

12.4.

25.17.

]

VARIATIONS IN THE COMPOSITION OF COWS' MILK AND THE
EFFECT OF ACIDITY AND HEAT TREATMENT ON THE
SALTS, HEAT STABILITY AND CURD
TENSION OF MIXED MILK

By

JOHN THOMAS OSBORNE

A THESIS

Submitted to the School of Graduate Studies of
Michigan State College of Agriculture and
Applied Science in partial fulfillment
of the requirements for the
degree of

MASTER OF SCIENCE

Department of Dairy

1953

THESIS

THESIS

ACKNOWLEDGMENTS

The writer desires to express his sincerest appreciation to Dr. J. R. Brunner, Assistant Professor of Dairying, for his encouragement and constructive criticism in the preparation of this thesis and for his guidance in the direction and scope of the material covered in this study.

The author is also greatly appreciative to Dr. Earl Weaver, Professor of Dairying and Head of the Dairy Department, and to Michigan State College for providing the facilities and equipment used in this study. Grateful appreciation is also due to the College Creamery for furnishing the raw milk from different herds which was used in this study.



TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
<u>Natural Variations in the Composition and Properties of Cows' Milk</u>	3
Influence of Breed	3
Fat and total solids	3
Protein	3
Minerals	4
Curd tension	4
Heat stability	5
Influence of Seasons	7
Fat and total solids	7
Protein	8
Minerals	8
Curd tension	10
Heat stability	10
<u>Natural Variations in the Composition and Properties of Cows' Milk Resulting from Induced Factors</u>	11
Influence of Heat Treatment	11
Mineral distribution	11
Nitrogen distribution	12
Heat stability	13

THESIS

J

Curd tension	14
Influence of Developed Acidity	14
Mineral distribution	14
Nitrogen distribution	16
Heat stability	16
Curd tension	17
EXPERIMENTAL PROCEDURE	18
<u>Preparation and Selection of Whole Milk Samples to Show Effect of Seasonal Variations Due to Acidity and Heat Upon the Properties and Constituents of Milk</u>	18
<u>Preparation of the Milk Serum - the Dialyzable Portion of the Milk</u>	21
<u>Chemical and Physical Methods Used to Estimate Changes in the Properties and Constituents of the Selected Milk Samples and Their Respective Dialyzable Portions</u>	22
Fat and total solids	22
Alcohol number	22
Acidity (Titratable)	22
Curd tension	22
pH	23
Heat stability	23
Citric acid	23
Total nitrogen	24
Minerals	24
RESULTS	27

THESIS

J

Variations in Some of the Constituents and Physical Properties of Cows' Milk in Relation to Seasons of the Year	27
Titratable acidity and pH	28
Curd tension	28
Heat stability	29
Citric acid	29
Nitrogen	29
Minerals	30
Variations in Constituents and Some of the Properties of Whole Milk When Subjected to Various Periods of Heating	33
Fat and total solids	33
Alcohol number	33
Titratable acidity and pH	33
Heat stability	33
Citric acid	33
Total nitrogen	34
Minerals	34
The Effect of Heat Upon Some of the Con- stituents and Properties of Milk with Abnormal Acidity	36
Fat and total solids	36
Alcohol number	36
Titratable acidity	36
pH	37
Curd tension	37
Heat stability	37

THESIS

J

Citric acid	38
Nitrogen	38
Minerals	39
The Effect of Induced Acidity Upon Some of the Constituents and Properties of heated Milk	41
Fat and total solids	41
Alcohol number	41
Titratable acidity	41
pH	41
Curd tension	41
Heat stability	42
Citric acid	42
Nitrogen	42
Minerals	43
Effect of Changes in Titratable Acidity Upon Some of the Constituents and Properties of Raw Whole Milk	45
Fat and total solids	45
Titratable acidity and pH	45
Alcohol number and heat stability	45
Curd tension	45
Nitrogen	45
Citric acid	46
Minerals	46
DISCUSSION	48

1

Influence of Breed and the Seasons of the Year Upon Some Properties and Distribu- tion of Various Normal Constituents of Milk	49
Effect of Heat Upon Some of the Constituents and Properties of Cows' Milk	54
Effect of Various Degrees of Acidity Upon Some of the Properties and Constituents of Cows' Milk	59
Effect of Heat-Treatment Upon Some Proper- ties and Constituents of Milk With Various Degrees of Acidity	62
Effect of Various Degrees of Acidity Upon Some of the Constituents and Proper- ties of Heated Milk	65
SUMMARY AND CONCLUSIONS	68
Influence of Breed and The Seasons of the Year Upon Some Properties and Distribu- tion of Various Constituents of Milk . .	68
Effect of Heat Upon Some of the Constitu- ents and Properties of Normal Cows' Milk	70
Effect of Various Degrees of Acidity Upon Some of the Properties and Constituents Of Cows' Milk	71
Effect of Heat-Treatment Upon Some Proper- ties and Constituents of Milk With Various Degrees of Acidity	72
Effect of Various Degrees of Acidity Upon Some of the Constituents and Properties of Heated Milk	73
LITERATURE CITED	97

]

LIST OF TABLES

TABLE	Page
1. Seasonal analyses of pooled herds milk . .	75
2. Seasonal analyses of milk from a Guernsey herd	77
3. Seasonal analyses of milk from a Holstein herd	79
4. Effect of heat upon some of the constituents and properties of milk . .	81
5. The effect of various heat-treatments upon some of the constituents and properties of milk with various degrees of acidity	83
6. Effect of induced acidity upon some of the constituents and properties of heated milk	85
7. Variations in some of the normal con- stituents and properties of whole milk as the acidity is increased . . .	87

1

LIST OF FIGURES

FIGURE	Page
1. The fat content of milk as influenced by seasons of the year	89
2. Total solids of milk as influenced by seasons of the year.	89
3. Variations in the total nitrogen content of milk with seasons of the year	90
4. Comparison of the Ca, P and the P/Ca ratio in whole milk with seasons of the year.	91
5. Variations in colloidal phosphorous with seasons of the year	92
6. Variations in the total and serum nitrogen contents of milk heated at 143°F. for different periods of time.	93
7. The influence of heat on the Ca, P and the P/Ca ratio of milk	94
8. Variations in the total and serum nitrogen contents in milk of vary- ing acidities.	95
9. Comparison of Ca and P and the P/Ca ratio in milk of various acidities . .	96

INTRODUCTION

For many years the instability of cows' milk toward heat has at times been of great concern to the evaporated milk industry. Some constituent or combination of constituents normally present in cows' milk is believed to be responsible for the heat coagulation of milk. This problem was alleviated to some extent by the use of high temperature preheating treatments or by the addition of protein stabilizing salts.

The first basic research pertaining to the coagulation of milk by heat was reported by Sommer and Hart (1926). Since then, various reports have been published on different phases of the heat coagulation problem. Most of the workers have associated the instability of milk toward heat with the seasonal effect, the effect of acidity, and the effect of an improper cation-anion balance.

The purpose of this study was to determine the changes which occur in the composition of milk due to seasonal and breed differences and the effect of these changes on the chemical and physical properties of the milk. The study was subdivided into three parts. The object of the first part of this study was to determine the variations in the composition of milk with respect

1

to the seasonal influence. The purpose of the second part of this study was to determine the effects of various heat treatments upon the constituents of cows' milk. The last part of the investigation was devoted to the changes which occur in composition and properties of cows' milk due to the combined effects of heat and acidity.

]

REVIEW OF LITERATURE

Natural Variations in the Composition and Properties of Cows' Milk

Influence of Breed

Fat and total solids. There are differences in the fat content of milk from different breeds of cows. Ragsdale and Turner (1922) reported that a Guernsey herd gave milk with a consistently higher fat content than did a Holstein herd. This observation was further supported by the work of Jacobsen and Wallis (1939) who found that the milk of the smaller breeds had a higher fat content than milk from the larger breeds.

Since the per cent of fat is encompassed in the per cent of total solids, any variation in the fat content of milk will show up as a variation in total solids. Jacobsen and Wallis (1939) showed that the milk of the smaller breeds had a higher percentage of total solids than did the milk of the larger breeds.

Protein. Very little information has been reported on the differences in the total protein content of milk of individuals or breeds. A report published by Shahani and Sommer (1951a) showed a slightly higher average for total protein in Guernsey milk than that found in Holstein

J

4

milk. For mixed herds milk, the average total protein content was lower than that reported for the individual breeds. Rowland (1938), under English conditions, found that the protein content of a high fat, high solids milk was consistently higher than the protein content of a low fat, low solids milk.

Minerals. The salts which are most generally believed to influence the properties of milk are calcium, magnesium, phosphorous and citric acid. Holm, Webb and Deysher (1932), from complete milk analyses, concluded that the smaller breeds of cows showed a consistently higher calcium content, a higher total phosphorous content and very little difference in magnesium content when compared to similar analyses of the larger herds. However, in a comparison of the citric acid content of high fat milk with that of low fat milk, the citric acid content was consistently higher in the low fat milk. Rice and Markley (1924) reported data which agreed favorably with the observations of Holm et al (1932).

Curd tension. Hill (1923), during his classical research on the curd tension of milk, observed that if a milk has a curd tension of less than 30 grams then it usually produces a curd of soft character. The average curd tension of Holstein milk was lower than that of Guernsey milk. He also pointed out that the fat content of the milk has no direct relationship with a high or

THESIS

low curd tension, but that the salt content may be responsible for the differences in curd tension. This is in partial agreement with research carried out by Weisberg, Johnson and McCollum (1933) who found that the softer curd milks contained less salts than did the harder curd milks.

Riddell, Caulfield and Whitnah (1936) showed that the curd tension of milk could be correlated with its protein content. Therefore, the different breeds could be ranked in curd tension in the same order as their total protein content. In this manner, the breeds would rank from the lowest to the highest in the following order: Holstein, Ayrshire, Guernsey, Jersey.

Heat stability. The heat stability of milk may be defined as that length of time at which milk will resist coagulation at a specified temperature. The influence of the breed on the heat stability of milk is related to the composition of the milk from the individuals. There are few specific references to the effect of any certain breed upon heat stability.

Sommer and Hart (1919) found that the stability of milk toward heat was influenced by the concentration of the salts which were present. Their experiments indicated that in heated-milk, coagulation will not occur when the calcium is removed, but that small amounts of calcium salts, when added to the milk, will cause coagu-

]

lation. The calcium and magnesium ions were found to be balanced by phosphates and citrates in almost gram-equivalent amounts. Optimum heat stability in the milk is achieved when the proper balance is present between the calcium and magnesium ions and phosphate and citrate radicals. However, they concluded that coagulation would result if one of the salt combinations was higher than the other.

The data presented by Holm et al (1932) showed, generally, that the higher the calcium and phosphorous content the longer was the time required to coagulate milk at 130° C. These workers found no definite correlation between the salt balance and heat stability.

Webb and Holm (1932) reported that as the temperature of the milk was increased, the time required for coagulation decreased logarithmically. They determined that a temperature of 120° C. was the most optimum temperature to use in evaluating the heat stability of milk.

Holm, Deysher and Evans (1923) eliminated the presence of a high fat content as being a factor in the coagulation of milk. They reported that the presence of the high fat content raised the coagulation temperature only slightly and deduced from this observation that the difference was probably due to heat absorbed by the fat.

Influence of Seasons

Fat and total solids. The season of the year is known to influence the fat content of milk. In research carried out by Overman (1945) with the Guernsey breed, the fat content decreased during the summer season but gradually increased during the fall and remained at a higher level during the winter months. Overman also reported that in the Holstein breed the fat content of the milk was high in late summer but remained at a lower level during the rest of the year. These results were in general agreement with data reported by Jacobsen and Wallis (1939) who demonstrated a downward trend in fat content for all breeds during the summer months and an increase during the fall and winter months. The work by Ragsdale and Turner (1922) further verified these trends in fat content.

As stated earlier, since the fat is included in the total solids, any variances in the fat content will result in a corresponding apparent variation in the total solids. Overman (1945) observed this relationship when he found greater differences in the total solids of Guernsey milk than in Holstein milk over a period of a year. He attributed this to the greater variations in the fat content of Guernsey milk as compared to the lower range of variations in fat content

THESIS

of Holstein milk.

Protein. Davis, Harland, Caster and Kellner (1947a) conducted experiments under Arizona conditions and observed that the protein content of Guernsey milk was consistently higher than the protein content of Holstein milk. Their results showed fluctuations in protein content for Guernsey milk during the early summer months while the period of greatest variances for Holstein milk occurred in the later summer months. The Holstein milk showed a leveled decrease period. From later experiments on mixed herds, Davis, Harland, Caster and Kellner (1947b) found that the protein content of mixed herds milk remained at one level over a period of one year.

In experiments upon the use of antiscorbutic-free fodder and pasture feeding of Holstein cows, Hess, Unger and Supplee (1920) found that the protein content of the milk produced on pasture was inherently higher, but that an increase was also noted in the fat and total solids of the milk.

Minerals. In analyses of mixed herd milk, Jacobsen and Wallis (1939) reported that the mineral content generally showed slightly lower values during the summer months while winter milk showed a slightly higher mineral content. Sommer and Hart (1926) reported that there was no change in the calcium or phosphorous content of milk from cows fed on dry winter roughage

and green pasture grass. Lenstrup's (1926) data showed that the total phosphorous remained at a constant value during the year but that the values were slightly lower during the summer months. Investigations by Golding, Mackintosh and Mattick (1932) showed lower mineral values for the summer months followed by an increase and eventual leveling off during the late fall and winter seasons.

Supplee and Bellis (1921) reported only a slight increase in the citric acid content of milk when one mixed herd was changed from winter feed to green pasturage. Holm et al (1932) found a slight decrease in the citric acid content of milk produced in March over that in milk produced in February. Their data is inconclusive as to the effect of feed on the salt composition of milk since their cows were fed dry hay. Hunziker (1949) stated that, "green feeds increase the citric acid content of milk; dry winter feeds decrease it." This statement was supported by Sommer and Hart (1926) who believed that there was a significant increase in the citric acid content of milk when a cow was changed from winter feed to green pasture. Hess et al (1920) observed that when a Holstein cow was changed from vitamin C-free fodder to green pasture the citric acid content of the milk showed a very significant increase.

THESI

]

Curd tension. There are very few research reports related to the influence of seasonal variations on the curd tension of milk. Hill (1923) observed no significant differences in curd tension over a six months period. He believed that the more important salts were responsible for variations in curd tension and that a decrease in the insoluble calcium might tend to cause a softer curd or reduced curd tension in milk. This statement is supported by Weisberg, Johnson and McCollum (1933) who observed that a soft curd milk contained less calcium and phosphorous than a hard curd milk.

Heat stability. The investigations of Holm et al (1932) showed that the heat stability of milk increased and reached a maximum during March and April. According to their data, there is a decrease in citric acid content of milk and a slight decrease in calcium and phosphorous. Cole and Tarassuk (1946) observed that when a high stability of milk to heat occurred that the best stability was encountered during March and April. In general, the same assumptions as stated earlier, under the heat stability as affected by breed, would also be true when applied to the influence of seasons on the heat stability of milk.

1HES1

J

Variations in the Composition and Properties
of Cows' Milk Resulting from
Induced Factors

Influence of Heat Treatment

Mineral distribution. Verma and Sommer (1950) observed, from determinations of both total and soluble salts in heated milk, that there was a significant decrease in soluble calcium after pasteurization but that following cool aging for 24 hours the amount of soluble calcium was higher than that determined in the raw milk. In analyses of fresh skim milk heated to 200°, 250°, 300° and 325° F., Bernardoni and Tuckey (1950) determined that the contents of calcium and phosphorous in the ultra-filtrates of the heated milk samples decreased as the temperature increased. Sommer and Hart (1926) observed that there were larger amounts of soluble calcium in concentrated skimmilk which had been heated at 180° F. for 5 minutes. This was accounted for, in part, to the precipitation of albumin.

Harman and Slater (1950) observed that in all samples of heated milk, regardless of the temperature used, the rates of diffusion of calcium, phosphorous and magnesium were significantly reduced and that citric acid appeared to be unaffected.

Whittier and Benton (1926) observed from their



experiments that as milk was heated the titratable acidity decreased momentarily and then rose, while the hydrogen-ion concentration increased consistently. In later experiments, Whittier and Benton (1927) concluded that the acid formed, upon the heating of milk, was in direct correlation with the time and temperature used as well as with the concentration of lactose. Gyorgy (1923) found that as the pH was lowered the amounts of calcium and phosphorous in solution were increased and that as the iso-electric point of casein was reached no calcium remained in combination with casein.

Nitrogen distribution. Few works have been reported on the effect of heat upon the nitrogen distribution in milk. Menefee, Overman and Tracy (1941) showed no significant changes between the total nitrogen content of raw milk and the same milk pasteurized at 145° F. for 30 minutes. However, when the milk was heated to 203° F. there was an immediate decrease in total nitrogen which was deduced to be due to a partial hydrolysis of proteins.

