

VISCOSITY OF SKIMMILK AS AFFECTED  
BY ION CONCENTRATION

by

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## INTRODUCTION

Although skimmilk is utilized in production of chocolate drinks, condensed milk, milk powder, and cottage cheese, it is a well known fact to the American dairymen that millions of pounds of the product are wasted each year. The inefficient utilization of this valuable source of protein, carbohydrate, and minerals results in economic and nutritional losses to both producers and consumers of milk.

Relatively little research has been done to minimize the "waste" problem through economical use of skimmilk on a commercial scale. Since whole milk is more palatable than skimmilk the problem, therefore, is to increase the palatability and human consumption of skimmilk by some technique of processing without imparting objectionable off flavors to the product. Reduced palatability of skimmilk as compared to whole milk, appears to result from lower viscosity and a less rich flavor. This investigation is an attempt to increase the viscosity of skimmilk, and although flavor was considered it was of secondary importance.

What can be done to increase the viscosity of skimmilk? Will the addition of ions affect viscosity, and if so, to what degree? In 1956, Burton (10) reported a change in particle size of the caseinate molecule(s) as a result of adding certain ions to skimmilk. His results were based on changes in light reflectance. However, he stated, "As far as is known, there are no published data on the effect of calcium ions and of citrate and phosphate ions on the viscosity of separated milk which would confirm the

conclusion that calcium ions increase and other ions decrease the size of casein particles." This clue indicated that a change in concentration of some ions in skimmilk might alter its viscosity. Therefore, the purpose of this thesis is to: (a) determine the effects of added and removed ions on the viscosity of skimmilk, (b) to determine the effects of added ions on the relative particle size of casein in relation to hydration and viscosity, and (c) to determine the effects of added and removed ions on flavor and palatability of skimmilk.

Accordingly, a review of the effect of salts (ions) on milk in regard to certain physical and chemical properties of milk, and a discussion of the composition of milk as to ion concentration has been considered imperative. In addition, the literature review includes formulae, types of viscometers, and reported viscosity values of whole and skimmilk. Considerable emphasis has been placed on the effects of ions on the relative particle size of casein as interpreted by viscosity determinations, since literature regarding this change is fragmentary.

## REVIEW OF LITERATURE

### Viscosity of Whole Milk

Skimmilk may be considered as whole milk from which fat only has been removed. Therefore, it appeared desirable to review factors which affect the viscosity of both whole and skimmilk. These factors include the following: (a) effect of previous heat

treatment on viscosity, (b) effect of storage period on viscosity, (c) effect of temperature determination on viscosity, (d) effect of homogenization pressures on viscosity and (e) effect of storage temperatures on viscosity. Since each factor may influence the ratio of dissociated salts or ions to associated salts or ions, a review of current knowledge was considered important.

Effect of Previous Heat Treatment on Viscosity. The temperature at which milk is pasteurized has a pronounced effect of viscosity of whole milk. Taylor (56), in 1913, found that heating milk to a pasteurization temperature of about  $65^{\circ}$  C., and then cooling to  $20^{\circ}$  C., decreased the viscosity of the milk slightly. He also noted that an increase in pasteurization temperature of milk produced a slight decrease in viscosity.

Caffyn (11) observed that viscosity of whole milk increases when held at  $60^{\circ}$  C. for a long enough time. He attributed this increase to changes occurring in the molecular structure of the proteins of milk. Evenson and Ferris (21) heated milks for 30 minutes at various temperatures. The samples were cooled to  $20^{\circ}$  C. and the viscosity determined. The results of these studies showed that a decrease in viscosity was apparent when the milks were heated to  $60-65^{\circ}$  C. Conversely, the heating of milks to  $75-80^{\circ}$  C. increased the viscosity.

Woll (68), in 1886, pasteurized samples by heating for 20 minutes at  $67^{\circ}$  C., and also by heating in an Arnold steam sterilizer for 30-35 minutes. He observed that pasteurization under

these conditions decreased the viscosity of both milk and cream. The decrease in viscosity of milk was about 4 percent and of cream about 16-17 percent. Woll also noted that the viscosity of whey was increased by pasteurization indicating a denaturation of the serum proteins.

Effect of Storage Period on Viscosity. The age at which viscosity of milk is determined has a pronounced effect on this physical property of milk. Dahlberg and Hening (16) showed that storage of milk at 3-4° C. for several days increased its viscosity. Evenson and Ferris (21) in a later study, confirmed this fact by storing milk for one week at 3° C. and determining its viscosity. Their results showed that there was an increase in viscosity over milk that had been held for 1 hour at 30° C. Whitnah, et al. (66), in 1956, probably demonstrated more clearly than previous workers that viscosity of both homogenized and un-homogenized milk increased with age in a linear relationship.

Effect of Temperature of Determination on Viscosity. The effect of the temperature of determination on viscosity of milk has been discussed by Caffyn (11). He noted that viscosity decreased with an increase in temperature of measurement from 20-80°C. Similar observations, in a more recent study, were made by Whitnah, et al. (66) when they determined the values for viscosity at 4, 8, 18, 22.7, 29, 34, and 44°C. Tapernaux and Villaume (55) presented the following table in support of the influence of temperature of determination on the viscosity of milk.

Table 1. Influence of temperature of determination on the viscosity of milk.

Temperature (degrees C.)	:	Viscosity in Poises
0		0.0344
5		0.0305
10		0.0264
15		0.0231
20		0.0199
25		0.0170
30		0.0149
35		0.0134
40		0.0123

It is evident from Table 1 that as the temperature of determination increases the viscosity decreases. Sommer (43) made reference to the work of Sohlet, who in 1876, studied the viscosity of milk at temperatures between 0 and 30° C. The results of Sohlet's studies are as follows:

Table 2. Viscosity of whole milk versus water at various temperatures.

Temperature degrees C.	:	Viscosity of whole milk: centipoises	:	Viscosity of water centipoises
0		4.291		0.0
5		3.254		1.519
10		2.809		1.308
15		2.463		1.140
20		2.127		1.005
25		1.857		0.894
30		1.640		0.801

As the temperatures of milk and water are raised the viscosity of milk decreased more rapidly than the viscosity of water. It appears likely that this is due to a decreased hydration of the



milk proteins.

Effect of Homogenization Pressures on Viscosity. The effect of homogenization pressure on the viscosity of whole milk has been discussed by several authors (9, 66, 67). Wiegner (67) and Buglia (9) were the first to report that viscosity of whole milk was increased by homogenization. Evenson and Ferris (21) pointed out that homogenization pressure of 3,500 p.s.i. increased the viscosity of whole milk. Trout (57), however, reported that homogenization decreased the viscosity of whole milk. Whitnah, et al. (66), in 1956, confirmed the work of Wiegner (67), Buglia (9) and Evenson and Ferris (21) without question, and it can be concluded from their studies that homogenization from 300 to 3500 p.s.i. increased the viscosity of whole milk.

Effect of Storage Temperatures on Viscosity. Puri and Gupta (37) considered the effect of storage temperature on the viscosity of milk a necessary factor in understanding and interpreting viscosity values. They stored fresh samples of milk at 15, 20, 25, 30, and 35° C. Storage for ten hours at 15-25° C., six hours at 30° C., or three hours at 35° C. produced no noticeable change in viscosity, but longer periods of storage at these temperatures increased viscosity.

#### Viscosity of Skimmilk

Relatively few studies on the viscosity of skimmilk have been reported. Bateman and Sharp (6) recognized this and stated that, "It is surprising that very little study has been done to deter-

mine if the changes in milk viscosity are due to the skimmilk phase." It appears that the factors which influence the viscosity of whole milk likewise influence the viscosity of skimmilk. However, the viscosity of skimmilk is undoubtedly influenced by the removal of the fat.

Effect of Previous Heat Treatment on Viscosity. As in the instance of whole milk the effect of previous heat treatment has a pronounced effect on the viscosity of skimmilk. Whitaker, et al. (63) showed conclusively that previous heat treatment markedly affects the viscosity of skimmilk. Table 3 shows the results of their study.

Table 3. Effect on viscosity determined at 24° C. of pasteurizing skimmilk for 30 minutes at various temperatures.

Pasteurization Temp. degrees C.	Viscosity cp.	Number of samples
Unheated	1.472	62
50	1.434	9
62.5	1.440	13
70	1.455	5
72.5	1.475	4
75	1.490	5
78	1.515	3
79	1.524	3
80	1.527	8
82	1.540	3
85	1.548	3
90	1.553	4
100	1.603	4
110	1.647	3
120	1.717	3

When samples heated at 50, 62.5 and 70° C. are compared it is noted that their viscosities were lower than those of the other

samples. This may be attributed to the fact that the hydration of proteins has not fully returned in 30 minutes to a point comparable to the unheated sample.

Bateman and Sharp (6) noted that pasteurizing skimmilk at 62° C. for 30 minutes slightly decreased viscosity compared with an unheated sample. They stated that this decrease may be attributed to a slow denaturation of the milk proteins. Woll (67) also reported that heating skimmilk to 67° C. for 20 minutes decreased viscosity of skimmilk, but he published little data to substantiate that statement.

Effect of Storage Period on Viscosity. The effect of storing skimmilk on viscosity has been noted by Bateman and Sharp (6) who stated that viscosity of skimmilk increases with age. Other literature to substantiate this statement has not been found.

Effect of Homogenization on Viscosity. Homogenization of skimmilk, as in the case of whole milk, has an effect on viscosity (6). However, the shift is toward a decrease in viscosity rather than an increase. Bateman and Sharp (6) who made this study in 1928, attributed the decrease in viscosity of the homogenized skimmilk to the state of subdivision of the protein clots which may be present in the milk. A distinct rise in viscosity was noted in the case of whole milk, and a slight decrease in the case of skimmilk was observed when the samples were homogenized. Unpublished data by the author of this thesis confirm the research of Bateman and Sharp.

Effect of Temperature of Determination on Viscosity. Whitaker,

et al. (63) have studied the relationship between temperature of determination and the relative viscosity of skimmilk. The following table shows the results of that study.

Table 4. The viscosity of skimmilk at various temperatures as compared with water.

Temperature : degrees C. :	Viscosity in cp. :	Viscosity relative to water	
	<u>Water</u>	<u>Skimmilk</u>	
5	1.519	2.96	1.95
10	1.308	2.47	1.89
15	1.140	2.10	1.84
20	1.005	1.70	1.78
25	0.894	1.54	1.72
30	0.801	1.33	1.66
35	0.723	1.17	1.62
40	0.656	1.04	1.58
45	0.599	0.93	1.55
50	0.549	0.85	1.54
55	0.506	0.77	1.53
60	0.469	0.71	1.52
65	0.436	0.66	1.52
70	0.406	0.62	1.52
75	0.380	0.59	1.54
80	0.357	0.57	1.59

When both the temperature of water and skimmilk is raised it is observed that their viscosities decrease. However, careful observation of Table 4 indicates that the decrease in the viscosity of skimmilk is more pronounced than water.

Other authors have compared the viscosity values of skimmilk and whole milk. Bateman and Sharp (6), in 1928, reported the viscosity value for whole milk at 25° C. and 1306.9 (gm/sq.cm.) pressure to be 1.584 cp., while the viscosity of skimmilk at the

same temperature and 682.9 (gm/sq.cm.) pressure was 1.425 cp. Puri and Gupta (37), in 1955, reported the viscosity values for whole and skimmed milk from the same lot as shown in Table 5.

Table 5. Effect of removal of fat from milk on its viscosity at 20° C.

Serial Number:	Viscosity of whole milk Millipoise	Viscosity of skim- milk, mp.
1	19.43	18.74
2	20.82	19.00
3	19.87	18.83
4	22.42	21.24

In summarizing the effects of several factors on the viscosity of both whole milk and skimmilk, it is evident that previous heat treatment, storage period, temperature of determination, and homogenization affect the viscosities of both. All of the above factors must be considered in evaluating viscosity determinations.

#### Effect of Protein Hydration and Particle Size on Viscosity

Heat. The viscosity of skimmilk, as previously stated, is markedly affected by previous heat treatment. Whitaker, *et al.* (63), in 1927, studied the relationship between heat treatment and relative viscosity of skimmilk. They reported that viscosity decreased with increasing heat treatment up to 70° C. However, at pasteurization temperatures above 70° C., the relative viscosity showed an upward trend. The lesser viscosities up to 60° C. are probably caused by an increase in the size of the caseinate

particles resulting in a decrease in viscosity (43). At temperatures above 70° C. it appears that the particle size is decreased with subsequent increase in viscosity and protein hydration (43). However, Eilers (19) pointed out that the rise in viscosity may be attributed to denaturation of soluble protein, mainly albumin. Mumm (36) stated that increased temperature decreased hydration of casein, but showed no figures in regard to viscosity changes.

Whitaker, et al. (63) showed that as the temperature of measurement was increased, viscosity of skimmilk decreased more rapidly than did water at the same temperature. This decrease may be attributed to a decrease in hydration or the percent of bound water present in the casein molecules as observed by Pyenson and Dahle (38). They noted that heat has a marked effect on the bound water in the protein molecule. In their studies on bound water in milk, Pyenson and Dahle discovered that the bound water in whole milk was 2.0 - 3.5 percent, while skimmilk contained 1.5 - 2.5 percent. They reported the distribution of the bound water was among casein, albumin, the fat membrane, and the serum proteins with casein and albumin containing the most bound water. An increase in bound water over that in fresh milk resulted in an increased viscosity, and a decrease in protein stability. High heat treatment decreased the bound water present in the proteins, and subsequently decreased viscosity.

It may be summarized from the literature presented that increasing heat treatment of the skim or whole milk up to 70° C.

decreased viscosity. At 70° C., the viscosity of skimmilk and whole milk begins to rise. It has been suggested that the increase in viscosity may be due to either decreasing particle size with subsequent increase in viscosity (43), and/or denaturation of soluble protein (19).

pH. The addition of salts to milk tends to change the pH depending upon the nature of the salt added. Holm, et al. (27) claimed that the addition of phosphates and citrates to milk increased the pH and increased the tendency for milk caseinate to pass into solution. Verma (60) substantiated the fact that phosphates and citrates increase pH. He stated that the addition of small amounts of calcium to milk increased hydrogen ion concentration (decreased pH) and phosphates decreased hydrogen ion concentration (increased pH). He further explains that addition of soluble calcium tends to shift the reactions (in milk) in favor of  $\text{Ca}_3(\text{PO}_4)_2$  precipitation with a simultaneous decrease in pH and soluble phosphate. The addition of phosphates, on the other hand, tends to decrease soluble calcium and magnesium with subsequent increase in pH.

Eilers (19) reported that, when milks had a pH above 6.7, viscosity increased. He attributed the increase in viscosity to an increased dispersion of the colloidal material, mainly casein. Burton (10) also found that the change of particle size of casein is affected by pH. He stated that "casein particle size is independent of pH between approximately 5.8 and 6.6, but increased rapidly above 6.6". Pyenson and Dahle (38) noted that increasing pH toward neutrality and slight alkalinity decreased bound water,

but that decreasing pH toward the isoelectric point decreased the bound water to a greater extent than increasing pH toward neutrality.

Salts. The classical work of Sommer and Hart (47) on salt balance in milk has drawn considerable attention and stimulated much study on the occurrence and functions of salts in milk. The addition of some salts to milks to be condensed has found wide use in the condensing industry as a technique for controlling the viscosity of condensed and evaporated milk. Sommer and Hart (47) have shown that the addition of positive and negative ions have a marked effect on the heat stability of milk. The effect of the added salts to milk for evaporation is influenced by many factors. They are: (a) relative stability of milk, (b) addition of either positive or negative ions, (c) the amount of salt (ion) added, (d) temperature to which milk is heated, and (e) the effect of the salt on reaction or pH of milk. These factors and their actual effects on the stability of milks are given by Sommer and Hart (47).

Sommer (43) explains the function of the various salts as follows. The colloidal milk proteins carry a negative charge. The divalent ions (such as calcium and magnesium) were effective in reducing the charge on the proteins, and consequently decreased stability of the milk. The trivalent ions are even more effective in neutralizing the charge on the proteins. Citrate and phosphate ions, yielding di and trivalent ions, respectively, were instrumental in increasing the negative charge on the milk proteins



and consequently, increased protein stability. Sommer and Binney (45) demonstrated that the addition of either calcium, magnesium, citrates or phosphates to milk affected the relative stability of the milk to the alcohol test. A slight increase in the calcium or magnesium content of milk resulted in a positive alcohol test which indicated less stability to heat. They further stated that an increase in the potassium, sodium, chloride, citrate, and phosphate ions did not cause a positive alcohol test which indicated greater stability to heat than when calcium or magnesium were added. Furthermore, the effect of calcium and magnesium in the alcohol test may be counteracted by the addition of citrates and phosphates.

Holm, et al. (27) agreed with the above findings of Sommer and Hart in regard to the effects of added salts on the heat stability of milks. However, they preferred to refer to the "positive charge" effect rather than "salt balance" in relation to protein stability. They contended that the charge of the ion rather than the salt itself determines protein stability. Nevertheless, the term "salt balance" has found favor in the dairy industry.

Stebnitz and Sommer (50) demonstrated the effects of various salts on the coagulation resistance of milk. They added calcium acetate, sodium citrate and disodium phosphate to milks to be condensed. Their results revealed that calcium acetate addition in small amounts decreased the thickening of milk, and large amounts accelerate or produce coagulation. They state that the addition

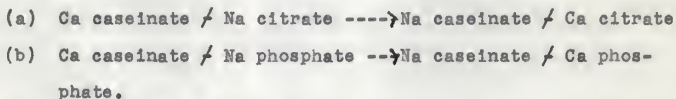
of small amounts of sodium citrate and disodium phosphate increased the thickening of condensed milk upon aging, while the addition of the calcium compounds decreased thickening of the condensed milk upon aging.

With an understanding of the effects of various ions on the heat stability of milks, the relationship between heat stability and protein hydration and viscosity will be discussed. It has been noted that calcium decreased thickening of condensed milk or, in excessive amounts, produced coagulation. Conversely, citrates and phosphates increase the tendency for age thickening. What then is the relationship between salts in milk, protein hydration and viscosity?

