N° 28/02.
Chimie 1

von Milchproteinen. Die erfolgreiche Trennung und Identifizierung von Molkenproteinen und Caseinen zeigt, daß mit diesem Verfahren komplexe biologische Systeme analysiert werden können.

FRANZEN, M., PABST, K., SCHULTE-COERNE, H., GRAVERT, H.O.: **Quantitative chromatographic separation of milk proteins.** Milchwissenschaft **50** (9) 483–488 (1995).

24 Milk proteins (separation)

Samples of milk were taken from 778 black pied cows and 368 cows of the Angler breed during the period from January to May 1990, and analysed concerning their protein fraction contents by means of HPLC. It was found that the protein fraction contents varied considerably between cows. They depended on the herd level, the stage of lactation and the cows' age as well. The High Performance Ion-Exchange Chromatography of the major bovine milk proteins is an alternative method to the electrophoresis with densitometry for the qualitative and quantitative analysis of milk proteins. The successful separation and identification of whey proteins and caseins showed us that with this method complex biological systems could be analysed.

Influence of proteolytic activity on agglutination behaviour of mesophilic starter cultures

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1. Introduction

The starter culture is an important element in the production of high quality dairy foods (1). Manufacturers are concerned about the selection of starter cultures which will perform properly in milk tanks, *i.e.* produce a sufficient amount of lactic acid within a reasonable time. Any delay in acid production will affect the processing steps and the quality of the final products.

Natural inhibitors in bovine milk have been recognized for their impact on starter culture performance. These inhibitors were originally called lactenins and were classified into 3 groups. Lactenin-1 was identified as the fat globule agglutinin (2) and lactenin-2 as lactoperoxidase (3). These two lactenins were thought to be active against certain strains of *Lactococcus* (4). Lactenin-3 includes the naturally occurring immunoglobulins "agglutinins" (5-9). Many researchers have postulated that these agglutinins are always present in milk and cause slow acid production of starter cultures. Agglutination of certain strains of *Lactococcus lactis* subsp. *cremoris* and subsp. *lactis* in pasteurized skim milk was associated with uneven or slow acid development (6), sludge formation and settling of bacteria to the bottom of the cheese vat, and a shattered grainy curd (5). Therefore, agglutination can affect quality and yield of dairy products.

Agglutination resulted from the interaction of starter culture cells with agglutinins to form long chains and clumps of cells. STOLLERMAN and EKSTEDT (10) reported that the formation of long chains of bacterial cells was a result of binding agglutinins into the bacterial cell wall. This binding action caused inhibition of protease leading to the formation of long bacterial chains. EKART *et al.* (11) found that many protease-negative (Prt^-) strains were more susceptible to agglutination than protease-positive (Prt^+) variants. Microscopic observations showed that agglutination-sensitive cultures occurred as long chains, whereas agglutination-resistant cultures were distributed as pairs or short chains (7, 11).

Fermented dairy foods are popular in the Middle East. The addition of starter culture gives the desired characteristics of these products (1). Starter cultures are usually grown and propagated in skim milk on a daily basis which affects their performance in fermentation tanks (12). Slow or uneven acid production of starter cultures has been observed recently in cheese industry. Bacteriophage infection were thought to be the problem. However, microscopic examination showed that these starter cultures formed considerably long chains and clumps of chains. Therefore, the objectives of this research were to evaluate a number of commercial lactic acid cultures used in dairy industry for their agglutination behavior and to understand the relationship between protease activity and starter culture-agglutination.

2. Materials and methods

2.1 Bacterial strains

A series of 24 commercially freeze-dried or concentrated freeze-dried mesophilic starter cultures of different bacterial compositions were provided by the following dairy starter manufacturers: Sanofi-Bio-Industries, Hamm, Germany; Laboratorium Visby, Tønder aps, Denmark; Miles-Marschall cultures, Frankfurt, Germany, and Chr. Hansen's Laboratorium A/S, Horsnolm, Denmark. These cultures are routinely used for cheese making. Also 3 single strains were obtained from Dr. M.S. Haddadin, University of Jordan. Cultures used in this research were coded A-ZI (Table 1).

2.2 Preparation of starter media

Nonfat dry milk (NDM) was reconstituted (12 % wt/vol) with distilled water and autoclaved at 106°C for 8 min. After cooling to room temperature (approxi-

mately 25 °C), the skim milk samples were stored at 4 °C until used for propagation of starter cultures, which was usually within 24 h.

