STUDIES ON FROZEN MILK WITH SPECIAL REFERENCE TO DENATURATION OF MILK PROTEINS

By

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INTRODUCTION

The storage of milk and milk products in frozen form has been commonly practiced in dairy industry. Particularly it was applied during and after World War II. Frozen homogenized milk was used to supply fresh milk to patients on U.S. hospital ships during World War II. Recently there is considerable interest in the development of frozen concentrated milk as a means of transporting milk to areas not adequately served by a fresh milk supply. Another frozen dairy product which attracts attention is frozen cream as a means of storage of summer cream to supplement winter production of butter. Many studies on these frozen products have been published with special reference to their defects.

Generally speaking frozen milk products seem to have the following three difficulties: a) development of oxidized flavor, b) breaking up of fat emulsion, and c) destabilization of milk proteins. BABCOCK et. al. (1-9) reported a series of experiments on frozen homogenized milk. They (1) detected a slight flat flavor and oxidized flavor in frozen homogenized milk stored under certain conditions. Development of the oxidized flavor in frozen milk products was also reported by many workers (10, 11, 12, 18, 19). Freezing damage of fat globule membrane and or destruction of fat emulsion was reported mainly on frozen cream (14, 16, 20).

Destabilization of milk proteins, and the resulting flocculation or precipitation when thawed, during frozen storage of milk or concentrated milk was reported by many investigators (6, 10, 17, 24, 25, 26, 29, 31). Tessier and ROSE (27) analysed ultrafiltrates of skim milk and the liquid portion of frozen ultrafiltrates for pH and salt concentration. They found that the concentrations were insufficient to coagulate casein but sufficient to lower appreciably the stability of casein suspensions. VAN DEN BERG (30) observed that pH of frozen milk

[Journ. Facul. Agr., Hokkaido Univ., Sapporo, Vol. 52, Pt. 2, 1962]

decreased to a minimum during the first 2 to 3 weeks of storage (at −7°C and at −12°C), and increased gradually thereafter. He presumed that the decrease in pH was due to precipitation of calcium phosphate and to a lesser extent in disodium phosphate and probably sodium carbonate, whereas the increase in pH may be caused by precipitation in such salts as monopotassium citrate. DOAN and WARREN (17) stated that the floc of frozen milk was calcium caseinate which had been thrown out of its normal colloidal dispersion by a "salting out" action of the high concentration of milk salts in the unfrozen water of the product. ROSE and BELEC (23) demonstrated that added sugars and glycerol increased the stability of casein. They also mentioned that the visicosity of the unfrozen portion played an important role in increasing the stability. ROSE and TESSIER (24) concluded that the destabilization was due to lactose crystallization in the frozen products. Their conclusion was supported by TUMERMAN et al. (29). TUMERMAN et al. suggested that the soluble lactose in the metacryotic fluid of partially frozen milk may sequester the concentrated calcium salts and thereby moderate their destabilizing influence on the colloidal casein. TESSIER et al. (28) stated in their report on lactose crystalization in frozen milk that both nucleation and crystal growth of lactose were retarded by low temperatures to a greater extent than being promoted by increased supersaturation. MILDASIN and DOAN (31) reported that nuclei of any type may act as a destabilizing influence on proteins in frozen milk. LAGONI and MERTEN (21) suggested that the fall in the vapor pressure during freezing caused dehydration of colloids, and it was responsible for the stabilization of cream.

These deffects were reduced or prevented to some extent by addition of antioxidants (6, 18), use of stabilizer (6, 8, 17, 23), improvement of manufacturing procedure (10, 25, 31), and improved storage conditions (1, 13). However, the destabilizing effect of continued frozen storage upon the normal dispersion of the milk solids still remain as one of the factors limiting the applications of freezing to the storage of cream and condensed milk. Furthermore, in future, it is considered that the manufacturing process including freezing of milk or milk products will play an increasingly important role in dairy industry. It follows that a fundamental understanding on deterioration of milk by freezing is still required.

In this paper some physicochemical studies on denaturation of casein and/or casein micelles by freezing were undertaken.

PROCEDURE

Materials:

a) Whole milk: Raw, fresh, mixed-herd, morning milk was used. The

milk was obtaind from the Hokkaido University herd, and was cooled immediately after sampling.

b) Skim milk: The whole milk was subjected to centrifugation (895 x g., for 10 min.) at room temperature. The cream layer was removed by means of spatula and the serum portion was siphoned. The serum portion was skimmed again in the same manner described above, and used as skim milk.

c) Acid casein and casein solution: Acid casein was prepared from the skim milk in the following manner: skim milk was diluted with 4 volumes of distilled water, and the mixture was acidified to pH 4.6 by addition of 5% acetic acid at 30°C under vigorous agitation. Precipitated casein was removed by centrifugation (895 x g., for 10 min.), and dispersed in equal volum of distilled water to the original volum of skim milk. The suspension was centrifuged at 895 x g. for 10 minutes. This washing procedure was repeated three times. The washed casein was treated with acetone (half volume of original skim milk) 4 times, and ethyl ether (the same volume as acetone) 4 times. Centrifugation at 895 x g. for 10 minutes was used throughout these treatment to separate casein. Thus fine powdered acid casein was prepared. Three parts of the casein powder was dispersed in 97 parts of distilled water. Normal natrium hydroxide solution was added gradually to the casein suspension to adjust pH to 6.6. Approximately 20 hours were required for this alkalifying procedure. The resuling casein solution was filtrated through Toyo Roshi No. 2 filter paper. YANAGIMOTO glass-electrode pH meter Model 41-A was used to adjust pH.

d) Freeze-concentrated skim milk: The skim milk was partially frozen at −2°C. The ice crystals formed in the skim milk were occasionally strained out through clean gauze. Finally a condensed skim milk containing 15% milk solids was prepared.

e) Lyophilized skim milk: The skim milk was placed into specially prepared glass ampules (15 mm in diameter), each ampule containing 3 ml. of the skim milk. The ampules were immersed in liquid nitrogen. The content was evenly distributed and frozen on the inside of the ampule which was rotated in the liquid nitrogen. Then it was dried by sublimation under high vacuum of 10^{-6} mmHg for approximately 5 hours.

f) Lyophilized casein glue: Casein micelles were removed from the skim milk by supercentrifugation at 51,000 x g. for 20 minutes. The casein micelles were dispersed in as small amount of distilled water as possible. Then it was lyophilized in the same manner as in the case of skim milk.

g) Sweetened condensed skim milk and powdered skim milk: Commercial products manufactured by the Snow Brand Milk Products Company were used.

Measuring of titratable acidity:

Standard method (22) recomended by the American Dairy Science Association was used.

Measuring of visicosity:

Ostwald viscosimeter placed in a water-bath at 15°C was used. The results were expressed as relative viscosity using distilled water as standard.

Electrophoretic analysis:

Two kinds of buffer solution were used, namely veronal buffer (pH 8.6, ionic strength of 0.10) (15) and phosphate buffer (pH 7.0, ionic strengte of 0.10, consisting of 0.02M KH_2PO_4, 0.03M Na_2HPO_4 and 0.05M NaCl). Samples were diluted with buffer solution to a protein concentration of 0.8 per cent. Ten ml. of the diluted samples were dialyzed at 10°C for 24 hours against 400 ml. of the buffer solution. The dialyzed samples were filtrated through Toyo Roshi No. 2 filter paper prior to electrophoresis. Electrophoretic analysis was carried out in the buffer solution at 10°C or 12°C for 90 minutes or for 60 minutes in a Tiselius type electrophoresis apparatus manufactured by Hitachi Seisakusho. A 2 ml. electrophoresis cell was used. Patterns were photographed using panchromatic plates. The area of components and mobilities were determined from enlarged patterns traced on section paper by means of a planimeter.

