Association of cheesemaking characteristics with genetic variants of κ-casein and β-lactoglobulin from milk of four breeds of dairy cattle

by

Xiaochun Wan

A thesis submitted to the Faculty of Graduate studies and Research

in partial fulfilment of the requirements for the degree of

Master of Science

Department of Animal Science

Macdonald Campus of McGill University

Montreal, Quebec

Canada

August, 1997

0-612-44311-6

ABSTRACT

Xiaochun Wan M. Sc. Animal Science

Association of cheesemaking characteristics with genetic variants of κ-casein and β-lactoglobulin from milk of four breeds of dairy cattle

Laboratory scale Cheddar type cheese were made from 411 milk samples originating from Ayrshire, Brown Swiss, Canadienne and Jersey with different phenotypes of κ-casein (κ-CN) and β-lactoglobulin (β-LG). From the milk input, cheese and whey outputs, the cheese yield, cheese composition and cheesemaking efficiency and other parameters were determined. The overall 37% moisture adjusted cheese yield and cheese yield efficiency were 11.19g, 10.19g, 11.97g, 11.46g and 98.91%, 90.10%, 90.76% and 90.92% for Ayrshire, Brown Swiss, Canadienne and Jersey respectively. The milk of the four respective breeds contained 12.91, 12.69,13.50,14.57% total solids; 3.97, 3.58, 4.40, 4.61 % fat; 3.61, 3.73, 3.81, 4.00% protein. In Ayrshire, the combination BB/BB and BB/AA (κ-CN/β-LG) were associated with higher 37% moisture adjusted cheese yield with the values of 12.51 and 12.83 g /100 g milk respectively. The cheese composition for these two types of milk were 62.28 and 63.96 % total solids, 24.22 and 20.40% protein; 32.75 and 37.92% fat. For Brown Swiss, type AA/BB was associated with higher cheese yield (11.18g) with composition of 62.15% total solids, 22.54% protein, 33.23% fat. The phenotype with the highest cheese yield for Canadienne is BB/AB (12.45g) with cheese composition of 63.61% total solids, 23.27% protein and 63.61% fat. In Jersey, the phenotype combination with higher cheese yield (14.59g) is BB/BB. The cheese composition corresponding to this phenotype was 58.64% total solids, 22.96% protein and 29.59% fat. Phenotypes associated with better coagulating properties for Ayrshire, Brown Swiss, Canadienne and Jersey were BB/AB, BB/BB, BB/BB and BB/BB for κ-CN/β-LG respectively.

Association des caractéristiques de la fabrication du fromage avec des variants génétiques de la k-caséine et la b-lactoglobuline à partir du lait provenant de quatre races de vaches

RESUME

Xiaochun WAN **M.Sc.** **Sciences Animales**

Du fromage du type Cheddar a été fabriqué à l'échelle du laboratoire à partir de 411 échantillons laitiers provenant de vaches Ayrshire, Brown Swiss, Canadiennes et Jerseys ayant différents phénotypes de κ-caséine (κ-CN) et β-lactoglobuline (β-LG). La production, la composition, le rendement de la fabrication du fromage ainsi que d'autres paramètres ont été déterminés à partir des données du lait, du lactosérum et du fromage.

La production fromagère obtenue à 37% d'humidité était de 11,19g pour les vaches Ayrshires, 10,18g pour les vaches Brown Swiss, 11,97g pour les vaches Canadiennes, et 11,47g pour les vaches Jerseys ce qui correspond à des rendements de 98,91%, 90,10%. 90,76%, et 90,92%.

Le lait des quatres races citées dans le même ordre que ci-dessus, contenait 12,91%, 12,69%, 13,50%, 14,57% de matière sèche totale; 3,97%, 3,58%, 4,40% et 4,61% de matière grasse et 3,61%, 3,73%, 3,81% et 4% de protéines. Chez les vaches Ayrshires de phénotypes BB/BB et BB/AA (k-CN/b-LG) la production fromagère obtenue à 37% d'humidité, était augmentée respectivement de 12,51 et 12,83g/100g de lait. La composition fromagère pour ces deux types de lait était respectivement 62,28% et 63,96% de matière solide totale, 24,22% et 20,40% de protéines 32,75% et 37,92% de matière grasse. Pour les vaches Brown Swiss, le phénotype AA/BB présente une production fromagère élevée (11,18g) dont la composition est 62,15% de matière sèche totale, 22,54% de protéines et 33,23% de matière grasse.

Le phénotype donnant le meilleur rendement fromager pour les vaches canadiennes est BB/AB (12,45g) avec une composition fromagère de 63,61% de matière sèche totale, 23,27% de protéines et 33,23% de matière grasse. Pour les vaches Jerseys, le phénotype BB/BB a donné le meilleur rendement fromager (14,5g). La composition fromagère correspondant à ce phénotype était 58,64% de matière sèche totale, 22,96% de protéines et 29,59% de matière grasse. Les phénotypes associés aux meilleures caractéristiques coagulantes pour les Ayrshires, Brown Swiss, Canadiennes et Jersey étaient BB/AB, BB/BB, BB/BB et BB/BB respectivement.

ACKNOWLEDGEMENTS

The author wishes to express appreciation to the following persons:

Dr. K. F. Ng-Kwai-Hang, my supervisor and the chairman of my thesis committee, for his encouragement, patience, advice and understanding during my graduate career, and also for his financial support.

Thanks to the supervisory committee, Dr. Monardes and Dr. B. H. Lee for their advice and for reviewing the thesis manuscript.

I'm grateful to Mr. Don Galbraith for his assistance in the laboratory analysis.

I would like to extend thanks to the staff in the laboratory of the Programme d'Analyse des Troupeaux Laitiers du Quebec (PATLQ) and " Le Centre de Controle et Recherche Agropur" in Granby for their cooperation and assistance in laboratory analysis.

Special thanks to Mr. and Mrs. Yvon Rioux for their excellent arrangement of milk sample transportation .

I would like to thank my fellow graduate students for their suggestions and discussions. Also, thanks to S. Hoi for translating the abstract into French.

I wish to extend my gratitude and appreciation to my girl friend Chin, for her patience, understanding and aid throughout the course of this study

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Cheesemaking is one of the oldest methods for preservation of milk as foodstuff. Throughout the world, there are as many as 2000 different types of cheeses. The International Dairy Federation published a catalogue in 1981 and formally confirmed 510 different kinds of cheese. In 1989, the world production figure for cheese was 14.48 million tons, the hard cheese such as Cheddar cheese and semi-hard cheese made up about 60% of total world output. Cheese and butter production were still well recognized as the major products for world milk utilization. They represented 26% and 37% respectively of total milk utilization as compared with 30% for liquid milk processing. Because of the increasing consumption, the world demand for cheese have increased each year and will continue to increase for the next decade at a rate in excess of that observed over the past decades.

The processes of cheesemaking have been extensively studied and many factors were identified by dairy scientists which could significantly influence the productivity of cheese manufacturing plants. Levels of milk components and overall cheesemaking are dependent on two categories of factors: genetic and environmental factors. Environmental factors that affect milk composition can be divided into two groups: (1) environmental factors which dairy farmers have little or no control over; and (2) those factors which dairy farmers can control. Factors that dairy farmers cannot influence are: season, stage of lactation and gestation, age of cow, and ambient temperature. Milk producers could alter milk composition primarily through control over the nutrition of cows. Feeding may result in quicker responses to increased milk composition levels than genetics. For genetic factors, there are two ways for dairy producers to influences milk composition. One is through selection of the breeds. An other way is selection of cows within the breed.

Improvements of cheesemaking procedures, as well as milk composition, have been shown to increase cheese yield. The primary factor which can influence cheese yield is milk composition. Increasing of milk majors components levels in the manufacturing milk does as much or more in increasing the plant's cheese yield potential as does fine tuning the manufacturing process. In very efficient operations, increasing the concentration of milk protein and fat is perhaps the only means for increasing the plant's product yield.

The effect of genetic variants of milk protein, as the potential influencing factor for cheesemaking, has been intensively investigated for the past 10 years. Genetic variants of individual milk proteins usually differ by only one or two amino acids. Studies have confirmed that some of the genetic variants of caseins and whey proteins are associated with improved curd forming properties of milk as well as increases in cheese yield and yield efficiencies. It was found that in Holstein milk, the effects of genetic variants have significant effects on cheesemaking properties. Previous work (Marziali and Ng-Kwai-Hang, 1986a, 1986b; Ng-Kwai-Hang *et al.*, 1986) in our laboratory have shown that genetic variants of milk protein, especially κ-casein (κ-CN) and β-lactoglobulin (β-LG), have profound effects on milk composition, cheese composition, cheese yield and other cheesemaking properties of Holstein milk.

The main objectives of this research project were:

1) To explore the relationships between phenotypes combination of κ-CN/β-LG variation of milk compositions from milk of four different breeds of cattle: Ayrshire, Brown Swiss, Canadienne and Jersey.

2) To study the effects of phenotype combinations of κ-CN/β-LG on cheese yields and cheese yield efficiencies for milk of the four breeds, as well as the differences for the effect of different phenotype combinations within each breed.

3) To investigate the effects of phenotype combinations of κ-CN/β-LG on coagulating properties of milk of the four breeds, as well as the different effects for phenotype combinations of κ-CN/β-LG in each breed.

4) To study the relationships between phenotype combinations of κ-CN and β-LG and the potential loses of major milk components of milk into whey during the process of cheese making in each of the four.

2. REVIEW OF LITERATURE

2. 1. Gross composition of milk

Milk is a complex biological fluid and is an important foodstuff for young mammals. The major constituents of milk are: water (87.2%), lipid (3.7%), protein (3.5%), and carbohydrate (4.9%). There are also small quantities of minerals (0.7%), enzymes, fat-soluble and water-soluble components (Jenness, 1974). The main composition of milk may vary from species to species and between breeds within a species.

2. 1. 1. Water

Water is quantitatively the most important component in milk of all species. It constitutes about 87% of bovine milk. Therefore it could be considered as a solvent or carrier of other components of milk.

2. 1. 2. Lipids

The composition of milk lipid or milk fat are quite complex. For a long time, milk fat percentage had been used as the most important parameter for the pricing of milk. Triacylglycerols are dominant and constitute 98% of milk fat. The remaining fractions are small amounts of di- and monoacylglycerols together with small amount of free fatty acids. There are some minor lipid components which play important roles such as phospholipids, cholesterol and fat-soluble vitamins. Lipid molecules in milk usually associate together to form large stable globules that are surrounded by a phospholipid-rich layer. Drastic physical and chemical treatment such as thermal shock or very low pH can rupture the membrane and release free fat as is encountered during milk processing. During the manufacture of Cheddar type cheese, approximately 93% of the milk fat are retained in the product.

2. 1. 3. Carbohydrates

The predominant constituent of milk carbohydrate is lactose which is a disaccharide, comprising of an α-D-glucose and a ß-D-galactose. The concentration of lactose in milk varies from 4.2 to 5%. Lactose contributes to the colligative properties of milk and it is also a significant source of dietary energy. Most of lactose is lost in whey during the cheesemaking process. In industry, it is used as food ingredient due to its protein stabilizing properties. Some other carbohydrates, such as monosaccharides, oligosaccharides and peptide-bound carbohydrates, are also found in trace amount in milk.

2. 1. 4. Minerals

The mineral or salt in milk consists mainly of the bicarbonates, chlorides, citrates and carbonates of calcium, magnesium, potassium and sodium. These minerals have important roles for the stability of milk and milk products. Colloidal calcium phosphate is important for the integrity of casein micelles. Calcium ions also bind to some caseins and compete strongly with citrate. In addition to the importance of minerals for the stability of casein, the monovalent ions together with lactose and other low molecular weight components play important role in inhibiting bacterial growth, maintaining pH, regulating and controlling cheese microflora.

2. 1. 5. Proteins

There are two main classes of milk proteins: caseins and whey proteins (serum proteins). Bovine milk normally contains 30-35g of protein per litre. Approximately 80% of milk protein are present as casein micelles which are large spherical complexes containing about 92% protein and 8% inorganic salts. The major component of the salts is calcium phosphate (Schmidt, 1980; Swaisgood, 1985; Whitney, 1988). Most of the whole casein (97%) are incorporated in the cheese. Whey proteins are water-soluble proteins which are usually lost in the whey fraction during cheese making. For a long time whey proteins had been ignored and presently, whey proteins have been used more and more in food and feed industry (Guinee and Fox, 1993).

2. 1. 5. 1. Caseins

The caseins comprise a group of proteins with a unique molecular structure (Swaisgood, 1973). The caseins, which are milk-specific proteins, occur as micelles containing calcium and inorganic phosphate and represent the few naturally occurring phosphoprotein. Caseins can be subdivided into five main groups, α_{s1}-, α_{s2}-, ß-, γ- and κ-caseins. All of these except γ-casein, are mammary gland gene products. γ-Casein is the product of hydrolysis of ß-casein. The relative proportions of α_{s1}-, α_{s2}-, ß- and κ-caseins are subject to genetic variation within individual herds and the casein composition are different from milk of different cows (Ng-Kwai-Hang *et al.*, 1984a; Kroeker *et al.*, 1985a, 1985b; Swaisgood, 1989).

2. 1. 5. 2. Casein micelle

Casein micelles are relatively large aggregation of the four casein components plus small amounts of calcium, phosphate, magnesium, sodium, potassium and citrate. The amphophilic nature of caseins and their phosphorylation facilitate interaction with each other and with calcium phosphate to form highly hydrated spherical complexes known as casein micelles. These casein micelles vary in size and the diameter ranges from 30 to 600 nm (McMahon and Brown, 1984; Farrell, 1988). The proteins which account for 92% of the casein micelle are composed of α_{s1}-, α_{s2}- ß- and κ-caseins which are in the proportions of 3:1:3:1. The remaining 8% of the micelle are composed of inorganic constituents, mainly colloidal calcium phosphate. It is believed that calcium phosphate is distributed throughout the micelle and the presence of calcium ions acts as an absolute essential factor for micelle formation. Phosphate appears to be important in the formation of temperature stable micelle (Payens, 1977). Electrostatic and steric repulsions opposing the Van de Waals force are responsible for the stability of micelles (Hill and Wake, 1969; Holt, 1975; Walstra, 1990). A number of models have been proposed for casein micelle structure. Among them, the most satisfactory one was first advanced by Slattery and Evard (1973). It is suggested that the casein micelle is composed of subunits of variable composition. Later, Schmidt (1979, 1982) adopted the variable subunit composition concept but with a more detailed description of

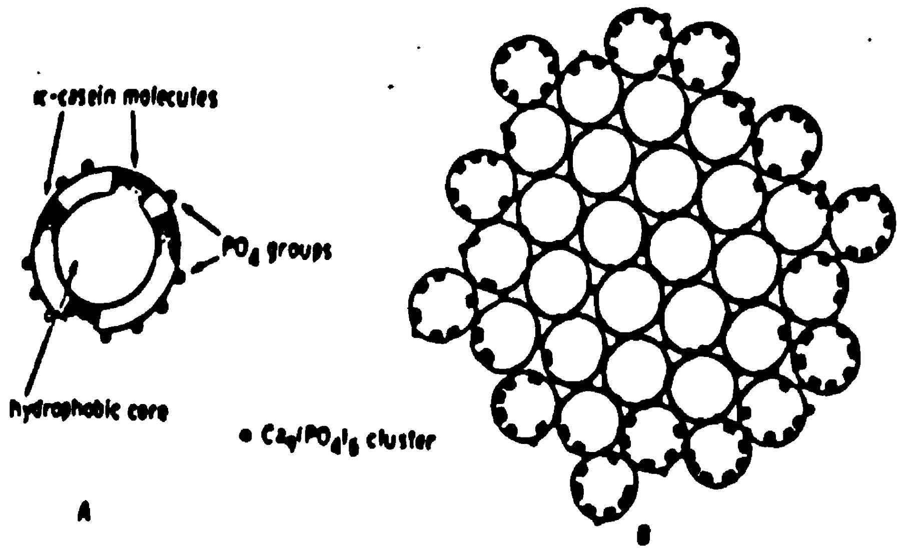

Figure 1. Schematic representation of a submicelle (A) and a casein micelle, composed of submicelles (B). It should be realized that the submicelles are only at a first approximation spherical (Schmidt, 1982).

inorganic phase of the micelles. It was assumed that a calcium ion is bound to virtually all of the ester phosphate groups and that some of this groups are linked in pairs by $Ca_9(PO_4)_6$ cluster.

A further modification of the subunit model was proposed by Walstra, in which the concept of steric stabilization of the micelle by κ-CN was incorporated (Walstra and Jenness, 1984; Walstra; 1990). In this model, the C-terminal parts of the κ-CN molecules are sticking out from the micelles surface into the surrounding solvent (Fig.1). Calcium-depleted aggregate of almost spherical sub-micelles, which in turn consists of more limited aggregates of casein molecules. α_s-casein and ß-caseins are linked by the involvement of the phosphoserine residues with the calcium phosphate. The κ-CN molecule is localized on or very close to the surface of the casein micelle. The hydrophobic part of the κ-CN molecule is bound to the core of the micelle, and the hydrophilic macropeptide forms a layer of highly hydrated "hairs" which project into the aqueous phase. The κ-CN hairs are responsible for the stabilization of casein micelles.

2. 1. 5. 3. Whey protein

Whey proteins represent a very heterogeneous group of proteins which consist of several fractions based on their electrophoretic mobility (Woodhouse and Lonnerdal, 1988). These proteins are made up of ß-LG, α-lactalbumin (α-la), bovine serum albumin (BSA), immunoglobulin (Ig), and the proteose-peptone fraction (Fox *et al.*,1982; Swaisgood, 1982). Of the four fractions studied, ß-LG, α-la, and possibly some components of the Ig fractions are synthesized in the mammary tissue. Many studies have shown that the amount of whey protein in milk had significant effect on its properties (Feagan, 1979). The effect of ß-LG on the heat stability of milk have been studied in some details (Haenlein, 1973; Euber, 1982; Choi, 1996).

Since the discovery of two variants of ß-LG in cow's milk by Aschaffenburg and Drewry in 1955, many studies have reported the findings of polymorphism in various milk proteins. It is now known that α_{s1}-casein, ß-casein, κ-CN, ß-LG and α-la are generally polymorphic. These major proteins are controlled by autosomal genes and are inherited according to Mendelian mode of inheritance (Aschaffenburg, 1968; Thompson *et al.*, 1974; Baker, 1980).

ß-Lactoglobulin, which constitutes approximately 50% of the whey protein, had been intensively studied for the past decades (Jenness, 1974, 1988; Schmidt and Morris, 1984). The primary structure of β-LG has both disulphide and a free sulfhdryl group that made it possible for the formation of intra- or inter- molecular thio-disulphide interchange. Bovine β-LG is known to interact with α-la (Hunziker and Tarassuk, 1965), and several of the caseins. For instance, it can react with κ-CN to form a 3 : 1 complex involving hydrophobic interactions. This complex is subsequently stabilized by covalent bonding, and a conformational change which makes the disulphide bridge less susceptible to attack (Haque *et al.*, 1988) Moreover, upon the formation of the complex, the amount of "free" κ-CN will be reduced and this leads to less κ-CN being used as substrate for rennet in cheese making and as a consequence, it will result in slowing down the coagulation of milk (Schmidt and Morris, 1984).

2.1.5.4. <u>Interaction of κ-CN and ß-LG</u>

As mention in the previous section, ß-LG is the milk protein which is most susceptible to heat denaturation. Therefore, upon heat treatment, pure ß-LG molecule will unfold and this results in breakage of some of the intramolecular bonds and rupture of the sulfhydryl and disulfide groups and thus leaving them free to interaction reactions (Dalgleish, 1982; Schmidt and Morris, 1984).

Tobias *et al.*(1952) showed that possibly, there was an interaction of the caseins with ß-LG. It was suggested as early as 1958 that -SH groups are involved in that reaction (Trautman and Swanson, 1958).

Over the years, many proofs of this interaction have been made. It was reported by many authors that when a mixture of κ-CN and ß-LG was heated, the formation of a complex between the two occurred.(Parry, 1974; Losi *et al.*, 1975; Schmit and Morris, 1984; Choi, 1996).

The existence of such a complex in milk was questioned by Tessier *et al.*(1969). However, more studies have supported the theory that interaction between κ-CN and ß-LG occurs also in the unpurified form of those proteins and that the association between them do take place in milk (Davies *et al.*, 1978; Elfagm and Wheelick, 1978; Smits and Van Brouwershaven, 1980). With this background information, it is much easier to understand why the formation of these κ-CN- ß-LG complexes reduce the amount of κ-CN available for attack by coagulating enzymes such as rennet (Losi *et al.*, 1979; Dalgleish, 1982; Schmits and Morris, 1984; Walstra and Jenness, 1984).

The complex formation has been shown to be dependent on the quantity of ß-LG present and also on the temperature applied. Some degree of denaturation must occur in order to totally expose the sulfhydryl residue which is usually partially buried in the molecule when it is in the native state. Not all the κ-CN present will interact with ß-LG and the quantity that do react is dependent on the temperature utilized (Parry, 1974; Losi *et al.*, 1979; Smits and Van Brouwershaven, 1980). It is also interesting to notice that the genetic variants of ß-LG influence the heat stability of the protein and therefore affect the formation of the complex. ß-LG B is more susceptible to heat denaturation than ß-LG A, which makes the latter better suited as fluid milk (Losi *et al.*, 1979; Morini *et al.*, 1982).

2. 2. Variation of bovine milk composition

The composition of bovine milk could be influenced by a number of factors. The variation of milk composition, especially of fat and protein, may result in the change in chemical and physical properties of milk as well as the manufacturing properties of milk.

It was found that physiological factors affect the gross composition of milk. Factors such as stage of lactation, age of cows, inflammation of the mammary gland, nutritional level and seasonal variations were reported to have influence on milk composition (Johnson, 1974; Rook, 1961; Grand and Patel, 1980; Grandison *et al.*, 1984a; Ng-Kwai-Hang *et al.*, 1984a).

2.2.1. Variation due to breeds

Genetically controlled factors such as breeds (Cerbulis and Farrell, 1975; Feagan, 1979; Storry *et al.*, 1983) have been found to influence gross composition of milk. For the four major dairy breeds Ayrshire, Brown Swiss, Holstein and Jersey in the US, milk protein are 3.39, 3.56, 3.19, 3.80% and milk fat are 3.93, 4.02, 3.66, 4.76%, respectively. Obviously, there is significant difference in milk main component concentrations among the major dairy breeds. The difference of the milk components among dairy breeds directly relates with the difference of cheese yield. The predicted Cheddar cheese yield for these four major dairy breeds are 10.69, 11.07, 9.99 and 12.36 g per 100 g of milk, respectively (Covington, 1994). The most recent data from Quebec (Programme d'Analyse des Troupeaux Laitiers du Quebec, 1996) show that protein content for Ayrshire, Brown Swiss, Canadienne and Jersey are: 3.38, 3.54, 3.65, 3.92% and fat content are 4.04, 4.13, 4.28 and 4.86%, respectively. The results of this research also confirmed the relationships of milk composition with both cheesemaking and physico-chemical properties of milk for different breeds of cow.

2. 2. 2. Variation due to genetic polymorphism of κ-casein and ß-lactoglobulin

2.2.2.1. Genetic polymorphism of κ-casein and ß-lactoglobulin

The term milk protein polymorphism refers to a small variation in the amino acid composition of polypeptide, mostly due to the substitution or deletion of certain amino acids in polypeptide chain. Up to now, genetic variants are found to exist in all major milk proteins: five for α_{s1}-casein, four for α_{s2}-casein; seven for ß-casein, four for κ-CN, three for α-lactalbumin and seven for ß-lactoglobulin (Ng-Kwai-Hang and Grosclaude, 1992). The genetic variants of κ-CN and ß-LG differ by only a few amino acid substitutions. These differences cause significant changes in renneting and cheesemaking.

The primary structures of the known genetic variants A and B of κ-CN were first identified by Grosclaude *et al.* (1972) and Mercier *et al.* (1973). κ-CN A differs from the B variant by the substitutions of a threonine for isoleucine at position 136 and an aspartic acid for alanine at position 148. Both amino acid substitutions occur in the macropeptide region between residues 106 to 169, the C terminal peptide resulting from the hydrolysis by chymosin. The difference between κ-CN A and B in Zebu involve similar amino acid substitutions at positions 136 and 148, plus a third substitution at position 135. For κ-CN A, it is isoleucine and for κ-CN B it is threonine (Grosclaude *et al.*, 1974a). Substitution of arginine in variants B for histidine at position 97 gives variant C. At position 155, variant A has serine while variant E has glycine.

Currently, it is known that ß-lactoglobulin, which contains 162 amino acids, has seven genetic variants. The primary structure had been determined by several studies (Braunitzer *et al.*, 1972; Braunitzer and Chen, 1972; Kolde and Braunitzer, 1983). The substitutions of aspartic acid at position 64 and valine at position 118 by glycine and alanine constitute differences between variant A and Variant B. Substitution of glutamine at position 59 of variant B by a histidine gives variant C, while the replacement of glutamic acid at position 45 of variant B by glutamine results in variant D (Brignon *et al* ., 1973). Grosclaude *et al* (1976) found variant E in yak's milk which differs from variant B by having glycine instead of glutamic acid at position 158. Bell *et al* (1981) found that variant F and G are restricted in Bali cows. However, the complete amino acid sequences still need to be established. It is suggested that variant F and G, like variant E, have glycine at position 78 instead of isoleucine as in variant B. In addition, ß-LG G has methionine at position 78 instead of isoleucine in variant B. Variant F differs from B by substitution of serine for proline at position 50 and tyrosine for aspartic acid either at position 129 or 130. The exact locations of amino acid substitution in ß-LG F and G need to be confirmed after the determination of the primary structures of these two variants.

