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Effects of Heat Treatment and Post-Treatment Holding Time on Rennet-Clotting Properties of Milk

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EFFECTS OF HEAT TREATMENT AND POST-TREATMENT HOLDING TIME ON
RENET-CLOTTING PROPERTIES OF MILK

by

Zeynep Ustunol

A thesis submitted in partial fulfillment
of the requirements for the degree
of
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in
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Approved:

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ABSTRACT

Effects of Heat Treatment and Post-Treatment Holding Time on Rennet-Clotting Properties of Milk

by

Zeynep Ustunol, Master of Science
Utah State University, 1983

Major Professor: Rodney J. Brown
Department: Nutrition and Food Sciences

Samples of raw whole milk were heated at 25, 50 and 75 °C for 0, 30, 60, 120 and 240 min. After heat treatment each sample was subdivided and portions held at 0 °C for 0, 30, 60 and 120 min. In a second experiment samples of milk were heated at 25 and 50 °C for the same lengths of time as before but this time at 75 °C for only 5, 10, 15 and 20 min. A constant holding time of 30 min was used after the heat treatments. Following the various heat treatments pH of each sample was measured and rennet clotting properties of milk at a constant rennet concentration were determined using the Formagraph instrument. The 75 °C heat treatment of both experiments was left out from the statistical analysis.

Results obtained in the first experiment indicate that cold
storage at 0 C for up to 120 min has no significant effect on pH or on rennet-clotting properties of milk. Milk that was heated at 75 C for 30 min or longer did not coagulate. Results of the second experiment show that increase from 25 C to 50 C and extended heating time both reduce pH and clotting time. However, curd firming rate and cutting time are only affected by the length of time that the milk is heated. When milk is heated at 75 C coagulation time, curd firming rate and cutting time of curd are severely retarded.

(66 pages)
INTRODUCTION

In modern dairy technology milk used for manufacturing of all dairy products is subjected to heat treatment. It is required by law that certain dairy products receive specified heat treatments; others, such as concentrated and dry milks, gain their identity through the heat treatments they receive. Milk is treated at a variety of temperatures for various lengths of time which may range from mild for cheese milk to severe for ultra high temperature (UHT) products and sterilized concentrated milks.

Because of its commercial significance and academic interest there has been steady research on heat stability of milk since 1919. Early studies on heat stability were done on milk used for manufacturing of evaporated milk which had to be sufficiently heat stable for the concentrated product to withstand heat sterilization without coagulation (Sommer and Hart, 1922). The general subject has been reviewed by Pyne (1945, 1962), Rose (1963, 1965) and Fox and Morrissey (1977).

Between 1919 and 1960 most of the attention was focused on the influence of milk salts on heat stability (Sommer and Hart, 1919, 1922; Miller and Sommer, 1940; Pyne and Mc Henry, 1955; Pyne, 1958, 1962). In general the results of these studies were inconclusive. During this time, some work was also done on heat coagulation time-pH (HCT/pH) curves, but it was not until the 1960's that its significance was appreciated (Rose, 1961). The influence of numerous experimental and compositional factors on the HCT/pH curve has been investigated.
Recent studies have been concerned with factors affecting heat stability of milk and changes that take place when milk is heated (Rose, 1962; Morrissey, 1969b; Sweetsur and White 1974; Fox and Hearn, 1978; Elfagm and Wheelock, 1977; Fox, 1981; Davies and Law, 1983).

Upon exposure of milk to elevated temperatures major changes take place which affect the stability of the milk colloids and have a bearing on the coagulation process to varying degrees (Fox, 1981). After heating, milk is often kept in cold storage until use. This is to preserve its physical, chemical and bacteriological properties. But cold storage causes modifications in the physico-chemical state of several of the milk components. As a result, the behavior of milk during cheese making is also modified (Ali et.al., 1980). However, coagulation of milk is a complex process; temperature, substrate composition and enzyme concentration all influence clotting. Effect of any of these parameters can be examined by holding all the others constant or by statistically accounting for all of them (Mc Mahon and Brown, 1982).

Most research in this area has been done on milk which has been subjected to severe heat treatments, often near 140 C, or over the range of 110-150 C (Sommer and Hart, 1919, 1922; Fox and Morrissey, 1977; Fox, 1981). There has been very little research on heat stability of cheese milk and few studies have been conducted on the effects of mild heat treatments, near the temperature of pasteurization, on enzymic coagulation. This study is concerned with the effect of mild heating in the temperature range of 0 to 75 C, for up to 240 min, and the effect of post-treatment holding time at 0 C.
for up to 120 min on clotting ability of cheese milk at a constant rennet concentration.
Enzymic Coagulation of Milk

Traditionally chymosin (E.C. 3.4.23.4) has been the enzyme used in the process of cheese making. The clotting of milk by chymosin is a complex process, which takes place in several overlapping steps. During the primary stage of the process the milk protein $\kappa$-casein is hydrolyzed by the enzyme at the phe$_{105}$-met$_{106}$ bond, to form para-$\kappa$-casein and a macropeptide.

$$\kappa\text{-casein} \rightarrow \text{Para-}\kappa\text{-casein} + \text{Macropeptide}$$

The macropeptide moiety (Residues 106-169) which is hydrophilic and therefore soluble diffuses away from the micelle after the enzyme action, whereas the para-$\kappa$-casein moiety (residues 1-105) is strongly hydrophobic and remains attached. The primary phase of the enzymic reaction, alters the structure of the casein micelles which makes them later susceptible to coagulation. During this stage the viscosity of the milk initially decreases, and increases again to its original as aggregation begins to take place (Scott-Blair and Oosthuizen, 1961). Decrease in viscosity is suggested to be due to the removal of the macropeptide which is accompanied by a change in the hydrodynamic radius of the micelle as well as to change in the molecular weight (Walstra et al., 1981).