It is a well known fact that high concentrations of certain salts cause a "salting out" effect which is merely a dehydrating of the milk proteins (43). Loeb (34) found that certain salts decreased the swelling of gelatin in water, and the depressing affect increased with the valence of the ions having a charge opposite to that of the gelatin. The ions which would have this effect would be those of calcium, and it can be reasoned that the calcium ions are actually decreasing hydration. However, the effect of cations in small amounts is in all probability not a dehydrating effect as such, for Burton (10) has shown that the addition of calcium to skim milk actually increased particle size of casein. He showed this by adding calcium to milk as  $\text{CaCl}_2$  and testing the ability of the milk to transmit light. His results revealed that light is reflected to a greater extent when calcium

is added to the milk as compared with the reflectance of the control milk. Hostettler and Imhof (29) and Imhof (30) have shown by electron microscopy, that calcium added to milk as  $\text{CaCl}_2$  increased the caseinate particle size. Furthermore, they noted that concentration of milk increased the calcium ion concentration, which resulted in an increase in protein particle size. When calcium was added to milk, several reactions occurred: (a) an increase in caseinate particle size, (b) decreased hydration because of less surface area, and (c) viscosity was less or decreased.

Sommer (43) pointed out that the hydrating effect of anions, citrates and phosphates was, in all probability, not a simple salt effect, but a combination of a salt effect and the result of a double decomposition reaction as follows:



Sodium caseinate is more readily soluble than calcium caseinate, and this may be interpreted to mean that sodium caseinate becomes more strongly hydrated than calcium caseinate. Some studies have been made in regard to the hydrating properties of milk proteins as affected by anions. Edmondson and Tarassuk (18) recently demonstrated that the addition of disodium phosphate decreased the sedimentation rate of milk proteins. Their studies revealed that disodium phosphate markedly increased the viscosity of the skim milk samples studied. They concluded that the addition

of phosphate as disodium phosphate causes a shift toward an average smaller particle size of the caseinate molecule or to an increase in protein hydration. If particle size is actually changed then the findings of Edmondson and Tarassuk are in accordance with the double decomposition reaction as suggested by Sommer (43).

From the studies of Pyenson and Dahle (38), in 1938, evidence is given to support the theory that certain salts affect the hydrating properties of the milk proteins. They stated that the so-called milk protein "stabilizing salts" (citrate and phosphates) increased bound water and protein stability, while the so-called "destabilizing salts" (calcium and magnesium) decreased bound water and protein stability.

#### Amounts of Calcium, Sodium, Phosphate, Citrate and Lecithin in Milk

The purpose of this section of the literature review concerning the salt composition of milk, is to establish the amount of salt (ion) that can be safely added before visible protein coagulation occurs by obtaining a knowledge of the amount of ion inherent to the milk.

Calcium. A review of the literature indicated that calcium has been by far the most extensively studied salt component of milk, with phosphorus a close second. Crichton (15) in a study of 220 samples from 20 Ayrshire cows gave a range of 0.120 to 0.212 percent CaO with an average of 0.166 percent. Sommer (43)

gave a range of 0.125 to 0.291 percent CaO. Cranfield, et al. (14) made a very extensive study of the calcium content of milk from 673 individual cows in 15 herds. They noted extremes of 0.135 to 0.23 percent CaO. However, 316 samples were in the range of 0.175 to 0.190 percent and they considered this the average range.

Jacobsen and Wallis (32) obtained one day composite samples from individual cows, at monthly intervals over a period of five years. Their calcium analysis showed respective averages at 114, 132, 146, and 146 mg. percent for Holstein, Ayrshire, Jersey and Guernsey cows. In an earlier, less extensive study, Van Slyke and Bosworth (59) analyzed 16 samples of milk from 13 individual cows at different stages of lactation. They reported 126 to 217 mg. percent of the milk to be calcium. Acharya and Devadatta (1) found an average of 235.5 mg. percent calcium in 10 samples.

Sodium. The percent sodium in milk is not of utmost importance in this study. Its only purpose in analysis was to determine the amount introduced by an ion exchange resin, which was used in some trials. This review, therefore, will mention only a few of the more extensive publications on the sodium content of milk.

Sommer (43) reported the average percent of Na<sub>2</sub>O in milk to be 0.70 percent. Crichton (15) analyzed 220 samples of milk from 20 Ayrshire cows. He found the maximum Na<sub>2</sub>O to be 0.155 percent, and the minimum 0.050 percent. Sherman (40) found an average of

0.069 percent  $\text{Na}_2\text{O}$  in the herd samples studied.

Phosphorus. In early literature (1913) it was noted by Sommer (43) that accurate investigations of phosphorus were particularly hampered by the lack of accurate methods of determination.

Seasonal fluctuations in phosphorus content of milk powders have been determined by Supplise (52), who reported that powders from New York and Wisconsin contained a maximum of phosphorus in January and a minimum in the summer. Acharya and Devadatta (1) reported an average value for  $\text{P}_2\text{O}_5$  content of ten samples of milk as 189.5 mg. percent. Graham and Kay (23) analyzed 18 samples; six each from Holstein-Friesian, Ayrshire, and Jersey. The average total phosphorus (P) was reported as 103.7 mg. percent. Jacobsen and Wallis (32) studied the phosphorus (P) content of milk from individual cows, based on one day composite samples, at monthly intervals for five years. The average phosphorus values reported were 87, 105, 112, and 108 mg. percent for Holstein, Ayrshire, Jersey, and Guernsey, respectively. Van Slyke and Bosworth (58, 59) gave the results of two analyses and reported 125.0 mg. percent phosphorus in milk.

Citric Acid. Citric acid was first discovered in milk by Scheibe and Hinkel, as cited by Davies (17). Its presence in milk was confirmed by Sommer and Hart (46), in 1918, but the determination of the exact amount in milk has been handicapped by the limitation of accuracy of techniques used. The following table is a summary of the citric acid content of milk as reported by some investigators.

Table 6. Citric acid content of milk as reported by several investigators.

Observers	: Basis of : Method	: Samples: : No.	: Citric Acid con- : tent in %		
			min. :	max. :	Av.
Bosworth and Frucha 1910 (8)	Deniges	2	0.224	0.203	0.212
Hess, et al. 1920 (26)	Ba salt	2	(a)0.130	0.180	0.150
Supplee and Bellis 1921 (52)	Stahre	17	0.182	0.121	0.145
Sommer and Hart 1918 (46)	Deniges	16	0.248	0.191	0.231
Sherwood and Hammer 1926 (41)	Deniges	335	0.330	0.070	0.180
Stewart	Deniges and Stahre	8	(b) ---	---	0.150

(a) Composite samples, each of five cows

(b) Dried milk calculated to liquid milk

The maximum amount of citric acid found in the above studies was 0.330 percent and the minimum was 0.070 percent.

Lecithin. The figures obtained by various authors for the lecithin content of milk vary greatly. Since the results, in practically all cases, are based on the phosphorus content of the extracted materials, these variations must be due to incompleteness of extraction of phospholipids in some cases, and to extraction of inorganic phosphorus in others (25). Table 7 shows average values for phospholipids in whole and skimmed milk as found by three authors.

Table 7. Phospholipids in some milk and skimmilk samples according to three authors.

Author	Percent phosphorus in milk	
	Whole Milk	Skimmilk
Hess and Helman 1925 (25)	0.1819 0.1377	0.1072 0.1156
Chapman 1928 (12)	0.0345 0.0709	0.0082 0.0290
Holm, et al. 1933 (28)	0.1580	0.1290

### Viscometry

After reviewing the literature pertaining to viscosity of whole and skimmilk, it was apparent that careful consideration to the type of viscometer used and other viscometric techniques should be given. The following review is considered necessary to assist in explaining the experimental design and evaluating the results. The following literature review consists of types of apparatus used to estimate or measure viscosity and the necessary equations for converting measured data to poise units or viscosity values.

Viscosity in its simplest terms may be defined as "\_\_\_ the resistance a liquid offers to flow "(43). A more technical definition is the " \_\_\_force per unit area necessary to maintain a unit velocity gradient between two parallel planes kept at a unit distance apart" (62), and:



$$\eta = T/(dv/dx)$$

where: T = force per unit area acting parallel to the planes equals shearing force in dynes per cc.<sup>2</sup>

dv/dx = velocity gradient perpendicular to the plates in centimeter second<sup>-1</sup>

$\eta$  = viscosity in g. centimeter<sup>-1</sup> second<sup>-1</sup>

The unit measurement for viscosity is the poise unit established by Poiseuille in 1846 (Bingham, 7). It is defined as "\_\_\_ the viscosity of a material which requires a shearing force of one dyne per centimeter<sup>2</sup> to maintain a velocity gradient of one centimeter per second between two planes one centimeter apart. In reported data the poise unit or centipoise unit (1/100 of a poise) with modifications are used (37, 65).

Viscosity is expressed in many fashions. The following review of these terms by Weissberger (62) indicate the many ways that viscosity may be expressed.

Absolute Viscosity. The coefficient of absolute viscosity of a liquid,  $\eta_b$ , can be defined as the force per unit area necessary to maintain a velocity gradient between two parallel planes separated by a unit distance or  $\eta = T/(dv/dx)$ . A material is said to have a viscosity of one poise when the force required to maintain a relative viscosity of one centimeter per second between two parallel planes one centimeter apart in one dyne per centimeter<sup>-2</sup>.

Kinematic Viscosity. The Kinematic viscosity,  $\nu$ , is the absolute viscosity divided by the density,  $D$ , of the liquid. Its unit is called the stoke. Kinematic viscosity is expressed as:

$$\nu = \eta/D$$

Fluidity. The fluidity of a liquid,  $\zeta$ , is defined as:

$$\zeta = 1/\eta$$

Fluidity is a more convenient and satisfactory characterization than the absolute viscosity for interpretive purposes. The unit of measurement is the rhe (from the Greek rhein, to flow).

Relative Viscosity. Relative viscosity may be defined simply as:

$$\eta \text{ rel.} = \frac{\eta_{\text{solution}}}{\eta_{\text{solvent}}} \text{ or } \frac{\eta_1}{\eta_2} = \frac{D_2}{D_1} \cdot \frac{T_2}{T_1}$$

Specific Viscosity. Specific viscosity may be defined as:

$$\eta_{\text{sp.}} = \frac{\eta_{\text{solution}} - \eta_{\text{solvent}}}{\text{SOLVENT}} = (\eta \text{ rel.} - 1)$$

Reduced Viscosity. Reduced viscosity is shown by the equation:  $\text{Red. } \eta = \text{sp.}/c$

where:  $c$  = concentration as g. solute/100 cc. solution.

Intrinsic Viscosity. Intrinsic viscosity is a more complex term. Its formula is presented as follows:

$$\text{Int. } \eta = [\eta] \equiv \lim_{c \rightarrow 0} \frac{(\eta_{sp})}{(c)}$$

$$\equiv \lim_{c \rightarrow 0} \frac{(\text{In. rel.})}{(c)} \equiv \lim_{c \rightarrow 0} \frac{(d\eta_{rel.})}{(dc)}$$

The different formulae pertaining to the calculation of viscosity are helpful in appraising experimental data on the actual viscosity of some liquids. Several methods of determining viscosity, methods of operation and the falacies of the techniques are described as follows:

Capillary Viscometer. Many modifications of capillary viscometers are now in use, depending upon the sensitivity of measurement desired or the liquid to be measured (7, 62).

The capillary viscometer measures the rate of flow of a liquid through a capillary under constant pressure. There is a direct relationship between liquid flow in a capillary and the viscosity of the liquid. In the flow of a Newtonian liquid (such as water) the equation of Poiseuille is adequate to describe this relationship. Poiseuille's equation is as follows:

$$\eta = \frac{hg\rho r^4 t \pi}{8lv}$$

where: = viscosity in poise

h = liquid head in centimeters

g = gravitational constant in centimeters  
per second<sup>-2</sup>

ρ = density in g/cc.

r = radius of capillary in centimeters

t = flow time in seconds

$v$  = volume of flow in cc.

$l$  = length of capillary in centimeters.

The precision with which a capillary viscometer may be operated, after the usual precautions of cleanliness of a sample are observed, depends upon the control and/or measurement of the following variables: (a) temperature, (b) time, and (c) the driving head on the liquid which includes vertical or constant alignment of the viscometer, volume of sample, and temperature (62).

Operation of a capillary viscometer is relatively simple, and with knowledge of the following steps the determinations can be rapidly made.

1. Align the viscometer in a vertical position to insure constant driving head of the liquid in the instrument.

2. Maintain constant temperature. Temperature has a two-fold implication in viscosity measurement. The lesser effect is the influence on the driving head of the liquid, for although the linear change with change in temperature in a uniform bore capillary is compensated by density change, the liquid is not confined to such a condition. The greater effect, and of more importance is the temperature dependence of viscosity as expressed by the equation:  $\eta = Ae^{B/Rt}$

3. Bring the temperature of the sample to equilibrium with the cooling or heating media. This takes only a few minutes.

4. Timing the flow of a liquid through the capillary is made possible with a mechanical stopwatch to  $\pm 0.2$  seconds. With photo-

graphic methods one can obtain flow time reading to  $\pm$  0.001 seconds.

5. Charging the viscometer is accomplished (with an Ostwald pipette) by inverting the capillary arm into the sample and filling to a designated mark by slight suction on the side arm. A rubber tube, of short length, is attached to the capillary arm to prevent the sample from flowing when determinations are not being made.

Falling Ball Viscometer. The falling ball technique for measuring viscosity, which is based on the principal of Stokes law to the fall of spheres through a liquid (69), is used when the viscosity of a liquid exceeds  $10^4$  centipoises (62). In the technique the rate of fall of a steel ball of measured diameter is made at a constant temperature. The falling ball method has two distinct advantages: (a) simplicity of design, and (b) flexibility of operating conditions. The disadvantages of its use are (a) the difficulty with which temperature may be maintained and (b) the difficulty of observing the fall of a steel ball through an opaque liquid.

The falling ball viscometer is operated in the following manner (69). A glass jar, as tall as possible, is filled with a liquid, such as glycerin, whose temperature is obtained by inserting a thermometer in it. The liquid should be maintained at constant temperature.

Steel ball bearings are measured with a screw gauge and placed in a small amount of the liquid to be tested in a watch glass.

The ball bearings are transferred to the glass jar by means of a spatula, and after each dropping, a few centimeters, their terminal velocity is obtained by timing a measured fall. To avoid parallel errors paper collars are slipped around the glass jar and the upper edge of these collars is used as points of reference for the determinations.

According to Stoke's law, the spheres move in a terminal velocity,  $V_0$ , expressed by the formula:

$$F = 6\pi\eta V_0 r$$

where:  $F$  = viscous force acting on spheres of radius,  $r$ , centimeters.

In a steady state  $F$  is equal to the net downward force:

$$\text{i.e., } 6\pi\eta V_0 r = 4/3\pi r^3 (\rho - \delta) g$$

$$\text{i.e., } V_0 = 2/9 \cdot \frac{r^2}{\eta} (\rho - \delta) g$$

where:  $\rho$  = density of the steel ball

$\delta$  = density of the liquid

Coaxial Cylinder Viscometer. The coaxial cylinder viscometer is also known as the conette viscometer. Its operation is not extremely complex, but is hampered by lack of accurate temperature control. The formula for expressing the variables and determination of laminar flow of a Newtonian liquid is:

$$M = 4\pi L \frac{R_2^2 R_1^2 \eta \omega}{R_2^2 - R_1^2} = K \eta \omega$$

where:  $K$  = constant for a given apparatus

In this apparatus the liquid exists in a thin cylindrical layer between two coaxial cylinders of height  $L$ , which have the radii,  $R$  and  $R_2$  ( $R_2 > R_1$ ). Usually the outer cylinder, is connected to a torsion measuring device to have imparted to it the angular moment,  $M$ .

The sample is placed in the outer rotating cup which is then driven at a constant speed by a motor arrangement. The deflection of the inner cylinder or the torque exerted thereon, is then measured by a suitable scale against a liquid of known viscosity.

This method is quite simple and accurate regardless of the flow characteristics of the liquid. The reason for this is that the velocity gradient across the smaller space is very nearly constant and approaches consistency as the ratio of the annular space to the radius of the inner cylinder becomes smaller. When the velocity gradient is constant, the velocity distribution is linear, and the viscosity of various shearing rates of non Newtonian liquids can be correctly evaluated.

Regardless of the type of viscometer used for measurements of viscosity, it is essential to take into account two factors which alter accurate viscosity determinations. They are summarized as follows (62).

1. Temperature (previously explained)

2. Velocity gradient. If for any reason there is no strict proportionality between shearing force,  $r$ , and the velocity gradient,  $dv/dx$ , the observed ratio of the two quantities will not be constant, and therefore, the viscosities corresponding to

these conditions will not be identical. If this is found, then the system is non Newtonian; it may be either a liquid or solid depending on whether a yield point exists, i.e., failure to flow under extremely small but physically finite stress.

#### DESCRIPTION OF EQUIPMENT AND MATERIALS

##### Construction and Operation of Viscometer Used

In this study a falling ball viscometer was not used for two reasons: (a) the inability of maintaining temperature control of  $4^{\circ}\text{C}.$   $\pm$   $0.01^{\circ}\text{C}.$ , and (b) the difficulty in observing the fall of a steel ball through an opaque liquid like milk. Likewise, the coaxial cylinder was not used because of the lack of temperature control that could be attained. However, the capillary or Ostwald viscometer offered many advantages in operation, and therefore, was used in this study.

Among the types of viscometers available for viscosity determinations on milk, the capillary viscometer has been used more extensively than any other type. Bateman and Sharp (6), Caffyn (11), and Cox (13) among others have employed the use of the capillary or Ostwald pipette. Bateman and Sharp (6) maintained that pressure control to obtain reproducible results is essential.

The factors which influenced the use of an Ostwald pipette in this study were as follows: (a) the simplicity with which the Ostwald pipette was charged and discharged with a milk sample; (b) the extreme ease with which temperature control could



be attained by suitable apparatus; (c) the uniformity with which flow times (viscosity) could be determined on a stock supply of distilled water (at different periods, flow times could be reproduced within 0.01 seconds); (d) the convenient flow times offered by an Ostwald pipette which permitted determination of many samples; (e) the visibility with which flow times could be observed; (f) the relatively small amount of sample that could be used, and (g) the low cost of viscosity determinations.

The slightly modified Ostwald viscometer used in this study was specially constructed and has been described by Whitnah, et al. (65). In the design of this viscometer the effect of driving head pressure was taken into consideration. However, a pressure regulating device as used by Bateman and Sharp (6) was not employed in this study. But in order to compensate for atmospheric pressure changes the following points were observed:

- (a) the viscometer was always placed in a vertical position;
- (b) the bulb (Plate 1, A-1) was constructed at an angle of about  $40^{\circ}$  to the capillary in order to reduce the driving head, and
- (c) the constancy of temperature control must be exact.

