Incorporation of Native and Denatured Whey Proteins into Cheese Curd for Manufacture of Reduced Fat, Havarti-type Cheese[1]

C. G. LO and E. D. BASTIAN
Minnesota-South Dakota Dairy Foods Research Center,
Department of Food Science and Nutrition,
University of Minnesota, St. Paul 55108-6099

ABSTRACT

Undenatured and heat-denatured whey proteins were incorporated into Havarti-type cheese by manufacturing cheese from ultrafiltered milk that had been heated to 72°C for 17 s or 85°C for 17 s. Traditional Havarti also was manufactured and used as a control. Cheeses were ripened for 12 wk. High heat treatment resulted in significantly greater percentages of denatured whey proteins in milk. The rate of α_{s1}-CN hydrolysis, as determined by urea-PAGE, was significantly lower in cheese that had been manufactured from UF milk heated to 72°C than in control cheese and cheese made from UF milk heated to 85°C. More whey proteins, mostly denatured, were recovered in cheese manufactured from milk heated to 85°C than in cheeses manufactured from milk heated to 72°C. The overall flavor intensity among cheeses was indistinguishable by the sensory panel; however, control cheeses were more bitter than UF cheeses.

(**Key words**: cheese, whey proteins, native protein, denatured protein)

Abbreviation key: **RU** = rennin units.

INTRODUCTION

Whey proteins have been incorporated into cheese using a variety of techniques that fall under two general methodologies: heat treatment, resulting in complexation of whey proteins with casein, and concentration of milk before cheese manufacture, using such techniques as UF, reverse osmosis, and evaporation. In the first case, whey proteins are primarily denatured, but, in the second situation, native whey proteins can be included in the cheese matrix. The consequences of whey proteins being part of the cheese matrix has received some attention by researchers, but many questions remain.

The motivations for incorporating whey proteins into cheese have included increased cheese yield and, more recently, replacement of milk fat in reduced-fat cheese products. Regardless of the reason, greater understanding of how whey proteins impact cheese systems seems warranted.

Fat content of traditional cheese products varies from 14 to 35% (27). Low or reduced fat cheese can be made by replacing fat with ingredients that bind water. Several fat replacers have been developed and used in the dairy industry with mixed success. However, these added ingredients increase the cost of production.

Intrinsically, the water-holding capacity of native whey proteins can be enhanced by heat treatments that partially denature these proteins. Such heat-treated milk may not be suitable for manufacturing full-fat, hard cheese because of the high moisture content of the resulting cheese (24, 29). Some manipulations have been made by Law et al. (20) and Banks et al. (4, 5) to improve sensory and functional properties of Cheddar-type cheese that contains heat-denatured whey proteins.

Ultrafiltration of milk has been used to manufacture many types of cheese (8, 19, 30). Incorporation of whey proteins into cheese usually results in increased yield and improved nutritional value. Cheese yield, however, is not improved if whey removal is extensive after the cheese curd is formed because native whey proteins are removed with whey; thus, this process generally limits the yield advantage of UF technology to semi-soft and soft cheese types.

Several researchers (15, 17, 28) have found that slow hydrolysis of α_{s1}-CN in cheese was responsible for abnormal flavor and texture development during cheese ripening. Guinee et al. (15) reported that higher heat treatment of milk before UF resulted in cheese that exhibited a higher degree of primary proteolysis than did UF cheese made from milk heated at

Received December 23, 1996.
Accepted August 20, 1997.
[1]Published as Paper Number 22,571 of the contribution series of the Minnesota Agricultural Experiment Station and based on research conducted under Project 18-036. This research was sponsored, in part, by the Minnesota-South Dakota Dairy Foods Research Center and Dairy Management Inc.

lower temperatures, which suggested that denatured whey proteins do not impede the hydrolysis of casein in cheese. The results of Guinee et al. (15) support the earlier work of Iyer and Lelievre (18) who studied the effect of whey proteins on proteolysis of casein in cheese slurries. They (18) proposed that native whey proteins reduced the rate of α_{s1}-CN hydrolysis, but denatured whey proteins had little effect on α_{s1}-CN degradation. Green et al. (13) and de Koning et al. (9) suggested that low residual rennet concentrations might be related to texture and flavor problems in UF cheese. However, Creamer et al. (7) added various concentrations of rennet and observed that, when the residual rennet concentrations in the control and UF Cheddar cheese were the same, conventional Cheddar cheese ripened more quickly. When rennet is added to retentate to manufacture higher moisture cheese, such that little whey drainage occurs after curd formation, most of the rennet remains in the curd, resulting in higher than normal residual rennet activity.

In an earlier study (21), we tested the influence of isolated native and denatured α-LA and β-LG on the hydrolysis of α_{s1}-CN by chymosin in a model system at pH 5.5 with simulated milk ultrafiltrate included in the solution. Those conditions did not completely mimic cheese because proteins were in solution, whey proteins were heat-denatured individually rather than with casein, moisture content was higher than in cheese, and the reactions were carried out at 20°C. Yet, under conditions of the study, we observed that both native and denatured β-LG interfered with hydrolysis of α_{s1}-CN by chymosin. The other major whey protein, α-LA, was only inhibitory when it was denatured and in the presence of milk salts. These experiments showed that, under specified conditions, whey proteins, particularly β-LG, can inhibit the action of chymosin, which might partially explain why the degree of α_{s1}-CN hydrolysis in cheese fortified with whey protein is lower than that in traditional cheese.