Hydrolysis of $\kappa$-casein by the enzyme destabilizes the casein
micelle, and causes its aggregation by random diffusion. This aggregative phase is referred to as the secondary stage of the clotting process. In the overall clotting reaction these stages are not strictly separable, aggregation of the casein micelles starts before all of the $\kappa$-casein is cleaved by the enzyme. Dalgleish (1979) postulated that 96% of the $\kappa$-casein must be hydrolyzed for a micelle to participate in the aggregation process. Payens et al. (1977) suggested instead that the two stages start simultaneously, and the lag time is only due to different orders of the enzymic and aggregation reaction. Aggregation rate of the micelles is slow at the beginning, but increases as more $\kappa$-casein is split (Darling and Hooydonk, 1981).

During this phase the aggregate material is produced by the bumping together of the renneted micelles which link together to form an open network (Mc Mahon et al., 1983a). Exactly how the micelles interact to produce coagulum has not yet been fully explained. It is suggested that when a considerable amount of $\kappa$-casein has been hydrolyzed, it creates suitable circumstances for specific interactions to take place between the micelles. Whether there indeed exist specific sites for precise interaction is not clear, it has only been suggested (Slattery, 1976; Green and Marshall, 1977; Payens, 1977). However, it is not necessary to postulate specific interactive sites. As another possibility, the free energy for interaction can be thought of as being made up of factors such as overall charge, hydrophobicity, and entropic change resulting from the loss of macropptide moieties.

As the stabilizing power of the $\kappa$-casein is destroyed by the action of the enzyme, the micelles in milk become progressively more
susceptible to clotting in the presence of calcium. A clot will not form in the absence of calcium. When calcium ions are not present, para-κ-casein seems to interact with $\alpha_{S1}$- and $\beta$-caseins and therefore does not precipitate (Lawrence and Creamer, 1969; Berry and Creamer, 1976).

Coagulation of milk is the most obvious aspect of the enzyme action. Although aggregation is observed before coagulation takes place, enzymic coagulation only results after a critical amount of aggregation has occurred. During coagulation the system changes from independent aggregating particles into an extended gel network of interconnected casein micelle chains. The structure rearranges itself into a more stable position (Goodarz-nia, 1978) and the milk is no longer a fluid of constant viscosity but rather transforms into gel (Tuszynski et al., 1968). After the gel network has been formed aggregation is no longer entirely by random diffusion. Links between the casein micelles rearrange themselves to produce a more dense structure (Sutherland and Goodarz-nia, 1971). This stage is considered the tertiary stage of the process, although it is not as clearly defined. It involves processes such as syneresis (the expulsion of water and shrinkage of the gel due to structural rearrangement of the curd) and non-specific proteolysis of the caseins in the curd by the enzymes that are incorporated.

Coagulation of the milk system by chymosin is due to the destabilization of the casein micelle. However, any factor contributing to this destabilization ultimately effects the coagulation of milk. The end result may be a summation of many minute changes in
the colloidal system.

One of the most important factors in manufacturing of cheese is the production of a satisfactory curd. The nature of the coagulum formed determines to a great extent the quality of the final product. It is desired to have a firm curd for production of good cheese. Although pasteurization destroys the undesirable gas and flavor forming microorganisms and inactivates some of their enzymes, longer coagulation time is observed in milk that has been overheated (Moir, 1930a, 1931a). There is also softening of curd, and of the resulting cheese. The making process is delayed, the curd is weak and retains excessive moisture (Moir, 1930b, 1931b). It has been reported in early research on making Cheddar cheese that the addition of a small percentage of calcium chloride improves the coagulation of pasteurized milk (Sommer and Hart, 1922). Addition of up to .05 M reduces coagulation time to a minimum. At very high calcium chloride levels (.4 M) coagulation of milk is severely retarded and only a weak curd is formed (McManon et al., 1933b).

These facts seem to indicate that the composition of milk is altered in some definite way by heating and the constituents which have been affected are among those which are involved in enzymic coagulation of milk. Since production of an almost normal curd results after adding calcium salts, it is reasonable to suppose that the heating partially affects the calcium salts of milk.
Major Changes That Take Place in Milk Upon Heating

Change in Salt Balance and pH

Salt balance and acidity are two of the most important factors in heat stability of milk. Colloidal and serum salt levels are important in maintaining casein integrity and lowering or raising the temperature of the system affects this equilibrium. Stability of milk is similarly sensitive to pH.

Upon heating, both total soluble and ionic calcium concentrations are reduced (Evenhuis and De Vries, 1956a,b; Rose and Tessier, 1959). However, this reduction seems to be slight under pasteurizing conditions. Heating for half an hour, in the temperature range 57-60°C causes 0.6% of the total calcium to become insoluble. This value increases to about 2% when milk is heated in the temperature range 63-65°C. At higher temperatures the amount that becomes insoluble varies from 2.5 to 3.6%. About 3.5% of the total phosphorus is changed to the insoluble state in the temperature range 79-81°C. At temperatures lower that this no significant change is observed (Mattick and Hallet, 1929). Hilgemann and Jenness (1951) instead, reported an initial loss of about 25% of soluble calcium in milk heated to 78°C for 30 min, and indicated that soluble phosphorus undergoes a similar change. Values reported for changes in salt distribution in heated milk show large discrepancies. This might be due to different methods used or to the variations in the time lapse between heat treatment and analysis.

Reduction of soluble and ionic calcium in heated milk is partly
due to conversion of soluble calcium phosphate to the colloidal state. The solubility of calcium phosphate decreases with increasing temperature and increasing pH. It is suggested that calcium phosphate precipitates on heating as hydroxyapatite (Evenhuis and De Vries, 1956a,b) and that it is different from indigenous colloidal calcium phosphate (CCP), which is thought to be a calcium phosphate/calcium citrate complex of the apatite form. Citrate does not precipitate on heating which may account for the differences in composition (Evenhuis and De Vries, 1956b; Fox et al., 1967). Because of its association with the casein micelles, heat precipitated calcium phosphate in milk does not sediment (Evenhuis and De Vries, 1956a) and it is also less soluble upon cooling compare to indigenous CCP (Fox et al., 1967). When heated milk is cooled it becomes unsaturated with respect to calcium and phosphate. However, the indigenous CCP slowly dissolves to restore equilibrium (Pyne, 1958; Kannan and Jenness, 1961).