2.3 Preparation of starter culture

Each culture was inoculated (1 %) into 10-ml aliquot of sterilized reconstituted NDM. All cultures were incubated for 8 h at 25 °C. The cultures were then propagated 3 times. First propagation was achieved in 10-ml reconstituted NDM, and this propagation was transferred into 50-ml of sterilized NDM. Five milliliters of the second propagation were transferred into 100-ml of sterilized NDM and incubated at 25 °C for 15 h, or until a pH of at least 5.1 was achieved.

2.4 Preparation of skim milk

Fresh raw bovine milk was obtained from the University of Jordan dairy farm prior to each trial. Milk was separated (about 0.1 % fat) and pasteurized at 62 °C for 30 min. After pasteurization, skim milk was cooled and refrigerated at 4 °C for 24 h. immediately before each trial, milk was warmed to 31 °C.

2.5 Agglutination testing procedure

The extent of culture agglutination was monitored by the determination of pH differential in skim milk and by direct microscopic examination (simple stain) for each fermented starter culture. Each fermented starter culture was inoculated at 5 % level into fresh pasteurized skim milk kept in a 2000-ml graduated cylinder and incubated at 31 °C. Agglutination was monitored by measuring pH (pH meter Model HI 8416, Hanna Instruments, Limena, Italy) at top and bottom of the graduated cylinder at the end of 5 h of incubation. The pH differential was computed by subtracting the bottom pH from the top pH. A pH difference of 0.1 or greater between top and bottom was used as an evidence of agglutination. Agglutination was confirmed by determining cells per chain distribution in the cylinder using direct microscopic count. Samples were taken from the bottom of each graduated cylinder, stained and examined under the microscope for cell morphology. Starter culture which had clumps of cells or long chains (more than 30 cells per chain) was considered agglutination-positive (+). A culture that did not form long chains or clumps of cells was agglutination-negative (–) (7). This experiment was replicated at least 3 times.

2.6 Determination of proteolytic activity of starter culture

Five agglutination-sensitive cultures and 2 agglutination-resistant cultures were selected for further study on agglutination behavior in skim milk. These selected cultures showed consistent response to agglutination in all replications. The purpose was to determine the relationship between culture agglutination and protease activity of starter cultures. The proteolytic activity of selected cultures was determined by measuring free amino groups using trinitrobenzene sulfonic acid test (TNBS) (13). Absorbances were converted to micro moles of free amino groups per ml of milk using a standard curve of glycine solution (0.1N).

2.7 Statistical analysis

Data were analyzed using General Linear Model (GLM) of SAS (14) to determine differences in pH and proteolytic with respect to agglutination behavior of various starter cultures.

3. Results and discussion

3.1 Agglutination model

In order to study culture agglutination, it was important to optimize conditions for this phenomenon. Therefore, this research was undertaken using skim milk as a bulk medium and several commercial starter cultures which were commonly used in dairy industry. To maximize the severity of agglutination, starter cultures were propagated several times. Sensitive starter cultures produced by this technique developed extended chains and clumps of cells when inoculated into cheese vats. These chains and clumps of cells fall to the bottom of the cheese vat causing severe agglutination problems which affect the quality of cheese curd.

3.2 Classification of starter culture according to agglutination response

Table 1 shows culture classification according to agglutination behavior of mesophilic lactic starter

Table 1: Culture classification according to agglutination reaction

Culture	Agglutination response[1,2]				Classification
Mesophilic homofermentative cultures[3]					
A	+	+	+		Sensitive
B	+	+	+		Sensitive
C	+	+	+	+	Sensitive
D	+	+	+	+	Sensitive
E	+	+	+	+	Sensitive
F	+	+	+	+	Sensitive
G	*	*	–		Moderate
H	+	+	+	–	Moderate
I	+	+	+	+	Sensitive
J	–	+	+		Moderate
K	+	–	+	–	Moderate
L	–	–	+	–	Moderate
M	+	–	+	+	Moderate
N	–	–	–	–	Resistant
O	+	+	–		Moderate
P	–	–	+		Moderate
Q	+	+	+		Sensitive
R	–	–	+		Moderate
Mesophilic aromatic cultures[4]					
S	+	+	–		Moderate
T	+	+	+		Sensitive
U	–	+	+		Moderate
Single strain cultures					
Lactococcus lactis ssp. lactis					
X	–	–	+		Moderate
Y	–	–	–		Resistant
Z	–	+	+		Moderate
Lactococcus lactis ssp. cremoris					
X1	+	+	–	+	Moderate
Y1	+	+	+	–	Moderate
Z1	+	+	+		Sensitive

