



Title	Studies on Frozen Milk : . Frozen Storage of Whole Milk, Homogenized Milk, Skim Milk and Their Concentrates
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# STUDIES ON FROZEN MILK

## I. Frozen Storage of Whole Milk, Homogenized Milk, Skim Milk and Their Concentrates.

By

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### Introduction

The application of deep freeze techniques on the preservation of fluid milk products is a common idea in dairy industry. However, there are still some practical problems, which remain to be solved such as destabilization of milk proteins for example. SAITO and HASHIMOTO<sup>7)</sup> made a brief review concerning this subject in their preliminary report on frozen milk. They also made some observations on milk frozen and stored at  $-10^{\circ}\text{C}$  in thin layers, suggesting that no remarkable denaturation of milk proteins may occur by this storage condition. Because of the increasing interest in frozen milk, information on properties of milk and its concentrate in frozen storage should be reexamined and further details should be made available. In this paper the following experiments were undertaken: whole milk, skim milk, homogenized milk and their concentrates were frozen in cylindrical shape and stored at  $-20^{\circ}\text{C}$ . After storage of certain periods, some properties of the frozen materials were observed.

### Procedure

#### Materials:

a) Whole milk: Raw, fresh, mixed herd, morning milk was used unless otherwise stated. The milk was obtained from the Hokkaido University herd, and was cooled in ice water bath immediately after sampling.

b) Skim milk: The whole milk was subjected to centrifugation ( $895 \times G$ , for 10 min.) at room temperature. The cream layer was removed by means of a 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) Homogenized milk: Commercial homogenized city milk was used.

d) Concentrated milk: Whole milk in a storage tank (capacity 3700 Kg) was divided into three portions. The first portion was whole milk. The second portion was skimmed by Westphalia cream separator. The third portion was homogenized at 140 Kg/cm<sup>2</sup> at 60°C, and pasteurized at 85°C for 15 minutes. Thus whole milk, skim milk and homogenized milk originating in the same batch were prepared. One liter of each milk was condensed by Precision evaporator at 93°C to about half of the original volume. The time required for the condensing procedure were 8, 8 and 25 minutes for whole milk, homogenized milk, and skim milk respectively.

#### **Method of frozen storage and thawing:**

Two hundred ml. of each material was placed in polyethylene bottles (6 cm in diameter and 12 cm in high). Three to five bottles were prepared for a single kind of material. The bottled materials were placed in a deep freezer (-20°C) and stored. After storage for a certain period a bottle of each material was thawed by immersion in flowing tap water (11°C) with gentle shaking every 10 minutes. Approximately 2 hours were required for the thawing procedure. Frozen condensed milk was diluted to its original volume with deionized water prior to being subjected to examinations.

#### **Measuring of titratable acidity:**

Ten ml. of sample was titrated by 0.1 N NaOH using 5 drops of 1% phenolphthalein as indicator. Results were expressed in lactic acid percentage.

#### **Measuring of ethanol stability:**

Ethanol of various concentrations (68—86%) were prepared by diluting of neutral 95% ethanol with deionized water. One ml. of aqueous ethanol was added to 1 ml. of sample in a small petri dish (3 cm. in diameter) by means of a 1 ml. pipette. The sample was constantly kept in motion during the addition of aqueous ethanol to ensure a thorough and rapid mixing. The addition of ethanol and judging of curd formation was completed within 20 seconds. Both ethanol and sample were adjusted to 15°C prior to the examination.

#### **Measuring of viscosity:**

Relative viscosity was measured in the same manner as in the previous paper<sup>7</sup>.

#### **Salting out analysis:**

SNELLMAN and TENOW's method<sup>8</sup> for tropomyosine was applied with slight

modifications. Ammonium sulfate solution of various grades of saturation were prepared by diluting saturated ammonium sulfate solution with phosphate buffer solution. One half ml. of sample was added to 5 ml of this solution. The mixture was filtered at 1°C through Toyo Roshi No. 2 filter paper after left standing for 15 hours at 1°C. The protein concentration of the filtrate was measured by FOLIN's phenol reagent method<sup>1)</sup>.

**Precipitability analysis around isoelectric point:**

One half ml. of sample was added to 5 ml. of citrate-phosphate buffers<sup>2)</sup> varying their pH from 4.0 to 5.0 with intervals of 0.1. After keeping the mixture at 1°C for 3 hours, it was filtered using Toyo Roshi No. 2 filter paper. The protein concentration of the filtrate was determined in the same manner as the salting out analysis.

**Observation of fat globules:**

One drop of sample was mixed with approximately a 10 fold volume of glycerin. A small amount of the mixture was mounted on a slide glass, and covered with a cover glass. Small pieces of cover glass were placed between the slide glass and cover glass so that fat globules were not compressed. The prepared slide was kept for at least 24 hours and observed with a phase contrast microscope manufactured by Olympus Kogaku Kogyo Co.

**Electrophoretic analysis:**

Five ml. of sample was mixed with the same volume of veronal buffer solution which had a two fold concentration of standard veronal buffer solution<sup>3)</sup>. The mixture was dialyzed against 400 ml. of the standard veronal buffer solution (pH 8.6, ionic strength of 0.10) at 1°C for 72 hours using cellophane as the dialysis membrane. Following the dialysis, it was filtered through Toyo Roshi No. 5c filter paper. The filtrate was subjected to electrophoresis at 10 °C for 60 minutes in the same manner as in the previous paper<sup>7)</sup>.

**Electron microscopic observation:**

The same technique as described in the previous paper<sup>7)</sup> was used.

**Measuring of curd tension:**

Fifty ml. of sample was pipetted into a 50 ml. beaker and adjusted to 38°C by placing the beaker in a water bath at 50°C. The sample in the beaker was occasionally stirred with a glass rod thermometer. Five ml of the coagulant (1/4 tablet of HANSEN's rennet was diluted to 200 ml. with 100 ml. of 5% NaCl solution and deionized water, and was kept in an ice water bath avoiding light) was introduced into the sample under rapid stirring. The mix-

ture was immersed in a water bath at 35°C for 30 minutes without disturbance. A suitable petri dish was placed over the beaker immediately. Curd tension of the clotted mixture was measured by a Nakamura curd tension meter manufactured by Nakamura Iryo Rikakikai Co., using a curd knife for yogurt. Elevating speed of the beaker containing the clotted mixture was adjusted at a rate of 3 cm in 13 seconds. The titer of the coagulant was determined by measuring the curd tension of standard sample consisting of 12 grams of skim milk powder, 68 ml. of deionized water, and 20 ml. of 1%  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  solution. The same batch of skim milk powder was used through the entire course of the experiment. The results were expressed in percentage of the curd tension of the standard sample.

#### **Determination of precipitate amount :**

a) Skim milk, whole milk and homogenized milk : Forty ml. of sample was centrifuged at  $5000 \times G$ . for 30 minutes at 2°C. The nitrogen amount of the precipitate was determined by micro Kjeldahl method after dispersing the precipitate in deionized water and diluting to 50 ml. Two ml. of the diluted material was subjected to nitrogen determination procedure.

b) Condensed milk : The precipitate in 100 ml. of sample, diluted previously to the original concentration, was removed by centrifugation at  $1,000 \times G$ . for 30 minutes, at room temperature. The precipitate was dispersed in small amount of deionized water and diluted to the final volume of 50 ml. or 100 ml. according to amount of the precipitate. Five ml. of the dispersed precipitate was subjected to determination of total solid. The official method<sup>11)</sup> for determination of total solid in milk was applied.

#### **Chemical analysis of precipitate :**

The dispersed precipitate used for determination of the amount of precipitate was subjected to chemical analysis. The following methods were used for the analysis :-

a) Protein : One half ml. of sample was used for nitrogen determination by micro Kjeldahl method<sup>9)</sup>. Protein amount was calculated by multiplying the nitrogen amount with 6.38.

b) Lactose : Bertrand method<sup>9)</sup> was applied using 10 ml. of sample.

c) Fat : Roese-Gottlieb method<sup>11)</sup> was applied using 10 ml. of sample.

d) Ash : Five ml. of sample was ashed according to the official method<sup>11)</sup> using a porcelain dish instead of a platinum dish.

e) Calcium : The ash used for determination of ash was dissolved with 1 ml. of N/2 HCl and diluted to 50 ml. Its calcium content was determined by YANAGIZAWA's method<sup>12)</sup>.

f) Phosphorus: Two ml. of sample was digested by 2 ml. of 5 N  $H_2SO_4$  at  $10^\circ C$  under occasional addition of 20%  $H_2O_2$ . Then MARTIN and DOTY's method<sup>5)</sup> was applied.

g) Soluble nitrogen at pH 4.6: Five ml. of sample was mixed with an equal volume of pH 4.6 acetate buffer solution<sup>3)</sup>. The mixture was heated to  $40^\circ C$  and filtered through Toyo Roshi No. 3 filter paper. The nitrogen amount of the filtrate was determined by the micro Kjeldahl method.

## Results

### Effect on acidity:

The results were presented in Table 1, supporting the results reported in the previous paper<sup>7)</sup>. Decrease of acidity by frozen storage was more pronounced in concentrated milk products than in unconcentrated milk.