Shahani and Sommer (1951b) observed that there were no significant changes between the total nitrogen or serum nitrogen of raw milk and of milk pasteurized at 155° F. for 30 minutes although there were changes in a few of the nitrogen supplying compounds. They also observed a similar pattern in the nitrogen distribution

of milk heated at 143° F. for 30 minutes. The nitrogen compounds most affected were the albumin and globulin.

Heat stability. Most of the research on heat stability has been done on evaporated milk. Webb and Bell (1943) found that high-temperature short-time heating of a concentrated milk caused an increase in heat stability. Further increases in temperature or time resulted in a decrease in the heat stability of concentrated milk. They also observed that when a raw milk had been forewarmed to 120° C. for 0 to 10 minutes the milk attained a heat stability conducive to the production of a good evaporated milk. The forewarming treatment increased the stability of the milk toward heat. Webb, Bell, Deysher and Holm (1943) also found that milk forewarmed from 110° C. to 150° C. for one-half to 5 minutes exhibited better heat stabilization than did milk forewarmed to 95° C. for 10 minutes. Further, they observed that a forewarming temperature and holding time of 120° C. for 3 to 4 minutes to be optimum.

In heat-treated milk, Powell (1935) observed that when a milk was heated to 85° C. the time required for rennet coagulation was increased many times over that required to cause coagulation of the raw milk before heating.

THESE

Curd tension. Menefee et al (1941) showed that the curd tension of milk was lowered when it was pasteurized at 145° F. for 30 minutes. Mortenson, Espe and Cannon (1935) observed from curd tension experiments that in all samples of milk tested the curd tension of milk was reduced by all temperatures of heating but to different degrees. At pasteurizing conditions of 142° F. for 30 minutes the curd tension was reduced approximately one-third, the curd tension of boiled milk was reduced about three times, while the curd tension of milk heated in an autoclave at 242° F. for 15 minutes was so reduced that no curd tension value could be made. The work of Hill (1931) substantiates the work of Mortenson et al (1935) since he also reported a decrease in the curd tension of milk samples when they were heated to increasingly higher temperatures. Miller (1935), in his experiments on the curd tension of heated Holstein and Jersey milk, observed that heating caused a decrease in the curd tension of both milks and that the decreases were similar for both milks.

Influence of Developed Acidity

Mineral distribution. Sommer and Hart (1926) concluded that when milk coagulates due to a lack of calcium the development of acid will reduce the excess phosphate by reverting some of the phosphates to primary

phosphates. In turn, they stated that the development of acid would increase the amount of soluble calcium due to the acid action upon any di-calcium or tri-calcium phosphate present in the milk.

Van Slyke (1928) stated that the formation of lactic acid on the heating of milk converts insoluble di-calcium phosphate salts into a soluble form such as mono-calcium phosphate. The higher the acidity is allowed to develop, the lower will be the minerals left in the curd. He also determined that insoluble di-calcium phosphate was converted completely by lactic acid into soluble mono-calcium phosphate in approximately 12 hours. However, the calcium in calcium caseinate combined with the lactic acid less rapidly than did the calcium of di-calcium phosphate. In earlier work, Van Slyke and Bosworth (1916) determined that insoluble magnesium went into solution completely in 11.5 hours and that the citric acid was completely decomposed into acetic acid and carbon dioxide. McCammon, Caulfield and Kramer (1933) observed that rennet-type cheddar cheese showed a higher calcium content than did the acid types. This was due to the formation of insoluble calcium paracaseinate which was formed before an increase in acidity took place.

Khambatta and Dastur, (1950) working with milk from Indian cows, found that as souring progressed there

was a consistent decrease in total solids and that this decrease was more pronounced in raw milk than in boiled milk. They also determined that there was little change in total calcium and phosphorous between the original milk and the soured milk. However, they noted that the calcium and phosphorous content of the dialyzable portion of the soured milk was significantly increased over the dialyzable portion of the original milk. These results are similar to those of Rice and Markley (1924) who found that as acidity decreased there was a general decrease in total solids as well as in phosphorous.

Nitrogen distribution. Khambatta and Dastur (1951) reported that as the process of souring occurred the total nitrogen content of milk did not change, but that the nitrogen content of the dialyzable portion of the soured milk showed a marked increase during souring.

Heat stability. Webb and Bell (1942) found that excessive acidity was conducive to the coagulation of milk during high temperature and that normal, good quality milk could be treated with temperatures of 150° C.-160° C. without inducing coagulation. McInerney (1920) reported that as the acidity of milk increases the temperature required for coagulation of the milk sample will decrease, and that this characteristic was due to a transformation of the calcium salts in milk.

Rogers, Deysher and Evans (1921) supported these reports when they found that a low pH in milk decreased the time required to coagulate a sample of milk at a given temperature. Steuart (1920) has shown that a milk of three per cent acidity will curdle when boiled and if the same milk is allowed to attain 0.6 to 0.8 per cent acidity it will curdle spontaneously. He also demonstrated that milk, pasteurized at 165° F. for 5 minutes and held 3 weeks, would curdle at 0.18 per cent acidity.

Sommer and Hart (1926) concluded that the developement of acidity, in milk which coagulated in the heat test due to a low calcium content or citrate and phosphate excess, prevented the coagulation of the milk because of the liberation of soluble calcium and an accompanying reduction in secondary phosphate in the milk.

Curd tension. Very little has been reported upon the curd tension of soured milk. However, a common observation in cheesemaking has been that as more acid is produced the curd will become harder. Weisberg et al (1933) supported this observation with their experiments on soft curd milk. They concluded that in addition to the possible effect of salts on the curd tension, the amount of fat present in the milk may also affect the curd tension by interrupting the growth of micellar threads in the protein.

THES

EXPERIMENTAL PROCEDURE

The milk samples used in this study were obtained from two individual herds and the pooled herds milk as received by the college creamery. One of the individual milk sources was from a Holstein herd and the other source was from a Guernsey herd. The herd samples were obtained from the weigh tank as they were received at the college creamery. The pooled milk samples were obtained from the raw-milk bulk-storage tank. All samples were immediately cooled to 45° F. after collection.

Preparation and Selection of Whole Milk Samples to Show Effect of Seasonal Variations and Variations Due to Acidity and Heat Upon the Properties and Constituents of Milk

The samples of milk used for seasonal variations were obtained frequently throughout the various seasons of the year. They were collected as stated above. Analyses of the samples were started on the same day that the samples were taken.

To study the effect of heat upon milk, raw pooled herds milk was divided into five one-quart portions. One portion of raw milk was kept as a control. The other four portions of milk were put into covered containers

WES

which were placed in a hot water bath and were heated to 143° F. and succeeding portions were removed after intervals of five minutes, ten minutes, thirty minutes and sixty minutes of holding. Each portion of milk was immediately cooled to at least 45° F. after its removal from the water bath. The analyses of the control and the heated portions of milk were started at once.

To study the effect of heat upon milk of different acidities six one-quart portions of raw pooled herds whole milk were obtained by the means stated herein. One portion of the raw milk of normal acidity was analyzed as a control. Two other portions of the normal acidity milk were subjected to various degrees of heat treatment. The heating was accomplished as before, with a circulating hot water bath. The second portion was heated to 145° F. and held at this temperature for thirty minutes and the third portion was heated to 190° F. and held at this temperature for ten minutes. Analyses of these three portions were started immediately after cooling to 45° F. The remaining three portions of milk were put into one container with three ml. of fresh buttermilk starter and the acidity was allowed to develop, at room temperature to an arbitrarily chosen acidity of three-tenths per cent acidity after reaching the desired acidity the milk was immediately cooled to



40° F. or below. The acidity induced milk was again divided into three portions; the first portion analyzed as a control and the remaining portions subjected to different degrees of heat treatment. The second portion was heated in the hot water bath to a temperature of 145° F. and held for thirty minutes and the third portion of acid-milk was heated to 190° F. and held for ten minutes at this temperature. Analyses were started immediately after cooling.

Pooled herds milk was also used to study the effect of acidity upon milk which had been subjected to heat treatment. A six-quart portion of the normal milk was divided into three two-quart portions. The first of these portions was further divided into two one-quart portions and an analysis made upon the normal milk which served as a control. The second portion was allowed to develop acid, in the manner described earlier, to a titratable acidity of three-tenths per cent prior to being analyzed. The second two-quart portion was subjected to a temperature of 145° F. and held for thirty minutes. This portion was then divided into two one-quart portions and an analysis made of one of the normal heated portions and the other portion was analyzed after it had acquired an induced acidity of three-tenths per cent. The third two-quart portion was subjected to a temperature of 190° F. for ten minutes and then carried through in the same

manner as before. A one-quart portion of the normal milk was analyzed as a control. The remaining milk was inoculated with three ml. of fresh buttermilk starter and the acidity was allowed to develop at room temperature. One-quart portions were taken from the acid-induced milk at arbitrary acidities of 0.22 per cent, 0.30 per cent and 0.40 per cent. Analyses of the individual portions were started immediately after they had been cooled.

Preparation of the Milk Serum-the Dialyzable Portion of the Milk

From the portions of milk used in this study, 35 ml. aliquots were pipetted into prepared 28 mm. cellophane dialyzing sacs. The dialyzing bags containing the samples of milk were suspended in erlenmeyer flasks which were evacuated by a water vacuum pump. The flasks were partially immersed in a water bath at 50° F. or below to retard the development of acidity during the experimental period. The ultrafiltration of the milk samples was allowed to continue for not longer than twelve hours. Immediately after removing the dialyzing bags from the erlenmeyer flasks, the ultrafiltration-serum-portions were put into test tubes which were then stoppered and placed in the refrigerator. Analyses of the serum portions were carried out as soon as possible after their removal from the ultrafiltration setup. In

the dialyzing of milk samples with abnormal induced acidity present, several drops of chloroform were added to prevent further bacterial action and acidity increases.

Chemical and Physical Methods Used to Estimate
Changes in the Properties and Constituents
of the Selected Milk Samples and Their
Respective Dialyzable Portions

All samples of milk and their corresponding ultrafiltrates were examined in duplicate by each of the following tests:

Fat and total solids. All fat and total solids determinations of the whole milk samples were made by the Mojonnier method (1925).

Alcohol number. This test was run on all whole milk samples using five ml. whole milk and an equal amount of 70-75 per cent alcohol as suggested by Hunziker (1949).

Acidity (titratable). The titratable acidities of all whole milk samples were determined by the acidity method adopted by Hunziker (1949).

Curd tension. Curd tensions of the whole milk samples were determined according to the recommendations of the American Dairy Science Association committee on methods of determining curd tension (1941). The apparatus used was the Submarine Signal Company's curd tensiometer calibrated to read curd tension directly in grams.

pH. The pH of the whole milk samples were determined by use of a model G, Beckman glass electrode pH meter.

Heat stability. Heat stability determinations were run on all whole milk samples. Glass tubes of 6 mm. inside diameter and approximately 100 mm. in length were made with one end sealed. A two ml. aliquot of whole milk was pipetted into the tube and the open end was sealed. The tubes were then inserted in specially designed holders mounted on a rotary shaft which was submerged in a constant temperature oil bath at 120° C. The shaft was allowed to rotate at a slow but constant rate of speed until the first sign of coagulation was noticed. Observations were made at regular five minute intervals.

Citric acid. A new method devised by Babad and Shtrikman (1951) for determining citric acid in milk was used in determining the citric acid contents of the whole milk samples and the corresponding dialyzable portions of the milk. A calibration curve was prepared for the procedure as it was used in this study. This was necessary because a Cenco-Sheard Photelometer and a 450 mu filter was used whereas Babad and Shtrikman (1951) used a Photoelectric Lumitron Colorimeter with a 420 mu filter. Otherwise, the procedure was followed exactly as outlined.

Citric acid contents of the serum portions were determined by the same procedure except that one ml. aliquots of the serum samples were used directly in the determinations.

Total nitrogen. Total nitrogen contents of the whole milk samples were determined by a semi-micro Kjeldahl method similar to one reported by Menefee and Overman (1940). A five ml. aliquot of the whole milk sample was pipetted into a 100 ml. flask, diluted to volume and thoroughly mixed. A ten ml. aliquot, representing 0.5 ml. of milk, was digested in a 300 ml. Kjeldahl flask. Serum nitrogen was determined by the same procedure except that a two ml. aliquot of the serum was used directly in the nitrogen determination.

Minerals. Samples of whole milk were prepared for total calcium, total phosphorous and total magnesium assay by using the ash from 25 grams of milk. The ash was dissolved in hydrochloric acid and evaporated to dryness, then dissolved in dilute hydrochloric acid and hot water, made up to a volume of 50 ml. and filtered. Ten ml. of the filtrate was pipetted into a 100 ml. flask and made to volume. This dilution represents 0.05 grams of milk.

The total calcium content of whole milk was determined, using a ten ml. aliquot of the final ashing-dilution, by a modified method of Kramer-Tisdall as



suggested by Shohl (1922). Soluble calcium was determined in a two ml. aliquot of the serum according to a modification of the Collip and Clark method as advanced by Hawk, Oser and Summerson (1949).

Total phosphorous of whole milk was determined by a modification of a method of Fiske and Subbarow (1925) using a ten ml. aliquot of the ash filtrate made to a volume of 250 ml. and representing 0.06 gm. of milk. Soluble inorganic phosphorous found in the serum portion was determined using one ml. serum diluted to 50 ml. and five ml. of this dilution in 25 ml. distilled water, giving a representation of 0.1 ml. of serum. The same procedure was used as for total phosphorous.

Colloidal phosphorous was determined as the difference between total and soluble inorganic phosphorous according to the definition given by Pyne and Ryan (1950). To determine the total inorganic phosphorous in milk, a ten ml. aliquot of milk was added to forty ml. of a ten per cent solution of trichloroacetic acid and allowed to stand at room temperature for two hours in order to allow for complete precipitation of the protein. The solution was filtered and a five ml. aliquot of the filtrate made to 50 ml. and 5 ml. of this dilution was pipetted into a 25 ml. flask for the final dilution which represented 0.1 gram of milk. The modified procedure of Fiske and Subbarow (1925) for phosphorous estimation was then followed.

The magnesium content of whole milk was determined according to a method advanced by Briggs (1924) using fifteen ml. of calcium-free solution from the calcium determination. The final aliquot represented 0.25 gram of milk. Soluble magnesium in the serum was determined by the same method but using two ml. of calcium-free solution, from the soluble calcium determination, representing an aliquot of 0.5 ml. of serum.

RESULTS

Variations in Some of the Constituents and Physical Properties of Cows' Milk in Relation to Seasons of the Year

Fat and total solids. The data graphically presented (Figure 1) shows a moderately level fat content in pooled herds milk over the entire seasonal range. A high in fat per cent occurred in April with a decrease to a lower level in the summer and a slight rise again in the fall. The total solids content of pooled herds milk (Figure 2) followed approximately the same seasonal trend as the fat but with a smaller range of variations.

The data (Figure 1) also shows the seasonal variations of the fat content observed for milk from a Guernsey herd. For the Guernsey milk, a wider range of variations was observed. The high point for the fat per cent was noted to occur in the winter followed by a decrease in the spring which showed some variations during the summer but at a lower level than that for the winter. A slight increase occurred in the fall. It was apparent from the data (Figure 2) that the total solids followed an identical trend of the fat content, the only difference being in the range of variations.

The seasonal variations in the fat content of milk

from a Holstein herd are also shown (Figure 1). The fat content showed only slight variations during the seasons until late summer when an increase occurred to show a higher level of fat which was maintained through the fall season. The total solids content of the Holstein herd's milk (Figure 2) was found to parallel the trend for the fat over the entire seasonal period.

The fat and total solids contents of the Guernsey herd's milk showed (Figures 1 and 2) consistently higher values and wider variations, than the other milks tested, for all the seasons.

Titrateable acidity and pH. From the data gathered (Tables 1,2 and 3) there appeared to be little evidence of a direct relationship between the titrateable acidity and pH of the milks with seasons of the year. However, a marked degree of difference was noted in the titrateable acidities of normal milk between breeds. The milk of the Guernsey herd showed consistently higher titrateable acidities than either the pooled herds' or Holstein herd's milks.

Curd tension. The experimental data tabulated in Tables 1,2 and 3 showed no indication that the curd tensions of the cows' milk tested were affected by changes in seasons. It was observed that the curd tension of milk from the Guernsey herd was higher than that of pooled herds or of the Holstein herd's milk.

Heat stability. The heat stabilities of all three milks showed little relationship to the season of the year (Tables 1,2 and 3). However, all samples of milks were most stable to heat during the late spring and early summer.

Citric Acid. The data shows a wide range of values for the citric acid content of the milks studied. The data for the milk from a Guernsey herd (Table 2) shows a slight decrease during the late winter and a very material increase in citric acid content in April which was followed by a decrease during the summer and a slight rise in the fall. The citric acid contents of the pooled herds and Holstein herd's milks (Tables 1 and 3) showed a gradual decrease during late winter and early spring which was followed by an irregular level until late summer when a slight upward trend was noted.

