Article

Effects of pH, Calcium and Unheated Casein Micelle on Physical Properties of High-Temperature Heated Milk Rennet Curd

Tomio Ohashi*, Seiichi Haga*, Kiyoshi Yamauchi* and N.F. Olson**

*Faculty of Agriculture, Miyazaki University, Miyazaki-shi, Miyazaki-ken, 880
**Department of Food Science, University of Wisconsin-Madison, Madison, Wisconsin 53706 U.S.A.

Skim milk, separated from bulk cow’s milk gotten from a dairy district in Miyazaki Prefecture, was heated at 85°C for 5 min, immediately followed by heating at 135°C in a moment on an experimental scale. The effects of pH, Ca and intact casein micelle on the physical properties (hardness, breaking energy and elastic modulus) of the rennet curd prepared from this high-temperature heated milk were investigated using a rheometer.

The results obtained were summarized as follows:

1. The physical properties of rennet curd of high-temperature heated and pH-adjusted (6.7-6.0) milk could not be improved up to those of unheated milk.
2. Using Ca-added (80 mg/100 ml) and pH-adjusted (6.7-6.0) milk, it could be seen that the physical properties of high-temperature heated milk were improved with pH lowering, whereas even the highest values of the physical properties obtained for pH 6.0-adjusted milk rennet curd could not come up to those of unheated one.
3. When unheated casein micelle (360 mgN/100 ml) of 0.35 or above as unheated casein micelle ratio were added to high-temperature heated milk at 6.6 or 6.2, the physical properties of milk rennet curd were greater than those of unheated one and the rennet curd of pH 6.2-adjusted milk was somewhat better than those of pH 6.6-adjusted one.
4. Rennet curd of high-temperature heated milk at pH 6.6 or 6.2 was remarkably improved by the combination of Ca addition (80 mg/100 ml) and unheated casein micelle fortification; pH 6.6 or 6.2-adjusted milk rennet curd was attained nearly same as unheated one by fortifying to 0.2 or 0.15 respectively, as unheated casein micelle ratio.
5. From those results it was confirmed that an adequate combination of both Ca addition and native casein micelle fortification as well as suitable acidification was essential for the improvement of high-temperature heated milk rennet curd.

All cheese manufacture depends upon the formation of milk curd by rennin (chymosin) or similar enzymes. Cheese milk is pasteurized under heating conditions that do not alter casein significantly the reaction with milk-clotting enzymes. Usually, pasteurization at 72°C for 15 sec and 63°C for 30 min or equivalent treatments have been selected for most cheese production1). However, in the recent dairy field, ultra high-temperature (UHT) heating treatment has been employed as one of the new methods for extending the properties of milk. Chemical changes and interactions among the constituents of UHT-processed milk are important in retarding enzymatic milk-clotting and in causing subsequent formation of a weak curd2)–8). On the other hand, an economical advantage plus recovery of more nutrients and reduction of the biological load of waste effluents could prompt the use of UHT heated milk to make Mozzarella cheese by the direct acidification procedure9).

In our previous paper9), skim milk was heated at 85°C for 5 min, immediately followed...
by heating at 135°C in a moment, and the effects of Ca and intact casein micelle on the physical properties (hardness, breaking energy and elastic modulus) of the rennet curd prepared from this high-temperature heated milk were investigated by the use of a rheometer. From the results obtained it was confirmed that the increase of soluble Ca and native micellar casein was essential for the improvement of rennet curd of high-temperature heated milk.

Furthermore, it is known that the rennet coagulation of milk depends very markedly on the hydrogen ion concentration and is stronger as the pH is lowered below that of milk14). However, there is no attempt to investigate the effect of pH value on the improvement of high-temperature heated milk rennet curd. Accordingly, the present study was conducted to investigate the effect of pH on the physical properties of high-temperature heated milk rennet curd in connection with the addition of Ca and/or unheated casein micelle.

Methods

Preparation of high-temperature heated skim milk

Fresh raw milk was obtained from a dairy district in Miyazaki Prefecture. Cold centrifugation at about 4°C was employed at 2,700 x g for 30 min to obtain skim milk with less than 0.05% milk fat. The skim milk was heated at 85°C for 5 min in a water bath, next at 135°C in a moment in a silicone oil bath and then at once cooled to below 4°C in an ice water according to previous paper10). This procedure was carried out on a small scale in a laboratory, and differed from that in a dairy factory.

