# Influence of genetic variants of κ-casein and β-lactoglobulin in milk on proteolysis in Cheddar cheese

By G.I. IMAFIDON[1,3], N.Y. FARKYE[1,4], P.S. TONG[1] and V.R. HARWALKAR[2]

[1] Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, CA 93407
[2] Centre for Food and Animal Research, Agriculture Canada, Ottawa, Ontario, Canada
[3] Present address: USDA, ARS, SRRC, 1100 Robert E. Lee Blvd, New Orleans, LA 70124
[4] Corresponding author

## 1. Introduction

Genetic variants of all the major milk proteins influence the composition (3, 13) and processing characteristics of milk (8, 11, 19), and the composition and yield of cheese therefrom (11, 13, 23). Differences in milk protein variants are reported (14, 18) to affect the sensory qualities of cheese also. SCHAAR *et al.* (18) reported that soluble-N levels in cheese made from β-LG AA milk were higher than in cheese from β-LG AB or BB milks. However, van den BERG *et al.* (19) found that genetic variants of β-LG or κ-CN in milk did not significantly affect the levels of soluble-N or amino-N in Gouda cheese during ripening. Because flavor development of rennet-coagulated cheeses, like Cheddar, is greatly influenced by the rate and extent of proteolysis that occur during ripening (5), it is necessary to ascertain the effects of genetic variants of milk proteins on cheese proteolysis. Therefore, the purpose of this study was to evaluate proteolysis in Cheddar cheese manufactured from milk containing different genetic variants of β-LG and κ-CN.

## 2. Materials and methods

### 2.1 Cheddar cheese samples

Three replicates of Cheddar cheese manufactured for another study (21) were obtained and used for this investigation. The cheese was manufactured by the traditional 4.25 h method (20) using single-strength calf rennet as coagulant. The milk used for cheese manufacture contained the following milk protein variants: κ-CN A + β-LG A, κ-CN A + β-LG B, κ-CN A + β-LG AB, κ-CN AB + β-LG A, κ-CN AB + β-LG AB, or κ-CN AB + β-LG B. Cheese made from bulk milk obtained from a mixed herd served as control. The cheeses were ripened at 8°C, and sampled after 1 d, and 1, 3 or 6 mo of ripening for assessment of proteolysis.

### 2.2 Assessment of proteolysis

Proteolysis in cheese was followed by polyacrylamide gel electrophoresis (PAGE), and by analysis of water-soluble extract (WSN) from the cheese. The WSN was prepared as described by KUCHROO and FOX (9). The % N in the extract was quantified by the semi-macro-Kjeldahl method (1). Freeze-dried portions of the WSN were analyzed by PAGE, high-performance liquid chromatography (HPLC), or fast protein liquid chromatography (FPLC).

PAGE of the cheese was performed as described by FARKYE *et al.* (4) using the Mini-PROTEAN II™ (Bio-Rad, Hercules, CA) system. The gels were run for 1 h 10 min at 200 V. The gels were fixed and stained in acetic acid/methanol/ water, 7/40/53, containing 1.4 mM of Coomassie brilliant blue R250. Gels were destained in acetic acid/methanol/water, 7/40/53.

Reversed-phase (RP) HPLC was run on the System Gol™ (Beckman Instruments, Fullerton, CA) setup, which consisted of 2 model 110B solvent delivery pumps, sample injector, 20 µl loop, and a model 116 UV/VIS detector. An Ultraspher™-ODS guard column, 4.6 mm x 4.5 cm, attached to an Ultraspher™-ODS (5 µm, 80 Å) analytical column, 4.6 mm x 25 cm (Beckman), were used for the analysis. A binary solvent system: A, 0.1 % (v/v) trifluoroacetic acid (TFA) in water, and B, 0.1 % TFA (v/v) in 75 % acetonitrile was used. Samples were eluted by a stepwise gradient from 0 → 18 → 35 → 45 → 80 → 0 % B for 5, 5, 15, 30, 5, and 40 min, respectively, at a flow rate of 0.75 ml/min. Total run time was 100 min. The eluate was monitored at 230 nm.