Qvist et al. (30) found that α_{s1}-CN was almost completely degraded in both UF and traditional Havarti cheese after 120 d of storage. The slower flavor development in UF Havarti cheese was associated with retarded degradation of β-CN.

The objective of this research was to incorporate both native and denatured whey proteins into cheese and to compare the chemical and sensory properties of such cheese to traditionally manufactured control cheese.

MATERIALS AND METHODS

Milk Treatment

Whole milk was obtained from Mid-America Dairymen, Inc. (Roseville, MN). The milk was standardized to 1.5% fat and pasteurized in the pilot plant of the University of Minnesota, Department of Food Science and Nutrition. To incorporate undenatured whey proteins into UF cheese and for manufacturing control cheese, milk was heated to 72°C for 17 s. A higher heat treatment (85°C for 17 s) was applied to milk for manufacturing UF cheese that contained denatured whey proteins. After pasteurization, milk samples were stored overnight in a cooler at 5°C.

UF

After pasteurization and storage, the milk for UF cheese containing undenatured or denatured whey proteins was concentrated in a batch ultrafiltration system to approximately 42 to 44% solids (5.5× concentration) at 50°C using a polyether-sulfone membrane (Fluid Systems, San Diego, CA) with a designated 10,000 nominal molecular mass cutoff and 5 m^2 of surface area. Diafiltration (30%) with deionized water was carried out when fat and casein in retentate had been concentrated threefold, and water was slowly added to maintain a constant retentate volume during the diafiltration step.

Cheese Making

Control and UF cheeses were manufactured according to the procedure of Qvist et al. (30) with a few modifications. Retentate (45 to 50 kg) was collected and maintained in a 38°C water bath and agitated. Five hundred grams of retentate were removed and inoculated with 15 g of direct-vet-set B11 culture (Chr. Hansen's Laboratories, Milwaukee, WI). Inoculated retentate was incubated at 38°C for 1 h to facilitate the repair of damaged cells. This inoculated retentate was then stirred back into the remaining retentate, and chymosin (Chymax®; Pfizer, Inc., Milwaukee, WI) was added (5 ml of chymosin/100 kg of retentate). This amount of rennet produced the firmness needed for cutting the curd in 35 ± 3 min at 38°C. Coagulation and cutting were carried out in an ALCURD continuous cheese coagulator (model MK IV; Alfa Laval, Lund, Sweden). After cutting, the curd was dropped directly into molds and handled as described by Qvist et al. (30). After brining, the cheeses were vacuum-sealed and ripened at 10°C.

Composition Analysis

The moisture, protein, ash, and salt contents of cheese were determined after 2 wk of storage using AOAC methods (2). The fat content was measured by the Mojonnier method (3), and the pH of cheese was measured in cheese slurries (cheese to water, 2:1, vol/vol) from comminuted cheese samples.

Denaturation of Whey Proteins in Milk

The solubility of whey proteins at pH 4.6 was used to determine the extent of whey protein denaturation in heated milk. Whey proteins remaining soluble in milk adjusted to pH 4.6 were quantified using ion-exchange chromatography. Milk samples were taken before and after pasteurization and analyzed for protein profiles using ion-exchange chromatography (FPLC®; Pharmacia LKB, Uppsala, Sweden). The pH of 100 ml of milk (unheated, low heat, and high heat) was reduced to 4.6 using 5*N* HCl at 20°C. Casein and denatured whey proteins were precipitated and were filtered using Whatman number 4 filter paper (Fisher Scientific, Pittsburgh, PA). The filtrate was centrifuged at 8000 × *g* for 45 min at 10°C, and the pellet was discarded. The supernatant was diluted in buffer A (0.02 *M* Tris·HCl, pH 7.0) at a rate of 1 ml of supernatant in 3 ml of buffer A. The diluted samples were filtered again through a 0.2-μm polyvinylidene difluoride (PVDF; Chrom Tech, Inc., Apple Valley, MN) syringe filter and injected into a Mono-Q HR 5/5 (5 mm diameter × 500 mm long) ion-exchange column (Pharmacia Biotech, Uppsala, Sweden). The separation conditions used were adapted from the published literature (1).

Total Whey Proteins in Cheese

Amino acid composition analysis. Whey proteins in cheese were estimated according to the method of Greenberg and Dower (14) as modified by Walsh (35) for measuring whey proteins in cheese. This method allows measurement of the total amount of whey protein in cheese, regardless of whey protein denaturation, complexation with casein, and aggregation. The first step in the analysis involved hydrolysis of all proteins in the cheese (6*N* HCl for 18 h) to release amino acids for compositional analysis; thus, the state of the various proteins in the cheese would not influence the measurement. This method is based on determination of the amino acid profiles of several standard protein mixtures that contain different amounts of casein and whey protein; the focus then is on amino acids with large differences in content between casein and whey protein, such as cysteine and proline. Although a single amino acid was initially used to estimate the amount of whey protein in cheese, accuracy improved when multiple linear regression was applied in a stepwise fashion to select several amino acids for building a prediction equation (35).