The citric acid contents of the serum portions of the three milks studied (Tables 1,2 and 3) were observed to follow the same general trend with a decrease to a lower irregular level through the spring and a gradual rise occurring in the summer and fall.

Nitrogen. The seasons of the year were found to exhibit identical general trends of total nitrogen contents in all the milks studied. The data (Figure 3) showed a slight decrease in total nitrogen during the late winter months followed by an irregular level during

THE

the spring and early summer. However, a steady rise in total nitrogen contents of the milks began in the summer and continued into the fall. A marked difference was noted in the total nitrogen contents of milk from the different breeds. The Guernsey herd's milk showed consistently higher total nitrogen values than the other two milks studied. A similarity was noted between the trends observed for the total solids and the total nitrogen contents of the milks studied for the seasons of the year.

The results for the serum nitrogen (Tables 1,2 and 3) for the milks studied show little relationship with any particular season of the year. There were large increases and decreases in serum nitrogen noted throughout the entire testing period.

Minerals. Figure 4 shows the effect of seasons upon the calcium and total phosphorous contents of the milk samples studied. The calcium contents for all milks tested were observed to parallel each other over the entire seasonal range. A slight decrease in calcium content was noted in the late winter followed by a sharp rise in the spring which reached a high in April. The calcium contents of the milks then decreased during the late spring until a point was reached which approximated the results for the winter season. The trend then remained fairly level during the summer and fall seasons. The total phosphorous contents of the three milks in-



investigated (Figure 4) followed a trend similar to the one observed for the calcium contents except that a more gradual decrease occurred in the summer and fall.

The curves for the P/Ca ratios of the three milks (Figure 4) show approximately the same trend as for the calcium and phosphorous contents in the milks but the peaks of the ratios came in mid-summer followed by a gradual decrease in the late summer and fall.

The magnesium contents of the milks studied (Tables 1,2 and 3) were found to be within the same range of results over the entire seasonal trend. However, a marked decrease was noticed for the mid-summer season and another low value was observed for March.

There were few variations found in the calcium content of the serum of the milks studied. However, from the results observed for calcium in the serum of the pooled herds and Guernsey herd's milks (Tables 1 and 2), a slight increase was noted in the spring followed by a uniform level in the summer with a slight decrease in the fall. The serum calcium of the Holstein herd's milk was noticed (Table 3) to remain at a fairly uniform level over the entire seasonal range except for a slightly higher value than the normal observed in the fall season. The serum phosphorous contents of the three milks (Table 1,2 and 3) were found to exhibit the same trend but at differing concentration levels. A

general increase in serum phosphorous was noted in the late winter with a high peak reached in the summer and followed by a gradual decrease in late summer and fall. The serum magnesium contents from the three milks studied (Tables 1,2 and 3) were observed to be almost the reverse of the trend noted for magnesium in the whole milk. The lowest values of serum magnesium were found in the winter season milks and highest values were found in the summer season milks.

The total inorganic phosphorous contents of all three milks were observed to follow an identical trend (Tables 1,2 and 3). A low was observed in the late winter followed by a very marked increase in the spring. A sharp decrease was noted in the summer followed by an increase which remained somewhat level through the fall season.

The data presented for the calculated values for colloidal phosphorous (Figure 5) shows a seasonal trend to occur with a high peak observed in the spring followed by a gradual decrease during the later seasons of the year.

Variations in Constituents and Some of the
Properties of Whole Milk When Subjected
to Various Periods of Heating

Fat and total solids. Changes in some of the constituents and properties of whole mixed milk as a result of heating the milk to 143° F. for varying lengths of time are recorded in table 4.

The percentages of fat and total solids were not changed as a result of heating the milk.

Alcohol number. The alcohol number of the milk was observed (Table 4) to become less as the milk was subjected to longer periods of heating.

Titrateable acidity and pH. The titrateable acidities of the heated milk (Table 4) were observed to be only slightly higher than the original titrateable acidity of the unheated milk. In the case of the milk heated at 143° F. for five minutes, the titrateable acidity showed a slightly lower value than the original.

The pH of the milk was observed to decrease gradually as the milk was heated for succeeding longer periods of time.

Heat stability. The heat stability determination (Table 4) demonstrated that the times required to cause coagulation of the milk samples were almost directly proportional to the degree of heat treatment.

Citric acid. Citric acid determinations on the

heated milk (Table 4) showed slightly lower citric acid values than the original citric acid contents of the unheated milk. The citric acid contents of the heated milk were generally a little lower. The citric acid determination of the serum portion of the heated milk showed a generally lower level of citric acid than was found in the serum of the unheated milk.

Total nitrogen. Total nitrogen and serum nitrogen values were expressed graphically (Figure 6) and showed that as the milk was heated the total nitrogen increased, at first, then decreased as the heating period lengthened. Serum nitrogen values for the heated milk showed slightly lower values than those observed for the original unheated milk.

Minerals. Figure 7 shows the values for calcium in raw milk and heated milk. No change in the calcium content occurred between the raw milk and the milk heated for five minutes at 143° F. Milk heated for ten minutes at the same temperature showed a sharp decrease in calcium with little change observed in the calcium content of milk heated longer than ten minutes. Serum calcium results followed the same trend as that observed for the total calcium but with a smaller degree of change. A decrease in serum calcium was shown to occur in the milk which had been heated at 143° F. for five minutes, after which very little variation occurred in

THE

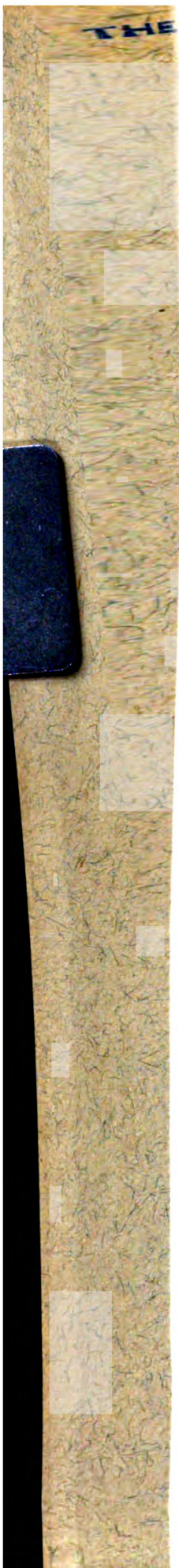
the serum calcium of milk heated for longer times.

Total phosphorous determinations (Figure 7) show a slight decrease in milk heated for five minutes but no further change until the milk had been heated for thirty minutes when a sharp decrease was observed. A marked increase was noted in the milk heated for sixty minutes. Serum phosphorous determinations shown in the same graph followed almost the same pattern except that there was no apparent change in serum phosphorous prior to the decrease in serum phosphorous recorded for milk heated for thirty minutes.

From the calculated P/Ca ratio of the milk studied (Figure 7) little change was noted prior to the period of heating for ten minutes when a rise occurred, followed by only slight changes as the milk was heated for longer periods of time. The calculated P/Ca ratio for the serum increased markedly for the milk heated for five minutes followed by slight variations as the milk was subjected to longer periods of heating.

The data in Table 4 showed a steady increase in magnesium as the milk was heated for succeeding longer intervals, except for the milk heated for ten minutes when a slight decrease resulted. The magnesium contents of the serums obtained from the raw and heated milks showed but small variations.

The total inorganic phosphorous (Table 4) was



found to decrease in milk heated for five minutes when compared with the original value for the raw milk. The results for inorganic phosphorous then increased gradually as the milk was subjected to longer heating periods.

An increase in colloidal phosphorous (Table 4) was shown in milk heated for five minutes, but the data then showed a gradual decrease as the period of heating was lengthened.

The Effect of Heat Upon Some of the
Constituents and Properties of
Milk with Abnormal Acidity

Fat and total solids. All values and data for the determinations made in this section of the study are recorded in Table 5. There was no apparent change in the per cent fat and total solids between the normal and the acid-milks.

Alcohol number. The alcohol number increased for normal milk which had been heat-treated (Table 5) while the alcohol number decreased for the heated milk with developed acidity. The alcohol numbers of the acid-milk samples were much lower than those of the non-acid-milk samples.

Titratable acidity. The titratable acidity of the normal milk was noted to decrease slightly as the milk was heated, there being no difference between the milk heated at 145° F. for thirty minutes and that heated at

1

2

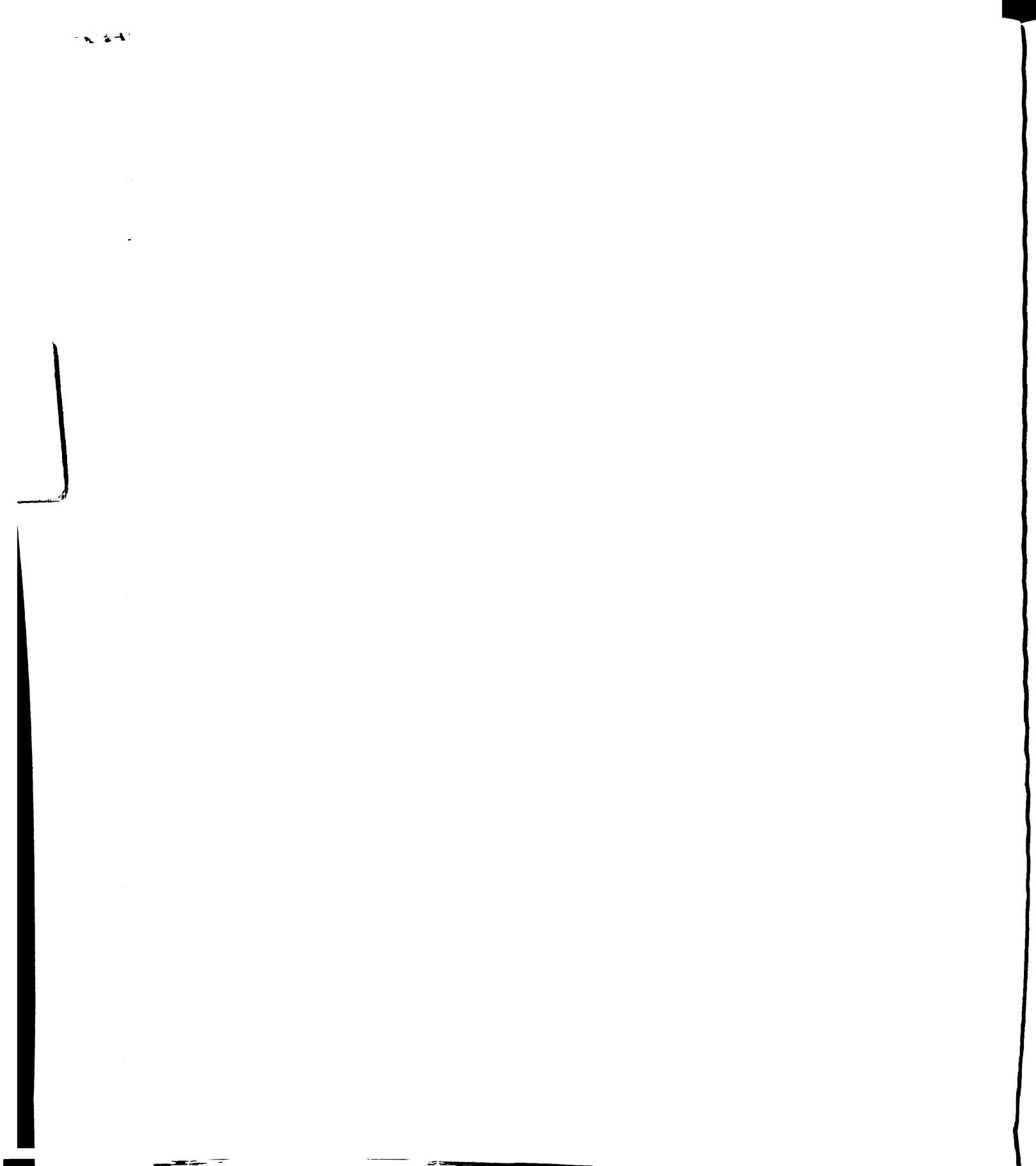
.

190° F. for ten minutes. However, the titratable acidity of the acid-milk showed an increase when heated with the highest acidity occurring in the acid-milk heated at 145° F. for thirty minutes.

pH. The pH of the normal milk decreased when heated, with the higher heat-treated milk showing a larger pH decrease. The pH of the acid-milk samples showed lower values than the normal milk samples. However, the pH of the heated acid-milk showed a slight increase over that of the control when heated at 145° F. for thirty minutes while the acid-milk heated at 190° F. for ten minutes showed a decrease over the original acid-milk value.

Curd tension. The curd tension of normal heated milk showed a decrease and the milk heated at 190° F. for ten minutes showed the lowest curd tension. The same trend was noted for the heated acid-milk, but the curd of the acid-milk heated at 190° F. was gassy and flocculent in character. This curd was not cut by the curd knives but it showed no resistance to the pressure applied by the tensiometer.

Heat stability. The heat stability of the normal milk showed an increase in the time required for coagulation when that milk was heated. However, when an acidity was allowed to develop in the milk, that milk showed no stability toward heat. Heating of the acid-milk failed to



show any change in the heat stability of that milk.

Citric acid. Data in Table 5 shows an increase in citric acid content of normal milk when heated at 190° F. for ten minutes. The citric acid content of the unheated acid-milk was slightly higher than that of the normal unheated milk. A decrease in citric acid was noted for the acid-milk heated at 145° F. for thirty minutes while citric acid in the acid-milk heated at 190° F. for ten minutes was the same as for the normal unheated milk.

Nitrogen. When normal milk was heated the total nitrogen increased, with the largest increase occurring in the milk heated to 190° F. for ten minutes. The total nitrogen of the unheated acid-milk was found to be higher than that for the unheated normal milk. A decrease was noted in total nitrogen of the acid-milk heated at 145° F. for thirty minutes but a marked increase was noted in the acid-milk heated at 190° F. for ten minutes.

The nitrogen contents of the sera from the normal heated milks showed an increase over that of the original milk. The serum of the normal milk heated at 190° F. showed the greatest increase in nitrogen. The serum nitrogen of the unheated acid-milk was higher than that of the unheated normal milk. The sera of the heated acid-milks showed an increase over the unheated acid-milk. The increases in serum nitrogen of the heated acid-milk

1

were of less magnitude than those for the heated normal milk.

Minerals. The calcium content of normal heated milk increased over that of the unheated milk. The calcium showed a higher increase in the milk heated to the higher temperature. The calcium contents of the heated and unheated acid-milks showed little variation between each other and calcium value reported for the original unheated normal milk. The phosphorous content of the normal milk increased as the milk was subjected to a higher temperature. The calculated P/Ca ratios for the acid-milk samples were found to be closer to 1:1 than the ratios for the normal milk samples. The magnesium contents of the normal milk showed an increase when the milk was heated to 190° F. for ten minutes but a slight decrease in magnesium was noted in the milk heated at 145° F. for thirty minutes. Magnesium in the acid-milk showed an increase when the acid-milk was heated. The acid-milk heated at 145° F. for thirty showed the largest increase. Inorganic phosphorous of the normal heated milk increased over the value of the original milk irrespective of the degree of heat-treatment. The inorganic phosphorous of the acid-milk showed a succeedingly higher increase as a higher heat-treatment was used. The inorganic phosphorous values of the heated and unheated acid-milk were higher than the values for

the normal milk samples.

The serum calcium of the normal milk showed a succeedingly larger decrease as the milk was heated to a higher temperature. The serum calcium of the acid-milk decreased as the acid-milk was subjected to heat and the greatest decrease occurred when the acid-milk was heated to 190° F. for ten minutes. It was also noted that the serum calcium values of the heated and unheated acid-milks were higher than those recorded for the sera of the normal milk samples. The phosphorous contents of the sera of the normal heated and unheated milks showed little change, but the serum phosphorous of the acid-milk showed higher values than the normal and these values were noted to decrease as the milk received a higher heat-treatment. The data showed slight decreases in serum magnesium as the normal milk was heated with the largest decrease occurring in the serum of the milk heated to 145° F. for thirty minutes. The magnesium of the sera from the heated acid-milk showed a decrease over the value for the unheated acid-milk. A greater decrease was noted in the serum magnesium of the acid milk heated to 145° F. for thirty minutes.

The Effect of Induced Acidity Upon
Some of the Constituents and
Properties of Heated Milk

Fat and total solids. Analyses of the constituents and observations on the properties of the milk samples studied in this part of the experiment are tabulated in Table 6. The fat and total solids showed no change between the normal milk and the acid-milk.

Alcohol number. The data showed an increase in alcohol number as the normal milk was heated. The acid-milk showed no stability to the alcohol test but the acid-milk heated to 190° F. for ten minutes showed a slight increase in alcohol number.