For HPLC, 5.0 mg of the freeze-dried WSN were dissolved in 1 ml of solvent A. The resultant solution was filtered successively through 0.2 µm membrane filter (Gelman Sciences, Ann Arbor, MI), and a Sep-pak™ C18 plus cartridge (Waters Chromatography Division, Milford, MA), and 20 µl of the filtrate was loaded onto the column.

FPLC of the WSN extracts was done using a Mono-Q HR5/5 anion exchange column (Pharmacia Ltd, Montréal, Canada). The freeze-dried WSN extracts were re-hydrated to 6.3 % solids, and 200 µl of the solution was loaded onto the column. Chromatography was run using a nonlinear gradient of buffers A (0.01 M Imidazole/HCl (pH 7) containing 8 M urea) and B (buffer A plus 1 M NaCl). The gradient was varied as follows: A = 0. 4.0, 7.5, 10.0, 24.0, 28.0, 32.0, 32.0, and 35.0 ml which corresponded to % B = 0, 0, 12.5, 14.8, 35.0, 100.0, 100.0, 0, and 0, respectively. Samples were eluted at a flow rate of 1 ml/min and the eluate monitored at 280 nm.

### 2.3 Statistical analysis

The Kjeldahl WSN data were analyzed by least-squares analysis of variance using the SAS computer software package (SAS Institute Inc., Cary, NC).

## 3. Results and discussion

### 3.1 Water-soluble nitrogen

Table 1 shows the mean ± S.E. of the WSN content of the cheeses. Average WSN levels increased at similar rates in all cheeses. At each ripening period, the differences in % WSN were not statistically significant ($P > 0.05$), suggesting that the levels of WSN in the cheeses were not affected by the genetic types of κ-CN (A or AB) or β-LG (A, AB or B). Similar results were reported by van den BERG *et al.* (19) who found that genetic variants of κ-CN or β-LG did not influence the levels of soluble-N or amino-N in Gouda cheese.

**Table 1: Water-soluble N[1] levels during ripening of Cheddar cheese containing different variants of κ-casein and β-lactoglobulin**

| Type of protein variants in cheese | Water-soluble N/total N (%) 1 d | 1 mo | 3 mo | 6 mo |
|---|---|---|---|---|
| Control (bulk milk) | 4.56±0.45 | 13.69±0.25 | 20.45±0.33 | 29.34±0.74 |
| κ-CN A+β-LG A | 6.15±0.59 | 13.18±0.35 | 19.83±0.67 | 27.74±0.85 |
| κ-CN A+β-LG AB | 5.19±0.61 | 11.90±0.63 | 18.37±0.47 | 25.85±0.43 |
| κ-CN A+β-LG B | 5.50±0.76 | 12.06±0.62 | 19.36±0.65 | 27.17±0.49 |
| κ-CN AB+β-LG A | 4.92±0.20 | 11.39±0.09 | 19.51±0.65 | 29.32±2.15 |
| κ-CN AB+β-LG AB | 5.43±0.21 | 12.52±0.30 | 21.49±0.78 | 27.06±2.10 |
| κ-CN AB+β-LG B | 5.67±0.28 | 12.05±0.09 | 21.43±0.31 | 28.08±1.07 |

[1]Means ± SE of duplicate determinations on 3 cheeses

Because of scarcity, enough milk containing κ-CN B could not be obtained for inclusion in this study. From a limited number of cheese making trials using milk containing κ-CN B, we found that the % WSN in κ-CN BB cheeses were slightly lower (15.44 to 16.29 % ) than those in κ-CN AA (18.83 to 19.83 % ) or κ-CN AB (19.51 to 21.49 % ) cheeses after 3 mo of ripening. MORINI *et al.* (13) and SCHAAR *et al.* (18) also reported that cheese made from κ-CN BB milk had a slower rate of proteolysis than that made from κ-CN AA milk.