[1] All evaluations were replicated at least 3 times
[2] Average pH differential, for sensitive cultures 0.28, for moderate cultures 0.14, and for resistant cultures 0.04.
[3] Contain selected strains of L. lactis ssp. lactis and ssp. cremoris
[4] Contain selected strains of L. lactis ssp lactis and ssp. cremoris, and Leuconostoc cremoris or lactis

cultures. Each culture could be classified into 1 of 3 groups according to agglutination reaction. Sensitive cultures were those which always agglutinated (all replications) in all skim milk samples. Cultures that agglutinated in some replications were classified as moderate strains. The third group, resistant cultures, had no agglutination problem (7–9). Two cultures (N and Y) (7.4 %) were agglutination-resistant cultures (Table 1).

Cultures N and Y, mesophilic homofermentative cultures, produced either single cells or short chains when inoculated into skim milk. Ten cultures (A, B, C, D, E, F, I, Q, T, and ZI) (37 %) caused severe agglutination and formed clumps of cells and long chains when grown in skim milk. Fifteen cultures (56 %) showed variable reaction to agglutination and were called moderate cultures (Table 1) .

3.3 Agglutination response using pH differential

Previous research defined agglutination as top/bottom pH differential greater than 0.09 units ($P < 0.05$) (7, 15). MILTON *et al.* (8) defined agglutination by a top/bottom differential greater than 0.20. In this work the degree of agglutination was determined when pH differential between top and bottom of the graduated cylinder of skim milk was greater than 0.10 ($P < 0.01$).

After 5 h of incubation at 31 °C, agglutination resistant cultures produced a top/bottom pH differential of 0.06 and –0.03 for N and Y, respectively (Table 2).

Table 2: Relationship between pH differentials and protease activity of some starter cultures[1]

Cultures	pH differentials[2]	Protease activity[2, 3]
A	0.20[a]	0.26[a,d]
B	0.16[a]	0.22[a]
C	0.17[a]	0.31[b,d]
D	0.15[a]	0.20[a]
Zi	0.28[a]	0.36[b]
N	0.06[b]	0.52[c]
Y	–0.03[b]	0.66[c]

[1] Overall means of duplicate experiments (Standard error=0.04)
[2] Means in the same column followed by the same letter are not significantly different ($P < 0.05$)
[3] µmoles/ml

Agglutination-sensitive cultures, on the other hand, had a top/bottom pH differential greater than 0.1 ($P<0.01$). All agglutination-sensitive and -resistant cultures had average pH differentials that characterized their sensivity to agglutinin in milk (Table 1).

3.4 Microscopic examination

After skim milk was inoculated with starter culture and incubated for 5 h in a water bath at 31 °C, samples taken from the bottom of the graduated cylinders were Gram-stained and examined under the microscope for cell morphology. The microscopic examination of the agglutination-resistant cultures showed that these cultures formed short chains (Fig. 1), whereas agglutination-sensitive cultures formed considerably long chains (Fig. 2). When agglutination resistant culture was grown in skim milk, very

