INFLUENCE OF MASTITIS ON CURD STRENGTH OF MILK

A Thesis

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ABSTRACT

Influence of Mastitis on Curd Strength of Milk. (August 1970)

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The effect of dialysis, calcium chloride, and low heat nonfat dry milk on the curd strength of abnormal milk was investigated, using quarter samples from cows in the University herd. Samples were selected to obtain a Wisconsin Mastitis Test negative (WMT<10 mm) and positive (WMT≥20 mm) sample for each cow. Curd tension was determined initially and after dialysis of 1:10 v/v ratio of positive to negative milk for 36 hr at 4 C. Skimmilk from the WMT positive and negative samples was dialyzed at a positive to negative ratio of 1:10 v/v in a multiple dialyzer at 4 C for 6 to 36 hours. Varying concentrations of $CaCl_2$ and nonfat dry milk were added to skimmilk before and after pasteurization. Mineral analysis was determined by atomic absorption and auto analyzer techniques. Acid produced curd firmness in pasteurized skimmilk was determined, using single-strain lactic cultures.

Initial curd tension of the WMT positive samples was approximately 45% lower than the negative samples. Curd tension of the positive samples was increased, and the negative samples decreased by dialysis. The difference after dialysis was less than 20%. Dialysis caused an increase in the curd firmness of the positive skimmilk and a decrease in the curd firmness of the negative skimmilk.

Initial curd firmness of the positive samples was approximately 70% lower than the negative samples. The difference after dialysis was less than 20%. The major change occurred in the first 6 hr of dialysis. Changes in curd firmness appeared to be associated with partial equilibration of unbound calcium and inorganic phosphorous. Curd firmness in the positive skimmilk was increased by the addition of $CaCl_2$ and decreased by fortification with nonfat dry milk. The combined addition of $CaCl_2$ and nonfat dry milk caused variable changes in curd firmness.

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INTRODUCTION

The problem of mastitis is of major importance to the dairy industry. Inflammation of the mammary glands, due primarily to Streptococcus agalatiae and Staphylococcus aureus, causes decreases in milk production and changes the composition and properties of milk. The cost to producers, due to losses in milk production and decreases in milk fat and total solids content, amounts to millions of dollars annually. Losses to the manufacturer because of changes in composition and properties are no doubt excessive. In addition to these losses, it is not known how much mastitis or abnormal milk costs the dairy industry through customers lost because of inferior products.

Extensive research has shown that milk fat, solids-not-fat, casein, and total solids contents of milk are decreased, while the whey protein and chloride contents are increased by mastitis. Limited research has been conducted on the effect of mastitis on the properties of milk. Recent research has indicated that mastitis alters the curd forming properties of milk. Curd tension of milk decreases as the Wisconsin Mastitis Test (WMT) values increase. Production of acid by lactic cultures is slower in WMT positive skimmilk, and the strength of coagulum is much weaker than that formed in WMT negative skimmilk. It has been suggested that these changes in curd forming

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properties might adversely affect the suitability of milk for manufacture of certain cultured products.

The causes for changes in curd forming properties of mastitic milk have not been investigated extensively. Mastitis is known to affect mineral balance, pH, and casein content of milk. These factors influence the coagulation properties of milk and could partially explain the decrease in curd strength of abnormal milk. The objectives of this study were to investigate causes for the decrease in curd strength and to explore means of restoring the curd forming properties.

REVIEW OF LITERATURE

Mastitis is an inflammation of the udder caused primarily by Streptococcus agalactiae and Staphylococcus aureus. It results in costly decreases in milk production and changes in the composition and properties of milk. Much research has been devoted to the effect of mastitis on milk production and gross composition. Limited attention has been given to the influence of mastitis on the minor constituents and properties of milk. This review deals with the effect of mastitis on production, composition, and properties, with emphasis on changes in curd forming characteristics.

Effect on Production and Yield

Numerous studies (3, 17, 23, 29, 35, 36, 37, 40, 43, 45, 56, 61, 70, 72 , 73, 78, 81, 83, 94, 100, 119, 120, 121, 126) reveal that mastitis causes a definite decrease in milk production. Shaw and Beam (100) studied the effect of mastitis on milk production and reported a 22% decrease. McLeod and Wilson (70) found that mastitis resulted in a 24% loss. Rowland et al. (94) found that milk yield was lowered approximately 15% due to udder infection and did not return to normal after the infection disappeared. There was no apparent recovery in milk yield until the following lactation; and even at that time, the quarters with prior infection gave approximately 10% less milk than the quarters that had not been infected. O'Donovan et al. (81) reported a depression of 10% in lactation yield due to mastitis, but they suggested that the reduction in yield would have been greater in the absence of antibiotic therapy.

Wheelock et al. (126) concluded that the decrease in production is related to the severity of udder infection. In relating the California Mastitis Test (CMT) reactions to milk production, Forster et al. (36) reported average decreases in yield of 9, 20, 32, and 43% for CMT trace (T), 1, 2, and 3, respectively. Philpot (83) reported reductions of approximately 3, 11, 26, and 46% in quarters with CMT reactions of T, 1, 2, and 3. In trials where _Aerobacter aerogenes_ were induced to produce acute mastitis, Carroll et al. (17) reported that a reduction in milk yield occurred, but production returned to normal within a two-week period. Hansson (45) suggested that when milk secretion ceased in one quarter, there was a tendency for the remaining quarter to increase in yield to compensate for the loss; but the compensation was not complete. Crossman et al. (23) found that individual quarters infected with mastitis show little recovery in proportionate yield even after treatment. Not until the following lactation could complete recovery be observed.

Total Solids. It has been established (5, 6, 7, 68) that mastitis causes a decrease in the total solids content of milk. The extent of the decrease is related to the severity of the infection. Ashworth and Blosser (5) studied the relationship between the CMT reaction and chemical composition of milk from opposite quarters and concluded that there is a consistent inverse relationship between the CMT reaction level and total solids content.