Whole casein was prepared by isoelectric precipitation from skimmed bovine milk obtained from the same source as the milk used for cheese manufacture. Whey protein was prepared from the filtrate (acid whey) of this milk. Both fractions were dialyzed against deionized water and freeze-dried. Six samples containing 0 to 100% casein and 100 to 0% whey protein were prepared for developing a standard curve. Each sample contained 80 mg of total protein and was analyzed for amino acid composition using an amino acid analyzer (model 6300; Beckman Instruments, Inc., Fullerton, CA). Multiple regression was done to develop an equation for predicting the percentage of total whey proteins in cheese. Cheese samples were prepared by mixing 20 g of ground cheese with 100 ml of 0.2 *M* sodium citrate, and this mixture was placed in a 40°C water bath for 2 h with frequent shaking. The resulting cheese suspension was centrifuged at 5000 × *g* for 30 min at 4°C and, after the fat layer was removed, the remaining cheese mixture was analyzed for amino acid composition. The amino acid composition of the cheese was used in the prediction equation to estimate the amount of whey protein in cheese.

Spectrophotometric method. The method of Meisel (23) also was examined as a way to predict whey proteins in cheese. We modified this method by measuring absorbance spectra and by using the highest peak absorbance for quantification of protein rather than using the fourth derivative of the spectra. Casein and whey protein standards that were similar to those made for amino acid analysis were prepared, except that each standard contained 30 mg instead of 80 mg of total protein. Cheese samples that had been dissolved in 0.2 *M* sodium citrate as described were further diluted by the addition of the cheese suspension (0.45 to 0.5 ml; the volume was adjusted so that 30 mg of protein from the cheese were added) into 25-ml volumetric flasks followed by dilution with 6 *M* guanidine·HCl and 0.1 *M* sodium acetate buffer at pH 5.0. The contents of the flasks were thoroughly mixed. Using a single beam spectrophotometer (model DU 650; Beckman Instruments, Inc., Fullerton, CA), the absorbance at 280 nm was recorded for each standard and cheese sample. Absorbance readings from the

standards were used to predict the whey protein content of cheese samples.

Native Whey Proteins in Cheese

The pH of cheese samples, dissolved in 100 ml of 0.2 *M* sodium citrate, was adjusted to 4.6. Undenatured whey protein was defined as cheese protein soluble at pH 4.6 and 20°C. After the precipitate was removed, the supernatant was filtered and injected into the ion-exchange chromatography system for quantification of whey proteins according to the method described by Andrews et al. (1). Denatured whey proteins were determined by the difference between total and native whey protein concentrations.

Hydrolysis of α_{s1}-CN

Proteolysis rates of α_{s1}-CN in all cheese samples were compared at 2, 4, 8, and 12 wk using the urea-PAGE method described by Farkye (11). The chemicals used for this method were Readymix [(DNA-PAGE gelling agent (95% acrylamide, 5% bisacrylamide); Pharmacia Biotech], ammonium persulfate, Commassie blue, glycine, TEMED (Sigma Chemical Co., St. Louis, MO), acetic acid, methanol, THAM, and urea (Fisher Scientific, Pittsburgh, PA). The bands of α_{s1}-CN were quantified (Personal Densitometer SI; Molecular Dynamics, Sunnyvale, CA).

Sensory Evaluation

Cheese was evaluated after 4, 8, and 12 wk of ripening by 10 judges (5 women and 5 men) who had experience in judging dairy products. The characteristics or descriptors used for sensory analysis were developed by these experienced judges during preliminary evaluation sessions. Seven characteristics were chosen: openness, rubberiness, curdiness, acid flavor, cooked flavor, bitterness, and overall flavor. Intensities were scored using a nine-point scale (0 = none and 9 = extremely high). Samples, labeled with three-digit random codes, were served at 22°C to panelists in individual booths. A completely randomized design was used to determine the serving order for cheese. Six cheeses were tested in each of 12 half sessions. Two replicate judgments of each cheese were made by each panelist. All panelists were present for all testing sessions. Panelists were given distilled water to neutralize the effect of preceding cheese samples.

Statistical Analysis

The experimental design used in this study was a split-plot design (25). The whole plot consisted of the different cheeses and the replicates, which represented a blocking effect. The effect of the different cheeses in the whole plot was tested using the whole-plot error term (cheese × replication). The effect of time, as well as the interaction between time and cheeses, was tested with the subplot error term, composed of the combined effect of the three-way interaction (heat × replicate × time) and the interaction between time and replicates. The resulting data from these experiments were analyzed according to the following statistical model. The last four terms in the model were used only for analyzing sensory data.

$$Y_{ijkl} = \mu + R_i + H_j + \epsilon_{ij} + T_k + (HT)_{jk} + \epsilon_{sub} + J_l + (HJ)_{jl} + (TJ)_{kl} + (HIJ)_{jkl} + \epsilon_{sub\text{-}sub} \quad [5]$$

where Y_{ijkl} = each individual observation; μ = overall mean; R_i = effect of replicate (i = 1, 2, 3, and 4); H_j = effect of heat treatment (j = 1, 2, and 3 representing traditional cheese, cheese manufactured from UF milk given a low heat treatment, and cheese manufactured from UF milk given a high heat treatment); ϵ_{ij} = whole-plot error term (its mean square was used to test effect of replicate and heat); T_k = effect of time (k = 1, 2, 3, and 4 representing 3 d and 4, 8, and 12 wk); $(HT)_{jk}$ = interaction of heat and time; ϵ_{sub} = subplot error term (its mean square was used to test effect of time and interaction of heat and time; J_l = effect of judge (l = 1 to 10); $(HJ)_{jl}$ = interaction of heat and judge; $(TJ)_{kl}$ = time by judge interaction; $(HTJ)_{jkl}$ = interaction of heat, time, and judge; and ϵ_{judges} = error term used to test effect of judge, interaction of heat and judge, interaction of time and judge, and interaction of heat, time, and judge.