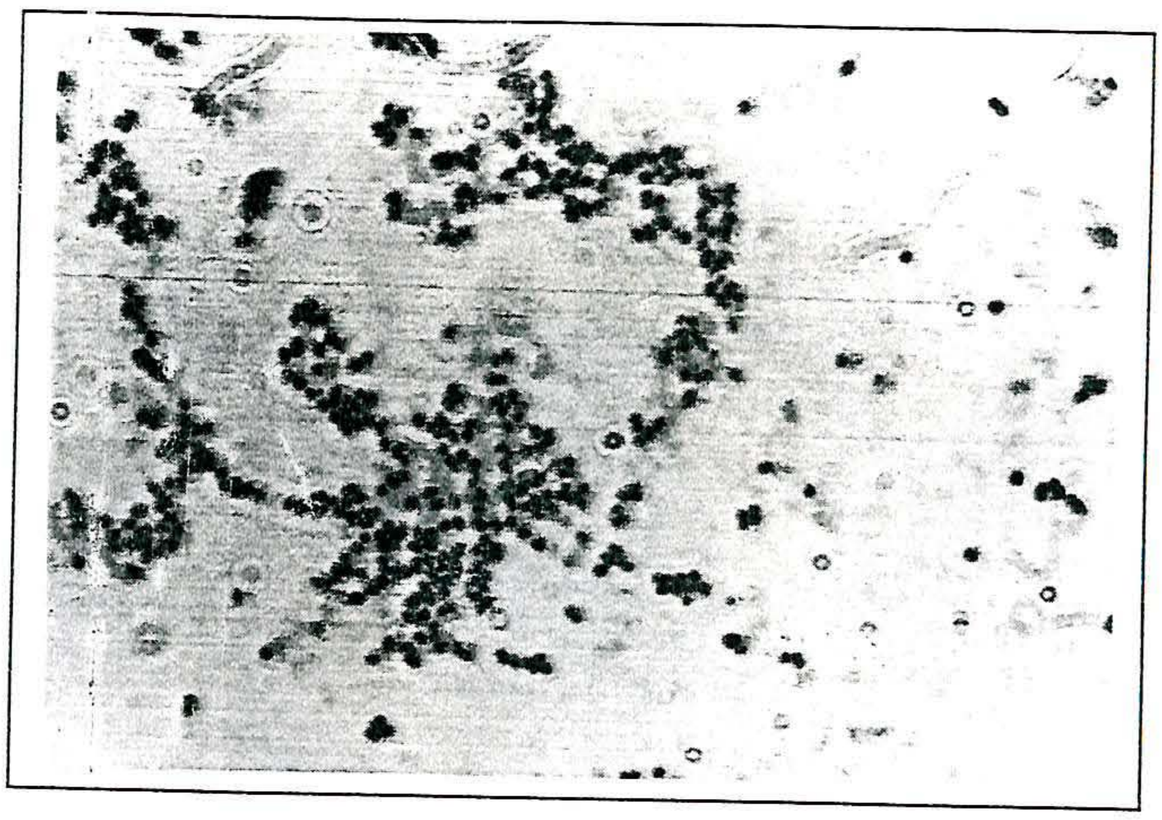

Fig. 1: Microscopy of agglutination-resistant culture showing single cells and short chains grown in skim milk after 5 h of incubation at 31 °C. (X 1000)

Fig. 2: Microscopy of agglutination-sensitive culture showing chains and clumps of cells grown in skim milk after 5 h of incubation at 31 °C. (X 1 000)

short chains or diplococci were observed. These short chains and cells were evenly distributed in the milk.

EKART *et al.* (11) and HICKS and IBRAHIM (7) indicated that the degree of starter agglutination was associated with the length of bacterial chains. The formation of long chains was associated with agglutination-sensitive cultures, whereas short chains were associated with agglutination-resistant cultures (Fig. 1 and 2). These agglutination resistant cultures were evenly distributed throughout skim milk and improved acid production, particularly at the top of the graduated cylinders.

Our microscopic examinations also support the data on pH differential discussed previously and show that microscopic examination can be considered as a simple and rapid method for testing starter agglutination.

3.5 Protease activity of selected starter cultures

After 5 h of incubation at 31 °C, the proteolytic activities of selected cultures (A-D, Z1, N, and Y) were measured using TNBS assay and recorded as mg glycine liberated per ml of fermented milk (Table 2). The amount of glycine released varied from 0.2

µg/ml for culture D to 0.66 µg/ml for culture Y. These values agreed with the values obtained from the pH differential measurements and cell morphology. The level of TCA-soluble free amino groups in agglutination-sensitive cultures were lower ($P < 0.1$) than those of resistant cultures (N and Y). Agglutination-sensitive cultures (A, B, C, D and Z1) formed long chains and had low protease activity (Table 2). Increased chain lengths is believed to result from lowered proteolytic activity (Table 2). MCDONALD (16) indicated that long chains of lactococci resulted from the failure of protease to separate daughter cells. STOLLERMAN and EKSTEDT (10) reported that long chains of group A streptococci resulted from the interaction between bacterial cells and agglutinins. This reaction resulted in the inhibition of some enzymes involved in cell division leading to the formation of long chains. PEARCE *et al.* (17) also found that low proteolytic cultures were more susceptible to agglutination than high proteolytic cultures. This suggests that protease could be involved in agglutination behavior of starter cultures. It is believed that the presence of active protease is required for cell growth and division, and therefore, could be an important element in prevention of culture agglutination.

