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Effects of Heat Treatment of Ultrafiltered Milk on its Rennet Coagulation Time and on Whey Protein Denaturation

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EFFECTS OF HEAT TREATMENT OF ULTRAFILTERED MILK ON ITS RENNET COAGULATION TIME AND ON WHEY PROTEIN DENATURATION

by

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ABSTRACT

Effects of Heat Treatment of Ultrafiltered Milk on its Rennet Coagulation Time and on Whey Protein Denaturation

by

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The purpose of this research was to determine the effects of heating (including heating to ultra-high temperatures) homogenized ultrafiltered whole and skim milks on whey protein denaturation and milk's subsequent rennet coagulation properties: coagulation time, curd firmness, and microstructure.

Whole and skim milk samples were ultrafiltered using a spiral wound ultrafiltration membrane system. Samples were preheated to 72°C for 58 s, held for 8 s then heated to 72, 89, 106, 123, or 140°C for more than 97 s and held for 4 s. The milk was then cooled to 60°C and homogenized, further cooled to 30°C, packaged into 120 ml sterile containers, and refrigerated overnight. Rennet coagulation time and curd firmness were monitored using a Formagraph. Milk and gel samples were fixed in 2.5% glutaraldehyde solution and examined by electron microscopy. Whey protein denaturation was determined by precipitating casein at pH 4.6 with .1N HCl and measuring protein content in the filtrate by the Kjeldahl procedure.

Rennet coagulation time of milk increased as processing temperature was increased. Gel strength decreased with an increase in processing temperature. Ultrafiltration shortened rennet coagulation time and increased gel firmness. Ultra-high- temperature-

heated whole and skim milks did not coagulate upon addition of rennet, but their concentrated counterparts did. Rennet coagulation of the concentrated milks was delayed by heating. Samples treated with ultra-high-temperature formed only a weak gel. The casein micelles in milk increased in size as a function of increasing processing temperature and concentration by ultrafiltration. Additional protein material adhered to the casein micelles after high-temperature processing and was especially noticeable in the samples treated with ultra-high-temperature. Whey protein denaturation increased as a function of increased heating temperature. The heated concentrated milks had higher levels of protein denaturation than the heated unconcentrated ones. (81 pages)

INTRODUCTION

Heating is a critical operation in the processing of milk and other dairy products. Milk is heated at various time-temperature combinations. Depending on the kind of dairy product intended, such heating conditions range from pasteurization, which kills harmful microorganisms and inactivates some enzymes, to sterilization sufficient to kill all microorganisms (12, 21).

There are many changes that occur in milk when it is heated. These depend on the temperature and the time it is held at that temperature. Heating milk at high temperatures for long times can cause heat-induced coagulation (21). Whey proteins, especially β-lactoglobulin, are denatured when milk is heated at high temperatures and are deposited on the surfaces of casein micelles. This increases rennet coagulation time because κ-casein becomes less accessible to enzyme action (88), and the denatured whey proteins hinder the interactions between micelles that cause them to aggregate. Excessive heating also leads to Maillard browning and subsequent losses in milk's nutritive and organoleptic value. Ultra-high-temperature (UHT) heating was designed to produce sterilized milk of better quality than retort sterilized milk. With UHT heating, bacteria can be destroyed without adversely affecting the organoleptic or nutritive properties of milk (56). However, age gelation can occur during prolonged storage of UHT milk at ambient temperatures. Much research has been conducted to determine the causes and methods of age gelation (80).

Concentrating milk by ultrafiltration (UF) has become of interest as a unit operation for modern dairy processing. Milk produced by UF is more stable than that produced by conventional evaporation methods (81). Many attempts have been made to produce cheese from ultrafiltered milks. Some cheese types can be successfully made from ultrafiltered milk, but making Cheddar or other hard cheeses is more difficult (10).

Use of UF to concentrate milk before UHT processing opens an array of possibilities for developing new dairy foods. Heating milk affects its microstructure

because of the physical changes that occur in milk during heating. An increase in casein micelle size is evident, as would be expected because of denatured whey proteins adhering to the casein micelles by complexing with surface κ–casein (12).

The objectives of this research were to determine the effects of heating (including heating to ultra-high temperatures) of homogenized ultrafiltered whole and skim milks on whey protein denaturation, and the milk's subsequent rennet coagulation properties: coagulation time, curd firmness, and microstructure.

LITERATURE REVIEW

Heat Treatments of Milk

In modern dairy technology, milk is heated to mild temperatures, as in cheese making, or to severe temperatures as in the making of UHT milk (21). Heating temperatures and times vary from pasteurization (73°C for 15 s) to UHT sterilization (120°C-140°C for several seconds) to retort sterilization (110-120°C for 20-40 min) (12). Because many enzymes present in milk are inactivated by pasteurization, their inactivation can be used to measure pasteurization efficiency. While pasteurization is effective against pathogenic and most spoilage microorganisms in milk, sterilization destroys all microorganisms (9).

Many reversible and irreversible changes occur when milk is heated. Examples of some reversible changes are mutarotation of lactose, changes in ionic equilibria, pH shifts, conformation changes of proteins, cold agglutination of fat, association of caseins, and association of fat. It is the irreversible changes that occur during heating of milk that are most important (85).

When milk is heated during sterilization, evaporation, or drying the following events occur: whey proteins are denatured and aggregate, ionic and soluble calcium and magnesium phosphates and citrates are converted to colloidal phosphates and citrates, and these colloidal phosphates are deposited onto casein micelles. Also Maillard browning reactions occur between proteins and lactose or other added reducing sugars (56). These changes are irreversible. Heating milk can also lead to nutritive losses, changes in milk color, and development of off-flavors (87).

Heat-induced coagulation can also occur when milk is heated at high temperatures for a long time. It is primarily caused by a lowering of pH during heating as well as a decrease in ζ–potential, casein hydration, and hydrolysis of κ-casein. Acidity is increased because of production of organic acids (especially formic acid) from lactose and of release of hydrogen ions from calcium phosphate or casein-bound phosphate (21).

When β-lactoglobulin is heated, it is denatured, probably through exposure of its SH groups so that they interact with other protein molecules that are at lower temperatures (89). Denatured β-lactoglobulin can then complex with κ-casein. Heating of κ-casein alone does not lead to complex formation when it is mixed with β-lactoglobulin. Adding β-lactoglobulin to a solution of κ-casein retards coagulation of κ-casein by chymosin. This may be because of competition between the proteins for binding to chymosin, which tends to associate with all proteins before hydrolyzing a peptide. Heating a mixture of β-lactoglobulin and κ-casein brings about a further increase in clotting time because spatial interference by complexed β-lactoglobulin prevents binding of the enzyme to the Phe_{105}–Met_{106} region of κ-casein.

Effects of heating milk on subsequent processing

During the manufacture of evaporated milk, a forewarming treatment is applied before retort sterilization. If a high forewarming temperature (140°C, 25 s) is used, the initial heat stability of the evaporated milk is increased. Forewarming at or below 65°C has little effect on heat stability (59). Also, milk heated above pasteurization conditions has increased stability against coagulation by rennet. This increases (cheese make time) and a curd is formed that retains excessive moisture and results in a soft-bodied cheese (88).

The yield of Cheddar cheese from milk can be increased by heating milk at 97°C for 15 s. This denatures 30% of whey proteins but does not inhibit rennet action. It is necessary to add extra calcium and acidify the milk to pH 6.4 to obtain normal coagulation and curd firming (48). Cheese made from such overheated milk does not fuse well; so further adjustments to the make procedure (such as increasing cook temperature and cheddaring time) must be made.

Cheese made from milk heated at 85°C for 20-30 s contains an increased amount of soluble nitrogenous compounds compared to cheese made from milk heated to 74°C for the same time. There are, however, no qualitative differences in electrophoretic patterns of

cheese proteins. When Cheddar cheese is made from milk heated above pasteurization temperatures, its flavor development is abnormal; a sulfide cooked flavor develops (55). The reduction of S-S bonds of β-lactoglobulin to SH groups during heating gives rise to the observed changes in flavor of such cheeses and causes alterations in the properties of κ-casein (45). The heat–induced association of β-lactoglobulin and κ-casein of casein micelles occurs mainly between 85—90°C. This association is caused by formation of intermolecular S–S bonds and hydrophobic interactions (79).

Ultra-High Temperature Processing

UHT treatment of milk produces a sterile milk of better organoleptic quality than retort sterilized milk. It takes advantage of the higher thermal coefficient of biological reactions leading to faster destruction of bacteria compared to detrimental chemical reactions, that lead to undesirable browning, flavor changes, and nutrient degradation (87). However, UHT processing still causes a severe to mild "cooked-flavor" defect in milk. Although this cooked flavor is much less intense in UHT milk than in retort-sterilized milk products, such as evaporated milk, it is replaced by the characteristic UHT stale flavor (56). There are also some denaturation and aggregation of whey proteins that occur during UHT sterilization of milk. The denatured whey proteins bond with κ-casein causing the casein micelles to increase in size. Some Maillard browning reactions between lactose and proteins take place and some vitamins are also destroyed (22).

An undesirable consequence of UHT sterilization of milk products is that an irreversible gelation can occur during prolonged storage. The chemical and physical nature of this age gelation phenomenon has not been explained well. Enzymic hydrolysis of proteins by heat stable proteinases that survive UHT treatment (or proteinase precursors, such as plasminogen, that are activated during storage) have been implicated as a cause of age gelation (11). However, it has yet to be shown whether age gelation is solely initiated by enzymic processes or whether it can occur by physico-chemical effects (80).

Various time and temperature combinations have been used in the manufacture of UHT milk products, and these vary between countries. In the United Kingdom, heating conditions of 132°C for more than 1 s are required for UHT processing of milk and of 132°C for more than 2 s for creams (5). In the United States, temperatures in the range of 135–150°C for various times are being used to produce commercially sterile milk products that are then aseptically packaged to retain sterility. UHT systems are approved for use, taking into account the equipment being used and the products being processed. UHT products can be heated either directly by steam injection or infusion or indirectly in a plate-, tubular-, or scraped-surface heat exchanger. Raw milk is commonly preheated to 80–85°C in an indirect heat exchanger then heated to the final UHT temperature (58).

Direct heating provides almost instantaneous distribution of heat throughout the product with the UHT temperature being achieved in a fraction of a second. The product is then held at that temperature for a set time before being cooled in a vacuum evaporator. Direct heating at 142°C for 4 s is insufficient to inactivate native milk proteinases (such as plasminogen) or proteinases produced by pyschotrophic bacteria during cold storage of raw milk. Directly heated UHT milk is therefore more susceptible to age gelation than the indirectly heated UHT milk (80). When milk is heated by addition of steam, it is diluted. This is corrected by the removal of condensate during evaporative cooling of the sterile product (58).

Indirect heating of milk requires a longer time to reach the UHT temperature and therefore causes more product deterioration than direct UHT processing. It can also cause product "burn on" on the heat exchanger, and that reduces the heat exchange rate (22).

Homogenization

Homogenization is the process of subjecting milk to a large pressure drop across a pressure valve. It is typically carried out at a temperature of 60°C or more in a one- or two-stage process at a pressure of 14–18 MPa. As milk passes across the homogenizer's first

stage valve, its pressure rapidly drops causing intense cavitation. The turbulence of this cavitation shears the milk fat globules and subdivides them into smaller droplets (6, 27).

Homogenization of UHT milk is necessary to prevent creaming during storage. This occurs because the fat globules are reduced from their natural size of 2–10 μm in diameter to less than 1 μm. Fat globules less than 7 μm will not rise and form a cream layer (or ring) because of the overriding influence of Brownian movement (59). In conjunction with this size reduction, the formation of a new protein layer on the surface of the fat droplets prevents their aggregation.

Parry (68) found that homogenization causes a six-fold increase in the fat globule surface area and increases the viscosity and foaming properties of milk. He also reported that susceptibility to light-activated flavor change and to oxidized flavor are decreased in homogenized milk while susceptibility to lipolysis is increased. Curd firmness of milk gels is reduced when milk is homogenized, and heat stability of high fat milk is lowered. Homogenization also alters the physical condition of the proteins in milk and makes them more easily coagulated by acids. The increased number of fat globules, greater surface area, and the break up of the milk fat globule membrane make homogenized milk susceptible to enzymic lipolysis and consequent development of rancidity. Therefore, homogenized milk must be made from pasteurized milk, or milk must be pasteurized promptly after homogenization (42).

Homogenization affects milk fat emulsion, whey protein, and casein dispersion in milk (26, ,63). The interfacial membrane between fat droplets and serum is composed of casein micelles, micellar subunits, and nonmicellar proteins (64, 76). There is a preferential adsorption of large casein micelles by small fat globules. Quantity of protein adsorbed on the fat droplets depends upon milk temperature during homogenization. It does not vary with protein/fat ratio unless less protein is present to coat the fat droplets.

Homogenization conditions are important in determining the initial heat stability of concentrated milk. Across a wide pH range, heat coagulation time is shortened as pressure

of homogenization is increased (81). Homogenizing milk before preheating reduces the heat stability of evaporated milk. This is because of the adsorption of casein micelles onto the newly formed milk fat globule surfaces (61, 68). Homogenizing milk after sterilization minimizes the effect on heat stability (59).

The efficiency of homogenization can be improved by increasing homogenization pressure and temperature. Within the range 22–65°C, temperature has no effect on heat stability, but it is recommended that homogenization be at the highest temperature compatible with individual plant operation and with other changes in the product (42).

Concentration by Ultrafiltration

Concentration is an important principle in milk processing. It is accomplished by removing water from milk by evaporation, or by membrane processes such as ultrafiltration (UF) or reverse osmosis (RO). UF is differentiated from RO by the relative porosity of the membranes. In UF, large molecules are retained and most of the small solute molecules such as salts, sugars, and most flavor compounds can pass through the membrane and are lost with the water. Osmotic pressure decreases because it depends upon the total number of particles in solution (41).

Longerman (43) observed that UF does not affect serum casein, Ca, or P content of casein micelles, or the size distribution of casein micelles . However, UF combined with diafiltration (addition of water while ultrafiltering) can remove up to 99% of the lactose, 36% of the Ca, and 42% of the P from skim milk. There is a limited permeation of Ca and P because of their association with casein micelles (86). Depending upon the pore size of the UF membrane, there is a 90% or higher retention of protein (8). Fat is 100% retained. Even though homogenization may increase the number of fat globules by more than three times, their comparatively large size still prevents their permeation through the membrane. Lactose passes freely through UF membranes due to its relatively low molecular weight and high solubility in water. Fat soluble vitamins are retained in

concentrated milk along with the fat. Vitamin B12 and folic acid, in spite of being water soluble, are also completely retained because of their association with proteins (86).

Properties of UF milk

Skim milk concentrate prepared by UF is more heat stable than that prepared by evaporation and is not appreciably affected by forewarming or addition of permitted stabilizers (81). UF concentrated milk has been suggested as a means of supplying milk proteins in a form acceptable to some members of every race who are lactose intolerant. Because of the reduced lactose content, Maillard reactions occur to a lesser extent in UF milks than in evaporated milks. Newstead et al. (61) observed that concentrating milk before preheating and homogenizing has a stabilizing effect on evaporated milk; while homogenizing before preheating causes the preheating to have a destabilizing effect .

Although heat-induced-coagulation depends on pH within the range of 6.2—6.8, concentrated milk remains very stable as pH is increased above 6.8. β-lactoglobulin induces more pH sensitivity on concentrated caseinates than on their unconcentrated counterparts (60). Dalgleish (13) showed that although rennet clotting time (RCT) is little affected by UF concentration of milk, the proportion of casein which is soluble at RCT depends upon the concentration of both milk and rennet.

