

July 16, 1957

To the Graduate Council:

I am submitting herewith a thesis written by Bobby Ross Mauk entitled "A Study of Some Factors Influencing the Rennet Curd Tension of Milk and Casein Sols." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

B. J. Bennett

We have read this thesis
and recommend its acceptance:

Thos B. Hansen

C. B. Gyle

W. M. Overcast

Accepted for the Council:

Walter Hantling
Dean of the Graduate School

A STUDY OF SOME FACTORS INFLUENCING THE RENNET CURD
TENSION OF MILK AND CASEIN SOLS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

by

Bobby Ross Mauk

August 1957

2nd
33

ACKNOWLEDGEMENTS

The author wishes to take this opportunity to express his sincere appreciation to Dr. T. W. Albrecht for his advice and assistance in beginning this work and to Dr. B. J. Demott for his guidance in the preparation of this manuscript. The appreciation of the writer is also extended to Dr. W. W. Overcast for his many helpful suggestions throughout the course of this study.

TABLE OF CONTENTS

	PAGE
INTRODUCTION.	1
REVIEW OF LITERATURE.	2
Properties of rennin	2
Theories of rennin coagulation	3
Factors affecting rennet coagulation time and curd tension .	9
EXPERIMENTAL PROCEDURE.	27
Rennet curd tension measurements	27
Preparation of casein.	30
Preparation of whey protein.	31
Preparation of salt solutions.	32
Composition of casein sols	32
Blending	33
RESULTS	36
Influence of homogenization and pasteurization upon the rennet curd tension of whole milk.	36
Influence of heating and cool-aging upon the rennet curd tension of skimmilk.	36
Influence of heating and cool-aging upon the pH of skimmilk.	40
Casein and whey protein preparations	42
Influence of sodium chloride and heat treatment upon the rennet curd tension and pH of 3% casein sols	42
Influence of sodium citrate and heat treatment upon the rennet curd tension and pH of 3% casein sols	43

RESULTS (continued)

Influence of tribasic sodium phosphate and heat treatment upon the rennet curd tension and pH of 3% casein sols. . .	46
Influence of magnesium sulfate and heat treatment upon the rennet curd tension and pH of 3% casein sols	48
Influence of whey protein and heat treatment upon the rennet curd tension and pH of 3% casein sols	50
Influence of whey protein, heat treatment and added salts upon the pH and rennet curd tension of 3% casein sols. . .	50
DISCUSSION.	63
SUMMARY	69
BIBLIOGRAPHY.	71

LIST OF TABLES

TABLE	PAGE
I. The Composition of 3 Per Cent Casein Sols Containing Added Salts and Whey Protein at the Rate of 5 Per Cent of the Weight of the Casein	34
II. Rennet Curd Tension of Raw, Heated and Homogenized Whole Milk	37
III. Influence of Heating (62° C. for 30 Minutes) and Cool- Aging (2° C.) Upon the Rennet Curd Tension of Skimmilk .	38
IV. Influence of Heating (70° C. for 30 Minutes) and Cool- Aging (2° C.) Upon the Rennet Curd Tension of Skimmilk .	39
V. Influence of Heating and Cool-Aging Upon the pH of Skim- milk	41
VI. Influence of Sodium Chloride and Heat Treatment Upon the Rennet Curd Tension and pH of 3 Per Cent Casein Sols . .	44
VII. Influence of Sodium Citrate and Heat Treatment Upon the Rennet Curd Tension and pH of 3 Per Cent Casein Sols . .	45
VIII. Influence of Tribasic Sodium Phosphate and Heat Treatment Upon the Rennet Curd Tension and pH of 3 Per Cent Casein Sols	47
IX. Influence of Magnesium Sulfate and Heat Treatment Upon the Rennet Curd Tension and pH of 3 Per Cent Casein Sols . .	49
X. Influence of Added Whey Protein (Dry Basis) and Heat Treat- ment Upon the Rennet Curd Tension and pH of 3 Per Cent Casein Sols.	51

TABLE

PAGE

XI.	Influence of Added Whey Protein at the Rate of 5 Per Cent of the Weight of the Casein (Dry Basis), Heat Treatment and Added Salts Upon the pH and Rennet Curd Tension of 3 Per Cent Casein Sols	52
XII.	Influence of Added Whey Protein at the Rate of 10 Per Cent of the Weight of the Casein (Dry Basis), Heat Treatment and Added Salts Upon the pH and Rennet Curd Tension of 3 Per Cent Casein Sols	54
XIII.	Influence of Added Whey Protein at the Rate of 5 Per Cent of the Weight of the Casein (Dry Basis), Heat Treatment and Added Salts Upon the pH and Rennet Curd Tension of 3 Per Cent Casein Sols	56
XIV.	Influence of Added Whey Protein at the Rate of 10 Per Cent of the Weight of the Casein (Dry Basis), Heat Treatment and Added Salts Upon the pH and Rennet Curd Tension of 3 Per Cent Casein Sols	58



LIST OF FIGURES

FIGURE	PAGE
1. Cherry-Burrell Corporation Curd Tension Meter.	28

INTRODUCTION

The physical character of the curd of milk is of primary importance with reference to the quality, texture and body of some dairy products. Curd tension measurements may indicate the chemical and physical conditions of certain proteins in milk and aid in determining the curd making properties of casein.

Much research and investigation has been devoted to the use of the curd tension test as a measure of the curd forming properties of milk. The test has been important in the study of soft curd milk for infant feeding and has indicated to some extent the efficiency of homogenization of milk.

Recent studies reported by Merrill (41) have shown that the Hill (4) curd tension test (with pepsin-hydrochloric acid coagulant) is capable of predicting the cottage cheese making qualities of nonfat dry milk solids. The rennet curd tension test (14) was found to be more reliable than the Hill curd tension test for indicating heat treatments of skimmilks and nonfat dry milk solids. Comparison of test results (14) showed that the rennet curd tension test is as reliable as the Harland-Ashworth (24) method for determining the heat treatments.

With this information at hand the rennet curd tension test has been used in this study to evaluate the influence of certain factors affecting the curd firmness of milks and caseinate sols.

REVIEW OF LITERATURE

Properties of Rennin

Rennin is the hydrolytic clotting enzyme which is secreted from the inner lining of the fourth stomach (abomasum) of young calves and lambs. It is also known by the general name "chymase" (15). Rennin is used commercially in the form of rennet extract to produce coagulation of milk in various cheese making processes. Davies (15) states that the mucosa of calves contain a small amount of pepsin-like proteinase which is also present in the final rennet extract.

Rennin is an extremely powerful enzyme but a method for measuring its primary activity, conversion of casein to paracasein, has not been devised (33). Tauber and Kleiner (60) report that one part of highly purified rennin coagulates 4 to 5 million parts of milk in less than 15 minutes. Kelley (33) states that the activity values reported in the literature are based upon arbitrarily selected conditions with respect to time, temperature, pH, added calcium salts and others but serve to demonstrate the extreme biocatalytic potency of rennin.

Davies (15) states that the optimum temperature for rennin action is 40° to 42° C. and that the rate of coagulation decreases on either side with no coagulation above 60° to 65° C. or below 10° to 15° C. The temperature of heat inactivation depends upon the pH and the concentration of the enzyme. It is more heat stable in concentrated and neutral solutions.

Sommer (57) states that rennin is irreversibly inactivated by alkaline reactions, usually above pH 6.9. Also, rennin is partially

deactivated by vigorous shaking, "apparently because of the coagulation and the resultant denaturation when rennin forms a concentrated adsorption film in the foam." The enzyme is also sensitive to light and elevated temperatures and therefore is stored in opaque jugs or kegs at low temperatures.

Dilute rennet extract may lose its strength rapidly and is more susceptible to light, heat and bacterial deactivation than concentrated rennet extract (33).

Pepsin and rennin were considered to be identical for many years. Both enzymes are proteins and have high milk clotting properties. Pepsin has much higher proteolytic power than rennin. Davies (15) reports that pepsin has an optimum pH near 2.75 while rennin has been reported most active at pH 5.4, and also in the range of pH 6.0 to 6.5, with the latter value most widely accepted. The enzyme is quickly destroyed at pH 9.0. The crystallization of pepsin by Northrop (44) in 1929 and the crystallization of rennin by Hankinson (23) in 1943 has established the identity of these two enzymes.

Theories of Rennin Coagulation

The principal method of coagulating milk in the making of cheese is by the action of rennet. There have been a number of theories developed to explain the action of rennin on milk. Unfortunately, none of the known theories are fully adequate or universally accepted. A theory to be adequate must be in harmony with all the well-known facts of the nature of rennin and casein. It is generally accepted that

clotting consists of two stages and that the enzyme plays a part only in the first stage. According to Kelley (33), the first stage involves the adsorption of the enzyme on the casein particle and a subsequent change in the state of the casein. In the second stage the changed casein (paracasein) precipitates as a calcium salt or complex. The loss of suspension stability of the colloidal calcium caseinate causes coagulation. Berridge (6) suggested that the second phase of the coagulation of milk by rennin is a denaturation (unfolding and linking of polypeptide chains) or partial denaturation of the casein, which has been rendered heat sensitive by the enzyme.

Kelley (33) states that when rennin acts on casein it changes it to soluble paracasein and in the presence of a necessary quantity of soluble calcium ions the insoluble gel calcium paracaseinate precipitates. Without agitation a continuous gel structure is formed. He also states that an ample concentration of paracasein, a suitable pH, favorable ion concentrations and no agitation are essential for the separation of paracasein as a continuous gel.

The concept of the physical nature of casein, provided by Rogers (51), was that a colloidal calcium phosphate sol is protected by a calcium caseinate sol in a manner not well understood. Calcium caseinate is probably calcium phospho-caseinate. He suggested that the secondary and tertiary hydrogens of the orthophosphoric acid esterified with certain amino acids react with calcium. The work of van der Burg (62) supports this view. He concluded that only the free amino and carboxyl groups are left as centers of charge for colloidal calcium and

phosphate, and that the colloidal calcium and phosphate are split off from the casein by heating.

The chemical nature of casein has been studied in great detail by many scientists. Much work was required before casein was successfully fractionated. Cherbuliez and Baudet (11) employed a procedure which yielded four fractions which were designated α , β , γ , and δ and were found to appear in 60, 25, 10, and 5% proportions, respectively. Cherbuliez and Baudet (12) used the fractionation procedure of Warner and obtained pure α_1 and pure α_2 fractions. The α fraction was found to be the only one which gave a normal clot with rennin (12, 47). Pyne (47) reported that a mixture of α - and β -caseinates required a higher calcium concentration to ensure rennet coagulation than is needed for α -casein alone where renneting is carried out below 20° to 25° C. Calcium β -caseinate apparently protects the rennet altered α -caseinate.

Kelley (33) stated that casein is the only constituent of milk altered by rennin action; however, there appeared to be no pronounced physical-chemical changes occurring when casein is converted to paracasein. He also stated that casein once clotted with rennet cannot be reprecipitated by the enzyme. Sommer (57) states, on the other hand, that when a paracasein sol is exposed to the pH and salts as in milk, the paracasein precipitates.

Wright (69) studied the optical rotation properties of casein and paracasein in the presence of alkali and found the resulting racemization curves to be coincident. He concluded that no radical

changes occur within the casein molecule. This is in harmony with the knowledge of the action of other enzymes. Wright also postulated that a colloidal phenomena was responsible for the transformation of casein to paracasein. Calcium caseinate and calcium paracaseinate both belong to the class of negatively charged suspensoid sols which are stabilized by hydrogen ion concentration in milk. The replacement of hydrogen ions with cations of a higher charge induces precipitation at a rate proportional to the rate of ionic exchange.

Sommer (57) states that, within limits, the clotting of milk by rennet follows the Segelcke-Storch law ($t \times m = k$) which states that the time required for coagulation is inversely proportional to the amount of enzyme used. Kelley (33) pointed out, however, that the coagulation is secondary to changes produced by the enzyme, and it is uncertain whether the primary action of the enzyme obeys this law.

In addition to a knowledge of casein and the action of rennin upon it, there are other inherent facts associated with the clotting process which must be kept in mind when considering the adequacy of a theory. For example, the amount of rennin required to produce the coagulation is extremely small, and the coagulation of milk by an enzyme is not confined to rennin (33). The enzymes pepsin and trypsin can also coagulate milk (57). Mattick and Hallett (37) and Albrecht (2) found that the previous heat treatment of the milk has an important effect on the rate of coagulation by rennin. When milk is heated and subsequently cooled, they found that the rate of rennet coagulation is most rapid immediately after cooling. Mattick and Hallett (37) reported that

coagulation time increases until it shows no further increase after about 5 hours cool-aging. They also stated that rennin coagulation becomes progressively slower as heat treatments above 145° to 151° F. for 30 minutes are applied, and fails to form a clot when heated to about 195° for 30 minutes.

Kelley (33) states that rennet acts on casein to produce paracasein even if no coagulation occurs. Actual coagulation requires the presence of soluble calcium salts or other di- or tri-valent ions. Other ions such as Ba^{++} , Mg^{++} , Mn^{++} , Ni^{++} , Co^{++} and Fe^{+++} can be used instead of calcium ions (15).

