure of α_{s2}-Cn is shown in Fig. 2. α_{s2}-Casein consists of 207 amino acid residues with a calculated molecular weight of 25,230 and was sequenced by Brignon and coworkers (1976; 1977). The α_{s2}-casein D variant involves deletion of 9 amino acid residues in one of the following sequences: 50-58, 51-59 or 52-60 (Grosclaude *et al.*, 1978, 1979). The difference between the primary structures of variants A and C of α_{s2}-Cn is due to the substitution of glutamic acid, alanine and threonine at positions 33, 47

Table 1 Positions and amino acid differences in genetic variants of milk proteins[a]

Protein[b]	Variants	Position and amino acid in the protein						
			14-26		53		59		192
α_{s1}-Cn	A		Deleted						
(199)	B				Ala		Gln		Glu
	C								Gly
	D				Thr				
	E						Lys		Gly
			33	47		50-58		130	
α_{s2}-Cn	A		Glu	Ala				Thr	
(207)	B								
	C		Gly	Thr				Ile	
	D					Deleted			
		18	35	36		37	67	106	122
ß-Cn	A^1						His		
(209)	A^2	SerP	SerP	Glu		Glu	Pro	His	Ser
	A^3							Gln	
	B						His		Arg
	C		Ser			Lys	His		
	D	Lys							
	E			Lys					
			97		136		148		155
κ-Cn	A				Thr		Asp		Ser
(169)	B		Arg		Ile		Ala		
	C		His						
	E								Gly
								129 or	
		45	50	59	64	78	118	130	158
ß-Lg	A				Asp		Val		
(162)	B	Glu	Pro	Gln	Gly	Ile	Ala	Asp	Glu
	C			His					
	D	Gln							
	E								Gly
	F		Ser					Tyr	Gly
	G					Met			Gly
			10			?			
α-La	A		Gln						
(123)	B		Arg			Asp			
	C					Asn			

a: Adapted from Genetic Polymorphism of Milk Proteins, Ng-Kwai-Hang and Grosclaude, 1992
b: Numbers in parentheses indicate the total number of amino acid residues in the protein

or 130 for glycine, threonine and isoleucine, respectively (Mahé and Grosclaude, 1982).

The recommended designations for the different variants of ß-Cn are: A^1, A^2, A^3, B, C, D and E (Eigel *et al.*, 1984). Aschaffenburg (1961) discovered three variants, denoted as A, B and C. The A variant of ß-Cn could be resolved into A^1, A^2 and A^3 under acidic conditions of gel electrophoresis (Peterson and Kopfler, 1966). D is a rare variant which was found in the Deshis breed of India, and the Borans of Kenya (Aschaffenburg, 1968). The variant E was discovered by Voglino (1972) in the Piedmont breed of Italy. In alkaline gels containing urea, the decreasing order of electrophoretic mobility for the seven variants of ß-Cn is: $A^1 = A^2 = A^3 > B > D, E > C$ (Kiddy, 1975). Within ß-Cn, the A^2 variant was the first to be completely sequenced (Ribadeau-Dumas *et al.*, 1972). The locations along this polypeptide chain of 209 amino acids where mutations have occurred to produce several genetic variants were later elucidated by the same group of researchers (Grosclaude *et al.*, 1972a, b, 1974a, b). Figure 3 shows the primary structure and the mutation positions for ß-Cn. Compared to the sequence of ß-Cn A^2, variant A^1, B, and C differ by having a histidine instead of proline at position 67. Variant B also differs from A^2 by having an arginine instead of a serine at position 122. ß-Casein C also differs from A^2 by a glutamic acid to lysine substitution at position 37 and the absence of glutamic acid at position 37 leads to a non-phosphorylated serine at position 35 in variant C. The rare variant A^3 is the result of a substitution of histidine in variant A^2 at position 106 for a glutamine. Variant D of ß-Cn is produced from variant A^2 by a replacement of serine at position 18 of A^2 by a lysine. Substitution of lysine for glutamic acid in variant A^2 at position 36 leads to the variant E. The fractions denoted as γ^1-, γ^2- and γ^3-Cn which were observed as gel electrophoretic bands or chromatographic peaks are now recognized as proteolytic breakdown fragments of ß-Cn, and represent amino acid residues at position 29-209, 106-209 and 108-209 respectively (Tripathi and Gehrke, 1969; Gordon *et al.*, 1972; Groves *et al.*, 1972, 1973). κ-Casein is the only phosphoglycoprotein in the casein family and the carbohydrate component is

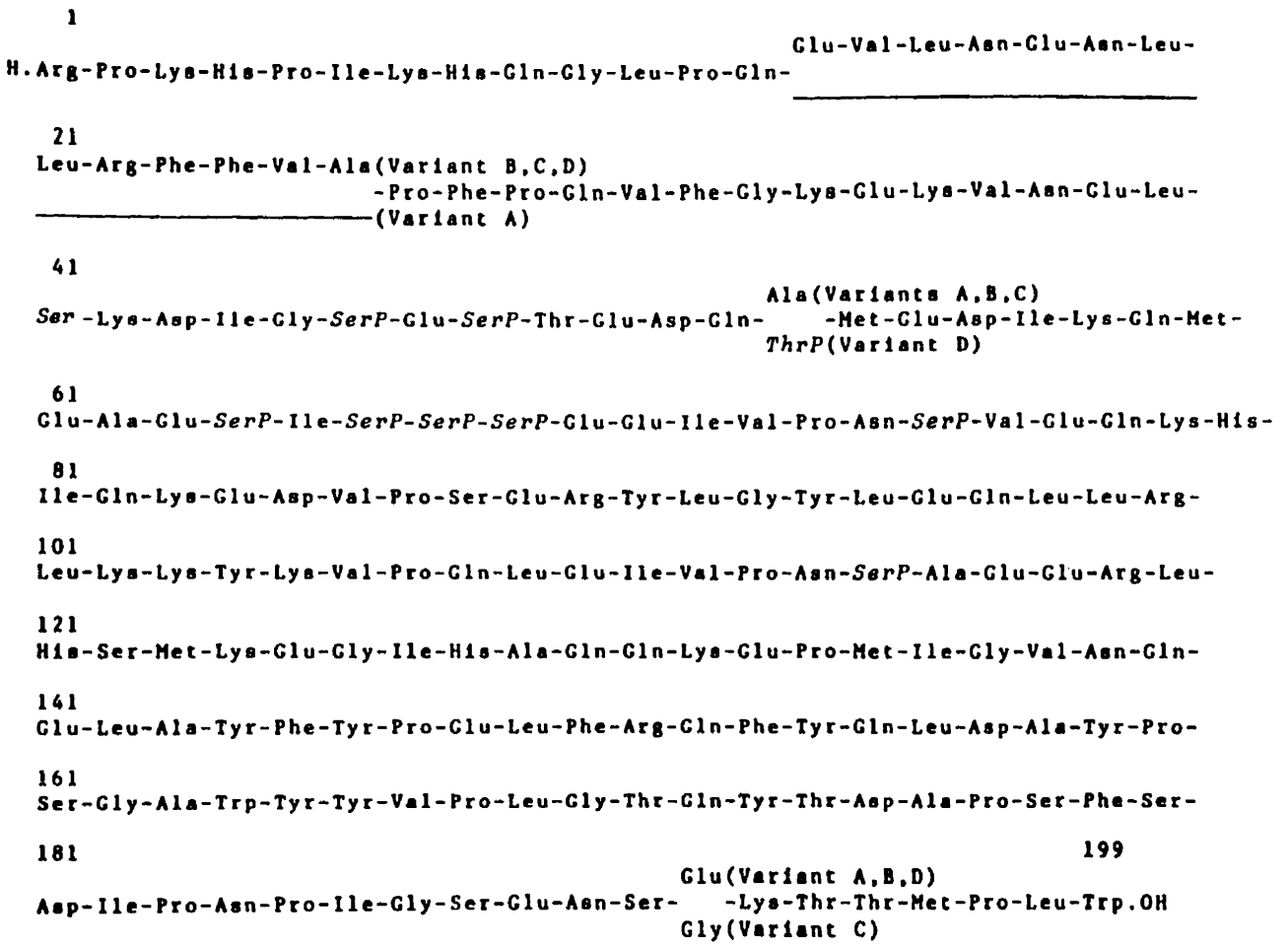

Fig. 1 Primary structure of known genetic variants of α_{s1}-casein. The enclosed amino acid residues are sites corresponding to mutational differences in the genetic variants: A, B, C and D. Sites of post-translational phosphorylation are in italics (P. F. Fox, 1989).

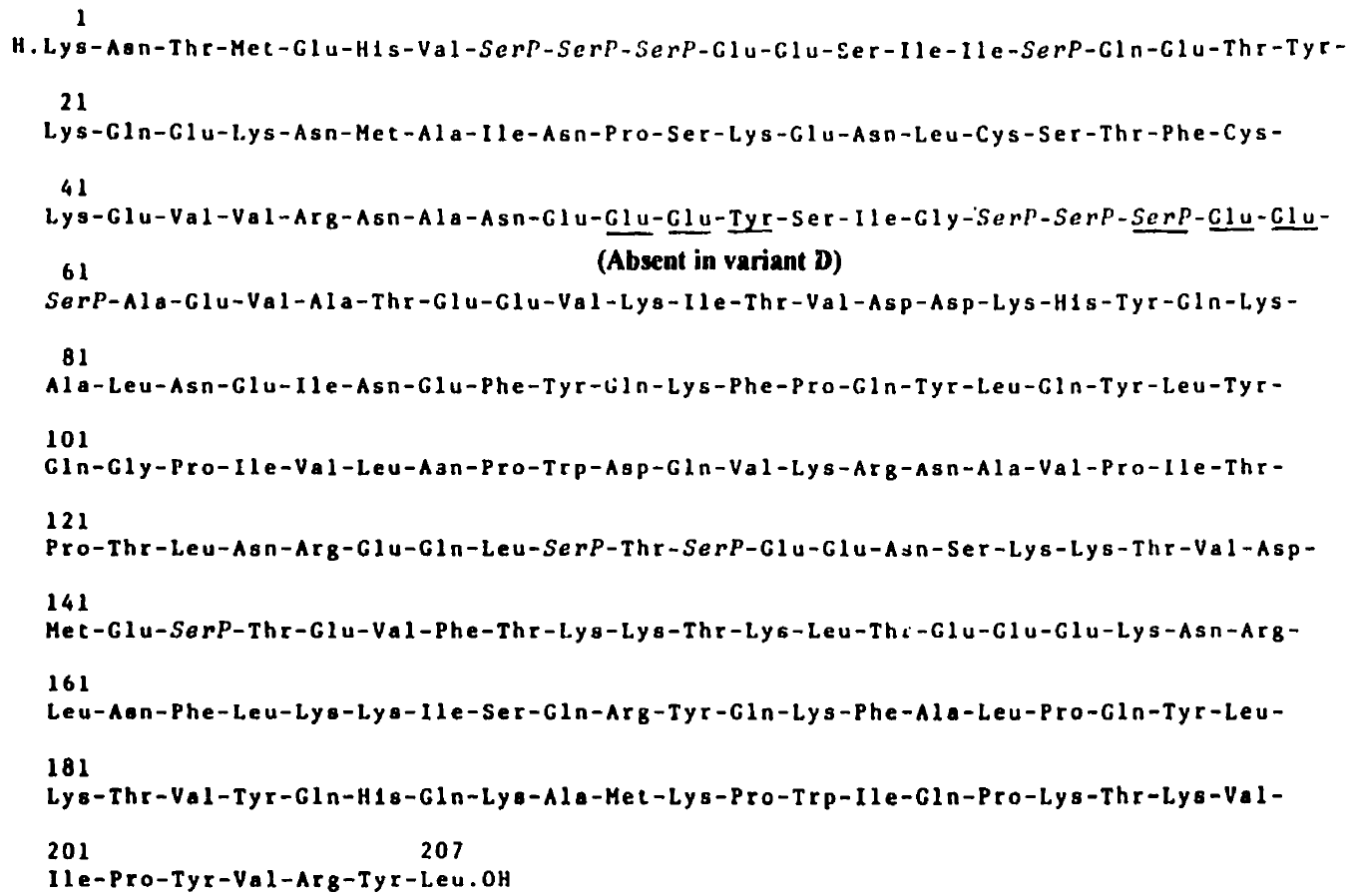

Fig. 2 Primary structure of known genetic variants of α_{s2}-casein. The amino acid sequences in bracket represent the possible amino acid sequence missing in the D variant. Sites of post-translational phosphorylation identified in the species containing 11 orthophosphate are in italics (Eigel *et al.*, 1984).

represented by N-acetyl neuraminic acid (Fournet *et al.*, 1979; van Halbeek *et al.*, 1980). The inclusion of 2-mercaptoethanol in urea-containing gels disclosed the presence of genetic polymorphism in κ-Cn (Grosclaude *et al.*, 1966b). Each of the two variants A and B of κ-Cn revealed multiple bands when subjected to alkaline electrophoresis due to differences in degree of glycoslation (Pujolle *et al.*, 1966; Doi *et al.*, 1979). The most common variants of κ-Cn are A and B. The third variant C was described by Di Stasio and Merlin (1979). Two additional variants, D and E, were detected by Seibert *et al.* (1987) and Erhardt (1989) through the techniques of isoelectric focusing. Results of amino aid sequencing of variant C, D and E revealed that variants C and D are the same (Erhardt, 1989). The primary structure of κ-Cn shown in Fig.4 was elucidated by Mercier *et al.*, (1973). κ-Casein contains 169 amino acid residues and A variant differs from B by the substitutions of threonine for isoleucine at position 136 and of aspartic acid for alanine at position 148 (Whitney *et al.*, 1976). Substitution of arginine in variant B for histidine at position 97 yield variant C. The variant E is the result of a glycine being replaced by serine at position 155 of variant A.

Consisting of seven genetic variants, ß-Lg is the main component of whey proteins and is the first milk protein where genetic polymorphism was found in cows' milk. The first two variants, A and B, were discovered by Aschffenburg and Drewry (1955). The presence of variant C was later detected in the milk of Jersey cows (Bell, 1962). Grosclaude *et al.* (1966a) identified the fourth variant, D, in the French Montbeliarde breed. Another variant designated as Dr which is unique to the Droughtmaster cattle was described by Bell *et al.* (1970a). Variant Dr differs from variant A by the substitution of asparagine for aspartic acid at position 28, the site of attachment for the carbohydrate moiety. Yak milk has been shown to contain only one type of ß-Lg, D_{yak} (Grosclaude *et al.*, 1976a, 1982), which has the same electrophoretic mobility as bovine ß-Lg D. In order to avoid the use of subscript and superscript in milk protein nomenclature, Eigel *et al.*(1984) suggested that the variant in yak be labeled as E due to its order of discovery. The primary structure of known genetic variants of ß-Lg is

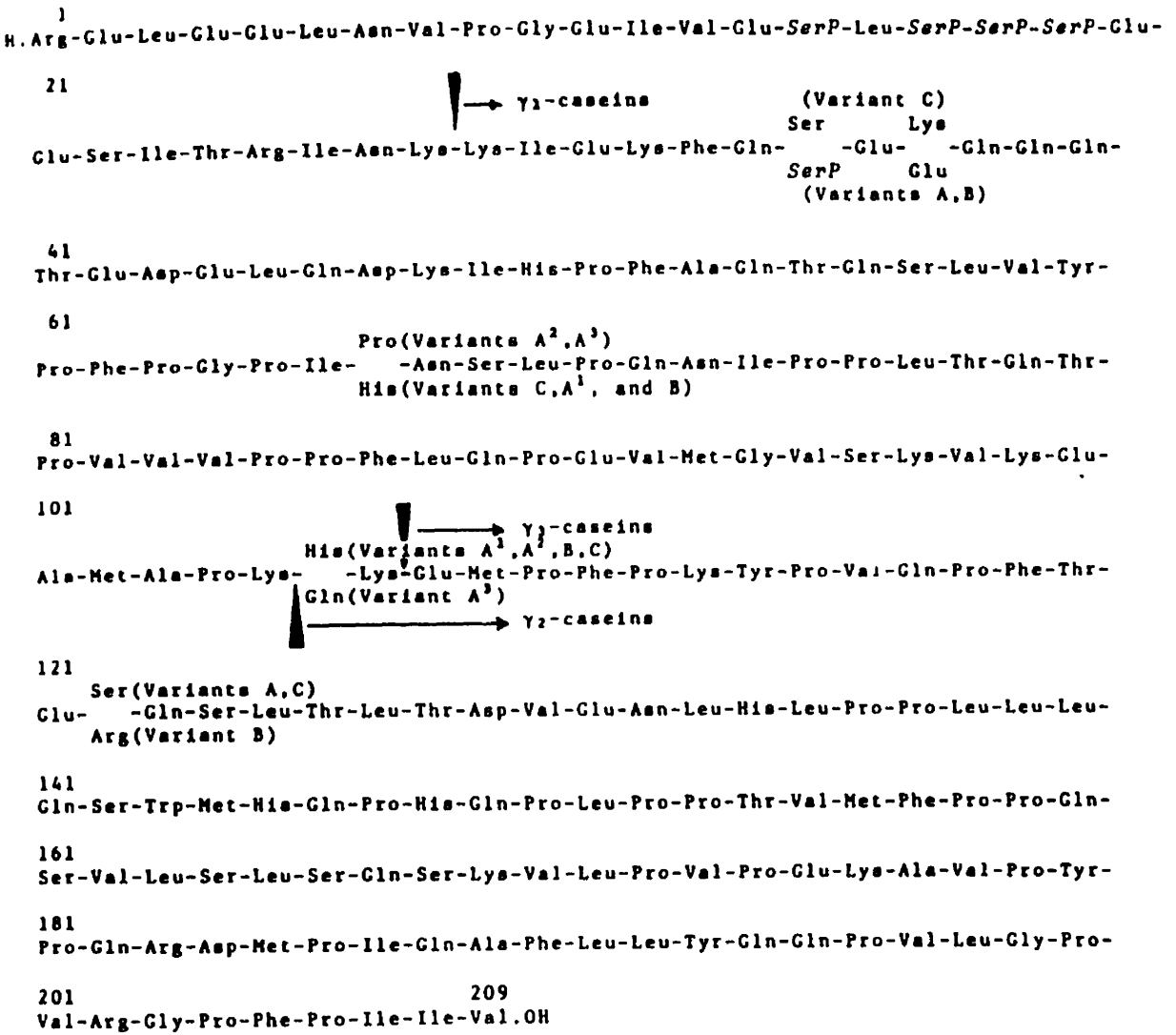

Fig. 3 Primary structure of known genetic variants of ß-cascin A^1, A^2, A^3, B and C. Sites of post-translational phosphorylation are in italics. Sites of post-translational hydrolysis by plasmin yield the resulting γ-cascins and components 5 and 8 are also indicated (P. F. Fox, 1989).

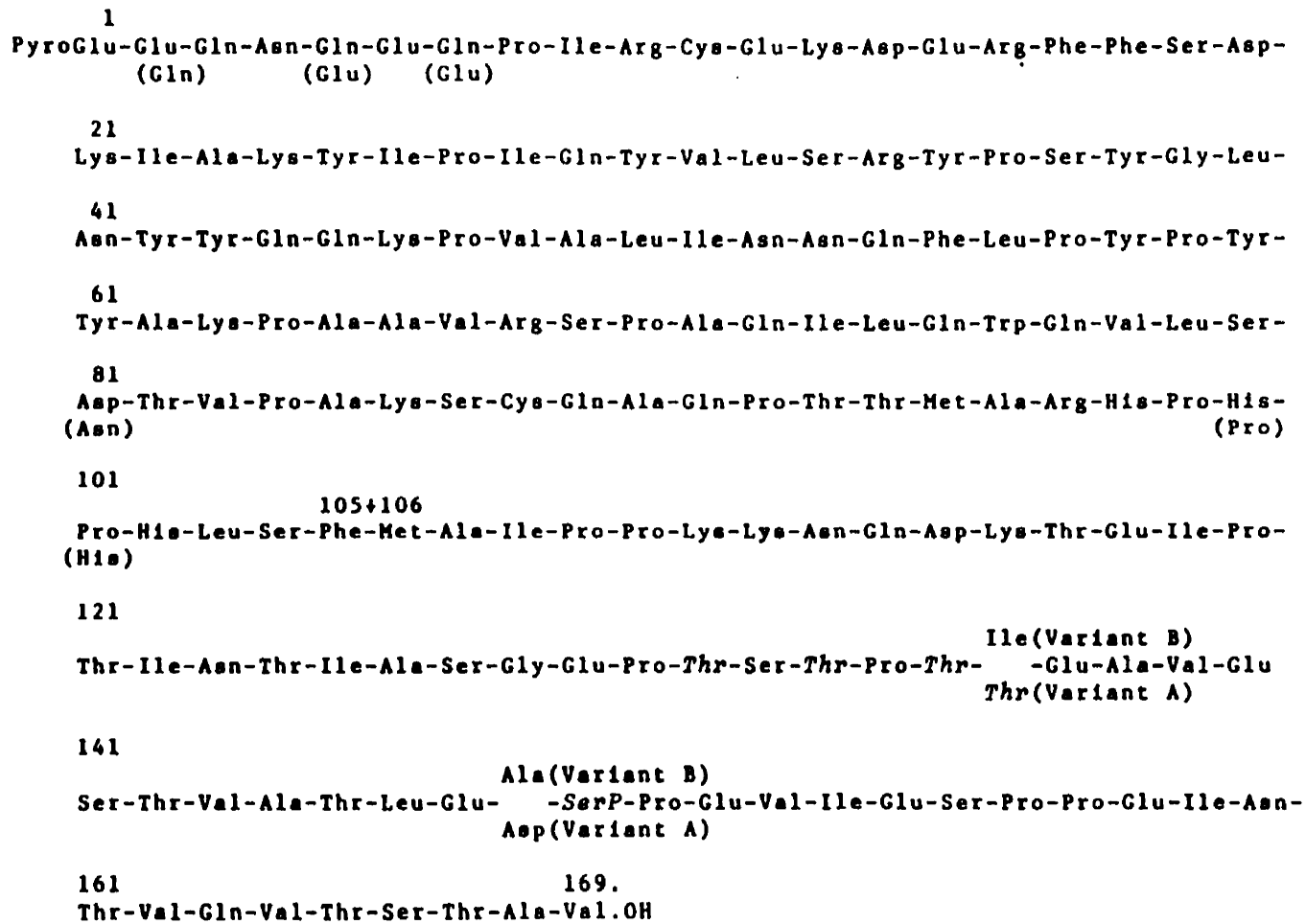

Fig. 4 Primary structure of known genetic variants of κ-casein. The enclosed amino acid residues are sites corresponding to mutational differences in the A variant. Sites of post-translational phosphorylation and glycosylation are in italics (P. F. Fox, 1989).

shown in Fig. 5. The substitution of aspartic acid at position 64 and valine at position 118 by glycine and alanine, respectively, constitute the difference between variant A and B. Substitution of glutamine at position 59 of variant B by a histidine yield variant C, while replacement of glutamic acid at position 45 of variant B by a glutamine results in variant D (Brignon and Ribadeau-Dumas, 1973). In yak milk, variant E differs from variant B in the substitution of glycine at position 158 for glutamic acid. Both variants F and G have glycine at position 158 replacing glutamic acid in variant B, but F has one additional substitution of serine for proline at position 50 and tyrosine for aspartic acid either at position 129 or 130. ß-Lactoglobulin exists as a noncovalent dimer and is sensitive to heat denaturation (Schmidt and Morris, 1984).

With a calculated molecular weight of 14175 (Brew *et al.*, 1970; Eigel *et al.*, 1984), α-La contains 123 amino acid residues. The most common breeds of cattle have the B variant of α-La, and the A variant is present among the Zebu cattle in Nigeria (Blumberg and Tombs, 1958). The third variant of α-La designated as C, moves slower than variant B, was reported by Bell *et al.* (1981a) in Bali cattle. Brew *et al.* (1970) determined the complete amino acid sequence for α-La A and B variants. The difference between these two variants is just by a single substitution of glutamine for arginine at position 10. The exact amino acid substitutions involved that differentiate variant C, observed in Bos javanicus (Bell *et al.*, 1980a), from A or B have not yet been established. It was tentatively proposed that variant C differs from B by an amide group, most probably by a substitution of asparagine for aspartic acid (Ng-Kwai-Hang and Grosclaude, 1992).Three genetic variants of A, B and C caused by amino acid substitutions are illustrated in Fig. 6.

2.2.2 Methods for detecting genetic polymorphism of milk proteins

Paper electrophoresis under alkaline conditions was the original method developed to detect the occurrence of genetic polymorphism of milk protein by Aschaffenburg and Drewry (1955). Subsequently several other techniques including electrophoresis with other support media, isoeletric focusing, and column chromatography were developed for identification of genetic variants.

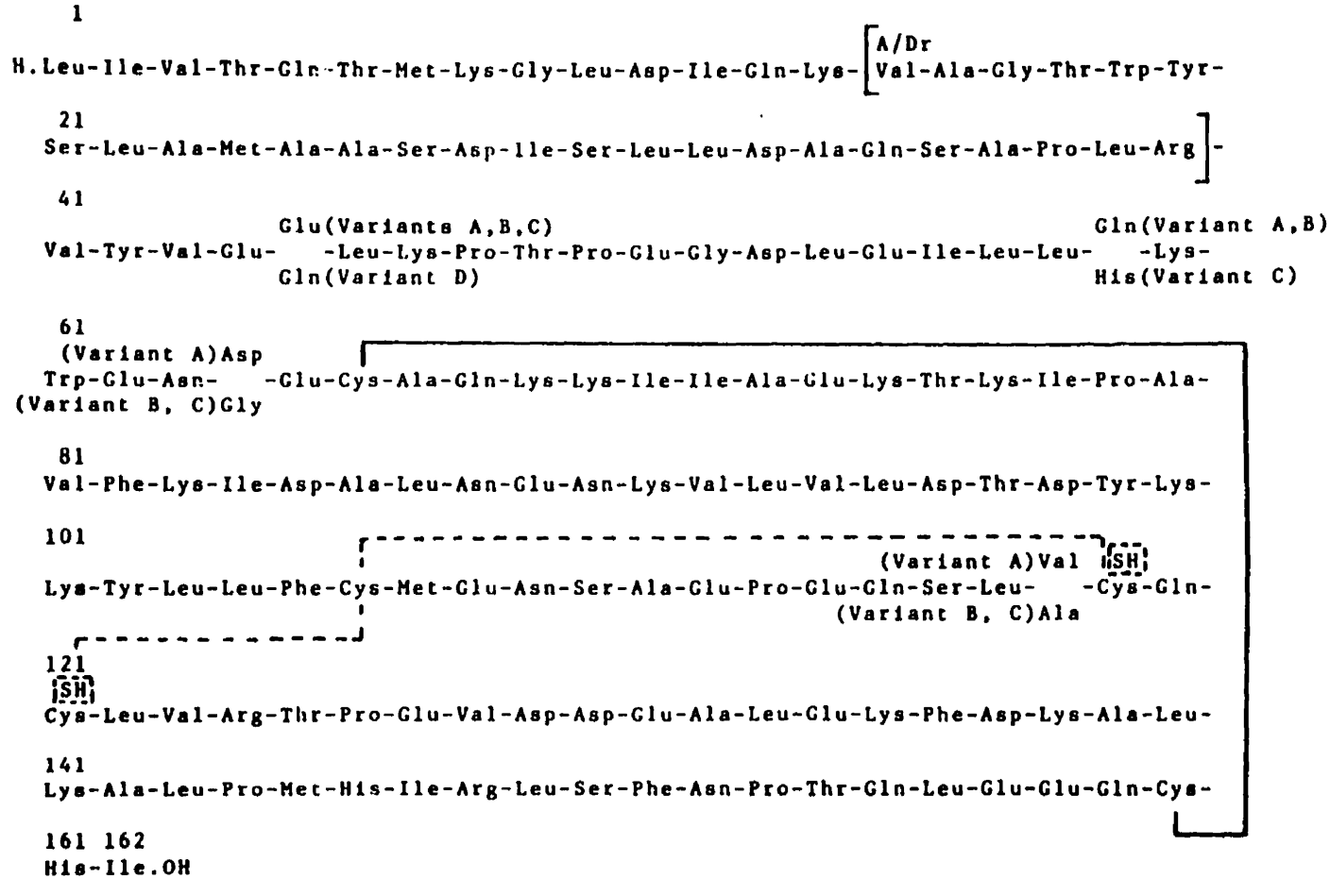

Fig. 5 Primary structure of known genetic variants of ß-lactoglobulin. The enclosed amino acid residues are sites corresponding to mutational differences in the genetic variants (Eigel *et al.*, 1984). The dashes indicate that some molecules may have the single sulphydryl group at position 119 and 121 (P. F. Fox, 1989).

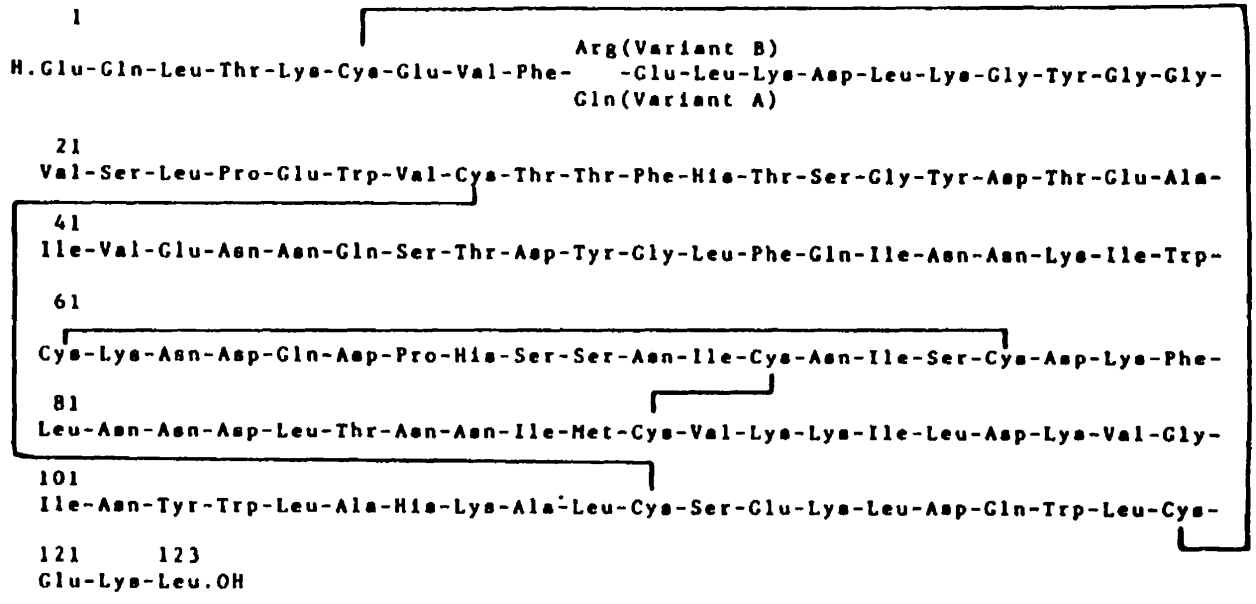

Fig. 6 Primary structure of known genetic variants of α-lactalbumin. The enclosed amino acid residues are sites corresponding to mutational differences in the genetic variants (P. F. Fox, 1989). Residues 46 and 49 may be reversed (Shewale et al., 1984).

2.2.2.1 Electrophoresis

The original paper electrophoretic technique of Aschaffenburg and Drewry (1955) was performed in barbitone buffer at pH 8.6 under 230 V for 16 h. By applying this method, Blumberg and Tombs (1958) identified the genetic variants of α-La in Nigerian White Fulani cattle. Thompson *et al.* (1962) introduced the use of starch gel electrophoresis with urea at pH 8.6 and they detected three variants denoted as A, B and C for α_{s1}-Cn in the Holstein, Brown Swiss and Ayrshire breeds. Bell (1962) used this method without the addition of urea in the starch gel and achieved the resolution of variants A, B and C for ß-Lg. Grosclaude *et al.* (1966a) modified the method of Wake and Baldwin (1961) by including 2-mercaptoethanol in the starch gel for the separation of A, B and D variants of ß-Lg. Thompson *et al.* (1964) and Michalak (1967) isolated the variants A, B and C in ß-Cn successfully by applying the starch gel electrophoresis. The polyacrylamide gel electrophoresis was introduced by Aschaffenburg (1964) and Thompson *et al.* (1964) to identify the genetic polymorphism of milk proteins. Under alkaline conditions, the variants of α_{s1}-Cn, ß-Cn and κ-Cn could be well separated except for the A^1, A^2 and A^3 variants of ß-Cn which were not resolved. By employing polyacrylamide gel containing 4.5 M urea at pH 2.8, Peterson and Kopfler (1966) found that the previously identifiable A variant of ß-Cn could be separated into three variants now known as A^1, A^2 and A^3. Other supporting media for electrophoresis include agar gels which have been used by Aschaffenburg (1965) for the phenotyping of ß-Lg in pH 8.6 veronal buffer. Different zone electrophoretic techniques for the separation of major milk proteins has contributed enormously to the study of genetic polymorphism. Consequently, selection of any one of the above methods depends on the protein variants to be detected and the availability of equipment (Aschaffenburg, 1968; Thompson, 1970).

2.2.2.2 Isoelectric focusing

Isoelectric focusing can be considered as a special type of gel electrophoresis whereby each protein migrates to a specific zone that is its isoelectric point across a pH gradient (Ng-Kwai-Hang and Grosclaude, 1992). Although a longer running time is

required for isoelectric focusing than for polyacrylamide gel electrophoresis, this separation method has the capability of resolving proteins differing by only 0.02 unit in their isoelectric points (Peterson, 1969). Whole casein has been shown to produce as many as 20 bands when subjected to electrophoresis across a pH gradient of 3.0 to 10.0 in 5% polyacrylamide gels containing urea. By using isoelectric focusing technique, A^1, A^2, A^3, B and C variants of ß-Cn can be separated simultaneously with one running. Josephson (1972) reported successful identification of κ-Cn A and B through the use of pH gradient electrophoresis of bovine milk caseins. Pearce and Zadow (1978) separated variant A and B of ß-Lg by employing this technique at a pH range of 3.5 to 5.0 in 5% polyacrylamide gel containing 6 M urea. A procedure for simultaneously phenotyping the four major caseins by isoelectric focusing was described by Addeo *et al.* (1983). The use of agarose gel containing 7 M urea as the support medium with a pH gradient of 2.5 to 7.0 could also produce good results for the phenotyping of α_{s1}-Cn, ß-Cn, κ-Cn and ß-Lg (Bech and Munk, 1988). The use of isoelectric focusing for the phenotyping of milk proteins can be considered as an alternative to starch gel or polyacrylamide gel electrophoresis (Ng-Kwai-Hang and Grosclaude, 1992).

2.2.2.3 Column chromatography

Yaguchi and Rose (1971) reviewed the application of column chromatographic techniques for the separation and purification of milk proteins. The most commonly used stationary phase in column chromatography is based on the principle of either ion exchange or size exclusion. For the purpose of phenotyping milk proteins, both ion exchange and size exclusion are of limited value. The former technique is tedious and time consuming because it requires the process of equilibrating the column, doing the actual separation, and regenerating the column for the next sample. The latter method is not sensitive enough to detect the small differences in molecular weights between the several variants of a specific protein. Nevertheless, it is interesting to note the possibility of separating the ß-Cn variant A from C in a DEAE-cellulose system containing urea (Thompson and Pepper, 1964), and by QAE-cellulose ion exchange

chromatography (Ng-Kwai-Hang and Pélissier, 1989). Chromatography on a DEAE-cellulose column using imidazole-HCl-urea buffer at pH 7.0 (Thompson, 1966) could resolve variants A and B of κ-Cn. With the development of high performance liquid chromatography (HPLC), the analysis time required for milk proteins analysis has been shortened. For instance, the HPLC system described by Pearce (1983) was able to resolve α-La and the variants A and B of ß-Lg within 30 minutes. In the process of fractionating bovine caseins by HPLC on a reversed phase column, Carles (1986) discovered a new variant of ß-Cn which is more hydrophobic than A^1 by having a leucine instead of a proline in the 114-169 region of ß-Cn. This newly found variant is one of the examples of the so called 'silent variants' because the substitution of an amino acid in this case would not result in a change in the net electrical charge of the protein and hence such variants would not be resolved by electrophoresis or ion exchange chromatography.