The capillary size of the viscometer (0.47 cm.) was selected for three reasons: (a) to facilitate a convenient flow of milk; (b) to permit the flow or passage of small aggregates of particles that may appear in the sample, and (c) to give laminar instead of turbulent flow (64).

The Ostwald pipette was constructed of Pyrex glass as shown in Plate I. The upper bulb (A-1) was a double cone type with the

## EXPLANATION OF PLATE I

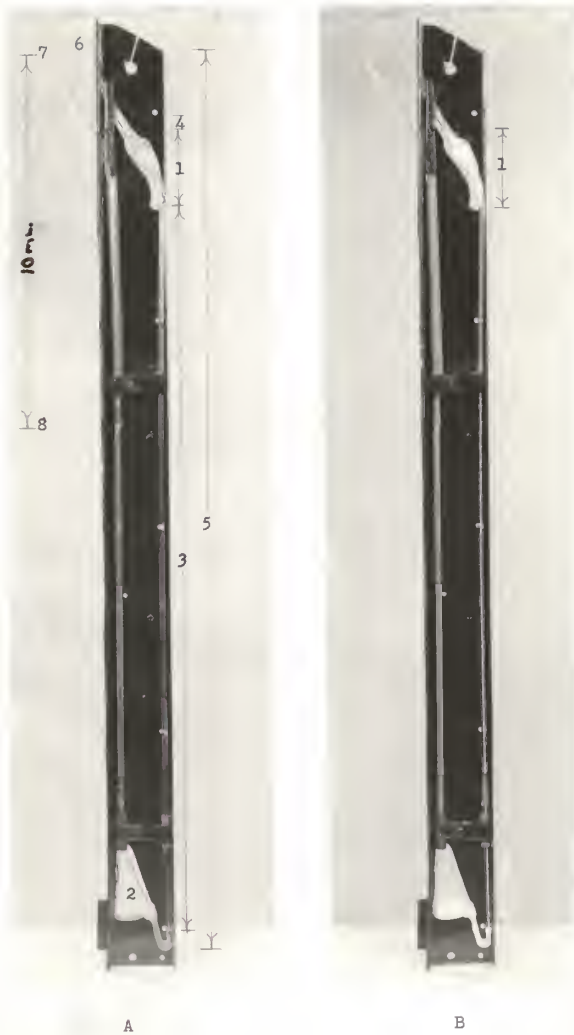
### A. Empty viscometer

A-1, upper bulb; A-2, lower bulb; A-3, capillary (72 cm. x .047 cm.); A-4, small capillary (1.2 cm. x .047 cm.); A-5, total length of viscometer (89.2 cm.); A-6, upper arm; A-7, lower arm; A-7 to A-8, area of charging.

### B. Viscometer charged with milk

B-1, area of flow time measurement.

PLATE I



A

B

common axis of the cone at an angle of about  $40^\circ$  to the capillary (A-3). This type structure provided good drainage of the bulb while in position. The bulb had a capacity of 5 ml. The lower bulb (A-2) was a 25 ml. Erlenmeyer flask which served as a reservoir for the liquid.

The length of the capillary (A-3) was approximately 72 cm. in length and the bore, 0.047 cm. in width. The length of the small capillary (A-4) had a measurement of 1.2 cm. The total length of the viscometer (A-5) was 89.2 cm. A metal rack protected the viscometer from breakage. Vertical alignment of the viscometer was assured by suspending the viscometer in its metal frame from a lug located above the glass jar (to be described).

Charging the viscometer with sample was accomplished by the following technique similarly described by Weissberger (62). A clear, Tygon hose was attached to the arm of the upper tube (A-6) of the viscometer. By creating suction on the tube of the lower arm (A-7) while immersed in the liquid, exactly 10 ml of sample (at the temperature of viscosity determination) was drawn to the indicated mark (A-7 to A-8). While still creating suction on the hose, the viscometer was lowered to a vertical position and inserted in the water bath. Plate I (B-1) indicates the area in which timed flows were measured.

Absolute cleanliness of the viscometer was necessary. The viscometer was rinsed by removing the sample and washing twice with successive rinsings of the next sample to be studied. The

procedure for rinsing was the same as for charging. However, the pipette was shaken gently while rinsing to insure exposure of the glass surface to the milk.

While the viscometer was not in use it was filled with distilled water to remove any milk particles adhering to the surface of the viscometer. Once weekly, the viscometer was filled with a strong chromic acid solution to completely remove any milk adhering to the glass.

Several considerations for satisfactory operation of capillary type viscometers are as follows: (a) Charge the viscometer with the same amount of sample for each determination. (b) Charge the viscometer with sample at the temperature at which the viscosity will be determined. If these precautions are not followed, contraction or expansion of the liquid will result (62). (c) Insert the viscometer in a constant vertical position in the water bath. (d) Keep the viscometer absolutely clean. (e) Maintain purity of the samples being determined. (f) Keep the temperature exactly at the temperature of determination.

#### Description of Temperature Control Apparatus

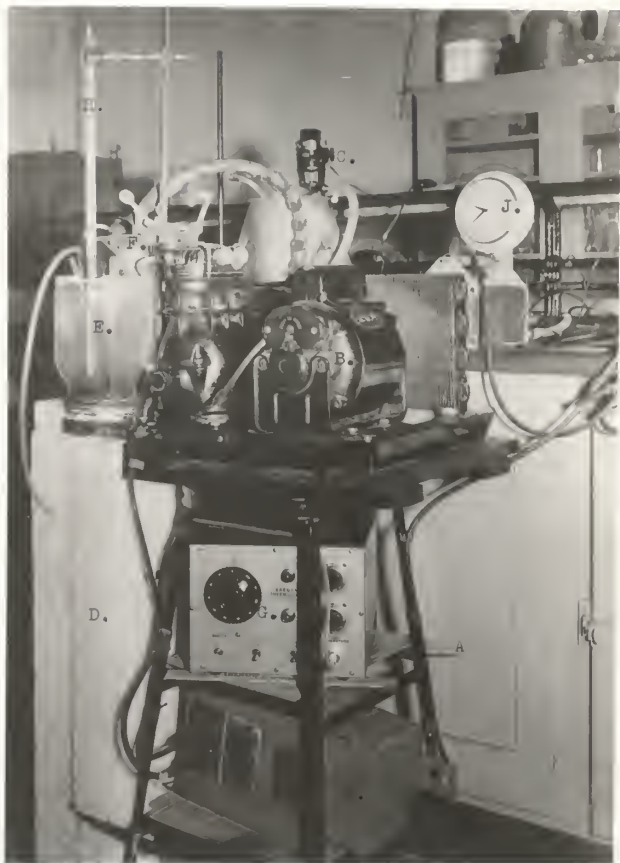
The apparatus described in this study (Plate II) was constructed to maintain temperature at  $4^{\circ}\text{C} \pm 0.01^{\circ}\text{C}$ . Observations by the author indicated that temperature changes of  $\pm 0.02^{\circ}\text{C}$  may cause flow time variations of 0.5 seconds or more with the type viscometer used. Therefore, precise temperature must be maintained. Similarly constructed apparatus without the use of a temperature control unit has been described by Whitnah, et al. (64).

EXPLANATION OF PLATE II

Temperature control unit for viscometer

- A. Stool base
- B. Freon compressor
- C. 1/100 h.p. centrifugal pump
- D. Copper cylinder insulated with Styrofoam
- E. Glass jar
- F. Viscometer support
- G. Sargent, Model S, Thermonitor
- H. Beckman thermometer
- I. KVA transformer (220 to 110 volts)
- J. Household clock

## PLATE II



The apparatus was designed with a laboratory stool as its base (Plate II, A). The compressor, (B) which was the primary source of cooling for the water bath, was a sulphur dioxide ( $\text{SO}_2$ ) machine converted to Freon-12. It was mounted on top of the stool by means of a wooden frame, which was bolted to the stool to minimize vibration of the viscometer. The evaporator section of the compressor was immersed to a depth of 24 inches in the water bath.

Sometimes during periods of high temperature and high humidity temperature control of  $4^\circ \text{C}$ . could not be attained. In order to compensate for lack of cooling, ice water was circulated through copper tubing, inserted in the water bath, at a rate controllable by a screw clamp adjustment on the outlet of a 1/100 h.p. pump (C). The circulating pump and compressor were used simultaneously.

The water bath, (D, covered with Styrofoam insulation) in which the viscometer was inserted, consisted of a copper cylinder four inches in diameter and 30 inches in length. It was bolted on the top to the frame of the compressor, and on the bottom to the stool supports. A wide mouth jar (E) was mounted in a vertical position on the open or upper end of the copper cylinder so the upper bulb of the pipette was visible in the water bath. The entire bottom of the jar was removed so that the viscometer could be inserted in a vertical position in the water bath. The middle of the jar cover was removed and the rim soldered to the open end of the copper cylinder, and the jar screwed on. The connection was sealed with glass cement of prevent leakage.



The water bath had a capacity of 10 liters of water at  $4^{\circ}$  C., and the water was circulated by means of compressed air at a constant rate. Styrofoam served as insulation for all except the glass section of the bath. A 15-watt tungsten bulb was mounted behind the glass jar to provide optimum illumination of the viscometer bulb.

The viscometer (Plate I) was mounted on a steel frame, (F) attached to the compressor, so that it could be adjusted to an exact position in the water bath. Behind the viscometer were mounted the thermistor assembly of the Thermonitor and the immersion heaters.

The temperature was controlled by the use of a Sargent, Model S, Thermonitor, thermistor actuated, with a sensitivity of  $\pm 0.01^{\circ}$  C. A sensitivity of  $\pm 0.01^{\circ}$  C. was continually maintained in this study, and when temperature and humidity conditions were ideal, no change could be observed on a Beckman differential thermometer, which was calibrated to  $0.01^{\circ}$  C. The Beckman thermometer (H) was supported in position by a burette clamp attached to a metal rod. The Sargent Thermonitor was supported in position by a plywood frame, and vibration was always at a minimum.

The source of power for the apparatus was a model KVA, constant voltage transformer I (with two, 110 volt outlet switches). The transformer was supported in position in the same manner as was the Thermonitor, but on the lower section of the stool. The KVA transformer was desirable for operation of the Thermonitor because of the constant voltage it supplied.

A clock (J) with sweeping second hand was mounted behind the viscosity apparatus. The clock was checked twice daily for correct time and adjusted accordingly so that log of the storage period could be plotted correctly against viscosity.

#### EXPERIMENTAL PROCEDURE

##### Preparation of Skimmilk

The general procedure described below was to add to skimmilk various ions, in the form of salts, to determine whether their addition would affect the viscosity of skimmilk. Before adding any salt, however, a review of the literature was necessary to determine the approximate amount of salts present in milk. The ideal condition would have been to determine the amount of ion present in the milk before the addition of salts was made, but since viscosity was determined soon after separation of the whole milk, this was impossible. Therefore, all samples were analyzed following viscosity determination.

The milk used in this study was obtained from the Kansas State College herd during the period from February 19, 1956 to September 6, 1956. All milk studied consisted of the previous night's milk plus the milk obtained the morning of initial viscosity determination. An aliquot sample was obtained for study by filling a batch pasteurizer to capacity (200 gallons) and withdrawing five gallons of milk after it had been thoroughly blended. Ample time was permitted to insure uniform mixing of the milk in the pasteurizer, and the milk was not heated higher than 30° C.

The five-gallon sample of milk was then heated to 32° C. and separated in a farm type De Laval separator a minimum of four times to insure a low fat test, as skimmilk in this study was considered to contain less than 0.20 percent fat by weight. Immediately following separation, 600 gram samples of the skim-milk were transferred to ground glass stoppered Erlenmeyer flasks, cooled to 4°C. and held at that temperature until ion additions were made. The number of samples weighed out depended upon the number of samples in a trial.

All samples were pasteurized at either 61 or 71° C. in an American Instrument Company, Model 4-01 thermostatically controlled water bath. The heating unit had a sensitivity of  $\pm$  0.02° C. Approximately 19 minutes were required for the milk to reach pasteurization temperature. The ground glass stoppered Erlenmeyer flasks minimized the loss of moisture by evaporation during the pasteurization process (18). Immediately following pasteurization the skimmilk samples were cooled to as close to 4°C. as possible in a circulated alcohol-water bath maintained at 0°C.

All samples were stored at 4°C.  $\pm$  1°C. for the duration of viscosity study. The sample transfer at each flowtime determination was from the Erlenmeyer flask in which the sample was pasteurized. Before each transfer the milk was agitated thoroughly but not so much that air was incorporated in it.

Enough sample was prepared initially so that viscosity, density, pH, and chemical analyses could be made.

Method of Determination of Flow Times. The viscometer was charged, as previously described, and several untimed flow periods of not less than  $4\frac{1}{2}$  minute durations were made to insure temperature equilibrium between the milk and the water bath. At no time were readings made if the temperature of the cooling media was not  $4^{\circ}\text{C} \pm 0.01^{\circ}\text{C}$ .

A minimum of four timed flows were made with a Waltham, Type A-8 of U.S. Ordinance Spec. No. 94-277A9, AM#3, stopwatch with which the flow time of distilled water in the viscometer was established to be 55.75 seconds. The viscosity of water at  $20^{\circ}\text{C}$ . is  $0.010019 \pm 0.000003$  (54). After calculating the average of 4 timed flows and obtaining the density of skimmilk at  $4^{\circ}\text{C}$ ., as previously described by Rutz, et al. (39), viscosity was determined by the equation (33):

$$\eta \text{ at } 4^{\circ}\text{C} = \frac{\eta_m}{\eta_w} = \frac{F_m}{F_w} \frac{d_m}{d_w}$$

where:  $\eta$  = viscosity

F = flow time

d = density

Viscosity was reported as poises  $\times 10^5$ .

Two flow time readers were employed in this study. Each was capable of reproducing flow times on the same sample within 0.05 seconds. Viscosity determinations were made after storage periods of 0 hours, 6, 24, 48, 96, and 144 hours insofar as possible. These periods of storage were selected so that an ample number of points could be plotted on semi-log paper.

Viscosity versus storage periods was plotted on semi-log paper for each individual trial. After the study was complete the means of viscosity values were plotted as they appear in a future section.

#### Addition of Ions to Skimmilk

The purpose of this section of the study is to explain in detail the methods of addition of ions, amounts added, pasteurization temperatures, and observations made on the effects of the addition of certain ions to skimmilk.

Calcium Added as  $\text{CaCl}_2$  Prior to Pasteurization. Pilot studies on the effect of the addition of calcium to skimmilk revealed that additions of more than 0.06 g. of calcium per 100 g. skimmilk produced visible coagulation. Therefore, Calcium was not added in excess of 0.06 g. per 100 g. skimmilk.

Calcium was added as  $\text{CaCl}_2$  in a 39 percent solution to prevent localized precipitation of casein (18), in amounts corresponding to 0.02, 0.03, 0.04, 0.05, and 0.06 g. calcium per 100 g. skimmilk. These amounts are equivalent to adding 0.028, 0.042, 0.056, 0.070, and 0.084 g.  $\text{CaO}$  per 100 g. skimmilk, respectively.

Control samples consisted of an untreated skimmilk sample and sample to which distilled water was added equivalent to the amount of calcium chloride solution added at the 0.06 level. All additions below the 0.06 level of calcium addition were likewise diluted with distilled water in amounts which corresponded

to the difference between the amount of calcium chloride solution at one level and the 0.06 level. This technique was followed to eliminate a dilution factor since the amount of  $\text{CaCl}_2$  solution added at each level was not the same.

The skimmilk samples were pasteurized at  $61^\circ\text{C}$ .  $\pm$   $0.02^\circ\text{C}$ . for thirty minutes. The procedure used in pasteurization has been discussed previously. After a storage period of 96 hours at  $4^\circ\text{C}$ . a small amount of white precipitate, presumably  $\text{Ca}_3(\text{PO}_4)_2$ , had collected in the bottom of the Erlenmeyer flasks. This phenomenon had previously been reported by Verma (60).

Calcium Added as Calcium Acetate Prior to Pasteurization.

It was of interest to determine whether the form in which the ion was added would have some effect on the viscosity of skimmilk. Therefore, calcium was added as calcium acetate,  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ , and the effect on viscosity studied. Pilot studies revealed that calcium acetate added in the dry form showed no localized precipitating effects. Consequently, calcium acetate was added in the dry form in the necessary amounts to furnish 0.02, 0.03, 0.04, and 0.05 g. calcium per 100 g. skimmilk. These amounts are equivalent to adding 0.028, 0.042, 0.056, 0.070, and 0.084 g.  $\text{CaO}$  to 100 g. skimmilk. The calcium acetate was weighed on an analytical balance to  $\pm$  3 mg.

The calcium acetate was added just prior to pasteurization at  $61^\circ\text{C}$ . for 30 minutes. No marked amount of precipitate occurred on the bottom of the Erlenmeyer flasks. Thus, the form of the salt may have some affect on the state of solution of salts in milk.

Calcium Added as  $\text{CaCl}_2$  following Pasteurization. It was conjectured that the addition of calcium ion as  $\text{CaCl}_2$  following pasteurization might show different responses, when graphed, than the addition of calcium as  $\text{CaCl}_2$  prior to pasteurization. Therefore, this section of the study involves the addition of calcium following pasteurization.

The samples were pasteurized at  $61^\circ \text{C}$ . for 30 minutes as in the two previous studies. After cooling to  $4^\circ \text{C}$ .,  $\text{CaCl}_2$  was added to the samples at rates which supplied 0.02, 0.03, 0.03, and 0.05 g. calcium/100 g. skimmilk. Pilot studies showed that  $\text{CaCl}_2$  could be added to skimmilk at  $4^\circ \text{C}$ . in infinite amounts with no visible coagulation occurring. However, it was desirable to add calcium in amounts equivalent to the previous studies for the sake of comparison. From the time  $\text{CaCl}_2$  was added, storage periods were recorded. As in the addition of  $\text{CaCl}_2$  prior to pasteurization the dilution factor was considered and distilled water was added in similar manner in this study. Flow time determinations were begun immediately following calcium addition after pasteurization.

Unlike the study of calcium addition prior to pasteurization no visible precipitate was noted on the bottom of the Erlenmeyer flasks even after storage period of 144 hours. This indicated that heat had a profound effect on the state of existence of the soluble salts in milk (60).