Electron microscopic observation:

Samples were diluted 400 times by addition of distilled water. Immediately after dilution, a drop of the diluted sample was placed upon a film-covered grid (collodion was used as the supporting film) by means of capilary pipette, and the suspending liquid was then allowed to evaporate at 40°C. Then electron microscopic observation was made using a Nihon Denshi Kagaku JEM-Model 5L electron microscope.

RESULTS

Effect of pH on the preparation of acid casein:

Throughout this study, acid casein was prepared in many cases, to prepare casein solution, and to observe the electrophoretic pattern of casein. It was considered that a slight variation in the pH, at which casein was precipitated, might exert an effect on the composition of precipitated casein. Therefore, the effect of varied pH on the precipitation of casein was studied.

Skim milk was adjusted to pH 4.7. Precipitation (pH 4.7-casein) was removed by centrifugation (895 x g., 10 min.). The supernatant obtained was further adjusted to pH 4.4 to precipitate residual casein (pH 4.4-casein). Both caseins precipitated at pH 4.7 and pH 4.4 were subjected to electrophoresis

after treatment in the same way as in the preparation of acid casein. In this experiment, a pH test paper (BCG) was used for adjusting pH.

The results are presented in Figure 1, revealing a remarkable difference in the distribution of α- and β-casein between pH 4.7-casein and pH 4.4-casein. The β peak in pH 4.7-casein was very small, and it adhered or associated somewhat to α peak. On the other hand, the β peak was enlarged and competely sepatated from α peak in pH 4.4-casein. The mixture of pH 4.7-casein and pH 4.4-casein indicated that both β peak in the two caseins represented the

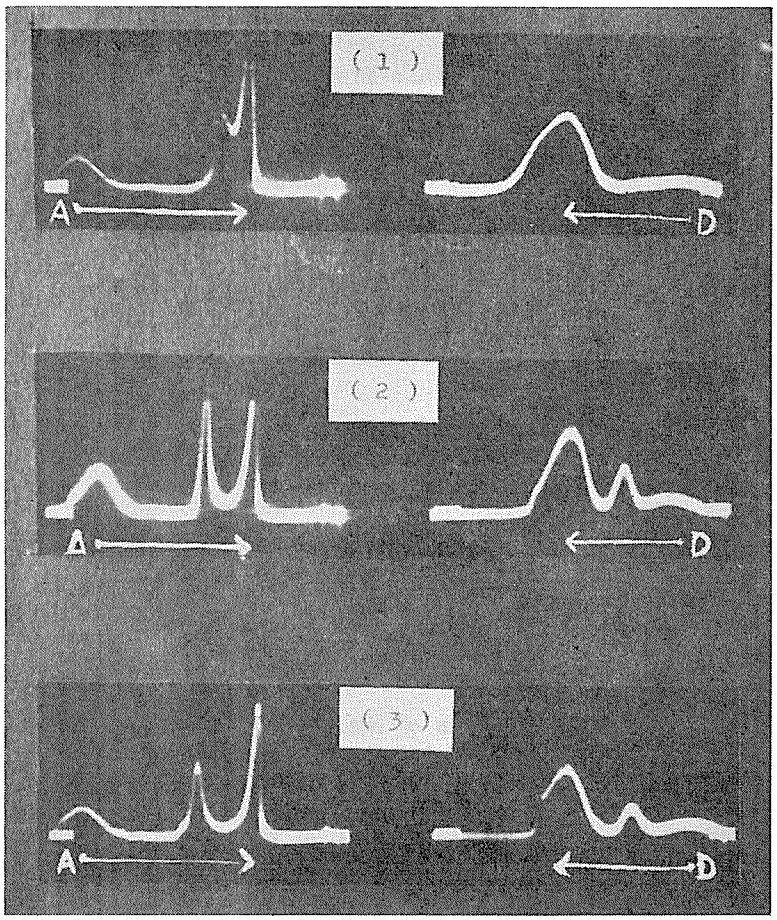

Fig. 1. Electrophoretic pattern of acid casein.
(1) precipitated at pH 4.7, (2) precipitated at pH 4.4, but not at pH 4.7, and (3) mixture of (1) and (2). Phosphate buffer (pH 7.0, μ=0.1), 5,400 sec. at 12°C.

TABLE 1. Effect of freezing and frozen storage on relative viscosity and acidity of frozen milk and casein solution

Sample	Treatment	Relative viscosity*				Acidity	
		Trial I	Trial II	Trial III	Trial IV	Trial I	Trial II
Whole milk	Non (Control)	1.70	—	—	—	0.125	—
	Repeated thawing (once a day)	1.84	—	—	—	—	—
	Frozen storage for 50 ds.	1.84	—	—	—	—	—
	70 ds.	1.78	—	—	—	0.118	—
Skim milk	None (Control)	1.45	1.48	1.46	1.38	0.169	0.125
Protein %;	Repeated thawing (once a day)	1.50	1.48	—	1.48	0.153	—
Trial I: 2.69	" " (once a week)	1.47	—	—	—	0.153	—
" II: 2.70	Frozen storage including a thawing procedure	1.47	—	1.61	—	0.151	—
" III: 2.78	Frozen storage for 12 ds.	1.45	—	1.54	—	0.156	—
" IV: 2.62	24 ds.	—	—	1.76	—	—	—
	30 ds.	1.45	1.47	—	—	0.156	—
	50 ds.	—	1.55	—	—	—	—
	70 ds.	—	—	—	—	—	0.116
Pasteurized skim milk	None (Control)	1.42	—	—	—	0.167	—
	Repeated thawing (once a day	1.48	—	—	—	0.153	—
Protein %;	" " (once a week)	1.46	—	—	—	0.151	—
Trial I: 2.69	Frozen storage including a thawing procedure	1.44	—	—	—	0.153	—
	Frozen storage for 12 ds.	1.47	—	—	—	0.148	—
	30 ds.	1.47	—	—	—	0.150	—
Casein solution	None (Control)	2.16	2.13	—	—	—	—
Protein %;	Repeated thawing (once a day)	2.22	—	—	—	—	—
Trial I: 2.36	" " (once a week)	2.23	—	—	—	—	—
" II: 2.33	Frozen storage including a thawing procedure	2.23	2.15	—	—	—	—
" III: 2.43	Frozen storage for 12 ds.	2.25	2.11	—	—	—	—
	24 ds.	—	2.73	—	—	—	—
	30 ds.	2.29	—	—	—	—	—
Pasteurized casein solution	None (Control)	2.05	—	—	—	—	—
	Repeated thawing (once a day)	2.14	—	—	—	—	—
Protein %;	" " (once a week)	2.09	—	—	—	—	—
Trial I: 2.36	Frozen storage including a thawing procedure	2.15	—	—	—	—	—
	Frozen storage for 12 ds.	2.09	—	—	—	—	—
	30 ds.	2.15	—	—	—	—	—

* at 15°C, standard: distilled water.

