Delactosed, High Milk Protein Powder. 1. Manufacture and Composition[1]

V. V. MISTRY[2] and H. N. HASSAN[3]
Minnesota-South Dakota Dairy Foods Research Center
Dairy Science Department
South Dakota State University
Brookings 57007-0647

ABSTRACT

The objective of this research was to develop a new method for the production of delactosed, high milk protein powder without pH adjustment. Skim milk containing approximately 3.2% protein and 4.91% lactose was ultrafiltered at 38°C to approximately 15% protein and 3.8% lactose. The ultrafiltered milk was batch diafiltered three times at 32°C with water to approximately 18.9% protein and .08% lactose and then spray dried. Inlet and outlet air temperatures during drying were 120 to 125°C and 75 to 80°C, respectively. The average composition of the dried product was 5.3% moisture, 83.9% protein, 2.27% fat, .73% lactose, and 7.05% ash. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the powders showed that protein composition was similar to that of skim milk. This powder may have potential in the manufacture of new products or in the improvement of existing products such as low fat yogurts.

(Key words: protein, powder, lactose)

Abbreviation key: DF = diafiltration, TEMED = .1% N,N,N′,N′-tetramethylenediamine, VCR = volumetric concentration ratio.

INTRODUCTION

Milk protein products are widely used in the food and dairy industries as valuable ingredients and have made a major contribution in the development of new food products (10, 15, 21, 23). Milk proteins account for approximately one quarter of total dietary proteins in the industrialized nations (21). These dietary proteins are consumed not only in the form of milk products but also as numerous nondairy food products that contain milk proteins as ingredients (24). Milk proteins possess excellent nutritional and functional characteristics (12, 13, 14, 16); thus, processing techniques should be such that inherent characteristics are not lost during manufacture.

Several methods for producing high protein products are currently available (Table 1). Most of these products are manufactured with the aid of pH or temperature adjustment of milk. Thus, whey proteins may be denatured, as in coprecipitates, or may not exist at all, as in caseinates. Development of procedures that either minimize or prevent denaturation would be beneficial to the dairy industry. Cryodestabilization (18) is an example of such a procedure by which casein is destabilized by freezing prior to drying.

Membrane technology is widely accepted by the dairy industry as a means for processing milk and milk products (5, 6, 11, 26), and composition of products produced with the aid of membrane processing is well established (6). For instance, it is known that when milk is ultrafiltered, a portion of the lactose and minerals is removed. This knowledge has been used in the production of low lactose milk powders (5, 11). In addition to UF, milk may also be diafiltered to remove additional lactose and minerals prior to cheese making (6, 25). No information except for preliminary reports (9, 19) is available on the use of diafiltration (DF) technology to remove lactose completely from milk for the production of high protein powders. This approach for the isolation of milk proteins and removal of lactose may make it possible to produce a high protein product at

Received August 27, 1990.
Accepted November 13, 1990.
[1]Published with the approval of director of the South Dakota Agricultural Experiment Station as Publication Number 2524 of the Journal Series.
[2]Reprint requests.
[3]Present address: Department of Dairy Science, Faculty of Agriculture, Tanta University, Kafr-Elsheikh, Egypt.

TABLE 1. High protein products derived from milk.

Product	Reference
Casein, caseinates	(14, 15, 22)
Isolates	(22)
Coprecipitates	(22, 25)
Whey protein concentrates	(12, 23)
Cryocasein	(18)

relatively low temperatures with no pH adjustment.

The objective of this research was to develop a procedure for manufacturing a high milk protein powder using membrane technology to remove lactose from milk and concentrate proteins prior to drying.

MATERIALS AND METHODS

Concentration of Milk

Approximately 180 kg of pasteurized skim milk was obtained from a commercial dairy. It was tempered to 38°C and ultrafiltered to approximately 5:1 volumetric concentration ratio (VCR) in an Abcor Spiral wound UF model 1/1 sanitary pilot plant unit equipped with a 5.6 m^2 spiral wound membrane (Wilmington, MA). Thirty-six kilograms of retentate and 144 kg of permeate were produced. For the first diafiltration (DF1), 144 kg of tap water at 32°C were added to the 5:1 UF milk to commence DF. The water diluted concentrate was ultrafiltered again to 5:1 VCR (36 kg of retentate and 144 kg of permeate). Diafiltration (dilution of UF milk with 144 kg water followed by UF) was conducted two more times. The VCR after the final DF was 6:1, producing 30 kg of concentrate. Membrane inlet pressure of 3.15 $kg \cdot cm^{-2}$ and outlet pressure of 1.05 $kg \cdot cm^{-2}$ were maintained throughout the operation. Flux rates were determined after UF, DF1, DF2, and DF3 by measuring the quantity of permeate produced in a given time at the above operating pressures. Flux rate was then expressed as $L \cdot m^2 \cdot h^{-1}$.