Preparation of milk dialysate and casein micelle

Milk dialysate was prepared by dialysis against water according to the partly modified method of Fox and Morkiss16), as described in the previous paper10). Casein micelle was prepared by centrifuging unheated skim milk at 30,000 x g for 60 min at 20°C. The micelle was suspended in milk dialysate and again centrifuged as above. The pellet of casein micelle in a centrifuge tube was resuspended in milk dialysate followed by centrifugation again at 2,000 x g for 15 min at 20°C in order to obtain an unheated casein micelle suspension.

Adjustment of pH

The pH of high-temperature heated milk was adjusted at room temperature to values ranging between 6.0 to 6.7 by addition of either 1N HCl or 1N NaOH. The milk was vigorously stirred during acidification to avoid localized coagulation.

Addition of Ca and casein micelle to milk

Calcium chloride solution containing 100mg of Ca per ml was added dropwise to high-temperature heated milk, to give the concentration of 80mg-added Ca/ml. Unheated casein micelle suspension was also added to high-temperature heated milk in the range of 0.05 to 0.5 as unheated casein micelle ratio. This ratio represents (casein micelle suspension added) / (casein micelle suspension added + high-temperature heated milk) (v/v) as described by the previous paper10).

Determination of various nitrogens in milk

Protein fractions in milk were separated by the method of Rowland16) and analyzed for nitrogen by micro-Kjeldal method same as the previous paper10).

Determination of P and Ca

P was determined by Gomori method17), and Ca by atomic absorption spectrophotometry18). Total P and Ca in milk were determined for the milk samples, whereas soluble P and Ca for the supernatant liquids prepared by centrifuging at 109,800 x g for one hr at 20°C.

Measurement of milk rennet curd

Hardness of milk rennet curd was measured by the method of Ohashi et al.13); 100 ml portions of the milk samples were introduced into 100 ml beakers, brought to 35°C and then mixed with 5 ml of 0.5% rennet solution pre-warmed at 35°C. The rennet solution was prepared using powdered commercial rennet from Difco Laboratories Inc. After 30 min of incubation at 35°C in a water bath, curd hardness in grams was measured with Sanwariken JK-T 264 type rheometer as a curd knife cut the surface of milk rennet curd. These measurement conditions were 1.44mm/sec in penetration speed of the curd knife, 18 cm/min in chart speed and 0.1V in sensitivity of a recorder.

Breaking energy of milk rennet curd (stress x strain, dyne/cm²) was estimated on the basis
of curd hardness (g), cross section of the curd knife, length of milk curd and penetration depth of the knife. Elastic modulus, stress/strain of milk rennet curd (dyne/cm²) was calculated as a measure of elasticity in the instance of solid matter. The detailed calculation of breaking energy and elastic modulus for milk rennet curd was described previously19).

Results and Discussion

Chemical characteristics of high-temperature heated milk

The distribution of nitrogen, P and Ca in three skim milk samples (A, B and C) employed in this study are shown in Table 1. It was accepted from these results that the milk composition was very similar among three milk samples and the amounts of total N, protein N, total P and total Ca in milk were not affected by high-temperature heating process. On the other hand, casein N, albumin·globulin N, soluble P and soluble Ca in high-temperature heated milk were altered in comparison with unheated one as reported previously10). Casein N was calculated from the difference between total N and non-casein N. Albumin·globulin N was calculated as the difference between non-casein N and non-protein N. An increase in casein N and a decrease in albumin·globulin N were observed during high-temperature heating process as in Table 1. These changes were attributed to denaturation of whey protein fractions in milk as the result of heating, the formation of complexes with casein and whey proteins and their recovery in the acid precipitation procedure. Whey proteins are susceptible to denaturation while casein is least affected, and the conjugate between $\beta$-lactoglobulin and $\kappa$-casein is formed through disulfide bridges after heat treatment5). P and Ca in milk were less soluble at high temperature as given in Table 1. Free P and Ca lost after high-temperature process might have been transformed into colloidal form or precipitated with other milk constituents20).

