*KOREAN J. FOOD SCI. TECHNOL.*
*Vol.* 18, *No.* 5 (1986)

# Effects of Dairy Processing Operations on Milk Proteins

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Important operations in dairy manufacturing involve heat treatments, acidification usually by bacterial fermentation of lactose, action of enzymes, and ultrafiltration. In this communication, some effects of each of these treatments on the proteins of milk are discussed.

## I. What are the Milk Proteins?

The proteins of cows' milk consist of two principal groups caseins and whey proteins, caseins consist of four different kinds of polypeptide chains celled $\alpha s_1$-, $\alpha s_2$-, ß- and *k*-caseins. They are all phosphorylated to some extent (*i.e.* contain phosphate groups attached to serine residues) and the *k*-casein has some attached carbohydrate groups. Ca eins associate together and in milk they are in the form of micelles of about 100*nm* diameter. These contain all 4 kinds of the caseins together with some inorganic calcium and phosphate. The micelles are structured in such a way that the portion of the *k*-casein carrying the carbohydrate sticks out of them. It is hydrophilic (water-loving) and thus stabilizes the micelles.

The whey proteins of milk are in solution, not in micelles. They consist of four principal kinds of proteins, $\alpha$-lactalbumin, ß-lactoglobulin, serum albumin, and immunoglobulins. Table 1 and 2 give properties and content of the principal milk proteins.

## II. Heat Treatment

Milk is heated (1) to kill pathogenic bacteria, (2) to preserve the product by killing microorganisms and inactivating enzymes, and (3) to change composition or properties. Some of the effects of heat are undesirable, the processing must be adjusted to minimize undesirable and maximize desirable effects.

### Heat treatments may be classified as:

1. Thermization - for example a few seconds at 65°C to kill psychotropic bacteria.
2. Low Pasteurization - 30 min at 63°C or 15 sec. at 72°C to kill all pathogenic bacteria.
3. High Pasteurization - 20 sec. at 85°C or higher to kill most bacteria and inactivate most enzymes. Ultra high temperature (UHT) treatment is included here.
4. Sterilization - 30 min at 110°C, 30 sec. at 130°C or 1 sec. at 145°C to kill all microorganisms including spores.

**Table 1. Properties and content of some milk proteins**

| Protein | Mol. Wt. | Residues/mole | | | | | | | Content (*g/l*) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Pro | Cys | -S-S- | P | pI | $A_{280}$ | Bovine | Human |
| $\alpha s_1$-Casein | 23,614 | 199 | 17 | 0 | 0 | 8 | 5.0 | 10.1 | 10.0 | 3.3 |
| $\alpha s_2$-Casein | 25,230 | 207 | 10 | 2 | ? | 11 | 5.3 | 14.0 | 2.6 | |
| ß-Casein | 23,983 | 209 | 35 | 0 | 0 | 5 | 5.2 | 4.5 | 9.3 | |
| *k*-Casein | 19,023 | 169 | 20 | 2 | ? | 1 | 5.5 | 10.5 | 3.3 | |
| $\alpha$-Lactalbumin | 14,176 | 123 | 2 | 8 | 4 | 0 | 5.4 | 20.9 | 1.2 | 1.5 |
| ß-Lactoglobulin | 18,363 | 162 | 8 | 5 | 2 | 0 | 5.3 | 9.5 | 3.2 | neg. |
| Serum albumin | 66,267 | 582 | 34 | 35 | 17 | 0 | | 6.6 | 0.4 | 0.4 |
| Lactoferrin | ca 90,000 | ca 700 | 28 | | | | | | <0.1 | 1.5 |
| Lysozyme | 14,701 | 130 | 2 | 8 | 4 | 0 | | | neg. | 0.4 |
| Immunoglobulins | | | | | See Table 2 | | | | | |

**Table 2. Concentrations of immunoglobulins in bovine blood serum, colostrum, and milk**

| Immuno-globulin | $g \cdot kg^{-1}$ | | | Percentage of Total Ig | | |
|---|---|---|---|---|---|---|
| | Serum | Colostrum | Milk | Serum | Colostrum | Milk |
| IgG1 | 11.00 | 47.60 | 0.60 | 50.0 | 81.0 | 73.0 |
| IgG2 | 7.90 | 2.90 | 0.02[a] | 36.0 | 5.0 | 2.5 |
| IgA | 0.50 | 3.90 | 0.14 | 2.0 | 7.0 | 18.0 |
| IgM | 2.60 | 4.20 | 0.05 | 12.0 | 7.0 | 6.5 |
| FSC | — | 0.20 | 0.05 | — | — | — |

[a] Some authors report IgG2 contents up to 10 times this amount.