TABLE 1. Effect of frozen storage on acidity of whole milk, homogenized milk, skim milk and their concentrates\*

Sample	Control (%)	Time in storage			
		24 hrs. (%)	1 months (%)	2 months (%)	3 months (%)
Whole milk	0.142	0.143	0.135	0.131	—
Homogenized milk	0.151	0.143	0.146	0.137	—
Skim milk	0.134	0.122	0.140	0.128	—
Concentrated whole milk	0.146	—	0.132	—	0.110
Concentrated homogenized milk	0.143	—	0.134	—	0.121
Concentrated skim milk	0.135	—	0.142	—	0.106

\* Concentrates were diluted to original volume prior to determination.

TABLE 2. Effect of frozen storage on ethanol concentration which gave positive results at ethanol test

Sample	Control (%)	Time in storage		
		24 hrs. (%)	1 month (%)	2 months (%)
Whole milk	74	78	78	80
Homogenized milk	80	78	80	80
Skim milk	78	70	78	80

**Effect on ethanol stability :**

Final ethanol concentrations which caused curdling of milk were shown in Table 2. A slight increase of ethanol stability by frozen storage was observed in whole milk.

**Effect on viscosity :**

The results were presented in Table 3. Viscosity of homogenized milk increased remarkably after 2 months storage. However, such increase of viscosity was not observed in whole milk and skim milk. A more remarkable increase in viscosity was demonstrated in concentrated milk. Precipitate formation and some aggregation of casein micelles, both reported in the latter section of this paper, might be responsible for these results.

TABLE 3. Effect of frozen storage on relative viscosity\* of whole milk, homogenized milk, skim milk and their concentrates

Sample	Control	Time in storage			
		24 hrs.	1 month	2 months	3 months
Whole milk	1.75	1.76	1.82	1.79	—
Homogenized milk	1.89	1.75	1.97	2.32	—
Skim milk	1.50	1.48	1.55	1.44	—
Concentrated whole milk	2.45	—	2.75	—	3.42
Concentrated homogenized milk	2.42	—	2.63	—	3.31
Concentrated skim milk	2.10	—	2.22	—	2.59

\* at 15°C, standard: deionized water.

**Effect on salting out :**

Skim milk and its concentrate were subjected to salting out analysis. The resulting salting out curves were demonstrated in Figure 1 and 2. The curve of skim milk was not remarkably affected by frozen storage, even though proteins in frozen skim milk seemed to precipitate within a rather narrow range of percentage saturation. In the case of concentrated skim milk, the salting out curves were not as simple as that of skim milk. Although a detailed explanation of each peak is not available at the present stage of the study, it is considered that precipitation by salting out tends to start at lower saturation of ammonium sulfate after frozen storage. However, this tendency

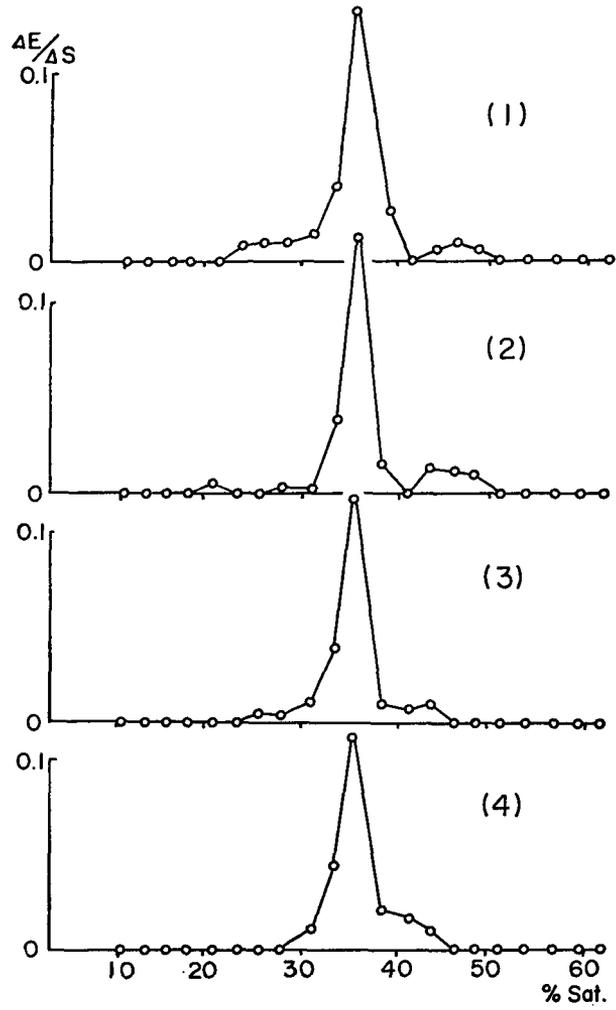


Fig. 1. Salting out curves of skim milk.

- (1) control
- (2) frozen for 24 hours
- (3) frozen for month
- (4) frozen for 2 months

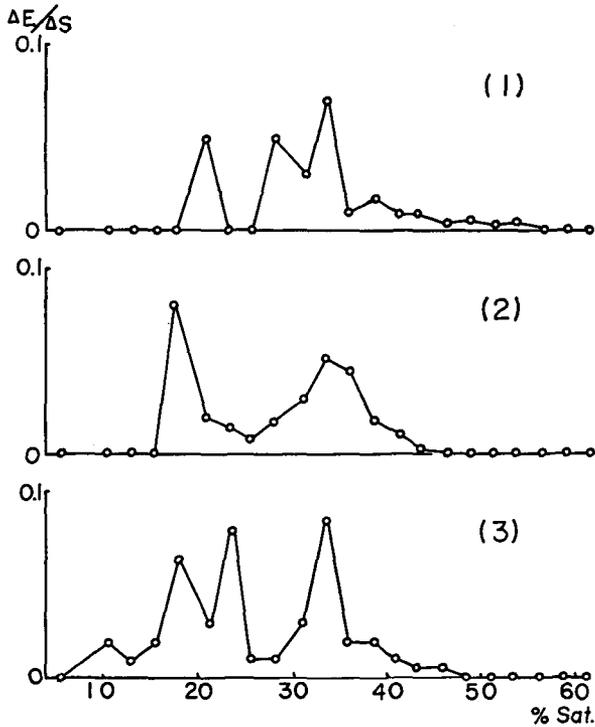


Fig. 2. Salting out curves of concentrated skim milk.

- (1) control
- (2) frozen for 1 month
- (3) frozen for 3 months

was probably accompanied slightly by precipitate formation during frozen storage.

#### Effects on isoelectric point precipitation:

Precipitation curves around the isoelectric point were shown in Figure 3, revealing rather complicated curves of concentrated milk. Both unconcentrated and concentrated milk seemed to elevate their optimum pH for acid precipitation approximately 0.1 after subjected to frozen storage.

#### Effect on electrophoresis:

Electrophoretic patterns of skim milk and its concentrate were demonstrated in Figure 4 and 5 and Table 4 and 5. The results revealed no remarkable effect of frozen storage on electrophoretic pattern of skim milk. On the other hand, frozen storage for 3 months somewhat affected on ascending pattern of concentrated skim milk, i. e. component A tended to decrease

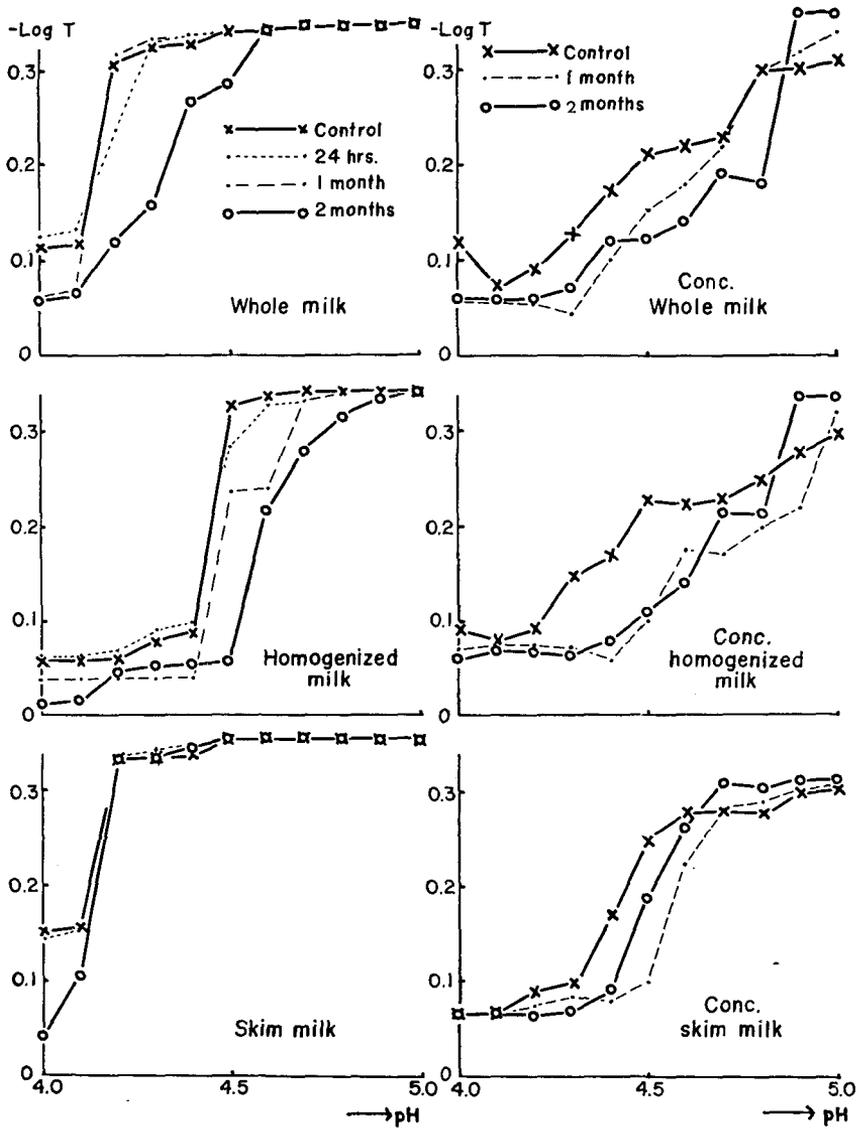


Fig. 3. Effect of frozen storage on isoelectric point precipitation.  
 Time in storage: 24 hours, 1 month and 2 months for unconcentrated milk.  
 1 month and 3 months for concentrated milk.

