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DRAB MEETING
July 23, 1986

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Tony Ernstrom	Utah State Univ.

DAIRY RESEARCH ADVISORY BOARD

Eccles Conference Center 303-305
Utah State University

July 23, 1986

A G E N D A

8:30 am Welcome: Dr. Doyle J. Matthews
Director, Utah State University Agricultural
Experiment Station

8:40 am PROJECT REVIEWS

1. Natural cheese curd from prefermented UF retenate.

C. A. Ernstrom

2. Factors affecting performance of lactic cultures in
UF retenate.

Brent Pope

3. Role of chymosin and porcine pepsin in Cheddar cheese curing.

Nana Yiadom-Farkye

4. Changes during vacuum treatment, pressing and cooling of 640
lbs. Cheddar cheese blocks.

Robert Reinbold

5. Cottage cheese from ultrafiltered skim milk.

C. A. Ernstrom and
J. Ocampo

Lunch: Center Colony Room - Taggart Student Center

6. Biological value of proteins in traditional vs UF cottage
cheese.

Deloy Hendricks

7. Gene transfer systems in group N streptococci.

Jeffery Kondo

8. Agglutination behavior of S. lactis and S. cremoris.

Todd Scheuble

9. Survey of coliform counts in cheese in the Western U.S.

Fahad Khayat

10. Enzyme action during the initial stages of milk clotting.

R. J. Brown

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Utah State University

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CHEESE CURD FROM PREFERMENTED 5X WHOLE MILK RETENTATE

C.G. BROWN AND C.A. ERNSTROM

Mechanization of Cheddar cheese manufacture has brought us a long way from procedures that were used 35 years ago that required open vats, hand cutting, hand cheddaring, hand milling and hand hooping. Machinery to handle curd between draining and hooping has appeared in numerous forms such as the Bell-Siro Cheese Maker II, Lactomatic, Cheddaring Towers, Drain Tables, Cheddaring Boxes, and Cheddaring Belts. Except for drain tables and cheddaring boxes, the other systems represent attempts to make the process continuous. Continuous manufacture of Cheddar cheese suffers from the problem that a significant amount of time is needed after draining to develop the required amount of acid in the curd. Continuous equipment has therefore been large and expensive. Much of the time the product spends in such equipment is incubation time wherein the curd is held for acid development and whey drainage.

If it were possible to manufacture Cheddar curd from milk that was already at the final pH of the cheese the continuous nature of the process ought to be much simpler since we would be concerned only with moisture removal.

Ultrafiltration of whole milk to 5X volume concentration ratio (VCR) could provide good starting material with approximately 60% moisture, 18-20% fat and 20-22% solids-not-fat.

The purpose of this study was to find a way to continuously form and cook curd made from prefermented 5X whole milk retentate. The experimental process is outlined in figure 1.

Procedures

Whole milk was pasteurized at 145°F for 30 min, cooled to 40°F and acidified to pH 5.8 with HCl. It was then warmed to 130°F and ultrafiltered with an Abcor spiral wound UF membrane with a molecular weight cut off of 10,000 (Fig. 2). After 60% of the original milk weight had been removed as permeate, diafiltration with an appropriate amount of deionized water was used to reduce the lactose concentration. Several experiments were conducted to determine the amount of diafiltration water needed so the amount of residual lactose, when fermented, would result in a final pH of 5.1-5.2. The effect of diafiltration on the lactose content of the final retentate, and the pH achieved following fermentation are shown in Figures 3 and 4. Diafiltration was carried out at constant volume so that water was added at the same rate permeate was removed. Following diafiltration, ultrafiltration was continued until the final volume of retentate was one-fifth that of the original milk.

The retentate was then adjusted to 77°F and inoculated with .7% lactic starter culture. The retentate was incubated until it reached pH 5.0-5.2. The incubation temperature was quite critical since acid development at higher temperatures frequently caused coagulation of the retentate by the time it reached the desired pH. Lower temperatures extended the incubation times. At 77°F the incubation time was approximately 24-26 hours.

The fermented retentate was pumped to a continuous curd forming device illustrated in figure 5. A diluted rennet solution was pumped into the retentate and mixed with a mechanical mixer just ahead of the

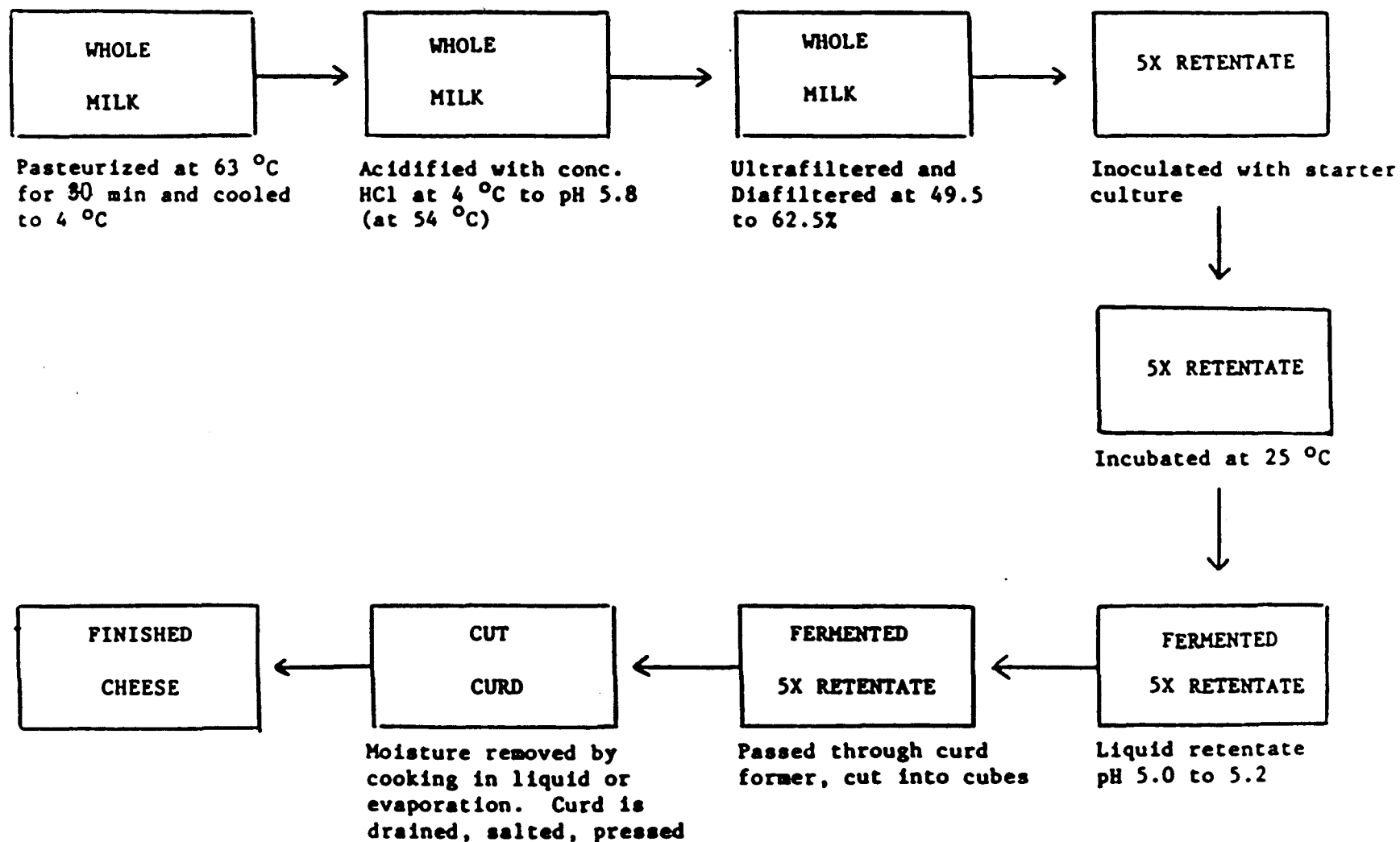


FIGURE 1

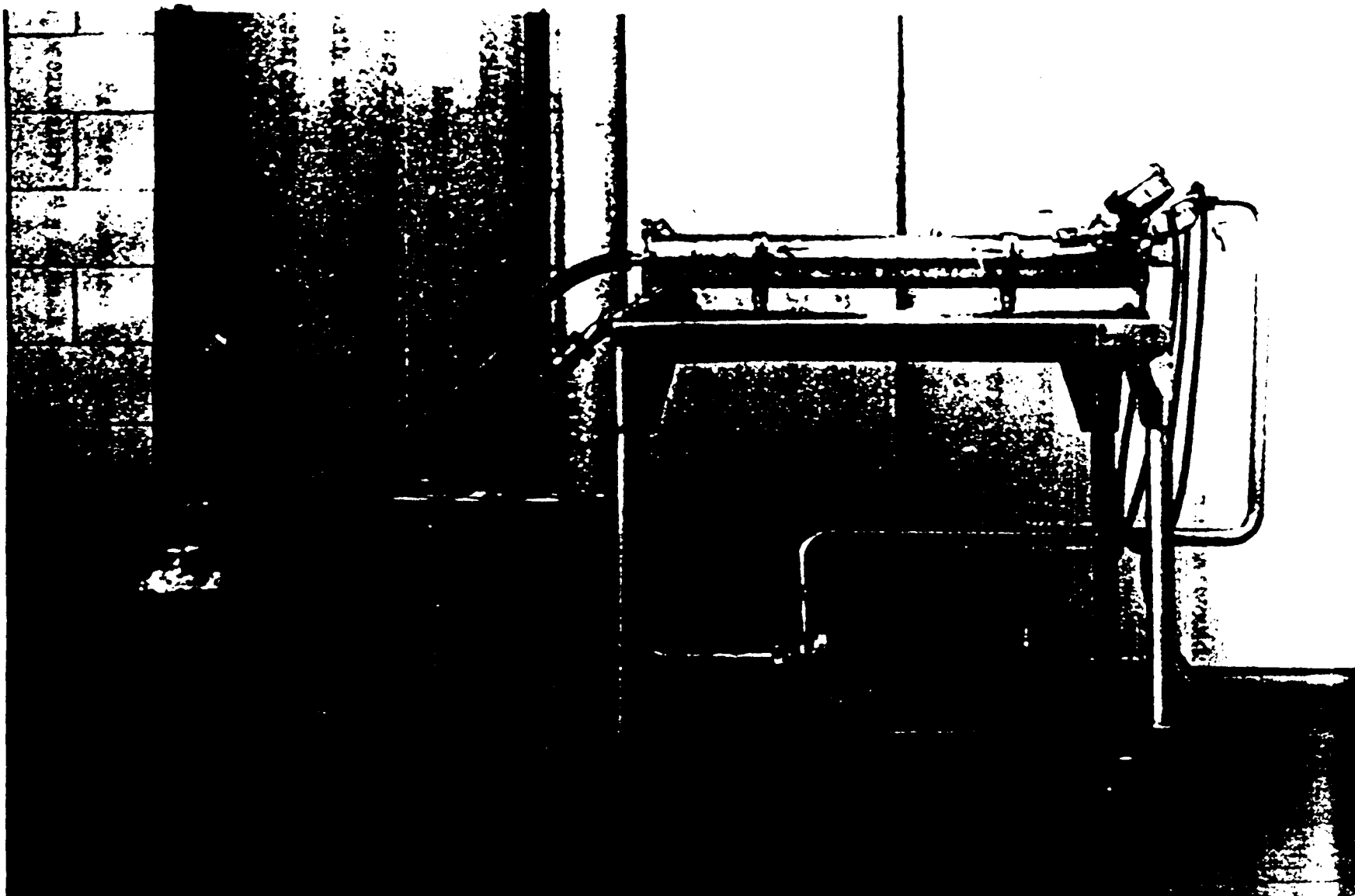


FIGURE 2

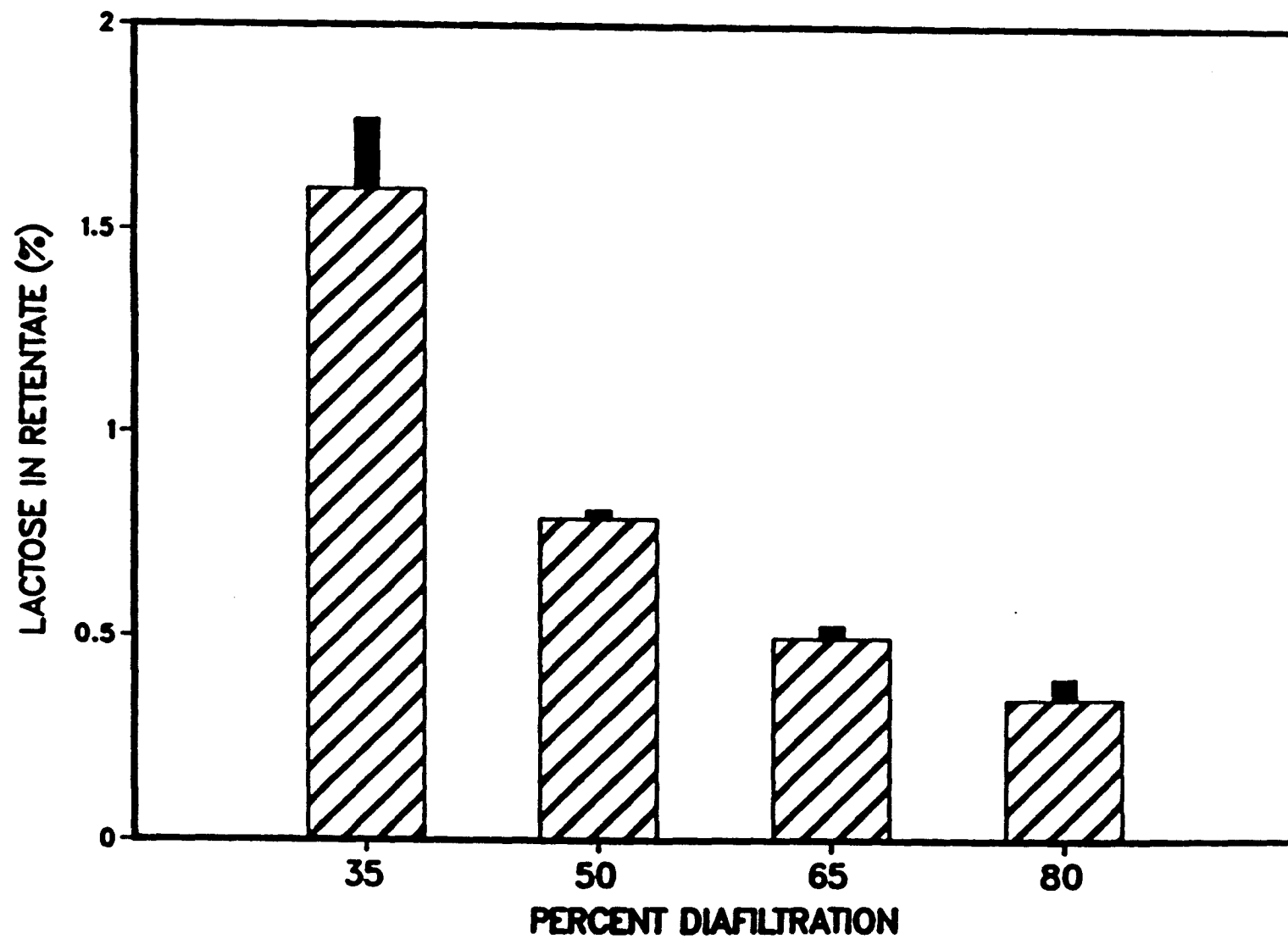


FIGURE 3

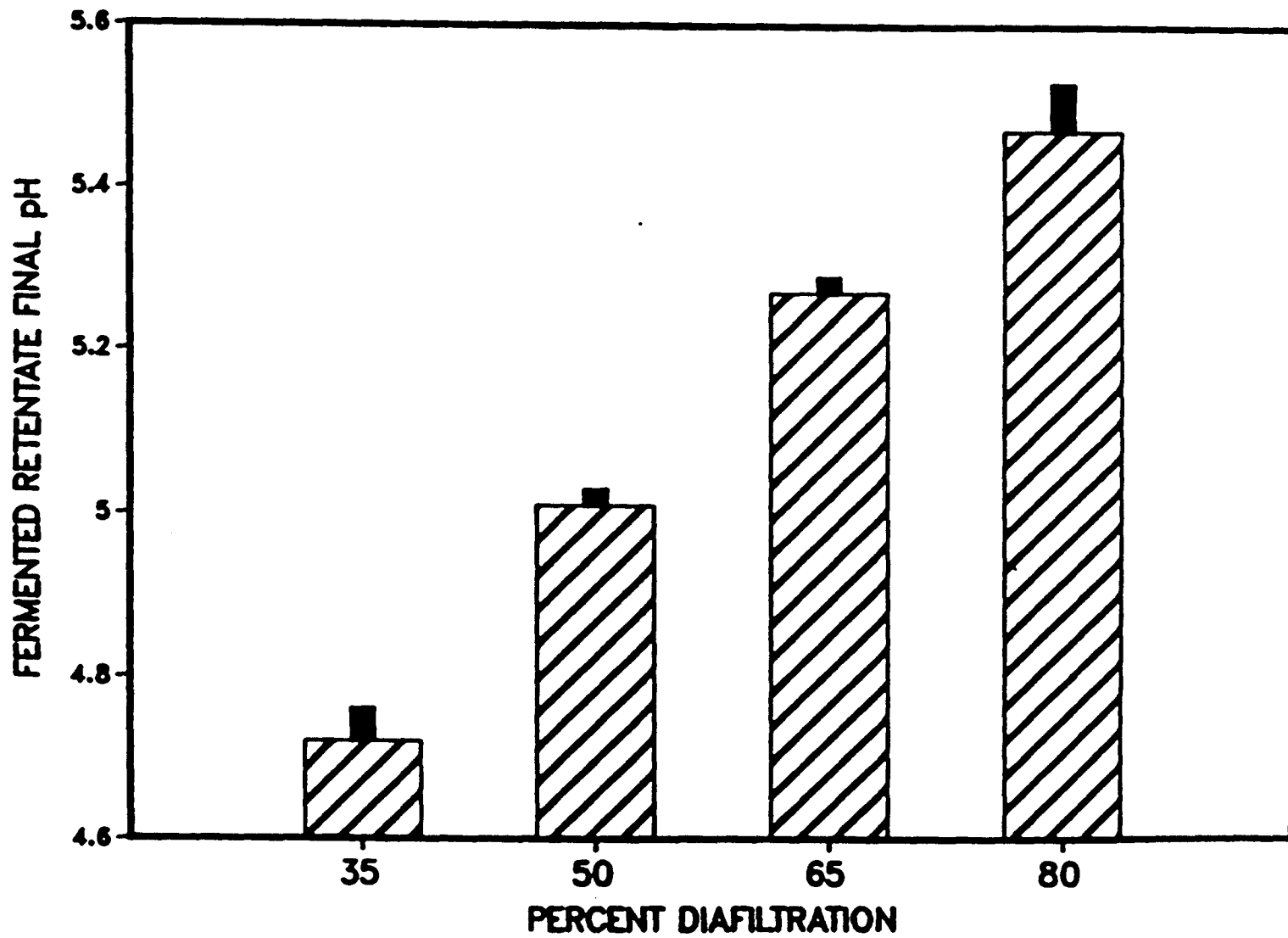


FIGURE 4

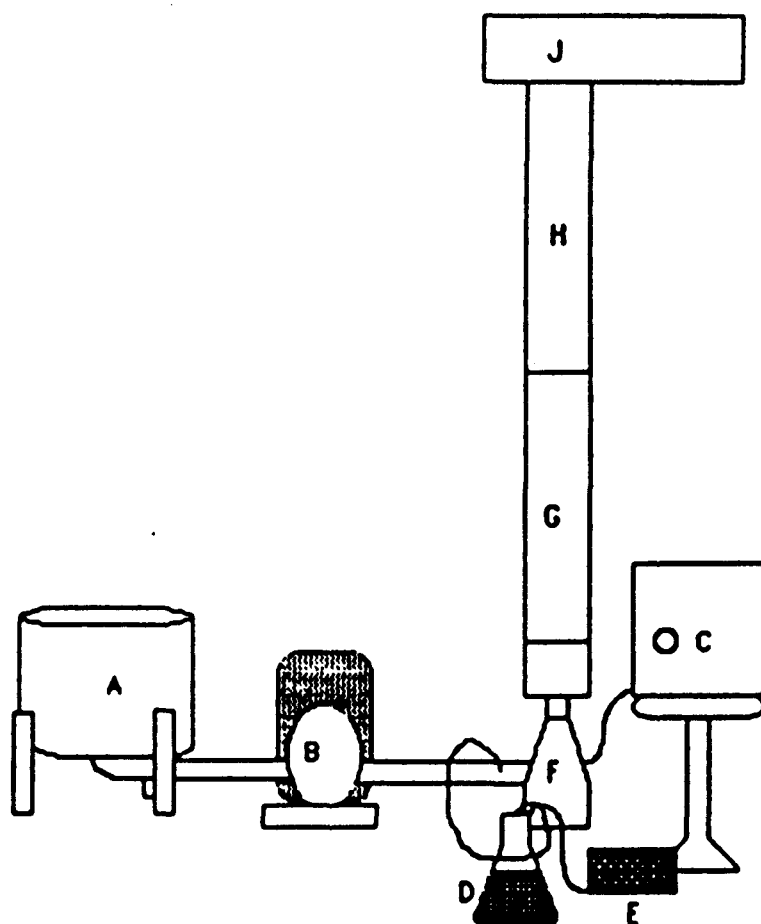


Figure 5 Assembled curd forming apparatus. Fermented retentate is poured into supply tank, A. Retentate pump, B, with converter, C, pumps retentate from supply tank to primary column. Dilute rennet, D, is introduced into retentate line by peristaltic pump, E. The retentate-rennet solution is thoroughly blended by in-line mixer, F. The mixture forms a curd as it travels up the primary column, composed of cylinders G and H. Curd emerges from exit hood, J.

curd former as illustrated in figure 6. The curd coagulated as it moved slowly up the curd former tube (Fig. 7) and was cut by a mechanical cutter as it emerged (Figures 8,9,10,11). Figure 12 shows the appearance of freshly cut curd.

The curd was then placed in either permeate or water that had been acidified to the pH of the retentate. The temperature was raised from 25°C to 38°C over time periods that varied between experiments.

Very firm curd was formed with this arrangement which operated well as long as plug flow was maintained in the system and coagulation in the mixer was avoided. The curd forming cylinder was 60 inches long and 3 inches in diameter. Residence time in the curd former was 6-8 min with rennet usage comparable to the 90 ml per 1000 lbs of original cheese milk. In some experiments residence time was reduced to about 2-3 min with a reduction in rennet usage of 25%.

When the curd was placed in either acidified permeate or water for cooking substantial fat loss was experienced. Figure 13 shows the milky appearance of the cooking medium due to fat leaching. Some protein also was lost but no curd fines were evident.

During cooking, the temperature was increased from 77°F to 100°F in 30 min.

Cooking in Acidified Permeate

5X UF retentate that had been diafiltered 55% (diafiltration water equal to 55% of original milk weight) was inoculated with .7% culture to a pH of 5.17. Figure 14 shows the decrease in percent lactose during 20 hours fermentation of the retentate. Moisture in the curd during cooking is shown in figure 13. After 1 hour cooking some of the curd

was removed and placed in a vacuum chamber under 25 inches of vacuum for 30 min. without application of heat. The lower curve shows the effect of vacuum treatment on moisture loss.

Composition of the curd after 7 weeks at 45°F is given in Table 1. It is obvious that cooking in permeate resulted in reabsorption of lactose and a final pH that was much too low.

Cooking in Acidified Water

Use of acidified water as a cooking medium solved the pH problem as indicated in table 2. Note that the final pH of the curd was at 5.2.

It was apparent that permeate as a cooking medium was not satisfactory since control of the lactose content in the cured was lost.

When acidified water was used as a cooking medium the pH problem was brought back under control, as shown in Table 2.

Cooking the curd in a liquid medium does not seem like a promising procedure. Reduction of moisture to a satisfactory level was not achieved. Higher cooking temperatures might be possible, but care must be taken to avoid too rapid heating or the curd will fuse and mat together.

An even greater problem with cooking in liquid is the loss of fat and protein. 26.8% of the fat in the original curd was lost during cooking along with 19% of the protein. This more than accounted for the serum protein gained by ultrafiltration.

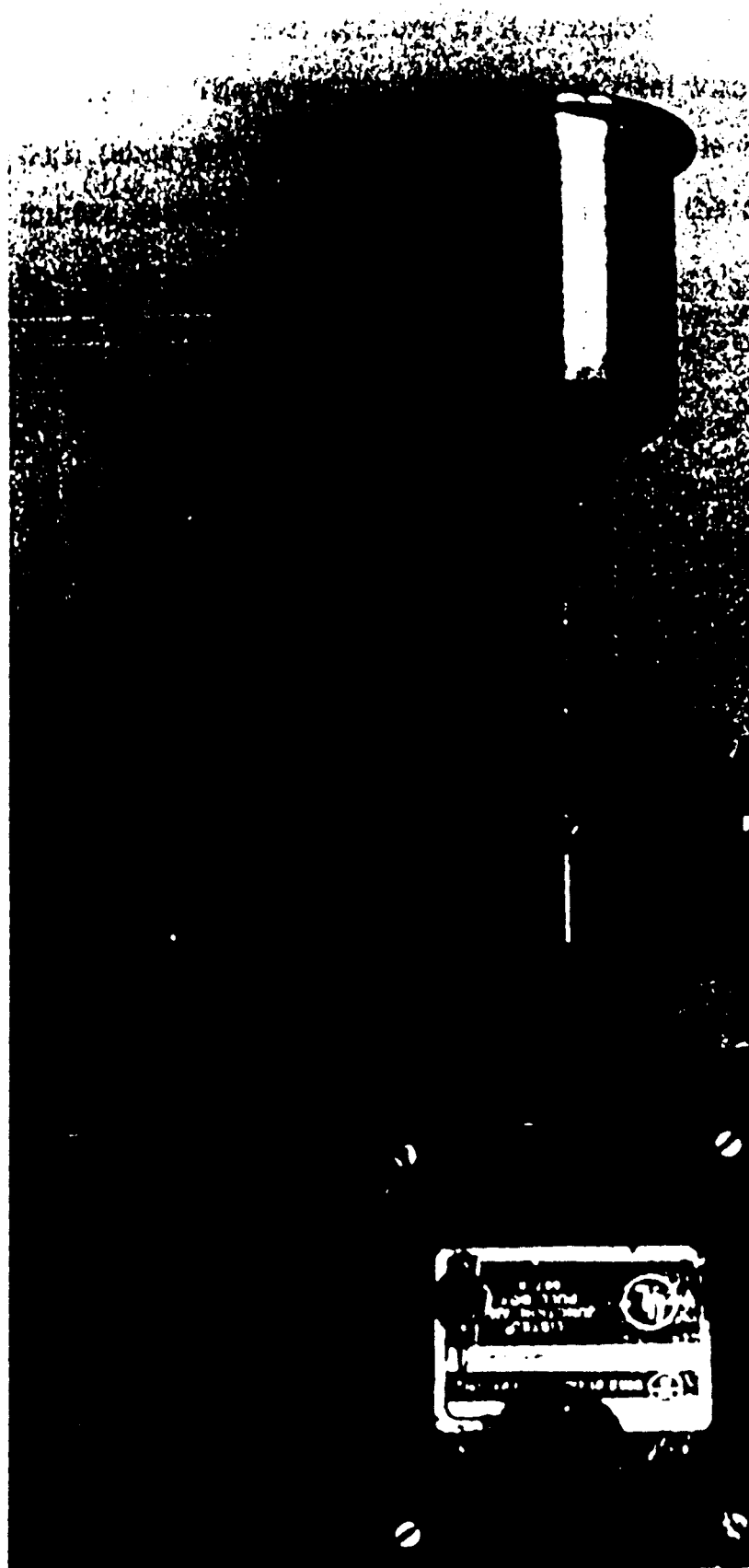






FIGURE 8



FIGURE 9



FIGURE 10



FIGURE 11



70

FIGURE 12

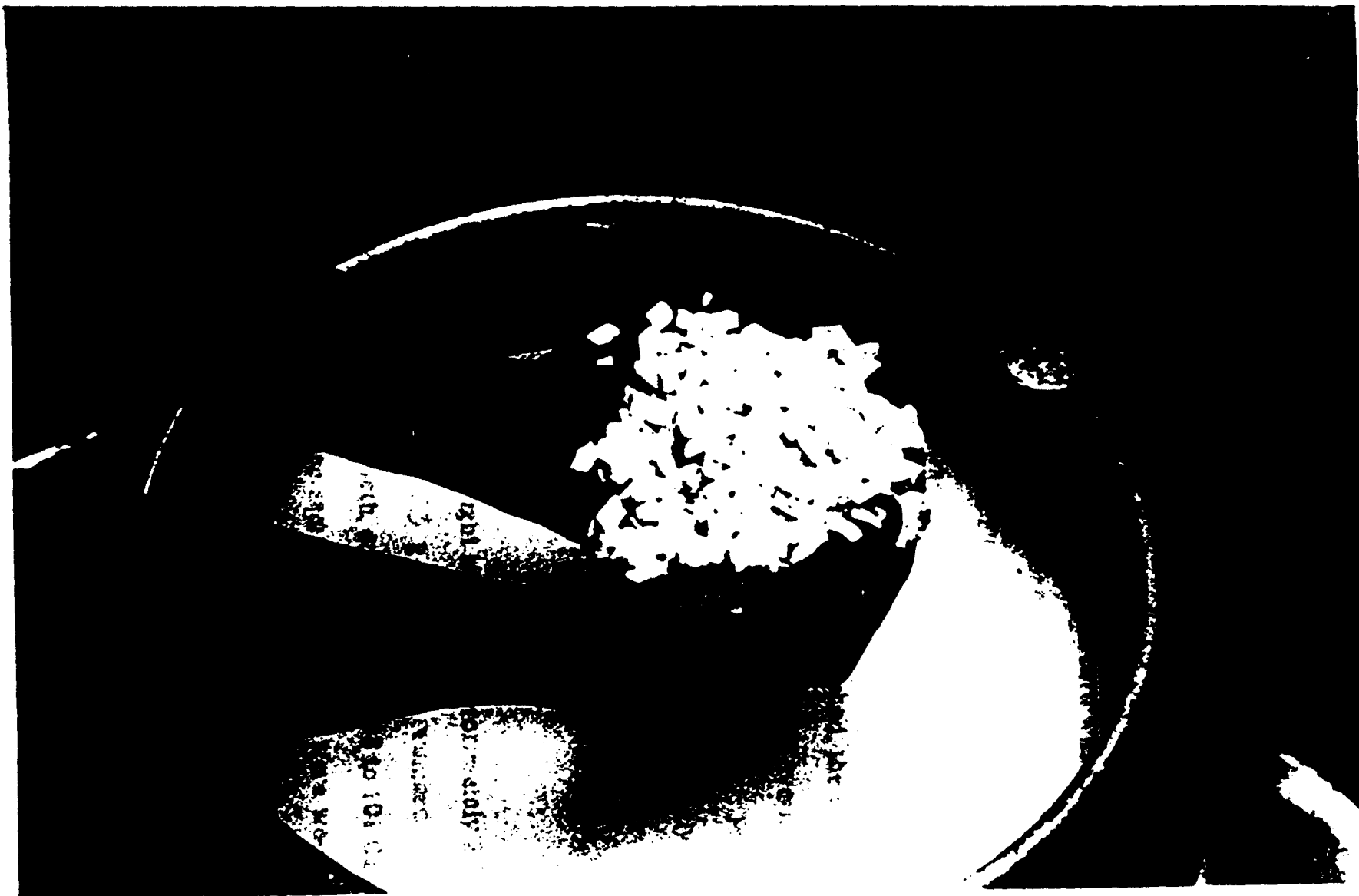


FIGURE 13

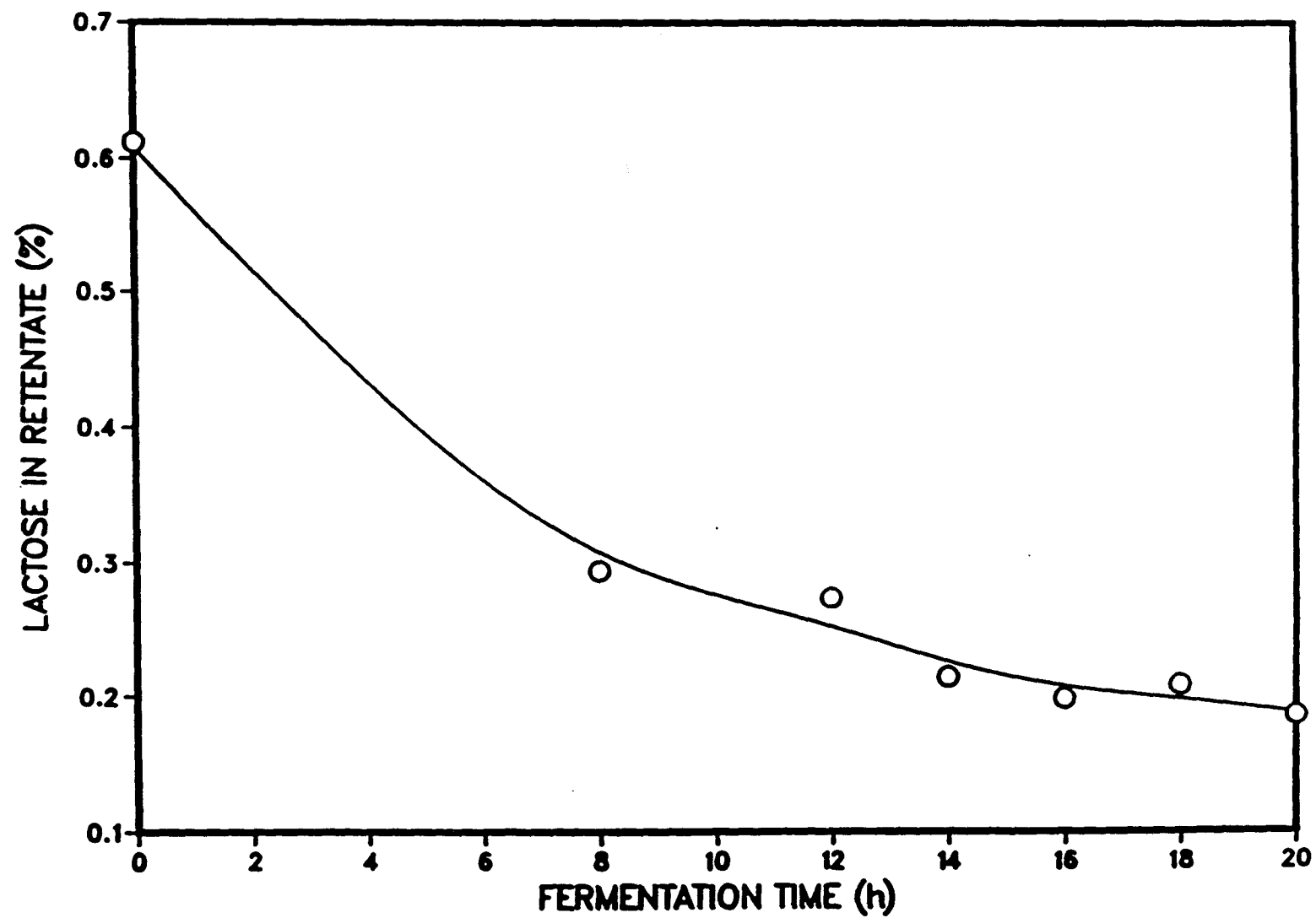


FIGURE 14

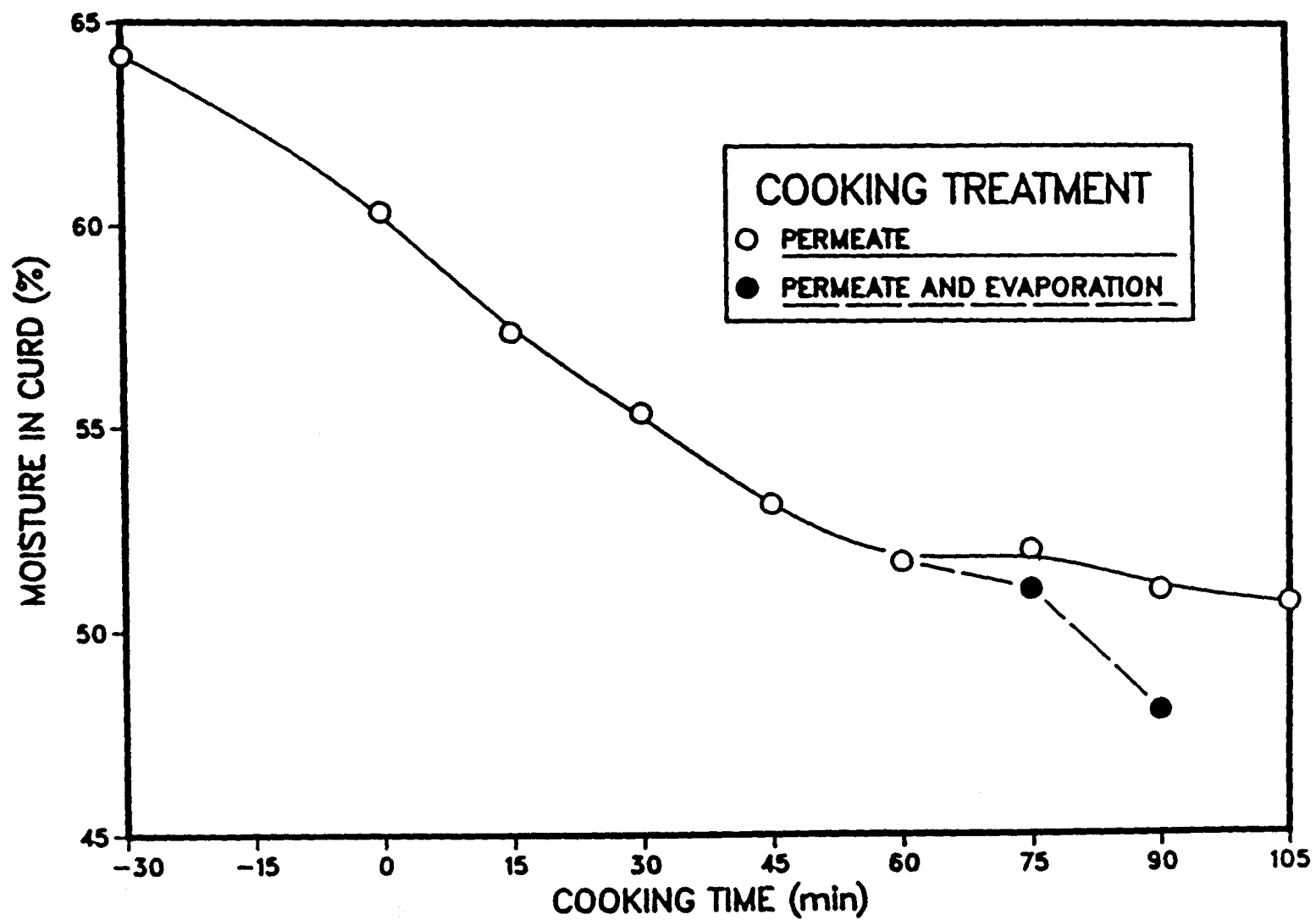


FIGURE 15

Table 1. Data for final cheese. Curd cooked in acidified permeate only, or with evaporation. (Moisture, fat, and protein are percentages)

	Moisture (%)	Fat (%)	Protein (%)	pH	pH after 7 weeks
Permeate	45.7	26.4	24.5	4.8	4.7
Permeate and evaporation	44.3	27.4	24.0	4.8	4.6

Table 2. Comparison of curd cooked in acidified, deionized water only, or with subsequent evaporation.