There are several theories on the nature of rennin action. One of the oldest and one of the most widely accepted is the classic theory developed by Hammersten in 1877, as cited by Sommer (57). He concluded that the action of rennin on casein was proteolytic and resulted in a hydrolysis of the casein into paracasein and a soluble whey albumin. Van Slyke and Bosworth (65) also believed that the process involved in rennin coagulation was proteolytic, but postulated that rennin cleaved casein into two molecules of paracasein. Their conclusion was based on the results of base combining studies of casein and paracasein. They considered Hammersten's "whey protein" as a proteose that would develop only if the action of rennin were allowed to proceed beyond the paracasein state. Spur and Wolman (59) found in their digestibility studies that milks treated with rennin were assimilated more rapidly in the stomach than either soft curd or normal curd milks which helped to substantiate the proteolytic view of rennin action.

Alais et al. (1) reported that rennin clotting of milk is preceded by the splitting of unidentified peptides (non protein nitrogen) not precipitated by 12% trichloroacetic acid. Results showed that the NPN stem from the α -casein and that the process which sets it free is probably identical with the primary reaction of the rennet curdling of milk.

Sommer (57) states that some research workers lean toward theories involving association or polymerization of casein into larger molecular structures. In 1904, Loevenhart, as cited by Davies (15), proposed a theory that rennin caused an association of casein particles into paracasein and thereby formed a less highly dispersed colloid. He suggested that this could be brought about by the cleavage of secondary valency bonds of the amino and carboxyl groups within the casein molecule. Beau (5) elaborated upon Loevenhart's theory. He suggests that rennin coagulation proceeds by a polymerization, physico-chemical condensation, or rearrangement of casein and "calcium-phospho-caseinate." This occurs through a process which involves the union of the secondary valences (which had been opened by rennin action) through calcium and phosphate radicals. Physical aggregates arise which increase in size by polymerization until a clot is formed.

Another theory developed concerning the nature of the action of rennin upon casein is that rennin, carrying a positive charge, combines with casein carrying a negative charge, thus causing precipitation (57).

In 1947, Nitschmann and Lehmann (43) concluded that the nature of rennin action is unknown but that it most likely acts as a "denaturase"

and precipitates casein by unfolding the molecule rather than by removing a protective colloid or proteolytic splitting of the molecule.

Complete acid precipitation of casein requires a pH of 4.7 while rennin coagulation occurs near neutrality. The gel formed by paracasein is firmer and tougher than the gel formed by casein. Also, the casein gel occurs at the isoelectric point of casein and is almost completely free of minerals (57). Sommer also (57) states that the difference is only a matter of degree rather than differences in basic principles. In support of this view he states that they differ only in degree because of the large number of reactive groups freed by rennin action. The effects are additive with respect to speed of coagulation and strength and firmness of the curd.

Factors Affecting Rennet Coagulation Time and Curd Tension

A discussion of the individual factors which influence rennin is difficult because of the wide difference of opinion held regarding the number and character of changes produced when rennin acts on casein. The rate of coagulation and strength of the curd are influenced by several important factors such as homogenization, heat treatments, hydrogen-ion concentration, concentration of casein and calcium in the milk, the temperature employed for coagulation and agitation (33). Kelley (33) also pointed out that some factors affect the enzyme while others affect the chemical and physical properties of the substrate which make it difficult to determine which is the more dominant. Literature which is directly related to those factors which influence the coagulation or

setting rate and the curd tension are limited while references to related subjects are in abundance.

The coagulation properties of cow's milk are known to shift somewhat due to natural causes such as breed, individuality, season of the year, stage of lactation, and mastitis (8, 3, 7). In general, any natural cause which affects milk composition will be reflected in the curd properties.

When milk from individual cows is coagulated with rennin, there is a wide variation in the rate of coagulation and toughness of the curd. Some milks coagulate in 5 minutes while others require over one-half hour (33). The curd may be soft and friable or it may be extremely tough and rubbery. A soft curd milk which is more desirable in market milk has been defined by Hill (29) as milk with a curd tension of less than 30 grams. Normally, individual cow's milk will vary in curd tension from 15 grams to about 150 grams by the Hill method (28).

Hill (29) found on tests over a 10-year period that the curd tension is quite uniform from lactation to lactation. Berry (8) reported that there may be wide differences in the curd tension of milks in succeeding lactation periods of the same cow. Differences reported may have been due to udder infection. Allemann and Schmid (3) found that individuality of the cow was the greatest factor influencing rennet coagulation. They also found great variations in the nature and strength of the curd.

Because of its effect on milk composition, breed is probably the most important factor in determining the curd tension of milk. Jersey

milk has the highest rennet curd tension, followed in order by Guernsey, Brown Swiss, Ayrshire and Holstein (3).

Berry (7) reported that curd tension is higher during early and late stages of lactation than during the middle stages, and that the curd tension remains fairly constant during the greater middle stage of lactation. Allemann and Schmid (3) reported that shortly after calving the coagulation time was at a minimum and after a few days increased to a fairly constant value. They also found that milk from cows in oestrus had a greatly retarded rate of coagulation and produced a rennet curd of reduced elasticity. The Hill curd tension of colostrum is, in general, higher than that of the later or normal milk.

Seasonal and atmospheric conditions seem to affect coagulation properties in a fashion parallel to composition. Doan and Welch (16) reported that the curd tension is highest in November, December and January and lowest in May, June and July. Drastic or sudden weather conditions cause a hardening of the curd (3, 27). This effect is probably due to an increased solids which occurs in severe cold weather.

Kelley (33) stated that soft curd milk is invariably low in fat, total solids, solid-not-fat, casein and probably ash. The ingredients of soft and hard curd milk appeared to be the same, the only variable being the relative amounts of each. Human milk has a lower protein and ash content than cow's milk and also yields a very low curd tension.

Casein is the important constituent in determining the curd character. Espe and Dye (20) concluded that casein content accounted for about 81% of the differences in curd tensions of milk. Welch and Doan (68)

stated that soft curd milk contains less than 2% casein and that hard curd milk (curd tension over 60 grams) usually contains 2.6% casein.

Weisberg et al. (67) found that because of its dilute composition, soft curd milk is lower than hard curd milk in buffer capacity, calcium and phosphorus content, and titratable acidity. They found no significant difference with respect to pH, flocculation point of casein, freezing point, relative viscosity and casein composition. They also found that milk with the same casein content does not necessarily have the same curd tension. Kelley (33) states that casein with small colloidal particles should have tougher curd since filaments will be more numerous and interlocking at more points.

Hostettler and Ruegger (32), in an effort to find why some milks coagulate slowly or not at all, precipitated the casein from soft curd milk and normal milk with acid. The rennet coagulation times were compared to caseinate sols prepared from them and that of the original milks. The time of coagulation was in good agreement from which they concluded that differing coagulation times are not attributable to peculiar differences in the casein, but rather, to differences in the structural linkage of the calcium caseinate-calcium phosphate complex.

Pyne (48), who believes that calcium may be the chief factor in the diverse renneting abilities of various milks, found a close relationship between the renneting times of individual milks and the renneting times of the corresponding artificial milks prepared from them by dialysis of relatively small volumes of reconstituted spray dried skim-milk against the individual samples. These results indicated that

differences in the composition of milk serum and not differences in the composition of the caseinate-phosphate complex is the essential factor controlling rennet coagulation time. He stated that differences in the composition of the caseinate-phosphate complex could be due to varying portions of colloidal phosphate as can occur between milks, or to variation in degree of casein heterogeneity, but that casein heterogeneity is unlikely.

There seems to be no definite relationship between fat content and the curd tension. Generally, however, there is a trend for curd tension to increase with fat content. This is misleading as it would lead one to infer that fat has tendency to harden the curd. Hill (27) and Allemann and Schmid (3) verified the fact that fat has a softening effect when they reported that the curd tension of skimmilk is higher than that of the corresponding whole milk. Kelley (33) states that the increased curd tension of high fat milk is apparently due to the corresponding increase in casein content which usually accompanies an increase in fat content.

Generally, high curd tension milk has a high phosphorous and calcium content (67). Holm, Webb and Deysher (31) found a tendency toward a shortening of the time of rennet coagulation as the calcium content of milk increased. The salt composition of milk is an important factor in the coagulation of milk by rennin, pepsin or pepsin-hydrochloric acid coagulant. Small additions of sodium citrate and disodium phosphate are known to retard rennet coagulation and to produce a softer curd.

Ling (35) reported accelerated rennet coagulation of milk when calcium lactate, calcium acetate, monosodium citrate and monopotassium phosphate were added to milk. Addition of trisodium citrate, disodium citrate and disodium phosphate retarded rennet coagulation. He stated, however, that changes in pH and ionic equilibria occur when mineral salts are added to milk, but the combined evidence supports the view that the phosphate-citrate to calcium ratio influences rennet action as well as heat coagulation and the alcohol test. Low percentages of soluble organic phosphorous are associated with shorter rennet coagulation times. Further studies revealed that caseinogen is associated with tricalcium phosphate in a physical rather than a chemical manner.

Bosworth (9) stated that the addition of soluble salts of ammonium, sodium or potassium to milk results in a double decomposition whereby calcium caseinate is changed to a caseinate of the base added. These are converted to paracaseinates by rennin but no coagulation results due to the fact that all the caseinates of these bases are soluble.

Eilers et al. (19) explained the addition of citrate and phosphate on the equilibria of the dispersed calcium caseinate phase. They state that citrate withdraws calcium from the dispersed phase by the formation of complex calcium citrate ions. On the other hand, addition of phosphate seems to form a rather insoluble calcium phosphate, probably as an additional product to calcium caseinate, thus causing an increase in the amount of inorganic components in the dispersed phase.

Sommer and Menos (58) state that tricalcium phosphate exists in milk in a finely divided colloidal form. The solubility of tricalcium

phosphate decreases with rise in temperature which seems typical of salts for which the solubility product involves second and third power of the ion concentrations. Verma (66) states that similar diminutions in solubility with a rise in temperature are likely to apply with respect to calcium and magnesium citrate and magnesium phosphate.

Analysis of the sediment of supercentrifuged fresh and heated milks by van der Burg (62) showed that there were 8 mg. more tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) per gram of casein after heating than before heating. He concluded that calcium united with the organic phosphate of the casein is in an equivalent amount to form the caseinate. He also stated that the free amino and carboxyl groups are left as centers of charge for the colloidal calcium and phosphate.

Maze (39) reported that the addition of 1 to 4% of sodium chloride to milk greatly prolongs the rennin coagulation time because of partial replacement of calcium in the casein molecule by sodium. Maze and Maze (40) added salts to skim milk in 0.001 M concentrations. Manganese chloride doubled the coagulation velocity; zinc chloride, ferric chloride and chromium chloride increased the rate 20 to 30%. Cobalt chloride and copper chloride retarded coagulation appreciably, and nickel chloride slowed it to about one-fifth of the normal.

Pishawikar and Kamat (45) studied the effects of adding halides and monovalent salts to reconstituted dried milk containing 0.0225, 0.0175 and 0.0125 M calcium chloride. As the calcium chloride content decreased the pepsin coagulation time increased. At a given calcium

chloride content bromides inhibited coagulation time more than chlorides. Sodium, potassium, and NH_4 inhibited coagulation in that order.

Mauri (38) stated that the poor coagulability of boiled casein under the influence of rennin does not depend upon an increased calcium binding power of the casein through heating. He prepared an artificial "milk" which coagulated normally with rennin by using casein isolated from a caseinate solution which had been boiled. Also, Mauri (38) stated that the effect of calcium in the rennin action cannot be reproduced through univalent action. All bivalent and trivalent cations behave more or less like calcium. Difficulty is met when the effects of multivalent cations are studied quantitatively because most of them produce precipitation in milk. One must work within the range of concentration where these cations do not cause precipitation except in the presence of rennet.

Bosworth and Van Slyke (10) added 0.400 grams of sodium citrate per 100 ml. of milk and no curd formed. Curd produced by rennin in the presence of smaller amounts of sodium citrate increased in softness as the amount of sodium citrate in the milk increased. They concluded that a double salt, calcium-sodium caseinate is formed from the calcium caseinate of normal milk. When rennin is added calcium-sodium caseinate is changed to a calcium-sodium paracaseinate, which owing to the presence of sodium is not curdled.

In 1940, Schwartz et al. (55) reported the use of sodium metaphosphate for the preparation of soft curd milk, and indicated that it may be important in certain commercial operations. They found that the

amount of metaphosphate required to soften milk with added calcium is about 4.8 times as much metaphosphate as calcium, plus an amount of metaphosphate such as will soften milk itself. Heating, in part, nullified the addition of metaphosphate. They concluded that it was not known whether these results were due to an alteration in the state of the milk constituents themselves or whether the increase was due to a partial revision of the metaphosphate to orthophosphate. They suggested, however, that the peculiar behavior of the curd tension of milk heated with 50 mg. of metaphosphate per 100 ml. of milk might indicate a rearrangement of the milk constituents.

Tracy and Corbett (61) reported that the curd tension of milk to which sodium citrate had been added was higher after pasteurization. They also observed that up until zero curd tension was obtained the proportional decrease in curd tension caused by sodium citrate was greater in the case of raw and non homogenized milk than it was in homogenized milk. Titratable acidity of all milks to which sodium citrate was added was reduced from 0.15 to 0.145 per cent. A salty flavor was noticeable when sodium citrate was added but was less noticeable in the case of homogenized milk.

These workers (61) reported that addition of sodium pyrophosphate to milk caused a greater reduction of curd tension than equivalent amounts of sodium citrate. A lower curd tension resulted when the sodium salts were added after pasteurization to the cooled milk than when added before heating. After adding the salt the curd tension decreased until an equilibrium was reached after about 48 hours.

Van Slyke and Bosworth (64) reported that when casein is precipitated by rennet solution the precipitated casein or paracasein carries down part of the albumin with it. When casein is precipitated with acid little or no albumin is absorbed.