2.2.2.4 Identification of genetic variants at the DNA level

Since genetic polymorphism observed in the milk proteins is a consequence of mutations which result in changes in the nucleotide sequence of the particular gene, it is possible to detect genetic variants at the DNA level. Prior to genotyping milk protein in the genes, information is required regarding the primary structure and the positions along the peptide chain where amino acid substitutions occur for the coded protein. Based on these observations, the polymerase chain reaction could be used to amplify the region of interests in an *in-vitro* system containing DNA extract isolated from various cells such as blood or semen samples. The amplification then is followed by digestion with restriction enzymes and analysis of the resulting fragments by electrophoresis. The number of electrophoresis bands, obtained from specific restriction endonucleases, identify the variant in question (Ng-Kwai-Hang and Grosclaude, 1992). By using this technique, Zadworny *et al.* (1990) isolated DNA from semen samples of Holstein bulls and determined their genotypes for κ-Cn.

2.2.3 Distribution of milk protein genetic variants

All the sensitive variants of milk proteins are not universally distributed among cattle breeds (Aschaffenburg, 1968; Baker and Manwell, 1980; Li and Gaunt, 1972; Thompson and Farrell, 1974; Zikakis *et. al.*, 1974). Data summarized in Table 2 show the gene frequencies of the polymorphs of the major milk protein for some of the common dairy breeds like Holstein-Friesian, Jersey, Ayrshire and Brown Swiss.

In most Western breeds, α_{s1}-Cn B, α_{s2}-Cn A and ß-Cn A^1 and A^2 occur with a much higher frequency than the other genetic variants. For instance, the gene frequency for the B variant of α_{s1}-Cn ranged from 0.87 to 0.99 in Holstein Friesian; 0.70 to 0.79 in Guernsey; 0.72 to 0.92 in Jersey; 1.00 in Ayrshire; and 0.94 to 0.98 in Brown Swiss (Aschaffenburg, 1968; Hoogendoorn *et al.*, 1969; Ng-Kwai-Hang *et al.*, 1984b; Li and Gaunt, 1972). Unlike other Western breeds, Jersey in Canada has the frequency of 0.43 for C variant which is much higher than 0.26 for Jersey in United States and 0.08 for Jersey in Germany (Kim, 1994). With frequency of 0.67 in Indian Zebu and 0.84 in African Boran, the C variant is more prevalent than the B variant of α_{s1}-Cn (Aschaffenburg, 1968).

The four recognized genetic variants A, B, C, and D of α_{s2}-Cn are breed specific. It was observed that A is the major variant in European breeds *(Bos taurus)*; D in the Vosgienne and Montbeliarde breeds (Grosclaude *et al.*, 1978); B in *Bos taurus*, and C in *Bos indicus* and *Bos grunniens* (Grosclaude *et al.*, 1978). The milk from Zebu cows also contains the B variant of α_{s2}-Cn (Grosclaude *et. al.*, 1979).

The A^1 and A^2 variants of ß-Cn occur with respective gene frequencies of: 0.31 to 0.66 and 0.46 to 0.62 in Holstein-Friesian (Aschaffenburg, 1968; Han *et al.*, 1985; Hoogendoorn *et al.* 1969; Li and Graunt, 1972; Ng-Kwai-Hang *et al.*, 1984b). The A^2 gene is most prevalent among Guernsey (Zikakis *et al.*, 1974), Indian Zebu and Jersey (Aschaffenburg, 1968). The A^1 variant predominates in Ayrshire while the A^2 predominates in Brown Swiss (Aschaffenburg, 1968, Li and Graunt, 1972). The B variant of ß-Cn is very rare in Holstein-Friesian and Ayrshire but occurs at much higher frequencies among Jersey which has the frequency of 0.31 in Canada, 0.37 in

Table 2 Gene frequencies of the polymorphs of the major milk protein for common breeds

Protein	Holstein-Friesian			Jersey			Ayrshire			Brown Swiss	
α_{s1}-Casein											
Location	US[a]	Canada[b]	Holland[a]	US[a]	Canada[c]	Germany[a]	US[a]	Canada[c]	UK[a]	US[a]	Canada[c]
Number tested	542	6788	693	287	1711	148	257	2610	52	282	1034
Gene frequency											
A	0.05	0.001	0	0	0	0	0	0	0	0	0
B	0.87	0.985	0.99	0.74	0.569	0.92	1.00	>0.999	1.00	0.98	0.973
C	0.08	0.004	0.01	0.26	0.431	0.08	0	<0.001	0	0.02	0.027
D	0	0	0	0	0	0	0	0	0	0	0
ß-Casein											
Location	US[a]	Canada[b]	UK[a]	US[a]	Canada[c]	UK[a]	US[a]	Canada[c]	UK[a]	US[a]	Canada[c]
Number tested	632	6460	85	298	1688	47	202	3596	29	235	1027
Gene frequency											
A^1	0.31	0.536	0.66	0.09	0.193	0.09	0.67	0.601	0.60	0.15	0.316
A^2	0.62	0.443	0.24	0.54	0.499	0.63	0.32	0.396	0.40	0.72	0.518
A^3	0.05	0.006	0.04	0	0.001	0	0.01	<0.001	0	0	0.005
A	0.98	0.986	0.94	0.63	0.692	0.72	1.00	0.998	1.00	0.82	0.939
B	0.02	0.014	0.06	0.37	0.308	0.28	0	0.002	0	0.18	0.161
C	0.001	0	0.06	0.003	0	0	0	0	0	0.03	0
D	0	0	0	0	0	0	0	0	0	0	0
κ-Casein											
Location	US[a]	Canada[b]	UK[a]	US[a]	Canada[c]	UK[a]	US[a]	Canada[c]	UK[a]	SA[a]	Canada[c]
Number tested	138	6509	189	297	1709	70	211	3607	52	30	1018
Gene frequency											
A	0.85	0.753	0.83	0.12	0.26	0.24	0.70	0.881	0.80	0.70	0.704
B	0.15	0.247	0.17	0.88	0.74	0.76	0.30	0.119	0.20	0.30	0.293
C	0	0	0	0	0	0	0	0	0	0	<0.003
ß-Lactoglobulin											
Location	US[a]	Canada[b]	Australia[a]	US[a]	Canada[c]	Australia[a]	US[a]	Canada[c]	Australia[a]	US[a]	Canada[c]
Number tested	406	8469	108	270	1708	448	191	3603	104	259	1033
Gene frequency											
A	0.46	0.354	0.51	0.36	0.442	0.26	0.17	0.240	0.38	0.33	0.417
B	0.54	0.646	0.49	0.64	0.558	0.64	0.83	0.760	0.62	0.67	0.583
C	0	0	0	0	0	0.10	0	0	0	0	0

a: Swaisgood, 1992
b: Ng-Kwai-Hang *et al.*, 1990
c: Kim, 1994

U.S.A. and 0.28 in Germany (Kim, 1994). Compared to Holstein-Friesian, Ayrshire and Jersey, the frequency of B variant in Brown Swiss is intermediate with values of 0.16 to 0.18 (Kim, 1994).

The A allele of κ-Cn tends to predominate in the majority of breeds with a gene frequency of 0.41 to 0.84. In Jersey, Normande and Zebu, the κ-Cn B allele occurs with higher frequencies of 0.99, 0.66, and 0.74, respectively (Aschaffenburg, 1968).

The pattern of distribution of the B variant of ß-Lg tends to be more consistent than the other variants. In Western breeds of cattle, the gene frequency of ß-Lg B is higher than A, C and D variant (Swaisgood, 1992; Ng-Kwai-Hang and Grosclaude, 1992). The occurrence of the variants E, F and G of ß-Lg is unique to Banteng, *Bos (Bibos) javanicus* (Bell *et al.*, 1981b). The data for the gene frequencies of the variants are not available for comparative purposes.

Only the B variant of α-La has been observed in milk of the Western breeds of cattle, whereas both A and B variants occur in milk from African Fulani, African and Indian Zebu cattle (Aschaffenburg, 1963; Bhattacharya *et al.*, 1963). The C variant, occurs only in Banteng, *Bos javanicus* (Bell *et al.*, 1981c).

2.2.4 Significance of milk protein polymorphism

2.2.4.1 Association with milk composition

Various workers have found significant association of genetic variants of proteins with milk composition. McLean *et al.* (1984), Golikova and Panin (1972) reported higher concentration and relative proportion of α_{s1}-Cn and lower concentration and proportion of κ-Cn associated with α_{s1}-Cn C when compared with type B. Ng-Kwai-Hang *et al.* (1986) found the highest concentrations of fat, protein, and casein in Holstein-Friesian cows' milk of phenotype A^1B for ß-Cn. It was found that ß-Cn A^1B was superior to other phenotypes in both concentration and proportion of ß-Cn in Jersey and Friesian cows (McLean *et al.*, 1984). In this study, a higher κ-Cn concentration and lower α_{s1}-Cn concentration were associated with ß-Cn B milk as compared with type A milk. Higher fat concentration was associated with ß-Cn A^2A^2 and BB milk when compared to A^1A^2 milk (McLean *et al.*, 1984). The research of

Kiddy *et al.*(1970) demonstrated that ß-Cn A^2 was superior to A^1 in terms of Holstein-Friesian cows. Munro (1978) found that A^1 of ß-Cn for fat concentration was superior to other types in Jersey cows.

The B variant of κ-Cn was found to be associated with higher concentrations and relative proportions of κ-Cn and lower proportions of α_{s1}-Cn when compared to κ-Cn A cows (Mariani *et al.*, 1976; McLean *et al.*, 1984; Michalak, 1973). Significantly higher total protein concentration for κ-Cn B was reported in several investigations (Hoogendoorn *et al.*, 1969; Munro, 1978). Phenotype BB of κ-Cn was shown to be associated with higher total casein and lower serum proteins than with the AA or AB type (Ng-Kwai-Hang *et al.*, 1984b). κ-Casein BB was also demonstrated to be associated with higher concentration of α_{s1}-Cn, κ-Cn and lower concentration of ß-Lg and α-La (Ng-Kwai-Hang *et al.*, 1987). It has been noted that κ-Cn BB milk contained a lower concentration of citric acid (Mariani *et al.*, 1979; Schaar, 1985) but a higher concentration of calcium and phosphorus when compared to AA phenotype (Mariani *et al.*, 1976). Milk from ß-Lg AA cows contained the highest concentration of ß-Lg and the lowest concentrations of α-La, BSA and Ig when compared with milk from ß-Lg BB cows; values for heterozygous AB were intermediate (Ng-Kwai-Hang *et al.*, 1987). An association between ß-Lg variants and concentration of ß-Lg has also been reported earlier by several investigators (Aschaffenburg and Drewry, 1955; Feagan, 1979; McLean *et al.*, 1984). The superiority of the ß-Lg B allele for fat concentration has been noted (Hoogendoorn *et al.*, 1969; McLean *et al.*, 1984; Ng-Kwai-Hang *et al.*, 1987).

Association of genetic polymorphism of milk proteins with milk composition and their physicochemical properties prompted speculation that there is also an indirect relationship between genetic variants of milk proteins and technological characteristics of milk.

2.2.4.2 Association with cheese yielding capacity and cheese composition

Several groups of workers have investigated whether the differences in milk composition, coagulation time and curd firmness associated with genetic polymorphism

produce the expected differences in cheese yielding capacity (Graham *et al.*, 1984; Losi *et al.*, 1979; Marziali and Ng-Kwai-Hang, 1986a,b; Morini *et al.*, 1979). Graham *et al.*(1984) have reported a considerable influence of the combined genotypes of α_{s1}-Cn, ß-Cn and κ-Cn and ß-Lg on cheddar cheese yield. This study compared the cheesemaking properties and cheese yield of milk from cows with the milk protein phenotype combination of α_{s1}-Cn BB, ß-Cn AB or BB, κ-Cn BB and ß-Lg AB or BB (B type) with matched α_{s1}-Cn BB, ß-Cn AA, κ-Cn AA, ß-Lg AA cows (A type). Cheese dry matter yield was higher with type B milk. Other studies which compared κ-Cn genetic variants for Parmesan cheesemaking had found that κ-Cn B milk had a superior yield of cheese due to a greater proportion of fat being converted to cheese (Mariani *et al.*, 1976; Morini *et al.*, 1979). Marziali and Ng-Kwai-Hang (1986a) obtained higher cheese yields with ß-Cn A^1A^1, κ-Cn BB and ß-Lg BB when compared to ß-Cn A^1A^2, κ-Cn AA and AB and ß-Lg AA and AB.

The physicochemical and sensory characteristics and yield of cheese are one of the main concerns for the cheesemaker to maximize his profits. In the process of making cheddar-type cheese, most of the fat and casein, approximately 50% of the minerals, some whey proteins and lactose from milk contribute to cheese solids (Belanger, 1975) and hence affect cheese composition. Curd firmness at cutting influences quality, texture and yield of cheese by influencing recovery of milk components, which determines the final cheese composition (Ali *et al.*, 1980a, b; Bynum and Olson, 1982). Different genetic variants of certain milk proteins influence processing properties of milk as discussed in section 2.2.4.3.

Graham *et al.* (1984) and Schaar (1985) reported that cheese yield and composition of cheese from κ-Cn A and κ-Cn B milk were not significantly different. However, the protein recovery in the B-type cheese was significantly ($P < 0.01$) different from that of the A-type. Cheese made from ß-Lg BB contained higher fat, dry matter and crude protein percentage when compared with milk from AA and AB phenotypes (Schaar, 1985). A comprehensive study on cheddar cheese composition (Marziali and Ng-Kwai-Hang, 1986c) showed that milk with ß-Cn A^1A^1 phenotype

produced cheese with 62.45, 33.54 and 24.17% of total solids, fat and protein compared with 62.73, 33.17 and 24.14% for milk from A^1A^2 phenotype. Comparable values for κ-Cn were 62.57, 33.64, 24.07% for phenotype AA; 62.49, 33.51, 23.83% for phenotype AB; and 62.71, 32.92, 24.56% for phenotypes BB. Cheese made from ß-Lg BB milk retained more fat, protein and total solids in cheese than that made from milk with AA and AB phenotypes. Lactose content in cheese was also affected by phenotype of ß-Lg (Marziali and Ng-Kwai-Hang, 1986c).

2.2.4.3 Association with technological properties of milk

Cheesemaking involves the retention of most of fat and casein of milk. Rennin (chymosin) hydrolyses the specific bond between Met 105 - Phe 106 of κ-Cn, followed by aggregation of the caseins and formation of the curd. Many investigators have shown the effects of genetic variants of α_{s1}-Cn, ß-Cn, κ-Cn and ß-Lg on rennet coagulation time of milk, rate of firming of curd and firmness of the curd (Graham *et al.*, 1984; Losi and Mariani, 1985; Marziali and Ng-Kwai-Hang, 1986b; McLean, 1982). ß-Lactoglobulin A milk coagulates faster than ß-Lg B milk but ß-Lg B milk forms a firmer curd than ß-Lg A milk (Marziali and Ng-Kwai-Hang, 1986b). The B variant of ß-Cn and κ-Cn produced a firmer rennet curd than the A variant (Feagan *et al.*, 1972; Graham *et al.*, 1984; Losi *et al.*, 1975; Mariani *et al.*, 1979; Schaar, 1984; Sherbon *et al.*, 1967). In a study by Marziali and Ng-Kwai-Hang (1986b), when the effects of milk components were adjusted, the effect of genetic variants on coagulation properties of milk tended to be non significant. However, phenotype A^1A^1 of ß-Cn and BB of κ-Cn were associated with a shorter rennet clotting time and a firmer curd at cutting time than the other variants in the casein systems. McLean (1986) postulated that the repulsive forces between casein micelles containing variants such as α_{s1}-Cn C, ß-Cn B and κ-Cn B in which amino acids substitution resulted in a lower negative charge are decreased compared to micelles containing more negatively charged variants such as α_{s1}-Cn B, ß-Cn A, κ-Cn A. This enhances aggregation of hydrolyzed micelles, decreasing coagulation time and increasing curd firmness. These effects could also be explained by differences in: genetic variants of ß-Cn and κ-Cn, concentrations of ß-Cn

and κ-Cn, soluble casein and calcium:citrate ratio in milk. Mariani *et al.* (1976) demonstrated that curd from milk of κ-Cn BB cows had a better consistency during the different stages of curd cooking, and better rheological features of the cheese mass with greater contraction and expulsion of whey than from milk of the AA type.

2.2.4.4 Association with heat stability of milk

Heat stability is one of the most important properties having an influence on the processing of milk and dairy products. Schmidt and Koops (1965) found that higher heat stability was achieved for milk containing κ-Cn A than for milk with κ-Cn B at high pH. Jenness and Parkash (1967) were unable to confirm this result. The effect of preheating milk samples at various temperature-time combinations were investigated on milk of different genetic types of ß-Lg during yogurt manufacture (Parnell-Clunies *et al.*, 1988). The regression analysis of the firmness versus the degree of denaturation indicated that ß-Lg B milk was superior to ß-Lg A milk. There was a significant relationship between apparent viscosity and denaturation. McLean *et al.* (1987) carried out a comprehensive study on the effects of milk protein variants on heat stability of evaporated milk, viz., using BB, BC, CC for α_{s1}-Cn; A^1A^1, A^1A^2, A^2A^2, A^1B, A^2B for ß-Cn; AA, AB, BB for κ-Cn; and AA, AB, BB, AC, BC for ß-Lg. Maximum heat stability was significantly affected by genetic variants of κ-Cn and ß-Lg. In both proteins, the B variant was more heat stable than the A variant. Natural heat stability was affected by κ-Cn variants (BB>AB>AA) but not by ß-Lg variant. Maximum and natural heat stability were positively correlated with ß-Cn, and κ-Cn concentrations and were negatively correlated with α_{s1}-Cn and ß-Lg concentrations. A brief report of this work has been presented (McLean *et al*, 1987). The recent report on the effects of ß-Lg and κ-Cn genetic variants and concentrations on syneresis of gels from renneted heated milk, indicated that ß-Lg B and κ-Cn A milk will be more desirable for yogurt manufacture (McLean and Schaar, 1989). With the use of Differential Scanning Calorimetry, Imafidon *et al.* (1991a, b) reported influence in heat stability of different genetic variants of ß-Lg in the presence of calcium salt. They demonstrated that κ-Cn

AB was more effective than either κ-Cn AA or BB in stabilizing ß-Lg against heat denaturation.

2.3 Isolation and purification of milk proteins

The major milk proteins, casein and whey proteins, can be separated by isoelectric precipitation of caseins at pH 4.0 - 4.6 and 20 - 37 °C or by treating skim milk with chymosin (Larsson-Raznitkieuicz and Mohamed, 1986; Ng-Kwai-Hang *et al.*, 1982; Swaisgood, 1982). After separating the precipitated casein, the supernatant known as whey is the source of ß-Lg, α-La, bovine serum albumin and immunoglobulins.

2.3.1 Casein fractions

Casein micelles are relatively large aggregates of four protein components which are α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn, and several minerals including calcium, phosphate, magnesium, sodium, potassium and citrate (Rollema, 1992). In the micelle complex, α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn exist in strong association with one another and their association is stabilized by van der Waal forces, hydrophobic interactions, hydrogen bonding, electrostatic and the so-called steric stabilization (Schmidt, 1982). Disaggregation of the micelles is the first step for the individual casein fractionation. To eliminate these protein-protein interactions, and to ensure reduction of polymeric κ-Cn which would otherwise behave as a polydisperse component, various concentrations of urea or dimethylformamide and ß-mercaptoethanol or dithiothreitol are used (Donnelly, 1977; Swaisgood and Brunner, 1962; Zittle and Custer, 1963).

Hipp *et al.* (1952) was the first to develop a sequential urea dilution method for the separation of different caseins and found that ß-Cn precipitated out of the solution when the urea concentration was 4.6 M at pH 4.7. Both Swaisgood and Brunner (1962) and Zittle and Custer (1963) modified this method with the use of 12% TCA and 7 N H_2SO_4, respectively, to fractionate casein components, particularly, κ-Cn. The two groups obtained preparation of κ-Cn with the purity of approximate by 77% and 55% respectively fractionated from isoelectric whole casein at 4 °C and 20 to 24 °C. A procedure that is more sparing in urea and more effective in the initial isolation of the ß-Cn fraction than the original method developed by Hipp *et al.* (1952) was described

by Aschaffenburg (1963). He successfully isolated the variants of A, B and C of ß-Cn from the milk of appropriate homozygous ß-Cn by precipitation of casein in 3.3 M urea and reducing the pH to 4.6. Under these conditions, the bulk of the ß-Cn remains in solution while virtually all the other caseins were precipitated out. However, this preparation was still contaminated by some impurities such as α_s-Cn and κ-Cn.

Several fractionation techniques have been developed for the preparation of enriched fractions of individual caseins. To obtain homogeneous fractions, the use of chromatography is necessary. It is quite common to use differential solubility of casein fractions in urea solution to achieve separation of the various components (Swaisgood, 1982). In 6.6 M urea solutions, acid whole casein dissociated into whole α_s-Cn, ß-Cn and κ-Cn. By sequential dilution of this dispersion through 4.6 M to 1.7 M with respect to urea concentration at pH 4.7, ß-Cn was precipitated out of solution (Hipp *et al.*, 1952). Relatively good yields of α_s-Cn and ß-Cn were obtained with this procedure.

With the development of chromatographic techniques, individual casein fractions of high purity were easily obtained by the use of ion exchange chromatography (Thompson, 1966; Mercier *et al.*, 1968: Rose *et al.*, 1969; Annan and Manson, 1969b). Column chromatography on hydroxyapatite (Donnelly, 1977), covalent chromatography on thiolsepharose (Chobert *et al.*, 1981), and more recently, mass ion-exchange chromatography (Ng-Kwai-Hang and Pelissier, 1989) have been successfully used to prepare the different casein fractions.

Swaisgood (1982) reviewed ion exchange chromatographic separation of bovine milk proteins. With open columns, caseins were separated on cation or anion exchangers (Annan and Manson, 1969a; Mercier *et al.* 1968) in the presence of urea and 2-mercaptoethanol. According to the reports, anion exchange chromatography is now by far the most frequently employed method of fractionation. A procedure was developed by Davies and Law (1977) with the use of DEAE-cellulose, for quantitative analysis of components of reduced and alkylated whole casein. Satisfactory separation of γ^1-Cn, γ^2-Cn, γ^3-Cn, κ-Cn, ß-Cn, α_{s2}-Cn and α_{s1}-Cn, eluted in that order by a NaCl

gradient in Tris buffer at pH 8.6, containing 6 M urea, was obtained. Quite similar fractionations were obtained from reduced non-alkylated whole casein by high performance anion exchange on DEAE-TSK-5PW column (Humphrey and Newsome, 1984; Visser *et al.*, 1986) or Mono Q HR 5/5 column (Guillou and Pélissier, 1987) under conditions similar to those mentioned above but with the addition of a reducing agent to buffers. An excellent separation of whole casein was obtained by Aoki *et al.* (1985) using a DEAE-TSK-5PW column and modifying the condition used by Humphrey and Newsome (1984). The buffer was 0.02 M imidazole, 3.3 M urea, 0.08 M NaCl at pH 8.0, and elution was achieved by linear NaCl gradient from 0.08 to 0.28 M over 70 min at 0.5 ml/min.

The use of hydroxyapatite column chromatographic separation of the variants of α_s-Cn, ß-Cn and κ-Cn has been re-examined (Donnelly, 1977). This stationary phase is especially interesting for fractionating caseins, since the separation is according to their phosphate content. By using 5 mM phosphate buffer with 6 M urea at pH 6.8 as solvent, the whole casein solution was loaded into the column and eluted first with 100 mM phosphate-6 M urea, followed by linear gradient of 100 to 450 mM phosphate, 6 M urea at a temperature of 20 °C. The proteins eluted according to their phosphate content: viz. κ-Cn, ß-Cn and finally, α_s-Cn with α_{s1}-Cn eluting before the α_{s2}-Cn. While α_s-group of casein fractions eluted with a trace of ß-Cn, the method will not resolve individual genetic variants.

Since only κ-Cn and α_{s2}-Cn contain thiol groups when whole casein is reduced, they can be isolated with covalent chromatography on thiolsepharose (Chobert *et al.*, 1981). A strong positively charged cluster on κ-Cn allows its selective adsorption on cation exchange resins (Snoeren *et al.*, 1977). Using 1 g of human whole casein as starting material, Chobert *et al.* (1981) separated different fractions of κ-Cn, after reduction with 300 mM 1,4-dithiothreitol in a 100 mM Tris-HCl buffer of pH 7.0 containing 7 M urea, 300 mM NaCl and 1 mM EDTA. The fraction, retained on the column and bearing the SH-groups, was then separated on hydroxyapatite, by using 5 mM phosphate buffer, pH 6.8. The eluate from this column was then purified by

chromatography on DEAE-cellulose in a 6.6 M urea, 0.02 M imidazole/0.02 M ß-mercaptoethanol buffer, pH 7.0. The final yield of κ-Cn-like fraction was 12.2% of whole casein. There is no report in the literature that this procedure is extended to the purification of bovine κ-Cn.

Mass ion-exchange chromatography was developed for rapid separation of milk proteins in gram quantities and is increasingly being used (Chaplin and Anderson, 1988; Ng-Kwai-Hang and Pelissier, 1989). Isoelectrically precipitated whole casein was dissolved in a pH 8.0 and 20 mM imidazole buffer containing 4.5 M urea and 30 mM ß-mercaptoethanol for the isolation of α_s-, ß-, κ- and γ-Cn by using QAE-ZetaPrep 250 anion exchanger cartridge. Loading 500 ml of skim milk, Ng-Kwai-Hang and Pelissier (1989) recovered 1.01 g of dry γ-Cn, 1.25 g of κ-Cn, 2.59 g of ß- Cn and 2.62 g of α_s-Cn.

2.3.2 Major whey proteins

The isolation of ß-Lg involves four steps: removal of fat, separation of the casein, fractionation of whey proteins and the purification of ß-Lg. Three main fractionation procedures have been used for this purpose, viz, precipitation of caseins with sodium sulfate, hydrochloric acid or ammonium sulfate.

Aschaffenburg and Drewry (1957a) prepared ß-Lg by cooling the whey to 25 °C and adjusting its pH to 2.0 with 1 M HCl after precipitating the caseins at 40 °C by addition of 200 g/l of anhydrous sodium sulfate. On further addition of 200 g/l ammonium sulfate, ß-Lg was salted out of the solution. Crystalline ß-Lg was obtained by subsequent dialysis against distilled water and buffer of pH 5.2. A yield of 1.3-1.5 g/l of filtered whey for both ß-Lg AA and AB was obtained. Lower ß-Lg BB yield was obtained due to its lower concentration in the milk of cows producing this type of ß-Lg.

ß-Lactoglobulin can also be isolated from acid whey by addition of 34.2 g TCA/l of whey (Armstrong *et al.*, 1967). The yield of ß-Lg per liter from whey was 3.5 to 4.25 g. The specific rotation, intrinsic viscosity, and electophoretic mobility in veronal buffer at pH 8.6 of the ß-Lg were in close agreement with those obtained in similar

analyses of commercially available samples of the three times crystallized ß-Lg prepared by salt fractionation. However, further purification of ß-Lg obtained by this method is needed (de Wit and Swinkels, 1980). Monaco *et al.* (1987) separated ß-Lg by DEAE-cellulose chromatography at pH lower than 6.6. Crystals grown from this ß-Lg diffracted to a higher resolution than those grown from ß-Lg purified by the method of Aschaffenburg and Drewry (1957b), suggesting that acid precipitation at pH 2.0 damaged the protein in some undefined way. Despite this, most methods for the commercial preparation of bovine ß-Lg still use the procedure described by Aschffenburg and Drewry (1957a, b). Préaux *et al.* (1979) and Godovac-Zimmerman and Shaw (1987) isolated caprine ß-Lg and bovine ß-Lg by a non-salt precipitation method in which the fat and caseins were removed by centrifugation and then the whey protein mixture was dialyzed, lyophilized and separated by gel filtration (Davies, 1974).

The solubility methods of fractionation of whey proteins will not separate the various genetic variants. In addition, the precipitated ß-Lg fraction is rich in α-La, and hence high purity of ß-Lg can not be achieved readily by salting-out procedures (Armstrong *et al.*, 1967). Hence various chromatographic techniques have been developed as final purification steps (Swaisgood, 1982).

Armstrong *et al.*, (1970) and McKenzie (1971) reported on the procedure for purification of ß-Lg and α-La and the resolution of various genetic variants separated by DEAE-Sephadex A-50 chromatography at pH 6.3 in imidazole-HCl buffer using a 0.0 to 0.15 M NaCl gradient. The use of a particular cellulose based ion-exchanged medium for whey protein recovery has been criticized by Skudder (1985) based on the extensive investigation of Jones (1974) and Palmer (1977). Whey proteins were adsorbed on to DEAE-cellulose at pH 8.0 to 9.0 and subsequently eluted using NaCl. This process suffered from the formation of precipitates containing calcium phosphate at alkaline pH values and reduction in efficiency. However, this problem can be overcome to some extent by diafiltration of the whey to remove milk salts before pH adjustments (Evans, 1980). Alternatively, proteins can be adsorbed using the

carboxymethyl cellulose form of exchanger at pH 3.0, although the uptake is lower in this system.

Skudder (1985) evaluated the potential use of the strongly basic form of silica ion exchange medium for the recovery of proteins from whey. The ion exchanger, spherosil QMA, is based on porous silica which is copolymerized with styrene-vinyl triethoxysilane carrying the functional groups, $-N(CH_3)_3^+Cl^-$. Using large quantities of acid whey and rennet whey, as starting materials, and 0.1 M HCl as eluate, quantitative fractionation of ß-Lg variants A and B, and α-La were achieved.

Manji *et al.* (1985) developed an anion exchange chromatographic procedure to fractionate the major proteins from 50 to 500 ml of acid and sweet whey. The method employed a Pharmacia FPLC system fitted with a "MONO Q" anion exchange column and operated at a pressure of 3.5 to 4.0 mPa and flow rate of 2.0 ml/min at room temperature. The whey proteins including ß-Lg A and B, were completely resolved by a stepwise gradient of water and 0.7 M sodium acetate buffer.

Imafidon and Ng-Kwai-Hang (1992) developed an effective method for isolation and purification of ß-Lg by mass ion-exchange chromatography which is relatively fast for preparation of ß-Lg. It is possible to prepare ß-Lg of known genetic types by prescreening the milk samples used for isolation of the protein.

2.4 Surface activities of milk proteins

The surface activity of proteins is important for the functional applications especially those requiring the formation of continuous, viscoelastic cohesive film, such as in foams and emulsions (Kinsella, 1981; Halling, 1981; Kinsella and Whitehead, 1987). Many proteins are surface active, because they adsorb at the surface or interface of liquids at relatively low concentrations and reduce the surface tension, even at concentration as low as 0.02 ppm for casein.

2.4.1 Surface activity and protein film

Materials which have polar or hydrophilic moiety (e.g. COOH and OH) and non-polar or lipophilic groups are soluble in both water and liquids that are immiscible with water. These substances will orient themselves at the interface between the two phases

with the polar group dissolved in the aqueous phase, and the non-polar group dissolved in the oil phase. The degree of adsorption of these materials at the surface or interface, often as a monomolecular layer, is a measure of the surface activity. Surface active components or surfactants will decrease the surface tension of water at very low concentrations. Many naturally occurring food constituents exhibit surface activity, e.g. alcohols, fatty acids, phospholipids, protein and tannins (Lewis, 1987). The formation of an adsorbed layer is not an instantaneous process but is governed by the rate of diffusion of the surfactant through the solution to the interface. If π is the expanding or surface pressure of an adsorbed layer, then the surface tension will be lowered to a value γ_0, the $\gamma = \gamma_0 - \pi$ or $\pi = \gamma_0 - \gamma$, thus the surface pressure of a monolayer is the relation in surface tension due to the monolayer. For dilute solutions there is a relationship between the surface tension γ, the bulk concentration C_b and the surface excess concentration τ_b (Lewis, 1987):

$$\tau_b = (C_b/RT) \times (d\gamma/dC_b)$$

Proteins form a continuous cohesive film which is characterized with the mechanical and rheological properties upon their adsorption at an interface. Such films are important in relation and stabilization of foams and emulsions.

Film formation by proteins is dependent upon a number of sequential events: such as diffusion, adsorption, spreading and partial unfolding to permit segmental rearrangement and reorientation at the interfacial surface (Kinsella and Whitehead, 1987). The migration of proteins from solution to the interface is thermodynamically favorable because some of the conformational and hydration energy of the protein is lost at the interface. Once at the interface, most proteins unfold to some extent, reorient, and rearrange with the apolar and polar residues in the air and aqueous phase respectively. Initially, there is no barrier to adsorption for protein molecules that are readily adsorbed at the interface, as the surface pressure increases, an electrostatic barrier develops on the water side of the interface. Subsequent protein molecules must

have the energy to overcome this barrier and compress the molecules already at the interface before the protein adsorbs. The distribution of segments of the protein at the interface depends upon the free energy of adsorption of the various segments and the flexibility of the polypeptide chain (Kinsella, 1981; Tornberg, 1978b; Kitabatake and Doi, 1982). All these events are influenced by the inherent structural characteristics of the protein, such as composition, conformation, molecular flexibility and extrinsic factors, e.g. pH, temperature, protein concentration, type of ion species, etc. (Kinsella and Whitehead, 1987).

Proteins migrate to and adsorb at an interface to form a film very rapidly. The kinetics of protein adsorption at an interface can be measured by monitoring surface concentration and surface pressure, i.e., depression of surface tension as a function of time.

Bull (1947) estimated that a surface film of ovalbumin at 1.8 mg/m^2 was adsorbed from a 0.03% solution in 0.36 seconds. Benjamin *et al.* (1975) reported the diffusion rates ranging from 3.3 to 0.7 x 10^{-10} m^2s^2 for several proteins with ß-Cn being adsorbed much more rapidly than κ-Cn, serum albumin, or lysozyme. At similar concentration, ß-Cn is more surface active than κ-Cn (Graham and Phillips, 1976a, b; Bull, 1947; Benjamin *et al.*, 1975). The surface pressure is markedly affected by surface concentration of protein and it increases with protein concentration to a critical level above which little further change occurs. At the same surface concentration at 1.5 mg/m^2, κ-Cn and ß-Cn have surface pressures of 5 and 15 mNm^{-1} respectively, this simply means that ß-Cn is a more effective surfactant (Kinsella, 1981).

The type of film formed by proteins adsorbed at an interface depends upon the concentration and structure of the molecules. Extended films formed at dilute concentrations while condensed films formed with concentrated solutions. Some surface denaturation occur at the interface because of conformational unfolding and spreading. The extent to which this occurs depends upon the tertiary structure of protein and the surface concentration (Graham and Phillips, 1976a, b). In general, the rate of diffusion and the tendency of the protein to adsorb and unfold at the interface

determines the rate of surface pressure increase. The flexibility and hydrophobicity of the molecule also affects this rate. The addition of salts by decreasing electrostatic repulsion may enhance adsorption. The rate of adsorption tends to increase near the iosoelectric pH, the surface hydrophobicity of the protein tends to enhance its surface activity (Benjiamin, 1975; Kinsella, 1981).

2.4.2 Protein structure and surface activity

Hydrophobic proteins with a random-structure, are generally regarded as having good interfacial properties. Horiuchi *et al.* (1978) showed that there was a good relationship between the stability of foams produced from pepsin-hydrolyzed proteins and the surface hydrophobicity but not with internal hydrophobicity because the hydrophobic groups were buried in crevices in the molecular structure and were therefore ineffective at interfaces. Kato and Nakai (1980) used cis-parinaric acid as a probe to determine the effective hydrophobicity and found that the hydrophobicity was highly correlated with surface activity and emulsifying capacity. Effective hydrophobicity is usually increased by denaturation but decreased for whey proteins presumably due to intermolecular hydrophobic interactions.

Molecular flexibility is another important characteristic that is highly correlated with both foaming and emulsifying properties (Kato *et.al.*, 1985). This was confirmed by the reduced emulsifying activity and stability and foam properties when bifunctional reagents were used (Kato *et. al.*, 1986). Since foamability is related to the rate of decrease of surface tension at the air-water interface by adsorbed protein, flexible proteins like ß-Cn which reduce surface tension rapidly and exhibit a large volume increase (overrun) with large air cells while highly-structured proteins like lysozyme yield low overrun and small air cells and the stabilized foams are creamier than ß-Cn foams (Phillips, 1981; Graham and Phillips, 1976b). ß-Casein foams are unstable and drain rapidly because ß-Cn films have lower resistance to the shear and lower dilatational modules than lysozme films. The importance of hydrophobicity to the emulsifying properties of proteins was illustrated by Shimizu *et al.* (1983, 1984, 1986) and they indicated that removal of the hydrophobic N-terminal segment of α_{s1}-Cn

residue 1-23 by treatment with pepsin, reduced the surface hydrophobicity of the remainder of the molecule.