TABLE 4. Effect of frozen storage on electrophoretic properties of skim milk

Time in storage	Component	Ascending		Descending	
		% Relative area	Mobility at 10°C $1 \times 10^{-5}$ cm/sec. volt	% Relative area	Mobility at 10°C $1 \times 10^{-5}$ cm/sec. volt
Control	A	60.7	-11.76	82.9	- 9.50
	B	33.7	- 8.36	16.3	- 5.44
	C	—	—	0.8	- 3.66
	C'	5.6	- 2.96	—	—
24 hrs.	A	67.5	-11.37	76.5	- 9.34
	B	32.5	- 7.80	20.1	- 5.44
	C	—	—	3.4	- 3.94
1 month	A	60.8	-11.40	79.5	-10.02
	B	33.6	- 8.40	18.6	- 5.68
	C	1.7	- 4.50	1.9	- 3.41
	D	3.9	- 0.97	—	—
2 months	A	59.6	-11.69	87.4	- 9.46
	B	33.5	- 8.60	12.6	- 5.60
	C	2.8	- 4.47	—	—
	D	4.1	- 1.14	—	—

TABLE 5. Effect of frozen storage on electrophoretic properties of concentrated skim milk

Time in storage	Component	Ascending		Descending	
		% Relative area	Mobility at 10°C $1 \times 10^{-5}$ cm/sec. volt	% Relative area	Mobility at 10°C $1 \times 10^{-5}$ cm/sec. volt
Control	A	70.7	-11.61	84.5	- 9.01
	B	24.7	- 8.08	15.5	- 5.77
	C	4.6	- 4.83	—	—
1 month	A	73.3	-11.15	84.8	- 8.93
	B	26.7	- 8.20	15.2	- 5.80
3 months	A	60.4	-13.03	85.6	- 9.91
	B	36.2	- 8.89	14.4	- 6.11
	C	3.4	- 5.06	—	—

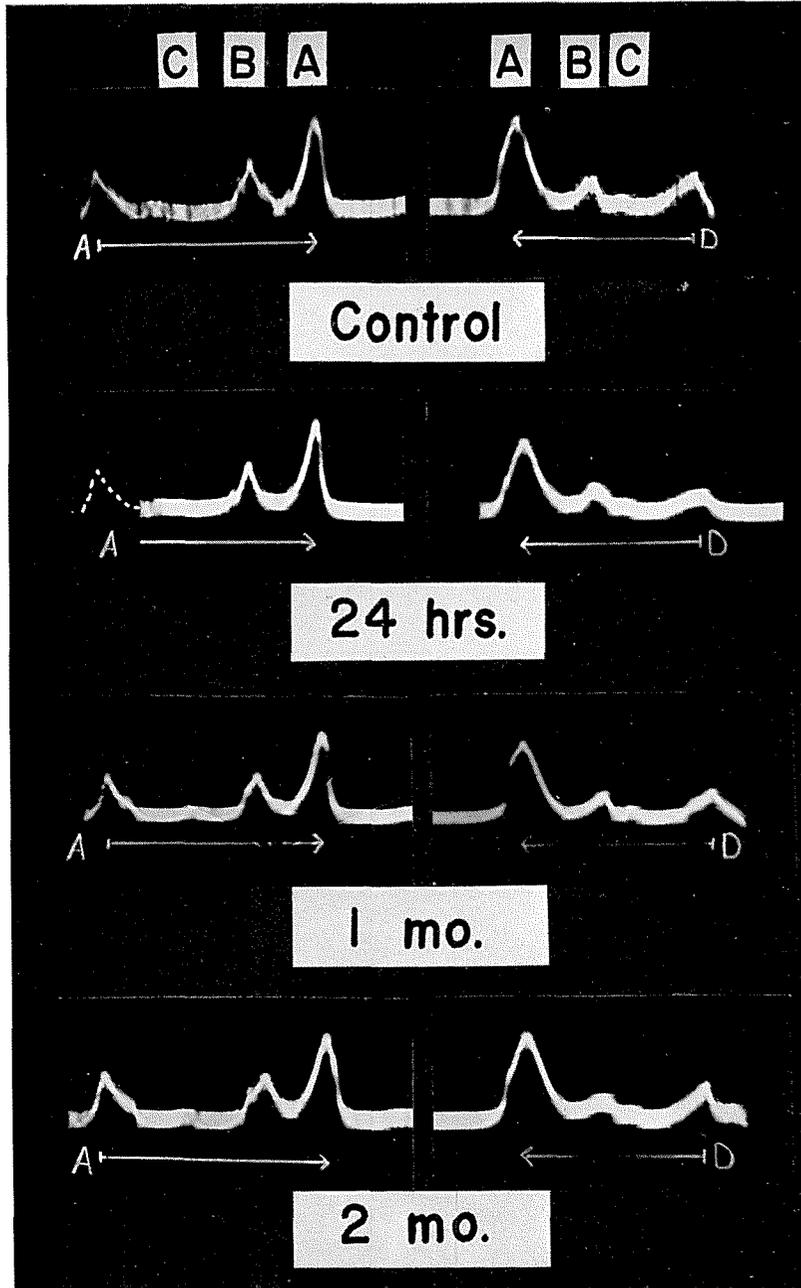


Fig. 4. Electrophoretic patterns of skim milk.  
 24 hrs.: frozen for 24 hours, 1 mo.: frozen for 1 month,  
 2 mo.: frozen for 2 months.

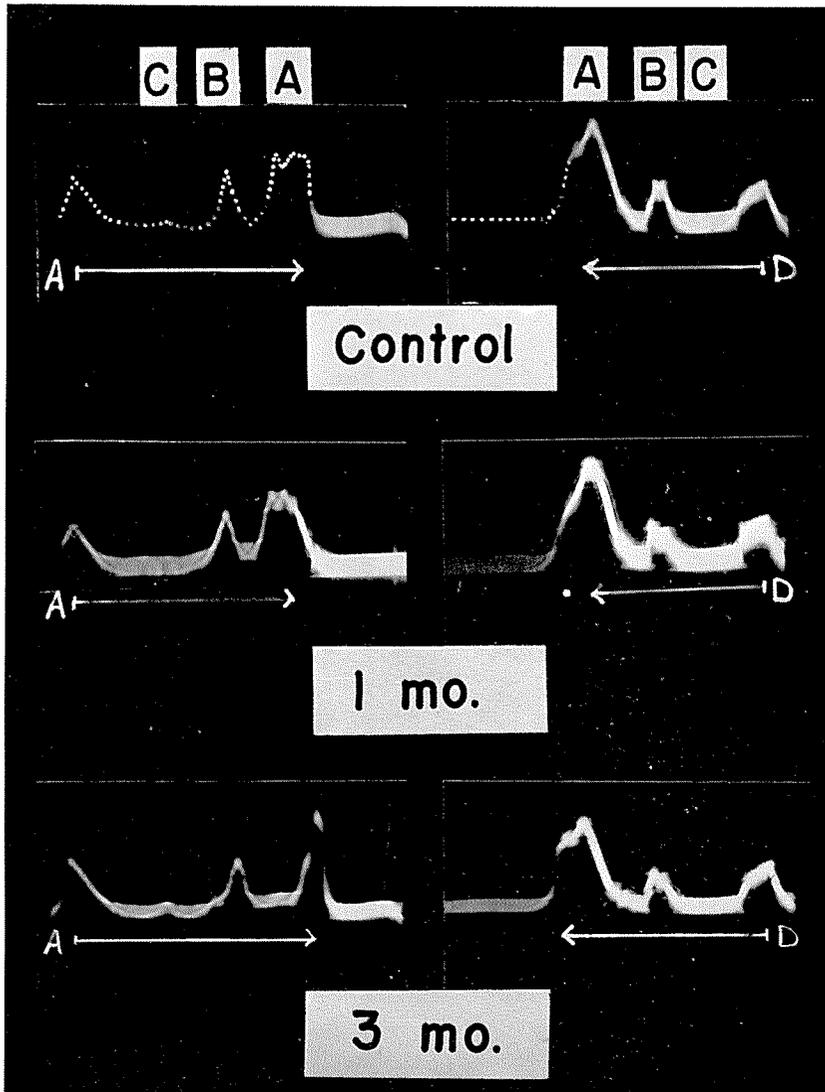


Fig. 5. Electrophoretic patterns of concentrated skim milk.  
 1 mo.: frozen for 1 month, 3 mo.: frozen for 3 months.