### 3.2 Electrophoresis

Figs. 1 A, B and C show typical electrophoretic patterns of the cheeses after 1 d, 3 mo or 6 mo of ripening, respectively. The most distinct difference is the band, x, which was present between the bands corresponding to $\gamma_1$- and $\gamma_3$-CN in the gels of κ-CN AB cheeses (lanes 5, 6 and 7), but absent from the gels of κ-CN AA cheeses (lanes 1 to 4). Similarly, electophoretograms of the WSN fraction from κ-CN AA or AB cheeses showed differences in the intensities of some bands (Fig. 2). For example, after 3 mo of ripening, bands a to e (Fig. 2) were of higher or lower intensities in the gels of the WSN from κ-CN AA cheeses than in κ-CN AB cheeses. This suggests that the specificities of the major proteolytic enzymes (*i.e.*, residual rennet or plasmin) toward caseins differ in cheese containing different milk protein variants.

### 3.3 FPLC of water-soluble fraction

Results of FPLC analysis of the WSN in the cheeses are shown in Table 2. The peptide peaks

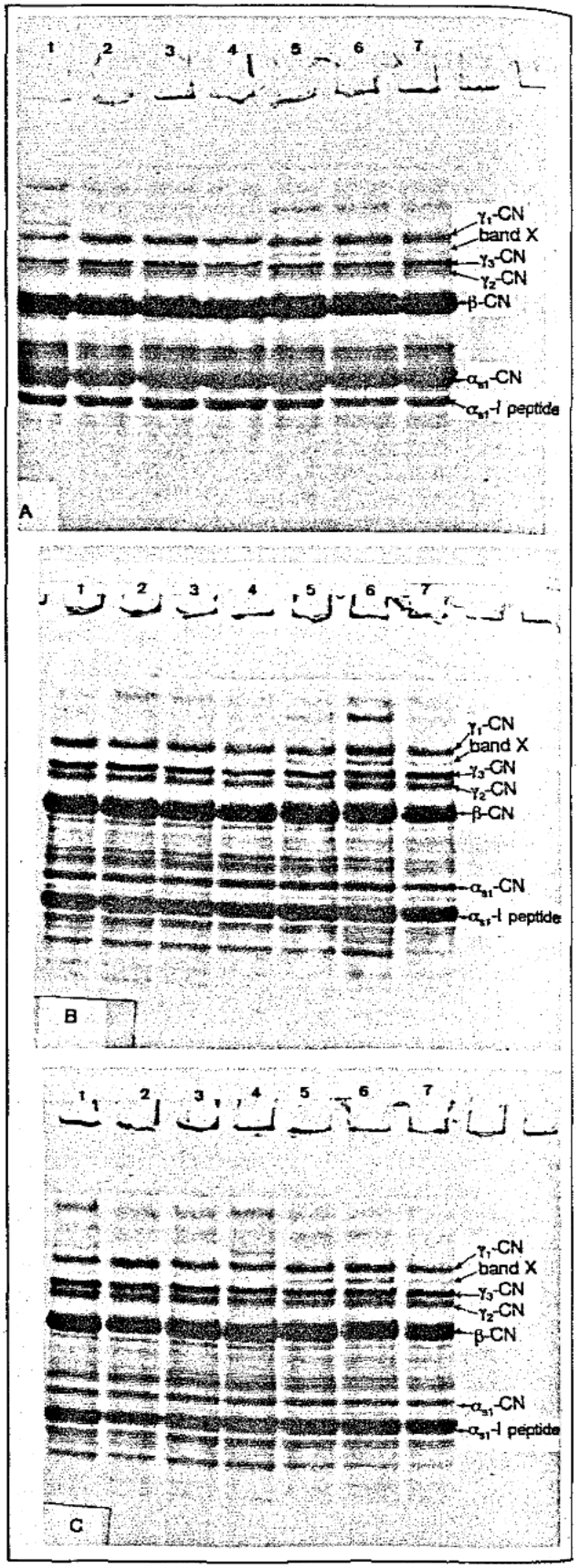


Fig. 1: Urea-PAGE (12 % T, 4 % C, pH 8.3) of Cheddar cheese made from control milk (1), or milk containing κ-CN A + β-LG A (2), κ-CN A + β-LG AB (2) , κ-CN A + β-LG B (4), κ-CN AB + β-LG A (5), κ-CN AB + β-LG AB (6), κ-CN AB + β-LG B (7) after ripening 1 d (A), 3 mo (B), or 6 mo (C) of ripening.