2.4.3 Surface activity of milk proteins

The surfactant properties of milk proteins have been extensively studied for the past 50 years (MacPitchie, 1978) because milk proteins have been available in pure form and have good surface activity. ß-Casein and BSA are regarded as typical random and globular proteins respectively and are frequently used as references in surface activity studies.

The first study on the interfacial properties of individual milk proteins was undertaken by Jackson and Pallansch (1961) who showed that the activity of the milk proteins at butter-oil/water interfaces at 40 °C decreased in the order of ß-Cn > monodispered casein micelles > BSA > α-La > α_S-Cn > ß-Lg > euglobulin.

Using a surface tension apparatus based on the drop volume principle, Tornberg (1978a) studied the behavior of surface tension/time, surface pressure/protein concentration relationships on soy protein, whey protein concentrate and caseinate at air/water interface and found that the protein concentration was the main factor affecting the surface tension. Sodium caseinate in 0.2 M NaCl at pH 7 was the most effective as an interfacial tension depressor and the effectiveness of soy protein decreased markedly with decreasing protein concentration from 1.0 to 0.001% while WPC occupied an intermediate position. The effectiveness of WPC and Na-caseinate was improved by the addition of NaCl and the WPC was almost as effective as the caseinate when made to 0.2 M NaCl. At or below 0.0005%, the surface activity of the three proteins was very low in the time scale of the experiment.

The surface properties of the individual lactoproteins have been characterized in considerable detail (Mulvihill and Fox, 1989). A comparative study was performed on the surface pressure (π), measured by a Wilhelmy Surface Balance, developed by the six principle lactoproteins at air-phosphate buffer interfaces as a function of area. The effect of concentration and time was reported by Mitchell *et al.*(1970). The surface area (m^2) per mg protein at a pressure of 5 dynes/cm (5 mN/m) for the six

lactoproteins, were ß-Lg = 1.0, α-La = 0.9, BSA = 0.09, ß-Cn = 1.0, α_{s1} -Cn = 0.75, κ-Cn = 0.65. The π-t isotherms strongly depend on protein structure and show that the caseins, especially α_{s1}-Cn and ß-Cn, cause a large and more rapid lowering of the surface tension than the whey protein; the order of effectiveness was ß-Cn > α_{s1}-Cn > κ-Cn > ß-Lg > α-La > BSA (Mitchell *et al.*, 1970). The surface tension lowering effectiveness of the whey proteins were increased by heat or urea denaturation but they were less effective than ß-Cn. The effectiveness of κ-Cn to decrease the surface tension was also increased by denaturation or by alkylation, supporting the view that κ-Cn has considerable secondary structure.

The viscosity (η) of interfacial films (oil/water) of the individual caseins is in the order of κ-Cn >> sodium caseinate > α_{s1}-Cn > ß-Cn. The viscosity η, after 30 h, was 200, 7.5, 5.0 and 0.5 $mNm^{-1}s$ for κ-Cn, sodium caseinate, α_{s1}-Cn, and ß-Cn, respectively. (Dickinson *et al.*,1987). Intermolecular disulfide linkages may be responsible for the very high viscosity of κ-Cn films.

The enzymatic modification of the casein may alter the surface activity, and change the properties. For example, dephosphorylation or treatment of sodium caseinate with plasmin greatly increased the surface activity (WiLson *et al.*, 1989). Enzymatic hydrolysis of ß-Cn produced γ^1-Cn, γ^2-Cn and γ^3-Cn. The γ^2-Cn and γ^3-Cn are smaller peptides with MW of 12,000 and very hydrophobic, considerably more active than ß-Cn. γ^1-Casein which represents residues 29-209 of ß-Cn was less active than ß-Cn at low concentrations but approximately similar at higher concentrations (Mulvihill and Fox, 1989).

Lysozyme and BSA are more highly structured than ß-Cn. For the flexible ß-Cn molecule, the film pressure (π) and surface concentration (τ) at air/water interfaces increased simultaneously and attained steady-state conditions at about the same time, but the surface concentration (τ) for BSA and lysozyme is higher, indicating that the more highly structured proteins lead to very large loops.

As determined by the drop volume technique, ß-Lg was more surface active than BSA but the reverse was true when the Wilhelmy plate method was used; both proteins

were much more active than lysozyme (Tornberg and Lundh, 1978).Since lysozyme is a very small molecule (MW 14,000), higher surface activity might be expected, however, the molecule has a high net positive charge at pH 7 and presumably causes a high interfacial barrier.

Using ellipsometry and radioactively labeled protein, Arnebrant and Nylander (1986) compared the surface activity such as surface pressure (π), rates of adsorption and rearrangement of ß-Lg and κ-Cn at pH values close to isoelectric point. The results showed that κ-Cn was more readily adsorbed on both hydrophobic and hydrophillic chromium surface than ß-Lg.

Although α-La was shown to be more surface active (Jackson and Pallansch, 1961), some studies (Caslte *et al.*, 1987; Mitchell *et al.*, 1970) indicate that it is among the least effective, with a surface activity similar to that of lysozyme (Caslte *et al.*, 1987). These two proteins are expected in view of the structural similarity, although lysozyme is strongly positively charged at pH 7 while α-La carries a small negative charge. The surface activity of α-La could be markedly improved by heat denaturation (Mitchell *et al.*, 1970). Dickinson (1989) also examined the surface activity (interfacial tension) of individual caseins and mixtures of caseins. When the bulk protein concentraction was 0.001%, ionic strength was 0.005 M and pH = 7, the surface activity was in the following order of gelatin> α-La > α_s-Cn > κ-Cn > ß-Cn = sodium caseinate.

2.5 Voluminosity and hydration of milk proteins

The ability of proteins to bind or entrap water is responsible for many of their desirable functional properties. Hydration, defined as the grams of water associated with or occluded by 1 gram of protein (dry weight), is the most representative term when compared to others reviewed by Kinsella (1976).

One of the earliest attempts to study quantitatively the hydration of casein micelles is that of Whitaker *et al.*(1927). These workers showed that the change in relative viscosity (η_{rel}) of skimmilk from 1.95 at 5 °C to 1.52 at 60 °C is due mainly to protein

and slightly due to lactose meaning the significance of the structure on the hydration and voluminosity properties of proteins.

The degree of hydration (g H_2O/g protein) of the principal caseins measured by Swaisgood (1982), showed that it was 3.3 for α_{s1}-Cn, 3.9 for α_{s2}-Cn, 8.4 for ß-Cn and 3.1 for κ-Cn. According to the relationship $V = V_2 + \delta_1 V_1^0$ (V is voluminosity, V_2 is the partial specific volume of the dry protein, V_1^0 is the specific volume of pure water) the partial specific volumes given by Swaisgood (1973) were 0.735, 0.749, 0.736 and 0.740 for α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn. The voluminosity (V) can be calculated for these four caseins: α_{s1}-Cn = 4.035; α_{s2}-Cn = 4.640; ß-Cn = 9.15 and κ-Cn = 3.836 cm^3/g. Berlin *et al.* (1969) reported that the water sorption of α_{s1}-Cn C is slightly less than that of α_{s1}-Cn A which is missing a strongly hydrophobic N-terminal sequence. Reported values for the degree of hydration of the principal whey proteins depend on the methods used for determination. Kuntz and Kaufmann (1974) reported values for ß-Lg of 0.55, 0.54, 0.49 and 0.45 g H_2O/g based on calorimetry, viscosity, sedimentation and a combination of hydrodynamic functions, respectively; corresponding value for BSA are 0.32, 0.33, 0.52 and 0.43 g H_2O/g, respectively. Townend *et al.* (1960) reported a value of 0.40 g H_2O/g for monomeric/dimeric ß-Lg while Swaisgood (1982) assumed a hydration of 0.60 g H_2O/g in calculating the fractional ratio of octomeric ß-Lg. The hydration of α-La is reported to be 0.45 g H_2O/g based on a spherical conformation (Wetlaufer, 1961) while Swaisgood (1982) assumed a hydration of 0.57 g H_2O/g which is similar to lysozyme when calculating the fractional ratio. Reported values for the partial specific volumes (cm^3/g) of the whey proteins are 0.751 for ß-Lg, 0.34 for BSA (Kuntz and Kaufmann, 1974) and 0.735 for α-La (Gordon and Semmett, 1953). Using these values for partial specific volume and those of Townend *et al.*(1960) and Wetlaufer (1973) for hydration, the voluminosity of the principal whey proteins are 1.51 cm^3/g for ß-Lg (monomer), 1.135 for α-La and 1.064 for BSA. The lower values for the voluminosity of the whey proteins compared to the caseins reflected the much higher levels of secondary structures of the whey proteins.

2.6 Food emulsion and milk proteins

From a physicochemical point of view, an emulsion is traditionally defined as "a colloidal dispersion of liquid droplets in a second immiscible liquid phase" (Dickinson and Stainsby, 1988). Emulsions are thermodynamically unstable mixtures of immiscible liquid such as vegetable oil and water. If energy is applied the system may be dispersed, but increased surface energy causes the phases to rapidly coalesce unless an energy barrier is established (Mangino, 1989). Emulsified droplets can be stabilized against coalescence by the addition of molecules that are partially soluble in both phases. A number of small molecules can serve this function. Proteins capable of unfolding at the interface may also function as emulsifiers since protein coats the lipid droplets and provides an energy barrier to both particle association and to phase separation.

2.6.1 Factors involved in emulsions and proteins at interface

Generation of an emulsion involves the mixing of two immiscible liquids with different polarities. When the nonpolar domains of a protein are exposed to the aqueous phase, the protein tends to spontaneously associate in a manner that minimizes contact with water (Mangino *et al.*, 1985). Measurements of the enthalpy of hydration of nonpolar molecule yield values that are similar and negative. This suggests that interaction between nonpolar molecules and water should be favorable. When solubility data are examined, however, it is found that nonpolar molecules are only slightly soluble in water. Measurements of the free energy of the transfer of nonpolar molecules from organic solvents to water give values that are positive. The negative values for the enthalpies of hydration and the positive free energy of transfer to the aqueous phase suggested that an entropically driven aggregation of nonpolar molecules occurs in an attempt to minimize their contact with water. When a liquid of low polarity, such as fat, is mixed with water, there is a strong driving force to limit the contact between two liquids. Work is required to increase the interface area and the energy of the system. When two immisible liquids are forced into contact by the application of work, a number of spherical droplets within the dispersed phase will be formed. The larger spheres have a smaller ratio of surface to volume than do smaller spheres and hence a

lower surface energy. If there is no energy barrier to prevent coalescence, the system will continue to lower its total energy content by the formation of larger droplets from smaller ones. The dispersed system can be stabilized against coalescence and phase separation if another component that is partially soluble in both phases is added. Molecules that are composed of portions that are soluble in water and portions that are soluble in lipids can serve as emulsifiers. Phospholipid, a simple emulsifier, are a class of naturally occurring compounds that can serve this function. Proteins are much larger and more complex than simple emulsifier molecules and the formation of a protein stabilized emulsion requires that the protein molecule must first reach the water/lipid interface and then unfold so that its hydrophobic groups can contact the lipid phase.

In native proteins, most of the nonpolar amino acid side chains are located in the interior of the molecules. It has been estimated that the removal of one mole of hydrophobic groups from the surface results in an energy gain of 12 kJ (Kinsella, 1982). Any hydrophobic groups that remain at the surface increase the total energy of the system. Protein have charged groups at the surface of the molecules and contact with water molecules. The favorable interaction of water with surface charges lowers the total energy of the protein molecule. As a protein molecule approaches the interface, there is less opportunity for the charged groups to interact with solvent. In the extreme case, charged groups are removed from the aqueous phase and enter the lipid phase. This is energetically unfavorable and these groups are repelled from the interfacial area. If the groups nearing the interface are in a region of the protein molecule that contains some flexible structure, then the molecule may begin to unfold. This unfolding causes the exposure of hydrophobic groups to the surface. If these groups are exposed to the aqueous environment, there is an increase in total energy. The random fluctuations in protein structure will cause these groups to return to the interior of the molecule. If the exposure occurs at an interface, the state of lowest free energy depends on the nature of the interface. In the case of a protein unfolding near a lipid, the hydrophobic groups are inserted into the lipid phase. This insertion has a very low energy of activation and proceeds spontaneously (Tanford, 1980). For most of the proteins studied, the size of the hydrophobic region inserted corresponds to six to

eight amino acid residues (MacRitchie and Alexander, 1963). When proteins are used for the generation of emulsions, they must be able to unfold enough to expose hydrophobic groups. Molecules like the various caseins are extremely flexible and contain little secondary structure (Farrell, 1973). Caseins are excellent emulsifiers because of their ability to easily unfold at interfaces. Molecules that contain crosslinks such as disulfide bonds are rigid and less able to unfold. They are less effective in emulsion formation (Tanford, 1980). Small highly crosslinked protein molecules tend to perform poorly as emulsifiers (Mangino, 1989).

2.6.2 Milk protein as the stabilizer of emulsions

The emulsifying activities of milk proteins are important in several products. It is difficult to compare the published data concerning milk protein emulsion properties because of the multitude of factors involved. Milk proteins, especially caseins, are excellent emulsifying agents in a wide variety of food, such as coffee whiteners, beverages, salad dressings and margarines. The low heat non fat milk was superior to caseinate and whey protein in emulsion properties (Kinsella, 1984). The stable tertiary structure of undenatured whey protein enhances the strength of the interfacial films formed during emulsification. Caseins are somewhat unique in that they have no tertiary structure and little secondary structure. Extensive whipping or emulsification does not result in excessive denaturation or overemulsification. Therefore, caseins are useful in systems where repeated emulsification may be desirable. Sodium and potassium caseinates are good emulsifiers because they are soluble and possess the ability to form interfacial films, and exhibit resistance to thermal denaturation and coagulation. These protein films remain stable over a wide range of pH, temperature and salt concentration (Schut, 1982). Calcium caseinate performed poorly in most emulsions (Tornberg and Lundh, 1978) because it is rather insoluble. Southward and Goldman (1978) studied the emulsifying and emulsion-stabilizing capacities of various coprecipitates by using the method of Inklaar and Fortuin (1969). The results showed that the high and medium soluble calcium coprecipitates exhibited good emulsion stabilizing properties. Emulsion made from soluble coprecipitates showed lower stability but compared favorably with commercial sodium caseinate.

Caseinate behaved quite differently from WPC and soy isolate during emulsification (Tornberg, 1978a, 1980). Caseinate was adsorbed from the bulk phase, with an increasing of interfacial area up to 8 m^2/ml, when 70 to 80% of the total protein had been adsorbed; in contrast, adsorption of WPC and soy protein reached a plateau at 3m^2/ml when 40% and 20% of total protein had been adsorbed. Soluble caseinates had greater emulsifying capacities than the more aggregated caseins. The viscosities of emulsions prepared in soy protein were much higher than those in WPC (Muvihill and Fox, 1989). Emulsions formed in the aggregated caseinates were more stable than those stabilized by the highly dispersed caseins and stability increased with increasing protein load. The emulsifying capacity of caseins was influenced by pH, because the solubility of casein is pH dependent. When pH was increased from 1.5 to 3.5 and from pH 5.5 to 7.0, the emulsifying capacity of casein decreased from 325 to 264 g oil/g protein and increased from 251 to 268 g oil/ g protein (Mohanty *et al.*, 1988).

Whey protein can form reasonable emulsions though they are very sensitive to heat denaturation. Morr *et al.* (1973, 1981) reported that WPC containing 27 to 62% protein all possessed comparable emulsifying activities which showed some relationship to their respective solubilities. Hayes *et al.* (1979) reported that heat-treated, ultrafiltered whey proteins formed emulsions the stability of which increased with degree of homogenization during emulsion preparation. The whey proteins were not as effective as egg white in stabilizing salad dressing.

In a comparative study (Pearson *et al.*, 1965) on the emulsifying activity of a number of proteins, the emulsifying activity index (EAI) of ß-Lg was found to be lower than sodium caseinates. It was found that ß-Lg forms smaller and more stable emulsion droplets than sodium caseinate (Graham and Phillips, 1976a). Yamauchi *et al.* (1980) reported that the creaming stability of emulsions prepared with whole whey protein was minimal and the viscosity, fat globule size and the amount of protein adsorbed were maximal at pH 5 over the pH range from 3 to 9.

2.7 Milk protein foams

Many food foams are formed by the entrapment of air by protein films (caseinate and WPC), which are used for whipped toppings, soufflé, mousse, meringue, angel -

food cake and leavened bread. The most important foaming characteristics of proteins are foam volume and foam stability.

2.7.1 Foaming properties

A foam is mostly air and is characterized by high viscosity, low density, high surface area, and high surface energy. The foaming capacity was expressed either by the percentage of conductivity decrease of protein solution after 30 seconds bubbling (Pearce and Kinsella, 1978) or by the percentage of foam volume increase (overrun). Foam stability is usually determined by monitoring the weight of liquid drained from the resulting foam as a function of time and expressed as time taken for 50% liquid drainage (Patel and Kilara, 1990).

For foam formation, the protein should be in solution and be capable of rapid migration and orientation to form an encapsulating interfacial film around the gas as it is bubbled or whipped to present coalescence of the nascent bubbles. As air bubbles are whipped into the liquid, they tend to coalesce to minimize surface exposure but the surfactant minimizes coalescence by forming an interfacial film. Surface viscosity and surface elasticity are related to the film property of the foam. High surface viscosity and high yield values of film are correlated with strong foams because they reflect strong cohesion between the protein molecules in the film (Graham and Philips, 1976a; Halling, 1981; Graham and Phillips, 1979a; Phillips, 1981). Film with high viscosity and possessing viscoelasticity forms more stable foams than high viscous but rigid film that tends to be brittle (Graham and Phillips, 1976a; Benjiamin *et al.*, 1975; Graham and Phillips, 1979a, b, c). Surface elasticity and the ability of molecules to adjust to interfacial pressure gradients enables the film to respond to shocks. A viscous thick film has better mechanical or thermal shock resistance than a thin film. The viscous drag and elasticity of the interfacial film decrease the rate of drainage and enhance stability of the foam (Graham and Phillips, 1976a, b; Phillips 1981).

Graham and Phillips (1976a, b; 1979c) studied the relationships between film formation and foaming properties by comparing the interfacial adsorption behavior and foaming properties of ß-Cn, BSA and lysozyme. They found that the loose flexible molecule ß-Cn rapidly adsorbed and spread at the air-water interface of new air

bubbles, reduced the interfacial free energy, and formed a continuous cohesive film around the air bubble. The globular proteins BSA and lysozyme, arrived at the surface as did ß-Cn but because of their globular tertiary structure, less of the molecule occupied the interface and the surface pressure did not build up as rapidly as it did with ß-Cn. Halling (1981) reported that α-Cn gave a slower surface pressure increase on adsorption and foamed poorly in bubbling chambers compared to other caseins.

The capacity of protein to form a foam is important while the ability of a protein to stabilize a foam is also critical. Because proteins differ in their capacity to stabilize foams and ironically some of the properties desired for facile foam formation do not ensure stability and molecular characteristics which convey foam stability, i.e., extensive intermolecular interactions, and excessive cohesiveness may not be compatible with rapid foam formation (Kinsella, 1981).

Foam stability is affected by the protein type, its concentration and solubility, pH, ionic strength and temperature (Kinsella, 1981, Halling, 1981). Soluble protein can aid diffusion, adsorption and spreading. The concentration of protein in solution directly affects stability by affecting thickness and cohesiveness of the film depending on the protein type, foaming methods and the condition of foam formation (Graham and Phillips, 1976a, b; Halling, 1981). By affecting the rigidity of the interfacial film, the pH affects foam stability. Maximum protein-protein interaction occurs close to the isoelectric pH. Addition of salts tends to enhance the foaming ability of protein at pH values away from the isoelectric point which may be due in part to some masking of electrostatic repulsions and minimize the extent of surface denaturation (Kinsella, 1981; Cumper, 1953).

2.7.2. Milk protein foams

Milk proteins possess good foaming properties. Morr *et al.* (1973) found that the overrun of sodium caseinate was 1120% compared to 0 to 760% for WPC by different methods. Graham and Philips (1976a, b) also found that caseins give large-volume but unstable foams and the stability was improved by addition of sugar. Lorient *et al.* (1989) reported that rapid increase of surface pressure appeared to be directly

correlated with an improvement in foam stability. The order of effectiveness of casein foaming capacity was: ß-Cn > α_{s1}-Cn > κ-Cn. With the bubbling method (Lorient *et al.* 1989), foam stability was found to be higher at pH 7 than at pH 4 for α_{s1}-Cn and ß-Cn whereas κ-Cn was not very pH dependent.

Whey proteins possess good foaming properties compared to caseins, but the foaming properties of a whey preparation are more affected by protein and solids concentration, pH, ions, extent of denaturation, whipping time, temperature, protease peptone levels and lipids (Bingham, 1971; Morr *et al.*, 1973; Haggett, 1976a, b) and foaming method. Using a standard whipping procedure and measuring overrun and drainage, Morr *et al.* (1973) and Morr (1976) reported that several WPC samples prepared by different methods showed widely different foam capacities and stability. Richert *et al.* (1974) reported overruns ranging from 500 to 1340% for different WPC preparations while De Vilbiss *et al.* (1974) observed a remarkable disparities in overrun (0 to 780%) and drainage, and these appeared to be independent of protein concentration, extent of denaturation, or pH of the dispersion.

The foaming properties of WPC generally increase with solids content with an optimum observed around 10% total solids. This is attributed to the increased viscosity effect of the solutes and the increased strength of the protein film at higher protein concentration. WPC samples overrun ranged from 300 to 1400% and foam stability increased with higher WPC solids. By influencing net charge, pH value affect the rate of adsorption and extent of protein-protein interaction in the interfacial film. Highest foam stability occurs in the isoelectric pH range (Cooney, 1974). Richert (1979), Richert *et al.* (1974) and Cooney (1974) reported that the optimum foaming properties are between pH 8 and 9. Salts influence solubility, conformational stability and film-forming properties of whey proteins films. In the case of WPC foams, stability decreased linearly with the square root of the ionic strength of the solution and maximum overruns occurred at 0.05 M NaCl (Cooney, 1974). Heat treatment affected foaming. Thus, as the treatment was increased from 65 to 85 °C, undenatured protein decreased from 80 to 40% and soluble protein decreased from 100 to 58%. The

maximum overrun decreased from 1500 to 800%. The addition of sucrose decreased overruns from 1500 to 250% of all WPC whips (Haggett, 1976a). The presence of fat in the range of 0.5 to 0.9% significantly depressed foaming of WPC (De Vilbiss *et al.*, 1974). This is by weakening at loci in the continuous protein film.

2.8 Milk protein gels

Gels are differentiated from other systems in which small proportions of solid are dispersed in relatively large proportions of liquid by the property of mechanical rigidity or the ability to support shearing stress at rest (Ferry, 1948). The remarkable property of gels is their ability to behave as solids while retaining many properties characteristic of the fluid component.

2.8.1 Gelation

Milk proteins undergo gelation when restricted protein unfolding yields polypeptide segments capable of specific interaction which forms a well-ordered, three-dimentional network able to entrain large amounts of water. This characteristic of milk protein to form a gel and provide a structure matrix for holding water and various food ingredients is very useful in food applications and in new product development.

The ability of unfolded proteins to associate and coagulate, precipitate or gel, depends on the protein, amino acid composition (net charge), molecular weight, net hydrophobicity and protein concentration (Shimada and Mattsushita, 1981), heating and cooling rate and a critical balance between attractive and repulsive forces (Schmidt, 1981). The pH range over which gelation occurs generally increases with increasing protein concentration (Mulvihill and Kinsella, 1987). The numerous hydrophobic and disulfide bonds formed at high protein concentration may compensate for repulsive electrostatic forces associated with pH values outside the isoelectric range (Shimada and Matsushita, 1981). Proteins with molecular weight higher than 60 KD and molar percentages of apolar amino acids lower than 30% such as that found in BSA, and the small proteins like ß-Lg, α-La with high molar percentages of apolar amino acids are concentraction-independent in gelling systems (Shimade and Matsushita, 1981). ß-Sheet hydrogen bonding was proposed to be important for aggregate formation. Facile

unfolding or molecular flexibility of the protein were also an important, albeit non-specific feature (Chen *et al.*, 1974).

Gelation is a two-step mechanism involving an initial unfolding or dissociation of the protein followed by aggregation to form a loose network and a gel. For the formation of a highly ordered gel it is necessary that the aggregation step proceedes at a rate slower than unfolding (Ferry, 1948). If aggregation occurs very rapidly then a coagulum is obtained. Such gels are characterized by higher opacity, lower elasticity, and more syneresis. When aggregation occurs more slowly, the gel would be more highly ordered, narrow-stranded, and fibrous. These gels are usually clear, firmer, and hold more water. Cross-linking is essential for gel formation together with the solvent which provides the fluidity, elasticity, and flow behavior of gels. The degree of cross-liking can be variable and provides a mechanism whereby the strength of gels can be manipulated. The protein-protein interactions which involve hydrogen bonding, ionic and hydrophobic interactions, and the balance of attractive and repulsive force are necessary to form a proper matrix. Excessive attraction may result in coagulation. The pH, ions and thiols can markedly affect gelation.

The term "gel" currently is imprecise, it includes soft viscous fluids, smooth paste, smooth curds, stiff or rubbery gels. These gels can vary tremendously in hardness, cohesiveness, stickiness, color and mouth-feel. Thus, in the context of dairy protein products, the gel can mean many things. Milk protein, especially whey protein can form very useful gels in different food systems, so a wide range of techniques for making and measuring gelation properties of milk protein has been developed. Heat-induced gel formation is the common method which is widely used in the food industry.

Properties of a gel such as strength, hardness, adhesiveness, cohesiveness, elasticity, and brittleness are frequently judged subjectively. The clarity, cloudiness, and opacity of gels are evaluated subjectively or by light transmission (Schmidt, 1981; Kalab and Emmons, 1971, 1972; Kalab *et al.*, 1971). Usually, the gel tester can be used to measure hardness, resilience using an electronically driven plunger, the texture analyser measures hardness, cohesiveness, elasticity. The Instron Universal Testing Machine is now most frequently used for measuring gel properties and texture profile

analysis (Bourne, 1968). Viscosity and gel strength are determined with Brookfield viscometer, using a range of spindles. Electron microscopy is also increasingly being used to study the structure and matrix of gels (Green, 1980). The capacity of a gel to hold water can be assessed by its ability to entrap moisture when the gel is squeezed. It can also be measured by methods based on capillary suction potential (Lewicki *et al.*, 1978) or by the amount of water imbibed by a filter paper in contact with a compressed gel (Schmidt, 1981).

2.8.2 Milk protein gels

Many important foods are gels in which gelling agents are proteins, starches, gums or pectins. Milk gels and milk protein gels are the basis of several dairy foods and gelling is an important functionality in several applications. For example, yogurt is a gel structure which, in good quality, should possess a smooth, thick constant body that retains moisture without syneresis.

Rennet-induced gels which are the basis of the manufacture of most cheese varieties are outstanding examples. The rennet coagulation of milk has been widely researched over the past 70 years. The milk protein system may be coagulated by 35 to 40% ethanol and could be gelled if milk and ethanol were mixed under quiescent conditions. Whey proteins are readily heat denaturable with gelation occurring at high protein concentration. Gelation during storage (age-gelation) may be a problem in sterilized milk, especially when prepared by the UHT process.

By heating non fat milk at concentration from 12 to 20% in salt solution, Kalab and Emmons (1971, 1972) obtained excellent milk gels. When the protein concentration increased the casein micelles were essentially fused together to form extensive bridging between casein micelles via denaturation of ß-Lg. Whey proteins have excellent thermal gelling characteristics especially above pH 7. Whey proteins can form both clear and opaque gels. Clear gels tend to be more elastic and hold water more effectively (Schmidt, 1981). Salt can markedly affect the whey protein gel structure (Schmidt, 1981). In the presence of 0.2 M sodium chloride, a coarse gel of large aggregates is formed, compared to a fine gel in absence of salt. Whey protein gel strength and hardness usually increases with protein concentration and temperature.

Gels formed at higher temperatures have a higher degree of intermolecular bonding which imparts higher mechanical strength (Schmidt, 1981; Kalab *et al.*, 1971).

Numerous studies concerning the gelation behavior of whey proteins show that a wide range of coagulate, curds, and gel types can be obtained from whey proteins (Schmidt, 1981; Hillier and Cheesman, 1979a, b; Johns and Ennis, 1981). This information is used to exploit the use of WPC in various types of foods or in fabrication of new foods, such as custards, puddings, sweet gels, yogurts, meat gels, confectioneries, cakes and so on. Whey proteins are also useful in the manufacture of liver pastes and meat spreads.

ß-Lactogloblin is considered to be the most important whey protein from a thermal gelation viewpoint (Mulvihill and Kinsella, 1987). Bovine serum albumin and immunoglobulins are also known to form stable gels on heating, but their contribution to the gelation of a whole whey protein system has remained unstudied. α-Lactalbumin gelling properties are generally regarded as inferior to those of ß-Lg since whey protein concentration enriched with α-La has poor heat-setting properties (Mulvihill and Kinsella, 1987). The gelation of BSA has been extensively studied (Clark and Lee-Tuffnell, 1986; Yasuda *et al.*, 1986). Proteose peptone did not influence gelation when added to whey protein isolates (Hillier and Cheesman, 1979a).

3. HYPOTHESIS AND OBJECTIVES

Genetic variants of milk protein due to amino acid substitutions or deletions have differences in their amino acid sequences, the primary structures of the milk proteins. Several studies have already demonstrated that the different genetic variants of ß-Lg were associated with different heat stability. The heat stability was also related to the different genetic variants of κ-Cn that influence the heat stability of ß-Lg. Some studies suggested that renneting time and cheese curd firmness were affected by the genetic variants of κ-Cn. It is assumed that other physicochemical properties like emulsifying properties and foaming properties could also be related to the primary structure of the protein, because these properties highly depend on the characteristics of protein films which are formed at the air-water or oil-water interface. Different protein films will yield entirely different emulsions or foams. Gelling properties, the texture characteristics of protein are also assumed to be highly associated with the primary structure of protein. Any changes in the protein structure might lead to differences in gels. Alteration in amino acid composition in the amino acid sequence of the protein due to the genetic variants will lead to the different properties of the protein. Therefore processing of milk and dairy products will be affected by these genetic changes in the milk proteins.

The objectives of this research project were to study the following:

1. Influence of genetic variants on surface properties of bovine caseins at the air-water interface.

2. Influence of genetic variants on voluminosity and hydration properties of bovine caseins.

3. Influence of genetic variants on emulsifying properties of isolated α_{s1}-Cn, ß-Cn and κ-Cn.

4. Influence of genetic variants on emulsifying properties of whole caseins with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn.

5. Influence of genetic variants on foaming properties of ß-lactoglobulin.

6. Influence of genetic variants on gelling properties of ß-lactoglobulin.

4. MATERIALS AND METHODS

4.1 Source of caseins and ß-lactoglobulin of different genetic variants

All the samples of milk proteins were prepared from the fresh milk of cows from Holstein and Jersey herds located within 100 km from Macdonald Campus. In two previous projects (Ng-Kwai-Hang *et al.*, 1990; Kim, 1994), cows milk was phenotyped for the genetic variants of caseins and ß-Lg by polyacrylamide gel electrophoresis as described by the method of Ng-Kwai-Hang and Kroeker (1984). From these studies cows could be identified to provide milk samples containing the following phenotypes: BB, BC, CC for α_{s1} -Cn; AA for α_{s2}-Cn; A^1A^1, A^1A^2, A^2A^2, A^1B, A^2B and BB for ß-Cn; AA, AB, BB for κ-Cn, and AA, AB, BB for ß-Lg. Only phenotype AA for α_{s2}-Cn and BB for α-La was found in the studied populations. For the purpose of this project, samples of α_{s1}-Cn, ß-Cn, κ-Cn and ß-Lg of specific phenotypes could be obtained from milk from the above identified cows.

4.2 Identification of milk proteins

Milk proteins were identified by vertical polyacrylamide gel electrophoresis described by Ng-Kwai-Hang *et al.* (1984b).

Phenotyping of α_{s1}-Cn and κ-Cn was performed in a polyacrylamide gel containing 5% of BIS (N,N'-methylene-bis-acrylamide) under alkaline (pH 8.3) condition. Variants of ß-Cn were resolved by electrophoresis in 10% polyacrylamide gel under acidic (pH 3.0) condition. Variants of ß-Lg and α-La were separated by electrophoresis in 12% polyacrylamide gel at pH 8.3 in TRIS (Tris-hydroxymethylaminomethane)-glycine buffer.

4.3 Preparation of milk proteins

Milk protein samples were prepared from the individual cow's milk in triplicate.

4.3.1 Isolation of whole casein and whey protein

Four liters of whole milk were obtained from individual cows of known genetic variants for the milk proteins and stored at 4 °C overnight. The top fat layer of the milk was removed and then the milk was centrifuged at 3000 x g for 20 min to separate out the residual fat. Whole casein was prepared by isoelectric precipitation of the fat-

free milk at 30 °C through adjusting the pH to 4.6 with 1N HCl. After centrifugation, the supernatant containing the whey proteins was used as the source of ß-Lg. The precipitated whole casein was washed three times with acidified water at pH 4.6 and then stored at -20 °C pending preparation of whole casein and isolation of α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn therefrom. Electrophoresis was performed on subsamples of the whole casein in order to ascertain the phenotypes of α_{s1}-, α_{s2}-, ß- and κ-Cn. Likewise the phenotype for ß-Lg was ascertained by electrophoresis of subsamples of the supernatant.

4.3.2 Preparation and analysis of whole casein

The whole casein previously stored at -20 °C was thawed out at room temperature. Approximately 30 g of wet casein were dispersed in 80 ml water. The pH was adjusted to 7.0 with 2 N NaOH. The prepared whole casein solution was then lyophilized. Total protein content of the whole casein was determined by Kjeldahl method. The contents of individual caseins in the whole casein were determined after the separation by HPLC (Dong, 1992).

4.3.3 Fractionation and purification of α_{s1}-, α_{s2}-, ß- and κ-casein

An ion-exchange chromatography system was developed in this laboratory for the purpose of individual casein fractionation. A 250 mm long and 45 mm diameter glass column was packed with Macro-prep Q50 (Biorad, Hercules, CA) anion exchanger. The column was connected to a Millipore Consep LC100 liquid chromatography system (Millipore, Milford, MA). In brief, the procedure involved solubilization of 5 g whole casein containing approximately 26% dry matter in 20 ml of a pH 7.0 buffer containing 20 mM Tris, 4.6 M urea and 0.04 g of dithioerythritol. After equilibration of the chromatography system with 5 column volumes of starting buffer, the column was loaded with 20 ml of the sample solution at a flow rate of 10 ml/min. The starting buffer was made of 4.6 M urea and 20 mM Tris-HCl and adjusted to pH 7.0. The elution of various fractions was done with a step-wise gradient of 0.10 M, 0.15 M, 0.20 M, 0.25 M and 0.50 M NaCl in the starting buffer. The eluate from the column was monitored with a UV detector according to its absorbance at 280 nm.

Fractions corresponding to each of the peaks were individually collected by a fraction collector. As a clean up procedure and for regeneration of the ion exchanger, the system was eluted with 200 ml of the Tris/urea buffer containing 0.50 M NaCl. This was followed by equilibration with 200 ml of buffer in the absence of NaCl prior to the introduction of the next sample.

The collected fractions of α_{s1}-, α_{s2}-, ß- and κ-Cn were then desalted and concentrated in a Model 8400 stirred ultrafiltration cell (Amicon, Danvers, MA) fitted with a YM 10 membrane. The operating pressure from a compressed nitrogen cylinder was adjusted to 2.8 kg/cm^2 for the stirred cell. This was followed by dialysis at 4 °C against a pH 7.0 phosphate buffer. The concentrated casein fractions were lyophilized and stored at -20 °C for future analysis.

4.3.4 Fractionation and purification of ß-lactoglobulin

ß-Lactoglobulin was isolated from the whey protein fraction by using an ion-exchange column chromatography system similar to that used for individual casein isolation, except that Macro-prep High Q (Biorad, Hercules, CA) was used as the matrix to fill the same glass column instead of Macro-prep Q 50 (Biorad, Hercules, CA). After equilibration of the liquid chromatography system with 5 column volumes of a pH 7.0 buffer containing 20 mM Tris-HCl, 50 ml of defatted whey solution was introduced into the column at a flow rate of 10 ml/min. Defatted whey proteins were eluted from the column by passing through a buffer containing 0.15 M, 0.20 M, 0.25 M, 0.35 M and 0.50 M NaCl. The eluate was channeled through a UV monitor and the presence of protein was detected at 280 nm. Peaks corresponding to Igs, BSA, α-La and ß-Lg were collected by a fraction collector. After collection of the last fraction, the column was regenerated and equilibrated with the starting buffer containing no salt. The system was then ready for the next sample fractionation. The fraction of ß-Lg was desalted and concentrated to a minimum volume and dialyzed against water for 24 h. The ß-Lg sample was lyophilized and stored at -20 °C for future use.