	Moisture (%)	Fat (%)	Protein (%)	pH (%)	FDM ^a (%)
Water	46.6	23.8	25.5	5.2	44.6
Water and Evaporation	43.0	26.1	25.4	5.2	45.8

^aFat as percent of dry matter

Factors Effecting Growth of Lactic Cultures in Ultrafiltered Retentate

by Brent Pope

Researchers working with retentates of ultrafiltered milk and have shown that it has economical potential for the dairy industry. In addition to energy savings in transportation and pasteurization it appears to have potential yield and efficiency advantages.¹

The practicality of manufacturing cheeses from highly concentrated retentates, however, has not yet been realized. Lactic cultures grow more slowly in retentates than in normal milk and some times cannot attain the proper pH. When protein ratios are greater than about 3:1 (retentate protein: normal milk protein) "lactic fermentation of skim milk retentates, in contrast to skim milk, places greater demands on starter bacteria for lactic acid production. This requirement lengthens cheese manufacturing times . . .² Koskiowski attributes this to the buffering effect stating that "when milk is ultrafiltered, these components [proteins, insoluble salts of calcium and phosphates] are concentrated and cause a concomitant increase in buffer capacity. As a result, pH reduction becomes difficult despite the presence of many active starter bacteria in their growth phase.³

Narasimhan attributed this time increase primarily to the effects of the colloidal calcium phosphates in milk. Above a pH of 5.2 phosphates are insoluble and are attached to the casein micelles. However as acid development progresses and the pH falls below 5.1 these phosphates become completely soluble and fall into solution. He showed that high concentrations of these salts is inhibitory to lactic cultures.⁴

I have been working with from whole milk that has been concentrated to 5x (1/5 its normal volume). This has been cultured using *Streptococcus*

Cremoris strain UC310 with both the protease positive and protease negative varieties in this retentate.

Since a long incubation time has been required to lower the pH sufficiently to make any cultured dairy product from retentate I have been looking at ways to shorten this time. To accomplish this I have been using different stimulants, levels of inoculum and levels of preacidification. My goal is to find an optimal combination of these three treatments to produce cultured dairy products from retentate.

I obtained 5x retentate and divided it into four lots. To three of the lots 0.5% of one of the stimulants was added. The fourth remained as a control. I then weighed out 50 g of this retentate into 100 ml beakers and inoculated each with 1% inoculum. These were incubated at 22° C for 24 hours and pH readings were taken at 0, 6, 9, 12, and 24 hours. Lactose appeared not to be a limiting factor while both yeast extract and casein hydrolysate had a positive effect on both the protease positive and protease negative cultures. These differences were more pronounced in those cultured with the protease negative bacteria. However, none of the samples reached a pH below 4.7 as shown in figure 1.

Since each stimulant that I tested had altered the initial pH of the retentate I repeated the same experiment. This time I adjusted the pH to 7 in one group and to pH 6 in the other group using 1N HCl and NaOH. This was to insure that the observed effects were due to the substrate and not to their inherent acidity. Similar results were obtained as shown in figures 2 and 3, however, the casein hydrolysate appeared not to have much of an effect.

It seemed that the effects of the pH might be important so I decided to test the effects of adding acid directly to the retentate. Again 50 g of retentate was weighed into 100 ml beakers. To each a different amount of acid or base was added to give an initial pH range from 5 to 7. These were inoculated with 5% culture and incubated for 24 hours with readings taken at the same

intervals. Those that had received the most acid were able to reach a pH even below 4.5. Figure 4 clearly shows that the inhibition of the acid production is not due to the effects of the proton concentration. It seems instead to be something that the bacteria produce as a metabolic by product that inhibits to their growth.

I then looked at the effects of two different acids. To four 100 ml beakers I added 25 ml of NDM. One was used as a control and I added 0.5 ml of one of the substrates (lactic acid, sodium lactate, and HCl were all 3N) to each of the others. From this experiment it appears that the lactate anion has a negative effect on the growth of lactic cultures (shown in figure 5).

Due to the buffer capacity of concentrated retentate Kosikowski showed that 5x retentate requires about 2.5% lactic acid to reach a pH of 4.6.⁵ A high concentration of this metabolite could easily be the reason that lactic cultures have difficulty growing in concentrated UF retentates.

At this point I have obtained three different batches of retentate that have been preacidified (acid added to cold milk before it is ultrafiltered) to pH 6.4, pH 6.2, and pH 6.0. Each of these has been cultured with varied levels of yeast extract and inoculum levels. Yeast extract at a concentration of 0.4% to 0.6% seems to be sufficient. Higher concentrations lower the final pH slightly, but not significantly. High inoculum levels also lower the final pH. This effect is seen in figures 6 through 8.

I plan now to look at some other stimulants to help decrease the time necessary to develop sufficient acid. I also want to repeat some of these experiments with a larger sample size for statistical analysis. Additionally, I would like to look for some bacteria that have a higher tolerance to lactate.

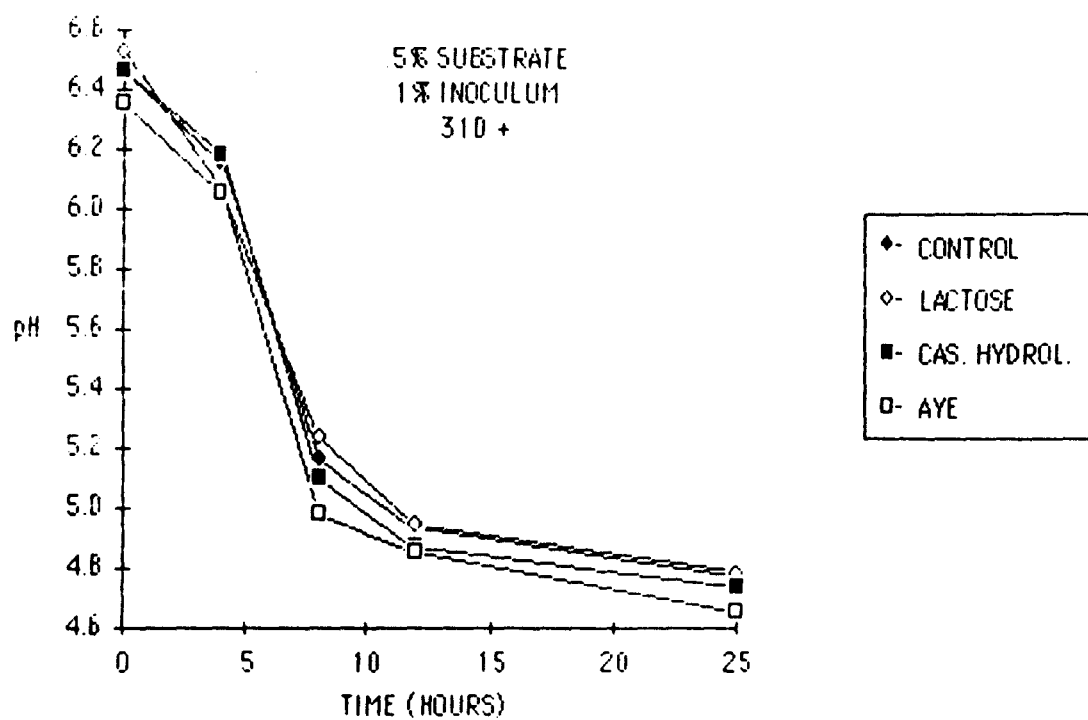


figure 1

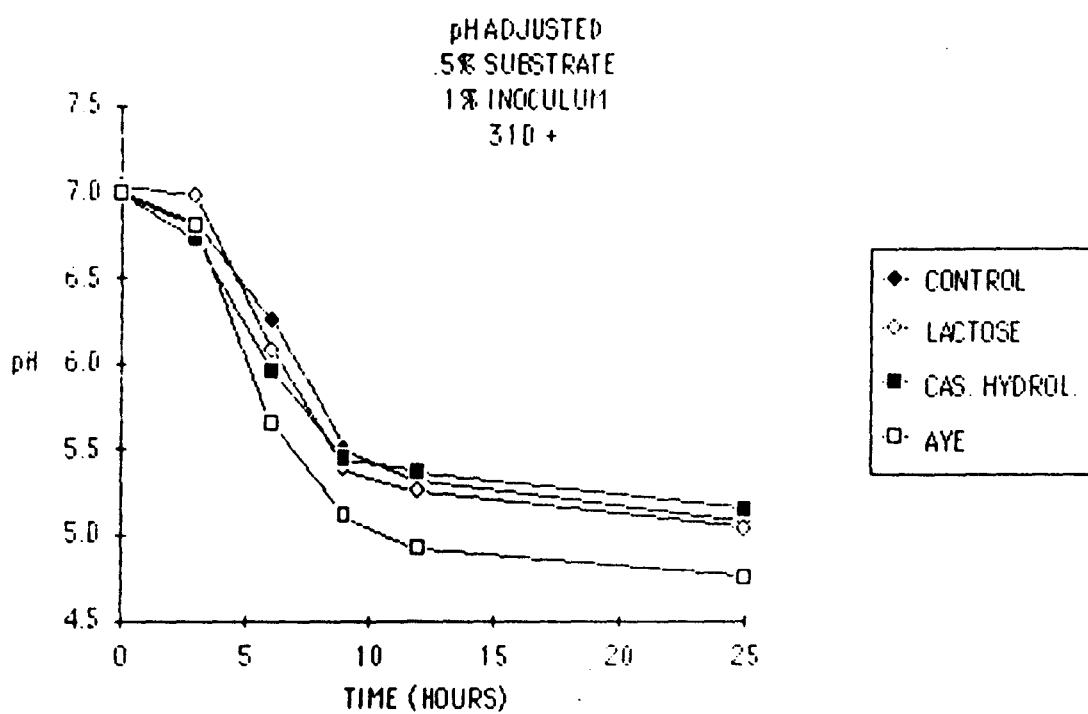


figure 2

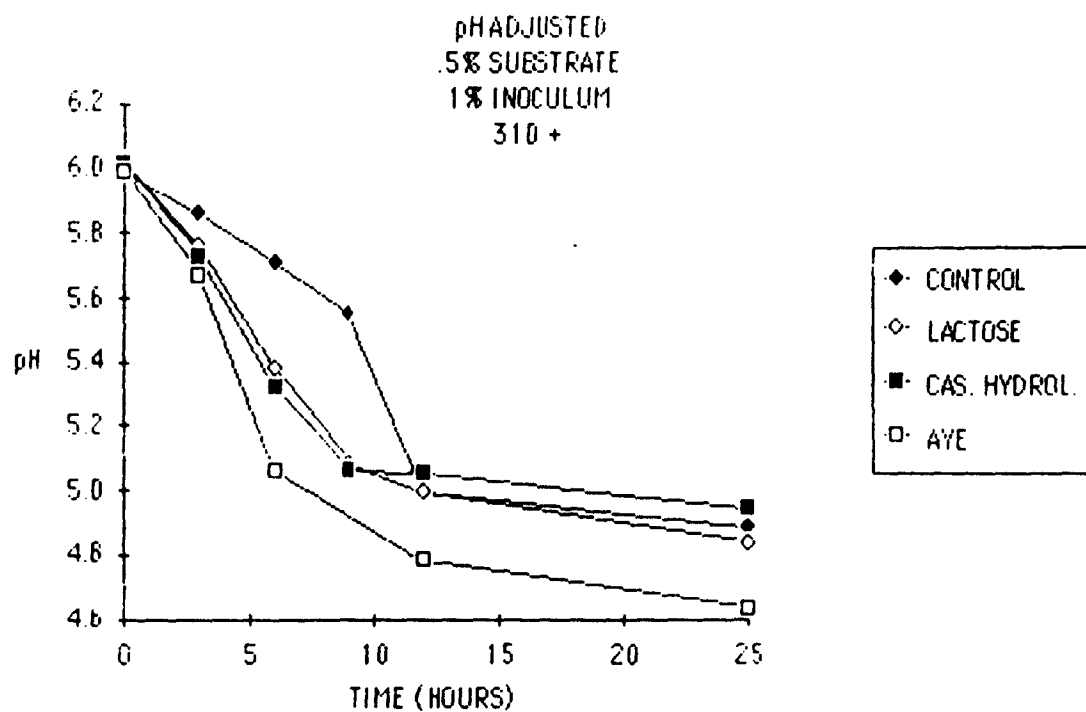


figure 3

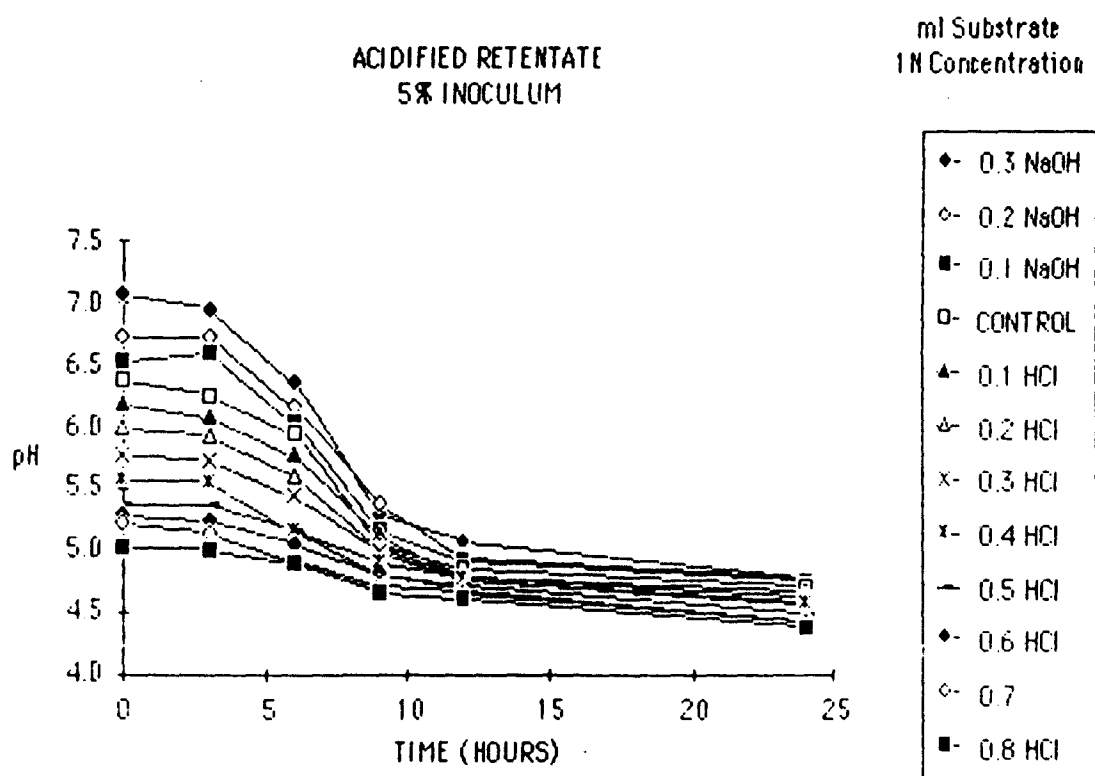


figure 4

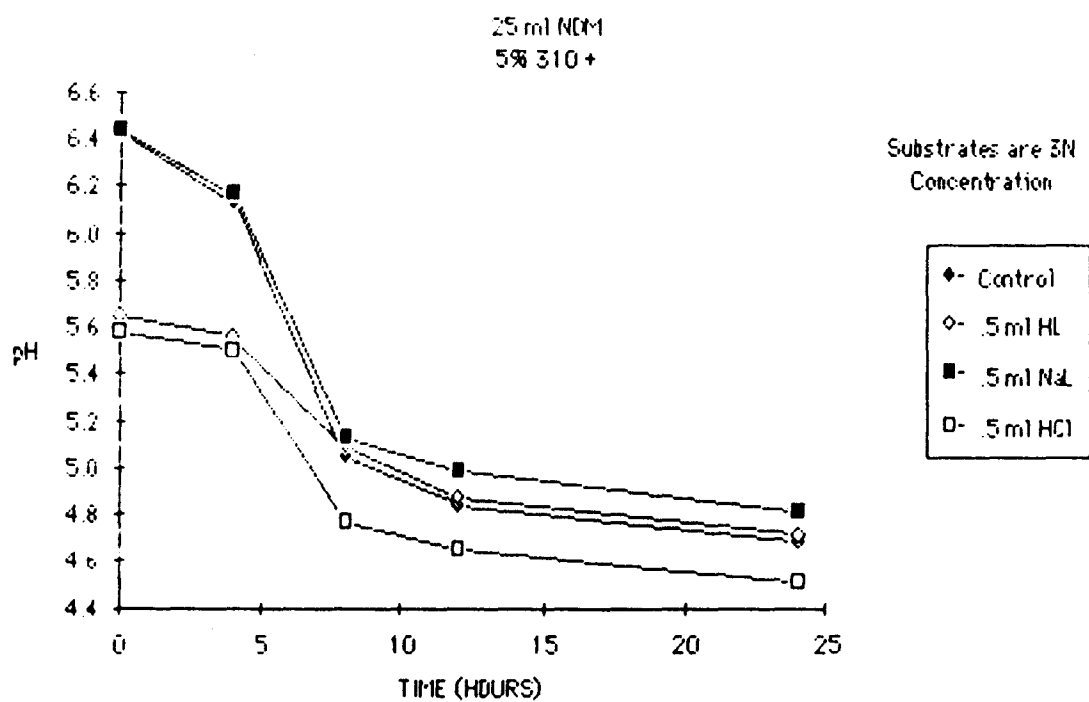


figure 5

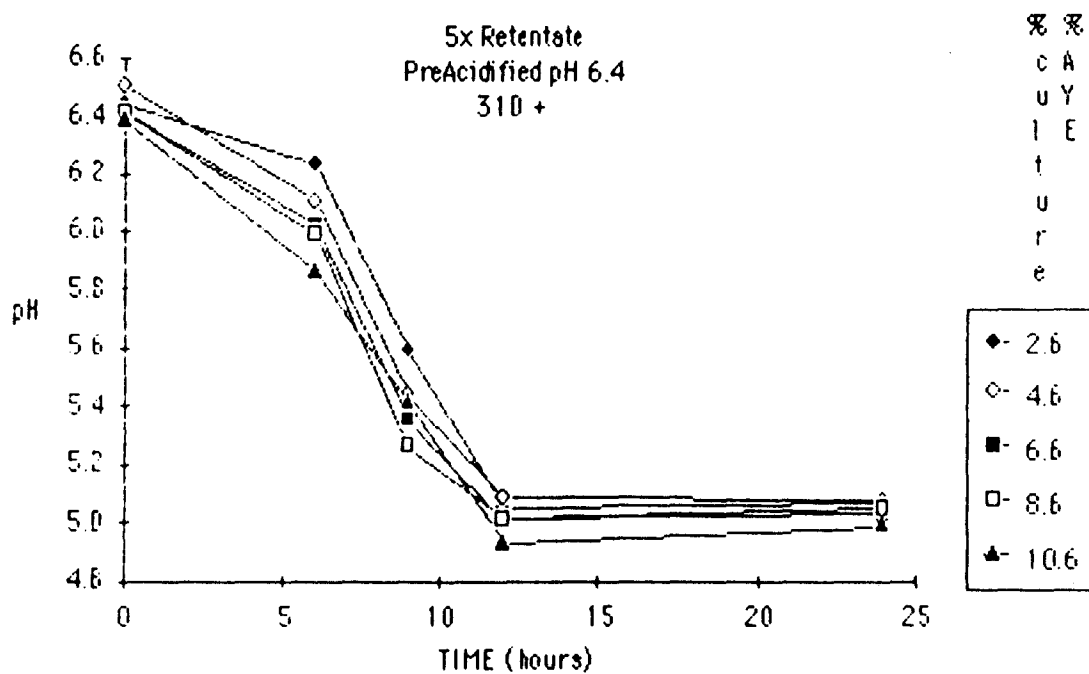


figure 6

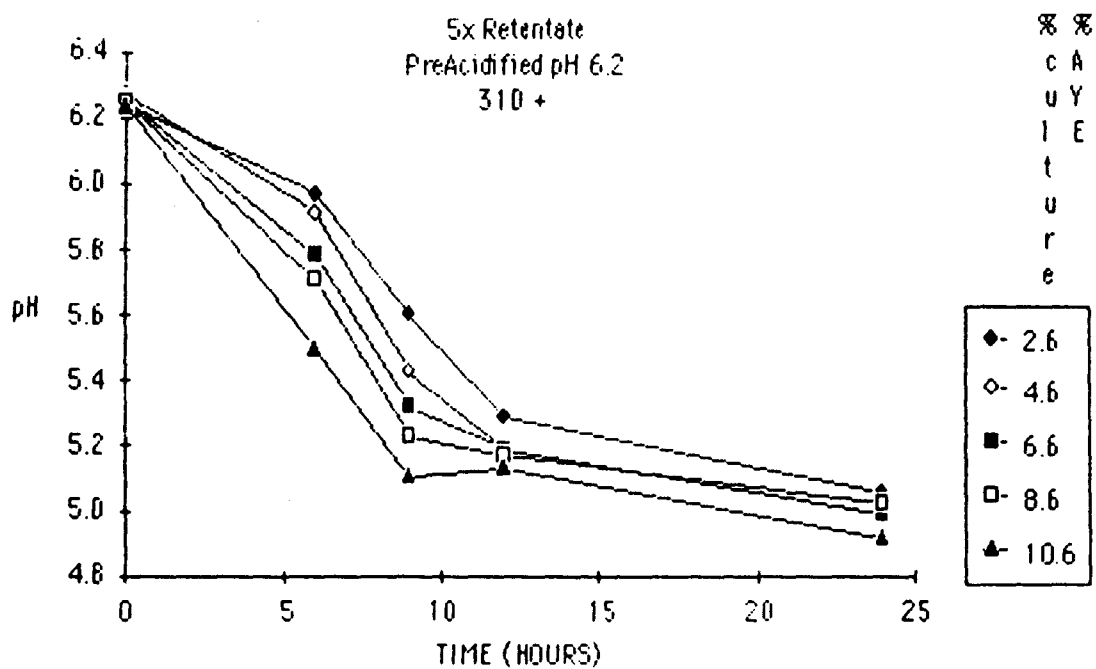


figure 7

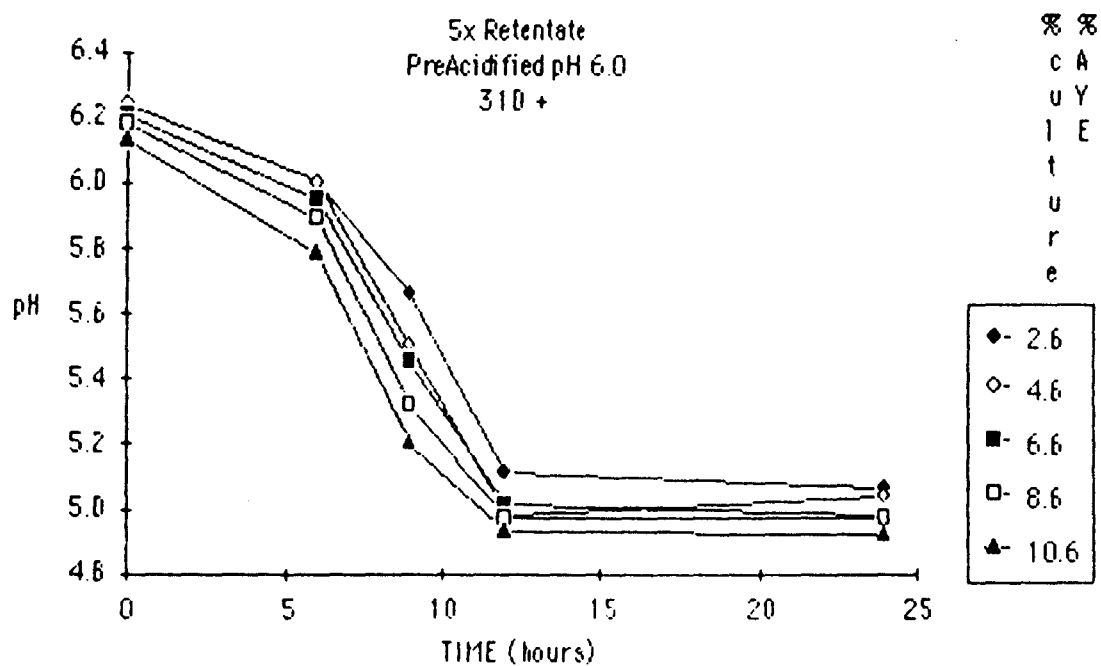


figure 8

¹Kealey, K. S. and F. V. Kosikowski. Journal of Dairy Science. December 1985, p. 3148.

²Mistry, V. V. and F. V. Kosikowski. Journal of Dairy Science. July 1985, p. 1616.

³Ibid. p. 1614.

⁴Narasimhan, Rajagopalan. An Evaluation of Heat Treatment and Ultrafiltration of skim milk for increasing cottage cheese yields. Dissertation for PhD at Utah State University, 1979.

⁵Ministry, V. V. and F. V. Kosikowski. Journal of Dairy Science. September 1985. p. 2540.

ROLE OF CHYMOSIN AND PORCINE PEPSIN
IN CHEDDAR CHEESE RIPENING

Nana Yiadom-Farkye

INTRODUCTION

Good Cheddar cheese is judged almost entirely on its flavor and body/texture. Though personal preferences exist, consumers accept Cheddar cheese with a wide range of flavors as long as objectionable flavors such as bitterness are absent. Hence, manufacture of cheese with good body and texture coupled with characteristic Cheddar flavor is the main concern of cheese makers and cheese technologists.

Many compounds important to Cheddar cheese flavor are derived from hydrolysis of casein fractions under various conditions. Release of proteolytic products is attributed to action of milk clotting enzymes, natural milk proteases and proteases from starter bacteria. The relative importance of these proteolytic enzymes is not well defined and evidence is inconclusive whether the role of milk clotting enzymes is contributory, detrimental or insignificant to overall cheese quality and flavor development.

Chymosin (EC 3.4.23.4), an extract from the abomasa of young milk fed calves is the most traditional milk clotting enzyme used for cheese manufacture. Compared to other proteases, it has a high ratio of milk clotting to proteolytic activity (28). Pepsins from bovine, porcine and chicken stomachs (30) have been studied for use in cheese manufacture. Of these, porcine pepsin (EC 3.4.23.1) is the most studied. Lucas (49), Melachoris and Tuckey (58), Emmons et al. (19) reported that cheese made with porcine pepsin has a harder body and develops flavor slower than cheese made with chymosin.

Kosikowski (42) reported that when porcine pepsin is used alone as a coagulant for cheese manufacture, it shows high sensitivity to heat and tends to create bitter cheese if concentrations added are not calculated accurately. Cheese made with chicken pepsin is of poor quality, has soft body, weak Cheddar flavor and intense off flavors (30). The rate and extent of hydrolysis of proteins during cheese ripening influence cheese quality and are related to stability of proteinases during cheese making. The pH of milk at setting (35) and moisture content of cheese (79) affect the amount of residual coagulant left in cheese. At a setting pH of 6.6, about 6% of residual chymosin remains active in Cheddar cheese curd (35,50) and no porcine pepsin is observed; showing complete inactivation of porcine pepsin during Cheddar cheese manufacture at normal pH of milk. Therefore, using porcine pepsin at pH greater or equal to 6.6 is a good control in studying the role of residual coagulant in cheese. Sherwood (75) reported that increasing the proportion of chymosin or a replacement of chymosin with pepsin influences the extent of proteolysis during ripening. Ernstrom et al. (22) found that reduction of chymosin concentration has little effect on development of flavor in cheese. Melachoris and Tuckey (58) did not observe any significant differences in proteolysis between cheese made with chymosin or pepsin (Metroclot) except for the first 30 days of ripening. Similar work by Green and Foster (29) showed that chymosin is active in cheese for at least 7 months of ripening and causes significant proteolysis whereas proteolysis in cheese made with porcine pepsin is slow and stops after 5 months of ripening.

Czulak (13) postulated that increased activity of chymosin at low pH and Stadhouders and Hup (80) reported that increase in amount of chymosin used for cheese manufacture result in bitter defect in cheese. However, Lawrence and Gilles (45) argued that bitterness results only when bitter strains of starters are used. These discrepancies have made it difficult to understand the role of chymosin or porcine pepsin in cheese ripening.

Therefore, the objectives of this study were:

- 1) to investigate the effect of chymosin and porcine pepsin on proteolysis and flavor development of Cheddar cheese during ripening.
- 2) show whether the role of milk clotting enzyme in cheese is contributory, detrimental or insignificant to overall cheese quality and flavor development.

MATERIALS AND METHODS

Enzymes: Ion-exchange purified calf chymosin was obtained from the New Zealand Rennet Co.

Lyophilized porcine pepsin (2640 units/mg solid) was obtained from Sigma Chemical Co.

Methods:

Two experiments were performed.

In the first experiment, 3-vat lots of Cheddar cheese were made in 3 replications. Each vat contained 410 kg (900 lb) of sweet milk pH 6.6. Milk in vat 1 was coagulated with 6070 clotting units (90 ml/454 kg milk) of chymosin. Milk in vat 2 was coagulated with 6070 clotting units of porcine pepsin. The amount of porcine pepsin used was based on its activity in sweet milk compared to chymosin. Milk in vat 3 was acidified to pH 6.2 with concentrated HCl and coagulated with 890 clotting units of pepsin. The amount of pepsin used in vat 3 was based on its milk clotting activity at pH 6.2.

The enzymes were added at levels to achieve a uniform set time of 30 min. Identical amounts (.7%) of a 4 strain commercial starter (*Streptococcus cremoris*) was used in each vat. A manufacturing schedule of 4 1/4 h (setting to milling pH of 5.4) was followed. After overnight pressing, cheese was ripened at 10°C (Figure 1).

The design of the second experiment was similar to the first except that various levels of chymosin was used in each vat (Figure 2). Milk in vat 1 was set with 6070 clotting units (90 ml/454 kg milk) of chymosin. Vat 2 was set with 18210 clotting units (270 ml)

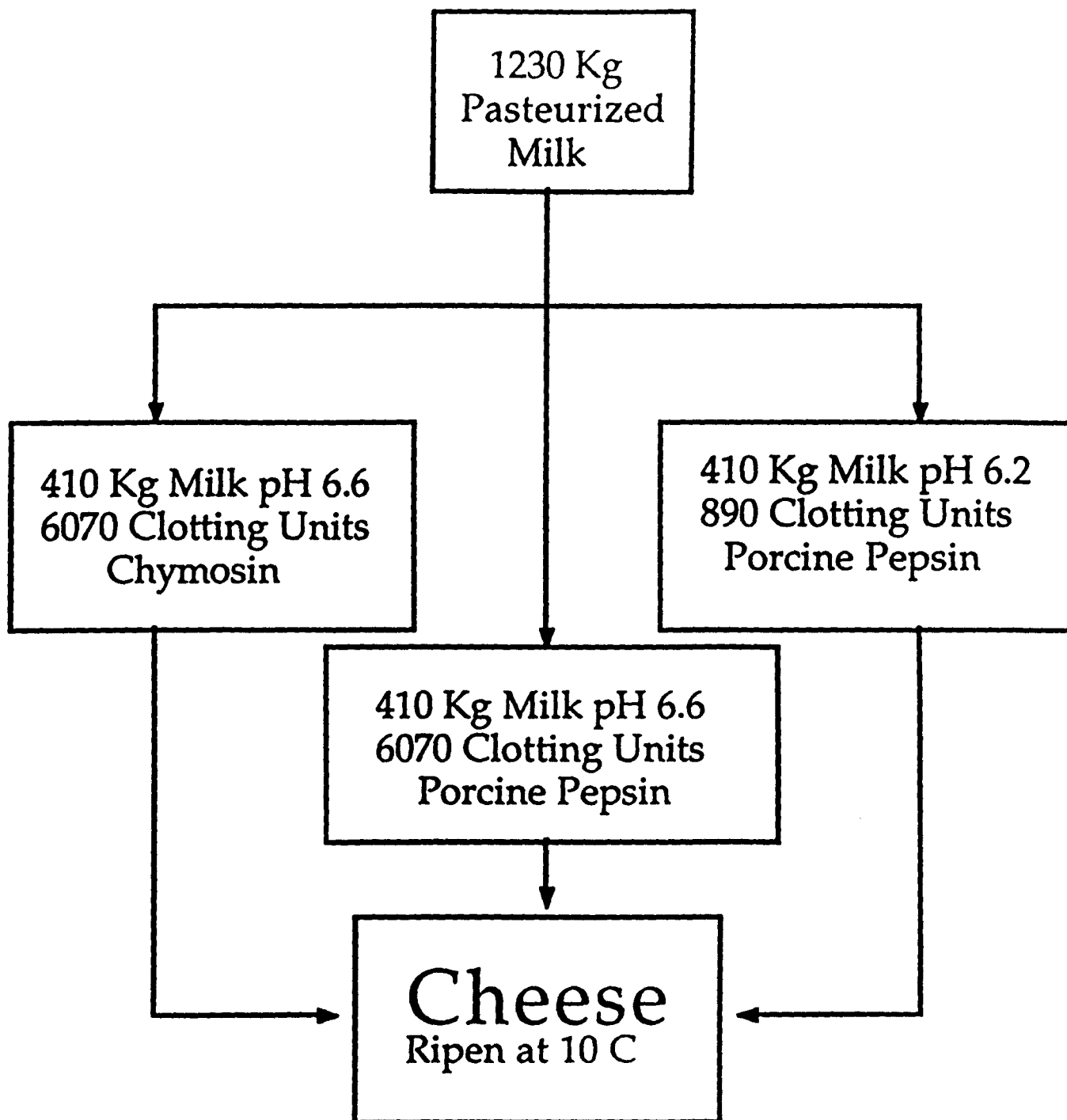


Figure 1. Schematic for manufacture of Cheddar cheese from milk set with chymosin or porcine pepsin.