A study of the precipitation of calcium from solutions of milk salts prepared in accordance with the composition and concentration occurring in the average of normal milk and at the reaction of normal milk by Zoller (73) indicated that the loss of calcium was progressive with the time and temperature of heat treatment. The hydrogen ion concentration was proportional to the removal of the buffer material (phosphates) by the calcium. Increasing the amounts of citrates, lactates, and malates greatly reduced the precipitation of calcium phosphate.

Verma (66) reported the average content of calcium, magnesium, phosphorous and citric acid as 132.5, 10.7, 94.7 and 159.1 mg. per 100 ml. of milk, respectively. He found the soluble fraction of calcium, magnesium and phosphorous to be 38.3, 75.7 and 48.7%, respectively, and 84.7% of the total citric acid existed in soluble form. The amounts of soluble calcium and magnesium decreased on pasteurization by the holding process. The average soluble calcium was lowered only slightly but a 2.8% decrease of soluble magnesium was reported. Pasteurization increased the soluble phosphorus and citric acid 0.3 and 0.2%, respectively.

Edmondson and Tarassuk (18) found a decrease in the soluble calcium and phosphorous when the milk was centrifuged immediately after

heating. On the other hand, Ling (35) found that a high soluble calcium content is accompanied by a longer time of rennet coagulation which is contrary to the generally accepted belief.

According to Magee and Harvey (36) using an ordinary filter, 26% of the total calcium is diffusible in fresh milk, about 20% when pasteurized at 65° C. for 30 minutes, and about 15% when kept at the boiling point one hour.

Mattick and Hallett (37), using parchment diffusion capsules, reported that temperatures above 175° F. for 30 minutes reduced the quantity of diffusible phosphorous in the skimmilk. At lower temperatures no difference was obtained. Heating skimmilk to 125° F. and above greatly reduced the diffusibility of the calcium content of milk. At all temperatures from 125° to 207° F. the amount of calcium which had diffused from the raw milk was definitely greater than that which had diffused from the same milk after heating.

Rupp (53), however, found that milk pasteurized at 62.8° C. (145° F.) for 30 minutes does not undergo any appreciable change. The soluble phosphates of lime and magnesia do not become insoluble at 68.3° C. (155° F.) and the quantity of phosphoric acid, lime and magnesia in the serum of both raw and pasteurized milk are practically the same.

Holland and Dahlberg (30) found no significant changes in the amounts of CaHPO_4 and $\text{Ca}_3(\text{PO}_4)_2$ when milk was heated at temperatures ranging from 140° to 165° F. for varying periods of time. There was no

relationship observed between the changes noted in the rennet coagulation time and the relative amounts of these salts.

Verma (66) concluded in his study that the lower pH on pasteurization and the increase in pH after cool-aging are an outcome of the shift in the equilibrium of tricalcium phosphate precipitation. He found that after cool-aging pasteurized milk 24 hours at 45° F. the amount of soluble calcium increased above the amount that was present as soluble calcium in raw milk. He also stated that magnesium plays a similar role but since the amounts of magnesium in milk are small the overall effect of this constituent is very little.

Zittle et al. (72) reported that small concentrations of calcium, not greater than 0.012 M, decrease the viscosity of sodium and calcium caseinate solutions but that by heating solutions containing higher concentrations of calcium, viscosities are produced which are greater than present before heating. This increase of viscosity is largely reversible and is due to the formation of precipitates of colloidal calcium caseinate which redissolve at lower temperatures. Also, the amount of precipitate is increased by high calcium concentration and low pH value. He found that the length of time held at a specific temperature, stirring and temperature after heating affect the precipitation and resolution of sodium and calcium caseinate solutions. Added quantities of sodium chloride reduced precipitation of sodium and calcium caseinate solutions (71). He suggested that this was due to; (1) reduction in activity of the calcium ion and (2) in part to the displacement of calcium

by the sodium ion. Further studies revealed that if sodium phosphate is present in a 1% casein solution containing calcium chloride (5 mM per liter) no phosphate is bound up to pH 6.0. Above pH 6.0 the binding of phosphate increases rapidly along with increased calcium binding (70).

Pasteurization is perhaps the most important modification or treatment to which milk for cheese making is subjected. Many studies (53, 37, 54, 30, 2) have been made regarding the effect of various pasteurization treatments on rennet coagulation. Most investigators paid little attention to the length of time of the heating treatment and the time allowed to elapse after pasteurization before determining the coagulation properties. Albrecht (2) suggested that the lack of agreement among early investigators was due to insufficient control of the cooling treatment following the heat treatment.

Pasteurization causes an increase in rennet coagulation time and a softer curd formation (54, 30). Rupp (53) found the time required for coagulating the casein by rennin to be slightly less in milk pasteurized at temperatures up to 65° C. than it is in raw milk. A marked retardation was observed at 70° C. and above.

Powell (46) found that heating milk at 65° C. for 30 minutes and cooling to 35° C. did not retard coagulability if rennet was added immediately after cooling. Aging the milk at either 5° or 35° C. for 3 hours, slightly retarded its coagulability. Heat treatments between 75° and 85° C. for 30 minutes definitely retarded its coagulability. The loss

increased as the heating temperature increased. When the milks were heat treated at 75° or 85° C. for 30 minutes and cool-aged at 5° or 35° C., the rate of coagulation became progressively slower as the temperature of heating increased. He found that flash pasteurization of milk at 75° C. slightly retarded the rate of coagulation. Flash pasteurization of milk at 85° C. decreased the rate of coagulation, but the retardation was not as great as milk heated at 75° C. by the holding process. Aging this milk at 5° or 35° C. caused further retardation of the rate of rennet coagulation.

Rowland (52) states that heat coagulation consists of two distinct processes: (1) denaturation, or the alteration of the protein under the influence of heat which must proceed, (2) flocculation, the rapid separation of the denaturated protein in the presence of a suitable electrolyte. The denaturation causes loss of solubility of the protein in water and in dilute salt solutions at its isoelectric point. He also states that denaturation is the term applied to the change which takes place in the albumin and globulin when milk is heated. The denaturation which takes place upon heating milk can be measured by the precipitation of albumin and globulin in addition to casein when heated milk is treated with those reagents which precipitate casein alone from unheated milk.

Moir (42) used flash pasteurization at 165° and 185° F. for 20 to 30 seconds. He stated that the increase in the rennin coagulation time caused by pasteurization is associated with a decrease in the

whey nitrogen so that one is approximately a linear function of the other.

Gould (21) reported that at 143° F. for 30 minutes or 160° F. for 15 seconds from 5 to 10% of the whey proteins are denatured. As the temperature and heating time are increased rapid denaturation occurs and at 176° F. for a few minutes it is essentially complete.

Harland and Ashworth (25) found that the amount of whey proteins coagulated reached a near maximum following treatment at 80° C. for 45 minutes. Higher temperatures for shorter periods of time denatured 93 to 95% of the whey proteins. Ramsdell and Whittier (49) heated milk to 100° C. for 10 minutes and reported that the albumin and globulin were denatured but were not coagulated or aggregated to a degree equal to that of the casein complex.

A very detailed study by Larson and Rolleri (34) of the denaturation of the specific serum proteins revealed that 29% of the total serum proteins of normal milk were denatured when heated to 70° C. for 30 minutes. This was due to the specific denaturation of 89% of the immune globulins, 32% of the β -lactoglobulins, 52% of the serum albumin and 6% of the α -lactalbumin. The immune globulins were the least, and α -lactalbumin, the most heat resistant. β -lactoglobulin and serum albumin showed an intermediate sensitivity.

Studies concerning the relationship between protein denaturation and curd tension of heated skimmilks revealed that the rennet curd tension test is capable of indicating the heat treatments that skimmilk has been subjected to before concentrating and drying. The higher the

percentage of denatured whey protein the lower the rennet curd tension (14).

Acidity is of considerable importance because of its effect on setting rate and curd firmness in cheese making. The effect of decreasing the pH on curd tension depends somewhat on the specific curd tension test used, but, in general, decreasing the pH increases the curd tension value until a point is reached where the opposite occurs. Doan and Welch (16) state that as the pH of the milk is reduced from the normal (6.4 to 6.7) a toughening of the enzyme curd is observed until the pH drops under approximately 5.9. From this point down to the isoelectric point of casein (pH 4.7) the curd tension decreases. Doan (17) states that this decrease is due to the progressive agglomeration or coagulation of casein in the acid which interferes with the formation of paracasein curd induced by enzyme action.

Sanders et al. (54) reported that the pH is a more important factor in determining coagulation time than it is in determining curd character. Their results showed that the influence of pH on coagulation time is most marked above 6.65, while pH effects are most marked for curd tension below pH 6.50. All acid additions though producing equivalent pH shifts, do not affect the coagulation process to the same degree. Schryver, as cited by Kelley (33), studied the effect of organic acid additions on the rapidity of rennet precipitation, and the character of rennet curd. He found that additions of 0.03 g. of lactic acid per 100 ml. of milk gave the best results. The acids valeric, formic, citric, malic and tartaric did not give an equally good

curd as compared to acetic and propionic. He pointed out that this indicates the importance of anions in determining curd character.

Using a curd tension meter to measure the firmness of the rennet curd, Heinemann (26) reported that the starter used in the manufacture of cottage cheese significantly influences the firmness of the curd at cutting time. This was presumed to be due to variations in the proteolytic activity of different types of organisms present in the different cultures. They also reported that the rate of curd firming by different starters varies linearly with the acid development.

Albrecht (2) concluded from his study that the effects of pasteurization, cool-aging and subsequent reheating are explainable to an important degree on the basis of the shift in pH caused by these treatments. A drop in pH of the milk occurred when it was heated to temperatures of 50° C. or higher for 50 minutes. Subsequent cool-aging was followed by a rapid rise in the pH with the change becoming progressively less until an equilibrium was established in 24 hours. Reheating of the heated cool-aged milk again lowered the pH but not to as low a level as observed when the milk was first heated.

Rapp and Calbert (50) reported that the average increase in the rennet coagulation time of mixed night's and morning's milk held at 36° F. was 4% after 1 day holding, 7.2% after 2 days holding and 12.5% after 3 days holding.

It is apparent that effects other than pH changes are involved in changes of rennet coagulation rates and curd firmness. These other effects center around the combination of bases, particularly calcium,

with milk proteins, magnesium, citrates and phosphates involving shifts in combinations under the influence of pasteurization and cool-aging. The object of this study is to determine the relationship of such treatments to the rennet curd tension of various milks and/or caseinate sols.

EXPERIMENTAL PROCEDURE

Rennet curd tension measurements. Freshly separated raw skim-milk samples were collected in 8-liter stainless steel containers from the University of Tennessee Creamery and stored from 1 to 6 hours at 2° C. The Cherry-Burrell Corporation curd tension meter (Figure 1) was used to measure the effects of heat treatment (62° and 70° C.) and cool-aging on the rennet curd tension.

The following procedure was used to prepare samples for curd tension measurements. Six 350-ml. portions of skimmilk were placed in 500-ml. Erlenmeyer flasks which were closed with rubber stoppers. The pH and the rennet curd tension of one sample was determined immediately. The second sample was held in a cooled condition (cool-aged) at 2° C. for 24 hours after which the rennet curd tension and pH were measured. The remaining four samples were heated at $62^{\circ} \pm \frac{1}{2}^{\circ}$ C. for 30 minutes without agitation in a thermostatically controlled water bath. Another series of samples from the same volume of skimmilk was treated similarly using $70^{\circ} \pm \frac{1}{2}^{\circ}$ C. for 30 minutes. After the heat treatment the samples were cooled immediately in an ice-water bath. When the samples were cooled to 35° C., one flask of skimmilk was removed from the water bath to measure the pH and the rennet curd tension as described later. The second sample was cool-aged one hour at 2° C.; the third for 24 hours at 2° C. The fourth sample was cool-aged 24 hours at 2° C., reheated to the same pasteurization temperature as was first used and held for 30 minutes. All samples were tempered to 35° C. for pH and rennet curd tension measurements.



Figure 1. Cherry-Burrell Corporation curd tension meter.

Rennet extract used in this investigation was obtained from the Marschall Dairy Laboratory, Inc. The pH measurements were made by a glass electrode model M Beckman pH meter.

The procedure for making the rennet curd tension tests was as follows (14). One hundred milliter portions of skimmilk, approximately 35° C., were measured into three 250-ml. beakers which were placed in a thermostatically controlled water bath equipped with an agitator, and the beakers were covered with watch glasses. Two milliliters of freshly prepared 2% rennet coagulant were placed in each of three 250-ml. beakers. After reaching 35° C. the tempered milk was poured rapidly into the beaker containing the coagulant, back into the original beaker and then into the coagulant beaker which was placed in the water bath without further agitation of the skimmilk. This mixing of the coagulant required about 10 seconds. Each beaker was again covered with a watch glass. Samples were set at intervals of 2 to 3 minutes to provide sufficient time between samples for measuring the curd tension of the preceding sample.

One sample at a time was removed from the water bath and placed on the scale of the curd tension meter about 29.5 minutes after setting so that the slow moving knife would strike the curd 30 minutes $\pm$ 15 seconds after adding the coagulant. The pointer on the rotating dial was set at zero graduation. The curd tension was the maximum reading in grams as the knife penetrated the curd surface.

Rennet curd tension measurements were made on raw whole milk, pasteurized whole milk and homogenized whole milk obtained from the

University creamery which was supplied solely from the University herd composed of approximately 75 Jersey and Holstein cows.