This study shows that culture agglutination could be a potential problem in both, bulk media and dairy products. The selection of agglutination-resistant cultures is a desirable and practical approach. Screening for agglutination-resistant starter cultures was the most effective technique for the prevention of agglutination problems (18). Agglutination-resistant cultures can improve the quality of dairy food and increase cheese yield, whereas agglutination-sensitive cultures can cause many problems in dairy industry. Low proteolytic activity of agglutination-sensitive cultures results in the development of long chains. When the long chains and clumps of bacterial cells become large enough, they fall to the bottom of the cheese vat forming culture-casein complex. This complex forms a sediment layer which lowers the quality and yield of the fermented dairy foods. ROBINSON (19) observed the presence of non-dispersible particles in yoghurt and named this defect nodulation. It is believed that this defect could be due to the presence of inhibitory substances, such as agglutinins. More research is needed to characterize the agglutination behavior of yoghurt culture.

4. Conclusions

This work revealed that proteolytic activity of agglutination-sensitive cultures was lower than those of agglutination-resistant cultures and shows that the direct microscopic examination can be considered as a rapid method for testing starter agglutination. Research continues in our laboratory to evaluate different methods for the detection of starter agglutination in both bulk media and skim milk.

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5. References

(1) HADDADIN, M.S.: In Developments in Food Microbiology. (Ed. R.K. Robinson) Elsevier, New York, N.Y. (1987)
(2) WRIGHT, R.C., TRAMMER, J.: Dairy Res. **24** 124-128 (1957)
(3) WRIGHT, R.C., TRAMMER, J.: Dairy Res. **25** 105-110 (1959)
(4) RANDOLPH, H.E., GOULD, I.A.: J. Dairy Sci. **51** 8-15 (1968)
(5) ELLIOTT, J.A., EMMONS, D.B.: Proc. 1st Biennial Marshall Internat. Cheese Conf., Madison, WI, p. 577 (1979)
(6) EMMONS, D.B., ELLIOTT, J.A., BECKETT, D. C.: J. Dairy Sci. **49** 1357-1362 (1966)
(7) HICKS, C.L., IBRAHIM, S.A.: J. Food Sci. **57** 1086-1092 (1992)
(8) MILTON, K.J., HICKS, C.L., O'LEARY, J., LANGLOIS, B.E.: J. Dairy Sci. **73** 2259-2268 (1990)
(9) SCHEUBLE, T.L., KONDO, J.K., SALIH, M.A.: J. Dairy Sci. **72** 1103-1111 (1989)
(10) STOLLERMAN, G.H., EKSTEDT, R.: J. Exp. Med. **106** 345-349 (1957)
(11) EKART, L.A., O'LEARY, J., HICKS, C.L.: J. Dairy Sci. **69** (Suppl. 1) 68 (Abstr.)(1986)
(12) ROBINSON, R.K., ALI, M.Z.: S. Afr. J. Dairy Technol. **17** 65-71 (1985)
(13) MCKELLAR, R.C.: J. Dairy Sci. **64** 2138-2145 (1981)
(14) SAS/StatTM User's Guide: Statistics, Version 5 Ed.. SAS Inst., Cary, NC (1985)
(15) IBRAHIM, S.A., HICKS, C.L.: J. Dairy Sci. **73** (Suppl. 1) 76 (Abstr.) (1990)
(16) MCDONALD, I.J.: Can. J. Microbiol. **17** 897-902 (1971)
(17) PEARCE, L.E., SKIPPER, N.A., JARVIS, B.D. : Appl. Microbiol. **27** 933-937(1975)
(18) THUNELL, R.K, SANDINE, W.E., BODYFELT, F.W.: J. Dairy Sci. **67** 11751180 (1984)
(19) ROBINSON, R.K.: Dairy Ind. Int. **46** 31-35 (1981)

6. Summary

IBRAHIM, S.A.: **Influence of proteolytic activity on agglutination behavior of mesophilic starter cultures.** Milchwissenschaft **50** (9) 488–492 (1995).

