J. Fac. Fish. Anim. Husb.,
Hiroshima Univ. (1977), **16**: 145～150

Effects of Cold-storage on the Changes in the Casein Complex in Sterilized Skim Milk

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Received September 30, 1977
(Figs. 1–4, Tables 1–3)

Sedimentation or gelation occurs during storage of high temperature-short time sterilized or ultra-high temperature sterilized milk (UHT milk). A lot of research works on this problem have been done.[1–10] However, the mechanism of sedimentation or gelation has not been elucidated sufficiently.

In the previous paper,[11] we reported the changes in the casein complex* during storage of the sterilized skim milk (SSM) prepared laboratorially. Cooling milk causes certain changes in the casein micelles; soluble casein releases from the casein micelles[12–16] and a part of the colloidal calcium phosphate is solubilized.[17] Accordingly, in the present study, the effects of cold storage on the changes in the casein complex in the SSM were examined.

MATERIALS AND METHODS

1. Preparation of the SSM

The milk sample used was raw herd milk of about 60 cows. Skim milk was separated from the milk by centrifugation at 1,000×g for 15 min without warming. The raw skim milk was poured into many glass tubes which had an inside diameter of 4 mm and their open ends were sealed. The sealed glass tubes were immersed in an oil bath at 135°C for 45 sec. Under the above heating conditions, the temperature of the inner skim milk is estimated to become 130°C as shown in the previous paper.[18] The SSM obtained was divided into two groups. One was stored at 30°C and termed sample I, and the other was stored at 5°C and termed Sample II.

2. Measurement of relative viscosity

The relative viscosity of the SSM was measured at 30°C with an Ostwald viscometer which took 17.6 sec of water flow time.

3. Measurement of pH

The pH of the SSM was measured with a Hitachi-Horiba pH meter (Type M-5) using a complex electrode (#6028-10T) at room temperature (20~25°C).

4. Determination of sedimentation-N by low speed centrifugation.

The SSM was centrifuged at 1,000×*g* for 15 min and then the upper 2/3 parts of the

* This term was used in the present paper to represent the calcium-caseinate-phosphate complex in milk.

contents in the centrifugal tubes were taken out. The difference of the N-contents between the upper part and the original skim milk was termed sedimentation-N by low speed centrifugation. Nitrogen was determined by the micro Kjeldahl method.

5. Determination of soluble casein-N and NCN

The SSM which had been cooled at 5°C for 4 hr was ultracentrifuged at 45,000 rpm (109,800×*g*) for 1 hr at 5°C with a Hitachi 55-P Type 2 ultracentrifuge. Noncasein nitrogen (NCN) was determined on the filtrate of 1:1 mixture of the ultracentrifugal supernatant and 1/10 M acetate buffer (pH 4.4). Nonprotein nitrogen (NPN) was determined on the filtrate of 1:4 mixture of the ultracentrifugal supernatant and 15% trichloroacetic acid (TCA) solution. The difference between the total nitrogen (total-N) in the ultracentrifugal supernatant and NCN was regarded as soluble casein-N.

6. Determination of Ca and P in the ultracentrifugal supernatant

Calcium was determined on the filtrate of 1:4 mixture of the ultracentrifugal supernatant and 15% TCA solution with a Hitachi atomic absorption spectrophotometer.[19, 20] Phosphorus was determined on the filtrate by Allen's method and regarded as inorganic phosphorus.

RESULTS

1. Stability of the SSM

The increment of the relative viscosity of Sample I was small. Visible sediment and "Whey off" were observed after 5 months of storage (Fig.1). In the case of Sample II, the relative viscosity increased markedly after 4 months of storage. Visible sediment and "whey off" were observed after 8 months of storage.

In order to examine the changes in the amount of destabilized protein of which the main component seemed to be composed of casein complex, the amount of destabilization-N by low speed centrifugation was determined. As shown in Fig. 2, the amount of sedimentation-N by low speed centrifugation in Samples I and II began to increase after 3 and 6 months of storage, respectively.

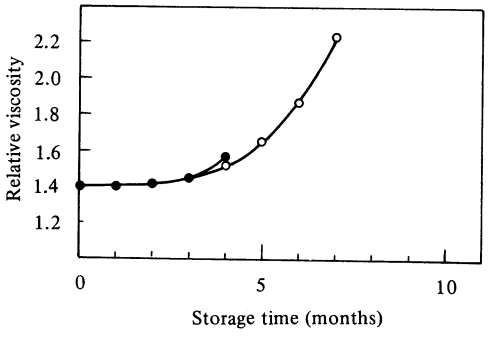

Fig. 1. Changes in the relative viscosity of Samples I and II during storage.
●, Sample I (stored at 30°C)
○, Sample II (stored at 5°C).