## IIA. Denaturation

Denaturation involves the disruption of secondary and tertiary structure of proteins so that they uncoil and unfold. The result is a reduction in solubility and loss of biological properties such as enzymatic power. Caseins are naturally in rather unfolded and uncoiled structures and are not subject to heat denaturation. Caseins are very insoluble at pH 4.6 and readily precipitate when milk is adjusted to that pH. Whey proteins are all readily denatured by heat treatments in the high pasteurization range. When denatured they are insoluble at pH 4.6 and precipitate at that pH with the caseins. Figure 1 illustrates the heat denaturation of whey proteins at various temperatures. These measurements were made by performing the heat treatment indicated, acidifying to pH 4.6 and analyzing the mixture of proteins remaining in solution. Note that the individual whey proteins differ increase of denaturation. The immunoglobulins are the most readily denatured and the $\alpha$-lactalbumin is the most resistant. A small fraction (called "proteose-peptone in this figure but later shown to be casein fragments) seems to be undenaturable. The net result of heat denaturation of whey proteins is to make them behave more like caseins. They will be included in cheese curd made by acidifying processes thus increasing the yield of cheese.

The enzymes in milk also differ increase of denaturation as will be seen in a later figure.

## IIB. Browning reaction

Heat treatment induces a reaction between free amino groups in proteins and aldehyde groups of sugars such as lactose as follows:

$$RNH_2 + O = \overset{\displaystyle H}{\overset{|}{C}} - R' \rightarrow RN = \overset{\displaystyle H}{\overset{|}{C}} - R' + H_2O$$

The resulting product rearranges and disintegrates, some of the products giving undesirable flavor and others brown color to the product. This series of reactions is called the Maillard or "browning" reaction. In milk the principal reactants are the sugar lactose and amino groups of lysine side chains of the proteins. Besides producing the undesirable brown color and associated flavors, this reaction results in loss of available lysine, an important dietary essential amino acid.

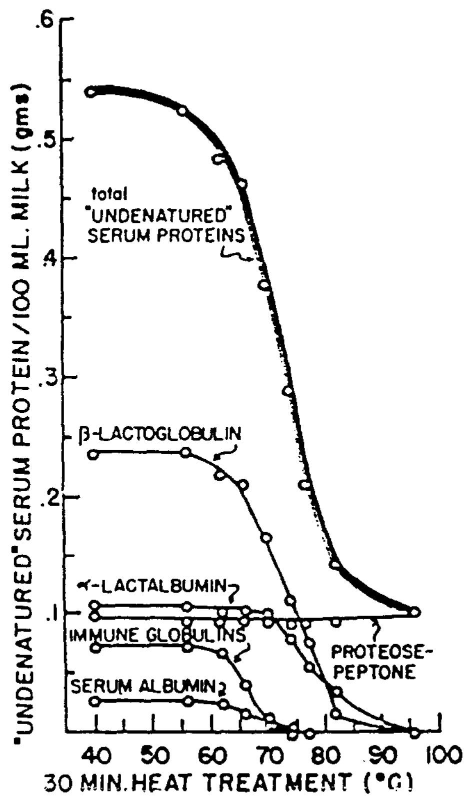


**Fig. 1. Heat denaturation of whey proteins at various temperatures**

### IIC. Reactions of side chain groups

Table 3 shows some important heat induced reactions of protein side chains. Among them are dephosphorization of the phosphoserine groups of caseins, disulfide interchange, and the formation of lysinoalanine. Table 4 shows the temperature dependence of some of these processes. They differ markedly in activation energy and $Q_{10}$. This may also be seen in Figure 2. Table 5 shows that loss of available lysine by heating is not serious except under drastic conditions of heating, as in sterilization.

