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Evaluation and Improvement of Coagulation Properties of Milk for Manufacturing

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EVALUATION AND IMPROVEMENT OF COAGULATION PROPERTIES OF MILK FOR MANUFACTURING

by

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Leslie M. Okigbo

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ABSTRACT

Evaluation and Improvement of Coagulation Properties of Milk for Manufacturing

by

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A rugged and sensitive instrument "Vatimer" was developed to evaluate the coagulation properties of milk in cheese vats. Microvolt signals from the instrument varied with standing-wave motion of milk, forces arising from coagulation, curd firming, curd syneresis and also clean-in-place spray forces. Curd firmness at cutting varied three to four fold in commercial Cheddar cheesemaking operations.

The Formagraph instrument was used to select individual cow milk samples with good and poor chymosin-coagulation characteristics from the Utah State University Holstein herd. Blending 50% good-, and 50% poor-coagulating samples did not improve average coagulation properties of poor samples. Higher chymosin concentration caused curd disintegration in poor samples. It was not neccesary to add

more than .02 rennin units per milliliter of milk, or .02% $CaCl_2$ to produce adequate curd firmness 30 min after chymosin addition if milk pH was reduced to at least 6.4 before coagulation.

Hydroxyapatite chromatography and polyacrylamide gel electrophoresis of whole casein from milk samples with good and poor chymosin-coagulation characteristics showed abnormally high contents of γ- and para-κ-caseins and lower κ- and β-casein concentrations in poor-coagulating samples. Poor samples showed higher deterioration in curd firmness than the good samples on chymosin coagulation after storage of milk samples at $4^{o}C$ for 24, 48, and 72 h with and without added .02% $CaCl_2$. Prolonged storage for 30 days at $-1^{o}C$ caused 7.6% and 68.2% losses in curd firmness for good and poor samples respectively.

Cheddar cheese was made in 9 liter laboratory cheese vats with various proportions of blended, and coagulation-modified poor-, and good chymosin-coagulating milk. One hundred percent poor-coagulating milk produced cheese with significantly higher moisture, lower yield, and very bitter flavor.

(217 pages)

GENERAL INTRODUCTION

There is a need for an objective method to evaluate curd firmness of chymosin treated milk to replace the current subjective methods used by cheesemakers. This would minimize variation in curd firmness at cutting that affects yield and quality of cheese. Many instruments have been developed for this purpose but some of them do not provide continuous data on measurement of milk coagulation so they conceal the kinetics of coagulum formation. Other instruments which provide continuous data are not rugged enough.

Improvement of the quality of coagulum from chymosin treated milk has been of great concern to cheesemakers. In addition to monitoring development of curd, it is also of interest to chemically or physically modify milk to improve the quality of the curd. To achieve this, optimum levels of various factors known to affect coagulum development have to be selected to coincide with changes in milk composition.

The purpose of this work was to:

1. Develop and evaluate a rugged and sensitive instrument for continuous monitoring of milk coagulation in open or closed cheese vats.
2. Select optimum levels of factors that affect milk coagulation properties to improve curd firmness of manufacturing milk, particularly milk that exhibits poor chymosin-coagulation characteristics.

3. Establish factors that relate to retardation of curd formation including possible casein compositional changes, effect of prolonged cold-storage of manufacturing milk, and inclusion of milk from quarters of udders with physiological abnormalities in manufacturing milk supplies.
4. Recommend steps to minimize retardation of curd development to optimize curd firmness to improve yield and quality of cheese.
5. Establish relationships between curd firmness, cheese yield and cheese moisture.

This dissertation consists of articles for publication that elucidate the design, fabrication and evaluation of a new instrument the "Vatimer" for measuring milk coagulation properties in cheese vats. A study of the factors associated with retardation of curd develpoment of chymosin treated milk, together with the improvement of chymosin coagulation properties of milk for manufacturing is also included.

PART 1A. INSTRUMENTS FOR MEASURING MILK COAGULATION TIME AND CURD FIRMNESS: A REVIEW

CURD-O-METER

Studies on measurement of physical changes that occur during milk coagulation was stimulated by Reuben Hill's development of a curd tester at Utah Agricultural Experiment Station. The need for manufacture of this instrument arose when there was great desire to modify cows' milk, which is hard curded, to resemble mother's milk which coagulates to fine, soft curd (9). Such modification is believed to render the curd more digestible by infants. Hill's test apparatus consists of a 10-pronged, star-shapped curd knife. At its center was attached a 6 1/2 inch rod with a loop at its free end. Milk to be tested is put into a jar, and calcium chloride-pepsin mixture is added. The tension required to pull the curd knife, which is attached to a spring balance, through the milk gel after coagulation is interpreted as the firmness of the curd. Hill was able to monitor up to ten fold differences in firmness between milk from individual cows. The instrument was manufactured under the trade name "American Curd-O-Meter" by the Heusser Instrument Manufacturing Company, Salt Lake City, Utah in 1932 and distributed by ZCMI wholesale druggists. The instrument was advertized as being capable of differentiating milk for infant food, market milk or cheese milk on the basis of curd firmness.

ROLLING BOTTLES

Interests in measurement of other changes that occur during milk coagulation was triggered by Hill's discovery (9), and a proliferation of many types of milk curd rheological instruments ensued. Sommer and Matsen (27) extended their studies to measuring flocculation time of renneted casein micelles. They (27) used rolling bottles immersed in a constant temperature water bath to measure clotting time of milk. Milk was put into the bottles, enzyme coagulant was added, the bottles rolled at a constant speed in a constant temperature water bath, and time required to observe the first signs of graininess or flaking was interpreted as coagulation time. Berridge (1,2) further improved the rolling bottle technique and emphasized reproducibility. He formulated a substrate which consisted of calcium chloride fortified reconstituted nonfat dry milk. Scott Blair and Burnett (25) in subsequent studies fabricated an instrument which detected earlier coagulation times than possible with the Sommer and Matsen method (27). Sommer and Matsen (27) also described use of a modified Hill test to measure curd firmness in milk. A top loader balance replaced the spring balance in Hill's test. The resistance encountered by a curd knife in passing through curd was read directly from the balance's dial.

SCOTT-BLAIR'S CURD TESTER MODELS

Scott-Blair (21) described the application of an apparatus for measuring the elastic and plastic properties of cheese curd under a compressive load at the time when it was ready for cutting. He concluded that the shear modulus of curd, which is inversely proportional to elastic deformation, is the most important single factor, and suggested that its measurement will prove useful in standardizing cutting conditions.

In a sequel to Scott-Blair's report Rowland and Soulides (19) simulated commercial cheese making on a small scale (100 mL milk samples) and developed a modified Scott-Blair apparatus. This apparatus was used to test the shear modulus of milk curd obtained from individual cows, and from separate quarters of the udder. They suggested that such tests could be used for preliminary testing of, and classification of milk supplies for cheesemaking on the basis of curd firmness. They also stated that it might be necessary to modify milk by acidification, increasing the amount of rennet added, and addition of calcium chloride to improve curd firmness.

Scott-Blair and Burnett (22) in their subsequent work stated that whereas much work had been done on elucidating the chemical changes which take place when rennet acts on casein in milk, very little had been reported on physical changes. They developed a U-tube gelometer with which they measured rigidity moduli and internal viscosities during

rennet-setting of milk and observed syneresis of curd. The principle of operation of the U-tube gelometer was based on application of air pressure to one end of a curd sample in a wide U-tube and measurement of displacements at the other end. It was basically a manometric measurement. The instrument showed reproducibility on replicate samples of reconstituted nonfat dry milk and fresh milk. The effects of changes in calcium levels on the rigidity moduli of rennet coagulated reconstituted nonfat dry milk was also investigated with the U-tube gelometer.

Further application of the U-tube gelometer to studies on rigidity moduli of rennet coagulated milk included studies of the effects of separation, homogenization, pasteurization, and variation of calcium content of milk on curd firmness (23). Scott-Blair and Burnett (24) also studied the effects of varying rennet concentration, and temperature on curd firmness.

Later developments in studies of curd rheology included direct application of curd firmness testers in cheese vats. Burnett and Scott-Blair (3) described a torsiometer which, when suspended in milk in a cheese vat after renneting, measured the increased rigidity of the curd until time to cut. In addition, it could signal the cheesemaker as soon as a pre-selected firmness was reached. Use of dynamic methods in place of previous static methods of measurements of the complex rigidity moduli (22) became the main study of Tuszynski et al. (31). They utilized the thrombelastograph,

an instrument previously used for measuring coagulation of blood, to measure milk coagulation in laboratory studies. They described apparent changes in setting and consequent consistency of curd as results of variation in pH, content of calcium, and addition of N-ethylmaleimide (NEME), a sulfur bond inhibitor. They proposed models which explained the nature of bonding induced by the action of rennet on milk and on the syneretic process which followed.

Recent developments in curd rheology studies have led to increased demand for replacement of the current subjective methods of determining curd cutting time after addition of enzyme coagulant in commercial operations with more objective and repeatable methods. Such demands have accentuated the design and manufacture of more rugged and accurate instruments. These instruments have been succesfully applied in the manufacture of different cheese varieties including Mozzarella, Swiss, Cheddar, Cottage etc. Emmons et al. (6) described the application of a curd tension meter in studying the effects of different lactic starters on acidity and firmness of cottage cheese coagulum. Williamson and Speck (33) utilized a curd tension meter to measure curd firmness of milk coagulated exclusively with lactic culture. Other authors (10,34) also utilized a curd tension meter to study the effects of some factors that influence the curd tension of rennet coagulated milk. Shehata et al. (26) utilized a penetrometer in measuring the variation in curd firmness of blue cheese as a result of

different acids for direct acidification of milk prior to rennet coagulation.

Curd firmness describes a complex of two elastic and two viscous moduli, and no instrument measures any one modulus specifically (13). Various methods have been developed but each has some drawback (30). Richardson et al. (17) successfully adapted an HVT helipath torsion viscometer to continuosly monitor changes in intrinsic viscosity that occured in renneted milk. Kowalchyk and Olson (11) utlized a modified helipath viscometer to monitor the rate of increase in firmness of milk coagula formed by rennet under various conditions of pH and temperature. McMahon et al. (15) also utilized a helipath viscometer in studying aggregation of chymosin treated casein micelles, and compared this technique to other previously described techniques.

OSCILLATORY DEFORMATION TECHNIQUE

Several principles have been employed in the manufacture and application of curd rheological instruments, and current models seem to emphasize the principle of oscillatory deformation (7,14,32).

Vanderheiden's Curd Firmness Tester

Vanderheiden (32) described the application of 2 juxta-positioned diaphragms; one oscillated and sent impulses through the milk gel to the receiving diaphragm. The extent of pulses or deformations transmitted through the gel was proportional to gel firmness. Kowalchyk and Olson (12)

subsequently applied this instrument in monitoring continuous changes in curd firmness under commercial cheesemaking conditions. They described the simplicity, sturdiness, ease of cleaning, non-disturbance of gel structure, and reproducibility of this instrument. Bynum and Olson (4) successfully standardized Vanderheiden's curd firmness tester for laboratory and commercial studies. Garnot and Olson (7) later utilized this instrument to determine clotting times and gel rigidities of milk treated with different concentrations of chymosin. The Vanderheiden curd firmness tester has been successfully applied in measuring variations in gel rigidities of ultrafiltered milk of different protein and fat contents treated with chymosin (8). The effect of curd firmness at cutting on cheese yield has also been studied (5).

Most curd firmness testers are almost entirely electronically controlled and depend on minimal mechanical movement. Simplicity of instrument operation is often emphasized, eventhough, the torsiometer (3,24) and thrombelastograph (16,31) are mechanically complex and are difficult to mantain (13). Other instruments such as penetrometers may be easy to operate and maintain, but often take single point readings only and may destroy the curd (26).

Torsiometer

The laboratory model (3) consists of a stainless steel cylinder (diam 8 cm, height 8 cm) which is very slowly

oscillated through a small angle (15^{o}), the cylinder being immersed in 3 L of renneted milk in a beaker, with temperature held at 32^{o}C. At the top of the cylinder is a soft, coiled spring, the distortion of which is measured on each swing. By accelerating the head, not only the amount but also the average rate of strain is kept constant. The readings on the dial which record the torque on the spring thus give a direct measure of the average (complex) modulus of the setting gel during the oscillation.

Thrombelastograph

This instrument (16,31) consists of three sets of two coaxial, open ended cylinders and a mechanism for recording rotation of the inner cylinder. Each outer cylinder or cup which holds a test sample (ca 0.36 mL) oscillates slowly through an angle of about 5^{o}. An oscillatory cycle consists of a foward rotation of 3.5 sec, a stationary period of 1 sec, return to original position in 3.5 sec and a stationary period of 1 sec. The inner cylinder is suspended freely in the sample by a 0.2 mm diameter wire. As the milk clots and the rigidity of the clot increases, the rotational motion of the outer cylinder is transmitted by the clot to the inner cylinder. A mirror on the suspension wire reflects a light beam to photosensitive recording paper producing a trace of oscillations of the inner cylinder.

Pressure Transmitting System

It (13) was designed after the Vanderheiden's curd firmness tester, and measures the rigidity of curd by its ability to transmit a pressure wave. The pressure wave (0.02 Hz, generated by a sinusoidal diaphragm movement of 0.5 mm) is generated electronically and propagated through the curd formed in the gap between the transmitting diaphragm and the receiving pressure transducer (pressure range ± 1.4 kPa). The signal from the transducer is amplified and displayed as a series of peaks of increasing height that correspond to development of curd firmness.

Ultra Viscoson

The ultra viscoson (18) is a vibrating reed viscometer. The electronic control unit sends pulses to the magneto-restrictive element of a narrow blade in the sensing probe, causing the blade to vibrate logitudinally at its natural frequency (28 kHz, maximum ampltiude 0.5 um) (13). When the probe is placed in a liquid, the damping effect increases the power required to keep the blade vibrating. A voltage proportional to the power is recorded on a chart to give a measure of the product viscosity (in poises) and density of the test sample.

Gelograph

The "Gelograph" (20) operates on a principle similar to that of the ultra viscoson. The instrument has been successfully adapted for laboratory and industrial use. It

is widely marketed in Europe for commercial cheesemaking operations.

The response produced by a particular curd firmness tester during milk coagulation depends on the parameter of the complex rigidity moduli the instrument is measuring. Thus, different instruments are expected to give different results depending on the principles upon which the particular instrument operate. A comparison of two curd firmness testers under identical conditions has shown that the same values are not always obtained by the instruments. The curd firming rate measured by the ultra viscoson was observed to be more affected by changes in temperature, calcium and rennet concentrations than a pressure transmitting instrument similar to the Vanderheiden's curd firmness tester (32). The ultra viscoson is primarily a viscometer and detects small variations in viscosity caused by changes during milk coagulation. Earlier viscometric studies have shown that aggregation of casein micelles by rennet in normal milk at 30^{o}C starts at about 60% of the rennet clotting time. The ultra viscoson detected increase in viscosity shortly after this time and before the pressure transmitting system (13). More recent studies have shown that aggregation of para-casein clusters occurs before this time. Marshall et al. (13) proposed that the ultra viscoson and pressure transmitting system are complementary since the former measured changes in viscosity caused by formation of a casein network whereas the latter measured changes in

rigidity of the network that had been formed. They (13) further stated that the ultra viscoson may be suitable for monitoring early stages of curd firming and the pressure transmitting system for monitoring curd development.

Instron Universal Testing Instrument

The Instron Universal Testing Instrument (28,29) consists of a machine cross-head which contains a 250 g load cell to which is attached a specially designed stainless steel wire probe of 5 cm diameter. The instrument continuously measures curd-firming rate without coagulum destruction.

Formagraph

The Formagraph (14) is a laboratory instrument that measures the parameters of milk curd formation in multiple samples. It is a multi-channel modular instrument system designed for recording the coagulation properties of milk. Sample results are recorded over a period of 30 min. A normal measuring cycle is completed within 30 min and the capacity is then 20 samples per hour when operated by one person. Samples are pre-heated and rennet added simultaneously to ten samples. Small stainless steel loop pendulums lowered into the samples detect the tiny forces induced when the gel of coagulating milk is exposed to linear movements. A timed strobe flash unit transmits the amplitude for the movements of the ten pendulums to a ten channel optical readout system. Results are displayed on a

"self-developing" photographic strip chart and represent an approximate 30 fold magnification of displacement. Firmness/time graphs are recorded under strict temperature control. The instrument can be used also for testing rennet activity and for simulating full scale cheese vat processes.

SPECTROPHOTOMETER

McMahon et al. (15) described the use of Beckman DU 8B UV/Vis spectrophotometer to observe milk coagulation through changes in turbidity of chymosin treated milk at a wavelength of 600 nm. They showed that turbidity measurements are sensitive to initial stages of micelle aggregation, and these demonstrate that aggregation begins well before any visual observation of coagulation. They established that the rate of increase in turbidity was greatest at high enzyme activity .

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PART 1B. FACTORS THAT AFFECT MILK COAGULATION PROPERTIES: A REVIEW

INTRODUCTION

Several factors which alter coagulation properties of milk have been identified (9,17,19,23,24,29,33,40,46). Proper adjustment of these factor levels can cause remarkable changes in curd characteristics which influence the yield and quality of dairy products. The need for these adjustments arises from variations in coagulation properties of milk (32). Some factors which influence these variations have been investigated (32) and include stage and number of lactations, season, feed, presence of mammary infection (45) and other environmental factors. Other sources of variation are those which can be directly controlled in the laboratory or under cheese making conditions.

Enzymic milk coagulation has been partly elucidated, and occurs in distinct but overlapping phases as follows: (a) cleavage of casein micelles by the enzyme coagulant (13), (b) initial rapid aggregation of the cleaved micelles to form a fibrous clot (7), (c) slow aggregation of the remaining, cleaved micelles including entrapment of uncleaved micelles in the fibrous clot (8), (d) syneresis of the fibrous network with consequent expulsion of whey (41). Rates of change in these phases can be altered by modifying the prevailing physical or chemical conditions. These rates are affected by the quantity (4,26) and type (15,39) of enzyme, temperature, pH, calcium ion concentration, ionic strength, and milk composition (9,23,24,29,46). However, the two major phases of milk coagulation viz: casein cleavage

and aggregation have attracted most attention of contemporary researchers. The enzymic phase is responsible for cleavage of κ-casein resulting in para-κ-casein and soluble macropeptide. In the aggregation phase para-casein micelles aggregate to form a visible clot whose firmness increases over time. The enzymic phase has been well characterized (13) and progress has been made recently in elucidating the aggregation phase (9,24,29,46). These authors indicate that coagulation occurs as a result of partial loss of repulsive potential allowing close approach of the micelles and accentuated by increased frequency of collisions. These interactions between casein micelles are enhanced by the formation of hydrophobic bonds and possibly calcium bridges between micelles (34).

EFFECT OF CONCENTRATION OF ENZYME COAGULANT

Enzyme coagulants of animal, microbial or plant origin are usable in coagulating milk (15,39). The differences in proteolytic and milk clotting activities of these enzymes have also been reported in detail (25,27). The effect of variation in concentration of the most acceptable coagulant, chymosin (calf rennet in its purified form) is of interest. Variations in coagulation times and changes in gel rigidity in consequence to changes in chymosin concentration are of primary interests.

The primary phase of enzymatically induced milk clotting proceeds via an initial limited proteolysis of one

of the casein fractions (κ-casein) obeying Michaelis-Menten kinetics (4). The extent of proteolysis of κ-casein required for clotting of normal milk is 80-90% (5,7). A recent report (29), however, indicated that clotting occurs much earlier. Variation in the concentration of chymosin is therefore expected to change the rate of this reaction as stipulated by the Michaelis-Menten equation. Different authors have reported the existence of excellent linearity between coagulation times and the inverse of chymosin concentration (16,26,28). Others have related variation in chymosin concentration to release of soluble nitrogen (13) from caseins. Relationship between variation in chymosin concentration and rate of gel formation is not similar to that between coagulation time and chymosin concentration. Gel firming appears to follow first order kinetics.

Garnot and Olson (16) studied the variation of chymosin concentration in nine trials, and all yielded values of theoretical maximum gel rigidity which were not significantly different. They also measured increase in milk gel rigidity after clotting milk with different concentrations of chymosin and reported the existence of excellent linearity between logarithm of gel rigidity and reciprocal of time after clotting. Early stages of curd development, that is, the stage described as the rapid micelle aggregation phase are significantly affected by chymosin concentration (16). The increase in rate of gel firming with increasing enzyme concentration indicates that

micelle aggregation is affected by enzymatic action, that is, rate of production of enzyme-modified micelles. Garnot and Olson (16) also indicated that the lack of linearity in rates of firming as affected by enzyme concentration suggests that gel assembly depends to a great extent on diffusion of modified micelles at the higher level of enzyme and less on rate of enzymatic action. The significance of their observations is that adding greater amounts of milk-clotting enzyme would not efficently accelarate gel formation under commercial conditions; adding lower amounts would markedly reduce it. The independence of maximum theoretical gel rigidity depends on interactions between casein micelles and the characteristics of milk rather the enzymatic rate. Other reporters (26,33,40,46) have similarly observed increase in coagulum firmness with increase in rennet concentration. Scott-Blair and Burnett (43) however, observed that the increase in rigidity with doubling of time after renneting varies inversely as the cube root of the rennet concentration.

EFFECT OF TEMPERATURE

The dependence of chemical reactions on temperature is well established. Increase in temperature is known to accelerate chemical reactions through attainment of the necessary activation energy as explained by the Arrhenius equation (30). Increased temperature speeds up attainment of adequate molecular energy and therefore increases

diffusional and collision frequencies. Increased rates of these two processes accelerate chemical reactions. In these respects, increased temperatures would be favorable for milk coagulation, because not only would it speed up the enzymatic phase but also the aggregation phase of the cleaved casein micelles. Kowalchyk and Olson (23) reported the dramatic effect of increases in temperature on casein micelle aggregation and suggested that hydrophobic interactions (31) played an important role in determining the rate of aggregation of micelles and cross-linking of micelles aggregates to form the casein gel. Dalgleish (9) showed the temperature dependence of diffusion and aggregation rate constants of rennet treated casein micelles. Dalgleish (9) also showed how hydrophobic interactions were weak at low temperatures and much stronger at elavated temperatures.

Doan (10), and Ernstrom and Wong (13) explained that curd tension of milk does not depend only on the temperature at which milk is clotted, but also on the temperatures and time at which the milk is held before clotting. Doan (10) indicated that pasteurization has a negligible effect, but heating at 160^{o}F for 30 min causes a distinct lowering of the tension. He (10) also reported that autoclaved milk, and evaporated milk frequently exhibit no curd tension. Ali et al. (1) reported that cold storage of milk for variable periods has pronounced effects on curd forming ability. They indicated that storage at 4^{o}C for 48 h causes solubilization

of certain casein variants from the micelle and caused less casein to be incorporated into the fibrous casein network on aggregation. Reimerdes (36,37) indicated that cold storage of milk was primarily responsible for the reversible conversion of the most temperature sensitive and hydrophobic β-casein into γ-caseins. Heat treatment of such milk restores the intergrity of β-casein (1). Ali et al. (1) indicated that solubilization of β-casein as a result of variation in temperature affects rennet clotting time and rate of aggregation of casein micelles. Conversely, Storry and Ford (46) observed that increased temperature reduces maximum rates of both phases of coagulum development. They indicated, however, that the first phase decreases mainly above 35°C whereas the second phase decreases over the whole temperature range studied, 25 to 40°C, but particularly at the lower end of the range. They (46) attributed the discrepancy of their results with those of other workers to differences existing in the parameter of the complex rigidity moduli measured. Scott-Blair and Burnett (42) had previously reported that some rheological parameters increase or decrease progressively as temperature is increased from 21 to 41°C; others pass through maxima or minima, all between 29 and 35°C.

EFFECT OF pH

Hydrogen ion concentration of milk has a remarkable effect on the activity of enzyme coagulants including

chymosin, through the influences it exerts on the ionic species of the enzymes. As the substrate is made more acidic, the enzyme becomes more protonated (6). Proteases such as chymosin, pepsin, fungal coagulants have higher activities at low pH (15,39) even though fungal rennet is less affected by variation in pH (39). Acidification of milk prior to cheesemaking is, therefore, commonly practised since this accelerates enzymic cleavage of κ-casein, thereby resulting in faster coagulation. Normal bovine milk has pH of about 6.6. Coagulation can be optimized if milk pH is reduced to 5.9 (47). Kowalchyk and Olson (23), Storry and Ford (46), and Tuszynski et al. (47), all observed increase in curd firmness of milk as the pH was reduced. Storry and Ford (46) however indicated that changes in pH particularly affected the first phase of coagulation more than the second phase.

Various milk acidulants are used for cheese making. Examples are phosphoric, acetic, hydrochloric, lactic and citric (33). The physical and chemical properties of the cheese variety to be manufactured often determine the type of acidulant used (44). Direct acidification may eliminate variability of acid production by bacteria (21). According to Keller et al. (21), in direct acidification, the pH of the milk and curd is adjusted to a value which remains constant during whey syneresis. Type of acid and pH at curd formation affect moisture, mineral content, and firmness of Mozzarella cheese. They (21) also observed that losses of

fat varied between curds made with different acids, but, in general soft curd which was formed at low pH lost more fat with any acid. In addition, they observed that acidification to pH 5.2 with citric, malic, hydrochloric, acetic, and phosphoric acids caused high calcium losses whereas at pH 5.6, malic, acetic, hydrochloric and phosphoric acids produced cheeses that contained more calcium.

Use of lactic starters to reduce milk pH is generally practised during manufacture of different cultured dairy products. Rheological changes due to acid production by starters after coagulating milk with chymosin compare with results obtained by direct acidification. However, proteolysis of curd by bacterial proteinases is often suspected. Emmons et al. (11) indicated that the extent of proteolysis and its effect on curd strength in a 5 h period is uncertain, but probably of a minor nature. Williamson and Speck (48) observed a two fold difference in proteolytic activities of different lactic cultures. In addition they observed a 60 g variation in curd strength between cultures but was not related directly to proteolytic activities of cultures. They (48) indicated that the incorporation of pancreatic extract, a growth stimulant to cheese milk caused a two fold reduction in proteolysis by the cultures and concomittant increase in the curd strength. Richardson et al. (38) proposed the exclusive use of proteinase negative lactic cultures for Cheddar cheese manufacture, because of

the anticipated increase in yield and reduced potential for producing bitter flavor.

EFFECT OF CALCIUM CHLORIDE

Calcium salts are naturally present in milk predominantly in the form of phosphates and citrates or complexed with serum proteins (3). Ionic species of calcium may also be found, depending on the prevalent conditions. The average calcium content of skim milk is 32 mM colloidal, 10 mM soluble and 3mM ionic. The available form of calcium in milk greatly influences the coagulation properties of milk. Colloidal calcium phosphate is known to increase the interaction between casein micelles (9,18,24) which facilitates curd formation. Addition of calcium chloride to milk has been practised in cheese making because of the known effect of calcium on coagulation. Federal regulations and standards of identity (14) limit the maximum level of calcium chloride added to .02% (w/w). McMahon et al. (29) indicated that increasing calcium concentration in milk may enhance aggregation while inhibiting gelation as evidenced by separation of these phases in turbidity experiments when calcium was added. They observed that coagulation time was severly retarded at high calcium concentration (4M), partly due to the effect of calcium upon enzyme activity. Retardation of coagulation time by high concentration of calcium can be overcome by increasing enzyme activity.

Increase in rate of micelle aggregation as a consequence of added calcium has been attributed by Knoop and Peters (22) to changes induced by the electrostatic attraction operating between negatively and positively charged areas on contiguous enzyme-altered casein micelles. Calcium bridging between two negatively charged areas is also believed to be involved (29). Kowalchyk and Olson (24) also observed decrease in clotting times with added calcium, and indicated that clot to cut time intervals decreased at a fairly uniform rate as calcium chloride concentration was increased. Storry and Ford (46) observed similar trends in coagulation time with added calcium. Scott-Blair and Burnett (42) had previously observed that increasing quantities of calcium chloride added to reconstituted, dried, fat-free milk progressively reduced the time needed to clot but did not greatly alter the final setting rates. Jen and Ashworth (19) observed increase in curd firmness with added calcium chloride until 10 mM after which curd tension decreased, but loss of calcium in whey, and retention of calcium in curd were directly linear with increased calcium addition.