At RCT of milk concentrated to over 20% total solids, the total nitrogen depletion curve changes from a single stage to two-stage process (60). Furthermore, addition of β-lactoglobulin to UF concentrated skim milk destabilizes the heated milk while the opposite effect is observed in presence of sulfhydryl group blocking agents. This suggests that the mechanism of coagulation in UF concentrated milk is similar to that which occurs within the RCT–pH profile of skim milk at normal levels of total solids.

Whey protein denaturation in skim milk is not significantly influenced by solids content up to 30% when heated at 65 and 82°C for 30 min (28). At 71 and 76°C, however, there is a decrease in whey protein denaturation with increase in milk

concentration. Whey protein denaturation is affected more by milk concentration than by temperature (49, 23). Although denaturation susceptibility of whey proteins in skim milk is reduced by milk concentration, actual rate of reaction is normal first order for heating times in excess of five minutes.

When the content of casein in milk is decreased, there is an increase in the amount of α-lactalbumin and β-lactoglobulin recovered after heating (18). Conversely, an increase in whey protein concentration decreases whey protein recovery after heating. Increasing the concentration of β-lactoglobulin in milk increases the proportion of α-lactalbumin in the complex. α-lactalbumin and β-lactoglobulin interact first and then complex with κ-casein.

Cheese from UF milk

Cheese processing from UF milk started in the early 1970's when the UF membranes were used to concentrate whey proteins. That was the standard application of UF in the dairy industry through most of the 1970's and early 1980's. Now, UF is used on milk streams and is becoming an essential technology in cheese making. In Europe, UF has been used in cheese making for almost twenty-five years (3).

Many soft cheeses are successfully made from UF milk. However, Cheddar cheese made from UF milk has coarse-textured-curd leading to excessive losses during cutting. Ripening is also delayed (10). Those problems may be caused, at least partially, by the smaller amount of rennet used to coagulate concentrated milks (13). However, there are some recent reports on making Cheddar cheese of good quality from UF milk. In collaborative studies by researchers in Australia, Cheddar cheese of good quality from UF milk has been produced (2). However, the method used in the production was not revealed. The technology has been transferred to the United States through agreements with some private dairy processors (35).

Primary benefits of UF to the processor are improved cheese yield, product consistency, continuous production, reduced labor and energy requirement, and greater

efficiency from reduced processing time (3). Higher yields result from the efficiency of the UF process. Also UF membranes allow concentration of the whey proteins that are lost in traditional cheese making. These whey proteins total about 20% of all the proteins in the milk, so capturing them increases yield.

Denaturation of Whey Proteins

As milk is heated, whey proteins are denatured to various levels depending upon the heating procedures used. These include batch, high temperature short time (HTST), and UHT sterilization heat treatments. In the native state, whey proteins have a definite conformation. When they are exposed to heat above a certain temperature, this conformation is disrupted and the proteins' characteristics are altered (38). Apparent casein content of UHT milk increases as a consequence of the denaturation of whey proteins (80).

The amount of heat-denatured β-lactoglobulin increases with temperature and is twice as high at pH 6.6 than at pH 5.5 (16). Rate of enzyme reaction on whey proteins is influenced by heat-induced β-lactoglobulin complex. Rate of proteolysis increases with increasing temperature. Extent of reduction rate depends on the amount of complex formed. β-lactoglobulin A and B variants are partially denatured by HTST and UHT and totally denatured by vat heating (9). Increasing residence time increases denaturation of both β-lactoglobulin variants in UHT and HTST and α-lactalbumin in vat heating systems. Surface hydrophobicity, which is related to heating, and sulfhydryl content are negatively correlated with whey protein denaturation.

Heating skim milk at 70°C for 30 min denatures 29% of total whey proteins. At 83°C, immunoglobulin is lost while α-lactalbumin is not affected because it is the most heat resistant fraction of whey proteins. Heating at 80°C for 10 min denatures up to 90% of whey proteins (1).

In UHT and HTST heating systems, the ratio of β-lactoglobulin to κ-casein increases linearly with increasing residence time (9). Denaturation of whey proteins in

milk normally precedes their interaction with casein. In presence of Ca and at temperatures above 65—75°C, β-lactoglobulin begins to unfold and reacts with κ-casein (75).

Denaturation of whey proteins is affected by solids more than by temperature variation. Whey protein denaturation at 80°C for 28 min decreases from 80 to 59% as total solids are increased from 28 to 44% (49). Increased total solids concentration slows denaturation of β-lactoglobulin A and B but hastens denaturation of α-lactalbumin in cheese whey (34). Removal of a large proportion of whey proteins (80%-90%) does not significantly alter the relative destabilizing effect of homogenization before preheating. Skim milks with reduced whey protein content are more heat stable than milks with normal whey protein contents (61).

Denaturation of α-lactalbumin appears to be first order, but is probably a second order reaction displaying pseudo-first order kinetics (34). Denaturation of both β-lactoglobulin A and B follows second order kinetics while that of serum albumin is more complex and can equally be described as first or second order. The relative heat stability of β-lactoglobulin A and B varies. Below 95°C, β-lactoglobulin A appears more thermostable than β-lactoglobulin B in skim milk. The same is observed in cheese whey below 100°C. Above this temperature β-lactoglobulin B appears more stable.

Morr and Josephson (57) showed that protein aggregation in heated whey systems is a multireaction process involving :

1) Aggregation to form intermediate sized protein particles.
2) Denaturation of whey protein through thiol-disulfide groups, hydrogen and hydrophobic bond reactions.
3) Gross aggregation of the above protein particles in the presence of Ca ions to larger sized particles.

Many studies suggest that when milk is heated, β-lactoglobulin complexes with κ-casein through disulfide bond (71, 44, 82). Presence of thiol and disulfide groups change the tertiary structure of proteins. This influences the adjacent molecules so a

complex will form without involvement of a covalent linkage between these proteins. Euber and Brunner (20) proved that thermally denatured β-lactoglobulin and κ-casein solution could only be released by disulfide reduction. However, their experiment was held in a model system which does not prove that such complex occurs in milk. Hae and Swaisgood (24) provided some evidence that these proteins complex through disulfide linkages. Despite extensive work by these researchers, unequivocal evidence of such disulfide bonds is still lacking.

The Kjeldahl method is the most widely used procedure for determining nitrogen in food systems. Rowlands (74) used it to measure the nitrogen content of different fractions of whey proteins, but his method is tedious and time consuming. Manji and Kakuda (46) compared the determination of whey proteins by different methods: fast performance liquid chromatography (FPLC), differential scanning calorimetry, whey protein nitrogen index, and Kjeldahl nitrogen . They found that these methods, except for differential scanning method, gave reproducible results. They found no difference between FPLC and Kjeldahl, but they found a significant difference between FPLC and whey protein nitrogen index.

Microstructure

The microstructure of many food products has been successfully investigated using microscopy (77). Although interesting results have been obtained with light microscopy, only the application of electron microscopy (EM), with its much higher resolution, has given a good picture into microstructure of foods. Each electron microscopic technique provides different information (47). Scanning electron microscopy (SEM) is appropriate to study the structure of surface morphology as well as internal structures of milk particles. However, transmission electron microscopy (TEM) techniques, such as thin-sectioning or freeze-fracturing, are used to identify individual constituents such as casein micelles and fat particles. SEM has been used more frequently than TEM in studying milk structure.

Casein micelles are nearly spherical conglomerates that are very polydisperse with respect to size, and their number decreases with increasing diameter. Different treatments on milk have different effects on casein micelle structure. An increase in casein micelle size is observed in heated milk and micelles coalesce preceding gelation (40). Evaporation increases casein micelle diameter from 1000 to 3900 Å. Casein micelles in UHT concentrated milks are two times larger than fresh milk micelles (7).

In milk gels containing 40 and 50% total solids (14 and 17% protein, respectively), casein micelles appear as individual entities linked by some bridging materials (40). At 60% total solids (20% protein), micelles are fused. Heating milk at high temperature denatures the whey proteins which are then precipitated on the micelles or as fine filaments in the serum at lower pH and higher pH respectively (12).

Microstructure determines some properties of dairy products such as viscosity, synersis, firmness, or mouthfeel. SEM is useful particularly in conjunction with other electromicroscopic techniques in studying microstructure of dairy products as it relates to the effects of manufacturing processes and properties of the products (39).

Microstructure of skim milk gels are markedly affected by type of acidulent used (29). A type of structure they described as "core and lining" was observed in milk acidified with glucono-δ-lactone (GDL) or oxalic acid and then heated at pH 5.5. The heated GDL milk has a fibrous microstructure and a gel network with most of the casein micelles associated in chains. Higher incidence of chains is observed in GDL milk gels than in HCl milk gels. Casein micelles in GDL milk gels are slightly larger than those from HCl or oxalic acid milk gels. Acid-induced milk gels also have a more open structure than rennet-induced milk gels which explains their greater susceptibility to syneresis (73).

Size measurements based on EM are subject to inaccuracies by possible artefacts resulting from fixture, staining, and dehydration processes (78). Care must be taken in preparing samples for EM. If such artefacts are observed, careful interpretation must be made to obtain meaningful results.

MATERIALS AND METHODS

Milk

Milk was obtained from the Utah State University Dairy Farm and was skimmed at the Utah State University Dairy Processing Plant.

UF Concentration

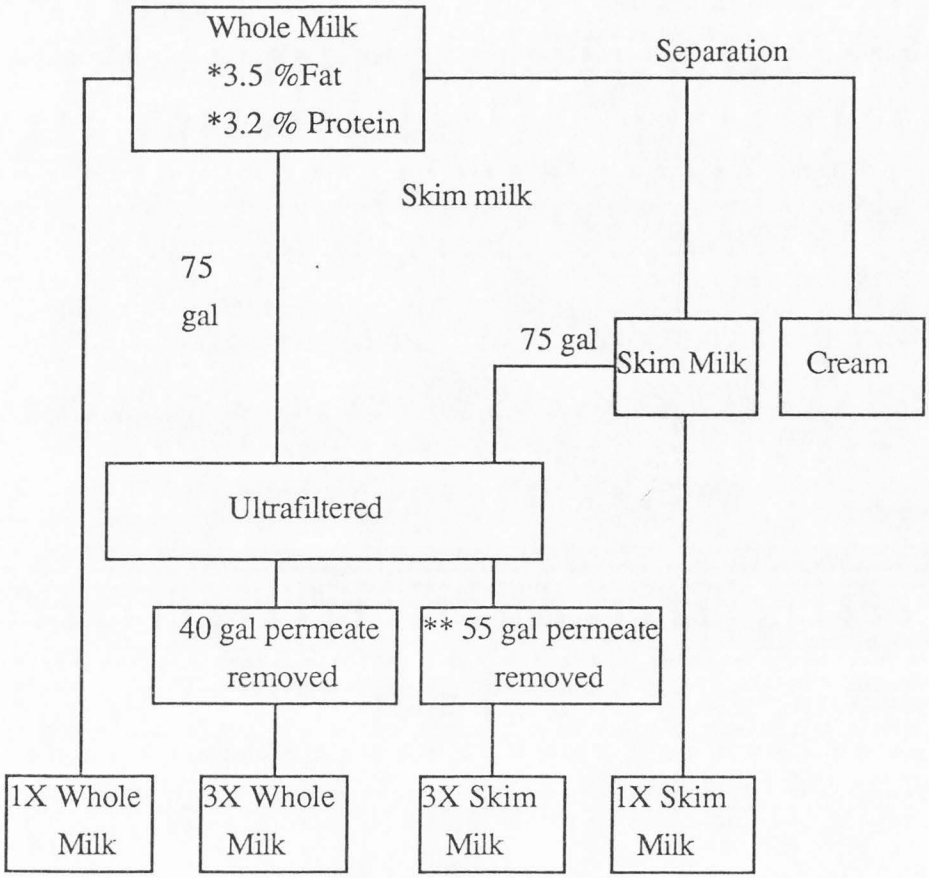
Whole milk was concentrated to three times (3X) by volume reduction to approximately 10% protein. For skim milk, the amount of permeate removed was calculated to give a concentrate of 3X with an equivalent protein concentration in serum as the 3X whole milk. A three-module UF system (Ladish Co., Tri-clover Division, Kensona, WI) with spiral wound membranes (20,000 daltons nominal MW cutoff, 15 m^2) in series was used to concentrate the milk. A scheme explaining milk sample preparation is shown in Figure 1.

Heat Treatment and Homogenization

Heat treatment of milk samples was conducted using pilot plant UHT equipment (Alfa-Laval, Lund, Sweden) operating with indirect heat exchange at a flow rate of 100 l/h. Milk was preheated to 72°C over 58 s then heated to 72, 89, 106, 123, or 140°C over 97 s with holding time of 4 s. The milk was cooled to 60°C and homogenized. Two-stage homogenization was used with 500 psi second stage pressure and 2000 psi first stage pressure. It was then cooled to 30°C packaged into disposable plastic containers, and refrigerated overnight. A scheme describing heat treatment is shown in Figure 2. Two control samples were used:

1) Raw milk heated to 60°C and homogenized (Homog).

2) Raw milk receiving no heat treatment and not homogenized (Control).



* Nominal fat and protein contents

** Amount of permeate removed was calculated to give 3X skim milk with equivalent percent protein in serum as 3X whole milk.

Figure 1. Schematic representation of milk sample preparation.

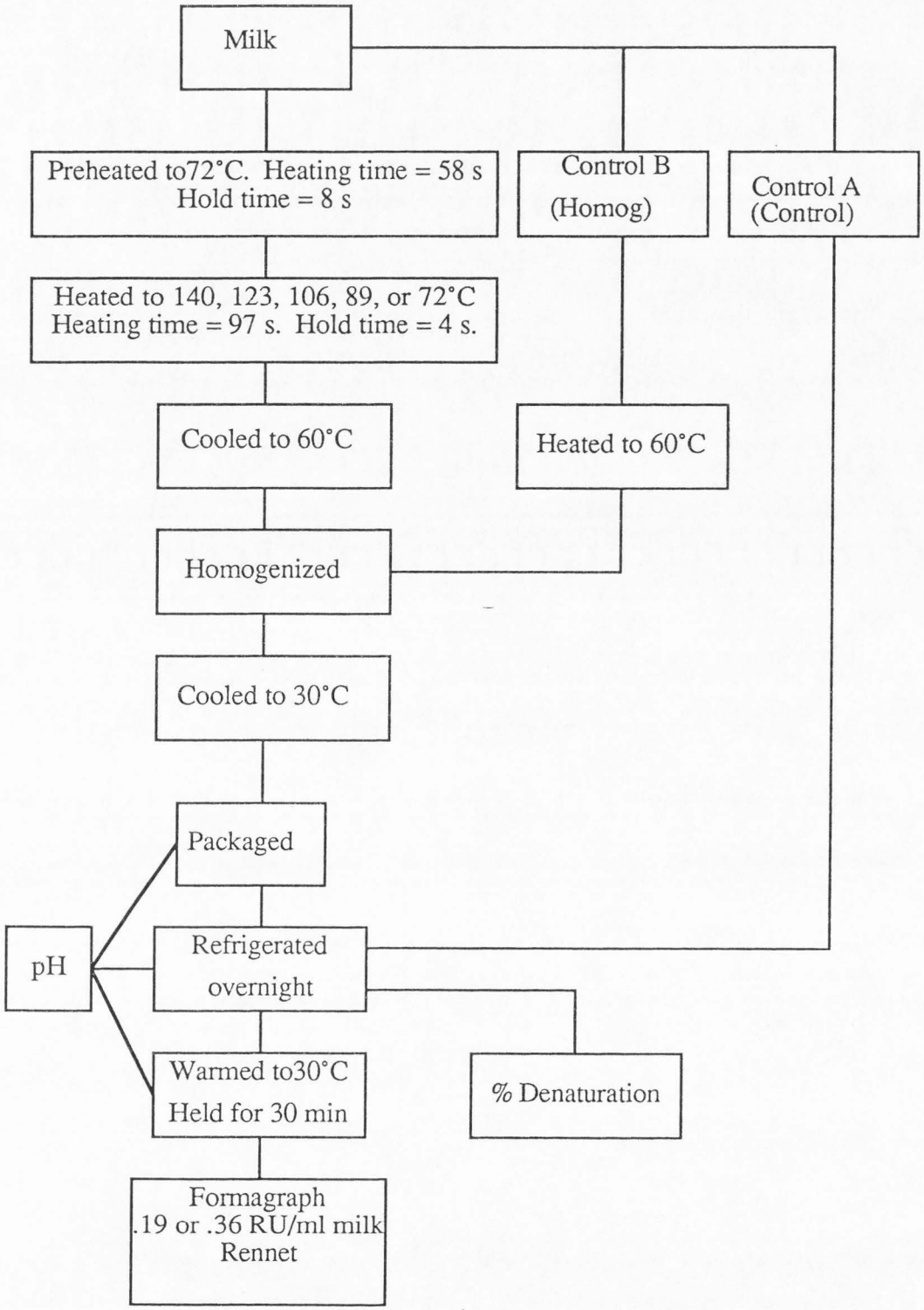


Figure 2. Schematic representation of the processing and analysis of the milk samples.