Spray Drying

Immediately after DF, 16 kg of 6:1 diafiltered milk at 38°C was spray dried in a Niro Atomizer pilot plant spray drier, model ASO 412/E (Columbia, MD), which was equipped with a rotary atomizer, a propane-fired heater, two exits for the powder, and the capacity of removing 16 to 18 kg $water \cdot h^{-1}$. Inlet air temperature was 120 to 125°C, and the feed rate was adjusted to attain an outlet temperature of 80 to 85°C. Powder obtained from the main exit of the drying chamber was sieved with a USA standard testing Sieve Number 18 (Tyler equivalent 16 mesh, Fisher Scientific Co., Minneapolis, MN) prior to analysis and applications.

Analyses

Total protein in skim milk, UF and DF milks, and powders was determined with the macro-Kjeldahl method (2). Total solids by oven drying and fat by the Mojonnier method were determined by the AOAC methods (2) and ash by using a muffle furnace at 600°C (3). Moisture in the powders was determined using the toluene distillation method of the American Dairy Products Institute (1). Lactose in skim milk, UF and DF milks, powders, and permeates was measured by the dialysis method (20).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was conducted on skim milks, powders, and commercial protein products. Powder (40 mg) or 1 g of skim milk was mixed with 10 or 9 ml of sample buffer, respectively, and immersed in a boiling water bath for 2 min (27). Samples prepared this way were used immediately or frozen (–10°C) until required. Sample buffer was 10 m*M* Tris-HCl pH 6.8, 1% SDS, 20% glycerol, .02% bromophenol blue tracking dye, and .77% dithiothreitol. For electrophoresis, 8.5 μl of the prepared samples were used. Stacking gels were 4% acrylamide, .1% N'N'-bis-methylene-acrylamide, .125 *M* Tris-HCl pH 6.8, .1% N,N,N',N'-tetramethylenediamine (TEMED), .1% SDS, and .05% ammonium persulfate. Separating gels were linear gradients (Bio-Rad Model 385 gradient former) of 10% acrylamide and .27% N'N'-bis-methylene-acrylamide to 20% acrylamide and .54% N'N'-bis-methylene-acrylamide, .375 *M* Tris-HCl pH 8.8, .1% SDS, .05% ammonium persulfate, .1% TEMED. Electrode buffer was 25 m*M* Tris-glycine pH 8.3, .5% SDS. Separating gels were allowed to polymerize for approximately 1.5 h before stacking gels

TABLE 2. Composition[1] of skim milk, UF, and diafiltered (DF)[2] skim milks.

Component	Skim milk	UF	DF1	DF2	DF3
	(%)				
Total solids	8.61	20.90	17.88	17.69	21.57
SD	.14	.24	.33	.22	.38
Total protein	3.22	15.16	15.31	15.51	18.90
SD	.05	.28	.20	.09	.51
Fat	.08	.39	.39	.42	.50
SD	.004	.001	.01	.01	.01
Lactose	4.91	3.81	.45	.09	.08
SD	.19	.15	.05	.05	.06
Ash	.73	1.71	1.40	1.37	1.67
SD	.04	.05	.05	.05	.03

[1]Each number is mean of three replicates.

[2]DF1, DF2, and DF3 = First, second, and third diafiltrations, respectively.

were poured. Gel thickness was 1.5 mm. Bio-Rad Protean II Slab Cell (Richmond, CA) was used for conducting electrophoresis. Gels were maintained at 13°C during the electrophoresis run, and a constant current of 20 mA per gel in the stacking gel and 30 mA per gel in the separating gel was applied (Bio-Rad 3000Xi Power Supply). Total running time was approximately 4 h. Gels were stained in a solution containing .1% Coomassie brilliant blue R250 solution, 40% methanol, and 10% glacial acetic acid for 18 h. Gels were destained in 40% methanol 10% glacial acetic acid mixture (Bio-Rad Model 556 Gel Destainer). Gels were photographed immediately and scanned with a Bio-Rad Model 620 video densitometer. Scans were transferred to a computer via a RS232 interface and integrated using 1-D Analyst software (Bio-Rad) to calculate the area under each peak, to establish percentages of each protein, and to determine molecular weights.