4.3.5 Determination of the concentration of milk protein

The concentration of whole casein was determined by Kjeldahl method and individual casein was determined by Bicinchoninic Acid (BCA) method (Smith *et al.*, 1985). BCA method is a modified protein assay procedure based on the method of Lowry *et al.* (1951). The BCA Protein Assay Reagent (Pierce, Rockford, IL) is a highly sensitive reagent for the spectrophotometric determination of protein concentration. This reagent system combines the well known reaction of protein with Cu^{2+} in an alkaline medium (yielding Cu^{1+}) with a highly sensitive and selective detection reagent for Cu^{1+} , namely Bicinchoninic Acid. The analysis procedure includes the preparation of a standard curve and measurement of protein samples. For the standard curve, a set of protein standards of known concentration is prepared by diluting a stock solution of casein or ß-Lg which was predetermined by Kjeldahl method. Standard solution of casein or ß-Lg were prepared to contain 0, 60, 120, 180 and 240 µg/ml of protein. All samples were analyzed in triplicate. A 100 µl sample of each standard and unknown protein sample was pipetted into the appropriately labeled test tube and 2.0 ml of working reagent consisting of reagent A and B in the ratio of 50:1 were subsequently added. The mixture was then incubated at 37 °C for 30 min, followed by ice cooling for 5 min. Absorbency was measured at 562 nm against a water reference. The protein concentration for each unknown protein sample was determined by comparing to the standard curve.

4.4 Determination of surface properties of caseins at the air-water interface

4.4.1 Measurement of surface tension

The lyophilized whole casein and individual casein samples were suspended in a 0.01 M, pH 6.8 sodium phosphate buffer at a concentration of 0.005% (w/v). The surface properties of milk protein were investigated by measuring the change in surface tension of the casein solutions over a period of 60 min. The Wilhelmy plate method (Davies and Rideal, 1963; Adamson, 1976) was employed for this measurement. The surface tension was determined by using a Sartorius M25D surface balance, and a Cahn Instruments glass sensor (17 x 17 x 0.2 mm, Goettingen, Germany). A programmed

computer was connected with the balance to record the surface tension changes as a function of time. The glassware was thoroughly cleaned with water, Nochromix Solution (Godax Laboratories Inc., New York, NY), water and acetone in sequence, and followed by fast drying with a stream of air.

4.4.2 Kinetic analysis of surface tension decay

The kinetics of surface tension decay of various caseins at the air-water interface were evaluated at different determining steps: surface enlargement, diffusion and penetration which were simply expressed as step 1, step 2 and step 3 in this thesis. The surface tension decay was plotted versus time. A plot of log $d\pi/dt$ versus π plot was constructed by computer differentiation as described by Tornberg (1978b). Each linear part of the log $d\pi/dt$ versus π plots was derived by linear regression analysis and represented the different stages of surface activity of the proteins at the air-water interface. The break points between any two linear parts were considered as the rate determining step during the surface tension decay. The surface pressures attained (π_{att}) during each rate determining step, i.e. the break point of linear regression, and the time of attainment (t_{att}) were derived from a plot of $\log(d\pi/dt)$ versus π and the surface tension decay curves. The maximum value of $\log(d\pi/dt)$ at the surface enlargement stage was presented for each casein. Other parameters which were also calculated were the quotients of $\Delta t/60$ and π_{att}/π_{60}. Quotients of $\Delta t/60$ indicate the relative contribution of time elapsed during each rate-determining step within 60 min. Quotients of π_{att}/π_{60} represents the π_{att} during each rate determining step in relation to the surface pressure attained after 60 min. At each rate-determining step, π_{att}, t_{att}, $\Delta t/60$ and π_{att}/π_{60} were analyzed.

4.5 Determination of voluminosity and hydration of caseins

A protein solution of 3% (w/v) was prepared by dispersing whole casein or individual caseins into a 0.01 M, pH 6.8 phosphate buffer. Prior to conducting the experiment, an extensive dialysis of the protein solution against the same phosphate buffer at 4 °C for 24 h was carried out and this was followed by the ultracentrifugation at 15,000 x g for 60 min. The fat residue layer and insoluble sediment were discarded

subsequently. The protein concentration was then determined by the BCA method (Smith *et al.*, 1985) and adjusted to 2.00% (w/v) by the phosphate buffer.

The Ubbelohde Viscometer (size 2B, International Research Glassware, Kenilworth, NJ) was used to measure the relative viscosity according to the method of Lee *et al.*(1969). This glassware was held in a 60 x 40 x 30 cm fish globe filled with water and equipped with an electronic heater and a thermometer. The water in the fish globe was heated and maintained at 25.0 ± 0.1 °C during the measurement. Protein solution was conditioned in the water bath at 25.0 °C for 20 min and then was transferred into the Ubbelohde viscometer. A stopwatch was used to determine the dropping speed of the protein solution in the viscometer under the gravity. Triplicate measurements were made for each of the protein solutions. The viscometer was then thoroughly cleaned with acetone and dried by passing air through before using it for the next sample.

Protein voluminosity (Ve, ml/g protein) was calculated by the formula of $Ve = \phi / C$ (Lee *et al.*, 1969), where C was the protein concentration and ϕ was a parameter that must be deduced from Lee's equation (1969): $\eta/\eta_0 = 1 + 2.5\phi + 7.031\phi^2 + 37.371\phi^3$, where η was the viscosity of protein solution, η_0 is the viscosity of phosphate buffer. Hydration was the result of Ve - 1 according to the method described by Lee *et al.* (1969).

4.6 Determination of emulsifying properties of caseins

4.6.1 Emulsion preparation

Emulsions containing 0.8% casein and 10% or 40% commercial soya oil (Crisco Ltd., Toronto, ON, Canada) were prepared. Exactly 20 ml of the emulsion was blended in a IKA-Labortechnik blender (Janke & Kunkel GMBH&CO. KG, Germany) at 24,000 rpm and then homogenized with the EmulsiFlex Model EF-B3 homogenizer (Avestin, Ottawa, ON, Canada) in two passes at the pressure of 70,000 kPa. The temperature during homogenization was held constant at 40 ± 2.0 °C. Before preparation of the emulsions, caseins were suspended (2.0 - 2.5% w/v) in 0.01 M, pH

residues. The solution was then dialyzed overnight at 4 °C. The final concentration of casein in the suspension was adjusted to exactly 0.8% (w/v) after the total protein content was determined by BCA method (Smith *et al.*, 1985). The measurements of emulsifying properties were conducted immediately after the preparation of emulsions.

4.6.2 Interfacial area of the emulsion

Interfacial area (IA) of the emulsion was described as square meter of surface area per milliliter of emulsion (m^2/ml). In order to measure IA, oil-in-water emulsions were diluted with 0.05 M, pH 7.0 sodium phosphate buffer containing 0.2% sodium dodecyl sulfate to give a final oil concentration of 1 in 10^5 phosphate buffer. Optical density of 2 ml of the above diluted emulsion was determined at 500 nm with a Beckman DU-7 spectrophotometer (Beckman Instruments, Palo Alto, CA) according to the method of Pearce and Kinsella (1978). Calculation of IA was based on the method of Cameron *et al.* (1991): IA = 2T, where T is the turbidity (T = 2.303 x OD_{500} x dilution factor/cm light path).

4.6.3 Protein load of the emulsion

Protein load of the emulsions was calculated from the amount of protein coated over the oil droplets. A 2 ml emulsion sample was transferred into a specific plastic tube and centrifuged at 12000 xg for 60 min. The serum phase was sucked out by a syringe after puncturing through the tube wall with a needle (Britten *et al.*, 1993). Protein of the serum phase was determined before and after the emulsion formation using a modified biuret reaction (BCA protein assay reagent, Pearce, Rockford, IL). The quotient of the protein concentration depletion in the aqueous phase over the interfacial area of the emulsion was the protein load. It was expressed as milligram protein per square meter of the interfacial area per milliliter of emulsion(m^2/ml). Kjeldahl method was used to obtain the calibration curve for the determination of protein concentration using sodium caseinate as standard.

4.6.4 Apparent viscosity of the emulsion

The apparent viscosity of the emulsion was evaluated immediately after the emulsion formation with a Brookfield Digital viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA). The viscometer was fitted with a SL-4 small-

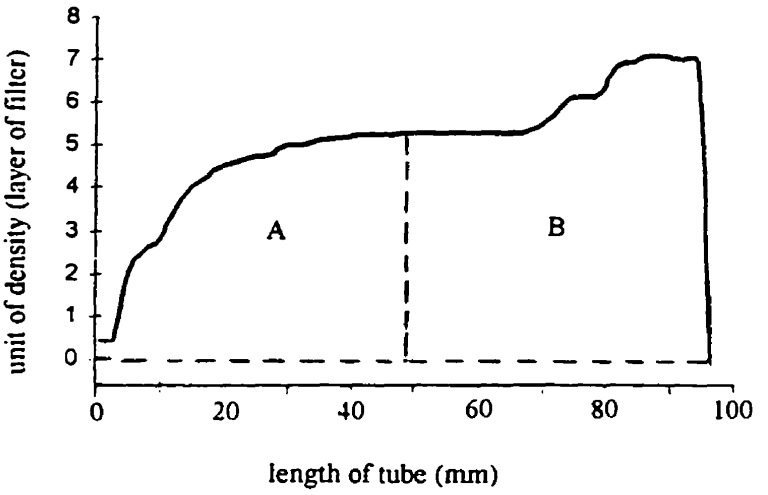

Fig. 7 Densitometry profile of oil-in-water emulsion.

sample adapter (type 18) and adjusted to a shear rate of 150/s. A volume of 8 ml emulsion was applied and measured with a setting of 60 rpm at 23 ± 1 °C for 60 s. The apparent viscosity was read directly from the window of the equipment.

4.6.5 Emulsion stability

Emulsion stability upon storage was evaluated over a thirty-day period by changes in densitometric profiles of the emulsions. According to the method of Britten and Giroux (1993), emulsion samples were placed in a 10 x 0.6 cm glass tubes, sealed on both ends and stored in a vertical position. At the end of the storage, the emulsion samples were scanned with a modified densitometer (Model CDS-200, Beckman Instruments, Palo, Alto. CA). The densitometer was calibrated with a series of opaque filters. These filters were prepared by stacking up layers of a reference filter. Opacity results were reported on an arbitrary scale and the units corresponded to the number of reference filter layers (Britten *et al.*, 1991a). Figure 7 shows a typical densitometer profile of the scanning result. In this figure, the whole area under the curve was divided into two parts by drawing a line in the middle: part A at left represents non-creamed portion of the emulsion and part B at right represents creamed portion of the emulsion. Part A and B can be integrated by Sigma Scan (Jandel Scientific Inc., Guelph, ON, Canada). The quotient of (B / (A + B)) x 100% is termed the creaming index which was used to describe the shelf life of emulsions.

4.7 Determination of foaming properties of ß-lactoglobulin of different phenotypes

4.7.1 Foam preparation

Model foams of ß-Lg AA, AB, BB and a mixture of the ß-Lg phenotypes from bulk tank milk were prepared with 100 ml protein solution. The purified ß-Lg (10 g) was dispersed in 40 ml of 0.2 M, pH 7.0 Tris-HCl buffer and dialyzed overnight to remove residual salt. The final concentration of ß-Lg was adjusted to 5.00% and then a 100 ml sample was whipped at 22.0 ± 1.0 °C in a mixer (model Mixmaster, Sunbeam, Corporation, Toronto, ON, Canada) operating at maximum speed for 5 min. The sample foam was then analyzed immediately.

4.7.2 Measurements of foaming properties

Foaming properties of ß-Lg were measured as foam capacity, stability, firmness and elasticity.

4.7.2.1 Foam capacity

Foam capacity is usually expressed as overrun (%) which was calculated from the density ratio between the solution and the foam (Britten and Pouliot, 1996):

$$\text{overrun (\%)} = \frac{V}{M - T} \times 100$$

Where, V is the volume of the container (100 ml); M is the weight (g) of foam plus container; T is the weight (g) of the empty container.

4.7.2.2 Foam firmness

Foam firmness was measured with a Model DV II Brookfield viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) mounted on a Helipath, supported and fitted with a "T" type spindle. Spindle rotational speed was set at 12 rpm, while vertical displacement was fixed at 2.5 cm per minute. The apparent viscosity was measured after 1-cm penetration. Figure 8 shows the profile of foam firmness recorded by the device. The foam firmness was calculated by the method of Britten and Pouliot (1996).

4.7.2.3 Foam drainage stability

Foam drainage stability was defined as the time required to drain half of the foam weight. For that purpose, a 50-ml funnel was filled with freshly prepared foam and placed over a balance. The balance was connected to a computer which collected weight data of drained liquid as a function of time.

4.7.2.4 Foam elasticity

Foam elasticity was expressed as foam relaxation that was also measured with a Model DV II Brookfield viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA). The calculatation was conducted as follows (Britten and Pouliot, 1996):

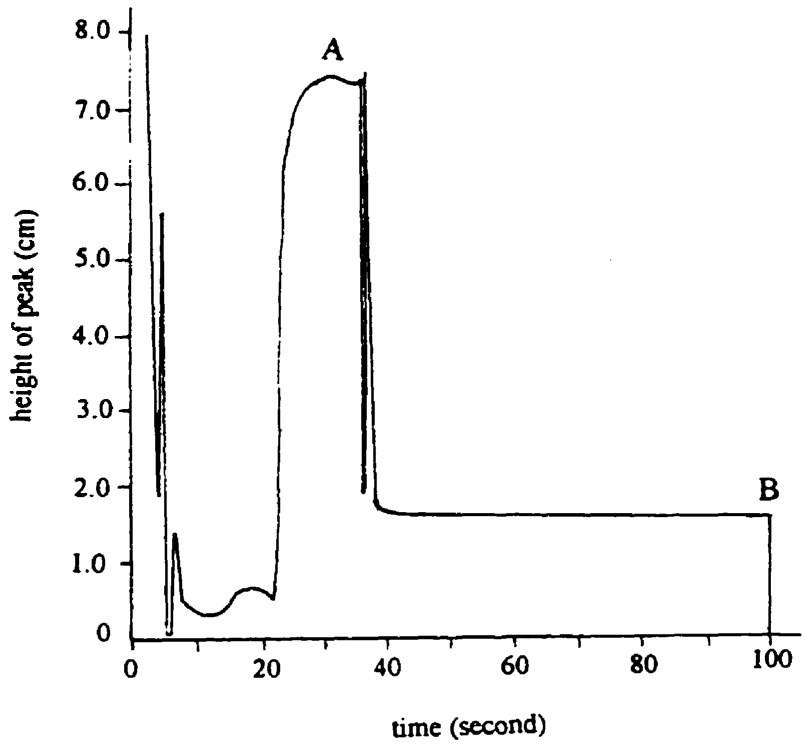

Fig. 8 Profile of foam firmness and relaxation measured with Brookfield Viscometer mounted on a Helipath, supported and fitted with "T" type spindle. Rotation speed: 12 rpm; vertical displacement: 2.5 cm/min., the apparent viscosity was measured after 1-cm penetration.

$$\text{foam relaxation (\%)} = \frac{A - B}{A} \times 100$$

From figure 8, A represents the maximum height of the peak, B is the height attained after 100 s. Both A and B were directly measured by a ruler from the profile.

4.8 Determination of gelling properties of ß-lactoglobulin of different phenotypes

4.8.1 Preparation of ß-lactoglobulin gels

Samples of ß-Lg AA, AB, BB and a mixture of phenotypes of ß-Lg were dispersed in a 0.2 M, pH 7.0 Tris-HCl buffer to a concentration of 10% (w/v) and dialyzed overnight at 4 °C to remove residual salt. Protein content of the solution was determined with BCA method and adjusted to a final concentration of 7.5%. A 2 ml sample of the above protein solution was transferred to a glass vial (inside diameter 11.0 mm) and placed on an ultrasound instrument for 30 min to remove the air bubbles. The ß-Lg solutions were heated at 90 °C for 30 min and then cooled in an ice bath for 5 min. The gels which were formed were allowed to stand overnight at 4 °C before analysis.

4.8.2 Measurements of gelling properties

4.8.2.1 Gel firmness

Gel firmness of ß-Lg was measured by a texture analyzer (model TA-XT2, Texture Technologies Corp., Scarsdale, NY). A cylinder of 6-mm in diameter and 50 mm in height was used as a probe which penetrated the gel at a speed of 0.8 mm/s. Maximal deformation was set at 15 mm. Gel strength was determined by the force required to fracture the gel. The system could automatically record the firmness measurements and the unit is represented in grams. Figure 9 shows the profile of the gel strength measured by the texture analyzer. The result of the gel firmness measurement was printed out on the record paper.

4.8.2.2 Gel relaxation

Relaxation of gel was measured by the same texture analyzer (model TA-XT2, Texture Technologies Corp., Scarsdale, NY) at a depth of 0.5 cm for the measuring

cylinder and calculated by the following formula:

$$\text{relaxation (\%)} = \frac{A - B}{B} \times 100$$

From figure 10, A is the value of the maximum peak height measured by the texture analyzer in centimeters; B is the height of the peak measured at the end of 30 s.

4.8.2.3 Gel syneresis

After completing the above measurements, the gel was weighed, broken and centrifuged at 2500 x g for 30 min. The liquid portion which separated out was decanted and weighed. Syneresis index was calculated as the proportion of liquid which separated out of the gel after centrifugation, viz:

$$\text{syneresis (\%)} = \frac{A}{B} \times 100$$

Where, A is the weight of discarded liquid and B is the weight of whole gel before centrifugation.

4.9 Statistical analysis

4.9.1 Individual caseins

After phenotyping the individual caseins, it was possible to select milk samples from which individual caseins of known phenotypes could be obtained. Those isolated caseins of known phenotypes were used for the physicochemical properties measurement described above. There were three phenotypes for α_{s1}-Cn: BB, BC and CC; one for α_{s2}-Cn: AA; six for ß-Cn: A^1A^1, A^1A^2, A^2A^2, A^1B, A^2B and BB; three for κ-Cn: AA, AB and BB. Each phenotype of the individual casein was prepared from milk of three different individual cows. Parameters of surface activity, voluminosity, hydration, emulsifying properties were all measured in triplicate for each phenotype of individual casein. The significance of differences between means of same casein of

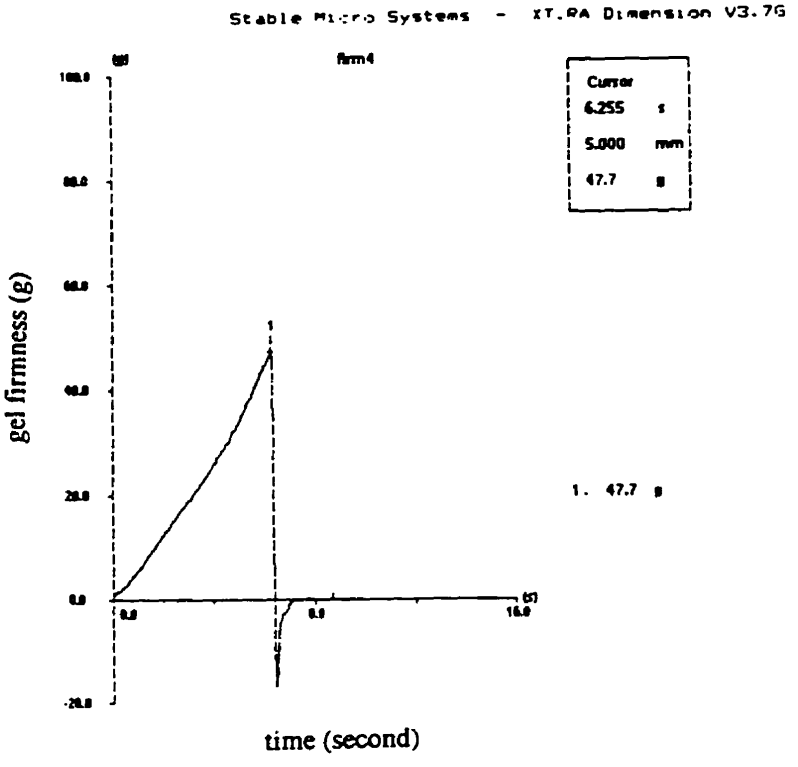

Fig. 9 Profile of firmness of ß-lactoglobulin gel measured by texture analyzer (model TA-XT2, Texture Technologies Corp., Scarsdale, N.Y., USA).

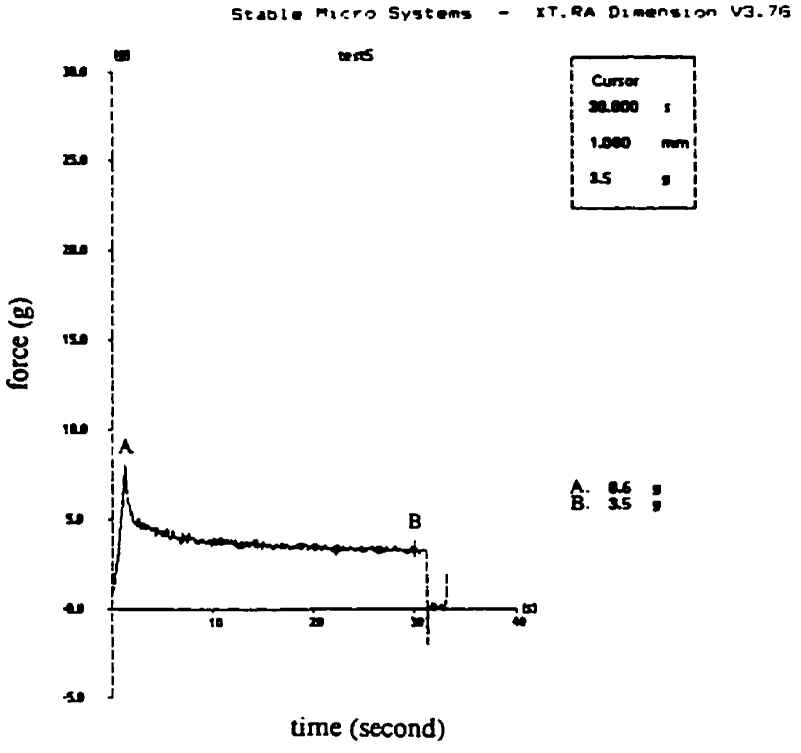

Fig. 10 Profile of relaxation of ß-lactoglobulin gel measured by texture analyzer (model TA-XT2, Texture Technologies Corp., Scarsdale, N.Y., USA).

different phenotypes were tested by the Duncan's multiple range test at the level of α = 0.05.

4.9.2 Whole caseins

Whole caseins were prepared in triplicate for each different phenotype combination of α_{s1}-Cn/ß-Cn/κ-Cn from milk of different individual cows. There was a total of twelve α_{s1}-Cn/ß-Cn/κ-Cn combinations including one bulk tank milk whole casein which represents a mixture of the all phenotypes of above three individual caseins. Parameters of surface activity, voluminosity, hydration, emulsifying properties were all measured in triplicate for each of the whole casein samples. The significance of differences between means of same casein of different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn was tested by the Duncan's multiple range test at the level of α = 0.05.

4.9.3 ß-Lactoglobulin

Phenotype AA, AB and BB of ß-Lg were prepared separately from the milk samples of three different individual cows. The mixture of these three phenotypes of ß-Lg was prepared from bulk tank milk samples on three different occasions. Isolated ß-Lg was prepared in triplicate for the foaming and gelling properties measurements. The significance of differences between means of ß-Lg of different phenotypes was tested by the Duncan's multiple range test at the level of α = 0.05.

5 RESULTS AND DISCUSSION

5.1 Source of individual casein of different phenotypes

5.1.1 Identification of individual caseins

Individual casein of different phenotypes was prepared from the fractionation of whole caseins. Figures 11 and 12 show the PAGE patterns of the genetic variants of α_{s1}-Cn, ß-Cn and κ-Cn under alkaline and acid conditions, respectively. Two genetic variants were found for both α_{s1}-Cn (B and C) and κ-Cn (A and B), three for ß-Cn (A^1, A^2 and B). In Fig.11, lanes 1, 5 and 6 show BB, 3 an 4 show CC, 7 and 8 show BC phenotypes for α_{s1}-Cn. The resolution for κ-Cn in Fig.11 was not clear enough to identify the phenotypes of κ-Cn. Another gel which was not presented in the thesis showed that κ-Cn in lanes 1, 3, 4, was AA; in lane 5, 6, 7 was AB; in lane 8 was BB phenotype. The clear identification of κ-Cn can be seen in Fig. 14 which shows the clear bands for the three phenotypes of κ-Cn. Polyacrylamide gel electrophoresis under alkaline conditions could resolve the genetic variants A, B, C, D and E but not A^1, A^2 and A^3 for ß-Cn. In Fig. 11, lanes 1, 6, 7 and 8 represent ß-Cn AB, lanes 3 and 4 represent ß-Cn AA and lane 5 represents ß-Cn BB, respectively. Differentiation between A^1, A^2 and A^3 of ß-Cn required PAGE under acidic conditions. Figure 12 shows phenotypes A^1A^1 (lanes 7 and 9), A^1A^2 (lanes 1, 3 and 5), A^2A^2 (lanes 2, 6, 8 and 10) and A^1B (lane 4) for ß-Cn.

5.1.2 Fractionation of individual caseins

The fractionation profile of individual caseins is shown in Fig.13. κ-Casein, due to the different degrees of glycosylation, is represented by a group of peaks (peaks 1, 2 and 3) which eluted between 37 and 75 min corresponding to 0.10 - 0.15 M NaCl in Tris-HCl urea buffer. Peaks 4, 5 and 6 are ß-Cn, α_{s2}-Cn and α_{s1}-Cn, respectively. These three individual caseins were eluted at 80-95, 110-116 and 117-140 min corresponding to 0.20, 0.25 and 0.25 M NaCl in Tris-HCl urea buffer. The fraction corresponding to each individual casein was collected. The different fractions of κ-Cn were collected as one sample.

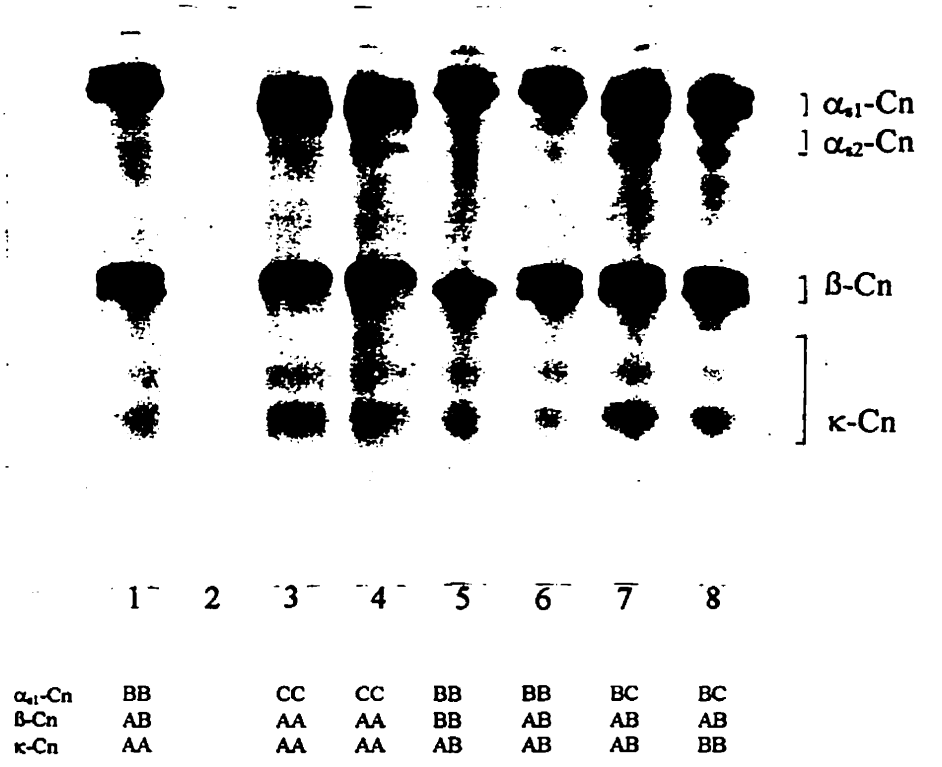

Fig. 11 PAGE patterns of α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn. Conditions: pH=8.3, 8% polyacrylamide gel containing Tris buffer, 4 M urea. Tris-Gly electrode buffer (Ng-Kwai-Hang *et al.*, 1984b).

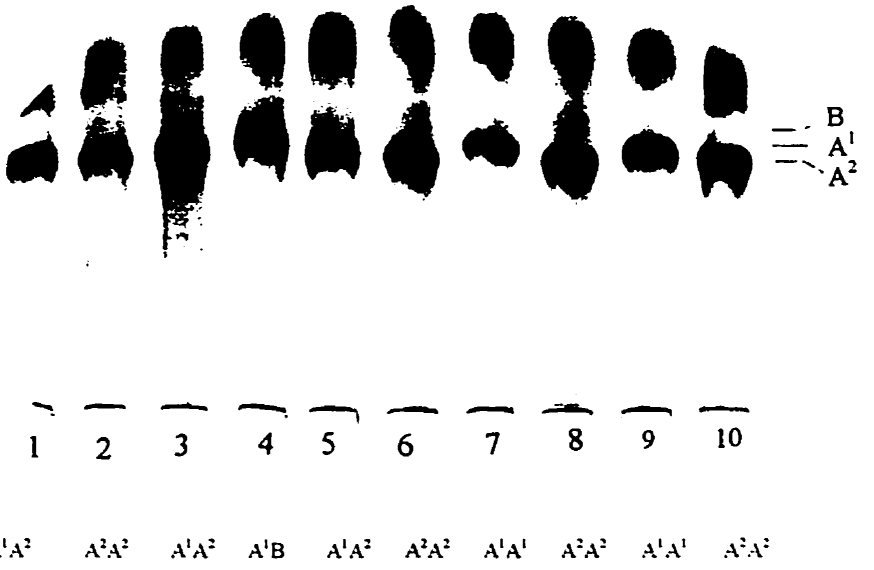

Fig. 12 PAGE patterns of A^1, A^2 and B variants of ß-Cn. Conditions: pH = 3.0, 10% polyacrylamide gel containing Tris buffer. 7.7% acetic acid electrode buffer (Ng-Kwai-Hang *et al.*, 1984b).

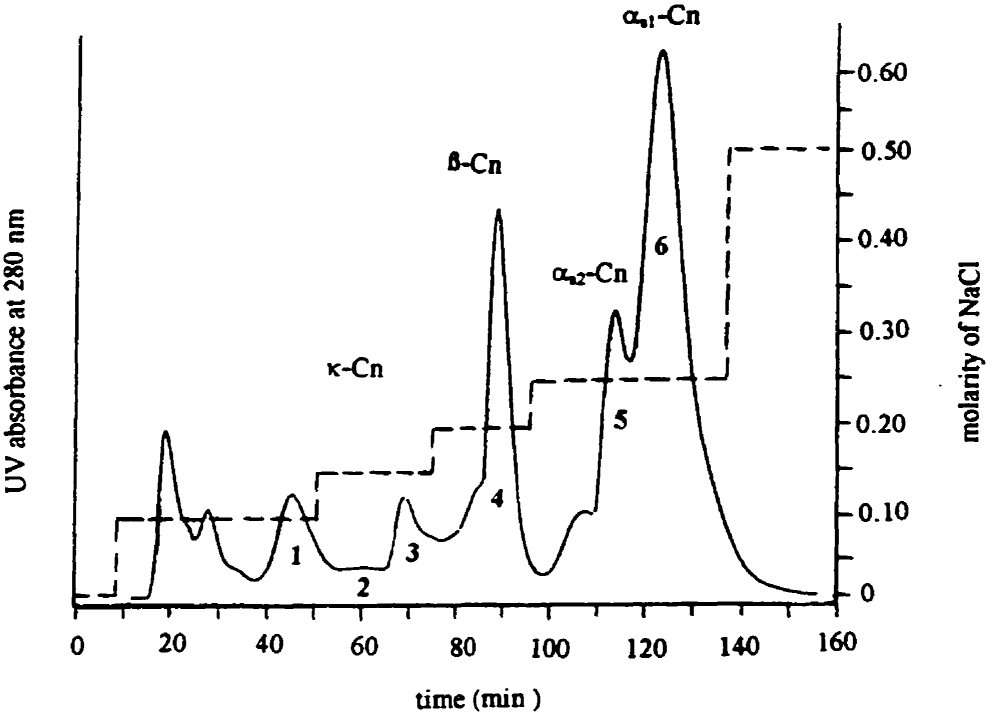

Fig.13 Profile of casein fractions separated by liquid chromatography, column supported with Macro-Q 50. Gradient: step-wise at 0.10 M, 0.15 M, 0.20 M, 0.25 M and 0.50 M NaCl in 20 mM, pH 7.0, 4.6 M urea Tris-HCl buffer.

5.1.3 Purity of individual caseins

The purity of the collected each individual casein fraction was examined by PAGE under alkaline condition (pH 8.3) and the result is shown in Fig. 14. Lanes 1, 2 and 3 show more than one bands that represent the different degrees of glycoslation of κ-Cn. Lane 4 shows a single band for ß-Cn. Lanes 5 and 6 show only one band for α_{s1}-Cn identified as CC and BB phenotypes, respectively. Lanes 7 and 8 were the whole casein loaded as the reference. No other protein contamination was found in the collected individual casein fractions, therefore the purity of the isolated κ-Cn, ß-Cn and α_{s1}-Cn was considered good enough for the physicochemical properties measurement of this research project.

5.2 Source of whole caseins

5.2.1 Haplotypes of whole caseins

Whole caseins were prepared from the individual cow's milk that were sampled from the Holstein dairy farms located around Macdonald Campus. In order to select as many different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn as possible, phenotyping of the milk protein of all individual cow's milk within the region was conducted at our laboratory. There were only BB and BC phenotypes found for α_{s1}-Cn in Holstein herds of the sampling area. Six phenotypes of ß-Cn were available: A^1A^1, A^1A^2, A^2A^2, A^1B, A^2B and BB. For κ-Cn there were three phenotypes: AA, AB and BB. Considering the combinations of α_{s1}-Cn/ß-Cn/κ-Cn, only eleven haplotypes as shown in Table 3 were found. Whole casein from bulk tank milk which contained a mixture of phenotypes of individual caseins was also prepared. There was an absence of the following combination of α_{s1}-Cn/ß-Cn/κ-Cn: BB/A^1B/AA, BB/A^2B/AA, BB/BB/AA, BB/A^2A^2/AB, BB/A^1B/AB, BB/A^2B/AB, BB/BB/AB and BB/BB/BB in the sampling area.

5.2.2 Quantitation of individual caseins

The content of four major individual caseins was determined after the separation of whole caseins by reverse-phase HPLC. The proportion of different fractions corresponding to κ-Cn, ß-Cn, α_{s2}-Cn and α_{s1}-Cn shown in Fig. 15 were integrated by a

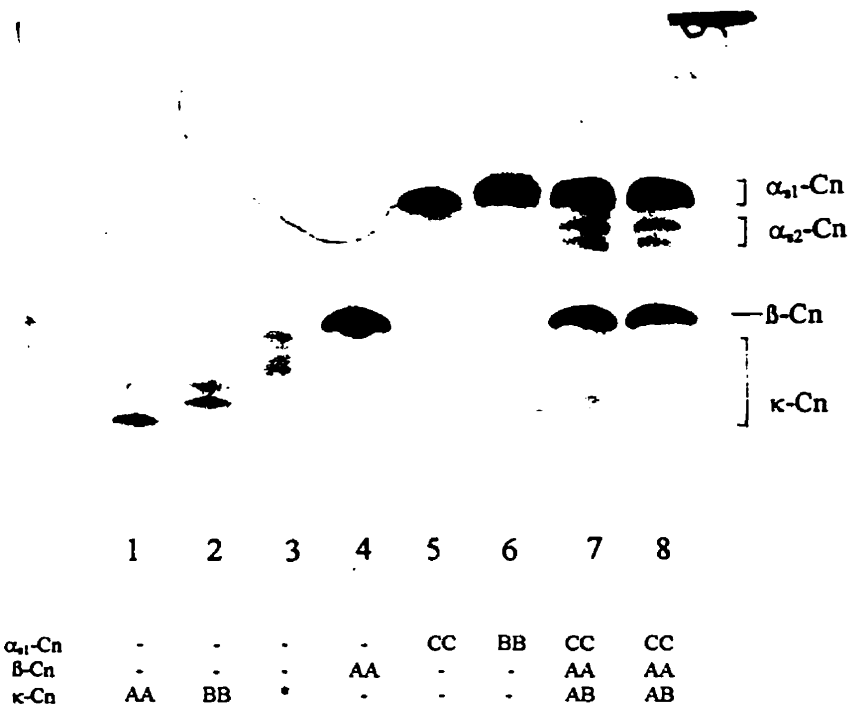

Fig. 14 PAGE patterns of purity of α_{s1}-Cn, ß-Cn and κ-Cn. Conditions: pH = 8.3, 8% polyacrylamide gel containing Tris buffer, 4 M urea. Tris-Gly electrode buffer (Ng-Kwai-Hang *et al.*, 1984b). * Glycoslyzation of κ-Cn. -, no band.