Milk Fat. There are conflicting reports on the effect of mastitis on milk fat, but the majority indicate that mastitis causes a decrease in fat content (5, 6, 22, 51, 79, 88, 114, 119, 122). Ashworth et al. (6) found that subclinical mastitis causes a decrease in fat content of 0.09 to 0.45%, depending upon the severity of infection. Walsh and Neave (122) found that udder infection reduced the percentage of fat by about 0.07%. Natzke et al. (79) reported that fat percentage of milk from infected quarters was higher than from normal quarters. Tanahashi (114) found that there was not a significant size difference in fat globules, but there was a proportional increase in globules less than 2μ in diameter in mastitic milk.

Solids-Not-Fat. There are numerous reports (1, 5, 6, 7, 22, 24, 68, 69, 79, 81, 92, 95, 118, 119, 122) showing that the solids-not-fat content of milk is lowered by mastitis. Alexander and Leech (1) reported that clinical mastitis caused a 0.19% decrease in the solids-not-fat content. McDowall (68) reported a decrease in solids-not-fat content of 0.31% in mastitic milk. Rowland (92) reported that mastitis caused a decrease in the solids-not-fat content of the affected quarters to an extent depending on the severity of the disease.

Proteins. Several reports (4, 5, 6, 14, 16, 17, 18, 21, 40, 46, 48, 54, 59, 60, 62, 63, 75, 79, 93, 103) show that mastitis causes an increase in the total protein content in milk. Ashworth et al. (4) reported that the increase was due to seepage of serum proteins from the blood system through the tissue of the damaged udder.

Heeschen (46) and Jones (49) found that the whey protein content is increased in abnormal milk. Lecce and Legates (62) concluded that the blood-serum albumin content of milk increases as the severity of the mastitic reaction increases.

Numerous researchers (4, 21, 24, 40, 54, 59, 60, 68, 75, 93, 119) have shown that mastitis causes a decrease in the casein content of milk. Ashworth (4) found that there was an inverse relationship between CMT reactions and casein content. Christ (21) reported a decrease in the β-casein fractions in mastitic milk. Nagasawa and Tanahashi (75), using electrophoretic studies on proteins in the milk of cows with mastitis, found increases in γ-casein, serum albumin, and immune globulins, and decreases in β-casein in mastitic milk. Rowland (93) reported that milk from cows with clinical mastitis is low in casein and has a higher content of albumin, globulin, and proteose-peptone substances, particularly globulin. Carroll (16) and Kisza (60) observed an increase in immune globulin and bovine serum albumin, while β-lactoglobulin and α-lactalbumin remained the same in mastitic milk.

Carbohydrates. Many studies (5, 6, 7, 18, 19, 22, 34, 48, 68, 79, 89, 90, 118, 122, 127) have shown that a definite decrease in lactose content of milk occurs due to mastitis. A decrease of approximately 35% in lactose content of milk has been reported by Ashworth and Blosser (5) and Cecil et al. (18, 19).

Some work (11, 18, 19) has shown that mastitis causes an increase in glycogen content of milk. Bitman et al. (11) stated that the glycogen response appeared to be correlated with leucocytic invasion. Cecil et al. (18, 19) reported that the glycogen concentration increased nine times in infected quarters. Mastitic milk yielded larger amounts of anthrone-positive material of which about 70% was glucose. Rosaschino (90) found little difference between normal and abnormal milk in the contents of nonglucosaminic polysaccharides or of mucopolysaccharides soluble in perchloric acid. Walsh and Neave (122) reported that the reductions of lactose content were significantly greater when infection was due to streptococci than to staphlococci.

Minerals. Many studies (2, 6, 11, 18, 19, 22, 47, 57, 68, 79, 86, 89, 102, 112, 118, 127) have been conducted on the influence of mastitis on the different minerals of milk. Pyne (85) stated that the practical importance of the milk salts arises largely due to their influence on milk proteins, especially casein. Relatively insignificant variations in the salt composition of milk can often cause quite disproportionate effects on the milk proteins. Allen (2) reported increases in the chloride and sodium contents and decreases in potassium, magnesium, calcium, and phosphate contents in mastitic milk.

Many researchers (6, 11, 18, 19, 47, 57, 79, 86, 118, 127) have shown an increase in the chloride content of mastitic milk. Bitman et al. (11) reported that sodium and chloride increased greatly and potassium decreased when mastitis was induced. Imamura et al. (47) reported similar increases in sodium and chloride and decreases in calcium, potassium, phosphorous and molybdenum in mastitic milk. Kisza et al. (57) found similar results and also reported a lower content of magnesium in mastitic milk. Tallamy and Randolph (113) found that total potassium, inorganic phosphorous, and calcium concentrations were lower; and total concentrations of iron, copper, zinc, magnesium, and sodium were higher in WMT positive samples. Unbound calcium and inorganic phosphorous concentrations were significantly lower in WMT positive milk. Whittier (129) concluded that the solubility of calcium phosphates in milk is affected by the amount of citrates present and that this effect is probably specific. White and Davies (127) stated that the increase in concentrations of sodium and chloride in mastitic milk is due to low total solids as a result of a low lactose content. They found that subclinical mastitic milk contained less citric acid and phosphorous than samples from bulk milk of a herd. The decrease in phosphorous was mainly in the soluble fraction, whereas the concentration of both colloidal and soluble citric acid was low. Wheelock et al. (125) stated that milk appears to be an osmotic equilibrium with blood flowing continuously through the udder during the period the milk remains within the udder. Milk secretion causes a slight alteration in the osmotic pressure of fluids within the immediate locality of the mammary gland.

Minor Constituents. Limited research has been devoted to the influence of mastitis on the minor constituents of milk. There are varying reports (64, 65, 66, 75, 76, 78) indicating that catalase activity increases during mastitic infection. Luedecke (64), Marquardt et al. (66), and Natzke et al. (78) reported that catalase activity increases as the level of the CMT reaction increases. Nageswarao et al. (77) reported that more free catalase is present in mastitic than in normal milk.