RESULTS AND DISCUSSION

Concentration of Milks and Composition of Concentrates and Permeates

The main objective of the initial part of this research was to develop optimum parameters for complete removal of lactose from milk at a low temperature using DF. Lactose is a low molecular weight, water-soluble constituent of milk (28); hence, it can be partially removed from milk by UF (6). Because lactose is a portion of milk serum, larger quantities of it can be removed with DF (6). Preliminary trials indicated that at least two batch DF would be necessary for maximal removal of lactose. Three replicates using three batch DF were conducted thereafter to produce the high protein powders.

Composition of skim milk and UF and DF concentrates is shown in Table 2. Lactose concentration of skim milk was reduced by UF from 4.91 to 3.81% for a corresponding increase in total protein from 3.22 to 15.16%. During DF (DF1, DF2, and DF3), lactose concentration was further reduced to a final concentration of .08%, which represents a 99.7% reduction in total lactose. During UF and DF, lactose that was removed could be found in permeate (Table 3). Permeate 1 corresponds to UF of skim milk and permeates 2, 3, and 4 correspond to DF1, DF2, and DF3, respectively. As shown in Tables 2 and 3, at least two DF were required to remove lactose from milk.

TABLE 3. Lactose concentration[1] in permeates of UF and diafiltration (DF) of skim milk.

Permeate	% Lactose	SD
1	5.18	.38
2	.63	.01
3	.11	.07
4	.11	.05

[1]Mean of three replicates. Permeate 1 = UF, Permeates 2, 3, 4 = DF1, 2, and 3, respectively.

TABLE 4. Flux rates[1] during UF and diafiltration (DF) of skim milk for the production of high milk protein powder.

	Initial flux	Final flux
	($L \cdot m^{-2} \cdot h^{-1}$)	
UF	31.15	19.28
SD	2.28	1.06
DF1	26.47	24.07
SD	1.20	.76
DF2	27.83	28.87
SD	0.00	1.80
DF3	25.53	23.61
SD	.76	2.81

[1]Mean of three replicates.

In the manufacture of casein and caseinates, pH of milk is adjusted to the isoelectric point of casein for precipitation. Proteins are separated by removing whey, which contains approximately 5% lactose (28) and which represents more than 95% of the lactose present in milk. In the present process, lactose is filtered out of milk using an UF membrane and without precipitating proteins.

Total protein concentration in DF1 and DF2 was approximately 15% but was increased to 18.9%, corresponding to a VCR of 6:1 in DF3 (Table 2). The final total solids content was 21.57%. In the manufacture of NDM, skim milk is condensed to approximately 45% total solids (7). This represents a total protein content of 16 to 18%. Using DF, milk could be concentrated to 18% protein with ease and with only a slight reduction in membrane flux rates. During UF, flux rate dropped from an initial of 31.15 $L \cdot m^{-2} \cdot h^{-1}$ to 19.28 $L \cdot m^{-2} \cdot h^{-1}$ after a VCR of 5:1 (Table 4). This reduction in flux rate is expected due to the increase in viscosity during concentration. When DF commenced, viscosity was reduced due to the addition of water, and part of the flux was recovered with only a slight drop at 5:1 VCR. It was possible to operate the UF unit continuously for approximately 5 h without cleaning and with a slight reduction in membrane flux rates.

Spray Drying of Concentrates and Composition of Powders

A single stage spray dryer was used to dry the skim milk concentrate, DF3. One of the goals was to minimize the drying temperature while maintaining a moisture content in the powder of approximately 5%. The inlet air temperature in spray dryers can be directly controlled, but the outlet air temperature depends on the feed rate and is related to the moisture content of the resulting powder (7, 17). It was determined from preliminary trials that the inlet air temperature could be lowered to 120 to 125°C. To maintain the moisture in powder at approximately 5%, feed rate had to be adjusted so that the outlet air temperature was 80 to 85°C. Inlet air temperatures for the manufacture of NDM can be as high as 200°C (7).

Due to the absence of lactose and reduction in mineral content, the high protein powder obtained was white and bland in flavor. There was no apparent color or flavor change after 1 yr of storage at room temperature. In the absence of lactose, Maillard reaction does not take place (28); hence, browning does not occur. Total protein content of the powder was approximately 84%, whereas lactose was .74% (Table 5). Conventional NDM made by the spray process contains approximately 35% total protein and 50% lactose (7, 28). A schematic diagram for the production of the delactosed, high milk protein powder is shown in Figure 1.