Milk Coagulation

Coagulation time was determined using a Formagraph as outlined by McMahon and Brown (50). A 10.5 g sample of milk was weighed into each sample well of the Formagraph. The Formagraph temperature was set at 30°C, and milk samples were warmed to 30°C for 30 min before adding rennet. Two hundred microliters of diluted calf rennet (New Zealand Cooperative Rennet Co., Eltham, NZ) were pipetted into each Formagraph well and mixed. Rennet was standardized in rennet units per ml (RU/ml) in which 90 RU/ml was equivalent to American single strength rennet (19). For 3X concentrated milk, 1, 4, and 10 RU/ml enzyme concentration were used. For the 1X milk, enzyme additions were 200 µl of 10 RU/ml enzyme and 50 or 200 µl of 188 RU/ml enzyme. RCT and curd firmness were determined from the Formagraph data. In the Formagraph, curd firmness of the coagulating milk is determined by deflection of a wire loop suspended in the milk. This deflection is recorded on photographic paper. Graphs of curd firmness versus time after rennet addition were prepared by measuring the deflection distance at specified intervals on the Formagraph paper and reproduced using Cricket 1.3 on a Macintosh computer.

Microstructure

Liquid milk samples were prepared for EM as follows: 5 ml of milk were added to 5 ml of 3% agar solution at 40-45°C. After the mixture solidified, the gel was cut into strips of 1x1x10 mm in size. These strips were then soaked in 2.5% glutaraldehyde solution for 3-4 h. They were then put into small vials filled with fresh 2.5% glutaraldehyde solution and sent to Dr. Miloslav Kalab at the Food Research Centre, Agriculture Canada, Ottawa. Transmission electron micrographs were prepared from each sample.

Milk gels were prepared as follows: 10.5 g of milk was weighed into a Formagraph sample well. For 1X milk, 200 µl of 188 RU/ml rennet were added, and for 3X milk, 200

µl of 10 RU/ml rennet were added. After twice the RCT, gels were taken out of the Formagraph well and cut into strips of the same approximate dimensions as those in the milk samples. These cubes were then soaked in 2.5% glutaraldehyde solution for 3-4 h and transferred to small vials containing fresh 2.5% glutaraldehyde solution. Samples were sent to Dr. Miloslav Kalab in Canada to conduct TEM.

The fixed samples were prefixed in a 2% osmium tetraoxide solution in a 0.05 M veronal-acetate buffer, pH 6.75. The postfixed samples were embedded in a Spurr's low viscosity medium (J.B. EM Service, Pointe Claire, Dorval, Quebec, Canada). Thin sections were stained with uranyl acetate and lead citrate solutions (73) and examined in a Philips EM-300 electron microscope operated at 60kV.

Protein Denaturation

Samples for whey protein denaturation were prepared by the procedure of Vakaleris and Price (83) with some modification. Ten milliliters of each milk sample were diluted with 40 ml distilled water. Twenty five milliliters of these diluted milk samples were brought to pH 4.6 by dropwise addition of 0.1N HCl and then filtered through Whatman filter paper # 5. Ten milliliters of the filtrate were used to determine whey protein nitrogen (WPN) content by a semi-micro Kjeldahl procedure using a Kjeltec Auto 1030 Analyzer, (Fischer Scientific Co.) (33). Percent protein denaturation was calculated by the following formula (46):

Denaturation (%) = ([WPN (raw milk) - WPN (heated milk)]100 / WPN (raw milk)

Statistical Analyses

The experimental design used was a split plot design with repeated measures. Each experiment was replicated twice. Data were analyzed using this design, and the appropriate ANOVA tables are provided in the results and discussion section.

RESULTS AND DISCUSSIONS

Whey Protein Denaturation

As temperature at which the milk samples were heated was increased, more whey proteins were denatured (Figure 3, Table 1). This agrees with the results of other reported experiments (72, 15, 62). Dargan and Savello (17) obtained similar results in yogurt samples heated at different temperatures held for different times. The most change in denaturation levels was observed as the temperature was increased from 72°C to 89°C. In this study, the heat treatment of 72°C produced more whey protein denaturation than that observed under milk pasteurization conditions. This was because this milk was held at 72°C for a total of 100 s compared to 16 s in milk pasteurization. Paluch et al. (66) reported that heat treatments of 73, 75, and 77°C for 16 s resulted in 4, 6, and 10% denaturation of β–lactoglobulin and 1, 6, and 10% denaturation of α-lactalbumin from Cheddar cheese.

More whey protein denaturation was measured in whole milk than in skim milk. This may be due to adherence of some of the whey proteins to the fat globules making less protein available in the serum for nitrogen measurement when casein was precipitated. McPherson et al. (53) observed that casein and whey proteins, mainly β–lactoglobulin, were the major protein components of fat globule membrane in homogenized milks. However, the UHT heated samples had the same level of whey protein denaturation irrespective of their fat content.

In the concentrated milks, there was more denaturation of the whey proteins than in the unconcentrated milks. This agrees with that observed by Dargan and Savello (17) who showed that yogurt had undergone higher whey protein denaturation as the total solids were increased from 11 to 15% and the yogurt heated to 100–140°C at different holding times. This effect of concentration may be due to the reduced volume of the concentrated milks forcing the proteins closer and therefore increasing the probability

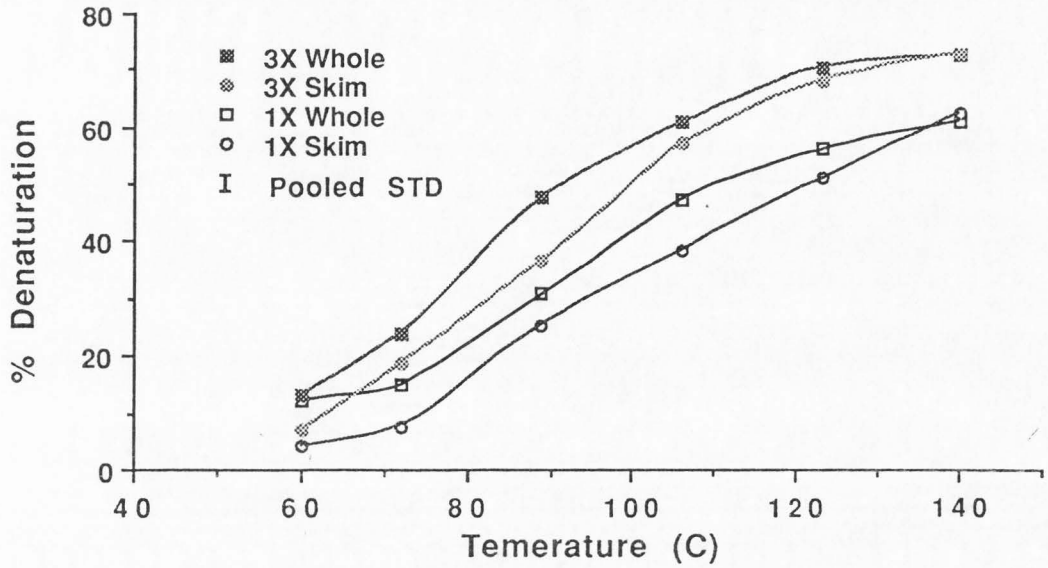


Figure 3. Percent whey protein denaturation in whole milk (1W), skim milk (1S) and their respective 3X, UF concentrates (3W and 3S) as a function of heating treatments.

Table 1. ANOVA of Whey Protein Denaturation of Milk Samples ($\alpha = 0.05$)

SV	df	MS	F	Significance
Rep	1	50.12	0.68	not sig.
Milk type (M)	1	633.8	8.71	SIG.
Conc (C)	1	1031	14.1	SIG.
MxC	1	41.01	0.564	not sig.
Error (a)	3	72.69		
Temp (T)	5	4494	584	SIG.
Error (b)	5	7.692		
MxT	5	22.96	8.93	SIG.
CxT	5	34.75	13.5	SIG.
MxCxT	5	12.03	4.68	SIG.
Error (c)	15	2.569		
Total	47			

might also be a factor as a slight decrease in pH occurred with concentration (Figure 4, Table 2). That agrees with Walstra (85) and Damicz and Dziuba (16) who reported that the amount of denatured β-lactoglobulin increased as pH decreased from 6.6 to 5.5. However, there are some contradictory results in that whey protein denaturation decreased as milk concentration increased in this experiment. That may be because of the different experimental conditions used.

Clotting Time

When milk is heated above pasteurization temperature, whey proteins, particularly β-lactoglobulin, are denatured and complex with κ-casein through hydrophobic and covalent interactions (25). Because most of the κ-casein is located on the surface of casein micelles, presence of this complex severely retards rennet coagulation of milk.

As the temperature at which milk was heated was increased, there was a resultant increase in that milk's RCT (Figure 5, Table 3). This was because more whey proteins were denatured at the higher temperatures causing more hindrance to renneting and aggregation of milk. Skim (1S) and whole (1W) milk samples, which were UHT treated, did not coagulate even though 200 µl of .36 RU/ml rennet was added to 10.5 g of milk samples. This again demonstrates that the amount of the complexed whey protein with the κ–casein is so great that it rendered the κ–casein either unaccessible to the enzyme action or incapable of aggregating. These results agree with what was observed by Reddy and Kinsella (72) who reported that heating milk to 60—90°C for 30 min reduced the initial chymosin hydrolysis and the release of glycomacropeptide. This increases RCT. An increase in S–S groups is also observed with increasing temperature. Since both hydrophobic and S–S interaction are involved in heat-induced association between β-lactoglobulin and κ-casein, it is more likely that this association is caused by increasing heating temperature. Dalgleish (15) also reported that the β-lactoglobulin and κ-casein

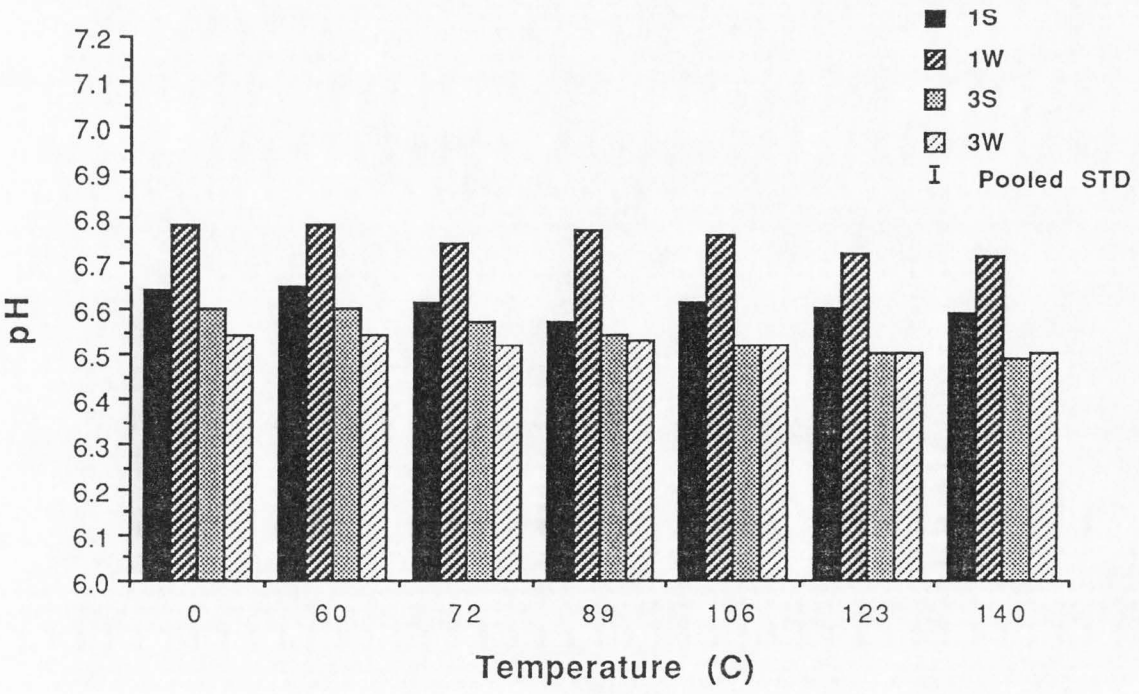


Figure 4. Effects of heating treatments in sterilab UHT system on pH. 1S, skim milk; 1W, whole milk; 3S, UF concentrated skim milk; 3W, UF concentrated whole milk.

Table 2. ANOVA of pH of Milk Samples ($\alpha = .05$)

SV	df	MS	F	Significance
Rep	1	0.00820	2.53	not sig.
Milk type (M)	1	0.09450	28.01	SIG.
Conc (C)	1	0.37276	110.4	SIG.
MxC	1	0.09630	28.53	SIG.
Error (a)	3	0.00337		
Heat (T)	5	0.00882	22.99	SIG.
Error (b)	5	0.00038		
MxT	5	0.00229	12.77	SIG.
CxT	5	0.00043	4.880	not sig.
MxCxT	5	0.00011	1.210	not sig.
Error (c)	15	0.00008		
Total	47			

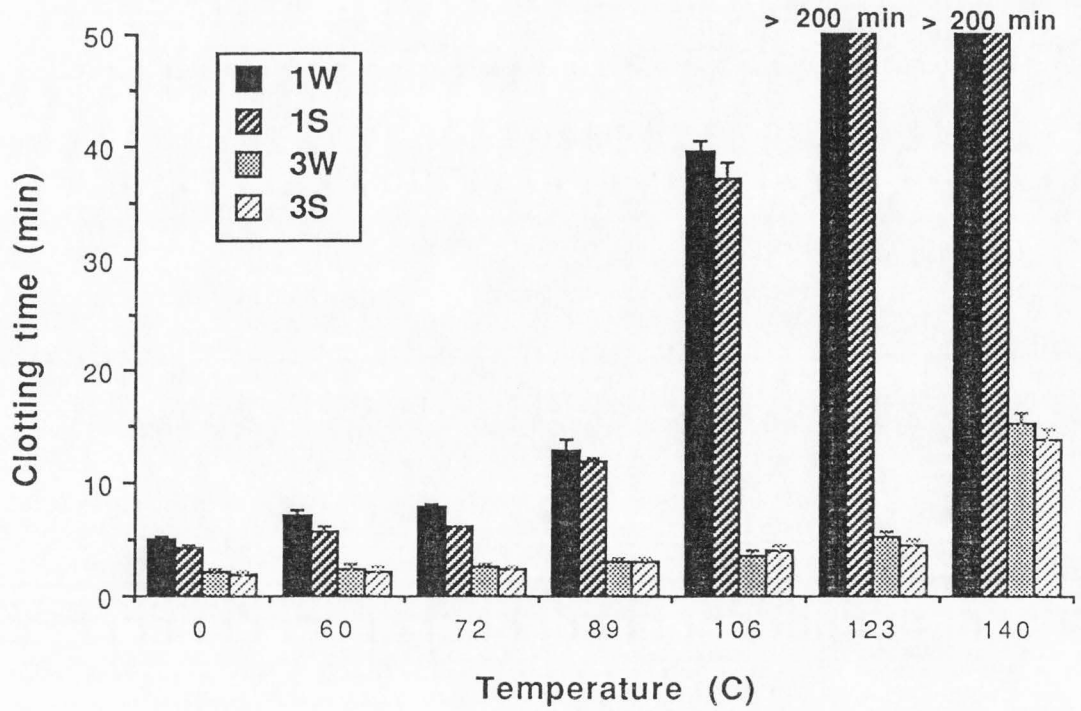


Figure 5. Effects of heating treatments on rennet clotting time. 1W, whole milk; 1S, skim milk; 3W, UF concentrated milk; 3S, UF concentrated skim milk. Error bar = standard deviation.