TABLE 2. Electrophoretic analysis

Sample	Component	Ascending		Descending	
		% Relative Area	Mobility at 10°C 1×10^{-5} cm²/sec. volt	% Relative Area	Mobility at 10°C 1×10^{-5} cm²/sec. volt
A-(1)	A	59.2	−10.31	76.4	− 8.76
	B	5.8	− 8.57	4.9	− 7.74
	C	29.5	− 6.24	18.7	− 5.08
	D	5.5	− 3.21	—	—
A-(2)	A	70.1	− 9.26	76.5	− 9.95
	B	4.2	− 6.98	2.6	− 7.80
	C	21.9	− 6.01	20.9	− 5.52
	D	4.4	− 3.00	—	—
A-(3)	A	61.7	− 8.98	75.1	− 9.45
	B	3.3	− 7.15	—	—
	C	35.0	− 5.97	24.1	− 5.28
B	A	62.3	−10.65	76.8	−9.67
	B	5.2	− 8.61	4.5	− 7.87
	C	29.1	− 6.98	18.7	− 5.76
	D	3.4	− 4.87	—	—
C-(1)	A	67.9	− 6.53	94.8	− 5.68
	B	32.1	− 5.11	—	—
	B′	—	—	5.2	− 2.85
C-(2)	A	67.8	− 6.53	94.7	− 5.65
	B	32.2	− 5.12	—	—
	B′	—	—	5.3	− 2.83
C-(3)	A	55.5	− 6.20	93.3	− 5.46
	B	43.0	− 5.36	—	—
	B′	—	—	6.7	− 2.52
	C	1.5	− 2.31	—	—
D-(1)	A	60.0	− 6.50	93.9	− 5.44
	B	40.0	− 5.08	—	—
	B′	—	—	6.1	− 2.72
D-(2)	A	56.0	− 6.51	94.6	− 5.44
	B	44.0	− 5.07	—	—
	B′	—	—	5.4	− 2.48
D-(3)	A	57.5	− 6.78	92.9	− 5.48
	B	42.5	− 5.49	—	—
	B′	—	—	7.1	− 2.60

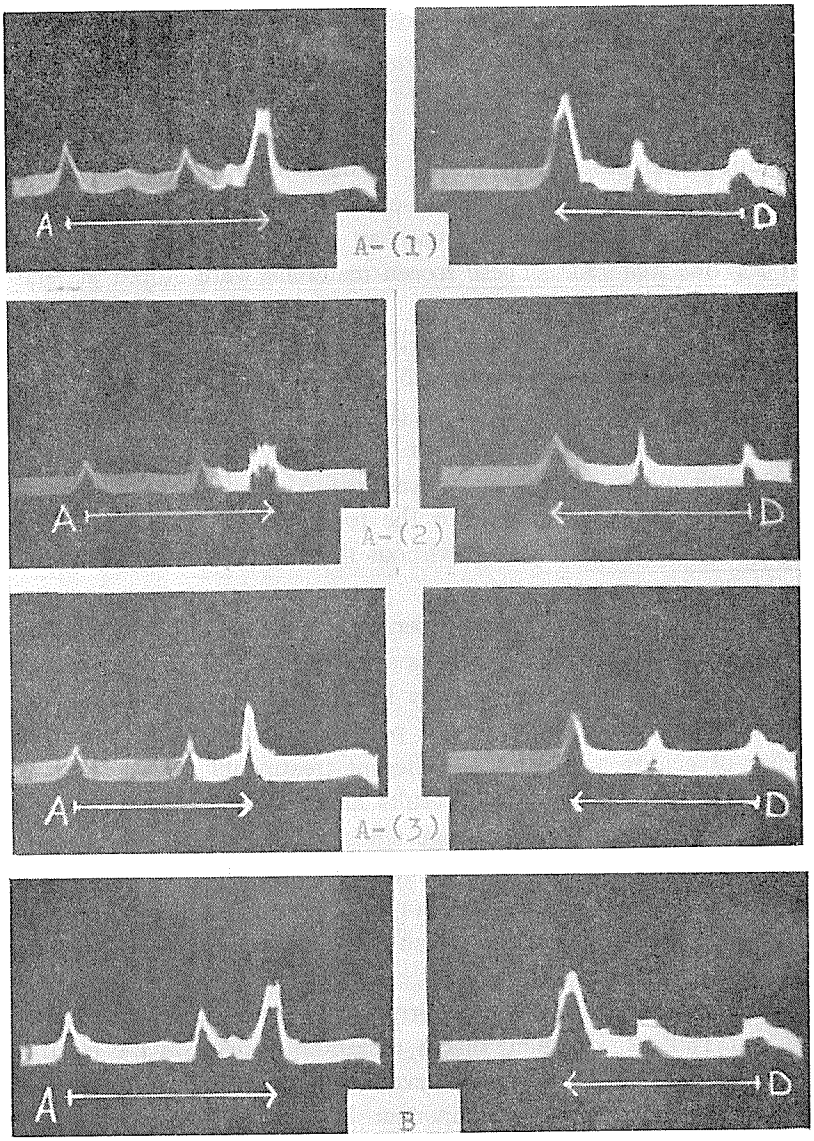

Fig. 2. Electrophoretic pattern of skim milk.

A. Skim milk; (1) control, (2) frozen and held at −10°C for 50 days, and (3) forzen and held at −10°C for 70 days.

B. Skim milk obtained from whole milk frozen and held at −10°C for 50 days. Veronal buffer (pH 8.6, μ=0.1), 3,600 sec. at 10°C.

same component, namely β-casein. These results emphasized the necessity of strict control for pH at which casein was precipitated.

Effect of freezing and frozen storage on the properties of whole milk, skim milk and casein solution:

Raw whole milk, raw skim milk, pasteurized (63°C, 30 min.) skim milk, casein solution and pasteurized (63°C, 30 min.) casein solution were used as experimental materials. Fifteen ml. of each material was placed in a sterilized petri dish (10 cm in diameter) and frozen at −10°C. Three to five dishes were prepared for one material. These frozen materials were stored at −10°C and subjected to the following treatments;

a. Ripetition of thawing-freezing (I); Thawing and freezing were carried out once a day and was repeated for 5 days.

b. Repeating of thawing-freezing (II); Thawing and freezing was carried out once a week and was repeated for 3 weeks.

c. Storage with one thawing procedure; After storage for 12 days, it was thawed, then refrozen and stored for the following 12 days.

d. Storage for certain period without thawing; Storage periods of 24, 30, 50, and 70 days were used.

Thawing was done at room temperature (15 to 20°C) without shaking.

1. Effect on acidity: The results are presented in Table 1, revealing that the freezing and frozen storage slightly lowered acidity of milk. The lowered acidity was observed in both raw and pasteurized skim milk. No differences in lowering of acidity was seen by different treatments during frozen storage.

2. Effect on viscosity: The results are showen in Table 1. The data in Table 1 revealed that frozen storage tends to increase viscosity, even though the increase was very slight. Two of three trials dealing with raw skim milk, demonstrated that the samples stored for long periods in frozen state possessed higher viscosity than tne samples which were subjected to thawing-freezing or to short storage.

3. Effect on electrophoretic pattern: The results are shown in Figure 2, Figure 3 and Table 2, revealing that a remarkable effect on the electrophoretic pattern by the treatments was not observed. Slight differences in electrophoretic pattern, particularly concerning component B and C of the ascending pattern, were observed in Figure 2. However, the differences were not considered to be sufficient to suggest denaturation or interaction of proteins.

4. Effect on electron microscopic pattern: Electron microscopic patterns were demonstrated in Figure 4 to Figure 6. These figures showed that the shape and size of casein micelles was not affected by the treatments applied in this study.