Morrissey (1969a,b) suggested that reversible heat induced changes in calcium phosphate equilibrium affect the secondary or calcium ion stage of enzymic coagulation. This investigation essentially confirms the views originally put forward by Pyne (1945).

The acidity of milk increases with temperature. The pH of skim milk decreases approximately 0.1 pH unit for each 10 °C temperature rise (Miller and Sommer, 1940). Decrease in pH upon heating is partially due to changes in the buffer capacity of the milk salts and release of CO₂ upon heating. According to Fox (1981), when milk is heated at elevated temperatures for prolonged periods of time additional acidity is developed as a result of:
1- Production of organic acids, principally formic from lactic
2- Release of hydrogen ions due to precipitation of primary and secondary calcium phosphate
3- Release of hydrogen ions due to hydrolysis of organic (casein) phosphate and its subsequent precipitation as Ca\(_{3}\)PO\(_{4}\)\(_2\)

These reactions contribute 50%, 20% and 30% respectively to the pH decline (Pyne and McHenry, 1955).

Enzymic coagulation of milk is inversely related to pH and is especially sensitive to pH changes in the pH range of 6.5-7.0 (Ernstrom, 1961).

**Casein Alteration**

The caseinate system is unique among major protein systems in its ability to withstand high processing temperatures. This is possibly due to the relatively small amounts of secondary and tertiary structure and a rather complex quaternary structure. Although casein is not denaturable within the strict definition of the term, it does undergo changes when subjected to heat. Following are the major changes that take place in casein upon heating:

**Dephosphorylation:** Liberation of inorganic phosphate from casein has been mentioned previously in the section on salt balance and pH. Dephosphorylated casein is much more heat labile than casein, and is capable of binding less calcium (Howat and Wright, 1934).

**Proteolysis:** Alais et.al. (1967) reported that peptides are split from whole casein or isolated \(\kappa\)-casein upon heating at 120 C for 20 min, and these peptides appear to be similar in their physical and chemical
properties to the macropeptides produced from κ-casein upon cleavage by chymosin. It has also been reported that glycopeptides are released when milk is heated at temperatures of 50°C and above. With the exception of D-mannose (which is located mainly in chymosin produced para-κ-casein) the amount of carbohydrate attached to the glycopeptides which are released is considerably less than that released by chymosin, and relative amounts of component sugars vary with heating temperature. These results suggest that chymosin and heat seem to hydrolyze similar bonds, and that additional bonds are also hydrolyzed by heat (Hindle and Wheelock, 1970).

**Aggregation and dissociation of casein micelles:** Studies involving ultracentrifugation, viscosity measurements and gel permeation chromatography show that micelles aggregate initially when heated but that later they dissociate until onset of coagulation, when rapid and extensive aggregation occurs (Fox, 1981).

**Whey Protein Denaturation**

In contrast to the caseins, the non-casein proteins of milk are relatively heat labile. Denaturation of whey proteins alters the course of milk coagulation and the rheological properties of the curd formed. Denatured whey proteins due to heating result in cooked flavor in milk, increased heat stability following concentration, reduced colloidal stability when frozen and resistance to clotting by rennin. Such changes are of practical significance in the production of cheese, milk powder and concentrated milk products.

The serum proteins make up approximately 0.6% of the milk or 20%
of the proteins in milk. The composition of the serum protein fraction based on electrophoretic analysis (Larson and Rolleri, 1955) is approximately 55% β-lactoglobulin, 12% α-lactalbumin, 10% proteose peptone and 10 to 15% casein with the remainder composed of globulins and enzymes.

Earlier methods of analysis based on acid precipitation have demonstrated that heat treatments at 77°C for 60 min, 80°C for 30 min or 90°C for 5 min completely denature whey proteins (Parry, 1974). In the temperature range 62 to 80°C the relationship of temperature to time for a constant level of serum denaturation is semilogarithmic. A temperature increase of 7.5°C reduces the time required for a fixed level of denaturation ten-fold (z value of 13.5) (Harland et al., 1952).

Quantitative electrophoretic analysis of the serum from heated milk place immunoglobulins, serum albumin, β-lactoglobulin, α-lactalbumin in order of increasing resistance to heat denaturation, α-lactalbumin being the most heat resistant component (Larson and Rolleri, 1955; Lyster, 1970). Heat treatment of skim milk at 70°C for 30 min denatures only 6% of the α-lactalbumin but 32% of the β-lactoglobulin, 52% of the serum albumin and 89% of the immunoglobulins. This represents cumulatively 29% of the total serum proteins upon heating to elevated temperatures. Denatured whey proteins do not precipitate in milk, but rather coprecipitate with the caseins on acidification, salting out or ultracentrifugation (Edmondson and Tarassuk, 1956a; Fox et al., 1967; Rowland, 1933; Sullivan et al., 1957).
An interesting hypothesis on the relationship between the heat stability of milk and thermal behavior of whey proteins as affected by pH and calcium ion concentration has been developed by De Wit (1981). De Wit analyzed some of the physico-chemical properties of the major whey proteins with differential scanning calorimetry, studying their thermal behavior up to 150 °C. He observed two distinct heat effects, the first near 70 °C denoted as the denaturation heat and the second near 130 °C ascribed to unfolding of the residual protein structure. Protein unfolding and protein aggregation are two different processes, which may behave differently from one another with respect to change in pH, protein concentration and the concentration of salts or other substances such as urea. Denaturation of the whey proteins is largely determined by the pH of the solution; the extent of aggregation however seems to be dependent on the presence of calcium ions (De Wit, 1981).

Denaturation results in structural modification of the protein molecule and an increase in reactivity of specific groups. The activation of sulfhydryl groups is one of the most readily detectable chemical changes that follows denaturation (Parry, 1974) and among the whey proteins β-lactoglobulin is known to be the most prominent sulfhydryl containing component. Sawyer (1969) and McKenzie (1971) observed the effect of heat on β-lactoglobulin. β-lactoglobulin dissociates from a dimer (36,000 daltons) to a monomer (18,000 daltons) as the temperature is increased from 20 to 45 °C. Amino acid analysis of β-lactoglobulin indicates that each 36,000 dalton dimer contains two -SH groups and four -S-S- linkages. The -SH groups of β-lactoglobulin are unreactive when the protein is in the native state, however, a
marked increase in reactivity is noticed after primary denaturation due to heat. This denaturation phase is identical to the reversible dimer to monomer dissociation mentioned previously. Prolonged exposure to denaturation conditions causes even more extensive protein unfolding, leading to cleavage of disulfide groups and/or other exchange reactions (Mc Kenzie, 1971).