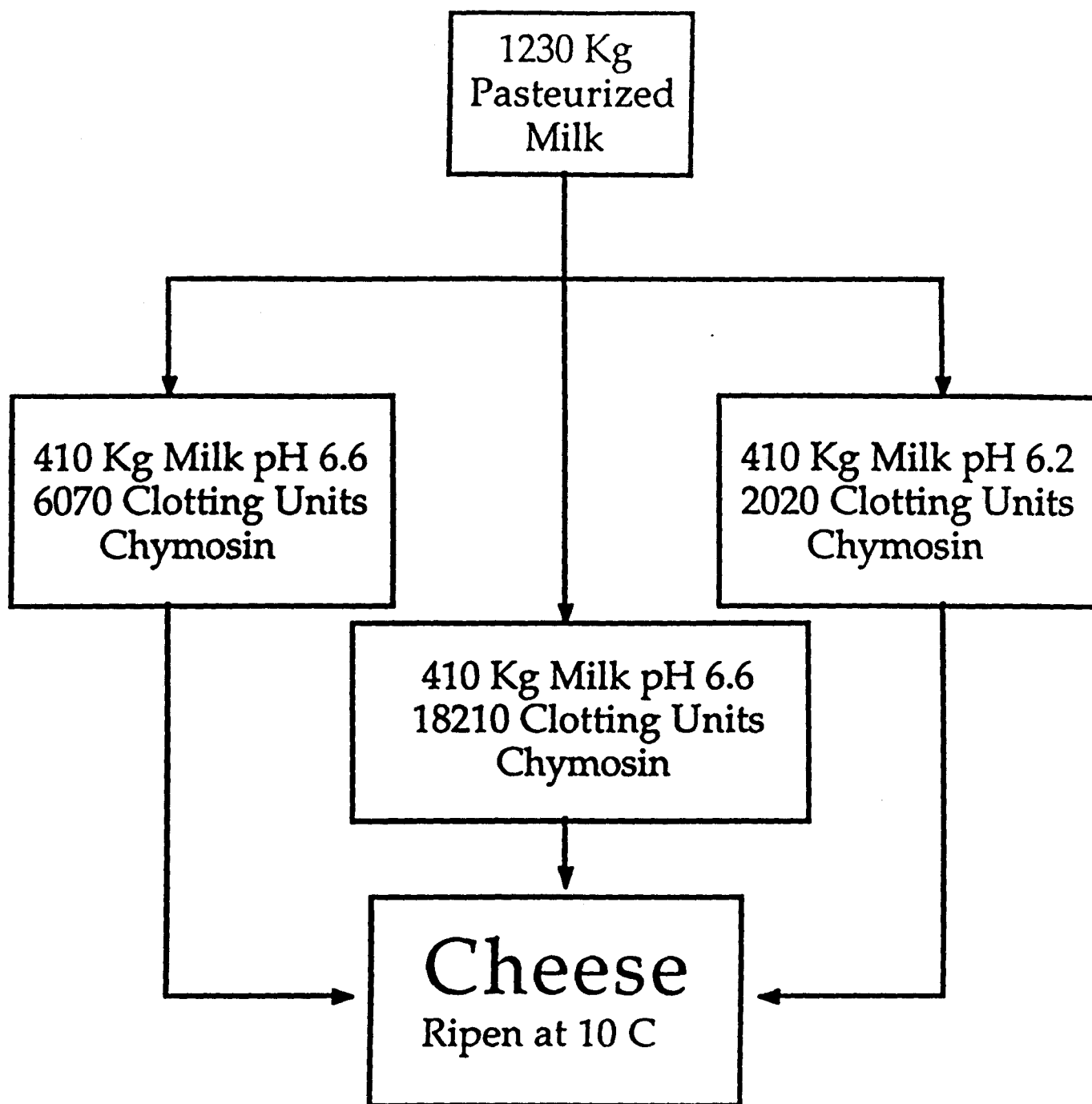


Figure 2. Schematic for manufacture of Cheddar cheese from milk set with different levels of chymosin.

chymosin. Milk in vat 3 was acidified to pH 6.2 with concentrated HCl and set with 2020 clotting units (30 ml) chymosin. Setting time in the first and third vats was 30 min. To insure uniform acid development in all 3 vats, starter was added 15 min prior to addition of 3X the normal level of coagulant in vat 2. Hence, setting time in this vat was 15 min.

A coagulant free, starter free cheese curd was also made with and without plasmin inhibitor.

Compositional analysis was done on cheese immediately after removal from the press. Moisture was in a forced-air oven maintained at 110°C for 16 h. Fat was by the Babcock procedure. Protein was determined by kjeldahl nitrogen x 6.38.

Residual chymosin in finished cheese was determined by the diffusion substrate technique reported by Holmes et al. (35). Residual pepsin was determined by the procedure outlined by Majeed and Ernstrom (50).

Primary proteolysis was followed by discontinuous polyacrylamide gel electrophoresis in urea.

The amount of water soluble nitrogen expressed as a percentage of total nitrogen was determined by Fox's procedure (43).

Cheeses were graded by a panel of 5 experienced graders at various ripening times. Grading was for flavor, body and texture on a linear scale of 1 (unsaleable), 5 (good) and 7 (superior). An overall score was assigned by the judges for each cheese.

RESULTS

I. Composition of Cheese

Percent fat, protein and moisture of cheese made with different levels of chymosin are shown in Table 1. Actual yields, actual yields adjusted to equal moisture of 37%, percent fat in the dry matter (FDM) and moisture in non-fat substance (MNFS) are shown also in Table 1.

No significant difference in yield or composition of cheese was obtained within and between treatment groups. All the cheese met U.S. legal standards of identity for Cheddar cheese (i.e. not more than 39% moisture and a minimum of 50% FDM).

Composition of cheese made with pepsin, pH 6.2; pepsin, pH 6.6 and chymosin, pH 6.6 are shown in Table 2. No significant difference ($\alpha = .05$) in composition or yield was obtained within and between treatment groups. All cheese in this treatment group met legal standards of identity for Cheddar.

II. Residual Coagulant in Cheese

Amount of residual chymosin recovered from cheese immediately after pressing (1 day after manufacture) represented about 13%, 7% and 13% of the original activity added to coagulate milk (2020 clotting units, 6070 clotting units and 18210 clotting units) respectively (Table 3). When data from Table 3 was transformed to give units of enzyme per kilogram of cheese, about 9.6 clotting units of chymosin was retained per kilogram of cheese during normal cheese manufacture. Trebling the amount of chymosin used to coagulate milk at pH 6.6 drastically increased the activity of

Table 1. Composition and yield of Cheddar cheese manufactured with various levels of chymosin.

Treatment	Trial	Composition (%)			FDM (%)	MNFS (%)	Yield (%)	Adj Yield ¹ (%)
		Fat	Protein	Moisture				
Chymosin, pH 6.2 (2020 clotting units)	1	33.5	25.18	37.69	53.76	56.68	9.67	9.49
	2	34.3	25.50	35.85	53.47	54.57	9.48	9.78
	3	34.5	25.13	36.36	54.21	55.51	9.56	9.73
Chymosin, pH 6.6 (6070 clotting units)	1	34.5	24.80	36.93	54.70	56.38	9.89	9.91
	2	33.8	25.49	37.90	54.43	57.25	9.76	9.53
	3	35.0	25.39	35.27	54.07	54.26	9.65	10.12
Chymosin, pH 6.6 (18210 clotting units)	1	34.5	25.36	36.48	54.31	55.69	9.78	9.92
	2	33.8	25.66	37.40	53.99	56.50	9.68	9.58
	3	34.5	25.67	36.57	54.39	55.83	9.68	9.79

¹Actual yields adjusted to 37% moisture.

Table 2. Composition and yield of Cheddar cheese manufactured with pepsin and chymosin.

Treatment	Trial	Composition (%)			FDM (%)	MNFS (%)	Yield (%)	Adj Yield ¹ (%)
		Fat	Protein	Moisture				
Pepsin, pH 6.2 (890 clotting units)	1	34.5	24.34	37.81	55.48	57.73	9.83	9.62
	2	34.0	25.16	37.09	54.05	56.20	9.62	9.60
	3	33.8	25.72	37.14	53.77	56.10	9.59	9.55
Pepsin, pH 6.6 (6070 clotting units)	1	35.0	24.31	37.78	56.25	58.12	9.84	9.64
	2	34.0	25.19	37.00	53.47	56.06	9.63	9.63
	3	34.0	25.75	36.39	53.45	55.13	9.53	9.69
Chymosin, pH 6.6 (6070 clotting units)	1	35.0	24.47	37.58	56.07	57.82	9.89	9.74
	2	34.0	25.48	37.20	54.14	56.36	9.57	9.52
	3	33.8	25.33	36.96	53.62	55.83	9.64	9.65

¹Actual yields adjusted to 37% moisture.

Table 3. Residual chymosin in cheese¹ at various times of ripening.

Treatment	% of original chymosin activity recovered at ...			
	1 day	3 mo	6 mo	9 mo
Chymosin, pH 6.2 (2020 clotting units)	12.7	10.8	10.3	10.1
Chymosin, pH 6.6 (6070 clotting units)	6.3	5.1	4.9	4.8
Chymosin, pH 6.6 (18210 clotting units)	13.1	11.0	10.0	9.7

1 = Mean of 4 assay on 3 trials.

Table 4. Activity of chymosin recovered per unit weight of cheese.

Treatment	Mean clotting units of chymosin recovered/kg cheese at ...			
	1 day	3 mo	6 mo	9 mo
Chymosin, pH 6.2 (2020 clotting units)	6.5	5.5	5.3	5.2
Chymosin, pH 6.6 (6070 clotting units)	9.6	7.8	7.4	7.2
Chymosin, pH 6.6 (18210 clotting units)	59.9	50.5	45.9	44.4

residual chymosin to about 60 clotting units per kilogram of cheese. About 7 clotting units was retained per kilogram of cheese when a third of the normal level of chymosin was used to coagulate milk at pH 6.2 (Table 4).

Table 5 shows amount of residual pepsin and chymosin detected in cheese at various times of ripening.

Table 5. Residual pepsin and chymosin in cheese¹ at various ripening times.

Treatment	% original activity of enzyme recovered at...			
	1 day	3 mo	6 mo	9 mo
Pepsin, pH 6.2 (890 clotting units)	5.0	4.2	4.0	3.8
Pepsin, pH 6.6 (6070 clotting units)	0.0	0.0	0.0	0.0
Chymosin, pH 6.6 (6070 clotting units)	7.0	5.5	5.2	5.0

1 = Mean of 4 assays on 3 trials.

When cheese milk (pH 6.6), was set with porcine pepsin, no residual pepsin activity remained in cheese curd. At a setting pH of 6.2, about 5% of original pepsin activity (890 clotting units/410 kg milk) added to coagulate cheese milk remained active in cheese.

Transformation of percent enzyme recovered to units of activity of enzyme recovered per kilogram of cheese gave 1.1 clotting unit of pepsin per kilogram of cheese when milk was set with porcine pepsin at pH 6.2 (Table 6). Cheese milk set at pH 6.6 with chymosin (6070

clotting units/410 kg milk) had 10.7 clotting units of residual chymosin per kilogram of cheese at 1 day of ripening and about 8 clotting units per kilogram of cheese at 9 month ripening.

Table 6. Activity of enzyme recovered per unit weight of cheese.

Treatment	Mean clotting units of enzyme recovered at...			
	1 day	3 mo	6 mo	9 mo
Pepsin, pH 6.2 (890 clotting units)	1.1	.9	.9	.8
Pepsin, pH 6.6 (6070 clotting units)	0	0	0	0
Chymosin, pH 6.6 (6070 clotting units)	10.7	8.3	7.9	7.6

III. Proteolysis of Cheeses During Ripening

a. Primary proteolysis in cheese containing various levels of residual chymosin

Primary proteolysis as determined by PAGE-urea for each treatment group at various ages of ripening are illustrated in Figures 3 through 5. Electrophoretic patterns of 1 day old cheese containing different levels of residual chymosin (Table 4) are shown in Figure 3. Cheese made with 3X normal level of chymosin and containing about 60 clotting units residual chymosin per kilogram cheese had distinct breakdown of α_{s1} - and β -casein. Slight proteolysis of α_{s1} -casein (not very evident in the figure) had occurred in cheese made with normal and reduced level of chymosin. Proteolysis of β -casein was not evident in this cheese at 1 day of

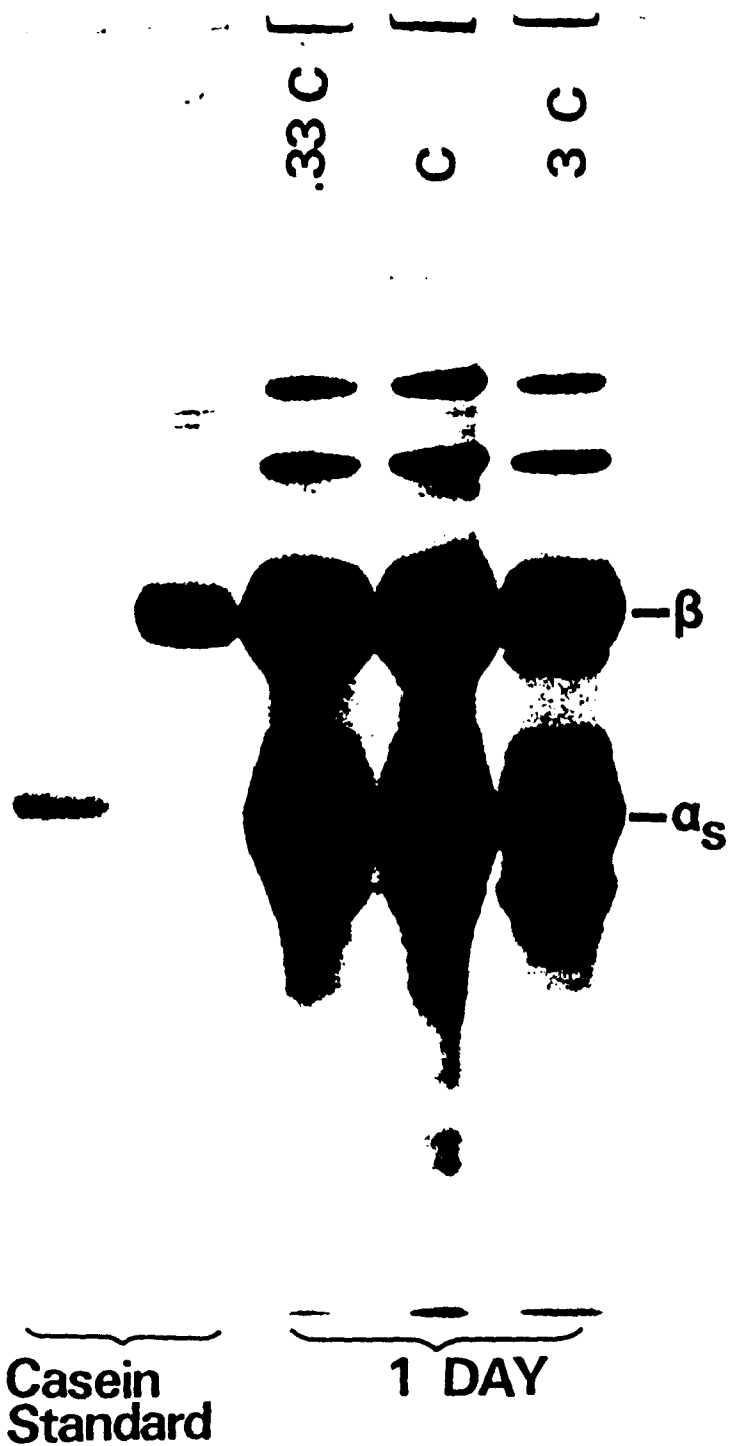


Figure 3. Electrophoretic patterns of cheese manufactured with chymosin

.33C = 30 ml/454 kg milk, C = 90 ml/454 kg milk,
3C = 270 ml/454 kg milk



Figure 4. Electrophoretic patterns of 1 and 3 month old cheese made with chymosin

.33C = 30 ml/454 kg milk, C = 90 ml/454 kg milk,
3C = 270 ml/454 kg milk

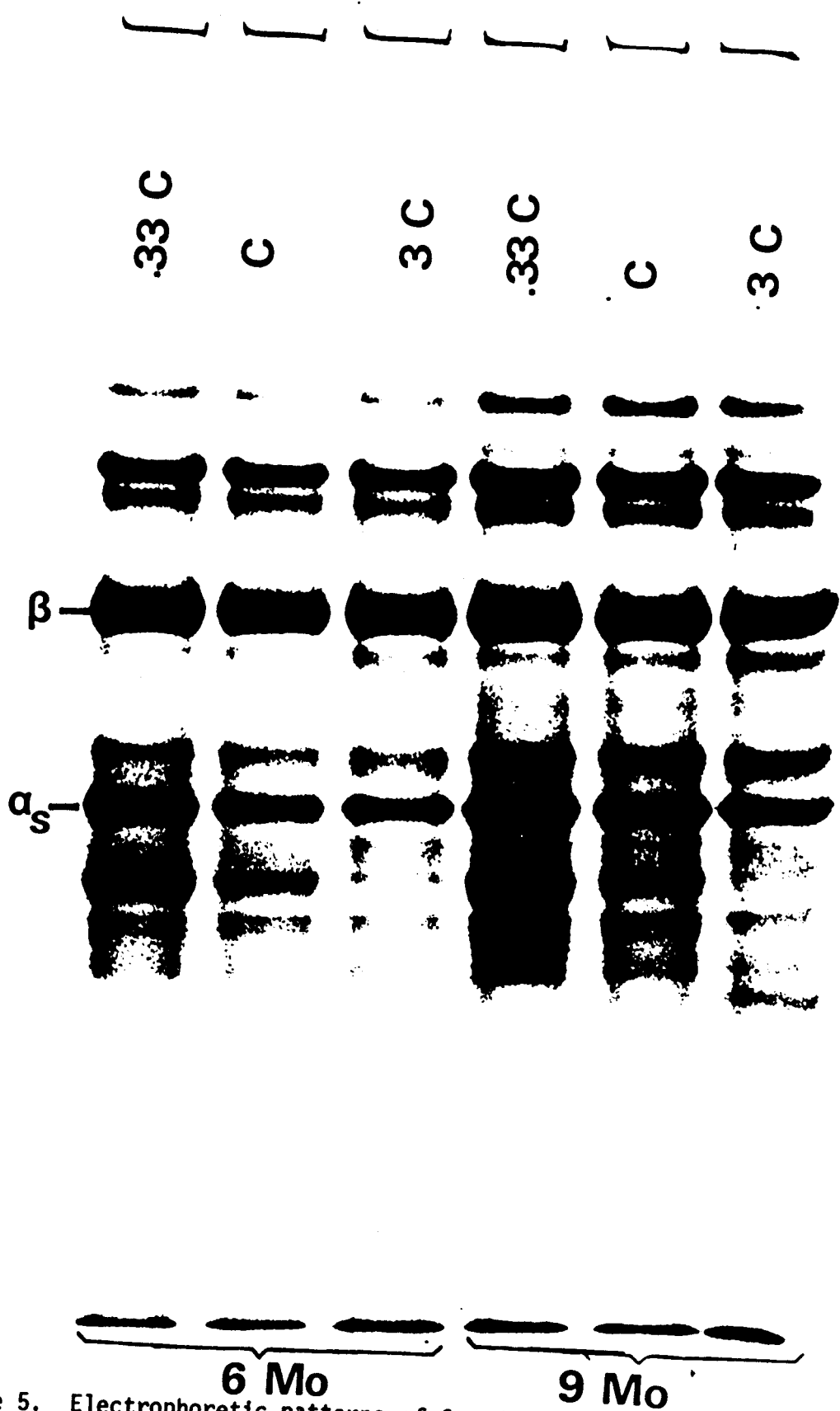


Figure 5. Electrophoretic patterns of 6 and 9 month cheese made with pepsin
 .33C = 30 ml/454 kg milk, C = 90 ml/454 kg milk, 3C = 270 ml/454 kg milk

ripening. The γ -caseins (γ_1 , and γ_2) were present in cheese of all treatment groups; γ_3 -casein appeared as a very faint band.

At 1 month of ripening, cheese containing highest level of residual chymosin had undergone extensive proteolysis of α_{s1} -casein (Figure 4). The primary breakdown product of α_{s1} -casein, α_{s1} -I peptide had also been degraded. Cheese containing normal levels of residual chymosin had large amounts of intact α_{s1} -casein and α_{s1} -I peptide. Slight breakdown of α_{s1} -I peptide was evident in this cheese (Figure 4). Extent of breakdown of α_{s1} -casein to give α_{s1} -I peptide in cheese containing lowest level of residual chymosin was smaller. This is shown by the small amount of α_{s1} -I peptide present and large amount of intact α_{s1} -casein (Figure 4). Beta-casein was only slightly degraded. The extent of degradation is obviously related to amount of residual chymosin present. This is evident by the intensity of β -I band present.

At 3 months ripening time, a small amount of α_{s1} -I peptide was present in cheese containing high levels of residual chymosin. Cheese containing normal and low levels of residual chymosin had large amount of α_{s1} -I peptide present. Intensity of γ_2 - and γ_3 -casein bands were high and there was a concomitant decrease in β -casein in all 3 treatment groups (Figure 5).

Electrophoretic patterns of 6 and 9 month old cheeses containing high (60 clotting units/kg cheese), normal (10 clotting units/kg cheese) and low (7 clotting units/kg cheese) levels of chymosin are shown in Figure 5. Almost complete disappearance of α_{s1} -I peptide was observed in cheese containing high levels of

residual chymosin at 6 and 9 months of ripening. Cheese containing low levels of residual chymosin had considerable amounts of α_{s1} -I peptide present.

b. Primary proteolysis in cheese made from milk set with chymosin or porcine pepsin

Electrophoretic patterns of cheese made with porcine pepsin and of cheese made with normal levels of chymosin are shown in Figures 6 and 7.

Cheese made from milk set with pepsin at pH 6.6 (containing no residual coagulant) pepsin and did not show any distinct proteolysis of α_{s1} -casein after 1 day of ripening (Figure 6). Proteolysis of α_{s1} -casein in cheese made from milk set with pepsin at pH 6.2 and containing about 1 clotting unit pepsin per kilogram cheese was less than in cheese containing normal levels of residual chymosin. There was no evidence of proteolysis of β -casein in 1 day old cheese for any of the treatment groups. The γ -caseins (γ_1 -, γ_2 - and γ_3 -) were present in all cheeses. Intensity of γ_2 -casein was highest and γ_3 -casein was present as a very faint band (Figure 6).

After 1 month ripening, cheese containing residual chymosin had a distinct α_{s1} -I peptide. The α_{s1} -I peptide also was present in cheese containing no residual pepsin. A β -I peptide, appeared as a very faint band in cheese containing residual chymosin (Figure 6).

Proteolysis in cheese at 3 months ripening are shown also in Figure 6. Major differences were observed in amount of intact α_{s1} - and β -caseins. Cheese containing no residual coagulant had large amounts of intact α_{s1} - and β casein. These cheeses also had more

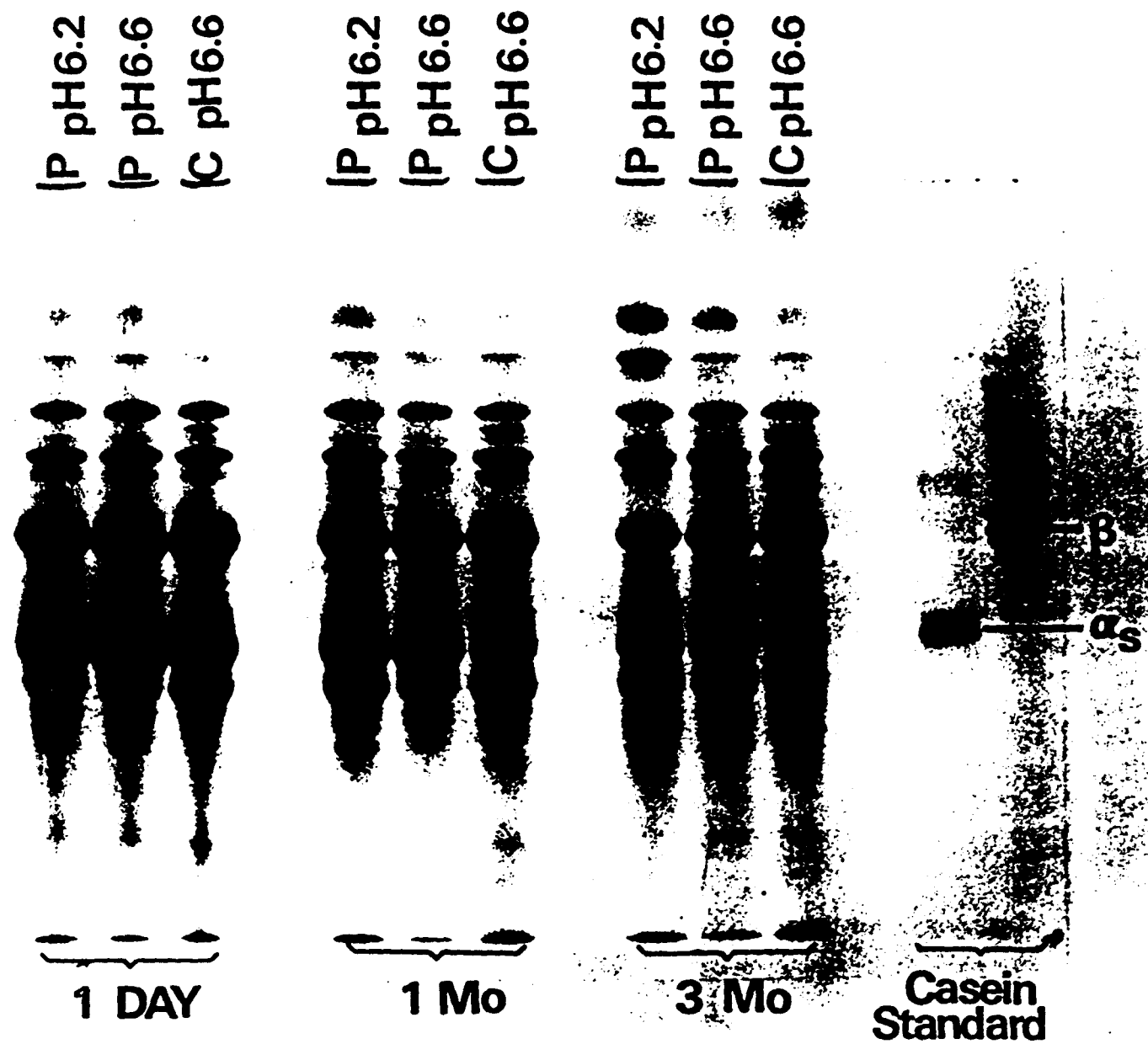


Figure 6. Electrophoretic patterns of cheese made with pepsin or chymosin.

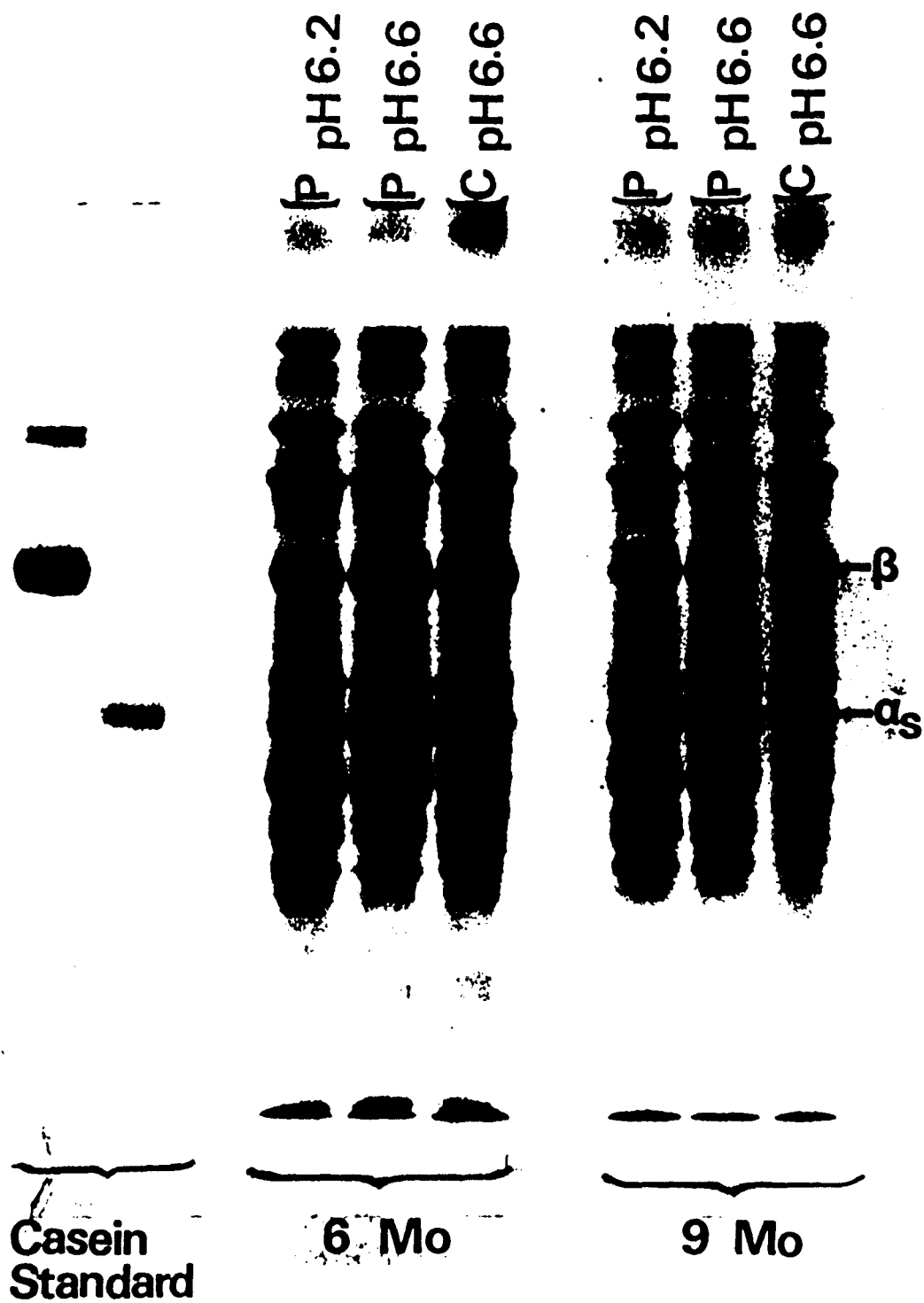


Figure 7. Electrophoretic patterns of cheese made with chymosin or pepsin

intense α_{s1} -I peptide. A striking decrease in β -casein was observed in cheese containing residual pepsin. These cheeses had 2 unidentified bands of lower electrophoretic mobilities than the γ -caseins. Peptides corresponding to these bands could have arisen from β -casein.

Six and 9 month old cheese containing residual chymosin showed extensive degradation of the α_{s1} -I peptide and increased intensities of γ_2 -casein and β -I peptide bands. Cheese containing residual pepsin did not show any further degradation of α_{s1} -I peptide at 6 and 9 months ripening. This cheese had smaller amounts of α_{s1} - and β -caseins present (Figure 7). At 6 and 9 months of age, cheeses without residual coagulant still had the largest amount of α_{s1} -casein, α_{s1} -I peptide, and β -casein.

Electrophoretic patterns of coagulant-free, starter-free cheese curd at 1 day, 1 month and 3 months of ripening are shown in Figure 8. No major differences were observed between control cheese curd and cheese-curd containing plasmin inhibitor ϵ -Amino-n-caproic acid at any ripening time.

Secondary Proteolysis

Percent of total nitrogen soluble in water increased from about 18% at 1 month of ripening to 44% at 9 months of ripening for cheese containing high levels of residual chymosin (60 CU/kg cheese). Cheese containing normal levels of residual coagulant had 14% water soluble nitrogen at 1 month ripening, 26% at 3 months, 31% at 6 months and 36% at 9 months. Eleven percent water soluble nitrogen was measured in cheese containing low levels of residual



Figure 8. Electrophoretic patterns of coagulant free, starter free cheese curd
A = control (no plasmin inhibitor), B = experimental with plasmin inhibitor
1 = 1 day, 2 = 1 month, 3 = 3 months ripening

chymosin (7 CU/kg cheese) at 1 month ripening. The amount of water soluble nitrogen increased to 31% at 9 months of ripening (Figure 9).

Cheese made with porcine pepsin, pH 6.6 (containing no residual coagulant) had an average of 8% water soluble nitrogen at 1 month of ripening. The amount of water soluble nitrogen doubled to 16% in 3 months, after which the rate of increase was gradual to 25% at 9 months of ripening. Cheese containing residual pepsin (1 CU/kg cheese) and residual chymosin (10 CU/kg cheese) had higher amounts of water soluble nitrogen at all ages of ripening than cheese without residual coagulant (Figure 10).

Cheese Flavor

Mean flavor scores of chymosin cheese at various times of ripening are shown in Figure 11. At $\alpha = .05$ level, no significant differences were observed between mean flavor scores of cheese for the various treatment groups at 15 days and 1 month of ripening. However, flavor scores were significantly different as ripening progressed. Cheese containing low levels of residual chymosin scored best in flavor, and cheese containing high levels of residual chymosin were poorest in flavor. Judges criticized the cheese with high levels of residual chymosin as slightly bitter at 3 months extremely bitter at 6 months and bitter at 9 months of age.

A comparison of mean flavor scores of cheese containing residual chymosin and no residual coagulant is shown in Figure 12.

Initial flavor scores at 15 days of ripening did not show any significant differences between the two treatment groups.