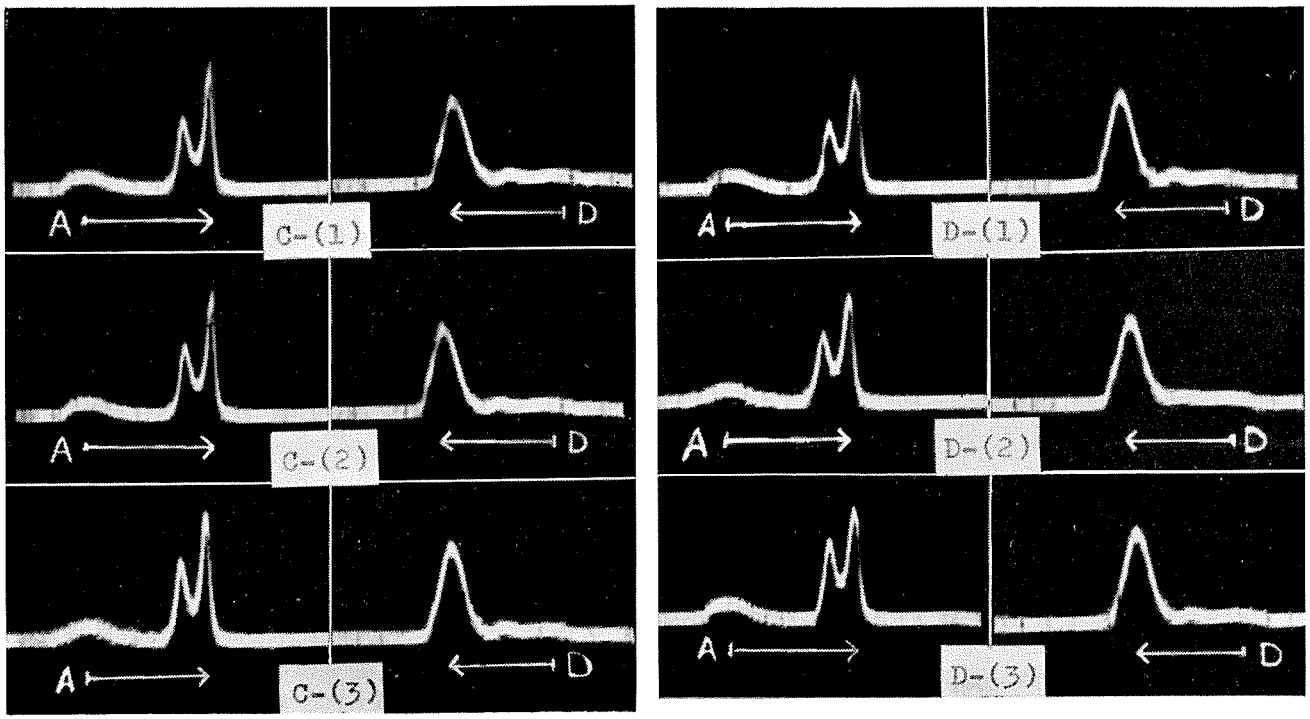

Fig. 3. Electrophoretic pattern of casein solution.

C. Casein solution; (1) control, (2) subjected to repeated freezing-thawing, and (3) frozen and held at −10°C for 30 days.

D. Pasteurized casein solution; (1) control, (2) subjected to repeated freezing, thawing, and (3) frozen and held at −10°C for 30 days. Veronal buffer (pH 8.6, μ=0.1), 3,600 sec. at 10°C.

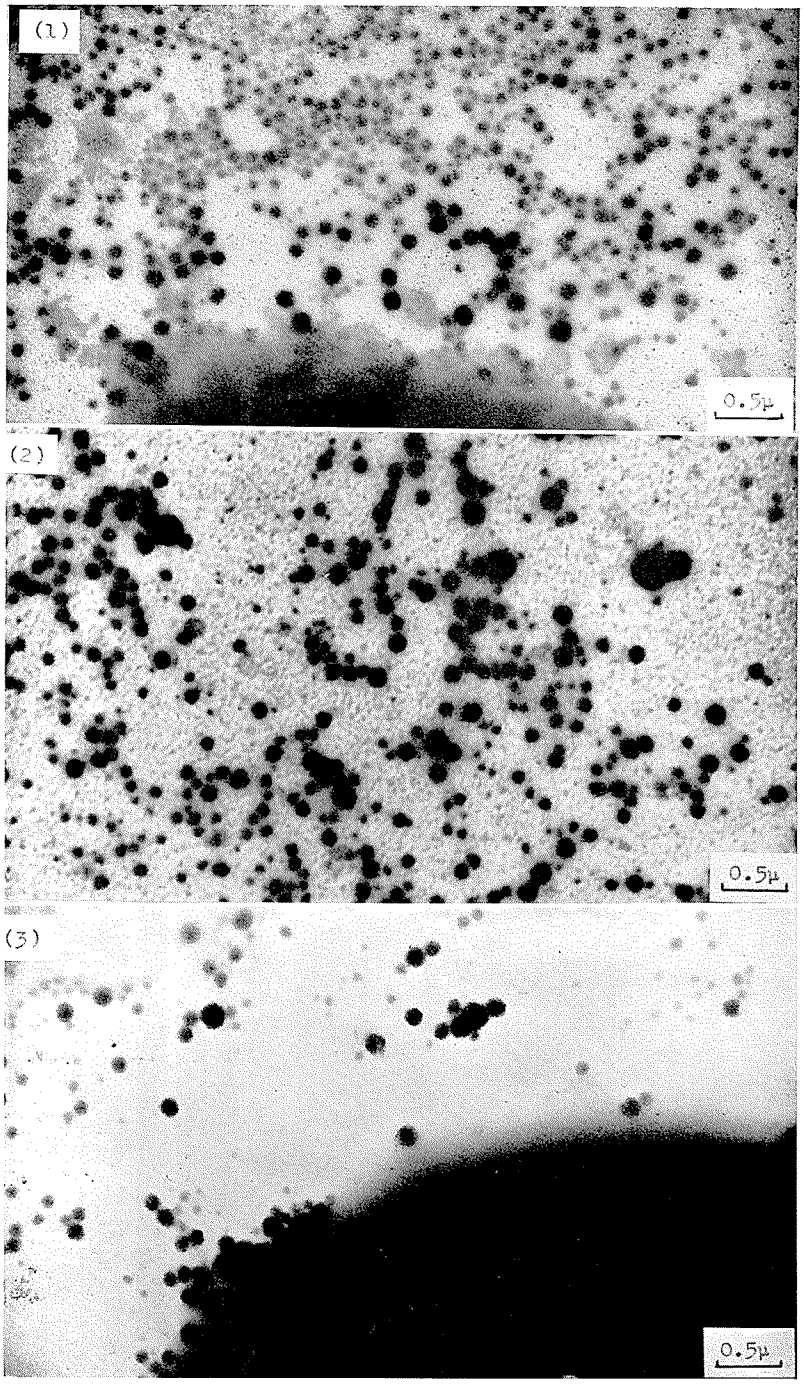

Fig. 4. Electron microscopic pattern (× 17,000) of whole milk. (1) control, (2) subjected to repeated freezing-thawing, and (3) forzen and held at −10°C for 50 days.