2.2.2.2. <u>Variation due to genetic polymorphism of κ-casein</u>

Many studies have been carried out to investigate the relationship between genetic variants and milk composition. It was found that the B type of κ-CN was associated with higher amount of κ-CN (Cerbulis and Farrell, 1975; Ng-Kwai-Hang *et al.*, 1984b, 1986; Ng-Kwai-Hang, 1990) and higher protein content (Mariani *et al.*, 1976; McLean *et al.*, 1984; Van Eenennaam and Medrano, 1991b; Van den Berg *et al.*, 1992; Sacchi *et al.*, 1993). However, the cause of higher expression of κ-CN B gene is still not well understood. It was reported that the replacement of A by B allele at the κ-CN locus would increase protein content in milk by 0.08, 0.06, and 0.04% respectively for the first, second and third lactations (Ng-Kwai-Hang, 1990). It was also reported that higher fat content was associated with κ-CN BB (Ng-Kwai-Hang *et al.*, 1986).

2.2.2.3. <u>Variation due to genetic polymorphism of β-lactoglobulin</u>

Since Aschaffenburg and Drewry (1957) discovered that milk from β-LG AA cows contains more protein than from those of AB or BB cows, many researchers have confirmed this observation (McLean *et al.*, 1984; Ng-Kwai-Hang *et al.*, 1986, Ng-Kwai-Hang, 1990; Sacchi *et al.*, 1993). The replacement of B by A allele at the β-LG locus was followed by an increase of 0.05, 0.07, and 0.08% protein for the first, second and third lactation (Ng-Kwai-Hang, 1990). Several studies reported that β-LG AA is associated with higher whey protein and lower casein, thus resulting in lower casein number than β-LG BB (Cerbulis and Farrell, 1975; Mariani *et al.*, 1979; Buchberger et al., Schaar *et al.*, 1985; Ng-Kwai-Hang *et al.*, 1986, 1987a). In most cases, either the homozygote or heterozygote form of β-LG B was associated with a higher fat percentage in the milk (McLean *et al.*, 1984; Ng-Kwai-Hang *et al.*, 1986).

It was found (Cerbulis and Farrell, 1975; Ng-Kwai-Hang *et al.*, 1987a) that β-LG B was associated with a higher casein number, which would leave less protein nitrogen in the whey. This is of great importance in cheese making, especially in improvement of cheese yield. Similar results were also found by Losi *et al.*(1975) , Mariani *et al.* (1979b), and Morini *et al.* (1982). These researchers all agreed that because of its casein content, β-LG B milk was best suited for cheese than milk homozygous for the A variant, since more casein would allow more aggregation of the micelles due to hydrophobic interactions during the coagulation process. This way, more fat and more whey can be trapped in the curd while it is formed, reducing the loss of those components which would decrease the yield of cheese.

These studies suggest that it would be possible to improve milk composition to suit dairy processing industries by the proper breeding and selection programme.

2. 3. Cheddar cheese making procedure

The basic technology for manufacturing all types of cheese are relatively similar. However, small modifications during cheesemaking procedures will result in large differences in the final cheese. As one of the most important variety of cheese, Cheddar cheese making technique is used on a large scale and on a world-wide basis. The special process used in Chedder cheese making is "Cheddaring" in which blocks of warm curd are piled and repiled every 20 min for a period of two hours. This traditional technique is well established and is usually performed normally for hundreds of years but in recent years there has been considerable degrees of refinement and automation.

2.3.1. Adding of culture

In modern cheese making practice, the commonly used bacteria are lactic acid producing bacteria which is added in milk as starter cultures. The key role for the culture is for the production of lactic acid by fermentation of lactose. Lactic acid is responsible for the fresh acidic flavor of unripened cheese and is of importance in the formation and texturizing of the curd. In addition, starters play other essential roles: the production of volatile flavor compounds such as diacetyl and aldehydes and the synthesis of proteolytic and lipolytic enzymes involved in the ripening of cheese and the suppression of pathogenic and some spoilage micro-organisms.

2. 3. 2. Enzymatic coagulation of milk

Cheddar cheesemaking start with milk coagulation which is the formation of a coagulum from liquid milk by rennet action that involves the hydrolysis of κ-CN and then followed by the aggregation of the casein micelles. The procedure of rennet coagulation of milk may be divided into primary or proteolysis, secondary or aggregation, and possibly tertiary or syneresis and structural rearrangement stages. During the primary stage, κ-CN is cleaved by rennet or chymosin substitute at the position of Phe_{105}-Met_{106} bond. The resulting peptides are: para-κ-casein for fragment of 1-105 and macropeptide moiety for fragment 106-169. The macropeptide is hydrophilic and soluble, and diffuses away from the micelle after hydrolysis, whereas para-κ-casein is strongly hydrophobic and remains attached to the micelle. The progressive hydrolysis of κ-CN during the primary stage alters the properties of the casein micelles and consequently they become susceptible to aggregation and this leads to the secondary stage of the reaction. Loss of the C-terminal segment (macropeptide) of κ-CN from the surface of the micelles reduces their surface charge by about 40% and destroys steric stabilization which permits the closer approach of rennet-altered micelles and facilitates aggregation (Walstra, 1990). The tertiary stage of the rennet coagulation process involves syneresis of the gel with the expulsion of water and structural rearrangement of the gel network. The rennet coagulation of milk has been the subject of several reviews (Dalgleish, 1982, 1987; McMahon and Brown, 1984). McMahon and Brown (1984) suggested that the process or coagulation could be subdivided into three phases: enzymatic proteolysis of κ-CN, aggregation and gelation. But these three phase are not distinctively separated and in fact some overlaps occur.

2. 4. Cheese yield expression and yield prediction formula

Cheese yield is the parameter which is directly related to profitability for the cheesemaker. There are several different ways for expressing cheese yield.

2. 4. 1. Expression of cheese yield

The basic expression of cheese yield is " kg of cheese per 100 kg of milk". This is the so-called actual cheese yield which is simple and the first step for all the other methods of expressing yield. Actual cheese yield is not widely used in practice because this expression does not take into account the moisture content of cheese. Therefore, it can not be used for valid comparison purposes.

The moisture adjusted yield is expressed as kg of 37% moisture cheese per 100 kg milk. It is a theoretical calculation of the weight of the cheese that would have been obtained if the moisture content in cheese was 37%. The value of 37% is the desired moisture content that is set for optimum quality of Cheddar cheese. This expression adjusts all the cheeses on the same moisture basis and thus gives a better indication of cheese yield than actual cheese yield. On the average, 37% moisture adjusted yield for Cheddar cheese would be in the range of 9.05 to 9.75 kg per 100 kg of 3.5% fat milk (Kosikowski, 1977). Barbano and Sherbon (1984) reported that the yearly average moisture adjusted yield was 9.24 kg per 100 kg milk in New York. Ng-Kwai-Hang *et al.* (1987b) found that the annual average moisture adjusted yield was 9.42 kg per 100 kg milk in 13 Cheddar cheese plants distributed across the province of Quebec.

2. 4. 2. Formula of yield prediction

In 1910, Van Slyke and Publow first presented a yield formula. From that time, many formulas have been developed. As the prediction of cheese yield, the formulas can be used as a tool for cheesemaking quality control. In a recent review (Emmons *et al.*, 1990), several different formulas were compared and it was concluded that Van Slyke's formula, which is expressed as Y= {[(0.93 x Fat) + (Casein-0.1)] x 1.09}/(1-moisture), is still very useful in optimising Cheddar cheese manufacturing practices. Lou and Ng-Kwai-Hang (1992a) also had similar conclusion.

Van Slyke assumed in 1949 that cheese producer should be able to recover 93% of milk fat and 99% of casein into cheese. The 1.09 factor takes into account the minerals in milk. The desired moisture is 37% for cheddar cheese. The formula could also be rearranged as: Y = {[(0.93 x Fat) + (0.96 x Casein)]x1.09}/(1-Moisture). This 4% is approximately equal to the glycomacropeptide lost by the action of chymosin. It is one of most widely adopted formula for expression of cheese yield. The above formula could be further modified as Y = (1.609 x Fat) + (1.661 x Casein) while the moisture is 37%.

2. 5. Factors influencing cheese yield

A number of factors have been found to affect cheese yields. The major factors that have been intensively investigated are milk composition and cheese making practice. Factors such as genetics and environmental factors which affect milk composition will consequently influence cheese yield,

2. 5. 1. Cheese making techniques

Although there are various types of cheesemaking, the basic principles are similar. First, milk from any species of mammal could be used as major raw ingredient. After the adding of bacterial culture or starter and coagulant, a soft, homogeneous curd is formed by rennet coagulation. Following cutting the cheese curd into small cubes, the curd is cooked in the whey and the whey is then removed from curd cubes which acquire more lactic acid. The following step which is specific for cheddar cheese is called "Cheddaring". It is a step of piling and repiling of the curd blocks for a prolong period. For the present project, cheese slabs were piled every 20 min for two hours. At optimum acidity, after removal of the whey, the curd blocks are cut into small cubes with size of 0.8 cm to 1.0 cm. A calculated amount of salt (about 1.5% of curd weight) is added uniformly for transforming insoluble curd and to flavour the cheese. The salted cubes are then put into the cheese mould and pressed to form a compact young cheese.

According to Moxley and Ng-Kwai-Hang (1984), cheese making procedure is one of the important factor which influenced both cheese yield and cheese composition. Barbano and Sherbon (1984) also confirmed that the differences of cheese yield were attributed to variation in the steps of cheese manufacturing.

2. 5. 2. Effects of heat treatment

For the past decades, more and more attention have focused on the heat treatment of milk for cheese-making. Heating of milk is not only for the purpose of hygiene, but also for transferring of more content of milk into cheese. Compared with other milk proteins, caseins are more thermostable. In contrast, ß-LG and other whey proteins are susceptible and can be easily denatured by heat treatment of milk. Furthermore, heat treatment will induce the interaction of milk protein and then produce many beneficial results as well as some adverse effects and some effects which are still unknown so far. Imafidon *et al.*, (1993) reported that heat treatment impaired rennet coagulation properties. Other researchers reported that heat treatment reduced curd syneresis (McLean *et al.*, 1989) and incorporated whey protein in cheese (Banks *et al.*, 1994; Jensen and Stapelfeldt, 1994). The interaction of κ-CN and ß-LG involves the formation of a specific disulphide-linked complex. When temperature is above 65^{0}C, whey protein are denatured thus exposing the sulfhydryl groups and non-polar residues. Thereafter, the denatured whey proteins will interact with casein micelles.

2. 5. 3. <u>Effect of calcium chloride</u>

It is commonly accepted that the addition of calcium chloride increases cheese yield by the incorporation of colloidal phosphate in curd. Walstra *et al.* (1987) reported that the adding of about 1 mM $CaCl_2$ in milk to enhance the clotting of milk would result in an increase of about 30 g of cheese from 100 kg milk. There is a negative correlation between calcium in curd and moisture retention. In general, more calcium in the curd will lead to lower moisture in the cheese (Keller *et al.*, 1974).

2. 5. 4. <u>Effect of starter culture</u>

The use of starter cultures which contain mainly proteinase-positive strains, inevitably results in the loss of casein to some degree during both the stage of preparation of bulk starter and cheesemaking. The extent of the loss is still not well defined up to now. Theoretically, use of proteinase-negative starter strains is expected to result in less loss of casein. This was demonstrated by Richardson (1985) in cottage cheese making. It has been found that exclusive use of proteinase-negative strains always results in highest cheese yield as well as the adverse effect of loss of typical flavour in long maturing cheese varieties.

2. 5. 5. <u>Effect of initial pH</u>

Many reports established that milk pH is closely related to cheese yield and properties. Kowalchyk *et al.* (1977) reported that the decrease of milk pH will accelerate micelle aggregation rate and result in shorter rennet clotting time. Okigbo *et al.* (1985a) reported that milk pH value was positively correlated with rennet clotting time (RCT) and negatively correlated with curd firmness. This may be due to increased accessibility of κ-CN and to hydrolysis at low pH (Chaplin and Green, 1981; Storry and Ford, 1983). Marziali and Ng-Kwai-Hang (1986a) reported that milk pH is negatively correlated with cheese yield. In contrast, the opposite results were reported by Grandison *et al.* (1984b) who also found negative correlation of milk pH with RCT. Okigbo *et al.* (1985c) proposed that pre-adjustment of milk pH to 6.3-6.4 was necessary, especially when milk with poor coagulating properties was used for cheesemaking.

2. 5. 6. Effect of milk-clotting enzymes

There are several conflicting reports which relate the effect of different commercial coagulants with cheese yield. Several studies demonstrated the lack of significant difference of cheese yield when enzymes from Mucor species and *Endothia parasitica* were compared with rennet extract or blends of rennet or porcine pepsin (Olson, 1977). On the other hand, Emmons *et al.* (1976) found that calf rennet was associated with slightly lower fat and protein levels in the whey and slightly higher yields when compared to the use of bovine pepsin. A detailed study by Olson (1982) also suggested that calf rennet was a better coagulant in terms of maximising cheese yield.

2. 5. 7. Effect of initial setting temperature

Usually the starting temperature employed in coagulation of the milk is between 22 and 35°C. For Cheddar cheesemaking, temperature is always set at 30-31°C (Wong, 1974). Different initial setting temperature influences the rate of the milk coagulation. Rennet clotting time decreased with increasing temperature, but low temperature still allows proteolysis of κ-CN without coagulation. Dalgleish (1982a, 1982b) reported that because changes in temperature influenced secondary phase of enzymatic coagulation, the aggregation rate will increase by as much as 5-fold when temperature is changed from 31 to 40°C. It was also reported that reduction of coagulum strength was associated with high temperature (Storry and Ford, 1983).

2. 5. 8. Effect of cutting time

Olson (1977) suggested that cutting a curd prior to the time for optimum firmness will cause disruption of the fragile curd during cutting and subsequent stirring. Inversely, cutting a coagulum which is too firm will also result in curd breakage. Ng-Kwai-Hang *et al.* (1989) found that the 37% moisture adjusted yield was correlated with the curd firmness (A30). The shorter clotting time was related with a faster rate of firming, and a harder curd resulted in a higher cheese yield. Similarly, A30 was found to be negatively correlated with losses of fat and total solids in the whey with coefficients of -0.31 and -0.23 respectively. Therefore, cutting firm curd when its formation is more complete will result in less fat and protein losses in the whey. However, it was reported (Banks and Muir, 1984) that higher retention of milk components in the cheese may not always be favourable since the increased curd firmness is usually associated with the corresponding loss of elasticity and thus leads to difficulty in further processing of the cheese.

2. 5. 9. Effect of curd stirring and cooking

It is reported that the moisture of cheese can be controlled by adjusting the temperature, the speed of stirring and the size of the cheese curd being cut. Banks (1988) found that extending the stirring time from 60 to 120 min resulted in reduced moisture in cheese. Banks and Muir (1984) showed that an increase in the speed of stirring did not affect the cheese yield or the fat and the casein recoveries in the cheese. It seems that variation in the curd stirring and cooking did not affect yield of cheese as much as previously expected.

2. 5. 10. Effect of Cheddaring and pressing

The temperature plays a critical role during "Cheddaring". It is known that high temperature and low milk pH will accelerate this step but part of the whey will be trapped into the curd and therefore gives a higher actual cheese yield with higher moisture content (Belanger, 1975). Another research showed that longer Cheddaring time could overcome the problem for cheese which was made from preheated milk at 97^{0}C for 15 s. (Marshall, 1986). High temperatures during the pressing period may result in reduced yield, caused by excessive losses of fat (Belanger, 1975).

2. 5. 11. Effect of mastitis

Mastitis is a general term that refers to as an inflammation the mammary gland caused by microbial infection. When an infection occurs, cellular damage at the site of the infection initiates chemical signals that attract white blood cells to the area of the tissue infection. Some of these white blood cells are transferred into the milk and therefore the somatic cell count (SCC) of milk increases during mastitis. Increased losses of both fat and casein into whey occur with increasing milk SCC (Ali *et al.*, 1980b; Grandison and Ford, 1986; Munro *et al.*, 1984; Ng-Kwai-Hang, 1984). In addition, milk somatic cells contain antibacterial compounds that can inhibit starter culture growth during cheesemaking. Politis and Ng-Kwai-Hang (1988a) reported that high SCC in milk due to mastitis was associated with longer RCT, softer cheese curd and excess losses of fat and protein in the whey. Less desirable coagulating properties resulted in lower cheese yield and poor cheese quality. Barbano *et al.* (1987) reported that fat and protein recoveries in cheese decrease with increasing level of SCC in milk. Because high SCC milk is associated with low casein number and high proportion of hydrolysed casein, especially β-casein which is converted to γ-casein and lost in the whey, cheese yield would decrease (Ng-Kwai-Hang, 1990). Rasmussen *et al.* (1987) also reported that cheese made from high SCC milk contains higher moisture than those made from low SCC milk.

2. 5. 12. Effects of milk composition

Milk compositions, especially casein and fat in milk, are the major factors in determining cheese yield, cheese composition, and coagulating properties (Gilles and Lawrence, 1985; Ng-Kwai-Hang *et al.*, 1987). According to Van Slyke's formula, for a given fat content of milk, one can expect an increase in cheese yield when the casein content of milk increases. Likewise, for a given casein content of milk, one should expect an increase in cheese yield with increasing fat content of milk. Although there are several predictors of cheese yield, Van Slyke's formula is still widely accepted as had been demonstrated by many studies (Barbano and Sherbon, 1984; Moxley and Ng-Kwai-

Hang, 1984; Gilles and Lawrence, 1985; Marziali and Ng-Kwai-Hang., 1986a; Lou and Ng-Kwai-Hang, 1992a).

It is known that aggregating of caseins form a cross-link net work structure that entrap most of the fat globules and some of whey solids during cheesemaking. Consequently, higher casein content in milk will help to entrap more fat and more whey solids and thus cheese yield will be increased. The influence of casein on cheese yield is also due to its association with water, colloidal salts and soluble solid in addition to the proportional transferring of casein and fat from milk to cheese. Lou and Ng-Kwai-Hang (1992a) reported that an increase of 1% of protein content in milk resulted in an increase of 1.8-2.04% moisture adjusted cheese yield with different levels of fat. Lou and Ng-Kwai-Hang (1992a) reported that an increase of 1% of fat in milk resulted in 1.23-1.37% increase in moisture adjusted yield in the different protein levels. Some previous studies had similar results (Barbano and Sherbon, 1984; Gilles and Lawrence, 1985; Marziali and Ng-Kwai-Hang, 1986a).

Cheese yield efficiency will slightly increase as well as the increase of protein content in milk. However, it will decrease as fat content increases. This is due to the gradual decrease in fat recovery in cheese linked with increasing fat content in milk. Since there is not enough casein in milk to associate with all of the fat, as a result, excess fat in milk will inevitably be lost in the whey and this caused lower fat recovery and lower efficiency.

Optimum cheese yield mainly depends on the optimum ratio of casein to fat. Ng-Kwai-Hang *et al.* (1987b) investigated 13 cheese factories in Quebec, and found that reduced cheese yields were mainly attributed to lower casein to fat ratio and poorer fat and casein recoveries in the cheese. Barbano and Sherbon (1984) had similar conclusion for their study in New York. Lou and Ng-Kwai-Hang (1992a) reported that higher yield efficiency was associated with higher protein to fat ratio or more precisely, casein to fat ratio. The efficiencies decrease as casein to fat ratio increase from < 0.6 to 0.73, and then gradually increase with an increase of ratio. The highest casein to fat ratio (>0.92) gives the best yield efficiency. However, it is reported that the casein to fat ratio in the range of

0.7 to 0.73 could still be considered in the optimal range for Cheddar cheese yield (Banks *et al.*, 1981,1987).

2. 5. 13. Effects of genetic variants of κ-CN and β-LG

Many researchers confirmed that B variant of both κ-CN and ß-LG are associated with higher cheese yields while A variant is related with lower cheese yield. It is possible that some effects may result directly from physical changes in the milk caused by the genetic variants and some may result indirectly from compositional differences influenced by genetic variants. Schaar *et al.* (1985) found that there was no relationship between κ-CN genotype and cheese yield when curd is cut at constant firmness, although milk with κ-CN B genotype has been confirmed to produce higher yield (9.42%) than A genotype (9.39%). Aleandri *et al.* (1990) found that Parmesan cheese yields based on total lactation were not associated with κ-CN, although cheese yield from κ-CN BB phenotype was 7.9% higher than that of AA phenotype. Marziali and Ng-Kwai-Hang (1986a) found that phenotypes for κ-CN do not influence 37% moisture adjusted yield. However, when casein contents of milk were adjusted, the adjusted yield is affected by κ-CN phenotype. Ng-Kwai-Hang (1994) reported that moisture adjusted yield for κ-casein AA and BB were 10.6g and 10.97g (per 100g of milk) respectively. Losi *et al.* (1979) and Politis and Ng-Kwai-Hang (1988b) also showed an association between κ-CN phenotype and moisture adjusted cheese yield. Vink *et al* (1993) and Tong *et al* (1994) selected individual types of milk of κ-CN AA or AB and ß-LG AA, AB or BB. Each type of milk was used to make cheese. The results showed that the 37% moisture adjusted yields were 1.5-2% higher for κ-CN AA/ß-LG BB (10.29%) than for κ-CN AA/ß-LG AB (8.45%) and κ-CN AA/ß-LG AA (8.03%). Moisture adjusted yields were significantly higher for κ-CN AB/ß-LG BB (10.5%) than for κ-CN AB/ß-LG AB (9.64%) and κ-CN AA/ß-LG AA (9.73%). Cheese yield efficiency for the κ-CN AB was significantly higher than that for κ-CN AA with the same ß-LG phynotype. Similar results were reported on Camembert type cheese as well (Rahali and Menard, 1991, 1992).

Several research groups have reported that higher cheese yield associated with the B gene for κ-CN was due to higher protein recovery in cheese. Kristiansen (1991a) observed that in Danbo cheese, effects of κ-CN and ß-LG on the protein recovery disappeared when corrections were made for the protein content of milk. Pabst *et al.* (1991) observed a 2.7% higher recovery for milk protein in Edam cheese manufactured from κ -CN BB compared with κ-CN AA. Van den Berg *et al.* (1992) also showed similar results in Gouda cheese. Marziali and Ng-Kwai-Hang (1986a) reported that there was no association between ß-LG phenotypes and Cheddar cheese yield when individual caseins and whey proteins were adjusted, but when milk casein was adjusted there was a definite association. The 37% moisture adjusted yields from ß-LG AA and BB were 10.46 g and 10.71 g, respectively (Ng-Kwai-Hang, 1994). Morini *et al.* (1982) and Politis and Ng-Kwai-Hang (1988b) also had similar reports. Schaar *et al.* (1985) found no difference between Svecia cheese yields and ß-lactoglobulin genotypes. Van den Berg *et al.* (1992) found the differences in casein number (ß-LG AA<AB<BB). In contrast, Aleandri *et al.* (1990) found that Parmesan cheese yield based on total lactation and constant fat content of milk was significantly related to ß-LG genotypes with ß-LG AA being best.

2. 6. Factors influencing cheese composition

The flavor and texture of Cheddar cheese are mainly determined by its chemical composition. In Canada, standard Cheddar cheese compositions are: maximum moisture 39%, minimum fat content 30%, moisture in non-fat substance 56% and fat in dry matter 49%.

Cheese composition is mainly influenced by milk composition, cheese making techniques and genetic variants of milk protein.

2. 6. 1. Effects of Cheesemaking technique

Cheese manufacturing technique is one major factor which influences cheese composition. Modifications of cheesemaking procedure could have profound influence on the final cheese composition.

Heat treatment of milk before cheesemaking has the potential of increasing cheese yield. However, this would induce the interaction between κ-CN and β-LG to form a complex, which causes physical entrapment of denatured whey proteins in the gel matrix and excess moisture of cheese (Wilson and Wheelock, 1972; Marshall, 1986).

Several researchers found that long term cooling and storage of milk at 3 ^{0}C to 4 ^{0}C could cause changes in cheesemaking. These changes resulted in weaker gels and excessive losses of protein and fat in the whey, subsequently leading to poor quality of cheese with lower protein and fat content and higher moisture. (Ali *et al.*, 1980a; Johnson *et al.*, 1983; Banks *et al.*, 1986).

It is important in cheese making to cut the curd at the correct firmness so that whey drains properly while loss of milk solids in minimized (Green, 1977). It is better to cut the curd when it is ready, but not too hard (Dumais *et al.*, 1985). Therefore, monitoring curd firmness during cheesemaking offers the potential for reducing cheese protein and fat losses by cutting at consistent curd firmness to optimize cheese manufacturing (Olson, 1982).

2. 6. 2. <u>Effects of milk composition</u>

Marziali and Ng-Kwai-Hang reported (1986c) an increase of 1% milk casein was accompanied by changes in cheese fat and protein of -1.55% and 0.79%, respectively. Politis and Ng-Kwai-Hang (1988a) reported that cheese fat decreased by 3.19% but cheese protein increased by 2.05% for every unit percentage increase in milk protein. Ng-Kwai-Hang (1988) observed that for every unit percentage increase in milk casein , both fat and protein in cheese will have an increase of 0.08% and 0.58%, respectively. A recent research conducted by Lou and Ng-Kwai-Hang (1992b) showed that higher protein level in milk was associated with higher protein and lower fat in cheese as well as lower total solid in cheese. Regression analysis showed that cheese protein increased by 2.35%, while cheese fat decreased by 6.14% for every unit increase in percentage in milk protein.

Moisture of cheddar cheese is also influenced to some extent by the casein to fat ratio of milk (Kosikowski, 1977). Acceptable cheese milk should have casein to fat ratio of 0.68. Raw milk with higher casein to fat ratio would produce a cheese with higher protein content and such a kind of cheese appears to be drier than what its moisture content will indicate. On the other hand, a lower ratio would produce a kind of cheese that is very soft. Some researchers (Banks *et al.*, 1984; Lou and Ng-Kwai-Hang, 1992b) indicated that milk with a protein to fat ratio greater than 0.95 would produce cheese containing less than 50% of fat in dry matter (FDM). In addition, milk with higher ratio will produce a cheese with higher moisture since protein in milk is positively correlated with cheese moisture. Furthermore, if milk with a protein to fat ratio much lower than 0.95 is used to make cheese, more fat would be lost in the whey since not enough protein is available to combine with the all fat during cheese curd formation. As a result, extra fat globules will get lost in the whey. Therefore, they suggested that the optimum protein to fat ratio in milk was 0.9 in order to get good quality cheese and maximum profits.