Composition of commercial high protein powders is indicated in Table 5. These products are low in lactose, and their protein contents are

TABLE 5. Composition of delactosed, high milk protein powders (HMPP).

		Commercial[1]	
Component	HMPP[2]	Na Caseinate	Isolate
	(%)		
Moisture	5.33	4.30	4.30
SD	.23		
Total protein	83.90	88.00	87.10
SD	.61		
Fat	2.27	1.10	1.10
SD	.12		
Lactose	.74	.10	.10
SD	.66		
Ash	7.05	6.30	6.50
SD	.47		

[1]Data from supplier.

[2]Mean of three replicates.

TABLE 6. Casein composition[1] of skim milk and delactosed, high milk protein powder (HMPP).

Product	Casein		
	α_s-	β- + κ-	γ-
	(%)		
Skim milk	49.43	47.17	3.40
SD	2.76	.76	2.34
HMPP	48.03	45.33	6.64
SD	1.14	2.07	2.71

[1]Mean of three replicates.

similar to those of the new delactosed, high milk protein powder. The composition of protein of the commercial products and that of the new delactosed, high milk protein powder are different from each other. The SDS-PAGE gels showed that the protein content of the high protein powders is similar to that of the original skim milk, except for a slightly higher γ-casein content in the powder (Figure 2, Table 6). γ-Caseins are by-products of β-casein (8) and may have formed due to proteinase activity during UF/DF. As indicated by others (4), casein mobilities in SDS-PAGE show a higher than actual molecular weight.

Sodium caseinate is a product free of whey protein (15, 22); hence, the majority of the protein present in sodium caseinate is casein (Figure 2). Milk protein isolate is a product containing casein as well as whey proteins (22). According to Figure 2, this product contains casein, whey proteins, as well as other smaller proteins having a molecular weight less than that of α-lactalbumin.

CONCLUSIONS

A new method for the production of delactosed, high milk protein powders has been developed. This method produces a protein product with no pH adjustment and at a relatively low temperature; hence, the protein composition is similar to that of the original skim milk. Conventional high protein products such as caseinates, coprecipitates, and isolates are prepared by adjusting pH or temperature of milk. The functional properties and other characteristics

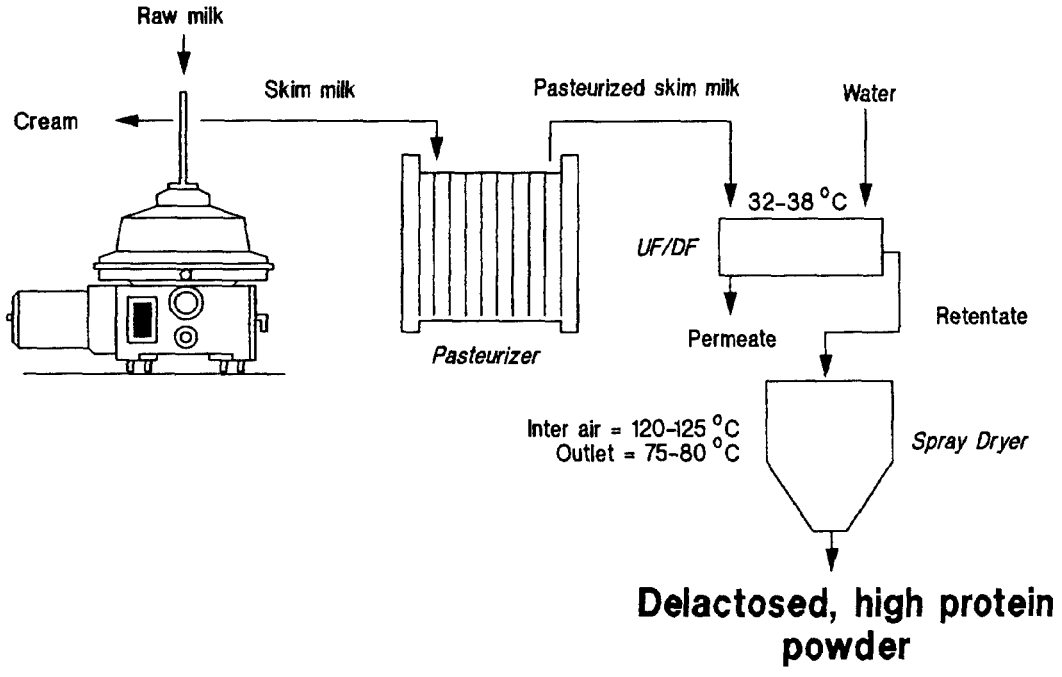

Figure 1. Schematic diagram for the production of delactosed, high milk protein powder. DF = Diafiltration.