### IID. Heat coagulation

Severe heating of milk destabilizes the casein particles of milk and they coagulate. This is not a serious problem except in concentrated milks. The susceptibility to coagulation increases greatly as the total solid content of the milk is increased. Considerable stabilization against coagulation can be brought about by preheating the milk before concentration. It is thought that this is due to an interaction between ß-lactoglobulin and *k*-casein through disulfide (-S-S-) linkages. Heat coagulation depends markedly on the pH as may be seen in Figure 3. A remarkable feature is that two types of milk are found as illustrated by A and B in the figure. Heating milk produces acid by the degradation of lactose as shown in Figure 4. The increase in acidity tends to destabilize the casein so that it coagulates more readily during the heating.

## III. Acidification

As mentioned earlier, the caseins are very insoluble

**Table 3. Possible reactions of side-chain residues of proteins at high temperature**

| | Reactants | | Products |
|---|---|---|---|
| 1. | $\vdash CH_2-CONH_2+H_2O$<br>Asparagine | → | $\vdash CH_2-COOH+NH_3$<br>Aspartic acid |
| 2. | $\vdash (CH_2)_2-CONH_2+H_2O$<br>Glutamine | → | $\vdash (CH_2)_2-COOH+NH_3$<br>Glutamic acid |
| 3. | $\vdash CH_2-O-PO_3^{2-}+H_2O$<br>Phosphoserine | → | $\vdash CH_2-OH+HPO_4^{2-}$<br>Serine |
| 4. | $\vdash CH_2-O-PO_3^{2-}$<br>Phosphoserine | → | $\models CH_2+HPO_4^{2-}$<br>Dehydroalanine |
| 5. | $\vdash CH_2-SH+OH$<br>Cysteine | → | $CH_2-S^-+H_2O$ |
| 6. | $\vdash CH_2-S-S-CH_2\dashv$ + $\vdash CH_2-S^-$ | → | $\vdash CH_2-S^-$ + $\vdash CH_2-S-S-CH_2\dashv$ |
| 7. | $\vdash CH_2-S^-+S^--CH_2\dashv$<br>Cysteine | → | $\vdash CH_2-S-S-CH_2\dashv$<br>Cysteine |
| 8. | $\vdash CH_2-S$<br>Cysteine | → | $\models CH_2+HS^-$<br>Dehydroalanine |
| 9. | $\models CH_2+HS-CH_2\dashv$ | → | $\vdash CH_2-S-CH_2\dashv$<br>Lanthionine |
| 10. | $\vdash (CH_2)_4-NH_3^++H_2C\dashv+OH$<br>Lysine | → | $\vdash (CH_2)_4-NH-CH_2\dashv+H_2O$<br>Lysinoalanine |
| 11. | $\vdash (CH_2)_4-NH_3^+ + O_2C-CH_2\dashv$<br>Lysine Aspartic acid | → | $\vdash (CH_2)_4-NH-CO-CH_2\dashv$<br>$+H_2O$<br>$\varepsilon$-N-($\beta$-aspartyl) lysine |
| 12. | $\vdash (CH_2)_4-NH_3^+ + O_2C\dashv (CH_2)_2\dashv$<br>Lysine Glutamic acid | → | $\vdash (CH_2)_4-NH-CO-(CH_2)_2\dashv$<br>$+H_2O$<br>$\varepsilon$-N-($\gamma$-glutamyl) lysine |

**Table 4. Approximate examples of the temperature dependence of some reactions**

| Reaction | Activation Energy ($kJ \cdot Mol^{-1}$) | $Q_{10}$ at 100°C |
|---|---|---|
| Many chemical reactions | 80-130 | 2.0-3.0 |
| Many enzyme-catalyzed reactions | 40- 60 | 1.4-1.7 |
| Hydrolysis, for example | 60 | 1.7 |
| Autoxidation of lipids | 40-100 | 1.4-2.5 |
| Maillard reactions (browning) | 100-180 | 2.4-5.0 |
| Dephosphorylation of caseinate | 110-120 | 2.6-2.8 |
| Heat coagulation of milk | 150 | 3.7 |
| Degradation of ascorbic acid | 60-120 | 1.7-2.8 |
| Heat denaturation of protein | 200-600 | 6.0-175.0 |
| Typical enzyme inactivation | 450 | 50.0 |
| Inactivation of milk proteinase | 75 | 1.9 |
| Killing vegetative bacteria | 200-600 | 6.0-175.0 |
| Killing of spores | 250-330 | 9.0-17.0 |