2. 6. 3. <u>Effects of genetic variants of milk protein</u>

Marziali and Ng-Kwai-Hang (1986c) found that phenotypes for κ-CN were not associated with total solid, fat and protein in Cheddar cheese when individual caseins and whey proteins were held constant. But when adjustments were made for milk casein, phenotypes for κ-CN affected cheese composition (Ng-Kwai-Hang, 1994). In contrast, Politis and Ng-Kwai-Hang (1988a) reported no relationship between κ-CN phenotypes and Cheddar cheese composition. Schaar *et al.* (1985) reported no relationship between κ-CN genotypes and fat, protein and moisture in young cheese. Van den Berg *et al.* (1992) made Gouda cheese with the addition of $CaCl_2$ and found that moisture content of the cheese was lower for κ-CN BB (44.5%) than for κ-CN AA (45.7%).

Marziali and Ng-Kwai-Hang (1986c) observed that ß-LG phenotypes influenced total solids and fat in cheese even after adjustments were made for individual casein and whey protein. ß-LG BB milk produced cheese with 37.51% moisture, 32.67% fat and 24.82%

protein and ß-LG AA milk produced cheese with 37.51%, 33.38% and 23.61% for the same three components respectively. Schaar *et al.* (1985) reported that ß-LG genotype affected protein but not fat and moisture contents in young Svecia cheese. However, Politis *et al.* (1988a) observed no association between ß-LG phenotypes and Cheddar cheese composition. Van de berg *et al.* (1992) also reported no relationship between moisture content of cheese and ß-LG phenotypes in Gouda cheese manufactured with addition of $CaCl_2$. Tong *et al.* (1994) selected 3 different kinds of milk containing ß-LG AA, AB and BB with the same phenotype AB for κ-CN. It was found that there were no significant differences in total solids, fat and protein contents among phenotypes for ß-LG. However, ß-LG BB cheese had the highest fat content and the lowest protein and moisture content among the three types.

It was found that the curd firmness influences the cheese yield and quality of cheese (Bynum and Olson, 1982; Ng-Kwai-Hang *et al.*, 1989). The cheese firmness is affected by the total caseins and the relative proportion of different caseins (Okigbo *et al*, 1985b; Marziali and Ng-Kwai-Hang, 1986b; Politis and Ng-Kwai-Hang, 1988c). Politis and Ng-Kwai-Hang (1988c) found that the rate of curd firming (K20) decreased by 5.42, 4.87, 5.16 and 6.59 min for every unit percentage increase in α_{s1}-casein, α_{s2}-casein, ß-casein and κ-casein respectively. The curd firmness (A30) increased by 12.92, 12.72, 13 and 18 mm for every unit percentage increase in the same four components respectively. Similar conclusion was confirmed by Marziali and Ng-Kwai-Hang (1986a).

Marshall (1982) found that increasing the fat content of milk resulted in slow syneresis of curd. On the other hand, several other studies (Storry *et al.*, 1983; Marziali and Ng-Kwai-Hang, 1986b; Politis and Ng-Kwai-Hang, 1988c) reported that fat does not influence the curd firmness. Storry *et al.*(1993), however, reported that coagulum strength is unaffected by casein to fat ratio.

2. 7. Effects of genetic variant κ-CN and ß-LG on cheesemaking

It was mentioned previously that fat and casein contents in milk are the determining factors for determination of cheese yield. However, Marziali and Ng-Kwai-Hang

(1986a) reported that genetic variants of certain milk proteins were also associated with Cheddar cheese yield, cheese composition and coagulating properties. Their work showed that the effect of such genetic variants are over and above the effects of milk composition. The genetic variants of milk proteins have been demonstrated to have profound influence on cheesemaking behaviour of milk (Imafidon *et al.*, 1991a; Ng-Kwai-Hang and Grosclaude, 1992; Puhan and Jakob, 1994). Therefore, the detection of genetic variants of milk protein offered new explanations for the variation from individual cows.

2.7.1. Coagulating properties of milk

Coagulating properties of milk are usually used for evaluating cheesemaking properties such as cheese yield, cheese composition (Aleandri., 1989). It was found that coagulating properties of milk are influenced by casein and mineral composition of milk (Storry *et al.*, 1983; Grandison *et al.*, 1984a; Okigbo *et al.*, 1985a; 1985b; 1985c).

The Formagraph is often used to measure the coagulating properties of milk which are expressed by three parameters: (1) rennet clotting time (RCT), (2) rate of curd firming (K20) and (3) curd firmness at cutting (A30). For detailed descriptions of the Formagraph see "Materials and Methods" section.

2.7.2. Effects of Environmental factors

Mariani *et al.*, (1982) worked on 9 Italian Friesian herds and found a negative relationship between curd firmness and coagulation time. O'Keeffe et al., (1982) found an increased RCT while curd strength and syneresis were reduced during late lactation period. A study by Auriol *et al.*, (1961) showed that renneting time of milk from heifer are slightly shorter than those of cows in their second of third lactation. These results make sense as it has been confirmed that the effects of age on various milk components affect clotting time.

Schaar (1984) found that renneting properties were strongly related to lactation number. RCT was slightly longer in the first two lactations than in later ones. He also observed stronger curd from cows in later lactations. These results were different with previous report (Auriol *et al.*, 1961)

Ohashi (1982) showed definite seasonal effects on clotting time of milk and rigidity of the curd associated with changes in milk composition. During the period when total solids, protein, fat and lactose were lower, they observed the lowest values for physical properties of the curd.

Ali et al., (1980b) showed that higher SCC was associated with prolonged RCT, increased fat loss and softer curd. A marked increase in bovine serum albumin (BSA) resulted in lower cheese yield and poor quality cheese. These results are consistent with previous work by Anderson and Andrews (1977) who also found an increased content of para-κ-casein and attributed their results to high levels of proteolytic activity in such milks. These findings were reconfirmed by Andrews (1983).

Increased losses of both fat and casein into whey occur with increasing milk SCC (Politis and Ng-Kwai-Hang, 1988a). In addition, milk somatic cells contain antibacterial compounds that can inhibit starter culture growth during cheesemaking. Politis and Ng-Kwai-Hang (1988a) reported that high SCC in milk due to mastitis was associated with longer RCT, softer cheese curd and excess losses of fat and protein in the whey. Less desirable coagulating properties resulted in lower cheese yield and poor cheese quality.

Studies on high SCC would lead us to believe that infected milk would have a longer RCT and would give a softer coagulum as reported by Mariani *et al.*, (1981) who found that high SCC were associated with higher frequency of poor clotting time. They also reported that slow coagulation resulted in weak curd obtained during that period.

2. 7. 3. Effects of genetic variants of κ-CN and ß-LG

The effects of genetic variants of κ-CN on coagulating properties are more consistent than those of genetic variants of ß-LG, presumably because ß-LG itself has no direct role in curd formation. Several possible explanations have been available for the effect of genetic variants on coagulating properties. These effects could be due to the variation in size of casein micelles which is influenced by genetic variants through the relative proportions of the casein fractions, such as the degree of glycosylation of κ-CN (Robitaille *et al.*, 1993) or salt content, i.e. calcium, citrate and phosphate contents (Puhan and Jakob, 1994). Aaltonen and Antila (1988) reported that milk from cows with κ-CN AA phenotype had a longer renneting time and gave a softer curd than milk from cows with κ-CN AB or BB. Some other studies had similar results (Davoli *et al.*, 1992; Kristiansen, 1991b; Losi and Mariani, 1985; Rahali and Menard, 1992; Rampilli *et al.* 1989; van den Berg *et al.* 1992). Jakob and Puhan (1987) reported that milk with κ-CN BB showed excellent rennetability and gave very firm curds, whereas milk with κ-CN AA milks have poorest rennetability, with width of the fork measured 10 min after RCT value of less than 18 mm and with RCT values exceeding 28 min. While pH was lower or $CaCl_2$ was added, RCT values were improved but differences between κ-CN phenotypes still remained. The κ-CN AA milk had poorest rennetability and was unsuitable for cheesemaking. Pagnacco and Caroli (1987) evaluated the effects of α_{s1}-CN, ß-CN, κ-CN and ß-LG genetic variants on RCT, K20 and A30 with adjustment for herd, pH and protein content of milk. The κ-CN BB phenotype showed the best coagulation parameters with AB being intermediate. Values of RCT, K20 and A30 for κ-CN AA phenotype were 12.52 min, 3.23 min and 36 mm and comparable values for in κ-CN BB phenotypes were 9.43, 2.14 and 42.7, respectively. Macheboef *et al.* (1993) reported that κ-CN genetic variants RCT, K20 and A30 after correction were made for year, breed, group and pH. Values of RCT (17.3, 15.8 and 14.2 min), K20 (14.3, 8.9 and 7.4 min) and A30 (39.3, 47 and 51 mm) for phenotypes AA, AB and BB were reported respectively. Politis and Ng-Kwai-Hang (1988c) reported an association among κ-CN phenotypes with K20 and A30 but no relationship was found between κ-CN phenotype and RCT. Schaar (1984) investigated the effects of genetic variants of κ-CN and lactation number on RCT, K20 and A30 with adjustments for period of sampling, stage of lactation, pH and casein content. The κ-CN genetic variants genotypes were not associated with RCT but with A30 and K20. Values in RCT, A30 and K20 of κ-CN B type were 11.6 min, 31.3 mm and 6.8 min and similar results in A type were 12.6, 23.1

and 11, respectively. The effect of κ-CN genetic variants on RCT could be eliminated by reducing pH and adding $CaCl_2$ to the milk samples, but the effect on A30 and K20 were not significantly different by this treatment. In contrast, Marziali and Ng-Kwai-Hang (1986b) found that κ-CN genetic variants did not affect RCT, K20 and A30 after adjustment were made for individual caseins and whey proteins. Values for RCT (7.24, 6.55 and 6.00 min), K20 (9.13, 9.00 and 8.37 min) and A30 (29.53, 32.5 and 35.38 mm) for phenotypes AA, AB and BB were reported for κ-CN, respectively.

Marziali and Ng-Kwai-Hang (1986b) reported that RCT and K20 were shorter in ß-LG AA phenotype (3.9 and 7.55 min) than for phenotype ß-LG AB (6.23 and 8.85 min) and BB (9.75 and 10.09 min) while A30 was significantly greater in phenotype ß-LG AA (36.3 mm) than in phenotypes AB (33.13 mm) and BB (27.98 mm). It was concluded that the association between ß-LG phenotypes and coagulating properties may be direct because adjustment were made for fat, casein and citric acid contents in milk. These results were in agreement with other studies (Kristiansen, 1991b; Rampilli *et al.*, 1989; van den Berg *et al.*, 1992).

So far it is confirmed that genetic polymorphism of milk protein is an important factor influencing various aspects of physical and chemical properties of milk and cheesemaking. The aim of the current research undertaken was to examine the effects of different phenotypes of κ-CN/β-LG on composition, cheesemaking properties, and formagraphic properties of milk from four different breeds: Ayrshire, Brown Swiss, Jersey and Canadienne.

3. MATERIALS AND METHODS

3.1. Selection of milk samples

In a previous study by Kim (1995), 3610 Ayrshire, 1034 Brown Swiss, 611 Canadienne and 1711 Jersey cows were phenotyped for the genetic variants of κ-CN and β-LG. There were 3 phenotypes: AA, AB and BB for each of the two milk proteins in the four breeds of cows. From this population it was possible to identify specific cows to provide milk samples of nine different κ-CN/β-LG phenotype combinations for the purpose of cheesemaking experiments involved in this project.

3.2. Overall experimental protocol

On a monthly basis, during the period of May 1994 to July, 1995, approximately 4 L of milk were collected from the above identified cows during the morning milking. All the milk samples were kept at 4 ^{0}C and transported to the research laboratory pending further analysis. The κ-CN/β-LG phenotype of all milk samples were analyzed by polyacrylamide gel electrophoresis methods. A subsample of the milk was taken for composition analysis. Another subsample was used for the determination of coagulating properties. A fourth subsample (2000 g) was used for cheesemaking experiment where cheese yield, cheese and whey compositions could be assessed. For each batch of cheese made from milk of selected cows from the four breeds, a bulk tank sample from the Macdonald Campus dairy herd consisting of Holstein cows, was used concurrently for the same procedure. Inclusion of the latter sample was to ensure that there were minimum day to day variation in cheesemaking procedures and in analysis of milk, cheese and whey.

3. 3. Phenotyping for κ-CN and β-LG

Polyacrylamide gel electrophoresis was used to determine the genetic variants of κ-CN and β-LG for individual milk sample from Ayrshire, Brown Swiss, Canadienne and Jersey which were used for cheesemaking. All electrophoresis were carried out in a vertical mini-gel system (BioRad Corp., Richmond, CA). The gel slabs measured 73 mm high x 102 mm wide x 1 mm thick. Whole casein was isolated from skimmed milk by adjusting the pH to 4.6 and centrifugation at 3000 x g. The precipitated whole casein was used for the phenotyping of κ-CN by the method described by Ng-Kwai-Hang *et al.* (1984). The resulting supernatant was used for phenotyping the β-LG according to the method of Ng-Kwai-Hang and Kroeker (1984). An aliquot of 4 μl sample volume of either whole casein solution or the above supernatant containing whey protein was applied in the well of the polyacryamide gel. After electrophoresis, the gel was fixed and stained in staining solution containing 40% methanol, 7% acetic acid and 0.1% coomassie brilliant blue R-250. After destaining the gel in a solution of 7% acetic acid, the gel was visualized to determine the phenotypes of κ-CN and β-LG.

For the separation of the phenotypes of κ-CN the gel contained 8.0 % polyacrylamide with 5% bisacrylamide, 4 M urea, 0.19 M Tris, 0.01 M EDTA, 0.037 M boric acid, 0.04 % dimethylaminopropionitrile, 0.04% β-mercaptoethanol and 0.25% ammonium persulfate. The same molarity of Tris, EDTA and boric acid as in the gel was used for the electrode buffer (pH 8.3). The A variant of κ-CN has a higher electrophoretic mobility than the B variant. The separation of β-LG variants was achieved in 12% polyacrylamide gel in 0.375 M Tris buffer (pH 8.9) containing 0.002% TEMED. An initial voltage of 25 V for 20 min was followed by 100 V for 90 min. The electrode buffer contained 0.005 M Tris and 0.04 M glycine. The A variant of β-LG moved faster than the B variant toward the anode.

3.4. Laboratory cheesemaking protocol

The method originally developed by Agropur was modified in our research project for the purpose of laboratory-scale cheesemaking. For the setup in our laboratory, a batch of 12 individual milk samples could be used to make cheese at the same time. A 2000 g milk sample was weighed into a plastic square container with the dimension of 20 cm x 20 cm x 7 cm and maintained in water bath at 30 °C-32 °C for 30 min. At time 0 min (T - 0), the pH was measured and 40 g of 2 % starter culture was added to each sample. The starter culture was prepared one day in advance from a stock of *Lactococcus cremoris* which was kindly provided by Agropur Research Laboratory. After 60 min incubation (T- 60), the pH was measured and 10 ml of freshly prepared 0.025 % diluted calf rennet solution was added. Milk samples were then thoroughly and gently mixed in order to initiate coagulation. After 30 min (T-90), the coagulum formed was cut with a set of horizontal and vertical wires to obtain cubes with side measuring approximately 5 mm and the pH of

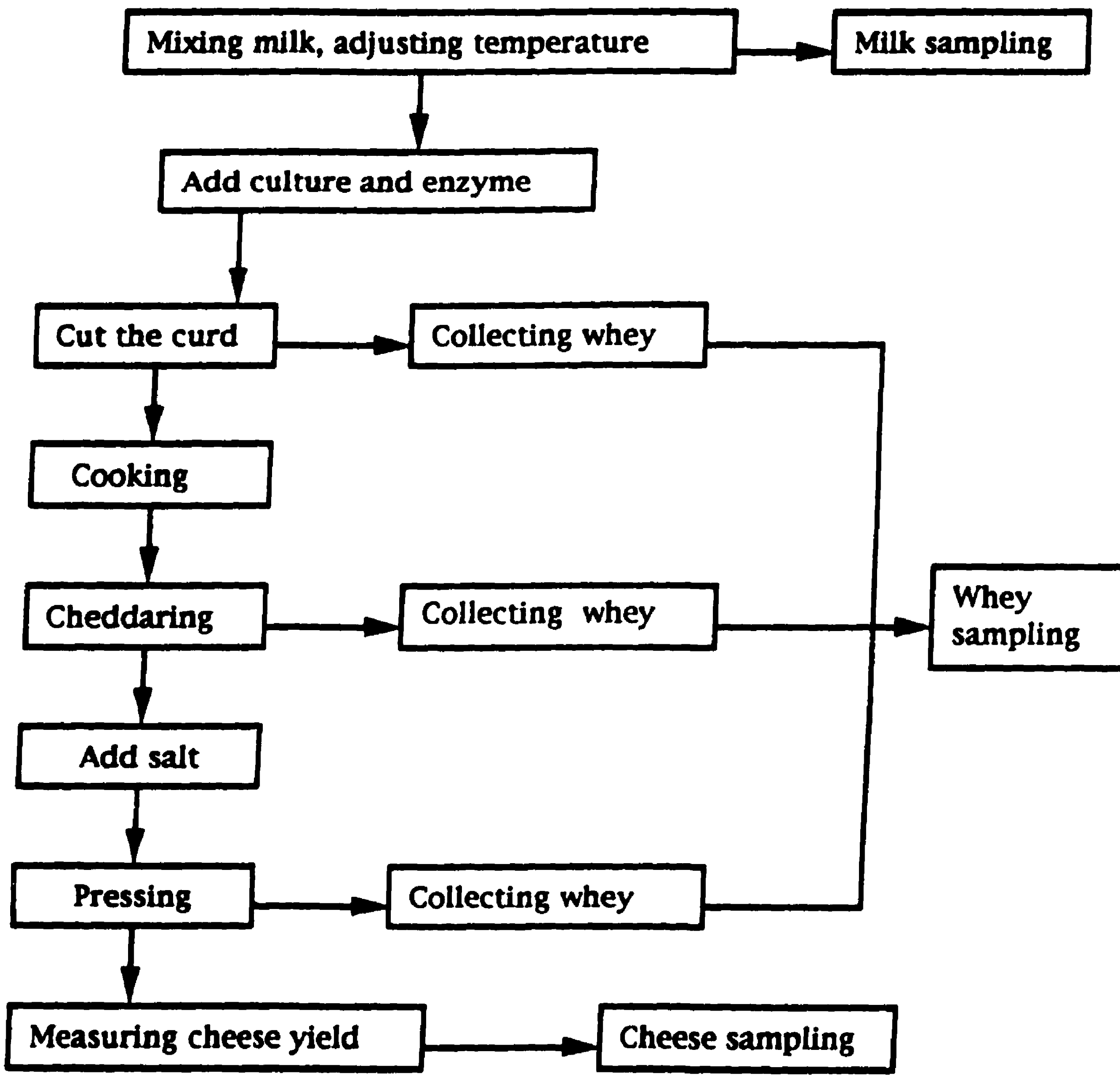

Figure 2. Flow chart of Laboratory-scale cheese making procedure. Milk, whey and cheese subsampling are shown in the procedure.

whey was measured. The cubes of coagulum were carefully stirred to prevent them from sticking back together and to allow the whey to move out of the curd. After 15 min (T-105), the temperature was increased from 30 ^{0}C to 39 ^{0}C within 30 min (T-135) and maintained at 38 ^{0}C for the next 30 min. This step is known as "cooking" the curd in the whey. After 30 min (T-165), approximately half of the volume of the whey had been removed from the containers and the pH was again measured. Then, samples were maintained at 38 ^{0}C for next 40 min. After remaining whey were removed (T-205), the pH of the curd was measured and the combined whey sample was weighed and stored in 4 ^{0}C refrigerator until the following day for sampling and compositional analysis. The curd was cut into four pieces and piled on one side in the container. The following Cheddaring step lasted for two hours. During which time, slabs were piled and repiled every 20 min and in each time the whey was removed. The curd was then cut into small cubes of approximately 1 cm and salt was added to a concentration of 1.5% of the curd weight. The salted curd was transferred into a standard plastic cheese mold and a 2 kg weight was placed on the top of the curd for 16 h. On the following day, cheese samples were weighed, grated, sealed in plastic bags and stored at -15 ^{0}C pending chemical analysis. Figure 2. shows the flow chart of laboratory Cheddar cheese making protocol. The pH value was measured at the end of each step in order to ascertain that the cheesemaking was proceeding according to specification.

3. 5. Chemical Analysis of milk, cheese and whey

3. 5. 1. Analyses of milk samples

The second day after cheesemaking, subsamples of all milk samples were subjected to the following analyses in duplicate. Total solids were determined by drying 5 g of milk sample overnight in an oven for at 100 ^{0}C to constant weight according to the method of AOAC (1980). Total protein, casein and whey protein were analyzed by the amido black dye-binding procedure outlined by Foss Electric (1973) and modified by Ng-Kwai-Hang and Hayes (1982). Fat was determined by the method of Rose-Gottlieb after extracting the sample with an alcohol-ether mixture (AOAC, 1980). Lactose was analyzed by the an infrared instrumental method using a Multispec II (Multispec, Inc. Wheldrake, York, England) after calibration with standards predetermined by the polarimetric method of AOAC (1980). Somatic cell counts of milk were determined by Fossomatic Cell Counter (A/S N. Foss Electric, Hillerod, Denmark.)

3. 5. 2. Analyses of cheese sample

All cheese samples were analyzed in duplicate for total solids, fat, protein and salt content. Total solids were determined by weighing 1 g cheese samples into aluminum dishes, drying for 24 h in an oven at 100^{0}C, followed by weighing according to AOAC (1980). Fat was analyzed by the Rose-Gottlieb method by using 1 g cheese samples weighed into 50 ml beakers containing 10 ml of 10% ammonium hydroxide. After thoroughly wetting of samples, 8 ml concentrated HCl were added and the mixture was boiled to dissolve the cheese samples. After cooling, the solutions were transferred into Mojonnier flasks and fat in the solutions was extracted with an alcohol-ether mixture

(AOAC, 1980) followed by drying and weighing. Protein was analyzed by the micro-Kjeldahl method using selenium as catalyst to digest cheese samples (0.3 g). Ammonia which was distilled from digested samples was titrated with 0.05 N HCl. Protein in cheese was calculated by multiplying total nitrogen by 6.38. Salt content of cheese was determined by the Volhard procedure as described in the standard methods for the examination of dairy products (American Public Health Association, 1978).

3. 5. 3. Analyses of whey samples

All of the whey samples derived from cheesemaking were analyzed in duplicate for total solids, fat and protein. Total solids and fat were analyzed by the methods as used for milk total solids and fat described previously. Whey protein was determined by Kjeldahl method as described above for cheese protein determination.

3.6. Cheese yield expression

Cheese yields were expressed on three different ways:

A) Actual yield was determined as a percentage by dividing the weight of cheese by the weight of milk.

B) Adjusted yield was determined by mathematically adjusting the actual cheese yield to 37% moisture in cheese.

C) Theoretical yield was calculated with Van Slyke and Price cheese yield formula (1949) using 93% recovery of fat and 37% moisture in cheese. The factor of 1.09 in the formula assumes that the cheese contains 1.7% salt.

$$\text{Yield} = \frac{0.93\ \text{fat} + (\text{casein} - 0.1)}{100 - 37} \times 1.09$$

Where: Fat and casein in the formula represent the percentage contents of these components in the milk.

3. 7. Recovery of major milk components during cheesemaking

Total accountability of total solids, fat and protein during cheesemaking experiments were determined. As the weight of milk, cheese and whey and their composition in total solids, fat and protein were known, it was possible to calculate the recovery of these components for each batch of cheese made under laboratory conditions. The equations used were as follows:

$$\text{Recovery of T.S.(\%)} = \frac{(\text{cheese wt. x cheese T.S.}) + (\text{whey wt. x whey T.S.})}{\text{milk wt. x milk T.S.}}$$

$$\text{Recovery of F (\%)} = \frac{(\text{cheese wt. x cheese F}) + (\text{whey wt. x whey F})}{\text{milk wt. x milk F}}$$

$$\text{Recovery of P (\%)} = \frac{(\text{cheese wt. x cheese P}) + (\text{whey wt. x whey P})}{\text{milk wt. x milk P}}$$

Where: T. S. = total solids, F = fat, P = protein, wt = weight

Total solids, fat and protein in milk, cheese and whey used in above equations are percentages.

3. 8. Determination of coagulation properties of milk

In order to determine the coagulating properties of milk, milk samples were kept in 0 °C and sent to Le Centre de Controle et Recherche Agropur in Granby where coagulating parameters were determined by a Formagraph (Foss Electric, Hillerod, Denmark).

The Formagraph, as described by Foss and Co. (1980), consists of two modules: a service module serves to heat the sample in a cuvette, and a recorder module that has a ten-channel recording system. Each channel consists of a pendulum with a counterbalance damper and an adjacent optical system.

The first step for calibration is sample loading. 10 ml of sample that has been pre-adjusted to pH 6.5 was introduced into one of the 10 sample wells. Samples were placed on the heating plate of the service module and maintained at 37 ^{0}C. Then, 0.2 ml of 1.6% Hansen's adult bovine extract plus porcine pepsin solution were added and samples were stirred. Later the cuvette was transferred to the heating plate of the recorder module. The pendulum loops were introduced into each sample cuvette as show in Figure 3. While milk samples remain in liquid state, there is no movement of the pendulum. As the coagulation starts, the formation of a curd caused synchronous movement of pendulum and light flashed reflected from the mirrors are recorded at different position on the light-sensitive photographic paper.