INTERACTIONS OF TEMPERATURE, pH, CALCIUM, IONIC STRENGTH AND CHYMOSIN CONCENTRATIONS

Dependence of milk coagulation on the main effects of temperature, pH, calcium content, ionic strength and concentration of rennet is well known. Limited information, however, has been reported recently on the interaction of

these factors. The relationship between calcium ion concentration and pH, and the effect of this interaction on coagulation has been described by Jen and Ashworth (19). They indicated that the pH of calcium-fortified milks decreased as calcium was increased, due to the binding of added calcium to the casein and the release of protons. Part of the increased curd tension obtained with such milk, they continued, could be attributed to pH change. In a similar experiment conducted by Jen and Ashworth (19) in which a mixture of calcium chloride and calcium hydroxide to keep pH of the milks constant was added, curd tension obtained was much less at high concentrations of calcium confirming that the effect of addition calcium was partly dependent on reduction of pH.

The interaction between pH and temperature was studied by Kowalchyk and Olson (23). Higher temperature reduces milk pH, and such increase in temperature accelerates micelle aggregation and cross-linking of micelle aggregates to form the casein gel (23). They (23) also suggested that charge effects may have some influence based on the changes in rates at various pH levels. Tuszynski et al. (47) indicated that lowering pH in milk causes changes in calcium equilibrium. They showed that lowering milk pH to 6.2-6.3 caused only very small changes in calcium equilibrium but between pH 6.2 and 5.9, the colloidal calcium phosphate of the serum was solubilized, and in lowering the pH from 5.9 to about 5.2, the casein bound calcium went into solution.

Kowalchyk and Olson (24) noticed significant effects of pH and temperature on the clot-to-cut times when firming rates of milk samples treated with rennet were compared to those treated with Mucor proteases at pH 6.5, 34°C and pH 6.7, 30°C. They observed a 3 min difference in the former and 7.8 to 9.2 min difference in the latter between the 2 enzymes in which Mucor enzymes required longer times. They (24) concluded that it may not be suitable to standardize various milk-clotting enzymes solely on clotting time since rates of firming of curd of commercial milk supplies may vary. Kowalchyk and Olson (24) also observed a consistent rate of firming of gels for all enzymes. Ernstrom et al. (12) showed that the amount of rennet used in coagulating cheesemilk could be reduced if more calcium chloride is added, but the effect of such changes manifest in increased curdiness. McMahon et al. (29) showed that the retardation of progressive reduction of coagulation time with increased calcium at high calcium levels was partly due to the effect of calcium (or high ionic strength) upon enzyme activity because retardation of coagulation time can be overcome by increasing enzyme activity. They (29) also observed that at high calcium concentrations curd had a very low firmness and very easily broken.

The interaction between temperature and calcium equilibrium in milk has been described. Heat treatment of skim milk causes the transfer of soluble calcium phosphate to the colloidal state, which is reversible on cooling (20).

They also showed that calcium addition after heat treatment partially restores coagulability. Wilson and Wheelcock (49) established that the addition of calcium ions to milk before pasteurization maintains the coagulability of the pasteurized milk when compared to raw milk. Poznanski et al. (35) explained this phenomenon by considering two possible factors: the protective action of calcium ions on the kinetics of denatured whey protein, and simultaneous uncovering of the κ-casein groups blocked by denatured whey proteins accessible to the rennet enzyme. By its divalent charge calcium intensifies the associatin of κ-casein molecules thereby increasing the stability of the complexes formed.

The overall effects of the interactions of some of these factors on coagulation of renneted bovine casein micelles was partly described by Dalgleish (9). He postulated that the surface charge of renneted casein micelles is strongly temperature dependent and that it is approximately zero at temperatures around 60°C, and hence, observed faster aggregation at that temperature. Moreover, he continued, the dependence of the aggregation rate upon ionic strength, is known, and increases in ionic strength should decrease repulsion between charged surfaces, and allow closer approach of renneted micelles. Thus, the effect of increasing calcium ion concentration is consistent with a general neutralization of negative charge on the surface. Dalgleish (9) observed lack of variation in aggregation

constant at high temperatures with increasing ionic strength, but apparent variation at low temperatures. He suggested that a specific charge interaction between micelle surfaces may operate, that is, that the aggregation is controlled by the formation of ion-pairs between the interacting micelles (18,22). Dalgleish (9) concluded that formation of coagula in renneted micelles arises from interaction of specific sites involving both hydrophobic interactions and ion-pair formation although the function of calcium ions in forming the aggregates is still somewhat obscure.

COWS' UDDER ABNORMALITIES

One of the most important contributors to softness of milk curd is the presence of mammary abnormalities, especially mastitis. Doan (10), in a review, reiterated that decreased curd tension of mastitis milk appeared to be primarily due to lowered casein concentration, although the high pH of such milk was a contributor. He (10) further commented on the lowered calcium and phosphorous content and particularly a change in the ratio of casein to calcium and phosphorous in mastitis milk. Sommer and Matsen (45) had previously made such observations.

More recent attributes of mammary abnormalities relate its effect on coagulation to other physiologic changes that occur in the udder. Predominant in such changes is the increased capillary permeation of blood constituents into

milk (2). The most reported of such constituents are immunoglobulin and some albumins but the components that greatly affect the structural and functional properties of caseins are the hydrolytic enzymes and enzymes originating from leucocytes (2). Bacterial proteinases, in cases where bacterial levels are high, might also be expected to contribute to compositional changes in mastitis milk, as it does in normal milk (25). Severe casein compositional changes have been strongly related to these proteolytic enzymes and result in the production of various casein fragments (2). Elevation of γ-caseins and para-κ-casein, and depletion of β-, α_s-, and κ-caseins are common observations related to such abnormalities. The cleavage of β-casein to fragments of known type (γ-caseins) clearly points to a trypsin-like enzyme specificity (2).

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PART 2. AN INSTRUMENT FOR MEASURING MILK COAGULATION IN CHEESE VATS (10)

INTRODUCTION

More objective determinations of cheese curd-cutting time should help refine cheese making and maximize yields (3). Such measurements should also help to optimize additions of acidifying agents, coagulants and calcium chloride.

The curd firmness tester designed by Vanderheiden has been successfully used by Olson and co-workers (3,4,5,6) to measure milk coagulation properties in cheese vats. The Gelograph (11) is marketed for the same purpose. This paper describes the development and application of a new instrument, tentatively called the "Vatimer", for continuous measurement of coagulation properties of milk in open or closed cheese vats.

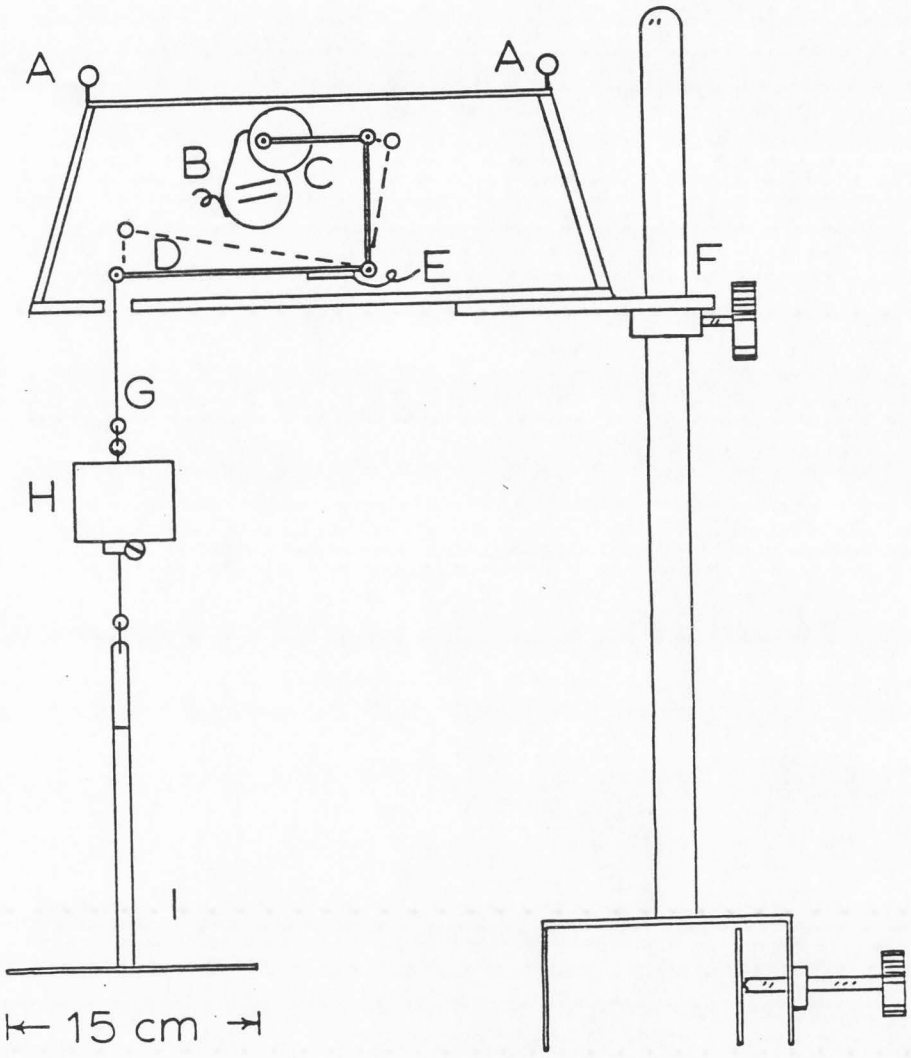
METHODS AND PROCEDURES

Probes were made from stainless steel rods, 0.64 in diameter, and attached perpendicularly to the center of stainless steel discs of various diameters (2.5, 5, 10, and 15 cm). Each rod was inscribed with a shallow groove around its circumference 15 cm from the disc to standardize the imersion depth of the probe in milk.

The probe was suspended in milk with a stainless steel line attached to the free end of the rod. The disc was parallel to the surface of the milk during immersion. The stainless steel line was attached to an adjustable take-up reel that allowed quick adjustment of the depth of the probe in milk (Figure 1). The take-up reel was attached to the end of a cantilever bar. A synchronous gear motor was used to move the end of the bar up and down vertically creating oscillating motions in the probe. The frequency of the cycle could be changed by changing motors. An adjustable eccentric permited the amplitude of the cycle to be changed. A strain gage was mounted on the cantilever bar to measure the forces that developed against the surface of the disc probe. A linear variable differential transformer or magnetic balance sensing system also could be used to detect force change. The instrument was mounted above the vat or 8 L vessel during measurement of milk coagulation properties.

The probe was set at the bottom of the oscillating cycle and immersed to the 15 cm mark in the milk. The synchronous

Figure 1. Schematic diagram of an early model "Vatimer" incorporated into a plastic box. A = Eyelets used to suspend instrument over manholes on closed cheese vats; B = Synchronous motor with gear drive to provide 6 cycles per min; C = Adjustable stroke offset gear; D = Cantilever bar with disc - probe drive to (I) suspended from the eyelet end; E = Strain gage to detect force changes on cantilever bar; F = Support bar to mount instrument on the side of open cheese vats. The bar was attached to an adjustable clamp mechanism; G = Stainless steel 27Kg test fishing line; H = Adjustable take-up reel; I = Instrument 15-cm diameter disc-probe.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motor was started and continuous strain measurements in the cantilever bar were obtained using a Vishay digital strain indicator (Micromeasurements, Raleigh, NC) and an analog recorder (Hewlett-Packard Corp., Corvallis, OR). A Micromac 4000 (Analog Devices, Norwood, MA) was interfaced with an Apple II (Apple Computer, Cupertino, CA) computer to provide read out, draw coagulation curves and calculate the desired parameters. Laboratory studies of the instrument were performed using reconstituted non fat dry milk (NDM) at 12% solids. The reconstituted milk was stored at 4°C for 18 h to insure complete salt equilibrium and hydration of casein micelles. The reconstituted NDM was then tempered at 37°C for 90 min and .2 rennin unit (RU)/mL was added to initiate enzymic coagulation in a 37°C water bath. Reconstituted NDM with identical treatment was also tested with the Formagraph (7). Values for milk coagulation parameters including curd firmness (G) at cutting and at other time points along the curve were easily obtained (8).

The instrument was mounted on the side of conventional cheese vats or suspended above open manholes of "Double O" vats (Damrow Co. Fund Du Lac, WI). Continuous readings were obtained from the Utah State University pilot and different commercial plants during manufacture of Cheddar, Swiss and direct-acid cottage cheeses.

RESULTS AND DISCUSSION

The probes with 10 and 15 cm diameter discs were the first evaluated in coagulating milk. The motor gear speed provided an oscillation frequency of 6 cycles per minute. Maximum probe amplitude was 2.4 cm. Clotting time indicated by the Formagraph was 15.7 min. The 10 and 15 cm probes indicated 19.6 and 17 min, respectively. The 15 cm probe provided an earlier estimate of clotting time than the 10 cm probe. The Formagraph provided the earliest estimate in this sample. In another trial, however, the 15 cm probe indicated a 15.3 min clotting time vs. 15.6 min for the Formagraph. Greater sensitivity could be obtained by installing a more sensitive device for measuring changes in force. A probe with a larger diameter disc, or a cantilever bar with a smaller cross section might be adequate.

Curd firmness 30 min after chymosin addition was 28 mm on the Formagraph and 1130 and 2687 mN for the 10 and 15 cm probes, respectively. The newton (N) is the unit of force in the SI system of units. The milli newton (mN) is 10^{-3} newtons. There are 4.448 N per poundforce or 4448 mN per pound force. The probes provided readings proportional to the surface areas of the attached discs. Four probes with disc diameters 2.5, 5.0, 10 and 15 cm provided readings of 75, 832, 1194, and 2687 mN. Probes with 15 to 25 cm diameter discs should give good estimates of curd cutting times in chese vats (3,8). A probe with a 5 cm disc was succesfully

used to measure curd formation in ultrafiltered milk retentate (C.G. Brown and C.A. Ernstrom, Unpublished).

During curd formation, the curd adhered tightly to the discs (12). No separation of the curd from the top plane surface was evident. The rod could be twisted manually and the entire mass of curd surrounding the probe would respond to the applied force. Further evidence of curd adhesion to the disc was observed when chymosin was added to 8 L of milk, the milk container placed on an electronic balance, and the probe oscillated. Forces on the strain gage were counter-weighed on the balance scale. No free whey was noted on the surface of the curd unless small milk containers and large discs were used. In these configurations the forces acting against the probes were not adequately dissipated and side-wall effects caused splitting of curd which resulted in uneven readings. The probe with 10 cm disc was required in small containers to eliminate side-wall effects. Free whey was observed around the rod when movement was too great to allow curd adhesion. Milk coagulum adheres firmly to some surfaces including stainless steel (12). The readings obtained with polished stainless steel probes were comparable to those from the unpolished probes. Thus curd adhesion was independent of degree of polish.

The probe with 15 cm disc was selected for further milk coagulation studies. It was calibrated in a static test by adding known weights. The output was linear through 1886 mN.

Motor-gear drives of 3, 6 and 12 cycles/min were evaluated. The 12 cycles/min motor was not powerful enough to drive the probe after coagulation began. Clotting times for the 3 and 6 cycle/min motors were 15 and 14 min repectively. Curd firmness readings at 30 min were 2543 and 2863 mN, respectively. The 6 cycles/min unit was selected because it gave shorter coagulation estimates, higher G values and twice the readings per minute of other devices (6,9).

The length of the stroke of the disc was varied from .3 to 2.4 cm. Stroke length in cm and corresponding forces in mN after 30 min were: .3:459, .6:896, 1.2:1674, 1.4:2250, 1.8:2495 and 2.4:2863, respectively. The 2.4 cm stroke was selected because it provided earlier estimates of clotting time and a broader scale.

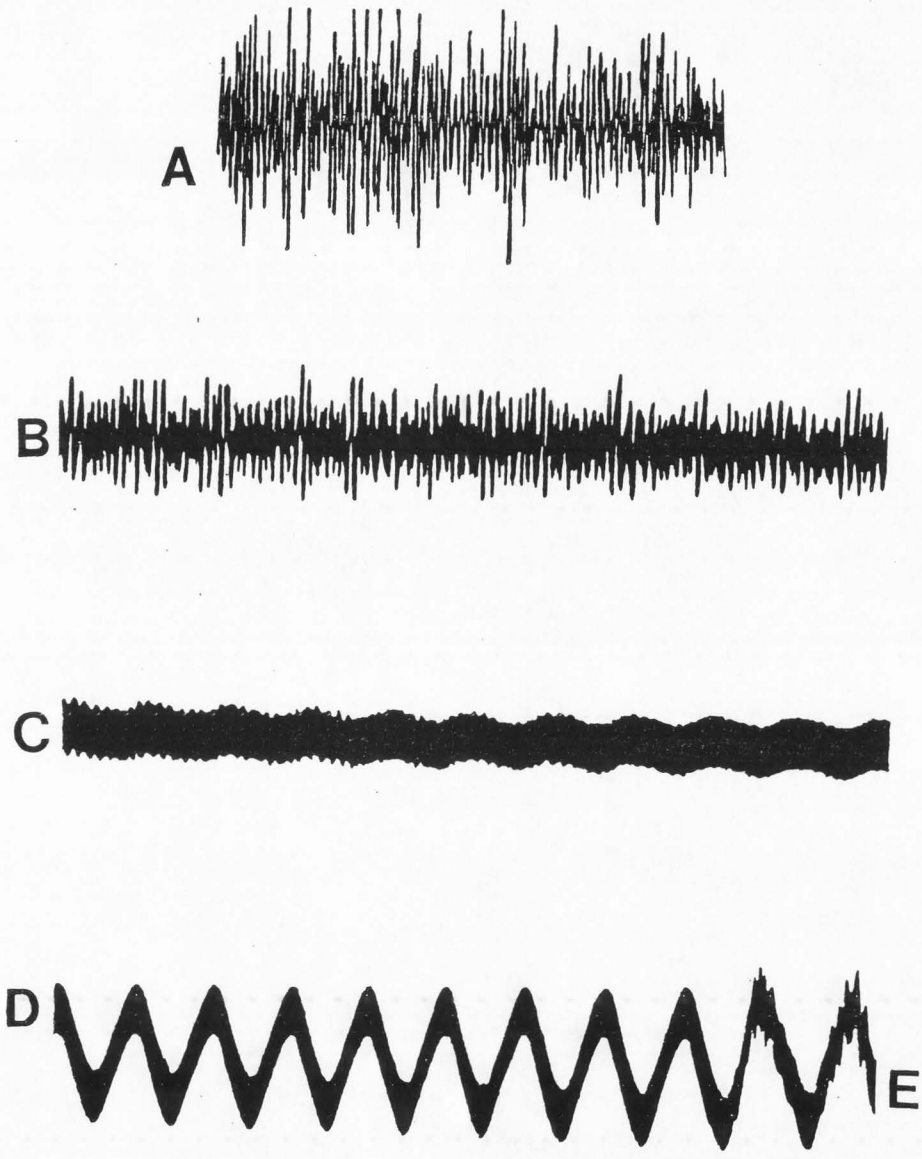
Berridge substrate (2) coagulated in 5 min with a 30 min force of 3338 mN when coagulated with .1 RU/mL. When 0.2 RU/mL was used the substrate coagulated in 1.75 min and the final reading was 4980 mN. Very high readings were thus possible eventhough the instrument tracing "bottomed out" when the resistance of the curd to the downward motion of the probe exceeded the probe mass (225 g for the probe with 15 cm disc). Reconstituted NDM without added calcium chloride was selected for further research because slower coagulation time permitted more points to be read during curd development. The data from NDM curves were adequate for estimation of the desired coagulation parameters.

The Coefficient of Variance (CV) for the Formagraph G values 30 min after chymosin addition was 3.7%. The Gelograph yielded a 5.8% CV 10 min after coagulation (11). Four replicates on reconstituted NDM yielded Vatimer readings of 2618, 2383, 2538, and 2522 mN 30 min for a mean/standard deviation of 2513.3 $\pm$ 97.7 mN. The CV was 3.9%. The curve shape corresponded to that obtained with Formagraph, however, a decrease in curd firmness after maximuim strength was not evident. Apparently there was less cutting of the coagulum by the moving plane of the probe than by the wire loop of the Formagraph.

The instrument was used in commercial and pilot plant cheese manufacturing operations. It produced excellent data under severe mechanical vibration and variations in line voltage. Width of the recorder tracing was a function of the low forces associated with non-coagulated milk against the vertical cycling of the disc (Figure 2C). Cutting strengths found in commercial Swiss cheese operations varied from 160 to 480 mN. Cheddar curd varied from 267 to 1066 mN at cutting. The probe was removed before curd was cut.

Reduction of the range of cutting strengths may reduce losses of curd fines, improve control and optimize cheese production (3). The instrument provided objective measurement and control not previously available for factory use. The instrument also provided data on the time required for milk standing-wave motion to cease after chymosin addition and agitation. Figure 2 shows tracings of the

Figure 2. Analog recorder tracings from the "Vatimer" during Swiss cheese manufacture in a conventional open cheese vat. The instrument was operated with a stroke of 2.4 cm and at 6 rpm. A = Signal immediately after cessation of agitation and probe insertion; B = Signal 20 min after probe insertion. Standing wave motion spikes were still evident and coagulation amplitude signal was beginning. The width of tracing subtended the signal extremes when probe was at the highest and lowest points of transverse; D = Signal just prior to cutting the coagulum, standing wave motion had ceased; E = The uneven signal resulting from insertion of the cutting harps. Background stray voltage in the plant caused the constant noise signal or thickness of the tracing.


A
B
C
D
E

instrument's ability to measure such wave motion in a vat of Swiss cheese after coagulation was well under way, over 20 min after chymosin addition. The instrument measured when vats were bumped and when harps were inserted into the vats (Figure 2, E).

After Cheddar cheese curd had been cut, the instrument was reinserted and shrinking curd allowed to settle on the probe. The entire recorded pattern drifted downward as the curd settled. This settling of curd on the probe allowed for measurement of curd syneresis and control of agitation after cutting. Similar data were obtained from cottage cheese production vats.

The coagulation data generated by the instrument were fed to a microprocessor controller to provide improved estimates of coagulation time, cutting time and other G values (8). Such values would be indicative of the effectiveness of casein in forming good quality curd (1,3,13). The G values and total protein values could be used to indicate when process variables needed adjustment to assure maximum cheese yield and optimum control of moisture.

A multiple channel laboratory model with similar probes and more sensitive detectors is being evaluated with 10 mL samples to measure G values for bulk milk samples.

SUMMARY

A rugged and sensitive instrument measured milk coagulation properties during cheese manufacture. The instrument consisted of a probe made from stainless-steel rod, 20 cm long and .62 cm in diameter, and attached perpendicularly to a 15 cm diameter stainles-steel disc. The probe is positioned with the disc 15 cm below the milk surface. The probe is raised and lowered 2.4 cm at 6 cycles per minute. The probe is attached to a stainless steel line, an adjustment reel and the end of a cantilever bar. A strain gage mounted on the cantilever bar measures the resistance of milk to the movement of the probe. Forces on the probe varied with standing-wave motion of milk, coagulation, curd firming, curd syneresis and clean-in-place operations. The curd adheres to the disc as continuous measurements are made, even in direct-acid cottage cheese curd. The instrument was successfully applied to Swiss, Cheddar and cottage cheese manufacturing processes in both open and closed cheese vats. Curd strength at cutting varied three to four fold in commercial operations. The instrument provided continuous curd firmness values. A 10-cm diameter probe was preferred in 8 L containers where side-wall proximity prevented adequate force dispersion from the 15 cm disc. A 5 cm diameter disc detected coagulation of ultrafiltered milk retentate.

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PART 3A. EFFECTS OF pH, CALCIUM CHLORIDE, AND CHYMOSIN CONCENTRATION ON COAGULATION PROPERTIES OF ABNORMAL AND NORMAL MILK

INTRODUCTION

Inability of milk from some individual cows to coagulate, especially in late lactation, has been reported (21). This phenomenon seemed more apparent in fall and winter months in the Northern hemisphere. The obvious impact is a loss of cheese yield resulting from non convertibility of milk solids (casein plus fat) into cheese. Addition of .02% (about 1.8 mM) $CaCl_2$ to cheese milk, especially during winter, improves coagulation and produces about 32% increase in curd firmness (12). Even though $CaCl_2$ produces firmer curd, other factors also may improve coagulation properties of poor chymosin-coagulating milk (PCM). Curd firmness may increase up to 81% by addition of about 10 mM $CaCl_2$, but higher levels cause a decrease (12,20). Such high levels are not practical at present in commercial operations because of legal limits (8). High levels of calcium also have been associated with curd meltability problems in processed cheese (23). Other modifications that would produce higher curd firmness without excessive use of $CaCl_2$ are desired.

This work summarizes the effects of variation of the levels of some factors that affect milk coagulation on coagulation properties of milk which exhibit PCM characteristics.

METHODS AND PROCEDURES

Selection of milk samples

Fifty milk samples from individual cows were obtained from Utah State University Holstein herd in December 1982 at one sampling. Cows were selected to cover all stages of lactation. Samples were stored overnight at 4°C to guarantee uniform temperature history. The pH of milk samples was measured at 37°C with a Beckman Model 60 pH meter (Beckman Inc., Fullerton, CA). Ten milliliters of each sample was then coagulated with 200 μL of diluted chymosin (.4 rennin units (RU)/mL) after tempering at 37°C for 90 min. Coagulation properties of the samples were recorded with a Formagraph instrument (18). Fifteen good chymosin-coagulating milk (GCM) and 15 PCM samples were selected based on their curd firmness and the source cows identified.

Fifty percent blends of selected individual GCM and PCM were prepared by adding 5 mL of one GCM to 5 mL of one PCM. This gave a total of 15 blends. The pH's of the blends were measured after tempering at 37°C for 90 min. They were then coagulated in the Formagraph.

Two milliliters of individual GCM samples were pooled together and the PCM samples were similarly pooled. Fifty percent of the pooled GCM was mixed with 50% of the pooled PCM and the final pH of each pool was measured after tempering at 37°C for min. Berridge substrate (4) and 12% low heat, spray dried, reconstituted nonfat dry milk (RNDM)

controls were tempered at 37°C for 90 min and were then compared with the pooled samples for chymosin-coagulation properties.

Modification of milk samples

The pH of each 10 mL aliquot of individual GCM and PCM samples was reduced to 6.3 by adding 1 to 3 drops of 2.1 N lactic acid solution during constant stirring at 37°C. The samples were left to equilibriate at 4°C for 24 h then minor shifts in pH were adjusted with lactic acid at 37°C. The samples were tempered in a water bath at 37°C for 90 min then coagulated in a Formagraph. Modification of pH was neccesary because previous work on variations in coagulation properties of milk from individual cows (21) showed that pH change was a significant factor in altering coagulation properties. In this study, preliminary observation with Berridge substrate on the effect of pH on coagulation time (Figure 3) showed that coagulation time was prolonged at pH 6.7. Optimum curd firmness was obtained at pH 6.2, 30 min after chymosin addition (Figure 4) at the concentration of chymosin used (.4 RU/mL).

Ten milliliters aliquots of individual GCM and PCM samples were adjusted to pH 6.3 and .02% $CaCl_2$ was added. The samples were tempered at 37°C for 90 min during which time minor shifts in pH were readjusted at 30 min intervals The samples were then coagulated in the Formagraph.