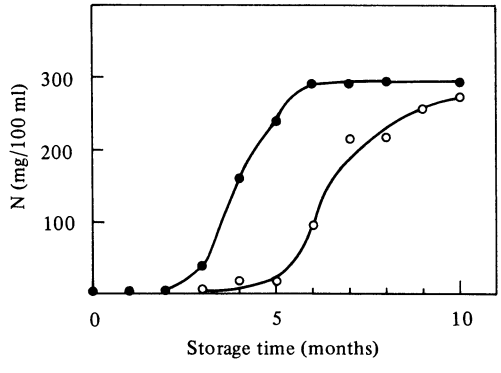

Fig. 2. Changes in the amount of sedimentation-N by low speed centrifugation during storage of Samples I and II.
●, Sample I (stored at 30°C)
○, Sample II (stored at 5°C).

Table 1. Changes in the pH of Samples I and II during storage.

Storage time (months)	Sample I*	Sample II**
0	6.61	6.61
5	6.51	6.57
10	6.48	6.58

* stored at 30°C.
** stored at 5°C.

As shown in Table 1, the pH of Samples I and II decreased slightly during storage. The decrease of pH was smaller in Sample II than in Sample I.

2. Changes in soluble casein and NPN

As shown in Fig. 3, the amount of the total-N in the ultracentrifugal supernatant of Samples I and II increased during storage. On the other hand, in Sample I the amount of soluble casein-N decreased during storage. In the case of Sample II, the amount of soluble casein-N increased at early stage of storage but decreased after 2 months of storage. The increase of the total-N in the ultracentrifugal supernatant seems to be caused by the increase of NCN. Since the soluble casein-N after sterilization contains heat-denatured whey protein-N, the net value of the soluble casein-N must be less than the one shown.

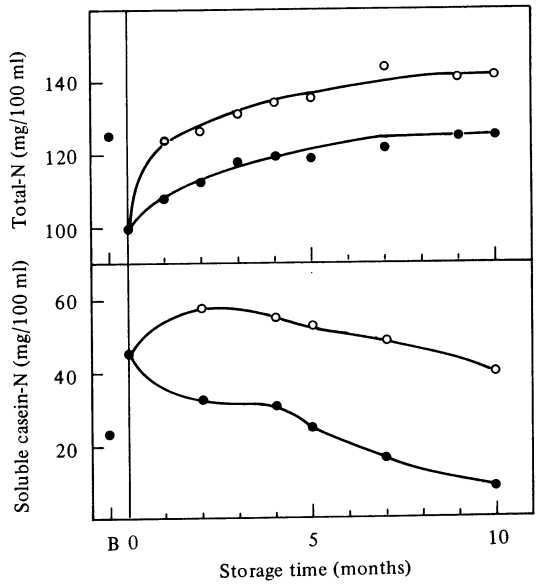

Fig. 3. Changes in the amounts of total-N in the ultracentrifugal supernatant and soluble casein-N during storage of Samples I and II.
●, Sample I (stored at 30°C)
○, Sample II (stored at 5°C)
B, before sterilization.

As shown in Table 2, the amount of NPN increased during storage in both Samples I and II. The in-

Table 2. Changes in the amount of NPN of Samples I and II during storage.

Storage time (months)	Sample I* (mg/100 ml)	Sample II**
0	26	26
5	35	30
10	46	35

* stored at 30°C.
** stored at 5°C.

crement of NPN was larger in Sample I than in Sample II.

3. Changes in the amount of Ca and P in the ultracentrifugal supernatant during storage

In order to examine the changes in the salts equilibrium during storage of the SSM, the amounts of calcium and inorganic phosphorus in the ultracentrifugal supernatant were determined.

As shown in Fig. 4, the amount of calcium in the ultracentrifugal supernatant of Sample I decreased gradually during storage, while that in the ultracentrifugal supernatant of Sample II was kept almost constant during storage. The amount of inorganic phosphorus in the ultracentrifugal supernatant of Sample I decreased during storage (Table 3). In the case of Sample II, the decrease of the inorganic phosphorus was very slight.

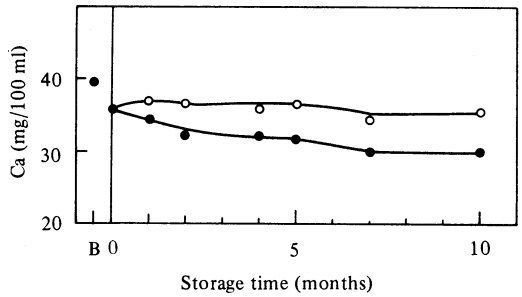

Fig.4. Changes in the amount of Ca in the ultracentrifugal supernatant of Samples I and II during storage.
●, Sample I (stored at 30°C)
○, Sample II (stored at 5°C).
B, before sterilization.