The coagulating properties of milk samples are measured by three formagrahic parameters as shown in Figure 4: (1) Rennet clotting time (RCT) represents time (min) from enzyme adding until the beginning of formation of a curd. It is measured by the distance from the origin to the point where the baseline begins to increase in width. (2) Rate of firming (K20) which is indicated by the time (min) from the start of gel development until the distance between the two branches on the fork researches 20 mm; (3) Curd firmness at 30 min(A30) which is obtained by measuring the width (mm) of the graph 30 min after enzyme was added.

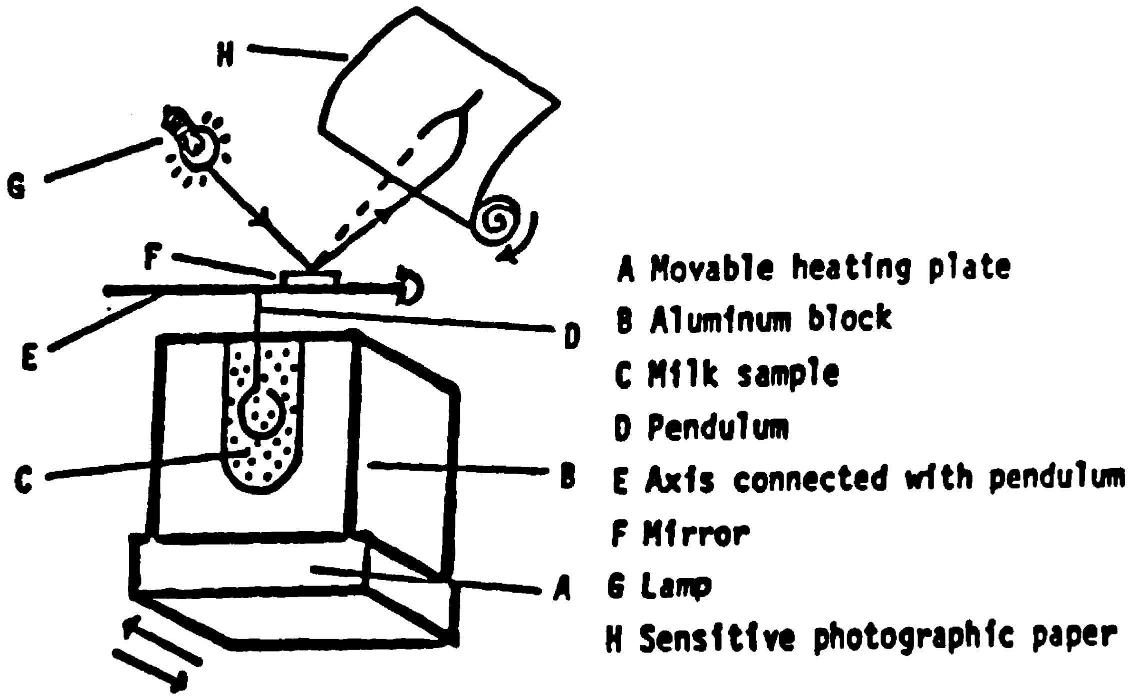

Figure 3. Diagrammatic representation of the principle of the formagraph

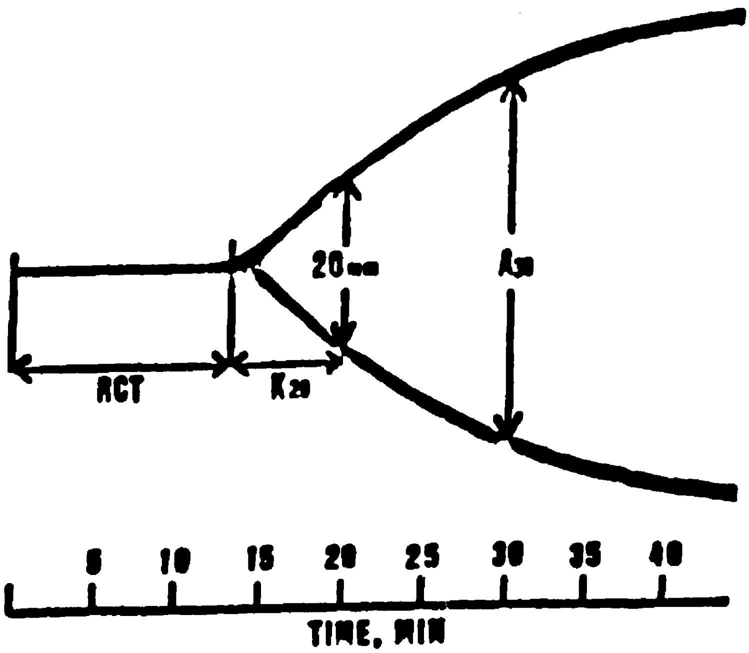

Figure 4. Formagraphic chart indicating the parameters of coagulating properties.

RCT=Rennet clotting time: interval (min) between enzyme addition and initiation of curd formation.

K20=Rate of curd firming: time (min) elapsed for the width of the fork to reach 20 mm.

A30=Curd firmness: width (mm) of the fork at 30 min after enzyme addition.

3.9. Statistical analysis:

Data collected for composition of milk, cheese and whey; yields of cheese expressed on different basis; coagulating properties of milk were analyzed separately for the four breeds of cattle (Ayrshire, Brown Swiss, Canadienne and Jersey). These procedures were followed because it is known that cheesemaking and coagulating properties of milk are breed dependent due to variation in milk composition (Kim, 1995). Analysis by least squares procedure (SAS, V 6.03) was performed to estimate the effect of κ-CN/β-LG phenotype combination on cheese yield, cheese and whey composition, and formagraphic parameters. The model used was:

$$\mathbf{Y_{ij}} = \mu + \boldsymbol{\kappa}\textbf{-CN / }\boldsymbol{\beta}\textbf{-LG}_i + \mathbf{b_1X_1} + \mathbf{b_2X_2} + \mathbf{e_{ij}}$$

Where:

$\mathbf{Y_{ij}}$ represents ijth observation of one of the following: actual yield, adjusted yield and cheese yield efficiency; total solids(%), fat(%), protein(%) and salt(%) in cheese; total solids(%), fat(%) and protein(%) in whey; RCT (min), K20 (min) and A30 (mm)

μ is the population mean,

κ-CN / β-LG$_i$ is the fixed effect of the ith milk type for κ-casein and ß-lactoglobulin,

$\mathbf{X_1}$ is fat (%) in the ith milk sample,

$\mathbf{b_1}$ is the regression coefficient of Y on X_1.

$\mathbf{X_2}$ is casein (%) in the ith milk sample,

$\mathbf{b_2}$ is the regression coefficient of Y on X_2.

$\mathbf{e_{ij}}$ is the random error NID ~ $(0, \sigma^2_e)$.

Significance levels were tested by both LSD and Duncan's test in order to verify the results.

4. RESULTS AND DISCUSSION

4. 1. Distribution of milk samples according to κ-CN / β-LG phenotypes

Table 1 shows the total number of milk samples used for cheesemaking in this project according to the phenotype combination of κ-CN/β-LG in the four different breeds. In addition, there were 45 bulk tank Holstein milk samples which were used in cheesemaking in order to ensure that the cheesemaking procedure was uniform throughout the project.

Not all nine different phenotype combinations of κ-CN/β-LG were found in each of the four breeds. These results show differences in frequency distributions of κ-CN/β-LG phenotypes in the four breeds. There were 115 milk samples for Ayrshire, in which AA/AB and AA/BB were most common phenotypes with 24 and 23 observations, respectively. In Brown Swiss, AB/AA was very abundant, 29 observations were found for this type. We did not find types AA/AA and AB/AB. Canadienne was the only breed in which observations for each of the nine different phenotypes were relatively evenly distributed and close to the average of 12 observation for each phenotype. BB/AB was the most common phenotype for Jersey milk which had 31 observations. In contrast, AA/AA and AA/AB were very rare. No observation was found for AA/AA and there was only one observation for AA/AB.

Previous work in our laboratory (Kim, 1995) investigated the population frequency distribution of both κ-CN and β-LG in the four breeds in Quebec. For κ-CN, the A

Table 1. Distribution of κ-CN/β-LG phenotype combination according to breeds

K-CN/ß-LG type	Breed			
	Ayrshire	Brown Swiss	Canadienne	Jersey
AA/AA	13	0	15	0
AA/AB	24	13	10	1
AA/BB	23	8	10	7
AB/AA	6	29	14	4
AB/AB	14	0	15	8
AB/BB	12	8	11	8
BB/AA	6	10	10	19
BB/AB	9	10	8	31
BB/BB	8	16	12	19
Total	115	94	105	97

variant was predominant among Ayrshire, Brown Swiss and Canadienne with a frequency distribution of 0.88, 0.70, 0.79, respectively, but was less frequent within Jersey with a frequency of 0.26. The frequency of variant B was high in Jersey (0.74). For β-LG, variant A and B were found at nearly equal frequencies of 0.44 v/s 0.56 in Jersey, 0.42 v/s 0.58 in Brown Swiss, and 0.43 v/s 0.58 in Canadienne. The B variant was dominant in Ayrshire with a frequency of 0.76.

Many researchers have reported that the occurrence and frequency distribution of genetic variants of milk protein vary among breeds (Aschaffenburg, 1968; Hoogendoorn *et al.*, 1969; Bech and Kristiansen, 1990; Ng-Kwai-Hang and Grosclaude, 1992). Changes of the occurrence and frequency distribution of genetic variants of milk are closely related to changes of milk composition of the breeds.

4. 2. <u>Composition of milk samples in five breeds irrespective of κ-CN / β-LG types</u>

Table 2 shows the unadjusted means and standard deviations of composition of milk which was used for cheesemaking without taking into consideration the κ-CN and β-LG phenotype combination. The means and standard deviations for total solids, fat, protein, casein, lactose, casein to protein ratio, casein to fat ratio, non-casein protein (NCP) and somatic cell count (SCC) for all the samples of the five breeds are summarized in Table 2. The composition of the Bulk tank Holstein milk was within the normal range and in agreements with the annual official result reported by PATLQ (1996). However, for the milk composition of the four breeds investigated, the results in Table 2 were different compared with PATLQ annual report for each of the four breeds. The main reason was the differences in frequency distribution of both κ-CN and β-LG for each breed.

Table 2. Overall means of milk composition (%) of different breeds

Component	Breed				
	Ayrshire	Brown Swiss	Canadienne	Jersey	Holstein
Total solids	$12.91^{c} \pm 1.03$	$12.69^{c} \pm 1.15$	$13.50^{b} \pm 1.31$	$14.57^{a} \pm 2.89$	$12.38^{c} \pm 0.48$
Fat	$3.97^{b} \pm 0.39$	$3.58^{c} \pm 0.65$	$4.40^{a} \pm 1.08$	$4.61^{a} \pm 1.14$	$3.75^{bc} \pm 0.50$
Protein	$3.61^{c} \pm 0.42$	$3.73^{bc} \pm 0.39$	$3.81^{b} \pm 0.49$	$4.00^{a} \pm 0.50$	$3.34^{d} \pm 0.41$
Casein	$2.93^{c} \pm 0.42$	$3.02^{bc} \pm 0.35$	$3.14^{b} \pm 0.51$	$3.35^{a} \pm 0.53$	$2.72^{d} \pm 0.46$
Non-Casein Protein (NCP)	$0.68^{ab} \pm 0.12$	$0.71^{a} \pm 0.16$	$0.68^{ab} \pm 0.13$	$0.65^{bc} \pm 0.14$	$0.62^{c} \pm 0.11$
Casein/Protein	$80.96^{b} \pm 3.74$	$81.07^{b} \pm 3.78$	$81.86^{b} \pm 3.91$	$83.49^{a} \pm 4.19$	$81.02^{b} \pm 4.41$
Protein/Fat	$93.58^{b} \pm 17.81$	$114.71^{a} \pm 42.08$	$91.38^{b} \pm 5.42$	$90.41^{b} \pm 21.04$	$93.06^{b} \pm 31.48$
Casein/Fat	$75.62^{b} \pm 14.81$	$92.78^{a} \pm 35.02$	$75.32^{b} \pm 18.11$	$75.12^{b} \pm 21.25$	$75.35^{b} \pm 17.48$
Lactose	$4.64^{ab} \pm 0.25$	$4.71^{a} \pm 0.24$	$4.68^{a} \pm 0.36$	$4.57^{b} \pm 0.28$	$4.68^{a} \pm 0.20$

[abcd] Values within a row with the same latters are not significantly different ($P < 0.05$).

In Ayrshire, the frequency of κ-CN B in present study was 20%, which was much higher than those of the population (1%). Therefore, it was expected that higher percentage of milk protein (3.61 v/s 3.38) could be obtained for the milk of the present study. The frequency of β-LG A in present study (20%) was higher than the population frequency (5%). Hence, it was expected that higher protein and lower fat content as well as higher casein, greater casein number and lower whey protein could be obtained for the Ayrshire milk.

For Brown Swiss, the frequency of κ-CN B in present study was higher (38%) than the population value (7%). Therefore higher milk protein percentage (3.73 v/s 3.54) was expected. The frequency of β-LG A in this study was higher (41%) compared with population frequency (14%). It was expected that higher milk protein and lower milk fat could be obtained for the present study. The higher frequency of β-LG A in this study was related with lower fat content (3.58) when comparing with the population value.

In Canadienne, the frequency of κ-CN B (29%) was higher than the population frequency (5%). The expectation was higher protein (3.81 v/s 3.54) and higher fat (4.40 v/s 4.20) for the milk of the present study. Frequency of β-LG A in present study was higher (37%) than that of the population (16%). Therefore, the expectation was higher protein and lower fat for milk of present study.

For Jersey breed, the frequency of κ-CN B in present study was higher (71%) than that of the population (55%). Hence the expectation was higher milk protein value (4.00 v/s 3.92) for milk of present study compared with that of the population. The frequency of β-

LG A in present study was higher (23%) than the value of the population (17%). The expectation was higher protein and less fat content in milk of the present study.

Significant differences were found in casein content for these five breeds. Jersey was the breed which has the highest value of casein content of 3.35 %, while Holstein has lowest value of 2.72%. Milk casein, which represents approximately 80% or more of the whole milk protein (Ng-Kwai-Hang *et al.*, 1986; Jenness, 1988), is directly proportional to milk protein content.

Milk protein content varied from 4.00% in Jerseys to 3.34% in Holsteins. Jersey and Canadienne have higher milk protein content than the other breeds. Holstein was the breed associated with the lowest milk protein content. When comparing the protein values in Table 2 with the corresponding population protein contents (PATLQ, 1996), it was found that protein content in breeds like Ayrshire and Jersey in Table 2 were higher than the population means. It could be explained by the relatively higher frequency of BB in these breeds in our study (Kim, 1995). κ-CN BB type had been confirmed to be related with higher milk casein and milk protein content. According to Table 1, breeds such as Jersey, Canadienne and Ayrshire have higher frequencies of BB than the frequency distribution of the population.

Milk fat ranged from 4.40% for Jersey to 3.58% for Brown Swiss. These values were lower than the population means except for Canadienne. The population values for Jersey and Ayrshire were 4.04 and 4.86%, respectively. The discrepancy could be explained by the fact that milk fat content is negatively associated with milk protein content for a given milk composition. Therefore, it could be expected that with higher casein in milk, fat content is slightly lower than normal level for the present study.

The protein to fat ratio in the five breeds differed from 0.90 of Jersey to 1.15 for Brown Swiss. The ratio of casein to fat for the five breeds followed the similar pattern, with the range from 0.93 for Brown Swiss to 0.75 for Jersey. NCP were in a range of 0.62 to 0.71% in five breeds, which were negatively related to casein/protein ratio.

As shown in Table 2, standard deviations for total solids and other milk components in Ayrshire, Brown Swiss, Canadienne and Jersey were greater than those for Holstein. This was because Holstein milk samples in this study were bulk tank milk, and as a mixture, they were less variable in composition.

The variations of the milk composition were caused by both environmental and genetic factors plus some undetected factors (Gaunt, 1980; Kroeker *et al.*, 1985a; Ng-Kwai-Hang *et al.*, 1982, 1987, 1988; Ozimek *et al.*, 1993).

Since all of the milk samples had not been standardized for cheesemaking, the obtained values of milk components could be taken as representative composition of milk used throughout the study.

From the wide range of differences in milk composition, especially in fat, protein and casein observed in 456 milk samples, differences could be expected for cheese yield, cheese composition and coagulating properties of milk (O'Keeffe *et al.*, 1982 Gilles and Lawrence, 1985; Ng-Kwai-Hang *et al.*, 1987b; Verret *et al.*, 1989; Lou and Ng-Kwai-Hang, 1992b).

Somatic cell count (SCC) of Ayrshire, Brown Swiss, Canadienne, Jersey and Holstein milk were 270840, 225700, 235490, 307020 and 222420 cells/ml milk, respectively.

These results were close to or well below the value of 300,000 cells/ml milk as indicated by Politis and Ng-Kwai-Hang (1988b). As a result, these SCC levels would not be expected to affect the cheesemaking process and coagulating properties (Politis and Ng-Kwai-Hang, 1988c; Barbano *et al.*, 1991).

4. 3. Cheese yield from milk of five breeds irrespective of κ-CN / β-LG types

It is known that cheese yield is influenced by many factors such as milk composition, especially fat and protein content, the casein to fat ratio, recovery efficiency of milk components in the cheesemaking technique, initial volume of milk used in cheesemaking. For the current study, cheesemaking was carried out under carefully controlled laboratory conditions, therefore, variation due to cheesemaking technique and the initial volume of milk were minimized. The cheese yield was expressed in three ways with the consideration of the influence of milk fat and milk protein on cheese yield.

Cheese yield of bulk tank Holstein milk is an indicator of cheese making procedure. The overall means for Holstein milk were: 10.79, 10.19 and 11.57 g for the three respective ways of expressing cheese yields. These results were in good agreement with previous results (11.15, 10.32 and 10.31g) under the same conditions (Lou and Ng-Kwai-Hang, 1992a). Therefore, it was indicated that these results were within the normal range and cheesemaking was under normal control.

Table 3. **Unadjusted means of cheese yield from milk of different breeds**

Cheese yield (g / 100g milk)	Breed				
	Ayrshire	Brown Swiss	Canadienne	Jersey	Holstein
Actual	$11.51^{c} \pm 1.75$	$11.79^{bc} \pm 1.72$	$12.37^{b} \pm 2.40$	$13.37^{a} \pm 2.21$	$10.79^{d} \pm 1.53$
37% moisture Adjusted	$11.19^{c} \pm 1.91$	$11.97^{bc} \pm 2.18$	$12.68^{b} \pm 2.25$	$11.46^{a} \pm 1.91$	$10.19^{d} \pm 2.13$
Theoretical	$11.46^{c} \pm 1.66$	$12.86^{b} \pm 2.47$	$13.40^{ab} \pm 3.33$	$14.11^{a} \pm 2.70$	$11.57^{c} \pm 2.00$
Efficiency (%)	$98.91^{a} \pm 17.42$	$90.76^{b} \pm 16.43$	$91.50^{b} \pm 14.62$	$90.92^{b} \pm 13.21$	$90.10^{b} \pm 22.31$

^{abcd}Values within a row with the same letters are not significantly different ($P < 0.05$).

The overall unadjusted means and standard deviations for cheese yields and yield efficiencies of the five breeds are presented in Table 3. Among the five breeds, Jersey milk was related with the highest actual yield. When comparing the 37% moisture adjusted yield and theoretical yield, Holstein and Jersey had the highest yields of 13.44 g and 14.11 g respectively.

Within each breed, actual, adjusted and theoretical yields were quite different. Actual yields for Ayrshire, Brown Swiss, Canadienne and Jersey milk were greater than their corresponding adjusted yields. In each of the five breeds, theoretical yield was greater than both actual and adjusted yields. these indicated that cheese yield efficiencies were slightly lower than 100% and values of cheese moisture were higher than 37%, and also possibly more milk components were lost in the whey during cheesemaking.

The discrepancy between actual and adjusted yields could be attributed to the differences of moisture in cheese. For instance, in Ayrshire, moisture in cheese was 39.65% as shown in Table 4 (60.35% of the total solids), and after adjusting to 37% moisture, the value of adjusted yield is 11.19 g, which is slightly lower than 11.51 g of actual yield. Theoretical yield is based on the mathematical calculation of the weight of the cheese which should be obtained with the moisture of 37%. Many other factors have been known as possible contributors to the differences between actual and adjusted yields. Factors such as milk composition, especially fat and casein contents (Lou and Ng-Kwai-Hang, 1992a), casein to fat ratio (Barbano and Sherbon, 1984), somatic cell count (Politis and Ng-Kwai-Hang, 1988b), efficiency of milk components recovered into cheese (Callanan, 1993; Emmons, 1993), cheesemaking techniques (Moxley and Ng-Kwai-Hang, 1984), initial volume of milk to be used for cheesemaking (Ng-Kwai-Hang, 1994), the final cheese composition

(Emmons, 1993), heat treatments (Marshall,1986: Lau *et al.*,1990,1991; Choi, 1996) and genetic variants of milk proteins (Marziali and Ng-Kwai-Hang, 1986a) were all reported to have possible influence. Since all cheesemaking for the current study were carried out under the standardized laboratory conditions. such as using the same volume of milk and using the same batch of starter culture and rennet. the variation due to cheesemaking techniques and initial volume of milk used to make cheese had been minimized. In Ayrshire, adjusted yield (11.19 g) is lower than theoretical yield of 11.46 g. Similar results could be observed for the other four breeds. The possible reason could be the losses of fat and casein in the whey being higher than 7% for fat and 4% for casein as been predicted by Van Slyke's formula. The efficiency of cheese yield is an expression of adjusted yield as a percentage of theoretical yield. The lower average value for yield efficiency further explains the lower 37% moisture adjusted yield. The difference between adjusted and theoretical yields was expected to be similar to that between actual and theoretical yield since, no correction was made with milk composition for actual cheese yield.

Efficiency of cheese yield is the adjusted yield as a percentage of the theoretical yield. The overall mean of efficiency for Ayrshire. Brown Swiss, Canadienne, Jersey and Holstein were: 98.91, 90.10, 90.76 and 90.92 and 91.50%, respectively. It was observed that with the increase of milk protein content. cheese yield efficiency will also increase slightly. This could be attributed to the addition of culture, which was perhaps denatured during cheesemaking. Although there are some exceptions. it still clearly shows that with the increase of milk fat, cheese yield efficiency would slightly decrease. This phenomenon may be explained by the reduction of milk fat recovery into cheese with the increasing of fat in milk. Since there is not enough casein in milk to uptake all milk fat, as a result, excessive milk fat is lost in the whey, therefore result in a low percentage of fat recovery in cheese and low cheese yield.

According to the analysis of variance, efficiency of cheese yield for Brown Swiss and Canadienne were significantly affected ($P<0.05$) by fat content, total solid and casein content of milk. Fat content and casein to fat ratio in milk influence the retention of fat and casein in cheese which were reflected in cheese yield and therefore caused the variation in cheese yield efficiency.

4. 4. <u>Variations in Cheese and whey compositions</u>

It is well accepted that changes in milk composition, especially fat and protein content, would influence the resulting cheese composition. The cheese and whey compositions for the five breeds are summarized in Table 4. As many factors are known to influence milk composition, it is expected that the changes in milk components would contribute to the variations in the compositions of the whey and cheese. Marziali (1985) reported that cheese with higher protein content (24.17%) and higher fat content (33.54%) could be made from Holstein milk with favorable phenotype. These results were close to the overall cheese composition of Holstein breed listed in Table 4. This indicated that cheese compositions of Holstein and other four breeds were within the normal range. Table 4 shows the variability in cheese composition in different breeds. Total solids in cheese differed from 58.95% for Jersey to 60.35% for Ayrshire. As shown in Table 4, overall means of total solids were statistically different for different breeds except Jersey v/s Holstein. Casein content in milk is positively related with the amount of protein, but negatively related with amount of fat incorporated in cheese. Since fat and protein

Table 4. Unadjusted means of cheese and whey composition from milk of five different breeds

Composition (%)	Breed				
	Ayrshire	Brown Swiss	Canadienne	Jersey	Holstein
Cheese					
Total solids	60.35[ab] ± 2.89	59.74[bc] ± 4.85	60.97[a] ± 3.48	58.95c ± 3.25	59.08[c] ± 3.07
Protein	23.16[b] ± 2.59	25.46[a] ± 4.01	23.40[b] ± 3.71	23.18b ± 3.04	22.83[b] ± 2.24
Fat	31.41[a] ± 4.23	27.55[c] ± 6.59	31.27[a] ± 4.66	29.60[b] ± 3.96	30.07[ab] ± 3.10
Salt	1.26[a] ± 0.19	1.32[a] ± 0.18	1.26[a] ± 0.19	1.26[a] ± 0.18	1.32[a] ± 0.18
Whey					
Total Solids	7.09[b] ± 0.38	7.03[bc] ± 0.28	7.41[a] ± 0.51	7.14[b] ± 0.87	6.87[c] ± 0.29
Protein	1.08[b] ± 0.20	1.19[a] ± 0.27	1.15[a] ± 0.21	1.23[a] ± 0.30	0.99[c] ± 0.15
Fat	0.26[b] ± 0.02	0.27[b] ± 0.21	0.38[a] ± 0.23	0.29[b] ± 0.28	0.23[b] ± 0.14

[abcd] Values within a row with the same letters are not significantly different ($P < 0.05$).

constitute more than 90% of cheese total solids, an increase in the protein content of cheese was accompanied by a decrease in fat content and vice versa.

Cheese protein ranged from 22.83% for Holstein to 25.46% for Brown Swiss. Cheese protein content for Brown Swiss was significantly higher (P< 0.005) than cheese protein of the other four breeds. No statistical differences (P < 0.05) were detected for the cheese protein among Ayrshire, Canadienne, Jersey and Holstein.