The effect of varying chymosin concentration on coagulation properties was investigated. In a preliminary

Figure 3. Effect of pH on chymosin coagulation time of Berridge substrate at pH 6.3

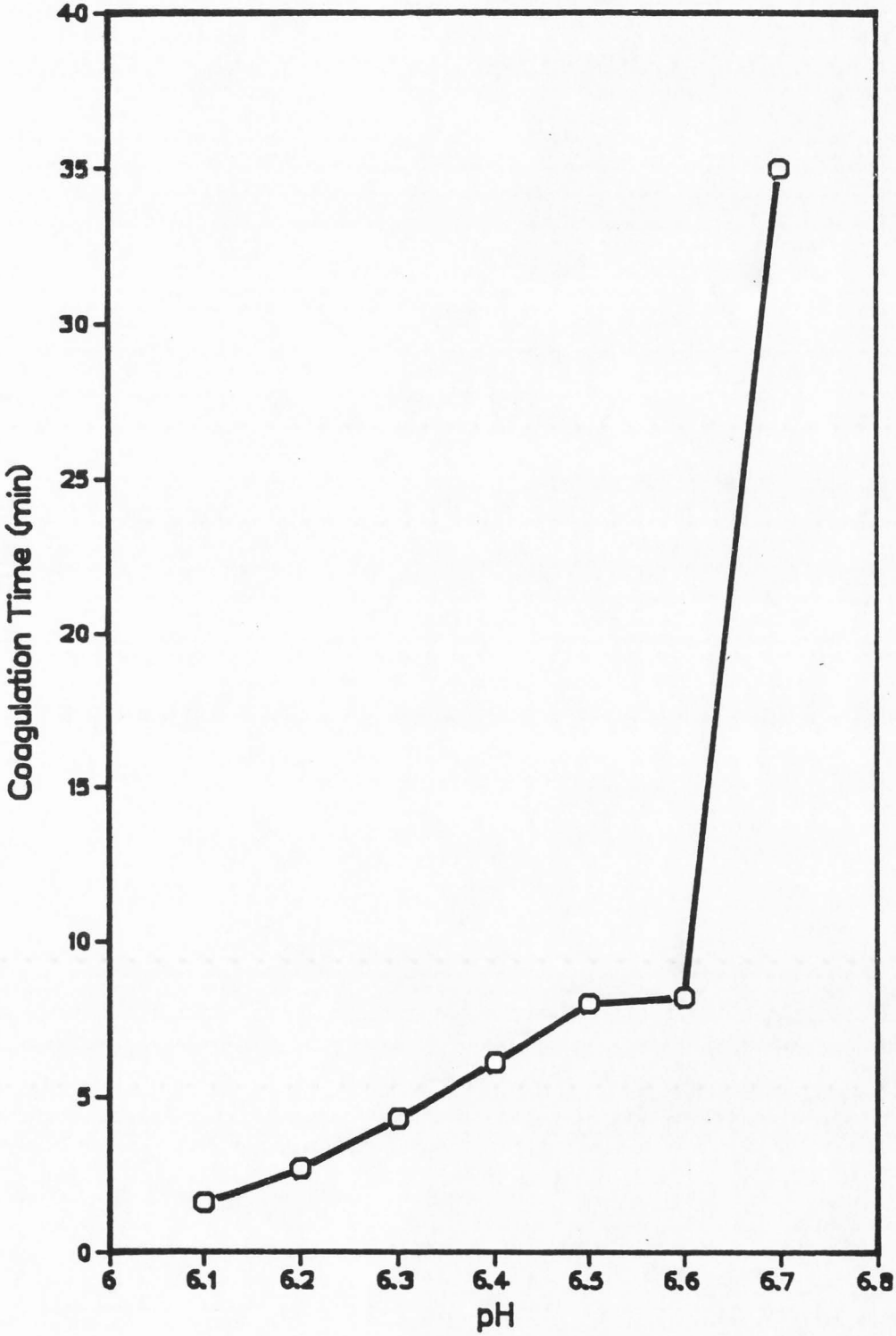
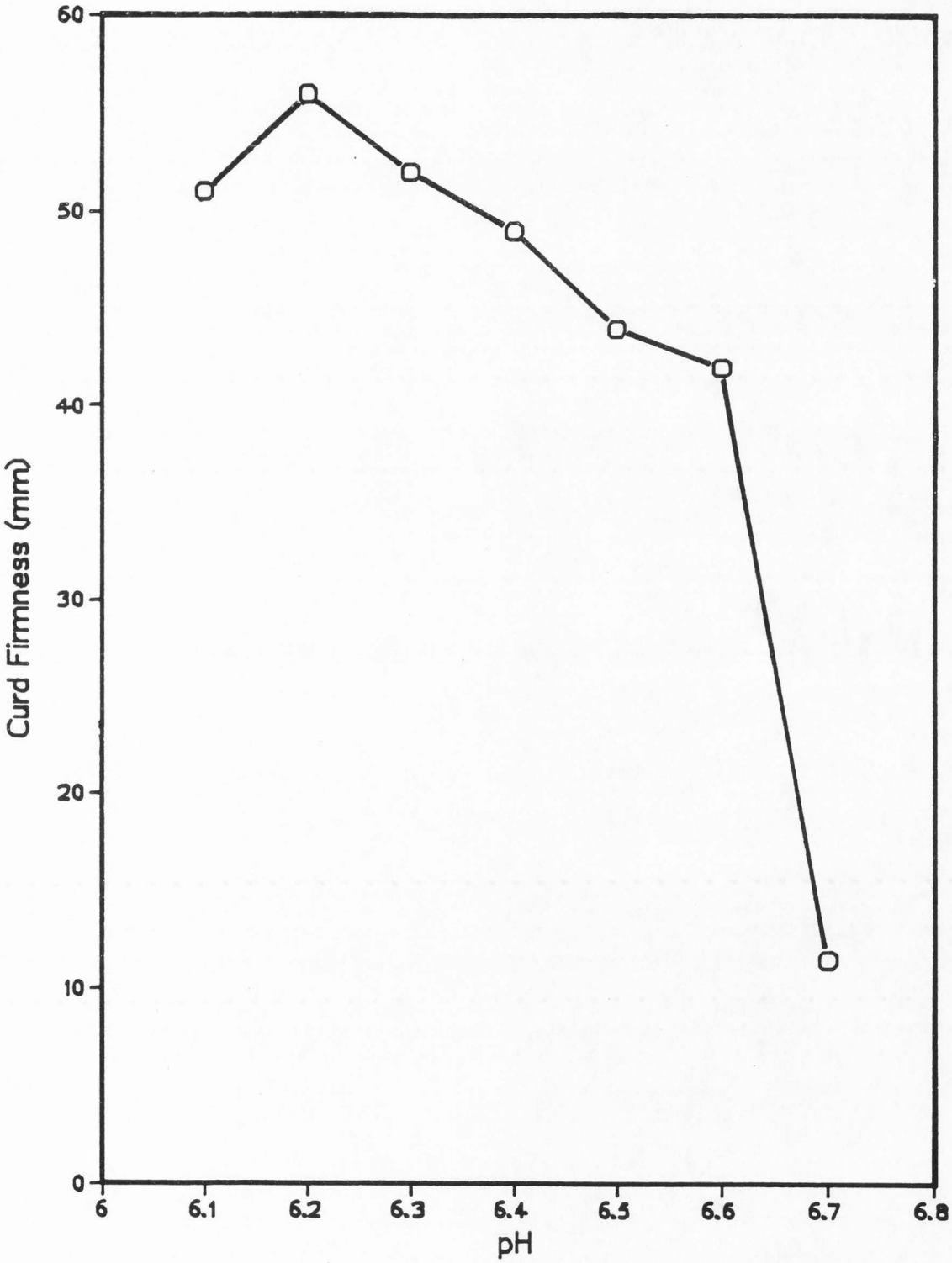

Coagulation Time (min)
40
35
30
25
20
15
10
5
0
6
6.1
6.2
6.3
6.4
6.5
6.6
6.7
6.8
pH

Figure 4. Effect of pH on curd firmness of Berridge substrate 30 min after chymosin addition.


60
50
40
30
20
10
0
Curd Firmness (mm)
6
6.1
6.2
6.3
6.4
6.5
6.6
6.7
6.8
pH

study with Berridge substrate at pH 6.3, low chymosin concentration (.2 RU/mL) produced curd firmness 30 min after chymosin addition which was not significantly different from curd firmness produced by .3 to .5 RU/mL (Table 17, Appendix 1). Chymosin concentration was then reduced from .4 to .2 RU/mL (18), the pH of each GCM and PCM sample was reduced to 6.3 from their natural pH, then minor shifts in pH readjusted while tempering at 37^{o}C for 90 min. $CaCl_2$ (.02%) was added before tempering. The milk samples were then coagulated in the Formagraph.

RESULTS AND DISCUSSION

Distribution of individual samples according to their coagulation properties

The distribution of 50 individual cow milk samples according to their coagulation properties is shown in Figures 5 and 6. The mean coagulation time for all samples which coagulated was 10.6 min, 15.9 min for 12% RNDM and 4.3 min for Berridge substrate. Jen and Ashworth (12) similarly observed a shorter coagulation time with fresh whole milk than RNDM. The distribution of the milk samples according to curd firmness showed 68% of the samples satisfactory (31 to 53 mm). The other samples had relatively weak curds (less than 30 mm). Mean curd firmness 30 min after chymosin addition for all the samples was 32.4 mm, 33 mm for 12% RNDM and 55 mm for Berridge substrate.

The 15 GCM and the 15 PCM samples selected on the basis of curd firmness are shown in Table 1 with correposnding pH values. Curd firmness tended to decrease as pH increased, but samples with pH greater than 6.85 generally did not coagulate. This observation is in agreement with previous findings (7,15,21) which indicate that pH change is highly significant in altering the firmness of milk curd. Changes in pH are known to affect enzyme activity (9,22). This is also illustrated in Figures 5 and 6.

Effect of blending individual samples

The effect of coagulating a blend of GCM and PCM is illustrated in Table 1. The PCM reduced the overall curd

Figure 5. Distribution frequency of milk samples from individual Holstein cows according to their coagulation times.

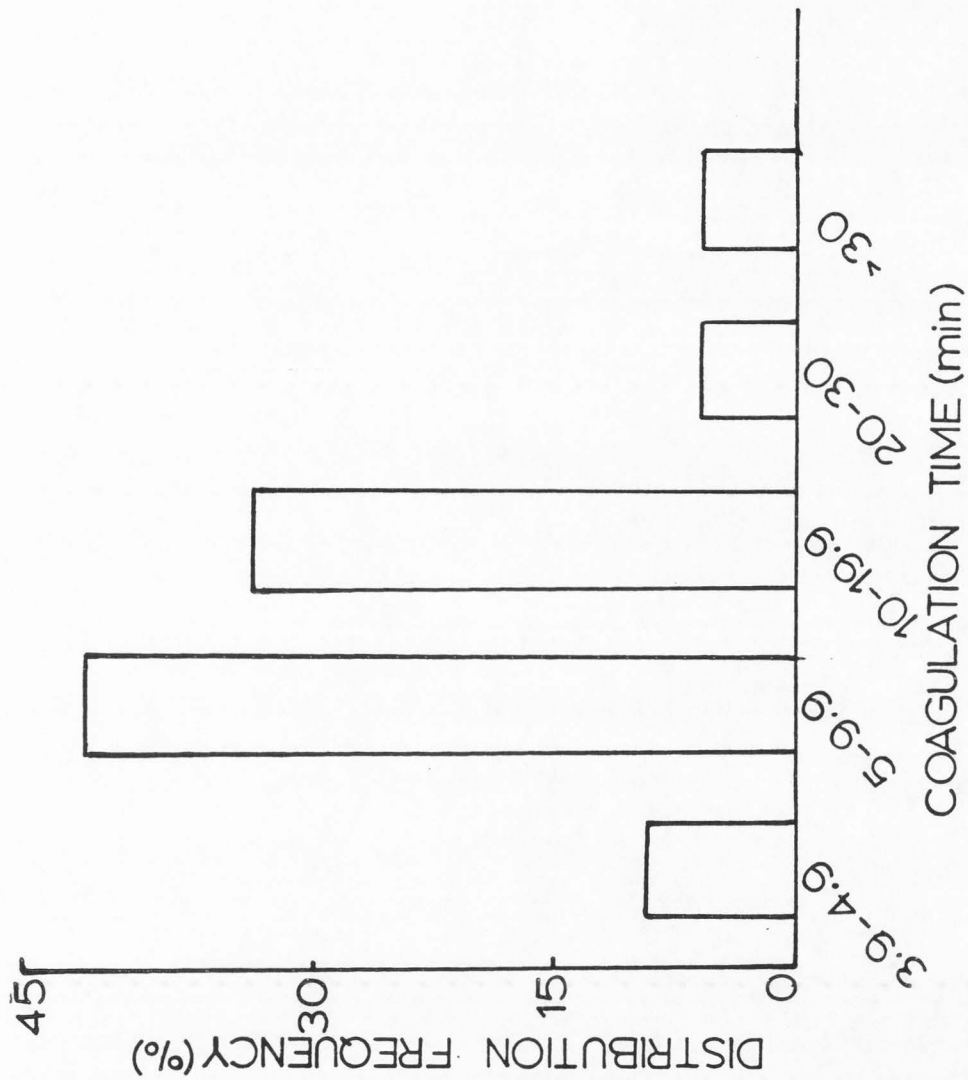

DISTRIBUTION FREQUENCY (%)
45
30
15
0
3.9-4.9
5-9.9
10-19.9
20-30
30-
COAGULATION TIME (min)

Figure 6. Distribution frequency of milk samples from individual Holstein cows according to their curd firmness. Curd firmness was determined 30 min after chymosin addition.

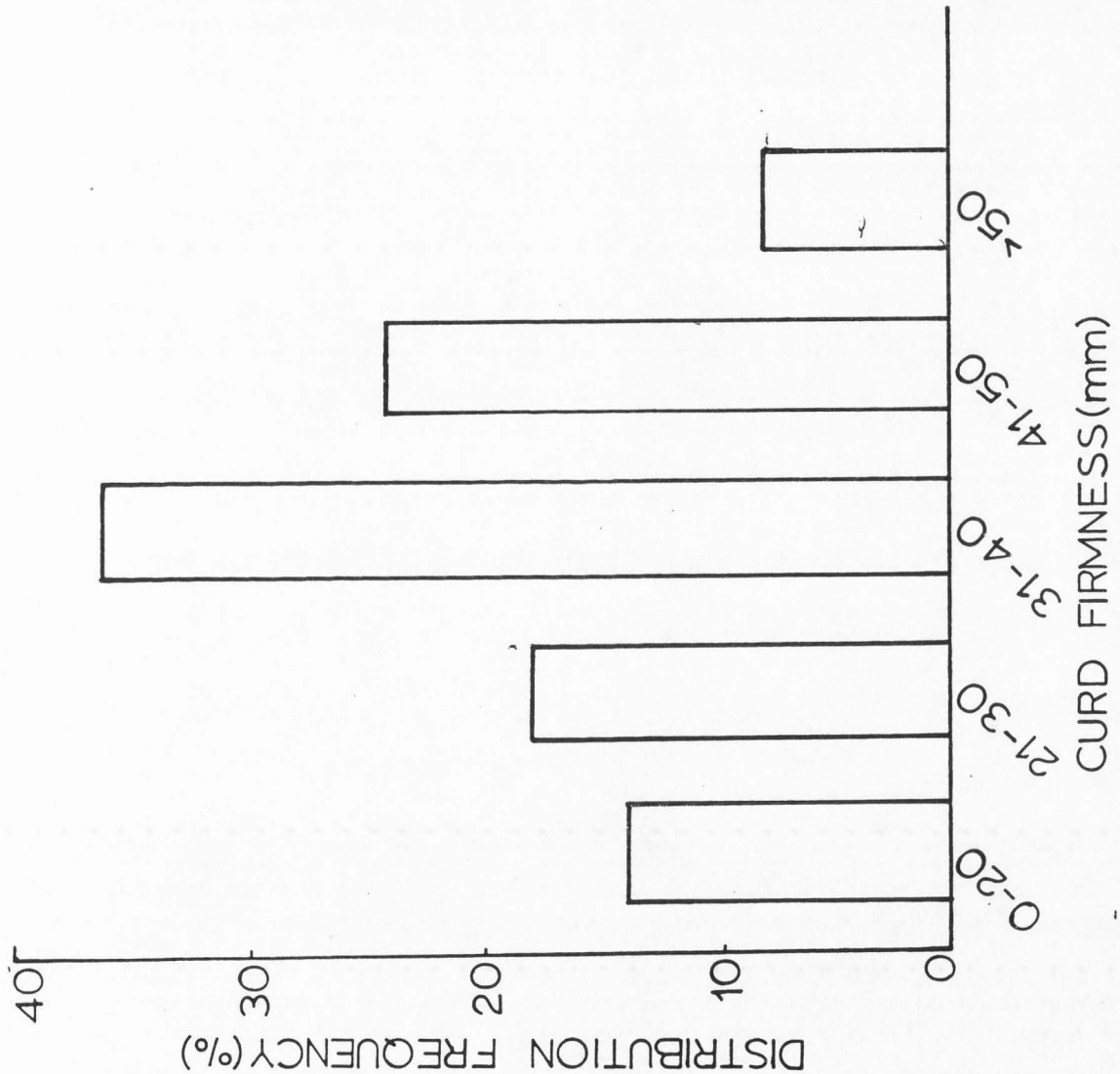

DISTRIBUTION FREQUENCY (%)
0
10
20
30
40
0-20
21-30
31-40
41-50
>50
CURD FIRMNESS (mm)

Table 1. Variation in curd firmness of individual cow milk samples with good-, and poor chymosin-coagulation characteristics (GCM and PCM) and also the results of blending them in 50% ratios.

GCM		PCM		50% blend	
Curd firmness (mm)	pH	Curd firmness (mm)	pH	Curd firmness after blending (mm)	pH
52	6.59	0	7.06	9	6.81
51	6.56	0	7.11	7	6.81
53	6.53	0	6.89	2	6.79
52	6.5	1	6.83	22	6.71
45	6.6	3	6.98	39	6.74
49	6.48	25	6.79	39	6.66
45	6.59	19	6.71	38	6.72
43	6.64	22	6.84	23	6.80
42	6.58	17	6.62	35	6.66
49	6.57	32	6.8	43	6.72
54	6.52	21	6.64	37	6.66
44	6.66	25	6.69	20	6.78
39	6.66	26	6.7	27	6.75
45	6.49	35	6.65	40	6.66
40	6.61	28	6.71	35	6.73
Mean 46.9	6.57	16.9	6.80	27.7	6.73
SD 4.88	.06	12.66	.15	13.34	.06

firmness of the blends. Some blends, however, were more affected than others in manifesting the negative influence. The mean curd firmness of the blends was 27.7 mm and mean pH was 6.73. This implies that GCM samples did not adequately compensate PCM samples as hoped since their mean curd firmness was below the overall mean of 32.4 mm for the herd.

The results of coagulating blends of all 15 GCM, all 15 PCM and 50% of each blend together are shown in Table 2. The effect of having equal proportions of GCM and PCM samples in a milk blend was evident. The latter overwhelmed the former and resulted in a non-coagulating condition. Milk of similar composition can be approximated when a predominant proportion of cows in a herd is in late lactation. Previous work (21) demonstrated that in one herd 38% of milk samples obtained from cows one month prior the end of their lactation period did not coagulate in 30 min. Bulk milk with similar or approximate characteristics would need modification, otherwise, the high protein and fat of such late lactation milk (13) would be under-utilized and produce less yield of cheese since substantial losses would occur in whey (11). Apart from yield loss, Lyall (17) and Lawrence and Gilles (16) indicated that Cheddar cheese made towards the end of the cheese-making season, when all cows are in late lactation, has unsatisfactory properties. High moisture is usually associated with such cheese (17) and the initial cheese pH tends to be high (16). Overall quality also deteriorates faster than cheese made with normal milk and

Table 2. Variation in coagulation properties of pooled milk from individual cows. Curd firmness was determined 30 min after chymosin addition.

Substrate	Coagulation time (min)	Curd firming time(min)	Curd firmness (mm)	pH
Pool of all the best coagulating samples	7.3	4.4	45	6.65
Pool of all the worst coagulating samples	>30	>30	0	6.9
50% each of both pools	>30	>30	0	6.79
Berridge	4.3	2.9	55	6.3
12% non fat dry milk	15.9	8.1	33	6.59

results in a shorter shelf life (11). Shorter shelf life of cheese made with such milk might be attributed to greater acid development as a result of increased lactose content in the higher moisture cheese. Further enzymic degradation of casein by indigeneous milk enzymes in finished cheese is also possible. Pasteurization of milk activates plasmin activity (2).

Effect of modification of individual samples

The effects of reducing milk pH, adding .02% $CaCl_2$, and reducing chymosin concentration, are summarized in Table 3.

The pH difference between unmodified GCM and unmodified PCM was quite wide (Table 1) considering the effect such a difference has on enzyme activity (Figures 3 and 4). Reducing the pH of each sample to 6.3 before coagulation caused remarkable decrease in coagulation time but not in curd firmness for both groups. After reducing pH of the PCM samples, two curd firming patterns were evident (Figure 7 (a)). Either (i) the curds began to firm but collapsed before the end of 30 min (samples B and D) or (ii) samples coagulated at higher chymosin concentration but did not firm in 30 min after chymosin addition (samples A and C). Four possibilities account for non gelation and gel decay observed at this pH:

1. Extreme proteolysis as is common with some protease enzymes other than chymosin (19), but chymosin was used in this experiment.
2. Extreme plasmin activity resulting in the conversion of some β- and α_s-casein into soluble proteose-peptones, γ- and λ-caseins as observed in late lactation milk (2,3,14).
3. Inappropriate salt balance (12) which might have resulted in poor casein micelle aggregation.
4. Autoclaved milk exhibited an identical coagulation pattern (Wright and Richardson, 1983, unpublished) as those samples that coagulated but never firmed. This suggests that the caseins were not very accessible to crosslinking by calcium phosphate.

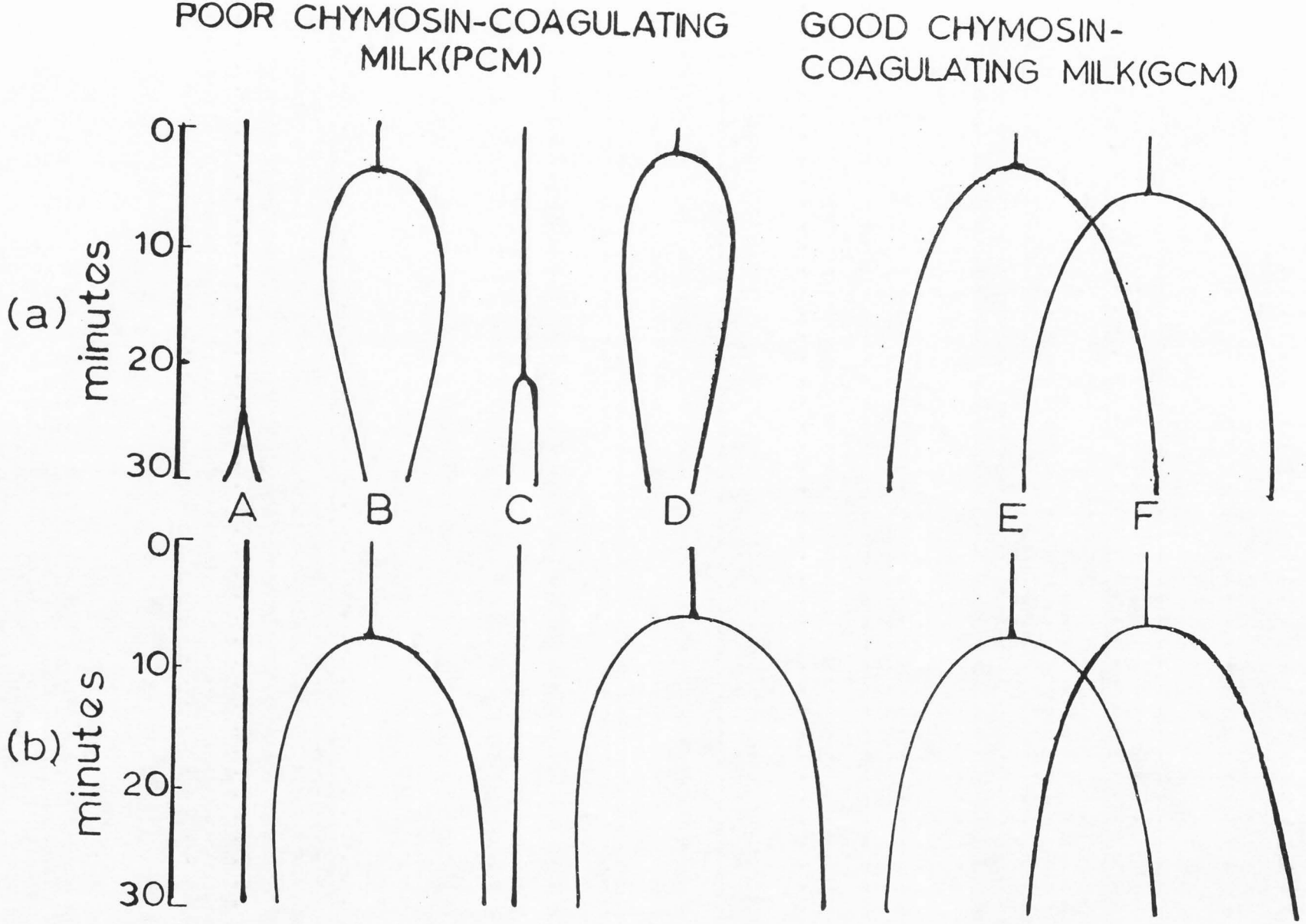
Table 3. Effects of adjustments of some cheesemaking parameters on mean chymosin coagulation properties of milk samples from individual cows. The pH of the samples was reduced to 6.3 with lactic acid before coagulation in the Formagraph.

Parameter(s) adjusted	Good coagulating samples			Poor coagulating samples		
	Coagulation time (min)	Curd firming time (min)	Curd firmness (mm)	Coagulation time (min)	Curd firming time (min)	Curd firmness (mm)
Unmodified samples	6.5 ±2.3	4 ±1.3	48.9 ±4.9	15.3 ±6.5	9.5 ±2.6	16.9 ±12.7
pH reduction	2.9 ± .7	2.1 ± .5	48.2 ±5.0	5.9 ±5.3	3.2 ±1.1	19.5 ±17.5
pH reduction + .02% $CaCl_2$ addition	3.8 ±1.7	2.3 ± .7	50.3 ±7.5	5.6 ±7.5	3.4 ±1.5	21 ±14.8
pH reduction + .02% $CaCl_2$ addition + reduced chymosin conc.	3.3 ±2.2	2 ±1	50.1 ±6	4.8 ±2.2	3.7 1.6	31.6 ±15.6

Effect of salt balance was considered by adding .02% $CaCl_2$ at a reduced pH of 6.3 before coagulation (Table 3). This did not adequately increase curd firmness. Thus, salt balance does not seem as important as pH in accounting for poor coagulation of PCM.

Insignificant increase in curd firmness of Berridge substrate observed by increasing chymosin concentration at decreased pH in the preliminary experiment suggested that the same curd firmness values would be obtained at lower chymosin concentration as at higher chymosin concentration in milk samples at decreased pH. Shorter coagulation times would, however, be obtained at high chymosin concentration when compared with coagulation times obtained at lower chymosin concentration. Chymosin concentration was then reduced to half its initial concentration (from .4 to .2 RU/mL). The result is also shown in Table 3 and Figure 7. A tremendous increase (86.9%) in curd firmness was observed among the PCM samples when all the 3 cheese making modifications (pH reduction, addition of .02% $CaCl_2$, and reducing chymosin concentration) were used at the same time. The PCM samples when unmodified were 65% weaker in curd firmness than the unmodified GCM. After all 3 modifiations of both groups, only 37% difference was apparent. Improvement in curd firmness for GCM after the 3 modifications was marginal 3%. The improvement observed for PCM as a result of reducing chymosin concentration was minimal curd disintergration. This agrees with the

Figure 7. Effect of varying chymosin concentration on the coagulation properties of good-, and poor chymosin-coagulating individual cow milk samples adjusted to pH 6.3 with lactic acid and .02% $CaCl_2$ added; (a) coagulated with 0.4 RU/mL of chymosin (b) coagulated with 0.2 RU/mL chymosin.


POOR CHYMOSIN-COAGULATING MILK(PCM)
GOOD CHYMOSIN-COAGULATING MILK(GCM)
(a)
minutes
0
10
20
30
A
B
C
D
E
F
(b)
minutes
0
10
20
30

preliminary observation that high chymosin concentration at reduced milk pH had the tendency to cause proteolysis of the caseins.

Carpenter and Brown (5) showed that addition of $CaCl_2$ to milk caused more incorporation of soluble caseins into casein micelles and hence led to increased content of total casein that could be measured by size exclusion chromatography. Ali et al. (1) showed that tempering (60^oC for 30 min) milk that had been in cold storage for 48 h caused greater incorporation of soluble caseins into micelles. When two variables only were considered (adding .02% $CaCl_2$, and tempering milk at 37^oC for 90 min) coagulation properties of PCM did not improve substantially. Berridge substrate tempered at different temperatures for different durations (Table 4) showed that tempering at 37^oC for 90 min caused better coagulation than tempering at 60^oC for 30 min. Storry and Ford (24) also showed that coagulum strength was markedly reduced by increased temperature. These observations partly suggest that increased plasmin degradation of β- and α_s -caseins to soluble proteose peptone, and to γ- and λ-caseins (2) in late lactation milk (3) caused substantial losses of caseins that would have participated in curd formation.