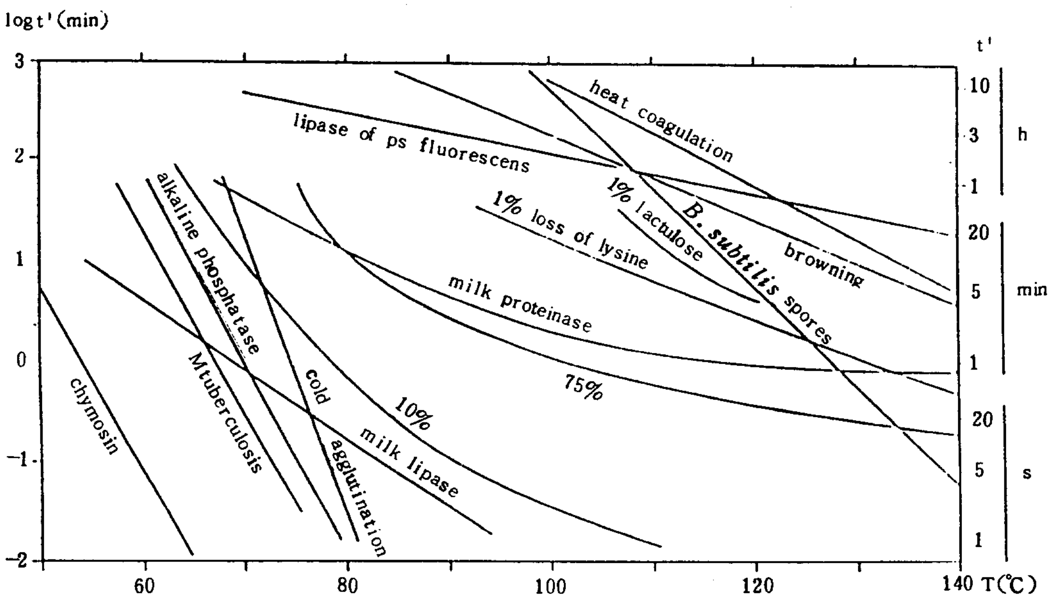


**Fig. 2. The time needed (t') at various temperatures (T) to inactivate some enzymes and cold agglutination;** to kill some bacteria and spores (*B. subtilus* reduced to 10%): to cause a certain degree of browning; to convert 1% of lactose to lactulose; to cause heat coagulation; to reduce available lysine by 1%; to make 10% and 75% of the whey proteins insoluble at pH 4.6 Approximate results after various sources.

**Table 5. Approximate loss(%) of some nutrients in milk by heat trearments**

| | Pasteurization | Boiling | UHT heating | Sterilization |
|---|---|---|---|---|
| Available lysine | <1 | ~5 | <1 | 2-10 |
| Vitamin $B_1$ | ~5 | 5-10 | 5-10 | 20-50 |
| Vitamin $B_6$ | ~0 | <5 | <10 | 5-20 |
| Folic acid | <5 | ~5 | <20 | <30 |
| Vitamin $B_{12}$ | 0-10 | 5-20 | 5-20 | 20-80 |
| Vitamin C | 5-25 | 5-50 | 5-30 | >50 |

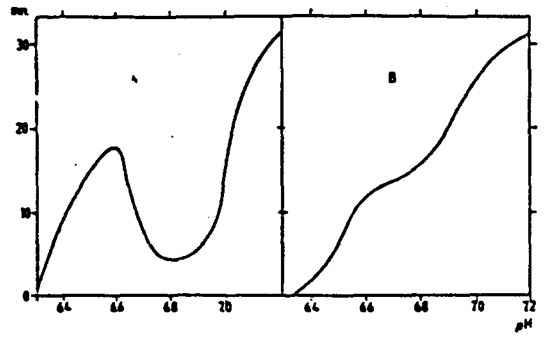


**Fig. 3. Examples of the heat coagulation time (minutes at 140°C) of milk as a function of pH**

at pH 4.6. Formation of lactic acid by bacterial fermentaion of lactose causes the casein to coagulate as the pH approaches 4.6. This is the basis of manufacture of various kinds of yogurts and cottage cheese products. If the milk is allowed to stand undisturbed as the acid develops a smooth coagulum forms. A wide variety of cultured milk products can be made by careful control of the fermentation process.