RESULTS AND DISCUSSION

Enzyme Coagulation Studies on Retentate

Because retentate produced in this study had 5.5 times more protein than regular milk, it was necessary to test the clotting ability of chymosin (Chymax®) on retentate to approximate the time and firmness that are typically used for cheese manufacture. A cutting time of 35 min was obtained from retentate (43% solids) to which half the normal concentration of chymosin had been added. Generally, the amount of rennet used in the cheese industry is based on the ratio of 2400 rennin units (**RU**)/100 kg of milk. Thus, in this study, 1200 RU/100 kg of retentate was used to coagulate UF retentate (single-

TABLE 1. Cheese composition.

Attribute	Treatment		
	Control	Low heat	High heat
Moisture (g/100 g of cheese)	47.86[b]	49.94[ab]	50.99[a]
Protein[1]	57.67[a]	59.16[a]	59.71[a]
Fat[1]	31.28[a]	31.46[ab]	30.81[b]
Ash[1]	7.52[a]	8.03[a]	8.10[a]
Salt[1]	3.41[a]	3.76[a]	3.92[a]
pH	5.23[a]	5.29[a]	5.26[a]

[a,b,c]Means within the same row without a common superscript differ ($P < 0.05$).

[1]Reported as a percentage of total cheese solids.

strength rennet has an approximate activity of 120 RU/ml).

Cheese Composition

Composition of the cheese produced in this study is given in Table 1. The cheese manufactured from UF milk given a high heat treatment had higher moisture and lower fat content than did traditional cheese. Cheeses manufactured from low and high heated UF milk were not significantly different in moisture and fat contents. No significant differences were observed in the concentrations of protein, ash, and salt or in the pH values among these cheeses.

Denaturation of Whey Proteins in Milk

Table 2 shows the percentage denaturation of α-LA, β-LG A, β-LG B, and total β-LG in milk given low (72°C for 17 s) or high (85°C for 17 s) heat treatment. Bovine serum albumin and Ig are more heat sensitive than α-LA and β-LG (34) and were not distinctly identifiable under the separation conditions of this experiment. Thus, the thermal denaturation of the two major whey protein fractions, α-LA and β-LG, were examined. We observed that α-LA was more heat stable than β-LG A and β-LG B, which is consistent with the reports of Mulvihill and Fox (26) and Vervaeck and Huyghebaert (34). High heat treatment denatured higher amounts of α-LA, β-LG A, and β-LG B than did low heat treatment.

Native and Total Whey Proteins in Cheese

Table 3 shows the percentages of native whey proteins (α-LA, β-LG A, β-LG B, total β-LG, and total native whey protein) and total whey proteins that were recovered in different cheeses. The difference between total whey protein and native whey protein would give an estimate of the levels of denatured whey proteins in these cheeses. The levels of native and total whey proteins retained in traditional cheese were lower than that retained in either of the UF cheeses. As the pasteurization temperature increased, the concentration of total whey proteins increased. The percentage of native whey protein was lower in UF cheese made from high heat milk than in UF cheese manufactured from low heat milk, but the UF cheese made from high heat milk had a higher percentage of total whey proteins than did the other UF cheese, indicating that most of the whey proteins incorporated into high heat cheese were denatured.

The concept of incorporating whey proteins into cheese by UF technology was introduced many years ago (22), but methods for measuring native and denatured whey proteins in cheese have not been standardized. Several researchers (10, 12, 18, 33) used the Rowland scheme (31, 32) to determine the concentration of native and total whey proteins in cheese. The procedures for preparation of nitrogen fractions were complex and based on several assumptions. Recently, the recovery of whey protein in cheese has been studied by gel permeation chromatography (4, 20), showing that the concentration of native whey protein fractions in whey can be accurately quantified from the peak area, and the difference in concentration of whey protein between starting milk and whey can be used to determine the total recovery of individual whey proteins in cheese. However, this methodology gives an indirect measurement of whey protein recovered in cheese, so we chose two methods to measure total whey proteins in cheese based on their ability to determine whey proteins, regardless of whether they were native, denatured, or complexed with casein. Both methods are based on differences in

TABLE 2. Effect of heat treatment on the percentage denaturation of α-LA, β-LG A, β-LG B, and total β-LG.[1]

Whey protein fraction	Denaturation			
	Low heat		High heat	
	(%)			
	$\bar{X}$	SE	$\bar{X}$	SE
α-LA	7.17	1.63	16.46	3.89
β-LG A	9.56	1.19	27.57	4.08
β-LG B	15.87	2.74	43.89	6.46
Total β-LG	25.43	1.92	71.46	5.24

[1]Values are the percentage of the protein that was denatured in milk given low (72°C for 17 s) or high (85°C for 17 s) heat treatment.

amino acid composition between casein and whey protein. The Walsh (35) method involves measuring 17 amino acids in protein mixtures with known amounts of casein and whey proteins and using multiple regression to select several amino acids that predict the amount of whey protein in cheese. Similarly, the modified Meisel (23) method involves denaturing cheese proteins in guanidine hydrochloride and measuring absorbance at 280 nm, thus measuring aromatic amino acids that cannot be detected by standard compositional analysis of amino acids, which are degraded during the hydrolysis step (35). Prediction of the amount of whey proteins in cheese by measuring aromatic amino acids at 280 nm also was based on running standards using known amounts of casein and whey proteins. Standards were used so that the extinction coefficients of variable mixtures of protein would not be required for calculating percentages of whey protein.