Table 3 Whole casein with different phenotype combinations of α_{s1}-casein, ß-casein and κ-casein selected for the study

Combination	α_{s1}-Casein	ß-Casein	κ-Casein
1	BB	A^1A^1	AA
2	BB	A^1A^2	AA
3	BB	A^2A^2	AA
4	BC	A^1A^2	AA
5	BB	A^1A^1	AB
6	BB	A^1A^2	AB
7	BB	A^1A^1	BB
8	BB	A^1A^2	BB
9	BB	A^2A^2	BB
10	BB	A^1B	BB
11	BB	A^2B	BB
12*	MIX	MIX	MIX

* Sample prepared from bulk tank milk which contains a mixture of the above phenotypes of the milk proteins

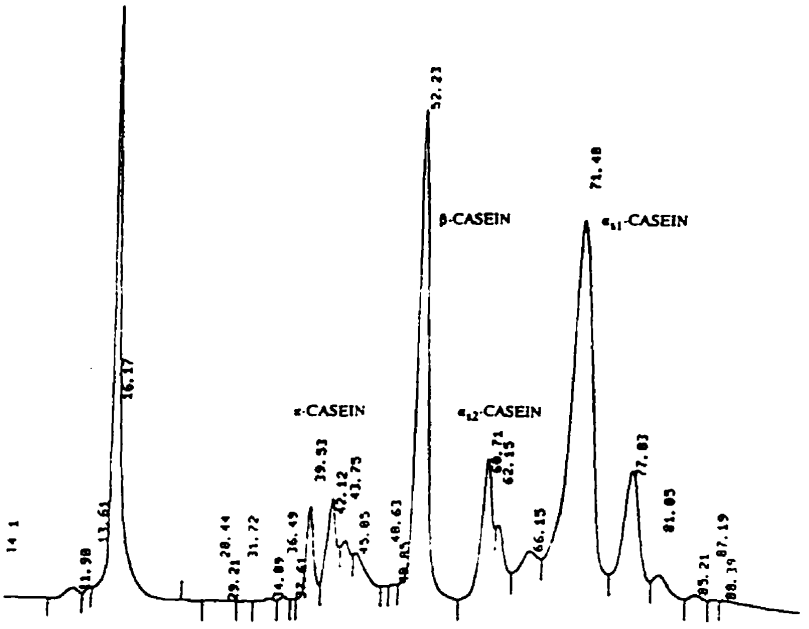

Fig.15 Separation profile of whole casein, HPLC, absorbance at 280 nm.

Table 4 Composition of whole casein with different phenotype combinations of α_{s1}-casein, ß-casein and κ-casein

Haplotype*	α_{s1}-Casein (%)	α_{s2}-Casein (%)	ß-Casein (%)	κ-Casein (%)
BB/A^1A^1/AA	40.96 ± 0.78	9.40 ± 0.96	37.82 ± 1.11	11.82 ± 1.16
BB/A^1A^2/AA	40.71 ± 0.73	9.16 ± 0.75	38.44 ± 0.43	11.69 ± 0.54
BB/A^2A^2/AA	40.13 ± 0.87	9.45 ± 1.21	38.50 ± 0.99	11.92 ± 1.15
BC/ A^1A^2/AA	40.35 ± 0.55	9.94 ± 0.46	37.97 ± 0.48	11.74 ± 0.57
BB/A^1A^1/AB	40.60 ± 1.34	9.27 ± 0.62	37.83 ± 1.01	11.94 ± 1.28
BB/A^1A^2/AB	40.65 ± 1.68	9.68 ± 0.66	38.80 ± 0.89	11.67 ± 1.26
BB/ A^1A^1/BB	40.37 ± 1.28	9.58 ± 0.77	38.13 ± 0.81	11.92 ± 0.88
BB/ A^1A^2/BB	40.33 ± 0.86	9.30 ± 0.38	38.30 ± 1.17	12.07 ± 0.94
BB/A^2A^2/BB	40.28 ± 0.58	9.80 ± 1.19	38.04 ± 1.20	11.88 ± 1.19
BB/A^1B/BB	40.75 ± 1.17	9.88 ± 0.79	38.16 ± 0.60	11.21 ± 0.86
BB/A^2B/BB	40.89 ± 1.21	9.32 ± 0.51	38.45 ± 0.85	11.34 ± 1.11
MIX**	40.47 ± 0.91	9.83 ± 0.71	38.29 ± 1.24	11.41 ± 1.03

All values are means ± s.e.

* Combination of α_{s1}-casein, ß-casein and κ-casein

** Sample prepared from bulk tank milk which contains a mixture of the above phenotypes of the milk proteins

recorder. The different peaks representing κ-Cn were integrated as one protein. Table 4 shows the relative percentage of α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn in each α_{s1}-Cn/ß-Cn/κ-Cn haplotypes. No significant differences ($P>0.05$) existed among α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn within these twelve triplicates of whole casein samples. The quantitation of individual caseins suggested that any differences of functional properties of whole casein would be due to the different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn because the relative percentage of individual caseins was the same ($P>0.05$).

5.3 Source of ß-lactoglobulin

5.3.1 Fractionation of ß-lactoglobulin

The precipitation of skim milk at pH 4.6 yielded two major fractions: whole casein as the precipitate and whey proteins in the supernatant. The Macro-High Q ion exchange chromatography was employed to fractionate whey proteins by passing the acid whey through the column with a loading volume of 50 ml. Figure 16 shows the separation profile of whey proteins. The immunoglobulins, α-La, BSA, ß-Lg A and B were eluted out at 0.15, 0.20, 0.22, 0.24 and 0.30 M of NaCl Tris-HCl buffer, respectively. The immunoglobulin was not separated as well as α-La and BSA. Since only ß-Lg was collected during the sample preparation, the other components were discarded thereafter.

5.3.2 Purity of ß-lactoglobulin

Figure 17 shows the purity of immunoglobulin, BSA, α-La and ß-Lg variant A and B isolated by the chromatography. Lanes 1 and 2 show the A and B variants of ß-Lg, respectively. Immunoglobulin, BSA and α-La was shown at lanes 7, 5 and 6, respectively. The protein at lane 8 shows the whole whey which was loaded as the reference. There were not any impurities in ß-Lg fractions, the purity was acceptable.

5.3.3 Content of variant A and B of ß-lactoglobulin

Analysis of whey protein from bulk tank milk on chromatography suggested that the mixture of AA, AB and BB phenotypes of ß-Lg contained proportionately more A variant than that in ß-Lg AB phenotype whey protein. The ratio of variant A to B of ß-Lg in bulk tank milk was 62.20 to 37.80. In ß-Lg AB phenotype whey protein, the ratio

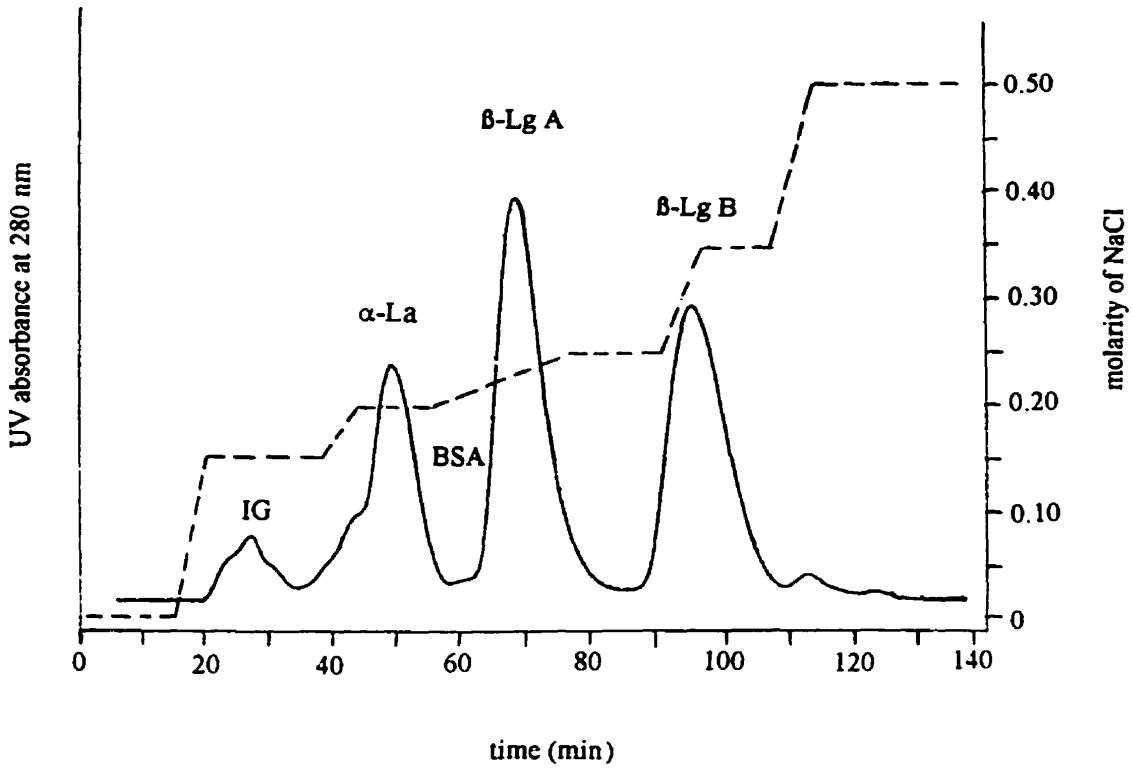

Fig. 16 Profile of whey protein fractions separated by liquid chromatography, column supported with Macro-High Q. Gradient: step-wise and linear at 0.15 M, 0.20 M, 0.25 M, 0.35 M and 0.50 M NaCl in 20 mM, pH 7.0 Tris-HCl buffer.

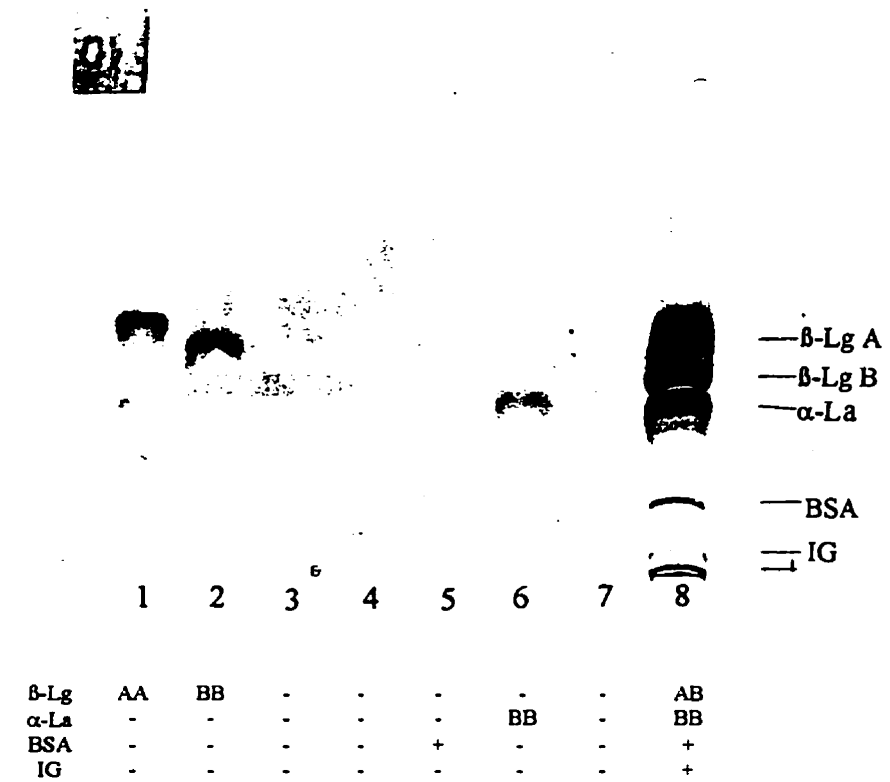

Fig. 17 PAGE patterns of purity of whey proteins. Conditions: pH=8.3, 12% polyacrylamide gel containing Tris buffer. Tris-Gly electrode buffer (Ng-Kwai-Hang *et al.*, 1984b). +, band; -, no band.

was 51.96 to 48.04. This result was in accordance with the separation by Yoshida (1990) with a Sephacry S-200 gel filtration column and with the separation by Ng-Kwai-Hang and Kim (1995).

5.4 Surface properties of caseins at the air-water interface

The initial interfacial tension (γ_0) of the buffer was found to be 72.4 ± 0.2 mN/m. In order to evaluate the kinetics of surface tension decay at the air-water interface in terms of different rate determining steps i.e., the effect of surface enlargement, diffusion and penetration, log($d\pi/dt$) versus π plots were constructed by computer differentiation of the fitted surface tension decay curves which were approximated in small sections using a second order polynomial function as described by Tornberg (1978b). Linear parts of the log($d\pi/dt$) versus π plots were derived by linear regression analysis. Break points, representing different rate-determining steps during surface tension decay, were evident. The correlation coefficients for the linear parts of the linear regression varied from 0.90 to 0.99. The surface pressure attained (π_{att}) during each rate-determining step and the times of attainment (t_{att}) were derived from the plot of log($d\pi/dt$) versus π in the surface tension decay curves. The maximum value of log($d\pi/dt$) that illustrates the highest rate of surface tension decay was also presented.

5.4.1 Individual caseins of different genetic variants

5.4.1.1 α_{s1}-Casein

The time-dependence of surface tension at the air-water interface of α_{s1}-Cn of different phenotypes are shown in Figure 18. The log($d\pi/dt$) as a function of π for the solution of α_{s1}-Cn of different phenotypes are shown in Fig. 19. Both of the above figures show the significant differences ($P < 0.05$) among the three phenotypes of α_{s1}-Cn in their surface tension decay profiles. The profile of time-dependent of surface tension at air-water interface shows that α_{s1}-Cn CC decreased the surface tension faster than BC and BB. The surface tension decay curve of phenotype BB of α_{s1}-Cn decreased more slowly than BC and CC. It took about 15 min for both BC and CC to reach a plateau whereas it needed 30 min for BB to get to the same level. The profile of log($d\pi/dt$) as a function of π (Fig. 19) looks somewhat similar for the three phenotypes

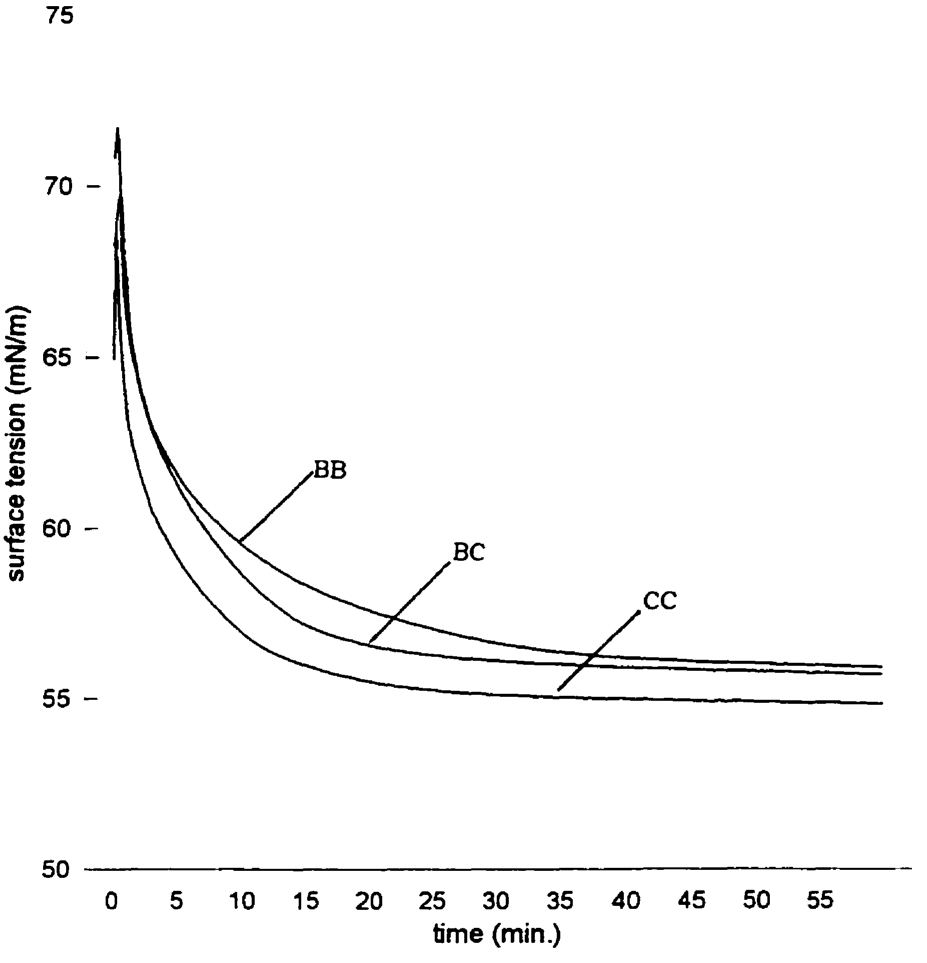

Fig. 18 Time-dependence of surface tension at the air-water interface for 0.005% (w/v) solution of α_{s1}-casein of different phenotypes

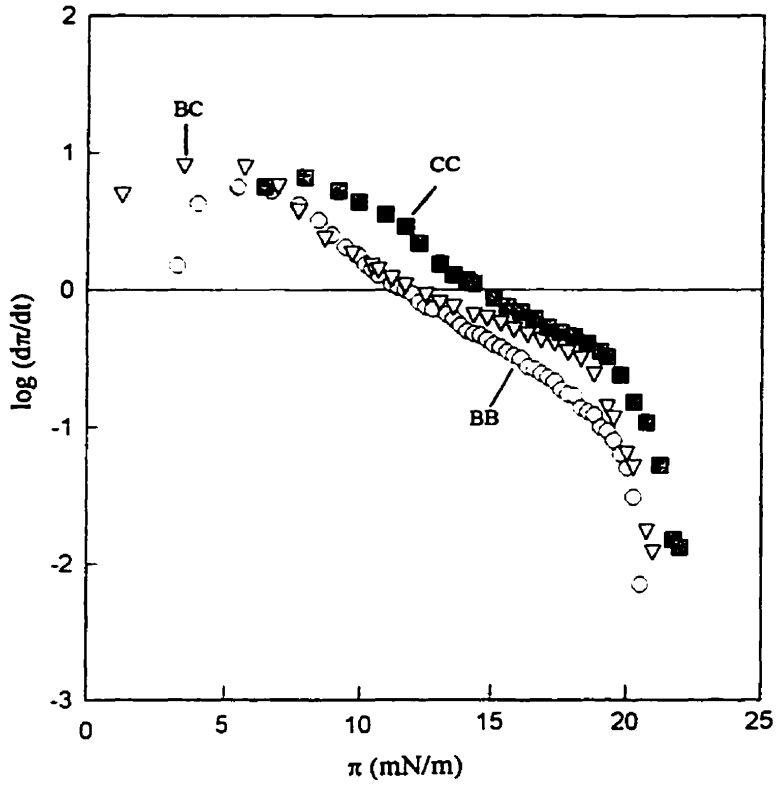

Fig.19 Log (dπ/dt) as a function of π for 0.005% (w/v) solution of α_{s1}- casein of different phenotypes.

Table 5 Kinetics of surface tension decay at the air-water interface of solutions of α_{s1}-casein of different phenotypes at bulk phase concentration of 0.005% (w/v)

Phenotype	Step	Max. log(dπ/dt)	$\pi_{att.}$ (mN/m)	Att.time (min)
BB	1	0.75 ± 0.02^{c}	4.46 ± 0.06^{b}	0.83 ± 0.02^{a}
	2		6.81 ± 0.18^{b}	1.33 ± 0.03^{a}
	3		16.48 ± 0.10^{c}	60.00
BC	1	0.90 ± 0.03^{a}	2.85 ± 0.06^{c}	0.50 ± 0.01^{c}
	2		6.71 ± 0.23^{c}	1.17 ± 0.03^{b}
	3		16.71 ± 0.05^{b}	60.00
CC	1	0.82 ± 0.02^{b}	6.42 ± 0.05^{a}	0.67 ± 0.02^{b}
	2		9.34 ± 0.06^{a}	1.33 ± 0.03^{a}
	3		17.56 ± 0.13^{a}	60.00

All values are means ± s.e.
Within the column and at the same step, values with different letters are significantly different ($P<0.05$)
Step 1, 2 and 3 represent surface enlargement, diffusion and penetration, respectively.

of α_{s1}-Cn at the diffusion and penetration steps of the protein molecules. There were significant differences ($P < 0.05$) at the surface enlargement step. Table 5 shows the differences of kinetics of surface tension decay at the air-water interface among three phenotypes of α_{s1}-Cn. The kinetics suggested that α_{s1}-Cn CC was more surface active than BB and BC phenotype during all the rate-determining steps. α_{s1}-Casein BC had a slightly larger surface tension than BB during the step 3 but lower surface pressure during surface enlargement and diffusion steps. Though the steps 1 and 2 were not the major period of the surface tension decay for the three phenotypes of α_{s1}-Cn, CC still had a proportion of 36.56% for the step 1 which was much higher than BB type (27.06%) and BC type (17.06%). The most reduction of the surface tension was during the penetration step for BB and BC type. During this stage, BB and BC decreased a portion of 58.68% and 59.85% of the surface tension. The maximum value of $\log(d\pi/dt)$ in Table 5 suggests the highest speed of the surface tension decay during the surface enlargement period. The order of this value for three phenotypes was BC > CC > BB. Phenotype BC reached a very high rate of surface tension decay in a short time and this rate was reduced rapidly.

The difference between variant B and C is at position 192 in the primary structure of α_{s1}-Cn. At this position, the amino acid residue is Glu for B variant and Gly for C variant. Variant B carries one more negative charge than variant C under the alkaline condition (> pI). This might be the source of the differences between the different phenotypes of α_{s1}-Cn.

5.4.1.2 ß-Casein

Figure 20 shows that the six phenotypes of ß-Cn performed quite differently in their surface tension decay at the air-in-water interface. Phenotype A^1A^1 and A^1A^2 reduced the surface tension and reached a plateau very fast. Phenotype A^2A^2 was associated with the lowest rate. Figure 21 suggestes that during the initial step, A^1A^1 was more active than the other five phenotypes. The ranking of surface tension decay for the all six phenotypes of ß-Cn was: $A^1A^1 > A^1A^2 = A^2A^2 > BB > A^2B > A^1B$. The initial step was very important for ß-Cn. Table 6 shows that 62 to 82% of surface

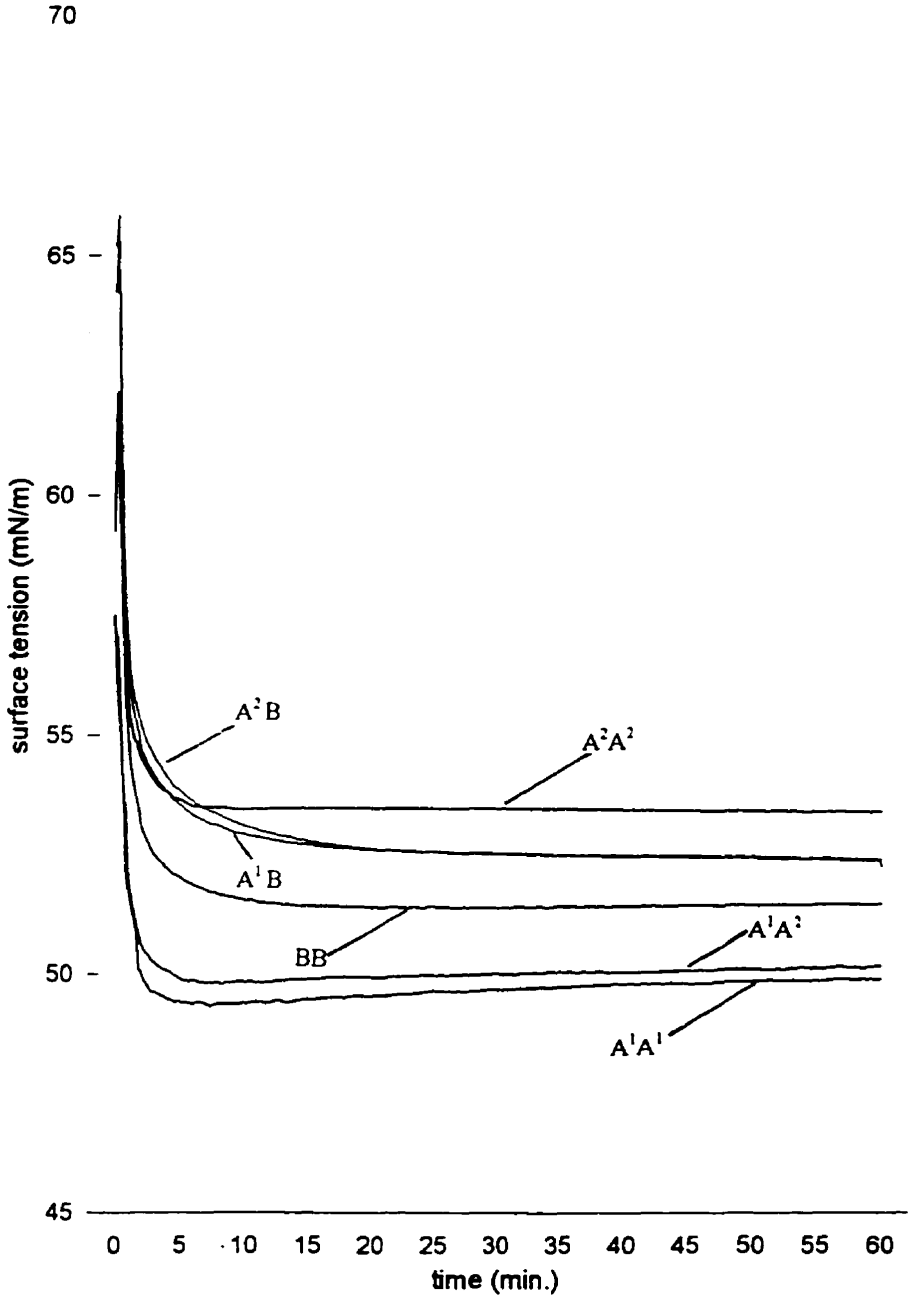

Fig. 20 Time-dependence of surface tension at the air-water interface for 0.005% (w/v) solution of ß-casein of different phenotypes

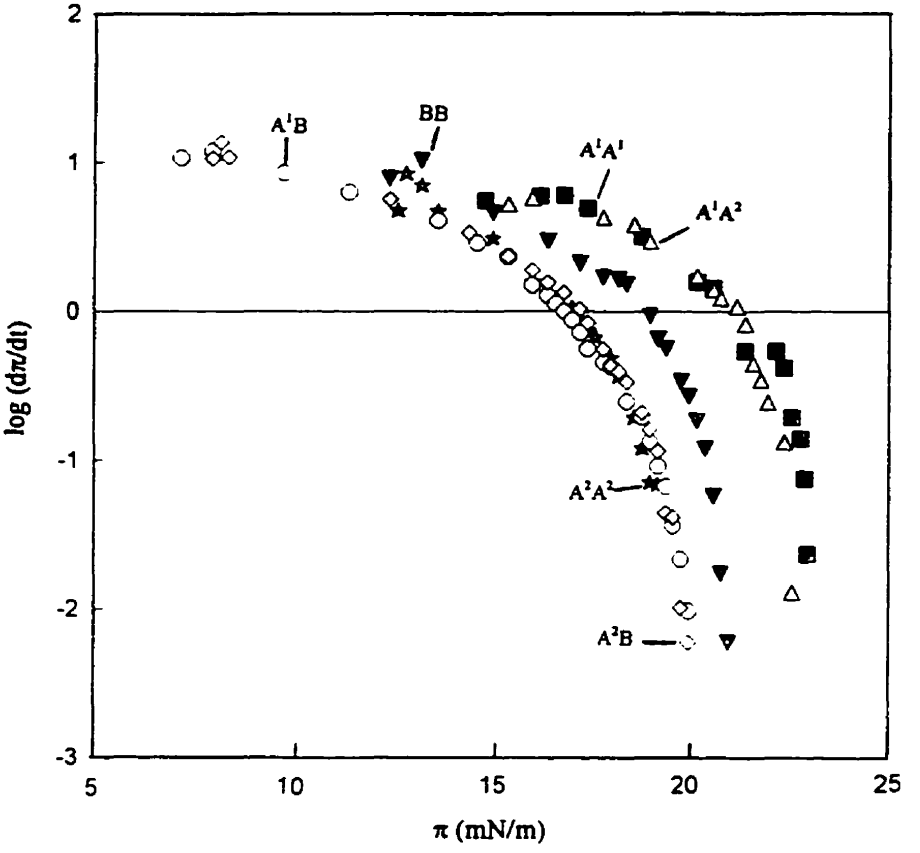

Fig.21 Log (dπ/dt) as a function of π for 0.005% (w/v) solution of ß - casein of different phenotypes.

Table 6 Kinetics of surface tension decay at the air-water interface of solutions of ß-casein of different phenotypes at bulk phase concentration of 0.005% (w/v)

Phenotype	Step	Max. log(dπ/dt)	$\pi_{att.}$ (mN/m)	Att.time (min)
A^1A^1	1	0.78 ± 0.02^{de}	18.78 ± 0.67^{a}	0.67 ± 0.02^{a}
	2		20.83 ± 0.69^{a}	1.67 ± 0.02^{a}
	3		22.98 ± 0.54^{a}	60.00
A^1A^2	1	0.78 ± 0.03^{de}	17.89 ± 0.58^{b}	0.50 ± 0.01^{b}
	2		20.61 ± 0.75^{a}	1.67 ± 0.03^{a}
	3		22.81 ± 0.31^{a}	60.00
A^2A^2	1	0.93 ± 0.03^{c}	17.75 ± 0.48^{b}	0.50 ± 0.01^{b}
	2		16.48 ± 0.60^{c}	1.00 ± 0.03^{c}
	3		19.01 ± 0.41^{c}	60.00
A^1B	1	1.08 ± 0.03^{ab}	6.52 ± 0.36^{e}	0.17 ± 0.01^{c}
	2		14.62 ± 0.48^{d}	0.83 ± 0.01^{d}
	3		20.01 ± 0.24^{b}	60.00
A^2B	1	1.13 ± 0.03^{a}	12.39 ± 0.48^{d}	0.50 ± 0.01^{b}
	2		16.64 ± 0.61^{c}	1.67 ± 0.02^{a}
	3		19.99 ± 0.05^{b}	60.00
BB	1	1.03 ± 0.02^{b}	15.05 ± 0.43^{c}	0.67 ± 0.02^{a}
	2		18.57 ± 0.43^{b}	1.33 ± 0.02^{b}
	3		20.93 ± 0.61^{b}	60.00

All values are means ± s.e.
Within the column and at the same step, values with different letters are significantly different ($P<0.05$)
Step 1, 2 and 3 represent surface enlargement, diffusion and penetration, respectively.

tension was decreased during this stage except for A^1B where there was a 32.58% decrease in the total surface tension. The changes in diffusion and penetration steps of ß-Cn were not as drastic when compared to the surface enlargement step, because at only approximately 25% of the total pressure was decreased at these two stages. The ranking for the step 2 of the six ß-Cn was: $A^1A^1 = A^1A^2 > BB > A^2A^2 = A^2B > A^1B$. The final surface pressure appeared to be relatively large as indicated by the highest value of 22.98 mN/m for A^1A^1 and the lowest value of 19.01 mN/m for A^2A^2. There were no significant differences ($P>0.05$) of the surface presure at steps 2 and 3 between A^1A^1 and A^1A^2; A^1B, A^2B and BB. As shown in Table 6, higher maximum values of $\log(d\pi/dt)$ were associated with ß-Cn A^1B, A^2B and BB and lower maximum values were associated with A^1A^1, A^1A^2 and A^2A^2 ($P < 0.05$).

The difference between variants A^1 and A^2 of ß-Cn is at position 67 of the polypipetide chain. The variant A^1 has a His residue instead a Pro residue for A^2. Both variant B and A^1 have a His residue at position 67, but B has an Arg at position 122 whereas A^1 has a Ser residue at this position. The differences of these three variants of ß-Cn in the amino acid sequence lead to a significant differences in the surface properties. With two positively charged amino acid residues (Arg and His), B variant has one and two more net positive charges than A^1 and A^2, respectively. The more charged residue could form a bond with the other functional group and make the structure less easy to open. Proteins with denser structures are less surface activity than proteins with a loose structure (Tornberg, 1978b). Mitchell *et al.* (1970) demonstrated this phenomenon with a study on random and globular proteins and found that random protein is more active than globular protein. In ß-Cn, phenotype A^1A^1 and A^1A^2 might be looser and more flexible than other phenotypes and therefore they showed a higher surface activity.

5.4.1.3 κ-Casein

For κ-Cn, Fig. 22 shows that phenotype AB lowered the surface tension faster than phenotype AA and BB during all the three steps. Phenotype AA was more surface active than phenotype BB. At the initial step, the kinetics of surface tension decay

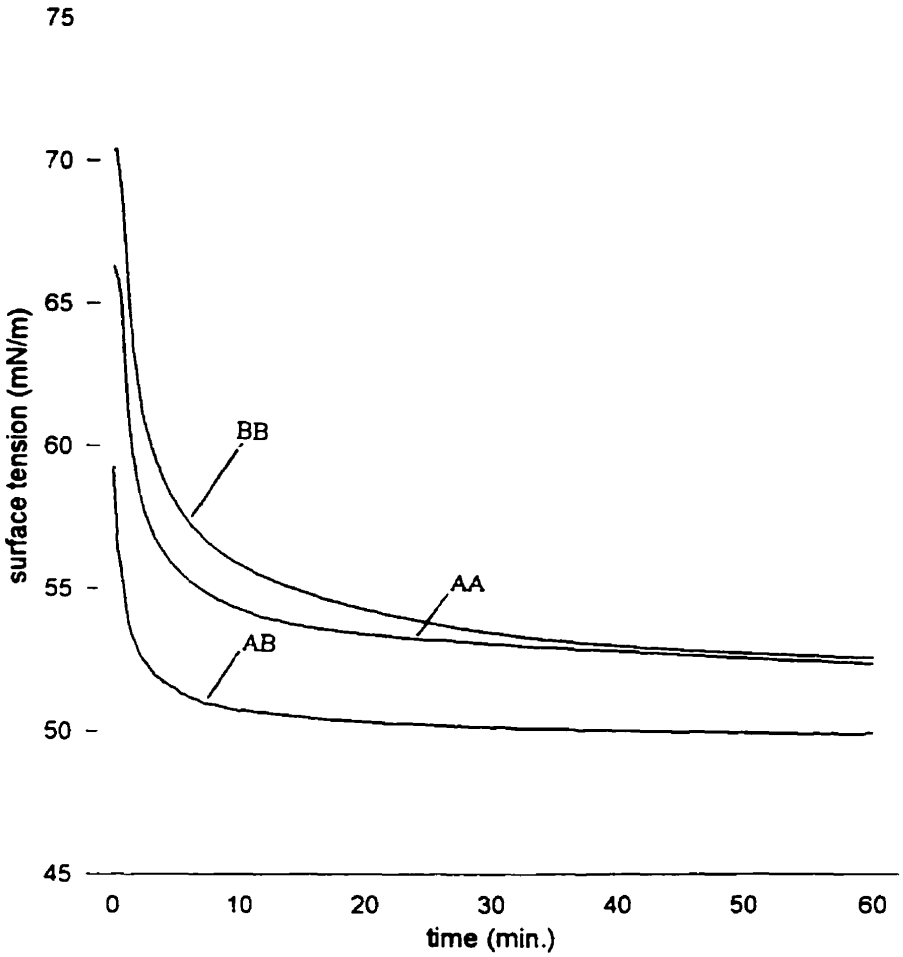

Fig. 22 Time-dependence of surface tension at the air-water interface for 0.005% (w/v) solution of κ-casein of different phenotypes

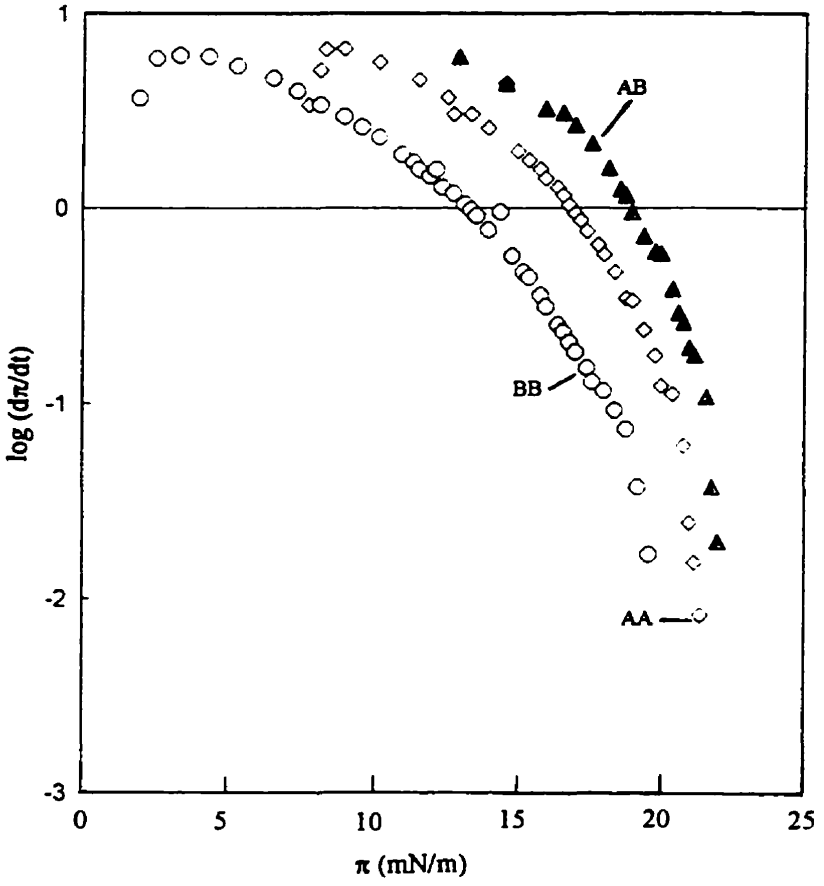

Fig.23 Log (dπ/dt) as a function of π for 0.005% (w/v) solution of κ-casein of different phenotypes.