There are reports (13, 64, 65, 66, 74) that A-esterase activity is higher in mastitic milk. Booth et al. (13) reported an increase in A-esterase activity of milk from cows exposed to Streptococcus agalactiae. This increase was closely associated with increases in leucocyte numbers and blood serum albumin levels. The A-esterase was thought to come directly from the blood during mastitic infection. Luedecke (64) and Marquardt et al. (65, 66) reported that A-esterase activity increased as the CMT reaction increased.

Mullen (74) found that mastitic milk has increased acid phosphatase titers. The highest values were shown when the infection was due to Streptococcus agalactiae. Neuman et al. (80) reported increased glutamic oxaloacetic transaminase and glutamic-pyruvic transaminase activities in mastitic milk.

Some work (18, 19, 131) indicates that mastitis causes an increase in the histamine content of milk. However, Bitman et al. (11) reported that there was a decrease in histamine concentrations of milk when mastitis was induced.

Rosaschino (90) reported increases in the sialic acid content of mastitic milk. The values for normal, chronic, and acute mastitis were 29.42, 30.75, and 48.41 mg per 100 ml, respectively.

As with the other minor constituents, very little work has been conducted on the effects of mastitis on vitamins in milk. Kisza et al. (58) found that there were higher concentrations of Vitamin A and particularly β-carotene in milk fat from cows with acute mastitis. The contents of thiamine, riboflavin, and ascorbic acid were lower in mastitic milk. These changes were less pronounced in chronic than acute mastitic cases.

Effect on Properties

pH. Numerous studies (5, 6, 7, 9, 16, 18, 20, 29, 32, 48, 51, 63, 68, 104, 106, 128) have shown that the pH of milk increases as the severity of the infection of mastitis increases. Ashworth and Blosser (5) found an increase of 0.186 pH units in the CMT positive milks compared to normal milk. Carroll (16) and Cecil et al. (18) found approximately the same results with mastitic milk increasing 0.2 pH units over normal milk. Cherrington et al. (20) reported that milk from cows with severe mastitic infection was usually neutral or slightly alkaline in reaction. Little et al. (63) reported similar results. Ashworth and Nebe (9) and White and Davies (128) reported that high pH values associated with subclinical mastitic milks may contribute to slow rennet coagulation time of milk.

Buffering Capacity. Lane et al. (61) studied the buffer capacity of Wisconsin Mastitis Test negative and positive quarter samples from individual cows. Maximum buffer capacity of the mastitic samples was observed in a slightly higher pH range. This shift appeared to be due to the higher initial pH of the positive samples. They concluded that mastitis does not significantly alter the buffer capacity of milk.

Heat Stability. There is little work available on the effect of mastitis on heat stability. Feagan et al. (32, 33), Kiermeier et al. (51) and Rose (91) concluded that the pH and mineral content of milk are the major factors that influence heat stability of milk. Feagan et al. (32, 33) studied heat stability of fluid milk and skimmilk and skimmilk powder and concluded that alteration of heat stability of milk was due mainly to the presence of milk from cows with subclinical mastitis.

Hydrolytic Rancidity. Guthrie and Herrington (38) studied the lipase activity of milk and concluded that mastitis seems to be a major factor in the rancidity problem. Tallamy and Randolph (112) reported that initial acid degree values for mastitic samples were 35 to 40% higher than for normal milk samples. Lipase activity of mastitic milk was approximately 7% higher than in normal milk.

Inhibition Titers. The influence of mastitis on the lactic culture inhibitory activity and inhibition titers was studied by Randolph (87). Mastitic positive skimmilks showed inhibition titers for cultures, Streptococcus cremoris R1 and HP, of approximately twice the magnitude as negative skimmilk. Mastitis did not affect the growth of Streptococcus lactis C2.

Rennet Coagulation. Considerable work is available (55, 68, 97, 104, 105, 106, 128) to show that mastitis causes increased rennet coagulation time. Sorokina and Slivko (106) reported that the rennet coagulation time for mastitic milk was 42 min 17 sec compared to 13 min 50 sec for normal milk. The growth of Streptococcus lactis, Streptococcus cremoris, Lactobacillus bulgaricus, and Lactobacillus acidophilus was retarded by the presence of mastitic milk, but growth of Streptococcus thermophilus was not affected. Sommer and Matsen (105) studied the relationship of mastitis to the rennet coagulability and curd strength of milk. They reported that even when mastitis was sufficiently mild so that the milk was still normal to casual observation, it usually caused a significant increase in the coagulation time of the milk. The coagulation time for mastitic milk was 41.69 min as compared to 9.53 min for normal milk. White and Davies (128) found that changes in chemical composition of sub-clinical mastitic milks were related to slow coagulation. The chemical composition of this milk indicated that it might be due to high pH values. They indicated that the slow coagulation was not due to a deficiency of calcium phosphate in the caseinate complex but suggested that it might have been caused by a low concentration of the complex. Kelley et al. (50) reported that rennet coagulation time was markedly affected by such factors as chemical composition and casein content. Kisza and Sobina (60) stated that the changes in the protein patterns in mastitic milk are considered to be the reason for the unsatisfactory renneting fermentation. Sebela and Suss (99) concluded that there was a significant correlation between the rennet time and protein content, acidity, and calcium content of the whey. There was less related significance between milk fat and total calcium content and rennet time.