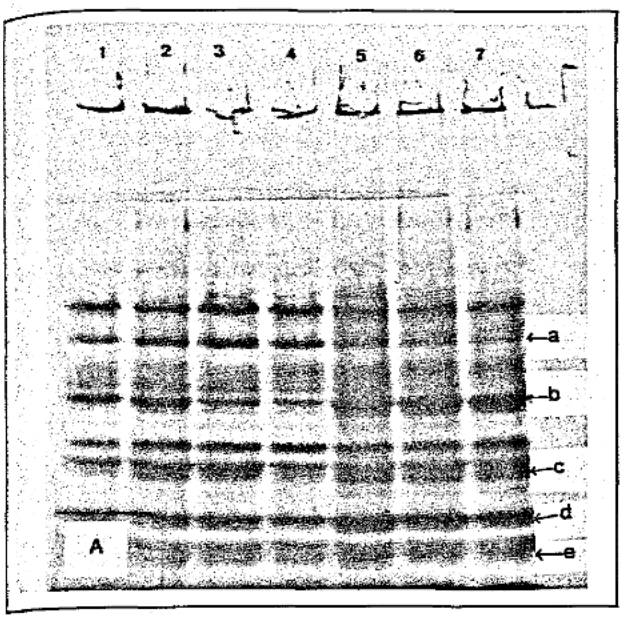


Fig. 2: Urea-PAGE (12 % T, 4 % C, pH 8.3) of water-soluble N extracts from Cheddar cheese made from control milk (1), or milk containing κ-CN A + β-LG A (2), κ-CN A + β-LG AB (2) , κ-CN A + β-LG B (4), κ-CN AB + β-LG A (5), κ-CN AB + β-LG AB (6), κ-CN AB + β-LG B (7) after 3 mo of ripening.

were subdivided into 3 groups, on the basis of their retention times (Rt) of 0 to 7.5 min, 7.5 to 16 min, and >16 min, which corresponds to peptides characterized as hydrophobic, intermediate, or very ionic, respectively. Most of the peaks had Rt of < 7.5 min, suggesting a high concentration of hydrophobic peptides in the WSN fraction of the cheeses. The differences between FPLC profiles of the WSN from cheeses containing the different milk protein variants were minimal. However, after 1 mo of ripening, peak areas for the intermediate peptides were lower in the WSN from cheese containing κ-CN A than in those containing κ-CN AB.

### 3.4 RP-HPLC of water-soluble fraction

Table 3 summarizes quantitative RP-HPLC data for the WSN from the cheeses. The number of peptides peaks and their corresponding areas were different among the different cheeses. In general, the average number of peptide peaks in the WSN from κ-CN AA cheeses were more than those from κ-CN AB cheeses, suggesting that proteolysis in κ-CN AA cheeses was more extensive than in κ-CN AB cheeses. Fig. 3 shows typical RP-HPLC chromatograms of the WSN from the cheeses. The most distinct difference in the chromatograms is the peak, c, which was prominent in 3- or 6-mo-old cheeses containing κ-CN A, but was almost lacking in cheeses containing κ-CN AB.

The percentage of peptide peaks that were eluted in 0.1 % TFA were 36 to 42 % after 1 d, and 10 to 11 % after 6 mo of ripening in cheese containing κ-CN A + β-LG variants. The corresponding values in cheeses containing κ-CN AB + β-LG variants were 29 to 38 % and 13 to 17 % , respectively, suggesting that the levels of hydrophilic peptides decreased during ripening, and the greatest decrease occurred in cheese containing κ-CN A.