Table 7 Kinetics of surface tension decay at the air-water interface of solutions of κ-casein of different phenotypes at bulk phase concentration of 0.005% (w/v)

Phenotype	Step	Max. log(dπ/dt)	π_{att} (mN/m)	Att.time (min)
AA	1	0.82 ± 0.03^{a}	9.72 ± 0.46^{b}	0.83 ± 0.01^{a}
	2		13.24 ± 0.32^{b}	1.67 ± 0.03^{b}
	3		20.05 ± 0.16^{b}	60.00
AB	1	0.78 ± 0.02^{b}	16.57 ± 0.46^{a}	0.33 ± 0.02^{b}
	2		18.16 ± 0.26^{a}	1.00 ± 0.03^{c}
	3		22.47 ± 0.57^{a}	60.00
BB	1	0.77 ± 0.02^{b}	5.45 ± 0.29^{c}	0.83 ± 0.02^{a}
	2		10.61 ± 0.62^{c}	2.00 ± 0.05^{a}
	3		19.85 ± 0.35^{b}	60.00

All values are means ± s.e.
Within the column and at the same step, values with different letters are significantly different ($P<0.05$)
Step 1, 2 and 3 represent surface enlargement, diffusion and penetration, respectively.

shown in Table 7 indicated that it only took 0.33 min for AB type of κ-Cn to reduce 73.74% of the surface tension but 0.83 min to reduce 48.48% and 27.46% for κ-Cn AA and BB, respectively. Considerably more reduction of surface tension occurred during the last two steps for phenotype AA and BB of κ-Cn. The maximum value of $\log(d\pi/dt)$ of phenotype AA was significantly higher than AB and BB. Figure 23 shows the differences of three phenotypes of κ-Cn in the profile of $\log(d\pi/dt)$ as a function of π. It is obvious that the $\log(d\pi/dt)$ value was obtained at higher pressure for AB phenotype than for AA and BB phenotypes.

The differences between variant A and B of κ-Cn are located at the positions 136 and 148. Variant A has Thr and Asp residues at these two positions instead of Ile and Ala for variant B. With an Asp residue, A variant is more negatively charged than B variant. This difference in amino acid composition might be the major factor which affected the surface activity at the air-water interface of this protein. The superiority of surface activity of AB phenotype of κ-Cn might attribute to the interaction between variant A and B.

5.4.2 Individual caseins

The data of the different phenotypes within α_{s1}-Cn, ß-Cn and κ-Cn were pooled, and the parameters measured for α_{s2}-Cn were also used for the analysis of the differences of surface properties between the four individual caseins. Figure 24 shows the different profiles of surface tension decay for the isolated α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn. It was found that ß-Cn reduced the surface tension faster than the other three caseins and the surface presure of ß-Cn reached a plateau at 5 minutes. α_{s1}-Casein was associated with lowest rate of surface tension decay. It took 20 min for α_{s1}-Cn to reach a plateau. The final surface tension level was significantly different ($P < 0.05$) among the four caseins. The surface pressure was 20.96 mN/m for ß-Cn; 20.79 mN/m for κ-Cn, 17.44 mN/m for α_{s2}-Cn and 16.92 mN/m for α_{s1}-Cn at the end of 60 min. This result suggested that ß-Cn and κ-Cn can decrease the surface tension further than α_{s1}-Cn and α_{s2}-Cn. During the surface enlargement and diffusion steps as shown in Fig. 25, ß-Cn had the fastest surface tension decay at highest surface pressure followed by

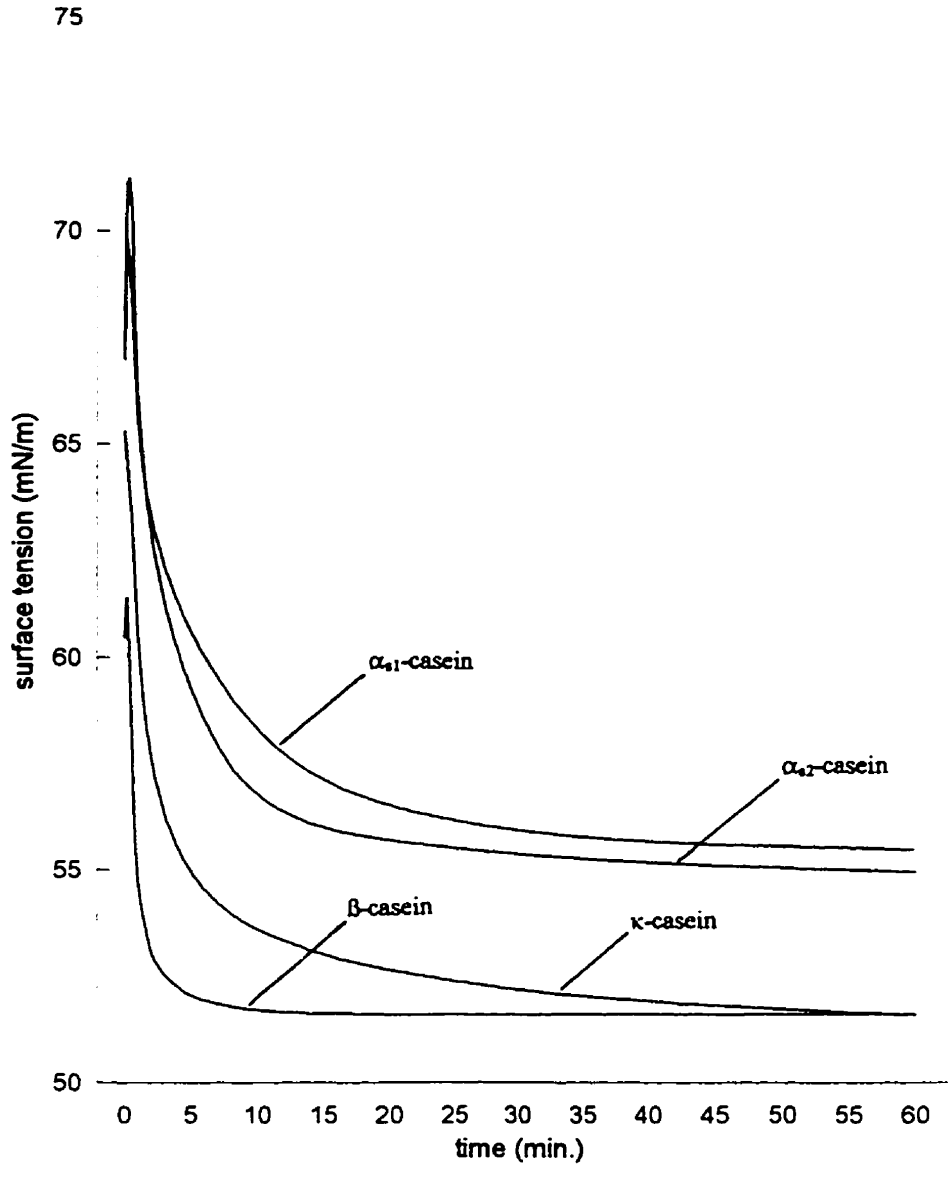

Fig. 24 Time-dependence of surface tension at the air-water interface for 0.005% (w/v) solution of α_{s1}-casein, α_{s2}-casein, ß-casein and κ-casein.

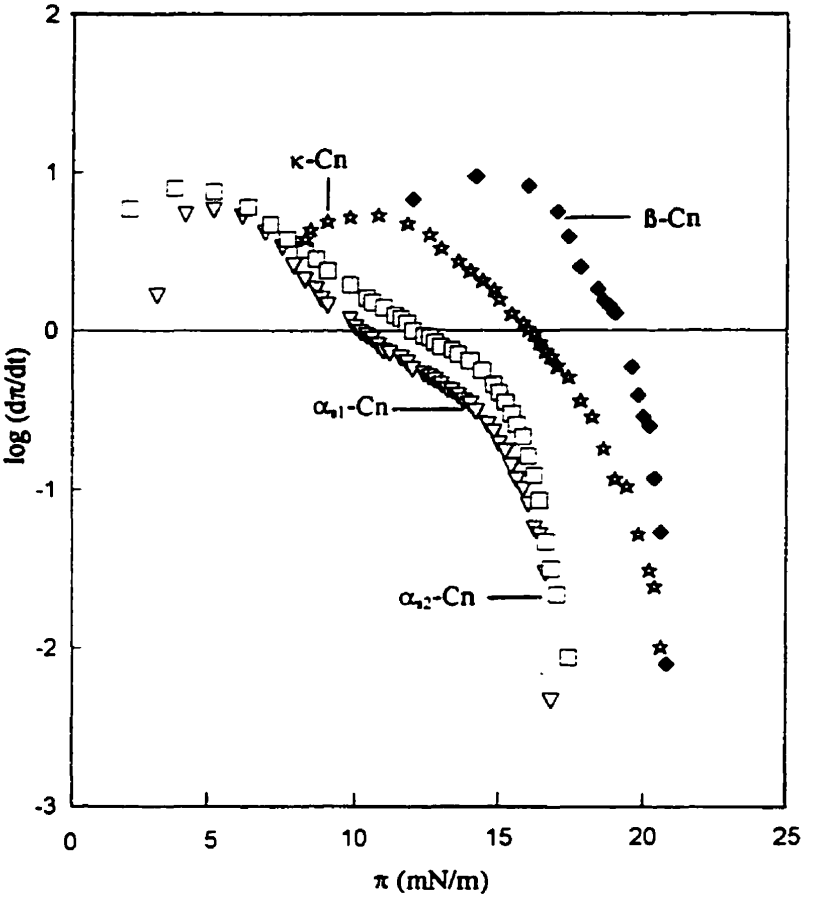

Fig.25 Log (dπ/dt) as a function of π for 0.005% (w/v) solution of α_{s1}-casein, α_{s2}- casein, ß- casein and κ- casein.

Table 8 Kinetics of surface tension decay at the air-water interface of solutions of individual caseins at bulk phase concentration of 0.005% (w/v)

Casein	Step	Max. log(dπ/dt)	$\pi_{att.}$ (mN/m)	Att. time (min)
α_{s1}-Cn	1	0.92 ± 0.08[a]	4.58 ± 1.79[c]	0.67 ± 0.17[b]
	2		7.62 ± 1.49[c]	1.28 ± 0.09[b]
	3		16.92 ± 0.57[c]	60.00
α_{s2}-Cn	1	0.90 ± 0.05[a]	6.78 ± 0.24[c]	0.67 ± 0.03[b]
	2		9.56 ± 0.20[c]	1.33 ± 0.07[ab]
	3		17.44 ± 0.20[b]	60.00
ß-Cn	1	0.96 ± 0.15[a]	14.73 ± 4.66[a]	0.50 ± 0.18[b]
	2		17.96 ± 2.48[a]	1.36 ± 0.37[ab]
	3		20.96 ± 1.62[a]	60.00
κ-Cn	1	0.79 ± 0.03[a]	10.58 ± 5.61[b]	0.83 ± 0.00[a]
	2		14.00 ± 3.83[b]	1.56 ± 0.51[a]
	3		20.79 ± 1.46[a]	60.00

All values are means ± s.e.
Within the column and at the same step, values with different letters are significantly different ($P<0.05$)
Step 1, 2 and 3 represent surface enlargement, diffusion and penetration, respectively.

κ-Cn, α_{s2}-Cn and α_{s1}-Cn. Table 8 summarises the kinetics of surface tension decay at the air-water interface for α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn. The initial step was the major surface tension decay part for ß-Cn and κ-Cn. During this step, 66.68% and 49.89% of the surface tension were decreased for ß-Cn and κ-Cn, respectively. In contrast, the diffusion step was the major decay part for α_{s1}-Cn and α_{s2}-Cn which reduced the surface tension by 55.12% and 45.18% during this stage, respectively. The time attainment over 60 min also showed this observations. It took 0.84% of the total time to reduce 66.68% of the total surface pressure for ß-Cn, which was significantly ($P < 0.05$) lower than for the other three caseins. Although there was no significant difference among the four caseins in the $\Delta\pi/\pi_{60}$, the time spent during the diffusion step was longer for ß-Cn and κ-Cn than that for α_{s1}-Cn and α_{s2}-Cn.

The differences in characteristics of surface activity at air-water interface of the four caseins can be interpreted by the difference of the protein structures. Swaisgood (1992) reported that ß-Cn does not have as much secondary conformation as α_{s1}-Cn and α_{s2}-Cn. ß-Casein is the most hydrophobic and contains more prolyl residues than other caseins. The polar domain has only one anionic cluster compared to α_{s1}-Cn and α_{s2}-Cn, so the molecular structure is dominated by hydrophobic interactions of its surface and is less sensitive to ionic strength than α_{s1}-Cn and α_{s2}-Cn. The monomer of ß-Cn is only observed at 0 to 4 °C (Payens and van Markwijk, 1963). As the temperature increases above 4 to 5 °C, ß-Cn undergoes a highly cooperative, reversible, rapidly equilibrating discrete self-association yielding large polymers with a narrow size distribution, similar to the formation of detergent micelles (Arima *et al.*, 1979; Buchheim and Schmidt, 1979; Takase *et al.*, 1980; Schmidt, 1985; Kajiwara *et al.*, 1988). These characteristics and its loose and flexible structure enable ß-Cn to diffuse and penetrate faster at the air-water interface when compared to the other three caseins. κ-Casein is a protein with an amphipathic structure but without the anionic phosphate cluster in its polar domain. The hydrophobic domain of κ-Cn as compared to that of ß-Cn is less hydrophobic. It has a lower frequency of prolyl residues and contains more secondary structure. The less hydrophobic, lower frequency of prolyl

residues and more secondary structure may be the reason why κ-Cn is less surface active than ß-Cn. α_{s1}-Casein exhibits progressive consecutive self-association to dimers, tetramers, hexamers, etc., with the degree of association being strongly dependent upon the pH and ionic strength of the solution (Schmidt, 1985). α_{s2}-Casein, when compared to α_{s1}-Cn, is more hydrophilic. It contains three anionic clusters and may contain inter or intramolecular disulphide bonds and has 40% fewer prolyl residues (Swaisgood, 1992). α_{s2}.Casein also associates less extensively than α_{s1}-Cn as a result of stronger electrostatic forces and lower hydrophobicity. The data of the kinetics of surface tension decay at the air-water interface of the solution of α_{s1}-Cn and α_{s2}-Cn indicated that α_{s2}-Cn was slightly more surface active than α_{s1}-Cn during surface enlargement and diffusion ($P>0.05$), and significantly more active at the stage of penetration ($P<0.05$). According to its structure, α_{s2}-Cn should be less surface active than α_{s1}-Cn at the air-water interface. α_{s2}-Casein is a protein with an amphipathic highly charged structure and the associative properties are very dependent on the ionic strength (Snoeren *et al.*, 1980). The conflicting observation under this measurement condition might be due to the change in the conformation of α_{s2}-Cn.

5.4.3 Whole caseins

The plot of time-dependence of surface tension at the air-water interface for whole casein with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn is shown in Fig. 26. The profile of $\log(d\pi/dt)$ as a function of π for whole casein with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn is shown in Fig. 27. Whole casein also displayed three major steps at the air-water interaface: surface enlargement, diffusion and penetration (Fig. 27). The previous experiment on surface active properties of individual caseins and individual caseins of different genetic variants suggested that there were significant differences ($P < 0.05$) among these proteins. Kinetic analysis on the surface tension decay curves and the plot of $\log(d\pi/dt)$ as a function of π at each rate-determining step (Fig. 27) shown in Table 9 revealed the magnitude of these differences. The twelve whole caseins were categorized into three groups according to the phenotype of ß-Cn for convenient analysis and discussion, because ß-Cn is the most

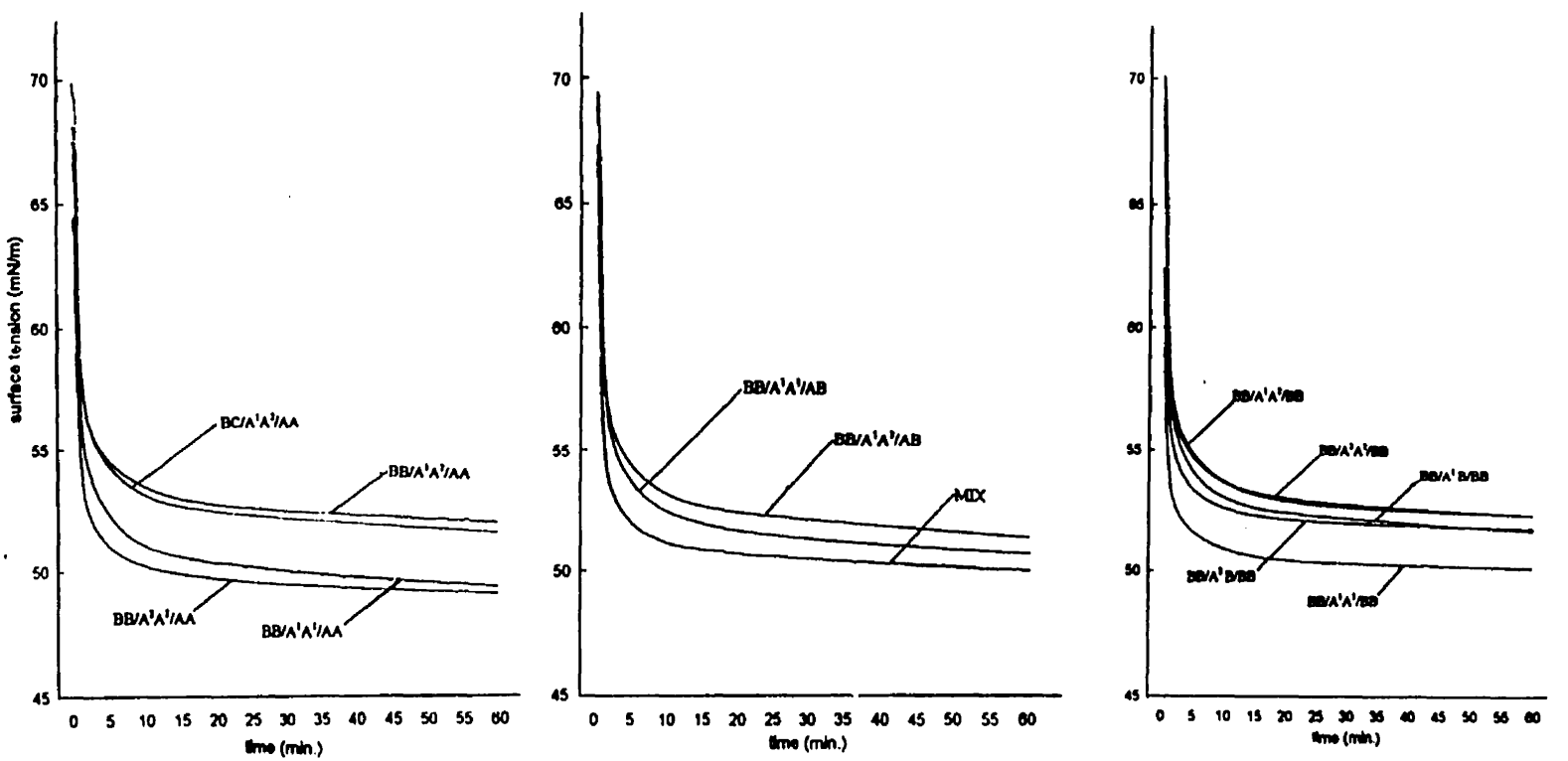

Fig. 26 Time-dependence of surface tension at the air-water interface for 0.005% (w/v) solutions of whole casein with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn.

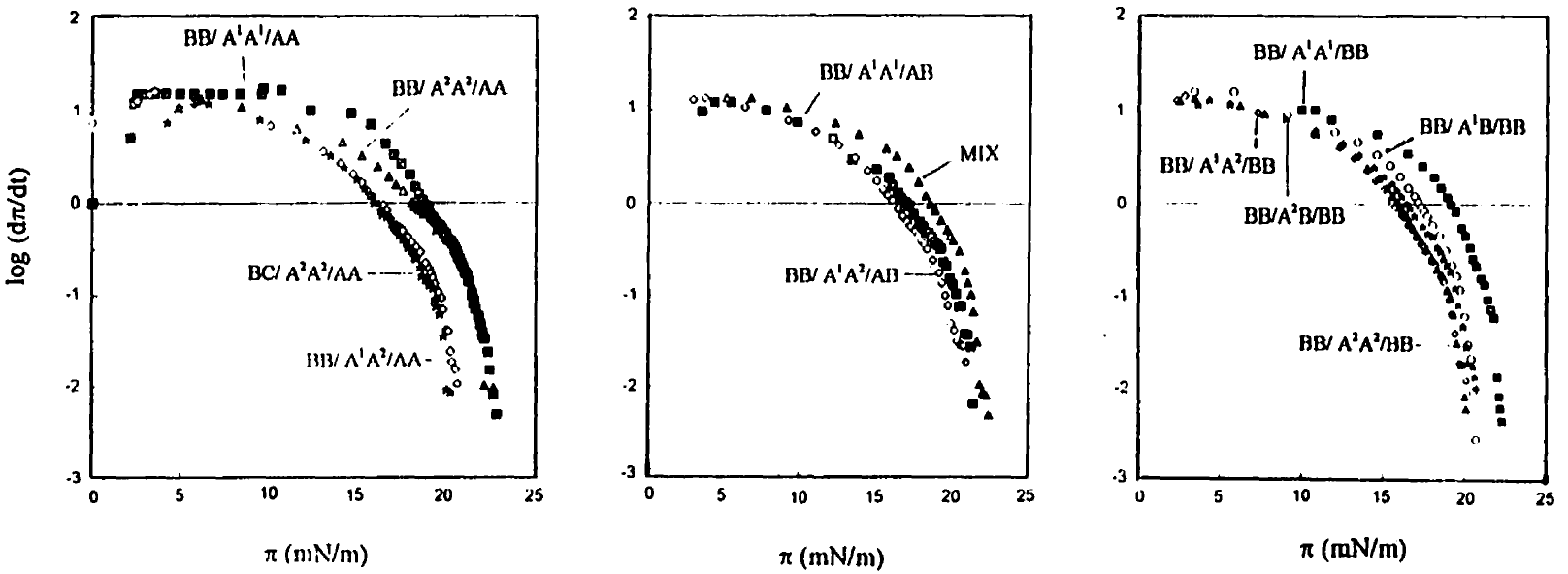

Fig. 27 Log (dπ/dt) as a function of π for 0.005% (w/v) solutions of whole casein with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn.

effective factor that influences the surface activity of the whole caseins. The phenotype of α_{s1}-Cn and κ-Cn were kept the same when the comparison was made between the phenotypes of ß-Cn.

Figure 26 shows that BB/A^2A^2/AA was the most surface active protein with the highest surface pressure value of 23.03 mN/m at the end of 60 minutes. Corresponding values were 20.90 mN/m for BB/A^1A^2/AA and 20.13 mN/m for BB/A^1A^1/AA. Comparing these three whole caseins that have the same phenotypes for α_{s1}-Cn and κ-Cn, the difference might be due to the genetic variants of ß-Cn. The effect of genetic variants of ß-Cn on surface activity of these three whole caseins was in the order of $A^2A^2 > A^1A^1 > A^1A^2$. This observation was in conflict with the results obtained from the individual ß-Cn of different phenotypes. In that study, the order of surface activity was $A^1A^2 > A^1A^1 > A^1B > A^2B > A^2A^2 >$BB. This difference might be due to the fact that the whole casein micelle influenced the properties of ß-Cn. We can also compare the difference between BC/A^1A^2/AA and BB/A^1A^2/AA. These two whole caseins have the same phenotypes for ß-Cn and κ-Cn and different phenotype for α_{s1}-Cn. Study on genetic variants of α_{s1}-Cn showed that BC was more surface active than BB phenotype. The final surface tension of BB/A^1A^2/AA was 20.90 mN/m which is not significantly ($P>0.05$) higher than that of BC/A^1A^2/AA. When the surface enlargement step was considered, BC/A^1A^2/AA decreased the surface tension faster ($P>0.05$) than BB/A^1A^2/AA. This observation is similar to what was previously observed for isolated α_{s1}-Cn.

The whole caseins in group 2 have almost the same phenotypes of α_{s1}-Cn and κ-Cn because the sample MIX contains the mixture of all phenotypes of the individual caseins. The kinetics of surface tension decay showed that BB/A^1A^2/AB was more surface active ($P<0.05$) than BB/A^1A^1/AB. This is in accordance with the study on individual ß-Cn of different genetic variants. The whole casein of MIX showed a superior ($P<0.05$) surface property than both BB/A^1A^2/AB and BB/A^1A^1/AB.

Group 3 included 5 whole caseins which all contain α_{s1}-Cn BB and κ-Cn BB, but the different phenotypes of ß-Cn. It was obviously shown in Fig. 26 that BB/A^1A^1/BB

combination was the most surface active protein with the highest attained surface pressure of 14.60 mN/m, 18.52 mN/m and 22.29 mN/m during the rate-determining step 1, 2 and 3, respectively. There was no significant difference between BB/A^1A^2/BB and BB/A^2A^2/BB in all the three steps. Haplotypes BB/A^1A^2/BB and BB/A^2A^2/BB were the least active compared to the other three proteins. Whole casein BB/A^1B/BB decreased the surface tension faster than BB/A^2B/BB only during the step 1. In the presence of α_{s1}-Cn BB and κ-Cn BB, the A^1A^1 phenotype of ß-Cn was superior to the other phenotypes and ranking of the activity was in the following order: $A^1A^1 > A^2B > A^1B > A^1A^2 = A^2A^2$.

The above discussion relates to whole caseins with the same phenotypes of α_{s1}-Cn and κ-Cn, but different phenotypes for ß-Cn. We can compare the difference among κ-Cn, if the same phenotypes of ß-Cn and α_{s1}-Cn were considered. There were significant differences ($P<0.05$) among the whole casein BB/A^1A^1/AA, BB/A^1A^1/AB and BB/A^1A^1/BB in all the three rate-determining steps. During the surface enlargement, BB/A^1A^1/BB decreased 65.50% of the total surface tension which was much higher than 47.54% for BB/A^1A^1/AA and 45.56% for BB/A^1A^1/AB. Similar trends were found during the diffusion step. Whole casein BB/A^1A^1/BB was also associated with the highest pressure at the end of 60 min with a value of 22.29 mN/m. The final pressure for BB/A^1A^1/AA and BB/A^1A^1/AB were 20.13 and 21.73 mN/m, respectively. The differences between these three whole caseins were all significant ($P<0.05$). When considering the other three whole caseins, BB/A^1A^2/AA, BB/A^1A^2/AB and BB/A^1A^2/BB, there were also significant differences ($P<0.05$). During the surface enlargement step, haplotype BB/A^1A^2/AB decreased the pressure faster ($P<0.05$) than BB/A^1A^2/AA and BB/A^1A^2/BB. The surface tension decay was very close for these three whole caseins during the second step. The pressure at the penetration step was 21.10 mN/m for BB/A^1A^2/AB which was significantly ($P<0.05$) higher than 20.90 mN/m for BB/A^1A^2/AA and 20.16 mN/m for BB/A^1A^2/BB. Since no combination of BB/A^2A^2/AB was found, there are only two combinations are available for the comparison: BB/A^2A^2/AA and BB/A^2A^2/BB. Table 9 shows that BB/A^2A^2/AA was

Table 9 Kinetics of surface tension decay at the air-water interface of solutions of whole caseins with different phenotype combinations of α_{s1}-casein, ß-casein and κ-casein at bulk phase concentration of 0.005% (w/v)

Haplotype of whole casein*	Step	Max. log(dπ/dt)	$\pi_{att.}$ (mN/m)	Att. time (min.)
BB/A^1A^1/AA	1	1.23 ± 0.03^a	9.57 ± 0.28^{cd}	0.67 ± 0.02^a
	2		17.04 ± 0.24^c	1.67 ± 0.03^a
	3		20.13 ± 0.54^f	60.00
BB/A^1A^2/AA	1	1.20 ± 0.02^a	3.13 ± 0.17^g	0.67 ± 0.02^a
	2		13.11 ± 0.35^i	1.33 ± 0.02^c
	3		20.90 ± 0.26^{de}	60.00
BB/A^2A^2/AA	1	1.14 ± 0.02^b	12.45 ± 0.75^b	0.67 ± 0.02^a
	2		16.70 ± 0.51^d	1.33 ± 0.02^c
	3		23.03 ± 0.10^a	60.00
BC/A^1A^2/AA	1	1.09 ± 0.03^c	9.40 ± 0.40^{cde}	0.67 ± 0.02^a
	2		14.87 ± 0.37^g	1.17 ± 0.03^{cd}
	3		20.54 ± 0.24^e	60.00
BB/A^1A^1/AB	1	1.09 ± 0.02^c	9.90 ± 0.36^c	0.67 ± 0.02^a
	2		15.58 ± 0.62^f	1.50 ± 0.03^{ab}
	3		21.73 ± 0.32^c	60.00
BB/A^1A^2/AB	1	1.13 ± 0.03^b	9.22 ± 0.52^{de}	0.50 ± 0.01^b
	2		14.99 ± 0.64^g	1.33 ± 0.03^c
	3		21.10 ± 0.16^d	60.00
BB/A^1A^1/BB	1	1.01 ± 0.03^d	14.60 ± 0.35^a	0.50 ± 0.01^b
	2		18.52 ± 0.28^a	1.17 ± 0.02^{cd}
	3		22.29 ± 0.21^b	60.00
BB/A^1A^2/BB	1	1.15 ± 0.03^b	7.35 ± 0.48^f	0.50 ± 0.02^b
	2		14.52 ± 0.20^h	1.33 ± 0.03^c
	3		20.16 ± 0.42^f	60.00
BB/A^2A^2/BB	1	1.13 ± 0.02^b	7.71 ± 0.33^f	0.50 ± 0.02^b
	2		14.70 ± 0.68^{gh}	1.33 ± 0.03^c
	3		20.14 ± 0.18^f	60.00
BB/A^1B/BB	1	1.20 ± 0.03^a	12.02 ± 0.55^b	0.50 ± 0.02^b
	2		15.99 ± 0.28^e	1.17 ± 0.02^{cd}
	3		20.70 ± 0.31^e	60.00
BB/A^2B/BB	1	1.11 ± 0.03^{bc}	8.98 ± 0.15^e	0.50 ± 0.02^b
	2		15.80 ± 0.41^{ef}	1.67 ± 0.02^a
	3		20.80 ± 0.49^{de}	60.00
MIX**	1	1.14 ± 0.03^b	9.15 ± 0.28^{de}	0.33 ± 0.01^c
	2		17.73 ± 0.48^b	1.33 ± 0.02^c
	3		22.39 ± 0.64^b	60.00

All values are means ± s.e. * Combination of α_{s1}-casein, ß-casein and κ-casein; ** Samples prepared from bulk tank milk
Within the column and at the same step, values with different letters are significantly different ($P < 0.05$).

much more surface active ($P<0.05$) than BB/A^2A^2/BB. The pressure attained during the surface enlargement, diffusion and penetration were 12.45, 16.70 and 23.03 mN/m for BB/A^2A^2/AA and only 7.71, 14.70 and 20.14 mN/m for BB/A^2A^2/BB. The order of the surface activity of different phenotypes of κ-Cn in whole caseins was corresponding to the results observed from the isolated κ-Cn of different phenotypes: AB >AA > BB.

According to the proposed casein submicelle structure (Walstra and Jenness, 1984), κ-Cn is located on the surface of the submicelles. The consistance between the results of individual κ-Cn and the κ-Cn in whole casein might be explained by the characteristics of the casein micelle structure. The surface active properties of κ-Cn were not influenced by the micelle structure because κ-Cn is on the surface of the submicelles. α_{s1}-Casein and ß-Cn are bound to each other and are situated inside of the submicelles. The surface activity of α_{s1}-Cn and ß-Cn in the whole casein therefore might depend on the location of casein micelle structure which is still not well established yet.

The maximum values of log(dπ/dt) presented in Table 9 show that there were significant differences ($P<0.05$) among the twelve whole caseins. The higher values were observed from the combination BB/A^1A^1/AA, BB/A^1A^2/AA and BB/A^1B/BB with the log(dπ/dt) of 1.23, 1.20 and 1.20, respectively. The lowest value was for haplotype BB/A^1A^1/BB. Different phenotype combination of α_{s1}-Cn/ß-Cn/κ-Cn showed the difference in surface active properties.

5.5 Voluminosity and hydration of caseins

The ability of proteins to bind or entrap water is responsible for many of their desirable functional properties (Mulvihill and Fox, 1989). Voluminosity and hydration of individual casein of different genetic variants and whole casein with different phenotype combinations of α_{s1}-/ß-/κ-Cn were studied in this section. Data of voluminosity and hydration from individual casein of different genetic variants was also pooled for α_{s1}-Cn, ß-Cn and κ-Cn and voluminosity and hydration were also measured for α_{s2}-Cn.