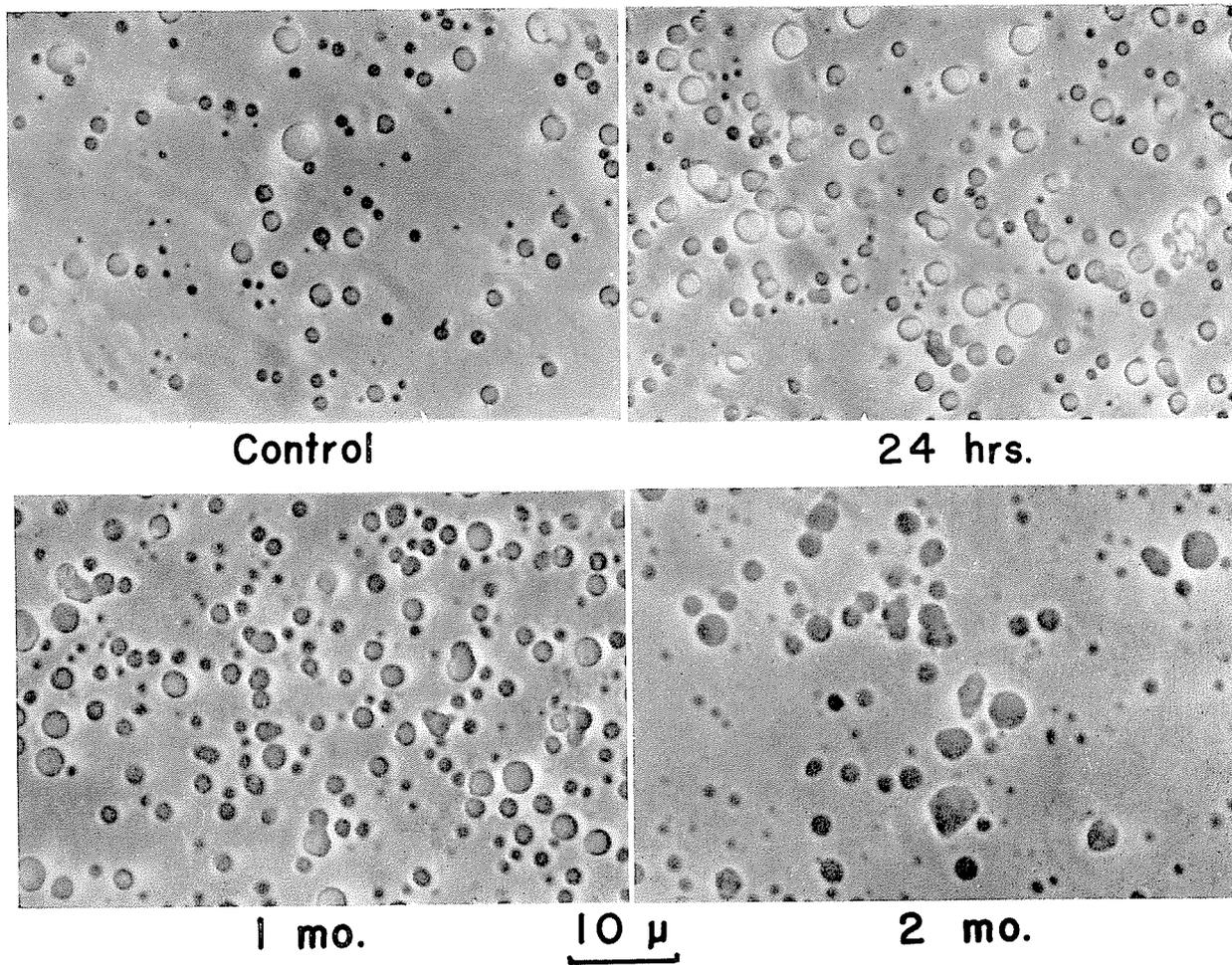
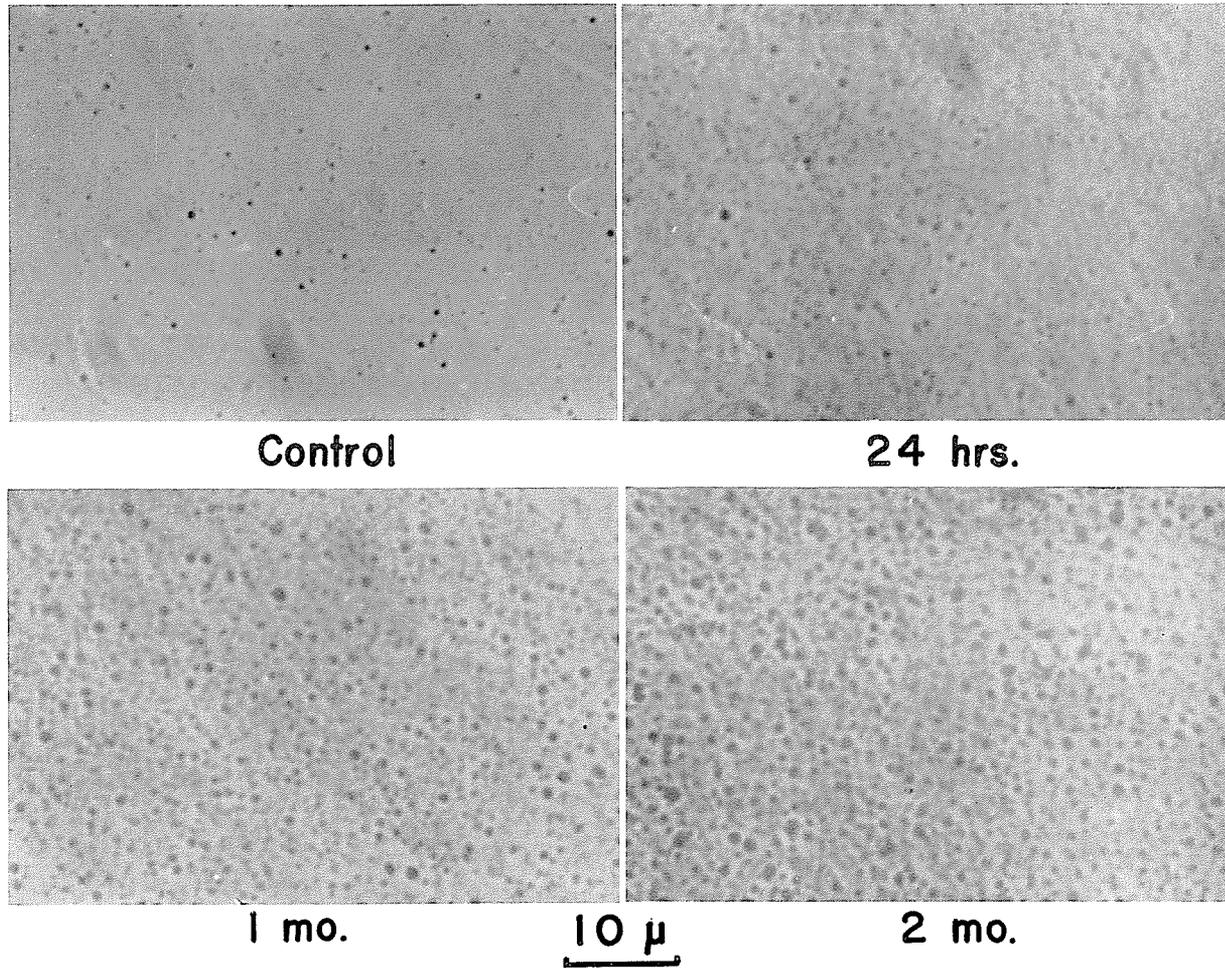
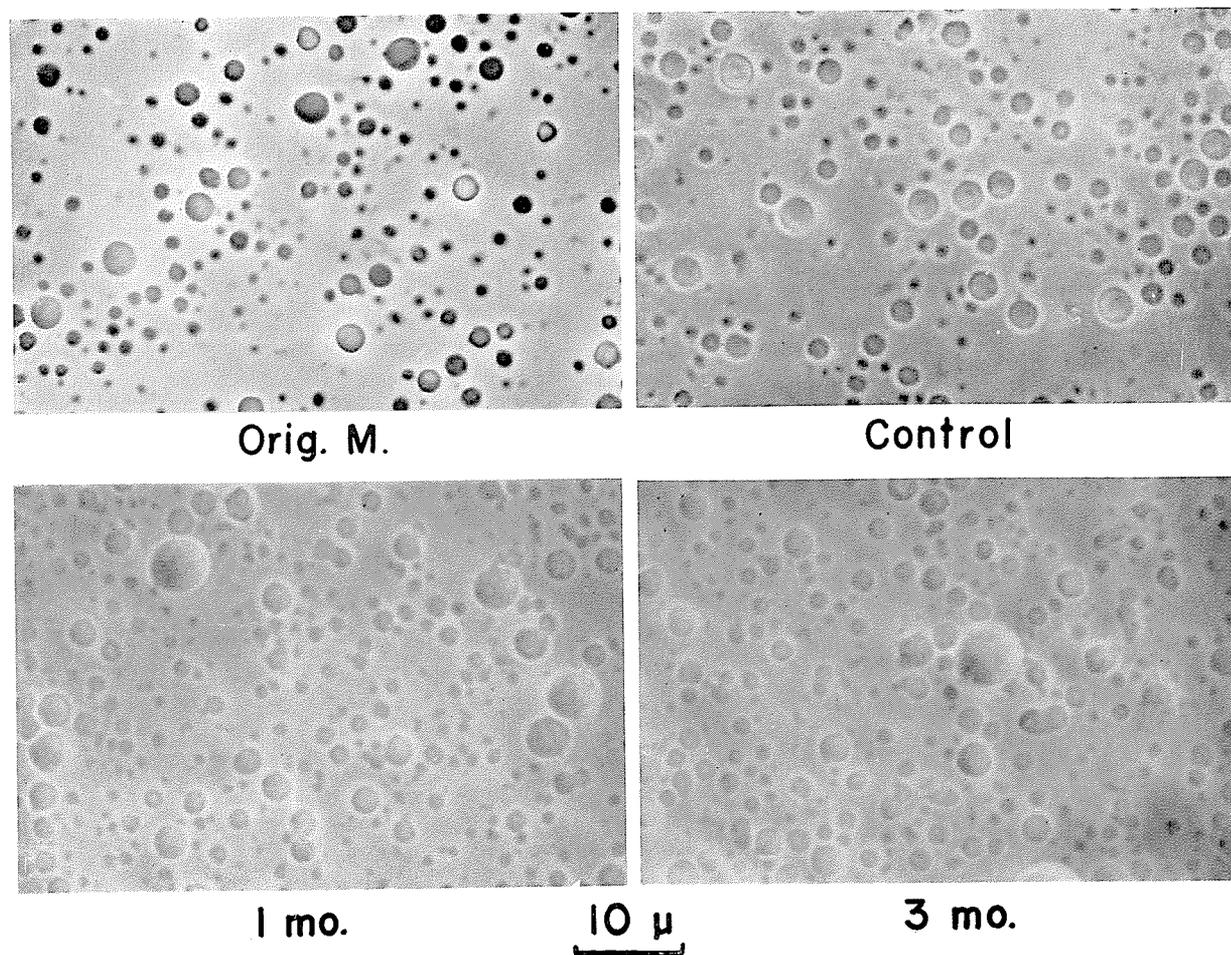