**Table 2: Summary of FPLC data on water-soluble N in cheeses made from milk containing different variants of β-lactoglobulin and κ-casein**

| Type of proteins in cheese | Cheese age | Peak areas[1] at retention times of < 7.5 min (hydrophobic) | 7.5 to 16.0 min (intermediate) | > 16.0 min (ionic) | Total area (arbitrary units) |
|---|---|---|---|---|---|
| κ-CN A+β-LG A | 1 d | 65.5 | 12.3 | — | 77.9 |
| κ-CN A+β-LG AB | 1 d | 53.9 | 15.6 | 1.1 | 77.9 |
| κ-CN A+β-LG B | 1 d | 71.3 | 10.9 | 0.2 | 82.4 |
| κ-CN AB+β-LG A | 1 d | 54.9 | 17.9 | 0.5 | 73.3 |
| κ-CN AB+β-LG AB | 1 d | 58.9 | 15.4 | 0.5 | 74.8 |
| κ-CN AB+β-LG B | 1 d | 55.1 | 13.3 | 0.7 | 69.1 |
| κ-CN A+β-LG A | 1 mo | 56.7 | 7.7 | 14.8 | 79.2 |
| κ-CN A+β-LG AB | 1 mo | 55.1 | 7.9 | 17.4 | 80.4 |
| κ-CN A+β-LG B | 1 mo | 52.7 | 8.8 | 15.0 | 76.5 |
| κ-CN AB+β-LG A | 1 mo | 53.7 | 11.5 | 14.8 | 80.0 |
| κ-CN AB+β-LG AB | 1 mo | 55.7 | 10.0 | 11.3 | 77.0 |
| κ-CN AB+β-LG B | 1 mo | 51.9 | 10.2 | 17.4 | 79.5 |
| κ-CN A+β-LG A | 3 mo | 51.1 | 4.4 | 28.3 | 83.8 |
| κ-CN A+β-LG AB | 3 mo | 48.5 | 6.4 | 22.6 | 77.5 |
| κ-CN A+β-LG B | 3 mo | 58.5 | 7.0 | 19.2 | 84.7 |
| κ-CN AB+β-LG A | 3 mo | 54.1 | 8.7 | 19.2 | 82.0 |
| κ-CN AB+β-LG AB | 3 mo | 59.2 | 10.2 | 17.2 | 86.6 |
| κ-CN AB+β-LG B | 3 mo | 52.5 | 7.4 | 18.5 | 78.4 |
| κ-CN A+β-LG A | 6 mo | 65.8 | 6.8 | 13.6 | 86.2 |
| κ-CN A+β-LG AB | 6 mo | 64.3 | 7.3 | 15.0 | 86.6 |
| κ-CN A+β-LG B | 6 mo | 59.8 | 6.8 | 18.3 | 84.9 |
| κ-CN AB+β-LG A | 6 mo | 61.6 | 6.2 | 15.3 | 83.1 |
| κ-CN AB+β-LG AB | 6 mo | 62.5 | 5.9 | 16.6 | 85.5 |
| κ-CN AB+β-LG B | 6 mo | 62.9 | 7.3 | 17.1 | 87.3 |