Table 3. ANOVA of Clotting Time of Milk Samples ($\alpha = .05$)

SV	df	MS	F	Significance
Rep	1	1078.87	0.6809	not sig.
Milk type (M)	1	11.16	0.0070	not sig.
Conc (C)	1	4594×10^3	2.9×10^5	SIG.
MxC	1	66.44	0.0422	not sig.
Error (a)	3	1574.16		
Heat (T)	6	1320×10^3	1.4×10^4	SIG.
Error (b)	6	92.45		
MxT	6	409.99	1.57	not sig.
CxT	6	8426×10^2	3.2×10^4	SIG.
MxCxT	6	887.61	3.413	SIG.
Error (c)	18	260.07		
Total	47			

association, caused by heating to 75—90°C for 30 min, was caused by disulfide bonding.

When milk gels, the casein micelles start to aggregate well before clotting is observed (52). The rate at which milk coagulates depends upon the rate of enzymic action and the rate of aggregation of the hydrolyzed products as characterized by their aggregation constant k_S (51). This suggests that when the micelles are drawn closer, the volume of the aqueous phase decreases leading to an increased number of effective collisions. It is the number of these effective collisions upon which aggregation velocity depends. Gel formation will occur with a lower degree of proteolysis of the κ-casein when the protein concentration is increased or when calcium chloride is added to milk although a minimum protein concentration is required for gelation to take place (31). In other words concentrating milk by UF reduces the water phase in milk so that casein micelles have shorter distances to travel before colliding with another micelle. Consequently, a shorter time is required for coagulation to occur. Total calcium concentration will also increase upon ultrafiltration of milk, and this also results in increasing coagulation rate (4).

Concentrated milk samples (3S and 3W) coagulated faster than the skim and whole milks (Figure 5). This confirms results reported by Orme and McMahon (65) that RCT of whole milk decreased with increasing protein concentration and leveled to a plateau at 8—11% protein. They also showed that increasing protein concentration above 11% then further decreased coagulation time. However, there are other controversial reports on the effect of milk concentration on RCT. Mehaia and Cheryan (54) showed that RCT decreases linearly as protein concentration of milk is increased up to about 12%. Dalgleish (14) reported that UF concentration of milk to 3X has little effect on RCT. Payens (69) and Inra (36) obtained similar results. There are no clear-cut explanations for these differences in experimental observations.

Concentrated skim and whole milks that were UHT treated still coagulated even though their unconcentrated counterparts did not coagulate within 200 minutes after adding the enzyme (Figure 5). This suggests that the effect of concentration in bringing the casein

micelles to closer proximity overcame the interference caused by whey proteins complexing with the κ-casein. The concentrated milks also had a lower pH than skim and whole milks (Figure 4) but this may have had a lesser impact on clotting time reduction. As the decrease in pH was not dramatic, the greater effect on CT was assumed to be from the smaller distance the renneted casein micelles had to travel before colliding with each other and the large number of aggregating casein micelles that can participate in gel formation.

Gel Firmness

Attachment of whey proteins onto the surface of the casein micelles, which is brought about by heating milk at high temperatures, causes steric hindrance between casein micelles. This, combined with their negative charge, reduces the ability of casein micelles to aggregate. Consequently, a soft gel is formed from milk that has received a high heat treatment (48). This occurred for all the milk samples studied. For 1X whole milk, the decrease in firmness as a function of increased heating was gradual and smooth (Figure 6). With 1X skim milk, a sharp decrease in firmness was observed in milk heated to 89°C (Figure 7). After UHT heating, the 1X whole and skim milk samples did not gel, so no data are shown for them in Figure 6 and 7.

A comparison of skim and whole milk (Figure 8) showed that unhomogenized 1X skim milk and 1X skim milk heated to 72, 89, or 106°C produced firmer gels than their counterpart 1X whole milk. This agrees with what was reported earlier in the literature (32, 70, 84). Dargan and Savello (17) reported that yogurt gels made from skim milk were firmer than those made from 1% fat milk. This might be due to homogenization, which was performed in all of these experiments, creating many small fat globules and, therefore,increasing the surface area of the fat/water interface. There was then a greater chance that casein micelles would adhere to these fat globules creating many weak points in the gel. This soft gel from whole milk might also occur because of fewer casein micelles in the serum phase and less chance of forming a strong gel network. On the other hand, 1X

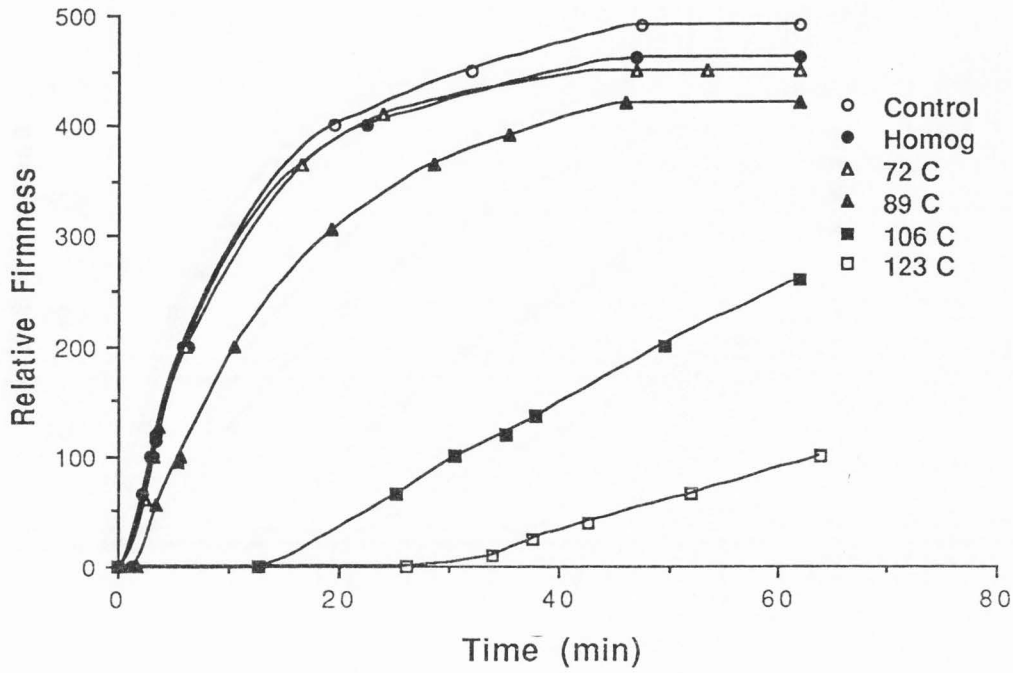


Figure 6. Change in gel firmness of skim milk upon addition of calf rennet (.36 RU/ml milk, 30°C) as measured using the Formagraph. Heat treatments of 72, 89, 106, and 123°C are compared to unheated, unhomogenized milk (Control) sample and milk heated to 60°C and homogenized (Homog).

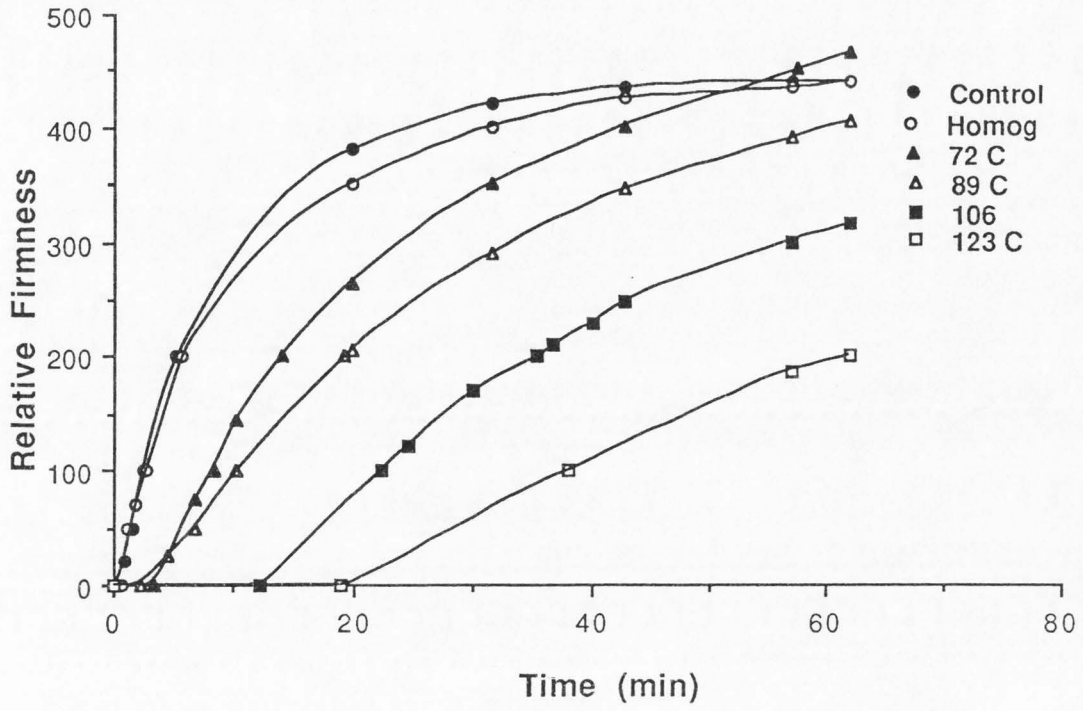


Figure 7. Change in gel firmness of whole milk upon addition addition of calf rennet (.36 RU/ml milk, 30°C) as measured using the Formagraph. Heat treatments of 72, 89, 106, and 123°C are compared to unheated, unhomogenized milk (Control) sample and milk heated to 60°C and homogenized (Homog).

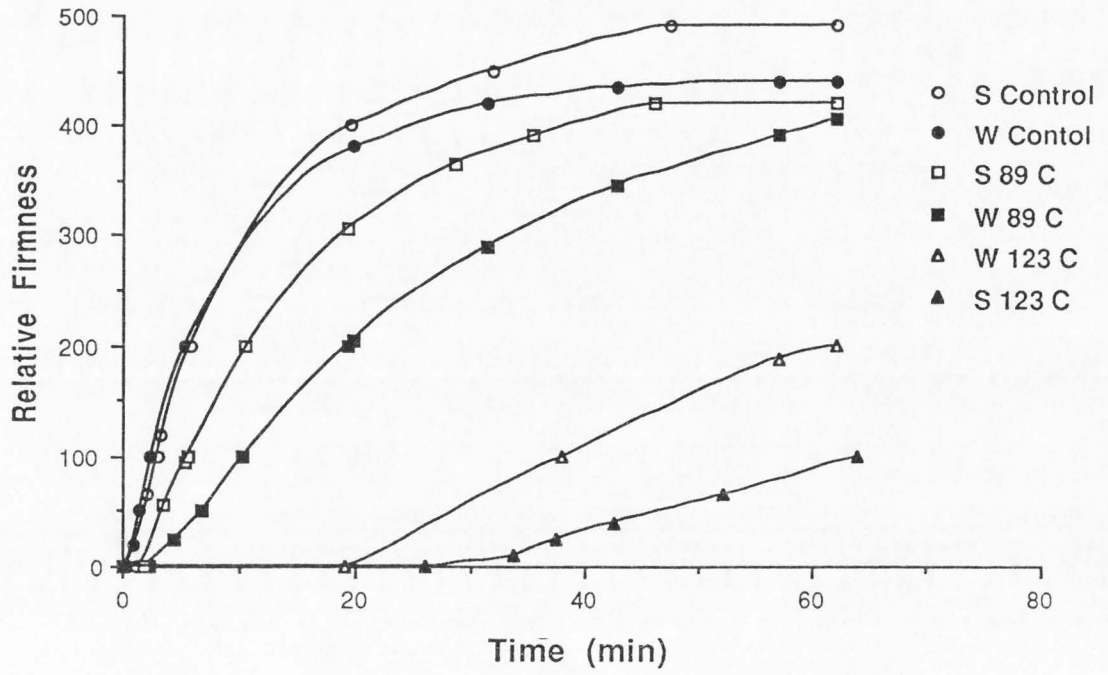


Figure 8. Change in gel firmness of skim milk (S) and whole milk (W) upon addition of calf rennet (.36 RU/ml milk, 30°C) as measured using the Formagraph. Heat treatments of 89°C or 123°C are compared to unheated unhomogenized (Control) samples.

whole milk heated to 123°C produced firmer gel than its skim counterpart which suggests that the impact of fat presence has an effect only to a certain limit of temperature.

For concentrated milk samples, gel firmness decreased gradually as a factor of increased process temperature (Figures 9 and 10). After UHT heating, the concentrated milk samples formed very weak gels as compared to the other heated milk samples. Except for UHT samples, gel firmness for all other samples was nearly the same after 75 min. Figure 11 shows that 3X whole milk samples formed firmer gels than their skim counterparts which was opposite to what was found in the 1X milk samples (Figure 8).

The concentrated milks formed firmer gels than their unconcentrated counterparts. A higher enzyme activity was needed to coagulate the heated, unconcentrated whole and skim milks (Figures 12 and 13). Rennet concentration normally has little effect on the secondary phase of milk coagulation. However, enzyme concentration affects the production of reactive micelles which does affect the aggregation rate (13). So, this increase in gel firmness could be primarily because of increase in milk concentration. This might be due to the effect of serum phase reduction which would result in more casein micelles per unit volume of milk and greater chances of gelation. Inra (36) also reported that the aggregation rate increased as the milk protein content increased by UF of milk.