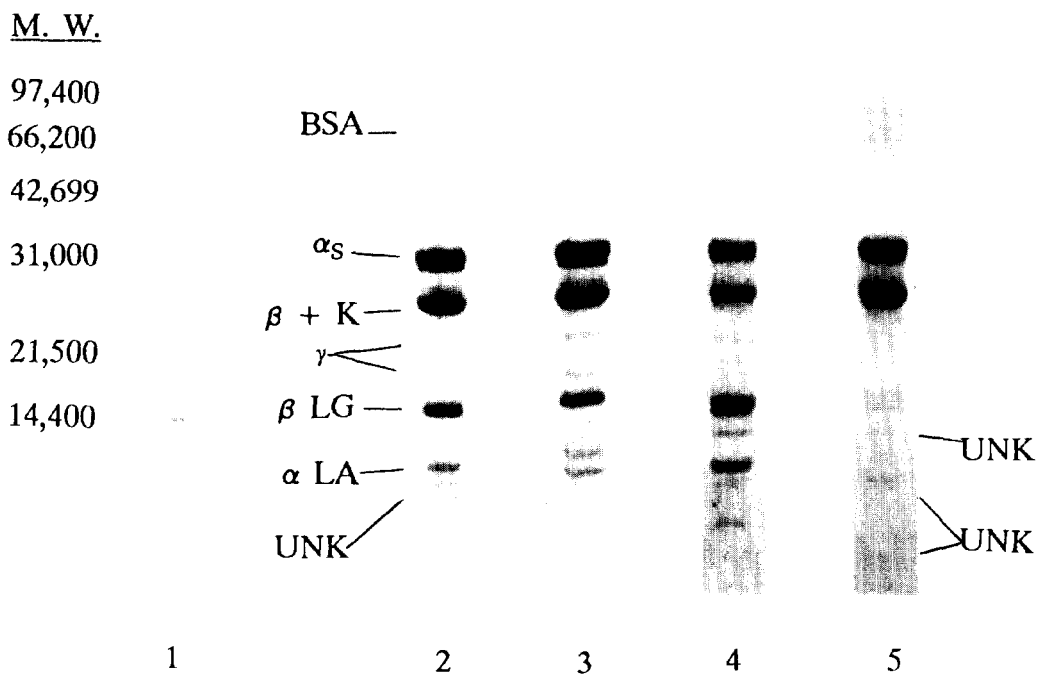

Figure 2. Sodium dodecyl sulfate-polyacrylamide gel of skim milk and delactosed high milk protein powder. Lane 1 contains the following molecular weight (MW) standards: phosphorylase b, bovine serum albumin, ovalbumin, bovine carbonic anhydrase, soybean trypsin inhibitor, egg white lysozyme. Lane 2 is skim milk, lane 3 is delactosed high milk protein powder, lane 4 is protein isolate, and lane 5 is sodium caseinate. BSA = Bovine serum albumin, α_s = α_s-casein, β + K = β- plus κ-caseins, γ = γ-casein, β-LG = β-lactoglobulin, α-LA = α-lactalbumin, and UNK = unknown.

of the new high protein powder are being evaluated. Due to the similarity of the proteins of this powder to those of skim milk and due to the absence of lactose, it has potential for use in the manufacture of new products or in the improvement of existing products such as low fat yogurts.

ACKNOWLEDGMENTS

The authors wish to thank the Minnesota-South Dakota Dairy Foods Research Center for supporting this project.

REFERENCES

1 American Dairy Products Institute. 1971. Standards for grades of dry milks including methods of analysis. Bull. 916, American Dairy Products Institute, Chicago, IL.

2 Association of Official Analytical Chemists. 1984. Official methods of analysis. 14th ed. AOAC, Arlington, VA.

3 Atherton, H. V., and J. A. Newlander. 1982. Chemistry and testing of dairy products. 4th ed. AVI Publ. Co., Westport, CT.

4 Basch, J. J., F. W. Douglas, Jr., L. G. Procino, V. H. Holsinger, and H. M. Farrell, Jr. 1985. Quantitation of caseins and whey proteins of processed milks and whey protein concentrates, application of gel electrophoresis, and comparison with Harland-Ashworth procedure. J. Dairy Sci. 68:23.