Although the whey protein content predicted by both of the methods examined in this study was similar for UF cheese manufactured from low heat milk, the two methods were substantially different in ability to predict whey protein content of UF cheese made from high heat milk. We do not know the reason for this difference because other data from the two methods were similar.

Protein Hydrolysis

In this study, cheese proteins were examined using urea-PAGE after 2, 4, 8, and 12 wk of ripening. The pattern for replicate 1 is shown in Figure 1; the other replicates had similar hydrolysis patterns. β-Casein was not significantly degraded over time except in control cheese, which showed increased production of γ_1-CN in cheese that had been ripened for 12 wk. Results of β-CN hydrolysis from this study agreed with those in the study by Qvist et al. (30) who observed limited hydrolysis of β-CN in UF Havarti cheese and suggested that plasmin might be inhibited by whey proteins. It was later verified that β-LG inhibits plasmin activity (6).

After 8 wk of ripening, the α_{s1}-CN band resulting from UF cheese made with low heat milk was more dense than that in traditional and UF cheese

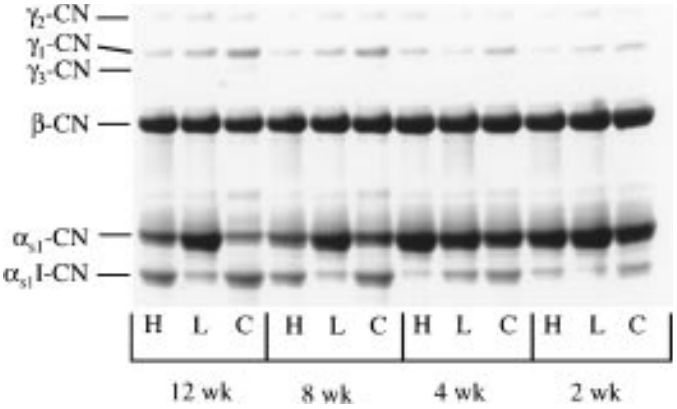

Figure 1. Urea-PAGE profile of cheese proteins. Cheese was manufactured by a traditional method (C) and from UF milk that had been given a low (L; 72°C for 17 s) or high (H; 85°C for 17 s) heat treatment. The ages of cheese are denoted below the letters.

TABLE 3. Native whey proteins (WP) (α-LA, β-LG A, β-LG B, total β-LG, and total native whey proteins) and total WP in different cheeses.[1]

Treatment	α-LA	β-LG A	β-LG B	Total β-LG[2]	Total native WP[3]	Total WP[4]	Total WP[5]
	(g/100 g of protein)						
Control							
X	0.18	0.55	0.30	0.85	1.03	1.16	1.59
SE	0.03	0.07	0.05	0.05	0.05	0.20	0.13
Low heat							
X	0.31	1.55	0.99	2.53	2.82	3.00	2.95
SE	0.04	0.14	0.11	0.12	0.08	0.38	0.28
High heat							
X	0.20	0.67	0.35	1.02	1.22	4.14	6.32
SE	0.02	0.08	0.05	0.05	0.04	0.87	0.86

[1]Values are means of four trials. Cheese was manufactured by a traditional method (control) and from UF milk that had been given a low (72°C for 17 s) or high (85°C for 17 s) heat treatment.

[2]Total β-LG = β-LG A + β-LG B.

[3]Total native WP = α-LA plus total β-LG.

[4]Method reported by Walsh (35).

[5]Modified method of Meisel (23).

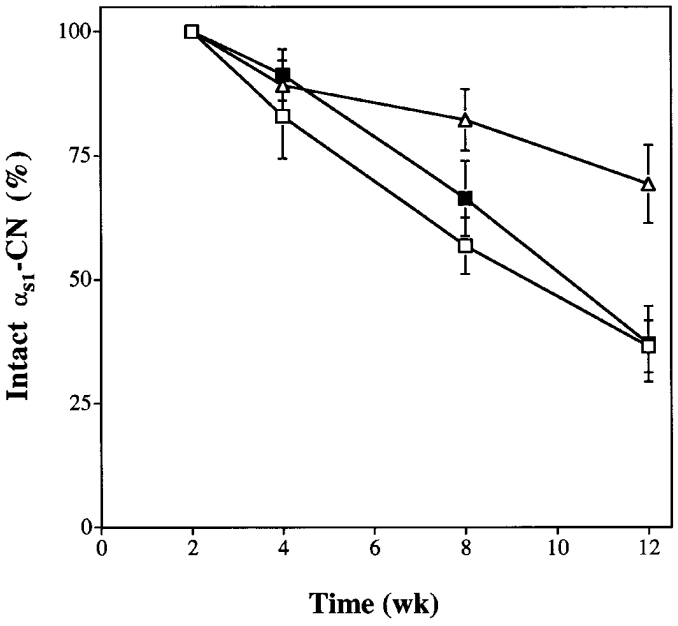

Figure 2. Hydrolysis of α_{s1}-CN during cheese ripening [□ = control cheese (C), △ = cheese manufactured from UF milk that had been given a low (72°C for 17 s) heat treatment (L), and ■ = cheese manufactured from UF milk that had been given a high (85°C for 17 s) heat treatment (H)].

manufactured from high heat milk. This phenomenon can be seen more clearly in Figure 2, which shows percentages of intact α_{s1}-CN based on the densitometry of electrophoretic bands of cheese samples taken during 12 wk of ripening. As ripening time increased, α_{s1}-CN in the traditional and UF cheese made from high heat milk was hydrolyzed more rapidly than in UF cheese manufactured from low heat milk. After 12 wk of ripening, approximately 37% of α_{s1}-CN remained intact in traditional cheese and UF cheese manufactured from high heat milk, but, in cheese made from low heat milk, 70% of α_{s1}-CN was not hydrolyzed. Guinee et al. (15) found that higher levels of native whey proteins in UF cheeses were associated with a lower degree of α_{s1}-CN hydrolysis, an observation that is consistent with our findings.