Preparation of casein. Casein was isolated from 20-gal. lots of raw skimmilk obtained from the University creamery. A modification of the Van Slyke and Baker (63) method of preparing isoelectric casein was used. The washed casein was not extracted with ether and alcohol and air dried; instead, the following procedure was used.

The cooled skimmilk (15° to 20° C.) was placed in a 30-l. Pyrex container diluted with an equal volume of distilled water. Ten per cent hydrochloric acid was added slowly with constant agitation with a high speed agitator until a pH between 4.7 and 4.5 was reached. The precipitated casein was allowed to settle and the whey was siphoned off. Distilled water was added to replace the whey. The mixture was agitated and left undisturbed approximately 30 minutes to remove calcium ions from the curd. After the curd settled the water was removed and replaced with distilled water. Dilute NH_4OH was added in small quantities with rapid stirring to dissolve casein, keeping pH below 7.5. This precipitation-washing-redissolving procedure was repeated three times. After the last washing the excess water was removed by pressing through fine mesh cloth until the curd contained about 75% moisture.

Moisture determinations on casein were made by drying 4-g. samples in aluminum foil sample pans at 90° C. for 16 ± 2 hours in a vacuum oven with 15 inches of vacuum.

The casein was placed in 600-ml. Pyrex beakers which were covered with a double layer of pliofilm and then frozen rapidly at -23.3° C. (-10° F.).

Freezing and storing of the casein in this manner had no obvious effect on the physical appearance or structure of the casein after defrosting. No detectable difference was found between the reaction of rennet of sols made from frozen and non frozen casein. Grindrod et al. (22) also found no difference in rennet action when freezing at -25° C. This method of preservation was chosen to avoid the hazards of possible chemical changes caused by drying, chemical dehydration or bacterial or enzymatic action at storage temperatures slightly above 0° C.

Preparation of whey protein. Whey protein was isolated from both freshly separated skimmilk and reconstituted Campa Lo-Temp (Mojonnier Process) dried skimmed milk obtained from the Chattanooga Area Milk Producers Association, made up to 15% total solids. The reconstituted skimmilk powder was stored at 2° C. for 3 hours before precipitating the casein by adding dilute (10%) hydrochloric acid until a pH of 4.6 was reached. The whey was removed, filtered through a fine mesh cloth and a Buckner funnel using Whatman No. 42 filter paper. The whey was concentrated by partial freezing in 8-l. stainless steel containers in a -10° F. hardening room. The whey was placed in 2 inch diameter cellulose dialyzing tubing with an average porous radius of 24 angstroms and dialyzed at 2° C. in a 30-l. Pyrex glass jar containing 25 l. of distilled water to remove salts. The water was changed once during the first 24 hours and twice during the next 4 days. After dialyzing 2

days a white flocculent precipitate settled to the bottom of the tubes. It was assumed to be one of the globulin fractions. The semi-micro Kjeldahl method for nitrogen determination as described by Shahani and Sommer (56) was used for total nitrogen analysis of the whey protein. The percentage whey protein was calculated by multiplying per cent nitrogen by the factor 6.38.

Preparation of salt solutions. Stock solutions of sodium chloride, tribasic sodium phosphate, sodium citrate and magnesium sulfate were prepared, containing 30 g. per liter of each salt. Ten solutions were prepared from each of these so that 10 ml. of the ten secondary solutions would accomplish the preparation of the corresponding casein sol prepared from it.

Composition of casein sols. Preliminary trials to determine the amount of casein and calcium to use in the casein sols for these rennet curd tension studies suggested the following percentages as the optimum for the most satisfactory rennet curd formation. Sols containing 3% casein (dry basis) and calcium hydroxide calculated as CaO at the rate of 3.5% of the weight of the casein were used throughout these studies. Two series of eleven casein sols were prepared containing 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%, respectively of each of the four salts listed above as per cent of the weight of the casein (dry basis).

Two series of calcium caseinate sols were prepared containing added whey protein at the rate of 0, 10, 12, 14, 16, 18, 20, 22 and 24% of the weight of the casein. The casein used for each series was prepared from different lots of raw skim milk. The whey protein for each

series was prepared independently from the same source of dried skimmed milk powder.

Two series each of 3% casein sols were prepared containing 5 and 10% whey protein as per cent of the weight of the casein. Raw skimmilk and Campa Lo-Temp dried skimmed milk powder were sources of whey protein. Two series of samples were prepared separately at each level of whey protein and from each of the two sources. The composition of the sols is illustrated in Table I.

Blending. Preliminary trials suggested the following procedure for compounding the casein sols used in this study. Three per cent casein sols were prepared by weighing into an 8-l. stainless steel container the calculated quantity of defrosted casein to make up a 6.3-l. volume of casein sol. The volume was made up to 6 l. by adding cold distilled water (2° C.). The calculated amount of $\text{Ca}(\text{OH})_2$ was added to 200 ml. of distilled water which formed a slurry. This slurry was slowly added to the mixture while stirring with a high speed agitator. Agitation was continued approximately 45 minutes until complete dispersion of the casein was attained, as evidenced by visual inspection.

The volume of the calcium-caseinate solution was remeasured at 20° C. and made up to 6300 ml. with distilled water. This sol was divided into 540-ml. portions and placed in 1-l. beakers. The addition of the salt solutions of the respective concentrations at the rate of 10 ml. per 100 ml. of calcium-caseinate sol made up the calculated composition of the sol. The salt solution was added slowly, and in order to disperse it more thoroughly, it was directed from the pipette

TABLE I

THE COMPOSITION OF 3 PER CENT CASEIN SOLS CONTAINING
ADDED SALTS AND WHEY PROTEIN AT THE RATE OF 5
PER CENT OF THE WEIGHT OF THE CASEIN

Sample Number	Added as Per Cent Casein (dry basis)				
	Whey Protein (%)	NaCl (%)	Na_3PO_4 (%)	MgSO_4 (%)	Ca(OH)_2 as CaO (%)
1	5	0	0	0	3.5
2	5	5	0	0	3.5
3	5	0	5	0	3.5
4	5	0	0	5	3.5
5	5	5	5	0	3.5
6	5	5	0	5	3.5
7	5	0	5	5	3.5

against the rotating propeller shaft where it entered the mixture to assure complete dispersion of the added salt.

The pH measurements were made of all solutions after the addition of salts. The pH was adjusted to 6.10 by slowly adding dilute (8%) phosphoric acid from a fine pointed pipette while stirring with a glass rod. Beakers were covered with parchment paper and stored at 2° C. for 16 ± 2 hours to allow the pH to reach an equilibrium.

After the storage period the samples were tempered to 25° C. and the pH readjusted to 6.10 with phosphoric acid or ammonium hydroxide. The caseinate sol was divided into three equal portions. Two portions were placed in 250-ml. Erlenmeyer flasks and heated to 62.2° C. (144° F.) and 71.1° C. (160° F.) for 30 minutes, respectively, temperatures commonly used for pasteurization. The samples were cooled quickly to 25° C. and resuspended with a Waring blender if precipitation occurred upon heating. The pH was measured and 100 ml. were placed in each of two 250-ml. beakers. The casein sols were tempered (35° C.) and the coagulant added. Ten-minute incubation periods were used for all casein sols because 30 minute periods allowed a tough wrinkled curd to form. The curd tension measurements were made as described.

Duplicate curd tension measurements were made on the unheated casein sols.

The whey protein was added to casein sols, along with, or in place of, the water prior to the addition of calcium hydroxide. This was the only difference in the blending procedure of the sols containing whey protein and those containing no whey protein.

RESULTS

Influence of homogenization and pasteurization upon the rennet curd tension of whole milk. In the preliminary investigations it seemed desirable to study the effects of pasteurization and homogenization upon the rennet curd tension of whole milk. Samples were collected on separate days from the University of Tennessee Creamery immediately after processing was completed. The milk was pasteurized by the holding process at 62.2° C. (144° F.) for 30 minutes. Homogenized milk was heated, after pasteurization and prior to homogenization with a single stage, Creamery Package homogenizer at a pressure of 2,200 p.s.i., to 65.5° C. (150° F.) and cooled immediately after homogenization. Table II shows the results of the rennet curd tension measurements of the twelve samples studied. The average curd tension of raw whole milk was 60.5 g. The rennet curd tension of heated whole milk and homogenized whole milk decreased 29.1 and 93.4%, respectively, as compared to the raw whole milk.

Influence of heating and cool-aging upon the rennet curd tension of skimmilk. Freshly separated raw skimmilk was divided into six 350-ml. portions for each heat treatment used. Heat treatment studies were made at 62° and 70° C. with a 30-minute holding period in each case. Rennet curd tension measurements were made on the raw skimmilk; heated; heated and cool-aged 1 hour; heated and cool-aged 24 hours; heated, cool-aged 24 hours and reheated to the previous pasteurization temperature and held 30 minutes; and the raw skimmilk cool-aged 24 hours. Data shown in Tables III and IV give the results of each treatment. Heating lowered

TABLE II

RENNET CURD TENSION OF RAW, HEATED
AND HOMOGENIZED WHOLE MILK^a

Trial Number	Raw Whole Milk (grams)	Milk Heated (62.2° C. for 30 Minutes) (grams)	Heated (62.2° C. for 30 Minutes) Homogenized (2,200 lbs./sq. in.) (grams)
1	67.3	41.6	4.3
2	62.0	40.3	4.0
3	66.6	49.0	3.6
4	55.3	47.3	4.6
5	58.6	36.3	3.6
6	55.3	36.0	4.0
7	60.3	48.6	4.3
8	61.6	49.3	3.3
9	55.3	36.6	3.6
10	64.3	40.6	3.6
11	58.3	47.6	4.3
12	60.6	41.6	4.0
Average	60.5	42.9	4.0
Range	52-68	35-49	3-5
Per Cent Decrease		29.1	93.4

^aAverage of triplicate determinations.

TABLE III

INFLUENCE OF HEATING (62° C. FOR 30 MINUTES) AND COOL-AGING
(2° C.) UPON THE RENNET CURD TENSION OF SKIMMILK^a

Trial No.	Rennet Curd Tension					
	Raw (control) (grams)	Heated (grams)	Heated, Aged 1 Hour (grams)	Heated, Aged 2½ Hours (grams)	Heated, Aged 2½ Hours, Reheated (grams)	Raw Skim- milk Aged 2½ Hours (grams)
1	70.3	64.7	67.0	55.3	56.7	65.3
2	67.0	59.3	59.0	54.0	57.3	64.3
3	77.3	65.7	65.3	64.3	65.0	67.0
4	77.0	68.3	67.7	59.7	61.0	73.3
5	81.3	74.3	74.0	67.7	69.3	78.7
6	74.0	71.0	68.3	57.3	63.7	70.3
7	69.0	66.3	62.0	57.3	58.3	67.0
8	80.3	75.0	73.7	66.7	68.0	76.0
9	84.3	63.0	62.7	56.3	51.0	79.0
10	78.0	72.0	70.7	66.3	67.3	75.7
Average	75.85	67.96	67.04	60.49	61.76	72.56
Range	65-85	58-76	58-75	53-68	50-70	63-80
Per Cent Decrease		10.41	11.62	20.26	18.58	4.34

^aAverage of triplicate determinations.

TABLE IV

INFLUENCE OF HEATING (70° C. FOR 30 MINUTES) AND COOL-AGING
(2° C.) UPON THE RENNET CURD TENSION OF SKIMMILK^a

Trial No.	Rennet Curd Tension					Raw Skim- milk Aged 2½ Hours (grams)
	Raw (control) (grams)	Heated (grams)	Heated, Aged 1 Hour (grams)	Heated, Aged 2½ Hours (grams)	Heated, Aged 2½ Hours, Reheated (grams)	
1	70.3	50.3	50.0	42.7	33.7	65.3
2	67.0	58.3	53.0	42.0	32.0	64.3
3	77.3	53.3	51.3	43.0	33.3	76.0
4	77.0	57.0	56.3	37.7	28.7	53.3
5	81.3	58.0	55.0	42.0	46.7	78.7
6	74.0	31.3	33.0	16.3	8.0	70.3
7	69.0	53.7	48.3	36.0	32.0	67.0
8	80.3	61.7	60.0	51.3	48.0	76.0
9	84.3	63.0	62.7	56.3	51.0	79.0
10	78.0	61.0	60.3	49.0	45.7	75.7
Average	75.85	54.76	52.99	41.63	35.91	72.56
Range	65-85	31-64	32-64	16-57	8-51	63-80
Per Cent Decrease		27.81	30.14	45.12	52.66	4.34

^aAverage of triplicate determinations.

the rennet curd tension in all cases, with the greatest reduction occurring at the higher temperature, 70° C. Cool-aging for 1 and 24 hours at 2° C. of skimmilk previously heated to 62° C. reduced the curd tension 1.21 and 9.85%, respectively, below the curd tension of the samples receiving the heat treatment but no cool-aging. Likewise, for the heat treatment at 70° C., the reduction due to cool-aging was 2.33 and 17.32%. Reheating (62° C. for 30 minutes) skimmilk heated for 30 minutes at 62° C. and aged for 24 hours increased the rennet curd tension 1.68% above that of the skimmilk heated 30 minutes and cool-aged 24 hours. No increase was observed when the heated skimmilk (70° C.) was reheated at 70° C. for 30 minutes, but a further decrease occurred. Cool-aging raw skimmilk 24 hours lowered the rennet curd tension 4.34% below that of the control. In no case did the difference of curd tension between triplicate determinations of the same sample exceed 5 g. The range was normally 3 g. or less.

Influence of heating and cool-aging upon the pH of skimmilk.