Curd Tension. Many reports (41, 42, 44, 52, 60, 68, 97, 105, 106, 108, 111, 123, 124, 128) are available showing that mastitis causes a decrease in the curd tension of milk. Halversen et al. (41) reported that acute mastitis often destroys the coagulating power of the milk and subclinical mastitis lowers the curd tension. Hansen et al. (44) studied the influence of mastitis on curd tension of milk and reported that a definite decrease occurred in mastitic milk as compared to normal milk. They found that mastitis caused by streptococci infection lowers the curd tension more than mastitis caused by staphylococci infection. Sanders et al. (97) reported that the curd of mastitic milk was usually but not always soft. However, some soft curd milks showed no evidence of mastitis. Tallamy et al. (111) reported that mastitis caused a 35% decrease in the curd tension of milk. This effect was more pronounced in milk from cows where the curd tension of the control was high. Wiesberg et al. (123) studied the properties of soft curd milk and concluded that the factors prevalent with mastitis are associated with soft curd. Welch and Doan (124) concluded that the probable reason for lowered curd tension in mastitic milk was the lower casein content and a salt balance changed by the disease. White and Davies (128) postulated that the softness of the coagula from subclinical mastitic milks was not caused by a deficiency of calcium phosphate in the caseinate complex but by a low concentration of the complex.

Acid Production and Curd Firmness. A very limited amount of work (42, 82, 84, 96) is available on the influence of mastitis on curd firmness and acid production. Hampton and Randolph (42) reported that mastitis caused a 70% decrease in strength of coagulum of skimmilk, and acid production was slower in WMT positive samples. This decrease in the rate of acid production and curd firmness of mastitic milk was similar to the influence of certain casein phenotypes (96). Sadler et al. (96) found that the casein phenotype significantly influences acid production and curd toughness in skimmilk. Pitkin et al. (84) studied the significance of lactic acid levels in mastitic and nonmastitic milk. A correlation was found between lactic acid level and leucocyte counts.

Product Manufacture. There is insufficient research available on the effect of mastitis on the manufacture of dairy products. The major research (15, 42, 43, 52, 53, 55, 60, 98, 106, 110) shows that use of mastitic milk in dairy products results in a poor quality product. Brus et al. (15) compared batches of butter prepared from milks with a low and a high cell count. They found that butter made from mastitic milk was inferior due to an oxidized taste. The amount of copper in butter made from mastitic milk was greater than in the butter made from normal milk. However, they did not find any difference in the copper content between mastitic and normal milks. They concluded that the copper in mastitic milk migrates to the butter more than in normal milk during manufacturing. Szakaly (110) found that mastitic cream showed slower acid development, had various flavor defects, and needed 30 to 37% longer churning time to reach 2 to 3 mm grain size. Butter made from mastitic cream was distinctly inferior in quality and during storage developed aroma and flavor defects.

Kiermeier and Keis (52) found that the use of mastitic milk in cheesemaking reduced the amount and rate of whey drainage from the curd. In another study Kiermeier and Keis (53) found that butter and cheese made from milk with the strongest mastitic values had the lowest quality. A later study by Kiermeier et al. (55) and Sorokina et al. (106) showed that cheese made from mastitic milk was also of inferior quality compared to cheese from normal milk. Schott (98) reported that yoghurt manufactured from mastitic milk was slightly yellow, but it did not differ organoleptically from the control milk yoghurt.

Factors Affecting Curd Strength

Mastitis. It has been shown that mastitis has a detrimental effect on curd tension and curd firmness of milk and skimmilk (42, 44, 111, 124). Hampton and Randolph (42) studied this specific problem and concluded that mastitis lowers the strength of coagulum of skimmilk. Tallamy et al. (111) reported that curd tension was lowered approximately 35% due to mastitis.

pH. There is a limited amount of work (9, 50, 71, 97, 109, 124) available comparing the relationship of pH to curd strength. Ashworth and Nebe (9) reported that when the pH of milk is lowered either by the slow addition of acid or by starter development, there is a marked increase in rennet curd tension. After reaching a maximum value, the curd tension decreases with further acidity development. Medrat and Ashworth (71) reported a correlation coefficient of 0.98 between increasing curd tension and decreasing pH.

Pretreatments. The pretreatment of whole milk or skimmilk has been shown to have a definite effect on curd strength (26). One pretreatment that influences curd strength is storage and processing temperature variations. Most reports (8, 12, 26, 27, 30, 39, 50, 67) show that extreme changes in temperature cause decreases in curd strength of the coagulum. Dill and Roberts (26) reported that curd tension was reduced when reconstituted skimmilk was heated to 165 F for 30 minutes. Doan (27), Eisele and Budny (30), and Hadary and Sommer (39) reported similar results with the pasteurization of milk causing a decrease in the firmness of the curd. Eisele and Budny (30) reported that slow freezing lowers curd firmness. Tessier and Rose (115) reported that heating of skimmilk causes precipitation of calcium phosphate and lowers the calcium ion concentration. Zittle et al. (130) reported that casein is precipitated by the heating of milk. This has been shown to have a detrimental effect on curd tension.

Another pretreatment is the preacidification of skimmilk. Baker and Stoll (10) reported that preacidification weakened the curd but increased the acid production time.

Homogenization is another pretreatment which has an effect on the curd strength of milk and skimmilk (27, 30, 56). There is some controversy on what actually happens upon homogenization. Doan (27) reported that homogenization lowers curd tension, but apparently improves digestibility very slightly. Eisele and Budny (30) found that homogenization increased the final curd firmness and decreased the clotting time.

One of the major pretreatments that has been studied is the use of additives to affect curd strength. Ashworth and Nebe (8) fortified skimmilk with 3% added low heat nonfat solids and found no significant change in curd tension. In an earlier study by Ashworth and Nebe (9), the addition of either $CaCl_2$ or NaH_2PO_4 to milk caused a more rapid curd tension increase than the control sample. When the molar ratio of monovalent to divalent cations became greater than 2.0, curd tension decreased. Hadary and Sommer (39) concluded that the curd tension of chocolate milk was decreased by the addition of cocoa itself. They stated that the effect of sugar and suspending agents such as starch, locust bean gum, and "cocoloid" is negligible. Hansen et al. (43) found that the use of antibiotics in the treatment of mastitis caused a decrease in curd firmness due to the restricted growth of lactic acid bacteria. However, when sulfanilamide, sulfamerazine, and aureomycin were used, a definite stimulating effect on the acid produced by lactic acid bacteria was found.