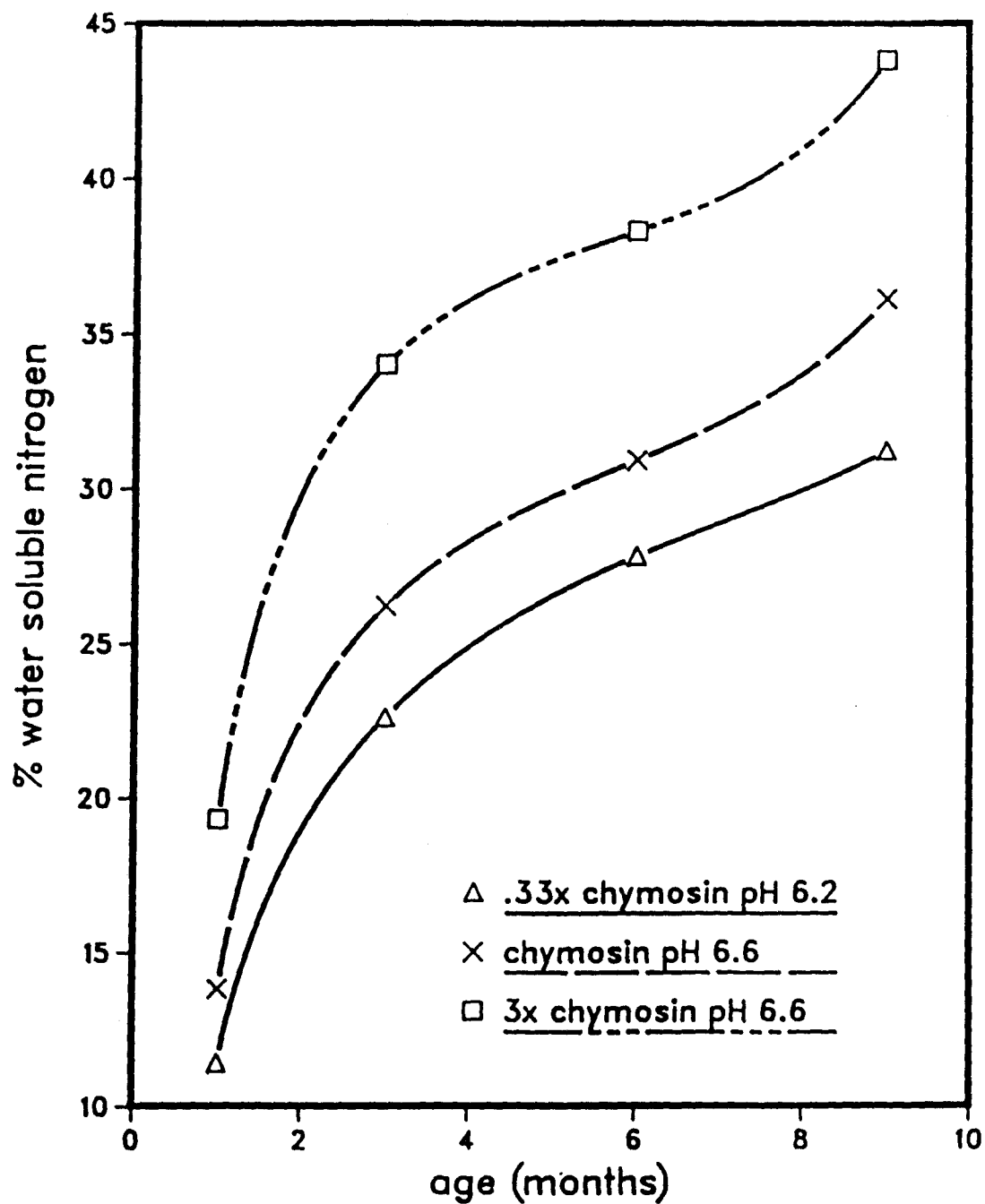


Figure 9. Comparison water soluble nitrogen in chymosin cheese at various ages of ripening.

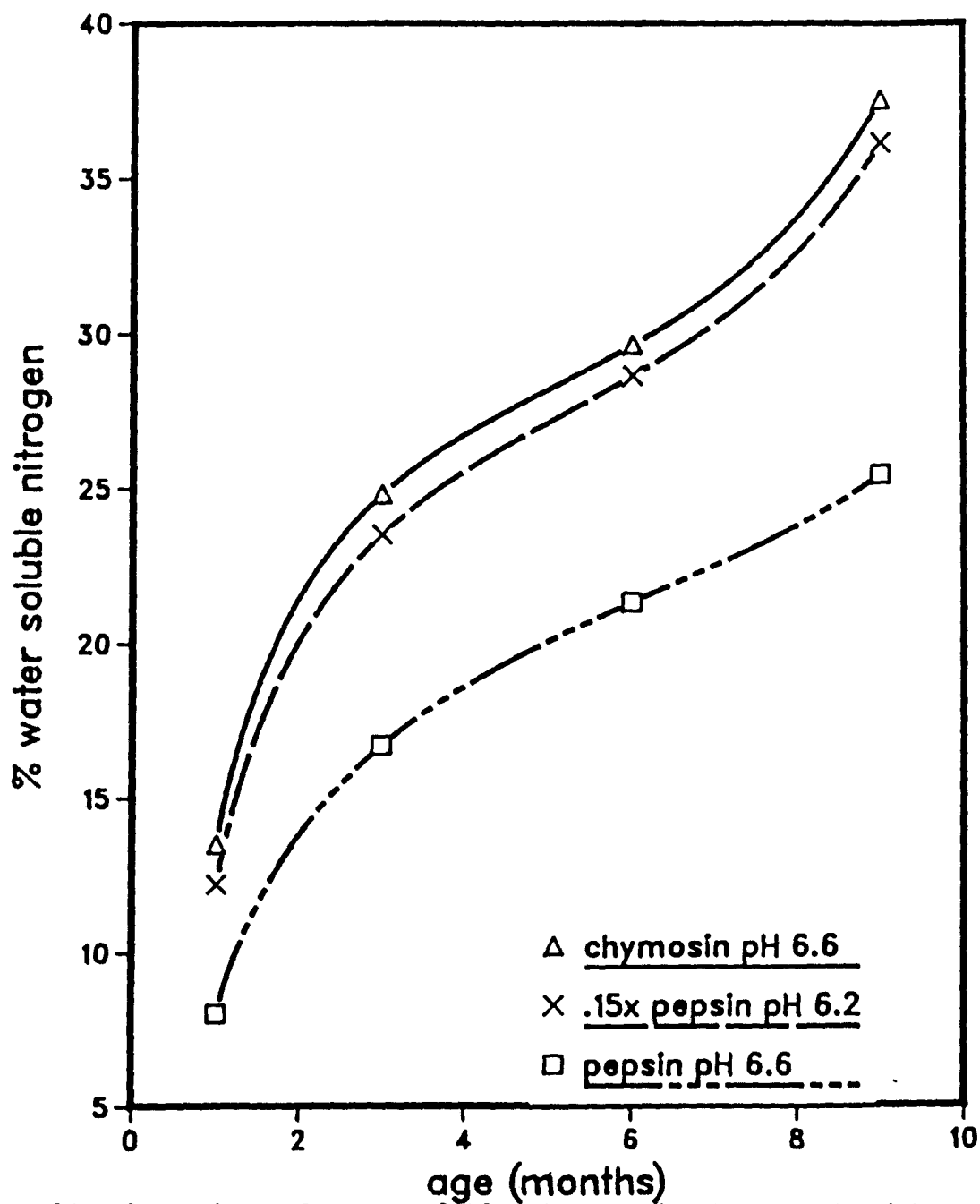


Figure 10. Comparison of water soluble nitrogen in cheese made with chymosin or pepsin at various ripening times.

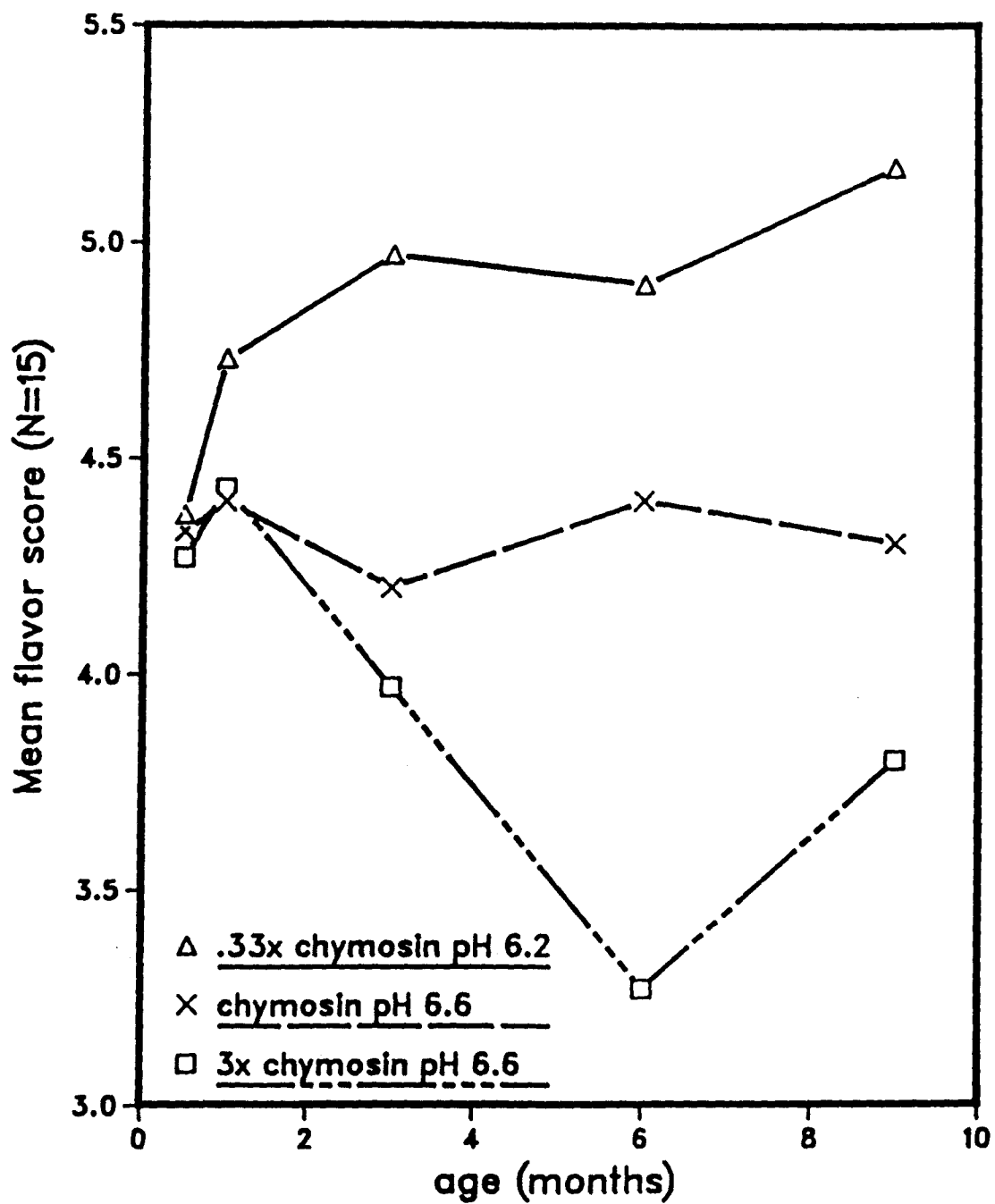


Figure 11. Comparison of flavor scores of chymosin cheese.

Significant difference in flavor scores between the two treatment groups were observed at 1 month of ripening, and cheese containing residual chymosin had the best flavor scores. At 3 months of Initial flavor scores at 15 days of ripening did not show any significant differences between the two treatment groups.

Significant differences in flavor scores between the two treatment groups were observed at 1 month of ripening and cheeses containing residual chymosin had the best flavor scores. At 3 months of ripening, even though cheeses containing residual chymosin had higher flavor scores (Figure 12), the difference between the scores of the two treatment groups was not statistically significant at an α -level of .05. At 6 and 9 months of ripening, cheese containing no residual coagulant were superior to cheeses containing residual chymosin. Significant differences in flavor scores were at an α -level of .05.

Cheese containing residual pepsin (1 CU/kg cheese) did not differ in flavor from cheese containing no residual coagulant at 15 days and 1 month of ripening (Figure 13). However, at 3 months and 6 months of ripening, cheese with residual pepsin had significantly better scores than cheese without residual coagulant. At 9 months ripening, the pattern reversed and cheese without residual coagulant scored better than cheese with residual pepsin.

Cheese Flavor Intensity

Figure 14 shows mean flavor intensity score at various ages for cheese made with and containing various levels of chymosin. At any age, no significant difference in mean flavor intensity score was

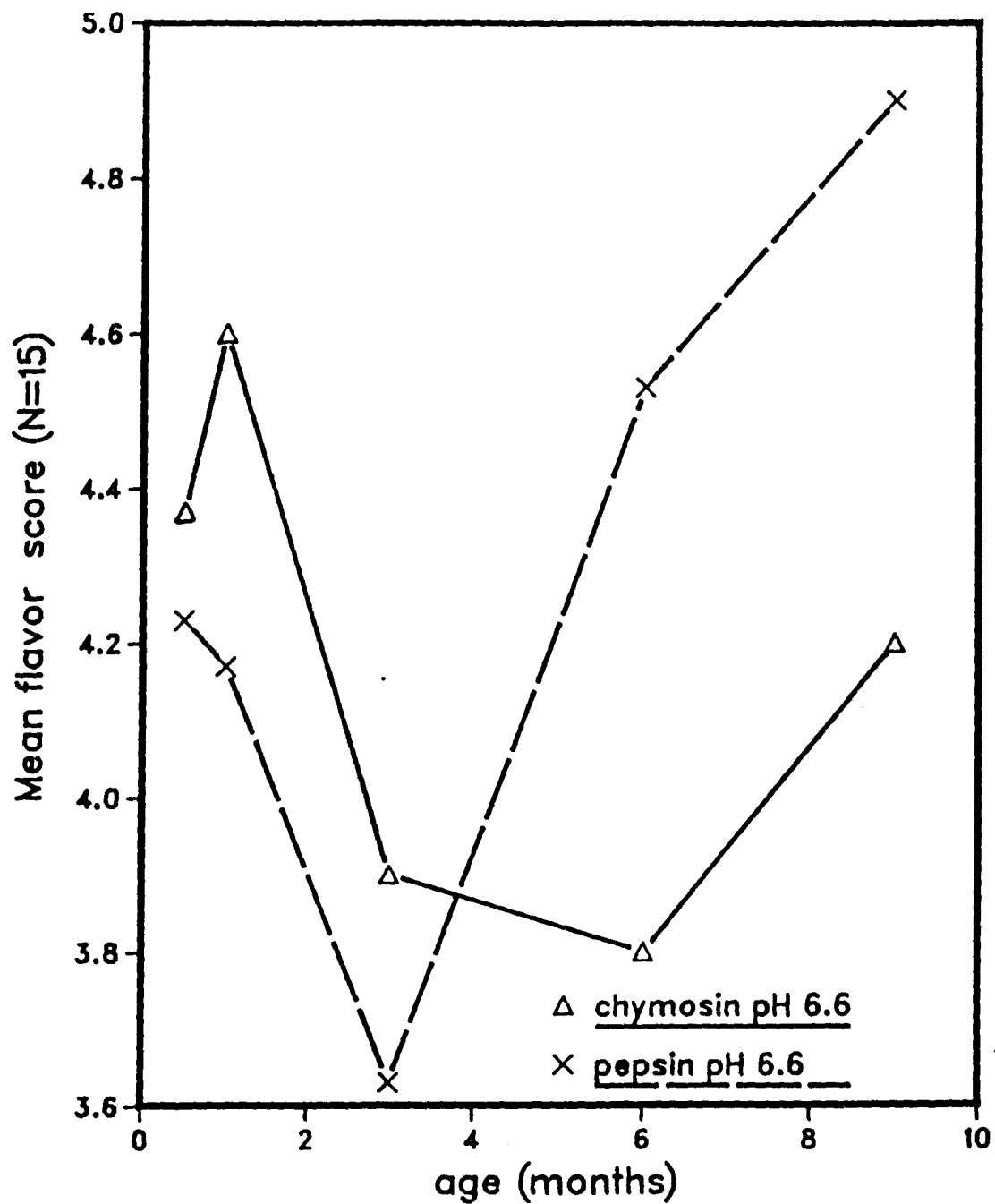


Figure 12. Comparison of flavor scores of cheese manufactured from milk set with porcine pepsin or chymosin at pH 6.6.

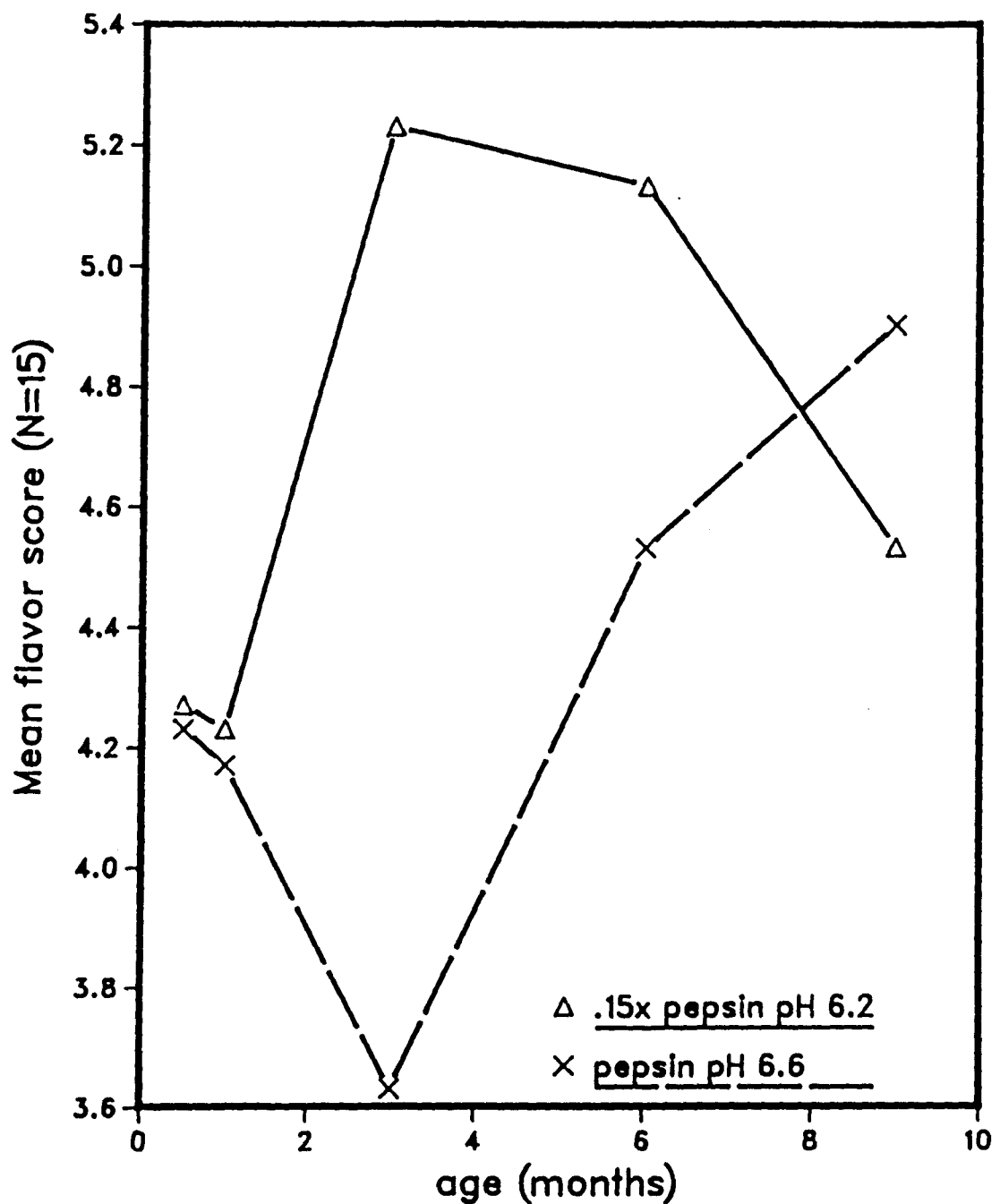


Figure 13. Comparison of flavor scores of cheese made from milk set with porcine pepsin at different pH values.

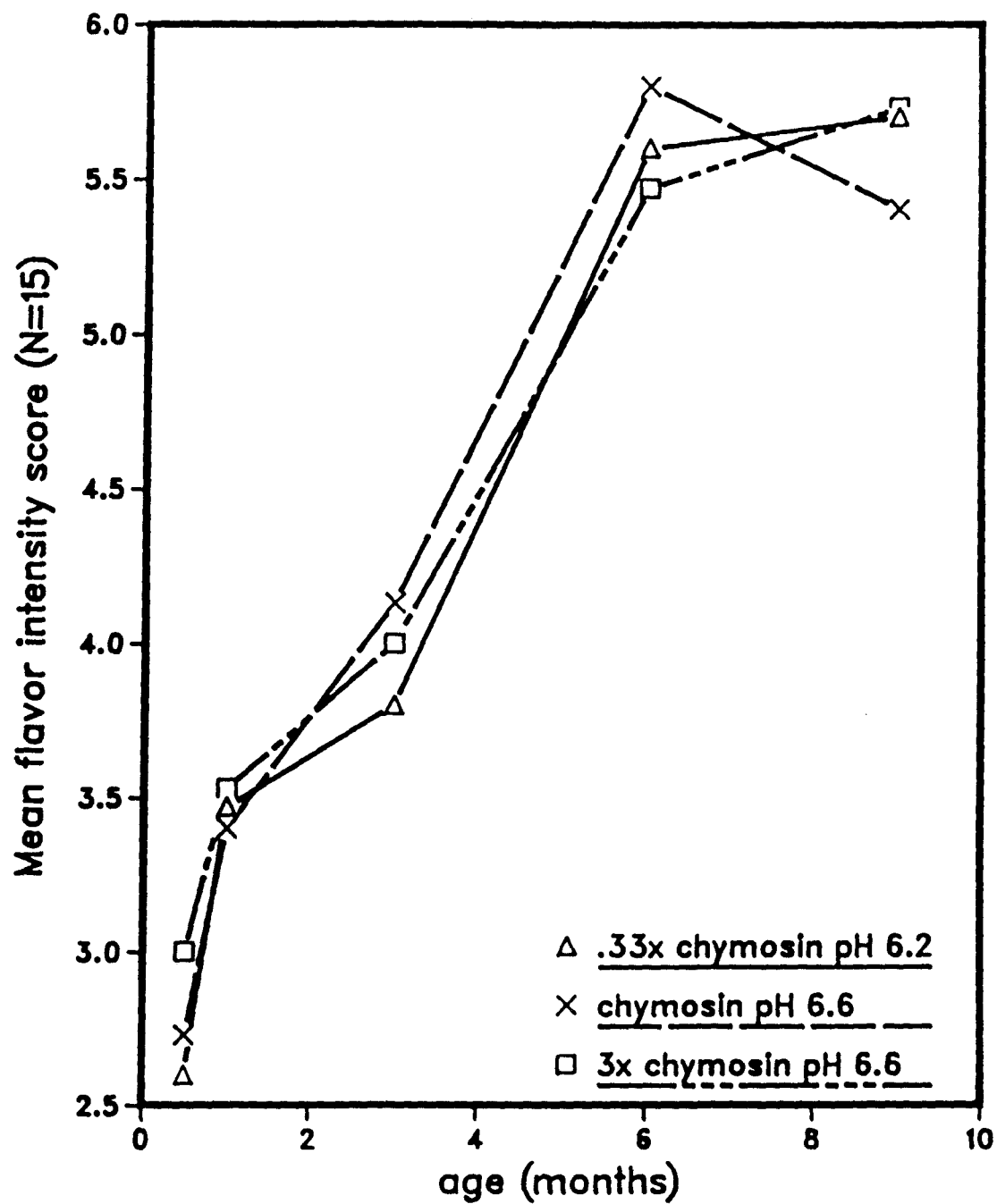


Figure 14. Flavor intensity of cheese made with chymosin.

observed. For each treatment cheese flavor intensity increased with age except for an unexpected decrease observed at 9 months of age for cheeses made with and containing normal levels of chymosin. This unexpected decrease was not statistically significant ($\alpha = .05$).

Flavor intensity scores for cheese containing residual chymosin and cheese without residual coagulant are compared in Figure 15. Intensity of flavor increased during ripening and paralleled each other for each treatment group. At no stage of ripening was there a significant difference in flavor intensity scores between the two treatment groups. Similar results were obtained when flavor intensity scores for cheeses containing residual pepsin were compared with cheese containing no residual coagulant (Figure 16).

Cheese Body

Major differences in body (as reflected by body scores) of cheese containing different levels of chymosin were evident at 15 days and 1 month of ripening (Figure 17). Cheese containing high levels of residual chymosin (60 CU/kg cheese) had highest body scores and those with low levels of residual chymosin (7 CU/kg cheese) had lowest body scores at 15 days and 1 month of ripening. Statistically, mean body scores of cheeses were significantly different ($\alpha = .05$) at 15 days and 1 month ripening. No significant differences in body scores were obtained between cheese of treatment groups at 3, 6 and 9 months of ripening.

A comparison of body scores of cheese containing residual chymosin and no residual coagulant is shown in Figure 18.

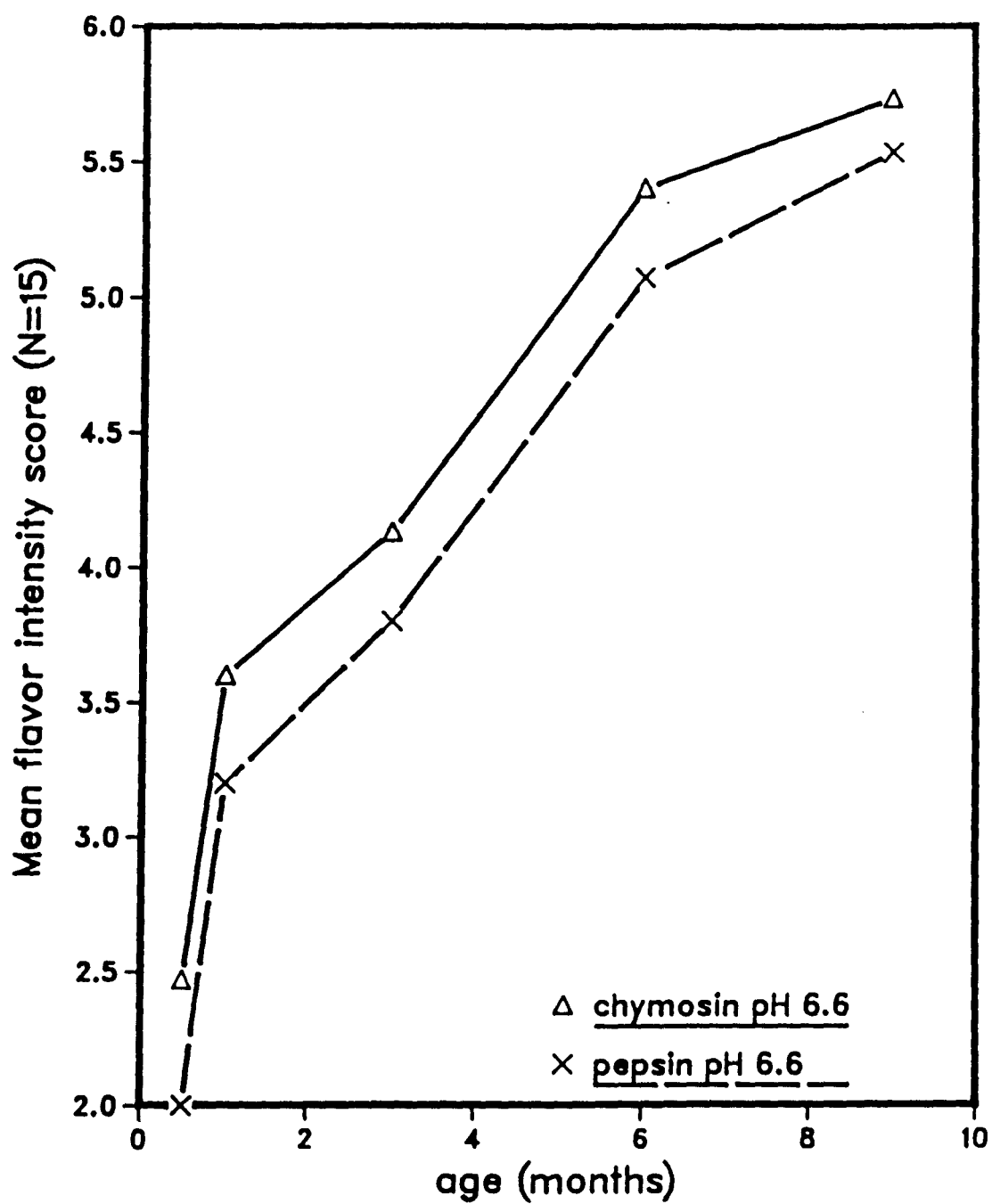


Figure 15. Flavor intensity of cheese made with porcine pepsin or chymosin.

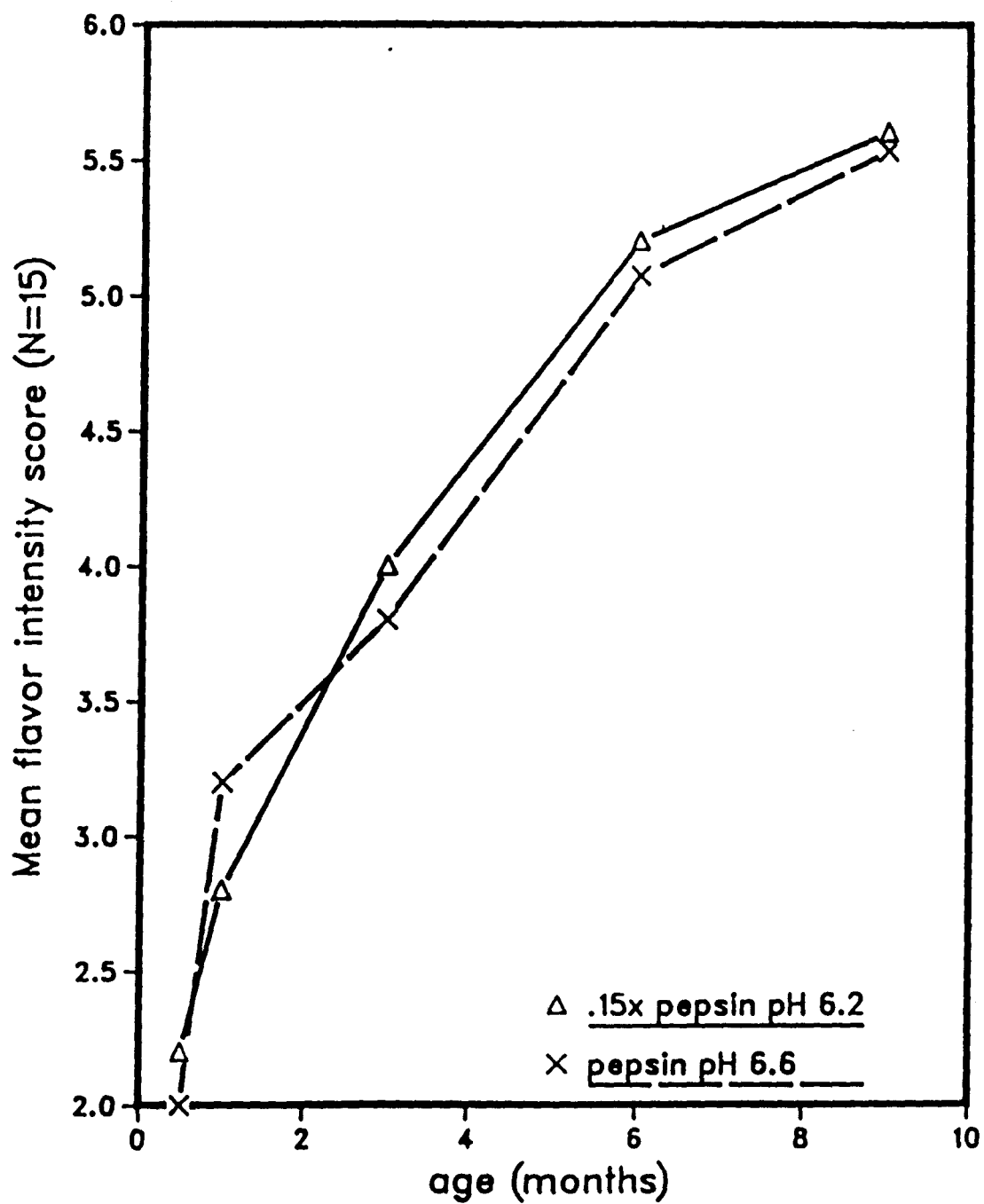


Figure 16. Flavor intensity score of cheese made from milk set at pH 6.2 or 6.6 with porcine pepsin.

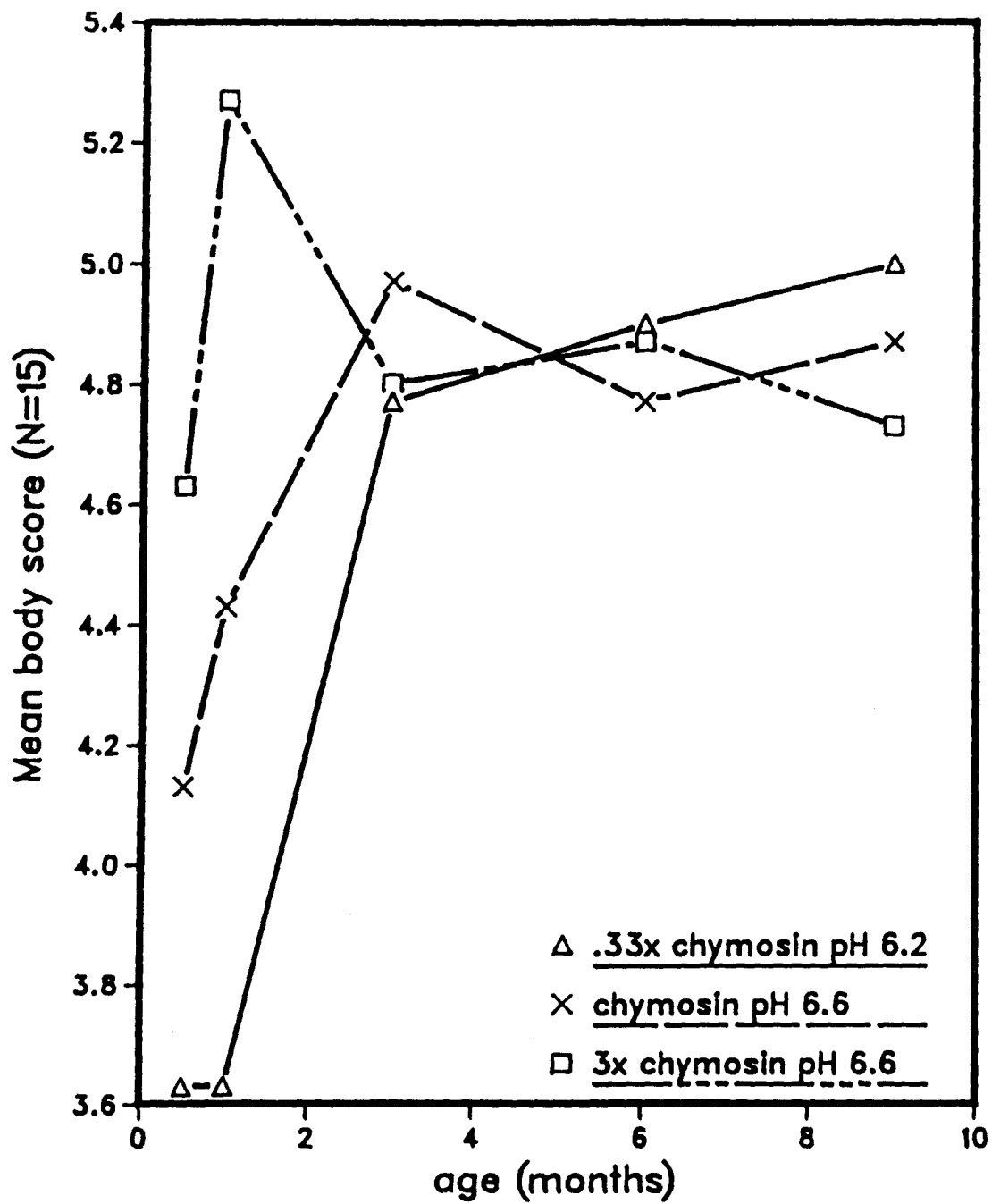


Figure 17. Body scores of cheese made from milk set with chymosin.