Storry et al. (25) correlated high curd firmness with increased α_s - and β-caseins. This suggests that low curd firmness in PCM is partly due to casein degradation into protein fractions that do not participate in curd formation

Table 4. Effect of different temperatures and tempering times on the coagulation properties of chymosin-coagulated Berridge substrate at pH 6.3.

Temperature and time	Coagulation time (min)	Curd firming time(min)	Curd firmness (mm)
37°C for 90 min	4.4	2.9	54.5
40°C for 75 min	5.9	3.9	49
45°C for 60 min	6.7	3.9	50
50°C for 45 min	7.9	4.5	47.5
60°C for 30 min	9.0	5.4	43

and poor curd formation has been associated with late lactation milk (6,21). Fox and Mulvihill (10) also indicated that rennet curd formed in late lactation milk shows poor syneresis characteristics. The curd disintergration observed at higher chymosin concentrations in PCM (Figure 7) but less apparent in GCM suggests that the caseins present in the former were more vulnerable to proteolysis by chymosin or synergism of chymosin and plasmin activities. Less susceptibility of Berridge substrate (which consists of pooled normal milk, spray-dried and reconstituted in .01 M $CaCl_2$ solution) in the preliminary experiment to such degradation supports this assertion. Further research is needed to explain the relative distribution of caseins in the PCM in order to detect if abnormal content of α_s- and β-caseins is partly responsible for their overall low curd

firmness. More investigation is needed to identify interactions between the coagulation properties of GCM and PCM with different pH, temperature and chymosin levels. Such interactions, if significant, will be highly applicable in optimizing the variables for obtaining optimum curd firmness and product yields.

SUMMARY

Individual Holstein cow milk samples were selected for good and poor chymosin-coagulation characteristics. The effect of pH adjustment, addition of .02% $CaCl_2$, and variation in chymosin concentration on coagulation properties of good and poor-coagulating samples was evaluated. Pooling 50% good and 50% poor samples did not improve the average coagulation properties of the poor samples. Reducing milk pH to 6.3 caused a significant decrease in coagulation time but a less marked increase in curd firmness. The greatest increase in curd firmness was obtained by combining reduction of milk pH, addition of .02% $CaCl_2$ and reduction of chymosin concentration. Higher chymosin concentration at reduced pH decreased coagulation time without substantially increasing curd firmness. Curd disintegration was more apparent at higher chymosin concentrations in the poor-coagulating samples.

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PART 3B. INTERACTIONS OF CALCIUM, pH, AND CHYMOSIN DURING MILK COAGULATION

INTRODUCTION

Much has been published on factors which affect enzymic milk coagulation. Most publications, however, emphasize main factor effects. Information on interactions of milk coagulation factors is limited (2,4,8,9,10,13). Furthermore most authors base their reports on bulk milk used as substrate. The use of bulk milk conceals some information because milk samples with good and poor chymosin-coagulation characteristics have shown different interactions with some of these factors (17). It is desired to know how different types of coagulating milk (11,16,17) respond to these factors. This would enable proper adjustment of the levels of these factors during seasons or stages of lactation when coagulation properties of milk are different in order to maximize yield and improve the quality of cheese.

METHODS AND PROCEDURES

Selection of milk samples

Individual milk samples with good and poor chymosin-coagulation characteristics (GCM and PCM) (17) were collected from six Holstein cows in the Utah State University dairy herd. One liter of milk collected from each cow was from a well-stirred, complete, evening-milking. Milk samples were stored overnight at $4^{o}C$ to maintain a uniform temperature history (15). Three temperatures, 32, 35 and $37^{o}C$ were confounded with cows (1 GCM and 1 PCM sample were confounded with each temperature level); two $CaCl_2$ levels 0 and .02% added; three pH levels, 6.3, 6.4 and 6.5; and five levels of chymosin, .1, .2, .3, .4 and .5 rennin units (RU) per milliliter (mL) of distilled water were selected (9,12,17,24). All levels of the unconfounded factors were then tested by varying one factor level at a time. The Formagraph (12) was used to record the coagulation properties of the milk.

Statistical analyses

The factorial design utilized in the analysis of variance (14,21) was a 2 (coagulating milk type) x 2 (presence or absence of added calcium) x 3 (pH levels) x 5 (rennin concentration) x 3 (temperature). Tukey multiple comparison (14) was used to compare mean coagulation properties produced by each factor level.

RESULTS AND DISCUSSION

Effect of factor levels

All main factor effects were significant ($p<.0001$) in altering both coagulation time (CT) and curd firmness (CF) (Tables 5 and 6) which agrees with previous reports (2,4,6,8,9,10,12,18,24,25).

Multiple comparisons of factor levels

Tukey multiple comparisons (14) of mean effects of factor levels showed that lower levels of chymosin (.1,.2, and .3 RU/mL) were significantly different from each other in altering CT (Table 7). Higher chymosin concentrations (.4 and .5 RU/mL), however, produced shorter CT which were insignificantly different from each other, but significantly different from the lower levels. The comparisons of mean CF produced by different levels of chymosin was different. The mean CF produced by .1 RU/mL was significantly lower than CF produced by .2 to .5 RU/mL levels. In practise, chymosin concentrations greater than .2 RU/mL might not be neccesary for adequate curd firmness to be produced 30 min after chymosin addition. Olson and Bottazzi (18) indicated that at high chymosin concentrations, in the presence of added phosphoric acid, curd firming rate was highly retarded. Shorter CT observed at higher chymosin concentrations could erroneously suggest that higher CF would be produced at high levels of chymosin. Care must be taken, however, to avoid

Table 5. Factorial analysis of variance of chymosin coagulation time of milk from individual cows. The R^2 = .993 and CV = 10.1%.

Source	DF	MS	F	Probability
Rennin unit (RU)	4	601.4	1875.2	.0001
Coagulating milk type (CMT)	1	145.6	454.1	.0001
RU*CMT	4	6.5	20.1	.0001
pH	2	154.5	481.8	.0001
RU*pH	8	12.5	39.1	.0001
CMT*pH	2	3.7	11.4	.0001
Calcium chloride(Ca)	1	111.2	346.9	.0001
RU*Ca	4	9.7	30.3	.0001
CMT*Ca	1	5	15.7	.0002
pH*Ca	2	7.4	22.9	.0001
Temperature (Temp)	2	27.8	86.5	.0001
RU*Temp	8	2.6	8.2	.0001
CMT*Temp	2	.2	.6	.5600
pH*Temp	4	2.9	8.9	.0001
Ca*Temp	2	2.1	6.3	.0029
RU*CMT*Temp	8	.5	1.4	.2143
RU*pH*Ca	8	4.8	14.9	.0001
RU*CMT*pH	8	1.4	4.2	.0003
RU*pH*Temp	16	.4	1.3	.2183
RU*CMT*Ca	4	.1	.2	.9550
CMT*pH*Temp	4	1.6	5.1	.0011
CMT*pH*Ca	2	5.2	16.2	.0001
CMT*Ca*Temp	2	1.5	4.5	.0145
pH*Ca*Temp	4	1.1	3.2	.0180
Error	76	.3		

Table 6. Factorial analysis of variance of curd firmness of milk from individual cows 30 min after chymosin addition. The R^2 = .953 and CV = 9.7%.

Source	DF	MS	F	Probability
Rennin unit (RU)	4	330.4	17.3	.0001
Coagulating milk type (CMT)	1	18548.1	969.7	.0001
RU*CMT	4	24	1.3	.2962
pH	2	256.9	13.4	.0001
RU*pH	8	90.4	4.7	.0001
CMT*pH	2	1.9	.1	.9084
Calcium chloride(Ca)	1	154.6	8.1	.0057
RU*Ca	4	177.9	9.3	.0001
CMT*Ca	1	2.5	.1	.7189
pH*Ca	2	29	1.5	.2266
Temperature (Temp)	2	771.2	40.3	.0001
RU*Temp	8	19.1	1.0	.4438
CMT*Temp	2	1654.9	86.5	.0001
PH*Temp	4	25.7	1.3	.2621
Ca*Temp	2	81.4	4.3	.0177
RU*CMT*Temp	8	26.6	1.4	.2141
RU*pH*Ca	8	65.2	3.4	.0021
RU*CMT*pH	8	25.3	1.3	.2448
RU*pH*Temp	16	16.5	.9	.6123
RU*CMT*Ca	4	32	1.7	.1653
CMT*pH*Temp	4	93.4	4.9	.0015
CMT*pH*Ca	2	12.1	.6	.5354
CMT*Ca*Temp	2	84.9	4.5	.0150
pH*Ca*Temp	4	8.1	.4	.7938
Error	76	19.1		

Table 7. Tuckey multiple comparison of mean chymosin coagulation properties and rennin units. Means with the same letter are not significantly different at alpha level = .05.

Grouping	Mean	Rennin units/mL
COAGULATION TIME (min):		
A	12.2	0.1
B	6.4	0.2
C	4.2	0.3
D	2.9	0.4
D		
D	2.4	0.5
CURD FIRMNESS (mm)[1]:		
A	47.3	0.5
A		
A B	46.8	0.2
B		
B	46.1	0.3
B		
B	45.8	0.4
C	39.9	0.1

[1]Curd firmness values 30 min after chymosin addition.

adding very low chymosin concentrations to high pH milk. Thus, if milk pH is adequately reduced before chymosin addition, optimum CF can be produced in 30 min after chymosin addition.

Comparisons of mean CT produced by different pH levels (Table 8) showed that each pH level was significantly different in affecting CT, in agreement with previous reports (8,17,20,24). Thus shorter CT was produced at lower pH. The effects of pH levels on CF were different. Insignificantly different CF values were observed at pH 6.3 and 6.4 but were different from the CF value at pH 6.5. This indicates that reduction of milk pH to at least 6.4 would assure production of firmer curds (3,8,15). This measure would be most valuable in PCM (11,15,17), for example when dairy cows are predominantly in late lactation (Appendix 2). Lower pH values accelerate enzyme cleavage of caseins (5,20). The PCM samples have high pH (17) and their casein would be slowly cleaved by chymosin unless the pH is reduced. A previous report (17) indicated that optimum CF could be produced at pH 6.3. Even though the mean CF value was higher at this pH, it was not different from the mean CF value at pH 6.4 (Table 8). Lactic starter activity is required to achieve significant pH reduction before chymosin is added to cheese milk. Williamson and Speck (26) and Emmons et al. (3) also emphasized the importance of pH reduction for optimum curd development.

Table 8. Tukey multiple comparison of mean chymosin coagulation properties and pH. Means with the same letter are not significantly different at alpha level = .05.

Grouping	Mean	pH
	COAGULATION TIME (min):	
A	7.2	6.5
B	5.6	6.4
C	4.1	6.3
	CURD FIRMNESS (mm)[1]:	
A	46.6	6.3
A		
A	46.1	6.4
B	42.9	6.5

[1]Curd firmness values 30 min after chymosin.

The effects of different temperature levels on coagulation properties is shown in Table 9. Mean coagulation times produced by the three temperature levels were not significantly different from each other. A different relationship was observed between CF and temperature where 37°C produced significantly higher CF than both 32 and 35°C. These observations support making cheese at higher temperatures (23). Higher temperatures would increase collisions of chymosin-treated hydrophobic casein micelles and hence a more rapid aggregation of micelles resulting in a faster gelling rate (2,9). Much higher temperatures will be unfavorable because of denaturation of β-lactoglobulin which will bind κ-casein resulting in a soft curd (19).

Table 9. Tukey multiple comparison of mean chymosin coagulation properties and temperature. Means with the same letter are not significantly different at alpha level = .05.

Grouping	Mean	Temperature (^{o}C)
	COAGULATION TIME (min):	
A	6.3	32
A		
A	5.7	37
A		
A	4.9	35
	CURD FIRMNESS (mm)[1]:	
A	49.4	37
B	43.5	32
B		
B	42.8	35

[1]Curd firmness values 30 min after chymosin addition.

Use of higher temperatures for cheese making together with high temperature lactic starters will not only reduce cheese making time but would also increase yield through retention of more milk fat as a result of production of firmer curds (1,7 and Appendix 3).

Effects of interactions

The 2-way interactions (Table 6) which significantly affected CT included, RU*CMT, RU*pH, RU*Ca, CMT*Ca, pH*Ca, RU*Temp, pH*Temp and CA*Temp. A curvilinear relationship existed between CT and chymosin levels (Figure 8). At the lowest chymosin level (0.1 RU/mL) the difference in mean CT

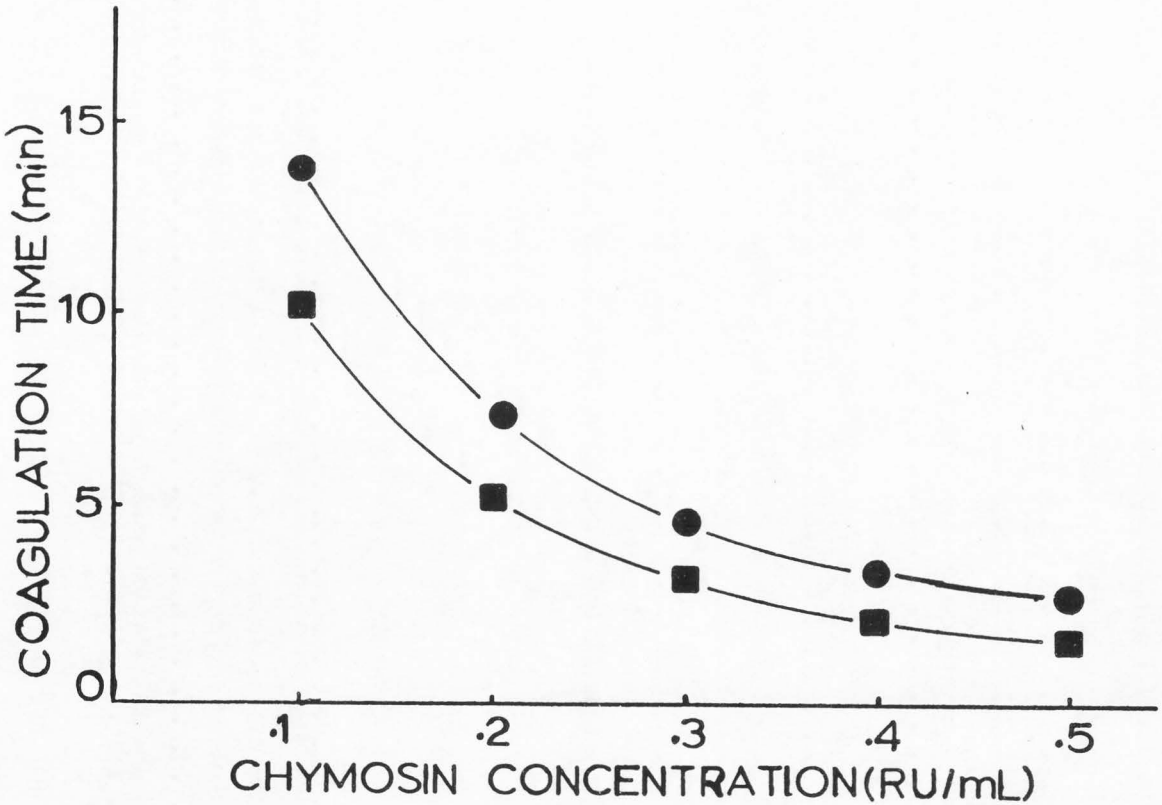

Figure 8. Interactive effect of chymosin and type of coagulating milk (■ good-, and ● poor chymosin-coagulating milk) on coagulation time.

between the two coagulating milk types (CMT) was 3.4 min, but at 0.5 RU/mL the difference in CT was 1.5 min. Thus, increasing chymosin concentrations decreased CT of the PCM more than it did for GCM. CF, however, was not significantly affected by the RU*CMT interaction (Table 6). This indicates that when coagulation properties of milk are poor, there will be no advantage to adding more chymosin. Addition of more chymosin only decreases CT but does not significantly increase CF. CF is more important than CT in cheese making because it affects cheese yield significantly (1,7 and Appendix 3).

The interaction between chymosin and pH was significant ($p<.0001$) in affecting CT (Table 5). The relationship is illustrated in Figure 9. Largest differences in mean CT between pH levels were apparent at the lowest chymosin level. At higher chymosin levels, however, pH 6.5 milk had the greatest decrease in CT when compared to pH 6.4 and 6.3 milks. Thus, higher chymosin levels produced more decrease in CT in the higher pH milk than in lower pH milks. CF in addition was significantly affected ($p < .0001$) by the RU*pH interaction (Figure 10). At 0.1 RU/mL the greatest differences were observed in mean CF of milk at different pH. But from 0.2 to 0.5 RU/mL chymosin concentrations, the differences in CF were not apparent. This agrees with the multiple comparisons of the effects of different chymosin levels on CF (Table 7). However, it appears that the curves of pH 6.3 and 6.4 milks had zero slopes between 0.4 and 0.5

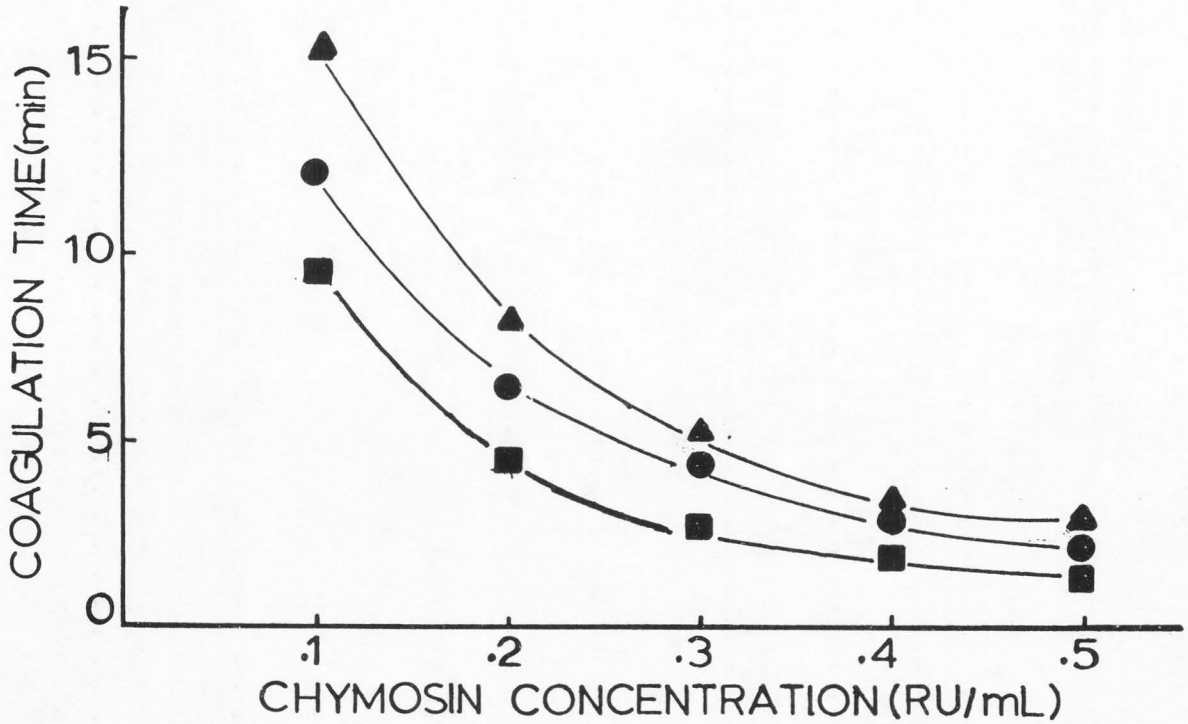

Figure 9. Interactive effect of chymosin and pH on coagulation time (■ pH 6.3, ● pH 6.4, and ▲ pH 6.5).

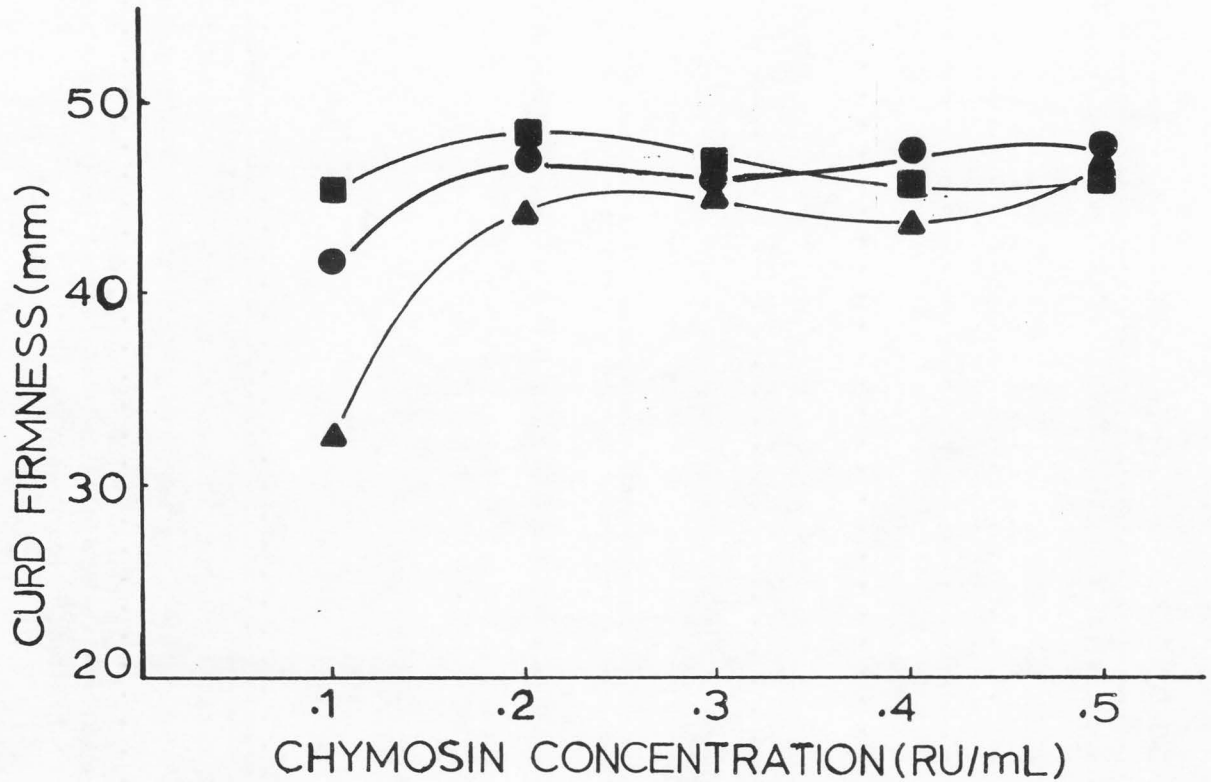

Figure 10. Interactive effect of chymosin and pH on curd firmness 30 min after chymosin addition (■ pH 6.3, ● pH 6.4, and ▲ pH 6.5).

RU/mL suggesting that additional chymosin would not have produced increased CF. On the other hand, pH 6.5 milk appeared to have the potential for increased CF at higher chymosin levels. In a previous study (17) high chymosin concentration (0.4 RU/mL) caused curd deformation or decay at pH 6.3 in PCM, but curd from the same milk was stronger at lower chymosin concentration (0.2 RU/mL). The production of firmer curds would be assured in GCM or PCM with low chymosin concentration, and low pH. Chymosin concentration of 0.2 RU/mL and pH 6.4 appears optimal for coagulating cheese milk.

The interaction between CMT and pH was significant ($p<.0001$) in affecting CT (Table 5). The data plot is similar to Figure 8. The interaction between CMT and pH was, however, insignificant in affecting CF. Thus, reducing milk pH before adding chymosin increased CF for both PCM and GCM at the same rate over the stated pH range. This confirms that pH reduction is necessary before chymosin is added to cheese milk even with GCM.

The interaction between chymosin concentration and .02% $CaCl_2$ was significant ($p<.0001$) in affecting CT (Table 5). This interaction is illustrated in Figure 11. Increasing chymosin concentration reduced the need for added .02% $CaCl_2$ (4). CF, however, was differently affected (Figure 12). At the lowest chymosin concentration (0.1 RU/mL), a wide difference was observed between the CF of milk in which $CaCl_2$ was added. From 0.2 to 0.5 RU/mL, there

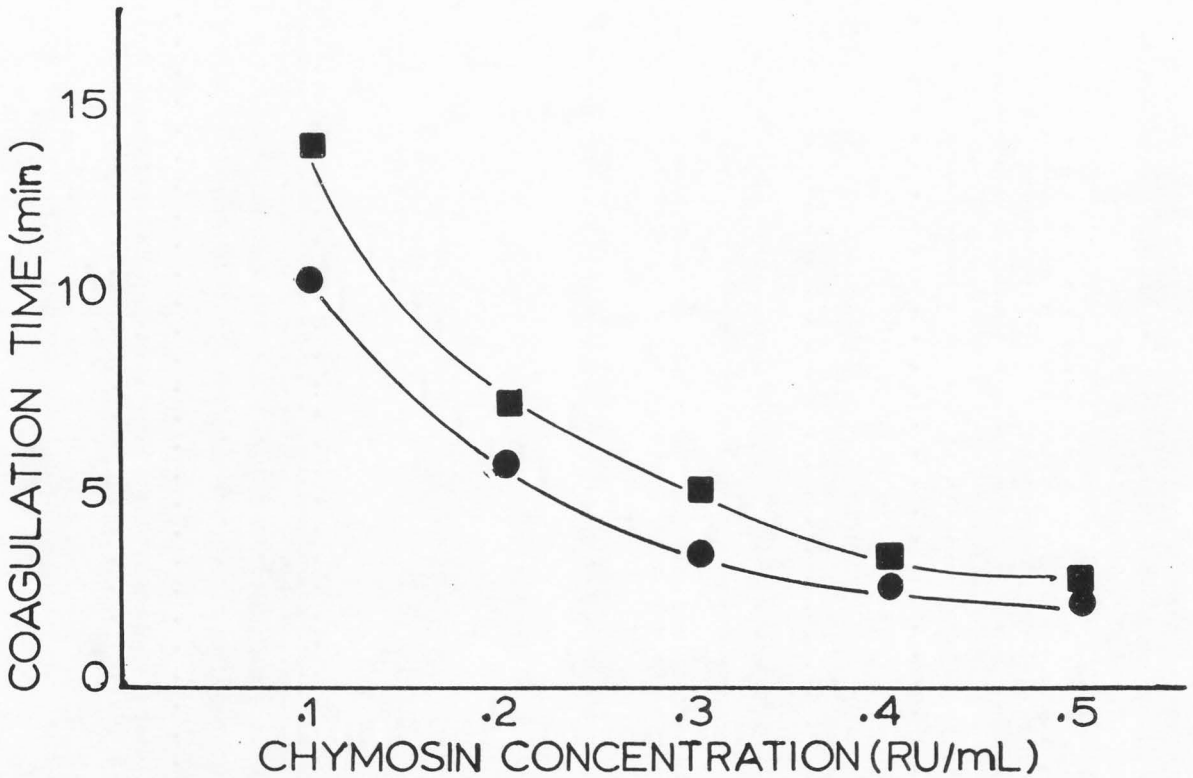

Figure 11. Interactive effect of chymosin and .02% $CaCl_2$ on coagulation time (● .02% $CaCl_2$ added, ■ no added $CaCl_2$).