Titratable acidity. As the normal milk was heated a decrease in acidity was noted with the larger decrease occurring in the milk heated to 190° F. for ten minutes. The acidities recorded for the acid-milk were chosen as the end-point for the study of acidity upon heated milk.

pH. From the data (Table 6) it was observed that the pH of the normal milk decreased as the milk was subjected to higher temperatures. The pH values of the acid-milks were lower than those of the normal milks, but there was no apparent trend related to the heat-treatment used.

Curd tension. The curd tension of the normal milk showed a marked decrease only in the milk heated to 190° F.

for ten minutes. The data showed lower curd tension values for the acid-milk which had been heated than those recorded for the normal milk. However, the curd formed in the acid-milk which had been heated to 190° F. was gassy and of a flocculent character. The curd was not cut by the curd knives and offered no resistance to the curd tensiometer.

Heat stability. The heat stability of the normal milk showed an increase as the milk was subjected to heat, but the acid-milk showed no stability towards heat.

Citric acid. The citric acid content of normal milk showed no change between the unheated milk and the milk heated to 145° F. but a lower value was observed for the milk heated to 190° F. The citric acid values for the acid-milks were found to decrease when the milk had been heated to a higher temperature. The citric acid values of the acid-milk samples were observed to be generally lower than those for the normal milk samples although the opposite was true for the milks heated at 190° F. The citric acid contents in the sera of the acid-milk samples were found to be generally lower than in the normal sera. However, the sera from the milk, both normal and acid-milks, showed higher values when the milk had been heated at 190° F.

Nitrogen. The data shows the total nitrogen of the normal milk to be higher as a higher heat-treatment

is used. The total nitrogen values for the acid-milk show a decrease as the milk was heated with a higher temperature and also lower values than those reported for the normal milk. The serum nitrogen of the normal milk was observed to decrease as higher heat-treatment was used while the serum nitrogen values of the heated acid-milks increased at equal rates. The serum nitrogen values of the acid-milk samples were markedly higher than those of the sera from the normal milk samples.

Minerals. The calcium contents of the normal milks showed no significant change as the milks underwent heat-treatment. The results for calcium in the acid-milk samples showed an increase only for the milk which had been heated at 190° F. The phosphorous values of the normal milk showed a slight increase as the milk was subjected to heat. The phosphorous content of the acid-milk was higher than the original value and as a higher temperature was used on the milk the phosphorous value increased. The calculated P/Ca ratio was noted to be higher in the milks which were heated at 145° F. Magnesium values in the normal milk decreased as higher temperatures were used. The magnesium contents of the acid-milk varied with no special trend noted for the heat-treated samples. The inorganic phosphorous results showed a slightly higher value in the normal milk heated at 145° F. but no other variation. The inorganic

phosphorous of the acid-milk increased as a higher temperature was used in heat-treating the milk. The calculated colloidal phosphorous values for the normal milk showed slight increases as higher temperatures were used. The calculated colloidal phosphorous values for the acid-milk showed an increase when the milk had been heated at 145° F. but a decrease when the milk had been heated at 190° F.

The calcium contents of the sera from the normal milks were observed to be lower as a higher heat-treating temperature was used. The data shows that the serum calcium of the acid-milk increased as the milk was heated at a higher temperature. These values for the serum calcium of the acid-milks were much higher than those recorded for the normal milks. Serum phosphorous decreased in the serum of the normal milk as the milk was subjected to higher heat-treatment while the phosphorous content of the serum from the acid-milk increased as the milk was heated at higher temperatures. The serum phosphorous contents of the acid-milks were higher than the reported normal values. The serum magnesium of the normal milk decreased as the milk was heated at higher temperatures but the serum magnesium of the acid-milk increased as higher temperatures were used in heat-treatment of the milk. The serum magnesium values of the heated acid-milk were higher than those

reported for the normal values.

Effect of Changes in Titratable Acidity Upon
Some of the Constituents and Properties
of Raw Whole Milk

Fat and total solids. All data for this section of the study is recorded in Table 7. There were no changes in per cent fat or total solids as the milk increased in titratable acidity.

Titratable acidity and pH. The milk was allowed to develop titratable acidity to the values recorded and samples of milk were analyzed at those acidities. The pH values of the milk decreased as the acidity increased.

Alcohol number and heat stability. The values for the alcohol number and heat stability of the milk were observed to decrease as the milk increased in titratable acidity.

Curd tension. The curd tension showed a narrow range of variations until the final stage of acidity when no curd resistance was encountered by the curd knives. The curd formed in the milk of 0.42 per cent acidity was noticed to be of a gassy, flocculent character.

Nitrogen. Figure 9 shows total nitrogen values for milk with induced acidity. It was apparent that the nitrogen values increased only slightly at the

first stage of developed acidity, but, as the acidity was allowed to develop further, the nitrogen values increased at a successingly higher rate. The serum nitrogen decreased at a fairly level rate as the acidity of the milk increased.

Citric acid. The citric acid content of the milk showed no change until the second stage of induced acidity when a sharp decrease occurred followed by a further decrease as the acidity increased in the milk. However, the citric acid values for the serum showed an increase as the milk developed a higher acidity.

Minerals. Figure 10 shows that the calcium of the milk increased almost proportionally with the degree of acidity developed in the milk. The phosphorous content showed a decrease in milk of the first and second stages of induced acidity followed by an increase to the original value in the final stage of acidity. The P/Ca ratio for whole milk decreased (Figure 10) as the acidity of the milk increased. The magnesium content of the milk (Table 7) showed a wide range of variations as acidity developed in the milk. A slight decrease occurred in magnesium present in milk of the first stage of induced acidity, but, an increase was observed in milk of the second induced acidity stage. The magnesium content showed a marked decrease in the final stage of acidity. Values for the inorganic phosphorous (Table 7) showed no

significant change. The calculated values for the colloidal phosphorous show a slight decrease in the milk of the first and second stages of developed acidity followed by an increase in the final stage to a value slightly higher than the normal.

The calcium in the serum portion of the milk steadily increased as the milk developed higher acidity. The phosphorous content of the serum also increased as the acidity increased but at a greater rate than the calcium. The P/Ca ratio for the serum paralleled the P/Ca ratio of the whole milk but with a smaller decrease as the acidity of the milk developed. The serum magnesium increased as the milk increased in acidity while the highest magnesium value occurred in the final stage of acid developement.

DISCUSSION

Milk becomes a very unstable product when various external influences are applied or when the normal balance among the constituents of milk is upset. By the same token, milk can be made more stable with the application of external influences and by restoring a more favorable balance among the constituents of the milk. These variations and adjustments have been shown to exist by such basic works as those by Sommer and Hart (1926).

Although the minerals of milk are in the lowest quantity, they seem to be the most important group of constituents to affect the stability of a milk toward the application of external factors. The three most common external influences which seem to affect the constituents of milk are the seasons of the year which cause natural occurring variations, the formation of acidity due to the presence of normal lactic acid fermenting bacteria and the application of heats used in processing milk for consumption. Other external factors such as health of the cow, the presence of bacteria normally not found in milk and the presence of chemicals in the milk are not considered as normal external factors.

It is generally agreed among dairy research workers

that a certain balance should be present among the mineral elements, calcium, magnesium, phosphorous, and citrate.

This study was undertaken to acquire information about the distribution of some of the normal constituents of milk when the milk was subjected to the three common external factors discussed, alone and in combination with each other.

Influence of Breed and the Seasons of the Year
Upon Some Properties and Distribution of
Various Normal Constituents of Milk

In this part of the study no attempt was made to control factors of lactation, type of feed other than to know when cows were feeding on pasture or winter rations or individual cows in a herd, but it was found from the producers that the two typed herds were put on pasture within the same week. The pooled herds milk was not checked but it was assumed from the general weather conditions that there would be little difference in the time that herds of the middle section of Michigan were put to pasture.

The trends observed in Figure 1 for the fat and total solids of Guernsey milk were found to coincide closely with results reported by Overman (1945) and Jacobsen and Wallis (1939) that lower values were observed during the summer months with an increase

in the fall and high values in the winter months. The results in Figure 2 for Holstein milk are also similar to those reported by Overman (1945) in that increases were observed in the late summer while a moderate level was maintained the rest of the year. Guernsey herds' milk is decidedly higher in fat and total solids when compared to values for Holstein milk. This was also in good agreement with work done by Ragsdale and Turner (1922).

Since the total solids of a milk includes the total nitrogen content it was assumed that it should vary as the total solids varied. This was found to be the case and correlated closely with work reported by Rowland (1938) and Shahani and Sommer (1951) that a milk with a high fat, high solids content showed consistently higher total nitrogen contents. Investigations of the total nitrogen content of cows' milk for seasons produced similar results to those found by Davis et al (1947a) over a period of one year. The nitrogen contents generally showed lower values in the winter months with increases noted when the cows were put to pasture, which was also in good agreement with works reported by Hess et al (1920).

Cranfield, Griffiths and Ling (1927) reported that CaO decreases in summer and the P_2O_5 increases for the summer period with $\text{CaO}:\text{P}_2\text{O}_5$ ratio reaching a

maximum in August. This is in part contradictory to results by Hess et al (1920), Ellenberger, Newlander and Jones (1950), who observed that both the calcium and phosphorous increased when cows were put on pasturage. Results obtained in this study indicate an agreement with the work done by Hess and co-workers in that the calcium and phosphorous increased markedly in the spring season. These values were shown to be lower during the late summer which is in agreement with results of Golding et al (1932). Since the total solids of milk from the smaller breeds are higher it was expected that the mineral content of high solids milk would be higher. This is in accord with works reported by Holm et al (1932) and Graham and Kay (1933-34).

In the past, investigators have differed as to the effect of seasons upon the citric acid content of milk. One main reason for this has been the lack of a satisfactory method for determining citric acid which gives undisputable results. Sommer and Hart (1926) believed, with Hunziker (1949), that the citric acid content increases when a cow goes onto green pasture in the spring. It appears from the data obtained in this study that the citric acid content may increase ~~or~~ decrease in milk from cows on pasture. An increase occurred in the Guernsey milk sampled in the spring but a decrease occurred in the Holstein milk. This

1

fact is in direct disagreement, as concerned with breed differences, with results of Holm et al (1932) who found decreases in high fat milk and increases in low fat milk. The disagreement in results could possibly be traced to the weather conditions or type of pasturage.

The results in this study upon the curd tension with seasons of the year show a relationship between increases in total nitrogen and higher values in curd tension. This is supported by investigations reported by Riddell et al (1936). No definite relationship was observed between the salt content and the curd tension, but it was observed in some cases that a higher curd tension resulted when a combination of high total nitrogen and high calcium contents were present. The degree of citric acid present in the samples appeared to make no difference upon the results of curd tension.

The stability of milk towards heat is an important factor in processing of the milk. The heat test is one of the best means advanced to give an indication of heat stability. From the results of this study the reasons for a better heat stable milk are not very clear but it seems to require a balance among the salts and the protein content of the milk. The best stability of the milk toward heat appeared to occur when the differences between the mineral contents and

the nitrogen contents of the milk were at their widest limits. This seems to follow the reasoning of Sommer and Hart (1926) that the coagulation of albumin is hastened when an increasing amount of electrolyte is added. They have also shown a lower heat stability of milk to occur when the calcium and albumin contents of milk are high. This may account for the occurrence of a lower trend in heat stability of the spring season milk when the calcium content was highest along with a relatively high protein content. (An assumption must be made that the albumin content of milk increases as the protein content increases as no determination was made in this study of the components of the total protein.) The idea of Sommer and Hart (1926), that an improvement is inherent in the stability of the milk toward heat when the cows go on pasture in the spring, is in disagreement with results in this study as the best stability was noticed to occur in the summer and fall.

Colloidal phosphorous appears to be affected by seasonal variations, as shown in results in Figure 5, with a peak noticeable in the spring and agrees favorably with data reported by Davies and Provan (1928) that colloidal phosphorous (total inorganic) showed an increase as a result of grazing. This is in disagreement with results stated by Ling (1937) that the total inorganic phosphorous did not appear to be affected by

changes in seasons.

As a result of work in this study and the studies of other investigators a closer control of factors which might influence variations of milk constituents and standardization of analytical procedures is an end to be desired if results are to be compared between investigations.

There seemed to be no relationship of the alcohol number and the season of the year. One interesting observation on all the milk studied was that the alcohol number improved as the total inorganic phosphorous increased. This follows the report by Sommer and Binney (1923) that a slight increase in a phosphate salt will increase the alcohol test but an increase in a calcium salt will give a lower alcohol test.

Effect of Heat Upon Some of the Constituents and Properties of Cows' Milk

There has been little work reported on the effect of pasteurizing temperature with various holding times upon changes in properties and constituents of milk. It was realized, after this part of the study was completed, that a forewarming temperature should have been run in conjunction with the pasteurizing temperature and holding times used in order to estimate the effects of the two more common processing heats. This will be covered to some extent in later investigations. Most of the temper-

atures reported in earlier studies by other investigators were for temperatures near the boiling point of milk and forewarming temperature of milk in preparation for concentration.

Davies (1936) relates that the application of heat to milk acts to cause a slight decrease in titratable acidity by driving off carbon dioxide and causing a partial decomposition of bicarbonates present, but that further heating of the milk will cause a slight increase in acidity due to the changing buffer index resulting from the precipitation of insoluble calcium salts. Results of titratable acidity as obtained in this study upon heating of milk follow the reasoning of Davies. Investigations by Whittier and Benton (1927) showed a similar trend in results of milk heated at 95° C. for varying time lengths but with greater increases. In later works Whittier and Benton reported the increase in acidity to be directly related to the amount of lactose present in the milk. No corresponding data as to the effect of lactose concentration was determined in this study. Following the reasoning established by Whittier and Benton it appears that the acidity of the milk investigated here should increase with a decrease in pH as milk is heated. Such was the case in this study when the pH was observed to follow a steady decrease as the milk was heated for longer

periods of time.

The variations in total nitrogen values reported for this study (Figure 6) on the effect of heat upon the total nitrogen content of milk is in disagreement with other investigators. Shahani and Sommer (1951) and Menefee et al (1941) have all shown that there were no significant changes in total nitrogen when milk was heated. Since no attempt was made to investigate the high values reported it is assumed that they are due to improper sampling or unsatisfactory analytical technique. However, since it is common knowledge that proteins are denatured by heat, it is possible that the low total nitrogen value obtained in the milk heated for sixty minutes at 143° F. may have been due to the denaturation and subsequent coating of coagulated proteins on the heating vessel. Davies (1936) has identified the albumin fraction of milk protein as conforming to the characteristics of a water-soluble protein and globulin as a colloidal protein. That albumin and globulin in milk are coagulated by heat in succeeding larger amounts as higher temperatures are used was reported by Rowland (1933). This may account for the small decreases in dialyzable nitrogen observed in this study which were possibly due to the coagulation of water-soluble proteins present in the raw milk. However, this is in contradiction to results reported by Mattick

and Hallet (1929) that no change occurred in the diffusible portion of milk heated for thirty minutes at a range of temperatures from 40-90° C.

The decrease in total calcium is unexplainable except that in the coagulation of some of the proteins, the calcium was used in the precipitation reaction caused by the heat and inaccurate sampling resulted. The loss in calcium may be due to the formation of tri-calcium phosphate shown by Soldner (1888) to occur in the coagulum which forms on the surface of the milk when heated. The soluble calcium was found to decrease slightly when the milk was heated at 143° F. for five minutes and remain level during further heating. This is in good agreement with works reported by Verma and Sommer (1950) and Lampitt and Bushill (1934) that soluble salts are precipitated with heat in the presence of water-soluble proteins.

Sommer and Hart (1919) have explained the heat stability of milk upon the basis of a balance among the major salts found in milk. From the graph in Figure 7 showing the P/Ca ratios for whole milk and the serum and comparing the increase in heat stability results (Table 4) and increase in the ratios as the milk was heated it is apparent that the ratio of phosphorous increased over the calcium at the same point that the heat stability increased. Following the reasoning of

Sommer and Hart it may be suppositioned that the change in the salt ratio has some effect upon the improvement in the heat stability of the milk. As stated earlier if some of the soluble calcium salts were precipitated to an insoluble form in the surface coagulum formed, the ratio of soluble salts would become higher for the phosphorous over the calcium, as the phosphorous shows little variation except for increases in inorganic phosphorous.

It has been generally agreed among investigators that the firmness of the curd formed between rennin and paracaseinate is subject to the presence of calcium salts in one form or another. The evidence available from this study points to the fact of a lower curd tension occurring when a lower calcium content was noticed. This follows the reasoning of Van Slyke and Bosworth (1916) that the firmness of a rennin formed curd of paracaseinate is due to the amount of calcium salts present, the more calcium present the firmer the curd.

When normal milk was heated for longer periods of time the alcohol number was observed to increase, but the heat stability of the heated milk increased along with an increase in acidity and a decrease in soluble calcium. These results are in agreement with reports of Sommer and Binney (1923) that the effect of

salts and acidity are additive and that increases in heat will cause a relative increase in stability due to the precipitation of soluble calcium. They did not, however, account for the relative increase and effect of acidity due to the heat.