Cheese fat varied from 27.55% for Brown Swiss to 31.41% for Ayrshire. Significant differences were found when compared with different breeds. Both cheese protein and cheese fat overall means were within the normal ranges for Holstein bulk tank milk control when comparing with previous studies (Marziali and Ng-Kwai-Hang, 1986b; Lou and Ng-Kwai-Hang, 1992a).

The salt content of cheese made from milk of the five breeds ranged from 1.26% for Ayrshire to 1.32% for Canadienne. No statistical difference was found among the salt content for the five breeds. These values were slightly higher than values of the previous report but close to 1.5%, which was considered as a desirable salt content for good quality of Cheddar-type cheese. Salt was added to a final concentration of 1.5% in cheese curd during cheesemaking. Due to the losses in the whey during the pressing process, the resulting cheese may contain less than 1.5% of salt.

The differences in cheese composition for the five breeds were mainly due to the differences in milk composition of the breeds. Whey compositions for the five breeds are also summarized in Table 4. The whey composition, which varied to some extent for different phenotypes of κ-CN/β-LG, is a good indicator of the ability to incorporate milk components into cheese. The whey total solids ranged from 6.87% for Holstein to 7.41% for Canadienne. From these results, it was found that whey total solids increased as protein content of milk increased. This may be explained by the fact that the increase of protein content in milk is accompanied by an increased loss of casein during the cheesemaking process. In contrast, increasing fat content in milk resulted in greater loss of fat in the whey because there is not enough casein to associate with fat.

An important factor which influence whey composition is casein to fat ratio of milk. It was reported (Lou and Ng-Kwai-Hang, 1992a) that total solids of whey increased as casein to fat ratio increased. This is due to the fact that casein to fat ratio is positively associated with the lactose content in milk. Most of the lactose does not contribute to the cheese composition since it will be lost in the whey. Therefore, greater cheese whey total solids could be expected from higher content of lactose in milk. As a consequence, total solids of cheese whey was higher when casein to fat ratio was high. Fat content in whey decreased as casein to fat ratio increased. During the cheesemaking process, milk fat is entrapped by casein networks. High amount of casein in milk will have the ability to entrap more fat in the curd and resulted in lower fat loss in the whey. Therefore, optimal casein to fat ratio is one of critical factor to cheese making industry. From Table 4 and Table 2, it was also suggested that protein content in whey increased with an increase of casein to fat ratio. This may be attributed to the positive relationship between non casein protein (NCP) and the casein to fat ratio. Recently, it is well accepted that most of the NCP will be lost in the whey during cheesemaking. In general, protein to fat ratio has the same effects as casein to fat ratio on whey composition.

Since the effects of major milk components on cheese yield have been reported by many studies on Holstein milk, therefore in present research, milk compositions, mainly milk fat and milk casein, were included as covariates in the models.

It could be concluded that milk protein has a greater contribution to cheese yield than milk fat for the four breeds. Specifically, the increase of casein components which constitute approximately 80% of the milk protein, are responsible for the observed increase in cheese yield. According to Van Slyke's cheese yield formula, under ideal conditions, 93% of milk fat and 96% of milk casein would be recovered into cheese. Hence, an increase in casein will influence cheese yield to a greater extent than those of milk fat.

4. 5. Recoveries of milk components into cheese for the five different breeds

The information on efficiency of the transformation milk components into cheese for the five breeds can be obtained by comparing the percentages of fat and casein recovered in cheese. Van Slyke's formula for theoretical cheese yield assumed that 93% of fat and approximately 96% of casein in milk would be recovered in the cheese. Table 5 shows that recoveries of milk total solids ranged from 95.88% in Jersey to 100.64% for Ayrshire. This indicated that there were no excessive losses of major milk components during cheesemaking process. The milk fat recoveries were different from 91.42% for Brown Swiss to 96.92% for Canadienne. Fat recovery for Holstein milk was 93.5%, and no significant differences ($P < 0.05$) were detected between this result and fat recoveries of the other four breeds. The recoveries of casein from milk of the five breeds ranged from 89.13% in Canadienne to 107.27% in Holstein. These results were lower than 96% except for Holstein when comparing with the standard value. Overall, the recovery of

Table 5. Recoveries of milk components into cheese for the five breeds

Breed	Recovery(%)		
	Total solids	Fat	Casein
Ayrshire	100.64[a] ±7.46	95.98[a] ±18.86	91.12[b] ±14.46
Brown Swiss	49.33[ab] ±3.05	91.42[a] ±19.92	90.94[b] ±10.84
Canadienne	99.54[a] ±7.04	96.92[a] ±27.79	89.13[b] ±17.20
Jersey	95.88[b] ±6.67	93.26[a] ±18.57	92.15[b] ±9.09
Holstein	100.15[a] ±9.01	93.50[a] ±18.11	107.27[a] ±26.23

[ab]Values within a column with the same letters are not significantly different ($P<0.05$).

milk casein into cheese was lower than the recovery of milk fat. This could be explained by an excessive amount of casein in milk to combine with the maximum amount of fat and thus leading to increased losses of casein during cheesemaking.

4. 6. Overall Unadjusted means of formagraphic properties of milk from the five different breeds

It is conceivable that factors responsible for the variations in milk composition may cause variability in clotting time (RCT), rate of firming (K20) and firmness of curd at cutting (A30).

Table 6 shows the results of formagraphic parameters for milk of the five breeds. The values of RCT, K20 and A30 were 8.86 min. 10.97 min and 26.07 mm for the Holstein milk. These results were very close to the values in previous reports from our laboratory under the same conditions (Marziali. 1985: Politis and Ng-Kwai-Hang, 1988c). Therefore, it was deduced that formagraphic parameters for milk from Holstein and other four breeds are within the normal range.

The overall means of RCT ranged from 8.86 min for Holstein to 9.10 min for Canadienne. No difference was found among Brown Swiss, Canadienne, Jersey and Holstein ($P< 0.05$). However, RCT of these four breeds were significantly different from RCT of Ayrshire milk ($P < 0.05$). No differences were found for overall means of K20 in the five breeds. Statistical differences ($P< 0.05$) were found within the five breeds for A30 except for Ayrshire v/s Holstein, in which no difference was detected.

According to Bynum and Olson (1982), better cheese yield was associated with firmer curd at cutting. Therefore, both κ-CN BB and β-LG AA were associated with better

Table 6. Overall means of formagraphic properties of milk from five different breeds

Formagraphic Parameter	Breed				
	Ayrshire	Brown Swiss	Canadienne	Jersey	Holstein
RCT (min)	9.93[a] ± 3.01	8.63[b] ± 1.81	9.10[b] ± 2.93	9.05[b] ± 2.37	8.86[b] ± 1.52
K20 (min)	10.39[a] ± 2.34	10.63[a] ± 2.88	10.04[a] ± 2.63	10.40[a] ± 3.62	10.97[a] ± 2.84
A30 (mm)	25.53[c] ± 7.29	30.60[ab] ± 6.49	28.71[b] ± 6.53	32.12[a] ± 8.06	26.07[c] ± 7.60

[abc] Values within a row with the same letters are not significantly different ($P < 0.05$).

cheese yield. The better clotting time was believed to be due to the association of κ-CN BB with higher content of κ-CN in milk (Kroeker *et al.*, 1985b). As more κ-CN is available as a substrate for the clotting enzyme, the faster the reaction would start and thus reduce the RCT.

The differences for the formagraphic properties observed for the milk of the five breeds were due to the different frequencies distribution of κ-CN and β-LG in these breeds. As shown in Table 1, phenotypes frequencies for BB and AA type for both κ-CN and β-LG were different for these four breeds, this would give rise to the differences in formagraphic parameters related with breeds.

4. 7. <u>Associations of κ-CN/β-LG phenotype combinations with milk composition within each breeds</u>

It is generally agreed that the B variants of κ-CN and β-LG are associated with higher milk casein and fat contents in the milk (Ng-Kwai-Hang and Grosclaude, 1992), but those association are breed specific. Hence, for the purpose of this project, it was more appropriate to analyze the effects of κ-CN/β-LG phenotype combination on milk composition and cheesemaking separately for each of the four breeds. The milk composition according to different κ-CN/β-LG phenotype combination in each of the four breeds are summarized in Table 7.

4. 7. 1. <u>Variation of milk composition according to κ-CN/β-LG types in Ayrshire</u>

All the nine possible different types of κ-CN/β-LG were available for Ayrshire milk. The SAS program was used to analyze the effect of κ-CN/β-LG type on milk composition. The results of means and standard deviations of Ayrshire milk composition are shown in

Table 7. Milk composition of the four breeds according to K-CN / ß-LG phenotypes

	K-CN / ß-LG Type	Component (%) Total solids	Fat		Protein	Casein	Lactose	
Ayrshire	AA/AA	$12.97^{bc} \pm 0.79$	3.94^{bcd}	± 0.69	$3.69^{b} \pm 0.44$	$2.99^{b} \pm 0.41$	4.66^{abcd}	± 0.16
	AA/AB	$12.39^{c} \pm 0.85$	3.79^{cd}	± 0.67	$3.42^{b} \pm 0.38$	$2.72^{b} \pm 0.36$	4.54^{cd}	± 0.27
	AA/BB	$12.90^{bc} \pm 0.98$	3.83^{cd}	± 0.78	$3.59^{b} \pm 0.28$	$2.94^{b} \pm 0.31$	4.65^{abcd}	± 0.22
	AB/AA	$12.75^{bc} \pm 0.97$	3.79^{cd}	± 0.67	$3.52^{b} \pm 0.48$	$2.78^{b} \pm 0.40$	4.74^{abc}	± 0.14
	AB/AB	$12.40^{c} \pm 1.08$	3.49^{d}	± 0.59	$3.52^{b} \pm 0.44$	$2.88^{b} \pm 0.42$	4.67^{abcd}	± 0.17
	AB/BB	$13.31^{bc} \pm 0.77$	4.40^{abc}	± 0.44	$3.64^{b} \pm 0.48$	$2.97^{b} \pm 0.47$	4.57^{bcd}	± 0.38
	BB/AA	$14.22^{a} \pm 1.33$	4.89^{bcd}	± 1.39	$4.13^{a} \pm 0.63$	$3.40^{a} \pm 0.66$	4.48^{d}	± 0.24
	BB/AB	$13.27^{bc} \pm 0.86$	3.93^{bcd}	± 0.71	$3.80^{ab} \pm 0.30$	$3.12^{ab} \pm 0.34$	4.78^{ab}	± 0.11
	BB/BB	$13.46^{ab} \pm 1.14$	4.58^{ab}	± 0.92	$3.67^{b} \pm 0.35$	$2.96^{b} \pm 0.38$	4.85^{a}	± 0.27
Brown Swiss	AA/AB	$12.70^{a} \pm 1.16$	3.76^{ab}	± 0.97	$3.62^{b} \pm 0.39$	$2.97^{a} \pm 0.39$	4.67^{a}	± 0.18
	AA/BB	$13.08^{a} \pm 0.48$	4.00^{a}	± 0.22	$3.58^{b} \pm 0.33$	$2.79^{a} \pm 0.27$	4.70^{a}	± 0.19
	AB/AA	$12.75^{a} \pm 1.15$	3.51^{ab}	± 1.09	$3.81^{a} \pm 0.33$	$3.07^{a} \pm 0.29$	4.75^{a}	± 0.26
	AB/BB	$12.87^{a} \pm 1.15$	4.07^{a}	± 1.30	$3.78^{ab} \pm 0.27$	$3.12^{a} \pm 0.14$	4.69^{a}	± 0.16
	BB/AA	$12.43^{a} \pm 1.05$	3.20^{b}	± 0.98	$3.85^{a} \pm 0.36$	$3.09^{a} \pm 0.32$	4.73^{a}	± 0.32
	BB/AB	$12.64^{a} \pm 1.57$	3.53^{ab}	± 1.62	$3.74^{ab} \pm 0.52$+	$3.02^{a} \pm 0.53$	4.71^{a}	± 0.30
	BB/BB	$12.46^{a} \pm 1.23$	3.36^{b}	± 0.86	$3.66^{b} \pm 0.50$	$3.02^{a} \pm 0.43$	4.67^{a}	± 0.26
Canadienne	AA/AA	$13.21^{a} \pm 1.19$	4.09^{ab}	± 0.98	$3.74^{a} \pm 0.41$	$3.02^{a} \pm 0.34$	4.70^{ab}	± 0.24
	AA/AB	$13.50^{a} \pm 1.85$	4.50^{ab}	± 1.34	$3.87^{a} \pm 0.56$	$3.21^{a} \pm 0.63$	4.38^{b}	± 0.59
	AA/BB	$13.19^{a} \pm 0.99$	4.35^{ab}	± 0.72	$3.55^{a} \pm 0.43$	$2.86^{a} \pm 0.50$	4.60^{ab}	± 0.58
	AB/AA	$13.72^{a} \pm 1.53$	4.69^{ab}	± 1.62	$3.86^{a} \pm 0.44$	$3.13^{a} \pm 0.47$	4.79^{a}	± 0.21
	AB/AB	$13.06^{a} \pm 0.95$	4.11^{ab}	± 0.89	$3.86^{a} \pm 0.41$	$3.18^{a} \pm 0.43$	4.80^{a}	± 0.29
	AB/BB	$13.57^{a} \pm 0.97$	4.47^{ab}	± 0.73	$3.76^{a} \pm 0.58$	$3.13^{a} \pm 0.59$	4.76^{a}	± 0.14
	BB/AA	$13.28^{a} \pm 0.79$	3.70^{b}	± 0.98	$3.97^{a} \pm 0.37$	$3.25^{a} \pm 0.36$	4.63^{ab}	± 0.45
	BB/AB	$13.97^{a} \pm 0.47$	4.95^{a}	± 0.40	$3.87^{a} \pm 0.89$	$3.20^{a} \pm 0.90$	4.59^{ab}	± 0.32
	BB/BB	$14.23^{a} \pm 2.03$	4.93^{a}	± 1.13	$3.91^{a} \pm 0.42$	$3.27^{a} \pm 0.44$	4.70^{ab}	± 0.21
Jersey	AA/AB	14.43^{a}	4.22^{a}		3.77^{ab}	3.21^{ab}	4.67^{a}	
	AA/BB	$12.67^{a} \pm 0.89$	4.02^{a}	± 0.72	$3.54^{b} \pm 0.39$	$2.90^{b} \pm 0.35$	4.54^{a}	± 0.15
	AB/AA	$13.29^{a} \pm 1.20$	4.38^{a}	± 1.25	$3.93^{ab} \pm 0.60$	$3.20^{ab} \pm 0.59$	4.68^{a}	± 0.08
	AB/AB	$13.28^{a} \pm 0.56$	4.09^{a}	± 0.68	$3.70^{ab} \pm 0.22$	$3.02^{ab} \pm 0.22$	4.64^{a}	± 0.11
	AB/BB	$14.25^{a} \pm 2.90$	3.96^{a}	± 1.16	$3.80^{ab} \pm 0.51$	$3.13^{ab} \pm 0.55$	4.53^{a}	± 0.37
	BB/AA	$14.18^{a} \pm 2.17$	4.40^{a}	± 1.22	$3.98^{ab} \pm 0.60$	$3.30^{ab} \pm 0.67$	4.54^{a}	± 0.37
	BB/AB	$15.34^{a} \pm 3.65$	4.78^{a}	± 0.71	$4.05^{ab} \pm 0.34$	$3.42^{ab} \pm 0.36$	4.63^{a}	± 0.18
	BB/BB	$15.34^{a} \pm 3.05$	5.32^{a}	± 1.54	$4.35^{a} \pm 0.50$	$3.72^{a} \pm 0.52$	4.45^{a}	± 0.39

[abcd] Values within a column in the same breed with the same letters are not significantly different ($P < 0.05$)

Table 7. Total solids of milk ranged from 12.39% for AA/AB to 14.22% for BB/AA. Significant differences (P < 0.05) were found for total solids of the milk with different κ-CN/β-LG types. The κ-CN BB group (BB/AA, BB/AB and BB/BB) had an average of 13.65%, which was higher than the average of 12.82% AB group (AB/AA, AB/AB and AB/BB) and 12.75% for AA group (AA/AA, AA/AB and AA/BB). When comparing the total solids of β-LG AA group (AA/AA, AB/AA and BB/AA) and the total solids of the β-LG BB group (AA/BB, AB/BB and BB/BB), the value of 13.22% for the average for BB was slightly lower than 13.31% for AA group. When the same method of comparison was applied for fat, protein and casein in different types, it was found that BB type for both κ-CN and β-LG were associated with higher values. Total solids for AB variants were usually intermediate. There were significant differences for lactose content for different types of milk.

4. 7. 2. <u>Variation of milk composition according to κ-CN / β-LG types in Brown Swiss</u>

Only seven κ-CN/β-LG phenotypes were found for Brown Swiss. No observations was available for the κ-CN/β-LG phenotype combinations AA/AA and AB/AB. The means and standard deviations of milk composition for different types of κ-CN/β-LG are presented in Table 7. No significant difference (P< 0.05) was found for total solids, casein and lactose when comparing the different types. However, significant differences (P< 0.05) were detected for both fat and protein for milk with different types. AA/BB was associated with higher fat content. BB/AA and BB/AB were two types related with higher milk protein content.

More desirable milk types were AA/BB and AB/BB, as well as BB/AA and BB/AB which were linked with higher total solids, fat, protein and casein content. Thus, higher cheese yield could be expected for Brown Swiss milk with these types.

4. 7. 3. <u>Variation of milk composition according to κ-CN/β-LG types in Canadienne</u>

There were nine different types of κ-CN/β-LG for Canadienne milk, and milk compositions for the different types of milk are listed in Table 7. No statistical difference ($P< 0.05$) was detected for both total solids and casein for different milk types. However, significant differences ($P< 0.05$) were detected for both milk fat and milk protein with different κ-CN/β-LG types. BB/AB and BB/BB were two types to be related with the highest content of fat and protein. Thus, higher cheese yield could be expected for milk with these two types. It has been confirmed that the B variant of both κ-CN and β-LG are associated with higher casein and fat contents in the milk (Ng-Kwai-Hang and Grosclaude, 1992). This point was confirmed in Canadienne milk. Although Table 7 shows differences of lactose for different milk types, it would be less likely to affect cheesemaking and formagraphic properties of milk.

4. 7. 4. <u>Variation of milk composition according to κ-CN/β-LG types in Jersey</u>

Table 7 shows the milk composition for eight different phenotypes of κ-CN/β-LG in Jersey. No statistical difference was detected for total solids, fat and lactose of milk with different κ-CN/β-LG types. However. significant differences were found for milk protein with different milk types. BB/AB and BB/BB were found to be related with the highest fat content, Thus, higher cheese yield should be associated with milk of these two types. Relationships were found in Holstein milk that BB type of κ-CN (BB/AB and BB/BB) is associated with higher contents of total solids, protein, fat and casein. In this study similar relationships were also found for BB type of β-LG (AB/BB and BB/BB). Therefore, it was confirmed that for Ayrshire, Brown Swiss, Canadienne and Jersey, B gene of both κ-CN and β-LG were associated with higher milk fat and casein content. This results were comparable with the results from Holstein milk (Marziali and Ng-Kwai-Hang, 1986a; Ng-Kwai-Hang *et al.*, 1990; Kim 1995; Choi 1996).

4. 8. Association of cheesemaking properties with different types of κ-CN/β-LG within each breed

In this study, three ways of expressing cheese yield were considered when explored the influence of different phenotypes of κ-CN/β-LG on cheese yields.

Because milk compositions were significantly ($P<0.05$) different according to breeds, the cheesemaking properties of milk from four breeds were analyzed separately to investigate the effects of κ-CN/β-LG types. Milk fat and protein were included in the models as covariates. Least squares means for the three ways of expressing of cheese yields for each κ-CN/β-LG types were obtained and could be used for comparison.

4. 8. 1. Association of cheesemaking properties with Ayrshire milk according to different types of κ-CN/β-LG

It is well accepted that cheesemaking is influenced by milk compositions. Most of the past and recent works have shown that factors contributing to variations in milk components could cause changes in cheese yielding capacity of milk as well as altering chemical and physical properties of milk

4. 8. 1. 1. Variation of cheese yield according to κ-CN / β-LG types in Ayrshire

For the milk with same phenotype combination of κ-CN/β-LG, actual yields was different from the corresponding 37% moisture adjusted yields as shown in Table 8. For AA/AA type, actual yield was less than the 37% moisture adjusted yields. The reason was that the moisture in cheese for this type was different from the standard moisture 37%. Higher cheese moisture could be expected for type BB/BB, therefore the actual cheese yield was greater than its adjusted cheese yield. The reason could be due to more casein in milk with κ-CN BB, and this is related to more capacity to associate with moisture in the cheese.

Theoretical cheese yields were different from 37% moisture adjusted yield in Table 8 for different types. These discrepancies reflected the low reliability for theoretical yield in predication of the cheese yield capacity of the Ayrshire milk in this study. In Table 8, significant differences were found for different κ-CN/β-LG types. The average of adjusted yield for BB group (BB/AA, BB/AB, and BB/BB) of κ-casein was 12.34 g, which was higher than the averages of κ-CN AB and AA groups. The average adjusted yield of BB group of β-lactoglobulin was 11.61 g, which was higher than 11.45 g for those of AA group. The highest cheese yield was related with BB group of κ-casein, especially BB/AA and BB/BB. These results were similar to previous study on Holstein milk (Aleandri *et al.*, 1990; Marziali and Ng-Kwai-Hang, 1986a; Morini *et al.*, 1979; Schaar *et al.*, 1985; van de Berg *et al.*, 1992). This indicated the association of B gene of κ-casein with the higher cheese yield for Ayrshire milk.

Table 8. Cheesemaking properties of Ayrshire milk with different κ-CN/β-LG types

Cheese yield

K-CN/ß-LG type	Type of yield (g / 100 g milk)		
	Actual	37% moisture adjusted	Theoretical
AA/AA	$10.99^{c} \pm 1.80$	$11.28^{abc} \pm 1.80$	$11.22^{cde} \pm 1.52$
AA/AB	$10.65^{c} \pm 1.79$	$9.99^{c} \pm 1.65$	$10.74^{e} \pm 1.37$
AA/BB	$11.80^{abc} \pm 1.54$	$11.21^{abc} \pm 1.82$	$11.60^{bcde} \pm 1.44$
AB/AA	$11.43^{bc} \pm 1.04$	$11.25^{abc} \pm 1.50$	$10.81^{e} \pm 1.34$
AB/AB	$10.98^{c} \pm 1.71$	$10.50^{bc} \pm 1.56$	$10.98^{de} \pm 1.79$
AB/BB	$11.95^{abc} \pm 1.26$	$12.11^{ab} \pm 2.17$	$11.89^{bcd} \pm 1.48$
BB/AA	$13.04^{a} \pm 1.99$	$12.83^{a} \pm 2.15$	$13.12^{a} \pm 2.13$
BB/AB	$11.99^{abc} \pm 1.94$	$11.68^{ab} \pm 1.45$	$12.04^{bc} \pm 1.75$
BB/BB	$12.67^{ab} \pm 1.93$	$12.51^{a} \pm 1.90$.	$12.53^{ab} \pm 1.84$

Cheese composition

K-CN/ß-LG type	Cheese component (%)			
	Total solids	Fat	Protein	Salt
AA/AA	$60.06^{bc} \pm 1.47$	$30.72^{b} \pm 2.31$	$22.61^{ab} \pm 2.31$	$1.35^{a} \pm 0.19$
AA/AB	$58.43^{c} \pm 2.95$	$29.78^{b} \pm 4.04$	$23.68^{ab} \pm 2.29$	$1.28^{ab} \pm 0.19$
AA/BB	$60.22^{bc} \pm 2.75$	$30.50^{b} \pm 3.68$	$23.44^{ab} \pm 1.92$	$1.30^{ab} \pm 0.16$
AB/AA	$61.32^{b} \pm 2.41$	$33.14^{b} \pm 7.12$	$22.85^{ab} \pm 3.40$	$1.34^{a} \pm 0.24$
AB/AB	$60.32^{bc} \pm 2.01$	$31.90^{b} \pm 3.61$	$21.90^{bc} \pm 2.06$	$1.27^{abc} \pm 0.18$
AB/BB	$61.44^{b} \pm 3.52$	$32.78^{b} \pm 3.25$	$22.07^{bc} \pm 1.99$	$1.14^{bc} \pm 0.16$
BB/AA	$63.96^{a} \pm 1.60$	$37.92^{a} \pm 3.75$	$20.40^{c} \pm 3.20$	$1.09^{c} \pm 0.19$
BB/AB	$59.91^{bc} \pm 2.80$	$29.74^{b} \pm 4.38$	$24.93^{a} \pm 2.23$	$1.21^{abc} \pm 0.22$
BB/BB	$62.48^{ab} \pm 1.25$	$32.75^{b} \pm 2.95$	$24.22^{ab} \pm 4.18$	$1.22^{abc} \pm 0.18$

Whey composition

K-CN/ß-LG type	Component (%)		
	Total solids	Fat	Protein
AA/AA	$7.29^{b} \pm 0.28$	$0.38^{a} \pm 0.18$	$1.18^{a} \pm 0.17$
AA/AB	$6.80^{b} \pm 0.35$	$0.21^{ab} \pm 0.12$	$1.14^{ab} \pm 0.24$
AA/BB	$6.99^{b} \pm 0.26$	$0.22^{ab} \pm 0.15$	$0.98^{b} \pm 0.19$
AB/AA	$7.20^{b} \pm 0.27$	$0.28^{ab} \pm 0.11$	$1.04^{ab} \pm 0.21$
AB/AB	$7.05^{b} \pm 0.35$	$0.42^{a} \pm 0.38$	$1.02^{ab} \pm 0.17$
AB/BB	$7.17^{b} \pm 0.28$	$0.33^{ab} \pm 0.31$	$1.11^{ab} \pm 0.21$
BB/AA	$8.23^{a} \pm 1.82$	$0.21^{ab} \pm 0.13$	$1.18^{a} \pm 0.23$
BB/AB	$7.15^{b} \pm 0.21$	$0.15^{b} \pm 0.16$	$1.13^{ab} \pm 0.11$
BB/BB	$7.27^{b} \pm 0.25$	$0.32^{ab} \pm 0.26$	$0.98^{b} \pm 0.17$

^{ab} Values within a column with the same letters are not significantly different ($P < 0.05$).