Data showing the shifts in the pH of skimmilk due to cool-aging are recorded in Table V. On an average, upon heating at 62° and 70° C. the pH lowered 0.064 and 0.081, respectively. On subsequent cool-aging this was followed by a rapid rise in the pH over the 24-hour period. The pH did not rise above the pH of the unheated skimmilk. Upon cool-aging 24 hours the pH of the raw skimmilk increased an average of 0.008. The changes in the pH of both the heated and unheated samples on cool-aging were similar to the changes in the rennet curd tension, with the curd tension decreasing as the pH increased. Reheating of the heated,

TABLE V

INFLUENCE OF HEATING AND COOL-AGING UPON THE pH OF SKIMMILK

Trial No.	pH					
	Raw (control)	Heated	Heated, Aged 1 Hour	Heated, Aged 24 Hours	Heated, Aged 24 Hours, Reheated	Raw Skim-milk Aged 24 Hours
<u>62° C. for 30 Minutes</u>						
1	6.86	6.70	6.70	6.73	6.61	6.77
2	6.79	6.78	6.78	6.80	6.71	6.83
3	6.79	6.71	6.74	6.75	6.76	6.80
4	6.81	6.79	6.79	6.76	6.74	6.85
5	6.80	6.74	6.75	6.79	6.75	6.81
6	6.76	6.69	6.70	6.74	6.70	6.79
7	6.71	6.67	6.67	6.70	6.67	6.71
8	6.74	6.69	6.71	6.75	6.68	6.76
9	6.80	6.71	6.73	6.79	6.74	6.81
10	6.78	6.72	6.72	6.79	6.73	6.79
Average	6.784	6.720	6.729	6.760	6.709	6.792
<u>70° C. for 30 Minutes</u>						
1	6.86	6.72	6.73	6.79	6.71	6.77
2	6.79	6.75	6.76	6.78	6.71	6.83
3	6.79	6.68	6.71	6.74	6.73	6.80
4	6.81	6.77	6.77	6.81	6.75	6.85
5	6.80	6.72	6.74	6.76	6.72	6.81
6	6.76	6.67	6.69	6.71	6.66	6.79
7	6.71	6.65	6.66	6.68	6.65	6.71
8	6.74	6.65	6.68	6.70	6.67	6.76
9	6.80	6.70	6.70	6.74	6.72	6.81
10	6.78	6.72	6.74	6.75	6.71	6.79
Average	6.784	6.703	6.718	6.746	6.703	6.792

cool-aged skimmilk again lowered the pH to a level approaching that of the heated skimmilk that was not cool-aged. Reheating at 62° C. for 30 minutes lowered the pH and increased slightly the curd tension. A decrease of curd tension was noted after reheating 30 minutes at 70° C.

Casein and whey protein preparations. Four lots of acid precipitated casein used in the preparation of casein sols for curd tension studies contained 26.13, 25.35, 25.25, and 26.45% total solids as determined by drying samples in a vacuum oven.

Attempts to prepare whey proteins by freeze-drying were unsuccessful because of the lack of a satisfactory method to concentrate the whey proteins before placing in the lyophilizer. An alternate procedure was used. After precipitating the casein from the skimmilk and reconstituted dried skimmilk powder, the resulting whey was concentrated by partial freezing, dialyzed, analyzed for its nitrogen content and the protein content calculated. Three preparations of whey protein from raw skimmilk contained 0.416, 0.432 and 0.789% protein, respectively. Two preparations from reconstituted dried skimmilk powder contained 1.027 and 1.014% protein, respectively. The whey was yellow after the casein was precipitated. Upon dialysis to remove salts a white precipitate formed, assumed to be a globulin fraction. The whiteness of the whey proteins was probably due to the reflection and scattering of light by the particles in suspension.

Influence of sodium chloride and heat treatment upon the rennet curd tension and pH of 3% casein sols. The influence of sodium chloride and heat treatment upon the rennet curd tension and pH of 3% casein sols

is shown in Table VI. The data show that after adjusting with phosphoric acid cool-aging decreased the pH of freshly prepared casein sols with a greater decrease occurring at the higher sodium chloride concentrations. Heat treatments of the casein sols after the pH was readjusted to 6.10 caused a lowering of the pH, especially, at higher sodium chloride concentrations.

After heat treatment the sols were whiter in all cases. A white aggregate was visible, with decreasing amounts at greater sodium chloride concentrations.

Higher concentrations of sodium chloride produced a softer curd in unheated sols. Sols heated to 62.2° C. for 30 minutes, containing low concentrations of sodium chloride, formed a tough rubbery curd which stuck to the curd knife on its upward motion and had no distinct breaking point when the curd tension was measured.

The curd tension was higher in the heated sols than in the corresponding unheated sols, provided the sol contained 5 to 10% sodium chloride as per cent of the weight of the casein. The higher heat treatment (71.1° C. for 30 minutes) further enhanced the curd tension of most samples within this range of salt concentration. The control sample containing no sodium chloride coagulated when heated at this temperature. After re-suspending the clot with a Waring blender, rennet formed a soft friable clot with a cloudy whey layer.

Influence of sodium citrate and heat treatment upon the rennet curd tension and pH of 3% casein sols. Data presented in Table VII show the influence of sodium citrate and heat treatment upon the rennet curd

TABLE VI

INFLUENCE OF SODIUM CHLORIDE AND HEAT TREATMENT UPON THE
RENNET CURD TENSION AND pH OF 3 PER CENT CASEIN SOLS^a

Salt Added as % Casein (dry basis) (%)	pH		Heat Treatment ^c				
	After Adding Salt	After Holding 16-18 Hrs. at 2° C. ^b	None Curd Tension (grams)	62.2° C. for 30 Min.		71.1° for 30 Min.	
				pH	Curd Tension (grams)	pH	Curd Tension (grams)
<u>Trial I</u>							
None	9.69	6.06	208.0	5.95	d	5.85	e
1	9.68	6.08	206.5	5.94	d	5.90	e
2	9.68	6.09	203.0	5.93	d	5.92	e
3	9.68	6.05	204.5	5.91	d	5.91	169.0
4	9.66	6.03	187.5	5.90	d	5.89	184.0
5	9.64	6.02	179.5	5.90	d	5.90	184.0
6	9.66	5.98	160.5	5.95	197.0	5.89	195.5
7	9.66	6.00	157.5	5.89	182.5	5.88	214.0
8	9.63	6.00	151.0	5.87	170.5	5.90	186.5
9	9.67	5.99	142.0	5.88	155.0	5.89	172.5
10	9.63	6.01	130.5	5.90	144.0	5.89	166.5
<u>Trial II</u>							
None	9.72	6.04	188.0	6.04	d	6.00	e
1	9.73	6.03	188.5	5.96	d	5.92	e
2	9.71	6.01	180.5	5.97	d	5.85	e
3	9.71	5.98	176.5	5.93	d	5.90	19.5
4	9.68	5.95	173.0	5.92	139.5	5.87	121.0
5	9.68	5.98	176.5	5.92	198.0	5.88	194.0
6	9.67	5.92	175.0	5.95	180.5	5.90	189.5
7	9.65	5.98	160.5	5.98	182.0	5.91	185.5
8	9.66	5.93	151.5	5.97	179.0	5.91	187.5
9	9.65	5.90	145.5	5.96	166.0	5.91	163.0
10	9.64	5.91	132.5	5.98	154.0	5.92	169.0

^a Raw skim milk as source of casein.

^b The pH of the casein sols was adjusted to 6.10 with 8% phosphoric acid before storage.

^c The pH was readjusted after storage and before heating or making rennet curd tension measurements of unheated casein sols.

^d Rubbery clot.

^e Friable clot.

TABLE VII

INFLUENCE OF SODIUM CITRATE AND HEAT TREATMENT UPON THE
RENNET CURD TENSION AND pH OF 3 PER CENT CASEIN SOLS^a

Salt Added as % Casein (dry basis)	pH		Heat Treatment ^c				
	After Adding Salt	After Holding 16-18 Hrs. at 2° C. ^b	None Curd Tension (grams)	62.2° C. for 30 Min.		71.1° C. for 30 Min.	
				pH	Curd Tension (grams)	pH	Curd Tension (grams)
<u>Trial I</u>							
None	9.64	6.01	206.5	6.01	d	5.99	e
1	9.62	6.05	190.0	6.06	d	5.93	59.5
2	9.57	6.08	171.5	6.10	213.0	5.91	d
3	9.56	6.11	157.5	6.13	218.5	5.97	d
4	9.54	6.15	132.0	6.16	155.0	5.98	101.5
5	9.51	6.15	No clot	6.15	e	6.00	No clot
6	9.49	6.18	No clot	6.15	No clot	6.00	No clot
7	9.49	6.19	No clot	6.14	No clot	6.00	No clot
8	9.48	6.20	No clot	6.14	No clot	5.99	No clot
9	9.48	6.22	No clot	6.17	No clot	6.00	No clot
10	9.48	6.23	No clot	6.18	No clot	6.00	No clot
<u>Trial II</u>							
None	9.67	6.06	195.5	6.03	d	6.00	e
1	9.64	6.06	188.0	6.04	d	5.97	63.0
2	9.62	6.09	175.0	6.07	207.0	5.99	d
3	9.61	6.10	160.5	6.09	200.5	6.00	d
4	9.58	6.12	127.5	6.07	146.0	6.00	96.5
5	9.55	6.14	No clot	6.11	No clot	6.02	No clot
6	9.52	6.17	No clot	6.14	No clot	6.01	No clot
7	9.51	6.15	No clot	6.15	No clot	6.02	No clot
8	9.50	6.19	No clot	6.19	No clot	6.04	No clot
9	9.48	6.20	No clot	6.19	No clot	6.04	No clot
10	9.49	6.21	No clot	6.21	No clot	6.05	No clot

^a Raw skim milk as source of casein.

^b The pH of the casein sols was adjusted to 6.10 with 8% phosphoric acid before storage.

^c The pH was readjusted after storage and before heating or making rennet curd tension measurements of unheated casein sols.

^d Rubbery clot.

^e Friable clot.

tension of casein sols. As the amount of salt increased the sols possessed a bluish hue. The casein sols were more opaque upon heating and less opaque upon subsequent cooling than unheated casein sols as evidenced by visual inspection. This increase of opalescence probably was due to the formation of a colloidal precipitate of casein which redissolved at lower temperatures.

These data show an increased pH at the higher concentrations of the salt in both the heated and unheated casein sols. Heating (62.2° C. for 30 minutes) increased the pH of the casein sols containing over 5% sodium citrate as per cent of the weight of the casein. Higher heat treatment (71.1° C. for 30 minutes) lowered the pH of all casein sols.

The curd tension of unheated casein sols decreased as the concentration of sodium citrate increased until a concentration of 4% of the weight of the casein was reached. Sols containing higher levels of the salt formed no clot when the rennet was added and the casein sol held 1 hour. Heating casein sols 30 minutes at 62.2° C. containing added sodium citrate equal to 2, 3 and 4% of the weight of the casein increased the curd tension. A slight clot with a cloudy whey layer formed at the 5% level, but it was impossible to obtain a reading. Higher levels of salt formed no clot at either heat treatment.

Influence of tribasic sodium phosphate and heat treatment upon the rennet curd tension and pH of 3% casein sols. The data reported in Table VIII shows the influence of tribasic sodium phosphate additions and heat treatment upon the curd tension and pH of 3% casein sols. These data show an increase of the pH after adjusting to 6.10 and storing in a cooled condition. A similar alteration of salt equilibria probably

TABLE VIII

INFLUENCE OF TRIBASIC SODIUM PHOSPHATE AND HEAT TREATMENT UPON THE
RENNET CURD TENSION AND pH OF 3 PER CENT CASEIN SOLS^a

Salt Added as % Casein (dry basis)	pH		Heat Treatment ^c				
	After Adding Salt	After Holding 16-18 Hrs. at 2° C. ^b	None Curd Tension (grams)	62.2° C. for 30 Min.		71.1° C. for 30 Min.	
				pH	Curd Tension (grams)	pH	Curd Tension (grams)
<u>Trial I</u>							
None	9.75	6.11	187.0	6.02	d	5.95	e
1	9.79	6.13	156.5	5.99	d	5.92	e
2	9.78	6.16	147.5	6.05	178.5	5.96	e
3	9.75	6.10	147.5	6.03	153.0	5.91	3.5
4	9.68	6.20	137.5	6.03	152.0	5.86	54.0
5	9.66	6.23	134.5	6.00	140.0	5.85	57.5
6	9.66	6.29	132.0	6.05	149.5	5.88	92.0
7	9.65	6.28	131.5	6.09	135.5	5.90	110.5
8	9.65	6.27	131.0	6.02	127.0	5.88	65.0
9	9.63	6.30	108.0	6.05	113.0	5.88	69.0
10	9.63	6.35	99.5	6.04	107.0	5.90	48.0
<u>Trial II</u>							
None	9.72	6.09	181.5	5.96	d	5.95	e
1	9.73	6.11	150.5	5.96	d	5.94	e
2	9.73	6.10	141.0	5.98	159.0	5.95	4.0
3	9.71	6.12	136.5	6.04	145.5	6.03	46.0
4	9.70	6.15	130.0	6.00	139.0	6.01	55.5
5	9.68	6.13	130.5	6.03	137.5	6.03	56.0
6	9.68	6.15	128.0	6.03	136.5	6.01	81.0
7	9.66	6.17	123.0	6.01	129.5	6.00	94.5
8	9.68	6.17	114.5	6.02	118.0	6.01	91.0
9	9.65	6.20	96.5	6.03	107.0	6.01	83.5
10	9.62	6.23	90.0	6.04	99.0	6.03	71.5

^a Raw skimmilk as source of casein.