Effect of Various Degrees of Acidity Upon Some
of the Properties and Constituents
of Cows' Milk

An unusual increase in total nitrogen, when acidity is allowed to develop in milk, was noted which was unaccountable. Other investigators have found no change in the total nitrogen of fresh and acid milks. This was shown in data reported by Khambatta and Dastur (1951). However, they reported an increase in the ammonia nitrogen content of soured milk. Roy and Bhatnagar (1948) believed that increases in the total nitrogen were due to microbial action. These reports may aid in accounting for the unusual increase in total nitrogen found in the acidified milk in this study. The decreases in the dialyzable nitrogen contents as reported in these experiments disagree with the reports of Khambatta and Dastur (1951) that dialyzable nitrogen increases in souring milk.

The increases in calcium with increasing acidity are not in agreement with results reported by Khambatta and Dastur (1950) although results on the total phosphorous

were found to be similar in that no large variations occurred with changes in acidity. The results for the serum calcium and phosphorous agree with works of Van Slyke (1928) in that these dialyzable minerals increase as the acidity is allowed to increase. Van Slyke and Bosworth (1916) also report results similar to those found in this study. Dialyzable magnesium was observed to increase with increases in acidity. The decrease in the citric acid content of souring milk occurred due to the fermentation of citric acid by various organisms. The increase in dialyzable citric acid is unaccountable and in disagreement with a general opinion of other workers that a decrease should occur due to fermentation. More data is needed on this constituent with emphasis upon the formation of a suitable analytical determination procedure.

The results of this experiment appear to lend some support to the "salt balance" theory of Sommer and Hart (1926) that a change in the balance will cause a decrease in the stability of milk.

When the heat stability results are compared with changes in the P/Ca ratio shown in Figure 9, the heat stability decreased very markedly with a decrease in the ratio of total phosphorous to the total calcium. Of course, it must also be realized that the presence of the casein and minor proteins should also be included in

explaining the heat stability of an acid-milk. Such a relationship has been demonstrated by Van Slyke and Bosworth (1916) when they found the maximum solubility of calcium to occur when casein was at its isoelectric point. Sommer and Hart (1926) expressed the same reasoning when they observed that an optimum calcium content will furnish optimum stability to the casein.

Hammarsten (1896) and Palmer and Richardson (1925) observed from experiments on rennin coagulation that a flaky precipitate, such as was observed in the rennet coagulated curd of milk at 0.42 per cent acidity in this study, is found when only calcium paracaseinate is present and no calcium phosphate; but a clot will form when calcium phosphate is present. It is possible to assume that the flocculent curd observed in this study was due largely to the absence of calcium phosphate which is thought to be soluble in the presence of increased acidity. The factors responsible for varying curds were too varied and incomplete to express any definite conclusions at this point.

In this portion of the study the alcohol number of the normal milk was found to decrease as the acidity was allowed to develop. Along with the decrease in alcohol number with decreasing acidity an increase in soluble salts was noted. The lowered alcohol numbers may then be explained by reports of Sommer and Binney

(1923) that addition of soluble salts will decrease the alcohol test.

Effect of Heat-Treatment Upon Some Properties
and Constituents of Milk With
Various Degrees of Acidity

The effect of heat and acidity upon milk was studied and discussed earlier. In this part of the study the effect of heat upon milk of high acidity was investigated to determine changes which might occur under processing conditions.

The increases obtained for the nitrogen values of the heated milk are regarded with suspicion. According to other investigators, Verma and Sommer (1950) and Mattick and Hallet (1929), no increases should occur when normal milk is heated. The increases in nitrogen values for acid-milk may be considered valid following the reasoning of Roy and Bhatnagar (1948) that nitrogen increases may be expected as a result of increased bacterial action. The heat-treatments serve to increase certain bacterial action. This probably takes place as the temperature of the milk is being increased to the desired processing temperature and during the period of acid developement in the milk preliminary to the heat-treatments. Another reason for the increases may have been due to poor sampling technique of the flocculent proteins which formed due to the acid-heat denaturation. Nelson (1953)

has observed that the serum nitrogen found in whey will decrease as heat is applied. He also stated that the denaturation may play an important part in the heat stability because of a serious loss of charge resulting in a larger particle size and aiding in causing an instability between the calcium and phosphorous salts. In this study the dialyzable nitrogen was found to increase in the milk as higher heat-treatments were used. When acid development was allowed to take place the dialyzable nitrogen increased but when the milk was subjected to heat the dialyzable nitrogen was lower than those recorded for the normal heated milks. These results are in fair agreement with results reported by Khambatta and Dastur (1951).

From results discussed earlier upon the effect of heat on milk, a decrease in soluble calcium was expected with no change in the other soluble salts. Also, from an earlier discussion of effects of pH (acidity) upon milk, a marked increase in soluble salts could be expected to be found in a milk with a lowered pH. These trends occurred in the heat-treated acid-milk. However, the increases of soluble salts were found to be checked by the application of heat to the acid-milk. Sommer and Hart (1926) reasoned that acid development aided the heat stability by the fact that the soluble calcium increased from the insoluble di- and tri-calcium phosphate of milk. However, it

appears that since the increase in soluble (primary) phosphates was of such a marked rise compared to the increase of soluble calcium, the P/Ca ratio increased at such a rate as to cause a lowered heat stability test due to a lack of secondary phosphates. This also adheres to the reasoning of Sommer and Hart (1915) that acid development in milk will cause a decrease in secondary phosphates and increase in primary phosphates which have no effect on the salt balance. But in this study, since the calcium and phosphorous did not increase at the same rate the difference between the two salts was too great to be in good balance.

Since few changes in citric acid contents were observed, it would appear that there were few citric acid fermenting bacteria present or active at the time of sampling. Probably, the application of heat stopped the action of these bacteria.

The curd tensions of the normal and acid-milk appear to show the same effect whether high acidity was present or not. However, the lower curd tension values observed for the heated acid-milk may be attributed to a lower insoluble salts content in combination with the coagulum formed by the rennet used in the determination.

The alcohol number decreased in milk with developed acidity and also with an increase in soluble salts. An increase in alcohol number was also noted as the heat

stability increased in the heated milk. These results follow the reasoning of Sommer and Binney (1923) that acidity increases will bring about increased amounts of soluble salts and it is the relation of soluble salts which are responsible for the reaction in an alcohol test.

Effect of Various Degrees of Acidity Upon
Some of the Constituents and
Properties of Heated Milk

This section of the study was concerned with the effect of heat and of changes which occur when acidity is allowed to develop in the heated milk. The increase in total nitrogen of the heated milk is unaccountable but the decrease in serum or dialyzable nitrogen might be attributed to the denaturation and coagulation of soluble proteins in the serum. This is in accord with reports by Rowland (1933-34) that water soluble protein of milk are coagulated by heat. The decrease in total nitrogen found to occur in the acid-milk as temperatures of processing were increased are in agreement with observations of Roy and Bhatnagar (1948) who stated that the decrease could be due to the breakdown of the colloidal protein by proteolytic bacteria. This may also aid in explaining the marked decrease in total nitrogen along with the fact that inaccurate sampling would result due to coagulated proteins.

The results for total amounts of the salts show

slight increases which might be attributed to a concentration of the milk during heating although the volumes of the heated milk and the unheated milk were not compared to determine the amount of evaporation which had taken place.

The statement above would also hold for the increases noted for the results observed. The dialyzable calcium and phosphorous both showed marked increases in heated milk where a lowering of pH occurred. This was in good agreement with works reported by Lampitt and Bushill (1934).

The citric acid contents show little variation with heat-treatment of the milk samples, which follows an observation reported by Arup (1938) who stated that there were negligible changes in citrates when milk was heated in an autoclave at succeeding higher temperatures. The decreases in citric acid values in the acid-milk are similar in trend to decreases shown to occur in experiments by Van Slyke and Bosworth (1916). The decreases are possibly due to the utilization of the citrates by the bacterial flora.

The presence of a lower pH in the acid-milk appears to be the deciding factor in the lower curd tension of the milk because an increase in acidity lowered the curd tension to a value less than that observed in normal heated milk. This is probably due to the changing of

insoluble salts to a soluble form from the denatured proteins. That the soluble salts are precipitated by heat has been shown in reports of Sommer and Hart (1926) while insoluble calcium and phosphorous salts are shown to be solubilized by the work reported by Lampitt and Bushill.

In general, the alcohol number of the milk samples in this portion of the study follow the same trends discussed in earlier parts of this investigation. The alcohol test may be an effective tool in forecasting the heat stability of a milk as it appears that the test shows whether an abnormal reaction has occurred in the salt and acidity present in the milk.

SUMMARY AND CONCLUSIONS

Studies were made upon the changes which occur in some of the normal constituents and properties of Guernsey, Holstein and mixed herds milk during seasons of the year. Experiments were also conducted upon normal mixed herds milk to determine the effects of heat-treatment and developed acidity upon some of the constituents and properties of cows' milk.

Influence of Breed and the Seasons of the Year Upon Some Properties and Distribution of Various Constituents of Milk

The seasons of the year were found to show definite trends for some of the milk constituents. The trends were similar in almost all cases for the milks investigated. It was found that the fat and total solids were generally lower in the summer season and higher during the winter months. There was a tendency for the total and dialyzable nitrogen values to increase steadily through the spring and summer seasons after the cows were put on pasture. The nitrogen values also varied as the total solids contents varied. The citric acid showed no steady trends but the values were generally higher for milk produced in the summer months. Minerals of the milks were found to increase markedly in

the spring with lower values found in the winter months. The heat stability and curd tension were found to be indirectly related to seasons of the year by an association with the seasonal changes in the calcium and total nitrogen present in the milk. High curd tension values were found in the milk when high calcium and total nitrogen contents were present in the milk due to increases in the spring season. The heat stability of the milks showed an improvement in the summer when the calcium and the total nitrogen contents were found to be at their widest difference. The alcohol number was found to have no definite relationship to seasonal change but when the total inorganic phosphorous increases were noted, there was a decided improvement in the alcohol test.

In comparing analyses of the Guernsey and Holstein milks, the Guernsey milk showed consistently higher average concentrations of fat, total solids, total nitrogen, citric acid, calcium, but lower average concentrations of phosphorous and magnesium salts. The curd tensions of the Guernsey milk were decidedly higher than those of the Holstein milk.

Effect of Heat Upon Some of the
Constituents and Properties of
Normal Cows' Milk

Heating of milk causes an increase in acidity and a decrease in pH. The variations in total nitrogen values were discussed. The total nitrogen content of heated milk showed an increase during the initial stage of heating with decreases as the milk was heated for longer periods of time at 143° F. No marked variations were observed for the dialyzable nitrogen. Total calcium and phosphorous decreased slightly as heat was applied, but the magnesium content increased. The dialyzable salts in heated milk showed little variation over the normal values, except for the dialyzable calcium which decreased slightly during the initial stage of heating. As the milk was subjected to longer periods of heating the heat stability of the milk was improved. The curd tensions of the heated milk were found to be lower when lower levels of dialyzable calcium were found. The stability of the milk toward the alcohol test increased as the milk was subjected to longer periods of heating.

Effect of Various Degrees of Acidity
Upon Some of the Properties and
Constituents of Cows' Milk

A decreasing pH accompanied an increasing titratable acidity. The total nitrogen content increased at a steady rate as the acidity increased while the dialyzable nitrogen decreased. The total calcium increased slightly with increases in acidity but the other salts showed little variation from the original values. The dialyzable salts increased with increasing acidity with soluble phosphorous showing the greatest increases. Citrates are known to be utilized by certain bacteria, therefore the resulting decreases in citric acid of whole milk were expected. But the increases in dialyzable citric acid cannot be accounted for. The stability of the milk toward heat may be indirectly related to the acidity present in the milk but directly related to the disturbing of the equilibrium among the salts and proteins of milk. The curd tension varied little in the early stages of developed acidity but at the highest acidity the character of the curd appeared to be completely changed thus showing to some extent the range of equilibrium which will tolerate the destabilizing effect of acidity.

Effect of Heat-Treatment Upon Some Properties
and Constituents of Milk With Various
Degrees of Acidity

In studying the effect of processing heat-treatments upon acid-milk it was found that the total nitrogen values increased as the normal and acid-milks were heated. The total nitrogen values of acid-milk showed a smaller increase with heat-treatment. The dialyzable nitrogen also increased as higher processing temperatures were used. The dialyzable nitrogen of the acid-milk showed only slightly higher values after heat-treatment. Slight increases in calcium and phosphorous were noted in the heated normal milk but little change occurred in these salts when the acid-milk was heated, although the level of salt contents were higher than for the normal heated milk. The concentration of dialyzable salts increased markedly with acidity but decreased slightly when the milk was heat-treated. Evidently the acidity had not increased to a point where the citrates were affected or there was a destruction of citric acid fermenting bacteria present in the acid-milk at the processing temperatures. Slightly lower curd tensions were observed in the acid-milk than in the normal milk, but both milks showed decreasing curd tensions with higher processing temperatures. The lower values might be attributed to the decrease in the in-

soluble salts in the acid-milk.

Effect of Various Degrees of Acidity Upon
Some of the Constituents and
Properties of Heated Milk

It was found that when heated milk is allowed to develop acidity the total nitrogen content decreased as a higher processing temperature was used, but the dialyzable nitrogen increased in the acid-milk when heated. The dialyzable nitrogen was higher in heated acid-milk than in normal heated milk. The calcium, phosphorous and magnesium varied little upon heat-treatment of the milk but the total salts of the acid-milk were higher than those of the normal milk. The dialyzable calcium and phosphorous values were higher in the acid-milk than in the normal milks; increasing proportionally with the heat treatment. However, the dialyzable calcium and phosphorous of the normal milk decreased with higher temperatures of heat-treatments. The citric acid contents were found to vary little with reference to the processing temperatures but a marked decrease was found to occur in the milk when the acidity was allowed to develop. The development of acidity in milk appears to lower the curd tension of milk.

From all indications, the most important factors found to cause detrimental effects in milk in relation to heat stability are the salts and proteins present in

milk and the developement of an abnormal acidity. The salt and protein contents are definitely related to seasons of the year and may be altered to form an unbalanced combination of constituents in the milk. The heating of milk will cause a change in distribution of some of the constituents but will also increase the stability of the milk toward heat. Therefore, the most important factor to be concerned with upon the receiving platform appears to be an abnormal acidity, due to developed acidity and not to natural acidity, along with a possible detrimental change in salt distribution. The alcohol test might be considered instrumental as a platform test in this respect.

TABLE 1

Seasonal analyses of pooled herds milk.

Determination	Sampling Period					
	Jan.	Mar.	Apr.	May	June	July
Butterfat (%)	3.78	3.55	4.21	3.88	3.56	3.61
Total solids (%)	12.74	12.41	12.47	12.69	12.60	12.42
Alcohol number	8.00	8.00	8.00	8.00	8.00	8.50
Titratable acidity (%)	0.21	0.15	0.17	0.17	0.19	0.18
Curd tension (g.)	30.00	30.00	30.00	35.00	24.00	30.00
pH	6.73	6.68	6.40	6.37	6.47	6.85
Heat stability (min.)	100.00	50.00	58.00	90.00	120.00	115.00
Citric acid (mg. %)	128.00	86.00	72.00*	80.00	62.00	87.00*
Whole milk	54.50	10.00	----	10.00	32.30	----
Serum	602.00	598.00	625.00	643.00	593.00	623.00
Total nitrogen (mg. %)	31.00	42.80	14.40	40.00	30.00	35.80
Serum nitrogen (mg. %)	107.20	103.20	156.60	130.80	102.40	110.80
Minerals (mg. %)	45.00	33.30	125.10	114.50	95.00	68.70
Calcium	17.30	7.20	15.30	12.20	3.90	4.50
Phosphorous	25.00	20.00	48.20	50.00	38.80	53.80
Magnesium	20.00	13.30	76.90	64.50	56.20	15.00
Total inorganic						
phosphorous						
Colloidal						
phosphorous						

* Values not obtained.

TABLE 1 (Continued)

Determination	Sampling Period						
	Jan.	Mar.	Apr.	May	June	July	Sept.
Minerals (mg. %)							
Serum							
Calcium	3.60	4.00	4.50	4.50	4.00	4.60	2.70
Phosphorous	37.50	40.90	42.50	45.0	71.30	52.10	15.00
Magnesium	6.00	2.90	7.50	7.50	14.70	6.70	4.10

Seasonal analyses of milk from a Guernsey herd.

TABLE 2
Seasonal analyses of milk from a Guernsey herd.