In earlier studies with model systems (pH 5.5) (21), we found that both native and denatured β-LG retarded α_{s1}-CN hydrolysis by chymosin in systems containing simulated milk ultrafiltrate. In the model system, whey proteins were heat-denatured in deionized water and then added to α_{s1}-CN solutions before addition of chymosin. Consequently, the native and denatured forms of β-LG were free to diffuse and interact in solution with chymosin, but, in this study, most of the whey protein in cheese made from high heat milk was probably associated with casein and was not free to diffuse. Thus, the results from research on model systems do not reflect the whey protein and casein complex that probably exits in the cheese manufactured from highly heated milk. Both the previous work and this study provide some evidence that the slow hydrolysis of α_{s1}-CN in UF cheese made from low heat milk, may be caused by higher levels of native whey proteins; however, this suggestion needs further verification.

Sensory Evaluation

Table 4 shows scores for openness and rubberiness for cheese from the different treatments. Both UF cheeses were more open than the control cheese, and cheese manufactured from milk given a high heat treatment was more open than cheese manufactured from milk given a low heat treatment although the difference was not significant. This observation agrees with results of sensory analysis by Guinee et al. (15) who found that, as the intensity of the heat treatment to which the milk was subjected increased, the cheese exhibited more mechanical openings.

Rubberiness is a common texture defect in most low fat cheeses. In this study, both UF cheeses were less rubbery than the control cheese, indicating that the UF technique is valuable in manufacturing reduced-fat Havarti cheese. The heat treatment applied in this study was not severe enough to cause a noticeable change in flavor after cooking.

Statistical analysis of the sensory data showed that two textural attributes (openness and rubberiness) were influenced ($P < 0.05$) by treatment. Scores for acid and overall flavor depended on time but not on treatment. Both treatment and time influenced ($P < 0.05$) curdiness and bitterness attributes.

Table 5 shows mean scores for acid and overall flavor of cheese during ripening. Significant flavor

TABLE 4. Openness and rubberiness of control (traditional) cheese, cheese manufactured from UF milk given a low heat treatment (72°C for 17 s), or cheese manufactured from UF milk given a high heat treatment (85°C for 17 s).[1]

	Treatment		
Attribute	Control	Low heat	High heat
Openness	2.03[b]	3.69[a]	4.33[a]
Rubberiness	5.99[a]	4.58[b]	4.35[b]

[a,b]Means within the same row without a common superscript differ ($P < 0.05$).

[1]Characteristics were evaluated by an experienced, 10-member sensory panel using a nine-point scale (0 = no openness or rubberiness to 9 = very open or rubbery). Values represent treatment means.

TABLE 5. Acid and overall flavor intensity scores of control (traditional) cheese, cheese manufactured from UF milk given a low heat treatment (72°C for 17 s), and cheese manufactured from UF milk given a high heat treatment (85°C for 17 s).[1]

Flavor attribute	Time		
	wk 4	wk 8	wk 12
Acid	4.21[b]	4.48[ab]	4.56[a]
Overall	4.17[b]	4.64[a]	4.99[a]

[a,b]Means within the same row without a common superscript differ ($P < 0.05$).

[1]Characteristics were evaluated by an experienced, 10-member sensory panel using a nine-point scale (0 = no acid or overall flavor; 9 = very acid or very intense overall flavor). Values represent means over all treatments.

differences between cheeses were not found; Harper et al. (16) also reported that whey proteins did not significantly influence the intensity of flavor in cheese slurries. The numerical differences in acid scores over time were less marked than in overall flavor. Although overall flavor scores increased during ripening, the intensity of overall flavor was only 4.99 after 12 wk of ripening (based on nine-point scale).

Figures 3 and 4 demonstrate curdiness and bitterness scores of the experimental cheeses during ripening. As expected, curdiness of all cheeses decreased with age, and UF cheeses manufactured from heat treated milk were more curdy ($P < 0.05$) than the control cheese throughout ripening. In our study, bitterness intensity reached its peak at 12 wk for all cheeses, and the control cheese was more bitter ($P < 0.05$) than the two experimental UF cheeses. Although the UF cheese manufactured from milk given a high heat treatment and control cheeses had similar α_{s1}-CN hydrolysis rates, the hydrolysis of β-CN was lower in cheese manufactured from milk given a high heat treatment than in control cheese and may explain the difference observed in bitterness intensity.