^b The pH of the casein sols was adjusted to 6.10 with 8% phosphoric acid before storage.

^c The pH was readjusted after storage and before heating or making rennet curd tension measurements of unheated casein sols.

^d Rubbery clot.

^e Friable clot.

occurred in the casein sols containing added tribasic sodium phosphate that occurred in the sols containing added sodium citrate when stored in a cooled condition. Again, heating caused a lowering of the pH as the temperature of heating increased.

The data show that the curd tension of the unheated sols decreased as the per cent tribasic sodium phosphate increased. Heating the sols 30 minutes at 62.2° C. increased the curd tension while the higher temperature produced a distinct drop of curd tension. Increased concentrations of the salt reduced the curd tension of unheated casein sols.

Influence of magnesium sulfate and heat treatment upon the rennet curd tension and pH of 3% casein sols. Results of the investigation of the effect of magnesium sulfate and heat treatment, reported in Table IX, show only slight changes of pH after cooling 16 to 18 hours. After re-adjusting the pH to 6.10 the rennet curd tension decreased rapidly as the per cent of added magnesium sulfate increased. Higher concentrations of magnesium sulfate within the range studied formed a white precipitate which was expected since saturated magnesium sulfate solutions are known to precipitate certain proteins. Heating the casein sols (62.2° C. for 30 minutes) caused increased precipitation, lowered pH and rennet curd tension.

The data show a drastic reduction of curd tension when the casein sols were heated to 71.1° C. for 30 minutes. The precipitation formed in the sols was resuspended with a Waring blender and pH measurements taken. Soft friable clots and cloudy whey were characteristic of these sols after addition of rennet. Curd tension measurements were not possible in all cases.

TABLE IX

INFLUENCE OF MAGNESIUM SULFATE AND HEAT TREATMENT UPON THE
 RENNET CURD TENSION AND pH OF 3 PER CENT CASEIN SOLS^a

Salt Added as % Casein (dry basis)	pH		Heat Treatment ^c				
	After Adding Salt	After Holding 16-18 Hrs. at 2° C. ^b	None Curd Tension (grams)	62.2° C. for 30 Min.		71.1° C. for 30 Min.	
				pH	Curd Tension (grams)	pH	Curd Tension (grams)
<u>Trial I</u>							
None	9.90	6.07	202.5	5.97	d	5.96	e
1	9.83	6.07	188.0	5.95	d	5.92	e
2	9.80	6.04	162.0	5.93	d	5.88	e
3	9.78	6.06	128.0	5.92	d	5.90	e
4	9.75	6.07	115.0	5.93	d	5.90	4.5
5	9.72	6.00	107.0	5.91	73.5	5.88	6.0
6	9.71	6.06	84.0	5.92	74.5	5.90	6.5
7	9.70	6.01	79.0	5.92	75.5	5.89	7.5
8	9.70	6.00	65.5	5.93	86.5	5.91	7.5
9	9.69	6.03	57.0	5.91	85.5	5.92	7.5
10	9.68	6.00	56.5	5.91	88.0	5.91	8.0
<u>Trial II</u>							
None	9.88	6.05	198.5	5.94	d	5.93	e
1	9.85	6.08	195.0	5.91	d	5.90	e
2	9.83	6.09	135.0	5.92	d	5.91	e
3	9.79	6.06	187.0	5.93	d	5.92	e
4	9.76	6.07	154.0	5.94	8.5	5.92	2.0
5	9.74	6.10	135.0	5.91	63.0	5.92	3.5
6	9.73	6.10	109.5	5.90	69.0	5.90	4.5
7	9.72	6.08	95.5	5.92	76.0	5.92	6.0
8	9.71	6.11	85.0	5.95	71.5	5.94	5.5
9	9.69	6.09	75.0	5.93	79.0	5.94	7.5
10	9.69	6.10	64.5	5.92	75.5	5.92	6.0

^a Raw skim milk as source of casein.

^b The pH of the casein sols was adjusted to 6.10 with 8% phosphoric acid before storage.

^c The pH was readjusted after storage and before heating or making rennet curd tension measurements of unheated casein sols.

^d Rubbery clot.

^e Friable clot.

The influence of whey protein and heat treatment upon the rennet curd tension and pH of 3% casein sols. The influence of whey protein and heat treatment upon the pH and curd tension of 3% casein sols has been investigated (Table X). Holding in a cooled condition had but little effect on the pH of the casein sols. After readjustment heating lowered the pH with slightly greater reductions occurring at the lower levels of whey protein. As with the salts previously mentioned, the higher the heat treatment, the lower the pH becomes.

The data in Table X show that all unheated casein sols formed a hard curd (above 60 g.). Heating (62.2° C. for 30 minutes) lowered the curd tension of all samples. Aggregation of the whey protein due to heating probably influenced this lowering. Heating 30 minutes at 71.1° C. formed a precipitate in all cases. Samples containing 0 and 10% whey protein (dry basis) as per cent of the weight of the casein formed a soft friable clot when rennet was added, but curd tension measurements could not be made. Sols containing higher amounts of whey protein formed a soft rennet clot after heating and resuspending the precipitate with a Waring blender.

Influence of whey protein, heat treatment and added salts upon the pH and rennet curd tension of 3% casein sols. The previous investigations of the effects of separately added salts and heat treatment on the pH and rennet curd tension of 3% casein sols led to a further investigation of the additive effects of these factors on casein sols more nearly approaching the composition of skimmilk. Data reported in Tables XI, XII, XIII and XIV show results of these investigations.

TABLE X

INFLUENCE OF ADDED WHEY PROTEIN (DRY BASIS) AND HEAT TREATMENT UPON
THE RENNET CURD TENSION AND pH OF 3 PER CENT CASEIN SOLS^a

Whey Protein Added as % Casein ^e	pH		Heat Treatment ^c				
	After Adding Whey Protein	After Holding 16-18 Hrs. at 2° C. ^b	None Curd Tension (grams)	62.2° C. for 30 Min.		71.1° C. for 30 Min.	
				pH	Curd Tension (grams)	pH	Curd Tension (grams)
<u>Trial I</u>							
None	10.05	6.09	179.0	6.06	77.5	6.00	d
10	9.65	6.02	119.0	6.02	93.5	6.00	d
12	9.32	6.03	117.5	6.02	111.0	6.00	2.0
14	9.31	5.98	142.5	6.03	122.5	6.02	1.5
16	9.28	6.04	127.0	6.00	120.0	6.03	2.0
18	9.25	6.03	142.5	6.05	129.5	6.00	11.5
20	9.20	6.06	154.0	6.05	138.5	6.04	2.0
22	9.18	6.08	152.5	6.06	137.0	6.05	1.5
24	9.15	6.07	173.0	6.08	154.0	6.05	3.5
<u>Trial II</u>							
None	9.54	6.10	170.0	6.01	10.0	6.00	d
10	9.49	6.09	121.0	5.94	65.5	5.93	d
12	9.46	6.08	129.0	5.93	113.0	5.96	d
14	9.41	6.09	136.5	5.94	119.5	5.97	2.5
16	9.37	6.10	142.5	5.97	129.5	5.98	3.5
18	9.32	6.08	149.0	5.96	130.0	5.98	3.0
20	9.25	6.10	169.5	5.97	140.5	5.97	6.5
22	9.18	6.08	183.5	5.98	134.5	5.98	3.5
24	9.12	6.07	186.0	5.98	143.0	5.98	4.5

^a Reconstituted skim milk powder as source of whey protein.

^b The pH of the casein sols was adjusted to 6.10 with 8% phosphoric acid before storage.

^c The pH was readjusted after storage and before heating or making rennet curd tension measurements of unheated casein sols.

^d Friable clot.

^e Per cent casein calculated on dry basis.

TABLE XI

INFLUENCE OF ADDED WHEY PROTEIN^a AT THE RATE OF 5 PER CENT OF THE WEIGHT OF THE CASEIN (DRY BASIS), HEAT TREATMENT AND ADDED SALTS UPON THE pH AND RENNET CURD TENSION OF 3 PER CENT CASEIN SOLS

Amount of Salt Added as Per Cent of Casein (dry basis)	After Adding Salt	pH After Holding 16-18 Hrs. at 20 C. ^b	Heat Treatment ^c					
			62.2° C. for 30 Min.			71.1° C. for 30 Min.		
			None	Curd	Tension	pH	Curd	Tension
			(grams)					(grams)
Trial I								
None	9.60	6.58	166.5	6.38	47.0	29.0		
5% NaCl	9.55	6.52	142.0	6.39	138.5	100.0		
5% Na ₃ PO ₄	9.73	6.57	130.0	6.43	105.5	38.5		
5% MgSO ₄	9.50	6.49	154.5	6.34	151.5	25.0		
5% NaCl + 5% Na ₃ PO ₄	9.71	6.50	89.5	6.47	95.0	64.0		
5% NaCl + 5% MgSO ₄	9.51	6.40	92.5	6.41	122.0	106.0		
5% Na ₃ PO ₄ + 5% MgSO ₄	9.53	6.52	93.0	6.42	117.5	112.0		

TABLE XII

INFLUENCE OF ADDED WHEY PROTEIN^a AT THE RATE OF 10 PER CENT OF THE WEIGHT OF THE CASEIN (DRY BASIS), HEAT TREATMENT AND ADDED SALTS UPON THE pH AND RENNET CURD TENSION OF 3 PER CENT CASEIN SOLS

Amount of Salt Added as Per Cent of Casein (dry basis)	pH		Heat Treatment ^c			
	After Adding Salt	After Holding 16-18 Hrs. at 20 C. ^b	62.2° C. for 30 Min.		71.1° C. for 30 Min.	
			None	Curd Tension (grams)	Curd Tension (grams)	Curd Tension (grams)
Trial I						
None	9.65	6.60	149.5	6.30	53.0	26.0
5% NaCl	9.58	6.52	119.5	6.32	111.5	58.0
5% Na ₃ PO ₄	9.78	6.58	100.5	6.45	66.0	17.5
5% MgSO ₄	9.55	6.50	96.5	6.34	83.0	1.0
5% NaCl + 5% Na ₃ PO ₄	9.70	6.58	76.5	6.43	55.5	38.5
5% NaCl + 5% MgSO ₄	9.51	6.40	82.0	6.39	101.5	31.0
5% Na ₃ PO ₄ + 5% MgSO ₄	9.53	6.50	71.0	6.43	67.0	38.0

TABLE XII (continued)

INFLUENCE OF ADDED WHEY PROTEIN^a AT THE RATE OF 10 PER CENT OF THE WEIGHT OF THE CASEIN (DRY BASIS), HEAT TREATMENT AND ADDED SALTS UPON THE pH AND RENNET CURD TENSION OF 3 PER CENT CASEIN SOLS

Amount of Salt Added as Per Cent of Casein (dry basis)	After Adding Salt	pH After Holding 16-18 Hrs. at 20° C. ^b	Heat Treatment ^c					
			None		62.2° C. for 30 Min.		71.1° C. for 30 Min.	
			Curd	Tension (grams)	pH	Tension (grams)	pH	Tension (grams)
			Trial II					
None	9.80	6.49	97.5	6.37	6.35	63.5		
5% NaCl	9.71	6.43	125.5	6.40	6.36	105.5		
5% Na ₃ PO ₄	9.90	6.50	47.5	6.48	6.45	51.0		
5% MgSO ₄	9.72	6.43	91.5	6.32	6.30	28.0		
5% NaCl + 5% Na ₃ PO ₄	9.85	6.49	84.0	6.45	6.40	88.0		
5% NaCl + 5% MgSO ₄	9.70	6.38	100.0	6.35	6.32	82.5		
5% Na ₃ PO ₄ + 5% MgSO ₄	9.72	6.45	92.0	6.38	6.40	103.0		

^a Raw skim milk as source of whey protein.

^b The pH of the calcium caseinate sols was adjusted to 6.50 with 8% H₃PO₄ before storage.

^c The pH was readjusted to 6.50 after storage and before heating or making rennet curd tension measurements of unheated sols.