26 Starter cultures (agglutination)

Twenty-seven mesophilic starter cultures were grown separately in reconstituted nonfat dried milk and then inoculated into pasteurized skim milk (2000 ml in a graduated cylinder) at 5 % inoculum level. Inoculated skim milk samples were incubated in a waterbath at 31 °C for 5 h. At the end of the incubation period samples were taken from top and bottom of each graduated cylinder and analyzed for agglutination response using pH differential and direct microscopic examination. The proteolytic activity of 5 selected agglutination-sensitive and 2 agglutination-resistant cultures was determined using trinitrobenzene sulfonic acid (TNBS). Ten cultures (37 %) showed severe agglutination and 15 cultures (56 %) had moderate agglutination problems. Proteolytic activity of agglutination-sensitive cultures was lower than that of agglutination-resistant cultures. It was concluded that high proteolytic activity might be an

important element in agglutination behavior of starter cultures.

IBRAHIM, S.: **Einfluß der proteolytischen Aktivität auf das Agglutinierungsverhalten mesophiler Säurewecker.** Milchwissenschaft **50** (9) 488–492 (1995).

26 Säurewecker (Agglutination)

27 mesophile Säurewecker wurden getrennt in rekonstiuierter Trockenmagermilch gezüchtet und sodann in pasteurisierte Magermilch (2000 ml in einem Meßzylinder) in einer Konzentration von 5 % inokuliert. Die inokulierten Magermilchproben wurden in einem Wasserbad bei 31 °C für 5 h inkubiert. Am Ende der Inkubationzeit wurden Proben oben und unten in jedem Meßzylinder genommen und auf Agglutinierungsreaktionen mit pH-Differential- und direkter mikroskopischer Untersuchung analysiert. Die proteolytische Aktivität der 5 ausgewählten agglutinationssensitiven und 2 agglutinationsresistenten Kulturen wurden mit Trinitrobenzol-sulfonsäure (TBNS) bestimmt. 10 Kulturen (37 %) wiesen eine starke Agglutination auf, 15 Kulturen (56 %) hatten ein mäßiges Agglutinierungsproblem. Die proteolytische Aktivität der agglutinationssensitiven Kulturen war niedriger als die von agglutinationsresistenten Kulturen. Es wurde gefolgert, daß eine hohe proteolytische Aktivität ein wichtiges Element im Agglutinationsverhalten von Säureweckern sein könnte.

Cheddar cheesemaking and rennet coagulation characteristics of bovine milks containing κ-casein AA or BB genetic variants

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1. Introduction

The association between genetic variants of milk protein and milk production and processing properties has been extensively reviewed (1, 2, 3, 4). Cheesemaking involving Parmigiano-Reggiano, Svecia, Edam and Gouda and rennet coagulation studies have reported higher cheese yields, shorter rennetting times, faster rates of curd formation and higher curd firmness with the κ-casein BB variant (2, 3, 5, 6, 7, 8).

Little information is available on the effect of κ-casein variants on Cheddar cheesemaking, apart from 3 reports on small-scale laboratory Cheddar cheesemaking experiments (9, 10, 11). Much attention is currently focussed on the genetic selection of cows for the production of milks suited to the manufacture of specific products including cheese (3). The objective of the current study was to investigate the rennet coagulation and Cheddar cheesemaking characteristics of bovine milks containing either κ-casein AA or BB variants.

2. Materials and methods

2.1 Milk supply

Milk was collected from 14 mid-lactation, spring-calving, Holstein Friesian cows from the Moorepark herds. Seven cows had genotype AA for κ-casein, and 7 had genotype BB. Cows were divided into 7 pairs matching each AA genotype cow with a corresponding BB genotype cow with respect to calving date, lactation number and milk yield. All were genotype AB for β-lactoglobulin. Milks from κ-casein AA or BB cows were separately collected over a 3 d period (given the low frequency occurrence of κ-casein BB phenotypes (1, 2, 3, 4)), bulked and stored at 4°C. Aseptically taken milk samples from the bulked milks were tested twice daily during the collection period for total bacterial count (TBC) (12).