CONCLUSIONS

The most significant finding in this study was the low hydrolysis rate of α_{s1}-CN in low fat UF cheese manufactured from milk heated to normal pasteurization temperatures. This phenomenon might be related to the levels of native whey proteins that were incorporated into UF cheese; however, such a suggestion needs further verification. The UF cheese manufactured from milk that had been heated to 85°C for 17 s

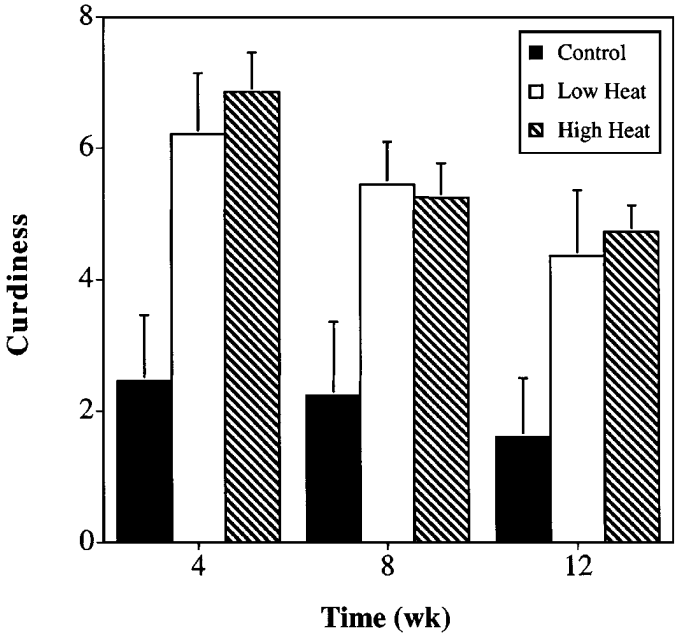

Figure 3. Curdiness scores of control (traditional) cheese, cheese manufactured from UF milk given a low heat treatment (72°C for 17 s), and cheese manufactured from UF milk given a high heat treatment (85°C for 17 s). Characteristics were evaluated during 12 wk ripening by an experienced, 10-member sensory panel using a nine-point scale (0 = not curdy to 9 = very curdy).

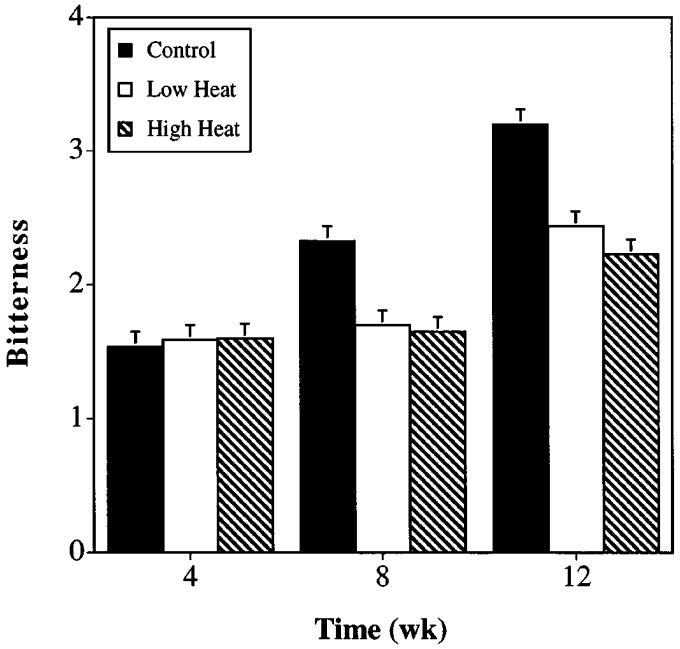

Figure 4. Bitterness intensity scores of control (traditional) cheese, cheese manufactured from UF milk given a low heat treatment (72°C for 17 s), and cheese manufactured for UF milk given a high heat treatment (85°C for 17 s). Characteristics were evaluated during 12 wk ripening by an experienced, 10-member sensory panel using a nine-point scale (0 = not bitter to 9 = very bitter).

and control cheese had comparable rates of α_{s1}-CN hydrolysis that were significantly faster than those for UF cheese manufactured from milk given standard pasteurization treatment. To attain similar coagulation and cutting times for UF and control cheeses, the amount of chymosin added to UF cheese was one-half the amount that was added to the control cheese. The UF and control cheeses had similar scores for acid and overall flavor, but the texture and body of the UF cheeses were different from those of the control cheese; the UF cheeses were softer and had more mechanical openings. In this study, high heat treatment resulted in higher moisture content and increased the amounts of whey protein recovered in UF cheese.

ACKNOWLEDGMENTS

We thank the Minnesota-South Dakota Dairy Foods Research Center for funding this project.