Phosphate Added as  $\text{Na}_2\text{HPO}_4$  Prior to Pasteurization at  $61^\circ \text{C}$ . Previously mentioned literature indicated that the addition of

anions would probably increase the viscosity of whole and skim-milk. It is well established that the addition of  $\text{Na}_2\text{HPO}_4$  has a marked effect on some physical and chemical properties of milk (38, 44). With careful consideration of previously reported literature,  $\text{Na}_2\text{HPO}_4$  was added to skimmilk with the understanding that this chemical compound would furnish the phosphate ion.

$\text{Na}_2\text{HPO}_4$  was added to skimmilk in the dry form after the proper amount had been weighed on an analytical balance to  $\pm$  3 mg. A small amount of the chemical was added, the milk agitated, and the process repeated until the addition was complete. The disodium hydrogen phosphate was sufficiently soluble to prevent any precipitation of the chemical.

$\text{Na}_2\text{HPO}_4$  was added at a rate which gave an exact addition of 0.04, 0.08, 0.12, 0.16 and 0.20 g. phosphate ion per 100 g. skim-milk which is equivalent to adding 0.0296, 0.0597, 0.0835, 0.110, and 0.149 g.  $\text{P}_2\text{O}_5$ . The milks were then pasteurized at 61°C. for 30 minutes, cooled to 4°C. and the flow times were determined. No precipitate formed in the Erlenmeyer flasks after settling for 144 hours, thus indicating that anions (phosphates) do not disrupt the chemical equilibrium in milk sufficiently to cause a visible precipitate to occur.

Phosphate Added as  $\text{Na}_2\text{HPO}_4$  Prior to Pasteurization at 71°C.  
Although examination of the literature indicates that the viscosity of skimmilk increases with increasing Pasteurization temperatures, beginning at approximately 70°C. (6), it is generally agreed that the increase in viscosity is due to complete physical



denaturation of whey proteins especially at temperatures at 88°C. or above (18). However, no studies have been reported to determine the combined effects of heat and the addition of an anion on the viscosity of skimmilk.

It was, therefore, the object of this phase of study to determine the combined effects of heat treatment at 71°C., and the addition of  $\text{Na}_2\text{HPO}_4$  on the viscosity of skimmilk. The addition of the phosphate ion is identical to the previous study; however, the temperature of pasteurization was increased from 61 to 71°C. In this study, as in the previous study, no precipitate formed in the bottom of the Erlenmeyer flasks.

Citrate Added as  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  Prior to Pasteurization at 61°C. Sodium citrate, like disodium hydrogen phosphate, has found widespread use in the condensing industry since the studies of Sommer and Hart (48). Its use for stabilizing milks to heat coagulation has previously been mentioned.

Since sodium citrate has marked effects on heat stability of milk it was decided to study its effects on viscosity of skimmilk. Sodium citrate was added in amounts equivalent to 0.04, 0.08, 0.12, 0.16, and 0.20 g. citrate ion per 100 g. skimmilk. The sodium citrate was weighed on an analytical balance to  $\pm 3$  mg. and transferred to an Erlenmeyer flask containing skimmilk. The sodium citrate was highly soluble when added to the skimmilk. Each sample being studied was pasteurized at 61° C. for 30 minutes and cooled to 4° C. following pasteurization at which time viscosity determinations were made. No precipitation was

evident on the bottom of the Erlenmeyer flasks.

Removal of Calcium. Thus far, the study had revealed two important factors: (a) that the addition of the cation, calcium, decreased viscosity below the control, and (b) that the addition of the anions, phosphate and citrate, increased viscosity of skimmilk above the control. It was conjectured that the removal of a cation, such as calcium, would have the same affect as the addition of an enion. It was deduced that the removal of an ion would increase the percentage of all others in relation to the total weight of the skimmilk.

Calcium was removed from the skimmilk by an ion exchange resin, Amberlite IR - 120, generate in the sodium cycle and washed free of excess sodium with the necessary amount of distilled water, depending upon the amount of resin used.

Previous workers (22) have used the column technique for removal of calcium, and it is understood that this procedure is used in commercial practice. But since only 2000 grams of milk, at the most, were used in this study a batch technique seemed more economically feasible. The general procedure as designed by the author is as follows:

1. 2000 grams (approximate) of milk were warmed to 8° C.
2. 100 grams of Amberlite IR - 120 ion exchange resin were added to the skimmilk. The exchange capacity of the resin when 100 grams are added is more than sufficient to remove the available calcium.
3. The milk was allowed exactly five minutes contact time with the resin while being constantly agitated.

4. The milk was then decanted to a filter and the resin particles removed.

It was assumed from the literature that at least 90 percent of the available calcium in milk could be removed by a suitable ion exchange resin. The treated skimmilk and the control skimmilk were mixed in proportions to give various amounts of calcium in each sample studied. They were blended as follows:

- a. Sample 1, control (untreated)
- b. Sample 2, treated skimmilk.
- c. Sample 3, 3 parts of control skimmilk plus 1 part of treated skimmilk.
- d. Sample 4, 1 part of control skimmilk plus 1 part of treated skimmilk.
- e. Sample 5, 1 part of control skimmilk plus 3 parts of treated skimmilk.

The mixtures of the control and the treated samples were made by actually weighing out the desired amounts into Erlenmeyer flasks. After the samples were weighed out they were pasteurized at 61°C. for 30 minutes, cooled to 4°C. and flow times were determined.

Lecithin Addition. Lecithin possesses some extremely important surface active properties (31). Among these properties are their reactions with water to form hydrates which are insoluble in fats and oils, and their ability to complex sugars and proteins resulting in properties that differ from the original ingredients. For these reasons it was conjectured that the addition

of lecithin to skimmilk might produce some marked effects on its viscosity.

As previously stated (28), lecithin constitutes about 0.76 to 1.05 percent of the total fat in milk. Since the literature is inconclusive regarding the exact amount of lecithin in skimmilk, the addition of lecithin was made on the basis of the total phospholipid content of skimmilk, which according to the literature is established to be less than 0.10 percent of the skimmilk. However, this figure was chosen arbitrarily because of lack of agreement in the literature. A commercial lecithin extract was obtained for experimental purposes<sup>1</sup>. The composition of this extract was as follows:

Commercial lecithin	-	60.0 percent
Chemical cephalin	-	30.0 percent
Inositol phosphatides	-	2.2 percent
Soybean oil	-	4.0 percent
Miscellaneous	-	3.8 percent

Pilot studies on the addition of lecithin showed that added amounts of 0.02 to 0.10 g. of lecithin to skimmilk in increments of 0.02 produced no change in viscosity of the skimmilk within 24 hours of storage.

The next step involved the addition of the extract in amounts equivalent to the addition of 0.02, 0.04, 0.06, 0.08, and 1.00 g. of lecithin per 100 g. skimmilk. These studies are reported in

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<sup>1</sup> Supplied by the Glidden Company.

the following sections.

After the correct amount of lecithin had been added to the skimmilk samples they were pasteurized at  $61^{\circ}$  C. for 30 minutes, cooled to  $4^{\circ}$ C., and the flow times determined. Some difficulty was encountered in dissolving the lecithin extract in skimmilk. Consequently, accurate measurement of flow time was sometimes hampered by the presence of clumps in the capillary of the viscometer.

#### Analysis of Skimmilk Samples

Chemical analyses were made in this study for two reasons: (a) to determine the salt composition of the skimmilk, and (b) to determine the total amount of ion present in the skimmilk after the addition of an ion.

All analyses, except pH, density, fat, total solid, and organoleptic determinations were made by the Chemical Service Department, Kansas State College, according to the official A.O.A.C. procedures (3). Fat and total solids were also determined by A.O.A.C. methods by the author.

Organoleptic Examination of Skimmilk. Although flavor determinations were of secondary importance in this study, the flavor of all samples was judged, but not scored. Two members of the Kansas State College staff (Department of Dairy Husbandry) and the author examined the skimmilk samples within 24 hours following pasteurization for flavor.

pH. As stated in the literature review, the addition of salts alter the normal pH of milk and affect protein hydration and viscosity. Therefore, pH was determined. Within two hours following pasteurization, pH values were recorded at 20°C., and estimated within 0.1 pH unit. A Leeds and Northrup battery type potentiometer was used in this study, and pH values were reported on all samples within a trial.

Density. The only purpose in determining density of the skimmilk samples in this study was to provide a factor in the following equation used to calculate viscosity:

$$\eta \text{ at } 4^{\circ}\text{C.} = \frac{\eta_m}{\eta_w} = \frac{F_m}{F_w} \cdot \frac{d_m}{d_w}$$

Density was determined at 4°C.  $\pm$  0.001°C. according to the procedure described by Rutz, et al. (39) and Medved (35). Density was determined within 48 hours after pasteurization, and the values recorded to the fifth decimal place.

Fat. Since skimmilk in this study was considered to contain less than 0.20 percent fat, analysis was made on each control sample according to procedure 15.25, Roese - Gottlieb, A. O.A.C. official methods.

Total Solids. To be positive of uniformity among trials as the percent total solids in the skimmilk, this determination was made on each control sample. Total solids were determined according to method I (15.14) A.O.A.C.

Protein. Protein hydration and viscosity are probably affected to a large extent, by the amount of protein present in

the milk. Therefore, protein determinations by the Kjehdahl method (2.23), A.O.A.C., were made on each control sample for each trial. Protein is reported as percent crude.

Calcium, Phosphorous, Citric Acid and Sodium. Since the addition of ions was the main aspect of this study, calcium, phosphorus, citric acid and sodium were determined in each control sample to which the particular ion(s) were added in the study. Sodium was the only ion that was not introduced by choice as it appeared in the milk because of the use of an ion exchange resin to remove calcium in one of the studies. All determinations were determined by the official A.O.A.C. procedures. Calcium was determined by method 6.11; phosphorus by method 2.12 - 2.14; citric acid by method 15.142, and sodium by a Beckman Du flame photometer. These determinations are reported as  $\text{CaO}$ ,  $\text{P}_2\text{O}_5$ , citric acid, and  $\text{Na}_2\text{O}$ , respectively for the above analyses. They are reported as such to keep in uniformity with previously reported literature.

#### EXPERIMENTAL RESULTS

The experimental results expressed in this study are given as both statistical results of the experimental data and the chemical analyses of the milk samples studied.

The experimental data was processed by the Kansas State College Statistical Laboratory. Analyses of variance were run on each study according to Snedecor (42). The purposes of the statistical treatment of the data were to determine the number

of trials necessary to obtain valid results, and to determine the relationship between viscosity and storage period, and viscosity and levels of ion addition.

Statistical treatment of the experimental data revealed that three trials within a study were ample to determine the response curve(s) for the variable being studied. Tables 16 - 31 show the results of the statistical analyses and viscosity values obtained for each study.

Tables 8 - 15 indicate the physical and chemical analyses of the milks studied. These analyses were an integral part of this thesis and the uniformity of the composition of the skim-milk among trials was essential to explain any variations in viscosity values.

#### Discussion of the Interpretation of Analysis of Variance

##### Calcium Added as $\text{CaCl}_2$ Prior to Pasteurization.

1. Treatment Effects. If it were hypothesized that there was no effect on viscosity of skimmilk caused by the addition of calcium ions, a result has been obtained in this study which would occur by chance less than 1 in 1000 times in repeated experiments under the same conditions. Hence, the above hypothesis is rejected and it is declared that the addition of calcium ion and variation in the amount added, produced a variation in viscosity of skimmilk significant at the  $P < .001$  level.

Further analysis reveals that the response curve for various



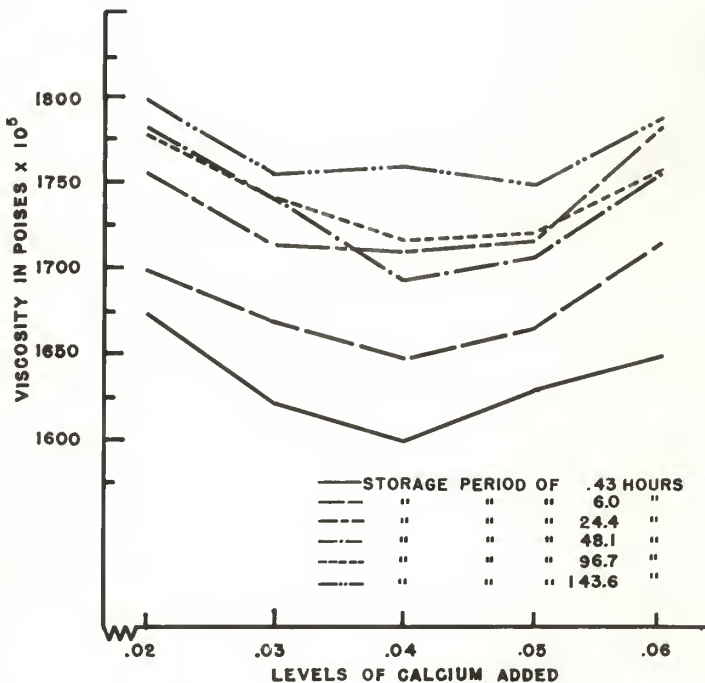


Fig. 1. Effect of addition of various levels of calcium (g./100 g. skimmilk) prior to pasteurization, on viscosity of skimmilk at selected storage periods.

levels of added calcium ions fits a quadratic curve (Fig. 1). This observation followed from a very highly significant value of  $F$  when testing the hypothesis that there was no quadratic effect (Table 10, Appendix). The linear, cubic, and quadratic responses were all non-significant. This means that the relation between viscosity of skimmilk and calcium ion concentration was not linear because a quadratic curve fitted the data.

In testing for a difference in the viscosity of the true means for the control and control with water added, there was insufficient evidence to reject the hypothesis that there was no difference in viscosity. Hence, one cannot say that the control with water added had a viscosity different from the untreated control.

A very highly significant value of  $F$  (Table 17, Appendix) was obtained when comparing the response from the control and control with water added with the treatments at various levels of calcium added. This means that there is less than 1 in 1000 chance for error in declaring that the effect of various levels of calcium are not different from that of the control or the control plus water added. In all cases the viscosity values for the samples with calcium added were below the control.

2. Storage periods. The hypothesis that storage period has no effect on viscosity of skimmilk may be rejected at the 0.1 percent level, for viscosity increased with increase in storage period (Fig. 2). Furthermore, statistical analysis showed that

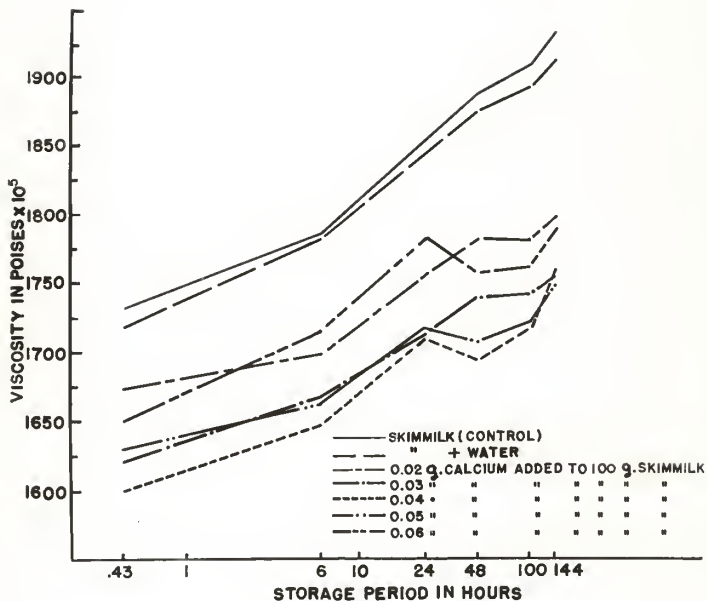


Fig. 2. Effect of storage period on viscosity of skim milk, to which calcium was added as  $\text{CaCl}_2$  prior to pasteurization.

the linear response at various storage periods was very highly significant. The deviations from linear response were also very highly significant indicating that a higher degree polynomial or exponential curve might fit the data better than a straight line (Fig. 2, Table 17, Appendix).

Calcium Added as  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$  Prior to Pasteurization.

1. Treatment effects. If it were hypothesized that there was no effect on viscosity of skimmilk due to the addition of various levels of  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ , then a result had been obtained in this study which would occur by chance less than 1 in 1000 times in repeated trials under the same experimental conditions. Hence, the above hypothesis was rejected and it was declared that the addition of calcium ion and variation in the amounts added produced a decrease in viscosity of skimmilk. This observation is significant at the  $P < .001$  level. Viscosity decreased below the control and control with water added at all levels of calcium addition (Fig. 3).

Further statistical analysis revealed that the response curves for the linear and quadratic responses are significant. The linear was significant at the  $P < .001$  level while the quadratic was significant at the  $P < .05$  level. Figure 3 and Appendix Table 19 indicate these relationships.

In testing for a difference between the viscosity of the true means for the control and all samples with calcium added a very highly significant value of F was obtained. This indicated

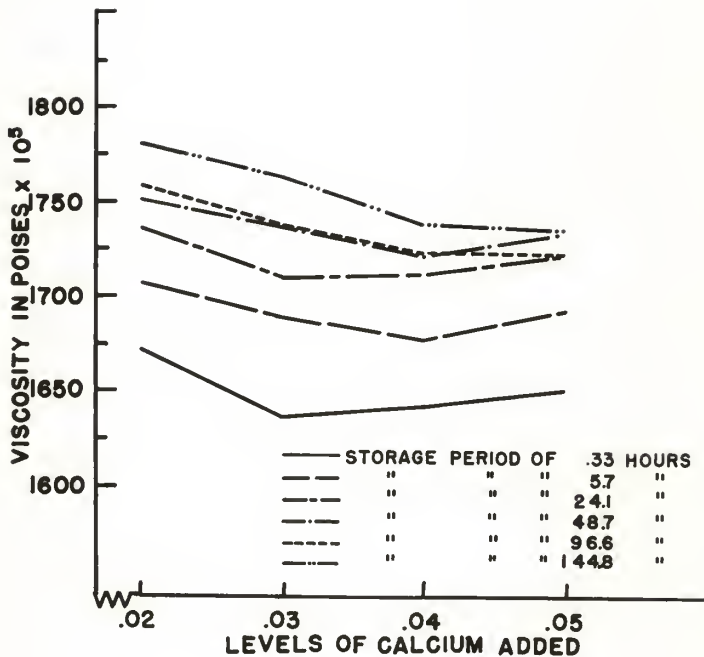


Fig. 3. Effect of addition of various levels of calcium (g./100 g. skimmilk) as  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ , prior to pasteurization, on viscosity of skimmilk at selected storage periods.

that there was less than 1 in 1000 chance for error in declaring that the effect of various added levels of calcium ion on viscosity was different from the control. In other words, the addition of calcium ion changed the viscosity of skim milk from that of the control.