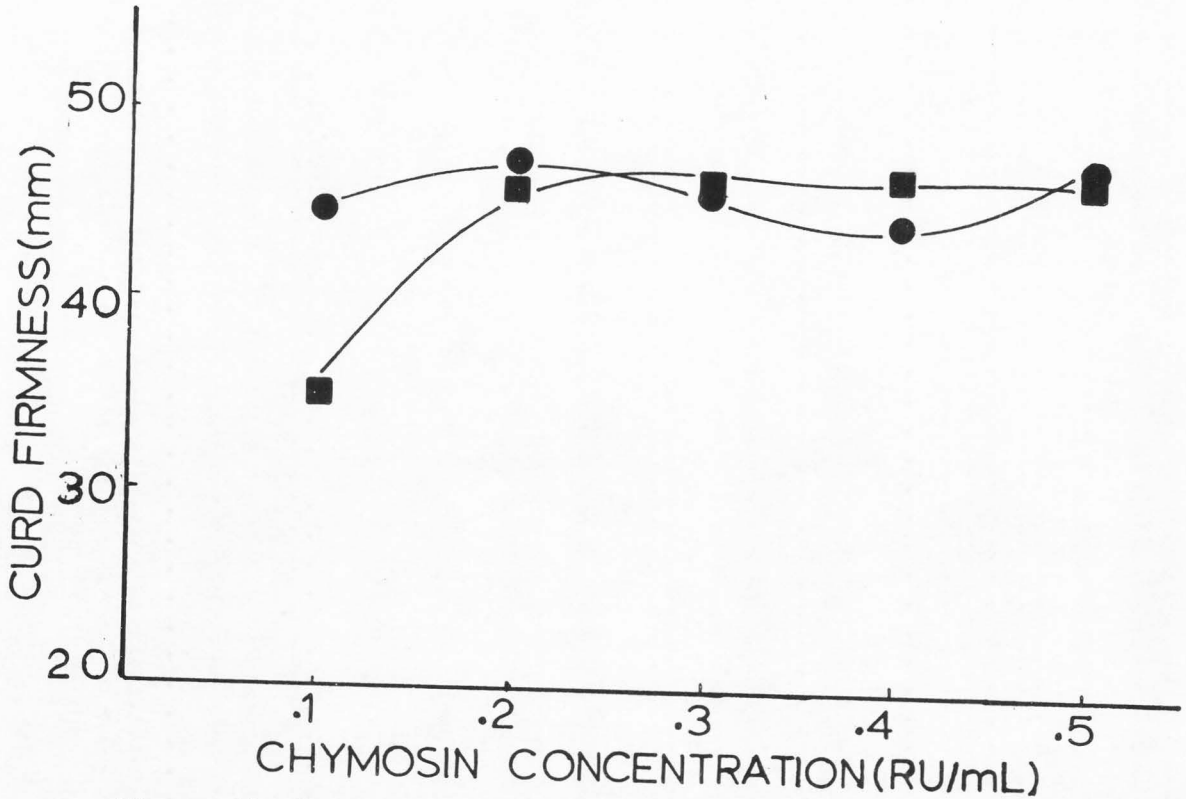

Figure 12. Interactive effect of chymosin and .02% $CaCl_2$ on curd firmness 30 min after chymosin addition (● .02% $CaCl_2$ added, ■ no $CaCl_2$ added).

was no significant difference in CF of both milks (Figure 12). This represented mean CF of milk between pH 6.3 and 6.5. Milk at pH 6.55 to 6.75 would not show similar curd firming patterns (17). For adequate CF to be produced in pH-unmodified milks, much higher level of chymosin would be needed, in addition to .02% $CaCl_2$ addition (17). Therefore increased CF would be obtained at significantly higher cost. Lower costs would be involved if milk pH and chymosin concentration can be reduced, and no $CaCl_2$ is added. Savello et al. (22) indicated that $CaCl_2$ was detrimental to meltability of process cheese made from ultrafiltered milk. Added $CaCl_2$ should be avoided if process cheese is the desired final product.

Optimization of curd firmness

Generally, there appeared no advantage to adding more than 0.2 RU/mL of chymosin (Table 7) for formation of adequate CF. CT was, however, significantly affected by increasing chymosin concentration. Apart from using a very low level of chymosin (0.1 RU/mL), CF was almost the same at all chymosin levels at 30 min when milk pH was reduced. Higher chymosin concentrations at reduced pH tended to cause curd decay and should be avoided (Figure 10). Thus, if pH is to be reduced, then low level of chymosin (0.2 RU/mL) should be added.

Addition of $CaCl_2$ to cheesemilk to optimize the production of adequate CF 30 min after chymosin addition

appears unneccesary if other factors (pH, temperature, and chymosin concentration) can be adequately adjusted (Figure 12). Jen and Ashworth (8) indicated that addition of $CaCl_2$ to milk partly increases CF through pH reduction, primarily due to binding of added calcium to casein micelles and release of protons. They established that if a mixture of $CaCl_2$ and $Ca(OH)_2$ are added to milk to keep pH constant then CF decreases compared to the situation when only $CaCl_2$ is added.

The difficulties encountered in cheese making when coagulation properties of milk are poor can be partly arrested by adjustment of all the main factor levels that affect milk coagulation. The most significant adjustments should include reducing pH to between 6.3 and 6.4, using between 0.2 to 0.3 RU/mL of chymosin, and increasing the temperature to $37^{o}C$. Addition of $CaCl_2$ might not be necessary. The above modifications would help increase CF at cutting. Increased CF at cutting increases retention of milk fat in curd (1,7 Appendix 3), and hence increases cheese yield. Further research is needed to determine if addition of .02% $CaCl_2$ to cheesemilk is neccesary, because loss of colloidal calcium phosphate from casein micelles occurs as pH is reduced during cooking of curd. Excess loss of colloidal calcium phosphate will retard syneresis of curd, and would cause high moisture retention and mealy body in finished cheese. Addition of $CaCl_2$ to milk might be expected to minimize the effect of this loss.

SUMMARY

Holstein milk samples with good and poor chymosin-coagulation characteristics were coagulated in the Formagraph using different combinations of five levels of chymosin, three pH and three temperatures in the presence and absence of .02% added $CaCl_2$.

All the main factor effects were highly significant ($p<.0001$) in altering both coagulation time and curd firmness. Multiple comparisons of mean coagulation times showed that lower levels of chymosin (.1, .2, and .3 rennin units) were significantly different from each other, and were different from higher levels (.4 and .5 rennin units). The three pH levels produced significantly different mean coagulation times. Addition of more than .2 rennin units per milliliter to milk was not necessary for adequate curd firmness to be produced 30 minutes after chymosin addition if milk pH was reduced to at least 6.4. Addition of .02% $CaCl_2$ to milk was not neccesary for adequate curd firmness to be produced 30 min after chymosin addition if other milk coagulation factors (pH, $CaCl_2$, and temperature) were adequately adjusted. Higher temperature (37°C) at reduced milk pH produced firmer curds.

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PART 4. CASEIN COMPOSITION OF COW'S MILK OF DIFFERENT CHYMOSIN COAGULATION PROPERTIES

INTRODUCTION

The relationship between chymosin coagulation properties and variability in casein composition of milk from individual cows has not been well described. Some authors (9,14,18) have shown that curd firmness is directly related to total casein in milk. Storry et al. (19) correlated high curd firmness with increased α_S - and β-caseins of milk from different breeds and species of ruminants. Davies and Law (3) showed that casein composition was constant in mid lactation but was highly variable in early and late lactation. Barry and Donnelly (2) showed that a high level of minor, unidentified protein, and γ-caseins and a reduced level of β-casein were associated with late lactation when compared to the low level of γ-casein in normal milk (4), and that these changes are expected to affect syneresis characteristics of curd.

Previous studies (15) showed variation in susceptibility to deformation of milk curds from certain cows made with chymosin. Some curds were highly deformed at high chymosin concentration, were weak, and not suitable for cheese manufacture. Milk from other cows coagulated early with chymosin but the curds never firmed. All indicate that probable variations occured in casein composition of milk from individual cows which affected their chymosin coagulation properties. The purpose of this study was to investigate possible variations in casein composition of

milk from individual Holstein cows and the effects on milk chymosin-coagulation properties.

METHODS AND PROCEDURES

Preparation of casein samples

Nine individual Holstein milk samples from the Utah State University dairy herd were selected from 33, and 17 milk samples which had good and poor chymosin-coagulation characteristics (GCM and PCM) respectively (12,15). The samples were filtered with glasswool to remove cell debris, centrifuged at 3000 x G at -1^{o}C to separate milk fat, and decanted to obtain skim milk. Caseins were precipitated from each 30 mL individual skim milk sample at pH 4.6 with 5 N HCl during constant stirring at 37^{o}C. Precipitated caseins were washed with distilled water, and resolubilized with 2 N NaOH at 20^{o}C. The pH of the sample did not exceed 8.0, and the final volume did not exceed the initial volume of milk (5). The process of precipitation and resolubilization was done twice and the final precipitate was frozen and stored at -20^{o}C.

Hydroxyapatite chromatography

Laboratory scale chromatographic grade hydroxyapatite was prepared as described by Atkinson et al. (1), equilibriated and stored in 5 mM sodium phosphate, pH 6.8 buffer at 4^{o}C. Frozen individual casein samples were thawed and 1 g of each sample was dissolved in 20 mL of 5 mM sodium phosphate in 6 M urea, pH 6.8 buffer and left overnight at 20^{o}C. Three elution buffers which consisted of 5, 80, and 310 mM sodium phosphate in 6 M urea all adjusted to pH 6.8

with 5 N HCl were prepared (2). A 16 cm x 1.5 cm glass column was packed with hydroxyapatite, and the elution rubber tubing attached to the flowcell of a Beckman DU-8 scanning spectrophotometer (Beckman Instruments Inc., Fullerton, CA) monitor, which was interfaced with a Tektronix 4052 computer (Tektronics Inc., Beaverton, OR). The computer was programmed to plot absorbance (280 nm) of the eluent at 1 min intervals. A 3 mL sample was applied gradually on top of the column packing with a pipette and elution was commenced with 5 mM sodium phosphate in 6 M urea buffer using a peristaltic pump (Scientific Industries Inc., Bohemia, NY) adjusted to deliver .5 mL of buffer per min. After 30 mL of 5 mM buffer was used it was replaced with 25 mL of 80 mM buffer. A linear gradient of 80 to 310 mM buffer was then set up with a gradient mixer, and elution was continued for 7 h.

Polyacrylamide electrophoresis

Polyacrylamide (10%) gels containing 4 M urea were prepared with tris-glycine pH 8.9 buffer as described (13) and stored at 4°C no longer than 96 h (11). Five hundred milligrams of thawed casein from each individual sample was dissolved in 5 mL of 6 M urea and was mixed with a Vortex mixer (Scientific Industries, Bohemia, NY) until completely dissolved. To 250 µL of each sample was added 10 µL of .25% bromophenol blue (tracking dye). The sample was mixed again and incubated at 45°C for 3 h. During incubation, a gel was pre-run for 3 h at 50 mA in an LKB electrophoresis apparatus

(11) (LKB-Producter, Broma, Sweden) with tris-glycine, pH 8.3 electrode buffer. After incubation, 20 μL of glycerol was added, each sample was mixed properly and 10 μL was pipetted into a sample well in the polyacrylamide gel. Also 50 mg each of freeze-dried α_s-, and β-casein standards (Sigma Chemical Co., St. Louis, Mo) were similarly treated. The gel was run for 10 min at 20 mA to concentrate the samples in the anode side of the wells in the gel after which the current was increased to 5mA/sample. This current was maintained until the tracking dye was monitored at the anode end of the gel, approximately 5 h. The gel was removed, fixed in a solution of trichloroacetic-sulphosalicyclic acid and methanol for 30 min, stained in comassie brilliant blue R-250 solution for 1 h, destained for 24 h in ethanol-acetic acid solution, and preserved in an ethanol-acetic acid solution (11). The main bands of the major casein variants were also over-loaded in a second gel by doubling the sample concentration in order to increase the color intensity of the minor bands for better evaluation, and electrophoresis was repeated.

Urea was added to individual 5 mL skim milk samples to a concentration of 6 M and the samples electrophoresed as described above. Acid whey samples collected after isolelectric precipitation of caseins from the individual samples were also electrophoresed.

RESULTS AND DISCUSSION

Variation in casein composition

Wide variations in casein composition were observed between the PCM and GCM samples. Figure 13 illustrates Formagraph (12) tracings of chymosin coagulating samples in both groups. The tracings presented in the figure were representative of all tracings not shown. Sample D (Figure 13) represents the Formagraph tracing of a typical GCM. The chromatograph of a normal clotting sample is shown in Figure 14. Sample A in Figure 13 coagulated early enough to permit adequate curd formation in 30 min, but this did not happen, indicating improper development of the secondary, non-enzymic phase of milk clotting (6). The chromatograph elution profile for casein variants of sample A is also shown in Figure 15. Abnormally high contents of para-κ- and γ-1,2 and 3- caseins (a)(2), were evident when compared to the κ-casein and unidentified chymosin-resistant minor protein peak (b) which had a smaller peak area. Remarkable in the casein composition of this sample, were the 2 substantial minor peaks of unidentified, minor proteins (c and e). The β-casein was also depleted (d). The α_s-casein content (f) appeared normal as evident from the peak area and when compared to a normal milk profile (2). Coagulation studies were repeated on all the milk samples in Figure 13 after storage at -1^{o}C for 1 mo. Sample A (Figure 13) showed a highly significant change in curd firmness, 95% reduction.

Figure 13. Formagraph tracings of good, and poor chymosin-coagulating milk samples from individual Holstein cows. Samples consisted of: A, milk which coagulated early but the curd did not firm adequately at 30 min; B, milk with long coagulation time and very weak curd; C, milk which could not coagulate 30 min after chymosin addition; D, milk with good chymosin-coagulation characteristics.

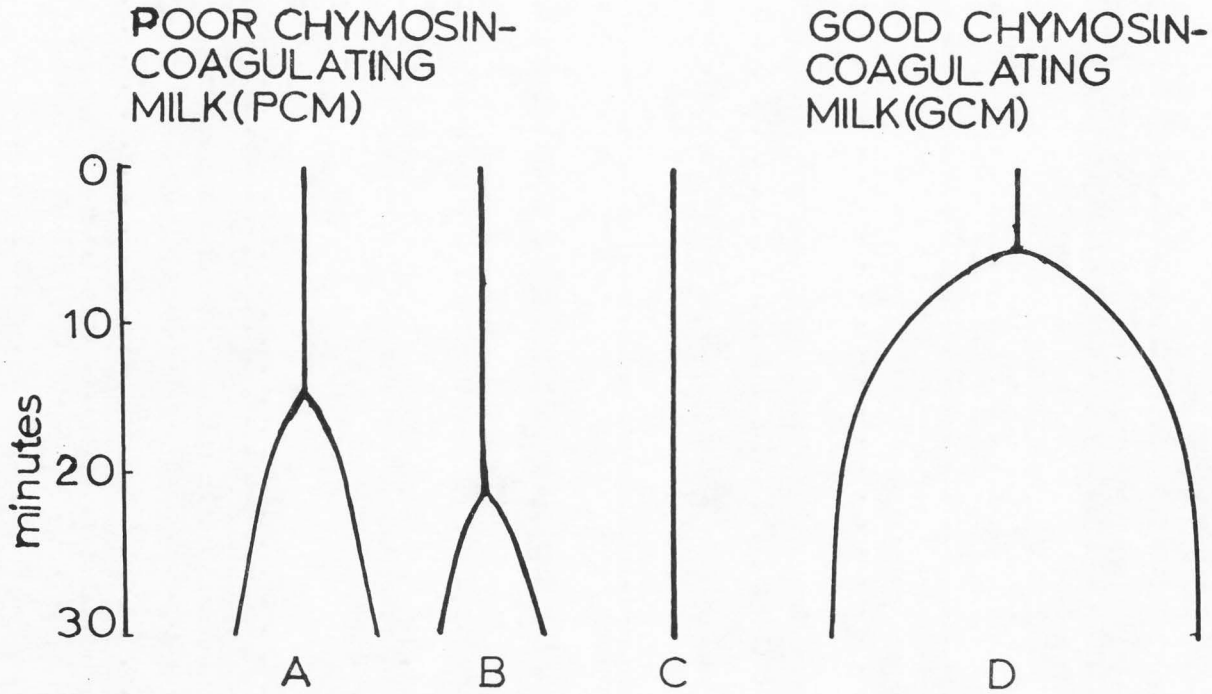

POOR CHYMOSIN-
COAGULATING
MILK(PCM)
GOOD CHYMOSIN-
COAGULATING
MILK(GCM)
0
10
20
30
minutes
A
B
C
D

Figure 14. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk with good chymosin-coagulation characteristics. Elution peaks consist of: a, γ - and para-κ-caseins; b, κ-casein; c, unidentified minor protein; d, β-casein; e, α_{s1}-casein; f, α_{s2}-casein.

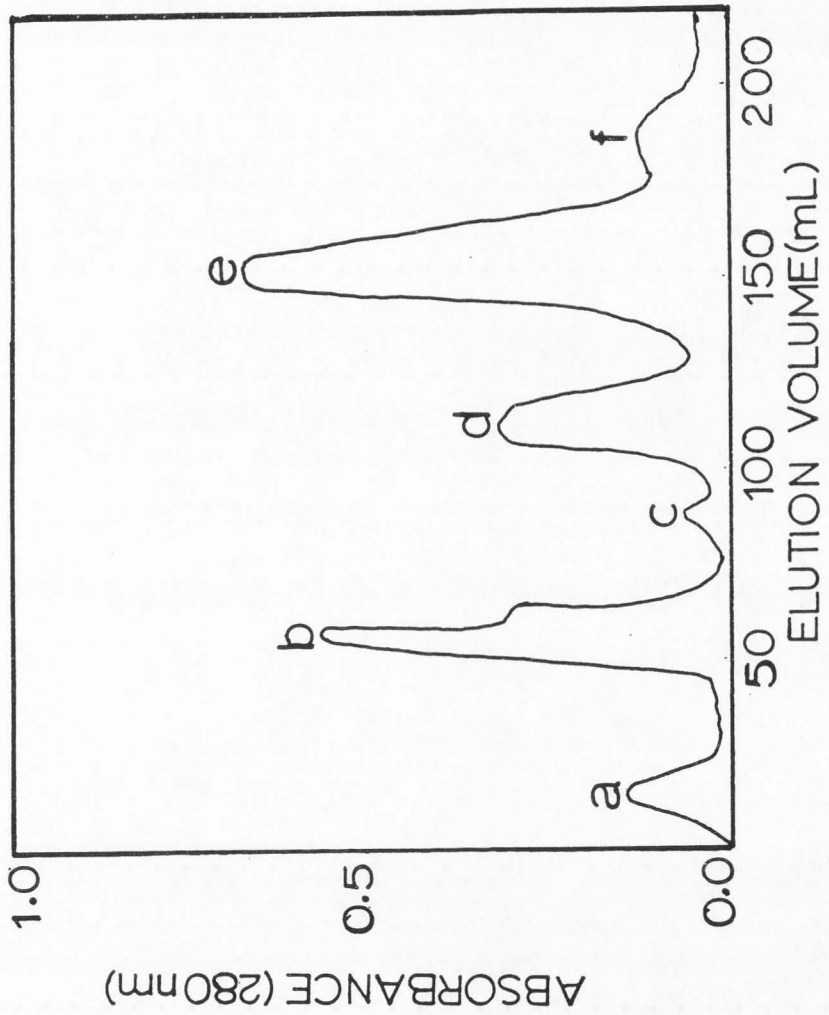
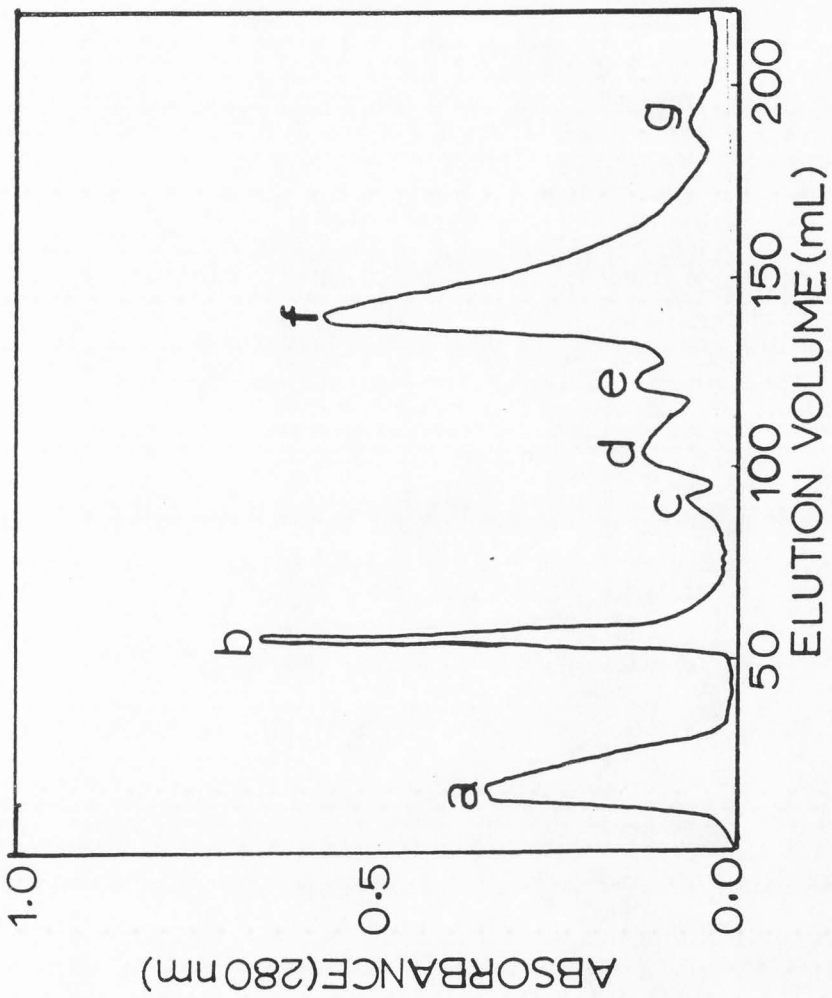

ABSORBANCE (280nm)
1.0
0.5
0.0
50
100
150
200
ELUTION VOLUME(mL)
a
b
c
d
e
f

Figure 15. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk which coagulated with chymosin but its curd did not firm adequately. Elution peaks consist of: a, γ- and para-κ-caseins; b, κ-casein; c and e, unidentified minor protein; d, β-casein; f, α_{s1}-casein; g, α_{s2}-casein.


ABSORBANCE(280nm)
1.0
0.5
0.0
50
100
150
200
ELUTION VOLUME(mL)
a
b
c
d
e
f
g

All other samples showed less significant differences but the coagulation time of one GCM sample increased 46% and curd firmness decreased 3.6%. This suggests that accumulation of unidentified minor proteins may be resposible for inability of sample 13A to complete secondary clotting phase. A Formagraph tracing similar to sample 13A was obtained when autoclaved milk was coagulated with chymosin. κ-casein is known to interact with β-lactoglobulin in milk upon high heat treatment (16). Such milk is known to produce soft curd when coagulated with chymosin. One possibility of soft curd formation, therefore, might be the presence of these unidentified minor proteins in a significant quantity as evidenced in sample 13A.

Sample B (Figure 13) also had a casein elution profile (Figure 16) which indicated abnormal content of para-κ- plus γ-1,2, and 3-caseins (a). The content of para-κ- plus γ-caseins which together form minor proteins, had almost peak area equal to its α_s-casein (e), the major casein. At four previous weekly samplings this sample consistently displayed the same coagulation pattern (Figure 13) and its pH was consistently near 6.8. The β-casein (d) content was low as was κ-casein (b).

Sample C (Figure 13) did not coagulate in 30 min. It had a pH of 7.1. The casein elution pattern is shown in Figure 17. All the casein variants were conspicuously low. It had an additional casein fraction (g) after the α_s-casein (f), which probably was λ-casein. Some authors (3,4,5) have

Figure 16. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk which had a long coagulation time with chymosin, and very weak curd. Elution peaks consist of: a, γ- and para-κ-caseins; b, κ-casein; c, unidentified minor protein; d, β-casein; e, α_S-casein.

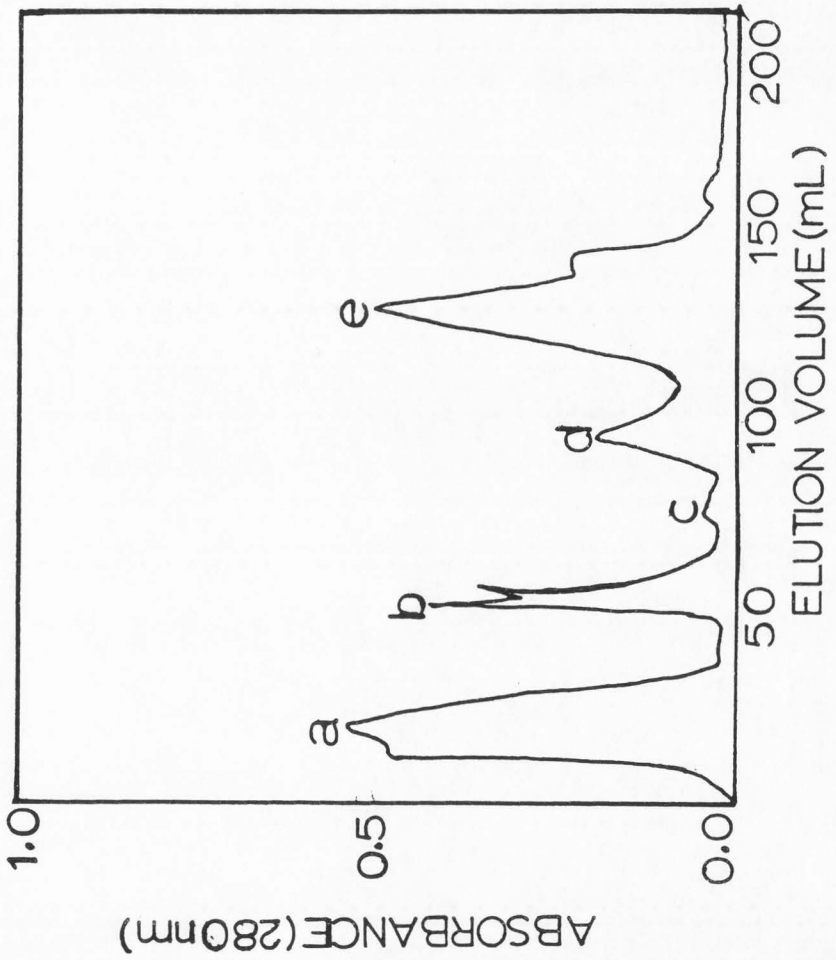
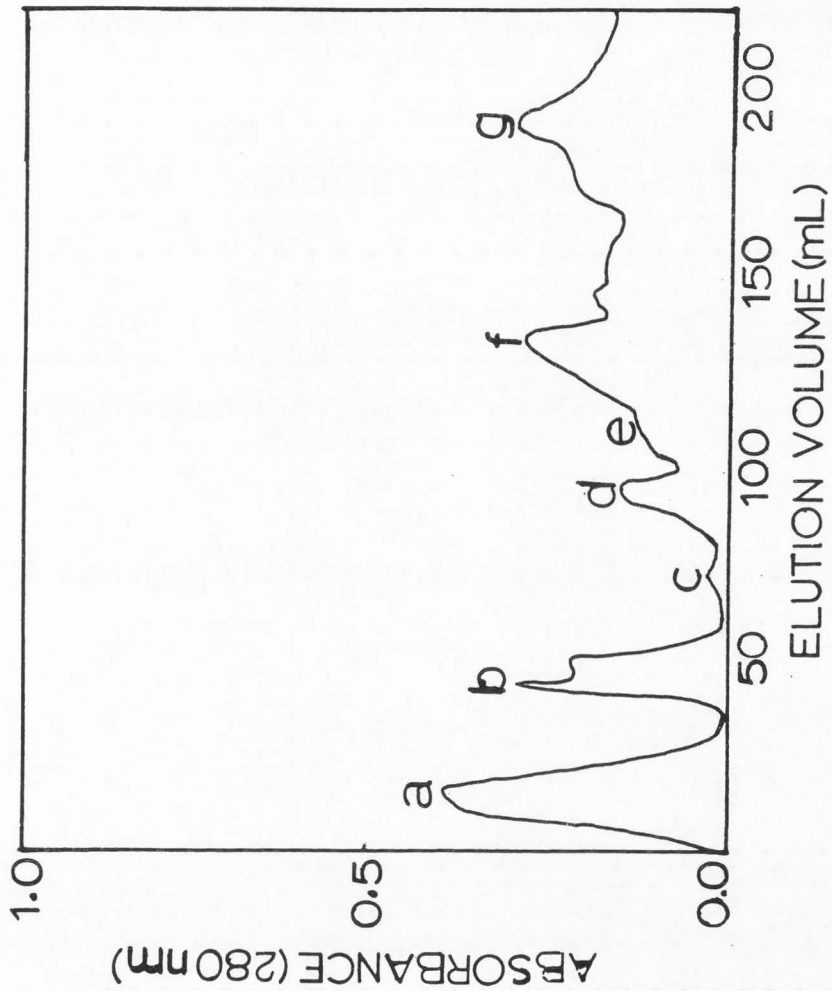

ABSORBANCE (280nm)
1.0
0.5
0.0
50
100
150
200
ELUTION VOLUME (mL)
a
b
c
d
e

Figure 17. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk which could not coagulate 30 min after chymosin addition. Elution peaks consist of: a, γ- and para-κ-caseins; b, κ-casein; c and e, unidentified minor protein; d, β-casein; f, α_s-casein; g, tentatively identified λ-casein.