[1] Average of 3 replicates

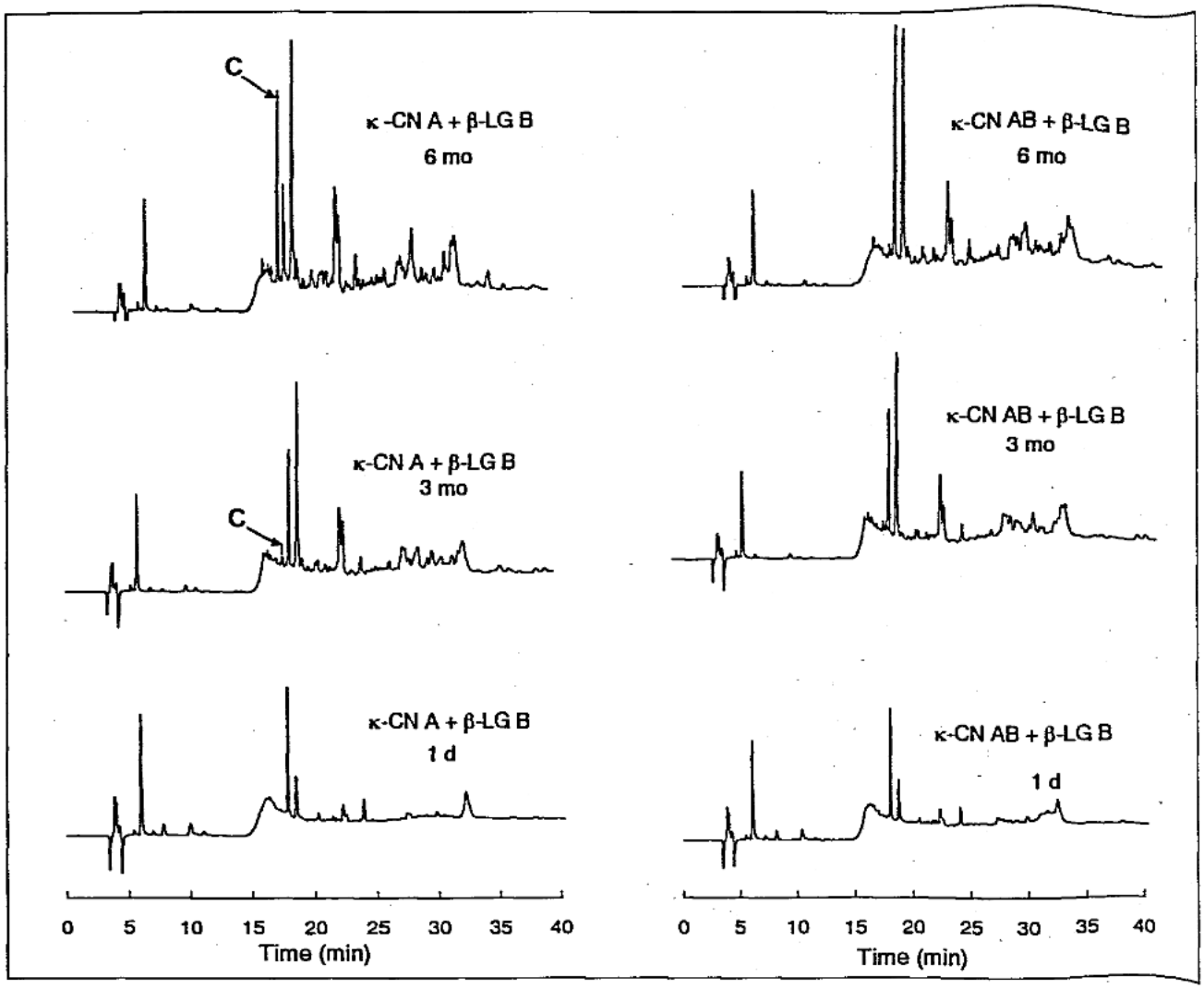


Fig. 3: Reversed-phase HPLC peptide profiles in WSN extracts from Cheddar cheese made from milk containing κ-CN A + β-LG A, κ-CN A + β-LG AB, κ-CN A + β-LG B, κ-CN AB + β-LG A, κ-CN AB + β-LG AB, or κ-CN AB + β-LG B after 1 d, 3 mo, or 6 mo of ripening. Peak C distinguishes κ-CN AA cheeses from κ-CN AB cheeses.