Table 10 Voluminosity and hydration of α_{s1}-casein, ß-casein and κ-casein of different phenotypes

Casein and phenotype	Voluminosity (cm^3/g)	Hydration (g H_2O/g)
α_{s1}-casein		
BB	4.9016 ± 0.0108^{a}	3.9016 ± 0.0108^{a}
BC	4.2939 ± 0.0372^{b}	3.2939 ± 0.0372^{b}
β-casein		
A^1A^1	8.2805 ± 0.0455^{c}	7.2805 ± 0.0455^{c}
A^1A^2	9.1048 ± 0.0469^{a}	8.1048 ± 0.0469^{a}
A^2A^2	9.1923 ± 0.0478^{a}	8.1923 ± 0.0478^{a}
A^1B	7.0720 ± 0.0379^{d}	6.0720 ± 0.0379^{d}
A^2B	-	-
BB	9.0271 ± 0.0689^{ab}	8.0271 ± 0.0689^{ab}
κ-casein		
AA	3.2796 ± 0.0331^{b}	2.2796 ± 0.0331^{b}
AB	3.6410 ± 0.0331^{a}	2.6410 ± 0.0331^{a}
BB	3.2183 ± 0.0372^{b}	2.2183 ± 0.0372^{b}

All values are means ± s.e.
Within the column and seam protein, values with different letters are significantly different (P<0 05)

5.5.1 Individual caseins of different genetic variants

The values of voluminosity and hydration measured for the different phenotypes of α_{s1}-Cn, ß-Cn and κ-Cn are shown in Table 10. Since there was not enough CC phenotype of α_{s1}-Cn sample available for the measurement, only two phenotypes of α_{s1}-Cn could be used for the comparison. Table 10 shows that α_{s1}-Cn BB phenotype was associated with higher ($P<0.05$) voluminosity and hydration than BC. The possible reason could be that the structure of BB is looser than that of BC phenotype. In one of studies, Berlin *et al.* (1969) reported that the water absorbed by α_{s1}-Cn C variant is slightly less than that of α_{s1}-Cn A variant which is missing a strongly hydrophobic N-terminal sequence. Therefore more hydrophilic cluster can entrap more water at this point. Variant B of α_{s1}-Cn has a Glu residue at position 192 in its amino acids sequence instead of a Gly residue as in C variant. One more negatively charged B variant due to Glu residue might enhanced the ability of water entrapping. In ß-Cn, A^2A^2 phenotype had the highest voluminosity and hydration compared to other phenotypes of the protein which were in the following order for the two parameters: $A^2A^2 > A^1A^2 > BB > A^1A^1 > A^1B$. ß-Casein A^2 variant has one less net positively charged amino acid residue than A^1 and two less net positively charged amino acids than B variant. The less negatively charged protein might be associated with higher ability of water entrapping. Higher value of voluminosity should be associated with higher surface activity at the air-water interface because of the looser structure. Phenotypes A^2A^2 and A^1A^2 possess higher voluminosity and hydration values than other phenotypes. These two phenotypes of ß-Cn also were shown to have a very different surface activity (Section 5.4.1.2) that might be attributed to the interaction of the two genetic variants of ß-Cn. In κ-Cn, AB phenotype entrapped more water than AA and BB. Perhaps the interaction between variant A and B of κ-Cn loosened the protein structure and enabled it to entrap more water than either AA or BB phenotype alone. This result is in agreement with the study on surface activity at air-water interface of individual κ-Cn of different genetic variants (Section 5.4.1.3). The kinetics of surface decay of κ-Cn suggested that AB was the most surface active protein, followed by the AA and BB phenotype.

Table 11. Voluminosity and hydration of individual caseins*

Casein	Voluminosity (cm^3/g)	Hydration (g H_2O/g)
α_{s1}-casein	4.5978 ± 0.4297^b	3.5978 ± 0.4297^b
α_{s2}-casein	4.2052 ± 0.0176^b	3.2052 ± 0.0176^b
ß-casein	8.5293 ± 0.8972^a	7.6153 ± 0.8972^a
κ-casein	3.3796 ± 0.2284^c	2.3796 ± 0.2284^c

All values are means ± s.e.
* Average of α_{s1}-casein, ß-casein and κ-casein of different phenotypes
Within the column, values with different letters are significantly different ($P<0.05$)

5.5.2 individual caseins

The data of voluminosity and hydration for α_{s1}-Cn, ß-Cn and κ-Cn were pooled from Table 10 to represent each of the individual caseins irrespectively of genetic types. Voluminosity and hydration measured for α_{s2}-Cn were also presented in Table 11.

The information shown in Table 11 reveals that ß-Cn was associated with the highest values for voluminosity (8.5293 cm^3/g) and hydration (7.7153 g H_2O/g). The value of voluminosity was 4.5978, 4.2052 and 3.3796 cm^3/g for α_{s1}-Cn, α_{s2}-Cn and κ-Cn, respectively. The hydration for α_{s1}-Cn, α_{s2}-Cn and κ-Cn were 3.5978, 3.2052 and 2.3796 g H_2O/g, respectively. It is known that ß-Cn has the loosest structure (Kinsella *et al.* 1989) among the four caseins. With this special characteristics in its structure, ß-Cn showed the strongest ability of water entrapping. κ-Casein showed the weakest ability to entrap water in this study. Voluminosity and hydration of α_{s1}-Cn and α_{s2}-Cn were intermediate between those of ß-Cn and κ-Cn. These measured parameters except that of κ-Cn were in accordance with those observed by Swaisgood (1982) who found the hydration value of 3.3 g H_2O/g for α_{s1}-Cn, 3.9 g H_2O/g for α_{s2}-Cn, 8.4 g H_2O/g for ß-Cn and 3.1 g H_2O/g for κ-Cn, respectively. The voluminosity measured by Swaisgood (1982) was 4.035 cm^3/g for α_{s1}-Cn, 4.640 cm^3/g for α_{s2}-Cn, 9.150 cm^3/g for ß-Cn and 3.836 cm^3/g for κ-Cn, respectively.

5.5.3 Whole caseins

The measurements of voluminosity and hydration for twelve whole caseins with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn are presented in Table 12. Whole casein of haplotype BB/A^1B/BB revealed the highest value for voluminosity and hydration. BB/A^1A^1/AA gave the lowest value for these two parameters. Considering BB/A^1A^1/AA, BB/A^1A^2/AA and BB/A^2A^2/AA, as having the same phenotype of α_{s1}-Cn BB and κ-Cn AA, the difference among them must be due to the influence of ß-Cn genetic variants. The difference between haplotype BB/A^1A^2/AA and BB/A^2A^2/AA was not significant ($P>0.05$). The whole casein containing A^2A^2 of ß-Cn was associated with a slightly higher ($P > 0.05$) value than the whole casein containing A^1A^1 and A^1A^2

Table 12 Voluminosity and hydration of whole caseins with different phenotype combination of α_{s1}-casein, ß -casein and κ-casein

Haplotype*	Voluminosity (cm^3/g)	Hydration (g H_2O/g)
$BB/A^1A^1/AA$	3.2972 ± 0.0409^{c}	2.2972 ± 0.0409^{c}
$BB/A^1A^2/AA$	3.3361 ± 0.0194^{c}	2.3361 ± 0.0194^{c}
$BB/A^2A^2/AA$	3.4195 ± 0.0190^{bc}	2.4195 ± 0.0190^{bc}
$BC/A^1A^2/AA$	3.6722 ± 0.0313^{b}	2.6722 ± 0.0313^{b}
$BB/A^1A^1/AB$	3.5361 ± 0.0506^{bc}	2.5361 ± 0.0506^{bc}
$BB/A^1A^2/AB$	3.5611 ± 0.0094^{bc}	2.5611 ± 0.0094^{bc}
$BB/A^1A^1/BB$	3.5917 ± 0.0528^{bc}	2.5917 ± 0.0528^{bc}
$BB/A^1A^2/BB$	3.4639 ± 0.0275^{bc}	2.4639 ± 0.0275^{bc}
$BB/A^2A^2/BB$	3.5861 ± 0.0140^{bc}	2.5861 ± 0.0140^{bc}
$BB/A^1B/BB$	3.9167 ± 0.1097^{a}	2.9167 ± 0.1097^{a}
$BB/A^2B/BB$	3.4834 ± 0.0197^{bc}	2.4834 ± 0.0197^{bc}
MIX**	3.5556 ± 0.0375^{b}	2.5556 ± 0.0375^{b}

All values are means ± s.e.
* Combination of α_{s1}-casein/ß-casein/κ-casein
** Samples prepared from bulk tank milk
Within the column, values with different letters are significantly different ($P<0.05$)

phenotypes. In the presence of α_{s1}-Cn BB and κ-Cn AA, the A^1 variant of ß-Cn seems to be associated with lower voluminosity. In comparing whole casein BB/A^1A^2/AA with BC/A^1A^2/AA, which have the same phenotypes for ß-Cn and κ-Cn, but different phenotypes for α_{s1}-Cn, these two whole caseins had different voluminosity with α_{s1}-Cn BC having higher value than α_{s1}-Cn BB. This means that phenotype BB and BC of α_{s1}-Cn influence its voluminosity and hydration significantly ($P<0.05$). In the presence of α_{s1}-Cn BB and κ-Cn AB, no difference ($P > 0.05$) of voluminosity was found between BB/A^1A^1/AB and BB/A^1A^2/AB. In combination with α_{s1}-Cn BB and κ-Cn BB, ß-Cn A^1B gave the highest value for voluminosity (3.9267 cm^3/g). There were no significant differences ($P>0.05$) between A^1A^1, A^1A^2, A^2A^2 and A^2B. The composition of the whole casein with a mixture of phenotypes was very close to that of BB/A^1A^2/AB. These two caseins showed almost the same value (3.5611 v.s. 3.5556 cm^3/g). If only the influence of κ-Cn is considered, AA type showed the lowest value ($P < 0.05$) when compared to AB and BB type in the presence of α_{s1}-Cn BB and ß-Cn A^1A^1. The AA phenotype of κ-Cn also appeared to have a lower value ($P < 0.05$) than AB and BB phenotype when A^1A^2 of ß-Cn replaced the A^1A^1 phenotype.

Hydration expressed the water entrapping ability of milk proteins. Since the calculation originated from the formula of Lee *et al.* (1969), the hydration is positively correlated with the voluminosity. Therefore the hydration showed the same trends as voluminosity for all the twelve caseins. The observation demonstrated that higher voluminosity was in accordance with higher hydration ability.

5.6 Emulsifying properties of caseins

Oil-in-water emulsions stabilized by individual casein of different genetic variants, individual caseins and whole casein with different phenotype combinations of α_{s1}-Cn, ß-Cn and κ-Cn were analyzed.

5.6.1 Individual caseins of different genetic variants

5.6.1.1 α_{s1}-Casein

Table 13 shows the differences of three phenotypes of α_{s1}-Cn in their emulsifying properties. Compared to BC and CC phenotype, BB phenotype of α_{s1}-Cn showed the

Table 13 Emulsifying properties of α_{s1}-casein of different phenotypes in 10% and 40% oil-in-water emulsions

Phenotype	Interfacial area (m^2/ml)	Viscosity (mPa.s)	Protein load (mg/m^2)	Creaming index (%)
10% oil				
BB	0.2986 ± 0.0114^a	5.17 ± 0.16^a	9.9897 ± 0.2945^b	57.61 ± 0.55^b
BC	0.2971 ± 0.0157^a	5.12 ± 0.18^a	9.3260 ± 0.2810^c	65.48 ± 0.71^a
CC	0.2763 ± 0.0013^b	5.16 ± 0.19^a	10.3590 ± 0.4194^a	64.00 ± 1.92^a
40% oil				
BB	2.5953 ± 0.0635^c	13.37 ± 0.22^c	1.2374 ± 0.0878^a	52.41 ± 0.56^a
BC	2.7379 ± 0.0709^a	19.27 ± 0.33^a	0.9910 ± 0.0392^b	52.36 ± 1.01^a
CC	2.6101 ± 0.0260^b	16.83 ± 0.31^b	1.2896 ± 0.0599^a	52.81 ± 1.39^a

All values are means ± s.e.
Within the oil level and the column, values with different letters are significantly different ($P<0.05$)

largest interfacial area of emulsion, apparent viscosity and lowest creaming index in 10% oil-water emulsions. The level of protein load of BB phenotype emulsion was intermediate between the other two phenotypes. Larger interfacial area of emulsion was usually related to higher quantity of protein load and more stable emulsions. The creaming index is an indication of the emulsion stability. Higher value of the creaming index means larger amount of cream was formed at the upper part of the emulsion after a 30-day of storage. Data presented in Table 13 show that BB phenotype of α_{s1}-Cn was associated with more desirable emulsion properties in 10% oil-in-water emulsions. In 40% oil-in-water-emulsions, BB phenotype had the smallest interfacial area and lowest apparent viscosity. The protein load of BB phenotype was intermediate between BC and CC phenotypes. The higher quantity of protein coated on a relatively smaller interfacial area of BB phenotype of 40% oil-in-water emulsion suggested that the layer of the coated protein was denser. BC phenotype showed the highest interfacial area but lowest protein load in the 40% oil-in-water emulsion. In this case, the layer coated on the oil droplets was looser compared to BB phenotype emulsions. The creaming index of 40% oil-in-water emulsions showed that no difference ($P > 0.05$) existed among the three phenotypes of α_{s1}-Cn. The effect of genetic variants of α_{s1}-Cn on emulsion stability was masked by the higher oil content in the emulsions. From the point of emulsion stability, BB phenotype of α_{s1}-Cn is the most desirable protein which can make more stable emulsions than BC and CC phenotype of α_{s1}-Cn. If we only compare the emulsifying properties of BC and CC type, we can find that BB phenotype of α_{s1}-Cn was associated with larger interfacial area and smaller creaming index in 10% oil emulsion even though the apparent viscosity and protein load were lower than that of CC phenotype. The previous studies on surface activity at air-water interface and voluminosity of α_{s1}-Cn of different genetic variants suggested that the structure of BB was looser but less effective than BC phenotype. Protein with looser structure would open faster and form a more cohesive film than the protein with a denser structure. The most stable 10% oil-in-water emulsion was formed by BB

phenotype of α_{s1}-Cn which might be attributed to its higher voluminosity and hydration ability.

When considering the oil content in the emulsions, it seems that as oil concentration increased, the protein layer of coating by α_{s1}-Cn on the oil droplets surface became thinner than it was in 10% oil emulsions. The interfacial area of emulsion increased about 10 fold. The apparent viscosity also increased dramatically and the protein load decreased very sharply. Within the range of oil content of 10 to 40%, increasing oil content in the emulsion yielded lower value of creaming index.

5.6.1.2 ß-Casein

Six phenotypes of ß-Cn were prepared for the analysis of the emulsion properties. It is obvious from Table 14 that differences exist among these six ß-Cn phenotypes. In 10% oil emulsions, A^1A^2 phenotype had the largest emulsion interfacial area but lowest quantity of protein load and therefore yielded an unstable emulsion with a larger creaming index of 62.94. Though A^2A^2 of ß-Cn had the smallest interfacial area and lower viscosity, the protein loading amount was the highest when compared to the other five phenotypes. The most stable emulsion with 10% oil for ß-Cn A^2A^2 had creaming index of 54.64. There was no significant differences ($P > 0.05$) among A^1A^1, A^2B and BB for interfacial area, but significant differences ($P < 0.05$) in protein load and creaming index were found among these phenotypes. The phenotype A^2A^2 was associated with the largest value of protein loading followed by A^1A^1, BB, A^1A^2 and A^2B. The emulsion stabilities were in the following decreasing order: $A^2A^2 > BB > A^1A^1 > A^2B > A^1A^2 > A^1B$. Higher interfacial area of emulsion should be related to higher value of protein load. These two parameters did not concur in the emulsions stabilized by different phenotypes of ß-Cn. Table 14 indicates that the coated protein layer on the oil droplets was very dense in A^2A^2 emulsion and very loose in A^1A^2 and A^2B emulsions. Opposite trends were observed in the 40% oil emulsions. The most desirable emulsion stabilizer was A^2B not the A^2A^2 phenotype of ß-Cn. In 40% oil emulsions, A^2A^2 was associated with smaller interfacial area and largest quantity of protein load, but most unstable emulsion with a higher creaming index of 53.74. The

Table 14. Emulsifying properties of ß-casein of different phenotypes in 10% and 40% oil-in-water emulsions

Phenotype	Interfacial area (m^2/ml)	Viscosity (mPa.s)	Protein load (mg/m^2)	Creaming index (%)
10% oil				
A^1A^1	0.2761 ± 0.0139^{bc}	5.79 ± 0.26^{a}	12.6333 ± 0.3198^{c}	60.84 ± 1.55^{cd}
A^1A^2	0.2908 ± 0.0031^{a}	5.50 ± 0.10^{b}	9.7939 ± 0.2841^{e}	62.94 ± 0.67^{bc}
A^2A^2	0.2619 ± 0.0033^{d}	5.16 ± 0.30^{d}	18.5976 ± 0.6786^{a}	54.64 ± 1.25^{e}
A^1B	0.2688 ± 0.0035^{cd}	4.85 ± 0.25^{e}	14.5055 ± 0.6663^{b}	63.41 ± 1.11^{ab}
A^2B	0.2799 ± 0.0052^{b}	5.38 ± 0.33^{c}	9.7938 ± 0.3924^{e}	62.73 ± 1.17^{bc}
BB	0.2754 ± 0.0037^{bc}	5.11 ± 0.27^{d}	11.6541 ± 0.2271^{d}	59.80 ± 1.02^{d}
40% oil				
A^1A^1	2.6482 ± 0.0458^{b}	9.91 ± 0.09^{a}	1.6402 ± 0.0579^{b}	52.36 ± 0.67^{abc}
A^1A^2	2.6934 ± 0.0876^{b}	9.75 ± 0.41^{c}	1.5111 ± 0.0607^{c}	52.29 ± 0.21^{bc}
A^2A^2	2.5794 ± 0.0363^{c}	9.90 ± 0.19^{a}	1.7217 ± 0.0307^{a}	53.74 ± 0.93^{abc}
A^1B	2.9492 ± 0.0259^{a}	9.84 ± 0.46^{b}	1.1474 ± 0.0582^{d}	52.69 ± 0.33^{abc}
A^2B	2.5794 ± 0.0545^{c}	9.78 ± 0.08^{c}	1.6142 ± 0.0362^{b}	51.88 ± 0.54^{c}
BB	2.5032 ± 0.0646^{d}	9.81 ± 0.39^{b}	1.6329 ± 0.0677^{b}	54.03 ± 1.89^{ab}

All values are means ± s.e.
Within the oil level and the column, values with different letters are significantly different ($P<0.05$)

probable reason could be that emulsion with high oil concentration, A^2A^2 ß-Cn would coalesce very easily during the shelf storage. Table 1 presented the location of amino acid substitution in genetic variants of milk proteins which showed that A^2 variant of ß-Cn is more negatively charged than A^1 and B variants. The stronger electric force pulling each other between A^2 variant protein molecules might have caused the coalescence. The emulsion stabilized by A^1A^1 was associated with higher protein load and emulsion stability. Phenotype A^1B gave the largest interfacial area but a lower protein load and subsequently the emulsion was also less stable than the other four phenotypes of ß-Cn emulsions. In considering the 40% oil-in-water emulsion stabilized by A^1B and A^2B of ß-Cn, phenotype A^1B emulsion was associated with higher interfacial area (2.9492 m^2/ml) and lower protein load (1.1474 mg/m^2). Phenotype A^2B emulsion was associated with lower interfacial area (2.5794 m^2/ml) and higher protein load (1.6142 mg/m^2). Both ß-Cn (A^1B and A^2B) emulsions had lower creaming index of 52.69 and 51.88, respectively. Surface tension and voluminosity measurement of ß-Cn of different genetic variants showed that A^2A^2 and A^1A^2 were more surface active with looser structure. This study also suggested that with increasing oil content in the emulsion, the influence of genetic variants of ß-Cn on emulsifying properties was less obvious.

5.6.1.3 κ-Casein

The creaming index of 10% oil-in-water emulsion of κ-Cn shown in Table 15 suggests that there was no significant difference ($P > 0.05$) among the emulsions prepared with AA, AB and BB phenotypes of κ-Cn. The interfacial area, apparent viscosity and protein load of the 10% oil-in-water emulsion were very closely related. Higher interfacial area of emulsion was associated with higher protein load and apparent viscosity. Among the three phenotypes of κ-Cn, BB possessed higher value of interfacial area, apparent viscosity and protein load than AA and AB phenotypes. Generally, BB also should yield a more stable emulsion than AA and AB phenotypes. The similar values of creaming index of 10% oil-in-water emulsions among the three phenotypes of κ-Cn suggested that the emulsion stabilized by these three phenotypes

Table 15 Emulsifying properties of κ-casein of different phenotypes in 10% and 40% oil-in-water emulsions

Phenotype	Interfacial area (m^2/ml)	Viscosity (mPa.s)	Protein load (mg/m^2)	Creaming index (%)
10% oil				
AA	0.3014 ± 0.0083^{b}	5.64 ± 0.08^{a}	10.9552 ± 0.1165^{b}	63.69 ± 0.92^{a}
AB	0.3125 ± 0.0028^{b}	5.68 ± 0.04^{a}	13.1258 ± 0.3681^{a}	62.25 ± 1.70^{b}
BB	0.3480 ± 0.0070^{a}	5.79 ± 0.07^{a}	13.2038 ± 0.3337^{a}	63.87 ± 0.28^{a}
40% oil				
AA	2.8385 ± 0.0416^{b}	12.03 ± 0.42^{b}	0.7736 ± 0.0503^{b}	54.34 ± 1.80^{a}
AB	2.7783 ± 0.0497^{b}	12.20 ± 0.51^{b}	0.6601 ± 0.0211^{c}	51.99 ± 1.73^{b}
BB	2.9269 ± 0.0604^{a}	13.52 ± 0.47^{a}	0.9270 ± 0.0656^{a}	53.63 ± 0.64^{ab}

All values are means ± s.e.
Within the oil level and the column, values with different letters are significantly different ($P<0.05$)

Table 16 Emulsifying properties of individual caseins in 10% and 40% oil-in-water emulsions

Casein	Interfacial area (m^2/ml)	Viscosity (mPa.s)	Protein load (mg/m^2)	Creaming index (%)
10% oil				
α_{s1}-casein	0.2898 ± 0.0140^{b}	5.15 ± 0.03^{c}	9.8902 ± 0.5231^{b}	63.27 ± 0.89^{a}
ß-casein	0.2755 ± 0.0098^{b}	5.30 ± 0.32^{b}	12.8311 ± 3.3446^{a}	60.73 ± 3.29^{b}
κ-casein	0.3208 ± 0.0246^{a}	5.70 ± 0.08^{a}	12.4308 ± 1.2764^{a}	62.36 ± 4.18^{ab}
40% oil				
α_{s1}-casein	2.6478 ± 0.0755^{b}	16.49 ± 2.96^{a}	1.1727 ± 0.1618^{b}	53.32 ± 1.20^{a}
ß-casein	2.6588 ± 0.1546^{b}	9.83 ± 0.06^{c}	1.5446 ± 0.2059^{a}	52.83 ± 0.87^{ab}
κ-casein	2.8479 ± 0.0747^{a}	12.58 ± 0.82^{b}	0.7800 ± 0.1339^{c}	52.53 ± 0.25^{b}

All values are means ± s.e.
Within the oil level and the column, values with different letters are significantly different ($P<0.05$)

of κ-Cn were not significantly different (P>0.05). In 40% oil-in-water emulsion, κ-Cn AB was associated with the lowest protein load (0.6601 mg/m^2 vs 0.7736 mg/m^2 for AA and 0.9270 mg/m^2 for BB) but the most stable emulsion (with lowest creaming index). The observation suggested that κ-Cn AB was a better emulsion stabilizer than AA and BB in emulsion.

The difference of amino acid composition in the κ-Cn sequence are at the positions 136 and 148 where *Thr* and *Asp* are located for variant A and *Ile* and *Ala* are located for variant B. Unlike ß-Cn which does not have the sulfide amino acid residues, κ-Cn has -SH groups from cysteine which might crosslink with the molecules and make the structure denser than ß-Cn and displays less surface active properties for emulsions.

5.6.2 Individual caseins

Data of emulsifying properties from individual caseins of different genetic variants were pooled for α_{s1}-Cn, ß-Cn and κ-Cn. The results presented in Table 16 showed that ß-Cn was associated with more desirable emulsifying properties than α_{s1}-Cn and κ-Cn in both 10% and 40% oil-in-water emulsions. The interfacial area, apparent viscosity and protein load of ß-Cn emulsion were not the highest, but the creaming index of ß-Cn emulsion was significantly lower ($P < 0.05$) in 10% and 40% oil-in-water emulsions. κ-Casein seems to show a better emulsifying properties than α_{s1}-Cn. It had larger interfacial area both in 10% and 40% oil emulsions, higher protein load in 10% oil emulsion but lower value in 40% oil emulsions. In considering the stability, κ-Cn emulsion was less stable than α_{s1}-Cn in 10% oil emulsion but not different in 40% oil emulsions. Voluminosity and hydration measurement of individual caseins showed that ß-Cn was associated with the highest value among the four caseins. The study on surface activity of individual casein also suggested that the highest active protein was ß-Cn. It is reasonable that the emulsion stabilized by ß-Cn was the most desirable one.

5.6.3 Whole caseins

Tables 17 and 18 show all the data analyzed and measured for the two oil level emulsions which were prepared with the whole caseins of different phenotype combinations of α_{s1}-Cn, ß-Cn and κ-Cn.

5.6.3.1 Interfacial area of emulsions

The total interfacial area of the emulsion was measured immediately by optical density method (Cameron *et al.*, 1991) after the emulsion formation. The differences of interfacial area of emulsions were significant ($P < 0.05$) among the twelve whole caseins. It was found that the whole casein with haplotype BB/A^1A^2/AA was associated with the highest value of 0.3138 m^2/ml and BB/A^1A^2/BB was associated with the lowest value of 0.1979 m^2/ml in 10% oil-in-water emulsion. The interfacial areas of most of the whole caseins in 40% oil-in-water emulsion were close to each other except for BB/A^2B/BB which had the highest value of 2.8300 m^2/ml and the whole casein with a mixture of phenotypes which had the lowest value of 2.0787 m^2/ml.

There were significant differences ($P<0.05$) among haplotype BB/A^1A^1/AA, BB/A^1A^2/AA and BB/A^2A^2/AA due to the different phenotypes of ß-Cn in 10% oil-in-water emulsions. The results suggested that in the presence of α_{s1}-Cn BB and κ-Cn AA, phenotype A^1A^2 strongly affected the interfacial area in 10% oil emulsions. The influence of the phenotypes of α_{s1}-Cn on the interfacial area was not significant ($P > 0.05$) when compared whole casein BB/A^1A^2/AA with BC/A^1A^2/AA. There was no difference ($P > 0.05$) between the haplotype BB/A^1A^1/AB and BB/ A^1A^2/AB which have the same phenotype of α_{s1}-Cn and κ-Cn. In the presence of BB phenotype for both of α_{s1}-Cn and κ-Cn, the phenotypes A^1A^1, A^2A^2 and A^2B were associated with higher values of interfacial areas of 10% oil emulsions than A^1B and A^1A^2.

Interfacial area of emulsion is an indication of the ability of the proteins to form a stabilizing film on the smaller oil droplets to prevent coalescence. During emulsification, oil droplets are fractionated and protein adsorbed to maintain the integrity of the newly formed interface. Uncovered oil droplets are unstable and undergo coalescence within the homogenization valve (Pearce and Kinsella, 1978). The larger the interfacial area of the emulsion, in principle, the more oil droplets were coated and the more stable the emulsions. Protein was partially or wholly denatured in an emulsification process, depending upon the type of protein, the source of protein could affect the interfacial properties (Walstra and Oortwijn, 1982). Casein and whey

Table 17 Emulsifying properties of whole caseins with different phenotype combinations of α_{s1}-casein, ß-casein and κ-casein, 10% oil-in-water emulsions

Haplotype*	Interfacial area (m^2/ml)	Viscosity (mPa.s)	Protein load (mg/m^2)	Creaming index (%)
BB/A^1A^1/AA	0.2231 ± 0.0036^{dc}	1.18 ± 0.03^{bc}	4.6645 ± 0.0652^{e}	58.83 ± 0.39^{ab}
BB/A^1A^2/AA	0.3138 ± 0.0034^{a}	1.40 ± 0.00^{a}	4.2734 ± 0.1767^{g}	56.55 ± 0.86^{d}
BB/A^2A^2/AA	0.2347 ± 0.0106^{dc}	1.15 ± 0.03^{bc}	4.6837 ± 0.0268^{e}	57.76 ± 0.97^{c}
BC/A^1A^2/AA	0.2831 ± 0.0064^{ab}	1.35 ± 0.02^{a}	5.2892 ± 0.0122^{c}	58.57 ± 0.33^{abc}
BB/A^1A^1/AB	0.2595 ± 0.0067^{bcd}	1.22 ± 0.03^{b}	3.8406 ± 0.0642^{i}	56.07 ± 0.03^{d}
BB/A^1A^2/AB	0.2802 ± 0.0026^{abc}	1.18 ± 0.03^{bc}	5.3678 ± 0.0522^{b}	54.72 ± 0.39^{e}
BB/A^1A^1/BB	0.2961 ± 0.0065^{ab}	1.18 ± 0.03^{bc}	5.0107 ± 0.0814^{d}	58.48 ± 0.43^{abc}
BB/A^1A^2/BB	0.1979 ± 0.0093^{e}	1.05 ± 0.02^{d}	5.8266 ± 0.0615^{a}	58.11 ± 0.64^{bc}
BB/A^2A^2/BB	0.2995 ± 0.0076^{a}	1.33 ± 0.03^{a}	5.3298 ± 0.0513^{bc}	58.86 ± 0.65^{ab}
BB/A^1B/BB	0.2451 ± 0.0023^{cd}	1.22 ± 0.03^{b}	4.1054 ± 0.1869^{h}	58.73 ± 0.44^{abc}
BB/A^2B/BB	0.2896 ± 0.0093^{ab}	1.18 ± 0.02^{bc}	3.2762 ± 0.0709^{j}	58.95 ± 0.79^{ab}
MIX**	0.2303 ± 0.0028^{dc}	1.10 ± 0.02^{cd}	4.5093 ± 0.0778^{f}	59.20 ± 0.87^{a}

All values are means ± s.e.

* Combination of α_{s1}-casein, ß-casein and κ-casein.

** Samples prepared from bulk tank milk

Within the column, values with different letters are significantly different ($P<0.05$).

Table 18 Emulsifying properties of whole caseins with different phenotype combinations of α_{s1}-casein, ß-casein and κ-casein, 40% oil-in-water emulsions

Haplotype*	Interfacial area (m^2/ml)	Viscosity (mPa.s)	Protein load (mg/m^2)	Creaming index (%)
BB/A^1A^1/AA	2.2819 ± 0.0573^{bcd}	3.15 ± 0.05^{ab}	1.6520 ± 0.0839^{d}	52.58 ± 0.31^{ab}
BB/A^1A^2/AA	2.4592 ± 0.0733^{b}	3.20 ± 0.05^{a}	1.6152 ± 0.0172^{f}	51.83 ± 0.42^{bc}
BB/A^2A^2/AA	2.3585 ± 0.0238^{bc}	3.12 ± 0.06^{ab}	1.6581 ± 0.0358^{d}	50.56 ± 0.19^{d}
BC/A^1A^2/AA	2.3477 ± 0.0838^{bc}	3.21 ± 0.05^{a}	1.7076 ± 0.0294^{c}	52.40 ± 0.95^{b}
BB/A^1A^1/AB	2.3973 ± 0.0590^{bc}	3.01 ± 0.02^{ab}	1.4952 ± 0.0600^{g}	51.00 ± 0.38^{cd}
BB/A^1A^2/AB	2.2384 ± 0.0769^{cdc}	2.99 ± 0.06^{b}	1.7728 ± 0.1176^{b}	51.85 ± 0.63^{bc}
BB/A^1A^1/BB	2.3748 ± 0.0646^{bc}	3.04 ± 0.06^{ab}	1.6045 ± 0.0379^{f}	52.20 ± 1.16^{bc}
BB/A^1A^2/BB	2.1180 ± 0.1161^{dc}	3.09 ± 0.07^{ab}	1.9016 ± 0.0877^{a}	51.85 ± 0.54^{bc}
BB/A^2A^2/BB	2.2716 ± 0.0858^{bcdc}	3.14 ± 0.03^{ab}	1.7756 ± 0.0683^{b}	52.37 ± 0.71^{b}
BB/A^1B/BB	2.3035 ± 0.0991^{bcd}	2.94 ± 0.06^{b}	1.6454 ± 0.0543^{e}	52.29 ± 1.40^{b}
BB/A^2B/BB	2.8279 ± 0.0535^{a}	3.20 ± 0.06^{a}	1.3236 ± 0.0313^{h}	53.75 ± 0.77^{a}
MIX**	2.0787 ± 0.0537^{e}	2.77 ± 0.05^{c}	1.7743 ± 0.0399^{b}	52.69 ± 0.28^{ab}

All values are means ± s.e.

* Combination of α_{s1}-casein, ß-casein and κ-casein.

** Samples prepared from bulk tank milk.

Within the column, values with different letters are significantly different ($P<0.05$).

protein are two different typical proteins with different structures that show a very interesting feature for comparison purpose. At low protein concentration (0.125 and 0.25%) emulsions from whey proteins showed larger interfacial area than emulsion from caseins (Britten and Giroux, 1993). Britten *et al.* (1993) reported that increasing the protein concentration resulted in the differences between protein fractions. For concentration higher than 0.5%, caseins stabilized a larger interface than whey proteins. It was suggested that at a higher protein concentration, newly formed interface was rapidly covered by a thin film and protein transport was no longer a key factor and further adsorption of protein molecules was required to produce a stable membrane resisting coalescence. The ability of protein molecules to adsorb and spread out at an interface already covered by a protein film is limited by the electric charge and the surface pressure of the existing film (MacRitchie and Alexander, 1963a,b). In this study, a protein concentration of 0.8% whole casein with different phenotype combinations of α_{s1}-, ß- and κ-Cn was used as the emulsion stabilizers for investigation of the influence of genetic variants of individual casein on its emulsifying properties. The concentration of 0.8% protein is slightly higher than 0.5% and this concentration could explore the difference of electric charge of caseins during stabilization of the emulsions.

5.6.3.2 Protein load of emulsions

Protein load was calculated from protein depletion in the phase after the emulsion formation by using a modified Biuret reaction (BCA protein assay reagent, Rockford, IL). Serum phase was separated from the emulsion by centrifugation at 21,000 xg for 60 min. Protein was determined in the aqueous phase before and after emulsion formation. The result was expressed as protein in mg coated per square meter of surface area of the emulsion. Table 17 and 18 show that the protein load was largely different between 10% and 40% oil-in-water emulsions. Higher oil content of emulsions which had lower protein load in emulsion meant that protein coating ability on the oil droplets was limited by large quantity of oil. Within 10% and 40% oil-in-water emulsions, BB/A^1A^2/BB, BB/A^1A^2/AB and BB/A^2A^2/BB in 10% oil emulsion

and BB/A^1A^2/BB, BB/A^2A^2/BB and BB/A^1A^2/AB in 40% oil emulsion showed larger amount of protein were coated on the droplets whereas BB/A^2B/BB and BB/A^1A^1/AB in both 10% and 40% oil emulsion showed lowest amount of coating protein. A close relationship existed between protein concentration and coating ability. As protein concentration increased, protein load (or membrane thickness) increased (Britten *et al.*, 1993). Strong interfacial activity of caseins allows penetrating and spreading out at a crowded interface (Kinsella, 1984). Lower interfacial activity of protein prevented the formation of thick protein films. Genetic variants due to changes in composition of some amino acids influenced the interfacial activity of the protein and resulted in the difference of membrane thickness of oil droplets.

In the presence of α_{s1}-Cn BB and κ-Cn AA, ß-Cn A^1A^2 gave a lower protein load than A^1A^1 and A^2A^2. With the same ß-Cn and κ-Cn phenotypes, the BC phenotype of α_{s1}-Cn showed a higher protein load than BB type. The ß-Cn A^1A^2 presented a much higher value than A^1A^1 phenotype when the whole casein has phenotype BB for α_{s1}-Cn and AB for κ-Cn. In the presence of BB phenotype for both α_{s1}-Cn and κ-Cn, ß-Cn A^1A^2 also showed the highest protein load among the five phenotypes.

The above results were in accordance with the previous studies on surface tension decay property, voluminosity and hydration of the milk proteins. With looser structure, ß-Cn A^1A^2, A^1A^1 and A^2A^2 were more surface active than A^1B, A^2B and BB both at air-in-water interface and in oil-in-water emulsions.

5.6.3.3 Apparent viscosity of emulsion

The apparent viscosity of the emulsions was evaluated immediately after emulsion formation at a shear rate of 73.8/s with a Brookfield Digital Viscometer fitted with SL-4 small-sample adapter type 18 operating at 23 ± 1 °C, 60 rpm. In 10% oil emulsion, it was found that higher viscosity was associated with whole casein with haplotype BC/A^1A^2/AA and BB/A^2B/BB; lower viscosity was associated with BB/A^1A^2/BB and the whole casein with mixture of phenotypes. When comparing BB/A^1A^1/AA, BB/A^1A^2/AA and BB/A^2A^2/AA, it was found that haplotype BB/A^1A^2/AA had the highest value of apparent viscosity which was significantly higher ($P<0.05$) than

BB/A^1A^2/AA and BB/A^2A^2/AA. These three caseins are composed of the same phenotype of α_{s1}-Cn and κ-Cn. The difference could be attributed to the different phenotypes of ß-Cn. It was also found that BC phenotype of α_{s1}-Cn was associated with higher apparent viscosity than BB phenotype when considering the comparison of whole casein BC/A^1A^2/AA and BB/A^1A^2/AA. There was no significant difference ($P<0.05$) between BB/A^1A^1/AB and BB/A^1A^2/AB. When α_{s1}-Cn BB and κ-Cn BB were presented in the whole casein, BB/A^2A^2/BB gave the largest value for the apparent viscosity, followed by BB/A^1B/BB, BB/A^1A^1/BB = BB/A^2B/BB and BB/A^1A^2/BB. In 40% oil emulsion, BC/A^1A^2/AA and BB/A^2B/BB were associated with higher viscosity, while the MIX and BB/A^1B/BB were associated with lower viscosity. There were no differences among the whole casein with α_{s1}-Cn BB/κ-Cn AA and α_{s1}-Cn BB/κ-Cn AB. In the presence of α_{s1}-Cn BB and κ-Cn BB, haplotype BB/A^2B/BB showed a value of 3.20 mPa.s which was higher ($P<0.05$) only than that of BB/A^1B/BB. The results suggested that the viscosity of 40% oil emulsion was not significantly influenced by the different phenotype of α_{s1}-Cn, ß-Cn and κ-Cn in the whole caseins. Apparent viscosity was associated with the emulsion stability. Higher value of apparent viscosity means that the emulsions is more cohesive and creaming of emulsion is more difficult to take place under this condition.