**Casein Whey Protein Interaction**

When β-lactoglobulin and κ-casein are heated together or when κ-casein is added to preheated β-lactoglobulin they interact by sulphydryl-disulfide interchange (Sawyer, 1969). Essentially all work on β-lactoglobulin and κ-casein interactions has been done in a temperature range of 80 to 90°C.

Creamer et.al. (1978), studied skimmilk heated at 100°C for 30 min, and observed these heat induced complexes with an electron microscope. Complexes appear to be large, containing hundreds of individual protein molecules attached to the micelles or incorporated into them. Their form changes at higher pH being more filamentous and showing less association with casein micelles.

Most of the proposed models for casein micelles suggest that κ-casein is on the outer surface of the micelle. One possibility for the formation of the heat induced complex in milk is for one of the whey proteins to interact with a κ-casein molecule at the micelle surface and other whey proteins to interact with this unit complex. Because almost all of the κ-casein in milk interacts with whey proteins, the κ-casein must be either on the outside of the micelle or
readily accessible to the whey proteins (Creamer et al., 1978).

The heat induced interaction between β-lactoglobulin and κ-casein by disulfide bonds may occur in a very narrow pH range between 6.7 and 7.0 with pH 6.8 being the critical value. This complex formation is sensitized by the presence of calcium salts. More severe heat treatments increase the sensitivity of the whey proteins to calcium ions (De Wit, 1981).

Chymosin is unable to release the macropeptide from κ-casein in heated milk. Isolation of the heat induced complex from heated milk, and the large size of this complex led Shalabi and Wheelock (1976) to believe that chymosin may not be able to react with κ-casein simply because κ-casein is physically inaccessible to the enzyme in heated milk.

Upon heating α-lactalbumin and κ-casein do not interact (Hartman and Swanson, 1965). However, Hunziker and Tarassuk (1965) stated, that α-lactalbumin and β-lactoglobulin do interact and that this complex appears to be able to interact with κ-casein (Baer et al., 1976; Elfagm and Wheelock, 1977).

It is not certain that casein/whey protein interactions take place in milk heated at temperatures lower than those indicated above, or that the complexes are similar in isolated and complex systems (Morr, 1965). But, it is suggested that similar interactions occur between β-lactoglobulin and casein upon heating milk at lower temperatures and these interactions seem to affect heat stability (Tessier and Rose, 1964; Fox and Hoynes, 1975) and rennet coagulability of milk (Morrissey, 1969a; Shalabi and Wheelock, 1977).
The Effect of Cold Storage on Milk

The main purpose of cold storing milk is to preserve its physical, chemical and bacteriological properties. However, cold storage modifies the physico-chemical state of several of the milk components; especially causing casein, calcium, magnesium and phosphorus to dissociate from the casein micelles.

Heat treatment causes reduction of both total soluble and ionic calcium and phosphorus. When milk is held at 5°C there is a gradual reversion toward the original soluble calcium level over a period of 24 to 48 h, soluble phosphorus is observed to undergo a similar change (Hilgemann and Jenness, 1961). This reversion on cool-aging has also been confirmed by analysis of calcium and phosphorus in centrifuged milk (Edmonson and Tarassuk, 1956b) as well as by ultrafiltration (Rose and Tessier, 1959). Changes in calcium and phosphate equilibrium due to heating and cooling of milk alters the pH of the milk; pH of heated milk increases upon cooling.

There is a change in micelle size with storage over a period of 4d h at 4°C. The smaller micelles (120 nm diameter or less) decrease in number. They are probably not entirely stable and are incorporated into larger micelles. During cold storage the relative amount of \( \beta \)-casein in the micellar phase decreases significantly. The amount of \( a_{s_2} \)-casein also tends to decrease, however, that of \( a_{s_1} \) and \( \gamma \) casein and an unidentified casein fraction show little variation. Serum casein concentration increases, reaching as much as 42% of the total casein during storage (Ali et al., 1980). The serum casein is very
rich in $\beta$-casein and comparatively poor in $\alpha_{S1}$ and $\alpha_{S2}$ caseins. The increase is due almost entirely to $\beta$-casein, with $\gamma$-casein also making a significant contribution (Davies and Law, 1983). After storage for 48 h, when maximum dissociation is observed, about 30-60% of the $\beta$-casein can be found in the serum phase (Ali et al., 1980).

Changes in the composition and distribution of micellar and serum caseins induced by cooling milk at 4°C are completely reversible when milk is re-equilibrated at 20°C for 18 h (Davies and Law, 1983). When storage time is extended to 7 days at 4°C, soluble casein, calcium and phosphorus concentrations show cycles of increase and decrease (Ali et al., 1980). Udagire and Nickerson (1965) suggested that the equilibrium between the colloidal and soluble calcium phosphate, although slow, affects the equilibrium of the casein constituents and their degree of association.

The changes in the physico-chemical properties of milk due to cold storage prolong rennet coagulation time as compared to the original uncooled milk. Cooling of milk prolongs primary as well as the secondary phase of the coagulation process (Qvist, 1979). According to Pyne (1945) dissolution of the colloidal calcium phosphate decreases sensitivity of the paracaseinate to precipitation by calcium ion. This increase in rennet coagulation upon cooling can be due to changes occurring in both the calcium phosphate equilibrium and the casein micelles.

As a result of the changes that take place in milk upon cooling, the behavior of the milk during cheese making is also modified. The effect of cold storage on cheese yield and quality are less clear, but
there is an increase in percent moisture of the curd, decrease in curd firmness and decrease in cheese yield by as much as 1.9% (Ali et al., 1980).
METHODS AND PROCEDURES

Sample Preparation and Heat Treatment

Raw whole milk was obtained from Utah State University Dairy. Milk was placed in test tubes and stored at 4°C overnight. Samples of each replicate were heated in water baths of 25, 50 and 75°C for 0, 30, 60, 120 and 240 min. Following the heat treatment each sample was subdivided and portions held in an ice bath for 0, 30, 60 and 120 min. After the heating and the holding treatments each sample was held an additional 30 min in the ice bath in order to standardize the temperatures of all samples to 0°C.