ABSORBANCE (280 nm)
1.0
0.5
0.0
50
100
150
200
ELUTION VOLUME (mL)
a
b
c
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f
g

indicated that α_s -casein is less susceptible to degradation by indigenious milk enzymes than β-casein. This observation agrees with our findings where less variability was observed in the α_s -casein peaks of the samples mentioned apart from the exceptional example of the non-coagulating sample (Figure 17).

The majority of the PCM were late lactation milk samples. A remarkable characteristic they possesed was high milk pH, consistent with previous observations (14,15). Some authors (2,3,10) have shown that increased capillary permeation of blood constituents into the mammary glands, as commonly observed in late lactation, was responsible for increased content of indigenious alkaline milk proteinases of which plasmin, a proteolytic enzyme similar to trypsin (7,10), has been identified. Plasmin cleavage sites (8) do not conform to chymosin cleavage sites. Plasmin is known to be inhibited by soybean trypsin inhibitor (7). Cleavage products have been identified as para-κ-, γ- and λ-casein-like substances in addition to proteose peptones 5 and 8 (8,20). These products have different electrophoretic mobilities than those of the major casein fractions, and precipitate together with the major caseins during isoelectric precipitation even though γ-caseins have isoelectric pH of about 6 (7,20).

Comparison of the contents of casein variants in the samples calculated from areas under the peaks of the chromatographs is shown in Table 10.

Table 10. Relative amounts (%) of casein variants in individual Holstein cow milk samples with good- and poor chymosin-coagulating characteristics (GCM and PCM).

Coagulating milk type	α_s	β	κ	para-$\kappa+\gamma$	unidentified minor protein	λ?
GCM	56.3	18.3	18.8	4.8	2.2	
PCM (A)	52.6	8.7	15.6	15.8	7.0	
PCM (B)	42.2	9.7	13.2	33.9	1.0	
PCM (C)	29.7	8.3	14.0	28.4	6.6	13.5

The poor chymosin-coagulating milk samples had higher content of γ- and para-κ-caseins, lower β- and α_s-caseins than the good chymosin-coagulating milk.

Plate 1 illustrates the electrophoretic distribution of the main casein fractions. Slots 1-5 contain PCM samples. The distribution is consistent with the elution profiles obtained with hydroxyapatite chromatography. The PCM showed a lot of variability in color intensity of their β-casein bands and little or no variability in the α_s-casein bands. The β-casein bands were generally lighter in color than the GCM indicating a lower content of β-casein. The minor casein bands of the PCM were numerous and faint and thus necessitated overloading of the main bands in another gel (Plate 2) to enable a better visualization of the minor bands. Slot 1 in Plate 2 contained the same sample as B in

Plate 1. Polyacrylamide electrophoresis in urea gels of whole casein from individual Holstein cow's milk. Slots 1-5, and 6-9 contained poor-, and good chymosin-coagulating milk samples respectively. Slots (i) and (ii) contained α_s- and β-caseins standards respectively.

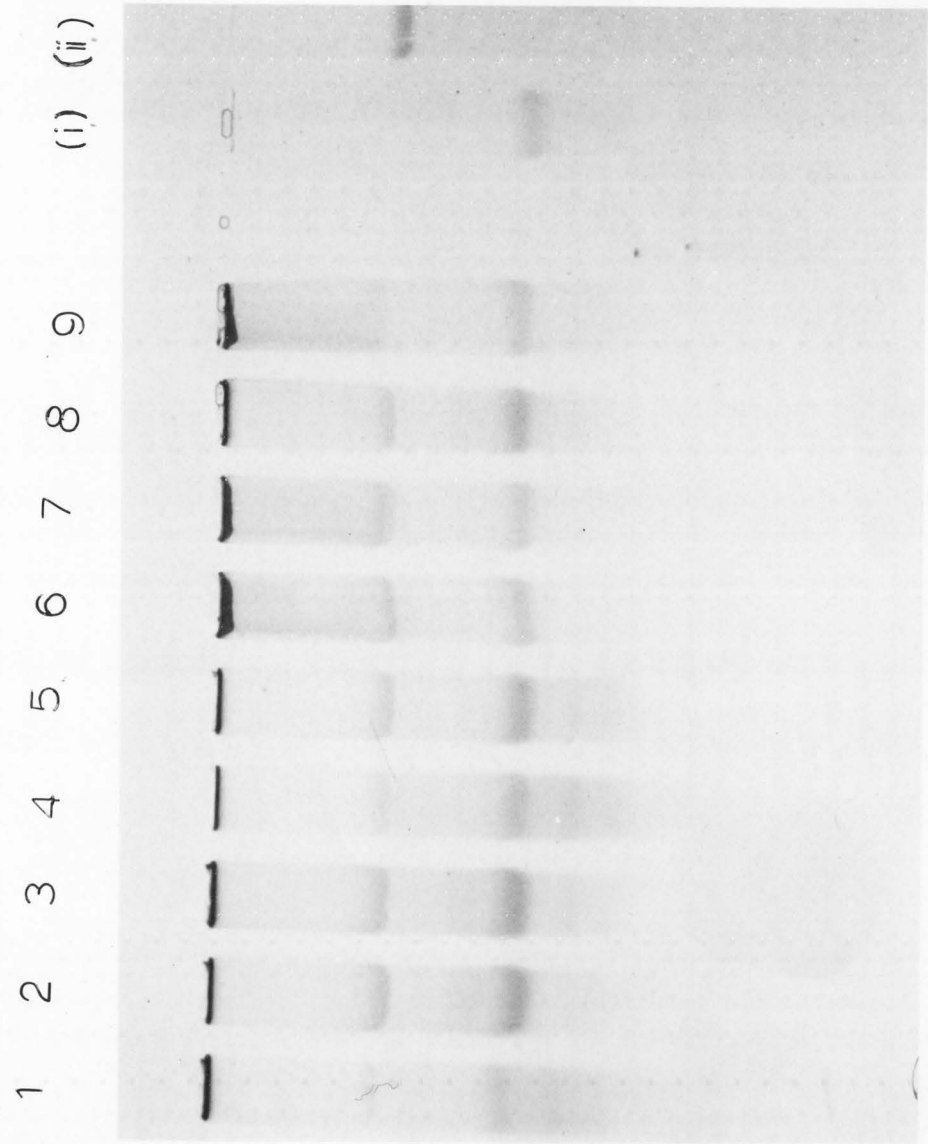
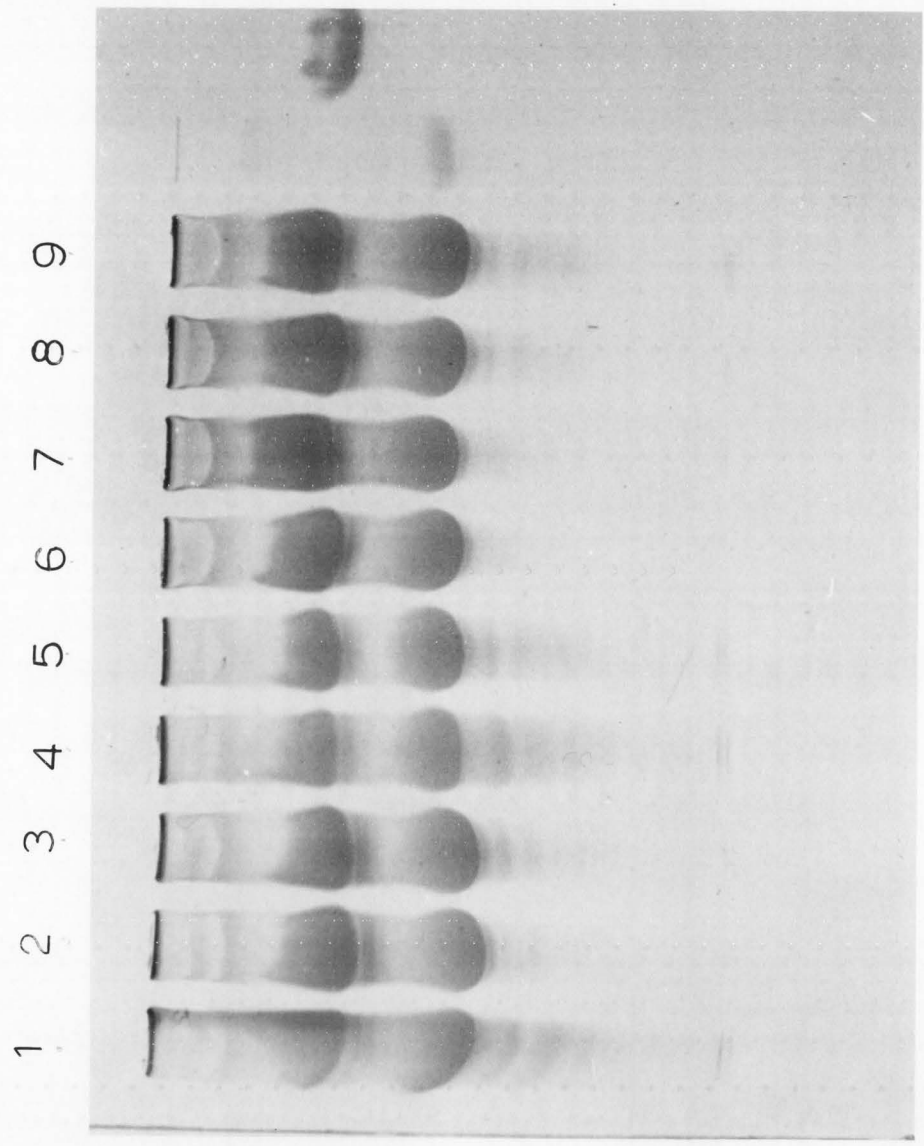

1
2
3
4
5
6
7
8
9
(i)
(ii)

Plate 2. Polyacrylamide electrophoresis in urea gels of whole casein from individual Holstein cow's milk to show the minor casein fractions as a result of overloading the main bands. Slots 1-5, and 6-9 contained poor-, and good chymosin-coagulating milk samples respectively.


β
d_S
1
2
3
4
5
6
7
8
9

Figures 13 and 16. Inumerable minor bands in this sample agree with the large peak area of para-κ- plus γ-caseins in the casein elution profile (Figure 16). Slot 4 in Plates 1 and 2 contained the sample which did not coagulate in 30 min (C in Figure 13). Absent in these plates for this particular sample was an intensely colored band in each plate that was close to the cathode indicating a cationic protein. This band disappeared during destaining of the gels, and was likely to coincide with (g), (Figure 17). It suggests a protein with isoelectric point greater than 8.9, the gel pH, hence a mobility towards the cathode.

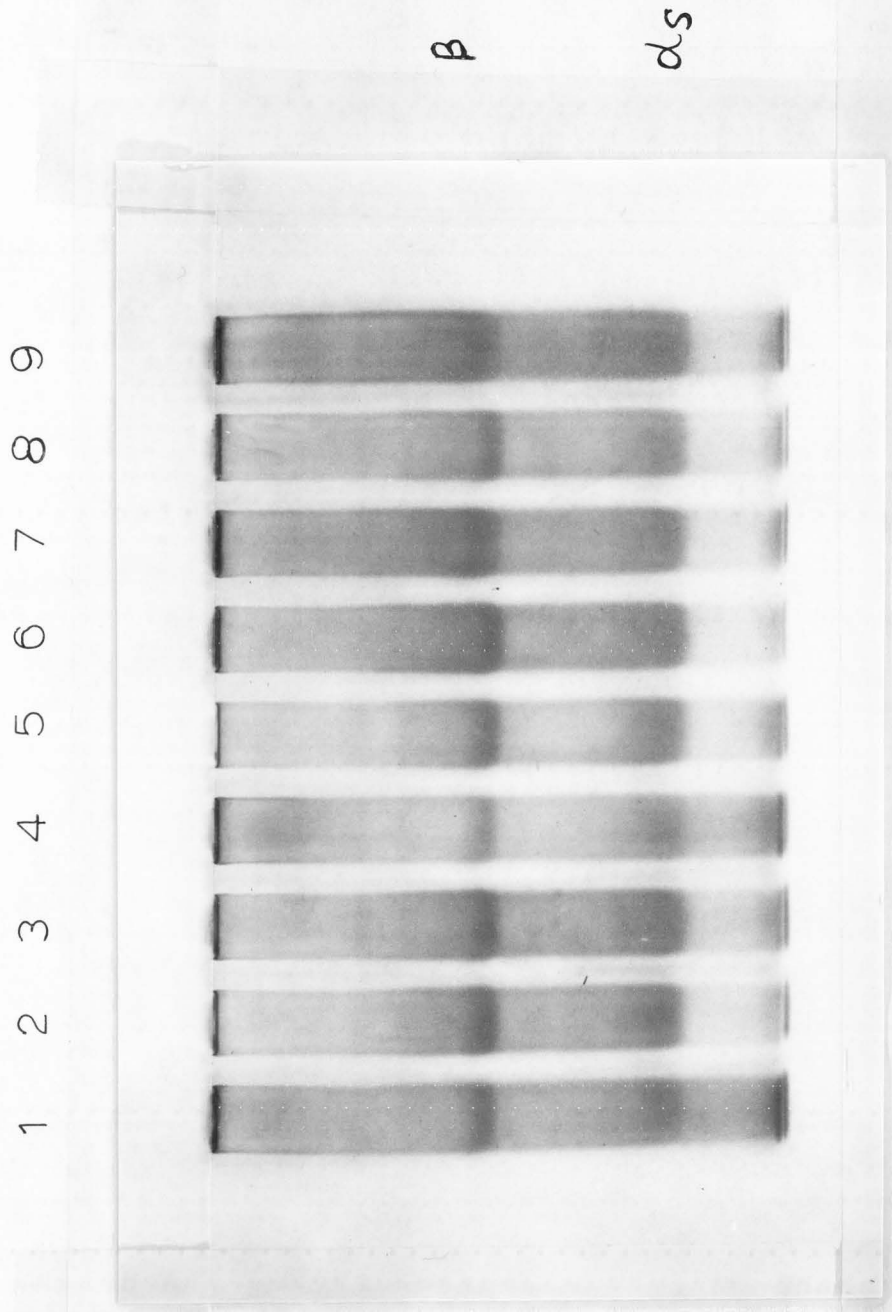
Slots 6-9 in Plates 1 and 2 contained GCM samples. Less variability was observed in their main casein bands apart from the sample in slot 9 where the β-casein band lost color intensity during destaining. Minor bands were observed among the GCM samples but were fewer when compared with the PCM. The elution profile of the casein fractions of a GCM sample (Figure 14) was consistent with previous finding for normal milk (2) in which low peak areas were observed for para-κ- plus γ-caseins. The other GCM samples had elution profiles consistent with Figure 14. Most of the GCM samples were mid-lactation milk. The pH of the samples were on the average near 6.6. Less indigenious milk proteinase activity has been observed in mid-lactation milk (10), and casein composition in mid-lactation tends to be more stable than in early or late lactation milk (3).

The major caseins in skim milk are shown in Plate 3. The slots contained samples from the same cows as in the two previous plates. Variation in color intensity of β-casein bands, and appearance of minor casein bands, however, were not apparent. Thus the PCM (slots 1-5) and GCM (slots 6-9) samples did not exhibit visible differences. Low casein concentration in the sample volumes of skim milk used in this experiment might account for the disparity. The gel which showed the electrophoretic distribution of the whey protein bands was not shown because there was little variation in their protein distribution apart from the non-coagulating sample which had an intensely colored -lactoglobulin band. Little variation in the whey protein bands agrees with the observations of Reimerdes and Herlitz (17), who reported a very slight increase in whey protein of milk that was stored at $4^{o}C$ for 100 h and was then coagulated with rennet.

Technological implications

There was a trend among the PCM samples; the milk shared some characteristics with mastitis milk, in agreement with the findings of Davies and Law (3), who indicated that late lactation milk and mastitis milk had similar properties. They established that a high positive correlation ($r = .885$) existed between milk sodium and γ-casein, a negative correlation ($r = -.75$) between sodium and β-casein, and also $r = -.51$ between sodium and α_s-casein existed. Late lactation milk is low in lactose and high in

Plate 3. Polyacrylamide electrophoresis in urea gels of skim milk from individual Holstein cows. Slots 1-5, and 6-9 contained poor-, and good chymosin-coagulating milk samples respectively.


B
ds
1 2 3 4 5 6 7 8 9

sodium, and such milk is comparatively poor in β- and α_s- and rich in γ- and para-κ-caseins (2,3). The PCM samples contained some blood constituents which might have reacted with the caseins and converted them to products which did not participate in curd structure formation but may have been trapped in the curd. Such curds were very weak and unsuitable for cheese production. Poor curd formation is not only attributed to the presence of these minor casein components in significantly large quantities, but also to increased content of monovalent cations (eg. sodium) as common in late lactation milk. Monovalent cations do not favor the secondary stage of milk clotting. They do not enhance rapid aggregation of casein micelles after chymosin cleavage of the phe 105 - met 106 bond (6).

Effect on the kinetics of milk coagulation

The para-κ-casein-like substance observed in significant quantities in PCM samples should have initiated aggregation of casein micelles, and should have caused better coagulation, but this never happened. Since para-κ- and γ-caseins were eluted under the same peak, the proportion of each variant under the peak could not be ascertained but γ-caseins content are expected to be larger. κ-casein, which is the substrate that is converted to para-κ-casein during chymosin cleavage generally has a lower content than the other major casein variants. Thus, stoichiometrically, the content of its cleavage product should be proportional to

its original substrate. In addition, if para-κ-casein was produced by plasmin cleavage, micelle aggregation might not have occured. This is because β-casein, which also participates in micelle aggregation, would be cleaved resulting in the loss of hydrophobicity to the new peptide products. Thus β-casein would remain in solution. Some hydrophobic bitter peptides have been implicated to be the products of β- and α_s-caseins cleavage (20). The possibility that bitterness in cheese is associated with these cleavage products of β- and α_s-caseins in late lactation milk should be considered.

SUMMARY

Five good chymosin-coagulating, and four poor chymosin-coagulating individual cow milk samples were analyzed for casein composition using hydroxyapatite chromatography and polyacrylamide gel electrophoresis to establish possible relationships between casein variants and differences in coagulation properties.

The samples exhibited a wide variation in casein composition. The poor chymosin-coagulating milk had higher content of γ- and para-κ-caseins, lower κ- and β-caseins than the good-coagulating milks. A poor-coagulating milk sample had an additional casein variant, tentatively identified as λ-casein. Substantial peaks of unidentified minor protein were apparent in a poor-coagulating milk sample which coagulated early but the coagulum did not firm in 30 min. Less variability was observed in the α_s-casein of all the samples studied.

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PART 5. DETERIORATION OF CURD FIRMNESS OF CHYMOSIN COAGULATED COLD-STORED BOVINE MILK

INTRODUCTION

Refrigeration is widely used to preserve raw milk if it cannot be processed immediately. The disadvantages of temporary cold storage, depending on the length of storage time, have been established. They include selection and propagation of psychrotrophic bacteria (3) and slow disruption of casein micelles due to reversible loss of temperature sensitive β-casein into the serum phase (11). The technological significance of these include reduction of cheese yield due to poor curd development and a concomittant loss of a substantial amount of fat in whey (8, Appendix 3). Deterioration of product quality due to high retention of moisture in cheese is an additional possibility. Measurement of the correlation of loss of cheese curd firmness with cold storage of milk was the objective of this study.

METHODS AND PROCEDURES

Selection of milk samples

Twenty-five cows were randomly selected from each of two pens of mid- and late-lactation cows in the Utah State University Holstein herd. These stages of lactation were used because the widest variation in curd firmness was observed between them in an earlier study (7). Evening milk samples were collected from each cow in August, 1983. The samples were equilibriated at 4°C for 24 h to insure a uniform temperature history for all the samples. Ten milliliters of each sample was tempered at 37°C for 90 min and was then coagulated with 200 μL of 0.4 rennet unit (RU)/mL of chymosin (Chris Hansens Laboratories Inc, Milwaukee, WI) in a Formagraph (6). Milk with good chymosin-coagulation characteristics (GCM) and milk with poor chymosin-coagulation characteristics (PCM) were selected on the basis of curd firmness (8).

Cold storage and coagulation of milk samples

Aliquots of GCM and PCM samples were stored as follows: (a) 4°C with added .02% $CaCl_2$ for 24 h, (b) 4°C for 48 h, (c) 4°C with added .02% $CaCl_2$ for 48 h, (d) 4°C with added .02% $CaCl_2$ for 72 h, and (e) stored at -1°C for 30 days. These aliquots were coagulated at the end of each storage period with chymosin as described above and curd firmness was measured.

Two GCM and two PCM samples were separately blended in equal volumes. $CaCl_2$ (.02%) was added to some aliquots of the blends and all were stored for different times (24, 48 and 72 h) at $4^{o}C$ and were coagulated with chymosin.

RESULTS AND DISCUSSION

Effect of normal versus abnormal milk

Average curd firmness decreased after a 48 h cold-storage period (Table 11). PCM samples had more decrease (22%) than GCM (2.3%). One GCM sample (4532) showed an increase in curd firmness, and another GCM sample (5198) did not show any change in curd firmness at the end of 48 h storage. Larger decrease in curd firmness for PCM suggested possibly more proteolysis of casein (5,7,15). GCM samples also exhibited average decreases in curd firmness, but less than the decrease among PCM samples. Greater decrease would be expected if milk was stored for a longer time at $4^{o}C$ (11). Pasteurization of milk before cold-storage might not prevent loss of curd firmness since it has been shown to activate the plasmin group of proteinases (4). Nevertheless, pasteurization reduces bacterial count, and minimize bacteria-derived proteolytic enzyme activity and pH change.

The effect of calcium ions on maintaining the intergrity of casein micelles through the formation of $Ca_9(PO_4)_6$ linkages between submicelles has been shown to limit disruption of casein micelles (14). β-casein dissociates from micelles into milk serum primarily because of its hydrophobicity and changes in the salt equilibrium (13).

The effect of calcium ions on keeping the micelles intact and maintaining curd firmness is shown in Table 12.

Table 11. Curd firmness of chymosin-coagulated raw milk samples from individual cows. Samples were stored at 4°C for different times.

Cow ident. No.	Curd firmness at 30 min (mm): Stored for 24 h[a]	Stored for 48 h	%Decrease in curd firmness
	GOOD CHYMOSIN-COAGULATING MILK		
5270	56	51	8.9
5198	44	44	0
5330	41	39	4.9
5318	40	38	5.0
4532	40	45	12.5[b]
5012	37	35	5.4
	POOR CHYMOSIN-COAGULATING MILK		
58	33	31	6.1
4800	30	28	6.7
5186	19	12	36.8
5068	18	7	61.1

[a] Storage for 24 h served as the equilibriation period for the samples to have uniform temperature history.
[b] Curd firmness increased in this sample.

Table 12. Effect of .02% $CaCl_2$ on curd firmness of chymosin-coagulated raw milk samples from individual cows. Samples were stored at 4°C for different times after addition of .02% $CaCl_2$. Blended samples consisted of 50% each of milk with good chymosin-coagulation (GCM) and poor chymosin-coagulation (PCM) characteristics.

Sample identity	Curd firmness at 30 min (mm): Stored for 24 h	Stored for 48 h	Stored for 72 h
A (GCM)	57	35	36
B (GCM)	44	27	43
C (PCM)	30	10	17
D (PCM)	30	30	23
Blend (A+C)	38	27	16
Blend (A+C)[a]	40	44	42
Blend (B+D)	39	24	22
Blend (B+D)[a]	46	46	30
Mean	40.5	30.4	28.6
Std. dev.	±8.82	±11.53	±10.77

[a] .02% $CaCl_2$ added.

Mean curd firmness for all storage periods were different from each other. Mean curd firmness continued to decrease with increased storage time.

The blends which did not contain $CaCl_2$ continued to decrease in curd firmness as storage time increased (Table 12). Blends with added $CaCl_2$ showed less decrease. Since milk in bulk tanks are blends of milk from individual cows, it might be advantageous to add .02% $CaCl_2$ before storage if processing cannot commence immediately. At low temperatures the naturally occuring enzymes in milk are in higher concentrations in the serum phase than they are with the casein micelles (13). Thus, enzymes are more liable to cleave proteins in the milk serum than in intact micelles. Since β-casein is highly dissociated from micelles into serum phase at low temperatures, cleavage to γ-caseins is anticipated.

Technological implications

Larger decrease in curd firmness observed for PCM than GCM samples (Table 11) suggests high proteolytic activities in PCM samples. The extent of such proteolysis was partly arrested by adding .02% $CaCl_2$ to blend milk. Reimerdes (11,12,13) showed that 3 distinct proteolytic reactions occur in cold stored milks. First is the reversible disruption of casein micelles. This reaction can be counteracted by warming milk before chymosin coagulation (1). Secondly, the caseins are proteolyzed irreversibly by plasmin, a trypsin-like enzyme that converts β-, κ- and

-caseins (2) into products that have proved detrimental to curd formation (9). Thirdly, enzymes produced by psychrotrophic bacteria proteolyze milk proteins non-specifically into soluble short peptides which impart undesirable taste to processed dairy products.

Deterioration of curd firmness of GCM and PCM samples stored at -1^{o} C for 30 days is shown in Table 13. Loss of firmness occured as a result of prolonged storage. Significantly greater losses were observed among PCM than GCM samples. Reduced curd firmness at cutting causes loss of fat in whey hence reduced yield of cheese (5, Appendix 3).

Possible methods of preventing decreases in curd firmness

Inhibition of proteinase activity in cold-stored milks can be partly acheived by seeding milk with active lactic starter (10). Reduction of pH and natural competetion by lactic acid bacteria in such milk limit the growth of psychrotrophs. This technique, however, may not limit the activity of the plasmin group of proteinases. Plasmin activtiy could be inhibited by soybean trypsin inhibitor (4). Further research is needed to study the effect of a combination of factors including addition of $CaCl_2$, active lactic starter and trypsin inhibitor to cheesemilk if it is to be cold-stored for long periods before processing. Further research is also needed to quantify plasmin and bacterial proteinase activities in cold stored milk in order to find their significance to loss of curd firmness.

Table 13. Deterioration of curd firmness of raw milk samples with good- and poor chymosin-coagulation characteristics from individual cows. Samples were stored at -1°C for 30 days before chymosin-coagulation.

Cow ID	Curd firmness at 30 min (mm): Equilibrated for 24 h at 4 C	Stored for 30 days at -1 C	%Decrease in curd firmness
	GOOD CHYMOSIN-COAGULATING MILK		
5270	56	48	14.3
5326	56	51	8.9
5518	55	53	3.6
5484	55	53	3.6
Mean Std. dev.	55.5 ±.58	51.25 ±2.36	
	POOR CHYMOSIN-COAGULATING MILK		
5278	17	7	58.8
5186	19	1	94.7
5138	12	0	100
5448	18	13	27.7
Mean Std. dev.	16.5 ±3.11	5.25 ±6.02	

SUMMARY

Individual cow raw-milk samples were stored at 4°C for 24, 48 and 72 h with and without added .02% $CaCl_2$. The samples were coagulated with chymosin after storage.

Decrease in curd firmness was observed among the samples after cold-storage. After 48 h of storage poor-coagulating milk samples had more significant decrease in curd firmness (22%) than good-coagulating milk samples (2.3%). Individual samples with $CaCl_2$ showed interactions between storage times and losses in curd firmness. Blended GCM plus PCM samples that contained added $CaCl_2$ showed less deterioration in curd firmness than non-$CaCl_2$ containing counterparts after cold storage. Prolonged storage for 30 days at -1°C caused 68.2% and 7.6% losses in curd firmness for PCM and GCM respectively.