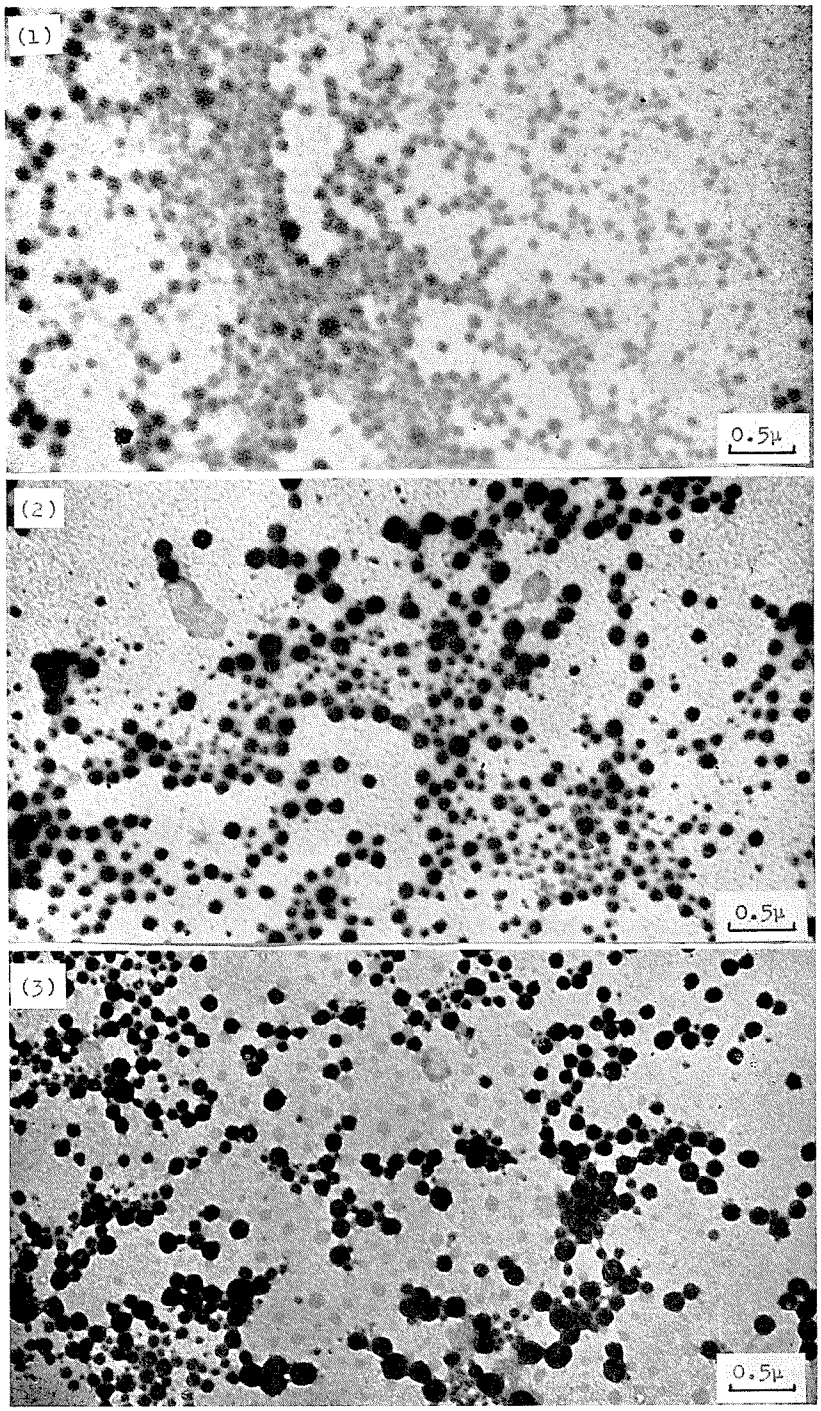

Fig. 5. Electron microscopic pattern (× 17,000) of skim milk.
(1) control, (2) subjected to repeated freezing-thawing, and (3) frozen and held at −10°C for 50 days.

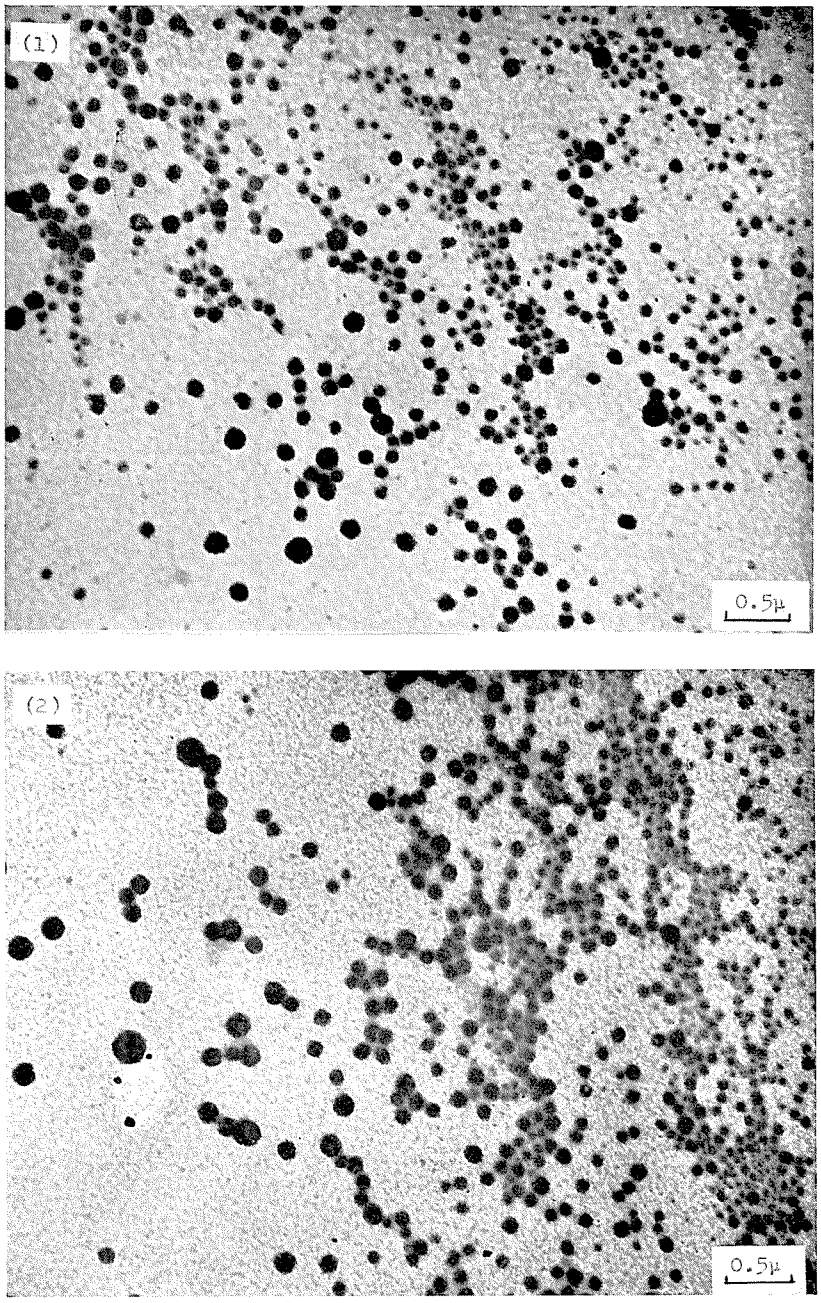

Fig. 6. Electron microscopic pattern (× 17,000) of skim milk obtained from treated whole milk.

(1) subjected to repeated freezing-thawing, and
(2) frozen and held at −10°C for 50 days.