Fig. 6. Phase contrast microscopic patterns of whole milk.

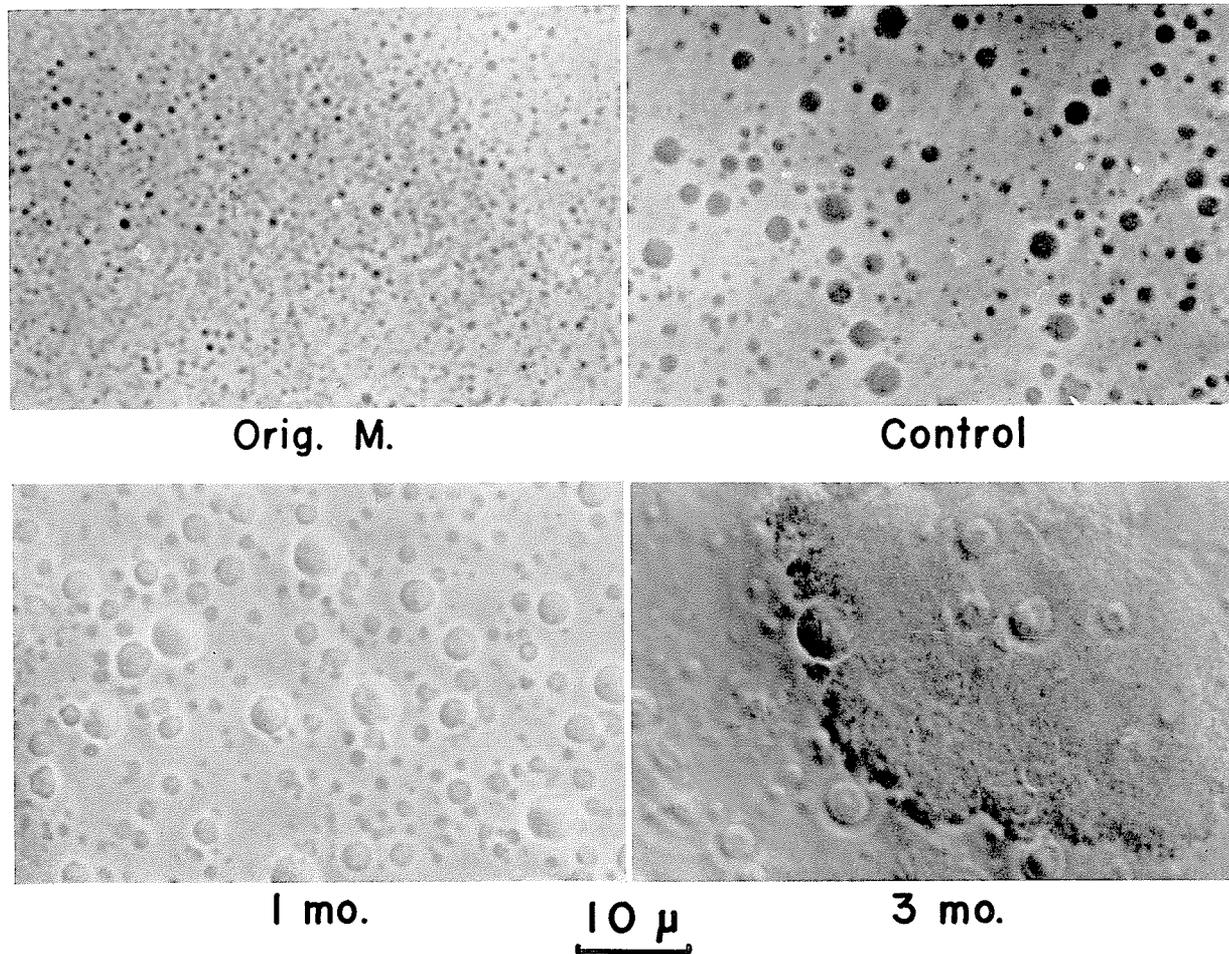
24 hrs. : frozen for 24 hours, 1 mo. : frozen for 1 month, 2 mo. : frozen for 2 months.



**Fig. 7.** Phase contrast microscopic patterns of homogenized milk.  
24 hrs. : frozen for 24 hours, 1 mo. : frozen for 1 month, 2 mo. : frozen for 2 months.



**Fig. 8.** Phase contrast microscopic patterns of concentrated whole milk.  
Orig. M.: original whole milk used for preparation of concentrated whole milk  
Control: concentrated whole milk before freezing  
1 mo.: frozen for 1 month, 3 mo.: frozen for 3 months.



**Fig. 9.** Phase contrast microscopic patterns of concentrated homogenized milk.  
 Orig. M.: original homogenized milk used for preparation of concentrated homogenized milk,  
 Control: concentrated homogenized milk before freezing  
 1 mo.: frozen for 1 month, 3 mo.: frozen for 3 months.