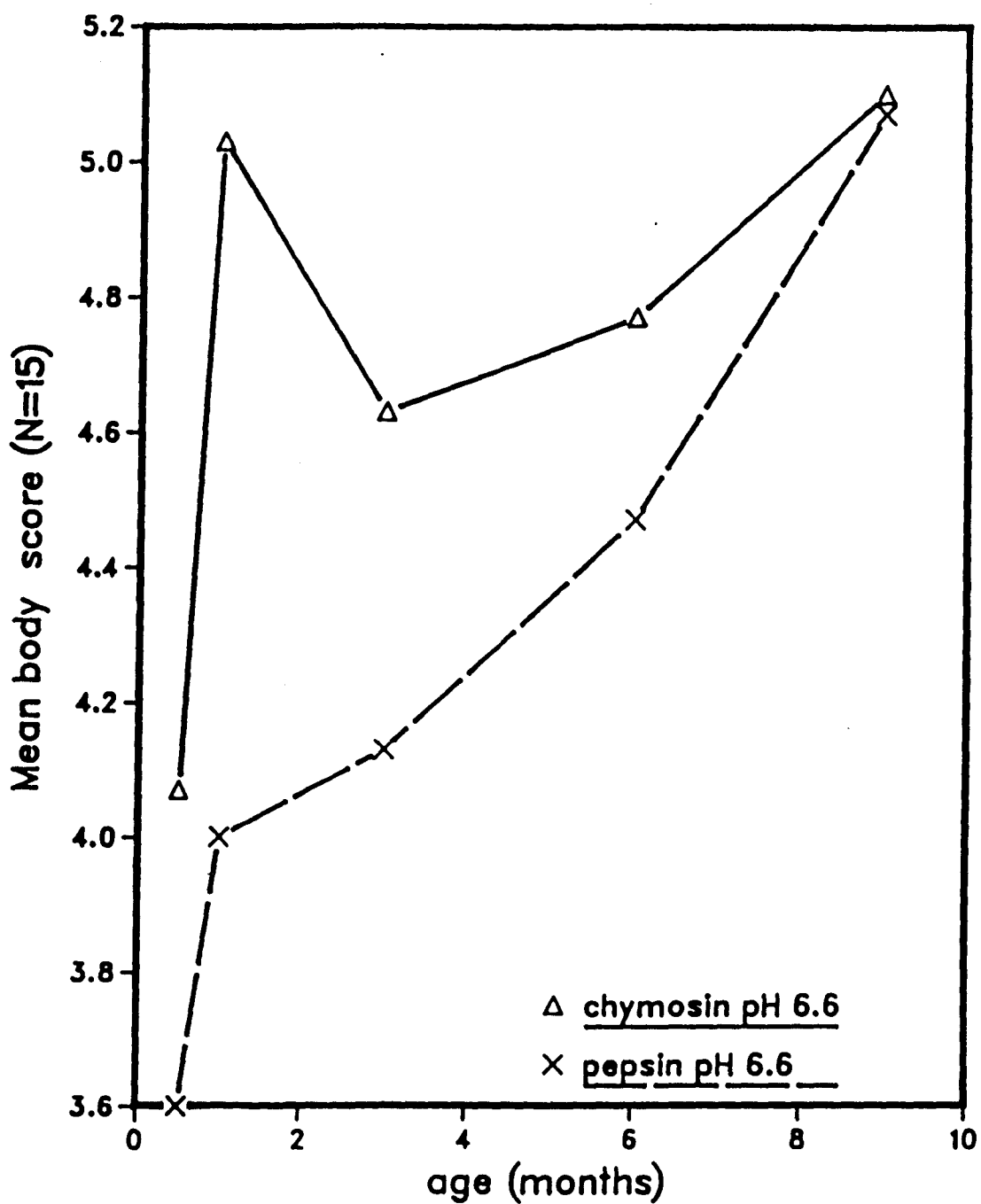


Figure 18. Body scores of cheese made from milk set at pH 6.6 with chymosin or porcine pepsin.

Significant differences in body scores at an alpha level of .05 was observed during the first 3 months of ripening. Cheeses containing residual chymosin exhibited a long body. Cheese containing no residual coagulant was soft and curdy. However, as it aged, cheese devoid of residual coagulant improved in body and at 6 months and 9 months of ripening no significant differences in body scores were noted.

Figure 19 shows mean body scores of cheese containing no residual coagulant and residual pepsin. Mean body scores of those cheeses were significantly different ($\alpha = .05$) at 3 and 6 months of ripening; cheese containing residual pepsin was most preferred by the judges. At 9 months ripening, mean body score of cheese without residual coagulant was better than that of cheese with residual pepsin.

Overall Quality of Cheese

Overall quality scores of cheese made from milk set with various levels of chymosin are shown in Figure 20. At 15 days and 1 month of age, cheese containing low levels of residual chymosin (7 CU/kg cheese) had overall quality scores which were significantly different from cheese containing normal (10 CU/kg cheese) and high (60 CU/kg cheese) levels of residual chymosin. After 3 months ripening, cheese containing low levels of residual chymosin were best in quality compared to cheese with normal and high amount of residual chymosin. Poorest quality scores were obtained in cheese containing high levels of residual chymosin.

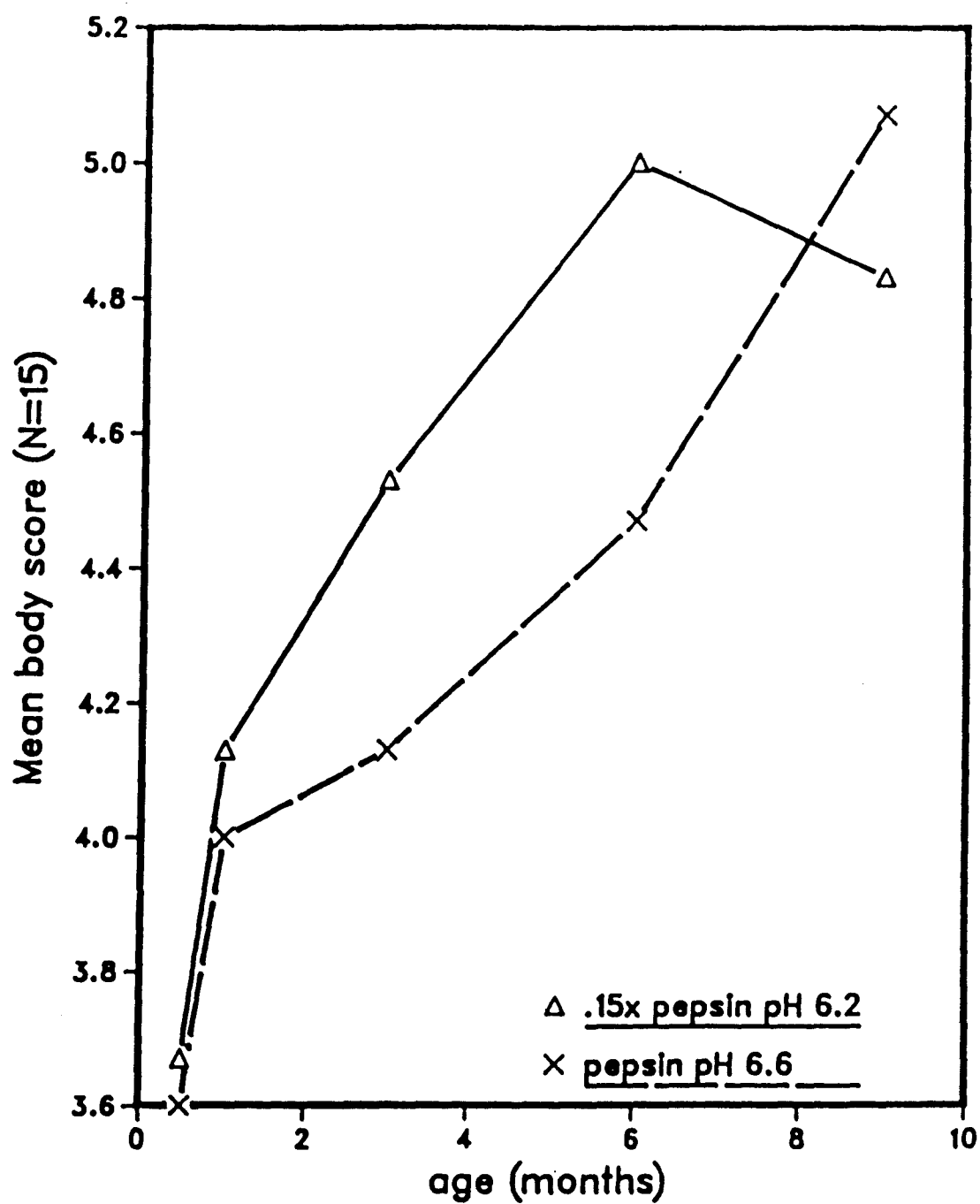


Figure 19. Body scores of cheese made from milk set at pH 6.2 or 6.6 with porcine pepsin.

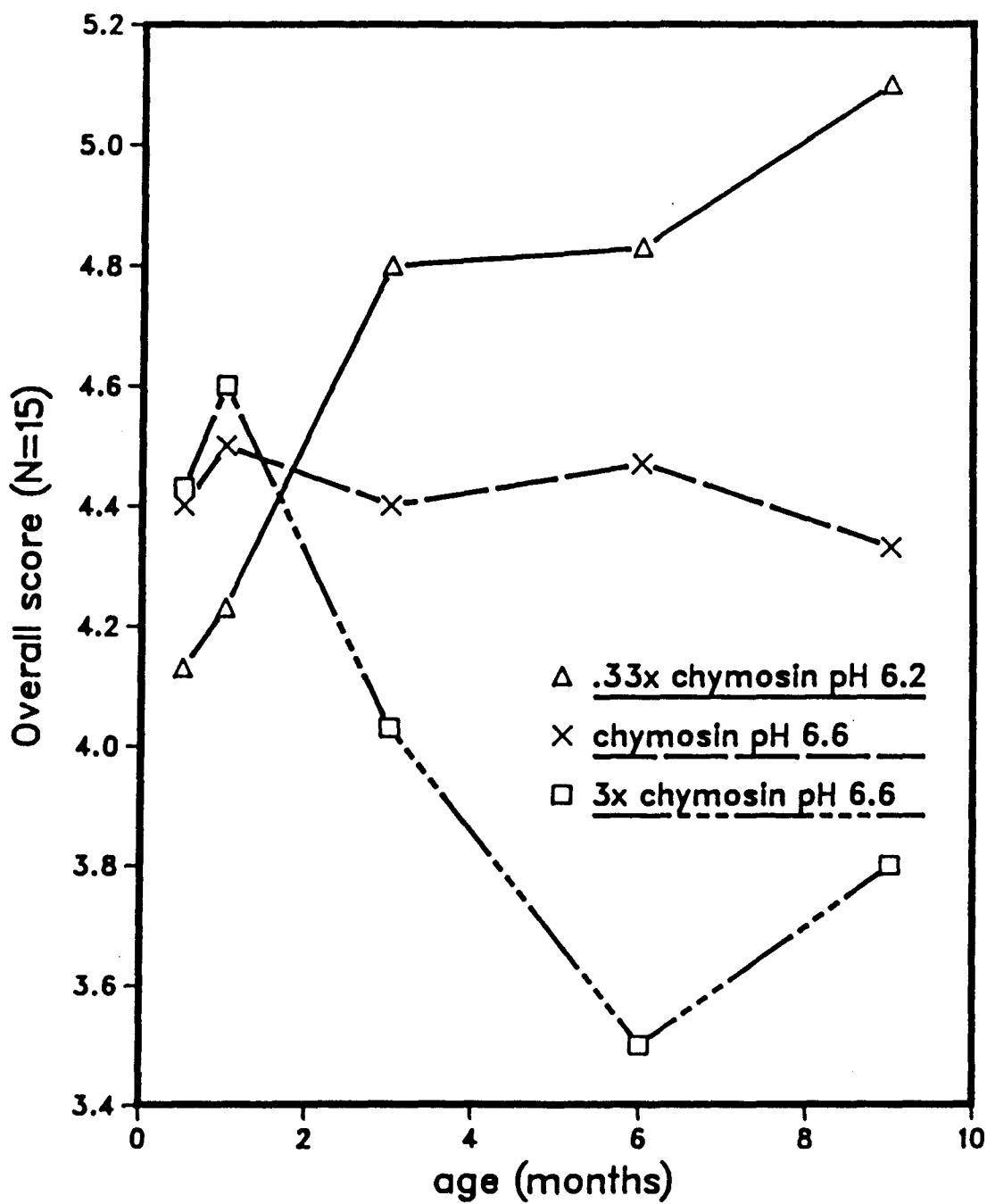


Figure 20. Overall quality of cheese made with chymosin.

Figure 21 shows overall quality scores of cheese made from milk set with chymosin or pepsin. In young cheese (15 days and 1 month old), chymosin cheese was best with overall scores significantly different from cheese made with pepsin. Cheese containing residual pepsin (1 CU/kg cheese) was best at 3 and 6 months ripening. As cheese aged, overall quality of cheese manufactured from milk set at pH 6.6 with porcine pepsin and containing no residual coagulant improved in its quality nad was best at 9 months. Cheese containing normal levels of residual chymosin was the poorest amongst the three treatment groups.

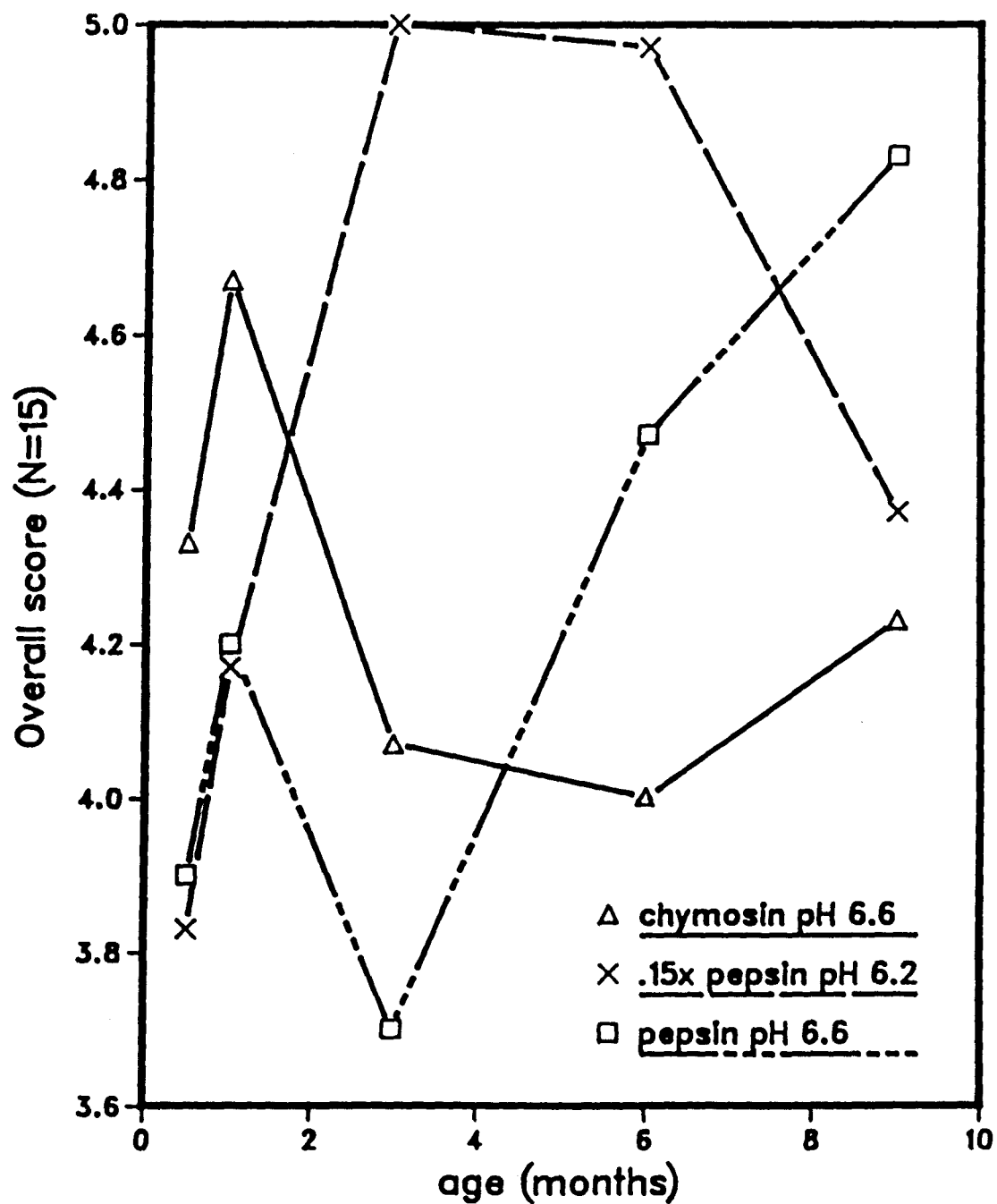


Figure 21. Overall quality of cheese made from milk set with chymosin.

DISCUSSION

I. Cheese Composition and Yield

No innovation in the manufacture of a product attracts the attention of entrepreneurs unless it meets legal standards and does not cause yield losses even if consumer acceptability is in "toto". Legal standards of identity of Cheddar cheese in the U.S. require that it contains not more than 39% moisture and not less than 50% fat in the dry matter. All cheese manufactured in this study met these legal standards. There was no significant difference in composition or yield between cheeses made with porcine pepsin or chymosin. Lucas (49) obtained slightly lower yields in cheese made with porcine pepsin compared to cheese made with chymosin. Cheese yield is determined by efficient recovery of fat, protein and moisture from cheese milk (88). Manufacturing conditions resulting in excessive losses of fat and casein in whey are undesirable. These effects are minimized when a firm coagulum is obtained during manufacture. Amount and type of coagulant used for manufacture affects firmness at cutting. It was therefore necessary to adjust manufacturing conditions when lower or higher than normal levels of chymosin was used in order to insure a product yield similar to what is normally obtained.

Use of porcine pepsin as a coagulant has not gained much popularity because it is very sensitive to pH and will not coagulant milk at pH greater than 6.8 (23). This problem can be alleviated by standardizing pepsin against chymosin in sweet milk before being

used as a coagulant. By doing this, enough pepsin can be used to provide a firm set in 30 min.

II. Residual Coagulant in Cheese

Holmes et al. (35) were the first to measure residual coagulant in cheese. Other methods, published by Stadhouders et al. (79), Dulley (17), and Carlson et al. (7) and Matheson (53) involve cumbersome laborious techniques and therefore, have not gained as much popularity in the dairy industry as the diffusion substrate technique of Holmes et al. (35). A recent improvement in Holmes' method (35) by Majeed and Ernstrom (50) has made the diffusion technique suitable for accurate determination of porcine pepsin activity in cheese hence, its use in this study.

Results of this study confirm earlier work (35,50) which shows that about 6% of original chymosin activity added to Cheddar cheese milk remains in cheese curd when 90 ml chymosin are used per 454 kg milk. Also, this work agrees with reports (35,50) that the amount of residual chymosin or porcine pepsin remaining active in cheese is pH dependent. However, there is no pH dependency of microbial rennets. Amount of chymosin required to give a firm 30 min set is less as setting pH is lowered, but a higher percentage of the enzyme will remain in the curd. Establishment of a numerical relationship involving setting pH and amount of chymosin required to give a desirable setting time of 30 min needs further study. In this experiment, a setting pH of 6.2 required a 67% reduction in chymosin from normal levels (90 ml/454 kg milk) to 32 ml/454 kg milk, but left 13% of the original activity in the curd. Numerical

relationship between amount of chymosin used to set cheese milk and its final activity remaining in the curd is also difficult to predict because as yet, no experiment involving a stepwise concentration of chymosin for Cheddar cheese manufacture is available in the literature. Though this is not of practical importance in the manufacture of Cheddar cheese as elucidated in a latter part of this discussion, it might be of concern in cheese requiring high chymosin levels for manufacture. Results from this study show that trebling the amount of chymosin used for manufacture results in a 6-fold increase in activity of residual chymosin per kilogram of cheese.

This study agrees with earlier work (35,50) that when porcine pepsin is used to set cheese milk at pH 6.6, it is totally inactivated leaving no residual activity in cheese. Activity of porcine pepsin is greater than that of chymosin at low pH values. Hence, when porcine pepsin was used as a coagulant for cheese milk at pH 6.2, 15% of the amount used in sweet milk was required for setting. This left about 5% (\approx 1 CU/kg cheese) of the original activity (890 clotting units/410 kg milk) in the cheese.

III. Proteolysis of α_{s1} -casein during ripening

It is well documented in the literature (60) that in cheese, α_{s1} -casein is degraded to produce α_{s1} -I peptide by the primary action of milk coagulants. However, there are conflicting reports on the rate and extent of breakdown of α_{s1} -casein and α_{s1} -I peptide. Ledford et al. (48) reported almost complete degradation of α_{s1} -casein at 55 days ripening while Phelan et al. (66) reported

that large amounts of α_{s1} -casein remained intact at 7 months of ripening. It is evident that the rate and extent of proteolysis of α_{s1} -casein during ripening depends on the type and amount of residual coagulant left in the cheese. High levels of residual chymosin in cheese resulted in rapid degradation of α_{s1} -casein and the α_{s1} -I peptide. At 3 months ripening, amount of α_{s1} -I peptide was very small in cheese containing high levels of residual chymosin. A linear relationship exists between rennet concentration and early ripening of Meshanger (15) and Gouda (86) type cheeses.

Residual porcine pepsin in cheese attacked α_{s1} -casein to produce α_{s1} -I peptide. While α_{s1} -casein continued to be extensively degraded by porcine pepsin, α_{s1} -I peptide appeared to resist further proteolysis by residual porcine pepsin. This was a major difference between proteolysis of α_{s1} -casein by chymosin and porcine pepsin in cheese.

Degradation of α_{s1} -casein occurred slower in cheese containing no residual coagulant. Breakdown of this protein in the absence of coagulant could be due to enzymes from starter bacteria or native milk proteases. It is evident from this study that if native milk proteases were involved, the alkaline milk protease, plasmin, was not responsible. This is because proteolysis of α_{s1} -casein occurred in coagulant-free, starter-free cheese curd containing a potent chemical inhibitor of plasmin. An acid milk protease was therefore responsible for degradation of α_{s1} -casein in the curd containing no coagulant and no starter organisms (exogenous bacteria was less than 1 cfu/g cheese). Kaminogawa and Yamauchi (41) have also suggested

an acid protease in milk analogous to cathepsin D which has a rennet like action on α_{s1} -casein.

IV. Proteolysis of β -casein During Cheese Ripening

Results of this study show that β -casein is degraded during Cheddar cheese ripening. This is contrary to an earlier report by Ledford et al. (48) indicating that β -casein is not hydrolyzed by rennet enzymes during Cheddar cheese ripening. Electrophoretic patterns of cheese proteins demonstrate that the extent of degradation of β -casein depends on the type and amount of residual coagulant in the cheese. Chymosin hydrolyzes β -casein to produce β -I peptide during early ripening. The increased intensities of γ -caseins with age of cheese and concomitant decrease in β -casein in cheese containing residual pepsin indicate that porcine pepsin hydrolyzes β -casein to produce the γ -caseins. Creamer (8) attributed the presence of γ -caseins in 11 month old Gouda and 30 month old Cheddar cheese to the action of a native milk protease which he later identified as plasmin. There is no doubt that plasmin hydrolyses β -casein into γ -caseins in solution. Whether or not this is the case in cheese is questionable. Results of this study do not agree with Creamer's (8) observation. Cheese manufactured from milk set with porcine pepsin at pH 6.6 and containing no residual coagulant did not show any increased intensity of the γ -caseins nor a decrease in β -casein during ripening. Similarly, cheese containing residual chymosin did not show increased intensity of γ -casein. Interestingly, all the

cheese was manufactured from the same lot of milk. Therefore the increased intensity of γ -casein observed in pepsin cheese was due to the action of residual porcine pepsin.

V. Secondary Proteolysis

Proteolytic products soluble in the aqueous phase of cheese arise from secondary proteolysis. In this study, percent of total nitrogen soluble in water increased with increased level of coagulant in cheese. Secondary proteolysis occurred even when residual coagulant was not present in cheese. Rate and extent of proteolysis in the cheese containing no residual coagulant were slow. This result indicates that natural milk proteases and proteases from starter organisms contribute to proteolysis in cheese during ripening. The relative contributions of these enzymes is yet to be elucidated.

VI. Effect of Residual Coagulant on Cheese Flavor

"Characteristic" Cheddar cheese flavor, if there is any, develops over the course of ripening. Hydrolysis of caseins in cheese contribute significantly to cheese flavor. It is very evident from this study that increased proteolysis due to presence of high levels of residual chymosin in cheese results in bitterness in cheese after 3 months of ripening. The fact that bitterness was detected in cheese made with normal and high levels of chymosin and not in cheese made with pepsin relates the extent and type of proteolysis to bitterness. The only major difference in electrophoretic patterns of proteins in the cheeses is that α_{s1} -I peptide was resistant to proteolysis by porcine pepsin but was

degraded by chymosin. Rate of degradation of this peptide increased with level of residual chymosin in cheese and hence bitterness. Pelissier and Manchon (65) reported that hydrolysis of cow α_{s1} -casein produces more bitterness than hydrolysis of β -casein. Results of this study therefore indicate that bitterness of cheese originated from α_{s1} -casein by the action of excessive amounts of residual chymosin. Belitz and Weisser (4) reported that goat and ewe cheeses are not bitter compared to cheese from cow's milk. The only known difference between cow milk protein and goat milk protein is in α_{s1} -casein (87). This result does not support the argument by Lowrie et al. (47) that rennet enzymes do not create bitterness in Cheddar cheese.

Cheese flavor intensity increased with age whether or not residual coagulant was present. Residual coagulant in cheese was not necessary for development of ultimate Cheddar cheese flavor.

VII. Effect of Residual Coagulant on Cheese Body

Like cheese flavor, rate and extent of proteolysis during ripening affected cheese body. Therefore type and amount of residual coagulant are necessary for rapid development of a long and smooth body during cheese ripening. When high levels of residual coagulant was present in cheese, a long body developed during early ripening. However, as cheese aged, excessive proteolysis resulted in a weak and pasty body. On the other hand, cheese containing low levels of residual chymosin and porcine pepsin, as well as cheese not containing residual coagulant had a relatively short body during

early ripening. A long body resulted only after the cheese aged. Relating proteolysis to cheese body, the amount of α_{s1} -I peptide seems to be very important in giving cheese a solid body and texture. A solid and firm body was observed in cheese containing no residual coagulant and cheese containing residual pepsin during sampling and grating the cheese for analyses. Creamer and Olson (9) also reported a relationship between Cheddar cheese texture and hydrolysis of α_{s1} -casein.

VIII. Cheddar Cheese Quality: Chymosin vs Pepsin

At any time of ripening, quality of Cheddar cheese as judged by its flavor, body and texture is of great importance to both the cheese maker and the consumer. Proteolysis during ripening is a significant parameter which determines cheese quality.

Incorporation of large amounts of chymosin in the curd during cheese manufacture resulted in extensive proteolysis of α_{s1} -casein to give young cheese a good flavor and body, and aged cheese a bitter flavor and pasty body. Porcine pepsin, due to its pH sensitivity and its inability to survive cheese making when used at the normal pH of milk, did not contribute to cheese ripening. Cheese made with porcine pepsin at normal pH of milk relied on the action of natural milk proteases and proteases from starter bacteria for protein breakdown. Very slow hydrolysis of α_{s1} -casein in pepsin cheese resulted in medium and aged cheese of better quality than cheese made with chymosin. Previous work by Lucas (49), Melachoris and Tuckey (58), Emmons et al. (19) and Sherwood (75) showed similar results.

CONCLUSIONS

1. Chymosin degraded α_{s1} -casein more extensively in cheese than porcine pepsin.
2. Porcine pepsin hydrolysed β -casein in cheese to produce γ -casein.
3. Extensive degradation of α_{s1} -casein resulted in bitterness in cheese containing normal and high levels of residual chymosin. Use of normal levels of chymosin (90 ml/454 kg milk) for Cheddar cheese manufacture leaves about 10 clotting units/kg of cheese. This appears to be borderline for the development of good flavor and quality of cheese. Any change in manufacturing conditions which leads to incorporation of more chymosin into cheese will result in a detrimental effect on cheese during long term ripening.
4. Porcine pepsin does not produce bitter defect in Cheddar cheese because it does not survive cheese manufacture when used at the normal pH of milk.
5. Presence of excess residual coagulant in cheese was only important for manufacture of young cheese. However, for the manufacture of medium and aged cheeses, it had a detrimental effect on cheese flavor and overall quality.
6. A reduction of chymosin or use of pepsin for Cheddar cheese manufacture resulted in better quality medium and aged cheeses.
7. Native milk proteases (not plasmin) and proteases from starter organisms contributed significantly to long term ripening of Cheddar cheese in absence of residual coagulant.

INTERNAL PRESSURE CHANGES DURING PRE-PRESS AND VACUUM TREATMENT OF 290 kg STIRRED-CURD CHEDDAR CHEESE BLOCKS

Robert S. Reinbold

Uneven moisture distribution in 290 kg blocks of stirred-curd Cheddar cheese is a problem that has not been fully resolved. Moisture contents may range from 33% in the center of blocks to 42% at the corners after only a few days of ripening. Naturally this is unacceptable to production and quality control personnel. In order to characterize this problem, we have looked at numerous factors affecting moisture distribution such as temperature profiles, pH changes, salt distribution, whey drainage and mechanical pressure application. This talk will focus on pressure profiles during a 2 h pre-press and 2 h vacuum treatment of cheese blocks. Before I discuss the experimental approach and results of the study, I would like to briefly describe the process of manufacture of stirred-curd Cheddar cheese in 290 kg blocks.

The granular curd is distributed to stainless-steel hoops of dimensions 56 cm x 71 cm x 75 cm with the aid of a cyclone hopper. The hoops are filled to a prescribed weight and then taken to press banks where they are pressed for 2 h. This is the 2 h pre-press I will be talking about. The 172 kPa provided by the 21 cm press cylinder translates to only 7.6 kPa exerted on the top of the cheese block and 15.2 kPa exerted on the bottom of the block.

After the 2 h pre-press, the blocks are vacuum treated at 80 kPa vacuum gage pressure for 2 h in a vacuum chamber. For the first h of vacuum treatment no mechanical pressure is applied, but for the final hour of vacuum, 275 kPa is applied. This translates to only 12.4 kPa exerted on the top of the block and 20.0 kPa exerted on the bottom of the block.

After the 2 h vacuum treatment, the blocks are inverted to retrieve a drain plate and then ripened in the 290 kg size at 5-10°C for 6-10 days. They are then removed from the hoop and cut into 18 kg sizes for further ripening.

Now I shall discuss the experimental design of the study. The pressure probe, which is a hard plastic screened cylinder, was attached to rigid tygon tubing and secured in either the center or at a depth of 1" from the side of blocks. This was done by attaching the probe and tubing, before the hoop was filled, to tennis string that was spanned across the hoop and bracketed to the sides of trier holes. The tubing was then connected to a Sensotech Super TJE pressure transducer positioned on the outside of the hoop. The transducer was then coupled to a Campbell Scientific 21X Datalogger and absolute pressure in either the center or the side of blocks was recorded every 30 seconds over the course of the whole process of the 2 hour pre-press and the 2 hour vacuum treatment. Here is the apparatus recording pressure during the 2 hour pre-press.

This slide shows that a whey trap had to be employed to keep the tubing open. If the tubes get clogged with whey, inaccurate readings result. This slide shows the technique used to determine pressure during the 2 hour vacuum treatment. The transducer was placed in the vacuum chamber with the block to be monitored and the leads from the transducer to the datalogger were drawn through the door. The heavy insulation surrounding the leads had to be removed to allow for a tight vacuum door seal during the 2 h vacuum treatment.

Now for the results of the study. The importance of pressure studies came to our attention when we observed that up to 25% more whey

could be released from blocks during the manufacturing process when the 2 h pre-press was eliminated. This suggested that mechanical pressure application might produce a pressure seal of compacted curd surrounding the outer regions of the block. If whey drainage was impeded by this pressure seal, then rapid evacuation of air during the 2 h vacuum treatment might be impeded as well. We therefore employed the pressure transducer to see if this were indeed the case.

This means then, that pressure profiles were determined for pre-pressed blocks, that is, blocks that were given both a 2 h pre-press and a 2 h vacuum treatment, and also for non-pre-pressed blocks, that is, blocks that went directly to the 2 h vacuum treatment without being pre-pressed.

This slide (Fig. 1) shows the pressure profiles for the center and side of blocks during the 2 h pre-press. Absolute P in kPa is on the "y" axis and time in hours is on the "x" axis. Note that the center P increases rapidly and then levels off while the side pressure increases gradually throughout the 2 h of pre-press. An observation should be noted at this time. When the probe is positioned in the center of the block, whey is always seen to escape through the tygon tubing, but when the probe is positioned on the side of the block, whey is never seen to escape through the tubing. This suggests that the hydraulic pressure of the whey contributes greatly to the total center pressure. The side pressure is more the result of curd compaction.

A moisture distribution analysis conducted at this stage revealed that the center of blocks possess up to 42% moisture while the side normally contains only 39% moisture. Therefore, it is concluded that a pressure seal of compacted curd surrounding the cheese block impedes

wey drainage. This pressure seal would conceivably also impede rapid air evacuation during the 2 h vacuum treatment.

We therefore decided to compare pressure profiles of pre-pressed blocks to pressure profiles of non-pre-pressed blocks during the 2 h vacuum treatment. Here are the results of that study (Fig. 2). The upper graph shows how pressure changes in the center and the side of pre-pressed blocks during the 2 h vacuum treatment. Vacuum chamber pressure is included as a reference. The lower graph shows how pressure changes in the side and center of non-pre-pressed blocks during the 2 h vacuum treatment. Absolute P in kPa is on the "y" axis and time in hours is on the "x" axis. This slide reveals that the pre-pressed block center pressure decreases to only 15 kPa while the non-pre-pressed block center decreases to 9 kPa. In fact, the center and side pressure of non-pre-pressed drops almost as rapidly as the vacuum chamber pressure. Since the pressure in non-pre-pressed blocks drops more rapidly and to a lower value than the pressure in pre-pressed blocks, this suggests that a pressure seal has indeed been formed in pre-pressed blocks. More importantly, it also suggests that more air has been evacuated from non-pre-pressed blocks.

Temperature profiles during the 2 h vacuum treatment were also determined for pre-pressed and non-pre-pressed blocks (Fig. 3). This slide shows how temperature changes in the center and side of either pre-pressed or non-pre-pressed blocks during the 2 h vacuum treatment. Temperature in °C is on the "y" axis and time in hours is on the "x" axis. Temperature decrease during vacuum treatment can be attributed to evaporative cooling of the curd as the pressure decreases and moisture goes into the vapor phase. Temperature increase is the result of the

evaporative cooling effect being counteracted by conduction of heat between compacted curd particles. Note that in non-pre-pressed blocks, the temperature in the center decreases to lower values than in the center of pre-pressed blocks. Since the evaporative cooling effect would be more pronounced in more loosely packed cheese and also more pronounced at lower pressures where moisture can go into the vapor phase more readily, it is concluded that eliminating the 2 h pre-press aids in reducing the strength of the pressure seal and also aids in allowing more rapid air evacuation during the 2 h vacuum treatment.