There are a few reports (97, 101, 103, 123) which show that addition of milk protein affects curd strength. Sherbon et al. (101) reported that β-lactoglobulin B may be associated with higher curd tension. They also stated that milks typed K-casein AA were different in both curd tension properties and rennet clotting time from type AB or BB. Smith (103) reported that tough curd is formed by the presence of casein in larger amounts. Weisberg et al. (123) reported similar results, stating that the concentration of casein is lower in soft curd milk than in hard curd milk.

The influence of calcium ion and calcium chloride concentrations on curd formation has been investigated extensively (12, 25, 26, 103, 107, 115, 117, 123, 130). Dill and Roberts (26) reported that reconstituted skimmilk powder after being heated had a low curd tension value. They found that this low value could be restored by the addition of solids-not-fat and calcium chloride. Calcium chloride concentrations as high as 0.08% gave no detectable off flavor to the heated skimmilk. Stoll and Morris (107) found that the addition of 1 or 2% NaCl increased syneresis of skimmilk rennet curd, but a 4% addition decreased syneresis. The addition of $CaCl_2$ to a NaCl suspension of the curd caused a filomentous structure to appear. This addition caused the curd strength to increase. They also found that ethylenediaminetetraacetate and sodium citrate decreased syneresis, thus causing a decrease in curd strength. Verma and Sommer (117) reported that the molar ratios of inorganic colloidal Ca-Mg to inorganic phosphorous in rennet curd indicate a predominance of calcium phosphate monobasic rather than tricalcium phosphate. Weisberg et al. (123) reported that soft curd milk contains less calcium and phosphorous than hard curd milks.

EXPERIMENTAL PROCEDURES

The causes for decreases in the curd strength of abnormal milk were investigated using quarter samples from individual cows. A major portion of the study dealt with changes in curd strength when Wisconsin Mastitis Test (WMT) positive milk or skimmilk was dialyzed against WMT negative milk or skimmilk. Calcium, magnesium and inorganic phosphorous concentrations were determined before and after dialysis. In addition, the influence of added calcium chloride and low heat Grade A nonfat dry milk on curd firmness was investigated.

Selection, Storage, and Handling of Samples

The samples were selected initially on the basis of the California Mastitis Test (CMT) (6). Approximately one liter of milk having negative and positive CMT values was obtained from Holstein and Jersey cows from the Texas A&M University herd using a quarter divided milking machine. The samples were cooled in ice water and stored at 4 C until used. Wisconsin Mastitis Test (WMT) values were determined for each sample using the procedure described by Thompson and Postle (116). Samples with WMT values of less than 10 mm were classified as negative, while those with WMT values greater than 20 mm were classified as positive for mastitis. Skimmilk was obtained by mechanical separation at 45 C.

Curd Tension

Curd tension was determined on whole milk samples with a Cherry

Burrell curd tension meter by the procedure recommended by the American Dairy Science Association Committee (28).

Acid Production and Curd Firmness

The method described by Hampton and Randolph (42) for the determination of acid production and curd firmness was used. Single strain cultures of Streptococcus lactis C2 or Streptococcus cremois R1 were used for development of acid. Culture R1 is susceptible, while Culture C2 is resistant to the inhibition and agglutination activity of milk (31, 42). The skimmilk was pasteurized at 63.8 C for 30 min and cooled to 32 C. Individual lots of the pasteurized skimmilk samples were innoculated at the rate of 6% with an active freshly transferred culture. Curd firmness was determined at pH 4.8 using a Cherry Burrell curd tension meter. Acid production was recorded as the time required to reach the desired pH.

Dialysis

All dialysis treatments were conducted at 4 C. Dialysis tubing (Union Carbide 27) was regenerated and washed with cold deionized water before use. During the initial investigation, samples were dialyzed at 1:10 v/v ratio of positive to negative milk or skimmilk without agitation. Subsequent dialysis treatments were conducted using an Oxford Multiple Dialyzer Model "C" which provided for continuous agitation. The initial ratio of WMT positive to WMT negative samples was 1:10 v/v.

Mineral Analyses

Total and unbound concentrations of calcium, magnesium, and inorganic phosphorous were determined before and after dialysis. Calcium and magnesium were determined by atomic absorption spectrophotometry, and inorganic phosphorous was determined by auto analyzer techniques (111). The total concentrations were those obtained for skimmilk and unbound values were those obtained on the ultracentrifugate (obtained by ultracentrifugation) of the skimmilk.

Ultracentrifugation

Approximately 15 ml of skimmilk were centrifuged for 3 hr at 144,880 x g at 5 C in a Spinco Ultracentrifuge to obtain the ultracentrifugate.

pH

A Beckman pH meter (Model Zeromatic) was used to determine pH. In the investigation of the influence of pH on curd formation, the pH of WMT negative milk was adjusted to that of the WMT positive milk by the addition of 0.5 N NaOH, and the pH of positive milk was adjusted to the pH of the negative sample by the addition of 0.5 N HCl.

Pretreatment Additives

Varying concentrations of calcium chloride ($CaCl_2$) and Grade A low heat nonfat dry milk were added to the WMT positive and negative samples before and after pasteurization. Calcium chloride was added at concentrations of 0.025 and 0.10%. Nonfat dry milk was added at levels of 1.0 and 2.0%. In addition, the combination of 0.025% $CaCl_2$ and 1.0% nonfat dry milk was added before and after pasteurization.

RESULTS AND DISCUSSIONS

Effect of Dialysis on Curd Tension

The first phase of this study dealt with the effect of dialysis on the curd tension of mastitic milk. The dialysis ratio was a 1:10 v/v of Wisconsin Mastitis Test (WMT) positive to negative milk for 36 hr at 4 C. Ten quarter samples of WMT negative and positive milk from individual cows were utilized.