Since in the above experiment holding time had no significant effect on pH or on rennet-clotting properties of milk and samples heated at 75°C did not coagulate when heated for 30 min or longer, a second experiment was conducted where samples of milk were heated at 25 and 50°C for the same length of time as before, but this time at 75°C for only 0, 5, 10, 15 and 20 min, and a constant holding time of 30 min was used after the heat treatments.

Following the various heat treatments pH of each sample was measured and the rennet-clotting properties of milk were determined using the Formagraph instrument.
pH Measurement

The pH measurements were done using an Orion Model 811 digital pH meter with an automatic temperature compensation (ATC) probe connected to it. The ATC probe automatically adjusts pH measurements for variation in solution temperature, and the instrument displays the corrected value. The temperature is measured with 0.1°C resolution over a range -5.0 to 105.0°C. The pH of each sample was recorded with an accuracy of ±0.01 pH units, after the samples are held at 0°C for 30 min.

The Formagraph (Model 20)

The Formagraph (Figure 1), which records coagulation properties of cheese milk, consists of two modules. A service module heats the sample cuvettes containing milk, controls the temperature of the instrument and contains the on/off switches for up to five recorder module. The recorder module produces the firmness versus time diagram; it contains a 10 channel recording system and each channel consists of a pendulum with a counterbalanced damper and an optical system. Common for all channels are the sample oscillation system, flashing unit and strip chart recorder.

The technique of measurement is based upon the movement of small stainless steel loop pendulums (Figure 2), which are immersed in linearly oscillating samples of coagulating milk. Previous to coagulation not enough force is applied to the pendulums from the milk
Figure 1. The Formagraph.
Figure 2. Pendulums of the Formagraph.
to cause them to move, but as coagulation takes place increase in viscosity and formation of the curd exerts a force on the pendulums causing them to tilt. A firmness versus time diagram results from light flashes at the ends of each sample oscillation, which transmits a measurement of the pendulum position onto self developing photographic paper (Figure 3). The light flashes are timed at four per minute to coincide with the limits of the oscillation stroke.

A typical firmness versus time diagram is shown in Figure 4. Variable $r$ indicates the time from the addition of enzyme until the point where two lines diverge, this is defined as the clotting time. Time in minutes from coagulation until a width of 20 mm is reached is represented by $k_{20}$. This approximates a curd firmness adequate for cutting. The time $r + k_{20}$ is then the cutting time.

Because of the density change caused by the heat treatments 10 g of each sample was weighed and transferred to the cuvettes of the Formagraph. The cuvettes were placed on the service module and allowed to equilibrate to the instrument temperature of 36.7 °C for 30 min; 200 µl of the diluted enzyme was pipeted into a multispoon apparatus and added simultaneously to each sample. The recorder module was turned on when the enzyme was added to the milk. After the samples were stirred with the spoons, cuvettes were transferred to the recorder module and pendulum loops are lowered into the milk. The instrument was run for 30 min in order to obtain the desired variables.
Figure 3. Schematic diagram of the Formagraph instrument for recording coagulation of milk (McMahon and Brown, 1982).
Figure 4. A typical firmness versus time diagram (McMahon and Brown, 1982).
Preparation of the Enzyme Solution

A purified calf rennet solution of clotting activity of 80 rennet units per ml (RU/ml) was obtained from the New Zealand Cooperative Rennet Co., Ltd., Eltham New Zealand. The enzyme was diluted to .80 RU/ml with .01 M Sodium Citrate buffer at pH 5.2 and maintained below 2 C for the duration of the experiment.

Statistical Design and Analysis

In order to eliminate the effect of possible changes in the composition of milk, a randomized block design was used. The first experiment was a crossed 4*2*5*4 factorial design including all possible interactions of the last three factors. Days were blocked. Each day consisted of one replicate, and a total of four replicates were run.

The second experiment was a crossed 2*2*5 factorial design including all possible interactions of the last two factors. The two replicates were run consecutively.

Data were analyzed by Analysis of Variance using the Statistical Analysis System (SAS) statistical package. The 75 C heat treatment of both experiments was left out of the analysis. Except for blocking, sums of squares for each effect were computed after removing the variability due to other effects. Sums of squares were therefore insensitive to the order of the effects. All effects were tested against the overall residual.
Correlation coefficients for main effects were also computed. Multiple comparisons were done using Duncan's multiple range test. Operating characteristics of this test somewhat resemble Fisher's unprotected LSD test. However, Duncan's test controls error rates at different levels depending on the number of means between each pair being compared.
RESULTS AND DISCUSSION

Effects of Cold Storage on pH and Coagulation Properties of Milk

Analysis of Variance for the first experiment shows that cold storage at 0°C for up to 120 min after heat treatments of 25, 50 and 75°C for 0, 30, 60, 120 and 240 min has no significant effect on pH (Table 1), clotting time (Table 2) or curd firming rate (Table 3) of cheese milk. Temperature/holding time interactions and temperature/heating time/holding time interactions in all cases were also insignificant. Milk that was heated at 75°C for 30 min or longer did not coagulate, even after one hour and was not included in these Analyses of Variance.

Measure of the degree of association between the main effects and the dependent variables is provided by the correlation coefficients in Table 4. There is no significant correlation between holding time at 0°C and pH, between holding time and coagulation time, or between holding time and curd firming rate. These correlation coefficients confirm the results of analysis of variance.
Table 1. Analysis of Variance for dependent variable pH.