4. 8. 1. 2. <u>Effects of κ-CN/β-LG types on cheese composition from Ayrshire milk</u>

Least squares means of cheese total solids, cheese fat, cheese protein and salt for each κ-CN/β-LG type of Ayrshire are summarized in Table 8. These values obtained for each types were similar when compared with reports conducted from Holstein (Ng-Kwai-Hang *et al.*, 1988; Politis and Ng-Kwai-Hang, 1988a; Lou and Ng-Kwai-Hang,1992b; Tong *et al.*, 1994). Total solids ranged from 58.43% for AA/AB type to 63.96% for BB/AA type, these values were basically close to the desirable total solids of 63% for Cheddar cheese (Barbano and Sherbon, 1984). Cheese total solids mainly consist of cheese fat and cheese protein. The variation of total solids was related with variations of both cheese fat and cheese protein contents.

Cheese fat for different milk types was less variable. No statistical differences ($P < 0.05$) were found for different κ-CN/β-LG types except BB/AA, which was associated with significantly higher ($P < 0.05$) cheese fat of 37.92% when comparing with the other types. Thus the variations of cheese total solids were mainly due to the variation of cheese protein content. Significant differences were found for cheese protein content for different κ-CN/β-LG types. Higher cheese protein were related with BB/AB and BB/BB types, which have cheese protein of 24.93% and 24.22%, respectively. Although salt content was different according to phenotypes, it was less likely to influence cheesemaking capacity.

4. 8. 1. 3. <u>Effects of κ-CN/β-LG types on whey compositions for Ayrshire milk</u>

Least squares means and standard deviations of whey fat, protein and total solids for cheese whey of Ayrshire milk are summarized in Table 8. Total solids in whey ranged from 6.80% of AA/AB type to 8.23% of BB/AA type. No statistical difference ($P < 0.05$) was found for different types except type BB/AA, which had significantly higher ($P < 0.05$) whey total solids than those of all other types. Most of whey total solids were accounted for by lactose. Whey fat had a wide spectrum of differences for different phenotypes. Statistical differences ($P < 0.05$) were found for different κ-CN/β-LG phenotypes. Type AA/AA and type AB/BB were related with higher whey fat. Statistical differences were ($P < 0.05$) also detected in whey protein with different κ-CN/β-LG phenotypes where type AA/AA and type AB/AA were linked with higher whey protein content. These results show that higher losses of milk components during cheesemaking were associated with these types which had higher whey fat or higher whey protein content.

4. 8. 2. Association of cheesemaking properties with Brown Swiss milk according to different types of κ-CN/β-LG

4. 8. 2.1. Variation of cheese yield according to different κ-CN/β-LG types

Three types of cheese yields for Brown Swiss milk with respect to different types of κ-CN/β-LG are presented in Table 9. Significant differences were detected for actual, adjusted and theoretical yields for different κ-CN/β-LG types. The actual and 37% moisture adjusted yields were different for the same phenotype. For instance, in type AA/AB, actual yield was less than 37% moisture adjusted yield, the moisture of cheese for this type was expected to be lower than 37% moisture. In contrast, greater moisture could be found for type BB/BB in which actual yield was greater than 37% moisture adjusted yield. This again confirmed that BB/BB type was associated with higher moisture of cheese.

AA/BB type was linked with the highest actual and adjusted yields of 11.41 g and 11.18g, respectively. There were some discrepancies between theoretical yield and 37% moisture adjusted yield. For instance, AB/BB was the type with the highest theoretical yield of 12.38 g, but actual and adjusted yields for AB/BB were 10.87 g and 9.90 g, respectively. These results were close to or less than the average values. The possible explanation was different moisture and different degree of milk components losses during the period of cheesemaking.

4. 8. 2. 2. Effects of κ-CN/β-LG types on cheese composition from Brown Swiss milk

Least squares means and standard deviations of cheese compositions for different κ-CN/β-LG types of the Brown Swiss milk are presented in Table 9. Cheese total solids ranged from 56.48% in BB/AA type to 63.15% in AA/AB types. When comparing with cheese total solids in Holstein milk (Lou and Ng-Kwai-Hang, 1992a), it was observed that cheese made from Brown Swiss milk have higher total solids values which resulted in higher cheese yield capacity and the cheese moisture. The moisture for different types of κ-CN/β-LG were more closer to standard moisture of 37%, hence better cheese quality and texture could be expected. AA/AB and AA/BB were two types to be related with the highest cheese total solids which were contributed by higher cheese fat content for these two types. Cheese fat contents were different from 23.26% of BB/AA type to 33.23% for AA/BB type. AA/BB was the type which had higher cheese fat content. Significant

Table 9. Cheesemaking properties of Brown Swiss milk with different κ-CN/β-LG types

Cheese yield

K-CN/ß-LG type	Type of yield (g / 100 g milk) Actual	37 % moisture adjusted	Theoretical
AA/AB	$10.67^{ab} \pm 1.63$	$10.75^{ab} \pm 2.46$	$11.50^{a} \pm 1.61$
AA/BB	$11.41^{a} \pm 0.59$	$11.18^{a} \pm 0.96$	$11.07^{a} \pm 0.72$
AB/AA	$10.81^{ab} \pm 1.29$	$10.37^{abc} \pm 2.13$	$11.38^{a} \pm 1.91$
AB/BB	$10.87^{ab} \pm 1.94$	$9.90^{abc} \pm 1.99$	$12.38^{ab} \pm 2.49$
BB/AA	$11.02^{ab} \pm 1.58$	$9.26^{c} \pm 1.73$	$11.77^{ab} \pm 1.80$
BB/AB	$10.86^{ab} \pm 2.30$	$10.11^{abc} \pm 2.78$	$11.93^{ab} \pm 2.99$
BB/BB	$10.32^{b} \pm 1.51$	$9.69^{bc} \pm 2.08$	$11.46^{b} \pm 2.07$

Cheese composition

k-CN/ß-LG type	Cheese composition (%) Total solids	Fat	Protein	Salt
AA/AB	$63.15^{a} \pm 4.30$	$32.48^{a} \pm 6.18$	$23.35^{bc} \pm 3.60$	$1.15^{b} \pm 0.22$
AA/BB	$62.15^{a} \pm 2.37$	$33.23^{a} \pm 2.03$	$22.54^{c} \pm 2.10$	$1.12^{b} \pm 0.12$
AB/AA	$60.05^{ab} \pm 4.39$	$27.48^{b} \pm 5.55$	$25.38^{ab} \pm 3.15$	$1.32^{a} \pm 0.13$
AB/BB	$60.87^{ab} \pm 2.77$	$27.73^{b} \pm 7.28$	$27.52^{a} \pm 6.05$	$1.33^{a} \pm 0.14$
BB/AA	$56.48^{c} \pm 3.02$	$23.26^{c} \pm 4.28$	$25.53^{ab} \pm 4.30$	$1.27^{a} \pm 0.14$
BB/AB	$60.11^{ab} \pm 4.27$	$26.43^{b} \pm 7.14$	$26.75^{a} \pm 4.48$	$1.33^{a} \pm 0.22$
BB/BB	$58.00^{bc} \pm 5.59$	$24.74^{bc} \pm 6.34$	$27.42^{a} \pm 4.11$	$1.34^{a} \pm 0.15$

Whey composition:

K-CN/ß-LG type	Component (%) Total solids	Fat	Protein
AA/AB	$6.94^{ab} \pm 0.14$	$0.39^{a} \pm 0.17$	$1.08^{a} \pm 0.18$
AA/BB	$7.13^{a} \pm 0.25$	$0.26^{ab} \pm 0.18$	$1.16^{a} \pm 0.25$
AB/AA	$7.15^{a} \pm 0.30$	$0.29^{ab} \pm 0.17$	$1.25^{a} \pm 0.27$
AB/BB	$6.94^{ab} \pm 0.29$	$0.26^{ab} \pm 0.19$	$1.22^{a} \pm 0.24$
BB/AA	$7.14^{a} \pm 0.30$	$0.14^{b} \pm 0.16$	$1.27^{a} \pm 0.49$
BB/AB	$6.89^{b} \pm 0.33$	$0.20^{b} \pm 0.13$	$1.28^{a} \pm 0.22$
BB/BB	$6.90^{b} \pm 0.19$	$0.25^{ab} \pm 0.18$	$1.14^{a} \pm 0.17$

^{ab} Values within a column with the same letters are not significantly different ($P < 0.05$).

differences ($P < 0.05$) were found for cheese protein for different κ-CN/β-LG types. AB/BB and BB/BB were two types which were linked with the highest cheese protein content. Hence, milk with these two types could have higher cheese yield potential.

4. 8. 2. 3. <u>Effect of κ-CN/β-LG types on whey compositions for Brown Swiss milk</u>

An analysis of variance was carried out to investigate the influence of milk composition and κ-CN/β-LG type on whey composition. Least squares means and standard deviations of major whey components based on different κ-CN/β-LG phenotype are presented in Table 9. The data of whey composition could provide useful information regarding milk components which were not incorporated into cheese. Therefore, the extent to which milk components were lost in the whey indicated the efficiency of milk components which were converted into cheese. For the seven κ-CN/β-LG types, total solids ranged from 6.89% for BB/AB type to 7.15% for AB/AA type. It was believed that during the process of cheesemaking, most of milk lactose should be lost in the whey. Hence, the total solids of cheese whey account mainly for lactose. Whey fat ranged from 0.14% of BB/AA type to 0.39% for AA/AB type. AA/AB type was associated with the highest whey fat content. This could be explained by lower casein/fat ration for this type of milk. Milk casein of this type was not enough to associate with all milk fat, hence some fat were lost in the whey. Whey protein ranged from 1.08% for AA/AB type to 1.28% for BB/AB type. No statistical difference was found for whey protein with different types of κ-CN/β-LG.

Both whey fat and whey protein of all seven types were below the ranges when compared with those of Holstein milk of the previous studies (Barbano and Sherbon 1984; Politis and Ng-Kwai-Hang, 1988a; Lau *et al.*, 1990; Barbano *et al.*, 1991). The possible reason could be the time from renneting to cutting was kept constant during cheesemaking and probably resulted in higher losses of milk components in the whey (Politis and Ng-Kwai-Hang, 1988a). This was due to the partial incorporation of whey protein into cheese. Since the cheese yield is closely related with the amount of milk components retained in the curd, any factor affecting cheese yield would consequently contribute to the variation of the whey composition. The ranges of standard deviations could be explained by the variations of milk fat, milk casein, non-optimal casein to fat ratio (Lou and Ng-Kwai-Hang, 1992b), somatic cell counts (Politis and Ng-Kwai-Hang, 1988a), curd firmness at time of cutting (Ng-Kwai-Hang *et al.*, 1989), certain genetic variants of milk protein (Marziali and Ng-Kwai-Hang, 1986c; Pabst *et al.*, 1991; van de Berg *et al.*, 1992) and heat treatments (Schafer and Olson, 1975; Smietana *et al.*, 1976; Marshall, 1986; Lau *et al.*, 1990; Thomsen and Stapelfeldt, 1990).

4. 8. 3. Association of cheesemaking properties with Canadienne milk according to different types of κ-CN/β-LG

4. 8. 3. 1. Variation of cheese yield according to different types of κ-CN/β-LG

Cheese yields for milk of the nine types of κ-CN/β-LG are presented in Table 10. The 37% moisture adjusted yields ranged from 11.03 g for AB/AB type to 12.45 g for BB/AB type. BB/AB type was related with the highest actual and adjusted yields. Type BB/BB was linked with the highest theoretical yield. This indicated a greater losses of main milk components into whey during cheesemaking process for BB/BB type milk. After statistical analysis, no difference ($P < 0.05$) was found for both actual and adjusted cheese yields for different κ-CN/β-LG types. Lou and Ng-Kwai-Hang (1992a) and Marziali and

Ng-Kwai-Hang (1986a) confirmed that the casein content of milk has a greater contribution to cheese yield than milk fat has due to its association with colloidal salts and soluble solids in addition to its higher water holding capacity. The 37% moisture adjusted yield was positively related with the changes in fat and casein of milk. Because adjusted yield is based on a constant moisture content of 37% in cheese, this measurement is expected to reflect the changes of milk fat or casein. When moisture content was made constant, the fat content has a greater contribution to adjusted yield than casein has (Marziali and Ng-Kwai-Hang, 1986a). The possible explanation for no significant differences for both actual and adjusted yield is milk composition. As shown in Table 6, there was no difference for milk total solids and milk protein with different κ-CN/β-LG types, these would possibly resulted in less differences for actual and adjusted cheese yields with different κ-CN/β-LG types.

4. 8. 3. 2. <u>Effects of κ-CN/β-LG types on cheese composition for Canadienne milk</u>

Table 10 shows the least squares means and standard deviations of cheese compositions of Canadienne milk with different κ-CN/β-LG types. The values of total solids varied from 59.33% for AA/AA type to 63.61% for BB/AB type. Significant differences were found ($P < 0.05$) for different κ-CN/β-LG types. Total solids for BB/AB type and BB/BB types were 63.61 and 62.16% which were close to the standard total solids of 63% for Cheddar cheese. No significant difference was detected for cheese protein and cheese salt with κ-CN/β-LG types. Type BB/AB and BB/BB were associated with the highest cheese fat. Therefore milk with these two types were associated with higher cheese yield capacity.

Table 10. Cheesemaking properties of Canadienne milk with different κ-CN/β-LG types

Cheese yield

K-CN/ß-LG type	Type of yield (g/ 100g milk)		
	Actual	37 % moisture adjusted	Theoretical
AA/AA	11.36^{a} ±1.77	11.11^{a} ±1.94	12.48^{bc} ±2.37
AA/AB	11.36^{a} ±2.67	11.25^{a} ±2.28	12.12^{bc} ±1.70
AA/BB	12.14^{a} ±1.51	11.64^{a} ±1.08	12.60^{bc} ±1.37
AB/AA	12.33^{a} ±2.09	11.93^{a} ±2.08	13.56^{ab} ±2.43
AB/AB	11.44^{a} ±1.41	11.03^{a} ±1.81	12.33^{bc} ±1.55
AB/BB	11.79^{a} ±1.31	11.09^{a} ±1.35	12.99^{bc} ±2.09
BB/AA	11.60^{a} ±1.93	11.15^{a} ±2.05	11.61^{c} ±1.96
BB/AB	12.24^{a} ±1.13	12.45^{a} ±1.11	13.12^{bc} ±1.93
BB/BB	12.04^{a} ±1.37	11.83^{a} ±1.71	14.80^{a} ±4.44

Cheese composition

k-CN/ß-LG type	Cheese composition (%)			
	Total solids	Fat	Protein	Salt
AA/AA	59.33^{b}	29.58^{b} ± 5.37	23.82^{a} ± 3.38	1.33^{a} ± 0.17
AA/AB	61.96^{ab} ± 2.99	30.83^{b} ± 5.16	24.31^{a} ± 3.15	1.27^{a} ± 0.19
AA/BB	60.31^{b} ± 2.81	32.54^{ab} ± 2.40	22.53^{a} ± 2.50	1.28^{a} ± 0.14
AB/AA	60.39^{b} ± 4.10	31.23^{b} ± 6.08	24.06^{a} ± 3.57	1.32^{a} ± 0.16
AB/AB	60.53^{ab} ± 3.62	30.29^{b} ± 5.29	24.03^{a} ± 2.03	1.25^{a} ± 0.13
AB/BB	61.35^{ab} ± 3.59	31.74^{ab} ± 3.28	23.70^{a} ± 2.04	1.38^{a} ± 0.13
BB/AA	60.55^{ab} ± 3.02	29.34^{b} ± 4.43	21.15^{a} ± 4.28	1.02^{b} ± 0.14
BB/AB	63.61^{a} ± 2.17	34.85^{a} ± 2.55	23.27^{a} ± 2.84	1.30^{a} ± 0.25
BB/BB	62.16^{ab} ± 2.37	32.66^{ab} ± 3.27	22.76^{a} ± 6.68	1.12^{b} ± 0.20

Whey composition

K-CN/ß-LG type	Component (%)		
	Total solids	Fat	Protein
AA/AA	7.19^{a} ± 0.62	0.44^{a} ± 0.23	1.22^{ab} ± 0.17
AA/AB	6.97^{a} ± 2.23	0.26^{a} ± 0.16	1.25^{ab} ± 0.26
AA/BB	7.27^{a} ± 0.47	0.40^{a} ± 0.31	1.10^{bcd} ± 0.16
AB/AA	7.45^{a} ± 0.59	0.33^{a} ± 0.21	1.16^{bc} ± 0.12
AB/AB	7.42^{a} ± 0.37	0.40^{a} ± 0.25	1.08^{bcd} ± 0.20
AB/BB	7.12^{a} ± 0.88	0.28^{a} ± 0.16	0.98^{d} ± 0.14
BB/AA	7.29^{a} ± 0.25	0.23^{a} ± 0.10	1.03^{cd} ± 0.26
BB/AB	7.28^{a} ± 0.17	0.41^{a} ± 0.24	1.15^{bcd} ± 0.14
BB/BB	7.54^{a} ± 0.25	0.35^{a} ± 0.28	1.34^{a} ± 0.33

^{abcd} Values within a column with the same letters are not significantly different ($P < 0.05$).

4. 8. 3. 3. Effect of κ-CN/β-LG types on whey compositions for Canadienne milk

The least squares means and standard deviations of major cheese whey components from Canadienne milk of different κ-CN/β-LG combinations are shown in Table 10. No significant difference ($P< 0.05$) was detected for both whey total solids and whey fat. Recall that in Table 6 and Table 10, no significant differences were detected for milk total solids, milk fat, actual and 37% moisture adjusted yields with different κ-CN/β-LG types as well. As a result, there should be no differences on the milk components losses during cheese making for different κ-CN/β-LG types. Significant differences ($P< 0.05$) were detected in whey protein content with different κ-CN/β-LG types. AA/AA and AA/AB were two types to be associated with higher whey protein content of 1.22% and 1.25%. These results indicated that more milk protein were lost in the whey from milk of these two types during cheesemaking.

4. 8. 4. Associations of κ-CN/β-LG phenotype combinations with cheesemaking properties of Jersey milk

4. 8. 4. 1. Variation of cheese yield according to different κ-CN/β-LG types for Jersey milk

Least squares means and standard deviations of cheese yields for the eight types of κ-CN/β-LG are listed in Table 11. No observation was available for AA/AA type. As shown in Table 11, significant differences were found for the three kinds of cheese yields with different κ-CN/β-LG types. Types BB/AB and BB/BB were linked with higher actual, 37% moisture adjusted and theoretical cheese yields. Especially for type BB/BB, which was associated with both the highest actual and adjusted cheese yields. Similar

results were also found in Ayrshire. Brown Swiss and Canadienne milk. However, BB/BB type of Jersey had the highest 37% moisture adjusted cheese yield of 14.59 g. Note that as shown in Table 2, Jersey milk was related with higher milk protein (4.00%) and higher milk fat content (4.61%). Consequently, it was predictable for higher cheese yield from Jersey milk than cheese yields of four other breeds.

4. 8. 4. 2. <u>Effects of κ-CN/β-LG types on cheese composition for Jersey milk</u>

The least squares means and standard deviations of cheese composition for Jersey with different κ-CN/β-LG types are presented in Table 11. No observation of AA/AA type was obtained. Total solids ranged from 57.58% for AB/AA type to 59.92% for BB/AB type. No statistical difference ($P < 0.05$) was detected for total solids with different κ-CN/β-LG types. Cheese fat ranged from 28.19% for BB/AA type to 30.56% for BB/AB type. No statistical difference was found for cheese fat with different κ-CN/β-LG types. However, significant differences ($P < 0.05$) were found for cheese protein with different κ-CN/β-LG types. Type AA/BB and type AB/BB were related with higher cheese protein content of 24.32 and 24.57%, respectively. Thus, milk with these two types have higher cheese yield potential. No significant difference was detected for cheese salt with different milk types.

4. 8. 4. 3. <u>Effect of κ-CN/β-LG types on whey compositions for Jersey milk</u>

Least squares means and standard deviations for cheese whey composition of Jersey milk with κ-CN/β-LG types after correcting for milk composition are shown in Table 11. No significant difference ($P < 0.05$) was found for both whey total solids and whey fat with different κ-CN/β-LG types. Significant differences ($P < 0.05$) were found for whey protein with different κ-CN/β-LG types. Whey protein content ranged from 0.8 % for AA/AB type to 1.39 % for BB/BB. These values were higher when comparing with those in previous study on Holstein (Lou and Ng-Kwai-Hang, 1992a). Because Jersey milk has higher milk fat, milk protein, milk casein and total solids when comparing with milk composition of Holstein (PATLQ, 1996). Hence, these results could be considered in the normal range. In other words, milk composition was the possible reason for the differences.

4. 9. <u>Association of coagulating properties of milk with different κ-CN/β-LG phenotype combinations</u>

It is known that factors responsible for the variations on milk composition may cause variations in rennet clotting (RCT), rate of firming (K20) and firmness of curd at cutting (A30). In the present project, the effects of phenotype combinations of κ-CN/β-LG were considered with respect to milk composition.

The rennetability of milk is essential to cheesemaking. When the coagulum was cut, some of the fat globules and casein particles were immediately released in the whey and the extent of these components losses depend on the degree of disruption of the curd during cutting. Therefore, the monitoring of the cheesemaking processes would be necessary and helpful to minimize milk components losses and enhance the cheese yield potential.

As described in section 3 Materials and Methods, parameters used for describing the coagulating properties of milk were: rennet clotting time (RCT), rate of curd firming (K20) and curd firmness at time of cutting (A30). These parameters could be measured by a formagraph.

Table 12. Formagraphic parameters of milk with different k-CN/ß-LG phenotypes for the four breeds

Breed	k-CN/ß-LG type	Formagraphic parameters RCT(min)	K20 (min)	A30 (mm)
Ayrshire	AA/AA	10.75^{ab} ±2.83	10.67^{a} ±2.74	24.46^{b} ±9.67
	AA/AB	11.06^{a} ±4.05	10.80^{a} ±2.40	24.47^{b} ±5.47
	AA/BB	10.41^{ab} ±2.70	10.37^{a} ±2.29	24.67^{b} ±6.95
	AB/AA	8.79^{ab} ±2.38	10.46^{a} ±1.04	22.92^{b} ±5.91
	AB/AB	8.13^{ab} ±1.53	9.13^{a} ±2.25	25.14^{b} ±5.92
	AB/BB	9.44^{ab} ±2.34	10.75^{a} ±3.31	25.29^{b} ±7.70
	BB/AA	10.46^{ab} ±3.81	9.63^{a} ±1.46	25.33^{b} ±8.77
	BB/AB	7.97^{b} ±2.44	10.47^{a} ±1.41	33.44^{a} ±9.[illegible]4
	BB/BB	10.13^{ab} ±2.27	11.53^{a} ±2.04	27.13^{ab} ±5.50
Brown Swiss	AA/AB	8.67^{ab} ± 1.80	11.14^{a} ± 2.95	28.89^{b} ± 4.35
	AA/BB	8.44^{ab} ± 2.43	11.47^{a} ± 4.00	29.22^{b} ± 4.33
	AB/AA	8.33^{ab} ± 1.52	10.10^{a} ± 2.84	27.62^{b} ± 5.37
	AB/BB	9.31^{ab} ± 2.12	9.25^{a} ± 1.28	29.13^{b} ± 5.74
	BB/AA	7.78^{b} ± 1.54	10.23^{a} ± 3.56	31.10^{ab} ± 8.38
	BB/AB	8.43^{ab} ± 1.50	11.80^{a} ±2 .69	35.00^{a} ± 7.49
	BB/BB	9.58^{a} ± 1.97	10.93^{a} ± 2.44	35.78^{a} ± 5.38
Canadienne	AA/AA	8.48^{ab} ± 1.81	10.08^{a} ± 3.34	29.80^{ab} ± 6.57
	AA/AB	9.93^{ab} ± 4.41	9.40^{a} ± 2.25	28.00^{ab} ± 4.13
	AA/BB	11.00^{a} ± 3.12	10.50^{a} ± 3.73	26.70^{ab} ± 5.05
	AB/AA	9.08^{ab} ± 1.80	9.33^{a} ± 2.32	30.95^{a} ± 6.11
	AB/AB	8.92^{ab} ± 2.38	10.16^{a} ± 2.58	26.50^{ab} ± 7.11
	AB/BB	9.77^{ab} ± 1.92	9.76^{a} ± 2.99	31.64^{a} ± 7.14
	BB/AA	8.05^{b} ± 1.44	11.45^{a} ± 2.08	23.95^{b} ± 5.39
	BB/AB	8.04^{b} ± 1.77	9.60^{a} ± 1.53	27.26^{ab} ± 6.26
	BB/BB	8.85^{ab} ± 2.28	10.18^{a} ± 2.08	32.00^{a} ± 6.98
Jersey	AA/AB	10.75^{ab}	12^{a}	28.5a
	AA/BB	11.21^{ab} ± 2.54	11.07^{a} ± 2.42	25.57^{a} ± 5.80
	AB/AA	13.13^{a} ± 5.80	10.75^{a} ± 1.54	28.63^{a} ± 1.97
	AB/AB	9.28^{b} ± 2.19	9.50^{a} ± 2.28	29.81^{a} ± 6.99
	AB/BB	8.84^{b} ± 1.92	11.28^{a} ± 2.02	26.75^{a} ± 5.52
	BB/AA	8.42^{b} ± 2.05	9.45^{a} ± 2.33	32.71^{a} ± 8.74
	BB/AB	8.20^{b} ± 1.63	9.81^{a} ± 2.34	32.87^{a} ± 8.12
	BB/BB	9.32^{b} ± 1.64	11.91^{a} ± 3.91	36.89^{a} ± 7.55

^{ab} Values within a column with the same letters are not significantly different (P<0.05).