The effect of dialysis on curd tension is shown in Table 1. The average curd tension for the WMT negative samples was 57 g before and 46 g after dialysis. The corresponding values for the WMT positive samples were 31 g before dialysis and 38 g after dialysis. These changes in curd tension values represent a 21% decrease in curd tension of the negative samples and a 23% increase in curd tension of the positive samples. Tallamy et al. (113) reported that mastitis caused a 35% decrease in curd tension of milk. These results indicate that factors responsible for variations of curd tension of WMT negative and positive milk were dialyzable.

Effect of pH Equilibration on Curd Tension

It has been shown that rennet curd tension is affected by pH (9). Since dialysis would be expected to influence the pH of normal and abnormal milk, studies were conducted on ten samples to determine if the difference in pH of normal and abnormal milk would account for differences in curd tension. The samples were divided into two aliquot

Table 1. Effect of dialysis on curd tension of Wisconsin Mastitis Test negative and positive quarter milk samples from individual cows.[a]

No. samples	Wisconsin Mastitis Test				Curd tension			
	Before dialysis		After dialysis		Before dialysis		After dialysis	
	Range	Average	Range	Average	Range	Average	Range	Average
	(mm)				(g)			
WMT negative[b]								
10	2to9	5	3to8	5	35to100	57	29to93	46
WMT positive[c]								
10	21to35	28	23to32	24	10to61	31	17to88	38

[a]WMT positive milk dialyzed against WMT negative milk at 1:10 v/v ratio for 36 hr at 4 C.

[b]WMT<10 mm.

[c]WMT>20 mm.

portions. The pH of the WMT negative milk was equilibrated to that of the positive milk and vice versa with 0.5 N NaOH or 0.5 N HCl. The effect of pH equilibration on curd tension is shown in Table 2. The average curd tension was changed from 51 to 49 g when the pH of the WMT negative milk was adjusted to that of the corresponding positive milk. This change did not appear to be significant. When the pH of the WMT positive milk was equilibrated to that of the negative milk, the average curd tension of 31 g was unchanged. These results indicate that the tendency for the curd tension values to equilibrate during dialysis could not be explained by pH equilibration.

Effect of Dialysis on Acid Production and Curd Firmness

Hampton and Randolph (42) reported that the effect of mastitis was more prominent on acid produced curd firmness than on curd tension. Therefore, the influence of dialysis on acid production and curd firmness of WMT positive and negative skimmilk was investigated. Three separate trials were conducted. Aliquot portions of WMT positive and negative milk samples from five individual cows were blended. This sampling technique was used to minimize biological variations between individual cows. The effect of dialysis on acid production and curd firmness is shown in Table 3. Acid production by either culture was not significantly altered by dialysis. Curd firmness of the skimmilk with Culture C2 before dialysis averaged 59 g in the WMT negative skimmilk compared to an average of 11 g for the corresponding positive skimmilk. After dialysis, the average curd

Table 2. Effect of pH equilibration on curd tension of Wisconsin Mastitis Test negative and positive quarter milk samples from individual cows.

No. Samples	pH				Curd tension			
	Initial		Equilibrated		Initial		Equilibrated	
	Range	Average	Range	Average	Range	Average	Range	Average
	(pH)				(g)			
WMT negative[a]								
10	6.65to6.95	6.80	6.75to7.05	6.91	15to96	51	15to93	49
WMT positive[b]								
10	6.75to7.05	6.91	6.65to6.95	6.80	12to64	31	11to62	31

[a]WMT<10 mm.

[b]WMT>20 mm.

Table 3. Effect of dialysis on acid production and curd firmness of Wisconsin Mastitis Test negative and positive skimmilk from individual cows.[a]

WMT mean	Acid production				Curd firmness			
	Before dialysis		After dialysis[b]		Before dialysis		After dialysis[b]	
	Range	Average	Range	Average	Range	Average	Range	Average
(mm)	(min)				(g)			
			Streptococcus lactis C2					
5	238to248	243	232to291	258	12to109	59	18to90	50
31	240to264	255	260to300	275	3to16	11	9to33	22
			Streptococcus cremoris R1					
6	245to275	263	250to280	270	60to74	66	42to52	47
30	250to295	280	252to290	276	16to26	19	30to46	37

[a]Values represent data for three trials.

[b]WMT positive skimmilk dialyzed against negative skimmilk to 1:10 v/v ratio at 4C.

firmness of the negative and positive samples was 50 and 22 g, respectively. These changes in curd firmness represent an average reduction of 15% in the negative and an increase of 100% in the positive skimmilks. Similar results were obtained with Culture R1. The average curd firmness obtained with Culture R1 in skimmilk before dialysis was 66 g for the negative and 19 g for the positive samples. After dialysis, the average curd firmness value of the negative skimmilk was 47 g compared to 37 g in the positive skimmilk. This represents an average reduction in curd firmness of 29% in the negative and an increase of 95% in the positive samples. These results indicate that dialysis alters the factors responsible for a decrease in the curd firmness of mastitic milk.

To further evaluate the effect of dialysis on acid production and curd firmness, aliquot samples were analyzed after dialysis for selected times. Three trials were conducted. Dialysis was accomplished with a multiple dialyzer at an initial 1:10 v/v ratio of positive to negative skimmilk at 4 C.

The effect of dialysis for various time intervals on acid production and curd firmness is shown in Table 4. Acid production was not significantly influenced by dialysis. The average initial curd firmness value was 63 g for the WMT negative samples compared to 21 g for the WMT positive samples. Major adjustment of the curd firmness values was observed after 6 hr when the average curd firmness was 50 g for the WMT negative samples and 35 g for the WMT positive samples. These changes in curd firmness represent a 21% decrease in the

Table 4. Effect of dialysis for selected time intervals on acid production and curd firmness of Wisconsin Mastitis Test negative and positive skimmilk from individual cows.[a]

Dialysis time[b]	Acid production		Curd firmness			
	WMT negative[c]	WMT positive[d]	WMT negative[c]		WMT positive[d]	
			Range	Average	Range	Average
(hr)	(min)		(g)			
0[e]	244	266	12to134	63	3to54	21
6	245	254	16to108	50	8to88	35
12	251	262	18to108	50	6to89	35
24	262	269	18to105	51	12to96	41
36	264	272	18to106	52	13to91	40
36[e]	249	284	16to138	66	3to46	19

[a]Values represent data for three trials.