Microstructure

Effects of heating on milk

As processing temperature was increased, the casein micelles in skim milk increased in size, and the number of small micelles decreased (Figure 14). The casein micelles were also coated with additional protein material. After UHT treatment, micelles were dramatically increased in size and had much adhered protein material. The origin of this adhered protein material was probably the denatured whey protein, small micelles, or fragments from micelles that had dissociated upon heating. This supports results found by many authors who have reported that whey protein, when denatured, precipitates onto the

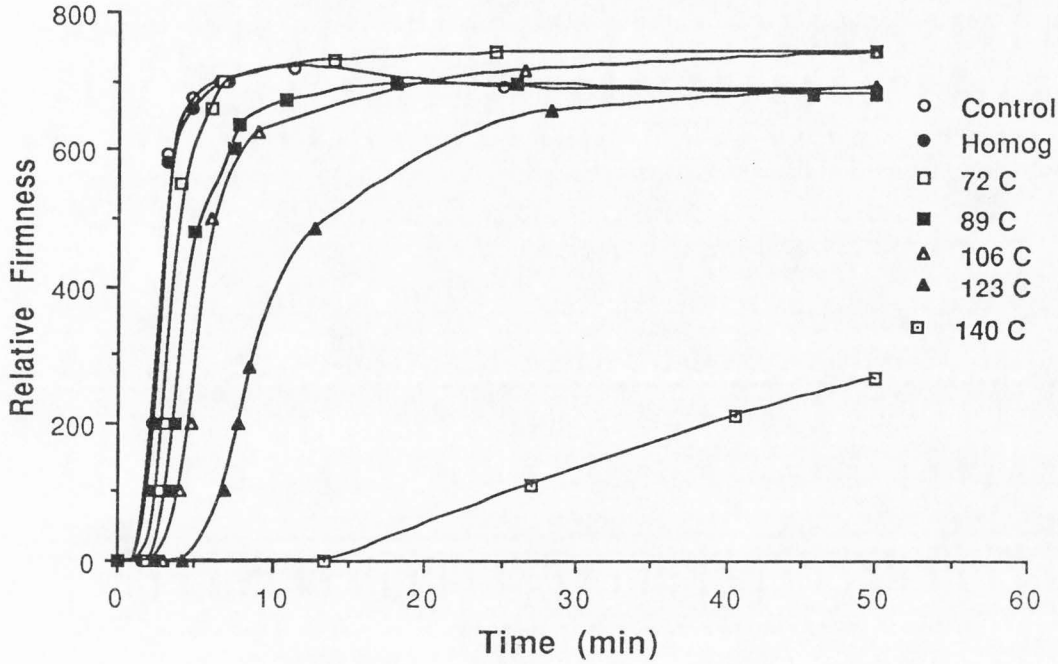


Figure 9. Change in gel firmness of 3X UF concentrated whole milk upon addition of calf rennet (.19 RU/ml milk, 30°C) as measured using the Formagraph. Heat treatments of 72, 89, 106, 123, or 140°C are compared to unheated, unhomogenized milk (Control) sample and milk heated to 60°C and homogenized (Homog).

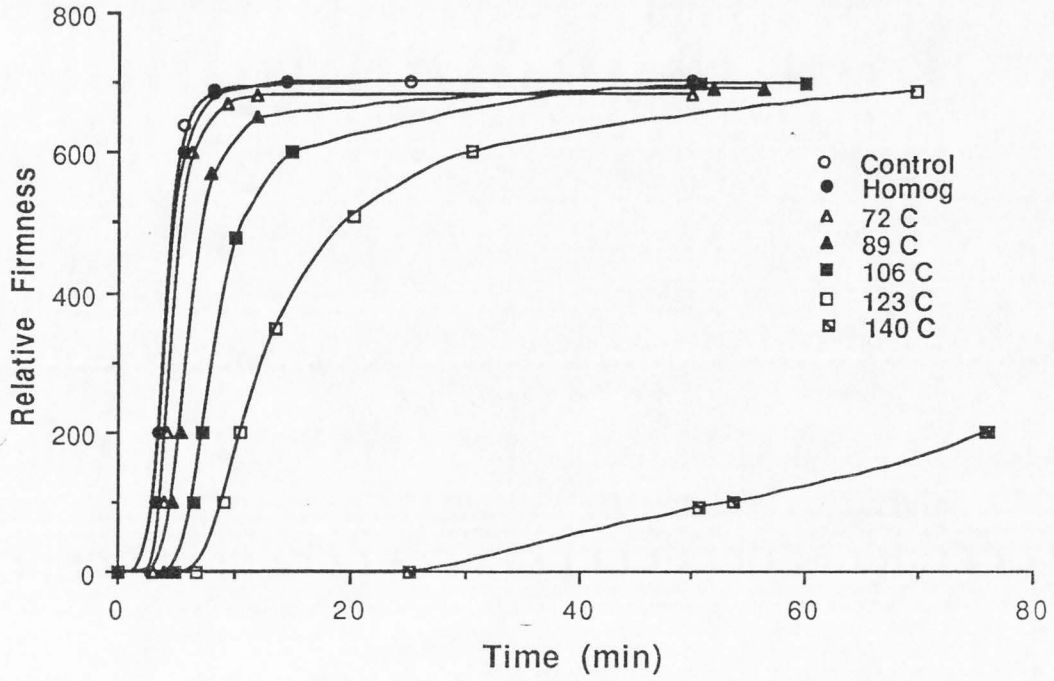


Figure 10. Changes in gel firmness of 3X UF concentrated skim milk upon addition of calf rennet (.19 RU/ml milk, 30°C) as measured using the Formagraph. Heat treatments of 72, 89, 106, and 140°C are compared to an unheated, unhomogenized (Control) sample and milk heated to 60°C and homogenized (Homog).

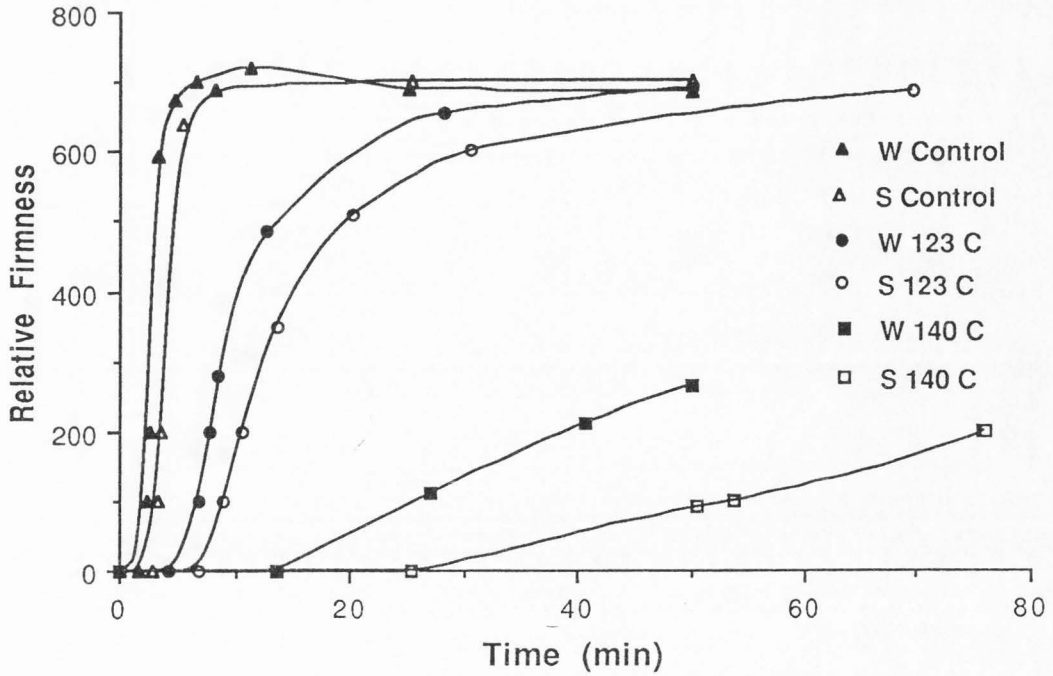


Figure 11. Changes in gel firmness of 3X UF concentrated skim milk (S) and whole milk (W) upon addition of calf rennet (.19 RU/ml milk, 30°C) as measured using the Formagraph. Heat treatments of 123°C and 140°C are compared to unheated unhomogenized (Control) samples.

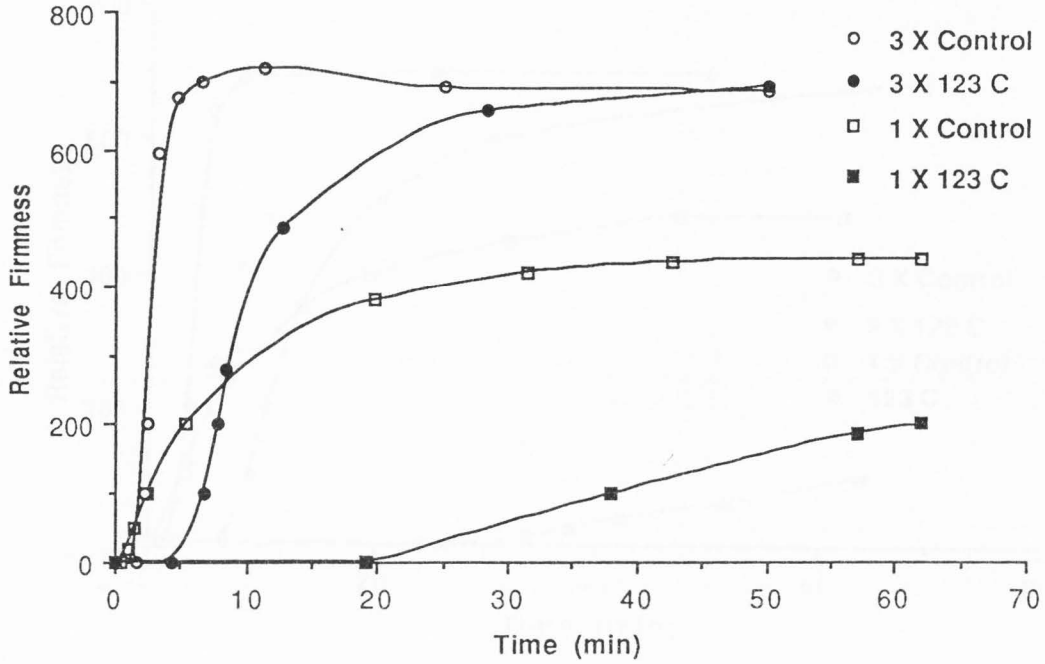


Figure 12. Comparison of effect of concentrating whole milk (1X) using ultrafiltration to 3X on curds firming after adding calf rennet (.36 RU/ml milk, for 1X; .19 RU/ml milk, 30°C, for 3X). The milk samples were either unheated, unhomogenized (Control) or heated to 123°C.

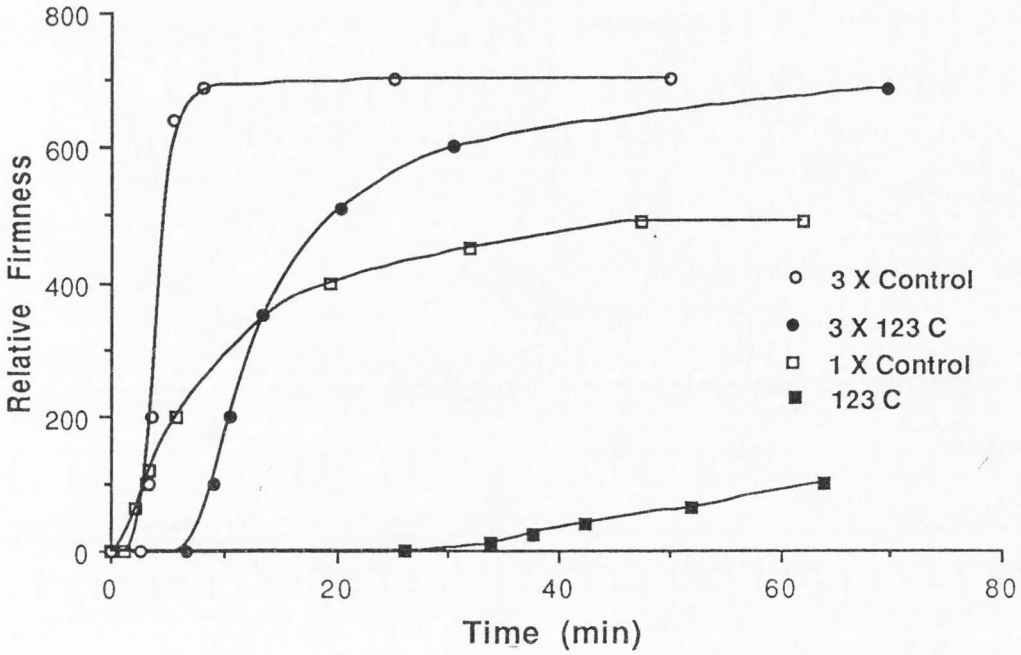
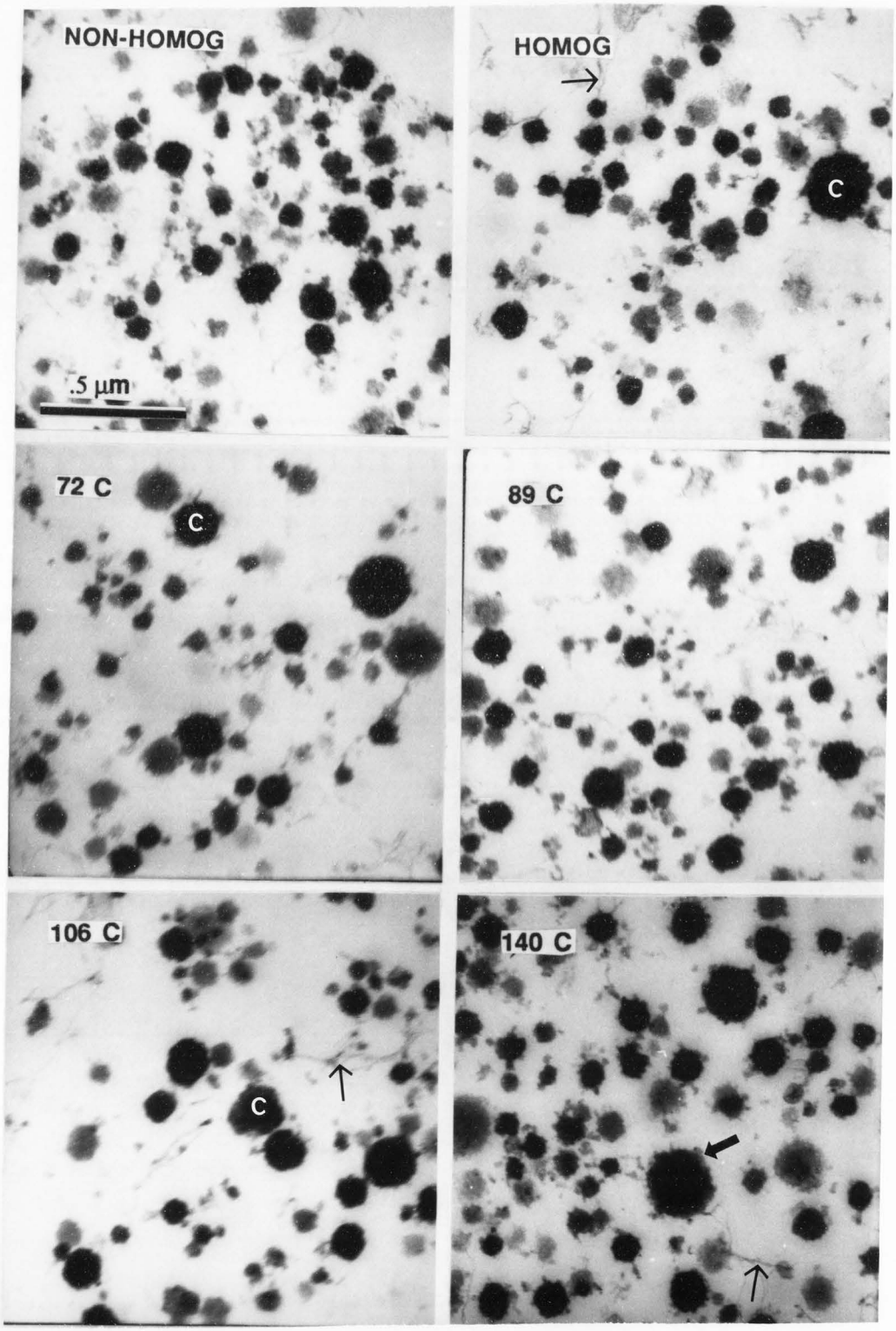


Figure 13. Comparison of effect of concentrating skim milk (1X) using ultrafiltration to 3X on curds firming after adding of calf rennet (.36 RU/ml milk for 1X; .19 RU/ml milk for 3X, 30°C). The milk samples were either unheated, unhomogenized (Control) or heated to 123°C.