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PART 6. COAGULATION PROPERTIES OF ABNORMAL AND NORMAL MILK FROM INDIVIDUAL COW QUARTERS

INTRODUCTION

Wide variations in coagulation properties primarily due to differences in milk composition have been reported to occur between milk samples from individual cows (8). Samples drawn from individual quarters have also shown compositional difference if all 4 quarters were not normal (4). Normal milk is hereby defined as milk secreted by quarters of udders that have no pathological disorder as determined by somatic cell count (SCC). The Formagraph (Foss America Inc. Fishkill, NY) requires only 10 mL milk samples, and thus permits evaluation of coagulation properties of individual quarter samples. The variations that occured in coagulation properties of Jersey and Holstein milks drawn from normal and abnormal quarters of the same cows are reported in this study.

METHODS AND PROCEDURES

Collection of milk samples

Six hundred and twenty-six, evening, quarter, foremilk samples from 162 cows were asceptically collected from two Jersey (J1 and J2) and two Holstein (H1 and H2) herds in Cache county, UT. Data were collected on somatic cell count, conductivity, pH and bacterial flora (9).

Coagulation of milk samples

Each 10 mL milk sample was then coagulated with 200 μL of .16 rennin unit per mL of chymosin in the Formagraph (6). SCC data was used to separate normal from abnormal quarter milk samples. Maximum modulus of rigidity of gels formed from some paired normal and abormal quarter milk samples from the same cow was calculated with the formula proposed by Scott-Blair and Burnett (11). The data used were obtained by measuring rigidity moduli on the Formagraph tracings.

RESULTS AND DISCUSSION

Identification of normal and abnormal quarter milk samples was based on SCC (Table 14). Mean conductivity and pH values of these samples are also included in Table 14. Mean SCC for normal quarters from all herds were below 2 x 10^5 cells/mL. Five hundred thousand cells/mL was used to identify normal from abnormal samples. Conductivity data were lower for the normal quarters when compared to the abnormal quarters. Mean pH values for normal quarters was closer to the 6.6 mark for bulk milk. Abnormal samples had significantly higher pH than normal samples. One hundred samples were considered normal and 80 were classified abnormal from the 626 quarter samples collected. Cows with all four quarters normal were excluded.

Comparison of coagulation properties of milk from normal versus abnormal quarters

Mean coagulation times and curd firmness for normal and abnormal quarter samples are shown in Figures 18 and 19. Mean coagulation times were significantly longer for abnormal than normal samples (Figure 18). Abnormal Holstein samples generally had longer coagulation times than abnormal Jersey samples. Normal Holstein samples had longer coagulation times than normal Jersey samples in all herds.

Mean curd firmness for individual samples 30 min after chymosin addition are shown in Figure 19. Abnormal samples had lower curd firmness than normal samples in all herds. Abnormal Jersey samples had higher mean curd firmness

Table 14. Mean and standard deviation (SD) data of somatic cell count, conductivity and pH of normal (N) and abnormal (A) quarter foremilk samples from Holstein (H) and Jersey (J) herds.

Herd	Number of quarters	Milk quality	Somatic cell count ($\times 10^3$)	Conductivity[1]	pH
			(Mean/SD)		
H1	24	N	138 ± 161	1.5 ± .93	6.59 ± .08
	20	A	697 ± 1668	3.9 ± 4.4	6.66 ± .1
H2	33	N	69 ± 121	1.2 ± .92	6.63 ± .08
	31	A	322 ± 534	3 ± 5.1	6.74 ± .13
J1	26	N	157 ± 199	2.8 ± .85	6.63 ± .06
	17	A	475 ± 603	3.7 ± 1.46	6.74 ± .09
J2	16	N	173 ± 139	3.0 ± .73	6.65 ± .08
	13	A	5644 ± 4635	5.46 ± 1.98	6.78 ± .17

[1] Instrument readings from a Mas-D-Tec (9).

Figure 18. Mean coagulation time for normal (N) and abnormal (A) milk samples from individual quarters. H1 and H2 = Holstein herd samples. J1 and J2 = Jersey herd samples.

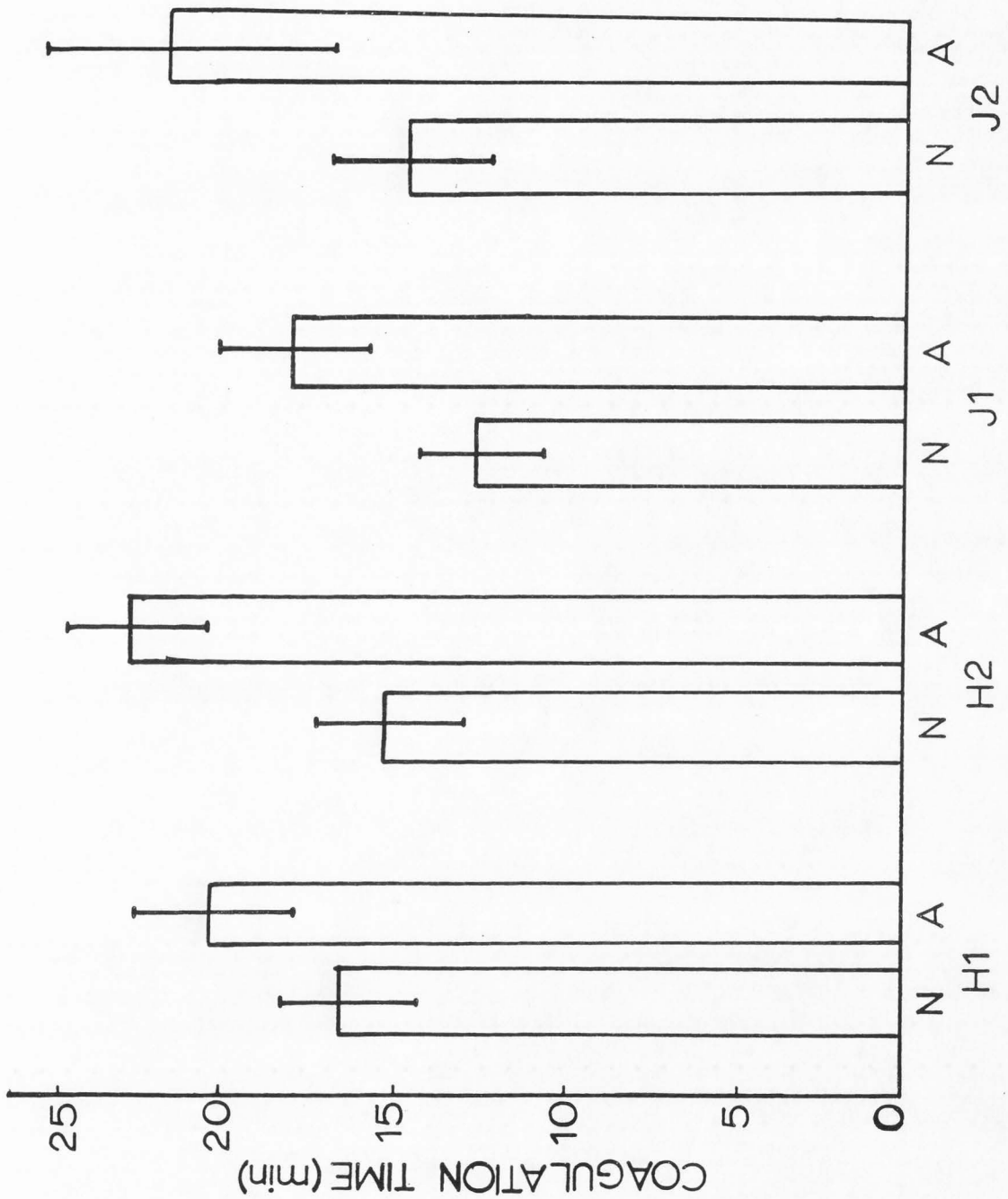
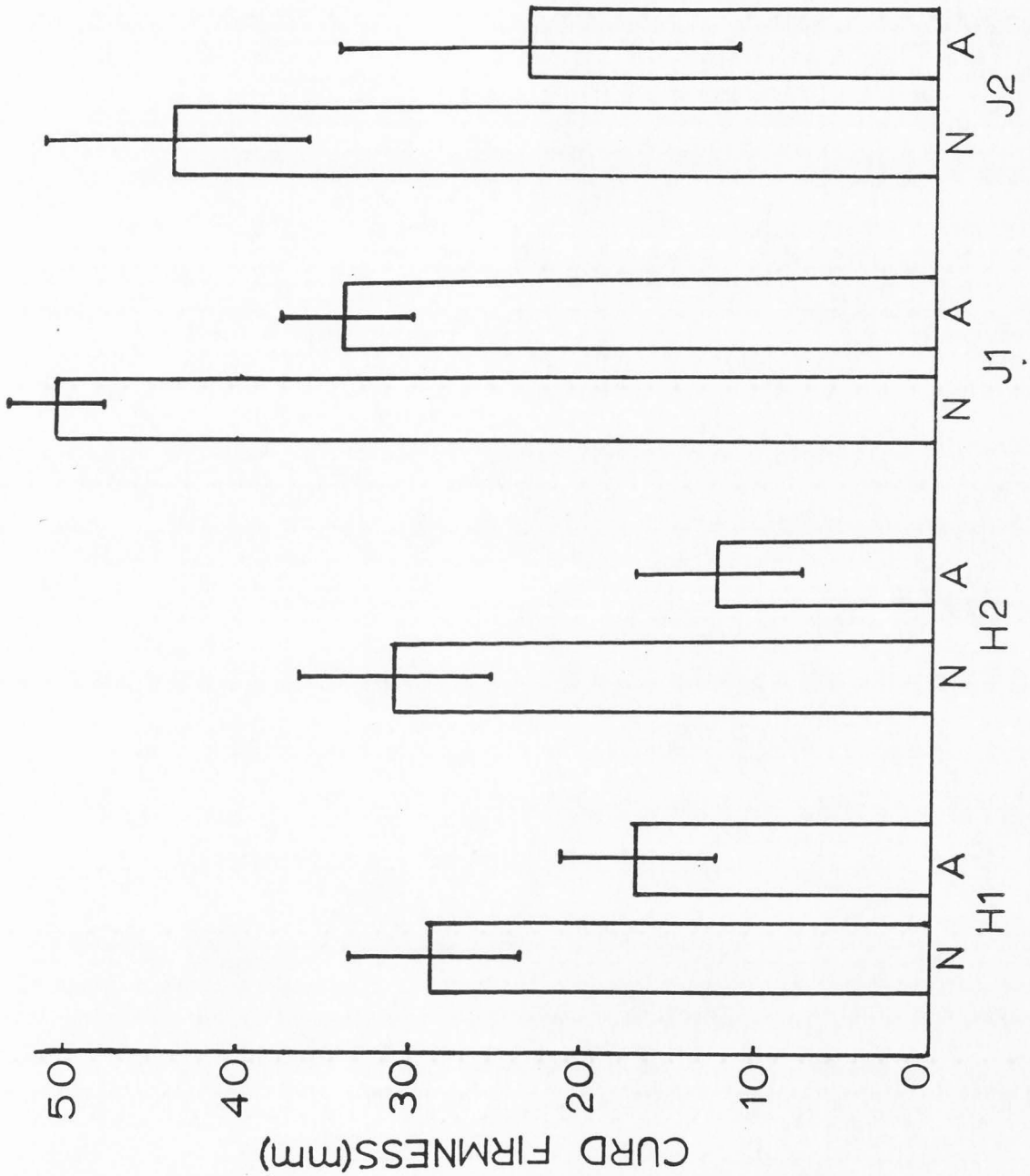

COAGULATION TIME (min)
0
5
10
15
20
25
N
A
H1
N
A
H2
N
A
J1
N
A
J2

Figure 19. Mean curd firmness 30 min after chymosin addition for normal (N) and abnormal (A) milk samples from individual quarters. H1 and H2 = Holstein herd samples. J1 and J2 = Jersey herd samples.


CURD FIRMNESS(mm)
0
10
20
30
40
50
N
A
H1
N
A
H2
N
A
J1
N
A
J2

than abnormal Holstein samples.

Long coagulation times and low curd firmness observed for abnormal samples confirm previous reports. Barry and Donnelly (4), and Andrews (2) showed that increased content of blood enzymes, the plasmin-like enzymes was responsible for cleavage of α_s-, β-, and κ-caseins (3). These caseins were converted to γ-caseins 1, 2, and 3, proteose peptones and unidentified minor proteins. Accumulation of cleavage products in composite milk samples from individual cows has been shown to be detrimental to coagulation of milk (10).

Cowside foremilk testing

Inclusion of milk from abnormal quarters in milk supplies could cause reduction of curd firmness of bulk milk supplies. This agrees with the findings of Ali et al. (1) who indicated that milk with high SCC had reduced yield of curd and made cheese of poor quality. The current practice of holding back milk from all four quarters of cows that have mastitis appears unnecessary. A better approach might be to withhold milk from quarters which show evidence of abnormality. Emphasis should be placed on quarter foremilk testing rather than testing composite samples. This would reduce losses in milk volume resulting from exclusion of milk from normal quarters. Cowside foremilk testing seems promising for achieving this objective (9, Appendix 4).

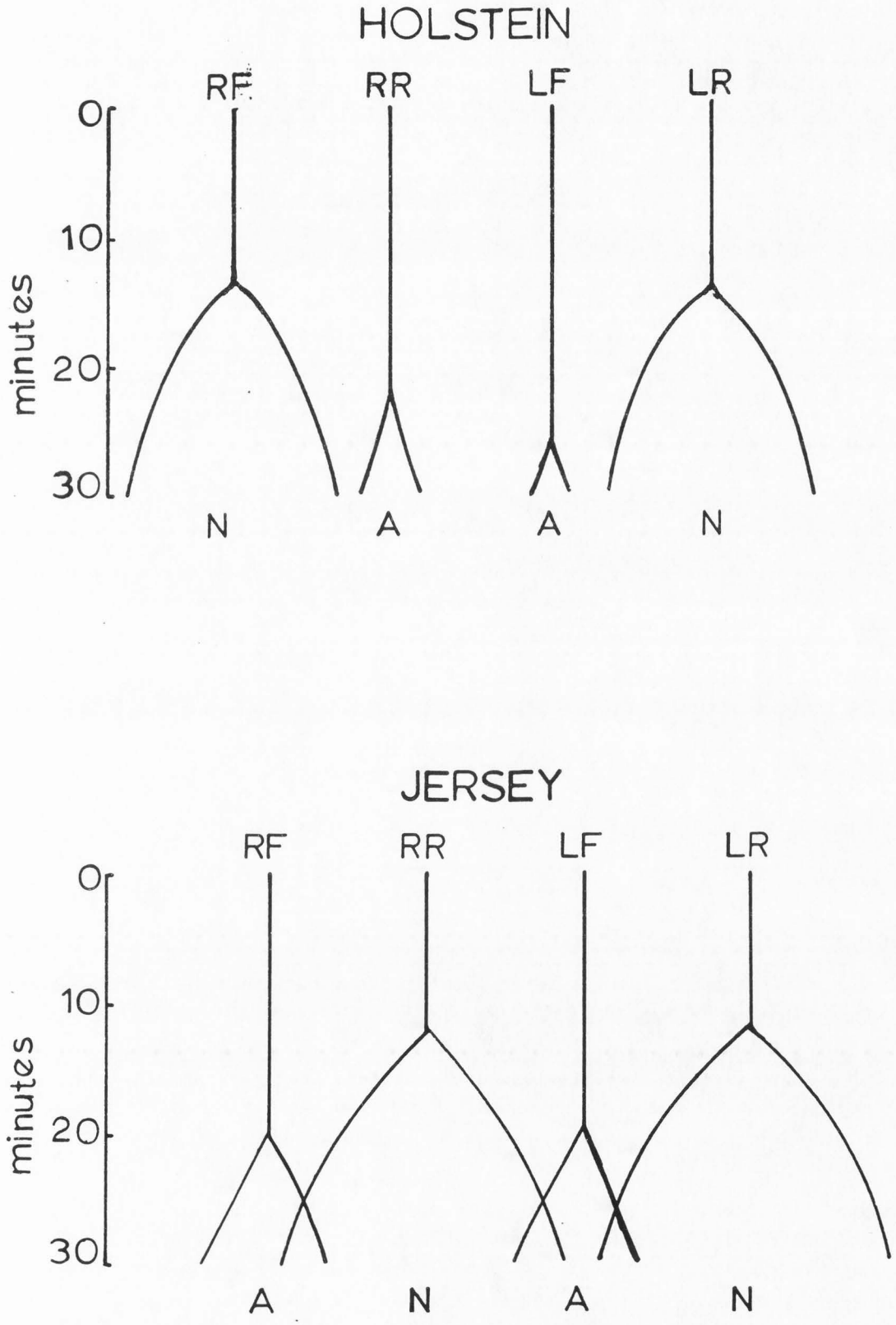
Higher mean curd firmness of Jersey samples as compared to Holstein samples supports the preference of Jersey milk for making some varieties of cheese (5,7). Mutzellburg et

al. (7) indicated that curd produced from milk of Jersey and Guernsey breeds shows little shattering during Cottage cheese manufacture while milk from other breeds, particularly Friesans, produce curd which shatter very easily. Thus cheese of better texture and increased yield will be produced with Jersey milk even if milk from abnormal quarters is included.

Typical Formagraph tracings of coagulating milk samples from normal and abnormal quarters from the same Holstein and Jersey animals are illustrated in Figure 20. Mean coagulation times for normal and abnormal Holstein quarter samples were 12.3 and 24.2 min; 11.8 and 19.8 min for Jersey normal and abnormal quarter samples repectively. Mean curd firmness 30 min after chymosin addition were 32.5 and 7 mm for normal and abnormal Holstein samples, and 46.5 and 20 mm for normal and abnormal Jersey samples respectively. Calculated mean maximum modulus of rigidity (G_{max}) for the normal and abnormal Holstein samples were 369 mm and 28 mm respectively. The values for the Jersey samples were 586 mm and 91 mm respectively.

Bacteriological analysis of quarter milk samples to establish the effect of degree of pathogenicity on coagulation properties showed that primary pathogens which included Streptococcus sp, coagulase positive Staphylococcus and Coliforms, and secondary pathogens which included Corynebacterium sp, coagulase negative Staphylococcus, Diplococcus, and Micrococcus were not significantly

Figure 20. Formagraph tracings of chymosin-coagulated paired normal and abnormal quarter milk samples from the same Holstein and Jersey cows. RF = right front, RR = right rear, LF = left front, and LR = left rear. N = normal, A = abnormal.


HOLSTEIN
RF
RR
LF
LR
0
10
20
30
minutes
N
A
A
N
JERSEY
RF
RR
LF
LR
0
10
20
30
minutes
A
N
A
N

different in altering coagulation time, in agreement with previous findings (Appendix 4), where primary and secondary pathogens were associated with normal and abnormal quarters. Quarter milk samples which contained primary, and secondary pathogens had mean coagulation times of 15.44 and 15.43 min respectively. Mean coagulation time was significantly different for quarter samples that contained no pathogens, 14.32 min. These samples contained Bacillus, Pseudomonas and also no bacteria. Curd firmness 30 min after chymosin addition was not significantly different for quarters that contained primary, and secondary pathogens, 36 and 35.57 mm respectively. Samples that did not contain pathogens had significantly higher mean curd firmness (39.7 mm) than the primary or secondary-pathogen containing samples.

SUMMARY

Individual quarter milk samples from two Jersey and two Holstein herds were tested for chymosin coagulation time and curd firmness in the Formagraph instrument. Normal and abnormal milk samples were identified using somatic cell count. Conductivity measurements and pH were determined.

Coagulation properties were significantly different for normal and abnormal milk samples drawn from individual quarters. Abnormal milk samples had longer coagulation times, weaker curd, and produced smaller maximum moduli of rigidity (G_{max}) than normal milk samples. Both normal and abnormal Jersey milk samples had shorter coagulation times and firmer curds than normal and abnormal Holstein milk. Milk samples that contained primary, and secondary bacterial pathogens were not significantly different from each other in coagulation properties, but were inferior to samples which contained no pathogens.

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PART 7. ESTIMATION OF SYNERESIS, AND CHEESE YIELD OF BOVINE MILK OF DIFFERENT CHYMOSIN COAGULATION PROPERTIES

INTRODUCTION

Numerous factors have been reported to affect milk coagulation, and curd firmness (1,4,9,10,25,29). The effect of curd firmness on cheese yield and quality, however, has not been fully evaluated. An early report (8) indicated that milk which forms a weak coagulum produces low cheese yield. They also indicated that such cheese has short shelf life as a result of high moisture retention, causing early development of off-flavors. Recent reports (2,3,15) support these observations (8) which relate high curd firmness with increased cheese yield. However, there are inconsistent observations on the relationship of curd firmness with syneresis and moisture retention. A CSIRO (3) annual report indicated that increased curd firmness causes high moisture retention in curd, but increases cheese yield primarily because of more entrapment of milk fat in curd. Storry et al. (26), in contradiction, observed that syneresis is inversely related to fat content of milk and is little affected by curd firmness. The observations of Bynum and Olson (2) also indicate that moisture content of cheese is not affected by curd firmness.

This report summarizes the effect of curd firmness on estimated syneresis, and yield of cheese made with bovine milk of good- and poor chymosin-coagulation characteristics.

METHODS AND PROCEDURES

Selection of milk samples

Fifty individual cow milk samples were obtained from the Utah State University Holstein herd in August 1983 at one sampling. Cows in mid- and late-lactation were selected because previous work (14) showed that mid-lactation cows gave milk that produced firmer curds on chymosin coagulation and late lactation cows gave soft curd milk. Ten milliliters of each sample was coagulated as described (15). Four best-coagulating samples (GCM) and four worst-coagulating samples (PCM) were selected based on curd firmness (15) and source cows were identified. Ten milliliters each of 4 GCM samples were pooled, and PCM samples were similarly treated. The pH of each pooled sample was measured at 37°C with a calibrated pH meter.

Estimation of syneresis

$CaCl_2$ (.02%) was added to some 10 mL aliquots of pooled GCM, and PCM. One percent each of active lactic starters, Streptococcus cremoris (UC 310) and S. cremoris (UC 77) was added to each aliquot, and all were tempered at 37°C for 90 min. Tempering also served as incubation period for lactic starters to enter their active growth phase. The pH of each sample was measured after tempering at 37°C, after which each sample was then coagulated in a Formagraph instrument (13) with 200 microliters of .4 rennin units (RU) per mL of chymosin. The coagulating milk samples were left

in the Formagraph for a total of 180 min during which the coagula syneresed.

Syneresis was estimated by continuous curd firmness measurements in the Formagraph and data obtained from the Formagraph's tracings at 30 min intervals from the time chymosin was added. Decrease in curd firmness that occurred with time of run was interpreted as syneresis. This decrease did not occur because of milk gel deformation by the Formagraph's pendulae, since reconstituted 10 and 14% nonfat dry milk coagulated with chymosin without starter culture did not undergo a similar loss of curd firmness with time.

Estimation of cheese yield

Cheese milk was obtained in October 1983 from each of the 8 identified cows which produced GCM, and PCM. GCM from 4 cows was pooled, and PCM was similarly treated. Prior to cheese making, aliquots of the GCM, and PCM were blended as follows:
(a) 100% PCM (b) 75% PCM + 25% GCM (c) 50% PCM + 50% GCM (d) 25% PCM + 75% GCM (e) 100% GCM. Duplicate sets of 5 blends were prepared. One set of blends had .02% $CaCl_2$ added. Protein and fat contents of milk blends were measured in the Utah Dairy Herd Improvement (D.H.I.A) laboratory with a Multispec Instrument (Multispec Inc, Newhall, CA). The pH of each blend was reduced to 6.3 with 1 to 3 drops of 2.1 N lactic acid solution (15). The blends were tempered at 37°C for 90 min during which shifts in pH were adjusted (15). The blends were then coagulated in the

Formagraph with 200 microliters of .2 RU/mL of chymosin. This concentration of chymosin was established to produce high curd firmness at pH 6.3 in GCM and PCM (15). Curd firmness of the blends are shown in Table 15. The same levels of chymosin, (.2 RU/mL), pH (6.3), temperature (37°C) and .02% $CaCl_2$ were then utilized in cheese making.

Cheese was made in 9 L laboratory cheese vats with five milk blends prepared as shown in Table 15. Each blend (7.5 L) was vat-pasteurized at 63°C for 30 min and .02% $CaCl_2$ was added. One percent each of lactic starters UC 310 and UC 77 was added. These had been separately incubated overnight in pH-controlled whey base medium with added stimulants (yeast extract and casein hydrolysate) was added. Each vat was tempered/ripened until milk pH was reduced to 6.3. One hundred and fifty milliliters of diluted (.2 RU/mL) chymosin (Chr Hansen, Milwaukee, WI.) was added. Each milk blend was well stirred and a 6 inch disc-diameter probe of the "Vatimer" (22), an instrument which measures milk coagulation in cheese vats was inserted. Each milk blend was left to coagulate under identical conditions at 37°C, and continuous curd firmness measurement was recorded till 30 min after chymosin addition. Traditional Cheddar cheese making procedures were then used. Cheese moisture was also determined (24).

Table 15. Curd firmness 30 min after chymosin addition of blended milk with good and poor chymosin-coagulation characteristics (GCM and PCM). Their pH was adjusted to 6.3 before coagulation in the Formagraph instrument.

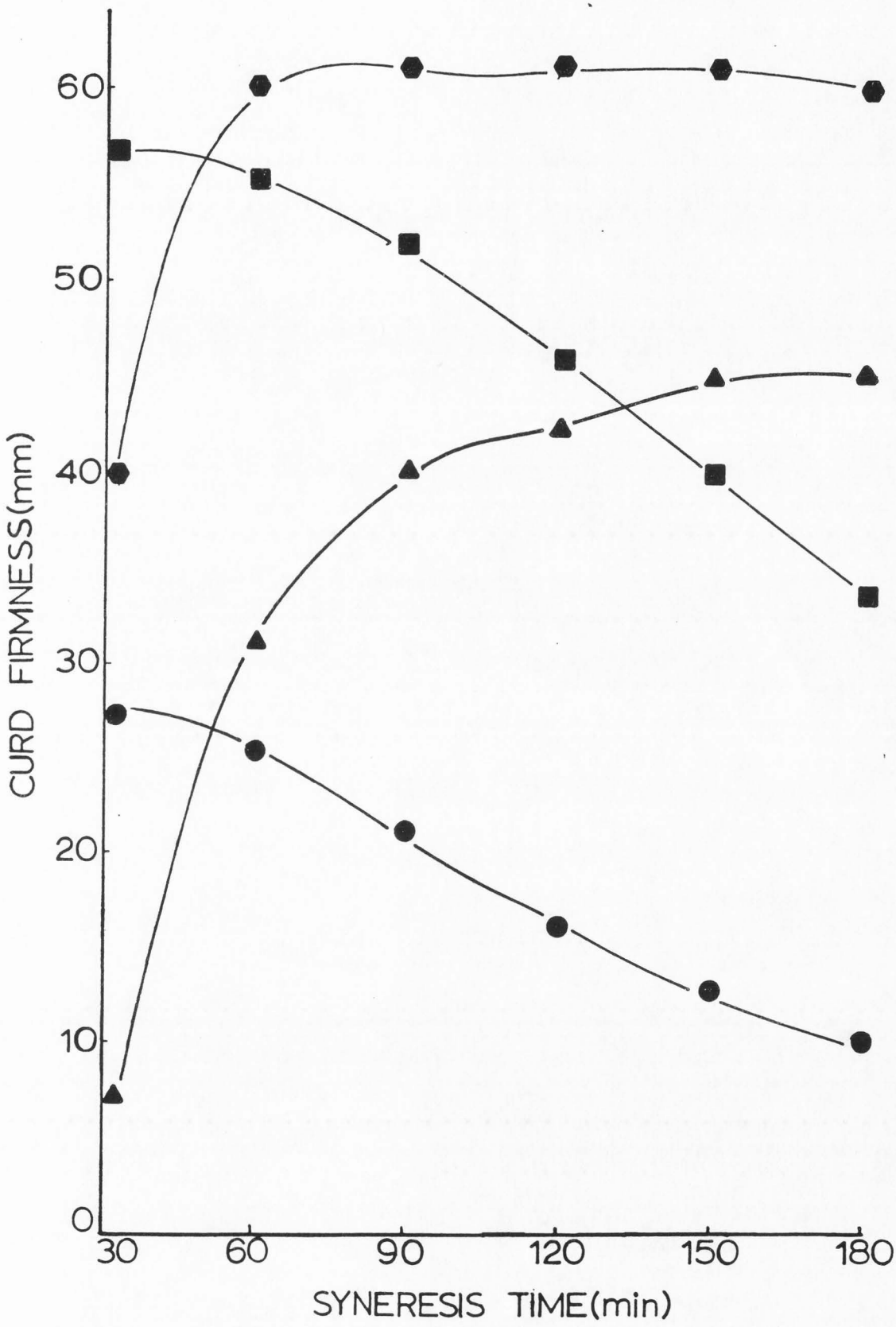
Milk blend	Curd firmness (mm): .02% $CaCl_2$ added	No $CaCl_2$ added
100% PCM	47	44
75% PCM + 25% GCM	49	47
50% PCM + 50% GCM	52	49
25% PCM + 75% GCM	49	50
100% GCM	54	52

RESULTS AND DISCUSSION

Estimation of syneresis

Syneresis rate was estimated from Formagraph tracings of chymosin-coagulated GCM and PCM as shown in Figure 21. Syneresis had a linear relationship with time, with correlation coefficients, r = .98 and .99 for GCM and PCM respectively. Curd shrinkage with exuded whey which partly collected in the interface of the Formagraph pendulae and curd, and partly in the interface of the curd and Formagraph cuvettes were observed. Curd firmness continued to decrease with progressive collection of whey in these interfaces. Reconstituted 10 and 14% nonfat dry milk that were not inoculated with lactic starter did not express whey in these interfaces, and a less apparent decrease in firmness occured later in the latter but not in the former. Expression of whey occurs as colloidal calcium phosphate is lost from rennet treated casein micelles in consequence to lowering of pH (19). Tuszynski et al. (27) had similarly estimated the softening of milk gels with thrombelastograph, and they also attributed it to "retraction" or syneresis. If oscillatory deformation technique is utilized, a continuous decrease in curd firmness with time should be expected in milk inoculated with lactic starter. Most continuous curd firmness testers utilize this principle of oscillatory deformation (7,19,22,23), and similar results would be anticipated for such instruments. Curd softening

Figure 21. Syneresis rate estimated from Formagraph tracings of chymosin-coagulating milk with lactic starter added (■ good, and ● poor chymosin-coagulating milk) ▲ 10%, and ● 14% reconstituted nonfat dry milk without starter.