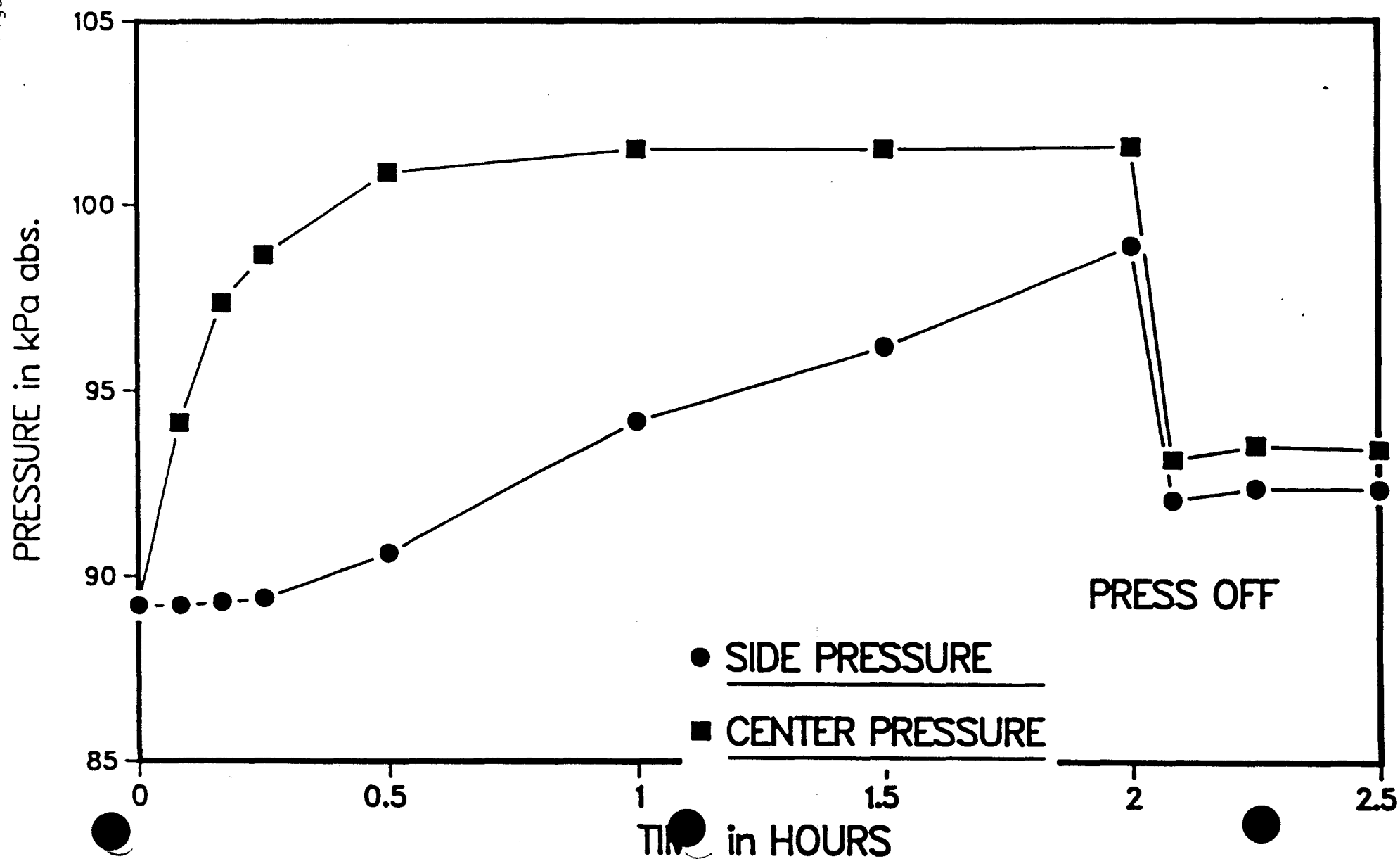
Finally, realizing that satisfactory vacuum treatment is helpful in eliminating mechanical openings in cheese, one would conclude that after a suitable amount of ripening, non-pre-pressed blocks would exhibit a tighter, firmer body than pre-pressed blocks. This slide shows portions of wire-cut cheese taken from the center of either pre-pressed or non-pre-pressed blocks after two months of ripening. The non-pre-pressed cheese exhibits a firmer, tighter body. Plugs show the difference more strikingly. Four plugs were taken from identical center positions in either pre-pressed or non-pre-pressed blocks. The worst side of each plug is shown. It appears that eliminating the pre-press aids in producing a cheese with a firm, tight body relatively free of mechanical openings.

In conclusion, a 2 h pre-press helps to develop a strong pressure seal that impedes both rapid whey drainage and air evacuation. Elimination of the pre-press aids in producing a cheese with a firmer, tighter body. However, we have found that even when the pre-press is eliminated, a pressure seal, although weak, is still produced, and the moisture distribution in non-pre-pressed blocks is similar to that in

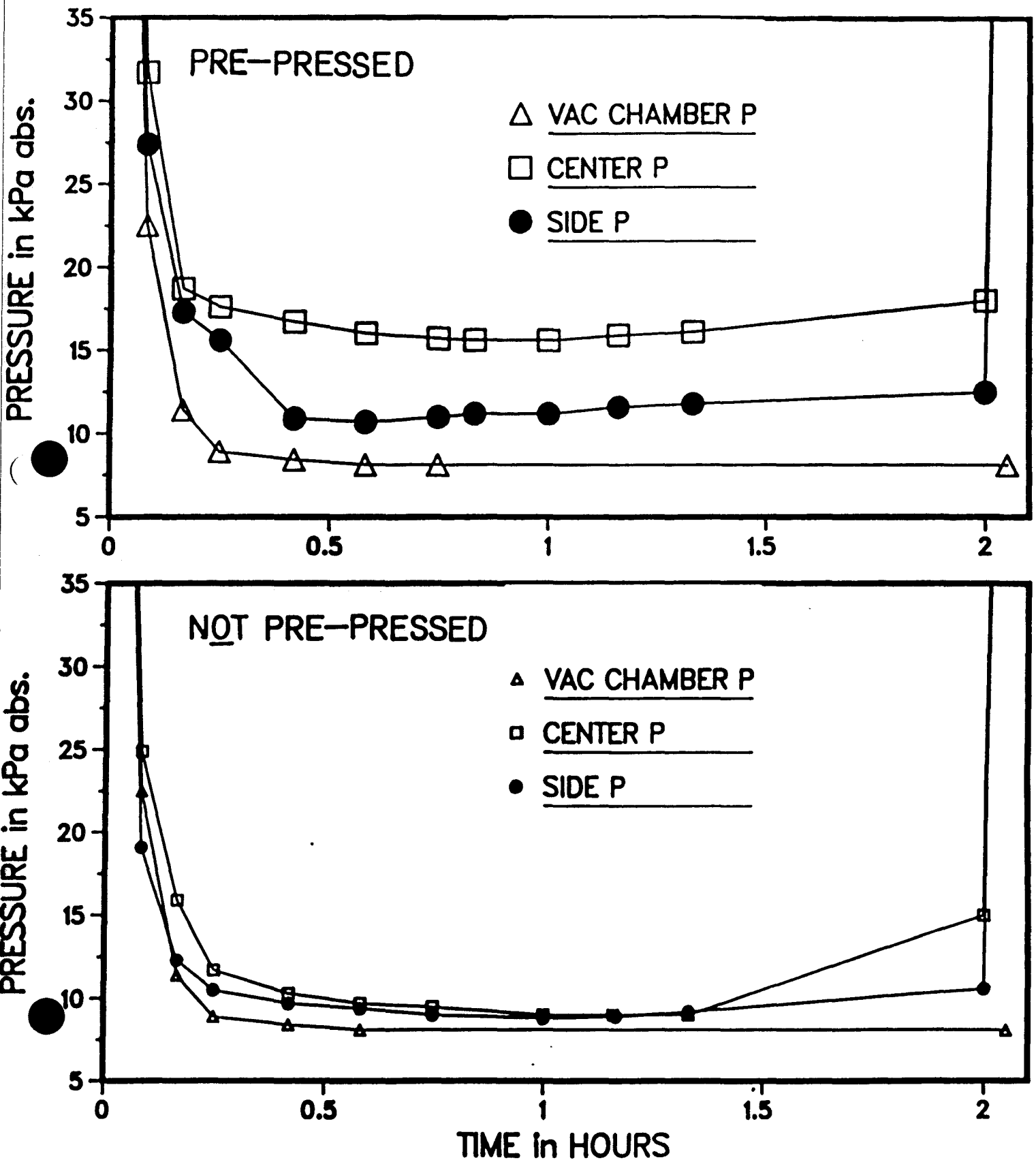
pre-pressed blocks. Therefore, if we wish to achieve maximum efficiency of whey drainage and air evacuation in cheese of such great size, we should investigate the feasibility of draining whey before the hoop is filled and also filling the hoop under vacuum.

CHANGE in SIDE and CENTER PRESSURE DURING PRE-PRESS

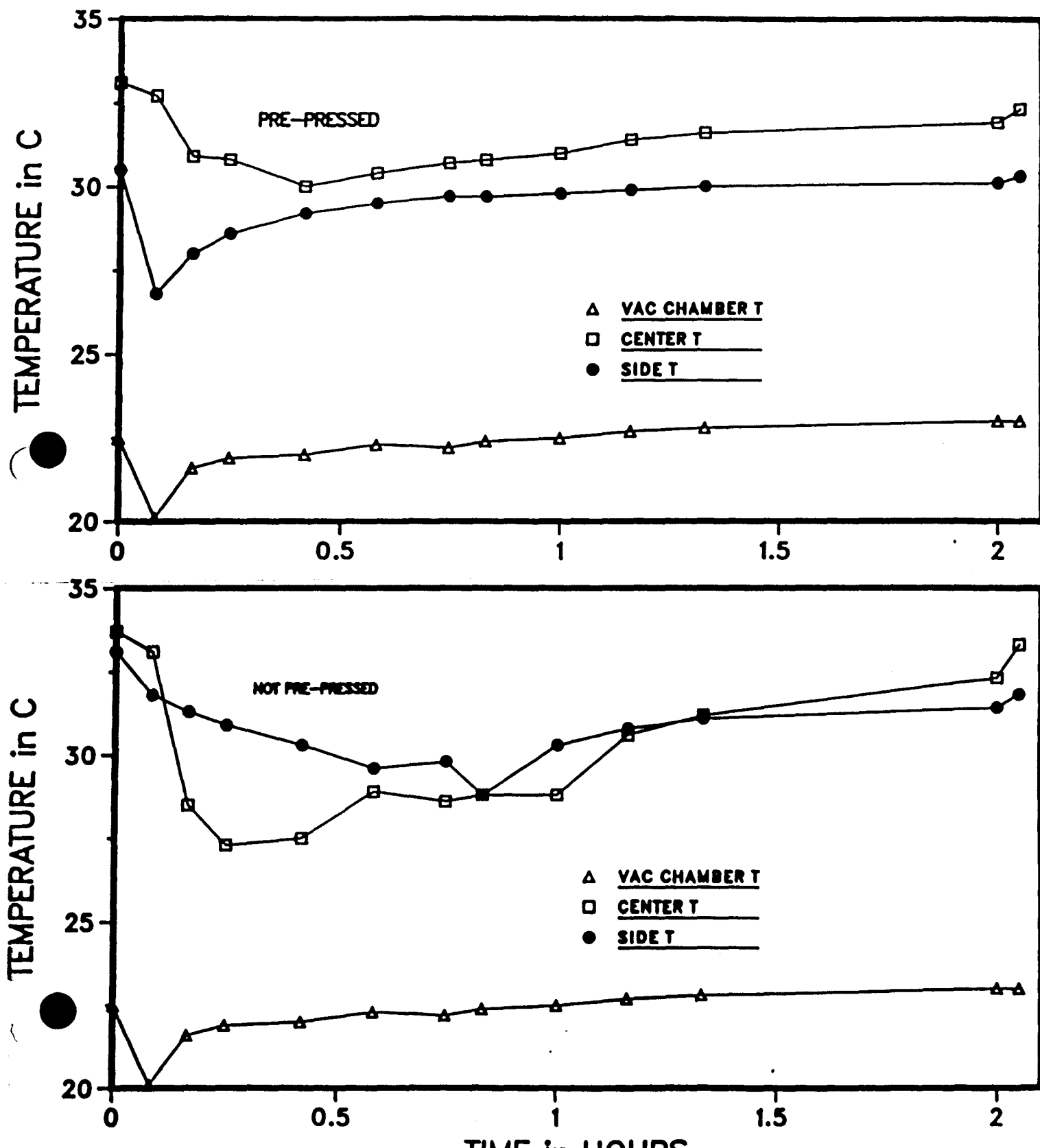
Figure 1



EFFECT of PRE-PRESS on PRESSURE CHANGES DURING VACUUM TREATMENT



EFFECT of PRE-PRESS on TEMPERATURE CHANGES DURING VACUUM TREATMENT



TEMPERATURE, pH, SALT AND MOISTURE PROFILES IN 290 kg
STIRRED-CURD CHEDDAR CHEESE BLOCKS

Robert S. Reinbold

Uneven moisture distribution in 290 kg blocks of stirred-curd Cheddar cheese is a problem that has not been completely resolved, and the extent to which it has been resolved is proprietary information. Therefore, the information presented in this talk with either help us solve the problem or help reveal how the problem has already been solved.

The subject of this talk concerns temperature, pH and salt profiles in 290 kg blocks and their effect on moisture distribution.

First, I shall review the process of manufacture of stirred-curd Cheddar cheese in 290 kg blocks.

The granular curd is placed in stainless steel hoops with dimensions 56 x 71 x 75 cm. The hoops are filled to a prescribed weight and then pressed for 2 hours at 172 kPa. They are then vacuum treated at 80 kPa vacuum gage pressure for 2 hours. No mechanical pressure is applied for the first hour, but for the final hour of vacuum, 275 kPa is applied. The blocks are then inverted to retrieve a drain plate and then cooled at 5-10°C for 6 to 10 days. They are then removed from the hoop and cut into 18 kg sizes for further ripening.

Now I shall discuss the experimental design of the study. This is a model of a 290 kg block. Looking at the top view we can see that only one quadrant was sampled. We can also see that the model's melting point is below average Sacramento Summer temperatures. Three positions, the center, mid-point, and side corner on the quadrant diagonal, were sampled for each of five different levels of the block. In this talk, I shall concentrate on the three positions in the middle level. For graphical depiction, I have designated them, the center, mid-point and side.

All positions were sampled immediately after the vacuum treatment, that is just before the block was placed in the cooler for ripening and then at various times over the course of cooling.

Since repeated sampling from one position in a block is impossible, the same block could not be employed for the whole study. Therefore, five blocks from the same vat of cheese were reserved for the study and were assumed to have identical characteristics throughout the study. Individual blocks were assigned a sampling time corresponding to 0 h, 12 h, 24 h, 48 h and 6 d of cooling, respectively, and eliminated from the study after samples were taken. For purposes of this talk, I will concentrate only on the time just before the block goes into the cooler, which is designated 0 h ripening, and on the time 12 and 24 hours after the start of cooling which are designated 12 h ripening and 24 h ripening, respectively.

Since the curd is very crumbly for the first few days of ripening and since samples must be obtained by going vertically from the top of the block down, a special cheese trier had to be designed. The trier was a stainless steel cylinder five cm in diameter and one meter long with retractable claws for grabbing and holding loose curds. Unfortunately, before a picture could be taken, the trier was run over a few times by a forklift and then mysteriously disappeared. If you're trying to picture what it looked like, envision a bazooka with jaws.

2.54 cm from each position were obtained at the designated time and immediately placed in an ice-water slurry to retard pH change. pH's were taken immediately thereafter with an Extech gel electrode and Altec pH meter. Duplicate moistures were determined by the atmospheric oven technique and had repeatability of .05%. Duplicate salt analyses were

determined by the Quantab Titrator method which had repeatability of $\pm .1\%$. The temperature profile is an estimate derived from previous studies employing a Leeds-Northrup Speedomax 250 Automatic Temperature Recorder.

Here are the results of the study accompanied by discussion. This slide (Fig. 1) shows the temperature in the center, mid-point and side positions for the middle level of the block at 0, 12 and 24 h of ripening at 2°C . Note that the center of the block does not cool down appreciably within 24 h, while the side temperature drops rapidly. At the end of 24 h, ripening, there is a 25°C temperature difference between the center and the side of the block.

This slide (Fig. 2) shows the pH in the center, mid-point and side positions for the middle level of the block at 0, 12 and 24 h of ripening. Note that 0 h ripening, the center pH is high, while the side pH is relatively low (i.e. 5.45 in the center versus 5.2 on the side). During 24 h of ripening, the center pH drops rapidly to 5.2, while the side pH drops only .1 more pH unit. The drop in pH in the center, and the stable pH on the side during the 24 h ripening can be attributed to the high temperature in the center, and the rapidly decreasing temperature on the side. What may not be as easy to explain is the difference in pH between the center and side of the block at 0 h ripening. Over the course of pressing and vacuum treatment, the center temperature increases to 35°C , while the side temperature remains at 30°C . The optimum growth temperature of the lactic starter culture organisms employed for production of stirred-curd Cheddar is 30°C , so this may explain why the pH would drop more rapidly on the side of the block during this stage of manufacture. However, in a study on pH

decrease versus temperature of incubation, little difference in pH change was observed when starter organisms were grown over the range of 30-35°C.

The next slide (Fig. 3) may help to explain these pH differences more accurately. This graph depicts the ratio of % salt in cheese to % moisture in cheese in the center, mid-point, and side positions for the middle level of the block at 0, 12, and 24 h of ripening. The high salt content in the center presumably inhibits the growth of the starter organisms, thereby slowing pH decrease during pressing and vacuum treatment. Another interesting point to note is that there was little change in the salt profile during the 24 h of ripening, and the profile for the 60 day old block looked very similar to these profiles. This suggests that salt diffuses rapidly into individual curd particles, but diffuses slowly throughout the total cheese mass. Also since a gradient of high salt content in the center to low salt content on the side exists, and salt promotes syneresis of cheese curd, one would expect that moisture would be expelled from the center to outer regions of the block. The next information supports this conclusion.

This slide (Fig. 4) shows the moisture content in the center, mid-point and side positions for the middle level of the block at 0, 12 and 24 h ripening. It shows that 0 h ripening moisture content is high in the center, but lower on the side of the block. At 24 h ripening, the moisture distribution reverses itself. There is now a low moisture content in the center and a high moisture content on the side of the block. The 60 d moisture profile is similar to the 24 h profile.

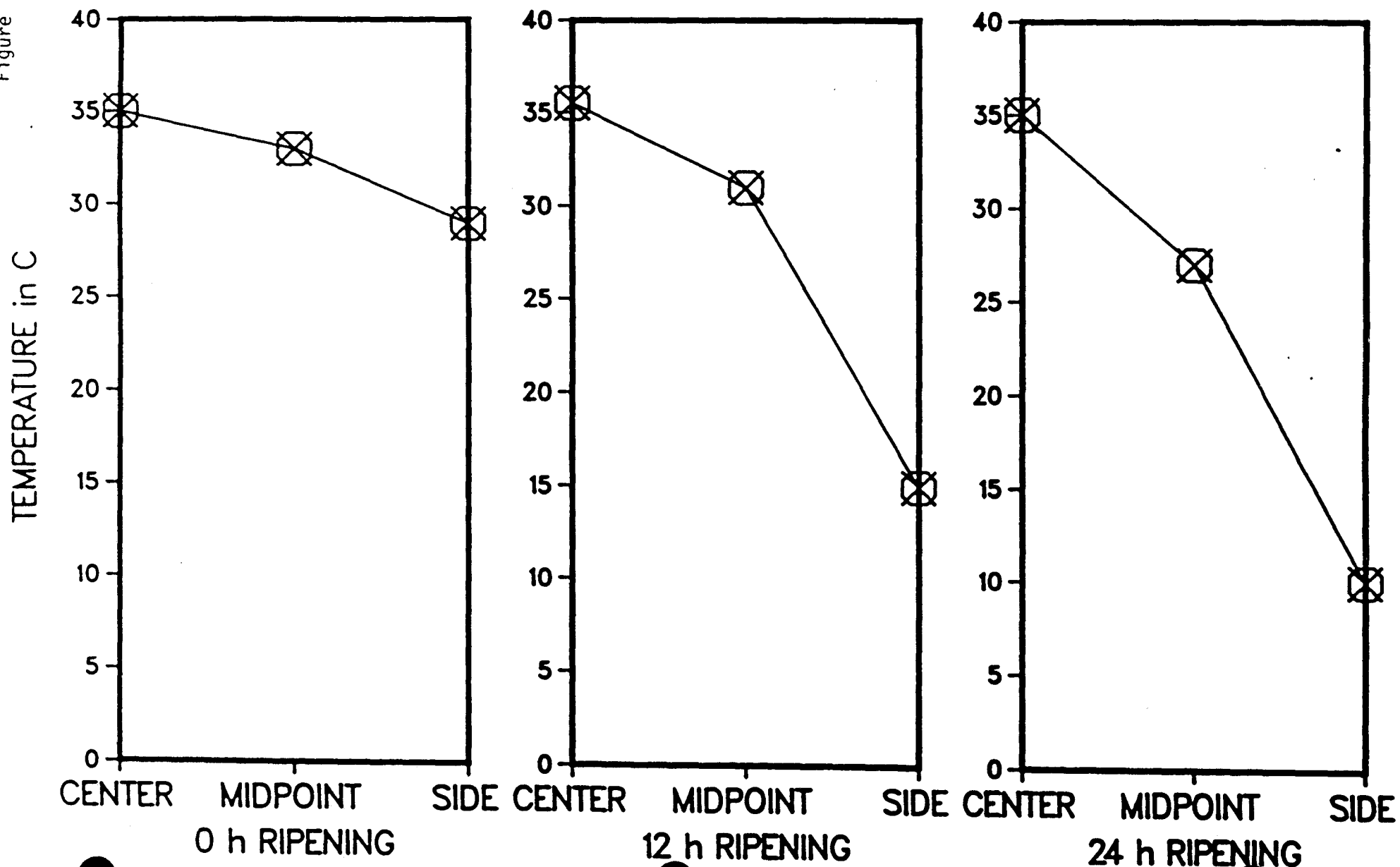
Summarizing these results, it appears that salt, pH, and temperature gradients may all combine to force moisture from the interior to the exterior of cheese blocks.

Since salt and pH are difficult variables to control, we tried to influence moisture distribution by varying ripening temperature. This slide (Fig. 5) depicts the results of one study on ripening temperature control. The control block was ripened in the normal fashion, that is immediately cooled to 2°C after the press and vacuum treatment. The test block was cooled in stages, that is for 2 days at 23°C, 2 days at 12°C and 2 days at 2°C. Moisture content for the positions indicated were then determined. The control block exhibited a maximum temperature difference of 3.28% between the center and exterior while the test block exhibited a maximum difference of only 1.17%. Two other such studies exhibited similar findings.

In conclusion, it appears that many variables may affect moisture distribution in 290 kg blocks. Minimizing differences in salt content and temperature between interior and exterior positions resulting during ripening may aid in a more even moisture distribution.

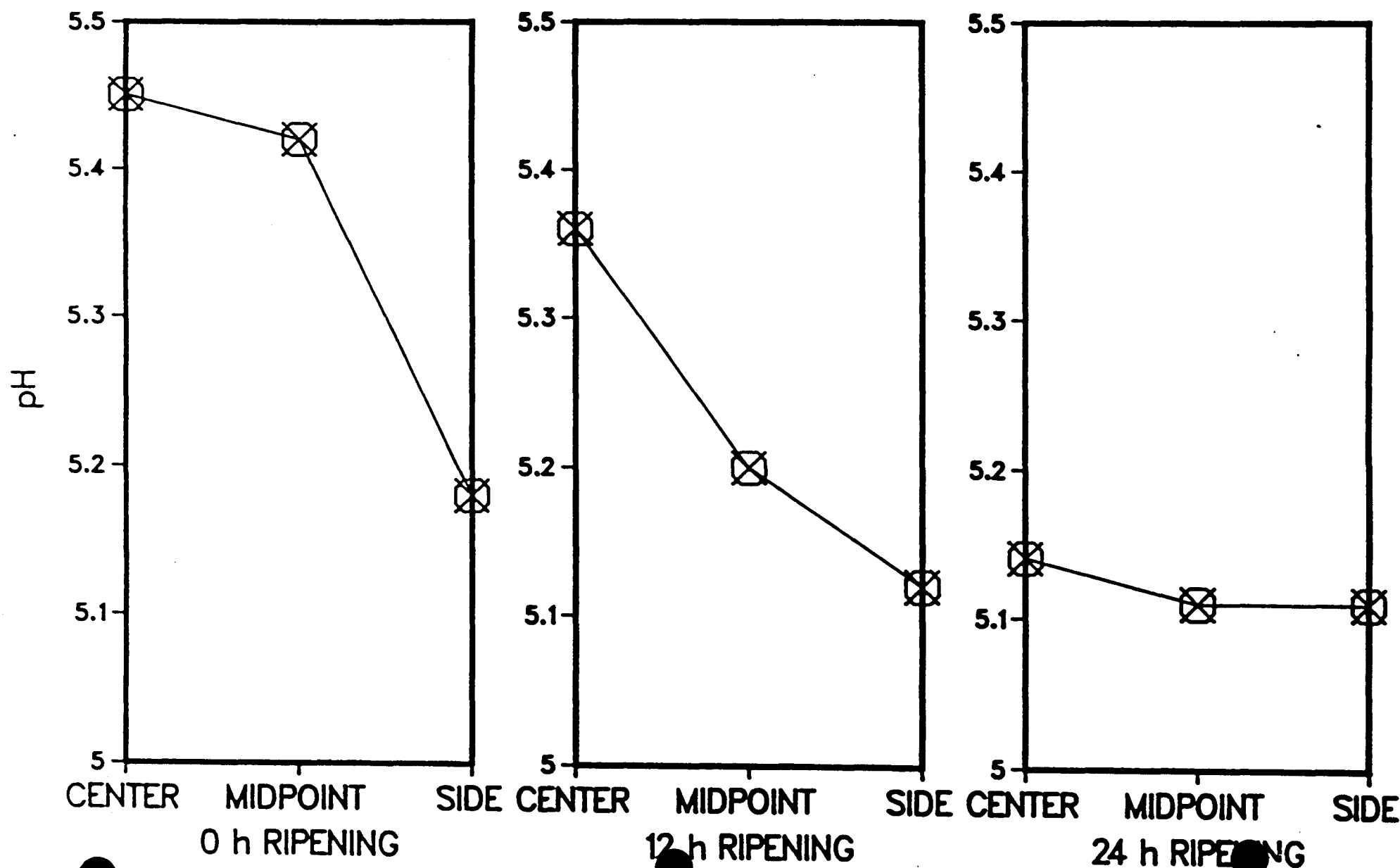
CHANGE in TEMPERATURE in CENTER, MIDPOINT and SIDE POSITIONS DURING RIPENING

Figure 1



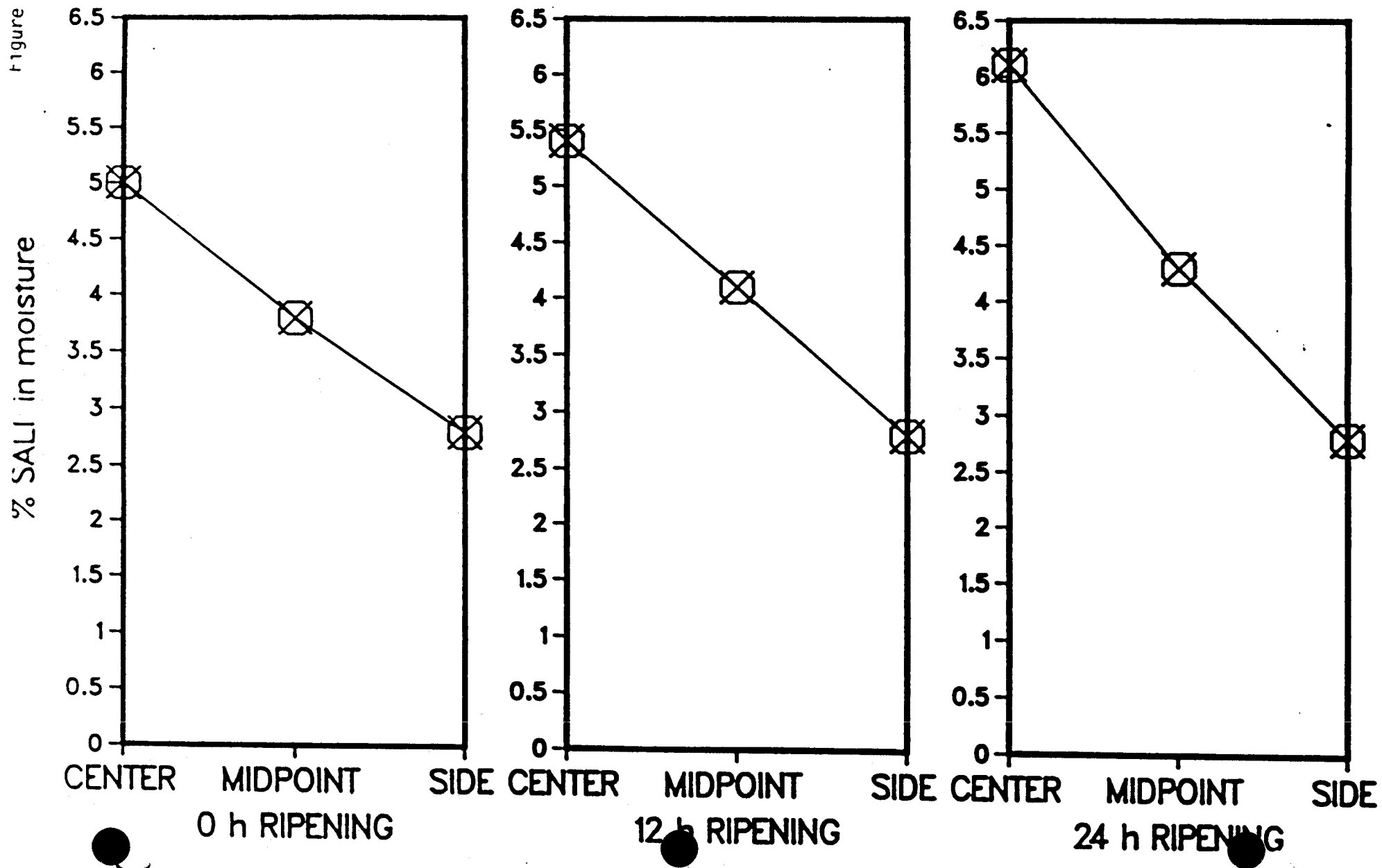
CHANGE in pH in CENTER, MIDPOINT and SIDE POSITIONS DURING RIPENING

Figure 2



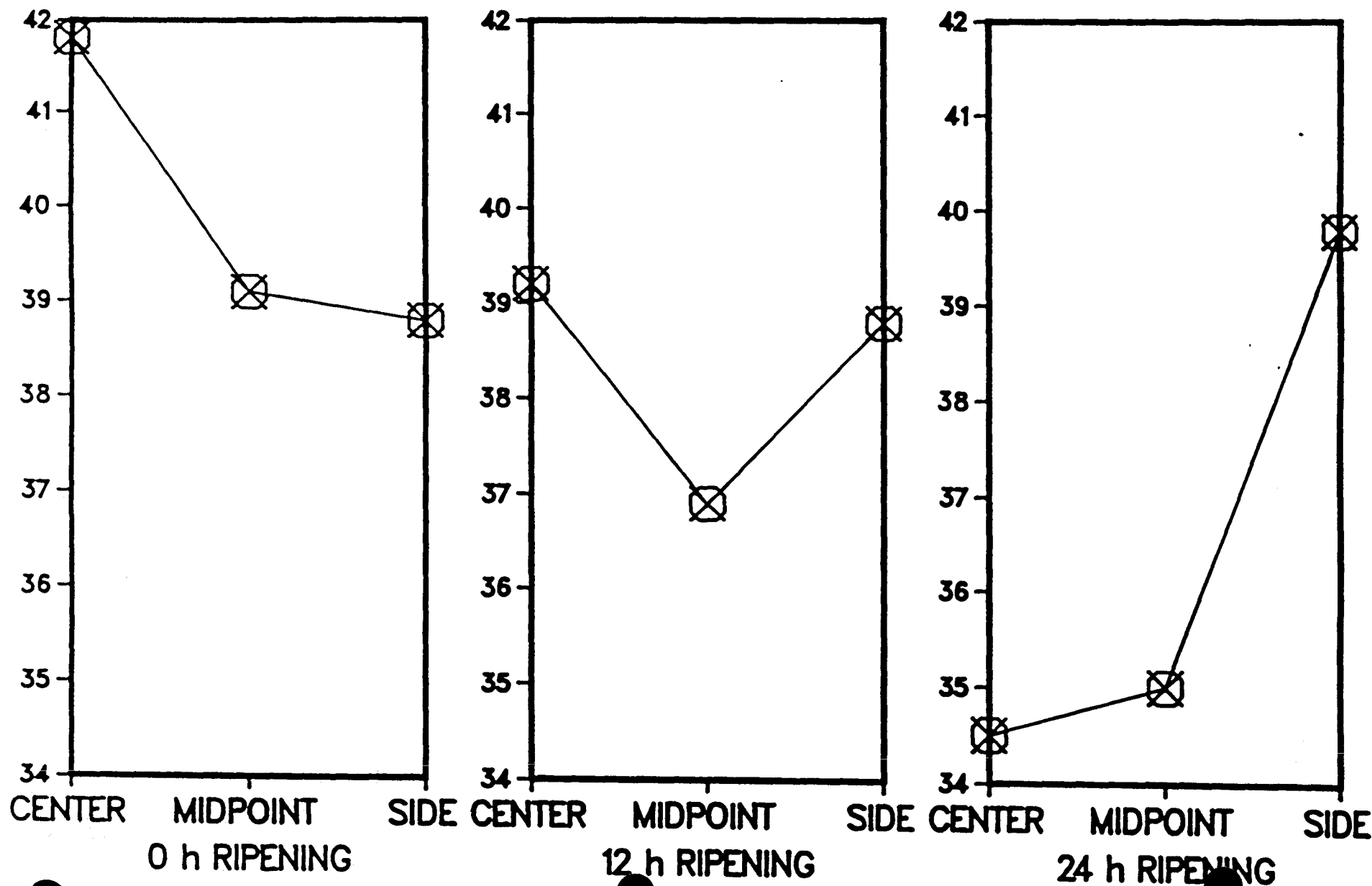
CHANGE in SALT CONTENT as % of cheese moisture in CENTER, MIDPOINT and SIDE POSITIONS DURING RIPENING

Figure 3



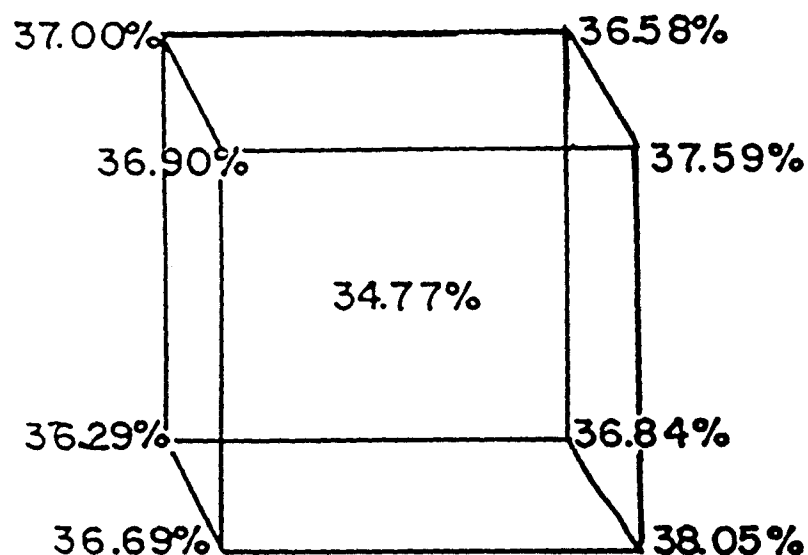
CHANGE in MOISTURE CONTENT in CENTER, MIDPOINT and SIDE POSITIONS DURING RIPENING

Figure 4



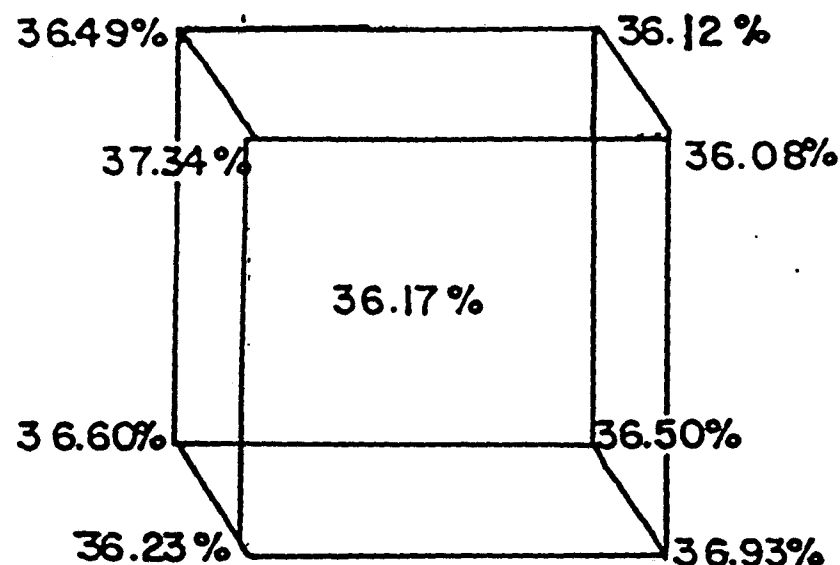
MOISTURE DISTRIBUTION STUDY^a

CONTROL BLOCK^b



$\bar{X} = 36.75$
 $S_d = 0.86$
Range = 3.28

TEST BLOCK^c



$\bar{X} = 36.50$
 $S_d = 0.39$
Range = 1.17

^a for cheese in stainless steel hoops
^b ripened for 6 days at 2°C
^c ripened for 2 days at 23°C, 2 days at 12°C,
and 2 days at 2°C

COTTAGE CHEESE FROM ULTRAFILTERED SKIMMILK

Jorge Ocampo and C.A. Ernstrom

A procedure for making cottage cheese curd from ultrafiltered skim milk was reported at the 1985 meeting.