Figure 14. Transmission electron micrographs of skim milk heated to 72, 89, 106 and 140°C, compared to unheated unhomogenized (NON–HOMOG) skim milk and skim milk heated to 60°C and homogenized (HOMOG). Casein micelles (C) were distributed throughout the serum. The irregular lines (small arrows) were artefacts produced while dispersing the milk in agar prior to fixing the samples in glutaraldehyde.


NON-HOMOG
.5 µm
HOMOG
C
72 C
C
89 C
106 C
C
140 C

casein micelles. They also reported that protein particles appear to be larger and more diffuse when higher temperatures are used (30, 12).

When milk is heated, some casein micelles aggregate, and the incidence of submicellar casein is somewhat increased. The shape of casein micelles is also altered. Severity of these changes depends upon the intensity of heat treatment applied. According to Harwalker et al.(30), there is also a formation of so-called "spikes" or "hair". These were evident in micrographs of UHT milks as can be seen in Figures 14 and 15. Changes also occurred in casein micelles ultrastructure; submicelles became more clearly visible than in casein micelles in unheated milk. This was probably the result of some loosening of the bonds between the submicelles that took place because of the effect of heat treatment. This loosening might be responsible for the enlargement of the casein micelles.

It was expected from what was observed in Figures 14 and 15 that the coagulation time would decrease because of the build-up of the protein material on the casein micelles. As shown in Figure 3, RCT increased as the processing temperature was increased. This inferred that the interactive sites on the casein micelles were related to κ-casein and these were being obscured by the coating of denatured protein on the micelles' surfaces.

Comparing unhomogenized sample with all other samples, it was observed that as expected, homogenization had decreased the fat droplet size (Figure 15). The fat globules had many casein micelles adhered to their surfaces. This supported what was found by Henstra and Schmidt (32) who attributed this to the homogenization process causing casein micelles to be broken into subunits which would ultimately adhere to the surface of the fat globules. In contrast, the fat globules in the unhomogenized sample, exist independently of the casein micelles (Figure 15).

Effects of heating on milk gels

When unheated milk was coagulated by rennet, a protein gel was formed that was made of chains of casein micelles (Figure 16). When the milk was heated, β-lactoglobulin

Figure 15. Transmission electron micrographs of whole milk heated to 72, 89, 106 and 140°C and homogenized, compared to unheated unhomogenized (NON-HOMOG) whole milk and whole milk heated to 60°C and homogenized (HOMOG). In homogenized samples the fat droplets (F) have become complexed with casein micelles (C). After heating milk to 140°C it appears that additional protein material has been deposited on the surface of the casein micelles (bold arrows). The same irregular lines (small arrows) as occurred in Figure 14 were present. The small electron-dense particles (arrow heads) are also artefacts caused by glutaraldehyde–osmium tetroxide complexing.

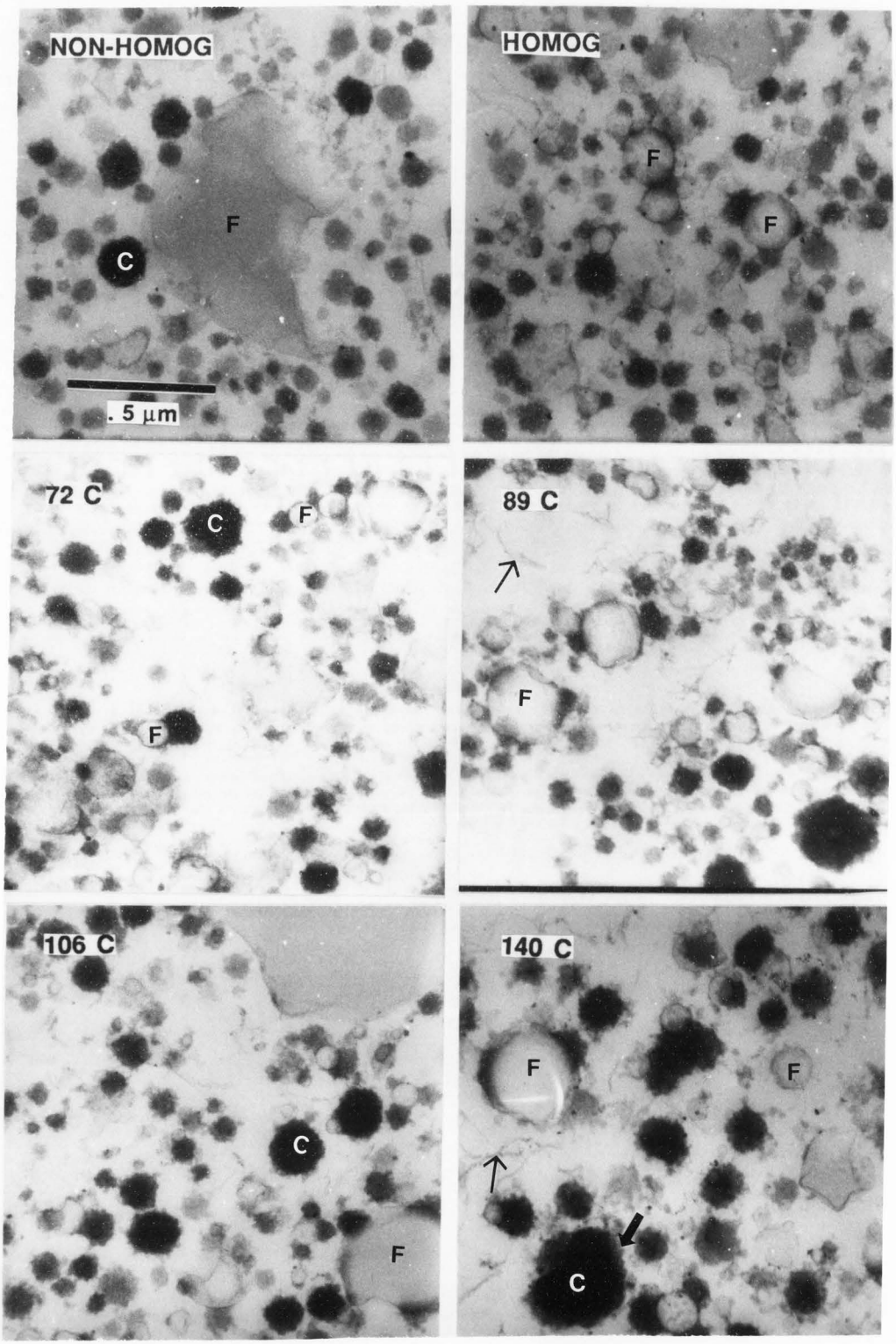
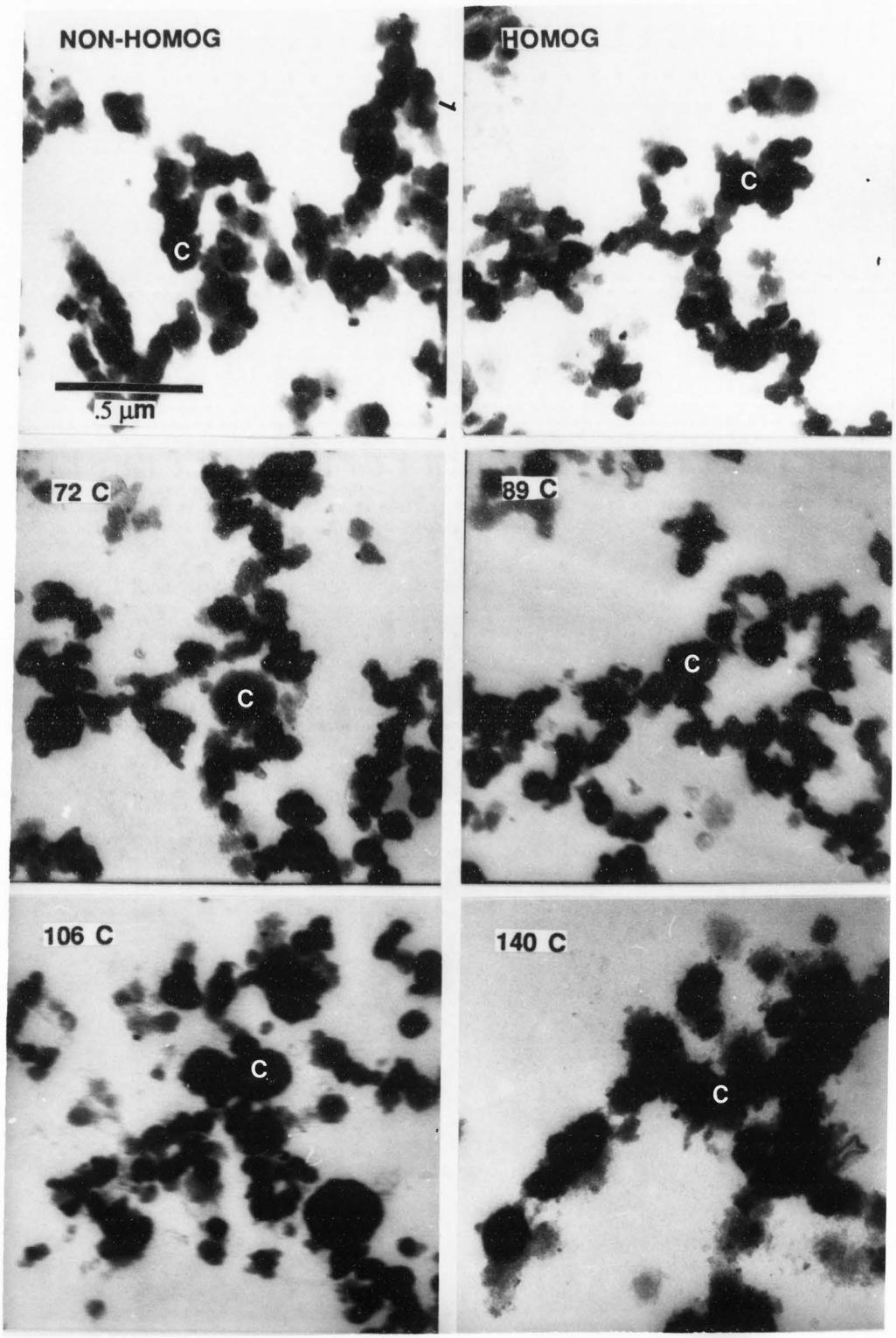

NON-HOMOG
F
C
.5 µm
HOMOG
F
F
72 C
C
F
F
89 C
F
106 C
C
F
140 C
F
F
C

Figure 16. Transmission electron micrograph of gels made from skim milk heated to 72, 106, 123, and 140°C compared to gels made from unheated nonhomogenized (NON-HOMG) skim milk heated to 60°C and homogenized (HOMOG). As the milk was heated at higher temperature, it was more difficult to identify individual casein micelles (C) in the gel network. In gels made from UHT milk (140°C), large separate casein micelles were observed.


NON-HOMOG
C
.5 µm
HOMOG
C
72 C
C
89 C
C
106 C
C
140 C
C

denatured and complexed with κ–casein on the micelle surface, so the number of individual casein micelles in the gel was reduced (Figure 17). This was because many of the small micelles and the submicellar casein material were incorporated into the β–lactoglobulin-κ-casein complex. So, when such milk was renneted, the resultant gel had fewer crosslinks and was not as firm.

Having fat in the milk tended to produce a gel network that had more crosslinks (Figure 17, 106°C). In the homogenized milk, the fat was separated from the protein network (Figure 17). As the milk was processed at higher temperatures the casein micelles lost their integrity in the gel network (Figures 16 and 17). In whole milks it can also be seen how homogenization caused the fat droplets to become incorporated into the gel network. For unheated milks this led to weaker gel structure (Figure 8) perhaps by adding weak points into the gel. However, for high heated milks (e.g 123°C) the whole milk gels were firmer than the skim milk gels (Figure 8). In this case, the presence of fat in the gel network strengthened the gel by increasing the volume of the gel network.

The small electron-dense particles observed in Figure 17 were characterized as difficult-to-avoid artefacts caused by formation of a glutaraldehyde–osmium tetroxide complex during TEM sample preparation (67).

Effects of homogenization

Fat globules decreased in size when milk was homogenized (Figures 15, 17, 18 and 19). This agrees with what was well established in the literature (32, 37). When milk was homogenized, the boundary of the fat droplets can be seen easily because of the protein adsorbed at the newly created fat/water interfaces. In all the homogenized samples, the fat globules and the casein micelles had complexed together.

Effects of concentration

All of the UF concentrated milks had larger casein micelles than their unconcentrated counterparts (Figures 18 and 20 compared to 14 and 15). This confirms

Figure 17. Transmission electron micrograph of gels made from whole milk heated to 72, 106, 123, and 140°C compared to gels made from unheated nonhomogenized (NON-HOMG) whole milk, and whole milk heated to 60°C and homogenized (HOMOG). In the gels made from the homogenized milks the fat droplets (F) had complexed with the proteins with many casein micelles (C) adsorbed on their surfaces. The same electron-dense artefacts (arrow heads) as described in Figure 15 are present.

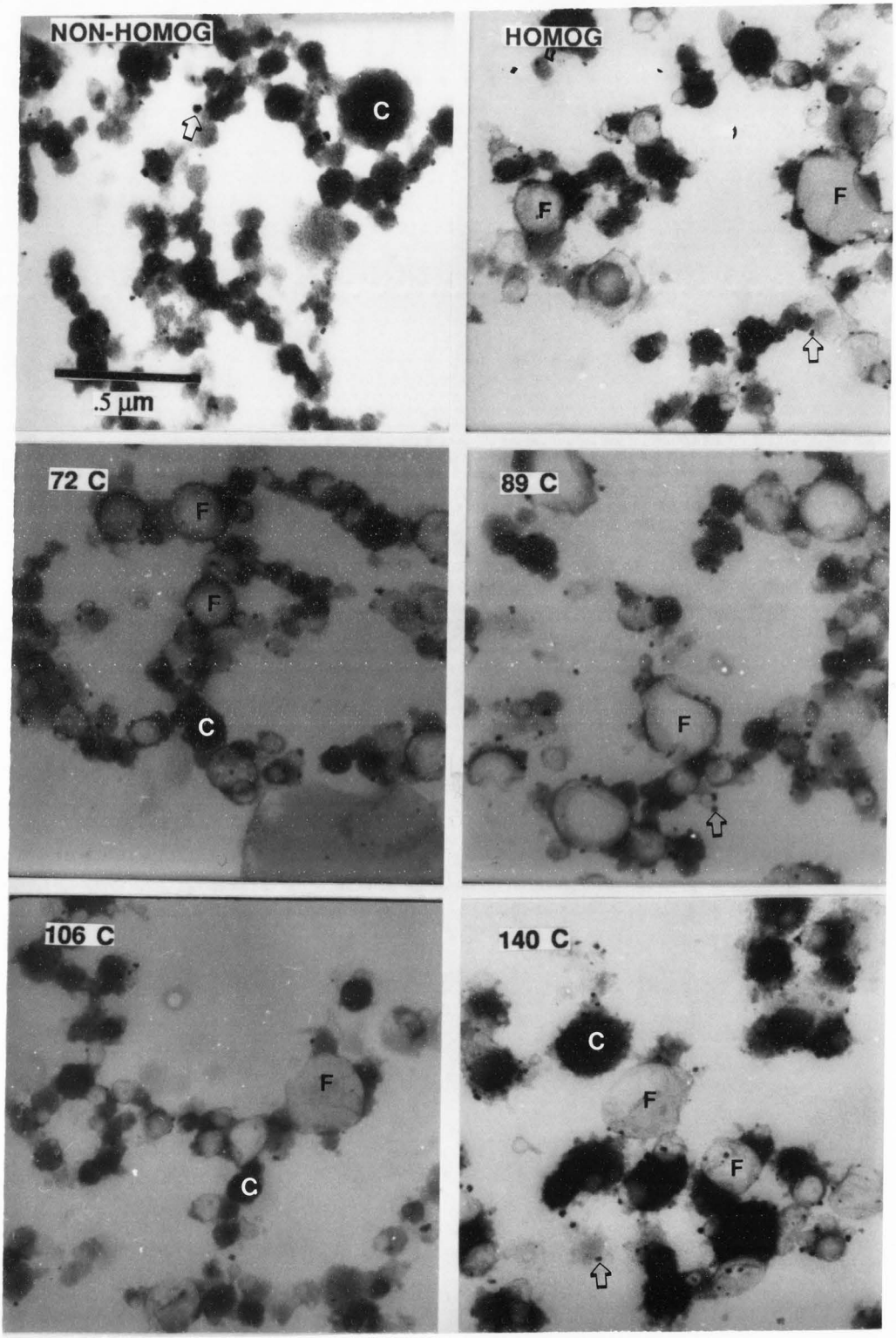

NON-HOMOG
C
.5 µm
HOMOG
F
F
72 C
F
F
C
89 C
F
106 C
F
C
140 C
C
F
F

Figure 18. Transmission electron micrographs of 3X UF concentrated whole milk heated to 72, 106, 123 and 140°C and homogenized, compared to unheated unhomogenized (NON-HOMOG) 3X UF concentrated whole milk and 3X UF concentrated whole milk heated to 60°C and homogenized (HOMOG). In homogenized samples the fat droplets (F) have become complexed with casein micelles (C). After heating milk to 140°C it appears that additional protein material has been deposited on the surface of the casein micelles (bold arrows). The same irregular lines (small arrows) as occurred in Figure 14 were present. The small electron-dense particles (arrow heads) as described in Figure 15 were also present.