Effect of pH on the physical properties of high-temperature heated milk rennet curd

In the manufacture of almost all varieties of cheese, milk proteins are coagulated to form continuous solid curd. The casein fraction of milk normally forms this curd and coagulation may be induced by adjusting the pH of milk to the isoelectric point of casein as one of major factors21). Therefore, first experiment was undertaken for examining the physical properties of high-temperature heated and pH-adjusted milk rennet curd. Using sample A (Table 1) adjusted to pH 6.0 to 6.7 region, physical properties of high-temperature heated milk rennet curd were determined as shown in Fig. 1(A). Amounts of soluble Ca in milk increased almost linearly with a decrease in pH: 31.6 mg/100 ml at pH 6.7 and 51.1 mg/100 ml at pH 6.0. Soluble Ca content in unheated milk, a control sample, was 35.0 mg/100 ml which was lower than that in high-temperature heated milk adjusted to pH 6.5. On the other hand, the physical properties of high-temperature heated milk could

<table>
<thead>
<tr>
<th>Item</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unheated</td>
<td>High temp.</td>
<td>Unheated</td>
</tr>
<tr>
<td>Total N</td>
<td>478</td>
<td>482</td>
<td>469</td>
</tr>
<tr>
<td>Protein N</td>
<td>435</td>
<td>438</td>
<td>430</td>
</tr>
<tr>
<td>Casein N</td>
<td>372</td>
<td>409</td>
<td>361</td>
</tr>
<tr>
<td>Albumin·globulin N</td>
<td>43</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>Total P</td>
<td>90.5</td>
<td>90.2</td>
<td>90.1</td>
</tr>
<tr>
<td>Soluble P</td>
<td>43.0</td>
<td>38.9</td>
<td>42.6</td>
</tr>
<tr>
<td>Total Ca</td>
<td>115.0</td>
<td>115.0</td>
<td>117.5</td>
</tr>
<tr>
<td>Soluble Ca</td>
<td>35.2</td>
<td>31.5</td>
<td>35.0</td>
</tr>
</tbody>
</table>

* Heated in the laboratory at 85°C for 5 min and immediately at 135°C in a moment.
not be estimated in the pH range between 6.4 and 6.7. A little formation of rennet curd was observed at pH 6.0 to 6.3; the highest values obtained at pH 6.0 were 8.8 g in hardness, 6.5 \times 10^4 \text{ dyne/cm}^2 in breaking energy and 12.5 \times 10^4 \text{ dyne/cm}^2 in elastic modulus. These values did not reach to those found in unheated milk, 43.5 g in hardness, 39.8 \times 10^5 \text{ dyne/cm}^2 in breaking energy and 49.6 \times 10^4 \text{ dyne/cm}^2 in elastic modulus. It was found from these results that the physical properties of rennet curd of high-temperature heated milk could not be improved up to those of unheated milk only by acidification.

Secondly, using high-temperature heated, Ca-added (80 mg/100 ml) and pH-adjusted (6.0 - 6.7) milk, soluble Ca in milk and physical properties of milk rennet curd were investigated. It could be seen from Fig. 1 (B) that the physical properties of this milk were improved with the decrease in pH and related to the amount of soluble Ca. Amounts of soluble Ca in milk were 91.3 mg/100 ml at pH 6.7 and 116.9 mg/100 ml at pH 6.0, and the difference amount of soluble Ca between pH 6.7 and 6.0 was 25.6 mg/100 ml. Physical properties of pH 6.7-adjusted milk rennet curd were 17.4 g in hardness, 11.6 \times 10^4 \text{ dyne/cm}^2 in breaking energy and 28.2 \times 10^4 \text{ dyne/cm}^2 in elastic modulus, while those of pH 6.0-adjusted milk rennet curd were 27.3 g, 19.9 \times 10^5 \text{ dyne/cm}^2 and 37.7 \times 10^4 \text{ dyne/cm}^2, respectively. Increasing values in physical properties with pH lowering from 6.7 to 6.0 were 6.9 g in hardness, 8.3 \times 10^5 \text{ dyne/cm}^2 in breaking energy and 9.3 \times 10^4 \text{ dyne/cm}^2 in elastic modulus. The highest values of physical properties obtained for pH 6.0-adjusted milk rennet curd could not attain to those of unheated milk rennet curd because of the denaturation of milk proteins by high-temperature heating.