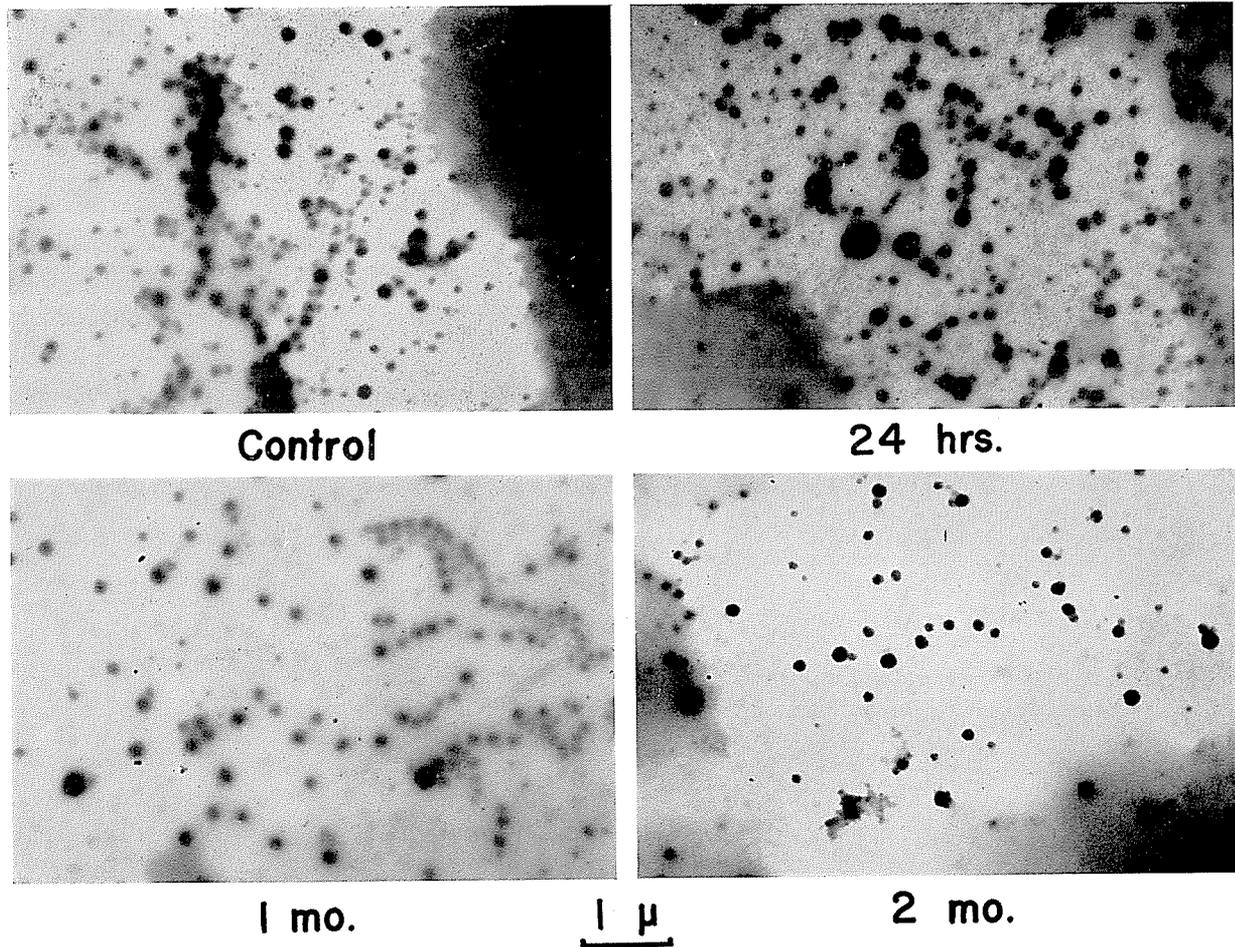


Fig. 10. Electron microscopic patterns of whole milk.  
24 hrs.: frozen for 24 hours, 1mo.: frozen for 1 month, 2mo.: frozen for 2 months.

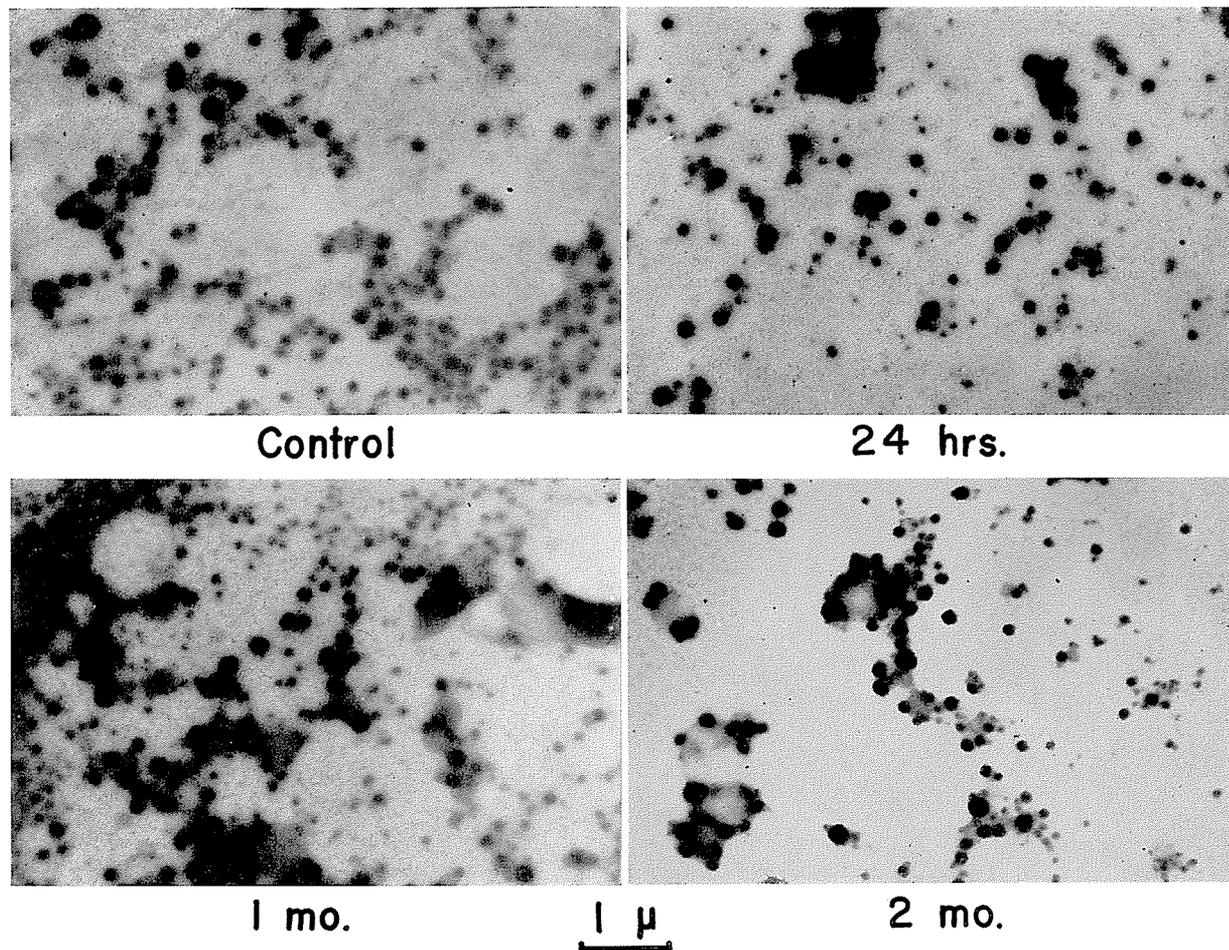


Fig. 11. Electron microscopic patterns of homogenized milk.  
24 hrs. : frozen for 24 hours, 1mo. : frozen for 1 month, 2 mo. : frozen for 2 months.

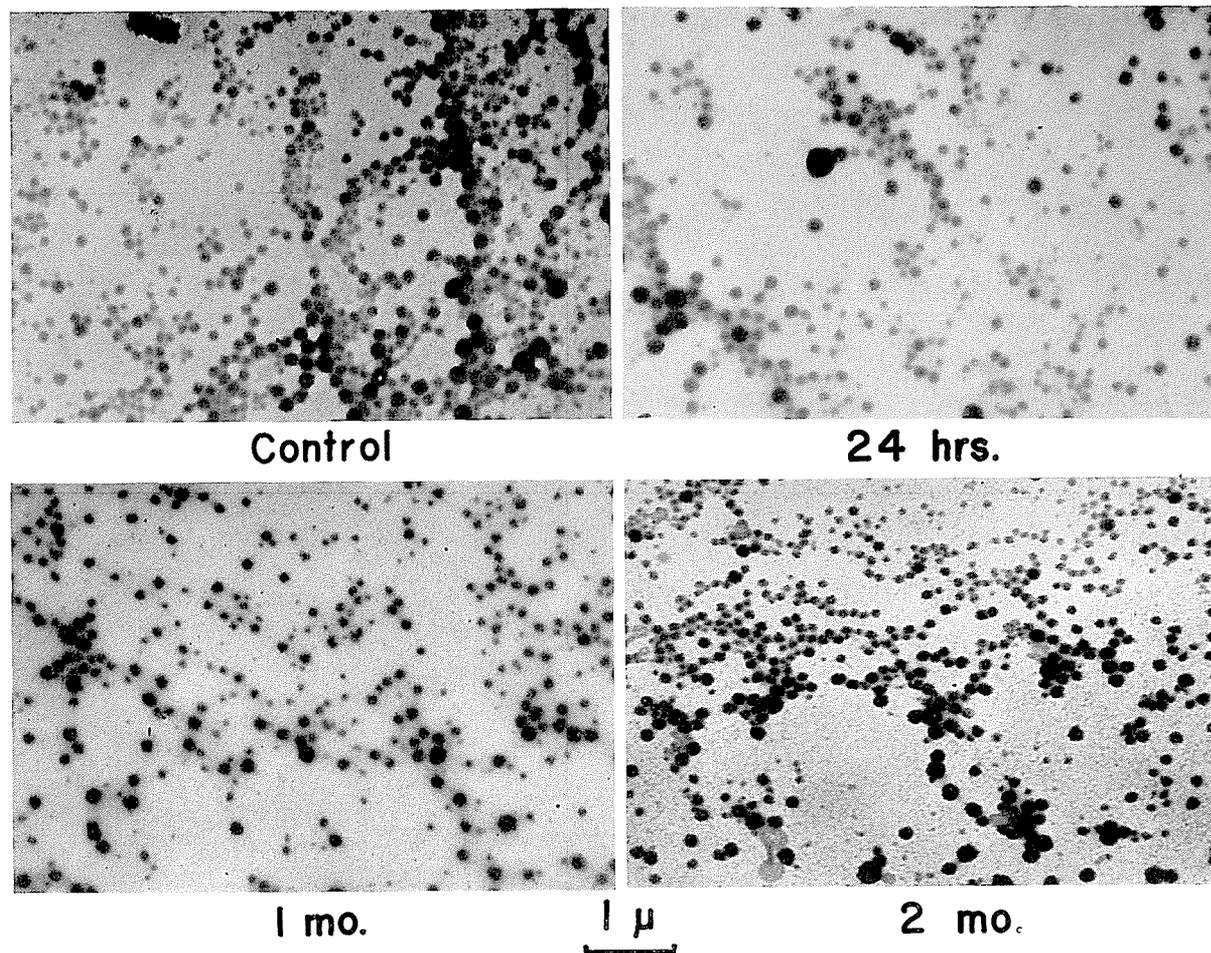
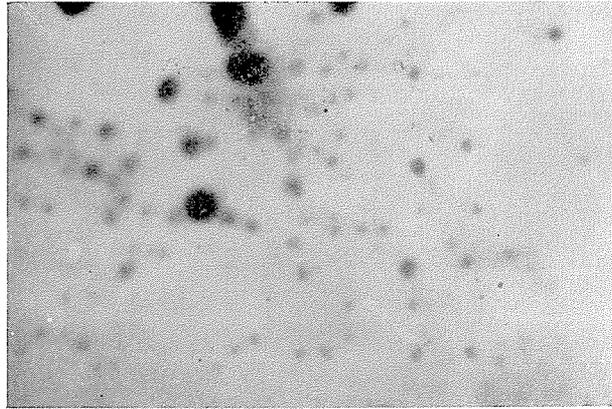
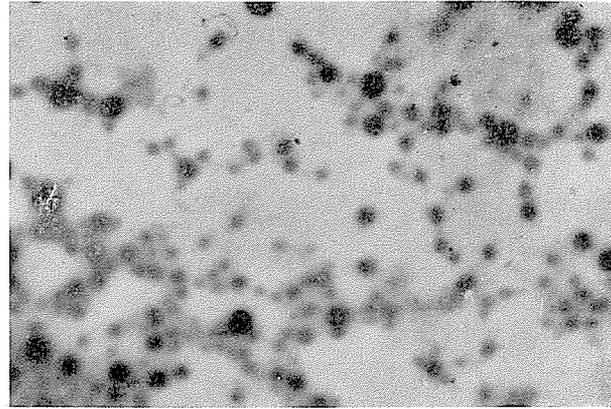