In that report, pasteurized skim milk was acidified to pH 5.8 with phosphoric acid, then ultrafiltered to a total solids concentration of 16%. In practice, the skim milk was over concentrated and adjusted to 16% total solids by adding back an appropriate amount of permeate. The protein content in the retentate was approximately 9.3%.

The pre-acidified retentate was then heated to 160°F (it required 25 min to reach that temperature), held for 3 min and cooled. Sufficient glucono-delta lactone was added to bring the pH to 4.8 in 75 min. The curd was cut and cooked in the usual way to produce good quality cottage cheese.

There seems to be two important factors involved in making good quality cottage cheese from UF retentate at 16% solids.

1. The skim milk must be acidified to approximately pH 5.8 prior to ultrafiltration.

Curd made from skim milk retentate that has not been preacidified suffers from the defect that after it is mixed with the cream dressing it becomes almost transparent and resembles tapioca. This defect can occur in regular cottage cheese when the final pH is too high. However, this particular defect could not be corrected by adjusting the pH of the curd or dressing. The problem is associated with the high concentration of mineral ions that develop upon acidification of concentrated caseinate micelles.

2. The retentate must be heated before it is made into cottage cheese curd.

Failure to heat the retentate results in a curd that is too firm to cut when it is at the appropriate pH. The heat treatment is extremely critical because if heated too much the curd may be too soft when cut and a lot of fines are lost in the whey.

A small high-temperature short-time pasteurizer was acquired in order to translate the batch heat treatment to the HTST process. An appropriate time and temperature of heating was determined to be 175°F for 16 seconds. Even with this heat treatment the curd is quite firm at cutting and could present some difficulty in cutting in a large cheese vat. Because of this problem, this process seems particularly attractive for use with direct acidification in a continuous curd former. The quality of the curd was as good as with the batch heating process.

Yield comparisons are difficult to make on small scale processes. However the combination of ultrafiltration to 16% total solids plus heat treatment of the retentate should result in increased retention of whey proteins in the curd.

Only one very preliminary attempt has been made to measure yield. In this case 455.8 pounds of skim milk testing 2.92% protein was ultrafiltered to yield 305.0 pounds of permeate plus 7.3 pounds rinse water. These solutions contained a total of .64 pounds protein.

If the rest of the milk protein went to the retentate which tested 9.28% there would have been 12.67 pounds protein in 136.49 pounds of retentate. From 25 pounds of retentate there was 9.9 pounds of curd containing 24.7% total solids. By adjusting to 20% solids, the yield would have been 14.63 pounds curd per 100 pounds of original skim milk.

A control batch made fomr the same milk yielded 12.78 pounds per 100 pounds when corrected to 20% solids. If these figures hold during subsequent comparisons, a 14.5% yield increase might be expected.

EFFECT OF ULTRAFILTRATION ON PROTEIN QUALITY OF MILK AND SKIMMILK PRODUCTS

Jorge Ocampo, Rita Tung, Deloy Hendricks, C.A. Ernstrom

Purpose:

Ultrafiltration of milk results in an increase in total solids in the retentate. Cheeses made from 3-5 times concentrated retentate have had yields 3-18% higher than cheeses made from regular milk. This increase has been suggested to be due to the trapping of whey proteins in the cheese structure instead of it being lost in the whey fraction.

Procedure:

Product preparation. All milk was from the same lot of skimmilk. One half of the milk was ultrafiltered to three times concentration of total solids. Part of the unfiltered and part of the ulfiltrated skim milk were then made into cottage cheese using conventional processing procedures.

Diet preparation. Skimmilk, retentate, regular cottage cheese and cottage cheese made from the retentate were freeze dried for incorporation into diets for the feeding trials for biological testing. Because lactose is lost in the filtrate lactose was determined on all products and in a second trial protein efficiency ratio (PER) was determined on diets in which lactose was added to the retentate and the cottage cheeses made from the retentate and the regular skimmilk at a level equal to the regular skimmilk diet. All diets were made up to meet all of the known nutrient needs of the rat except protein. In the first trial diets containing 5, 8, or 11 percent protein were made up from each dried product. In the second trial all diets contained 10 percent protein, provided by the test product.

Animals. Sprague-Dawley weanling male rats were purchased from Simonsen Laboratories, Gilroy, CA. After 3 days adaptation to a 12 hour light dark regimen in a temperature controlled room (78°F) rats were weighed and randomly assigned to dietary treatments.

Trial #1.

Five rats were assigned to each treatment in Trial 1. Trial 1 is given in Table 1.

Table 1

Dietary Treatment	Protein Level		
	5%	8%	11%
Initial kill, #	5		
No protein, #	5		
Skimmilk, #	5	5	5
Regular cottage cheese, #	5	5	5
Retentate (UF milk), #	5	5	5
UF cottage cheese, #	5	5	5
ANRC reference casein	5	5	5

All animals were fed ad libitum with fresh food and water provided every other day. Animals were housed in metabolism cages in which urine and feces could be collected separately. Food spilled or refused was weighed back from the amount given to quantitate food intake. At the end of 14 days all animals were weighed, killed, the carcasses put into pint canning jars with an equal volume of 5% acetic acid and autoclaved for 1 hour at 121°C. The autoclaved carcasses were mixed in a blender and samples taken for determination of carcass nitrogen. Diet, carcass, urine, and fecal nitrogen levels were determined in triplicate by micro kjeldahl analysis. Data obtained was used to determine nitrogen efficiency for growth, biological value and net protein utilization for each animal.

Trial #2.

Trial #2 was conducted over 28 days and involved only food intake and weight gain determinations used to determine protein efficiency ratios. The dietary treatments for trial #2 are given in Table 2. there were 10 rats per treatment.

Table 2. Dietary Treatments

Dietary Protein Source

ANRC protein
Skimmilk

Cottage cheese made from skimmilk
Cottage cheese made from skimmilk and lactose added

Cottage cheese made from retentate
Cottage cheese made from retentate and lactose added

Cottage cheese made from ultrafiltered milk
Cottage cheese made from ultrafiltered milk and lactose added

Results:

Weight gain (figure 1), nitrogen efficiency for growth (figure 2), and net protein utilization (figure 3) all were higher for the retentate than for the skimmilk and higher for the cottage cheese made from the retentate than for the cottage cheese normal skimmilk. The differences never achieved statistical significance but were consistent. Biological values, however, failed to follow the same pattern (figure 4).

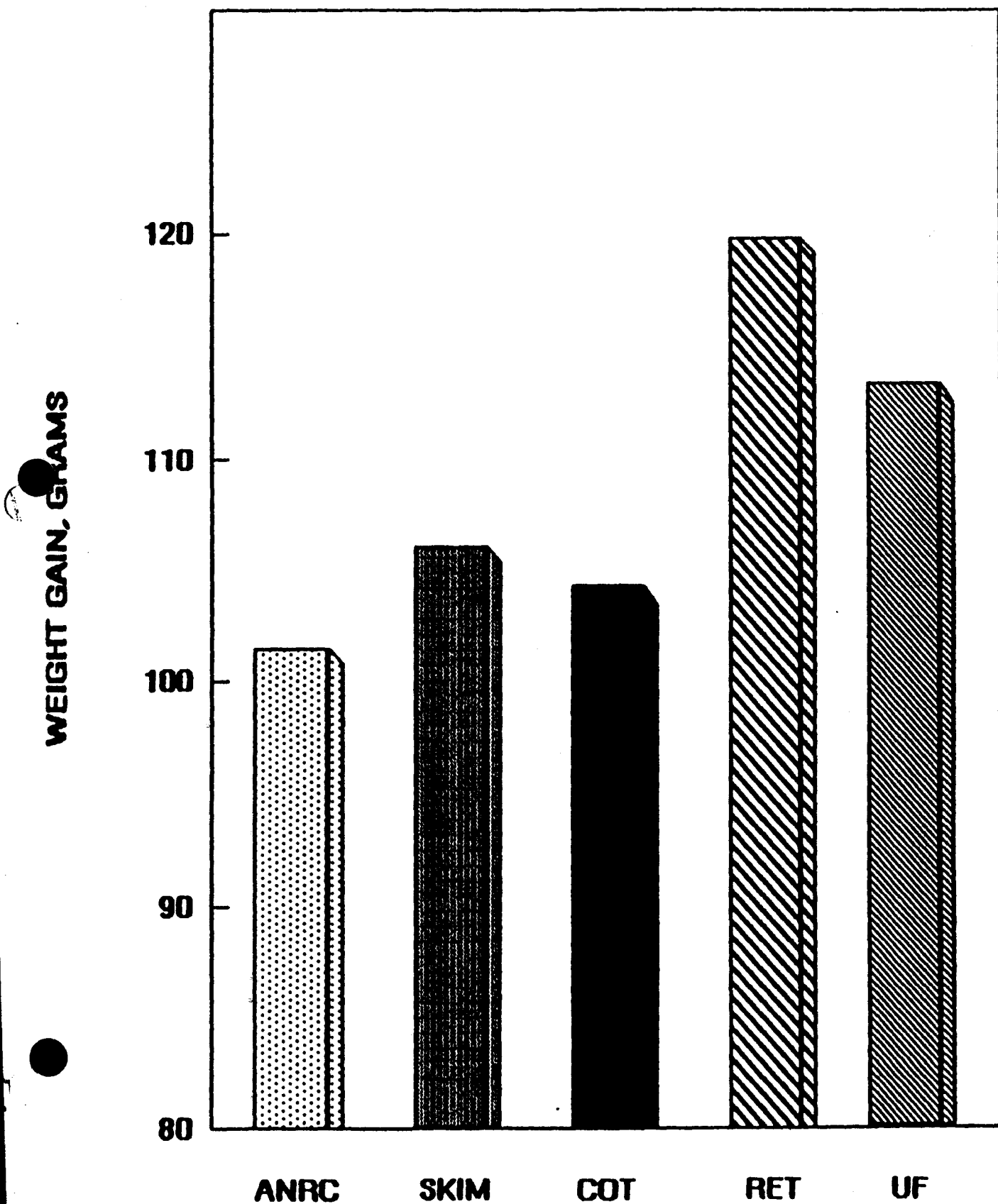
In trial 2, protein efficiency was calculated and these results showed a pattern similar to that observed for the different protein sources in trial 1 (figure 5). That is, the milk and products made from milk which had been ultrafiltrated had higher values for protein quality. Statistically, differences were again not significant.

Discussion:

Samples are currently being analyzed for amino acid patterns to determine chemical scores by the AOAC ion exchange method and the newer HPLC method. All measures for biological quality of the protein tend to favor the UF milk and products made from UF milk. This is in agreement with the theory that cheese yield increases when using the UF milk may be due to the incorporation of lactalbumin into the curd. Lactalbumin has a higher biological activity than casein; the major protein in most cheeses. If making cheese from ultrafiltrated milk does increase yields and does increase protein quality of the cheese the new processing method will have significant economic and nutritional benefits. Further studies are needed on other types of cheese.

figure 21

EFFECT OF SKIM MILK PRODUCTS ON WEIGHT GAIN IN THE RAT



**EFFECT OF SKIM MILK PRODUCTS ON
NITROGEN EFFICIENCY FOR GROWTH (NEG) IN THE RAT**

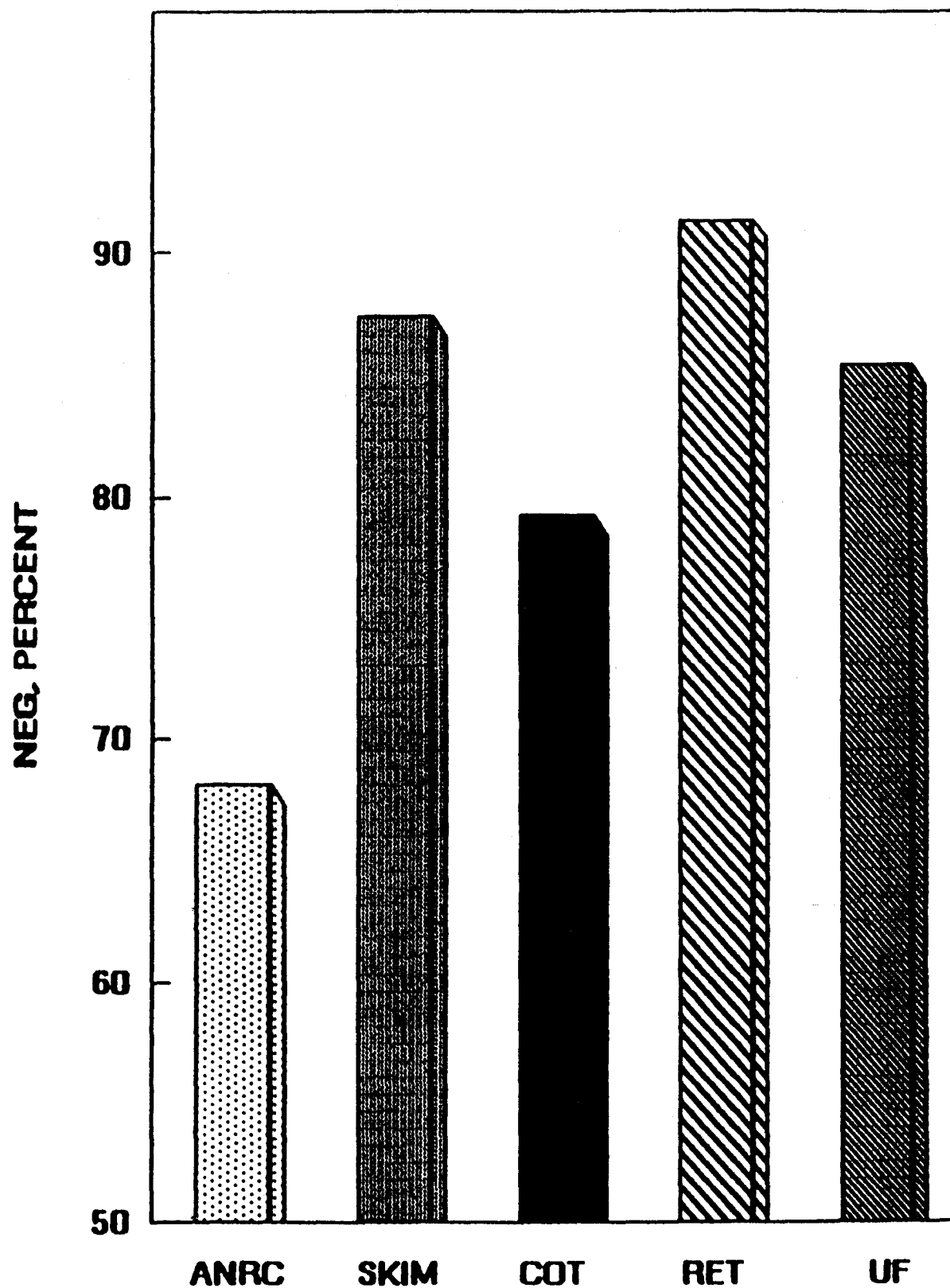


Figure 3

EFFECT OF SKIM MILK PRODUCTS ON NET PROTEIN UTILIZATION (NPU) IN THE RAT.

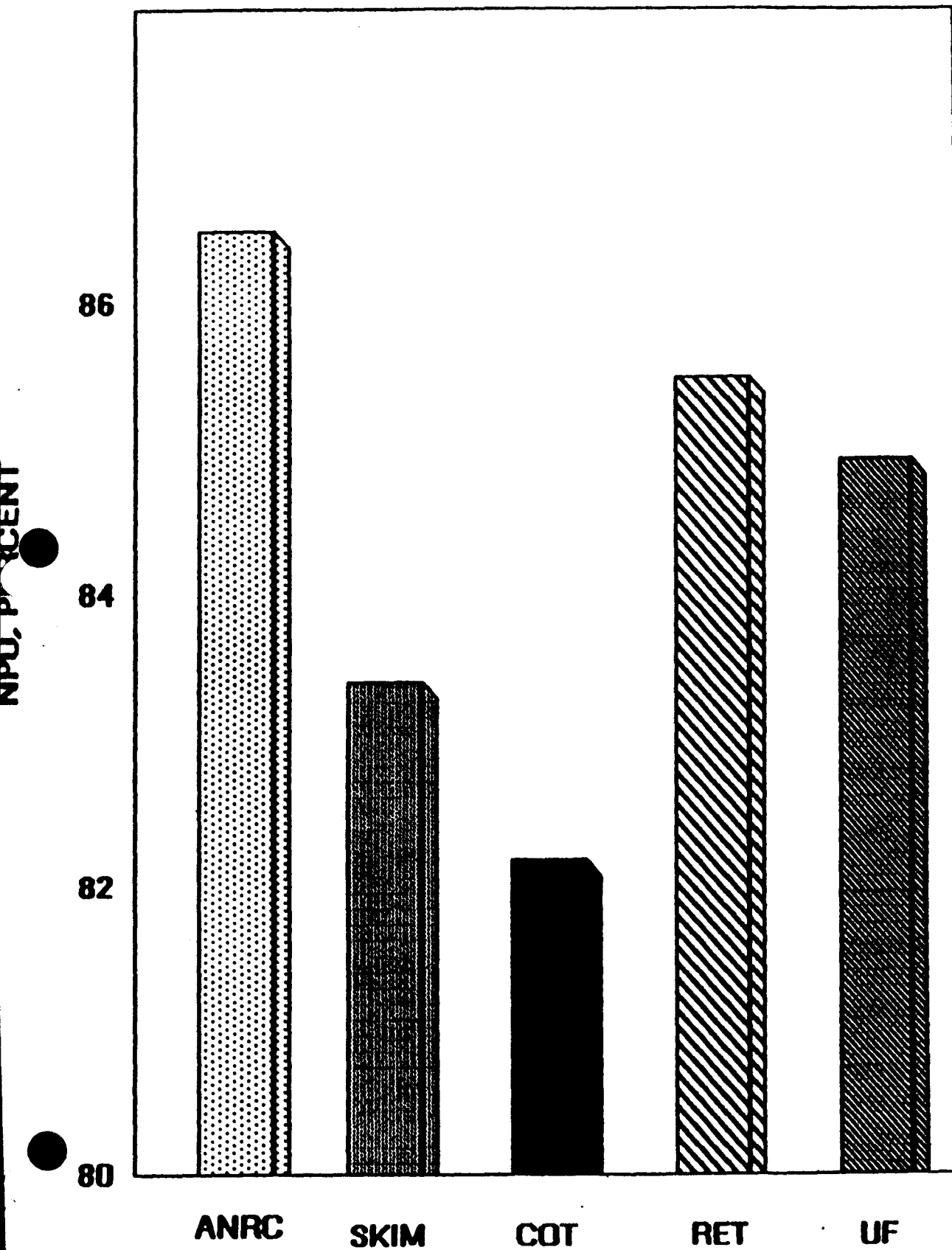


Figure 4

EFFECT OF SKIM MILK PRODUCTS ON BIOLOGICAL VALUE (BV) IN THE RAT

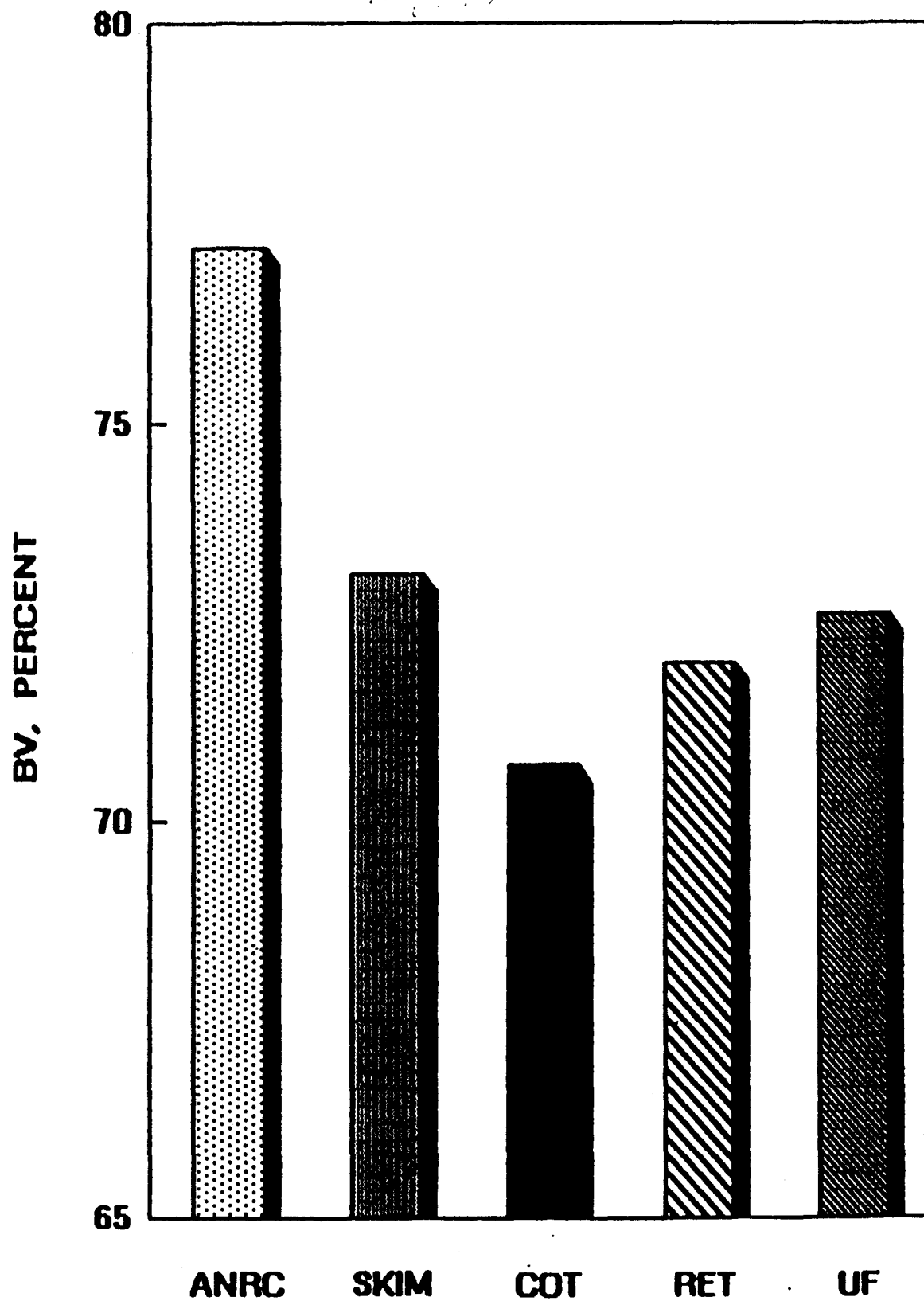
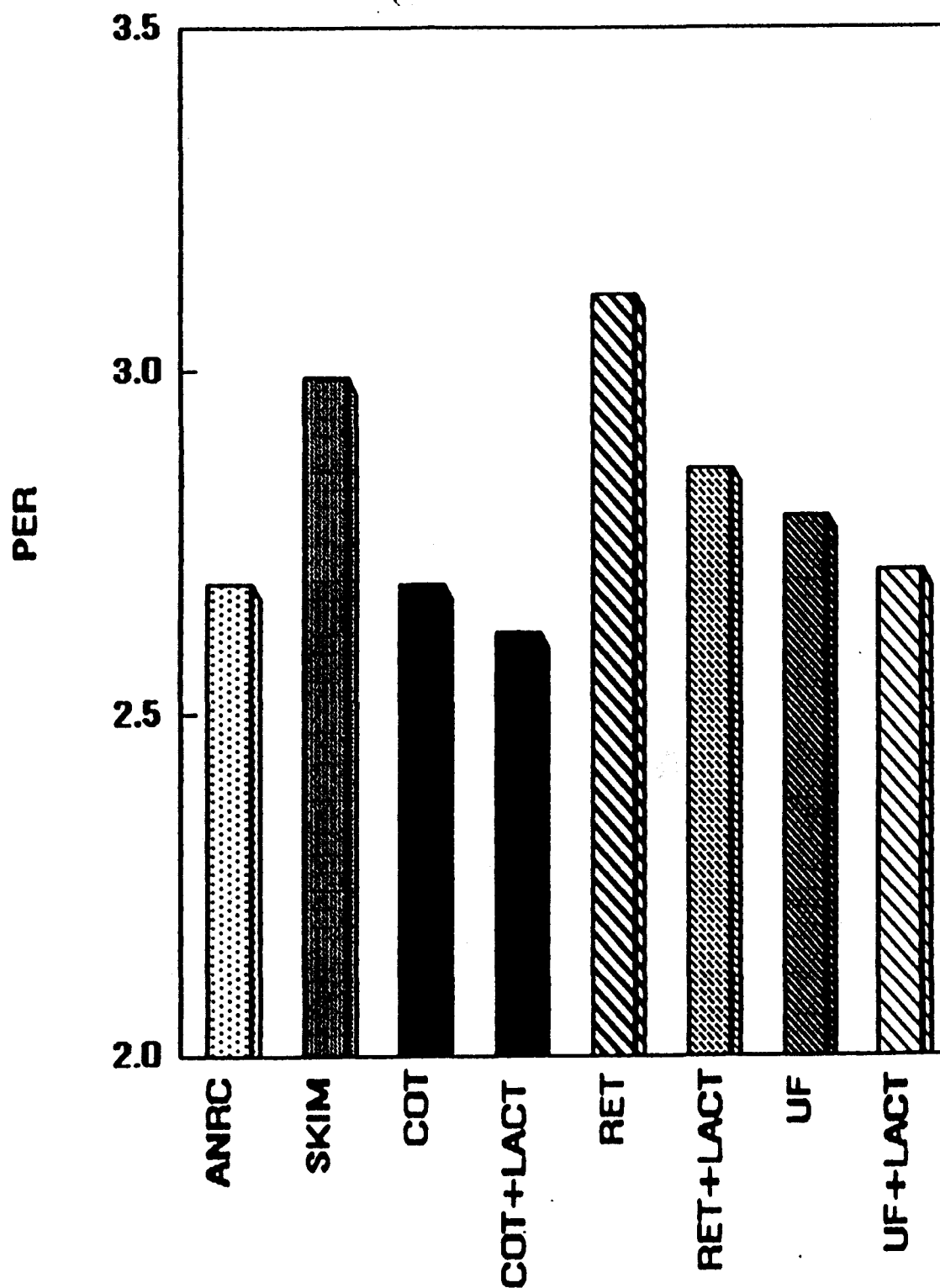


Figure 05

EFFECT OF SKIM MILK PRODUCTS ON PROTEIN EFFICIENCY RATIO (PER) IN THE RAT



GENE TRANSFER SYSTEMS IN GROUP N STREPTOCOCCI

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Use of genetic technology to develop improved lactic starter culture strains has great potential to aid the dairy starter culture industry. Since the 1930's, problems with variability in major starter associated metabolic functions such as lactose metabolism, proteinase activity, and citrate utilization have been observed. Many strains used in cheese manufacture have produced undesirable off-flavors such as bitterness, and most strains are susceptible to attack by bacteriophages. These factors, along with increased cheese production schedules and the appearance of large mechanized manufacturing plants, have placed increased stress on existing starter strains. Because of these factors, the number of suitable strains for use in cheese manufacture is limited and there is a continuing need to obtain new and improved strains that can perform efficiently and predictably for extended times.

Several strategies have been used to increase the number and efficiency of starter strains. Attempts have been made to isolate new strains from the natural habitat but this approach has been largely unsuccessful since most of these strains are unsuitable for cheesemaking. Mutagenesis of pre-existing strains followed by selection has been useful for developing bacteriophage resistant mutants. However, these mutants may revert to phage sensitivity or may, after a short time, become susceptible to new phages. Two new strategies involve genetic manipulations. One genetic strategy is to use gene transfer systems e.g. conjugation, to move desirable genes from one

strain to another. The other strategy is to use recombinant DNA techniques to alter metabolic capabilities. These genetic strategies have potential since strain improvement may be specifically directed and controlled. In addition, it has been well established that plasmids play an important metabolic role in the ability of lactic streptococci to be used for cheesemaking. Our increasing knowledge of the genetics and plasmid biology of these organisms is now beginning to facilitate the development of improved strains from pre-existing starter strains. For example, at the 1986 ADSA Annual Meeting in Davis, CA, Dr. Todd Klaenhammer from North Carolina State University in collaboration with Miles Laboratories presented data on improving the phage resistance of starter strains by transferring a phage-resistance plasmid to a phage sensitive starter strain via conjugation. This recipient strain showed enhanced phage resistance in pilot and field trials and is now being used very successfully commercially. The results exemplify the beneficial use of genetic technology to alter or enhance specific metabolic capabilities.

Our laboratory is actively involved in plasmid biology and the development of gene transfer systems. We are focusing on improvement of transformation techniques in Streptococcus lactis and on developing transformation systems for Streptococcus cremoris. Transformation, or the uptake of naked DNA, is an essential gene transfer step for using recombinant DNA techniques.

We have screened over 40 strains of S. cremoris for the ability to be transformed by plasmid DNA. Only two strains were proven to be transformable under the conditions used. Although many S. lactis strains are transformable, further research will be required to develop transformation systems in S. cremoris.

Other research in our lab centers around the development of other tools to perform recombinant DNA experiments, e.g. cloning vectors. Using recombinant DNA techniques we hope to study how genes are maintained and expressed, and to use these techniques in the directed improvement of starter strains.

Applied research includes using microbiological and genetic techniques to develop an inhibitor of psychrotrophs in milk products and to study and develop strains which are resistant to an agglutination reaction in milk, a frequent problem in cottage cheese manufacture. The research involving agglutination will be discussed by T.L. Scheuble in the next presentation.

In conclusion, genetic technology has potential to benefit cheesemakers through the development of improved starter strains, as shown by recent results in improving phage resistance using gene transfer systems. However, further research is needed to develop tools for using recombination DNA techniques in lactic streptococci. Once these techniques have been developed, the most powerful tool for the directed improvement of starter strains will be available.

AGGLUTINATION BEHAVIOR OF STREPTOCOCCUS LACTIS
AND STREPTOCOCCUS CREMORIS

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The inhibitory action of cow's milk on bacterial species has been well known since the early 1900's (12) and has been shown to be responsible for many defects in the manufacture of fermented dairy products (6). The agglutination of lactic streptococcal starter cultures is one such defect common to acid coagulated cheeses (1,2).

In 1966, Emmons (3) described the mechanism of agglutination as an interaction between bovine immunoglobulins present in the milk and the bacterial cells, resulting in aggregates of cells. Acid production is localized around these aggregates causing casein precipitation. Dense flecks of curd also form in the uncoagulated vat. These flecks, which contain high concentrations of casein and bacteria, either become entrapped in the coagulum or settle to the bottom of the vat.

When starter cultures agglutinate, a product of poor quality and low yield is produced. Due to uneven acid production throughout the vat, a curd that shatters easily upon cutting is formed which has a mealy body after cooking.

The severity of the agglutination defect is dependent upon two factors, the cheese milk and the strain of lactic acid starter culture used (4,5). Cheese milk which is high in colostral or mastitic secretions will favor agglutination whereas homogenized milk or milk which is pasteurized at higher than normal temperatures has been reported to reduce agglutination (2,3).

Strains of lactic streptococci have been observed to differ markedly in their agglutination behavior (5). Initially, we screened 34 strains of Streptococcus cremoris and 3 strains of Streptococcus lactis using the agglutination testing procedure of Salih and Sandine (11). Each strain was tested against pepsin coagulated colostrum whey samples. We found that each strain could be grouped into one of three classes according to its agglutination reaction as shown in Table 1.

Susceptible strains were those that agglutinated to some extent with all colostrum whey samples tested. Twenty-seven percent of the 37 strains surveyed were in this group.

Strains in the variable class gave different agglutination reactions with each sample tested, showing intense agglutination to some samples and no reaction to others. Fifty-nine percent of the strains surveyed belonged to this variable or whey dependent agglutination class.

Resistant strains proved to be the smallest group with only 14% of the strains surveyed giving no visible agglutination reaction to any of the whey samples tested.

Based on results obtained in this preliminary study we began an investigation of the molecular nature of lactic streptococcal agglutination.

The study has four main objectives: First, in order to study the microbial factors involved in agglutination, an assay method was needed that would allow rapid screening of large numbers of strains and their mutant derivatives for susceptibility to agglutination. The test also needed to be sensitive enough to detect small changes in agglutination behavior.

The second objective concerns the agglutination factor present in the milk. A great deal of variability exists in the ability of individual whey samples to cause agglutination. We have recently begun studies to purify and standardize the bovine agglutinating factor, which at this time are incomplete. For the data presented here, we have removed a great deal of the variability by using a composite whey sample made by pooling large numbers of individual colostrum whey samples together.

Our third objective is to study the bacterial genetic factors causing agglutination. It has been reported that resistance to agglutination is an unstable trait, lending to the possibility of plasmid involvement (10).

Our fourth objective stems from the observation of Kanno, et al. that the agglutination reaction is an antigen-antibody interaction (8). Thus the possibility exists that specific cell surface antigenic determinants involved in agglutination can be identified.

Agglutination Assay Procedure

We have developed a rapid agglutination testing procedure which is capable of screening up to 100 bacterial strains per day. The antigen preparation and testing procedures are shown in Table 2. The reaction is read on a glass plate by holding the plate over a dark background and illuminating by indirect light to give a darkfield effect. Best results occur when the illuminating light strikes the glass plate at near parallel or slightly below the plate.

Table 3 shows a comparison of our antigen preparation and testing procedure (method A) to the method of Salih and Sandine (Method B). In the latter method, the agglutination reaction is read on a Brewer's

Diagnostic Card. Agglutination titers observed with our method were consistently higher for all strains tested, providing the increased sensitivity needed to detect small changes in agglutination behavior.