2. Storage Period. The hypothesis that storage period had no effect on viscosity of skim milk may be rejected at the 0.1 percent level. Furthermore, the statistical analysis showed that the linear response at the various storage periods was very highly significant (Table 19, Appendix). The deviations from linear response are also very highly significant indicating that a higher degree polynomial or exponential curve might better fit the data. There is little question, therefore, that viscosity of skim milk increased with storage period (Fig. 4).

#### Calcium Added as CaCl<sub>2</sub> Following Pasteurization.

1. Treatment Effects. It is apparent that the addition of calcium ions following pasteurization also affects viscosity (Fig. 5). This can be substantiated at the  $P < .001$  level (Table 21, Appendix). Further statistical analysis showed that the relationship between viscosity of skim milk and various added amounts of calcium ions may be described as linear. This conclusion follows from a very highly significant value of  $F$ .

In testing for a difference in viscosity of the true means for the control and the control with water added, there was insufficient evidence to reject the hypothesis that there is no difference. Hence, there is no difference in viscosity of skim-

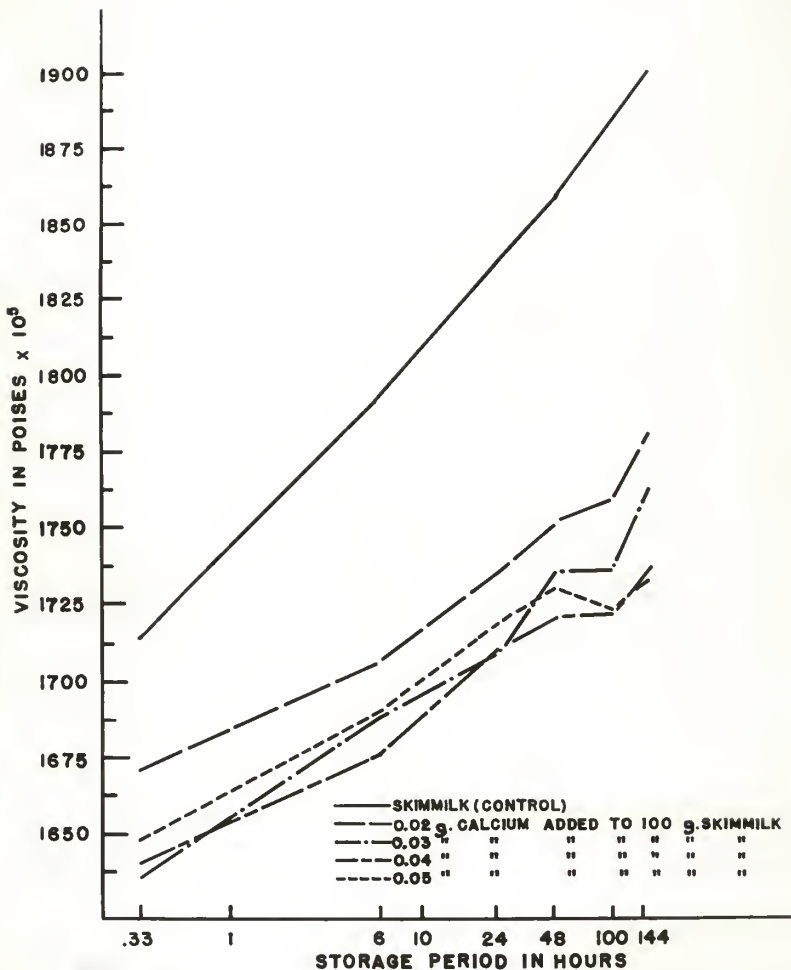


Fig. 4. Effect of storage period on viscosity of skimmilk to which calcium was added as  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ , prior to pasteurization.

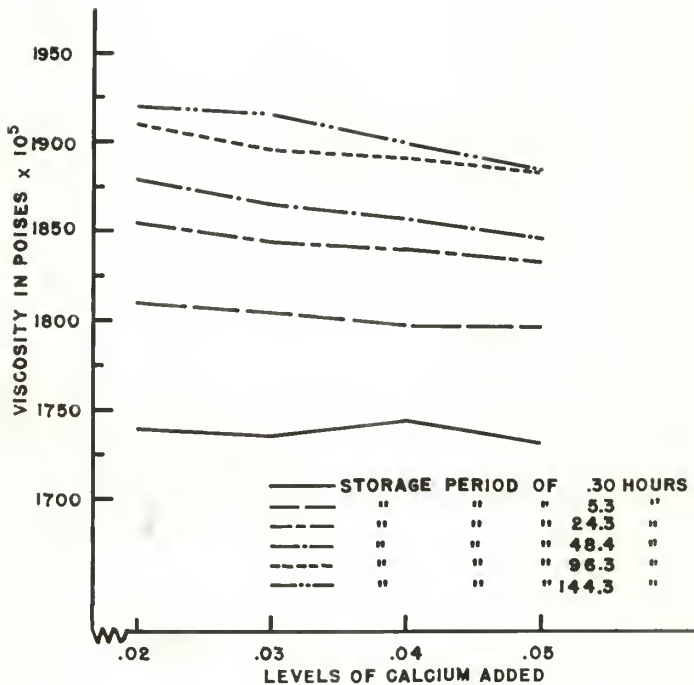


Fig. 5. Effect of addition of various levels of calcium (g./100 g. skimmilk) as  $\text{CaCl}_2$ , following pasteurization.



milk between the control and the control with water added.

A very highly significant value of F was obtained when comparing the responses from the control and control with water added with the treatment at various levels of calcium ion addition. This may be interpreted to mean that the addition of various levels of calcium ion following pasteurization causes a change in viscosity as compared to the control samples. This conclusion may be accepted at the  $P < .001$  level. The viscosity of skimmilk samples with various levels of calcium ions added were always below the control samples.

2. Storage Periods. The hypothesis that storage period has no effect on viscosity of skimmilk may be rejected at the 0.1 percent level. The statistical analysis also showed that the linear response at various storage periods was very highly significant (Table 21, Appendix). The deviations from linear response were also very highly significant indicating that a higher degree polynomial might fit the data better than a straight line (Fig. 6). However, it is apparent that regardless of the response, viscosity of the skimmilk samples increased with increasing storage period.

Phosphate Added as  $\text{Na}_2\text{HPO}_4$  Prior to Pasteurization.

1. Treatment Effect. If it were hypothesized that viscosity means of various levels of phosphate added were all equal when determined across storage periods, then a result had been obtained, in this study, which would occur by chance less than 1 in 1000 times. The data (Table 23, Appendix) show a very highly significant

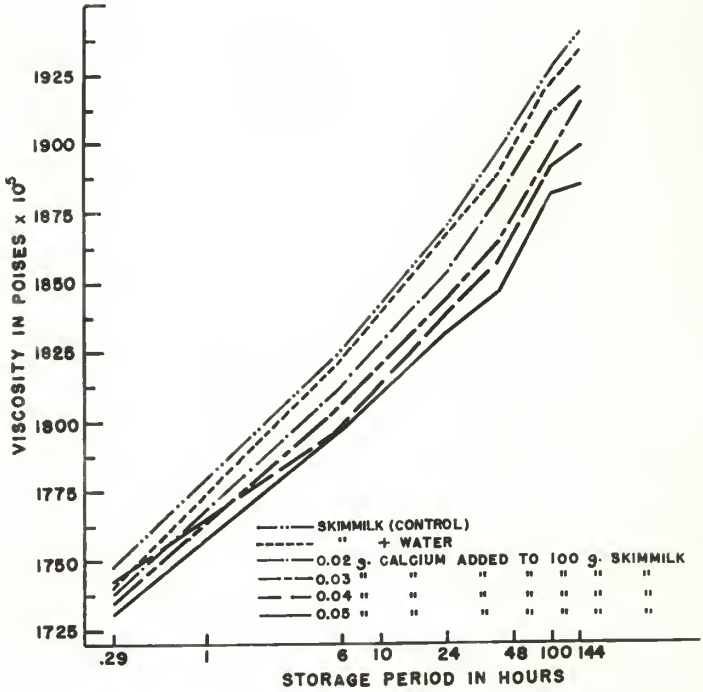


Fig. 6. Effect of storage period on viscosity of skim milk to which calcium was added as  $\text{CaCl}_2$  following pasteurization.

value of F. Therefore, the above hypothesis may be rejected at the  $P < .001$  level. Viscosity of the treated skimmilk samples always increased above the control sample.

The data also indicated the relationship between treatment levels and viscosity of skimmilk was linear. This may be accepted at the  $P < .001$  level. Therefore, it may be concluded that an added increment in the amount of phosphate ion added produced an added increment in viscosity of the skimmilk samples. The non-significance of the F values when testing for quadratic, cubic, and quintic responses indicated that the relation between treatment levels and viscosity of skimmilk was best described as linear (Fig. 7).

2. Storage Periods. If it were hypothesized that viscosity storage means were all equal when adding across treatment levels, then such a hypothesis may be rejected because of significance at the 0.1 level. Table 23, Appendix, indicates a very highly significant value of F for the linear response and also shows that an added increment in storage period produced an added increment in viscosity of skimmilk. Figure 8 indicates that viscosity of the skimmilk samples increased with increasing storage periods. Since the deviations from linear response are also very highly significant it is possible that a higher degree polynomial or exponential curve might better describe the relation between storage periods and viscosity of skimmilk rather than a linear curve.

Phosphate Added as  $\text{Na}_2\text{HPO}_4$  Prior to Pasteurization at  $71^\circ\text{C}$ .

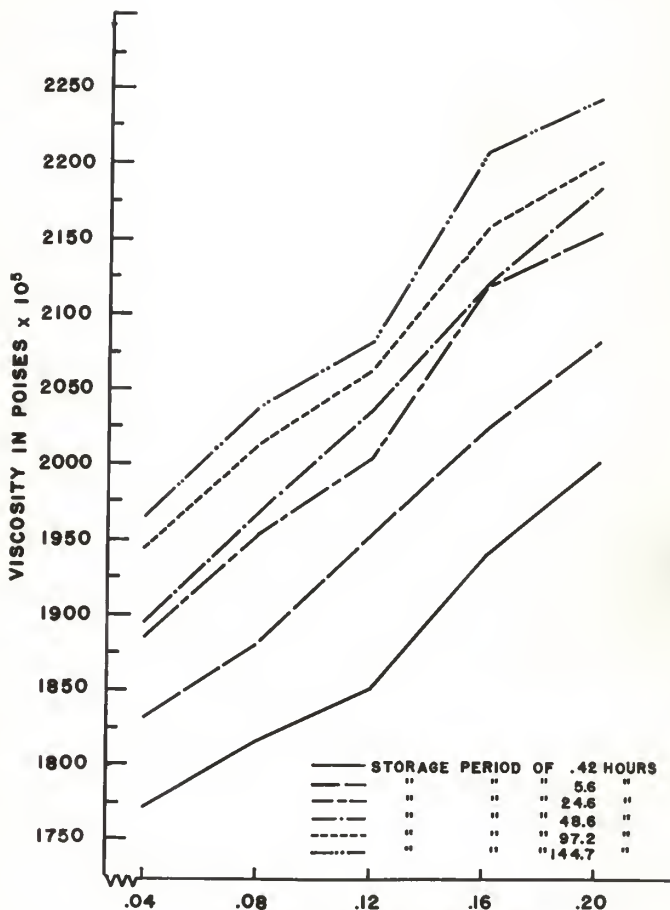


Fig. 7. Effect of addition of various levels of phosphate (g./100 g. skim milk) as  $\text{Na}_2\text{HPO}_4$ , prior to pasteurization, on viscosity of skim milk at selected storage periods.

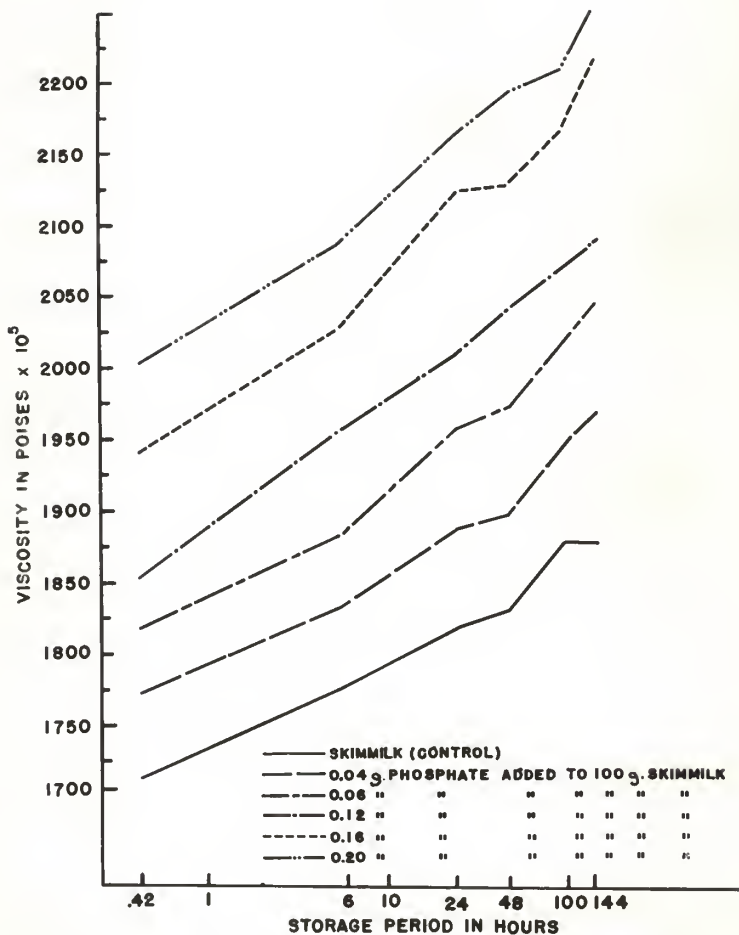


Fig. 8. Effect of storage period on viscosity of skim milk to which phosphate was added as  $\text{Na}_2\text{HPO}_4$  prior to pasteurization.

1. Treatment Effect. The data in Fig. 9 show that the addition of phosphate ion had a marked effect on the viscosity of skimmilk. The effect was very highly significant ( $P < .001$ ). The analysis of data in Table 25 of the appendix shows that the relationship between treatment levels and viscosity of skimmilk is linear, and that viscosity is always above the control (Fig.9). Therefore, it may be concluded that an added increment in the amount of phosphate ion added produced an added increment in viscosity. The values of F for the quadratic, cubic, quartic and quintic responses were all non significant, thus, justifying the conclusion that the relationship between viscosity of skimmilk and treatment levels is best described as linear (Table 25, Appendix).

2. Storage Period. If it were hypothesized that storage means were all equal when adding across treatment levels then such a hypothesis could be rejected at the 0.1 percent level. The very highly significant value of F for the linear response indicated that an added increment in storage period produced an added increment in viscosity (Fig. 10). The deviations from linear response were also significant, which indicated that a higher degree polynomial or exponential curve might better fit the data than a straight line.

Citrate Added as  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2 \text{H}_2\text{O}$  Prior to Pasteurization.

1. Treatment Effect. If it were hypothesized that treatment means were all equal when adding across storage levels, then a result had been obtained in this study which would occur

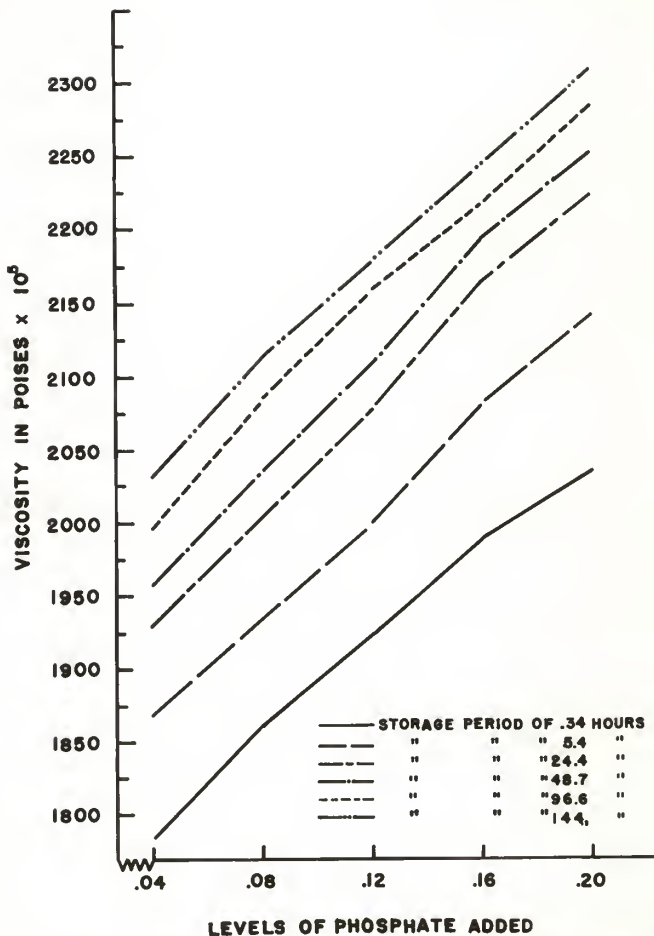


Fig. 9. Effect of addition of various levels of phosphate (g./100 g. skim milk) as  $\text{Na}_2\text{HPO}_4$ , prior to pasteurization at  $71^\circ\text{C}$ ., on viscosity of skim milk at selected storage periods.