[b]WMT positive skimmilk dialyzed against negative skimmilk at 1:10 v/v ratio at 4 C.

[c]WMT $<$10 mm.

[d]WMT $>$20 mm.

[e]Control - not dialyzed.

negative samples and a 67% increase in the positive samples. Curd firmness values of WMT positive samples continued to increase for 24 hr, but this could possibly be attributed to changes in the ratio of positive to negative skimmilk as well as equilibration. Aliquot portions of the samples were stored for 36 hr at 4 C without dialysis. Curd firmness values were 66 and 19 g for the WMT negative and positive samples, respectively. This indicates that the changes affected by dialysis were not due to aging.

Concurrent with the analysis of curd firmness after dialysis for selected time intervals, mineral concentrations were determined on the skimmilk and ultracentrifugate obtained from the samples. Mineral concentrations determined were calcium, magnesium, and inorganic phosphorous. The effect of dialysis for selected time intervals on mineral concentrations is shown in Table 5. At 0 hr dialysis time, the average total concentration of calcium and inorganic phosphorous was higher in the WMT negative samples than in the corresponding positive samples. There were only small differences in the magnesium concentrations. After 6 hr dialysis, there was a tendency for the mineral concentrations of the WMT negative and positive samples to equilibrate. Minerals which were not sedimented by ultracentrifugation were considered to be unbound. Unbound concentrations of calcium and inorganic phosphorous were higher, and magnesium concentrations were lower in the WMT negative samples before dialysis. The unbound mineral concentrations tended to equilibrate during dialysis similar to the equilibration observed

Table 5. Effect of dialysis for selected time intervals on mineral concentrations of skimmilk and ultracentrifugate of Wisconsin Mastitis Test negative and positive skimmilk from individual cows.[a]

Dialysis time[b]	Mineral concentration					
	WMT negative[c]			WMT positive[d]		
	Ca	Mg	P[e]	Ca	Mg	P[e]
—(hr)—	(ppm)					
			Skimmilk			
0[f]	1388	103	715	1328	109	680
6	1371	102	708	1356	110	684
12	1364	104	711	1352	108	684
24	1367	106	706	1355	108	695
36	1360	105	706	1351	109	699
36[f]	1385	104	713	1330	109	682
			Ultracentrifugate			
0[f]	534	82	428	496	87	382
6	531	84	422	502	86	388
12	512	83	421	505	86	389
24	512	83	416	510	86	392
36	512	84	416	509	85	397
36[f]	536	80	431	494	88	380

[a]Values represent data for three trials.

[b]WMT positive skimmilk dialyzed against negative skimmilk at 1:10 v/v ratio at 4 C.

[c]WMT<10 mm.

[d]WMT>20 mm.

[e]Inorganic phosphate.

[f]Control - not dialyzed.

for the total concentrations. Samples that were held for 36 hr at 4 C without dialysis did not vary from the initial total or unbound mineral concentrations. Equilibration was never fully accomplished, but the trend of the mineral equilibration was similar to that observed for curd firmness values.

In an attempt to obtain complete equilibration of the curd firmness values, trials were made dialyzing WMT positive to negative skimmilk at 1:10 v/v ratio for 12 hr and then placing the dialyzed positive skimmilk in a new volume of negative skimmilk for an additional 12 hours. Total and unbound calcium and inorganic phosphorous concentrations were determined before and after dialysis. Magnesium values were not determined since only slight differences were observed in previous trials. The effect of dialysis on curd firmness and calcium and inorganic phosphorous concentrations is shown in Table 6. Before dialysis, the average curd firmness value of the WMT negative skimmilk was 78 g compared to 36 g for the positive skimmilk. Total and unbound calcium and inorganic phosphorous concentrations were higher in the WMT negative samples than in the corresponding positive samples. The large differences in curd firmness and mineral concentration were partially equilibrated by 12 hr of dialysis. The curd firmness value of the WMT negative samples decreased by 8%, while the curd firmness value of the positive samples increased by 30%. The calcium and inorganic phosphorous concentrations tended to equilibrate as observed in the previous trials. During the 12 hr dialysis after the replacement of the WMT

Table 6. Effect of dialysis on curd firmness and calcium and phosphorous concentarations of skimmilk and ultracentrifugate of *Wisconsin Mastitis* Test negative and positive skimmilk from individual cows.[a]

Dialysis time[b]	Curd firmness		Mineral composition							
	WMT negative[c]	WMT postive[d]	Skimmilk				Ultracentrifugate			
			WMT negative[c]		WMT postive[d]		WMT negative[c]		WMT postive[d]	
			Ca	P[e]	Ca	P[e]	Ca	P[e]	Ca	P[e]
(hr)	(g)		(ppm)							
0	78	36	1366	615	1255	590	450	394	418	346
12	68	48	1354	610	1313	590	451	390	429	368
24[f]	60	51	1351	614	1320	605	447	384	437	369

[a]Values represent data for three trials.

[b]WMT postiive skimmilk dialyzed against negative skimmilk at 1:10 v/v ratio at 4 C.

[c]WMT<10 mm.

[d]WMT>20 mm.

[e]Inorganic phosphate.

[f]WMT negative skimmilk changed after 12 hr.

negative skimmilk, there was a 12% decrease in the curd firmness value of the negative sample. The curd firmness value of the WMT positive samples was increased a total of 41% by dialysis. However, total equilibration of the curd firmness values was not accomplished. Mineral concentrations tended to equilibrate further due to the addition of the new WMT negative skimmilk, but total equilibrium did not occur in either the total or unbound minerals. These results indicate that the minerals, calcium and inorganic phosphorous, have a definite influence on the curd firmness of skimmilk.