Determination	Sampling Period					
	Jan.	Mar.	Apr.	May	June	July
Butterfat (%)	5.78	5.29	4.62	5.09	5.14	4.21
Total solids (%)	15.68	15.20	14.19	14.62	15.28	13.71
Alcohol number	7.50	7.50	8.00	8.00	8.00	9.00
Titratable acidity (%)	0.21	0.17	0.18	0.19	0.18	0.20
Curd tension (g.)	55.00	70.00	35.00	55.00	40.00	65.00
pH	6.71	6.69	6.48	6.42	6.57	6.79
Heat stability (min.)	93.00	70.00	60.00	105.00	90.00	75.00
Citric acid (mg. %)						
Whole milk	170.00	100.00	235.00	155.00	93.00	107.00
Serum	61.00	12.30	-----*	13.50	29.60	-----*
Total nitrogen (mg. %)	769.00	714.00	704.00	769.00	724.00	788.00
Serum nitrogen (mg. %)	20.80	48.10	25.80	41.50	31.00	47.70
Minerals (mg. %)						
Whole milk						
Calcium	129.60	123.40	172.00	154.00	107.40	123.00
Phosphorous	59.60	43.30	116.60	110.40	100.00	97.00
Magnesium	17.80	8.70	15.70	14.10	4.30	5.00
Total inorganic phosphorous	30.00	24.30	51.00	57.50	42.50	60.00
Colloidal phosphorous	29.60	19.10	65.60	52.90	57.50	37.00

* Values not obtained.

70.

Determination	Sampling Period						
	Jan.	Mar.	Apr.	May	June	July	Sept.
Minerals (mg. %)							
Serum							
Calcium	4.00	3.90	4.50	4.00	4.10	5.00	3.80
Phosphorous	35.00	40.90	40.00	45.30	72.50	53.80	22.00
Magnesium	5.70	2.20	6.50	7.30	15.10	6.30	4.10

TABLE 3

Seasonal analyses of milk from a Holstein herd

Determination	Sampling Period					
	Jan.	Mar.	Apr.	May	June	July
Butterfat (%)	3.01	2.95	3.32	3.19	3.03	3.73
Total solids (%)	11.43	11.13	11.78	11.69	11.54	12.23
Alcohol number	6.50	7.00	7.00	8.50	8.00	8.00
Titratable acidity (%)	0.19	0.15	0.12	0.12	0.17	0.17
Curd tension (g.)	25.00	20.00	20.50	35.00	28.00	30.00
pH	6.64	6.67	6.40	6.38	6.43	6.79
Heat stability (min.)	75.00	105.00	70.00	90.00	50.00	125.00
Citric acid (mg. %)						
Whole milk	160.00	86.00	55.00	100.00	87.00	68.00
Serum	54.50	8.20	-----*	8.70	29.60	-----*
Total nitrogen (mg. %)	1490.00	1438.00	539.00	661.00	573.00	638.00
Serum nitrogen (mg. %)	19.50	35.50	14.40	39.00	23.00	26.60
Minerals (mg. %)						
Whole milk						
Calcium	92.00	60.40	141.80	119.80	98.80	105.80
Phosphorous	145.80	21.70	81.20	75.40	62.50	64.60
Magnesium	13.70	9.30	14.10	13.00	2.80	5.20
Total inorganic phosphorous	28.30	19.20	51.00	57.50	43.80	48.80
Colloidal phosphorous	17.60	2.40	30.30	17.90	18.70	15.80

* Values not obtained.

TABLE 3 (Continued)

Determination	Sampling Period						
	Jan.	Mar.	Apr.	May	June	July	Sept.
Minerals (mg. %)							
Serum							
Calcium	3.90	3.80	4.00	3.80	3.70	3.60	4.50
Phosphorous	40.00	43.50	35.00	43.80	63.90	49.80	18.30
Magnesium	5.90	2.60	7.50	9.40	17.50	5.50	4.90

1

TABLE 4

Effect of heat upon some constituents and properties of milk.

Conditions of Heat-treatment of the Milk					
Determination	Raw milk	143°F.- 5 min.	143°F.- 10 min.	143°F.- 30 min.	143°F.- 60 min.
Butterfat (%)	3.62	----	----	----	3.62
Totalsolids (%)	12.38	----	----	----	12.38
Alcohol number	6.00	7.00	7.50	8.00	8.50
Titratable acidity (%)	0.13	0.12	0.18	0.15	0.14
Curd tension (g.)	35.00	35.00	35.00	30.00	30.50
pH	6.62	6.53	6.50	6.48	6.44
Heat stability (min.)	100.00	100.00	105.00	115.00	125.00
Citric acid (mg.%)					
Whole milk	87.00	80.00	68.00	74.00	74.00
Serum	23.00	21.00	20.00	20.00	21.00
Total nitrogen (mg.%)	608.00	678.00	647.00	615.00	553.00
Serum nitrogen (mg.%)	31.40	29.80	26.80	28.80	29.40
Minerals (mg.%)					
Whole milk					
Calcium	87.49	87.49	71.60	65.60	74.20
Phosphorous	95.50	94.30	94.30	85.90	91.30
Magnesium	6.99	9.89	7.53	13.27	18.85
Total inorganic					
phosphorous	40.00	20.00	35.00	45.00	65.00
Colloidal					
phosphorous	55.50	74.30	59.30	40.90	25.30

*

Not determined.

TABLE 4 (Continued)

Conditions of Heat-treatment of the milk				
Determination	Raw milk	143°F.- 5 min.	143°F.- 10 min.	143°F.- 30 min.
				143°F.- 60 min.
Minerals (mg.%)				
Serum				
Calcium	3.72	3.45	3.39	3.36
Phosphorous	33.80	33.80	33.80	32.50
Magnesium	5.50	5.10	5.10	4.82
				3.56
				35.02
				5.10

TABLE 5

The effect of various heat-treatments upon some of the constituents and properties of milk with various degrees of acidity.

Conditions of Acidity and Heat-treatment Used					
Determination	Normal Acidity			0.3% Induced Acidity	
	Raw milk	145°F.- 30 min.	190°F.- 10 min.	Raw milk	145°F.- 30 min. 190°F.- 10 min.
Butterfat (%)	3.71	---	---	3.71	---
Total solids (%)	12.84	---	---	12.84	---
Alcohol number	8.50	10.00	10.00	3.00	2.00
Titratable acidity (%)	0.16	0.15	0.15	0.30	0.33
Curd tension (g.)	40.00	35.00	5.00	40.00	30.00
pH	6.83	6.78	6.68	5.80	5.88
Heat stability (min.)	115.00	135.00	140.00	0.00	0.00
Citric acid (mg.%)					
Whole milk	120.00	120.00	142.00	127.00	114.00
Serum	23.00	19.00	24.00	11.00	14.00
Total nitrogen (mg.%)	480.00	556.00	656.00	561.00	548.00
Serum nitrogen (mg.%)	39.10	78.90	171.40	45.00	60.20
Minerals (mg.%)					
Whole milk					
Calcium	101.20	108.80	110.40	102.40	104.40
Phosphorous	86.60	91.60	105.00	102.00	100.00
Magnesium	17.30	14.90	22.20	16.30	21.60

* Not determined.

TABLE 5 (Continued)

Conditions of Acidity and Heat-treatment Used						
Determination	Normal Acidity		0.3% Induced Acidity			
	Raw milk	145° F.- 30 min.	190° F.- 10 min.	Raw milk	145° F.- 30 min.	190° F.- 10 min.
Minerals (mg.%)						
Whole milk (cont.)						
Total inorganic phosphorous	13.00	15.30	15.30	29.30	33.30	40.50
Colloidal phosphorous	73.60	76.40	89.70	72.80	66.70	61.50
Serum						
Calcium	3.50	3.30	3.20	9.60	6.40	6.00
Phosphorous	27.00	29.30	25.00	46.00	42.00	40.50
Magnesium	14.60	3.90	4.20	7.10	5.60	5.90
P/Ca ratio (mg.%)	.86:1	.84:1	.95:1	.99:1	.96:1	.99:1

TABLE 6

Effect of induced acidity upon some of the constituents and properties of heated milk.

Conditions of Acidity and Heat-treatment Used						
Determination	Normal Acidity			0.3 % Induced Acidity		
	Raw milk	145°F.- 30 min.	190°F.- 10 min.	Raw milk	145°F.- 30 min.	190°F.- 10 min.
Butterfat (%)	3.48	---	---	3.48	---	---
Total solids (%)	11.74	---	---	11.74	---	---
Alcohol number	8.50	9.00	9.00	0.00	0.00	1.00
Titratable acidity (%)	0.16	0.14	0.13	0.30	0.30	0.30
Curd tension (g.)	30.00	30.00	7.00	10.50	15.00	0.00
pH	6.84	6.82	6.80	5.65	5.44	5.79
Heat stability (min.)	185.00	210.00	210.00	0.00	0.00	0.00
Citric acid (mg.%)						
Whole milk						
Serum	103.00	103.75	98.00	61.25	67.00	85.00
Total nitrogen (mg.%)	102.00	101.00	109.00	64.00	60.00	93.00
Serum nitrogen (mg.%)	520.00	547.00	567.00	538.00	468.00	312.00
Minerals (mg.%)	39.10	34.70	35.40	48.70	62.80	62.40
Whole milk						
Calcium	101.20	101.80	101.60	107.20	107.20	110.80
Phosphorous	86.20	94.10	91.20	99.50	105.00	117.50
Magnesium	6.30	7.80	9.60	11.00	8.60	12.50

* Not determined.

TABLE 6 (Continued)

Conditions of Acidity and Heat-treatment Used						
Determination	Normal Acidity		0.3 % Induced Acidity			
	Raw milk	145° F.- 30 min.	190° F.- 10 min.	Raw milk	145° F.- 30 min.	190° F.- 10 min.
Minerals (mg.%)						
Whole milk (cont.)						
Total inorganic phosphorous	37.00	43.50	37.00	42.00	43.50	76.60
Colloidal phosphorous	49.20	50.60	54.20	57.50	61.55	40.80
Serum						
Calcium	3.80	3.60	3.40	6.60	10.50	11.10
Phosphorous	38.80	36.80	35.80	43.30	61.50	67.00
Magnesium	6.00	5.85	5.60	5.00	7.70	7.75
P/Ca ratio (mg.%)	.85:1	.92:1	.90:1	.93:1	.98:1	.90:1

TABLE 7

Variations in some of the normal constituents and properties of whole milk as the acidity is increased.

Conditions of Acidity in the Milk			
Determination	Normal Acidity	Induced Acidities	
		0.24%	0.30%
Butterfat (%)	3.64	----	----
Total solids (%)	12.33	----	----
Titratable acidity (%)	0.14	0.24	0.30
Alcohol number	8.00	3.00	1.50
Curd tension (g.)	35.00	36.00	33.00
pH	6.75	6.21	5.87
Heat stability (min.)	160.00	5.00	0.00
Citric acid (mg.%)			
Whole milk	183.00	183.00	74.00
Serum	105.00	113.00	125.00
Total nitrogen (mg.%)	438.00	441.50	514.00
Serum nitrogen (mg.%)	40.70	39.30	38.00
Minerals (mg.%)			
Whole milk			35.50
Calcium	98.80	99.40	107.80
Phosphorous	94.60	91.60	94.60
Magnesium	7.50	6.30	4.60

* Not determined.

TABLE 7 (Continued)

Determination	Conditions of Acidity in the Milk		
	Normal Acidity	Induced Acidities	
		0.24%	0.30% 0.42%
Minerals (mg.%)			
Whole milk (cont.)			
Total inorganic	26.00	25.00	25.00
phosphorous			
Colloidal			
phosphorous	68.60	66.50	69.50
Serum			
Calcium	3.50	6.00	9.50
Phosphorous	36.00	48.00	63.50
Magnesium	5.90	6.70	7.30
P/Ca ratio (mg.%)	.96:1	.92:1	.88:1

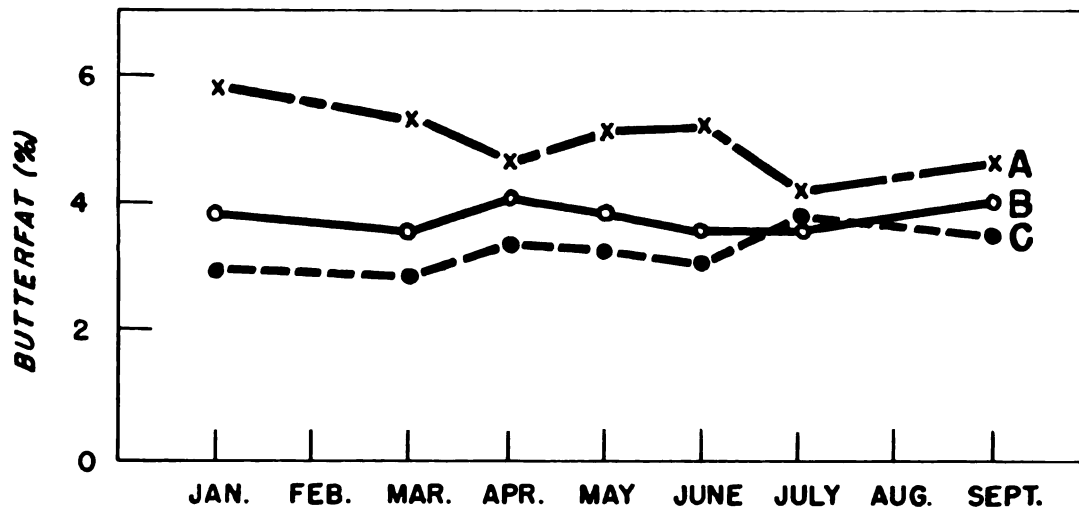


Figure 1. The fat content of milk as influenced by seasons of the year. A- Milk from a Guernsey herd. B- Pooled herds milk. C- Milk from a Holstein herd.

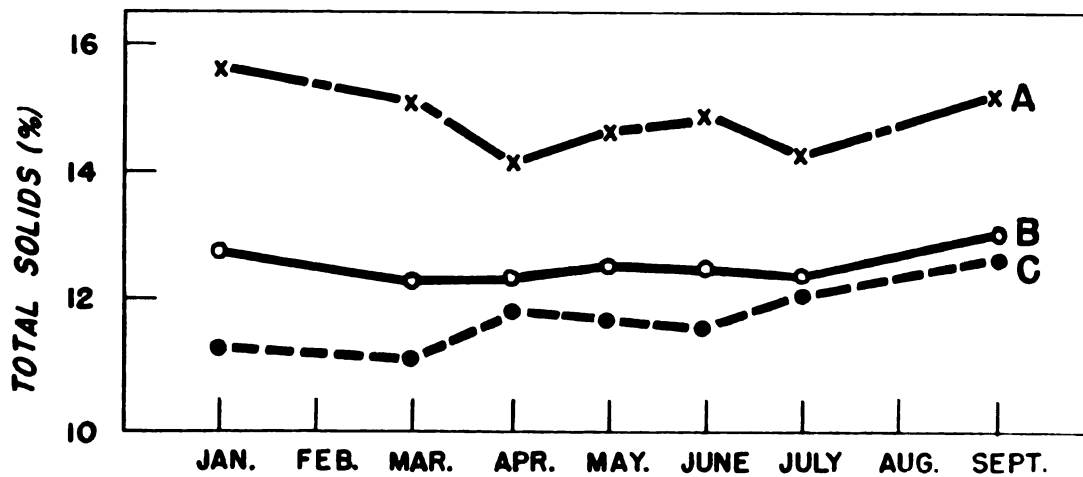


Figure 2. Total solids of milk as influenced by seasons of the year. A- Milk from a Guernsey herd. B- Pooled herds milk. C- Milk from a Holstein herd.

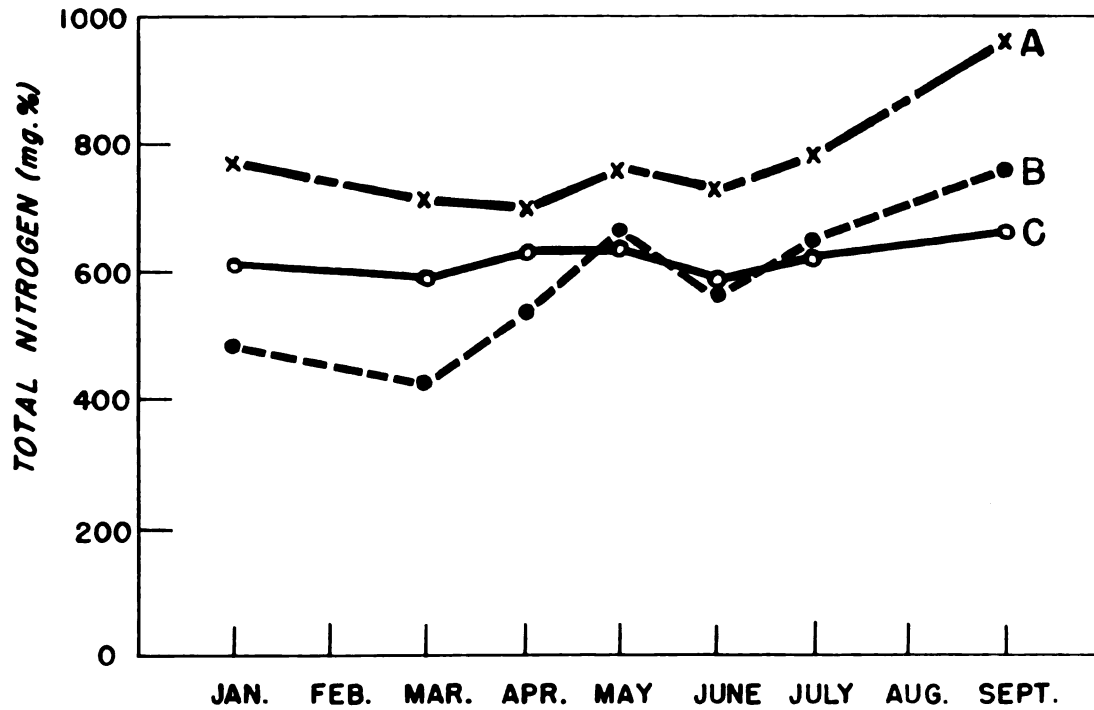


Figure 3. Variations in the total nitrogen content of milk with seasons of the year. A- Milk from a Guernsey herd. B- Milk from a Holstein herd. C- Pooled herds milk.