## 4. Conclusions

The % WSN in Cheddar cheese during ripening was not affected by the genetic variants of κ-CN plus β-LG in the milk used for manufacture. However, the peptide patterns in the cheeses made from milk containing different genetic types of κ-CN were different. Specific peptide bands with slower electrophoretic mobilities than β-CN were present in the electrophoretograms of cheeses made from milk containing κ-CN AB but were absent in cheese made from κ-CN AA milk. Similarly, HPLC and FPLC peptide profiles of the WSN from κ-CN AA cheese were different from those of κ-CN AB cheeses. The results suggest that the specificities of proteolytic enzymes towards milk proteins in cheese depend on the genetic type present.

The significance of the observed differences in proteolytic patterns in Cheddar cheese made from milk containing different genetic variants of β-LG and κ-CN to the quality of the cheeses is not known and needs further investigation. The relationship of κ-CN or β-LG variants to proteolysis in Cheddar cheese is difficult to explain because under normal conditions, very little of either protein is present in the cheese. Milk containing κ-CN A has more submicelles and larger micelles (12), that are less clustered or fused during coagulation (16), than that containing κ-CN AB or B. Therefore, differences in the rate of proteolysis in cheese made from κ-CN AA milk compared to κ-AB milk may be due to differences in the distribution of casein particles in cheese. An open protein structure in curd from κ-CN AA milk may result in more accessible peptide bonds for faster proteolysis, than in curd containing a compact structure.

*Acknowledgments*

This work was supported by California Dairy Research Foundation. The authors also thank Dr. Juan Medrano, University of California, Davis, for genetic typing of animals; Dr. Stan Henderson, Cal Poly State University, for animal care; Mr. Sean Vink, Cal Poly State University, for cheese making; and Mrs. Dorothy E. Raymond, Agriculture Canada, for assistance in FPLC analysis.

## 5. References


(1) Association of Official Analytical Chemists (AOAC): Official Methods of Analysis. 15th ed., Arlington, VA (1990).
(2) AALTONEM, M.L., ANITA, V.:Milchwissenschaft **42** 490-492 (1987)
(3) ALEANDRI, R., BUTTAZZONI, L.G., SCHNEIDER, J.C., CAROLI, A., DAVOLI, R.: J. Dairy Sci. **73** 241-255 (1990)
(4) FARKYE, N.Y., KIELY, L.J., ALLHOUSE, R.D., KINDSTEDT, P.S.: J Dairy Sci. **74** 1433-1438 (1991)
(5) FOX, P.F.: J. Dairy Sci. **72** 1379-1400 (1989).
(6) GRAHAM, E.R.B., MCLEAN, D.M., ZVIEDRANS, P.: Int. Dairy Congress, The Hague, Posters. D. Reidel Publ. Co., Dordrecht, Netherlands, p. 68. (1986)
(7) IMAFIDON, G.I., NG-KWAI-HANG, K.F.: Int. Dairy J. **2** 275-285 (1992)
(8) JAKOB, E., PUHAN, Z.: Int. Dairy J. **2** 157-178 (1992)
(9) KUCHROO, C.N., P.F. FOX, P.F.: Milchwissenschaft. **37** 331-335 (1982)
(10) MARZIALI, A.S., NG-KWAI-HANG, K.F.: J. Dairy Sci. **69** 1193-1201 (1986)
(11) MARZIALI, A.S., NG-KWAI-HANG, K.F.: J. Dairy Sci. **69** 2533-2542 (1986)
(12) MORINI, D., LOSI, G., CASTAGNETTI, G.B., BENEVELLI, M., RESMINI, P., VOLONTERIO, G.: Sci. e Tecn. Latt.-cas. **26** 437-444 (1975)
(13) MORINI, D., LOSI, G., CASTAGNETTI, G.B., MARIANI, P.: Sci. e Tecn. Latt.-cas. **30** 243-262 (1979)
(14) NG-KWAI-HANG, K.F.: Mod. Dairy **69** (2) 14-15 (1990)
(15) NG-KWAI-HANG, K.F., IMAFIDON, G.I.: Int. Dairy Congr., Posters and Brief Commun. Vol. 2. p. 359. Int. Dairy Fed., Brussels, Belgium (1990)
(16) NIKI, R., ARIMA, S.: Jap. J. Zootech. Sci. **55** 409-415 (1984)
(17) REHALI, V., MENARD, J.L.: Lait **71** 275-279 (1991)
(18) SCHAAR, J., HANSSON, B., PETTERSSON, H.-E.: J. Dairy Res. **52** 429-437 (1985)
(19) VAN DEN BERG, G., ESCHER, J.T.M., DE KONING, P.J., BOVENHUIS, H.: Neth. Milk Dairy J. **46** 145-168 1992
(20) VAN SLYKE, L.L., PRICE, W.V.: In Cheese. Orange Judd. Publ. Co., New York, NY. (1952)
(21) VINK, S., TONG, P.S., FARKYE, N.Y., IMAFIDON, G.I., MEDRANO, J.F.: J. Dairy Sci. **75** (Suppl. 1) 123 (Abstr.) (1992)