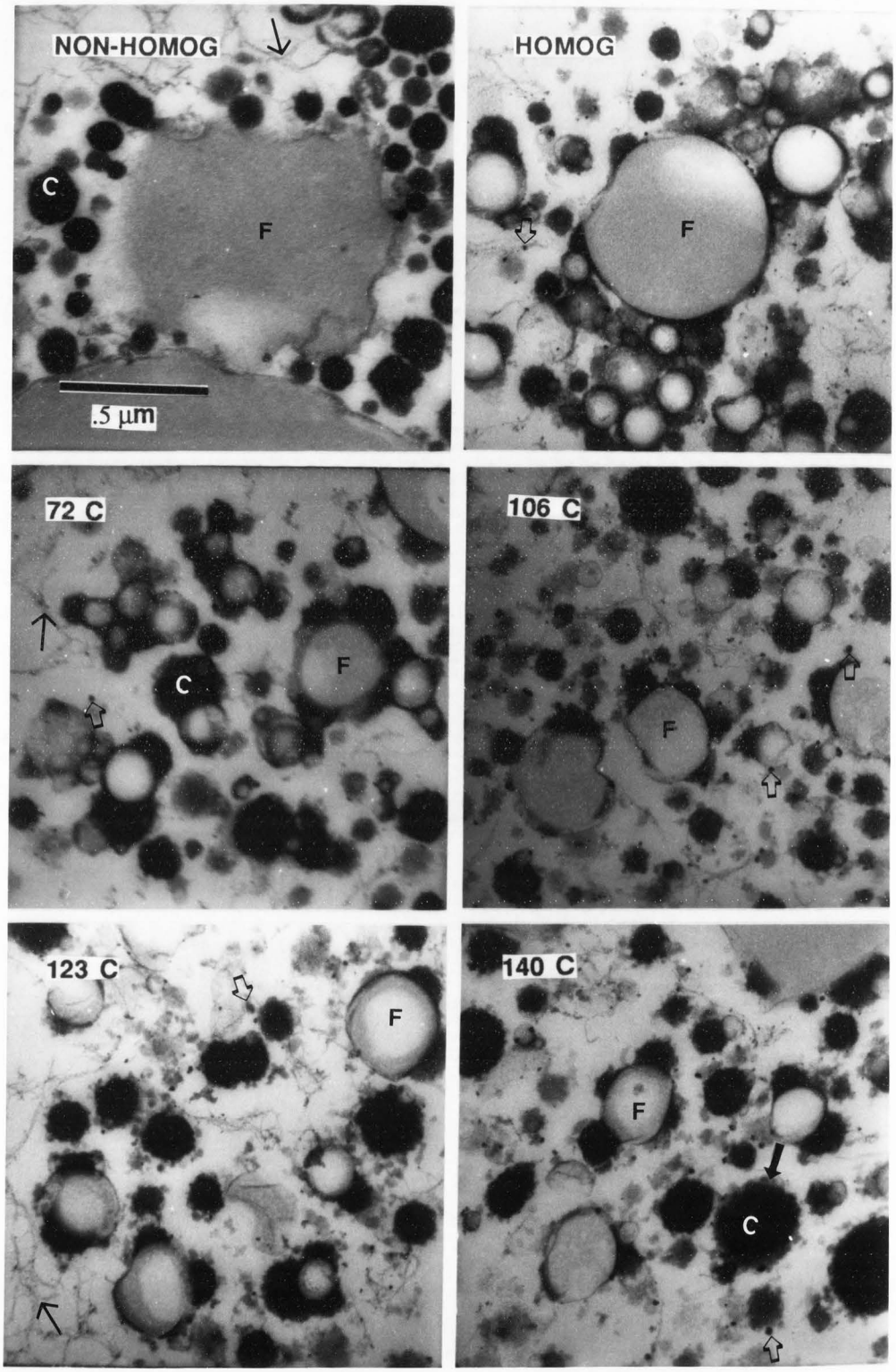

NON-HOMOG
C
F
.5 µm
HOMOG
F
72 C
C
F
106 C
F
123 C
F
140 C
F
C

Figure 19. Transmission electron micrograph of gels made from 3X UF concentrated whole milk heated to 72, 89, 123, and 140°C compared to gels made from unheated nonhomogenized (NON-HOMG) 3X UF concentrated whole milk, and 3X UF concentrated whole milk heated to 60°C and homogenized (HOMOG). In the gels made from the homogenized milks the fat droplets (F) had complexed with the proteins with many casein micelles (C) adsorbed on their surfaces. The same electron-dense artefacts (arrow heads) as described in Figure 15 are present.

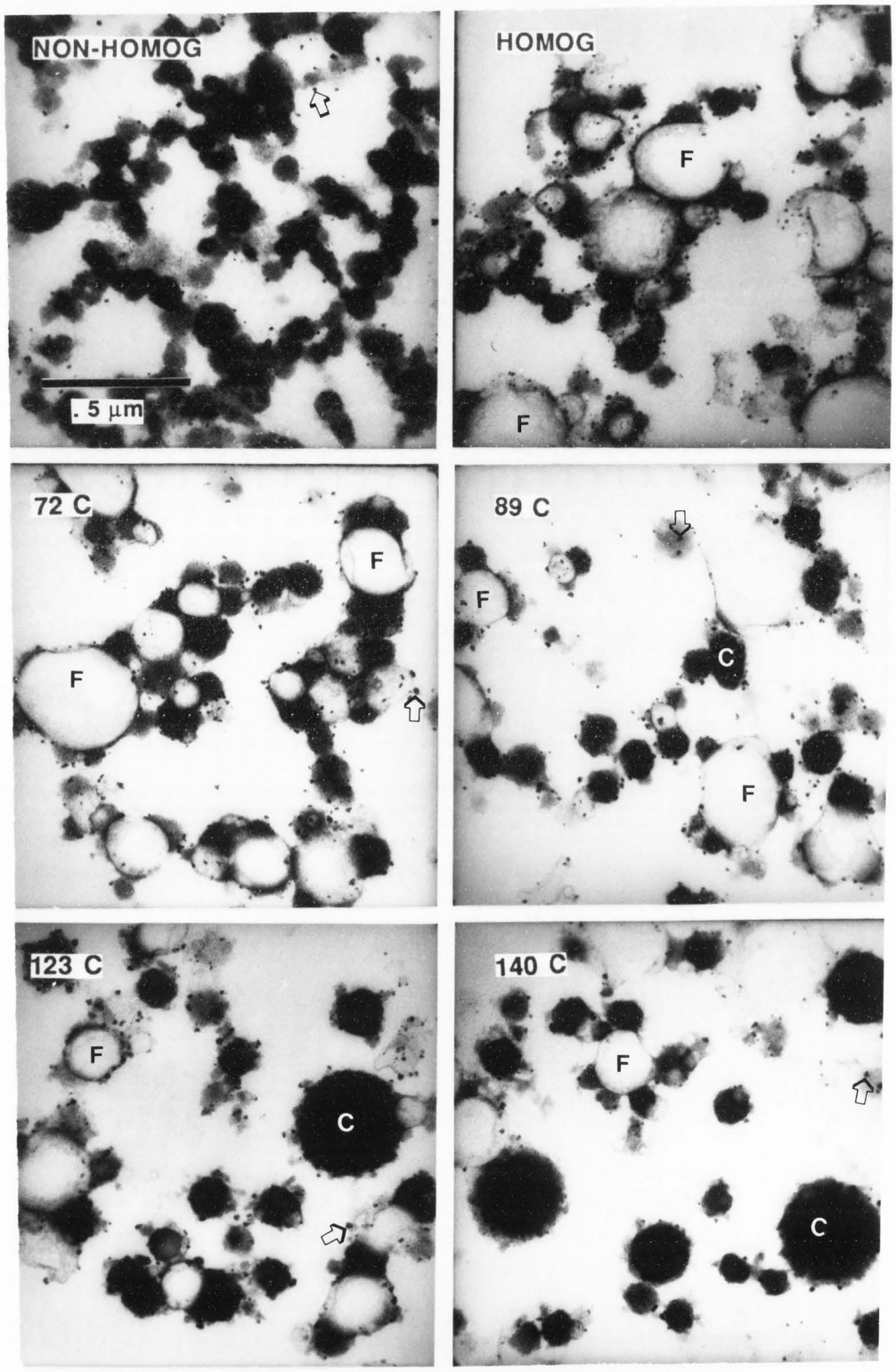
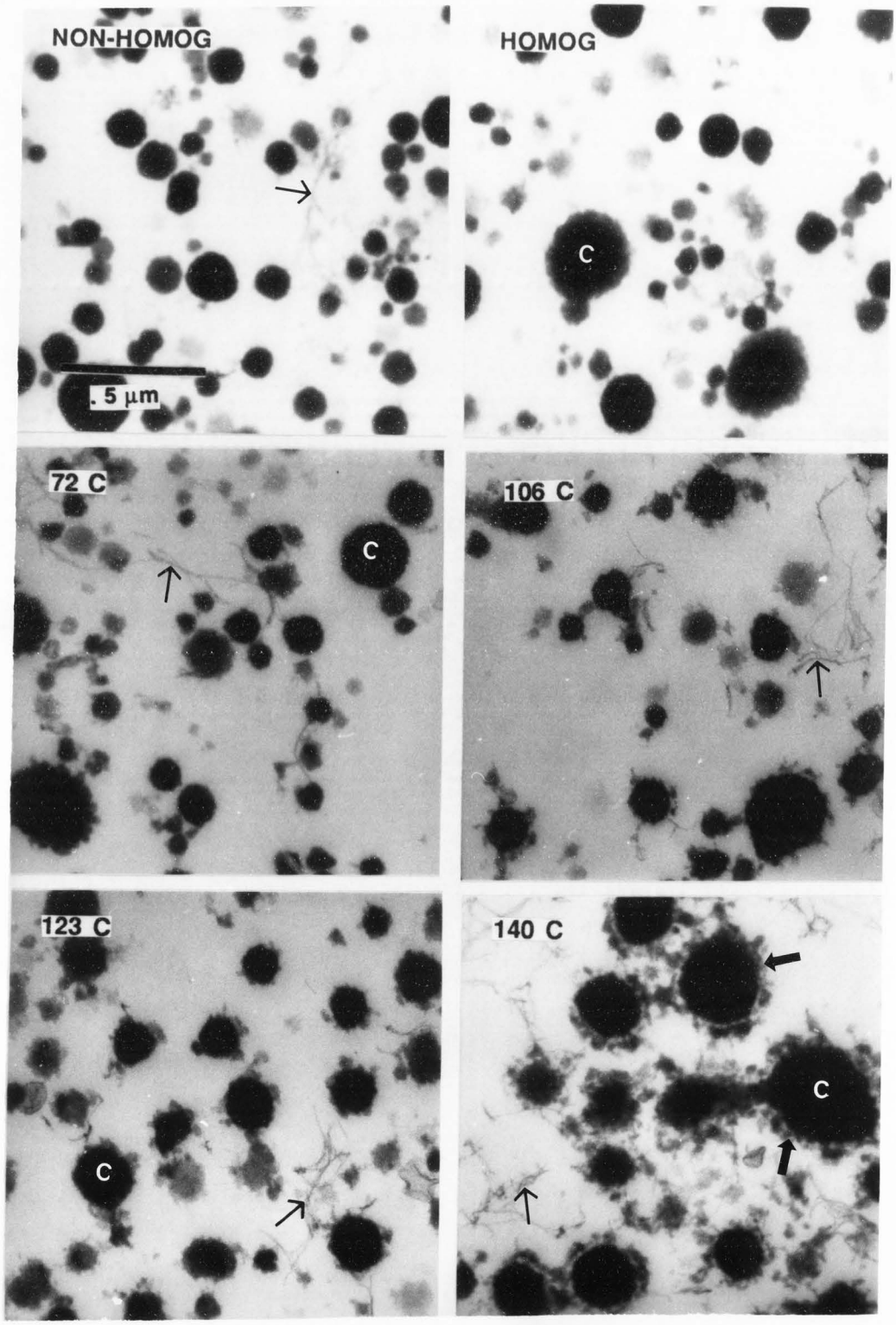

NON-HOMOG
. 5 µm
HOMOG
F
F
72 C
F
F
89 C
F
C
F
123 C
F
C
140 C
F
C

Figure 20. Transmission electron micrographs of 3X UF concentrated skim milk heated to 72, 106, 123, and 140°C, compared to unheated unhomogenized (NON-HOMOG) 3X UF concentrated skim milk and 3X UF concentrated skim milk heated to 60°C and homogenized (HOMOG). Casein micelles(C) were distributed throughout the serum. The same irregular lines (small arrows) as described in Figure 14 were present.


NON-HOMOG
.5 µm
HOMOG
C
72 C
C
106 C
123 C
C
140 C
C

resultobtained by Kalab and Harwalker (40) who reported that as milk total solids increæ, casein micelles are fused together, and their size increases. A high solid content implicly implies a low water content. This means that in concentrated milks there was less water vailable as a medium for the dispersion of the casein micelles. More protein material adherd to the casein micelles in concentrated milks thàn in their corresponding unconentrated counterparts, especially in UHT samples (Figures 21 and 22).

Adsorbed protein material was less fused with the casein micelles in skim milk than with vole milk. This may be because of the presence of fat in whole milk. More adsorbd protein material suggests that RCT was increased because of the steric interfeence which rendered the κ-casein unaccessible to the action of the enzymes. That was tre when comparing milks of the same concentration (Figure 3). But when compáng unconcentrated milks with concentrated milks, RCT decreased dramatically in the coentrated samples indicating that the effect of concentration was more drastic than that ofhe adhering protein material on the casein micelles. Comparing Figures 16 and 17 (gels c 1X whole and skim milks) with Figures 19 and 23 (gels of 3X whole and skim milks) hows that gels of concentrated samples were more dense than those of unconentrated ones. Also the casein micelles were larger in the concentrated samples which gain confirms the results obtained by Kalab and Harwalker (40). As temperature was inreased, the gels lost integrity, and the casein micelles increased in size. This was similaro the results obtained with unconcentrated milks.