Effect of Calcium Chloride and Nonfat Dry Milk Solids on Curd Firmness

Addition of calcium ions as a coagulate stimulator to low calcium content milk or high heat-treated milk is a common practice in cheese manufacturing. The legal allowance of calcium ion addition, in the form of calcium chloride ($CaCl_2$), to skimmilk is 0.02% (9). Due to the dialysis results dealing with curd firmness and mineral analysis, $CaCl_2$ was added to skimmilk to determine the effect it would have on curd firmness. Concentrations of 0.025 and 0.10% $CaCl_2$ were added to skimmilk samples at 25 C either before or after pasteurization.

The effect of $CaCl_2$ and nonfat dry milk solids on curd firmness is shown in Table 7. The average curd firmness values for the WMT negative and positive samples when 0.025% $CaCl_2$ was added before pasteurization were 96 and 63 g, respectively. This represents an 11% decrease in the WMT negative samples and a 31% increase in the

Table 7. Effect of $CaCl_2$ and nonfat dry milk solids on curd firmness of Wisconsin Mastitis Test negative and positive skimmilk from individual cows.[a]

Treatment	Curd firmness	
	WMT negative[b]	WMT positive[c]
	(g)	
Control	106	48
0.025% $CaCl_2$ added before past.	96	63
0.10% $CaCl_2$ added before past.	106	50
0.025% $CaCl_2$ added after past.	96	63
0.10% $CaCl_2$ added after past.	98	59
1% NFDM added before past.	93	37
2% NFDM added before past.	84	32
1% NFDM added after past.	95	38
2% NFDM added after past.	77	30
0.025% $CaCl_2$ and 1% NFDM added before past.	91	56
0.025% $CaCl_2$ and 1% NFDM added after past.	90	32

[a]Data represents average values for six trials.

[b]WMT<10 mm.

[c]WMT>20 mm.

positive samples from the initial curd firmness values. The addition of 0.025% $CaCl_2$ after pasteurization produced similar results. Adding 0.10% $CaCl_2$ before pasteurization to WMT negative and positive samples resulted in no change in the negative samples and a very small change in the positive samples. The addition of this level of $CaCl_2$ after pasteurization showed a slight decrease in the negative samples and a 23% increase in the positive samples as compared to the initial curd firmness value.

The data indicates that the addition of $CaCl_2$ increases curd firmness to a certain extent. However, in the WMT negative samples, the calcium concentration was higher than in the positive samples; and a slight lowering of curd firmness values resulted. Addition of calcium ions to skimmilk to improve curd formation is not caused by the actual presence of the calcium ion, but rather because it induces a shift in salt balance in milk. A shift in salt balance could affect the physical and chemical structure of the casein micelles in milk to a point where coagulation would be increased.

In nonfat dry milk, the total solids are contributed by milk proteins, carbohydrates (mainly lactose), inorganic and organic ions and salts, and other minor constituents. Lactose has practically no influence on curd tension (97). The ions and salts contribute a small amount of the total solids. Thus, the study of total solids content reflects mainly the total protein content effect on curd firmness. Commercial Grade A nonfat dry milk (NFDM) powder was added at 25 C either before or after pasteurization of the skimmilk. Levels of 1.0 and 2.0% were used. In both the before and after pasteurization fortification, there was a detrimental effect on the curd firmness due to the nonfat dry milk. However, there was a more pronounced decrease when the addition was after pasteurization than before pasteurization. Also, the 2% level caused a larger decrease than the 1% level.

A combination of calcium chloride (0.025%) and nonfat dry milk (1.0%) was used to further study the effect of additives on curd firmness. Both additions were made at 25 C before or after pasteurization of the WMT negative and positive skimmilk. This treatment caused a decrease in the negative skimmilk and an increase in the positive skimmilk. Fortification with $CaCl_2$ and nonfat dry milk before pasteurization resulted in similar findings as in the data from the addition of only $CaCl_2$. However, the addition of $CaCl_2$ and nonfat dry milk after pasteurization resulted in data similar to that of the addition of only nonfat dry milk. These results reveal a decrease in curd firmness values of both the negative and positive samples.

The physical and chemical characteristics of the casein micelles in milk and the exact mechanism of rennet action in milk are not thoroughly understood. Dill and Roberts (26) have reported that there may be an interaction between casein and whey proteins in milk. Whether the whey protein content in milk is involved in determining the curd firmness as the milk is being attacked by rennet is not known. Dill and Roberts (26) found that as heat treatment was increased, the amount of $CaCl_2$ required to increase the curd tension values increased. The effect of high heat treatments could not be overcome by fortification with nonfat dry milk.

The results obtained when WMT negative and positive skimmilk was fortified with $CaCl_2$ and nonfat dry milk cannot be fully explained. It can be postulated that pasteurization had an effect on the reaction of the casein micelles with the additives. Fortification of the samples with both additives before pasteurization minimized the decrease in curd firmness observed when the samples were fortified only with nonfat dry milk. However, nonfat dry milk prevented $CaCl_2$ from causing the increase in curd firmness values that $CaCl_2$ caused when added alone. This effect was not observed when fortification was after pasteurization of the skimmilk.

SUMMARY AND CONCLUSIONS

Factors responsible for the decrease in curd strength of abnormal milk were investigated. The influence of dialysis of Wisconsin Mastitis Test (WMT) positive milk or skimmilk against WMT negative samples and the addition of calcium chloride and nonfat dry milk before and after pasteurization were determined. Results may be concluded as follows:

1. The dialysis of WMT positive milk or skimmilk against WMT negative samples resulted in an increase in the curd strength of the positive samples and a decrease in the negative samples.

2. The changes in curd strength of WMT positive and negative samples during dialysis could not be explained by equilibration of pH, but appeared to be related to changes in unbound calcium and inorganic phosphorous concentrations.

3. Curd firmness of the positive skimmilk was increased by the addition of calcium chloride and decreased by fortification with nonfat dry milk. The combined addition of calcium chloride and nonfat dry milk caused variable changes in curd firmness.

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