## IV. Enzymatic Action

Proteases attack some of the peptide linkages in milk proteins more or less specifically. As a matter of fact there is an enzyme called plasmin in milk itself which attacks ß-casein at three specific linkages as shown in Figure 5. About one-tenth of the ß-casein is cleaved by the time milk is drawn from the udder. The small fragments constitute "the proteose-peptone" component of the whey mentioned above. The large fragments, called γ-caseins, remain in the micelles.

28 ↓ 29
-ASN - LYS - LYS - ILE -

104 105 ↓ 106 107 ↓ 108 109
- PRO - LYS - HIS - LYS - GLY - GLN

**Fig. 5. Cleavage of ß-caseins by plasmin**

By far the most important protease in the dairy industry is chymosin (sometimes called rennin). It is the enzyme secreted in the stomach of young calves that coagulates the milk. It is widely used in the manufacture of many kinds of cheese. Chymosin has a high specificity for the linkage between phenylalanine at position 105 and methionine at position 106 in the *k*-casein molecule (Figure 6). As previously mentioned the casein micelles are stabilized by *k*-casein chains projecting from them. The hydrophilic part of the *k*-casein molecule lies in the fragment 105-169, called caseinomacropeptide. When this is cleaved off by chymosin the *k*-casein no longer protects the micelles and they coagulate. If the enzymatic acid occurs while the milk sits quietly a smooth curd is formed. Upon cutting and warming the curd shrinks expelling the aqueous solution called whey.

Various other kinds of proteases are sometimes used in making cheese. These attack the Phe 105-Met 106

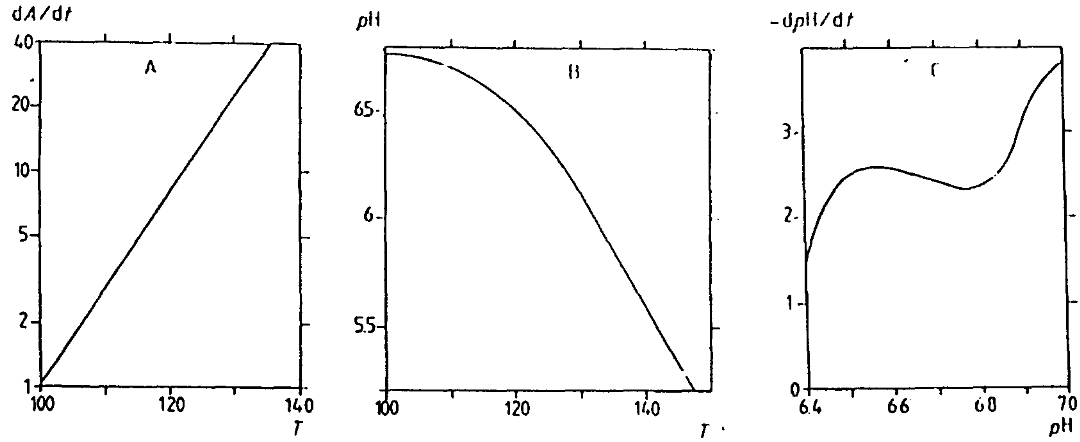


**Fig. 4. Effects of heating on the acidity of milk** (A) Acidity produced (mEq·$l^{-1}$·$h^{-1}$) at various temperatures (°C); (B) pH at room temperature after 30 min heating at various temperatures; (C) rate of change of pH (units per h) at 135°C as a function of initial pH. Examples from various sources.

BOVINE

HGLU --- SER -- PHE 105 ↓ 106 MET--ALA ---VALOH 169

HUMAN

HGLU --- SER -- PHE 93 ↓ 94 ILE -- ALA --- ALAOH 158

PARA-K-CASEINS CASEINOMACRO PEPTIDES

**Fig. 6. Cleavage of *k*-caseins by chymosin**

bond but some of them attack other bonds as well (Figure 7). This leads to the production of various peptides, some of which have a bitter flavor.