**Effect of casein micelle addition on the physical properties of high-temperature heated milk rennet curd**

As described above, the rennet curd of high-temperature heated and Ca-added (80 mg/100 ml) milk adjusted to the pH ranging from 6.7 to 6.2 was not match for unheated milk rennet curd. Therefore, in order to improve the physical properties of high-temperature heated milk rennet curd, various amounts of unheated casein micelle, the substrate for rennet in milk clotting, were added to high-temperature heated milk at pH 6.6. The effect of casein micelle addition on rennet curd of high-temperature heated milk at pH 6.6 was examined using sample B shown in Table 1. Unheated casein micelle (360 mg N/100 ml) in the range of 0.05 to 0.5 as unheated casein micelle ratio, was added to high-temperature heated milk,
Fig. 2 Effect of casein micelle addition on the physical properties of high temperature heated milk rennet curd

and the physical properties of milk rennet curd were measured [Fig. 2(A)]. Hardness, breaking energy and elastic modulus of unheated milk rennet curd were 41.3 g, \(38.5 \times 10^4\) dyne/cm\(^2\) and \(48.5 \times 10^4\) dyne/cm\(^2\), respectively, as shown in Fig. 2(A) C*. When casein micelle in the range of 0.05 to 0.2 as unheated casein micelle ratio was added to high-temperature heated milk at pH 6.6, the physical properties of milk rennet curd could not be estimated. However, with increasing the addition of casein micelle ratio from 0.25 to 0.5, a considerable increase in the formation of milk rennet curd was caused and the three curves in Fig. 2(A) were nearly linear. At the casein micelle ratio of 0.35, hardness, breaking energy and elastic modulus of milk rennet curd were 70.5 g, \(66.3 \times 10^2\) dyne/cm\(^2\) and \(78.5 \times 10^2\) dyne/cm\(^2\), respectively, and these values were greater than those of unheated milk rennet curd. In addition, the physical properties of rennet curd from the milk adjusted to 0.5 as unheated casein micelle ratio were 159.5 g in hardness, \(148.7 \times 10^4\) dyne/cm\(^2\) in breaking energy and \(176.1 \times 10^4\) dyne/cm\(^2\) in elastic modulus, which were about 3.8 times those of unheated milk rennet curd.

The same milk as in Fig. 2(A) (Sample B shown in Table 1) were adjusted to pH 6.2 and the physical properties of its rennet curd were measured [Fig. 2(B)]. In the range of 0.05 to 0.2 as unheated casein micelle ratio, the physical properties of milk rennet curd could not be measured, similar to the results from pH 6.6-adjusted milk in Fig. 2(A), whereas in the casein micelle ratio of 0.25 to 0.5, the physical properties of milk rennet curd were highly improved with the increase of unheated casein micelle. Hardness, breaking energy and elastic modulus of milk rennet curd produced from the casein micelle ratio of 0.35, were \(80.6\) g, \(75.6 \times 10^2\) dyne/cm\(^2\) and \(90.7 \times 10^4\) dyne/cm\(^2\), respectively. These values were nearly twice those of unheated milk and were higher than those of milk rennet curd formed in the same unheated casein micelle ratio, 0.35, and at pH 6.6 as shown in Fig. 2(A). The physical properties of milk rennet curd from unheated casein micelle ratio of 0.5 in Fig. 2(B) were 198.6 g in hardness, \(175.7 \times 10^4\) dyne/cm\(^2\) in breaking energy and \(215.8 \times 10^4\) dyne/cm\(^2\) in elastic modulus, respectively, which were about 4.6 times those of unheated milk rennet curd and higher than those of pH 6.6-adjusted milk rennet curd.

From the results in Fig. 2(A) and (B), a combination of the adequate addition of unheated casein micelle and the appropriate lowering of pH value was essential for the improvement of high-temperature heated milk rennet curd.