2.2 Manufacture of Cheddar cheese

Cheddar cheeses were made, in parallel, from bulked κ-casein AA and κ-casein BB milks on 4 separate occasions between the 24th of June and the 7th of July 1994. A total of 8 vats of Cheddar cheese were made (4 each for κ-casein AA and BB containing milks). Milks were standardised to a protein to fat ratio of 0.96:1.00. Milks (320–400 kg) were weighed to the nearest 0.10kg into 500 l cheese vats and inoculated with 1.4 % (w/w) of *Lactococcus lactis* subsp. *cremoris* 227 and *Lc. lactis* subsp. *cremoris* 303 (Chr. Hansen's Laboratory (Ireland) Ltd.). After a ripening period of 30 min, chymosin (Double Strength Chy-max, 50,000 MCU/ml, Pfizer Inc, Milwaukee, WI, USA) was added at a level of 0.07 ml/kg milk. Cheddar cheeses were made according to GUINEE *et al.* (13).

2.3 Sampling and mass balance

Representative samples (14) from the milk vats were taken immediately prior to the addition of starter and analysed for composition and rennet coagulation properties (within 2 h).

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

curds were collected, pooled, preserved (as above) and stored for subsequent analysis.

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

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

2.4 Milk analyses

Fat, protein and lactose were determined by the Infra-red Milk Analyser (Milkoscan, Foss Electric, Hillerod, Denmark) which was calibrated by the IDF method (15).

A formagraph instrument (Model 11700, Foss Electric, Hillerød, Denmark) (16) was used to assess the rennet coagulation properties of standardised, pasteurised (72°C, 15 s) milks with the different genetic variants. Milk (10 ml) was tempered at 31°C, and 66 µl of chymosin was added (1:100 dilution of Double Strength Chy-max; Pfizer Inc, Milwaukee, WI, USA). The rennetted milk was incubated at 31°C and the coagulation properties monitored over a 60 min period. Milks were also adjusted to pH 6.60 before measurement in order to eliminate the potential effects of different natural pH values on rennet coagulation properties (5). The pH values were adjusted using lactic acid (0.5 N) except in the case of trial 3 where the κ-casein AA milk was adjusted using NaOH (0.1N).

2.5 Whey analyses

Whey samples were analysed for fat by the Röse-Gottlieb method (17), protein by the Kjeldahl method (18) and fines by a modification of the NIZO method (19). In this modification the sediment was poured on to pre-dried and weighed Whatman GF/F filter paper which was then dried and reweighed.

2.6 Cheese analyses

Cheese was analysed for pH (20), moisture (21), fat (22), salt (23) and protein (24) at 14 d.

2.7 Statistical methods

Apart from the K_{20} and A_{60} values at natural pH (Table 2) which were analysed using Friedman's test, the rennet coagulation properties, composition, yield and recovery data (Tables 1, 2, 3, 4) were examined using the two-tailed paired t-test.

3. Results and discussion

3.1 Microbiological analyses

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

3.2 Milk composition

The composition of the raw milks is given in Table 1. No significant difference (p = 0.984) was observed in the fat content between κ-casein AA and κ-casein BB milks. These results are in general agreement with those of JAKOB (3). Similar to the findings of others (2, 3, 7), the protein content of the κ-casein AA and BB milks were numerically different, with the latter displaying a higher protein percentage (Table 1). However, the difference was not significant (p = 0.072).

Table 1: Composition of bovine milks containing κ-casein AA or BB genetic variants

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

SED, Standard error of difference
NS, not significant (p > 0.05)

3.3 Rennet coagulation properties

The effects of the κ-casein variants on the rennet coagulation properties of standardised, pasteurised milks at both natural and adjusted pH (*i.e.* pH 6.60) values are shown in Table 2. The rennet coagulation times (RCTs) were longer for κ-casein AA than κ-casein BB at both natural (p = 0.050) and adjusted pH (p=0.004). These observations are in agreement with those of others for raw milk at natural (1) and adjusted (25) pH values. Moreover, the RCT values for pH adjusted raw (data not shown) and pasteurised milks were generally shorter than those of the corresponding non-pH adjusted milks. When studying the effect of κ-casein variant on the RCT, it is

Table 2: Rennet coagulation properties of standardised, pasteurised milks with κ-casein AA or BB genetic variants at natural and adjusted (pH = 6.60) pH values

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

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