TABLE XIII

INFLUENCE OF ADDED WHEY PROTEIN^a AT THE RATE OF 5 PER CENT OF THE WEIGHT OF THE CASEIN (DRY BASIS), HEAT TREATMENT AND ADDED SALTS UPON THE pH AND RENNET CURD TENSION OF 3 PER CENT CASEIN SOLS

Amount of Salt Added as Per Cent of Casein (dry basis)	After Adding Salt	pH After Holding 16-18 Hrs. at 20 C. ^b	Heat Treatment ^c					
			62.2° C. for 30 Min.		71.1° C. for 30 Min.			
			None	Curd	pH	Tension	pH	Tension
			(grams)		(grams)		(grams)	
Trial I								
None	9.59	6.51	134.5	122.0	6.47	104.5		
5% NaCl	9.52	6.46	140.0	156.0	6.43	167.0		
5% Na ₃ PO ₄	9.71	6.52	90.0	119.5	6.49	117.0		
5% MgSO ₄	9.49	6.46	116.0	148.5	6.41	62.0		
5% NaCl + 5% Na ₃ PO ₄	9.63	6.50	87.5	103.5	6.50	105.0		
5% NaCl + 5% MgSO ₄	9.50	6.43	86.0	109.5	6.37	108.0		
5% Na ₃ PO ₄ + 5% MgSO ₄	9.52	6.48	91.0	127.0	6.41	124.5		

TABLE XIV

INFLUENCE OF ADDED WHEY PROTEIN² AT THE RATE OF 10 PER CENT OF THE WEIGHT OF THE CASEIN (DRY BASIS), HEAT TREATMENT AND ADDED SALTS UPON THE pH AND RENNET CURD TENSION OF 3 PER CENT CASEIN SOLS

Amount of Salt Added as Per Cent of Casein (dry basis)	pH		Heat Treatment ^c					
	After Adding Salt	After Holding 16-18 Hrs. at 20 C. ^b	None		62.20 C. for 30 Min.		71.10 C. for 30 Min.	
			Curd	Tension (grams)	Curd	Tension (grams)	Curd	Tension (grams)
Trial I								
None	9.50	6.43	130.5	6.47	118.5	6.46	87.5	
5% NaCl	9.42	6.40	113.0	6.46	120.0	6.45	117.5	
5% Na ₃ PO ₄	9.62	6.53	72.5	6.48	103.0	6.47	88.5	
5% MgSO ₄	9.38	6.47	106.5	6.45	88.5	6.41	43.5	
5% NaCl + 5% Na ₃ PO ₄	9.54	6.52	89.0	6.50	81.0	6.48	84.0	
5% NaCl + 5% MgSO ₄	9.36	6.46	72.5	6.40	76.5	6.35	62.0	
5% Na ₃ PO ₄ + 5% MgSO ₄	9.37	6.49	77.0	6.47	73.0	6.42	65.5	

Casein sols were prepared containing 5 and 10% whey proteins as per cent of the weight of the casein. Increased amounts of whey protein decreased the rennet curd tension, with lower readings occurring when the whey proteins were obtained from raw skim milk as compared to the casein sols prepared, using reconstituted dried skim milk powder as the source of whey protein. Also, casein sols containing whey protein obtained from reconstituted dried skim milk were the most stable to heat treatments, resulting in higher rennet curd tension measurements.

Casein sols containing sodium chloride as the only added salt, in six out of eight cases, showed an increased curd tension as heat treatment (62.2° C. for 30 minutes) was applied even though increased amounts of whey protein tended to have a retarding effect upon the curd firmness. Casein sols heated 30 minutes at 71.1° C. containing sodium chloride at the rate of 5% of the weight of the casein possessed, in most cases, a relatively high curd tension. These results substantiated the data shown in Table VI which indicated an increased curd tension of heated casein sols containing only added sodium chloride. The curd tension of the casein sols containing whey protein was lower than that of those containing no whey protein. It must be pointed out, however, that pH 6.10 was used when studying the casein sols containing only sodium chloride while pH 6.50 was used when studying those containing both sodium chloride and whey protein.

Addition of sodium chloride caused a drop in the pH of the casein sols. After readjusting the pH to 6.50 and aging at 2° C. the pH again dropped. Heat treatment caused a lowering of the pH but there seemed

to be no definite relationship between the pH changes and the changes of rennet curd tension.

Addition of tribasic sodium phosphate to casein sols containing whey proteins produced a relatively higher pH than sols containing other salts upon cool-aging, and produced softer curd than casein sols containing the same amounts of sodium chloride. Heating (62.2° C. for 30 minutes) lowered the pH and, generally, increased the rennet curd tension. Heating at 71.1° C. for 30 minutes, in most cases, caused a further reduction of pH. This treatment reduced the curd tension below that of those sols heated at 62.2° C. and in some cases below that of the unheated casein sols. Results indicated that changes in the rennet curd tension cannot be explained entirely on the basis of pH changes.

The pH of casein sols containing added magnesium sulfate showed a distinct drop upon aging in a cooled condition. Heating, in most cases, caused a greater reduction of pH in these casein sols than was observed in any of the casein sols studied (Tables XI, XII, XIII and XIV) which was probably due to precipitation.

In five of the eight cases the rennet curd tension was higher for the unheated casein sols which was probably due to the presence of the divalent cation, Mg^{++} . The presence of divalent cations such as Ca^{++} and Mg^{++} in milk are known to cause higher curd tension readings. Heating reduced the rennet curd tension which was due to a combination of factors.

Casein sols containing combinations of the added salts sodium chloride, tribasic sodium phosphate, and magnesium sulfate showed no

significant differences between the rennet curd tension when heated or unheated. Magnesium sulfate lowered the pH more than any other one salt used.



DISCUSSION

The rennet curd tension test has been used throughout this investigation to measure the change of curd firmness of various milks and casein sols when subjected to various treatments. The lowering of the curd tension by homogenization as reported in the data confirms the work of Sanders et al. (54) who believed that the softening effect was due to the dispersion of the fat which acts as a physical hinderance to the aggregation of the casein molecules. Weisberg et al. (67) believed that the softening was due to an increased absorption of the protein by the dispersed fat. Either theory offers a partial explanation of the lowered curd tension shown in the data. All the curd tensions of homogenized milk were below 6 g., sufficiently low for properly homogenized milk.

Heat treatment has long been known to reduce the curd tension of milk. Twelve samples of pasteurized whole milk were studied, and the average curd tension was found to be 42.9 g., a lowering of 29.1% due to pasteurization. Heating skim milk at 62° or 70° C. for 30 minutes lowered the curd tension 10.41 and 27.81%, respectively, below the raw skim milk reading. The reason for this has never been adequately explained, but Doan (17) states that it seems likely that the calcium ion content is decreased, the electrostatic charge of the casein micelles increased, some albumin rendered insoluble and some protein denatured.

The solubility of calcium phosphate is definitely known to be affected by temperature. Tricalcium phosphate decreases in solubility

with a rise in temperature which is contrary to the behavior of most solutes (66). This reduction in soluble calcium offers a partial explanation to the decreased curd tension. To further substantiate this fact Weisberg et al. (67) found that soft curd milk is lower than hard curd milk in both calcium and phosphorous content.

Subsequent cool-aging for 1 and 24 hours of the heated skimmilks reduced the curd tension. Reheating the same samples at 62° C. for 30 minutes after cool-aging 24 hours increased the curd tension slightly, but the higher temperature (70° C. for 30 minutes) lowered the curd tension. The shifts in the pH of both the heated and unheated skimmilks on cool-aging were in harmony with the differences in the rennet curd tension in that a sample having a high pH would be expected to have a lower curd tension. Thus, as the pH increased, the curd tension decreased. Reheating at 62° C. for 30 minutes again lowered the pH and at the same time caused an increased curd tension. At the higher temperature (70° C. for 30 minutes) the pH decreased which was accompanied by a further decrease of curd tension. Effects other than pH changes are involved in these curd tension changes. These are centered around the combination of bases, particularly calcium, with milk proteins, magnesium, citrates and phosphates.

The casein sols prepared in this investigation exhibited some properties of skim milk but were not as representative of skim milk as the casein sol prepared by Grindrod et al. (22), which reacted with rennet like skim milk in that it showed a similar rate of coagulation and curd tension. The present investigation revealed that addition of both whey

protein and sodium salts to 3% casein sols produced a softer curd than casein sols containing only one salt. These curd tension readings resembled those of skim milk when coagulated with rennet but were higher than normal for skim milk. This was probably due to the high per cent of casein in the sols and the lack of other ions such as K^+ , Cu^{++} , Mn^{++} , Zn^{++} and Cl^- normally found in milk. These may form various chemical combinations with casein or each other and thus, influence the rennet coagulation. Other workers (66, 2, 13) have prepared experimental casein sols, some of which more nearly represented the true salt composition of milk than the sols prepared in this investigation.

The casein sols used throughout this investigation were prepared using cold distilled water ($2^\circ C.$) to minimize hydrolysis of the casein when it was subjected to high pH for dispersion. It seems likely that lowering the pH by incorporating salts and phosphoric acid reduces the solubility of casein and forms insoluble milk salts, which condense or aggregate in the presence of protective colloids, the soluble caseinates. Zoller (13) stated that the soluble caseinates are as effective as gelatin and 20 times as effective as gum arabic in preventing the precipitation and flocculation of colloidal gold.

As evidenced by visual inspection, the casein sols turned whiter when the pH was lowered. Some precipitation was observed in the sols containing magnesium sulfate before heat was administered, which indicated that the soluble caseinates were not completely effective in preventing precipitation.

The lowered pH due to heating can be partially explained on the basis of tricalcium phosphate precipitation if tertiary phosphate ions are present. With the removal of the tertiary phosphate the third acid hydrogen must be neutralized as tertiary phosphate ions are removed which confirms the belief of Sommer and Meno (58). These ions were present since phosphoric acid was used to adjust the pH of all casein sols.

The addition of sodium chloride to casein sols reduced the curd tension. It is suggested that this results from a reaction between the sodium chloride and calcium caseinate of the casein sol, by which was formed sodium caseinate (or calcium-sodium caseinate) and calcium chloride. Upon the addition of rennet, the sodium caseinate forms sodium paracaseinate which is soluble and will not form a clot.

The pH of casein sols containing either added sodium citrate or tribasic sodium phosphate increased as the amount of salt increased. Shifts of the salt equilibria caused this increase. Results of work by Tracy and Corbett (61) adding sodium citrate and pyrophosphate to milk supports this observation.

The formation of soluble sodium paracaseinate when the sodium salts were added reduced the rennet curd tension of unheated casein sols. Sodium citrate was more effective in reducing the curd tension than either of the other sodium salts. Heating (62.2° C. for 30 minutes) the casein sols containing the sodium salts increased the curd tension which confirms the results of Tracy and Corbett (61) who added hexametaphosphate to milk before heating and found an increased curd

tension. They thought that this was due to the hydrolysis of a certain portion of the hexametaphosphate which formed the less active dihydrogen phosphate (NaH_2PO_4). Schwartz et al. (55) thought that heat reverted the metaphosphate to orthophosphate. Obviously, a rearrangement of the milk constituents occurred and these phenomena may offer a partial explanation for the changes occurring. A similar change probably occurred in the casein sol containing sodium chloride and heated to the higher temperature (71.1°C. for 30 minutes).

Addition of whey protein at the rate of 10% of the weight of the casein to casein sols adjusted to pH 6.10 and containing no sodium salts reduced the curd tension below casein sols containing no whey protein. As the amount of whey protein increased the curd tension increased until at the level of 24% of the weight of the casein the curd tension reached a point approaching or slightly above that of the casein sols containing no whey protein. At present no satisfactory explanation can be given for these results, but it is thought that the absence of certain salts normally present in milk and the pH may have some influence. It is interesting to note, however, that upon heating, the curd tension decreased as the temperature of heating increased. Denaturation of the whey proteins and the interaction of the calcium and phosphate ions may be related to this decrease.

The present studies of casein sols containing a combination of salts and whey proteins show that increased amounts of whey proteins decreased the remnet curd tension. Greater reductions of curd tension occurred when the whey proteins were obtained from raw skim milk than

when obtained from reconstituted dried skim milk powder. This indicated that the low temperature drying process had no detrimental effect upon the whey protein. Of interest was the fact that casein sols containing whey protein obtained from reconstituted dried skim milk were the most stable to heat treatments and possessed higher curd tensions.

Less precipitation occurred in the casein sols containing whey protein plus added salts at pH 6.50 when heated than in casein sols studied at pH 6.10. The precipitate could be easily dispersed by shaking. As a result the curd tension of all heated casein sols could be measured at this pH. These results, concerning the effect of pH on heat stability, confirms the work of Zittle et al. (71) who reported a twofold influence of pH on the heat stability of the casein complex. They reported that as the pH is raised more calcium is required to precipitate casein and at high pH values phosphate will bind calcium more strongly than at a lower pH. These facts offer an explanation to the firmer clots of the heated sols formed at pH 6.50 since more calcium could be bound at this pH.

The rennet curd tension test appears to be a satisfactory method to measure the efficiency of homogenization. Also, this test will indicate heat treatments of milk which is of importance to the cheese maker in his curd making processes. According to the present findings the avoidance of cool-aging of milks for cheese making is important since this practice will cause the formation of a softer curd. Addition of salts to milks before pasteurization may have some practical significance in the formation of a desirable clot.

SUMMARY

The rennet curd tension test has been used to measure the influence of homogenization, heat treatment, cool-aging and pH on the curd firmness of milks and the influence of whey protein, heat treatment and added salts upon the pH and curd firmness of 3% casein sols.

The additive effects of pasteurization and homogenization reduced the rennet curd tension of raw milk from 60.5 to 4.0 grams.

Heating skimmilk at 62° or 70° C. for 30 minutes lowered the curd tension 10.41 and 27.81%, respectively. Subsequent cool-aging of the heated or freshly separated skimmilks decreased the curd tension and at the same time increased the pH.

Reheating of the skimmilk previously heated at 62° C. and cool-aged 24 hours increased the curd tension and decreased the pH. Reheating skimmilk previously heated at 70° C. for 30 minutes lowered both the pH and curd tension.

The pH of acidified, cool-aged casein sols containing sodium chloride was found to decrease over a 16 to 18 hour period following adjustment, but the pH of the casein sols containing tribasic sodium phosphate and sodium citrate increased. Very little change of pH occurred in those sols containing only whey protein or magnesium sulfate upon aging.

Heating reduced the pH of all casein sols, with greater reductions occurring as the temperature of heating increased.

Addition of sodium chloride, sodium citrate, tribasic sodium phosphate and magnesium sulfate to casein sols reduced the curd tension.

Heating (62.2° or 71.1° C. for 30 minutes) increased the curd tension of casein sols containing 5 to 10% sodium chloride as per cent of the weight of the casein. Heating at 62.2° C. was found to increase the curd tension of casein sols containing sodium citrate or tribasic sodium phosphate. Heating reduced the curd tension of casein sols containing magnesium or whey protein.