## 6. Summary


IMAFIDON, G.I., FARKYE, N.Y., TONG, P.S., HARWALKAR, V.R.: **Influence of genetic variants of κ-casein and β-lactoglobulin in milk on proteolysis in Cheddar cheese.** Milchwissenschaft **50** (6) 321–325 (1995).

**54 Cheddar cheese** (proteolysis, genetic protein variants

Effects of genetic variants of κ-casein (A or AB) and β-lactoglobulin (A, AB or B) in milk on proteolysis in Cheddar cheese were evaluated. Average water-soluble nitrogen levels (expressed at % total nitrogen) in the cheeses did not depend, significantly ($p > 0.05$), on genetic types of κ-casein or β-lactoglobulin. However, qualitative differences were noted in the electrophoretic patterns of the cheese made from κ-CN AA or AB milks. Similarly, electrophoretic patterns or HPLC profiles of peptides in the water-soluble extracts from κ-casein AA cheeses were different from those from κ-CN AB cheeses.

The results suggest that proteolytic enzymes in cheese may have different specificities towards milk protein variants during cheese ripening.

IMAFIDON, G.I., FARKYE, N.Y., TONG, P.S., HARWALKAR, V.R.: **Einfluß genetischer Varianten von κ-Casein und β-Laktoglobulin in Milch auf die Proteolyse in Cheddarkäse.** Milchwissenschaft **50** (6) 321–325 (1995).

**54 Cheddarkäse** (Proteolyse, genetische Proteinvarianten

Es wurde der Einfluß genetischer Varianten von κ-Casein (A oder AB) und β-Laktoglobulin (A, AB oder B) in Milch auf die Proteolyse in Cheddarkäse bewertet. Der durchschnittliche Gehalt an wasserlöslichem N (ausgedrückt in % Gesamt-N) in den Käsen war nicht signifikant ($p > 0{,}05$) abhängig von den genetischen Typen des κ-Casein oder β-Laktoglobulin. Jedoch wurden qualitative Unterschiede in den elektrophoretischen Mustern der Käse festgestellt, die aus κ-Casein AA- oder AB-Milch hergestellt worden waren. Ähnlich unterschieden sich die elektrophoretischen Muster oder HPLC-Profile der Peptide in den wasserlöslichen Extrakten aus κ-Casein-AA-Käsen von denen aus κ-Casein-AB-Käsen.
Die Ergebnissen lassen vermuten, daß proteolytische Enzyme in Käse unterschiedliche Spezifitäten gegenüber Milchproteinvarianten während der Käsereifung haben könnten.


# Production and evaluation of a fresh soft cheese (quark type) from ewe and cow milk with selective cultures


By I. VLACHOS

Laboratory of Dairy Technology, Faculty of Agriculture, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece


## 1. Introduction

The fresh cheeses are products which are consumed immediately. Differences between the various types of cheeses include fat content, milk homogenization during cheese manufacturing, and the presence of other added ingredients. The objective of this study was the production and characterization of fresh cheese (quark type) obtained from ewe milk with selective lactic cultures.

## 2. Materials and methods

*Cheese milk*: Cow and ewe milk from evening and morning milkings was used for cheese production.