Comparison between freeze-concentrated skim milk and sweetened condensed skim milk:

The electrophoreticpatterns of acid caseins obtained from both freeze-concentrated skim milk and sweetened condensed skim milk at pH 4.7 are shown in Figure 7. A high similarity was observed between these two patterns. However, a slight difference was suggested when compared with acid casein prepared from skim milk at pH 4.7 (Fig. 1). The supernatant separated from the condensed milk at pH 4.7 was clear in appearance and no precipitation was obtained from the supernatant by further acidifying to pH 4.4. These results indicated that condensing of milk by freezing tend to make acid precipitation of casein easier, and the tendency was also shown in sweetened condensed skim milk.

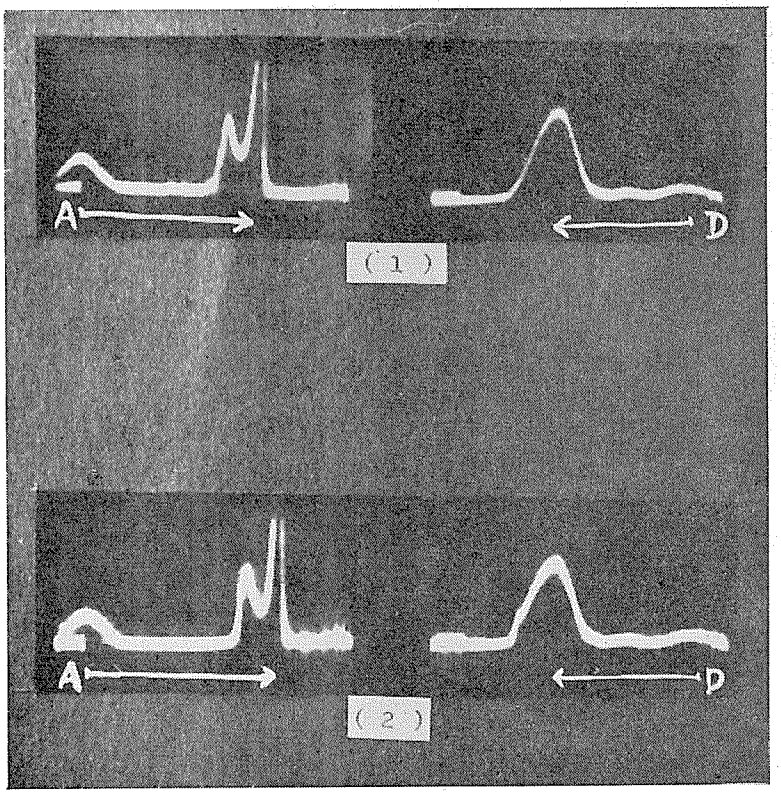

Fig. 7. Electrophoretic pattern of acid casein precipitated at pH 4.7.
(1) obtained from freeze-concentrated skim milk, and
(2) obtained from sweetened condensed skim milk.
Phosphate buffer (pH 7.0, μ=0.1), 5,400 sec. at 12°C.

Acid casein prepared from freeze-concentrated skim milk was compared with that of sweetened condensed skim milk in respect to the effect of varied pH, i. e. acidification and alkalification. For adjusting pH, pH test papers were used.

1. Acidification: A three per cent suspension of acid casein in distilled water was acidified to pH 2.6 by addition of N HCl. After keeping at the pH for 2 hours, it was neutralized to pH 6.8 by addition of N NaOH. Then casein was precipitated by acidifying. The most successful precipitation of casein was obtained at pH 5.2. The electrophoretic patterns of the acid casein

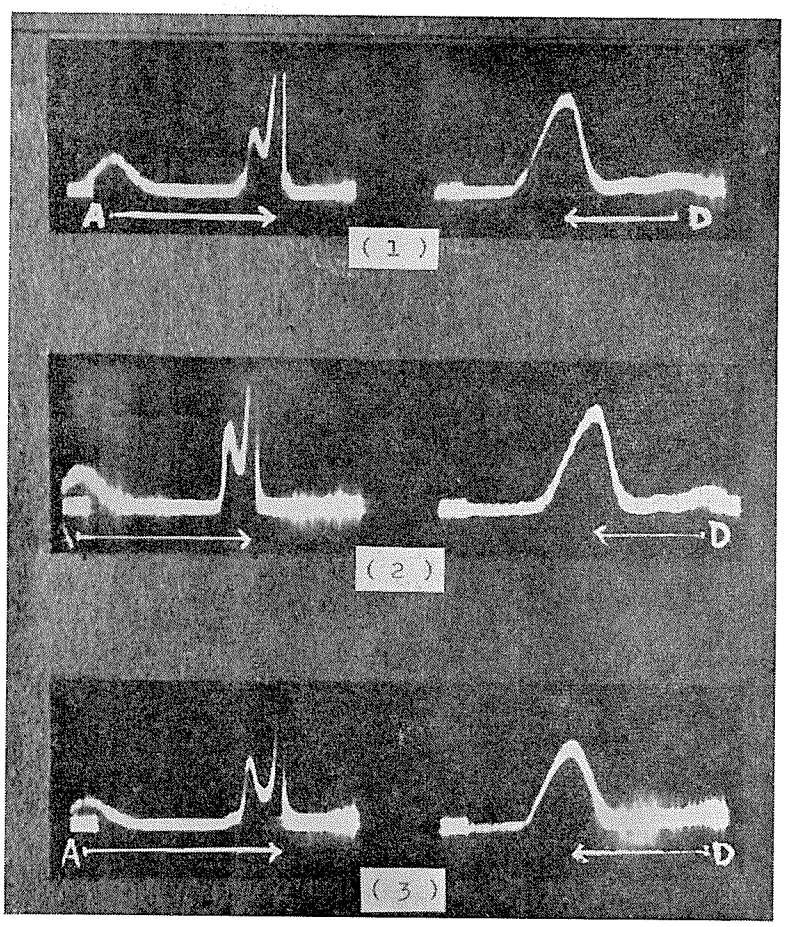

Fig. 8. Electrophoretic pattern of acid casein treated by acidification, obtained from (1) freeze-concentrated skim milk, (2) sweetened condensed skim milk, and (3) raw milk. Phosphate buffer pH 7.0, $\mu=0.1$), 5,400 sec. at 12°C.

separated at pH 5.2 is shown in Figure 8. No effect of the acidifying on the electrophoretic patterns was observed at acid casein prepared either from freeze-concent-rated milk or from sweetened condensed skim milk.

2. Alkalification: A three per cent suspension of acid casein in distilled water was alkalified to pH 9.0 by addition of N NaOH. After keeping at pH 9.0 for 2 hours, it was adjusted to pH 4.7 at which the most successful precipitation was occured. The precipitation formed was subjected to electrophoresis. The results are shown in Figure 9, revealing some difference in descending pattern.

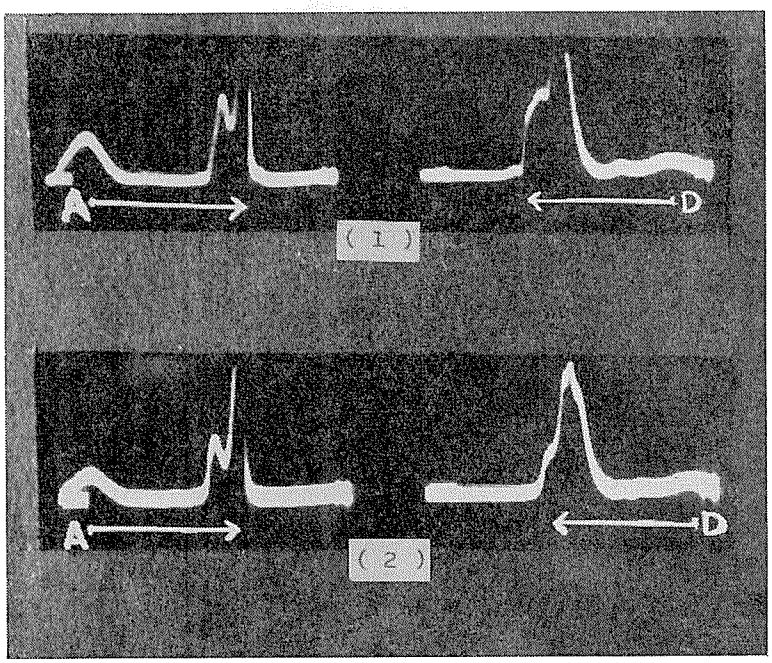

Fig. 9. Electrophoretic pattern of acid casein treated by alkalification, obtained from (1) freeze-concentrated skim milk, snd (2) sweetened condensed skim milk. Phosphate buffer (pH 7.0, μ=0.1), 5,400 sec., at 12°C.