CURD FIRMNESS(mm)
0
10
20
30
40
50
60
30
60
90
120
150
180
SYNERESIS TIME(min)

characteristically occurs at initial pH values of 6.02 to 5.94 (27). Higher rates of softening have been observed at pH 5.2 (27). Thus if milk is to be acidified with lactic starter before cheese making, the pH should not be reduced below 6.0 because curd produced by such milk will synerese poorly. Olson and Bottazzi (19) indicated that on chymosin coagulation, unacidified milk produced curd which continued to increase in firmness with time whereas samples that were acidified to pH 5.6 produced curd which either stabilized in firmness at 30 min or exhibited a decay. Since high losses of colloidal calcium phosphate occur from casein micelles at low pH (19,27), care must be taken to insure that pH reduction is optimal before cutting curd in order to enable development of high curd firmness. This would guarantee whey expulsion and minimize excessive cooking after cutting. The begining and final pH before coagulation and after syneresis were 6.22 and 5.11 for GCM; 6.51 and 5.55 for PCM after a 4 1/2 h incubation period with culture at 37^{o}C.

Addition of $CaCl_2$ to milk followed by equilibriation to counteract loss of colloidal calcium phosphate during syneresis was investigated (Figure 22). The results indicate that milk with added $CaCl_2$ syneresed better than milk without $CaCl_2$. Milk without $CaCl_2$ showed more curd fracture as observed from Formagraph tracings. Some reporters (11,12) have shown that excessive loss of colloidal calcium phosphate from casein micelles can cause defective body in cheese. Such cheese tend to be mealy. Observation of more

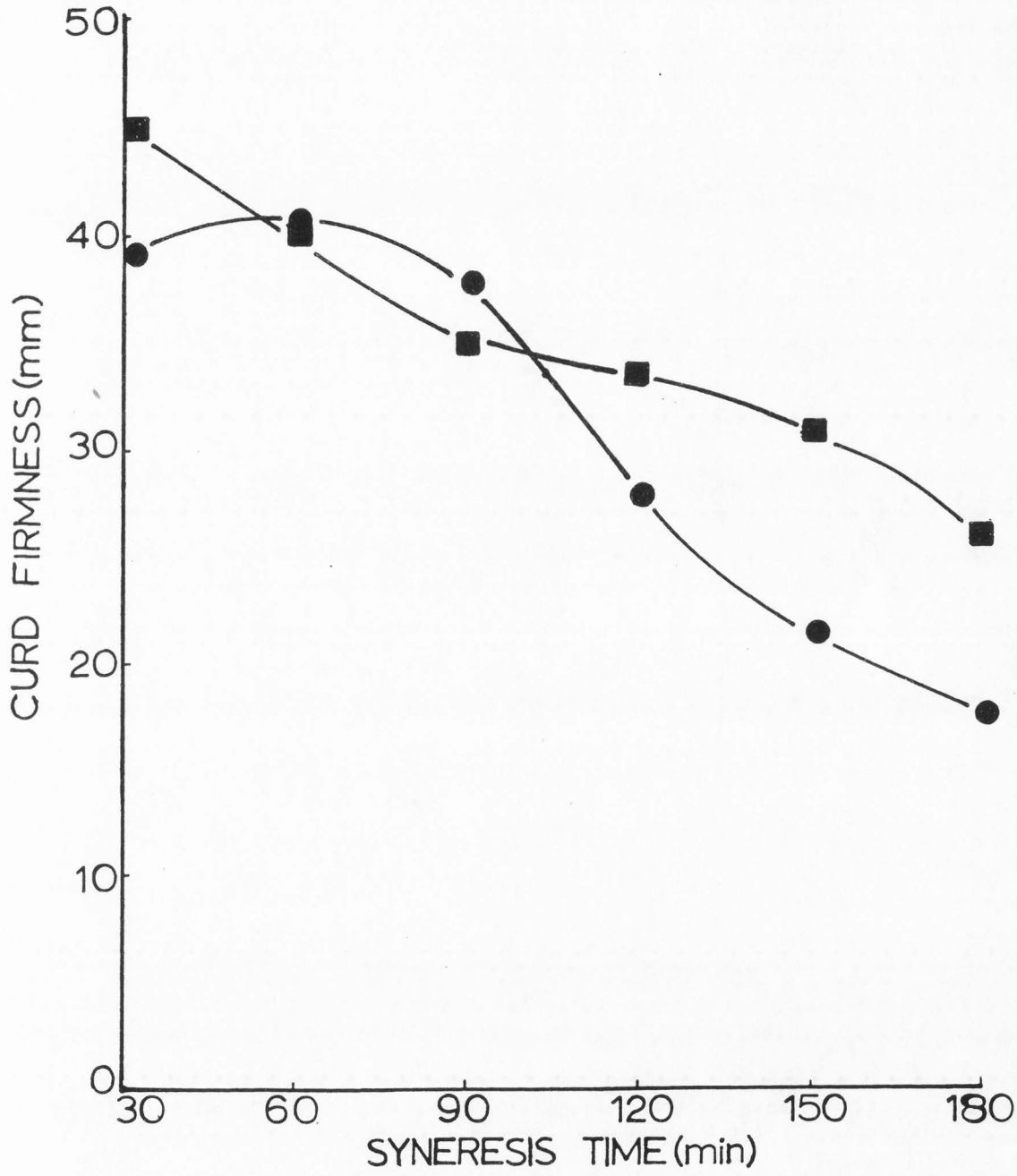


Figure 22. Effect of .02% $CaCl_2$ on syneresis rate of chymosin-coagulated milk with lactic starter (■ .02% $CaCl_2$ added, ● no added $CaCl_2$).

curd fracture in non-$CaCl_2$ containing samples confirms this assertion. Addition of .02% $CaCl_2$ to milk when coagulation properties of milk are poor will not only increase curd firmness at cutting, but will also improve syneresis of curd. Proper expulsion of whey from curd should be expected of milk which forms coagulum of high firmness because such curd would shrink when cut. PCM conversely will not shrink when cut. Improper coagulum formation by PCM has been attributed, primarily, to conversion of β-casein into cleavage products which include γ-caseins and proteose peptones probably by indigenous milk enzymes (16). These products were apparently absent in whey and therefore should be trapped in curd (16) together with milk fat globules. Improper bond formation including possible losses of hydrophobic interactions in such PCM caused poor curd formation and such curd could not syneresie. Hill and Merrill (8) attributed high moisture retention in cheese to such soft curded milk. Eventhough the pH of PCM was reduced from about 6.8 to 6.5 with lactic culture to permit chymosin cleavage of caseins, syneresis was still inhibited because partial loss of intact β-casein in such milk (16) would have, possibly, prevented the formation of adequate fibrous clots by casein micelles.

Estimation of cheese yield

Cheese yield estimates ranged from 8.3% for 100% PCM to 9.8% for 75% GCM + 25% PCM blend (Table 16). Mean cheese

Table 16. Variation in estimated cheese yield of coagulation-modified milk blends of different chymosin-coagulation properties.

Milk blend	Curd firmness[1]	Cheese moisture (%)	Cheese yield (%) (wet weight)	Cheese yield (%) (dry weight)
100% PCM[2]	50.7	59.2	8.32	3.39
75% PCM + 25% GCM[2]	45.8	41.6	9.61	5.61
50% PCM + 50% GCM	50.6	43.2	9.37	5.32
25% PCM + 75% GCM	54.3	40.0	9.79	5.87
100 GCM	50.7	41.4	8.94	5.24

[1] Instrument readings in millivolts from a "Vatimer" (23) 30 min after chymosin addition.
[2] PCM - poor chymosin coagulating milk
GCM - good chymosin coagulating milk.

yield value was 9.21% ± .59. This mean value agrees with 9.07% and 9.97% yield reported by Bynum and Olson (2) in 2 cheese plants which suggests that the laboratory method provided an unbiased estimate. Previous mean yield estimate for individual cow samples from the same herd (14) was 9.18%.

Linear relationships neither existed between cheese moisture and curd firmness (R^2 = 1.18E-14) nor cheese moisture and yield (R^2 = .16). A multiple linear regression model (R^2 = .98) related cheese moisture with curd firmness and cheese yield. The equation indicates that cheese moisture is a function of curd firmness and cheese yield combined and is shown as follows:

$$Y = a + .57X - .16Z \qquad (1)$$

where Y = cheese moisture (%), a = the intercept of the hyperplane on the Y-axis, X = cheese yield (%), Z = curd firmness at 30 min after chymosin addition. The equation indicates that cheese moisture was increased for each unit increase in cheese yield, and lowered for each unit increase in curd firmness. The effect of curd firmness on cheese moisture was, however, less than the effect of cheese yield as interpreted from the magnitudes of their coefficients in the regression equation. The results confirm that the relationship which exists between cheese moisture and curd firmness is nonlinear. Curd firmness should be considered together with other factors which affect cheese yield before its relationship with moisture can be deciphered.

The relationship between cheese moisture and other variables which significantly affect yield (milk protein, fat, and curd firmness) was then considered in a multiple regression model (R^2 = .77). The equation is

$$Y = a + 23.9W - 80.9X + 1.1Z \qquad (2)$$

where Y = cheese moisture (%), a = 251.3, the intercept of the hypothetical plane on Y-axis, W = milk fat (%), X = milk protein (%), and Z = curd firmness. This equation indicates that each unit increase in milk fat increased cheese moisture, each unit increase in milk protein decreased cheese moisture, and curd firmness in this model had insignificant effect on moisture. The equivalent regression model (R^2 = .63) in which "Vatimer" readings were replaced with Formagraph readings shown in Table 15, suggests that more dependable curd firmness measurements on cheese curd should be made in cheese vats, rather than outside it. Eventhough identical coagulation conditions were utilized, the Formagraph readings were not completely in phase with "Vatimer" readings. Insignificant effects of curd firmness on moisture (equation 2) was probably because of the modifications of the milk blends to optimize curd firmness in each blend. The range of curd firmness measured was therefore narrowed and allowed less variability. Insignificant effects of curd firmness on moisture in equation 2 agrees with Bynum and Olson (2) who reported that no correlation existed between curd firmness at cutting and Cheddar cheese moisture.

Elevated cheese moisture in GCM + PCM blends which were above the maximum allowed by federal regulations (39%) was probably due to the type of container the cheese samples were put during overnight press. Eventhough the containers were the same for every sample, they were different from conventional hoops. The samples were not large enough to put in normal 25 lb hoops. Overall the minimum moisture (40%) observed was not much greater than 39%. The results of Storry et al. (26) which related poor syneresis with increased milk fat agrees with our observations, but this relationship was not observed to be strictly linear. The effect of milk protein in decreasing cheese moisture was also not strictly linear.

Technological implications

Generally it is suggested that PCM be excluded from manufacturing milk. Eventhough that it was modified to produce curd ready to cut in 30 min after chymosin addition, subsequent syneresis after cutting was poor. It made cheese of reduced yield, high moisture, and very bitter flavor. Other authors (5,6,20,21) have established high proteolytic activities in late lactation milk. The cleavage products of such reactions similar to bitter peptides (28) would possibly, impart undesirable taste to finished dairy products. These cleavage products might be partly responsible for rapid deterioration of high moisture cheese made from soft curd milk as observed by Hill and Merrill (8). Significant quantities of casein cleavage products are

expected in bulk milk supplies containing substantial amounts of PCM. Some authors (6,21) have indicated that such products are also present in normal milk, for example, mid-lactation milk but in limited quantities. Higher concentration of such products accumilate with time as more plasminogen is activated and also by microbial activity (21,28). Deterioration of curd firmness of cold-stored bovine milk (17) has also been related to PCM (6). In bulk milk supplies these deteriorative effects are shared by GCM and are undesirable. PCM can be shunted for non-cheese uses depending on the extent of its inability to coagulate with chymosin. Periodic tests of individual cow's milk to detect poor-chymosin coagulation characteristics are recommended. Such tests would in addition detect abnormal milk since it is poor-coagualting with chymosin (18).

Exclusion of PCM from bulk milk supplies might be guaranteed by selective breeding of lactating cows. Some cows produced milk which exhibited this trait as early as in the 4^{th} month till the end of their lactation. This phenomenon was more apparent between the 8^{th} and 10^{th} month of lactation (14). Some other cows did not exhibit such a trait (14). Such cows in the latter category should be selected for breeding because such trait is, possibly, genetic eventhough it might be accentuated by environmental factors, especially, season and probably feed. Further research is needed to establish if genetic and environmental factors are related to the length of time which milk cannot

coagulate in late lactation. The presence of other mammary abnormalities such as mastitis should, however, be noted in such studies since they are likely to confound each other.

SUMMARY

Cows' milk with good, and poor chymosin-coagulation characteristics were inoculated with 2% active lactic culture with or without .02% added $CaCl_2$, and were coagulated with chymosin in the Formagraph instrument. Syneresis was estimated at 30 min intervals for 3 h from the Formagraph tracings. Poor-coagulating milk syneresed poorly when compared to good-coagulating milk, and syneresis rate was linear. Addition of $CaCl_2$ improved syneresis, and no-$CaCl_2$ containing milk showed more curd fracture.

Cheese was made in 9 L laboratory cheese vats with various proportions of blended, and coagulation-modified good-, and poor chymosin-coagulating milk. One hundred percent poor-coagulating milk blend produced cheese with significantly higher moisture, lower yield, and the most bitter flavor. A multiple linear regression model (R^2 = .98) showed that cheese moisture was neither linearly related to curd firmness nor to cheese yield. Increased milk protein caused decrease in cheese moisture while increased milk fat increased cheese moisture as were evident in a multiple linear regression model (R^2 = .77).

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GENERAL SUMMARY

A simple, rugged and sensitive instrument "Vatimer" was developed for monitoring milk coagulation in open or closed cheese vats. The instrument's probe is inserted in milk after adding chymosin and continuous measurements can be made on parameters of coagulating milk. It can detect coagulation time which closely approximates Formagraph coagulation time. Curd firming rate, curd firmness, curd syneresis and clean-in-place spray forces can be detected. The instrument fulfills the need for objective evaluation of curd firmness in cheese vats. Such evaluations minimize the variations which occur in curd firmness at cutting. Three to four fold differences in curd firmness were observed at different commercial Cheddar cheesemaking operations. These variations adversely affect yield and quality of cheese. The "Vatimer" can be used in monitoring curd development not only in American styled Cheddar cheese, but also in cottage and Swiss cheeses. Its utilization in monitoring curd development in ultra filtered milk retentate was also ascertained.

The quality of curd for Cheddar cheese making was improved by optimizing levels of factors which influence milk coagulation properties. These include temperature, pH, chymosin concentration and added calcium chloride. Addition of more than .02 rennin units of chymosin per mL milk in Cheddar cheese operations is not necessary for optimum curd

development 30 min after chymosin addition. Adding less chymosin prolongs time of cutting. Milk pH if reduced to at least 6.4 favors optimum curd development. Addition of .02% $CaCl_2$ to cheesemilk shortens coagulation time, and increases curd-firming but this addition is not necessary for optimum curd development 30 min after chymosin addition if milk pH is reduced to, at least, 6.4 at a chymosin concentration of .02 rennin units per mL milk and temperature of $37^{o}C$.

Individual Holstein cow milk samples with poor-, or no chymosin-coagulating charateristics showed interactions with pH and chymosin concentrations. While some poor chymosin-coagulating samples coagualted early at higher concentration of chymosin, their curd could not firm. Others which could not coagulate in 30 min at .02 rennin units per mL milk coagulated at higher chymosin concentration (.04 rennin units per mL milk) but the curd suffered a decay at 30 min after chymosin addition. Gel decay was minimized when chymosin concentration was reduced from .04 to .02 rennin unit/mL milk. Milk with good chymosin-coagulation characteristics did not exhibit gel decay at higher chymosin concentrations indicating interaction between chymosin and casein quality.

Casein quality was found to be a critical variable that affects coagulum development. Individual Holstein cow milk samples which were different in chymosin-coagulation properties showed wide variations in casein composition.

Samples with poor chymosin-coagulation characteristics had higher levels of γ- and para-κ-caseins, lower β - and α_s-casein concentrations, and slightly reduced κ-casein than samples with good chymosin-coagulation characteristics. High content of unidentified minor casein was observed in a sample that coagulated early but its curd never firmed at 30 min after chymosin addition. A sample which could not coagulate in 30 min had highly reduced content of α_s -casein and in addition, had a casein variant tentatively identified as λ-casein. Loss of hydrophobic interactions in samples with reduced β-casein was postulated as the primary reason for poor coagulum development in samples with poor chymosin-coagulation characteristics.

Cold storage at 4°C of individual cow raw milk samples of different chymosin-coagulation properties caused variations in losses of curd firmness. Generally samples with good chymosin-coagulation characteristics tended to retain their coagulum forming ability while the samples with poor chymosin-coagulation characteristics exhibited apparent losses in curd firmness with storage. Addition of .02% $CaCl_2$ tended to minimize losses of curd firmness with storage. It was postulated that losses of curd firmness in poor chymosin-coagulating milk with cold-storage was due to continued plasmin proteolysis of caseins, and also proteolytic activities of psychrotrophs. Addition of $CaCl_2$ before cold-storage of milk was also postulated to help

maintain intergrity of casein micelles through formation of calcium phosphate bridges between submicelles, and less β-casein was converted to the soluble phase where proteolytic activity is usually higher. Seeding cheese milk with lactic starters, addition of trypsin inhibitors and $CaCl_2$ to milk or a combination of these variables might extend cold storage life of manufacturing milk.

Individual quarter milk samples from Jersey and Holstein cows showed significant differences in coagulation properties on chymosin coagulation. Coagulation properties were also different for normal and abnormal samples. Abnormal milk had longer coagulation time, weaker curd and produced a smaller maximum modulus of rigidity (G_{max}) than normal milk. Both normal and abnormal Jersey milk had shorter coagulation times and firmer curds than abnormal and normal Holstein milk samples. Inclusion of abnormal milk in bulk milk supplies can cause apparent losses in curd firmness on chymosin-coagulation due to higher proteolysis of casein by plasmin, leucocytic and bacterial proteinases during storage. Such losses can be minimized by cowside quarter foremilk testing with a portable conductivity meter, Mas-D-Tec whose results correlate with lactose content, and the California Mastitis Test. Testing composite milk samples could be misleading since it produces average results for all four quarters.

Laboratory scale Cheddar cheese manufactured with different blends of coagulation-modified milk with good, and

poor chymosin-coagulation characteristics showed that 100% poor-coagulating milk produced cheese with higher moisture, lower yield and bitter flavor. Poor coagulum development primarily due to loss of intact β-casein was postulated to be the cause of poor syneresis in such cheese causing high cheese moisture. Low fat entrapment in curd due to improper matrix formation by chymosin-treated casein was the most probable cause of low yield of cheese made with such milk. Severe bitterness observed in cheese made with 100% poor chymosin-coagulating milk might be related to short bitter peptides that are usually associated with proteolysis of hydrophobic β-casein.

Linear relationships neither existed between cheese moisture and curd firmness nor cheese moisture and yield. A multiple linear regression model (R^2 = .98) related cheese moisture with curd firmness and cheese yield. This implies that cheese moisture is a function of curd firmness and cheese yield combined. Regression equation (R^2 = .77) that related cheese moisture with milk fat, milk protein, and curd firmness, showed that each unit increase in milk fat increases cheese moisture, while each unit increase in milk protein decreases cheese moisture. Curd firmness in this regression model model has insignificant effect on moisture.

Periodic tests of individual cow's milk to detect poor chymosin-coagulation characteristics are recommended. Poor chymosin-coagulating milk could be shunted for non-cheese uses depending on the extent of its inability to coagulate

with chymosin. Exclusion of poor chymosin-coagulating milk from bulk supplies is desirable and can be acheived by selective breeding of lactating cows. Cows which produce milk that coagulate with chymosin through their entire lactation will, possibly, give offsprings with similar genetic characteristics.

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APPENDICES

Appendix 1.

Table 17. Effect of chymosin concentration on coagulation properties of Berridge substrate at pH 6.3. Curd firmness was determined 30 min after chymosin addition.

Chymosin concentration (RU/mL)	Coagulation time (min)	Curd firmness (mm)
0.2	3.9	51.5
0.3	2.4	51
0.4	1.8	50.5
0.5	1.3	47.3

Appendix 2

Table 18. Means of factors that caused variations in curd firmness of milk samples from individual cows. Curd firmness was estimated at cutting 5 h after addition of 2% lactic starter, and .016 RU/mL milk and samples incubated at 37°C.

Lactation stage (months)	Milk lactose (%)	Initial pH	Final pH	Delta pH	Milk casein (%)	Milk protein (%)	Milk yield (lb/ cow/ day)
1 - 3	4.96	6.65	5.52	1.13	2.61	3.35	81.6
4 - 7	4.87	6.75	5.48	1.27	2.6	3.02	58.4
8 - 10	4.45	6.82	5.59	1.23	2.88	3.38	34.9
>10[a]	4.51	6.71	5.51	1.2	3.11	3.61	28
			OVERALL MEANS OF FACTORS				
6.6	4.73	6.73	5.51	1.22	2.77	3.28	52.7

[a] Includes cows which were not inseminated at the normal period because they were manipulated for increased milk production by the herd management.

Initial milk pH was highest (6.82) for normal late lactation milk. This pH is not favorable for chymosin activity, since chymosin cleavage of caseins has been observed to occur very slowly at pH 6.7 and greater. Final pH after incubation was highest for normal late lactation milk. Increased lactic starter activity is required in such milk if cheese of acceptable acidity and normal composition is to be made. Casein content was also high for late lactation milk. If pH is not adequately reduced, casein will not be cleaved sufficiently and so will not participate in curd formation.

Appendix 3

Table 19. Factorial analysis of variance of curd firmness of milk samples from individual cows. Curd firmness was estimated at cutting 5 h after addition of 2% lactic starter and chymosin, and samples incubated at 37°C.

Source	DF	SS	F
Milk fat	1	5.51	2.75
Milk casein	1	9.4	4.69*[1]
Milk lactose	1	6.88	3.44**
Initial pH	1	8.17	4.08*
Final pH	1	.1	.05
Lactation stage	3	2.08	.35
Lactation number	4	15.56	1.94
Whey fat	1	37.71	18.83***
Error	27	54.07	
Corrected total	40	214.39	

[1]*Significant.
**alpha = .04
***alpha = .08
alpha = .0002

Curd firmness was most correlated (r = -.6) with whey fat and its effect was highly significant in causing variation in whey fat. Thus, less fat was lost in whey when curd firmness was higher. In practice higher curd firmness would cause higher cheese yield because of greater entrapment of fat in curd. Higher curd firmness can be produced if milk pH is reduced. Milk pH can be reduced by increasing starter activity. Casein content of milk significantly affects curd

Appendix 3 (Continued)

firmness. The effect of lactose was significant in causing variation in curd firmness because lactose is an indicator of physiological normality of the udder. Thus milk with higher lactose content produced firmer curd because of the higher quality of the milk. Lactose itself does not affect curd firmness.

Appendix 3 (Continued).

Table 20. Factorial analysis of variance of estimated cheese solids from individual cow milk samples. Estimation was by difference between milk solids and whey solids.

Source	DF	SS	F
Milk fat	1	22.83	68.27***[1]
Milk casein	1	3.15	9.42**
Curd firmness	1	.08	.23
Initial pH	1	.05	.15
Final pH	1	1.87	5.58*
Lactation stage	3	1.15	1.15
Error	53	17.72	
Corrected total	61	74.72	

[1] Significant
*** alpha = .0001
** alpha = .003
* alpha = .02

The effect of curd firmness in causing variations in cheese solids was not significant because its effect on retention of milk fat was isolated. Thus the effect of milk fat on cheese solids was highly significant. This suggests that it is not the direct effect of increased curd firmness that increases yield but the high retention of milk fat by it. Less significance of protein in causing variation in cheese solids suggests that each unit of milk fat alters cheese yield more significantly than each unit of milk protein.

Appendix 3 (Continued)

Final pH had less significant effect in causing variations in cheese solids.

Appendix 4

Table 21. Correlation coefficients between conductivity and other parameters used for detecting abnormal milk. Quarter foremilk samples were used for the analyses.

Herd	Breed	Lactose	Somatic cells	pH	Total count
		(Correlation coefficient)			
		(Level of significance)#			
1	Holstein	-.6615	.3029	.0863	.1664
		.0001	.0001	.2338	.0914
2	Holstein	-.6543	.3654	.4213	.0617
		.0001	.0001	.0001	.3868
3	Jersey	-.6035	.3343	.2277	.1063
		.0001	.0001	.0016	.1444
4	Jersey	-.7303	.8223	.8034	.4542
		.0001	.0001	.0001	.0017

#Probability

The 4th herd showed the highest correlation coefficients and had samples with substantial evidence of abnormality. This herd exhibited a wide variation in milk abnormality and had the highest test sample with a somatic cell count of 14 million, conductivity of 9 and pH of 7.2.

Appendix 4 (Continued)

Table 22. Classification of quarter foremilk samples for abnormality according to pathogenic bacterial group isolated. Conductivity, log somatic cell count and lactose were combined as classification variables in a discriminant analysis.

Bacterial group	Cluster 1	Cluster 2	Cluster 3	Frequency of occurence
	(percent)			
Streptococcus	24	29	47	7
Staphylococcus (coagulase +)	19	27	54	7
Coliforms	0	0	100	1
Staphylococcus (coagulase -)	27	46	27	50
Corynebacterium	38	30	32	18
None[1]	41	26	33	18

[1]Includes no bacteria isolated, Bacillus and Pseudomonas sp.

The bacterial groups most commonly associated with intramammary infections, Streptococcus sp and coagulase positive Staphylococcus were consistent with the definition, eventhough they were reasonably associated with normal quarters. Previous workers suggested that a large proportion of infections detected by standard bacteriological methods are teat canal infections and that these infections caused no pathological changes in the cow. The absence of pathologic changes indicates that the composition of milk from such udders is normal. Generally erroneous conclusions

Appendix 4 (Continued)

would be drawn about the infection status of a quarter if only the genus or group bacteria isolated from it was considered.

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