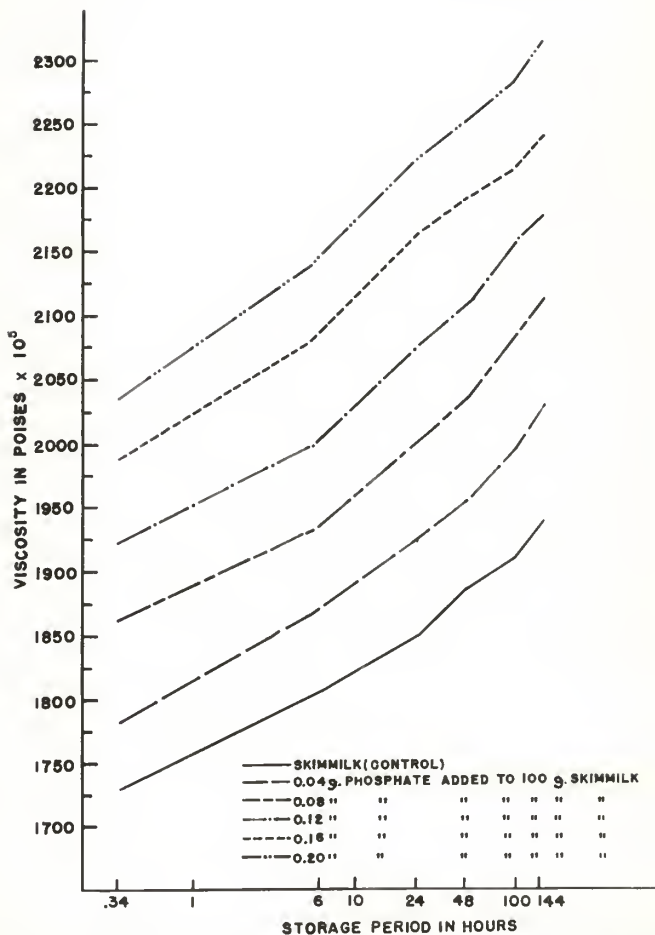


Fig. 10. Effect of storage period on viscosity of skim milk to which phosphate was added as  $\text{Na}_2\text{HPO}_4$  prior to pasteurization at  $71^\circ\text{C}$ .



by chance less than 1 in 1000 times. The very highly significant value of F (Table 27, Appendix) justifies rejection of the above hypothesis at the  $P < .001$  level because viscosity had increased with each added increment of phosphate ion. The data clearly show that one of two curves fit the data. The very highly significant value of F. indicated a linear response between levels of ion addition and viscosity (Fig. 11). However, the very highly significant value of F for a quadratic response also must be considered. Figure 11 shows that the quadratic response becomes apparent at the higher levels of citrate ion addition. The cubic response was significant at the  $P < 0.05$  level, while the quartic and quintic responses were non-significant.

2. Storage Period. The hypothesis that periods of storage have no effect on viscosity of skimmilk may be rejected at the 0.1 percent level. Further statistical analysis reveal that the linear response at various storage periods is very highly significant (Fig. 12). The deviations from linear are also very highly significant indicating a higher degree polynomial or exponential curve might fit the data better than a straight line (Fig. 12).

#### Calcium Removal.

1. Treatment Effect. If it were hypothesized that there was no effect on viscosity of skimmilk due to calcium removal, then a result had been obtained in this experiment which would occur by chance less than 1 in 1000 times in repeated trials under the same conditions. Hence, the hypothesis must be rejected and

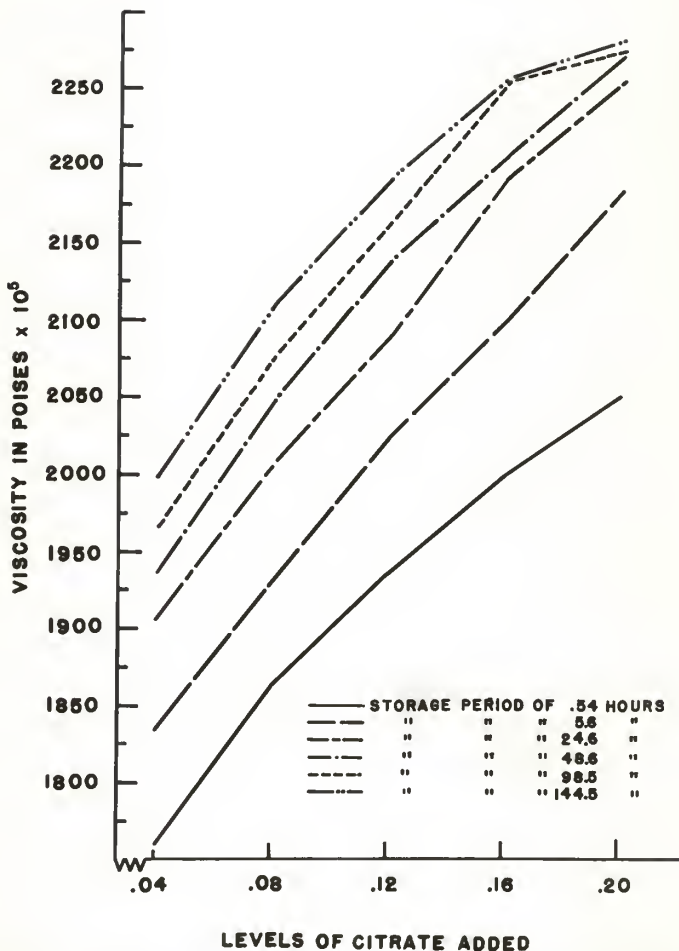


Fig. 11. Effect of addition of various levels of citrate (g./100 g. skimmilk) as  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ , prior to pasteurization, on viscosity of skimmilk at selected storage periods.

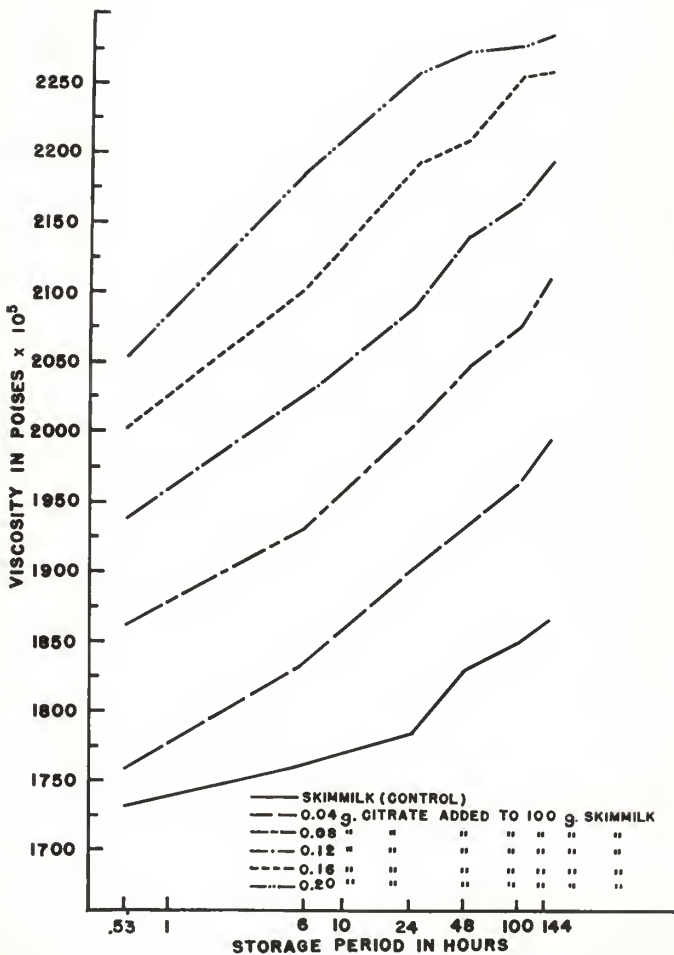


Fig. 12. Effect of storage period on viscosity of skim milk to which citrate was added as  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  prior to pasteurization.

the variation in the amount of calcium removed concluded to have produced a variation in viscosity of skimmilk. The viscosity of the treated milks increased above the control indicated by the very highly significant value of F (Table 29, Appendix).

Further statistical analysis revealed that the response curve for various levels of calcium removed fits a quadratic curve indicated by a very highly significant value of F. However, the linear and cubic responses were also very highly significant. The largest value of F, however, was that for the quadratic curve. Figure 13 indicates that as storage time increased deviations from linearity appeared.

In comparing the response for the treated samples with all others, a very highly significant value of F is obtained. This indicated that there was less than a chance of 1 in 1000 for error in declaring that various levels of calcium removal have an effect on the viscosity of skimmilk. The viscosity of skimmilk increased with increasing levels of calcium removal (Fig. 14).

2. Storage Period. The data reveals (Table 29, Appendix) that storage period had an effect on the viscosity of skimmilk. This conclusion may be accepted at the 0.1 percent level. Further analysis revealed that the linear response was very highly significant (Fig. 14), and that viscosity always increased with increased storage period. However, the deviations from linear were also very highly significant, indicating that a higher degree polynomial or exponential curve might fit the data better than a straight line.

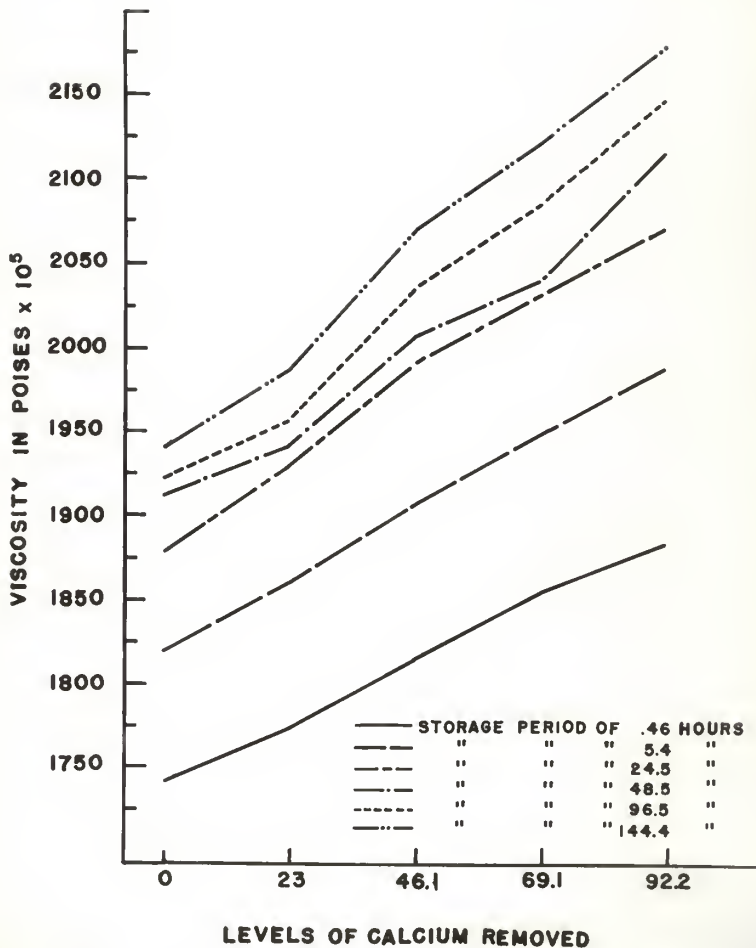


Fig. 13. Effect of removal of various levels of calcium (percent removed) prior to pasteurization on viscosity of skim milk at selected storage periods.

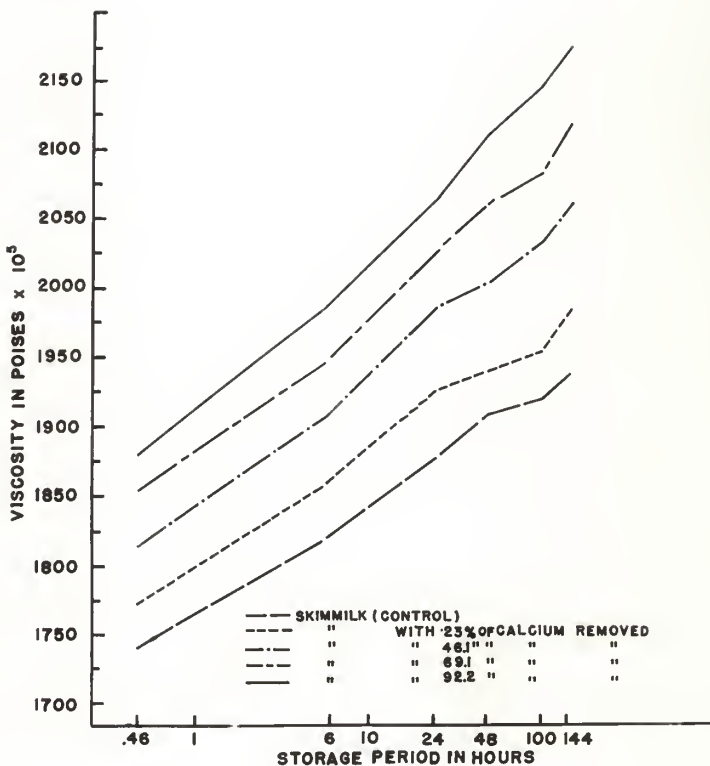


Fig. 14. Effect of storage period on viscosity of skim milk from which calcium was removed prior to pasteurization.

### Lecithin Addition.

1. Treatment Effect. If it were hypothesized that there was no effect on viscosity of skimmilk due to the addition of various levels of lecithin then the result obtained in this experiment would occur by chance less than 1 in 1000 times, in repeated experiments under the same conditions. The data (Table 31, Appendix) indicate that this hypothesis may be rejected at the  $P < .001$  level.

Further statistical analysis reveals that the relation between viscosity and lecithin addition may best be described as linear (Fig. 15). This was indicated by the large value obtained for  $F$  at the  $P < .001$  level. The viscosity of the samples treated with various levels of lecithin was always above the control.

2. Storage Period. The data revealed that storage period did have an effect on viscosity of skimmilk and the very highly significant value of  $F$  at the 0.1 percent level justified this conclusion. The effect is that viscosity of skimmilk increased with storage period changes. The analysis further showed that the linear response at various storage periods was very highly significant. The deviations from linear responses were also very highly significant indicating that a higher degree polynomial or exponential curve might better fit the data (Fig. 16).

### Chemical and Physical Analyses of Samples

In a study such as this, it is imperative that the chemical

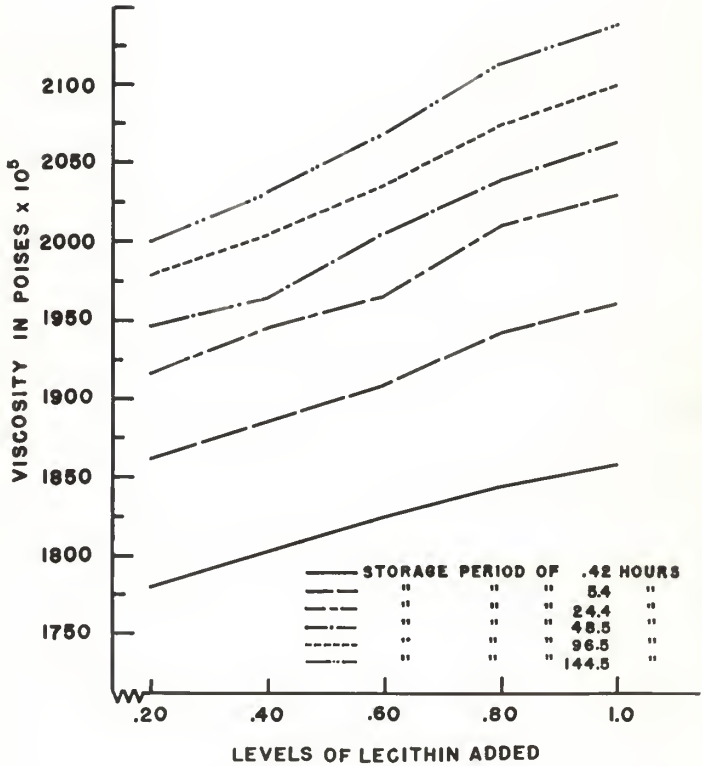


Fig. 15. Effect of addition of various levels of lecithin (g./100 g. skim milk) prior to pasteurization, on viscosity of skim milk at selected storage periods.



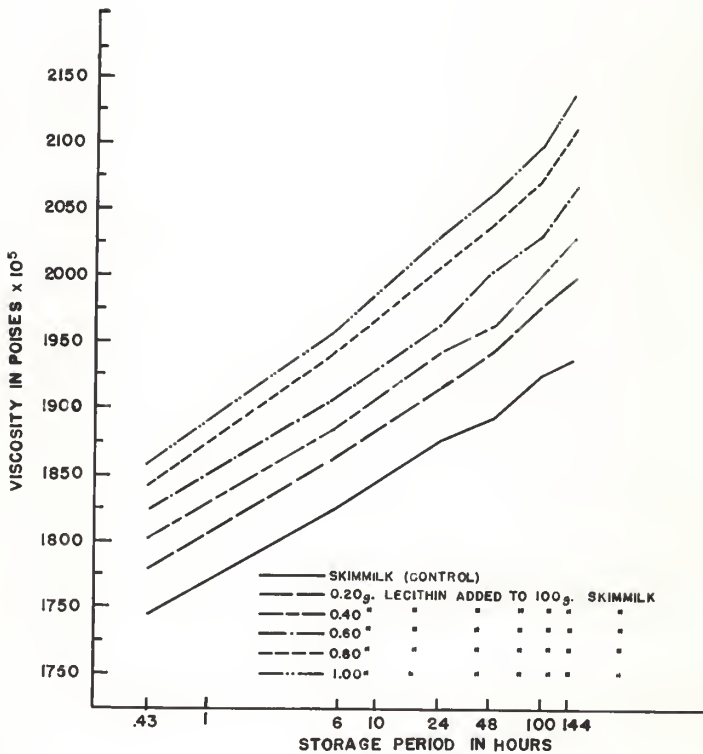


Fig. 16. Effect of storage period on viscosity of skimmilk to which lecithin was added prior to pasteurization.

and physical composition of the milks under study be known. Too often investigators have added various salts or ions to milk without knowing what the chemical composition of the milk being studied was. Such data appears of doubtful value from the standpoint of predicting what additions or reductions of a chemical compound might do to a definite chemical reaction. Therefore, the following discussion of the chemical and physical composition of the milks under study should be considered. They should show why consistent results have been obtained in all trials reported in this thesis.

Upon the addition of all ions a marked difference was noted in the flavor of the skimmilk samples. The addition of calcium at all levels produced an "off-flavor" best described as "chalky". This off-flavor is characteristic of high calcium milk and appeared as the concentration of calcium was increased.

Phosphates, citrates, and lecithin gave a "chemical" flavor to the milk samples. Phosphate, however, did not give an extremely objectionable off-flavor, and was considered the best flavored milk of these three additives. The removal of calcium imparted a slight salt flavor to the milks, which was introduced by the ion exchange resin. However, the salty flavor was not objectionable.

Examination of appendix Tables 8 - 15 indicate the chemical and physical values obtained for all analyses made. The density of the control samples was fairly constant. Of course, the

density is dependent, in part, upon the total solids in the milk. Variations in the total solids produced variations in density. The addition of ions, in all cases, increased the density of skimmilk while the addition of small amounts of water slightly decreased the density. Similar changes were noted in whole milk by Medved (35).

The pH value increased depending upon the amounts and nature of the salts added. The addition of calcium to the skimmilk samples decreased the pH, while the addition of phosphate, citrate, and lecithin increased the pH. An increase in pH was noted when calcium was removed. This is probably due to the increase in the percent of anions in the mixture as well as the introduction of small amounts of sodium into the milk by the ion exchange resin.