Sodium citrate was found to be more effective in reducing the curd tension than either of the other sodium salts used, with no clot forming when the sol contained sodium citrate at a level above 5% of the weight of the casein.

Casein sols containing whey protein plus added salts at pH 6.50 formed clots more nearly resembling a normal clot of skimmilk and were found to be more heat stable than sols adjusted to 6.10.



BIBLIOGRAPHY

BIBLIOGRAPHY

- (1) Alais, C., Mocquot, G., Nitschmann, H., and Zahler, P. Rennet and Its Action on the Casein of Milk. VII. Hydrolysis of Non Protein Nitrogen and Its Relation to the Primary Reaction of Rennet-Induced Curdling of Milk. Helv. Chim. Acta., 36: 1955. 1953. (From Chem. Abstr., 49: 527_a) 1955.
- (2) Albrecht, T. W. Factors in the Response of Milk to Coagulation by Rennin. Ph. D. thesis, University of Wisconsin, 1949.
- (3) Allemann, O. and Schmid, H. The Elasticity of the Coagulum Produced in Cow's Milk by Rennet. Landw. Jahrb. Schweiz., 30: 357. 1916. (From Chem. Abstr., 11: 3343) 1917.
- (4) American Dairy Science Association. Final Report of the Committee on Methods of Determining Curd Tension of Milk. J. Dairy Sci., 21: 353. 1938.
- (5) Beau, M. Casein and Rennet. Lait, 21: 113. 1941. (From Chem. Abstr., 37: 2350₆) 1943.
- (6) Berridge, N. J. Second Phase of Rennet Coagulation. Nature, 149: 194. 1942.
- (7) Berry, M. H. Soft Curd Milk Studies. Milk Plant Monthly, 23 (10): 30. 1934.
- (8) Berry, M. H. Soft Curd Milk Studies. Md. Agr. Expt. Sta., Bull. 388. 1935.
- (9) Bosworth, A. W. The Action of Rennin on Casein. N. Y. (Geneva) Agr. Expt. Sta., Tech. Bull. 31. 1913.
- (10) Bosworth, A. W. and Van Slyke, L. L. I. Why Sodium Citrate Prevents Curdling of Milk by Rennin. N. Y. (Geneva) Agr. Expt. Sta., Tech. Bull. 34. 1914.
- (11) Cherbuliez, E. and Baudet, P. Studies on Casein. V. The Components of Casein. Helv. Chim. Acta., 33: 398. 1950. (From Chem. Abstr., 44: 5403_a) 1950.
- (12) Cherbuliez, E. and Baudet, P. Studies on Casein. VI. The Transformation of Casein to Paracasein. Helv. Chim. Acta., 33: 1673. 1950. (From Chem. Abstr., 45: 2033_d) 1951.
- (13) Clark, W. M. Synthetic Milk as a Basis for Research. J. Dairy Sci., 10: 195. 1927.

- (14) Curd Tension Meter. Cherry-Burrell Corporation Bull. IP5132-M. Nov. 1955.
- (15) Davies, W. L. The Chemistry of Milk. Chapman and Hall, Ltd. London. p. 322. 1936.
- (16) Doan, F. J. and Welch, R. G. Soft Curd Milk. Pa. Agr. Expt. Sta., Bull. 312. 1934.
- (17) Doan, F. J. Soft Curd Milk. J. Dairy Sci., 21: 739. 1938.
- (18) Edmondson, L. F. and Tarassuk, N. P. Studies on the Colloidal Proteins of Skimmilk. II. The Effect of Heat and Disodium Phosphate on the Composition of the Casein Complex. J. Dairy Sci., 39: 123. 1956.
- (19) Eilers, H., Saal, R. N. J. and van der Waarden, M. Monographs on the Progress of Research in Holland During the War. Chemical and Physical Investigations on Dairy Products. Elsevier Publishing Co., Inc. p. 94. 1947.
- (20) Espe, D. L., and Dye, J. A. Effect of Curd Tension on the Digestibility of Milk. Am. J. Dis. Child., 43: 62. 1932.
- (21) Gould, I. A. Effect of Heat on Milk Constituents. (Abstr.) J. Dairy Sci., 35: 139. 1952.
- (22) Grindrod, P. E., Price, W. V. and Sommer, H. H. Synthetic Milk for the Study of Cheese Making. I. Preparation of a Casein Sol in Semblance of Skimmilk. J. Dairy Sci., 39: 499. 1956.
- (23) Hankinson, C. L. The Preparation of Crystalline Renmin. J. Dairy Sci., 26: 53. 1943.
- (24) Harland, H. A. and Ashworth, U. S. A Rapid Method for Estimation of Whey Proteins as an Indication of Baking Quality of Nonfat Dry Milk Solids. Food Research, 12: 247. 1947.
- (25) Harland, H. A. and Ashworth, U. S. The Preparation and Effect of Heat Treatment on the Whey Proteins of Milk. J. Dairy Sci., 28: 879. 1945.
- (26) Heinemann, B. Influence of Cottage Cheese Starter Organisms on Strength of Curd at Time of Cutting. J. Dairy Sci., 40: 437. 1957.
- (27) Hill, R. L. A Decade and a Half of Soft Curd Milk Studies. Utah Agr. Expt. Sta., Circ. 101. 1933.

- (28) Hill, R. L. A Test for Determining the Character of Curd from Cow's Milk and Its Application to the Study of Curd Variance as an Index to the Food Value of Milk for Infants. J. Dairy Sci., 6: 509. 1923.
- (29) Hill, R. L. Soft Curd Milk. Utah Agr. Expt. Sta., Bull. 227. 1931.
- (30) Holland, R. F. and Dahlberg, A. C. The Effect of the Time and Temperature of Pasteurization Upon Some of the Properties and Constituents of Milk. N. Y. (Geneva) Agr. Expt. Sta., Tech. Bull. 254. 1940.
- (31) Holm, G. E., Webb, B. H., and Deysher, F. F. The Heat Coagulation of Milk. I. Variations in the Compositions, Heat Stability and Other Tests of Milks from Four Cows During the Course of a Lactation Period. J. Dairy Sci., 15: 331. 1932.
- (32) Hostettler, H. and Ruegger, H. R. The Rennet Coagulation of Acid Casein with Observations on the Rennet Coagulation of Milk. Landw. Jahrb. Schweiz., 64: 667. 1950. (From Chem. Abstr., 45: 2597^e) 1951.
- (33) Kelley, L. A. The Factors Affecting Rennet Coagulation Time and Rennet Curd Tension. Ph. D. thesis, University of Wisconsin. 1951.
- (34) Larson, B. L. and Roller, G. D. Heat Denaturation of the Specific Serum Proteins in Milk. J. Dairy Sci., 38: 351. 1955.
- (35) Ling, E. R. The Composition of Milk and Whey with Special Reference to the Partition of Calcium and Phosphorous. J. Dairy Research, 8: 173. 1937.
- (36) Magee, H. E. and Harvey, D. Studies of the Effect of Heat on Milk. I. Some Physiochemical Changes Induced by Heat. Biochem. J., 20: 873. 1926.
- (37) Mattick, E. C. V. and Hallett, H. S. Effect of Heat on Milk (A) On the Coagulability of Rennet (B) On the Nitrogen, Phosphorous and Calcium Content. J. Agr. Sci., 19: 452. 1929.
- (38) Mauri, C. Rennin. I. The Effect of Heating on the Coagulability of Casein. Biochem. Z., 173: 363. 1926. (From Chem. Abstr., 21: 423) 1927.
- (39) Maze, P. Influence of Sodium Chloride on the Coagulant Power of Rennin. Compt. Rend. Soc. Biol., 134: 425. 1940. (From Chem. Abstr., 36: 833^o). 1942.

- (40) Maze, P. and Maze, P. J., Jr. Influence of Mineral Salts on the Coagulating Activity of Rennin. Compt. Rend. Soc. Biol., 135: 808. 1941. (From Chem. Abstr., 37: 3828₉) 1943.
- (41) Merrill, D. W. Evaluating the Heat Treatment of Skimmilk in the Manufacture of Nonfat Dry Milk Solids. Milk Products J., 46 (7): 56. 1955.
- (42) Moir, G. M. The Effect of Heat on Rennin Coagulation. J. Dairy Research, 2: 68. 1932.
- (43) Nitschmann, H. and Lehmann, W. Electrophoretic Differentiation Between Acid Casein and Labcasein. Experientia, 3: 153. 1947. (From Chem. Abstr., 41: 5562_f) 1947.
- (44) Northrop, J. L. Crystalline Pepsin. Science, 69: 580. 1929.
- (45) Pishawikar, S. D. and Kamat, D. N. Effect of Halogen-Ion Concentration on Milk Coagulation by Pepsin. Current Sci., 25 (8): 263. 1956. (From Chem. Abstr., 51: 2196_d) 1957.
- (46) Powell, M. E. Effects of the Time and Temperature of Holding Milk Heat-Treated at Various Temperatures Upon Its Subsequent Coagulation by Rennet. J. Dairy Sci., 19: 305. 1936.
- (47) Pyne, G. T. Casein Heterogeneity and the Rennet Coagulation. Chem. and Ind., (13) 171. 1951.
- (48) Pyne, G. T. Calcium Salts and the Rennet Coagulation of Milk. Chem. and Ind., (9) 302. 1953.
- (49) Ramsdell, G. A. and Whittier, E. O. The Effect of Heat on the Albumin and Globulin in Milk. J. Dairy Sci., 36: 437. 1953.
- (50) Rapp, H. and Calbert, H. E. Influence of the Bulk Handling of Raw Milk on Its Rennet Coagulation Time. (Abstr.) J. Dairy Sci., 37: 637. 1954.
- (51) Rogers, L. A. Associates of Fundamentals of Dairy Science. The Chemical Catalog Company, Inc. New York. p. 188. 1928.
- (52) Rowland, S. J. The Heat Denaturation of Albumin and Globulin in Milk. J. Dairy Research, 5: 46. 1933.
- (53) Rupp, P. Chemical Changes Produced in Cow's Milk by Pasteurization. U. S. Dept. of Agr. Bur. of Animal Ind., Bull. 166. 1913.
- (54) Sanders, G. P., Matheson, K. J. and Burkey, L. A. Curd Tension of Milk and Its Relationship to Firmness of Curd in Cheese Making. J. Dairy Sci., 19: 395. 1936.

- (55) Schwartz, C., Jones, K. K., Mack, T. W., and Vance, R. W. The Use of Sodium Metaphosphate for the Preparation of Soft-Curd Milk. J. Dairy Sci., 23: 19. 1940.
- (56) Shahani, K. M. and Sommer, H. H. The Protein and Non-Protein Nitrogen Fractions in Milk. I. Methods of Analysis. J. Dairy Sci., 34: 1003. 1951.
- (57) Sommer, H. H. Dairy Chemistry. (mimeographed). 1950.
- (58) Sommer, H. H. and Menos, J. The Effect of Dilution on the Titratable Acidity of Cow's Milk. J. Dairy Sci., 14: 136. 1931.
- (59) Spur, B. and Wolman, I. J. Peptic-Tryptic Digestion on Rennet-Custards in Vitro. J. Dairy Sci., 30: 13. 1947.
- (60) Tauber, H. and Kleiner, I. S. The Purification of Rennin and Its Separation from Pepsin. J. Biol. Chem., 96: 745. 1932.
- (61) Tracy, P. H. and Corbett, W. J. Reduction of Curd Tension of Milk by the Addition of Sodium Salts. J. Dairy Sci., 23: 289. 1940.
- (62) van der Burg, P. The Condition of Casein in Milk. Netherlands Milk Dairy J., 1: 69. 1947.
- (63) Van Slyke, L. L. and Baker, J. C. The Preparation of Pure Casein. J. Biol. Chem., 35: 127. 1918.
- (64) Van Slyke, L. L. and Bosworth, A. W. Conditions of Casein and Salts in Milk. J. Biol. Chem., 20: 135. 1915.
- (65) Van Slyke, L. L. and Bosworth, A. W. Studies Relating to the Chemistry of Milk and Casein. N. Y. (Geneva) Agr. Expt. Sta., Tech. Bull. 37. 1914.
- (66) Verma, I. S. A Study of the State of Solution of the Naturally Occurring Salts in Milk. Ph. D. thesis, University of Wisconsin. 1950.
- (67) Weisburg, S. M., Johnson, A. H., McCollum, E. V. Laboratory Studies on the Chemistry of Soft Curd Milk. J. Dairy Sci., 16: 225. 1933.
- (68) Welch, R. C. and Doan, F. J. Soft Curd Milk and Mastitis. Milk Plant Monthly, 22 (11): 30. 1933.
- (69) Wright, C. N. The Action of Rennet and of Heat on Milk. Biochem. J., 18: 245. 1924.

- (70) Zittle, C. A. Factors Influencing the Stability of the Milk Proteins. Report of Proceedings Conference on Milk Concentrates p. 1. March 1957.
- (71) Zittle, C. A., Dellamonica, E. S. and Custer, J. H. Effect of Certain Salts on Precipitation of Casein by Calcium Chloride and Heat. J. Dairy Sci., 40: 280. 1957.
- (72) Zittle, C. A., Dellamonica, E. C. and Custer, J. H. Precipitation of Calcium Caseinate by Heat and Subsequent Reversal. J. Dairy Sci., 39: 1651. 1956.
- (73) Zoller, F. H. The Buoying up of the Equilibrium of Milk Salts During Heat Treatment. Science, 52: 614. 1920.