Fig. 12. Electron microscopic patterns of skim milk.

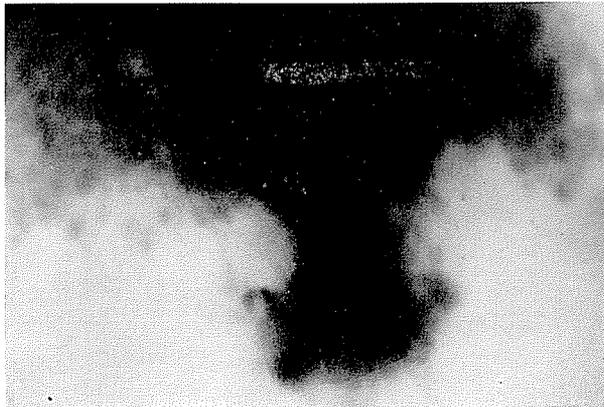
24 hrs. : frozen for 24 hours, 1 mo. : frozen for 1 month, 2 mo. : frozen for 2 months.



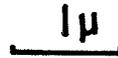
**Control**



**1 mo.**



**3 mo.**



**Fig. 13.** Electron microscopic patterns of concentrated whole milk.  
1 mo.: frozen for 1 month, 3 mo.: frozen for 3 months.

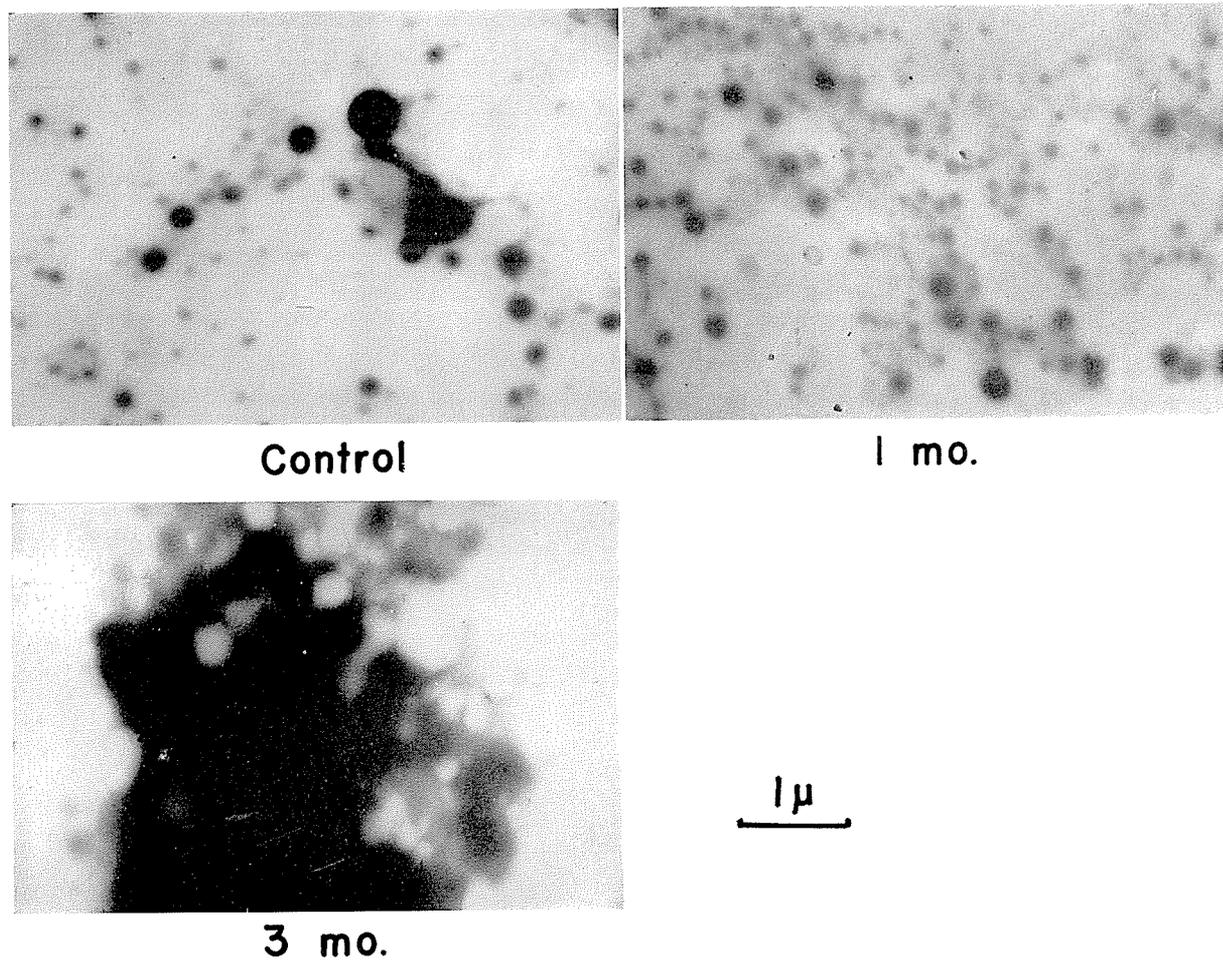
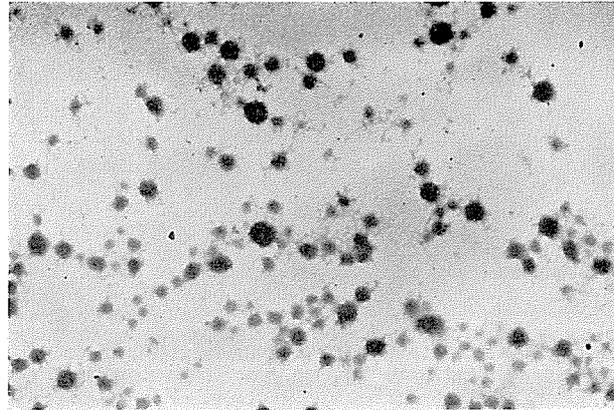
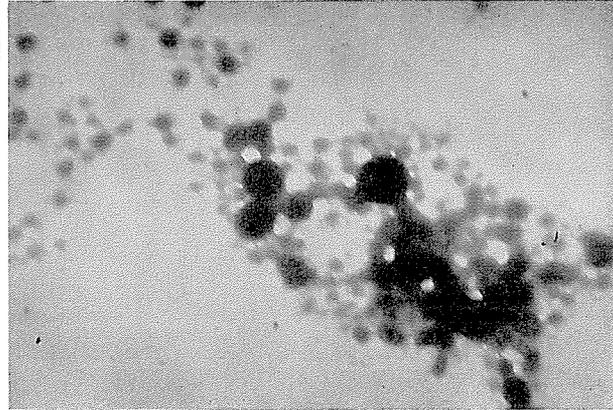


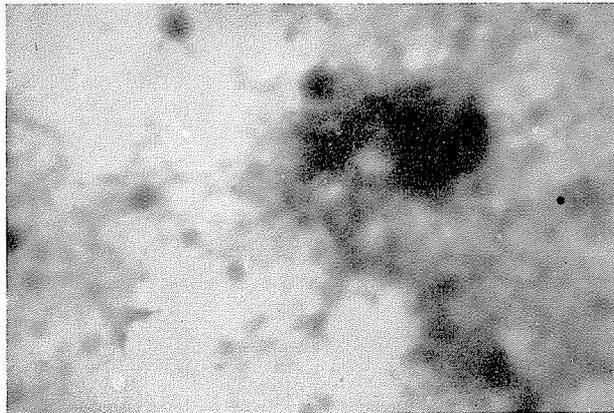
Fig. 14. Electron microscopic patterns of concentrated homogenized milk.  
1 mo.: frozen for 1 month, 3 mo.: frozen for 3 months.