Properties of sediment formed by repeated thewing-freezing of freeze-concentrated milk:

Sediment was formed in freeze-concentrated skim milk by repetition of thawing followed by refreezing for 4 times. The sediment was separated by centrifugation at 895 x g. for 10 minutes and washed with distilled water three times, then it was treated with acetone and ethyl ether. Thus the sediment

was isolated in a form of white powder. The isolated sediment was dissolved with remarkable difficulty. Visible precipitation was observed in the suspension of the sediment at either pH 8.0 and pH 2.0 unless highly diluted to 0.5 per cent. Electrophoretic properties of the isolated sediment was observed in the following manner :

a. Electrophoresis of the soluble part at pH 7.0 ; Three tenth grams of

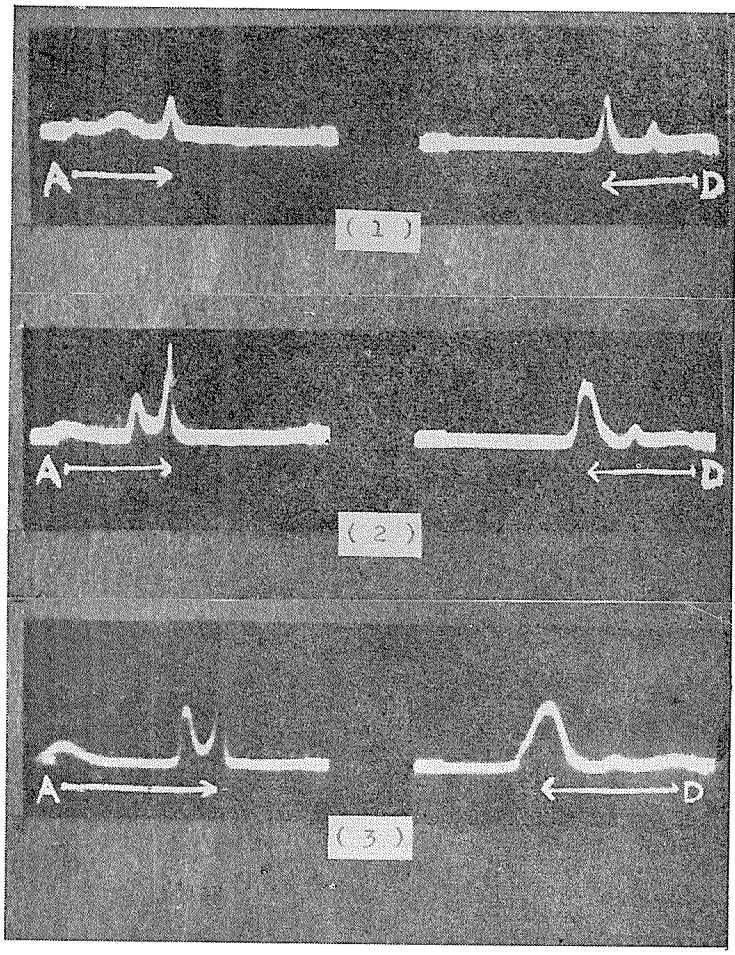

Fig. 10. Electrophoretic pattern of sediment formed in freeze-concentrrted skim milk by repeated thawing-freezing. (1) Soluble part at PH 7.0, (2) whole sediment after acidification and dialysis, and (3) precipitable part at pH. 4.7. Phoshate buffer (pH 7.0, μ=0.1), 5,400 sec., at 12°C.

the sediment was suspended in 10 ml. of phosphate buffer (pH 7.0). Then it was filtrated through Toyo Roshi No. 2 filter paper. The filtrate was subjected to electrophoresis using phosphate buffer solution.

b. Electrophortesis after acidification and dialysis; Three tenth grams of the sediment was suspended in 10 ml. of distilled water, then it was acidified to pH 2.4 by addition of N HCl. The acidified mixture was dialyzed for 24 hours against 500 ml. of distilled water using cellophane as the dialysis membrane. The dialyzed mixture was neutralized to pH 7.0 by addition of N NaOH. Then

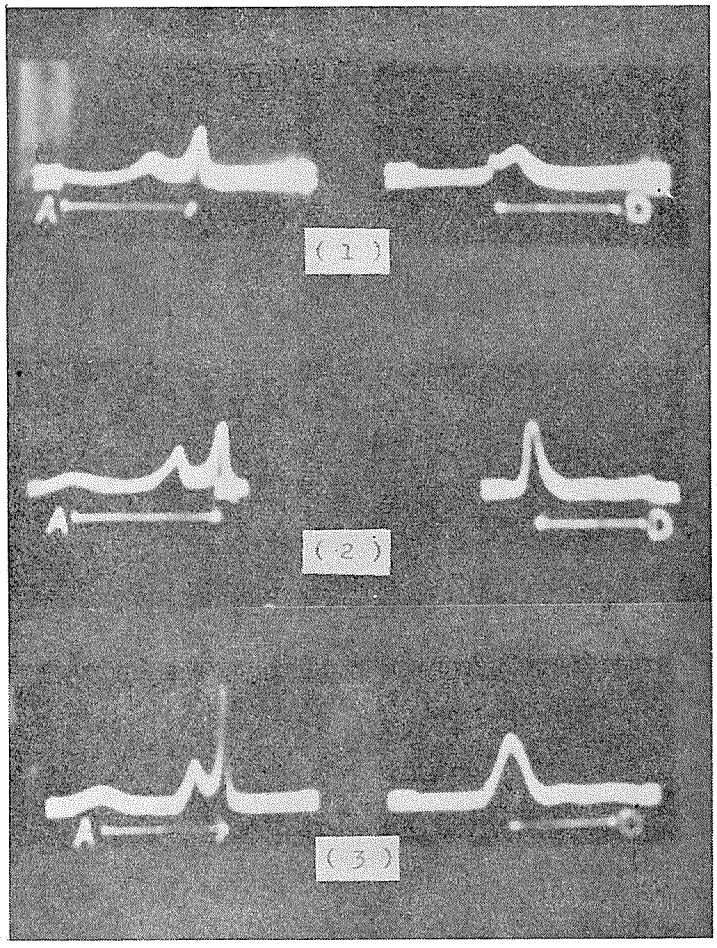

Fig. 11. Electrophoretic pattern of (1) lyophilized casein glue, (2) casein glue not liophilized, and (3) acid casein obtained from (2). Phosphate buffer (pH 7.0, μ=0.1), 5,400 sec., at 12°C.

it was subjected to electrophoresis using phosphate buffer solution.

c. Electrophoresis of the precipitable part at pH 4.7; One half grams of the sediment was suspended into 100 ml. of distilled water. The mixture was acidified to pH 2.8, then the pH was readjusted to pH 4.7. The precipitation formed at pH 4.7 was collected and subjected to electrophoresis using phosphate buffer solution.