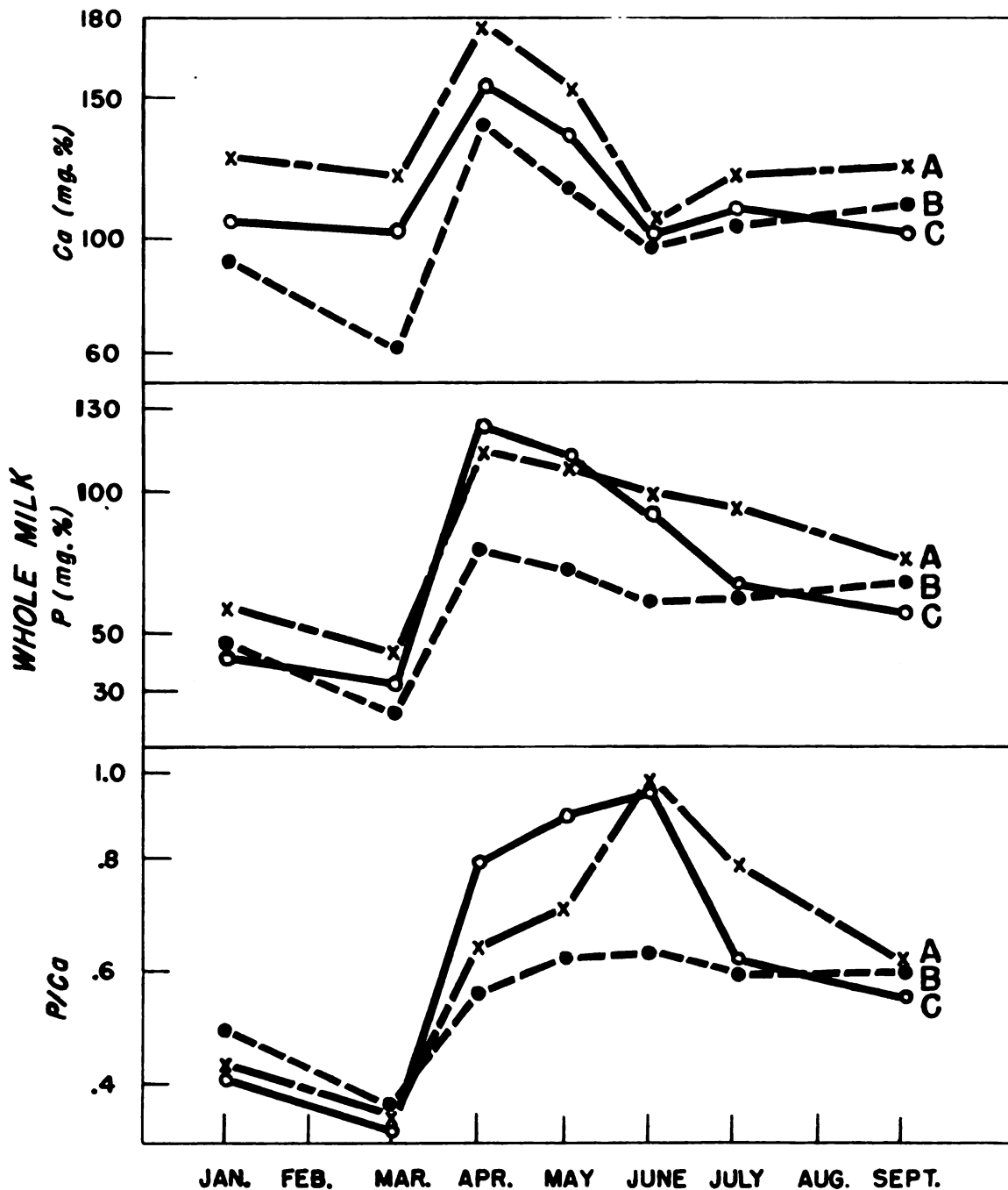


Figure 4. Comparison of the Ca, P and the P/Ca ratio in whole milk with seasons of the year. A- Milk from a Guernsey herd. B- Milk from a Holstein herd. C- Pooled herds milk.

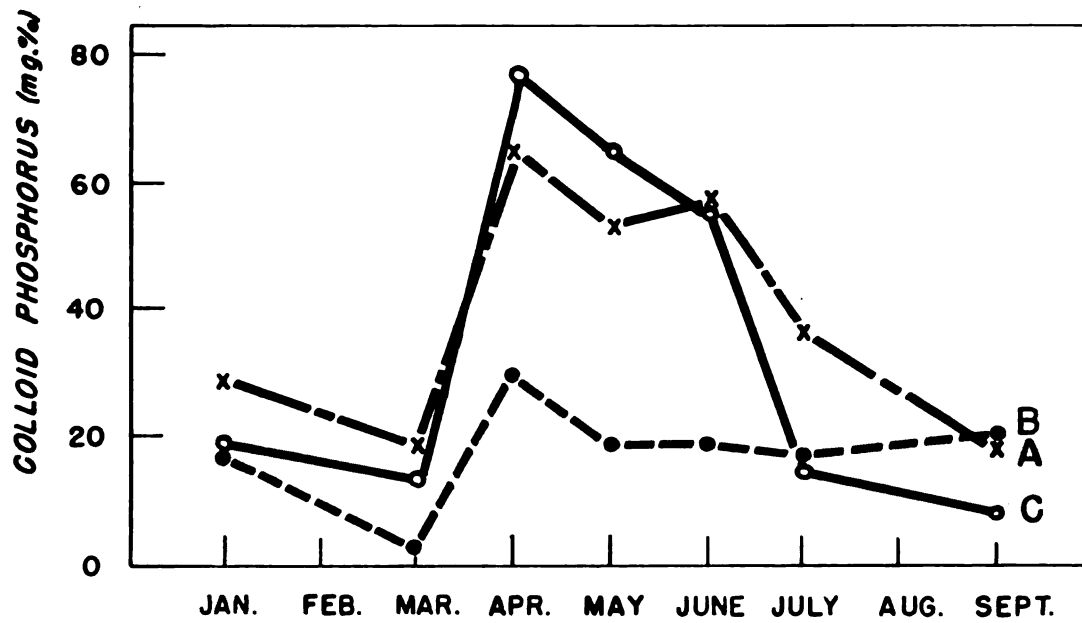


Figure 5. Variations in colloidal phosphorous with seasons of the year. A- Milk from a Guernsey herd. B- Milk from a Holstein herd. C- Pooled herds milk.

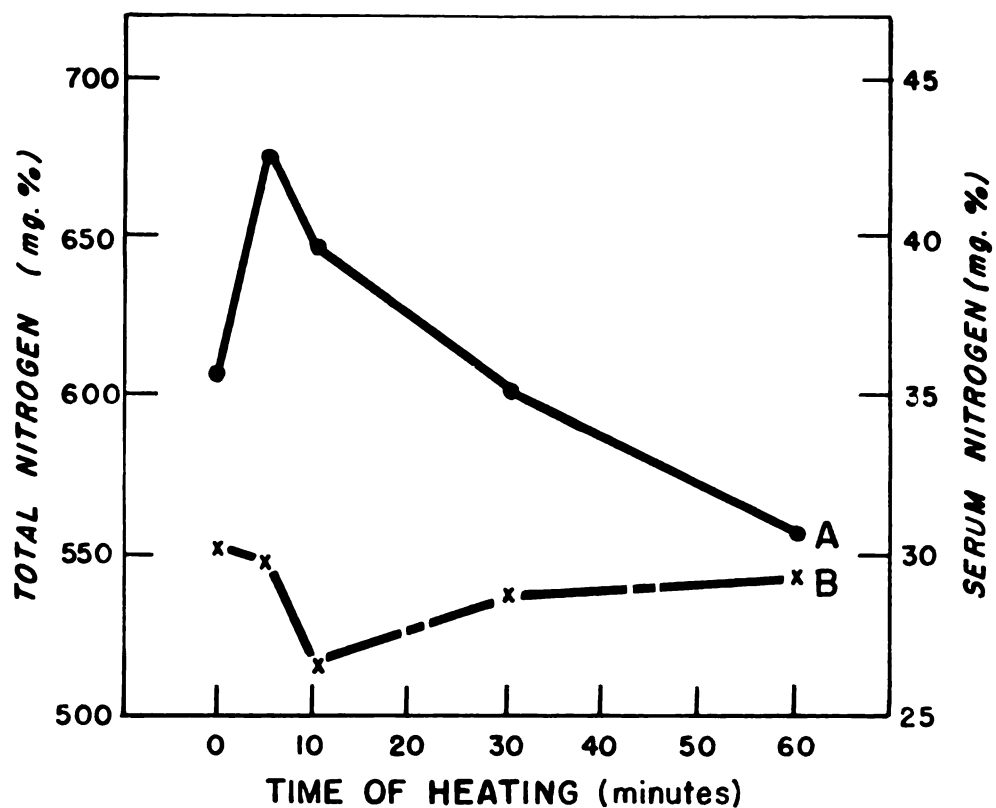


Figure 6. Variations in the total and serum nitrogen contents of milk heated at 143° F. for different periods of time. A- Whole milk. B- Serum portion of the milk.



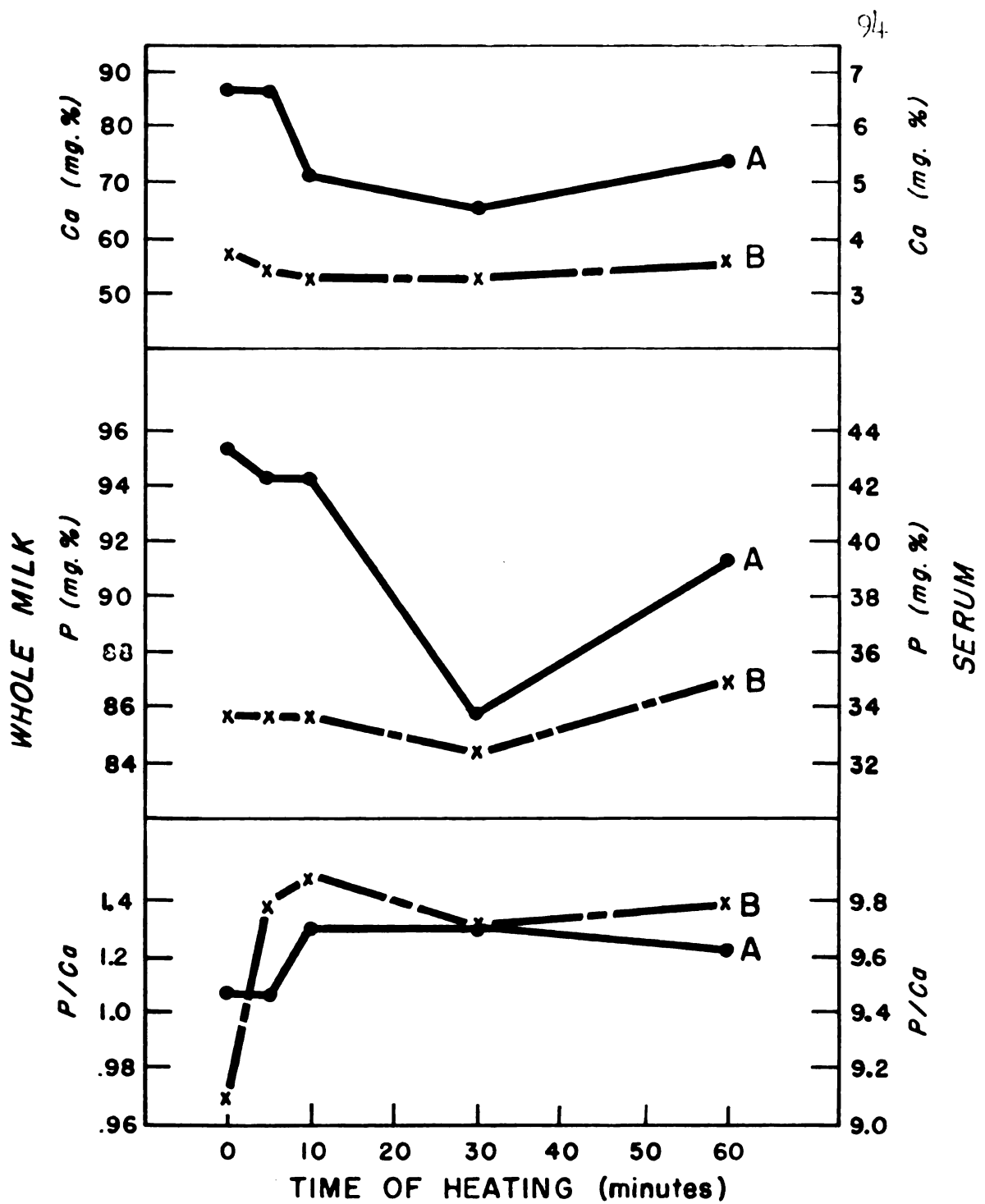


Figure 7. The influence of heat on the Ca, P and the P/Ca ratio of milk. A- Whole milk. B- Serum.

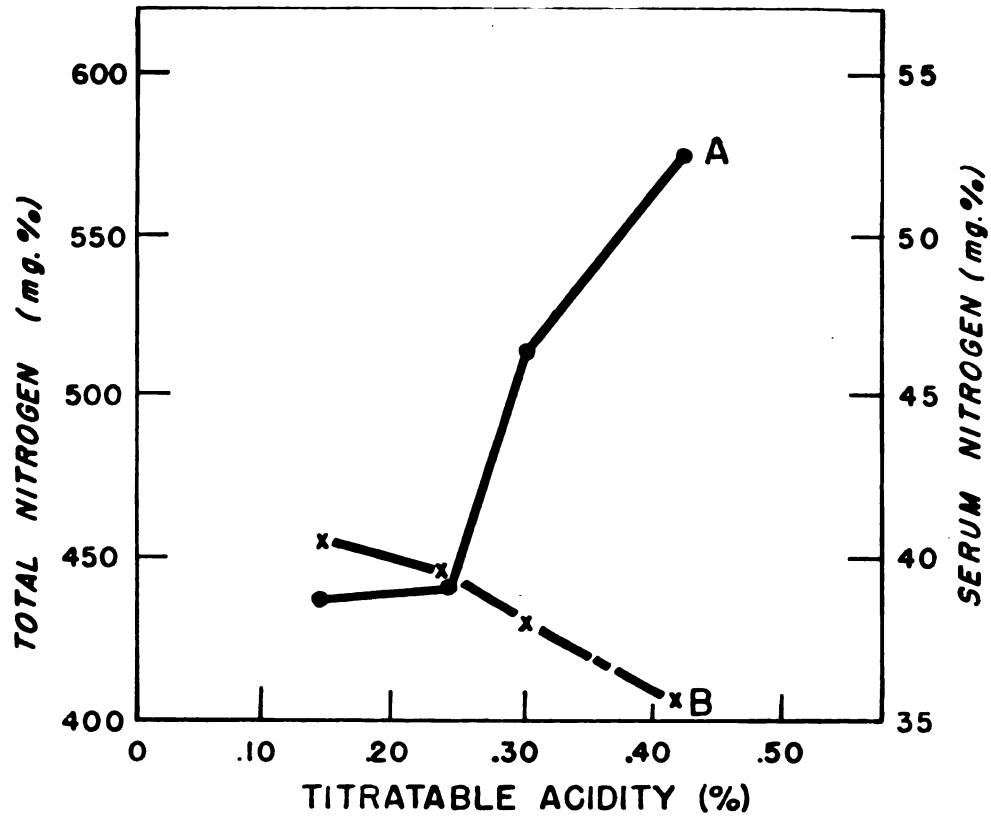


Figure 8. Variations in the total and serum nitrogen contents in milk of varying acidities. A- Whole milk. B- Serum portion of the milk.