Table 3. Changes in the amount of inorganic phosphorus in the ultracentrifugal supernatant.

Storage time (months)	Sample I* (mg/100 ml)	Sample II** (mg/100 ml)
0	33.2	33.2
1	32.4	33.9
5	30.9	32.8
10	31.0	32.9

* stored at 30°C.
** stored at 5°C.

DISCUSSION

Although many studies have been made on gelation or sedimentation during storage of UHT milk, the mechanism of gelation has not been elucidated sufficiently. The following causes have been presumed for gelation. Samuelsson and Holm[2] observed that there was an inverse relationship between the degree of protein decomposition and onset-time of gelation. They attributed gelation to the reactivation of proteolytic enzymes. On the contrary, Samel *et al.* [4] reported that proteolysis was not the primary cause of gelation during storage. Weelock and Hindel[5] reported that the amounts of carbohydrates attached to κ-casein decreased during storage, and considered that the loss of carbohydrates could be one of factors in the development of gelation during storage of UHT milk. Andrews and Cheeseman[6–9] reported that the polymerization of casein by the Maillard reaction during storage and sediment formation was due to covalent cross-linking of polypeptide chains.

When the SSM was stored at 5°C, its appearance differed from that of the SSM stored at 30°C. The relative viscosity of Sample II stored at 5°C increased markedly during storage, while the increase of the relative viscosity of Sample I stored at 30°C was small (Fig. 1). The sediment of Sample II looked like a soft gel and have a larger volume than that of Sample I. In the case of sterilized concentrated skim milk, cold-storage caused remarkable increase of viscosity and gelation.[21] Liang *et al.* [22] reported that the swelling of casein micelles occurred when skim milk was cooled. Accordingly, it is assumed that the increase of the relative viscosity during storage of Sample II may be due to the swelling of casein complex.

Cold-storage of the SSM depressed the increase of NPN and the decrease of the amounts of calcium and inorganic phosphorus in the ultracentrifugal supernatant (Tables 2 and 3, Fig. 4). In the previous paper, [11] the change in the salts equilibrium was assumed to be one of the causes of the destabilization of the casein complex during storage of the SSM. In the case of Sample II, the destabilization of the casein complex could not be explained by the salts equilibrium since almost no change occurred in this respect. As above-mentioned, the appearance of Sample II differed from that of Sample I. It is considered that the mechanism of the destabilization of the casein complex during cold-storage of the SSM may differ from that of the destabilization during storage at ambient temperature.

SUMMARY

In order to examine the effects of cold-storage on the changes in the casein complex in the sterilized skim milk (SSM), the SSM was stored at 30°C (Sample I) and 5°C (Sample II). The results obtained are summarized as follows.

1) The relative viscosity of Sample II increased markedly during storage, while the increase of the relative viscosity of Sample I was small.
2) Visible sediment and "Whey off" were observed in Samples I and II after 5 and 8 months of storage, respectively.
3) The increase of NPN of Sample II was smaller than that of Sample I.
4) The amount of calcium in the ultracentrifugal supernatant of Sample I decreased, while that in the ultracentrifugal supernatant of Sample II was kept almost constant.

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滅菌脱脂乳貯蔵中のカゼイン複化合物の変化に及ぼす低温貯蔵の影響

青木孝良・畑中千歳・今村経明

1）130℃で瞬間加熱することにより，滅菌脱脂乳を調製した。試料Ⅰは30℃で，試料Ⅱは5℃で貯蔵した。1月毎に10月間，相対粘度，不安定化カゼイン量，可溶性カゼイン量，非蛋白態窒素量および超遠心上澄液中のカルシウムと無機リン量を調べた。

2）試料Ⅱの相対粘度は貯蔵中著しく増加したが，試料Ⅰの粘度増加は小さかった。

3）試料Ⅰでは貯蔵5月後に，試料Ⅱでは貯蔵8月後にホエーの分離が認められた。

4）貯蔵中の非蛋白態窒素の増加は，試料Ⅰより試料Ⅱの方が少なかった。

5）超遠心上澄液中のカルシウム量は，試料Ⅰでは貯蔵中減少したが，試料Ⅱではほとんど減少しなかった。

6）これらの結果から，低温貯蔵により起きるカゼイン複化合物の不安定化と室温貯蔵の場合のそれとでは，その機構が異なるものと推察された。