The results are shown in Figure 10. These results revealed that the sediment consisted mostly from casein. Whole components of casein were included in the sediment and no selectivity in casein components was observed to consist

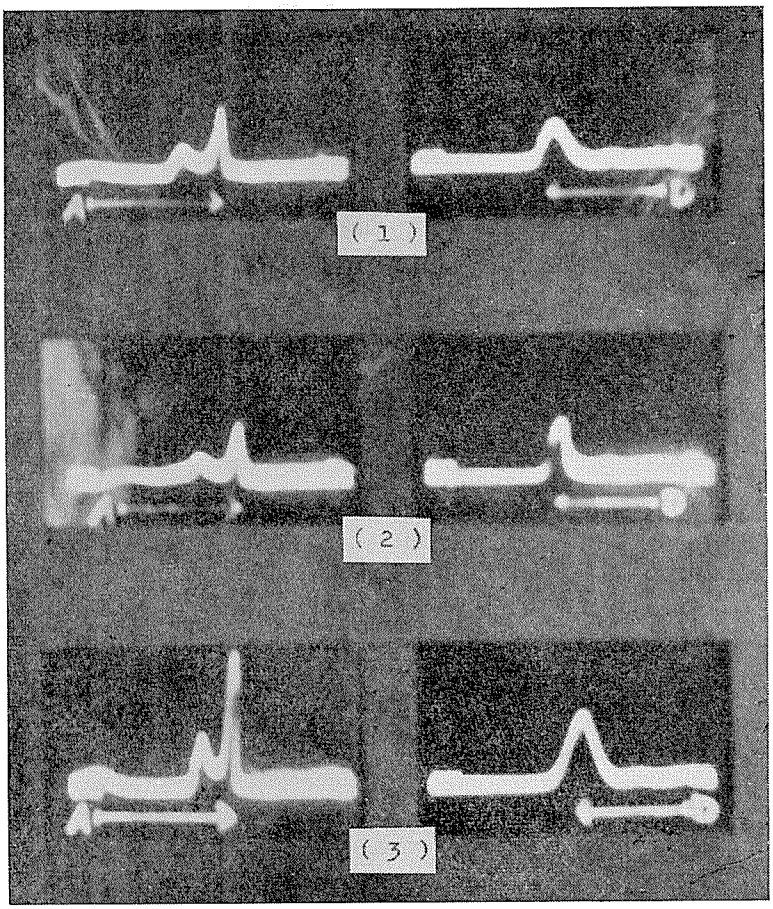

Fig. 12. Electrophoretic pattern of (1) lyophilized skim milk, (2) skim milk powder, and (3) acid casein obtained from skim milk powder. Phosphate buffer (pH 7.0, μ=0.1), 5,400 sec., at 12°C.

the sediment.

Effect of lyophilizing on casein:

1. Lyophilized casein glue: Electrophoretic pattern of lyophilized casein glue was compared with that of casein glue not lyophilized, and with that of acid casein obtained from the casein glue. The result was shown in Figure 11, revealing a difference in the major peak of the descending pattern.

2. Lyophilized skim milk: Electrophoretic pattern of lyophilized skim milk was studied in comparison with that of skim milk powder by pressure spray process. The result is shown in Figure 12. The result suggested that neither lyophilizing nor spray drying had any effect on the resulting electrophoretic pattern.

DISCUSSION

Acidity of milk was lowered by freezing and studied in this paper while no attempts were made on studying the mechanism. It is considered that precipitation of salts, as mentioned by VAN DEN BERG (30), probably caused the change in acidity. No precipitation was observed in frozen milk, under the condition studied, unless it had been concentrated prior to freezing. However, it is highly possible that destabilization of milk proteins, owing to the effect of freezing on colloidal state of milk, might occur in skim milk or whole milk as well as in concentrated milk, as reported by several workers (26, 30). An electrophoretic study and measuring of viscosity were applied unsuccessfully in the investigation of denaturation of milk proteins by freezing. Expected changes such as cohesion between casein micelles, for instance, were not observed by electron microscopic observation. These results suggested that the physical state of milk proteins might not be readily affected.

Both experiments on acid casein isolated from frozen milk and on frozen casein solution suggested that freezing or frozen storage of milk showed no remarkable effect on casein with respect to some properties studied in this paper, even though slight changes in electrophoretic pattern were observed in some trials. Physical interraction between casein and other components, namely whey proteins and fat globules, might be affected by freezing or frozen storage. However, the properties of isolated casein itself seemed to be unchanged by the treatment. Further studies on the above mentioned physical interraction will be expected.

Both evaporate-concentration followed by sweetening and freeze-concentration resulted in no difference between each other in electrophoretic pattern of acid casein obtained from both final products. The similarity in electrophoretic

pattern was not affected by acidification and alkalification prior to electrophoresis. The electrophoretic patterns of these acid caseins slightly differed from that of acid casein prepared from skim milk. However, it was considered that this change observed in electrophoretic pattern depended on the difference in precipitability of casein at pH 4.7. Practically the entire amount of casein in concentrated milk was precipitated at pH 4.7. On the other hand, some parts of casein in skim milk remained in supernatant at pH 4.7. The difference affected the ratio of α-casein and β-casein in samples subjected to electrophoresis. This result suggested that acid precipitability of casein shoud be studied in further work concerning stability of frozen milk.

No sediments were developped visually in frozen milk. However, a sediments was slightly formed in freeze-concentrated milk, brought about by repeated freezing-thawing. Electrophoretic analysis of the sediment proved that its major component was casein. It is highly possible, that casein micelles (calcium-caseinate) in milk combined with calcium and possibly magnesium in serum portion under high salt concentration of unfrozen part of concentrated milk, produce insoluble sediment.

SUMMARY

Some physicochemical studies on denaturation of casein and/or casein micelles by freezing or other treatments related to freezing were undertaken. The following results were obtained: (1) Freezing or frozen storage at $-10°C$ slightly lowered acidity and tended to increase viscosity of raw and pasteurized skim milk and raw whole milk. The tendency of increasing in viscosity was also observed in casein colution. Electrophoretic pattern and electron microscopic pattern was affected neither by freezing nor frozen storage. (2) Casein precipitated at pH 4.7 from freeze-concentrated skim milk and from evaporate-concentrated skim milk (sweetened) presented similar electrophoretic patterns. (3) The sediment was developed in freeze-concentrated skim milk. The major component of the sediment was casein. (4) Lyophilizing of skim milk resulted in no effect on its electrophoretic pattern. Lyophilizing of casein glue exerted an effect on the descending part of its electrophoretic pattern but not on the ascending part.

It was concluded that freezing of milk had no remarkable effect on its properties studied in this paper, unless it was concentrated prior to freezing.

ACKNOWLEDGMENT

The authors express their appreciation to Dr. M. MAENO, Morinaga Milk Industry Company, for his helpful suggestions and constructive criticism given during the early stages of this study. The authors also thank Mr. S. TAKAHASHI, Hokkaido University Tuberculosis Research Institute, for his aid and advice in the preparation of electron micrographs. Special recognition is made for financial support from the Hokkaido Kagaku Kenkyu-hi funds supplied by Hokkaido-cho. The financial support has made a part of this work possible. Appreciation is also expressed to the Snow Brand Milk Products Company for supplying the sweetened condensed skim milk and the skim milk powder.

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