Effect of casein micelle addition on the physical properties of high-temperature heated milk rennet curd

The physical properties of milk rennet curd could not be measured, similar to the results from pH 6.6-adjusted milk in Fig. 2(A), whereas in the casein micelle ratio of 0.25 to 0.5, the physical properties of milk rennet curd were highly improved with the increase of unheated casein micelle. Hardness, breaking energy and elastic modulus of milk rennet curd produced from the casein micelle ratio of 0.35, were \(80.6\) g, \(75.6 \times 10^2\) dyne/cm\(^2\) and \(90.7 \times 10^4\) dyne/cm\(^2\), respectively. These values were nearly twice those of unheated milk and were higher than those of milk rennet curd formed in the same unheated casein micelle ratio, 0.35, and at pH 6.6 as shown in Fig. 2(A). The physical properties of milk rennet curd from unheated casein micelle ratio of 0.5 in Fig. 2(B) were 198.6 g in hardness, \(175.7 \times 10^4\) dyne/cm\(^2\) in breaking energy and \(215.8 \times 10^4\) dyne/cm\(^2\) in elastic modulus, respectively, which were about 4.6 times those of unheated milk rennet curd and higher than those of pH 6.6-adjusted milk rennet curd.

From the results in Fig. 2(A) and (B), a combination of the adequate addition of unheated casein micelle and the appropriate lowering of pH value was essential for the improvement of high-temperature heated milk rennet curd.

Effect of casein micelle addition on the physical properties of high-temperature heated
Fig. 3 Effect of casein micelle addition on the physical properties of high temperature heated and Ca-added milk rennet curd

* Shown in Fig. 1 ** Added 80 mg/100 ml

and Ca-added milk rennet curd

The rennin-altered casein is very sensitive to coagulation by calcium ion\(^1\). On the other hand, high temperature heating had a fairly effect on the soluble Ca content in milk, as shown in Table 1. The decrease in the concentration of soluble Ca, especially Ca\(^{2+}\), may have altered some physicochemical properties of milk\(^2\). In the previous study\(^3\), it was shown that the physical properties of high-temperature heated milk rennet curd were maximal by the addition of 80 mg Ca/100 ml, but these were significantly inferior to those of unheated milk rennet curd because of the heat denaturation of milk proteins. In this study, the effect of the combination of unheated casein micelle fortification and Ca addition on the physical properties of high-temperature heated milk rennet curd was investigated at two different pH, 6.6 and 6.2, using sample C as shown in Table 1.

From the results in Fig. 3, the physical properties of milk rennet curd were remarkably improved in comparison with those for milk without added Ca in Fig. 2. When unheated casein micelle in the range of 0.05 to 0.2 as unheated casein micelle ratio was added to high-temperature heated milk, rennet curd of both casein micelle and Ca-added milk could be estimated. Three parameters indicating the physical properties of milk rennet curd, hardness, breaking energy and elastic modulus, were similarly increased with increasing unheated casein micelle up to unheated casein micelle ratio of 0.35. However, in the range of 0.35 to 0.5 as unheated casein micelle ratio, the increasing rate of elastic modulus was lower than that of hardness or breaking energy. It was shown that hardness and breaking energy of milk rennet curd displayed more similar behavior than elastic modulus of one. A detailed study concerning the relationship among those three parameters will be published in other paper using a number of milk samples.

In Fig. 3(A), when high-temperature heated milk was fortified to 0.2 as unheated casein micelle ratio and adjusted the pH to 6.6, the physical properties of milk rennet curd attained nearly same as those of unheated milk one. On the other hand, rennet curd of the high-temperature heated milk fortified to 0.15 as unheated casein micelle ratio and adjusted the pH to 6.2 was approximately similar to that of unheated one [Fig. 3(B)]. From those results, it was recognized that the physical properties of high-temperature heated, unheated casein micelle fortified and Ca-added milk rennet curd were remarkably improved by the pH lower-
ing of milk because of the release of Ca\(^{2+}\) from dissolved and colloidal complexes and the activation of rennin reaction.