The percent fat in the skimmilk, in this study was an important factor as skimmilk was considered to contain less than 0.20 percent fat by weight. Appendix Tables (8 - 15) show that the minimum amount of fat in any one trial was 0.047 percent and the maximum, 0.20 percent with an average of 0.0985 percent. In all cases, the percent fat was 0.20 percent or less indicating that the skimmilk samples were as desired.

The figures in Tables 8 - 15 also indicated that the total solids of the skimmilk samples were constant within trials. However, seasonal fluctuations were noted, with the lowest percent total solids observed in the month of August. The minimum total solids was 8.35 percent and the maximum, 9.13 percent, with an

average of 8.74 percent. The percent protein was 3.13 percent at the minimum, and 3.66 percent at the maximum, with an average of 3.35 percent.

CaO has shown to be a very uniform constituent of milk with an average value of 0.137 percent. The minimum percent CaO was observed to be 0.12 percent, and the maximum, 0.16 percent. On the basis of addition 0.02, 0.03, 0.04, and 0.05 and/or 0.06 g. of calcium were added to 100 g. skimmilk. These amounts were equivalent to adding 0.028, 0.042, 0.056, 0.070, and 0.084 g. of CaO per 100 g. skimmilk. On the basis of the total CaO inherent to the milk (0.137 percent) the addition of CaO amounted to 20.4, 30.6, 40.7, 51.1, and 61.3 percent, respectively, for the total for the 0.02, 0.03, 0.04, and 0.05 levels of calcium addition. Not more than 61.3 percent CaO could be added to the milk before coagulation of the proteins occurred. The amount of CaO found in this study is in close agreement to that found by Sommer (43).

The  $P_{25}O_5$  found present in the control skimmilk samples averaged 0.21 percent with a minimum of 0.19 percent and a maximum of 0.22 percent. Since phosphate was added to the milk samples as the ion the exact amount of  $P_{25}O_5$  could not be determined until the phosphate had been converted to  $P_{25}O_5$ . 0.04, 0.08, 0.12, 0.16 and 0.20 g. of phosphate ion were determined to be equivalent to .0296, .0597, .0835, 0.110 and 0.149 g.  $P_{25}O_5$ , respectively. With an average of 0.21 percent  $P_{25}O_5$  present in the milk samples the 0.04, 0.08, 0.12, 0.16, and 0.20 levels of addition of phosphate ion are equivalent to adding 14.11, 28.42, 39.77, 52.38, and

70.90 percent  $P_2O_5$  to the milk. The value, 0.21 percent  $P_2O_5$  found in the milk samples is in close agreement with the values found by Acharya and Devadatta (1).

It is apparent from Table 14 that sodium introduced into the milk samples by the ion exchange treatment in the sodium cycle. The average percent  $Na_2O$  for three trials of untreated milks was found to be 0.07 percent. The treated samples contained 0.09 percent. The 0.07 percent  $Na_2O$  found in the milk samples is in exact agreement to that found by Sommer (43). The ion exchange treatment removed approximately 92 percent of the calcium found in the milk samples.

The average value for citric acid was found to be 0.123 percent with a minimum of 0.12 percent and a maximum of 0.13 percent. The amounts of citrate ion added (0.04, 0.08, 0.12, 0.16 and 0.20 g. per 100 g. skimmilk) are equivalent to the amounts added in the phosphate studies.

#### Discussion of Results

The discussion of results will be treated under the headings "cations" and "anions" to avoid repetition in the discussion of results and theories postulated.

Cations. Addition of Calcium. Examination of Figs. 1, 3, and 5 show that in all cases the addition of the calcium ion reduced the viscosity of skimmilk below the control(s). When  $CaCl_2$  and  $Ca(C_2H_3O_2)_2$  were added prior to pasteurization the minimum viscosity was at the 0.04 level of calcium addition.

Beyond the 0.04 level the viscosity began to increase but never attained a viscosity equivalent to the control(s). However, when calcium ion was added as  $\text{CaCl}_2$ , following pasteurization, the minimum viscosity was observed at the 0.05 level of addition. Since higher levels were not made, there is no reason to suspect that infinite amounts of calcium could not be added before a minimum is attained in viscosity values.

In all cases of calcium addition (Figs. 2, 4, and 6) viscosity of skimmilk increased with increasing storage periods. The relationship between viscosity and storage period among the studies where calcium was added as  $\text{CaCl}_2$  and  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ , prior to pasteurization, and  $\text{CaCl}_2$ , following pasteurization, have been best described as linear. However, the deviations from linear are also significant. At the 96 hour storage period it was noted that the viscosity decreased when calcium ion was added at the 0.04, 0.05, and 0.06 levels prior to pasteurization. At this period of storage (96 hours), a white precipitate, presumably  $\text{Ca}_3(\text{PO}_4)_2$ , appeared on the bottom of the Erlenmeyer flasks. If this precipitate is  $\text{Ca}_3(\text{PO}_4)_2$  this finding is in accordance with Verma (59) who noted that  $\text{Ca}_3(\text{PO}_4)_2$  precipitated after several hours of standing when calcium was added to skimmilk.

The fact that viscosity of skimmilk increased with storage period in a response best described as linear, is in accordance with the findings of Whitnah, et al. (65) who, in 1956, stated that the relationship between viscosity and storage period of whole, homogenized milk, is best described as linear. The fact

that viscosity of skimmilk increases with age has previously been established by Dahlberg and Henig (16), Evenson and Ferris (21), and Bateman and Sharp (6) who stored skimmilk at 3-4°C. for several days and determined viscosity. The authors, however, did not store milks at specific periods as did Whitnah, et al. (65) and as reported in this thesis. The results of this thesis show that the viscosity of skimmilk increased with age confirming the research of previous workers.

Viscosity increased with storage period due to the hydration of the milk proteins (36). This seems to be the only logical explanation for the increase in viscosity of skimmilk for no other factors inherent to milk apparently affect this change.

The response curves for the relationship between viscosity of skimmilk and levels of calcium added as  $\text{CaCl}_2$  and  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$  prior to pasteurization, and  $\text{CaCl}_2$  following pasteurization have been best described as quadratic, linear, and linear, respectively. Although these responses were of interest, the point of consideration was that the addition of calcium to skimmilk decreased viscosity below the control(s) at all levels of addition. This imposes the problem as to the function of calcium in relation to viscosity of skimmilk.

Burton (10) in his studies on light reflectance observed that reflectance of light is increased through a sample of skimmilk when calcium chloride was added to skimmilk. The implication is that particle size of casein is increased permitting greater passage of light through the sample. Burton, therefore,

theorized that the addition of calcium to skimmilk would decrease its viscosity. He made the observation that no studies whatsoever had been reported on the effect of calcium on the viscosity of skimmilk. Previous studies to that of Burton had shown that calcium increased the particle size of casein. Hostettler and Imhof (29) and Imhof (30) have shown by electron microscopy that calcium added to skimmilk increased particle size of casein. With the results of previous workers in mind, and the fact that the addition calcium decreased the viscosity of skimmilk, it is felt that the results of this study confirm the theory that calcium increases the particle size of casein. This may further be substantiated by the fact that the addition beyond 0.06 g. per 100 g. skimmilk produced casein coagulation. It is assumed that as the amount of calcium ion added increases the particle size of casein also increases.

Although calcium ion increases particle size both in theory and in practice, no published literature has been found to substantiate the fact that it changes viscosity of skimmilk. The point of interest, however, is not necessarily the fact that protein particle size is changed, but the fact that the change in particle size affects the degree to which proteins hydrate. Large particles have less surface area than small particles, and therefore, there is less chance that they will hydrate as much as smaller particles. The result of less hydration would logically be less viscosity. Pyenson and Dahle (38) stated that the so-called "destabilizing" salts, calcium, and magnesium, when



added to milk, decrease the tendency for proteins to hydrate.

When calcium was added to skimmilk the pH decreased. This finding confirms the reports of Verma (60). Pyenson and Dahle (38) stated that a decrease in pH towards the isoelectric point decreased bound water. This leads one to believe that viscosity of skimmilk would decrease.

Several conclusions can be drawn from this study on the effect of calcium ions on the viscosity of skimmilk. They are: (a) Calcium ions added to skimmilk decrease its viscosity. (b) Calcium has the effect of decreasing viscosity of skimmilk by increasing particle size of casein and decreasing the hydration of casein. (c) The casein particle is increased by a chemical reaction not explained in this study.

The composition of the milks in this study (Appendix Tables 8, 9, and 10) did not vary markedly. Therefore, it was assumed that the viscosity of skimmilks in this study were not affected by composition.

It has been previously stated that one of the purposes of this thesis was to determine if the addition of ions would increase the palatability of skimmilk. The results of this study showed that there was no increase in palatability caused by the addition of calcium. Contrary, the palatability was far decreased because of a decreased viscosity, and a marked "chalky" flavor imparted to the skimmilk by the calcium chloride. The intensity of the off-flavor increased with increasing added amounts of

calcium.

This study has brought to light several important points of interest to the dairy industry. However, there is good reason to believe that other cations, such as Mg, K, and Na might alter the viscosity of skimmilk considerably. Much more research in regard to the effects of salts on the degree of hydration of proteins as well as the actual "bonding" formed when calcium is in the presence of casein would be of considerable academic and fundamental value.

Removal of Calcium. Examination of Fig. 14 indicates that when calcium was removed from skimmilk viscosity increased significantly above the control sample. When calcium was added, a decrease rather than an increase in viscosity occurred. The relationship between levels of calcium removed and viscosity probably fits a quadratic curve; however, the linear and cubic responses are also significant. Viscosity increased with increasing storage period in a linear response similar to the calcium addition studies.

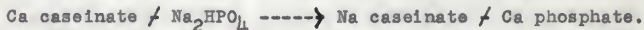
The chemical analysis (Table 14, Appendix) indicated that 92.2 percent of the available calcium in the skimmilk studies had been removed by the ion exchange resin. In accordance with the theory that the anions would increase in relation to the total mixture when calcium was removed, the chemical analyses showed that such was the case. The effect of removal of calcium appears to be the simultaneous increase of the anions; therefore, the increased concentration of the anions were probably responsible for

viscosity increase along with the removal of calcium. The effect of phosphate and citrate ions on viscosity of skimmilk will be discussed in the following section.

Anions. Examination of Figs. 7, 9, 11, and 15 indicate that the addition of phosphate ion, citrate ion, and lecithin increased the viscosity of skimmilk above the control at all levels of addition. The maximum viscosities were attained at the highest level of addition and at no time was a sharp decrease in viscosity noted. The analyses of variance showed that viscosity of the skimmilk samples increased with storage period in a response best described as linear. This increase in viscosity with storage period is for the same reasons presented in the discussion of anions.

The discussion of the effect of cations on the viscosity of skimmilk showed that calcium decreased viscosity. When anions were added, however, the opposite was observed. Why then do anions increase viscosity of skimmilk?

Sommer (43) stated that the effect of anions on the caseinate molecule is not merely a salt affect, but the combination of a salt affect and a double decomposition reaction as:



Sodium caseinate is much more soluble than calcium caseinate. It would, therefore, pass into solution more easily; consequently particle size of casein is decreased and hydration is increased. Burton (10) theorized that the addition of anions

(phosphates and citrates) would decrease particle size of casein and therefore, increase viscosity. Edmondson and Tarassuk (18) proved this when they added  $\text{Na}_2\text{HPO}_4$  to skim milk. The viscosity of the milk increased. They attributed the increase in viscosity to a shift toward an average smaller particle size of casein or to an increase in hydration. Why don't both factors appear simultaneously? It appears likely that both are possible at the same time. Imhof (30) noted that the addition of anions decreased particle size of casein when added to milk. These results were established by the use of an electron microscope. Sommer (43) pointed out that the addition of phosphates and citrates increased the hydrating capacity of casein. Fyenson and Dahle (38) indicated the same response when they stated that the so-called milk "stabilizing salts" (phosphates and citrates) increased the bound water found in the proteins.

Tables 11, 12, 13 and 15 of the appendix indicate that the addition of anions to skim milk increased the pH above the control. These findings are in accordance with Verma (60) who reported that anions increase pH above the control sample. Burton (10) stated that particle size of casein was independent of pH at 6.6, but when the pH was increased toward neutrality the particle size decreased. Fyenson and Dahle (38) showed conclusively that increasing pH toward neutrality decreased bound water in the milk proteins, but not to the extent of decreasing toward the isoelectric pH.

Two different temperatures (61 and 71°C.) were employed in the study of the effects of the phosphate ion upon the viscosity of skimmilk. Previous literature had reported that high temperature of pasteurization increased viscosity of skimmilk. In this study there were actually two factors which increased viscosity, phosphate ions and temperature.

In comparing Figs. 4 and 5 it can be observed that the viscosity values of the milks pasteurized at 71° C. (Fig. 5) are much higher than those at 61°C. (Fig.4). This discovery is in accordance with the work of Whitaker, et al. (63). They stated that temperature above 70° C. caused the relative viscosity of skimmilk to increase above the samples heated at lower temperatures. This may be due to a decrease in protein particle size and increased in hydration. Eilers (19) stated, however, that the rise in viscosity may be associated with denaturation of soluble proteins, while Pyenson and Dahle (38) associated the effect of temperature with increased bound water and increased viscosity. Higher temperatures than 90°C., however, decreased the percent of bound water and, consequently, decreased viscosity. Tables 11, 12, 13 and 15 of the appendix show that the composition of milk in this study had been relatively constant, and that any variations in viscosity values is not due to composition changes.

In view of previous literature and the findings in this study, statements of importance regarding particle size of casein, hydration and viscosity of skimmilk are as follows: (a) The viscosity of skimmilk is increased by the addition of phosphate,

citrate, and lecithin ions, as well as by the removal of calcium. (b) The particle size of casein is decreased by the addition of anions. (c) Anions added to skimmilk increase its pH. (d) The combination of decreased particle size of the casein molecule and an increase in hydration increase viscosity. (e) The particle size of casein is affected by anions in accordance with the double decomposition reaction as proposed by Sommer (43).

The addition of phosphates, citrates, and lecithin to skimmilk have not proven beneficial in increasing palatability of skimmilk by increasing viscosity, although viscosity was markedly increased. The presence of chemical "off-flavors" hinder their use. However, of the three ions added, the phosphates gave the least off-flavor, and future research on its possible usage in skimmilk may prove fruitful.

#### SUMMARY AND CONCLUSIONS

The purposes of this study were to determine the effects of certain cations and anions on the viscosity of skimmilk, and to state the effect of these ions on the relative particle size of the casein molecule. Emphasis was also placed on the effects of the added ions on the viscosity of skimmilk in relations to the palatability of the product.

The study began February, 1956, and was continued until early September, 1956. During this period milks were obtained from the Kansas State College herd, separated, and specific ions were added to the skimmilks to determine their effects on viscosity,

Calcium, citrate, phosphate, and lecithin ions were added in amounts comparable to that inherent to the milk. In one study the effect of the removal of calcium ions was reported upon.

Viscosity was determined after the ions were added, and the skimmilks pasteurized to 61 or 71°C. by a modified Ostwald viscometer. Viscosity values were reported in poises  $\times 10^5$ . In brief, the addition of calcium ions decreased the viscosity of skimmilk below the controls, while the addition of phosphate, citrate, and lecithin ions increased the viscosity of the skimmilk samples above the control. The removal of calcium increased the viscosity of the skimmilk samples.

The addition of all except the phosphate ions produced marked chemical or chalky flavored skimmilk.

Several conclusions are drawn from the results of the studies reported in this thesis. They appear as follows:

#### Cations

1. The addition of calcium ions decreased the viscosity of skimmilk below the control(s).
2. It appears that the addition of calcium ions to skimmilk decrease the hydrating properties of the casein molecule by increasing its size. Therefore, the viscosity of skimmilk is decreased.
3. Viscosity of skimmilk increased with age (storage period).
4. Addition of calcium to skimmilk decreased its pH.
5. The removal of calcium increased the viscosity of skimmilk.

6. The addition of calcium to skim milk did not increase its palatability, but decreased it by imparting an off-flavor described as "chalky", and by decreasing the viscosity.

#### Anions

1. The addition of the anions, phosphate, citrate, and lecithin, increased the viscosity of skim milk above the control skim milk.

2. The addition of anions decreased particle size of casein, and increased hydration indicated by an increase in viscosity of skim milk above the control sample.

3. The viscosity of skim milk is increased during storage period.

4. The addition of anions increased the pH of skim milk.

5. From these data it is postulated that the actual cause of a smaller casein molecule, and increased hydration, is the result of a double decomposition reaction where sodium replaces the calcium in the calcium caseinate molecule. This results in an average smaller size of the casein molecule which possess greater hydrating properties.

It is hoped that this study may stimulate future study on the viscosity of skim milk. The results found in this thesis have merely introduced a small phase in the study of the viscosity of skim milk.

Although the author examined specific ions at specific concentrations on the viscosity of skim milk, other approaches to



viscosity may be examined with the same ions or similarly reacting ions. Likewise, there are virgin fields which demand investigations such as the addition of magnesium to milk or the effect of removal of certain ions on the viscosity of skimmilk. In a closely allied field it would be of utmost interest to determine the actual existance of calcium in the casein molecule. Such a study would be of fundamental importance in determining the effects of certain ions on the viscosity of skimmilk.

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## APPENDIX

Table 8. Chemical and physical analyses of samples to which calcium was added as  $\text{CaCl}_2$  prior to pasteurization.

Analyses made	Trials and Dates			Means
	1. 2/14/56:	2. 2/28/56:	3. 3/13/56:	
Fat (%)	0.08	0.08	0.085	.081
T.S. (%)	8.90	9.08	9.14	9.04
Protein (%)	3.37	3.46	3.66	3.49
CaO (%)	0.13	0.13	0.14	0.133
Density				
control	1.03629	1.03664	1.03731	1.03674
control $\text{H}_2\text{O}$	1.03614	1.03646	1.03716	1.03658
.02 level $\text{H}_2\text{O}$	1.03642	1.03670	1.03742	1.03684
.03 "	1.03666	1.03692	1.03771	1.03709
.04 "	1.03693	1.03707	1.03778	1.03726
.05 "	1.03713	1.03743	1.03806	1.03754
.06 "	1.03719	1.03759	1.03824	1.03767
pH				
control	6.70	6.70	6.70	6.70
control $\text{H}_2\text{O}$	6.60	6.70	6.60	6.63
.02 level $\text{H}_2\text{O}$	6.50	6.50	6.50	6.50
.03 "	6.46	6.30	6.30	6.35
.04 "	6.33	6.30	6.30	6.31
.05 "	6.29	6.25	6.20	6.24
.06 "	6.20	6.10	6.20	6.16
Flavor				
control	clean	clean	clean	clean
control $\text{H}_2\text{O}$	clean	clean	clean	clean
.02 level $\text{H}_2\text{O}$	chalky	chalky	chalky	chalky
.03 "	"	"	"	"
.04 "	"	"	"	"
.05 "	"	"	"	"
.06 "	"	"	"	"

Table 9. Chemical and physical analyses of samples to which calcium was added as  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$  prior to pasteurization.