When 3X skim milk was UHT processed, there was more accumulation of protein onto th casein micelle surfaces than in 1X skim milk (Figure 21, 3XS). It appeared to be a two-ep process. First, a compact mass of material (presumably β-lactoglobulin) compleed with the casein micelles. This caused the sizes of the micelles to be increased in compason to those in unheated milk (Figure 20). Following this complexing of β-lactolobulin with κ-casein, additional material was adsorbed onto the micelles to form a porous oating encircling the micelles. This was not the case when fat was present because

Figure 21. Comparison of transmission electron micrographs of four milk samples showing effects of presence of fat and UF concentration on the protein complexes that are formed during UHT processing (140°C). 1XW, whole milk; 1XS, skim milk; 3XW, 3X UF whole milk; 3XS, 3X, UF skim milk. Fat droplets (F) have adsorbed casein micelles on their surfaces. Casein micelles (C) are larger in UF samples. Additional protein material deposited on the casein micelles (bold arrows). The same irregular lines (small arrows) as described in Figure 14 are present. The same electron-dense artefacts (arrow heads) as described in Figure 15 are present.

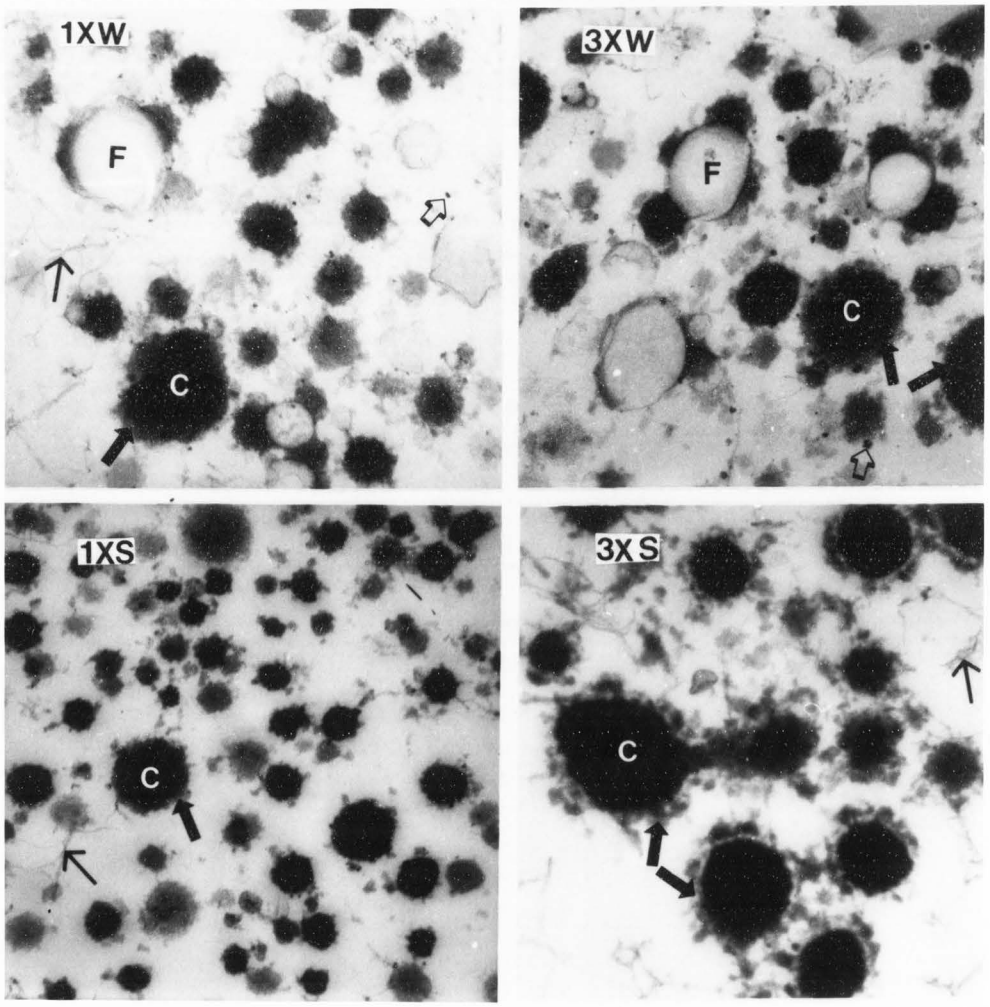

1XW
F
C
3XW
F
C
1XS
C
3XS
C

Figure 22. Comparison of transmission electron micrographs of gels made from four milk samples showing effects of presence of fat and UF concentration on the protein complexes that are formed during UHT processing (140°C). 1XW, gel made from whole milk; 1XS, gel made from skim milk; 3XW, gel made from 3X UF whole milk; 3XS, gel made from 3X UF skim milk. Fat droplets (F) have adsorbed casein micelles on their surfaces. Casein micelles (C) are larger in UF samples. Additional protein material deposited on the casein micelles (bold arrows). The same electron-dense artefacts (arrow heads) as described in Figure 15 are present.

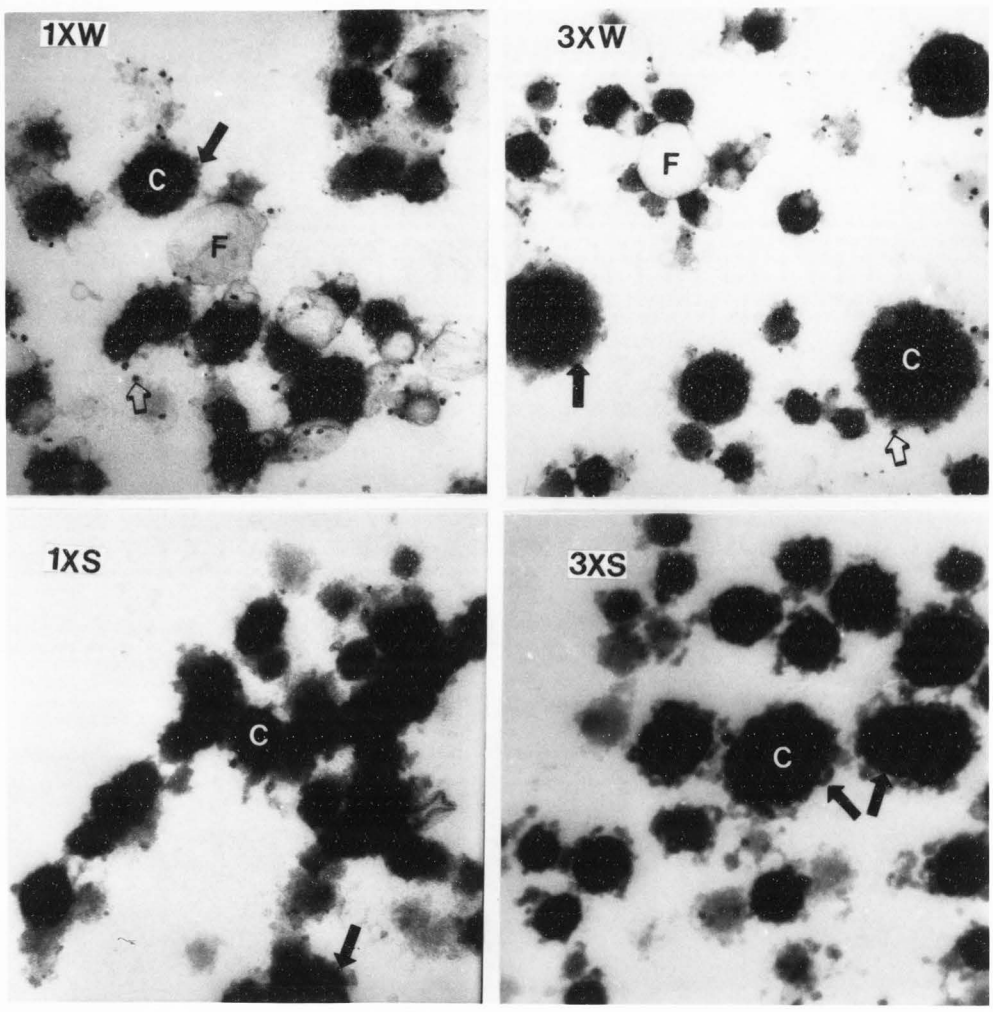
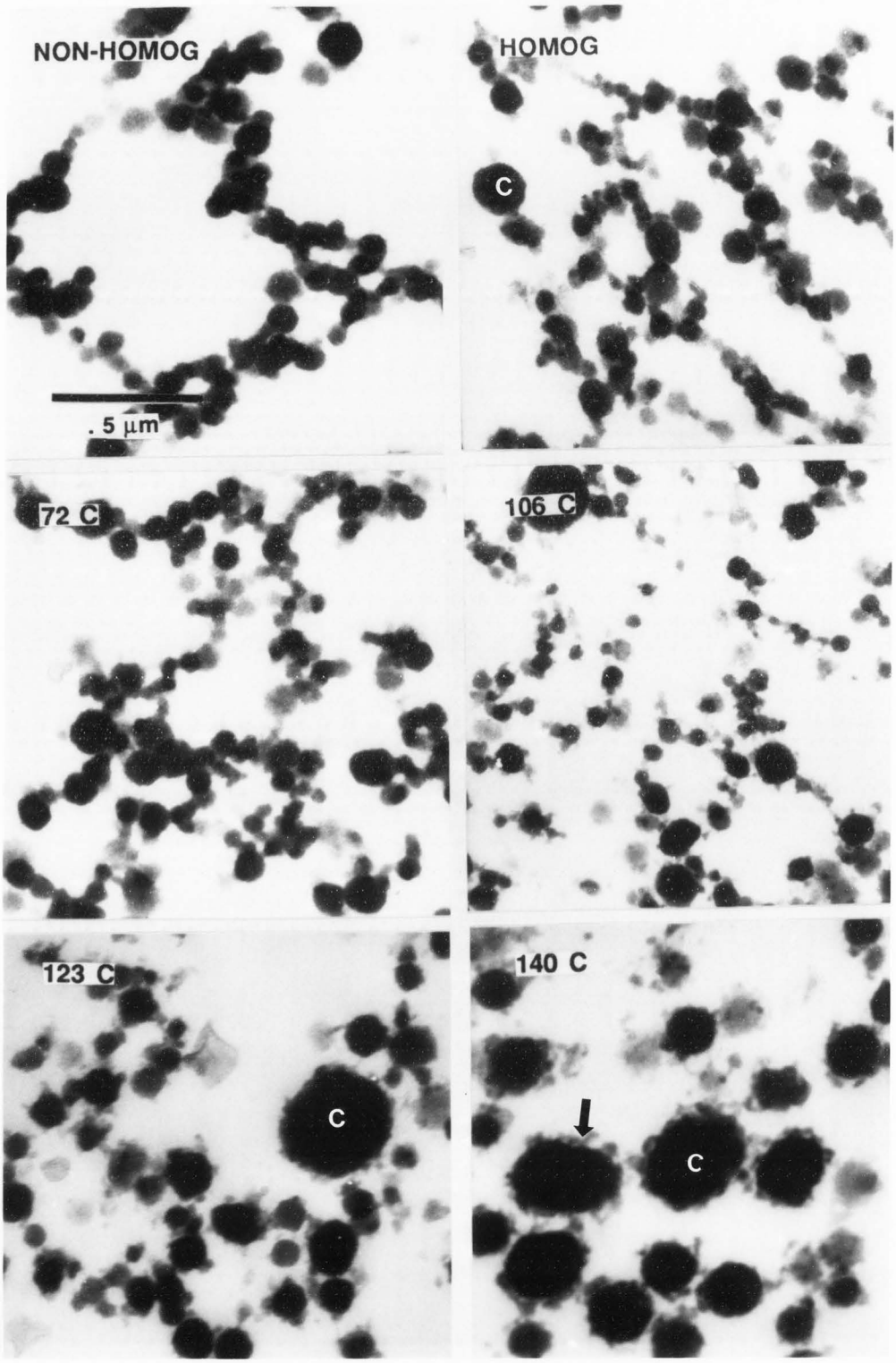

1XW
C
F
3XW
F
C
1XS
C
3XS
C

Figure 23. Transmission electron micrograph of gels made from 3X UF concentrated skim milk heated to 72, 106, 123, and 140°C compared to gels made from 3X UF concentrated unheated nonhomogenized (NON-HOMG) and 3X UF concentrated skim milk heated to 60°C and homogenized (HOMOG). As the milk was heated at higher temperature, it was more difficult to identify individual casein micelles (C) in the gel network. In gels made from UHT milk (140°C), large separate casein micelles were observed. After heating milk to 140°C it appears that additional protein material has been deposited on the surface of the casein micelles (bold arrows).


NON-HOMOG
. 5 μm
HOMOG
C
72 C
106 C
123 C
C
140 C
C

the extraneous protein material was adsorbed onto the fat droplet surfaces (Figure 21, 3XW). Because of the configuration of the UHT processing system, homogenization occurs after UHT heating; so it can be deduced that this secondary layer of micellar material was either:

1) Loosely bound to the micelles by ionic or hydrophobic interactions and was subsequently stripped from the micelles as a result of the interfacial forces generated by creation of the new fat–water interfaces upon homogenization of the fat globules.
2) Accumulated onto the micelle surfaces during storage of the milk after homogenization rather than during UHT heating.

The first hypothesis perhaps better explains these observations the best. Disulfide bonding was thought to occur between denatured β–lactoglobulin and κ–casein, and any β–lactoglobulin that complexed with the casein micelles would be able to withstand the forces imposed on them during homogenization. In contrast, the casein micelles were relatively flexible, open structured molecules with both lipophilic and hydrophilic domains. They, therefore, bound strongly to fat–water interfaces when new fat surfaces were exposed during homogenization. Without any covalent bonds to hold them to the micelles, they dissociated from the β–lactoglobulin–casein micelle complexes and acted to stabilize the fat droplets. From the levels of whey protein denaturation after the various heat treatments it was observed that concentrated milks gave more whey protein denaturation than their corresponding unconcentrated milks. This is further confirmed by having more protein material in concentrated samples as shown in Figures 22 and 23. Furthermore, the fat globule surfaces were more visible in concentrated milks and their corresponding gels than their unconcentrated counterparts, suggesting that more protein was adsorbed at the surface.

CONCLUSIONS

Whey protein denaturation in milk increased with increasing temperature. Milk heated to 140°C (UHT conditions) had the most whey protein denaturation. Concentrating milk by ultrafiltration increased the whey protein denaturation, although the reason for this has not been determined.

Rennet coagulation time of whole and skim milks and their ultrafiltered 3X counterparts, increased with increasing process temperature. Coagulation time was shorter for the concentrated milks. Unconcentrated milk which was UHT treated, did not coagulate within 200 minutes even when .36 RU/ml of milk was added. In contrast, the concentrated milks that were UHT heated did coagulate. Their coagulation was retarded and had only weak gel strength. Gel strength decreased with increased processing temperature, and increased with increased concentration.

Homogenization decreased the fat globule size, and many casein micelles were deposited on the fat globule surfaces of homogenized milk. Casein micelle size was observed to increase upon heating as well as a result of UF concentration. In milk samples that had been heated at high temperature (especially UHT milks), an accumulation of protein material adhered to the casein micelles. This adhered material was more fused with the micelles of whole milk than with that of skim milk.

Denatured β-lactoglobulin had complexed with κ-casein on the casein micelle surfaces and was covalently bound to the micelles through disulfide bonds. Further protein material can then complex noncovalently to this denatured β-lactoglobulin but can be removed by homogenization.

The gels formed by renneting unheated milk consisted of chains of casein micelles with many crosslinks between the chains. When milk was heated, there was less evidence of chain formation and fewer crosslinks. UHT treatment retarded coagulation, and only weak gels were formed from the concentrated milks. This was evident when the microstructure of UHT milk gels were compared to gels from unheated milk.

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