By replacing the rose-bengal stain with the fluorescent dye Rhodamine 123 and observing the agglutination reaction by fluorescent microscopy, we were able to correlate our plate test results with actual microscope examination of the behavior of live cells. Rhodamine 123 is a cationic dye which is brought into live cells by active membrane transport and exhibits no toxic effects to lactic streptococci at levels up to 15 ug/ml in broth. Our microscopic examinations showed cell-cell interactions taking place between cells as a direct result of exposure to colostrum whey.

Plasmid Involvement in Agglutination

To investigate the possibility of plasmid involvement in agglutination we screened a number of plasmid-cured mutants of S. lactis C₂ and S. lactis C₁₀. Table 4 shows our results for S. lactis C₂. The parent strain of C₂ was not susceptible to agglutination whereas the plasmid-cured derivative MS-27 exhibited strong agglutination. Each plasmid lost appears to have some effect on agglutination behavior, implicating plasmid involvement. No single plasmid appears to be responsible for susceptibility or resistance to agglutination.

Involvement of Cell-Wall Associated Proteins in Agglutination

Lactose utilization (Lac) and proteinase activity (Prt) of lactic streptococci are known to involve cell wall associated components (7,9). Experiments were performed to determine what effect loss of these traits would have on agglutination susceptibility. In most cases loss of Prt and, to a lesser extent, Lac, increased agglutination

susceptibility as shown in Table 5 with S. cremoris strain NJJ. Other strains were found which demonstrated decreased susceptibility to agglutination when either of Lac or Prt were lost.

CONCLUSIONS

We have developed a rapid agglutination testing procedure using live-stained streptococcal cells. This method allows preparation and testing of up to 100 strains per day. The sensitivity of the test permits detection of small changes in agglutination behavior.

Microscopic studies of live cells under phase contrast and fluorescence correlate well with reactions observed on the plate test.

Screening plasmid-cured mutants of S. lactis C₂ and S. lactis C₁₀ has indicated possible plasmid involvement in agglutination susceptibility.

Altering the activity of cell wall associated traits such as proteinase activity and lactose utilization does change agglutination behavior.

We are currently examining, in depth, the role of plasmid involvement and cell surface antigenic determinants in order to further define the microbial factors responsible for starter agglutination.

In light of recent trends toward genetic improvement of starter cultures and the interrelationship of starter agglutination to other characteristics presented in this study, testing strains for agglutination susceptibility may become an important consideration in the development of improved starters for the fermented dairy products industry.

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Table 1. STRAIN CLASSIFICATION ACCORDING TO AGGLUTINATION REACTION

GROUP	REPRESENTATIVE STRAIN IN GROUP	REACTION ^a	PERCENT OF STRAINS SURVEYED
I SUSCEPTIBLE	<u>S. cremoris</u> HP (ATCC 19257) <u>S. lactis</u> C ₁₀	4+ 3+ or 2+	27% (10)
II VARIABLE	<u>S. cremoris</u> H ₂ <u>S. lactis</u> C ₂	0 - 4+	59% (22)
III RESISTANT	<u>S. cremoris</u> C ₃ <u>S. cremoris</u> UC 77	0	14% (5)

^a4+, strong agglutination; 0, no visible reaction

TABLE 2

MODIFIED AGGLUTINATION ANTIGEN PREPARATION PROCEDURE

Inoculate 10 ml M17 broth with 0.1 ml fresh overnight culture
Grow at 30C to $OD_{600} = 0.5$
Centrifuge at 10,000 x g for 10 min
Wash with 0.5 M Tris-maleate buffer (pH 6.5)
Resuspend in 1.5 ml Eppendorf tube with 1.0 ml of same buffer
Stain with 20 ul Rose Bengal (5% w/v) for 1 min at room temperature
Centrifuge 1 minute in microfuge (12,000 x g)
Wash twice with 0.5 M Tris-maleate buffer (pH 6.5)
(or until supernatant is relatively free of pink color)
Resuspend in 1 ml same buffer for use in test

AGGLUTINATION TEST PROCEDURE

Prepare 1/2 dilutions of whey to 1/256
Keep whey dilutions and antigen on ice
On a glass plate (10" x 14" - 1/8" thick) mix 30 ul of antigen with 30
ul of each whey solution
Mix well with toothpick
Rotate plate 2-3 minute and observe reaction

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TABLE 3

COMPARISON OF ANTIGEN PREPARATION AND ASSAY METHOD

TEST STRAIN: S. cremoris HP (ATCC 19257)

ANTIGEN PREPARATION METHOD	ASSAY METHOD	WHEY DILUTION								
		$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$
A	PLATE	+	+	+	+	+	+	+	-	-
B	PLATE	+	+	+	+	+	-	-	-	-
B	CARD	+	+	+	+	-	-	-	-	-

+, visible agglutination reaction -, no reaction

TABLE 4

AGGLUTINATION BEHAVIOR OF *S. LACTIS* C₂ (U of M)
AND PLASMID-CURED MUTANTS

TEST STRAIN	PLASMID PROFILE ^a	PHENOTYPE	WHEY DILUTION								
			$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$
C ₂ (U of M)	30,18,12, 5,2,1	Lac ⁺ Prt ⁺	-	-	-	-	-	-	-	-	-
MS1	18,12,5,2,1	Lac ⁻ Prt ⁻	+	+	+	+	+	+	-	-	-
MS2	30,18,5,2,1	Lac ⁻ Prt ⁻	+	+	+	+	-	-	-	-	-
MS8	2	Lac ⁻ Prt ⁺	+	+	+	+	+	+	-	-	-
MS27	CURED	Lac ⁻ Prt ⁻	+	+	+	+	+	+	+	+	-
KA7	30,18,12,2	Lac ⁺ Prt ⁺	+	+	+	+	+	-	-	-	-
KA16	30,18,12	Lac ⁺ Prt ⁻	+	+	+	+	+	+	+	-	-

^aplasmid mass expressed in megadaltons (Mdal)

TABLE 5

AGGLUTINATION BEHAVIOR OF PROTEINASE AND LACTOSE
UTILIZATION VARIANTS OF S. CREMORIS NIJ

TEST STRAIN	PHENOTYPE	WHEY DILUTION								
		$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$
NIJ	Lac ⁺ Prt ⁺	-	-	-	-	-	-	-	-	-
TS020	Lac ⁻ Prt ⁻	+	+	+	+	+	+	-	-	-
TS022	Lac ⁺ Prt ⁻	+	+	+	+	+	+	-	-	-

⁺, visible agglutination reaction - , no reaction

SURVEY OF COLIFORM COUNTS AND S. AUREUS IN CHEESE IN THE WESTERN U.S.

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ABSTRACT

A total of 256 cheese samples were tested for coliforms using violet red bile agar medium and the Bactometer coliform medium and for S. aureus using Baird-Parker medium and the Bactometer Staphylococcus aureus medium. When different cheese samples were inoculated with 10^2 - 10^7 CFU/g of E. coli and S. aureus, both the coliform media and the Staphylococcus aureus media had good correlation between the Bactometer and plate methods. ($R^2 = -.94, -.96$). In cheese samples contaminated with coliforms the correlation was $R^2 = -.82$. About 52% of samples contained coliforms ranging 10^2 - 10^7 CFU/g which indicate that foodborne pathogens present in the cheese samples constitute a possible public health hazard. Staphylococcus aureus coagulase (+) above the screening level of 1000 per gram was found in five (2%) of the samples analyzed.

INTRODUCTION

Dairy products are an excellent vehicle for transmission of pathogenic microorganisms. The incidence of infantile diarrhea from foodborne diseases has been reported since the 1920's. In 1971, the first confirmed outbreak in the United States of food poisoning due to dairy products was reported from contaminated imported French Camembert and Brie cheese (8). Coliforms have been found in pasteurized dairy products (4,7), soft and semi soft cheese (6), imported and domestic cheeses (5,9,10) and market milk and dairy products (12). In 1985, a *Salmonella* outbreak occurred in Illinois in a pasteurized milk product.

In the summer of 1985 an outbreak of Listeria monocytogenes occurred in Mexican style cheese made in California, and a similar outbreak occurred in Massachusetts in 1983 (3). This study was undertaken to survey the coliform content of commercial cheese (as an indicator of proper sanitation) and examine the occurrence of staphylococcus aureus coagulase (+). This paper also examines the ability of the Bactometer monitoring system to detect coliforms and S. aureus in cheese samples as an alternative to total plate counts.

MATERIALS AND METHODS

Sample Collection

Samples were collected from grocery and cheese stores in Sacramento, CA and shipped overnight in a cold box to Logan, UT. Samples were stored at 3°C and tested within four days after arrival. Some freshly manufactured cheese samples were also collected from Cache Valley area, UT. Both sliced and unsliced cheeses were selected, and representative samples of a variety of brands were obtained. Types of soft-ripened and hispanic cheese that were sampled include Brie, Camembert, Feta, Havarti, Fresco, Asadero, Cotija, Enchilado, Panela, Ranchero, Oaxaca, Crema, Mexico, Teleme, and Chevre. The types of semisoft and hard cheeses that were sampled include Monterey Jack, Mozzarella, Muenster, Swiss, Cheddar and Cheddar made with goat milk.

Analysis for Coliforms

Ten grams of cheese was placed into a sterile plastic bag (Stomacher 400 Sterile Bags, Tekmar, P.O. Box 37202, Cincinnati, OH 45222) and 90 ml coliform medium (CM) (Bactomatic, Inc., P.O. Box 3103, Princeton, NJ 08540) was added to make 10^{-1} dilutions. The bags were

mixed for 1 min using the Stomacher model 400 (Dynatech Labs. Inc., 900 Slater Lane, Alexandria, VA 22314). Ten mililiters was transferred into sterile test tubes and preincubated for 2-3 h in a 35°C waterbath. Coliform counts were determined (11) using violet red bile agar media (VRBA) (Difco Lab. Inc., Detroit, MI 48201). Duplicate 1.0 ml samples were inoculated to module wells and incubated at 35°C in Bactometer monitoring system (Bactomatic, Inc.) for 14 h. A Bactometer system Code #2 was used for conductance measurements. The preincubation time was added to the Impedance Detection Time (IDT). Samples with short IDT were confirmed on Brilliant Green Bile tubes containing fermentation tubes with gas production in 48 h indicating the presence of coliforms (1,11).

Analysis for *S. aureus*

Ten grams of cheese was placed into a sterile plastic bag and 90 ml of Staphylococcus aureus medium (SAM) (Bactomatic, Inc.) was added to make 10^{-1} dilutions. The bags were mixed for 1 min using Stomacher model 400. Ten mililiters were transferred into sterile test tubes and preincubated for 2-3 h in a 38°C waterbath. Total counts (11) were determined using Baird Parker medium (Difco Inc.). Twenty four hours prior to analysis 0.9 ml SAM was inoculated into the module wells and left at room temperature in order to give enough time for equilibration of electrical double layer between the medium component and the electrode surface, so capacitance changes that take place after the addition of samples will be due to microbial growth. Duplicate 0.1 ml of the preincubated samples were inoculated into module wells containing 0.9 ml of sterile SAM. Modules were incubated in the Bactometer monitoring system at 38°C for 14 h (2). A Bactometer system code #3 was

used for capacitance measurements. The preincubation time was added to the IDT. Samples with short IDT were confirmed by using the coagulase test (2,11).

Results and Discussion

Cheese Inoculated with *E. coli*

Twenty seven cheese samples were inoculated with different concentrations (10^2 - 10^7 CFU/g) of *E. coli* and total coliform counts were performed on VRBA medium. One milliliter duplicates were inoculated into module wells and incubated in the Bactometer. The correlation between VRBA and CM media was $(R^2) = -0.94$. The correlation equation was $Y = -0.56 X + 6.73$ where $Y = \log \text{CFU/g}$, $X = \text{IDT}$. The Bactometer IDT could be used for detection of coliforms in cheese and CM medium correlated well with VRBA medium (Fig. 1).

Cheese Inoculated with *S. aureus*

Thirty four cheese samples were inoculated with different concentrations (10^2 - 10^7 CFU/g) of *S. aureus* and total counts were performed on Baird Parker medium. Duplicate samples of 0.1 ml from each test tube were inoculated into module wells containing 0.9 ml of sterile SAM and incubated in the Bactometer. The correlation between B.P. and SAM media was $(R^2) = -0.96$. The correlation equation was $Y = -0.62 X + 6.5$ where $Y = \log \text{cfu/g}$, $X = \text{IDT}$. The Bactometer IDT could be used for detection of *S. aureus* in cheese and SAM medium correlated well with BP medium (Fig. 2).

Analysis for Coliforms and *S. aureus* in Cheese Samples

A total of 256 samples were analyzed for coliforms. Coliform analysis showed (Fig. 3) that 48% of samples had less than 100 CFU/g, while the other 52% ranged from 10^2 - 10^7 CFU/g. The correlation

between VRBA and CM media of these 52% was (R^2) = -0.82. The correlation equation was $Y = -0.46 X + 7.88$ where $Y = \log \text{ CFU/g}$, $X = \text{IDT}$. The R^2 dropped from -0.94 to -0.82 because in the inoculated cheese, the growth was due to one strain of E. coli and gave the same generation time and hence activity was more uniform. In commercial cheese samples there are other microorganisms involved which act as a natural inhabitate of cheese. These microorganisms can inhibit or stimulate the growth of coliforms depending on the manufacturing procedure of the cheese and type and condition of the milk used in the cheese processing. The correlation dropped also because the microorganisms counted on VRBA medium as typical dark red colonies measuring at least 0.5 mm in diameter in uncrowded plate (11), so colonies do not fit with this description were not counted by the operator while in the bactometer all coliform microorganisms will be able to grow and the system has no way of distinguishing between colonies since every cell will contribute to the IDT. This problem would not reduce the benefit of using the Bactometer to detect coliforms in the food industry because a food plant is concerned only in the detection of coliforms in the product, not in the total coliform counts. The Bactometer will tell if the product is contaminated with coliforms within 4 h or if the product is free of coliforms within 14 h. Since $R^2 = -0.82$ was obtained, the Bactometer could be used for quality control during the processing of the cheese and will give a good indication of the bacteriological condition of the product.

Five samples (2%) were S. aureus coagulase (+) out of 224 samples tested above the screening level of 1000 per gram. Due to lower numbers of positive samples the statistical analysis was not performed. Other samples gave a short IDT but were coagulase (-). This indicates that

the occurrence of S. aureus coagulase (+) is very low and may not be of a concern as much as the coliforms in cheese.

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FIGURE CAPTIONS

- Figure 1. The correlation between log initial conc and impedance detection time (IDT) for an artificially contaminated cheese samples with E. coli.
- Figure 2. The correlation between log initial conc and impedance detection time (IDT) for an artificially contaminated cheese with S. aureus.
- Figure 3. The correlation between log initial conc and impedance detection time (IDT) for the coliform counts in 133 naturally contaminated cheese samples.
- Figure 4. The percent distribution for the coliform counts in cheese samples.

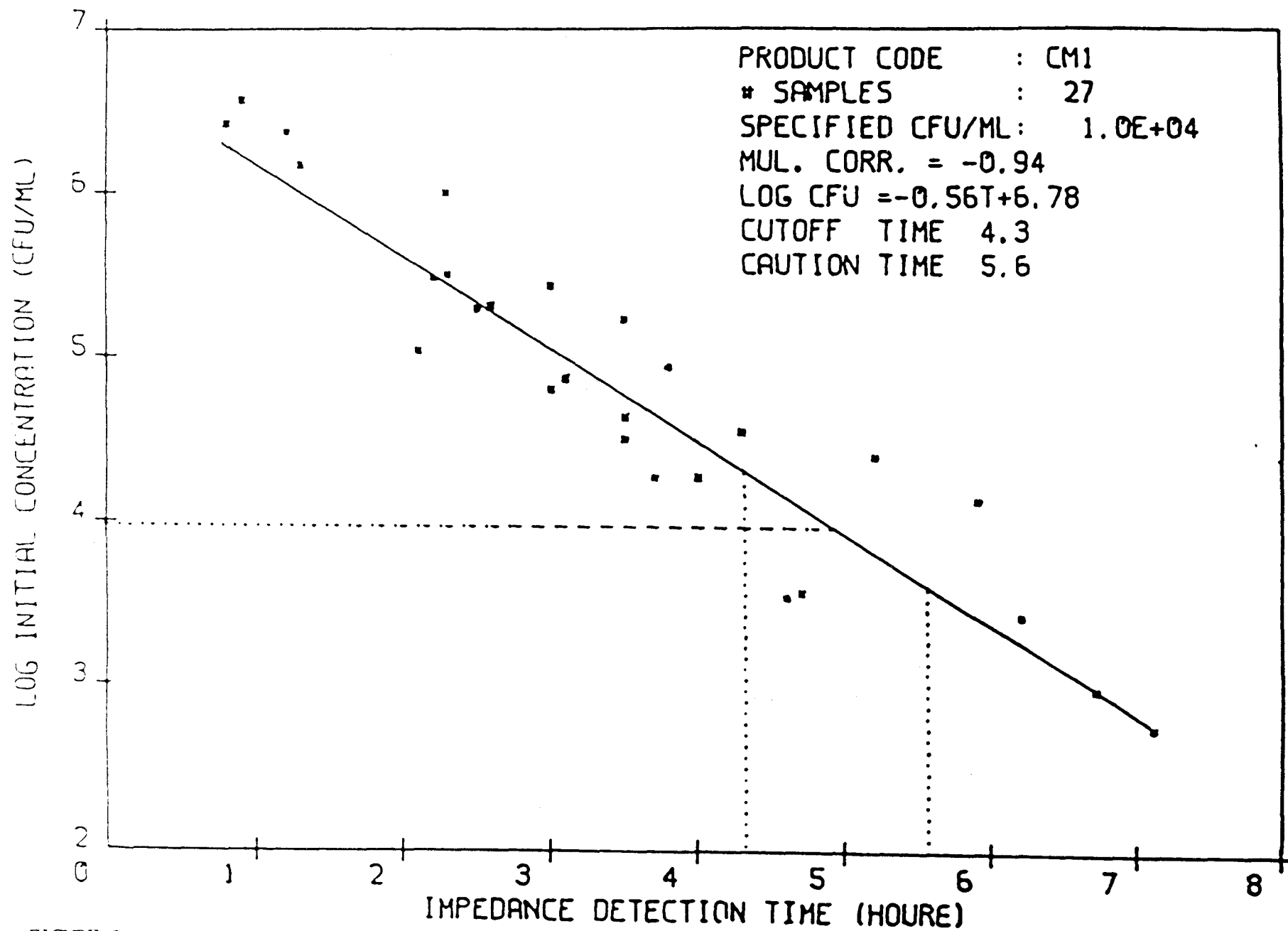


FIGURE 1

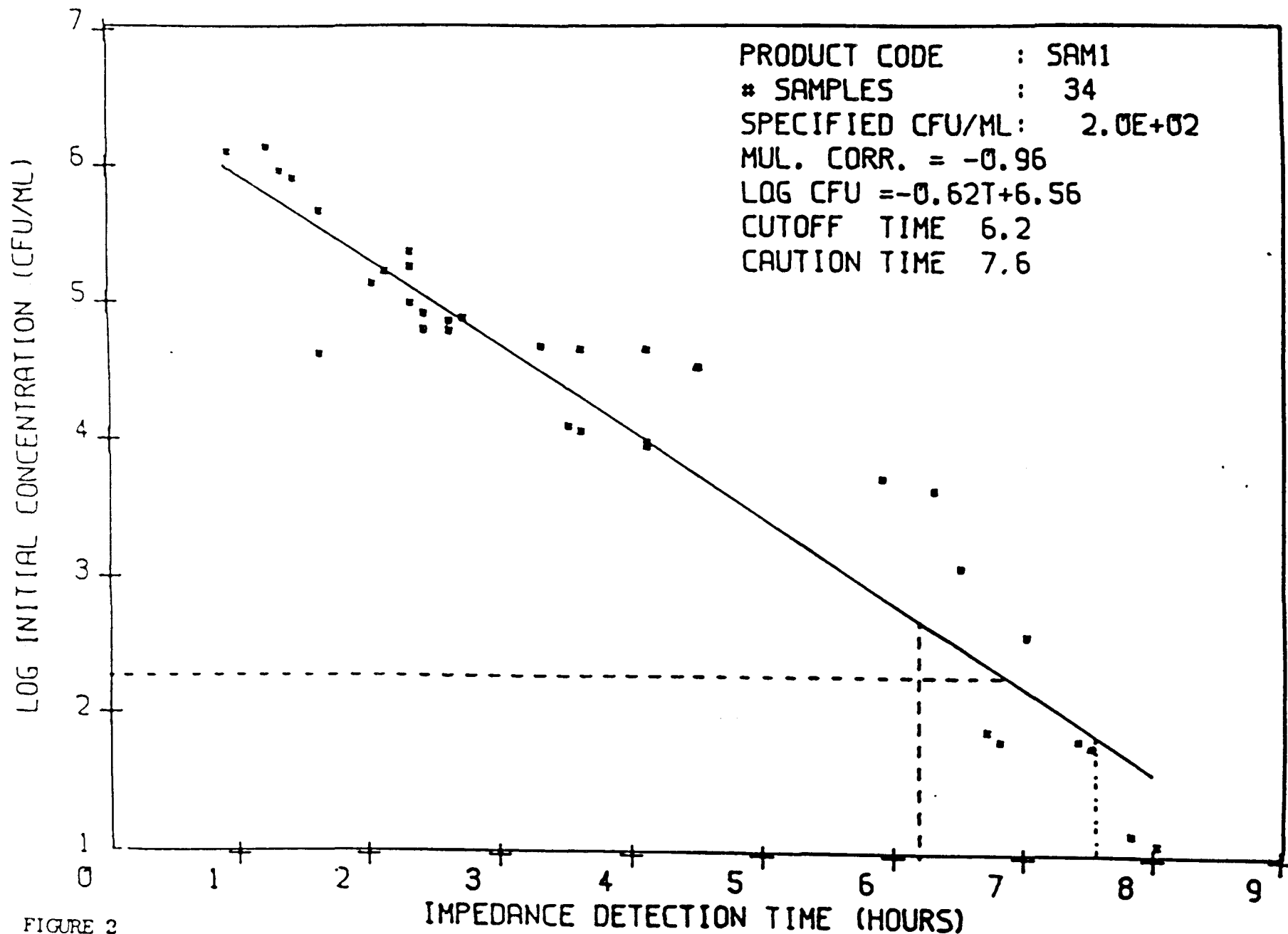


FIGURE 2

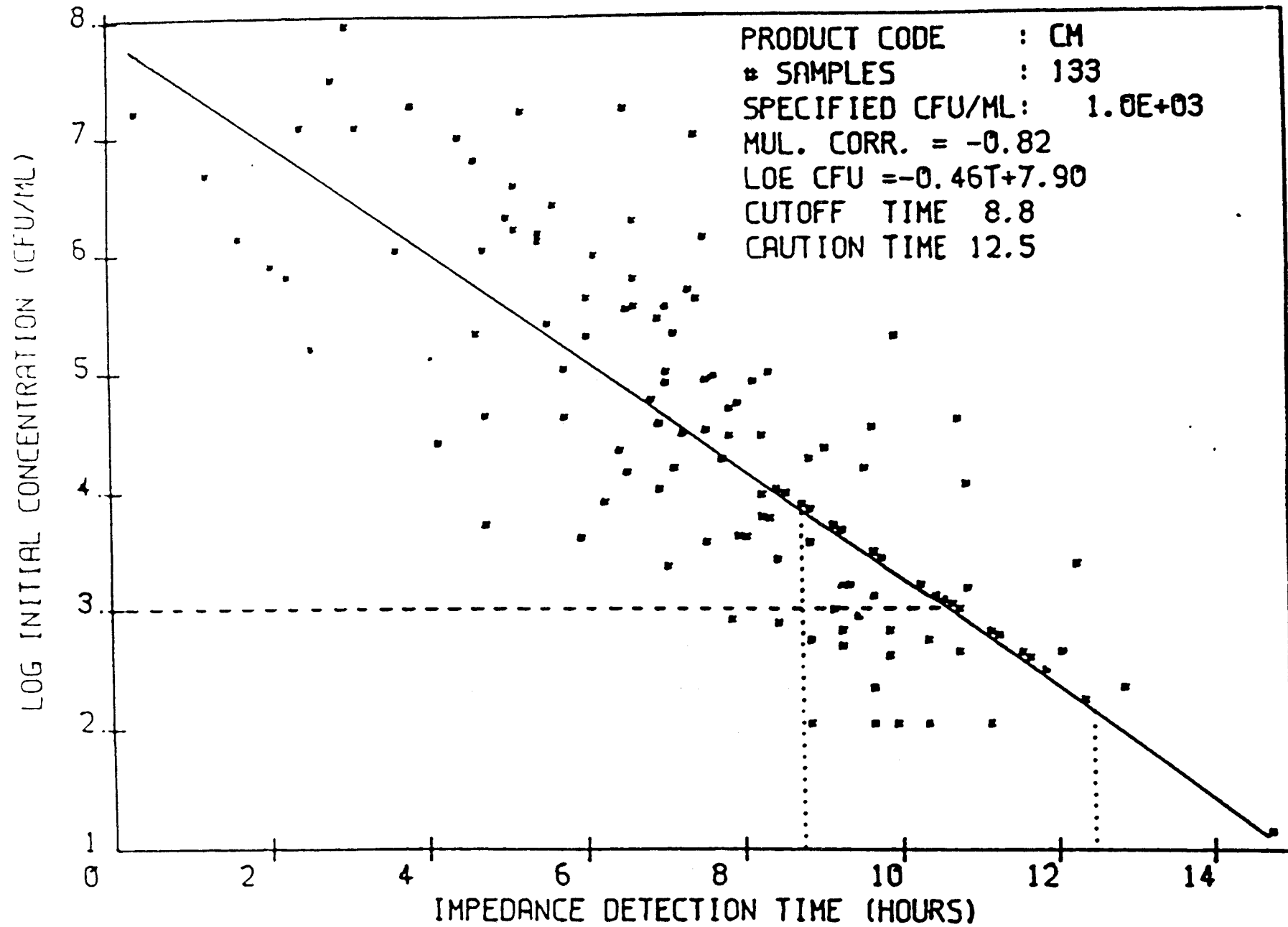


FIGURE 3

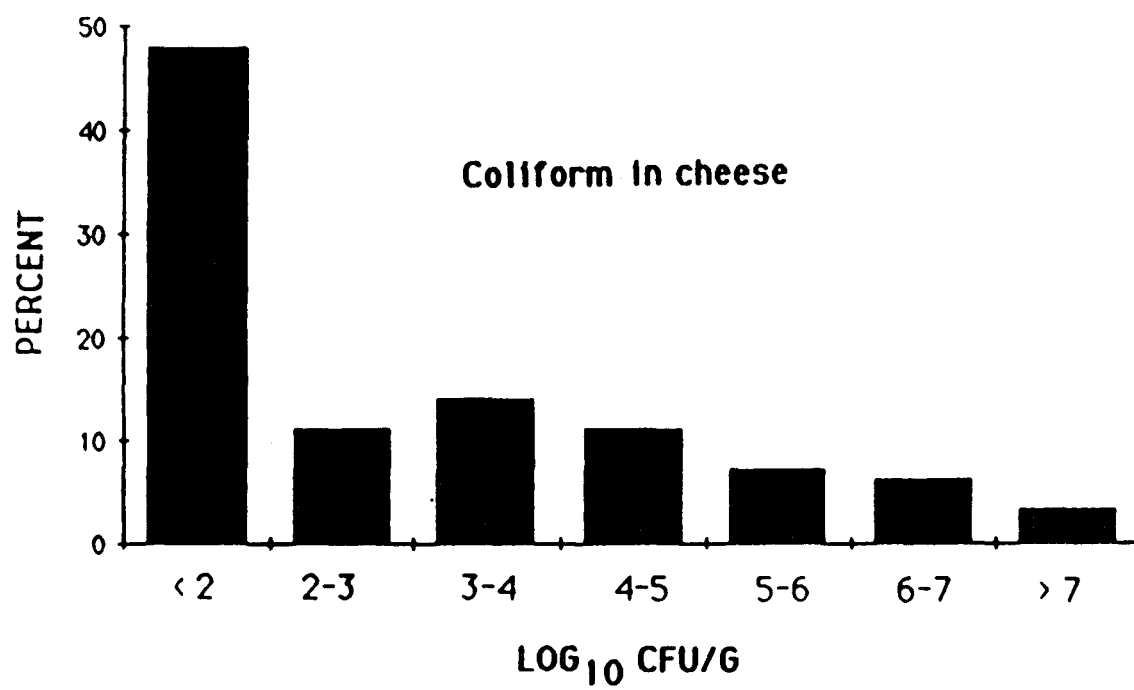


FIGURE 4

Enzyme Action During the Initial Stages of Milk Clotting

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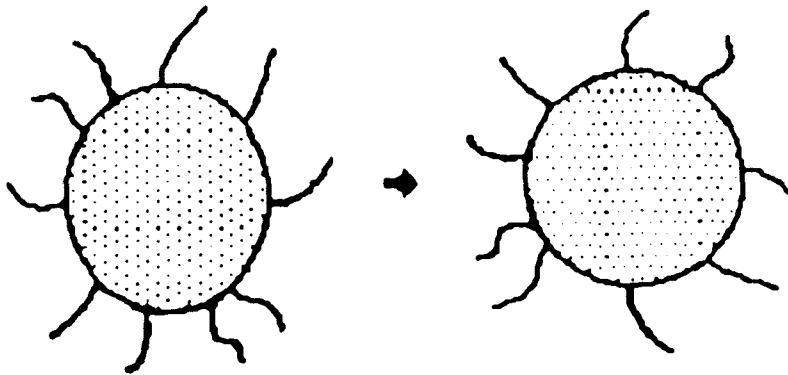
INTRODUCTION

Coagulation of milk to make cheese takes place in three distinct steps (McMahon and Brown, 1984b).

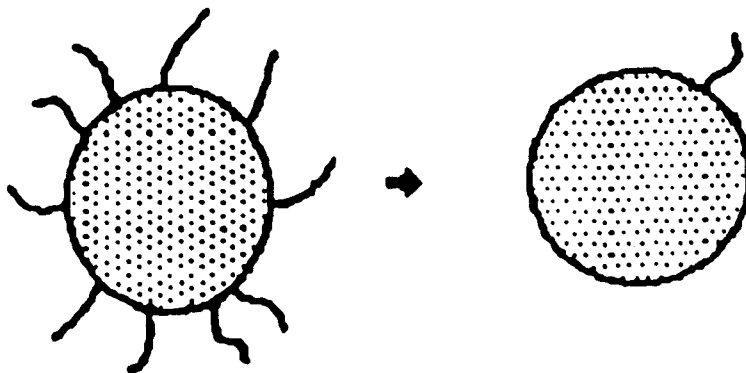
1. Enzymic proteolysis of κ -casein on the surface of casein micelles, removing a peptide from each κ -casein molecule and making the micelles "sticky".
2. Aggregation of the "sticky" micelles with each other.
3. Gelation to form cheese curd. (This stage continues on to syneresis of the curd.)

This stepwise description of milk coagulation leaves some questions unanswered. One of these is whether the κ -casein molecules on all of the micelles are hydrolyzed evenly. In normal cheese making, there is approximately one enzyme molecule for every one thousand casein micelles and each micelle contains thousands of κ -casein molecules (McMahon and Brown, 1984a). So, do the enzymes "graze" on one micelle then move to another or do they cleave a few molecules of κ -casein on one micelle then move to another? With a turnover number (k_{cat}) of $2-13 \text{ sec}^{-1}$ and considering the large effect of diffusion (micelles in milk are about 140 nm in diameter and 240 nm apart), the enzymes probably "graze". Otherwise, milk would take much longer to coagulate than it does.

Another question is whether the three steps overlap or occur one after the other. More specifically, how much of the κ -casein must be cleaved before aggregation commences? This figure represents a micelle with 10% of the κ -casein being cleaved.



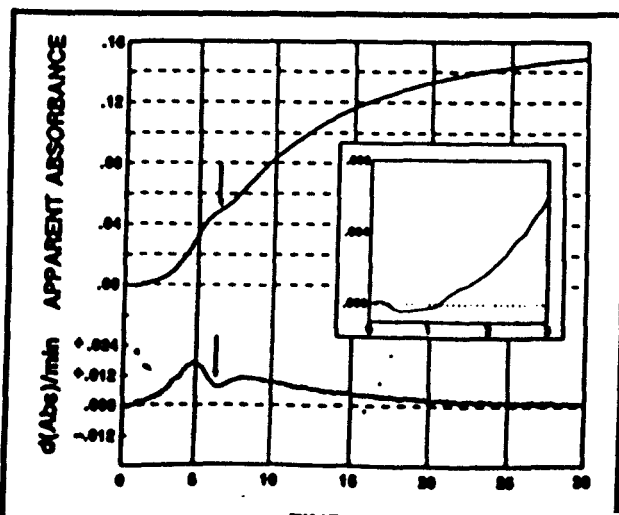
One theory is that aggregation cannot begin until proteolysis of the κ -casein is more than 90% completed (Dalglish, 1979). This figure represents 90% cleavage of κ -casein.



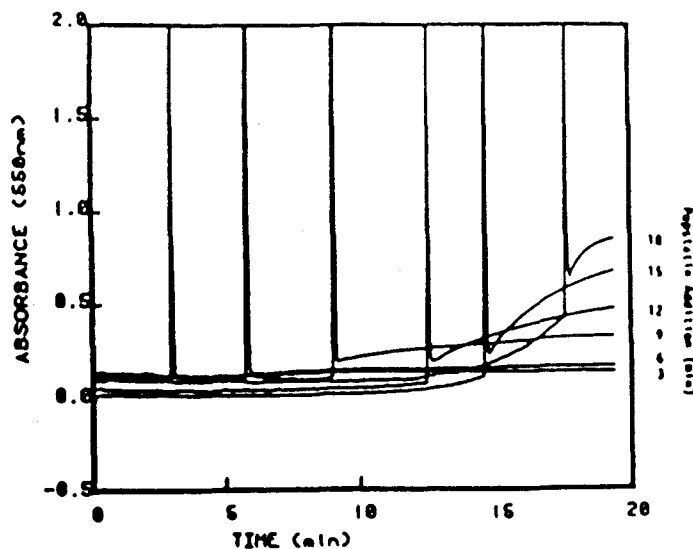
Experiments with immobilized proteolytic enzymes have indicated that aggregation can begin when a very small portion of the κ -casein has been hydrolyzed (Brown and Swaisgood, 1975). Some have criticized such experiments by saying that immobilized enzyme is lost into the milk (Green and Crutchfield, 1969). Others have used milk coagulation tests to follow aggregation with chymosin and have concluded that more than 90% of the κ -casein must be cleaved before aggregation can begin (Dalglish, 1979). These experiments probably measured gelation rather than aggregation.

EXPERIMENTAL METHODS

We tried another method to answer these questions. A spectrophotometer was coupled to a small laboratory computer. A cuvette containing milk (12g low heat nonfat dry milk per 100 ml 0.01 M CaCl_2) was put into the spectrophotometer and the instrument was zeroed on 600nm wavelength. Then chymosin (4.87×10^{-3} Rennin Units per ml) was added to the milk and A_{600} was followed over time to observe all three steps of milk clotting (McMahon, Brown and Ernstrom, 1984; McMahon, Brown, Richardson and Ernstrom, 1984).



In a series of experiments, 0.025 mg of pepstatin per milliliter of milk was added to stop the enzyme at different times. Clotting was observed even when enzymic proteolysis was stopped at a very early stage.



CONCLUSIONS

We concluded from these experiments that the first two steps of milk clotting overlap. It is also reasonable to assume that the third step overlaps with the second and probably with the first. Therefore, as we develop mathematical models of milk clotting kinetics, all steps must be integrated together (McMahon, Richardson and Brown, 1984; Brown and Collinge, 1986). The rate of the enzymic reaction changes as substrate (uncleaved κ -casein) decreases, causing the aggregation rate "constant" to not be a constant and so on through gelation.

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