Rennet coagulation of milk can be divided into two phases: the primary (or enzymic) phase, during which the proteolytic enzyme cleaves a phenylalanine–methionine bond of \(\kappa\)-casein creating a metastable state of the micelle, and the secondary (or nonenzymic) phase, where the milk subsequently gels and forms a clot. In addition to the perviously recognized mechanism of rennet coagulation involving calcium bridging and hydrophobic bonding, there may also be an interaction between the separate casein molecules through oppositely charged regions of suitable configuration, and arginine residues may be among those which contribute to the special configuration of the regions on the interaction of micelles\(^{23}\). In other experiment in which the lysine residues of \(\kappa\)-casein molecules were blocked there was a resultant loss of sensitivity to rennet coagulation\(^{24}\). All milk products are heated at some stages of production, and this heating may strongly influence the properties of the final products. This may depend in part for the effectiveness on the interaction of serum protein and casein micelles at high temperature, and the same reaction is normally to be avoided in pasteurizing milk for cheese manufacture. But, as suggested earlier, it may be possible to heat milk at high temperature to produce cheese containing whey protein as well as casein. From those results obtained in this study, it was confirmed that, besides the milk acidification and the addition of Ca, the increase of native micellar casein by the addition of unheated casein micelle was essential for the improvement of rennet curd of high-temperature heated milk.

This study was performed using high-temperature heated skim milk prepared on a small scale, as described above. Therefore, further investigation will have to be continued employing commercial, industrial equipment and machinery, so that the results obtained from this study may be utilized on the manufacture of cheese. A series experiments has been already designed in order to improve the physical properties of UHT milk rennet curd.

### Acknowledgment

The authors wish to thank the members of Miyazaki Prefecture Dairy Cooperative for supplying milk samples. This study was presented at the 28th Annual Meeting of the Japanese Society of Food Science and Technology, Fukuoka, Japan, April 4-6, 1981.

### References


(Received May. 11, 1981)

高温加熱牛乳のレンネットカード物性に及ぼす pH,
カルシウム及び未加熱ゼインミセルの影響

大橋登美男*・芳賀聖一*・山内 清*・N. F. オール
ソン**

*宮崎大学農学部
**米国ウィスコンシン大学食品科学部

宮崎県内の酪農地域で採取した牛乳を脱脂乳に調製
し、実験的な小さい規模において、まず 85℃で 5 分間、
その後直ちに 135℃で瞬間加熱を行い、高温加熱牛乳の
レンネットカード物性（硬度、破断エネルギー及び弾性
率）に及ぼす pH, Ca 及び未加熱カゼインミセルの
影響についてレオメーターを用いて検討した。
得られた結果の要点は次のとおりである。

(1) pH 6.7〜6.0 に調整した高温加熱牛乳のレンネット
カード物性は未加熱牛乳のレンネットカード物性に達
しなかった。
(2) Ca を添加し (80 mg/100 ml)，pH
6.7〜6.0 に調整した高温加熱牛乳のレンネットカード
物性は pH の低下に伴って改善されたが、最も改善さ
れた pH 6.0 においても未加熱牛乳のレンネットカード
物性に達しなかった。
(3) 高温加熱牛乳に未加熱カゼ
インミセル比として 0.35 またはそれ以上の未加熱カゼ
インミセル (360 mgN/100 ml) を添加し、pH 6.6 また
は 6.2 に調整すると、未加熱牛乳のレンネットカード物
性以上に改善され、pH 6.2 が pH 6.6 よりも幾分すぐ
れていることを認めた。
(4) Ca を添加し (80 mg/100 ml), さらに
未加熱カゼインミセルを添加した高温加熱
牛乳のレンネットカード物性は著しく向上した。この場
合、pH 6.6 または 6.2 に調整し、未加熱 カゼインミセ
ル比 として 0.2 または 0.15 の未加熱 カゼインミセル
(360 mg N/100 ml) をそれぞれ添加すると、未加熱牛
乳のレンネットカード物性近くまで改善することができ
た。
(5) 以上の結果から、高温加熱牛乳のレンネット
カード物性の改善には適切な酸性化とともに Ca と未加
熱カゼインミセルの添加を組み合せることが必要である
ことを認めた。