4. 9. 1. Effects of κ-CN/β-LG types on coagulating properties of Ayrshire milk

An analysis of variance was performed to investigate the influence of different κ-CN/β-LG combinations and milk composition on formagraphic properties of Ayrshire milk. Least squares means and standard deviations of RCT, K20 and A30 are presented in Table 12. For the nine types detected, RCT ranged from 8.13 min for AB/AB type to 11.06 min for AA/AB type. AA/AA, AA/AB, AA/BB and BB/AA were three types which have longer RCT ($P < 0.05$) than those for other types. These results indicated that AA type for both κ-CN and β-LG were related with longer RCT. The values of RCT in Table 19 were close to those for Holstein milk according to previous reports (Marziali and Ng-Kwai-Hang, 1986b; Paluch *et al.*, 1990; Imafidon *et al.*, 1993; Montilla *et al.*, 1995).

K20 ranged from 9.13 min for AB/AB type to 11.53 min for BB/BB type. No significant difference ($P < 0.05$) was found for K20 with different κ-CN/β-LG types.

Wide spectrum of variations were found for A30, with lower values of 25.57 mm for AA/BB type and higher value of 36.89 mm for BB/BB type. Type BB/BB and type BB/AB were associated with significant longer ($P < 0.05$) A30 values compared with those of other types. Thus, good cheesemaking properties could be expected for these two types.

4. 9. 2. Effects of κ-CN/β-LG types on coagulating properties of Brown Swiss milk

The formagraphic parameters of Brown Swiss milk with different κ-CN/β-LG types are presented in Table 12. RCT ranged from 7.78 min for BB/AA type to 9.58 min for

BB/BB type. Significant differences (P< 0.05) were found for milk with different κ-CN/β-LG types. No significant difference was found for K20 with different κ-CN/β-LG types.

A30 were different from 27.62 mm for AB/AA type to 35.78 mm for BB/BB type. BB group of κ-CN (BB/AA, BB/AB and BB/BB) had greater values (P< 0.05) when comparing with those of other types. These results indicated that in Brown milk, BB variant of κ-CN was linked with greater A30 than other types of variants. Cheese made from milk of these three types which have greater A30 would be related with better cheese yield and cheese quality.

4. 9. 3. <u>Effects of κ-CN/β-LG types on coagulating properties of Canadienne milk</u>

Table 12 shows formagraphic parameters of Canadienne milk with different κ-CN/β-LG types.

Significant differences were found for RCT with different κ-CN/β-LG types. RCT for the seven types ranged from 8.04 to 11.00 min, in which AA/AB and AA/BB were two types to be related with higher values of RCT. This indicated that κ-CN AA was associated with prolonged RCT.

No significant differences (P< 0.05) was detected for K20 with different κ-CN/β-LG types.

There were significant differences (P< 0.05) for A30 with different κ-CN/β-LG types. A30 varied in a range from 26.50 mm to 32.00 mm. AB/BB and BB/BB were two types to be linked with higher A30 values when comparing with those of other types. This reflected that milk with these two types have greater cheesemaking potential. These results were similar to those for Brown Swiss milk. The ranges of standard deviations presented in Table 12 could be attributed to casein and fat concentrations (Storry *et al.*, 1983; Grandison *et al.*, 1984a; Okigbo *et al.*, 1985b, 1985c), somatic cell counts (Politis and Ng-Kwai-Hang, 1988c), genetic polymorphism of milk protein (Schaar, 1984; Marziali and Ng-Kwai-Hang, 1986b: Pagnacco *et al.*, 1987) and heat treatments (Paluch *et al.*, 1990; Imafidon and Farkye, 1993; Montilla *et al.*, 1995).

4. 9. 4. Effects of κ-CN/β-LG types on coagulating properties of Jersey milk

An analysis of variance was carried out to study the effects of κ-CN/β-LG types and milk composition on RCT, K20 and A30 for Jersey milk and results are presented in Table 12. The effects of κ-CN/β-LG types on RCT were significant ($P< 0.01$). RCT for the eight types were different from 8.20 min to 13.13 min. AB/AA was the type which had longer RCT when comparing with other types. This again confirmed that for Jersey milk, AA of β-LG was associated with prolonged RCT. No significant difference was detected for both K20 and A30 with different κ-CN/β-LG types.

Generally, B gene of κ-CN, in milk from Ayrshire, Brown Swiss, Canadienne and Jersey, has faster rate of curd firming. The trends for the curd firmness at time of cutting were opposite to those of the rate of curd firming. These findings were also confirmed by other studies on Holstein (Schaar, 1984; Mariani and Leoni, 1985; van den Berg *et al.*, 1992; Horne *et al.*, 1994;)

The fact that the concentration of κ-CN and β-LG in milk is related to the particular genetic variants could in part account for differences observed in coagulating properties. In statistical analysis, adjustments were made for casein content in milk because casein would affect the overall structure and properties of casein micelles and thus influence coagulating properties of milk (Marziali and Ng-Kwai-Hang, 1986a). It was reported (Horne *et al.*, 1994) that κ-CN genotype does not influence the accessibility of the rennet-sensitive site in κ-CN and critical fraction of B variants than for A variant. It means that κ-CN B variant is a less effective stabilizer of the micelle than κ-CN A variant. This mechanism may partially explain rennet coagulation behavior affected by protein variants, possibly by difference of charge or degree of glycosylation of the macropeptide tail.

Type BB / BB, which was related with higher cheese yield, was also associated with faster rate of curd firming and firmer curd at cutting than milk of AA/AA. For instance, based on the results listed in Table 12, curd firmness of milk type BB/BB for Ayrshire, Brown Swiss, Canadienne were significantly greater than those of all other types. These were consistent with previous results conducted on Holstein milk (Marziali and Ng-Kwai-Hang, 1986a; Lou, 1992).

κ-CN B variant is a less effective stabilizer of the casein micelle than κ-CN A variant. This may partially explain rennet coagulation behavior affected by protein variants, possibly by difference of charge or degree of glycosylation of the macropeptide tail.

Type BB / BB, which was related with higher cheese yield, was also associated with faster rate of curd firming and firmer curd at cutting than milk of AA/AA. For instance, Based on Table 12, A30 of milk type BB/BB for Ayrshire, Brown Swiss, Canadienne were significantly greater than those of all other types. These were consistent with previous results conducted on Holstein milk (Marziali and Ng-Kwai-Hang, 1986a; Lou, 1992).

5. SUMMARY AND CONCLUSIONS

This research project was carried out to investigate the possible effects of genetic variants of κ-CN/β-LG on cheesemaking properties and coagulating properties of milk from Ayrshire, Brown Swiss, Canadienne and Jersey.

Cows from each of the four breeds were selected according to the nine possible phenotype combinations of κ-CN/β-LG. Electrophoresis techniques were employed to determine the phenotypes of κ-CN and β-LG for milk samples. After fresh milk samples were collected, Cheddar cheesemaking and chemical analysis were carried out for individual milk sample. In order to ensure that the cheesemaking processes for this project were comparable to previous studies, bulk tank Holstein milk samples were used as control for each batch of cheesemaking.

Because of differences in milk composition between breeds in this study, statistical analysis was performed separately for each of the four breeds. It was demonstrated that milk composition, cheesemaking and formagraphic properties were different between breeds in this project. The cheese yield capacity for milk of Ayrshire, Brown Swiss, Canadienne and Jersey were greater than that of Holstein milk.

Significant effects were detected ($P< 0.05$) for phenotype combinations of κ-CN/β-LG on cheesemaking and coagulating properties of Ayrshire milk. The combinations of BB/AA and BB/BB of κ-CN/β-LG were found to be associated with higher actual and 37% moisture adjusted cheese yields. They were also associated with higher cheese total solids and cheese fat content. BB/AB and BB/BB were found to be related with lower milk components losses during cheesemaking. κ-Casein/β-Lactoglobulin combination BB/AA, BB/AB and BB/BB were detected to be related with shorter rennet coagulation time and firmer curd. Therefore, good cheese quality and higher cheese yield could be expected for these three types of Ayrshire milk.

In Brown Swiss, significant effects ($P< 0.05$) were detected for phenotype combinations of κ-CN/β-LG on cheesemaking and coagulating properties of milk. Types AA/BB and BB/AA were found to be associated with higher actual and 37% moisture adjusted cheese yields. AA/AB and AA/BB were found to be linked to higher cheese total solids, cheese fat as well as cheese protein contents. BB/AB and BB/BB were related with lower milk components losses into the whey. BB/AA, BB/AB and BB/BB were found to associated with better formagraphic properties. Therefore, Brown Swiss milk with these three types are recommended for cheesemaking.

For Canadienne, it was found that BB/AB and BB/BB were associated with higher actual and 37% moisture adjusted cheese yields as well as higher theoretical cheese yield. BB/AB was detected to be associated with higher total solids, higher cheese fat and cheese protein content. BB/AB was found to be associated with lower loss of milk fat and AB/BB was found to be associated with lower milk protein loss during cheesemaking. BB/AB and BB/BB were found to be linked with better formagraphic properties.

In Jersey breed, significant effects ($P< 0.05$) were detected for phenotype combination of κ-CN/β-LG on cheesemaking and coagulating properties of milk. BB/AB and BB/BB were found to be related with higher actual and 37% moisture adjusted cheese yields. AB/BB and BB/AB were associated with higher cheese total solids, higher cheese fat and cheese protein contents. AB/AA and AB/BB were found to be associated with lower whey total solids, lower losses of milk fat and milk protein. BB/AA, BB/AB and BB/BB were found to be associated with better formagraphic properties of milk. These three types of Jersey milk were more favorable for cheesemaking.

The effects of phenotypes of κ-CN and β-LG on cheesemaking and coagulating properties when considered separately were found to be significant ($P < 0.05$) in Holstein milk. In the present study, the effects of phenotype combinations of κ-CN/β-LG on cheesemaking properties were demonstrated to be significant for milk of Ayrshire, Brown Swiss, Canadienne and Jersey.

Since it was found that several κ-CN/β-LG phenotype combinations in each of the four breeds were associated with better cheese quality and higher cheese yields, this study indicates that proper selection of genetic variants of κ-CN and β-LG in the four breeds could be of benefits to the dairy industry.

6. REFERENCES

Aaltonen, M. L. and V. Antila. 1988. Milk renneting properties and the genetic variants of protein. Dairy Sci. Abstr. 50: 3429.

Aleandri, R., L. G. Buttazzoni, J. C. Schneider. 1990. The effects of milk protein polymorphism on milk components and cheese-producing ability. J. Dairy Sci. 73: 241.

Aleandri, R., J. C. Schneider, and L. G. Buttazzoni. 1989. Evaluation of milk on cheese production based on milk characteristics and formagraph measures. J. Dairy Sci. 72: 1967.

Ali, A. E., A. T. Andrews and G. C. Cheeseman. 1980a. Influence of storage of milk on casein distribution between the micelle and soluble phases and its relationship to cheesemaking parameters. J. Dairy Res. 47: 393.

Ali, A. E., A. T. Andrews and G. C. Cheeseman. 1980b. Influence of elevated somatic cell count on casein distribution and cheese making. J. Dairy Res. 47: 371.

American Public Health Association. 1978. Standard methods for the examination of dairy products. 14th Edition. Amer. Publ. Health Assoc., Washington, D. C.

Anderson, M. and A. T. Andrews. 1977. Progressive changes in individual milk protein concentrations associated with high somatic cell counts. J. Dairy Res. 44: 223.

Andrews, A. T. 1983. Break down of caseins by proteinases in bovine milk with high somatic cell counts arising for mastitis or infusion with bacterial endotoxin. J. Dairy Res. 50: 507.

Aschaffenburg, R. 1961. Inherited casein variants in cow's milk. Nature. 192: 431.

Aschaffenburg, R. 1968. Genetic variants of milk protein, their breed distribution. J. Dairy Res. 35: 447.

Aschaffenburg, R. and J. Drewry. 1955. Occurrence of different beta-lactoglobulins in cow's milk. Nature. 176: 218.

Aschaffenburg, R. and J. Drewry. 1957. Genetics of the ß-lactoglobulins of cow's milk. Nature. 180: 376.

Aschaffenburg, R., A . Sen and M. P. Thompson. 1968. Genetic variants of casein in Indian and African zebu cattle. Comp. Biochem. Physio. l.25: 177.

Association of Official Analytical Chemists. 1980. Official Methods of Analysis. 13th Edition. Assoc. Off. Anal. Chem., Arlington, VA.

Auriol, P., M. Dupont and M. T. LeBorgne. 1961. Queques facteurs de variation du temps de coagulation des laits individuals de vaches. Ann. Biol. Anim. Biophys. 1: 152.

Baker, C. M. A., and C. Manwell. 1980. Chemical classification of cattle. 1. Breed groups. Anim. Blood Grps. Biochem. Genet., 11: 127.

Banks, J. M. 1988. Elimination of the development of bitter flavor in Cheddar cheese made from milk containing heat-denatured whey protein. J. Soc. Dairy Technol. 41: 37.

Banks, J. M., W. Banks, D. D. Muir and A. G. Wilson. 1981. Cheese yield: Composition does matter. Dairy I. Int. 46:15.

Banks, J. M., G. Stewart, D. D. Muir and I. G. West. 1987. Increasing the yield of Cheddar cheese by the acidification of milk containing heat-denatured whey protein. Milwissenschaft. 42: 212.

Banks, J. M., A. J. Law, J. Leaver and D. S. Horne. 1994. The inclusion of whey proteins in cheese - An overview. IDF Seminar on cheese yield and factors affecting it control. International Dairy Federation. Brussels, Belgium, pp.387.

Banks, J. M., W. Griffiths, J D. Phillips and D. D. Muir. 1986. The yield and quality of Cheddar produced from thermised milk. Dairy Ind. Int. 57: 31.

Banks, J. M., D. D. Muir, and A. Y. Tamime. 1984. Equations for estimation of the efficiency of cheddar cheese production. Dairy Ind. Int. 49: 14.

Banks, J. M., Stewart, D. D. Muir, and I. G. West. 1985. Increasing the yield of Cheddar cheese by the acidification of milk somatic cell count on Cheddar cheese yield. J. Dairy Sci. (Abstr) suppl. 1, 70: 91.

Barbano, D. M. and R. R. Rasmussen. 1987. Influence of milk somatic cell count on Cheddar cheese yield. J. Dairy Sci. (Abstr) suppl.1, 70: 91.

Barbano, D. M., R. R. Rasmussen and J. M. Lynch. 1991. Influence of milk somatic cell count and milk age on cheese yield. J. Dairy Sci. 74: 369.

Barbano D. M., and J. W. Sherbon. 1984. Cheddar cheese yield in New York. J. Dairy Sci. 67:1873.

Bech, A. M. and K. R. Kristiansen. 1990. Milk protein polymorphism in Danish dairy cattle and the influence of genetic variants on milk yield. J. Dairy Res., 57: 53.

Belanger, G. 1975. Nos fromages Cheddar et autres. St-Hyacinthe. Institut de technologie agricole Quebec, Agriculture Quebec.

Bell, K., H. A. McKenzie and D. C. Shaw. 1981. Bovine β-lactoglobulin E, F and G of Bali (Banteng) cattle, Bos (Bibos) Javanicus. Aust. J. Biol. Sci. 34: 133.

Braunitzer, G. and R. Chen. 1972. Die spaltung des βlactoglobulins AB mit bromcyan. Hoppe-Seyler's Z. Physiol. Chem. 353: 674.

Braunitzer G., R. Chen, B.Schrank and A. Strangl. 1972. Automatische sequenz analyse eines proteins (β-lactoglobulin AB). Hoppe-Seyler's Z. Physiol. Chem. 353: 832.

Brignon, G. and B. Ribadeau-Dumas. 1973. Localisation dans la chaine peptidique de la β-lactoglobuline bovine de la substitution Glu/Gln differenciants les variants genetique B et D. FEBS Lett. 33: 73.

Bynum, D. G. and Olson, N. F. 1982. Influence of curd firmness at cutting on Cheddar cheese yield and recovery of milk constituents. J. Dairy Sci. 65: 2281.

Callanan, T. 1994. Recovery of milk constituents in cheesemaking. In: IDF Seminar on Factors affecting yield of cheese. International Dairy Federation, Brussels, Belgium, pp. 48.

Carroll, R. J. and H. M. Farrell. 1983. Immunological approach to location of κ-casein the casein micelle by electron microscopy. J. Dairy Sci. 66: 679.

Cerbulis, J., and H. M. Farrell. 1975. Composition of milks of dairy cattle. I. Protein, lactose, and fat content and distribution of protein friction. J. Dairy Sci. 58:817.

Chaplin, B. and M. L. Green. 1980. Determination of the proportion of κ-casein hydrolyzed by rennet on coagulation of skim milk. J. Dairy Res. 47: 351.

Chaplin, B. and M. L. Green. 1982. Probing the location of casein fraction in the casein micelle using enzymes and enzyme-dextran conjugates. J. Dairy Res. 49: 631.

Cheryan, M., P. J. Van Wyk, N. F. Olson and T. Richardson. 1975. Secondary phase and mechanism of enzymatic milk coagulation. J. Dairy Sci. 58: 477.

Choi, J. 1996. Effects of genetic variants of κ-casein and β-lactoglobulin and heat treatments on cheese yielding capacity, cheese composition and coagulation properties of milk. M. Sc. Thesis. McGill University, Montreal, Canada.

Covington, C. 1994. Genetic and environmental factors affecting milk composition and their relation to cheese yield. In: Cheese Yield and Factors affecting its Control, IDF seminar. International Dairy Federation, Brussels, Belgium, pp. 76.

Cox, W. A. and G. Stanley. 1978. Starters: Purpose, production and problems. In: "Streptococci", Skinner, F. A. and Quesnel, L. B. (Eds.). Academic Press, London, pp.279-83.

Creamer, L. K., G. P. Berry and A. R. Matheson. 1978. The effect of pH on protein aggregation in heated skim milk. N. Z. J. Dairy Sci. Technol. 13: 9.

Dalgleish, D. G. 1980. A mechanism for the chymosin-induced flocculation of casein micelle. Biophys. Chem. 11: 147.

Dalgleish, D. G. 1982a. In: Food Protein , P. F. Fox and J. J. Condon (Ed.). Applied Science Publishers, London, pp.155.

Dalgleish, D. G. 1982b. The enzymatic coagulation of milk, In: Developments in Dairy Chemistry - I. P. F. Fox (Ed.). Applied Science Publishers, London, pp. 157.

Dalgleish, D. G. 1983 Coagulation of renneted bovine casein micelles: dependence on temperature, calcium ion concentration and ionic strength. J. Dairy Res. 50: 331.

Dalgleish, D G. 1987. In: Cheese: Chemistry, physics and Microbiology, Vol. 1, P. F. Fox (Ed.), Elsevier Applied Science, London, pp. 63.

Davies, D. T. and A. J. R. Law. 1977. The composition of whole casein from the milk of Ayrshire cows. J. Dairy Res. 44: 447.

Davies, D. T. and A. J. R. Law. 1980. The content and composition of protein in creamery milks in south-west Scotland. J. Dairy Res. 47: 83.

Davies, F. L., P. A. Shankar, B. E. Brooker and D. G. Hobbs. 1978. A heat-induced change in the ultrastructure of milk and its effects on gel formation in yogurt. J. Dairy Res. 45: 53.

Davoli, R., S. Dall'Olio and V. Russo. 1992. Effect of κ-casein genotype on the coagulation properties of milk. Dairy Sci. Abstr. 54: 2427.

Dumais., R. J. A. Blais and F. Conrad. 1985. Cheese. In: Dairy Science and Technology- Principles and applications. La foundation de technologie laitiere de Quebec. Inc. (Ed.). Les presses de l'universite Laval.

Elfam, A. A. and J. V. Wheelock. 1978. Heat interaction between α-la, ß-LG and casein in bovine milk. J. Dairy Sci. 61: 159.

Emmons, D. B. 1993. Definition and expression of cheese yield. IDF seminar on factor affecting yield of cheese. IDF. Brussels, Belgium, pp.12.

Emmons, D. B., C. A. Ernstrom, C. Lacroix and P. Verret. 1990. Predictive formulas for yield of cheese from composition of milk: A review. J. Dairy Sci. 73: 1365.

Emmons, D. B. B. Reiser, R N. Giroux and D. W. Stanley. 1976. Cheddar cheese made with bovine pepsin. I. Yield and quality of cheese. Can. Inst. Food Sci Technol. J. 9: 189.

Euber, J. R. and J. R. Brunner. 1982. Interaction of κ-casein with immobilized β-lactoglobulin. J. Dairy Sci. 65: 2384.

Farrell, H. M. 1988. Physical equilibria: protein. In Fundamentals of Dairy Chemistry. N. P. Wong, R. Jenness, M. Keeney and E. H. Marth (Eds). Van Nostrand Reinhold Company, New York, pp. 461-510.

Feagan, J. T. 1979. Factors affecting protein composition of milk and their significance to dairy processing. Austr. J. Dairy Technol. 34: 77.

Foss & Co. 1980 . Instruction manual for Formagraph 20-40-60-80-100. 66, Helleruprej - DK 2900 Hellerup - Denmark.

Foss Electric, A/A N. 1973. Application notes No 5 and 6 (E) Hillerod, Denmark.

Fox, P. F. 1987. Coagulation and their action. Proc. 22nd Int. Dairy Congr., The Hague, D. Reidel Publishing Co., Dordrecht, pp. 61-73.

Fox, P. F. 1989. The milk protein system. In: Food Gels. P. Harris (Ed.). Elsevier Applied Science, London.

Fox, K. K., M. K. Harper, V. H. Holsinger and M. J. Pallansch. 1967. Effects of high-heat treatment on stability of calcium caseinate aggregates in milk. J. Dairy Sci. 50: 443.

Fox, P. F. and D. M. Mulvihill. 1982. Milk proteins: molecular, colloidal and functional properties. J. Dairy Res. 49: 679.

Gaunt, S. N. 1980. Genetic variation in the yields and contents of milk constituents. In: Factors affecting the yield and contents of milk constituents of commercial importance. P. C. Moore and J. A. F. Rook (Eds.). Intl. Dairy Fed. Doc. 125.

Gilles, J., and R. C. Lawrence. 1985. The yield of cheese. N. Z. J. Dairy Sci. Technol. 20: 205.

Grandison, A. S. and G. D. Ford. 1986. Effects of variations on somatic cell count on the rennet coagulation properties of milk and on the yield, composition and quality of Cheddar cheese. J. Dairy Res. 53: 645.

Grandison, A. S., G. D. Ford, D. Millard and A. J. Owen. 1984a. Chemical composition and coagulation and coagulating properties of renneted milk from cows during the early lactation. J. Dairy Res. 51: 407.

Grandison, A. S., G. D. Ford, A. J. Owen and D. Millard. 1984b. Chemical composition and coagulating properties of renneted Friesian milk during the transition from winter rations to spring grazing. J. Dairy Res. 51: 69.

Green, M. L. 1977. Review of the progress of dairy science: milk coagulants. J. Dairy Res. 44: 159.

Grosclaude, F., M. F. Mahe and J. C. Mercier. 1974. La comparison du polymorphisme genetique des lactoproteines du Zebu et des bovins. Ann. Genet. Sel. Anim. 6: 305.

Grosclaude, F., M. F. Mahe, J. C. Mercier. J. Bonnemaire and J. H. Teissier. 1976. Polymorphisme des lactoproteines de bovines Nepalais. I. Mise en evidence, chez le yak, et caracterisation biochimique de deux nouveaux variants:β-lactoglobuline D (yak) et caseine α_{s1} E Ann. Genet. Sel. Anim. 8: 461.

Grosclaude, F., M. F. Mahe, J. C. Mercier and B. Ribadeau-Dumas. 1972. Localisation des substitutions d'acides amines differenciant les variants A et B de la caseine bovine. Ann. Genet. Anim. 4: 515.

Guinee, T. P. and P. F. Fox. 1993. Salt in Cheese: Physical, Chemical and Biological Aspects in Cheese: Chemistry, physics and microbiology, 2nd edition, Chapman and Hall, UK, pp. 257-302.

Haque, Z., and J. E. Kinsella. 1988. Interaction between heated κ-casein and ß-lactoglobulin: predominance of hydrophobic interaction in the initial stages of complex formation. J. Dairy Res. 55: 67.

Haque, Z., M. M. Kristjansson and J. E. Kinsella. 1987. Interaction between κ-casein and ß-lactoglobulin: possible mechanism. J. Agric. Food Chem. 35: 644.

Haenlein, G. F. W., L. H. Schultz and J. P. Zikakis. 1973. Composition of protein in milk with varying leucocyte counts. J. Dairy Sci. 56: 1017.

Hayes, J. F., K. F. Ng-Kwai-Hang and J. E. Moxley. 1984. Heritability of milk casein and genetic and phenotypic correlation with production traits. J. Dairy Sci. 67: 841.

Hill, R. J. and R. G. Wake. 1969. Amphiphile nature of κ-casein as the basis for its micelle stabilizing properties. Nature. 221: 635.

Hoogendoorn, M. P., J. E. Moxley, R. O. Hawes, and H. F. MacRae. 1969. Separation and gene frequencies of blood serum transferrin, casein and beta-lactoglobulin loci of dairy cattle and their effects on certain production traits. Can. J. Anim. Sci. 49: 331.

Holt, C. 1975. The stability of bovine casein micelle. In: Proceedings International Conference on Colloid and Interface Science. E. Wolfram (Ed). Akademia Kiado, Budapest, pp. 641.

Horne, D. S., D. D. Muir, J. Leaver, T. A. McCreight, J. M. Banks and A. J. R. Law. 1994. Influence of κ-casein and β-lactoglobulin on rennet coagulation of bovine milk. In: IDF Seminar on cheese yield and factors affecting its control. International Dairy Federation, Brussels, Belgium, pp. 433.

Hunziker, H. G. and Tarassuk, N. P. 1965. Chromatographic evidence for heat induced interaction of α-lactalbumin and β-lactoglobulin. J. Dairy Sci. 48: 733.