5.6.3.4 Emulsion stability

Emulsion stability upon storage was evaluated over a 30-day period from the change in densitometric profile of the emulsion according to the method of Britten and Giroux (1993). For 10% oil emulsion, the calculated creaming index which is presented in Table 17 indicated that whole casein with haplotype BB/A^1A^2/AB and BB/A^1A^1/AB formed the most stable emulsion with the smallest creaming index of 54.72 and 56.07, respectively. In whole casein with α_{s1}-Cn BB/κ-Cn AA, BB/A^1A^2/AA was more stable than BB/A^1A^1/AA and BB/A^2A^2/AA. Table 17 also shows that BC/A^1A^2/AA was less stable than BB/A^1A^2/AA. In 40% oil-in-water emulsions, BB/A^2A^2/AA was associated with the highest emulsion stability, BB/A^2B/BB was associated with the lowest

emulsion stability. There were no significant differences among the other whole caseins at this oil level of emulsions.

5.7 Foaming properties of ß-lactoglobulin

The properties of foams generated by different phenotypes of ß-Lg were analyzed and discussed below.

5.7.1 Foaming capacity

Foaming capacity was defined as overrun (Pearce and Kinsella, 1978) or expansion factor (Britten and Pouliot, 1996). Figure 28 shows the data of overrun for foams generated with ß-Lg AA, AB, BB and ß-Lg of mixed phenotypes. The highest volume of foam was produced by BB phenotype (1200%) and the lowest by AA phenotype (1093%) of ß-Lg. The order of overrun for the four phenotypes of ß-Lg is BB > MIX > AB > AA. Foaming capacity is mainly related to the flexibility of structure (Kato *et al.*, 1985, 1986), hydrophobicity (Tanford, 1980) and molecular weight of the structure (WiLson, 1989). For instance, it was found that ß-Cn formed coarse and high-volume foams while a globular protein like lysozyme gave foams with small air cells (Philips, 1981), because ß-Cn is more flexible, hydrophobic and relatively smaller than lysozyme. It was reported that B variant of ß-Lg was more soluble but less hydrophobic than A variant (Hambling *et al.*, 1992). Generally, the foam overrun for AA phenotype should be higher than for BB phenotype, because A variant of ß-Lg is more hydrophobic than B variant. The different result obtained at this study might due to the conformational changes in ß-Lg AA and BB structures.

5.7.2 Foam stability

Figure 29 shows the foam drainage half time of the four phenotypes of ß-Lg with a value of 18.56 min for AA, 20.14 min for AB, 20.78 min for BB and 20.50 min for the mixed phenotypes of ß-Lg, respectively. Foam with longer foam drainage half time will drain more slowly. The results suggested that foam generated with BB phenotype of ß-Lg exhibited the highest stability compared to other three phenotypes of ß-Lg. AA phenotype was associated with the most unstable foam. Drainage of foam reflects the liquid draining from the lamella into the plateau borders, and drainage under the influence of gravity which is accentuated following rupture of foam bubbles. Halling

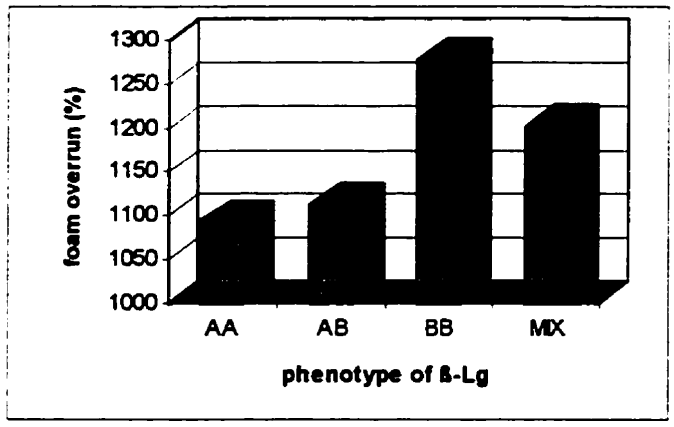

Fig. 28 Overrun of foams generated by ß-lactoglobulin of different phenotypes

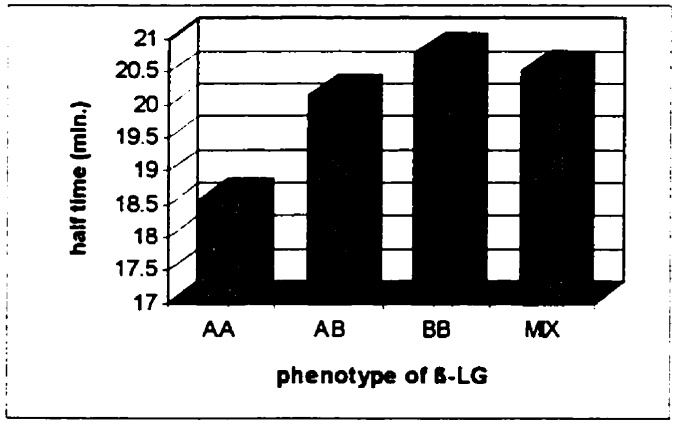

Fig.29 Stability of foams generated by ß-lactoglobulin of different phenotypes

(1981) indicated that drainage from lamella is impaired by capillary action and surface viscosity of the film. Drainage also resulted from thinning of the lamellae and reduction in viscosity of the film (Graham and Phillips, 1976a). As assumed above, the molecule of ß-Lg A variant became more compact due to the additional carboxyl at this experiment condition. The less flexible structure of ß-Lg AA led to a thin and less surface viscosity of the film which was less stable.

5.7.3 Foam firmness and elasticity

The measured foam firmness which is presented in Fig. 30 suggested that AA phenotype of ß-Lg was associated with the firmest foam. The value of foam firmness was 17.40 mP.s for AA, 15.97 mP.s for AB, 15.75 mP.s for BB and 16.60 mP.s for the mixture of the phenotypes of ß-Lg, respectively. With an increase of B variant in ß-Lg, the firmness of foams was decreased gradually. The relaxation of foam shown in Fig.31 reflects the elasticity of the foam. The higher the value of the relaxation of the foam the less elastic the foam will be. The relaxation for ß-Lg AA, AB, BB and the mixture of the phenotypes of ß-Lg was 65.72%, 70.18%, 74.84% and 68.21%, respectively. When compared the foam firmness and relaxation, it is not surprising to find that the elasticity of foam is highly related to the foam firmness. Foam generated by phenotype AA of ß-Lg foam was the firmest and also the most elastic. With a decrease of A variant proportion in the ß-Lg, the foam elasticity decreased gradually. This reflects that A variant is responsible for the foam firmness and elasticity of ß-Lg foam.

5.8 Gelling properties of ß-lactoglobulin

Gelling properties of ß-Lg are discussed in terms of gel strength, gel relaxation and water holding capacity of gels.

5.8.1 Gel strength

There were significant differences in the gel strength ($P < 0.05$) generated with four phenotypes of ß-Lg. Figure 32 shows that the gel formed with AA phenotype of ß-Lg was the strongest (52.77g) compared to the other three phenotypes of ß-Lg. The gel formed with BB phenotype was the weakest (33.57g). There was a tendency for the gel strength to increase as A variant proportion in ß-Lg increased. This might

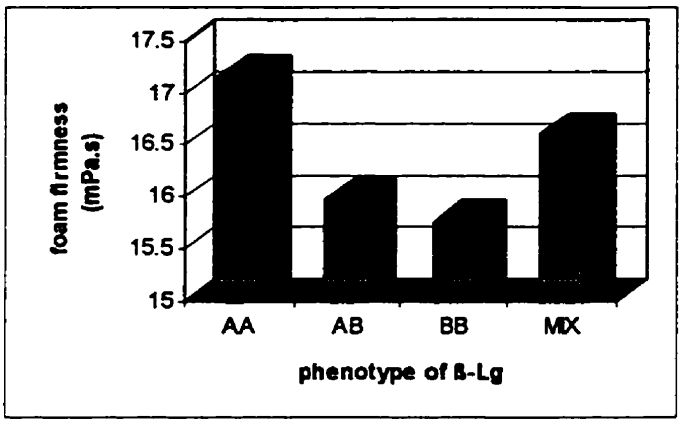

Fig. 30 Firmness of foams generated by ß-lactoglobulin of different phenotypes

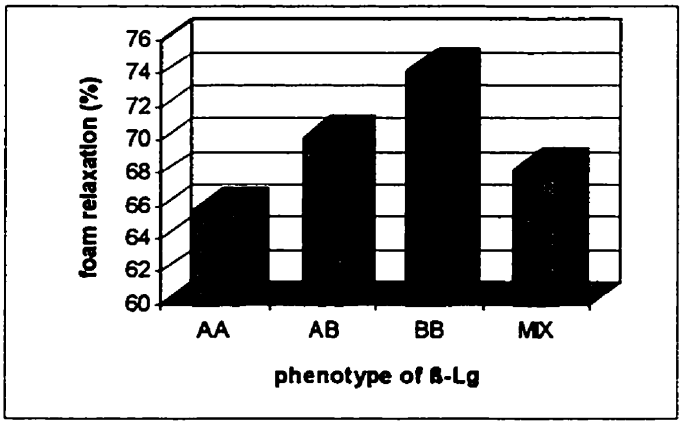

Fig.31 Relaxation of Foams by ß-lactoglobulin of different phenotypes

suggest that A variant had more effect on heat introduced gelling process than B variant.

There are many factors which could influence the gel strength such as pH, ion strength of protein solution and the hydrophobicity of the protein. The forces which are involved in maintenance of the native protein structure in solution will be also involved in network formation during protein gelation (Mulvihill and Kinsella, 1987). Protein with more hydrophobic structure yields a firmer texture of gel. The variant A of ß-Lg is more hydrophobic than variant B. It is reasonable that the gel formed with ß-Lg AA was firmer than with AB, BB and the mixture of the phenotypes of ß-Lg.

5.8.2 Gel relaxation

Gel relaxation reflects one of the elastic properties of protein gels. The higher the value of the relaxation, the less elastic the gel. Figure 33 shows the value of gel relaxation for the four phenotypes of ß-Lg. The observation suggested that BB phenotype of ß-Lg was associated with the highest relaxation (86.06%), followed by AB (81.83%), the mixture of phenotypes of ß-Lg (79.92%) and AA (78.65%). Therefore gel formed by BB phenotype of ß-Lg was the least elastic. The order of the gel elasticity was: BB<AB<MIX<AA. With more hydrophilic structure, BB phenotype of ß-Lg entrapped less water than other phenotypes and showed higher value of relaxation. The result also suggested that the relaxation is related to gel strength when considering the gelling properties. Stronger gel revealed a smaller gel relaxation index or better elasticity.

5.8.3 Water holding capacity of gels

Water holding capacity has been proposed for evaluating the ability of the 3-dimensional protein gel network to retain solvent (Hermansson and Akesson, 1975). Figure 34 shows the differences in water holding capacity of four types of ß-Lg gels. It was found that, the syneresis of gels formed with ß-Lg AA, AB, BB and the mixture of phenotypes of ß-Lg was 33.11%, 38.69%, 42.32% and 38.32%, respectively. According to the method of Britten and Pouliot (1996) and Kocher and Foegeding (1993), water holding capacity was defined as the free serum volume in the gels (gel syneresis, %). The larger the free serum volume, the weaker the water holding

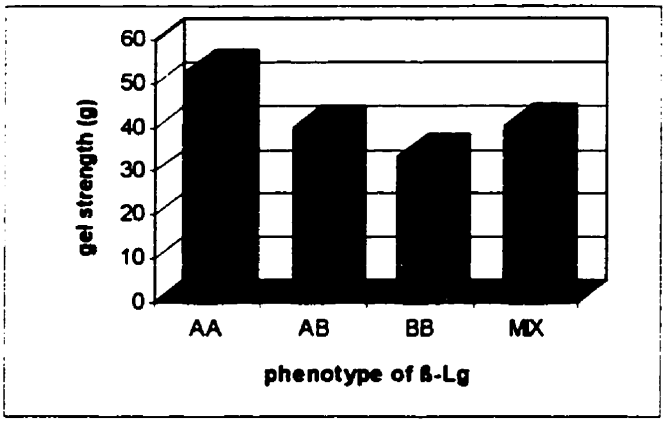

Fig.32 Strength of gels formed by ß-lactoglobulin of different phenotypes

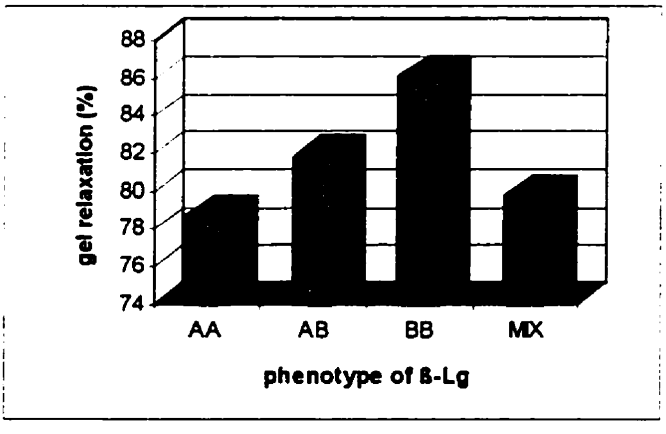

Fig. 33 Relaxation of gels formed by ß-lactoglobulin of different phenotypes

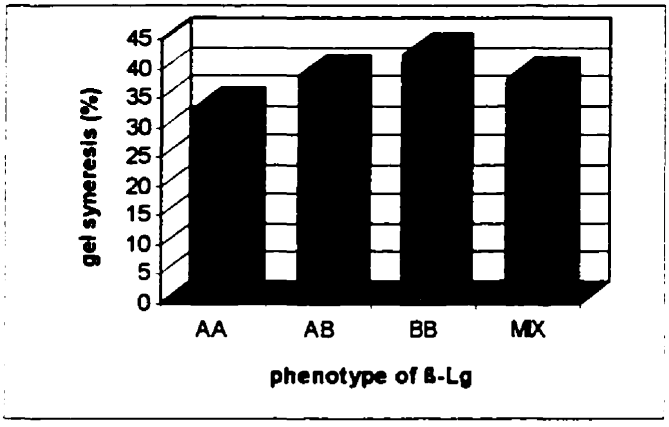

Fig. 34 Syneresis of gels formed by ß-lactoglobulin of different phenotypes

capacity of gels. In this study, ß-Lg AA had the least value of syneresis therefore it had the highest water holding capacity. The order for the four types of ß-Lg was: AA>MIX>AB>BB. Water holding capacity was not related to shear stress but to the microstructure (Karleskind *et al.*, 1995). Gels with high water holding capacity showed a stronger 3-dimensional network which could be formed through an adequate combination of hydrophobicity characteristics, lipids, SH and ion contents. Water holding capacity is important to characterize whey protein concentration that have the potential of producing strong gels, providing that the first stage of the gelation mechanism (protein unfolding stage) is adequate.

6. GENERAL DISCUSSION

In order to investigate the influence of genetic variants on functionalities of milk, all experiments were carried out under the same sets of conditions for the different phenotypes of the various milk proteins. The purity of isolated individual caseins and ß-Lg of different genetic phenotypes were proved by electrophoresis. Inorganic ions associated with the casein and ß-Lg preparation were removed by extensive dialysis. The contents of the four major caseins of whole casein were quantified with reverse phase HPLC. The statistical analysis suggested that differences in the amounts of α_{s1}-Cn, α_{s2}-Cn, ß-Cn and κ-Cn among the twelve whole caseins were not significant($P>0.05$), therefore any differences of physicochemical properties would be ascribed to the difference of genetic variants of milk proteins.

All results presented and discussed in section 5 significantly substantiated the hypothesis of this research project: genetic variants of milk proteins due to amino acids substitution or deletion in the polypeptide chain do alter the physicochemical properties.

Surface activities in terms of interfacial properties, emulsifying properties and foamability were highly related to the film characteristics of the proteins. Thickness and stability of protein film at the air-water interface depend on the ability of surface enlargement, diffusion and penetration of the protein molecules. Different hydrophobicity and molecular flexibility of the protein will perform very differently when they form the film at the surface or interface. The study on surface properties at air-water interface of 0.005% (w/v) protein solution verified that the kinetics of surface tension decay were unique for individual caseins of different genetic variants and whole caseins of different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn.

The unique physicochemical and functional properties of different proteins reflect differences in the primary structure, their folding behavior such as conformation and protein:protein interactions. Molecular shape and reactivity like charge, hydrophobicity, molecular flexibility all account for many properties of food proteins (Kinsella, 1981). Our observations on surface activity of caseins at air-water interface showed patterns

that were different to those of Tornberg's (1978a, b) measurements. She studied globular proteins (lysozyme, ß-Lg and BSA) by employing both drop volume and Wilhelmy plate method and reported that only lysozyme has a surface enlargement stage. Compared to her observations, all caseins including individual and whole caseins, have a very short initial stage but a relatively slower unfolding procedure. Kinsella and Phillips (1989) indicated that only a part of a protein absorbs initially and then the protein may subsequently unfold further as hydrophilic and hydrophobic relationships are altered at the interface. This initial procedure is related to the net charge, protein stabilizing forces, the relative amount of hydrophobic domains and flexibility of the molecules. Genetic variants of milk protein due to amino acids substitutions or deletions are different in their original structures and influenced the protein absorption at the interface.

Comparisons were made between the individual caseins of different genetic variants and between whole caseins of different phenotype combination of α_{s1}-Cn/ß-Cn/κ-Cn. In order to do so, the comparison in groups was made during the section of discussion. For instance, the comparison among the phenotype BB, BC and CC of α_{s1}-Cn was made when the phenotype of ß-Cn and κ-Cn were the same in the whole casein. The results presented in section 5 suggested that the observations from the individual ß-Cn and κ-Cn were in concordance with those from the whole casein. α_{s1}-Casein and α_{s2}-Cn performed very differently when they were in the isolated form as compared to when they were as components of whole casein in presence of ß-Cn and κ-Cn.

ß-Casein is the most surface active individual casein with the loosest and most flexible structure. Because it constitutes nearly 40% of the whole casein, ß-Cn has a major role in influencing the physicochemical properties of whole caseins. Six phenotypes of ß-Cn were used for this research project. It was found that A^1A^1, A^1A^2 and A^2A^2 were more surface active than A^1B, A^2B and BB phenotypes. ß-Casein A^1A^1, A^1A^2 and A^2A^2 were also found to be associated with higher values for voluminosity and a stronger ability of water entrapping than ß-Cn A^1B, A^2B and BB. This

observation could explain the differences among the oil-in-water emulsions prepared with individual ß-Cn of different phenotypes and whole casein with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn.

Emulsion and foam are two major forms reflecting the surface properties of proteins which were used as emulsion stabilizers and foam generators. Both these two forms are dispersed systems which contain two distinct phases. A liquid continuous phase surrounds a dispersed phase, and droplets of a second liquid immiscible with the first, or bubbles of gas. In the emulsion and foam situations, the total interfacial area between the phases becomes very large, so the characteristics of this interface have important effects on the whole system (Walstra, 1989). Proteins tend to accumulate at both oil-water and air-water interfaces, constituting an interfacial layer and thereby altering surface properties. The important initial step in the formation of protein based foams and emulsions is the adsorption and spreading of the protein at the surface or interface (Graham and Phillips, 1979a,b). Reduction of interfacial tension is the driving force for protein adsorption. Once adsorbed, protein molecules spread out and undergo rearrangement to form a stabilizing film (Waniska and Kinsella, 1985). Further adsorption to an existing film depends on protein hydrophobicity and flexibility to penetrate the film and compress already adsorbed proteins (Kinsella, 1984; Shimizu *et al.*, 1981). The study on the influence of genetic variants on surface active properties of bovine caseins at air-water interface gave the observations that the whole casein which contained A^1 variants of ß-Cn and AB phenotype of κ-Cn was more surface active than other whole caseins. In each individual casein, CC of α_{s1}-Cn, A^1A^1, A^1A^2 and A^2A^2 of ß-Cn, AB of κ-Cn were found to be the most surface active of the phenotypes. ß-Casein was confirmed to be the most surface active protein among the four caseins. Therefore ß-Cn is the main protein which determines the emulsifying properties of whole caseins. There are two important major factors that influence the kinetics of protein adsorption: the solution condition and the conformation of the protein. Graham and Phillips (1979a, b, c) studied the influence of protein conformation on adsorption at liquid interfaces with four structurally different proteins, lysozyme, BSA, ß-Cn and κ-

Cn. They demonstrated that ß-Cn adsorbed rapidly to the air-water interface and formed a dilute monolayer, whereas both lysozyme and BSA exhibited lower rates of adsorption but formed concentrated films. Because ß-Cn has a highly flexible random coil structure and contains no disulfide bonds, it is easier to unfold and to spread out on the surface. According to the study of this project, A^1A^1 and A^1A^2 of ß-Cn presented higher surface activity than the other phenotypes, possibly due to their more flexible structure.

Hydration and voluminosity are other important characteristics of protein related to emulsifying and foaming properties because the protein is easier to dissolve in the solution and disperse in the system if the protein has a higher capacity of hydration. Among the four major caseins, ß-Cn appeared to have a much higher capacity of hydration and voluminosity than α_{s1}-Cn, α_{s2}-Cn and κ-Cn. The phenotypes BB of α_{s1}-Cn, A^2A^2 and A^1A^2 of ß-Cn and AB of κ-Cn were superior to the other phenotypes within each individual caseins. Whole casein with phenotype combination of BB/A^1B/BB was associated with the highest value of hydration and voluminosity. The various phenotypes of individual casein and different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn which were associated with different water entrapping ability could be one of the reasons resulting in the differences in emulsion systems stabilized by whole caseins and individual caseins.

The ability to rapidly diffuse to the interface, reorient with limited aggregation and form a viscous film is critical for the formation of protein based foam (Kinsella and Phillips, 1989). The flexible, hydrophobic and small in size of protein molecules will generate large volume of foams. BB phenotype of ß-Lg was associated with the highest foam volume but the lowest foam stability. This could be due to the difference of a net electric charge in the molecular structure, because the net charge on protein molecules affects the solubility and extent of protein-protein interactions. BB phenotype of ß-Lg is more negatively charged than AB and AA phenotypes and this perhaps increases the repulsive effect causing a more flexible structure in BB than AB and AA ß-Lg. The

faster foam drainage rate of AA phenotype suggested that the film was thinner, and the mechanical strength was weaker.

The gelation mechanism suggested by Ferry (1948) is the most generally accepted theoretical model of heat-induced protein gelation. The process of gelation is assumed to follow a two stage process: an initiation step involving unfolding or dissociation of the protein molecules, followed by an aggregation step in which association or aggregation reactions occur, resulting in gel formation under appropriate conditions. The gelation reaction requires the proper balance between repulsive and attractive protein-protein and protein-water bonds (Shen, 1981) which in turn depends on solution pH, salt concentration and salt types (Mulvihill and Kinsella, 1988). Heat-induced gelation is the result of thermal denaturation of whey protein or individual ß-Lg. At higher temperatures, unfolding occurs concomitant with increased activity and oxidation of the thiol group (Larson and Jenness, 1952). Imafidon *et al.* (1991a) indicated that genetic polymorphism of ß-Lg is highly related to the heat stability and the results showed that BB phenotype of ß-Lg had the highest heat denaturation temperature and onset temperature followed by AB and AA phenotypes. From the measurement of gel strength and elasticity in the present study, it appeared that the highest value was associated with AA phenotype of ß-Lg. Lower heat stability of ß-Lg may be associated with stronger and more elastic gel properties. Since the dissociation of ß-Lg involves not only in the denaturation peak but also in the activity and oxidation of the thiol group, the hydrogen bond formation by imidazole amino acid plays an important role. The conformation of ß-Lg A is different from ß-Lg B and this could influence the exposure of the thiol group, the repulsion and attraction of the protein, and subsequently could influence the gelling properties.

7. SUMMARY AND CONCLUSIONS

Six different experiments were designed to investigate the influence of genetic variants of milk proteins on surface properties at the air-water interface, voluminosity and hydration, emulsifying, foaming and gelling properties. In order to investigate the relationships between the genetic variants and the above physicochemical properties, twelve whole caseins with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn, purified isolated individual caseins and ß-Lg with different genetic variants were collected and prepared from Holstein and Jersey milk samples. All prepared proteins were well washed, demonized and stored at -20 °C pending analysis.

Wilhelmy plate method was employed to measure the surface active properties at air-water interface for the whole caseins and the purified isolated individual caseins with different phenotypes in 0.005% (w/v) protein solution. The Ubbelohde Viscometer method was applied to measure the voluminosity and hydration of the all prepared casein samples. Model emulsions were prepared with 0.8% protein solution blended with 10% and 40% commercial soya oil, homogenized at the pressure of 70,000 kPa. in two passes. The emulsifying properties of twelve whole caseins with different phenotype combinations and of individual caseins were analyzed in terms of interfacial area of emulsion, protein load, apparent viscosity and emulsion stability. To investigate the influence of genetic variants on foaming properties of ß-Lg, four types of isolated ß-Lg were dispersed in phosphate buffer and whipped for five minutes. The generated foams were immediately used to measure the foam capacity, drainage half time, relaxation and firmness. Heat induced gels formed at 90 °C for 30 min and cooled in ice were used for investigating the influence of genetic variants on gelling properties of ß-Lg. The parameters measured were: strength, elasticity and water holding capacity of the gels. All collected data were statistically analyzed by SAS program with the Duncan's multiple range test at level of $\alpha = 0.05$.

From the results of this research project, it could be concluded that genetic variants do influence the physicochemical and functional properties of milk proteins.

This is due to alteration in the protein structure because of differences in amino acid composition in the different genetic variants. The higher surface activity of whole casein with different phenotype combinations of α_{s1}-Cn/ß-Cn/κ-Cn was associated with the content of A^1 and A^2 variant of ß-Cn and AB type of κ-Cn. Phenotype CC of α_{s1}-Cn, A^1A^1, A^1A^2 and A^2A^2 of ß-Cn, AB of κ-Cn decreased the surface tension faster than other phenotypes of the three proteins. Whole casein with the combination of BB/A^2B/BB had higher voluminosity and hydration value than the other eleven combinations. For individual caseins, α_{s1}-Cn BB, ß-Cn A^2A^2 and κ-Cn AB had greater value of voluminosity and hydration than the other phenotypes of the three proteins. ß-Casein shows the strongest water entrapping ability and the highest value of volumonisity, followed by α_{s1}-Cn, α_{s2}-Cn and κ-Cn. The emulsifying capacity and stability were affected by genetic variants of milk protein and by the oil concentration of the emulsions. Whole casein with the combination of BB/A^2A^2/BB and BB/A^1A^2/BB in 10% oil emulsion, BB/A^1A^1/BB in 40% oil emulsion were the most desirable emulsion stabilizers. ß-Casein A^2A^2 in 10% and A^1A^1 in 40% oil emulsions; κ-Cn BB and α_{s1}-Cn BB in both 10% and 40% oil emulsions were the best emulsion stabilizers when compared to other phenotypes of these three proteins. Among the four major caseins, ß-Cn shows the best performance in both 10% and 40% oil emulsions when compared to α_{s1}-Cn and κ-Cn. Foamability measurements revealed that the B variant for ß-Lg is responsible to higher foaming capacity and stability but less elastic and firm foam. AA phenotype of ß-Lg generated the strongest, more elastic and highest water holding capacity gels when compared to AB and BB phenotypes.

The information from the above conclusions contributed knowledge to the dairy industry and will help us make plans in our future research. If we select the most desirable genetic variant that is associated with the most desirable physicochemical properties and functionalities as the breeding trait, it will be possible to obtain dairy products of better quality.

8. COMMENTS ON FUTURE RESEARCH

Food emulsion is one of the most common and important systems in food industry and human daily life. Milk proteins have been extensively used as emulsion stabilizers. For this purpose, the combination with ß-Cn A^2A^2 or A^1A^2 and κ-Cn AB has been found to yield finer and more stable emulsion system. For breeding purpose, it is suggested that the frequencies of A^1 and A^2 variant of ß-Cn and B variant of κ-Cn should be increased not only because of their best surface activity as shown from this study, but also because of their association with better technological and manufacturing properties, especially in cheesemaking because shorter RCT and firmer curd are associated with κ-Cn B variant. The B variant of ß-Lg is associated with better foaming capacity and foam stability, whereas stronger and more elastic gel with higher water holding capacity is associated with A variant. Considering the future of food industry development, two directions of selection for ß-Lg is suggested: one population of cows ß-Lg AA and another for ß-Lg BB.

Research, up to now, concerning the relationships between genetic variants of milk protein and their physicochemical and functional properties is just in the initial steps. Separation of individual caseins and whey proteins has being done commercially. It is not surpring that some particular genetic variant of milk protein will function significantly better in some specific aspects of the commercial products. Further studies on these views are essential to provide more useful, and reliable information for new products development.

Casein, the major part of milk proteins, is used more and more as a specific source of raw material for production of functional and pharmaceutical materials by means of enzymatic, physical, and chemical modification methods (Fox *et al.*, 1982). Casomorphins derived from ß-Cn have different medical effects (Fox and Flym, 1992). Immunomodulating peptides that can stimulate the phagocytic activity of human macrophages in vitro and exert a protective effect in vivo (Migliore-Samour *et al.*, 1989; Gattergno *et al.*, 1988) were derived from casein. The residues of 106-116 and 106-112 of κ-Cn, known as platelet-modifying peptide were demonstrated to have the

function of inhibiting the aggregation of , and the binding of fibrinogen to ADP-treated platelets (Jolles *et al.*, 1986). One of the two-amino acids peptide, 23-24 from α_{s1}-Cn B variant was associated with the function of angiotension converting enzyme inhibitor that can block peptidyl-dipeptidase (EC 3.4.15.1). Other uses such as in infant formulas, special food preparations, specific drug preparations, cosmetics as well as food ingredients, are all the potential areas for the new products where developments in the genetic variant research could contribute.

The worldwide cheese and casein manufacturing industry produces an estimated 190 billion lb. of whey annually, which contains an estimated 1.3 billion lb. of whey protein (Zall, 1984; Morr, 1984; Van Hoogstraeten, 1987). ß-Lactoglobulin is the major fraction of whey proteins and contributes significatly to its functionality. The industry has spent considerable effort during last 30 years to develop whey protein concentrations for use as a food and animal feed ingredient (Moor and Ha, 1993). Unfortunately, not much information on the effects of genetic variants or their functionalities are available. Further exploration will be necessary to understand the relationships between the functionality and genetic variants of whey protein, to maximally use this huge resource.

9. CLAIMS TO ORIGINAL RESEARCH

1. First comparative study on the influence of genetic variants on surface properties of bovine whole casein with different phenotype combinations of α_{s1}-Cn, ß-Cn and κ-Cn.

2. First comparative study on the influence of genetic variants on surface properties of different phenotypes of isolated α_{s1}-Cn, ß-Cn and κ-Cn.

3. First comparative study on the influence of genetic variants on voluminosity and hydration properties of bovine whole casein with different phenotype combinations of α_{s1}-Cn, ß-Cn and κ-Cn.

4. First comparative study on the influence of genetic variants on voluminosity and hydration properties of different phenotypes of isolated α_{s1}-Cn, ß-Cn and κ-Cn.

5. First comparative study on the influence of genetic variants on emulsifying properties of bovine whole casein with different phenotype combinations of α_{s1}-Cn, ß-Cn and κ-Cn.

6. First comparative study on the influence of genetic variants on emulsifying properties of different phenotypes of isolated α_{s1}-Cn, ß-Cn and κ-Cn.

7. First comparative study on the influence of genetic variants on foaming properties of ß-Lg.

8. First comparative study on the influence of genetic variants on gelling properties of ß-Lg.

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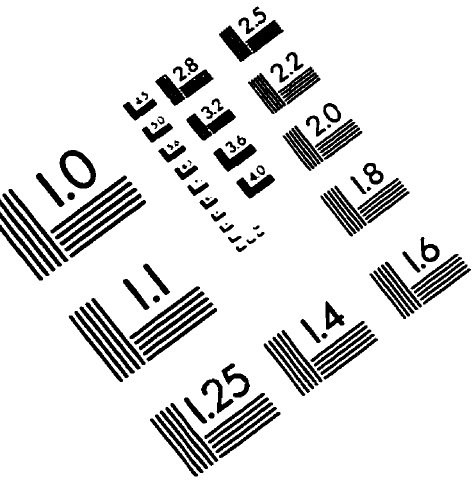
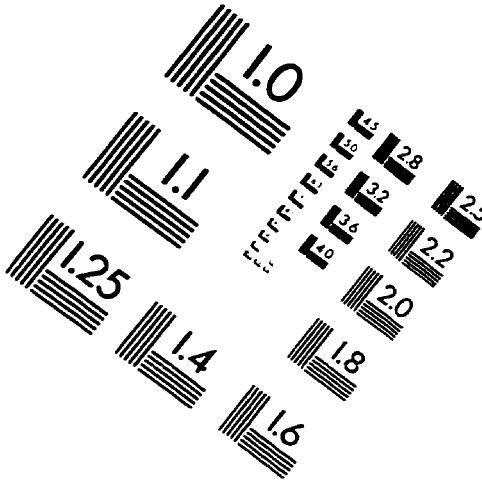
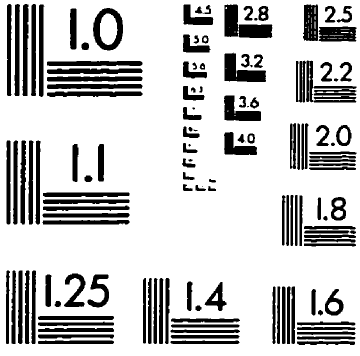
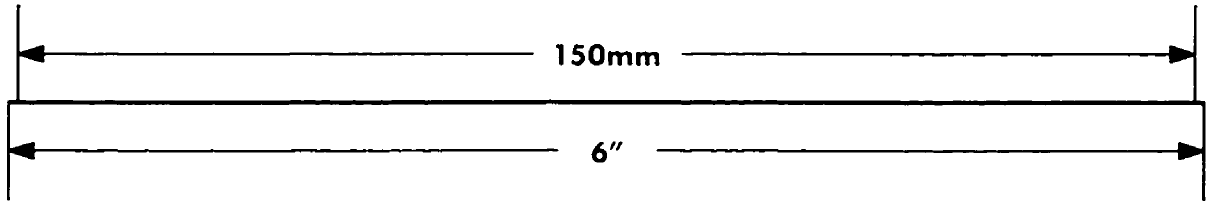
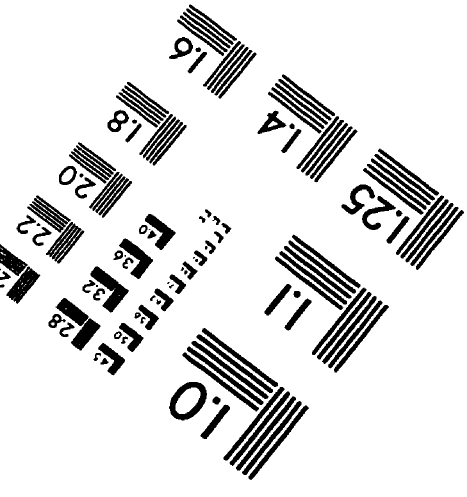
IMAGE EVALUATION
TEST TARGET (QA-3)

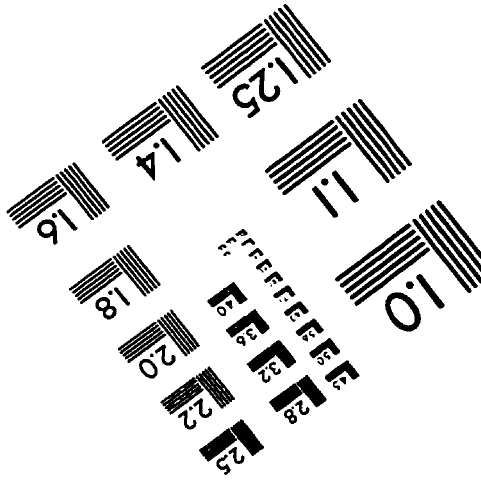












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