Another problem that may be caused in part by the action of proteases is age gelation of sterilized concentrated milk. The process is dependent on temperature (Figure 8). It evidently involves aggregation of casein micelles. In some cases, at least, it is caused by the slow attack of heat-resistant enzymes or enzymes from heat resistant organisms on the casein.

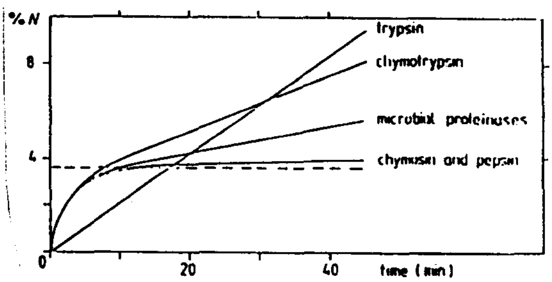


**Fig. 7. Action of various proteolytic enzymes on milk** Percentage of the protein N becoming soluble after adding the enzyme. The dotted line indicates the N in the "caseinomacropeptide." Approximate examples. Largely after C. Alais, *Science du Lait*. 3rd ed., 1974.

## V. Ultrafiltration

Filtration through membranes is used to fractionate and concentrate milk. The membrane is essentially a filter through which small molecules can pass while large molecules and colloidal micelles are retained. In

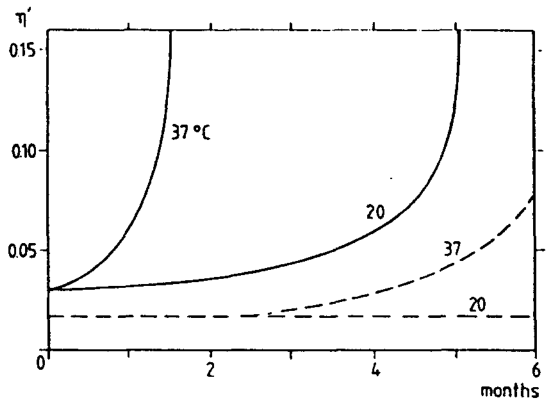


**Fig. 8. Age thickening of concentrated milk** Apparent viscosity (*n'*, Pa·s) as a function of time of storage at two temperatures (parameter). The broken lines concern milk to which polyphosphate had been added. Approximate result from various sources.

**Table 6. Examples of the composition of milk protein preparations**

| Product | Method of Preparation | Isolated from | Composition Protein (%) | Carbohydrate (%) | "Ash" (%) | "Fat" (%) |
|---|---|---|---|---|---|---|
| Acid casein | acidification | skim milk | 83-95 | 0.1-1.0 | 2.3-3.0 | ~2 |
| Na caseinate | acid-NaOH | skim milk | 81-88 | 0.1-0.5 | ~4.5 | ~2 |
| Rennet casein | renneting | skim milk | 79-83 | ~0.1 | 7.0-8.0 | ~1 |
| WP concentrate | ultrafiltration | whey | 50-75 | 8.0-40.0 | 1.0- 6.0 | 2-10 |
| WP concentrate | electrodialysis | whey | 27-37 | 40.0-60.0 | 1.0-10.0 | ~4 |
| Whey powder | spray drying | whey | 11 | 73.0 | 8.0 | 1 |
| WP complex | metaphosphate | whey | 55 | 13.0 | 13.0 | 5 |
| WP complex | CMC | whey | 50 | 20.0 | 8.0 | 1 |
| WP complex | Fe-polyphosphate | whey | 35 | 1.0 | 54.0 | 1 |
| Lactalbumin | heat-acid | whey | 78 | 10.0 | 5.0 | 1 |
| Coprecipitate | heat-acid | skim, buttermilk | 85 | 1.0 | 8.0 | 2 |

the ultrafiltration of milk the whey proteins partly pass the membrane along with lactose and various salts; the casein micelles are retained. Electrodialysis is sometimes applied to remove ions from milk through a membrane.

Reverse osmosis or hyperfiltration involves applying pressure to milk held by a membrane. Water containing some salts is forced through the membrane and the milk can be concentrated greatly. This process avoids heat damage to milk proteins that might occur in concentration by processes involving heating. Table 6 gives examples of some protein preparations that can be made from milk by various processes.

(Received June 16, 1986)