<table>
<thead>
<tr>
<th>SOURCE</th>
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<th>SS¹</th>
<th>F²</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Days)</td>
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<td>0.10</td>
<td>......</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.04</td>
<td>47.09</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>0.18</td>
<td>57.80</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Temp.*Heat.Time</td>
<td>4</td>
<td>0.01</td>
<td>3.98</td>
<td>0.0046 **</td>
</tr>
<tr>
<td>Holding Time</td>
<td>3</td>
<td>0.01</td>
<td>1.60</td>
<td>0.1919</td>
</tr>
<tr>
<td>Temp.*Hold.Time</td>
<td>3</td>
<td>0.001</td>
<td>0.48</td>
<td>0.7002</td>
</tr>
<tr>
<td>Temp.*Heat.T.*Hold.T.</td>
<td>24</td>
<td>0.02</td>
<td>0.64</td>
<td>0.8959</td>
</tr>
<tr>
<td>Error</td>
<td>117</td>
<td>0.09</td>
<td>......</td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>159</td>
<td>0.44</td>
<td>......</td>
<td></td>
</tr>
</tbody>
</table>

¹ Except for blocking, sums of squares for each effect were computed after removing the variability due to other effects. Sums of squares are therefore insensitive to the order of the effects.

² All effects are tested against the overall residual.
Table 2. Analysis of Variance for dependent variable r.

<table>
<thead>
<tr>
<th>SOURCE</th>
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<th>SS</th>
<th>$F^1$</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Days)</td>
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<td>818.51</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>4801.78</td>
<td>24.60</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>5905.72</td>
<td>7.56</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Temp.*Heat.Time</td>
<td>4</td>
<td>1130.57</td>
<td>1.45</td>
<td>0.2226</td>
</tr>
<tr>
<td>Holding Time</td>
<td>3</td>
<td>633.33</td>
<td>1.08</td>
<td>0.3604</td>
</tr>
<tr>
<td>Temp.*Hold.Time</td>
<td>3</td>
<td>2243.39</td>
<td>3.83</td>
<td>0.0117</td>
</tr>
<tr>
<td>Temp.*Heat.T.*Hold.T.</td>
<td>24</td>
<td>5328.87</td>
<td>1.14</td>
<td>0.3156</td>
</tr>
<tr>
<td>Error</td>
<td>116</td>
<td>22641.13</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>Corrected Total</td>
<td>158</td>
<td>43656.91</td>
<td>.....</td>
<td>.....</td>
</tr>
</tbody>
</table>

$^1$ All effects are tested against the overall residual.
Table 3. Analysis of Variance for dependent variable $k_{20}$.

<table>
<thead>
<tr>
<th>SOURCE</th>
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<th>SS</th>
<th>$F^1$</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Days)</td>
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<td>.....</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>20.98</td>
<td>0.07</td>
<td>0.7957</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>1396.41</td>
<td>1.12</td>
<td>0.3505</td>
</tr>
<tr>
<td>Temp.*Heat.Time</td>
<td>4</td>
<td>2928.17</td>
<td>2.35</td>
<td>0.0584</td>
</tr>
<tr>
<td>Holding Time</td>
<td>3</td>
<td>339.35</td>
<td>0.36</td>
<td>0.7826</td>
</tr>
<tr>
<td>Temp.*Hold.Time</td>
<td>3</td>
<td>2407.23</td>
<td>2.57</td>
<td>0.0564</td>
</tr>
<tr>
<td>Temp.*Heat.T.*Hold.T.</td>
<td>24</td>
<td>6693.55</td>
<td>0.89</td>
<td>0.6082</td>
</tr>
<tr>
<td>Error</td>
<td>116</td>
<td>36152.95</td>
<td>....</td>
<td>.....</td>
</tr>
<tr>
<td>Corrected Total</td>
<td>158</td>
<td>9145.96</td>
<td>....</td>
<td>.....</td>
</tr>
</tbody>
</table>

$^1$ All effects are tested against the overall residual.
Table 4. Correlation coefficients\textsuperscript{1}.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>r</th>
<th>$k_{20}$</th>
<th>$r+k_{20}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>-0.29</td>
<td>-0.33</td>
<td>-0.02</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.84</td>
<td>0.02</td>
</tr>
<tr>
<td>Heating Time</td>
<td>-0.53</td>
<td>-0.33</td>
<td>-0.11</td>
<td>-0.27</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.15</td>
<td>0.0006</td>
</tr>
<tr>
<td>Holding Time</td>
<td>0.05</td>
<td>0.09</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.53</td>
<td>0.26</td>
<td>0.73</td>
<td>0.38</td>
</tr>
<tr>
<td>pH</td>
<td>0.25</td>
<td>-0.08</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.32</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.09</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{20}$</td>
<td></td>
<td></td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} Correlation coefficients/Significance probabilities associated with the correlation
Heat treatment causes reduction of both total soluble and ionic calcium. When milk is held in cold storage there is a gradual reversion toward the original soluble calcium level. Soluble phosphorus undergoes a similar change (Hilgemann and Jenness, 1951). Changes in the state of calcium and phosphorus due to heating and cooling alter the pH of milk. Odagire and Nickerson (1965) suggested that the equilibrium between colloidal and soluble calcium phosphate, although slow, affects the equilibrium of casein constituents and their degree of association. Ali et al. (1980) reported that during cold storage soluble casein concentration increases, constituting as much as 42% of the total casein at maximum dissociation. Smaller micelles (120 diameter nm or less) decrease in number during storage, suggesting that they may be incorporated into larger micelles. Due to these changes, the rennet coagulation time of cheese milk is extended in comparison to the original uncooled milk (Qvist, 1979). As a result there is an increase in the percent moisture of the curd, decrease in curd firmness and decrease in cheese yield (Ali et al., 1980).

However, these reported changes occur after cold storing milk at 4-5 C over a period of 24-48 h. In our study cold storage up to 120 min at 0 C was observed to have no significant effect on either pH or on clotting properties of milk, which suggests that this length of time is not long enough to significantly alter the physico-chemical state of any of the milk components.
Effects of Heat Treatment on pH and Coagulation Properties of Milk

In the second experiment, both temperature and heating time had a significant effect on pH and on clotting time of cheese milk. However, curd firming rate and cutting time is only affected by the length of time that the milk is heated. Measure of the degree of association between the main effects and dependent variables is provided by correlation coefficients in Table 5. Since no main effect is by itself responsible for determining the effect on any dependent variable, the magnitude of the correlation coefficients is not very high. Therefore, their probabilities also need to be taken into account.