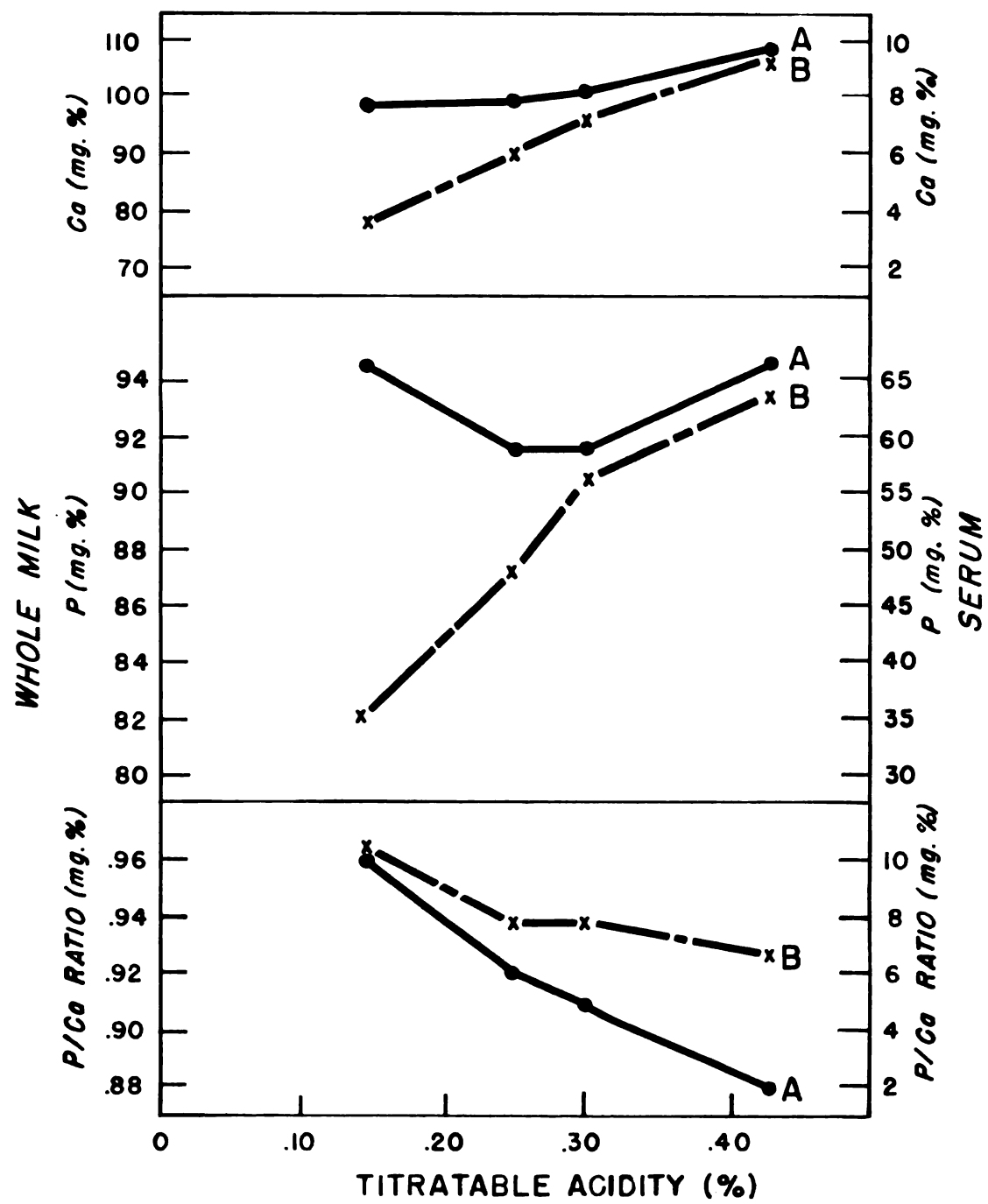


Figure 9. Comparison of Ca and P and the P/Ca ratio in milk of various acidities. A- Whole milk. B- Serum.

LITERATURE CITED

- (1) Arup, P. S.
1938. Citric acid Determinations in milk and milk products. *Analyst*, 63: 635-640.
- (2) Labad, J., and Shtrikman, M.
1951. The estimation of citric acid in dairy products. *J. Dairy Research*, 18: 72-76.
- (3) Bernardoni, E. A., and Tuckey, S. L.
1950. The changes produced in the ultrafilterable calcium, phosphorous, and nitrogen components of skim milk during processing in a Mallory heat exchanger. (Abst.) *J. Dairy Sci.*, 33: 409-410.
- (4) Briggs, A. P.
1924. Some applications of the colorimetric phosphate method. *J. Biol. Chem.*, 59: 225-264.
- (5) Committee on Methods of Determining the Curd Tension of Milk: American Dairy Science Association.
1941. Final report of committee on methods of determining the curd tension of milk. *J. Dairy Sci.*, 24: 825-827.
- (6) Cranfield, H. T., Griffiths, —, and Ling, E. R.
1927. *J. Agr. Sci.*, 17: 72. (Original not seen. Cited by Davies, W. L. *The Chemistry of Milk*. Vol. 10, 522 pp. New York: D. Van Nostrand Co., Inc.)
- (7) Davies, W. L.
1936. *The Chemistry of Milk*. Vol. 10, 522 pp. N.Y.: D. Van Nostrand Co., Inc.
- (8) Davies, W. L., and Provan, —.
1928. *Welsh J. Agr.* 4: 114. (Original not seen. Cited by Ling, E. R. *J. Dairy Research*, 8: 171-194. 1937.)

- (9) Davis, R. N., Harland, F. G., Caster, A. B., and Kellner, R. H.
1947a. Variations in the constituents of milk under Arizona conditions. I. Variations of individual cows within breeds by calendar months. J. Dairy Sci., 30: 415-424.
- (10) Davis, R. N., Harland, F. G., Caster, A. B., and Kellner, R. H.
1947b. Variations in the constituents of milk under Arizona conditions. III. Variations in milk from Jersey, Guernsey, Holstein and Mixed Herds. J. Dairy Sci., 30: 435-442.
- (11) Ellenberger, H. B., Newlander, J. A., and Jones, C. H.
1950. Variations in calcium and phosphorous contents of cow's milk. Univ. of Vermont and State Agr. College Agr. Expt. Sta. Bull. 556. 26 pp.
- (12) Fiske, C. H., and Subbarow, Y.
1925. The colorimetric determination of phosphorous. J. Biol. Chem., 66: 375-400.
- (13) Colding, J., Mackintosh, J., and Mattick, E. C. V.
1932. Investigations on the milk of a typical herd of shorthorn cows. I. J. Dairy Research, 4: 48-73.
- (14) Graham, W. R., Jr., and Kay, H. D.
1933. Phosphorous compounds in milk. I. The phosphorous partition in milk, with preliminary observations on milk phosphatase. J. Dairy Research, 5: 54-62.
- (15) Gyorgy, —.
1923. Biochem. Zeitsch. 142: 1. (Original not seen. Cited by Allen, L. A., J. Dairy Research, 3: 1-51. 1931.)

- (16) Hammarsten, O.
1896. Z. Physiol. Chem., 22: 103. (Original not seen. Cited by Associates of Lore A. Rogers. Fundamentals of Dairy Science. 2nd Ed. 616 pp. New York: Reinhold Publishing Corp. 1935.)
- (17) Harman, T. D., and Slatter, W. L.
1950. Effect of heating on the diffusion of calcium, magnesium, phosphorous and citric acid. (Abst.) J. Dairy Sci., 33: 409.
- (18) Hawk, P. B., Oser, B. L., and Summerson, W. H.
1949. Practical Physiological Chemistry. 12th Ed. 1323 pp. Phila. : Blakiston Co. 1949.
- (19) Hess, P. B., Unger, L. J., and Supplee, G. C.
1920. Relation of fodder to the anti-scorbutic potency and salt content of milk. J. Biol. Chem, 45: 229-235.
- (20) Hill, R. L.
1923. A test for determining the character of the curd from cows' 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: 509-526.
- (21) Hill, R. L.
1931. Soft curd milk. Utah Agr. Expt. Sta. Bull. 227. 24 pp.
- (22) Holm, G. E., Deysher, E. F., and Evans, F. R.
1923. The relationships of concentration and time to the temperature of coagulation of evaporated skim and whole milk. J. Dairy Sci., 6: 556-568.
- (23) Holm, G. E., Webb, B. H., and Deysher, E. F.
1932. The heat coagulation of milk. I. Variations in the compositions, heat stability and other tests of milk from four cows during the course of a lactation period. J. Dairy Sci., 15: 331-343.
- (24) Hunziker, O. F.
1949. Condensed Milk and Milk Powder. 7th Ed. 630 pp. La Grange: Author.

- (25) Jacobsen, D. H., and Wallis, G. C.
1939. Factors affecting the composition of milk.
South Dakota Agr. Expt. Sta. Bull. 331.
28 pp.
- (26) Khambatta, Miss Jer S., and Dastur, N.
1950. Changes in the chemical composition of
milk during souring. Indian J. Dairy Sci.,
3: 147-160.
- (27) Khambatta, Miss Jer S., and Dastur, N.
1951. Changes in the chemical composition of
milk during souring. II. Changes in nitrogen
constituents. Indian J. Dairy Sci., 4: 73-80.
- (28) Lampitt, L. H., and Bushill, J. H.
1934. Dialysis of milk. II. Some factors affecting
the distribution of calcium and phosphorous.
Biochem. J., 28 (pt. 2): 1305-1312.
- (29) Lenstrup, E.
1926. The phosphorous content of human milk and
cows' milk. J. Biol. Chem., 70: 193- 202.
- (30) Ling, E. R.
1937. The composition of milk and whey, with
special reference to the partition of
calcium and phosphorous. J. Dairy Research,
8: 173-194.
- (31) Mattick, E. C. V., and Hallet, H. S.
1929. J. Agr. Sci., 19: 452. (Original not seen.
Cited by Davies, W. L. The Chemistry of
Milk. Vol. 10. 522 pp. New York: D. Van
Nostrand Co., Inc. 1936.)
- (32) McCammon, R. B., Caulfield, W. J., and Kramer, M. M.
1933. Calcium and phosphorous of cheese made under
controlled conditions. J. Dairy Sci., 16:
253-263.
- (33) McInerney, T. J.
1920. A preliminary report on the study of the
temperatures at which milk of different per
cents of acidity will coagulate. J. Dairy
Sci., 3: 220-229.
- (34) Menefee, S. G., and Overman, O. R.
1940. A semimicro-Kjeldahl method for the deter-
mination of total nitrogen in milk. J. Dairy
Sci., 23: 1177-1185.

- (35) Menefee, S. G., Overman, O. R., and Tracy, P. H.
1941. The effect of processing on the nitrogen distribution in milk. J. Dairy Sci., 24: 953-968.
- (36) Miller, D.
1935. The determination of curd tension by the use of hydrochloric acid-pepsin coagulant. J. Dairy Sci., 18: 259-265.
- (37) Mojonnier, T., and Troy, H. C.
1925. Technical Control of Dairy Products. 2nd Ed. 936 pp. Chicago: Mojonnier Bros. Inc.
- (38) Mortenson, F. N., Espe, D. L., and Cannon, C. Y.
1935. Effect of heating milk on the time which the curds remain in the abomasum of calves. J. Dairy Sci., 18: 229-238.
- (39) Nelson, —
1953. Some observations on forewarming and heat stability of milk. Paper given before the Evaporated Milk Technical Association, Chicago, Ill. April 28.
- (40) Overman, O. R.
1945. Monthly variations in the composition of milk. J. Dairy Sci., 28: 305-309.
- (41) Palmer, L. S., and Richardson, G. A.
1925. Third Colloid Symposium Monograph, Chemical Catalog Co., Inc. 112. (Original not seen. Cited by Associates of Lore A. Rogers. Fundamentals of Dairy Science. 2nd Ed. 616 pp. New York: Reinhold Publishing Corp. 1935.
- (42) Powell, M. E.
1935. Effects of time and temperature of holding milk heat-treated at various temperatures upon its subsequent coagulation by rennet. (Abst.) J. Dairy Sci., 18: 462.
- (43) Pyne, G. T., and Ryan, J. J.
1950. The colloidal phosphate of milk. J. Dairy Research, 17: 200-205.
- (44) Ragsdale, A. C., and Turner, C. W.
1922. The seasonal variations of the per cent of fat in cows milk. J. Dairy Sci., 5: 545-554.

- (45) Rice, F. E., and Markley, A. L.
1924. The relation of natural acidity in milk to composition and physical properties. J. Dairy Sci., 7: 468-483.
- (46) Riddell, W. H., Caulfield, W. J., and Whitnah, C. H.
1936. Normal variations in the curd tension of milk. J. Dairy Sci., 19: 157-164.
- (47) Rogers, L. A., Deysher, E. F., and Evans, F. R.
1921. The relation of acidity to the coagulation temperature of evaporated milk. J. Dairy Sci., 4: 294-309.
- (48) Rowland, S. J.
1933. The heat denaturation of albumin and globulin in milk. J. Dairy Research, 5: 46-53.
- (49) Rowland, S. J.
1938. The protein distribution in normal and abnormal milk. J. Dairy Research, 9: 47-57.
- (50) Roy, S. C., and Bhatnagar, D. P.
1948. Current Sci., 17: 25. (Original not seen. Cited by Khambatta, Miss Jer S., and Dastur, N. Indian J. Dairy Sci., 4: 73-80. 1951.)
- (51) Shahani, K. M., and Sommer, H. H.
1951a. The protein and non-protein fractions in milk. II. Their content in fresh raw milk. J. Dairy Sci., 34: 1010-1013.
- (52) Shahani, K. M., and Sommer, H. H.
1951b. The protein and non-protein fractions in milk. III. The effect of heat treatments and homogenization. J. Dairy Sci., 34: 1035-1041.
- (53) Shohl, A. T.
1922. The effect of hydrogen ion concentration upon the determination of calcium. J. Biol. Chem., 50: 527-535.
- (54) Soldner,
1888. Landw. Verucht., 35: 351. (Original not seen. Cited by Davies, W. L. The Chemistry of Milk. Vol. 10. 522 pp. New York: D. Van Nostrand Co., Inc.)

- (55) Sommer, H. H. and Binney, T. H.
1923. A study of the factors that influence the coagulation of milk in the heat test. J. Dairy Sci., 6: 176-197.
- (56) Sommer, H. H., and Hart, E. B.
1919. The heat coagulation of milk. J. Biol. Chem., 40: 137-151.
- (57) Sommer, H. H., and Hart, E. B.
1926. Heat coagulation of evaporated milk. Univ. of Wis. Agr. Expt. Sta. Research Bull. 67. 47 pp.
- (58) Steuart, D. W.
1920. The developement of acidity in milk. J. Dairy Sci., 3: 52-59.
- (59) Supplee, G. C., and Bellis, B.
1921. Citric acid content of milk and milk products. J. Biol. Chem., 48: 453-461.
- (60) Van Slyke, L. L., and Bosworth, A. W.
1916. Chemical changes in the souring of milk. J. Biol. Chem., 24: 191-202.
- (61) Van Slyke, L. L.,
1928. Chemistry of sour milk. New York Expt. Sta. Tech. Bull. 140. 14 pp.
- (62) Verma, Indrapal S., and Sommer, H. H.
1950. State of solution of the naturally occurring salts in milk. (Abst.) J. Dairy Sci., 33: 397.
- (63) Webb, R. H., and Bell, R. W.
1942. The effect of high-temperature short-time forewarming of milk upon the heat stability of its evaporated product. J. Dairy Sci., 25: 301-311.
- (64) Webb, R. H., and Bell, R. W.
1943. The effect of high-temperature short-time heating of concentrated milk upon its heat stability. J. Dairy Sci., 26: 1071-1077.

- (65) Webb, B. H., Bell, R. W., Deysher, E. F., and Holm, G. E.
1913. The effect of various degrees of forewarming upon the heat stability of milks of different solids concentrations. J. Dairy Sci., 26: 571-578.
- (66) Webb, B. H., and Holm, G. E.
1932. The heat coagulation of milk. II. The influence of various added salts upon the heat stabilities of milks of different concentrations. J. Dairy Sci., 25: 355-366.
- (67) Weisberg, S. M., Johnson, A. H., and McCollum, E. V.
1933. Laboratory studies on the chemistry of soft curd milk. J. Dairy Sci., 16: 225-247.
- (68) Whittier, E. O., and Benton, Anne G.
1926. The effect of heating on the hydrogen-ion concentration and on the titratable acidity of milk. J. Dairy Sci., 10: 126-138.
- (69) Whittier, E. O., and Benton, Anne G.
1927. The formation of acid in milk by heating. J. Dairy Sci., 10: 126-138.

ROOM USE ONLY

JUN 7 1958

ROOM USE ONLY