Imafidon, G. I. and N. Y. Farkye. 1993. Rennet coagulability of high-heat treated milk influenced by time of pH adjustment. J. Food Sc. 58: 1300.

Imafidon, G. I., K. F. Ng-Kwai-Hang, V. R. Harwalkar and C. Y. Ma. 1991a. Effect of genetic polymorphism on the thermal stability of β-lactoglobulin and κ-casein mixture. J. Dairy Sci. 74: 791.

Imafidon, G. I., K. F. Ng-Kwai-Hang, V. R. Harwalkar and C. Y. Ma. 1991b. Differential scanning calorimetric study of different genetic variants of β-lactoglobulin. J. Dairy Sci. 74: 2416.

International Dairy Federation. 1964. Determination of the casein content of milk . Int. Dairy Fed. Stand. No. 29, Brussels, Belgium.

Jakob, E. and Z. Puhan. 1987. The significance of genetic variants of κ-casein for rennetability of milk. Dairy Sci. Abstr. 49: 3915.

Jang, H. D. and H. E. Swaisgood. 1990. Disulfide bond formation between thermally denatured κ-casein and β-lactoglobulin in casein micelles. J. Dairy Sci. 73: 900.

Jenness, R. 1974. The composition of milk. In: Lactation Vol. III, B. L. Larson and V. R. Smith (Eds.), Academic Press, New York, pp.3.

Jenness, R. 1988. Composition of milk. In: Fundamentals of Dairy Chemistry (3rd Edition). N. P. Wong (Ed.) Van Nostrand Reinhold Company. New York, pp.3.

Jenness, R., 1974. The composition of milk. In Lactation, Vol. III. B. L. Larson and V. R. Smith (Eds). Academic Press, New York, pp. 3-107.

Jenness, R.,1985. Biochemical and nutritional aspects of milk and colostrum. In Lactation. B. L. Larson (Ed). Iowa State University Press, pp. 164-197.

Jennsen, G. K. and H. Stapelfeldt. 1994. Incorporation of whey protein in cheese. In: IDF Seminar on factors affecting yield of cheese International Dairy Federation, Brussels, Belgium.

Johnson, D. E., R. J. Murphy and N. R. Whitaker. 1983. Effects of thermization and cold storage on some properties of rennet-induced milk gels. J. Dairy Res. 50: 231.

Jolles, J. P. and C. Alais. 1969. Present knowledge concerning the amino acid sequence of cow κ-casein. Nature. 222: 668.

Keller, B., N. F. Olson and T. Richardson. 1974. Mineral retention and rheological properties of Mozzarella cheese made by direct addification. J. Dairy Sci. 57: 174.

Kiddy, C. A. Rollins, R. E. and Zikakis, J. P. 1972. Discontinuous polyacrylamide electrophoresis for β-lactoglobulin of cow milk. J. Dairy Sci. 55: 1506.

Kim, S. 1995.Genetic polymorphism of milk proteins and their association with production traits in Ayrshire, Jersey, Brown Swiss, and Canadienne. M. Sc. Thesis. McGill University, Montreal, Canada.

Kolde, H. J. and G. Braunitzer. 1983. The primary structure of ovine β-lactoglobulin 1. Isolation of the peptides and sequence. Milchwissenschaft. 38:18.

Kowalchyk, A. W. and N. F. Olson. 1977. Effects of pH and temperature on the secondary phase of milk clotting by rennet. J. Dairy Sci. 60: 1256.

Kroeker, E. M., K. F. Ng-Kwai-Hang, J. F. Hayes and J. E. Moxley. 1985a. Effect of environmental factors and milk protein polymorphism on composition of casein fraction in bovine milk. J. Dairy Sci. 68: 1752.

Kroeker, E. M., K. F. Ng-Kwai-Hang, J. F. Hayes and J. E. Moxley. 1985b. Heritabilities of relative percentages of major bovine casein and serum proteins in test-day milk samples. J. Dairy Sci. 68: 1346.

Kirchmeir, O., A. Mehana, R. Graml and J. Buchberger. 1982. Influence of casein genotypes on the water-binding capacity and the heat stability of casein. In: XXI Int. Dairy Congress. Brief Communications. Moscow. Vol. 1. Book 1, pp.195.

Kristiansen, K. R. 1991a. Effects of genetic variants of the milk proteins on composition of milk and technological properties. Report from The Danish Government research Institute for Dairy Science, Hillerod, Denmark, pp. 46.

Kristiansen, K. R. 1991b. Influence of genetic variants on milk composition and technological properties. Dairy Sci Abstr. 53: 2849.

Kosikowski, F. 1977. Cheddar cheese and related types. In: Cheese and related types In: Cheese and fermented milk foods 2nd edition. Kosikowski, F. (Ed.). Edwards Brothers, Inc. Ann Arbor, Michigan, pp.228.

Kolde, H. J. and G. Braunitzer. 1983. The primary structure of ovine β-lactoglobulin 1. Isolation of the peptides and sequence. Milchwissenschaft. 38: 18.

Lau, K. Y., D. M. Barbano and R. R. Rasmussen. 1990. Influence of pasteurization on fat and nitrogen recoveries and Cheddar cheese yield. J. Dairy Sci. 73: 561.

Lau, K. Y., D. M. Barbano and R. R. Rasmussen. 1991. Influence of pasteurization of milk on protein breakdown in Cheddar cheese during aging. J. Dairy Sci. 74: 727.

Lin, C. Y., A. J. McAllister, K. F. Ng-Kwai-Hang , and J. F. Hayes. 1986. Effects of milk protein loci on first lactation production in dairy cattle. J. Dairy Sci. 69: 704.

Lin, C. Y., A. J. McAllister, K. F. Ng-Kwai-Hang , J. F. Hayes, T. R. Batra, A. J. Lee, G. L. Roy, J. A. Vesley, J. M. Wauthy and K. A. Winter. 1989. Relationships of milk protein types to lifetime performance loci on first lactation production in dairy cattle. J. Dairy Sci. 72: 604.

Losi, G., G. B. Castagnetti, and D. Morini. 1975. Importanza delle varianti genetiche della κ-caseina sulla formazione e sulla caratteristiche della cagliata. Sci. e Tecn. degli Alim. 3: 373.

Losi G., G. B. Castagnetti and Morini. 1979. Le varianti genetiche della caseina κ-e attitudine del latte alla coagulazione presamica. Il Latt. 4:1.

Losi, G. and P. Mariani. 1985. The technological significances of milk protein polymorphism for the manufacture of Grana cheese. Dairy Sci. Abstr. 47: 1570.

Lou, Y. 1991. Effects of milk composition on cheesemaking and coagulation properties. M. Sc. Thesis. McGill University, Montreal, Canada.

Lou, Y. and K. F. Ng-Kwai-Hang. 1992a. Effects of protein and fat levels in milk on Cheddar cheese yield. Food Res. Intl. 25: 437.

Lou, Y. and K. F. Ng-Kwai-Hang. 1992b. Effects of protein and fat levels in milk on cheese and whey compositions. Food Res. Intl. 25: 445.

Macheboeuf, D., J. B. Cloulon and P. d'Hour. 1993. Effects of breed, milk protein variants and feeding on cow's milk coagulation properties. J. Dairy Res. 60: 43.

Mariani, P. and M. Leoni. 1985. Il tempo di coagulazione del latte in rapporto alle varianti genetiche dell caseine β et κ. Anne. Fac. Med. Vet., Univ. Parma. 5: 195.

Mariani, P., G. Losi, D. Morini and G. B. Castagnetti. 1979a. Il contenuto di acido citrico nel latte di vacche con genotipo diverso nel locus κ-caseina. Scienza e Tecn. Latt. - Cas. 30 (5): 1.

Mariani, P., D. Mori, G. Losi, G. B. Castagnetti, E. Fossa and V. Russo. 1979b. Ripartitizatione delle frazioni azotate del latte in vacche caratterizzate da genotipo diverse nel locus β-lacttoglobulina. Scienza e Tecn. Latt.-Cas. 30 (3): 153.

Mariani, P., M. Pecorari and E. Fossa. 1982. Le caratteristiche di coagulazione del latte in rapporto allo stado della Lattaione ed ai livelli di produzione. Scienza e Tecn. Latt.-Cas. 33 (5): 409.

Mariani, P., M. Pecorari and E. Fossa and S. Fieni. 1981. Diffusione del latte a coagulazione anomala e rapporti con il cotenuto cellulare e l'acidit a titolabile. Scienza e Tecn. Latte.-Cas. 32 (4): 222.

Marshall, R. J. 1982. An improved method for measurement of the syneresis of curd formed by rennet action on milk. J. Dairy Res. 49: 329.

Marshall, R. J. 1986. Increasing cheese yields by high heat treatment of milk. J. Dairy Res. 53: 313.

Marziali, A. S. 1985. Effects of genetic variants of milk protein on cheese yield capacity, cheese composition and coagulation properties of milk. M. Sc. Thesis. McGill university, Montreal, Canada.

Marziali, A. S. and K. F. Ng-Kwai-Hang. 1986a. Relationship between milk protein polymorphism and cheese yield capacity. J. Dairy Sci. 69: 1193.

Marziali, A. S. and K. F. Ng-Kwai-Hang. 1986b. Effects of milk composition and genetic polymorphism on cheese on cheese composition. J. Dairy Sci. 69: 1793.

Marziali, A. S. and K. F. Ng-Kwai-Hang. 1986c. Effects of milk composition and genetic polymorphism on coagulating properties of milk. J. Dairy Sci. 69: 2533.

Mayes, J. J. and B. J. Sutherland. 1984. Coagulum firmness and yield in Cheddar cheese manufacture - The role of the curd firmness instrument in determining cutting time. Austr. J. Dairy Technol. 39: 69.

McLean, D. M. 1987. Influence of milk protein genetic variants on milk composition, yield and cheesemaking properties. Animal genetics 18 (Suppl. 1): 100.

McLean, D. M., E. R. B. Graham, R. W. Ponzoni and H. A. Mckenzie. 1984. Effects of milk protein genetic variants on milk yield and composition. J. Dairy Res. 51: 531.

McLean, D. M. and J. Schaar. 1989. Effects of ß-lactoglobulin and κ-casein genetic variants and concentrations on syneresis of gel from renneted heated milk. J. Dairy Res. 56: 297.

McMahon, D. J. and R. J. Brown. 1984. Enzymatic coagulation of casein micelles: A review. J. Dairy Sci. 67: 919.

McMahon, D. J. G. H. Richardson and R. J. Brown. 1984. Enzymatic milk coagulation: role of equations involving coagulation time and curd firmness in describing coagulation. J. Dairy Sci. 67: 1185.

Mercier, J. C., G. Brignon and B. Ribadeau-Dumas. 1973. Structure primaire de la casein B bovine. Sequence complete. Eur. J. Biochem. 35: 222.

Mercier, J. C. and J. Vilotte. 1993. Structure and function of milk protein gene. J. Dairy Sci. 76: 3097.

Montilla, A., E. Balcones, A. Olano and M. M. Calvo. 1995. Influence of heat treatments on whey protein denaturation and rennet clotting properties of cows and goat's milk. J. Agric. Food Chem. 43: 1908.

Morini, D., G. B. Castagnetti, C. Chiavari, L. Grazia, G. Losi, R. Davoli and P. Bosi. 1982. Prova di caseificazione con latte caratterizzato dalle varianti A e B della β-lattoglobulina nella produzione del formaggio parmigiano-regiano. Sci. Tecn. Latt. -Cas. 33: 475.

Morini D., G. Losi, G. B. Castagnetti, and P. Mariani. 1979. Prove di caseificazione con latte caratterizzato dalle varianti A e B dell κ-caseina: rilievi sul formaggio stagionato. Sci. Tecn. Latt.-Cas. 30: 243.

Moxley, J. E. and K. F. Ng-Kwai-Hang. 1984. A study of milk composition- cheese yield relationships in the Canadian cheddar cheese industry. Final report to Canadian Dairy Commission. Macdonald College of McGill University, Ste-Anne-de-Bellevue. Quebec.

Munro, G. L., P.A. Grieve and B. J. Kitchen. 1984. Effects of mastitis in milk yield, milk composition, processing properties and yield and quality of milk products. Austr. J. Dairy Technol. 39: 7.

Ng-Kwai-Hang, K. F. 1994. Genetic variants of milk proteins and cheese yield. In: IDF Seminar on cheese yield and factors affecting its control. International Dairy Federation, Brussels, Belgium, pp. 160.

Ng-Kwai-Hang, K. F. and F. Grosclaude. 1992. Genetic polymorphism of milk protein. In: Advanced Dairy Chemistry Vol. 1: Protein. P. F. Fox (Ed.), Elsevier Science Publishers Ltd., London.

Ng-Kwai-Hang, K. F., and J. F. Hayes, J. E. Moxley and H. G. Monardes. 1982. Environmental influences on protein content and composition of bovine milk. J. Dairy Sci. 65: 1993.

Ng-Kwai-Hang, K. F., and J. F. Hayes, J. E. Moxley and H. G. Monardes. 1984a. Association of genetic variants of casein and milk serum proteins with milk, fat and protein production by dairy cattle J. Dairy Sci. 67: 835.

Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley and H. G. Monardes. 1984b. Variability of Test-day milk production and composition and relation of somatic cell counts with yield and compositional changes in bovine milk. J. Dairy Sci. 67: 361.

Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley and H. G. Monardes. 1985. Percentage of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. J. Dairy Sci. 68: 1257.

Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley and H. G. Monardes. 1986. Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. J. Dairy Sci. 69: 22.

Ng-Kwai-Hang, K. F., J. F. Hayes , J. E. Moxley and H. G. Monardes. 1987a. Variation in milk protein concentration associated with genetic polymorphism and environmental factors. J. Dairy Sci. 70: 563.

Ng-Kwai-Hang, K. F., and E. M. Kroeker. 1984. Rapid separation and quantification of major caseins and whey proteins of bovine milk by polyacrylamide gel electrophoresis. J. Dairy Sci. 67: 3052.

Ng-Kwai-Hang, K. F., H. G. Monardes and J. F. Hayes. 1990. Association between genetic polymorphism of milk protein and production traits during three lactations. J. Dairy Sci. 73: 3414.

Ng-Kwai-Hang, K. F., J. E. Moxley and A. S. Marziali. 1987b. Gross composition of milk and Cheddar yields in some Quebec cheese factories. Can. Inst. Food Sci. Technol. J. 20: 372.

Ng-Kwai-Hang, K. F., J. E. Moxley and A. S. Marziali. 1988. Cheddar cheese composition in some Quebec cheese factories. Can. Inst. Food Sci. Technol. J. 21: 80.

Ng-Kwai-Hang, K. F., I. Politis, R. I. Cue and A. S. Marziali. 1989. Correlation between coagulation properties of milk and cheese yield capacities and cheese composition. Can. Inst. Food Sci. Technol. J. 22: 291.

Ohashi, T., S. Haga, K. Yamauchi, H. Katayama and N.F. Olson. 1982. Seasonal variations in physical properties of milk rennet curd. Japanese J. Zootech. Sci. 53: 445.

O'Keeffe, A. M., J. A. Phelan, K. Keogh and P. Kelly. 1982. Studies of milk composition and its relationship to some processing criteria. IV. Factors influencing the renneting properties of a seasonal milk supply.

Okigbo, L. M., G. H. Rechardson, R. J. Brown, and C. A. Emstrom. 1985a. Variation in coagulation properties of milk from individual cows. J. Dairy Sci. 68: 822.

Okigbo, L. M., G. H. Rechardson, R. J. Brown, and C. A. Emstrom. 1985b. Casein composition of cow's milk of different chymosin coagulation properties. J. Dairy Sci. 68: 1887.

Okigbo, L. M., G. H. Rechardson, R. J. Brown, and C. A. Emstrom. 1985c. Effects of pH, calcium chloride, and chymosin concentration on coagulation properties of abnormal and normal milk. J. Dairy Sci. 68: 2527.

Olson, N. F. 1977. Factors affecting cheese yields. Dairy Industries International. 42 (4): 14-15, 19.

Olson, N. F. 1982. Cheese making procedures that affect yield. 5th Bienn. Cheese Ind. Conf., Utah State Univ., Logan.

Pabst, K., D. Prokopek, U. Krusch, H. Wietbrauk and E. Krell. 1991. Composition of milk and milk quality. Bundesanstalt fur Milchforshung kiel, Germany-Annual Report 1991. B6-B7.

Pagnacco, G. and A. Caroli. 1987. Effects of casein and β-lactoglobulin genotypes on renneting properties of milks. J. Dairy Res. 54: 479.

Paluch L. T., M, E. Johnson, B. Riesterer and N.F. Olson. 1990. Cheddar cheese manufactured with milk HTST pasteurized at 73.3 ^{0}C, 75.6 ^{0}C and 77.8 ^{0}C. J. Dairy Sci. 73 (Suppl. 1): 115.

Parry, R., M., Jr. 1974. Milk coagulation and protein denaturation. Page 603 in Fundamentals of dairy Chemistry. B. H. Webb and A. H. Johnson (Eds.). AVI Publishing Co., Westport, Connecticut.

Payens, T. A. J. 1966. Association of caseins and their possible relation to structure of the casein micelle. J. Dairy Sci. 49: 1317.

Payens, T. A. J. , A. K. Wiersms, and J. Brinkhuis. 1977. Biophysical Chemistry. 6: 253.

Programme d'analyse des Troupeaux Laitiers du Quebec (PATLQ).1996. In: Rapport de production, pp.18.

Politis, I., and K. F. Ng-Kwai-Hang. 1988a. Effects of somatic cell counts and milk composition on cheese yield and cheese making efficiency. J. Dairy Sci. 71: 1711.

Politis, I., and K. F. Ng-Kwai-Hang. 1988b. Association between somatic cell count level of milk and cheese yielding capacity. J. Dairy Sci. 71: 1720.

Politis, I., and K. F. Ng-Kwai-Hang. 1988c. Effects of somatic cell counts and milk composition on coagulating properties of milk. J. Dairy Sci. 71: 1740.

Puhan, Z. and E. Jakob. 1994. Genetic variants of milk proteins and cheese yield. In: IDF seminar on cheese yield and factors affecting its control. International Dairy Federation. Brussels, Belgium, pp.111.

Rahali, V. and J. L. Menard. 1991. Effect of genetic variants of ß-lactoglobulin and κ-casein on the milk composition and suitability for cheesemaking. Dairy Sci Abstr. 53: 4017.

Rahali, V. and J. L. Menard. 1992. Influence of genetic variants of ß-lactoglobulin and K-casein on milk composition and suitability for cheesemaking. Dairy Sci. Abstr. 54: 2432.

Rahali, M., A. Caroli, P. Bolla and G. Pirlo. 1989. Relationships of milk protein genotypes with casein composition and rennetability of milk during lactation. Dairy Sci. Abstr. 51: 2430.

Rampilli, M., A. Caroli, P. Bolla and G. Pirlo. 1989. Relationships of milk protein genotypes with casein composition and rennetability of milk during lactation. Dairy Sci. Abstr. 51: 1321.

Rasmussen, R. R. and D. M. Barbano. 1987. Influence of milk somatic cell count on Cheddar cheese composition, starter culture activity, and make time. J. Dairy Sci. (Abstr.), suppl..1, 70: 91.

Richardson, G. H. 1985. Increasing cultured quality and yield through careful strain selection and propagation. Cultured Dairy Products J. 20: 20.

Robitaille, G., K. F. Ng-Kwai-Hang and H. G. Monardes. 1993. Effects of κ-casein glycosylation on cheese yielding capacity and coagulating properties of milk. Food Res. Intl. 26: 365.

Rollema, H. S. 1992. Casein association and micelle formation. In: Advanced Dairy Chemistry- Vol. 1: Proteins. P. F. Fox (Ed.), Elsevier Science Publishers Ltd., London, pp. 61.

Rook, J. A. F. 1961. Variation in the chemical composition of the milk of the cow. Dairy Sci. Abstr. 23: 251.

Rose, D. 1969. A proposed model of micelle structure in bovine milk. Dairy Sci. Abstr. 31: 171.

Sacchi, P., E. Cauvin and R. M. Turi. 1993. Relationships between milk protein polymorphism and production traits during two lactations in Friesian cows rared in Turin area. Scienza e Tecinca Latt.-Cas. 44: 20.

SAS. 1988. Statistical Analysis System Institute. SAS User's Guide: Statistic. SAS Institute, Cary, N C.

Schafer, H. W. and N. F. Olson. 1975. Characteristics of Mozzarella cheese made by direct acidification from ultra high temperature processed milk. J. Dairy Sci. 58: 494.

Sawyer, W. H., S. T. Coutler and R. Jenness. 1963. Role of sulfhydryl groups in the interaction of κ-casein and β-lactoglobulin. J. Dairy Sci. 46: 564.

Schaar, J. 1984. Effects of κ-casein genetic variants and lactation number on the renneting properties of individual milks J. Dairy Res. 51: 397.

Schaar, J., B. Hansson and H. E. Pettersson. 1985. Effects of genetic variants of κ-casein and β-lactoglobulin on cheesemaking. J. Dairy Res. 52: 429.

Schmidt, D. G. 1979. Properties of artificial casein micelles. J. Dairy Res. 46: 351.

Schmidt, D. G. 1982. Association of casein micelles and casein micelle structure. In: Developments in Dairy Chemistry - I. P. F. Fox (Ed.). Applied Science Pub., Essex. England, pp. 61.

Schmidt, R. H. and H. A. Morris. 1984. Gelation properties of milk proteins, soy proteins, and blended protein systems. Food Technol. 38: 85.

Singh, H. and P. F. Fox. 1987a. Heat stability of milk: influence of colloidal and soluble salts and protein modification on the pH-dependent dissociation of micellar κ-casein. J. Dairy Res. 54: 509

Singh, H. and P. F. Fox. 1987b. Heat stability of milk; role of β-lactoglobulin in the pH-dependent dissociation of micellar κ-casein. J. Dairy Res. 54: 523.

Slattery, C. W. and R. Evard. 1973. A model for the formation and structure of casein micelles from subunits of variable composition. Biochem. Biophys. Acta 317: 529.

Smietana, Z., S.Poznanski, J. Jakubowski, J. Rembaoz, Z.Rybka and J. Kowalewska. 1976. Technological and characteristics of Italian cheese and Halian servit. Dairy Sci. Abstr. 38: 1434.

Smits, P. and J. H. van Brouwershaven. 1980. Heat-induced association of β-lactoglobulin and casein micelles. J. Dairy Res. 47: 313.

Storry, J. E., A. S. Grandison, D. Millard, A. J. Owen, and G. D. Ford. 1983. Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant J. Dairy Res. 50: 215.

Swaisgood, H. E. 1973. The caseins. CRC Critical Reviews in Food Technology. 3: 375.

Swaisgood, H. E. 1982. Chemistry of milk protein. In: Developments in Dairy Chemistry - I. P. F. Fox (Ed.). Applied Science Publishers. London, pp.1.

Tessier, H., M. Yaguchi and D. Rose. 1969. Zonal ultra centirfugation of κ-CN and ß-LG complexes induced by heat. J. Dairy Sci. 52:139.

Thompson, M. P. and H. M. Farrell Jr. 1974. Genetic variants of milk proteins. Page 109 in Lactatin. Vol. III. B. L. Larson and V. R. Smith (Eds.). Academic Press, New York, NY.

Thomwen, D. and H. Stapelfeldt. 1990. Yield and quality of Danbo 45+ cheese from milk heat treated at temperatures between 72 and 95 ^{0}C. Dairy Sci. Abstr. 52: 7094.

Tobias, J., R. M. Whitney and P. Tracy. 1952. Electrophoretic properties of milk protein. II. Effect of heating to 300 F by means of the Mallory Small-tube heat exchanger on skim milk proteins. J. Dairy Sci. 35: 1036.

Tong, P. S., S. Vink, N. Y. Farkye and J. F. Medrado. 1994. Effect of genetic variants of milk protein on the yield of Cheddar cheese. IDF Seminar on cheese yield and factors affecting its control. International Dairy Federation, Brussels, Belgium, pp. 179.

Trautman, J. C. and A.M. Swanson. 1958. Additional evidence of a stable complex between ß-LG and κ-CN. J. Dairy Sci. 41: 715.

van den Berg, G., J. T. M. Escher, P. J. de Koning and H. Bovenhuis. 1992. Genetic polymorphism of κ-casein and β-lactoglobulin in relation to milk composition and processing properties. Neth. Milk. Dairy J. 46: 145.

Van Slyke, L. L. and W. V. Price. 1949. Cheese. Orange Judd Publishing Co. Inc. New York.

Verret, P., C. Lacroix, C. Toupin and D. B. Emmons. 1989. Relationships between the milk quality and the cheese quality and yield. J. Dairy Sci. (Abstr.) Suppl. 1. 72: 177.

Vink, S., P. S. Tong, N. Y. Farkye, G. I. Imafidon and J. Medrano. 1993. Genetic variants of K-casein and ß-lactoglobulin and their effect of Cheddar cheese manufacture. Dairy Sci. Abstr. 55: 3179.

Walstra, P. 1990. On the stability of casein micelle. J. Dairy Res. 73: 1965-79.

Walstra, P., A. Noomen and T. J. Geurts. 1987. Dutch-type varieties. In: Cheese: Chemistry, Physics and Microbiology. Vol. 2: Major Cheese Groups. P. F. Fox (Ed.), Elsevier Applied Science, London, pp. 45.

Walstra, P. and R. Jenness. 1984. Dairy Chemistry and Physics. John Wiley and Sons, Publishers, New York, NY.

Wong, N. P. 1974. Cheese Chemistry. In: Fundamentals of Dairy Chemistry 2nd. ed., p.722. Webb, B. H. Johnson, A. H. and Alford. J. A. (eds), Westport, Connecticut, The AVI Publishing Company, Inc.

Woodhouse, L. R. and Lonnerdal, B. 1988. Quantitation of the major whey proteins in human milk, and the development of a techniques to isolate minor whey proteins. Nutr. Res. 8: 853.