**Control**



**1 mo.**



**3 mo.**

**1 $\mu$**

**Fig. 15.** Electron microscopic patterns of concentrated skim milk.  
1 mo. : frozen for 1 month, 3 mo. : frozen for 3 months.

in its relative area.

**Effect on fat globules :**

Phase microscopic patterns of frozen milk were shown in Figure 6, 7, 8, and 9, showing the condition of fat globules. Slight cohesion of fat globules in whole milk occurred by long term storage. It was pronounced to some extent in the case of concentrated whole milk. On the other hand, no effect of frozen storage was observed in the fat globules in homogenized milk. However, large fat globules developed in homogenized milk during concentrating process. Curds including fat globules were observed in concentrated homogenized milk after long term storage in frozen form.

**Effect on electron microscopic pattern :**

Electron microscopic patterns were presented in Figure 10 to Figure 15. Effect of frozen storage on casein micelles was not observed in the case of unconcentrated milk. However, frozen storage of concentrated milk caused aggregation of casein micelles to some extent. It should be considered that the aggregation was somewhat emphasized in the electron microscopic pattern than in the original sample because of drying process applied for preparation of specimen.

**Effect on precipitate amount :**

1. Protein amount recovered in precipitate in frozen milk : Protein separated from frozen milk by high speed centrifugation was determined. The protein amount was shown in Table 6, revealing that no curdling of protein occurred during storage of unconcentrated milk in frozen form.

TABLE 6. Effect of frozen storage on sedimentary nitrogen of frozen milk

Sample	Control (mg/ml)	Time in storage		
		24 hrs. (mg/ml)	1 month (mg/ml)	2 months (mg/ml)
Whole milk	0.98	0.92	1.08	0.97
Homogenized milk	0.99	1.11	0.89	0.97
Skim milk	0.96	1.08	1.12	0.88

2. Precipitate amount in frozen concentrated milk : The amount of precipitate separated from frozen concentrated milk by low speed centrifugation was presented in Table 7. The results demonstrated that prolonged storage of 3 months in frozen form caused remarkable curdling particularly in the

TABLE 7. Effect of frozen storage on precipitate amount of concentrated milk

Sample	Control (mg/ml)	Time in storage		
		1 month (mg/ml)	3 months (mg/ml)	3 months (washed)* (mg/ml)
Concentrated whole milk	4.96	5.10	52.46	14.22
Concentrated homogenized milk	7.78	7.79	59.48	12.31
Concentrated skim milk	2.37	2.55	4.52	0.72

\* Precipitate washed 3 times with deionized water.

concentrated whole milk and the concentrated homogenized milk.

#### Chemical composition of precipitate in frozen concentrated milk :

The results of chemical analysis of the precipitate were shown in Table 8. The major component of the precipitate separated before freezing was protein. However, the fat content of precipitate in concentrated whole milk and concentrated homogenized milk increased after frozen storage for 3 months. This result indicates that casein formed curd including fat globules during frozen storage. Precipitation of calcium phosphate presumed by VAN DEN BERG<sup>11)</sup> was not suggested from the composition of precipitate.

TABLE 8. Chemical composition of precipitate removed from frozen concentrated milk by centrifugation

Sample	Concentrated whole milk			Concentrated homogenized milk			Concentrated skim milk		
	Time in storage (month)								
Component	1 (%)	3 (%)	3* (%)	1 (%)	3 (%)	3* (%)	1 (%)	3 (%)	3* (%)
Protein	54.0	38.5	64.9	46.5	33.7	40.9	66.8	61.8	37.8
Lactose	22.0	15.4	0.6	15.8	14.8	0.4	19.9	31.4	0.0
Fat	16.8	42.4	29.3	30.0	47.3	53.9	1.1	1.3	0.0
Ash	7.0	5.0	6.0	6.0	4.4	3.7	8.3	6.5	10.0
Ca	1.4	1.3	2.1	1.0	1.0	0.9	2.1	1.8	4.6
P	1.0	0.8	1.2	1.1	0.7	0.7	1.4	1.4	1.1

\* Washed precipitate.

#### Effect on curd tension of individual milk :

This item in the experiment has no direct connection with others concerning preparation of sample, i. e. individual milk was used instead of pooled

TABLE 9. Effect of frozen storage on curd tension\* of individual milk

Cow No.**	Milking	Trial I		Trial II	
		Control	Frozen for 6 hrs.	Control	Frozen for 1 week
G-1	morning	252	238	391	252
	evening	273	248	350	236
G-2	morning	164	156	198	173
	evening	192	204	166	143
G-3	morning	183	179	173	162
	evening	142	128	197	160
H-1	morning	116	119	118	186
	evening	161	155	105	157
H-2	morning	67	69	76	68
	evening	78	77	98	87
H-3	morning	103	112	122	107
	evening	139	139	114	110
H-4	morning	96	95	107	90
	evening	136	108	156	133
H-5	morning	69	70	81	68
	evening	99	92	101	92
H-6	morning	96	116	99	89
	evening	139	124	118	103
Average***	morning	127 (100)	128 (100.4)	152 (100)	133 (87.5)
	evening	151 (100)	142 (94.0)	156 (100)	136 (87.2)

\* Percentage of the curd tension of standard sample consisting of 12 g of skim milk powder, 68 ml. of deionized water and 20 ml. of 1%  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  solution.

\*\* "G" stands for Guernsey cow. "H" stands for Holstein cow.

\*\*\* Figures in ( ) show percentage of original curd tension.

milk. The results were shown in Table 9. Differences of curd tension among samples of individual milk were rather large, even though differences resulting from freezing were small. Frozen storage for 6 hours had no effect on curd tension in average. However, frozen storage for one week lowered the curd tension to approximately 87 per cent of the original in average.

### Discussion

Effects of frozen storage on milk and its concentrates suggested in the preliminary report<sup>7)</sup> were generally confirmed in this paper, even though some

experiments, which should be done to obtain conclusions on frozen milk, have remained unsettled. Skim milk was less affected by frozen storage, as would be expected, than whole milk and homogenized milk. The salting out curve and precipitability around pH 4.6 indicated that a slight change occurred in casein/or casein micelles of skim milk by frozen storage, even though the change was not detectable by electron microscopic observation and electrophoresis. In the case of concentrated skim milk, the change was a little more pronounced, and the aggregation of casein micelles was observed to some extent.

The effect of frozen storage on properties of whole milk was more complicated than in the case of skim milk because of fat globules. The effect was probably similar to the case of skim milk at least as far as casein was concerned. However, it is almost certain that fat globules were involved in the aggregation of casein micelles occurring in concentrated whole milk. Thus, a considerable amount of precipitate was formed during prolonged frozen storage of concentrated whole milk as mentioned in the analysis of the precipitate. Therefore, freezing of concentrated whole milk must be carefully treated. On the other hand, freezing or frozen storage of unconcentrated whole milk, raw milk for instance, might have no problem concerning precipitate formation. Such raw milk that is accidentally frozen during hauling in winter probably can be manufactured after a suitable thawing process is introduced. However, it should be mentioned that fresh pooled milk in high quality was used as experimental material. Milk from each cow has a high individuality as suggested by experiments on curd tension of individual milk. Therefore, special care should be taken to precipitate formation in frozen storage using large numbers of individual milk.

Frozen storage of concentrated homogenized milk was most troublesome. Visible curd developed during 3 months storage as reported by BELL and MUCHA<sup>2)</sup>. The curd separated as a deposit without centrifugation. Concentrated whole milk made precipitates of approximately the same amount as in concentrated homogenized milk. But the precipitate of whole milk was so fine that it was not visible as a deposit. It is considered that the destabilization of protein by the homogenization process was clearly indicated after frozen storage. Certain pretreatment, such as ultrasonic treatment used by WEARMOUTH<sup>10)</sup> with success, as well as improved condition of freezing and frozen storage will be required for frozen storage of homogenized milk and its concentrate. Body defects of frozen milk was studied mostly in this paper. Flavor study of frozen milk should be undertaken in the followed stage of this study.

### Summary

Whole milk, skim milk, homogenized milk and their concentrates were frozen and stored at  $-20^{\circ}\text{C}$  for 24 hours to 3 months. Some properties of the frozen milks were observed by means of phase contrast microscope and electron microscope as well as by measuring acidity, viscosity, electrophoretic property and precipitability by ethanol, salt and lowered pH. Analysis of precipitate formed in the frozen milk was also undertaken. The effect of frozen storage on milk was rather slight unless it was concentrated prior to freezing. The effect was least in skim milk, and was the most remarkable in homogenized milk. Increase of viscosity was rather remarkable, particularly in homogenized milk, i. e. relative viscosity of 1.89 increased to 2.32 after storage of 2 months. In the case of concentrates, precipitate formation was a remarkable defect caused by frozen storage for 3 months. The precipitate in concentrated homogenized milk separated as a deposit. Chemical composition of the precipitate was as follows: protein 33.7%, fat 47.3% lactose 14.8% and ash 4.4%. Aggregation of casein micelles to some extent was observed in all concentrates kept for 3 months in frozen form.

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