Effect of Heat Treatment on pH

Analysis of Variance (Table 6), indicates that both temperature and heating time had a significant effect on pH. Temperature heating time interaction was also significant.

Comparisons of means of the pHs at each heating time were done using Duncan's multiple range test. Duncan's test for dependent variable pH (Table 7), shows that for the second experiment with data of 25 and 50 °C, there is a significant difference in pH between 0 min and 30 min of heating, having taken out the effect of temperature before comparison. Heating milk for 30 min with a mean pH of 6.63 shows a significant decrease compared to 0 min with a mean pH of 6.66. Heating milk for 30, 60 or 120 min does not result in significant difference in pH. However, heating up to 240 min with a mean pH of 6.60 again results in a significant decrease in pH compare to the
Table 5. Correlation coefficients\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>r</th>
<th>k(_{20})</th>
<th>r+k(_{20})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>-0.55</td>
<td>-0.25</td>
<td>0.29</td>
<td>-0.08</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.29</td>
<td>0.22</td>
<td>0.75</td>
</tr>
<tr>
<td>Heating Time</td>
<td>-0.68</td>
<td>-0.75</td>
<td>0.45</td>
<td>-0.59</td>
</tr>
<tr>
<td></td>
<td>0.0009</td>
<td>0.0001</td>
<td>0.05</td>
<td>0.007</td>
</tr>
<tr>
<td>pH</td>
<td>0.54</td>
<td>-0.33</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.15</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td></td>
<td>-0.62</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.004</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>k(_{20})</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.88</td>
</tr>
</tbody>
</table>

\(^1\) Correlation coefficients/Significance probabilities associated with the correlations
Table 6. Analysis of Variance for dependent variable pH.

<table>
<thead>
<tr>
<th>SOURCE</th>
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<th>SS</th>
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<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
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<td>0.00002</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Temperature</td>
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<td>0.00392</td>
<td>60.83</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>0.00768</td>
<td>29.79</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Temp.*Heat.Time</td>
<td>4</td>
<td>0.00098</td>
<td>3.80</td>
<td>0.0446 *</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.00058</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Corrected Total</td>
<td>19</td>
<td>0.01318</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

$^1$ All effects are tested against the overall residual.
Table 7. Duncan's multiple range test for dependent variable pH.

<table>
<thead>
<tr>
<th>Heating Time (min)</th>
<th>Mean pH(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.66(^a)</td>
</tr>
<tr>
<td>30</td>
<td>6.63(^b)</td>
</tr>
<tr>
<td>60</td>
<td>6.63(^b)</td>
</tr>
<tr>
<td>120</td>
<td>6.62(^b)</td>
</tr>
<tr>
<td>240</td>
<td>6.60(^c)</td>
</tr>
</tbody>
</table>

\(^1\) Means with the same superscript are not significantly different at alpha level=0.05.
There is an inverse relationship between pH and temperature and between pH and heating time (Figure 5). Heating milk at 75°C reduces its pH from an initial pH of 6.67 to 6.54 in 20 min. At 25 and 50°C the initial pH drop is not as sharp, however, most significant decrease is again observed in the first 30 min of heating. Heating milk for 30 min at 25°C decreases its pH to 6.65. When milk is heated at 50°C for the same length of time its pH is decreased to 6.62. The decline in pH becomes more gradual over extended heating time. After 240 min of heating, pH of milk is lowered to 6.61 when heated at 25°C, and to 6.58 when heated at 50°C.

Correlation coefficients calculated with 25 and 50°C indicate that pH correlates better with heating time, $r = -0.68$ (prob>0.0009), than it does with temperature $r = -0.55$ (prob>0.0129). The negative correlation shows the inverse relationship between the two variables.

Miller and Sommer (1940) suggested that decrease in pH upon heating is partially due to changes in the buffer capacity of the milk salts, and release of CO$_2$ upon heating. According to Fox (1981), when milk is heated at elevated temperatures for prolonged periods of time additional acidity is developed as a result of production of organic acids (principally formic from lactic), release of hydrogen ions as a result of hydrolysis oforganic (casein) phosphate and precipitation of primary and secondary phosphate.

Effect of Heat Treatment on Clotting Time

Temperature and heating time also had a significant effect on
Figure 5. Effect of heat treatment on pH.
coagulation time of cheese milk. Temperature/heating time interaction was significant as well (Table 8).

Duncan's test (Table 9), shows that for data from 25 and 50°C heat treatments, after having removed the effect of temperature, the most significant difference in mean clotting time is observed after 60 min of heating. Heating for 120 and 240 min results in significantly shorter clotting time of the cheese milk compare to heating for 0, 30 or 60 min. However, mean clotting times of either 120 min or 240 min of heating are not significantly different from each other.

Milk that has been heated at 50°C for up to 180 min coagulates faster than milk heated at 25°C for the same length of time. Although mild heating initially shortens clotting time of cheese milk, extensive heating and elevated temperatures have a reverse effect. The coagulation time of cheese milk starts to increase after 180 min of heating at 50°C. When milk is heated for 240 min at this temperature it takes longer to coagulate than milk that has been heated at 25°C. Coagulation time of milk heated at 75°C is severely retarded (Figure 6).

Clotting properties of milk heated at 75°C for various lengths of time can be seen in Figure 7. Heating milk for 5 min at this temperature gives a coagulation time of 15 min. Heating for 20 min extends coagulation time to 26 min. No coagulation is observed when milk is heated for 30 min or longer at this temperature.

Clotting time also correlates better with heating time \( r = -0.75 \) (prob>0.0001), than it does with temperature
Table 8. Analysis of Variance for dependent variable r.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>$F^1$</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>0.0045</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.9245</td>
<td>10.83</td>
<td>0.0094 **</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>11.6750</td>
<td>34.20</td>
<td>0.0001 ***</td>
</tr>
<tr>
<td>Temp.*Heat.Time</td>
<td>4</td>
<td>1.8230</td>
<td>5.34</td>
<td>0.0175 *</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.7680</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Corrected Total</td>
<td>19</td>
<td>15.950</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

1 All effects are tested against the overall residual.
Table 9. Duncan's multiple range test for dependent variable r.

<table>
<thead>
<tr>
<th>Heating Time (min)</th>
<th>Mean r (min)</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>13.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>12.63&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>11.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>11.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Means with the same superscript are not significantly different at alpha level=0.05.
Figure 6. Effect of heat treatment on $r$. 
BEATING TIME (min)

- 25 C
- 50 C
- 75 C
Figure 7. Coagulation properties of milk heated at 75 C.
HEATING TIME (MIN.)