5 Butterick, J. K., and J. J. Higgins. 1982. Functional properties of milk powders from ultrafiltered skim milk. Brief Commun., 21st Int. Dairy Congr., Moscow, 1 (Book 2):439.

6 Glover, F. A. 1985. Ultrafiltration and reverse osmosis for the dairy industry. Tech. Bull. 5, Natl. Inst. Res. Dairying, Reading, Engl.

7 Hall, C. W., and T. I. Hedrick. 1971. Drying of milk and milk products. 2nd ed. AVI Publ. Co., Westport, CT.

8 Harwalkar, V. R. 1982. Age gelation of sterilized milks *in* Developments in dairy chemistry–1. Proteins. P. F. Fox, ed. Appl. Sci. Publ., New York, NY.

9 Hassan, H. N., and V. V. Mistry. 1990. High protein lactose-free milk powders. II. Functional, chemical, and physical properties. J. Dairy Sci. 73(Suppl. 1): 114.(Abstr.)

10 Hettinga, D. H. 1988. Processing technologies for improving nutritional value of dairy products *in* Design-

ing foods—animal product options in the marketplace. Natl. Acad. Press, Washington, DC.
11 Jiminez-Flores, R., and F. V. Kosikowski. 1986. Properties of ultrafiltered skim milk retentate powders. J. Dairy Sci. 69:329.
12 Kinsella, J. E. 1984. Proteins: physicochemical and functional properties. CRC Crit. Rev. Food Sci. 21:197.
13 Kinsella, J. E. 1987. Physical properties of food and milk components: research needs to expand uses. J. Dairy Sci. 70:2419.
14 Kirkpatrick, K. J., and R. M. Fenwick. 1987. Manufacture and general properties of dairy ingredients. Food Technol. 41(10):58.
15 Kirkpatrick, K., and N. J. Walker. 1985. Casein and caseinates. Proc. Int. Congr. Milk Proteins. T. E. Galeshoot and B. L. Tinbergen, ed. Pudoc, Wageningen, Neth.
16 Kjaergaard Jensen, G., R. H. Ipsen, and C. Illsoe. 1987. Functionality and application of dairy ingredients in dairy products. Food Technol. 41(10):66.
17 Kyle, W.S.A., and B. R. Rich. 1986. Principles of milk powder production. Victorian Coll. Agric. Horticult., East Melbourne, Aust.
18 Lonengran, D. A. 1983. Isolation of casein by ultrafiltration and cryodestabilization. J. Food Sci. 48: 1817.
19 Mistry, V. V., and H. N. Hassan. 1990. High protein lactose-free powders. I.-Production and composition. J. Dairy Sci. 73(Suppl. 1):113.(Abstr.)
20 Mistry, V. V., H. N. Hassan, and R. J. Baer. 1989. Spectrophotometric method for measurement of lactose in milk and milk products by dialysis. J. Assoc. Offic. Anal. Chem. 72:877.
21 Modler, H. W., and J. D. Jones. 1987. Selected processes to improve the functionality of dairy ingredients. Food Technol. 41(10):114.
22 Morr, C. V. 1985. Manufacture, functionality, and utilization of milk protein products. Proc. Int. Congr. Milk Proteins. T. E. Galeshoot and B. L. Tinbergen, ed. Pudoc, Wageningen, Neth.
23 Mortensen, B. K. 1985. Recent developments in the utilization of milk proteins in dairy products. Proc. Int. Congr. Milk Proteins. T. E. Galeshoot and B. L. Tinbergen, ed. Pudoc, Wageningen, Nether.
24 Renz-Schauen, and E. Renner. 1987. Fortification of nondairy foods with dairy ingredients. Food Technol. 41(10):122.
25 Southward, C. R., and R. M. Aird. 1978. Co-precipitates and their application in food products. I. Observations on the manufacture of co-precipitates. N.Z. J. Dairy Sci. Technol. 13:77.
26 Sutherland, B. J., and G. W. Jameson. 1981. Composition of hard cheese manufactured by ultrafiltration. Aust. J. Dairy Technol. 36:136.
27 Verdi, R. J., D. M. Barbano, M. E. Dellavalle, and G. F. Senyk. 1987. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milks. J. Dairy Sci. 70:230.
28 Walstra, P., and R. Jenness. 1984. Dairy chemistry and physics. John Wiley and Sons, Inc., New York, NY.