REFERENCES

1 Andrews, A. T., M. D. Taylor, and A. J. Owen. 1985. Rapid analysis of bovine milk proteins by fast protein liquid chromatography. J. Chromatogr. 18:177–185.
2 Association of Official Analytical Chemists International. 1990. Official Methods of Analysis. Vol. II. 15th ed. AOAC, Arlington, VA.
3 Atherton, H. V., and J. A. Newlander. 1977. Chemistry and Testing of Dairy Products. AVI Publ. Co. Inc., Westport, CT.
4 Banks, J. M., A.J.R. Law, J. Leaver, and D. S. Horne. 1994. Sensory and functional properties of cheese: incorporation of whey proteins by pH manipulation and heat treatment. J. Soc. Dairy Technol. 47:124–131.
5 Banks, J. M., G. Stewart, D. D. Muir, and I. G. West. 1987. Increasing the yield of Cheddar cheese by the acidification of milk containing heat-denatured whey protein. Milchwissenschaft 42:212–215.
6 Bastian, E. D., K. G. Hansen, and R. J. Brown. 1993. Inhibition of plasmin by β-lactoglobulin using casein and a synthetic substrate. J. Dairy Sci. 76:3354–3361.
7 Creamer, L. K., M. Iyer, and J. Lelievre. 1987. Effect of various levels of rennet addition on characteristics of Cheddar cheese made from ultrafiltered milk. N.Z. J. Dairy Sci. Technol. 22: 205–214.
8 de Boer, R., and P.F.C. Nooy. 1980. Low-fat semi-hard cheese from ultrafiltrated milk. Nordeuropaeisk Mejeritidsskrift 46: 52–63..
9 de Koning, P. J., R. de Boer, P. Both, and P.F.C. Nooy. 1981. Comparison of proteolysis in a low-fat semi-hard type of cheese manufactured by standard and by ultrafiltration techniques. Neth. Milk Dairy J. 35:35–46.
10 Dybing, S. T. 1994. Effect of whey protein incorporation into Cheddar cheese using ultrafiltration techniques on product yield, body, and texture. Ph.D. Diss., Univ. Minnesota, St. Paul.
11 Farkye, N. Y. 1986. Role of chymosin and porcine pepsin in Cheddar cheese ripening. Ph.D. Diss., Utah State Univ., Logan.
12 Green, M. L. 1990. The cheese making potential of milk concentrated up to four-fold by ultrafiltration and heated in the range 90–97°C. J. Dairy Res. 57:549–557.
13 Green, M. L., A. Turvey, and D. G. Hobbs. 1981. Development of structure and texture in Cheddar cheese. J. Dairy Res. 48: 343–355.
14 Greenberg, R., and H. J. Dower. 1986. Detection of added whey protein concentrate in nonfat dry milk by amino acid analysis. J. Agric. Food Chem. 34:30–32.
15 Guinee, T. P., P. D. Pudja, W. J. Reville, D. Harrington, E. O. Mulholland, M. Cotter, and T. M. Cogan. 1995. Composition, microstructure and maturation of semi-hard cheeses from high protein ultrafiltered milk retentates with different levels of denatured whey protein. Int. Dairy J. 5:543–568.
16 Harper, J., M. Iyer, D. Knighton, and J. Lelievre. 1989. Effects of whey proteins on the proteolysis of Cheddar cheese slurries (a model for the maturation of cheese made from ultrafiltered milk). J. Dairy Sci. 72:333–341.
17 Hickey, M. W., H. Van Leeuwen, A. J. Hillier, and G. R. Jago. 1983. Amino acid accumulation in Cheddar cheese manufactured from normal and ultrafiltered milk. Aust. J. Dairy Technol. 38:110–113.
18 Iyer, M., and J. Lelievre. 1987. Yield of Cheddar cheese manufactured from milk concentrated by ultrafiltration. J. Soc. Dairy Technol. 40:45–50.
19 Kyle, S., and M. W. Hickey. 1993. Feta cheese with ultrafiltration retentate. Aust. J. Dairy Technol. 48:47–48.
20 Law, A.J.R., J. M. Banks, D. S. Horne, J. Leaver, and I. G. West. 1994. Denaturation of the whey proteins in heated milk and their incorporation into Cheddar cheese. Milchwissenschaft 49:63–67.
21 Lo, C. G., and E. D. Bastian. 1996. Chymosin activity against α_{s1}-casein in model systems: influence of whey proteins. J. Dairy Sci. 80:615–619.
22 Maubois, J. L., G. Mocquot, and L. Vassal, inventors. 1969. Procede de traitment du lait et des souproduit laitiers. Fr. Pat. No. 2052121.
23 Meisel, H. 1995. Application of fourth derivative spectroscopy to quantitation of whey protein and casein in total milk protein. Milchwissenschaft 5:247–251.
24 Modler, H. W. 1988. Development of a continuous process for the production of Ricotta cheese. J. Dairy Sci. 71:2003–2009.
25 Montgomery, D. C. 1991. Design and Analysis of Experiments. 3rd ed. John Wiley & Sons, New York, NY.
26 Mulvihill, D. M., and P. F. Fox. 1989. Physico-chemical and functional properties of milk proteins. Page 131 *in* Developments in Dairy Chemistry. P. F. Fox, ed. Elsevier Sci. Publ., New York, NY.
27 National Dairy Council. 1992. Newer Knowledge of Cheese and Other Cheese Products. 4th ed. Natl. Dairy Counc., Rosemont, IL.
28 O'Keeffe, A. M., P. F. Fox, and C. Daly. 1978. Proteolysis in Cheddar cheese: role of coagulant and starter bacteria. J. Dairy Res. 45:465–477.
29 O'Keeffe, A. M., J. A. Phelan, and E. Mulholland. 1981. Effect of seasonality on the suitability of milk for cheese making. Ir. J. Food Sci. Technol. 5:73.
30 Qvist, K. B., D. Thomsen, and E. Hoier. 1987. Effect of ultrafiltered milk and use of different starters on the manufacture, fermentation and ripening of Havarti cheese. J. Dairy Res. 54:437–446.
31 Rowland, S. J. 1938. The determination of the nitrogen distribution in milk. J. Dairy Res. 9:42–46.
32 Rowland, S. J. 1938. The precipitation of the proteins in milk. J. Dairy Res. 9:30–41.
33 Van Dam, K. J. 1994. Whey protein concentrate as a partial caseinate replaced in imitation process cheese. M.S. Thesis, Univ. Minnesota, St. Paul.
34 Vervaeck, J., and A. Huyghebaert. 1992. HPLC of Food proteins. Page 141 *in* Food Analysis by HPLC. L.M.L. Nollet, ed. Marcel Dekker, Inc., New York, NY.
35 Walsh, M. 1988. Estimation of whey proteins in dairy products using amino acid analysis. M.S. Thesis, Utah State Univ., Logan.