A: 0
B: 5
C: 10
D: 15
E: 20
F: 30
Effect of Heat Treatment on Curd Firming Rate

Analysis of Variance (Table 10) indicate that once coagulation has taken place temperature has no significant effect on curd firming rate of milk, however the length of time that the milk is heated effects it significantly.

Although heating milk at 50°C results in faster coagulation time, 25°C gives faster curd firming rate up to 120 min heating (Figure 8). After 120 min of heating the effect of both temperatures on curd firming rate is the same.

Correlation coefficients calculated with 25 and 50°C data show that curd firmness does not correlate with temperature, but correlates better with heating time $r = 0.45$ (prob>0.048).

Elevated temperature of 75°C has a reverse effect on curd firmness. Heating milk at 75°C very significantly increases the time required to reach adequate curd firmness. Heating over 10 min at this temperature does not give adequate curd firmness for cutting (Figure 7).

Effect of Heat Treatment on Cutting Time

The variable $r+k_{20}$ represents time from addition of the enzyme until adequate firmness is reached to cut the curd, which can be

$r = -0.25$ (prob>0.29). Coagulation time also correlates significantly with pH $r = 0.54$ (prob>0.0143) which may suggest that for temperatures of 25 and 50°C reduced coagulation time is partly due to reduction in pH.
Table 10. Analysis of Variance for dependent variable $k_{20}$.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>$F^1$</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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<td>0.528125</td>
<td>2.93</td>
<td>0.1212</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>2.916750</td>
<td>4.04</td>
<td>0.0380 *</td>
</tr>
<tr>
<td>Temp.*Heat.Time</td>
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<td>1.258750</td>
<td>1.74</td>
<td>0.2241</td>
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<tr>
<td>Error</td>
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<td>............</td>
</tr>
<tr>
<td>Corrected Total</td>
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<td>6.327375</td>
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<td>............</td>
</tr>
</tbody>
</table>

$^1$ All effects are tested against the overall residual.
Figure 8. Effect of heat treatment on $k_{20}$.
referred to as the cutting time. Temperature had no significant effect on cutting time of cheese curd, but the length of time that the milk is heated effects cutting time significantly (Table 11).

Heating milk at 50 C gives a slightly shorter cutting time than 25 C does, however, extended heating at a lower temperature is observed to have an equivalent effect (Figure 9). Heating for 240 min at either 50 or 25 C gives the same cutting time. There is a significant correlation between $r+k_{20}$ and heating time $r= -0.59$ (prob>0.0067). Increasing heating time decreases $r+k_{20}$. Cutting time also correlates somewhat with pH, $r= 0.41$ (prob>0.07).

Heating milk at an elevated temperature of 75 C, as might be expected, significantly increases the cutting time of curd. Heating milk at 75 C for 10 min increases the cutting time from an initial of 21.4 to 35 min. This increase in cutting time is probably due to increase both in clotting time and curd firming rate. Heating cheese milk for 15 min or longer at this temperature does not give adequate firmness for cutting of the curd.

Heat treatment at 77 C for 1 h completely denatures whey proteins. Denaturation of whey proteins alters the course of milk coagulation. When $\beta$-lactoglobulin and $\kappa$-casein are heated together in the temperature range of 80-90 C they interact by sulfhydryl-disulfide interchange. It is not certain that such interactions take place in heated milk or that the complex is similar. Morrissey (1969a) suggested that some type of interaction occurs between $\beta$-lactoglobulin and casein upon heating that markedly affects the rennet-coagulability of milk. According to Hunziker and Tarassuk (1965) $\beta$-lactoglobulin and
Table 11. Analysis of Variance for dependent variable \( r+k_{20} \).

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>( F^1 )</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
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<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>Temperature</td>
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<td>0.055125</td>
<td>0.34</td>
<td>0.5743</td>
</tr>
<tr>
<td>Heating Time</td>
<td>4</td>
<td>7.481750</td>
<td>11.53</td>
<td>0.0014 **</td>
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<tr>
<td>Temp.*Heat.Time</td>
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<td>0.446750</td>
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<td>9.447375</td>
<td>.....</td>
<td>.....</td>
</tr>
</tbody>
</table>

1 All effects are tested against the overall residual.
Figure 9. Effect of heat treatment on $r+k_{20}$. 
α-lactalbumin also interact when heated together, and this complex appears to be able to interact with κ-casein. Perhaps it is this complex that prevents the enzyme from acting on κ-casein and therefore effects the coagulation reaction.

However, milk coagulation is a complex process. Anything that changes the ionic environment around the casein micelles affects coagulation. It is known that milk coagulation is affected by the amount of calcium and phosphate present and is also influenced by pH, ionic strength and temperature of the milk (Zittle, 1970). Changes in the solubility of calcium phosphate due to heating likewise influences this process.
CONCLUSION

1- Cold storage of milk at 0 C up to 120 min following heat treatments of 25 and 50 C up to 240 min, had no significant effect on pH or on clotting ability of cheese milk.

2- Extended heating and elevated temperatures resulted in reduction of pH, and this reduction seemed to contribute partially to decrease in coagulation time of cheese milk.

3- Mild heat treatments decreased the clotting time of milk significantly. But, once coagulation had taken place curd firming rate, was affected only by the length of time that the milk was heated.

4- Elevated temperatures appear to have altogether a different effect on the milk protein system. Coagulation time, curd firming rate and cutting time of cheese milk were severely retarded when heated at 75 C.

Therefore, it is recommended that milk used for manufacturing of cheese be treated at temperatures no higher than and heated no longer than those required for pasteurization, in order to obtain desirable cutting time and curd firmness.
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