Analyses made	Trials and Dates			
	1. 3/22/56:	2. 6/28/56:	3. 7/17/45:	Means
Fat (%)	.0715	0.49	.047	.056
T.S. (%)	8.86	8.87	8.47	8.73
Protein (%)	3.34	3.31	3.25	3.30
CaO (%)	0.13	0.12	0.16	0.137
Density				
control	1.03542	1.03587	1.03554	1.03561
.02 level	1.03553	1.03622	1.03571	1.03582
.03 "	1.03588	1.03636	1.03609	1.03611
.04 "	1.03594	1.03653	1.03617	1.03621
.05 "	1.03596	1.03662	1.03650	1.03636
pH				
control	6.70	6.55	6.60	6.61
.02 level	6.60	6.40	6.50	6.50
.03 level	6.50	6.30	6.50	6.43
.04 level	6.40	6.20	6.45	6.35
.05 level	6.40	6.10	6.40	6.30
Flavor				
control	feedy	clean	clean	clean
.02 level	chalky	chalky	chalky	chalky
.03 "	"	"	"	"
.04 "	"	"	"	"
.05 "	"	"	"	"

Table 10. Chemical and physical analyses of samples to which calcium was added as  $\text{CaCl}_2$  after pasteurization.

Analyses made	Trials and Dates				Means
	1. 8/21/56:	2. 8/23/56:	3. 8/28/56:		
Fat (%)	0.182	0.07	0.085	0.112	
T.S. (%)	8.45	8.35	9.02	8.61	
Protein (%)	3.32	3.51	3.45	3.43	
CaO (%)	0.15	0.14	0.15	0.15	
Density					
control	1.03610	1.03612	1.03606	1.03609	
control $\text{H}_2\text{O}$	1.03599	1.03564	1.03604	1.03589	
.02 level	1.03612	1.03612	1.03607	1.03613	
.03 "	1.03612	1.03623	1.03607	1.03614	
.04 "	1.03617	1.03633	1.03608	1.03619	
.05 "	1.03621	1.03639	1.03616	1.03625	
pH					
control	6.63	6.62	6.62	6.62	
control $\text{H}_2\text{O}$	6.60	6.62	6.61	6.61	
.02 level	6.60	6.60	6.60	6.60	
.03 "	6.58	6.60	6.60	6.59	
.04 "	6.58	6.58	6.58	6.58	
.05 "	6.58	6.57	6.57	6.57	
Flavor					
control	clean	clean	sl. feed	clean	
control $\text{H}_2\text{O}$	clean	clean	sl. feed	clean	
.02 level	chalky	chalky	chalky	chalky	
.03 "	"	"	"	"	
.04 "	"	"	"	"	
.05 "	"	"	"	"	

Table 11. Chemical and physical analyses of samples to which phosphate was added as  $\text{Na}_2\text{HPO}_4$  prior to pasteurization at 61°C.

Analyses made	Trials and Dates			
	1. 6/12/56	2. 6/14/56	3. 6/19/56	Means
Fat (%)	.082	.020	0.15	0.144
T.S. (%)	8.85	8.78	8.80	8.81
Protein (%)	3.27	3.22	3.29	3.26
$\text{P}_2\text{O}_5$ (%)	0.22	0.19	0.20	0.20
Density				
control	1.03545	1.03622	1.03612	1.03593
.04 level	1.03589	1.03662	1.03659	1.03636
.08 "	1.03636	1.03710	1.03707	1.03684
.12 "	1.03695	1.03767	1.03749	1.03737
.16 "	1.03744	1.03812	1.03799	1.03785
.20 "	1.03788	1.03875	1.03806	1.03841
pH				
control	6.60	6.50	6.60	6.53
.04 level	6.60	6.62	6.60	6.61
.08 "	6.70	6.70	6.60	6.67
.12 "	6.70	6.75	6.70	6.72
.16 "	6.80	6.80	6.70	6.77
.20 "	6.80	6.90	6.80	6.83
Flavor				
control	clean	sl. feed	clean	clean
.04 level	sl. chemical	sl. chemical	sl.chemical	sl. chemical
.08 "	pronounced	pronounced	pronounced	pronounced
.12 "	"	"	"	"
.16 "	"	"	"	"
.20 "	"	"	"	"

Table 12. Chemical and physical analyses of samples to which phosphate was added as  $\text{Na}_2\text{HPO}_4$  prior to pasteurization at  $71^\circ\text{C}$ .

Analyses made	Trials and Dates			Means
	1. 8/7/56:	2. 7/12/56:	3. 8/14/56:	
Fat (%)	.078	0.185	.085	0.116
T.S. (%)	8.50	8.82	8.48	8.60
Protein (%)	3.25	3.32	3.25	3.27
$\text{P}_2\text{O}_5$ (%)	0.21	0.21	0.21	0.21
Density				
control	1.03554	1.03619	1.03556	1.03576
.04 level	1.03604	1.03663	1.03614	1.03627
.08 "	1.03655	1.03711	1.03627	1.03664
.12 "	1.03706	1.03761	1.03698	1.03722
.16 "	1.03752	1.03803	1.03746	1.03767
.20 "	1.03798	1.03858	1.03797	1.03818
pH				
control	6.60	6.60	6.68	6.63
.04 level	6.70	6.67	6.70	6.69
.08 "	6.72	6.70	6.77	6.73
.12 "	6.77	6.75	6.82	6.78
.16 "	6.82	6.80	6.87	6.83
.20 "	6.87	6.85	6.95	6.89
Flavor				
control	clean	clean	clean	clean
.04 level	sl.chemical	sl.chemical	sl.chemical	sl.chemical
.08 "	pronounced	pronounced	pronounced	pronounced
.12 "	"	"	"	"
.16 "	"	"	"	"
.20 "	"	"	"	"

Table 13. Chemical and physical analyses of samples to which citrate was added as  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  prior to pasteurization.

Analyses made	Trials and Dates				Means
	:1. 8/7/56	:2. 8/9/56	:3. 8/14/56	:	
Fat (%)	0.078	0.135	0.085		0.099
T.S. (%)	8.50	8.50	8.49		8.50
Protein (%)	3.19	3.19	3.13		3.17
Citric Acid	0.13	0.12	0.12		0.12
Density					
control	1.03559	1.03557	1.03548		1.03555
.04 level	1.03602	1.03600	1.03589		1.03597
.08 "	1.03632	1.03657	1.03642		1.03644
.12 "	1.03688	1.03703	1.03700		1.03697
.16 "	1.03730	1.03746	1.03743		1.03740
.20 "	1.03771	1.03773	1.03771		1.03772
pH					
control	6.60	6.60	6.60		6.60
.04 level	6.65	6.67	7.67		6.66
.08 "	6.70	6.72	6.72		6.71
.12 "	6.75	6.75	6.75		6.75
.16 "	6.82	6.80	6.82		6.81
.20 "	6.87	6.85	6.87		6.86
Flavor					
control	clean	clean	clean		clean
.04 level	chem., puckery	chem., puckery	chem., puckery		chem., puckery
.08 "	"	"	"		"
.12 "	"	"	"		"
.16 "	"	"	"		"
.20 "	"	"	"		"

Table 14. Chemical and physical analyses of samples from which calcium was removed.

Analyses :	Trials and Dates			
	made	1. 9/4/56	2. 9/6/56	3. 9/8/56
Fat (%)	0.087	0.094	0.091	0.091
T.S. (%)				
control	8.65	8.67	9.13	8.82
treated	8.77	8.68	9.13	8.86
Protein (%)				
control	3.32	3.33	3.51	3.32
treated	3.32	3.33	3.45	3.37
CaO				
control	0.13	0.13	0.13	0.13
treated	0.01	0.02	0.01	0.01
Na <sub>2</sub> O				
control	0.07	0.07	0.07	0.07
treated	0.09	0.08	0.09	0.09
P <sub>2</sub> O <sub>5</sub>				
control	0.22	0.22	0.22	0.22
treated	0.23	0.23	0.23	0.23
Density				
control	1.03675	1.03670	1.03672	1.03672
% Ca re-				
moved	1.03677	1.03673	1.03676	1.03675
" "	1.03678	1.03675	1.03682	1.03678
" "	1.03681	1.03677	1.03689	1.03682
" "	1.03686	1.03710	1.03700	1.03698
pH				
control	6.65	6.60	6.62	6.62
% Ca removed	6.74	6.75	6.73	6.74
" " "	6.76	6.77	6.77	6.77
" " "	6.80	6.82	6.81	6.81
" " "	6.82	6.82	6.81	6.82
Flavor				
control	feedy	clean	clean	clean
%Ca removed	sl. salty	sl. salty	sl. salty	sl. salty
" " "	"	"	"	"
" " "	"	"	"	"
" " "	"	"	"	"



Table 15. Chemical and physical analyses of samples to which lecithin was added prior to pasteurization.

Analyses :	Trials and Dates			
	made :	1. 8/21/56 :	2. 8/28/56 :	3. 8/30/56 :
Fat (%)	0.182	0.072	0.12	0.089
T.S. (%)	8.45	9.02	9.01	8.83
Protein (%)	3.32	3.64	3.63	3.53
Density				
control	1.03602	1.03606	1.03618	1.03609
.20 level	1.03610	1.03618	1.03629	1.03619
.40 "	1.03614	1.03628	1.03632	1.03625
.60 "	1.03620	1.03629	1.03637	1.03629
.80 "	1.03633	1.03638	1.03639	1.03637
1.00 "	1.03638	1.03642	1.03633	1.03638
pH				
control	6.62	6.66	6.67	6.65
.20 level	6.60	6.65	6.67	6.64
.40 "	6.60	6.64	6.67	6.64
.60 "	6.57	6.62	6.65	6.61
.80 "	6.55	6.60	6.65	6.60
1.00 "	6.50	6.57	6.65	6.57
Flavor				
control	clean	clean	clean	clean
.20 level	chemical	chemical	chemical	chemical
.40 "	"	"	"	"
.60 "	"	"	"	"
.80 "	"	"	"	"
1.00 "	"	"	"	"

Table 16. Individual and mean values of viscosity (Poisex  $\times 10^5$ ) for three trials. Calcium added, as  $\text{CaCl}_2$ , prior to pasteurization.

Storage periods in hours:	Levels of Calcium Added (Grams/100 Grams Skimmilk)							
	control:	Control: % water:	.02	.03	.04	.05	.06	
.43	(1)	1710	1707	1677	1585	1566	1604	1632
	(2)	1743	1729	1673	1662	1633	1668	1687
	(3)	1746	1720	1668	1613	1601	1614	1631
		1733	1719	1673	1620	1600	1629	1650
6.0	(1)	1765	1760	1706	1644	1629	1671	1722
	(2)	1793	1787	1710	1683	1671	1682	1743
	(3)	1803	1803	1682	1679	1643	1643	1683
		1787	1783	1699	1668	1648	1665	1716
24.4	(1)	1841	1819	1756	1711	1699	1722	1794
	(2)	1877	1871	1791	1731	1738	1730	1780
	(3)	1847	1842	1722	1701	1693	1699	1774
		1855	1844	1756	1714	1710	1717	1783
48.1	(1)	1906	1856	1755	1694	1694	1695	1744
	(2)	1876	1896	1797	1788	1702	1727	1775
	(3)	1880	1873	1794	1725	1686	1699	1751
		1887	1875	1782	1740	1694	1707	1757
96.7	(1)	1887	1844	1753	1721	1715	1703	1740
	(2)	1913	1924	1810	1776	1736	1750	1794
	(3)	1920	1908	1781	1725	1698	1711	1748
		1907	1892	1781	1741	1716	1721	1761
143.6	(1)	1900	1854	1806	1731	1746	1759	1811
	(2)	1968	1950	1810	1798	1789	1757	1798
	(3)	1932	1931	1780	1735	1742	1720	1759
		1933	1912	1799	1755	1759	1749	1789

- (1) Trial 1, Feb. 14, 1956  
 (2) Trial 2, Feb. 28, 1956  
 (3) Trial 3, March 3, 1956

Table 17. Analysis of variance. Data compiled from Table 16.

Source of Variation	Degree of Freedom	Mean Square	F
<u>Treatments</u>	6	78,770.00	109.70***
Linear	1	760.56	n.s.
Quadratic	1	52,693.40	73.39***
Cubic	1	256.81	n.s.
Quartic	1	2.06	n.s.
Control vs. sample			
/ water	1	1,508.03	2.10 n.s.
Control / sample			
/ water vs. 0.02			
/ 0.03 / 0.04 /			
0.05 / 0.06	1	417,398.80	581.30***
 <u>Storage Periods</u>	5	67,862.00	94.51***
Linear	1	33,895.17	325.74***
Deviations from linear	4	26,353.71	36.70***
 Treatment and Storage Periods	30	804.1	1.17
 Error		687.30	
Total	$\frac{84}{125}$		
 Pooled for new error	114	718.04	

\*\*\* P &lt; .001

Table 18. Individual and mean values of viscosity (Poisex  $\times 10^5$ ) for three trials. Calcium added, as Ca  $(C_2H_3O_2)_2$ , prior to pasteurization.

Storage periods : in hours :		Levels of Calcium Added (Grams/100 Grams Skim-milk)				
		Control:	.02 :	.03 :	.04 :	.05
.33	(1)	1746	1669	1654	1666	1671
	(2)	1701	1698	1611	1623	1632
	(3)	1699	1651	1642	1632	1641
		1715	1673	1636	1640	1648
5.70	(1)	1842	1722	1708	1680	1698
	(2)	1778	1703	1662	1667	1678
	(3)	1760	1698	1694	1681	1695
		1793	1708	1688	1676	1690
24.1	(1)	1870	1751	1712	1716	1732
	(2)	1829	1716	1695	1702	1710
	(3)	1814	1741	1721	1712	1716
		1838	1736	1709	1710	1719
48.7	(1)	1905	1790	1770	1742	1764
	(2)	1837	1717	1707	1698	1706
	(3)	1849	1750	1732	1722	1724
		1864	1752	1736	1721	1731
96.6	(1)	1914	1794	1758	1750	1766
	(2)	1868	1720	1717	1706	1707
	(3)	1868	1758	1737	1709	1695
		1883	1759	1737	1722	1723
144.8	(1)	1928	1794	1779	1755	1751
	(2)	1881	1755	1730	1723	1723
	(3)	1889	1796	1784	1735	1725
		1899	1782	1764	1738	1733

- (1) Trial 1, March 22, 1956  
 (2) Trial 2, June 28, 1956  
 (3) Trial 3, July 17, 1956

Table 19. Analyses of variance. Data compiled from Table 18.

Source of Variation		Degree of Freedom	Mean Square	F
<hr/>				
<u>Treatments</u>		4	53,305.80	84.83***
Linear		1	7,793.40	12.40***
Quadratic		1	3,916.12	6.23**
Cubic		1	15.62	.02 n.s.
Control vs. others		1	201,498.02	320.67***
<u>Storage Periods</u>		5	146,113.53	46.51***
Linear		1	98,945.78	157.46***
Deviations from linear		4	47,167.75	18.77***
Treatments and Storage Periods		20	692.49	1.10 n.s.
Error		<u>60</u>	628.37	
Total		89		

---

\*\*\* P < .001  
 \*\* P < .05

Table 20. Individual and mean values of viscosity (Poises  $\times 10^5$ ) for three trials. Calcium added, as  $\text{CaCl}_2$ , following pasteurization.

Storage periods : in hours :	Levels of Calcium Added (Grams/100 Grams Skimmilk)						
	Control:	water:	.02	.03	.04	.05	
.29	(1)	1747	1738	1738	1739	1746	1737
	(2)	1747	1737	1733	1733	1739	1728
	(3)	1751	1749	1743	1734	1746	1729
		1748	1741	1738	1735	1744	1731
5.3	(1)	1826	1820	1817	1806	1799	1799
	(2)	1817	1813	1805	1800	1795	1794
	(3)	1828	1829	1810	1807	1795	1793
		1824	1821	1811	1804	1796	1795
24.3	(1)	1866	1861	1853	1838	1833	1833
	(2)	1870	1867	1852	1847	1847	1837
	(3)	1877	1876	1858	1848	1839	1828
		1871	1868	1854	1844	1840	1833
48.4	(1)	1844	1883	1868	1845	1846	1837
	(2)	1897	1891	1875	1879	1872	1868
	(3)	1905	1896	1894	1872	1853	1837
		1895	1890	1879	1865	1857	1847
96.3	(1)	1918	1909	1899	1877	1873	1864
	(2)	1925	1919	1905	1896	1896	1886
	(3)	1940	1937	1928	1916	1906	1895
		1928	1922	1911	1896	1892	1882
144.3	(1)	1937	1929	1923	1918	1897	1876
	(2)	1930	1925	1906	1902	1895	1892
	(3)	1955	1950	1934	1929	1905	1885
		1941	1935	1921	1916	1899	1884

- (1) Trial 1, 8/21/56  
 (2) Trial 2, 8/23/56  
 (3) Trial 3, 8/28/56

Table 21. Analyses of variance. Data compiled from Table 20.

Source of Variation:	Degree of Freedom:	Mean Square:	F
<u>Treatments</u>	5	4005.	36.41***
Linear	1	5236.	47.60***
Quadratic	1	1.	.01 n.s.
Cubic	1	34.	.31 n.s.
Control vs. sample / water	1	230.	2.09 n.s.
Control / sample / water vs. others	1	14521.	132.01***
 <u>Storage Periods</u>	5	78351.	712.28***
Linear	1	287786.	2616.24***
Deviations from linear	4	25993.	236.30***
 Treatments and Storage Periods	25	135.	1.23 n.s.
 Error	<u>72</u>	110.	
 Total	107		

\*\*\* P &lt; .001

Table 22. Individual and mean values of viscosity (Poises  $\times 10^5$ ) for three trials. Phosphate added as  $\text{Na}_2\text{HPO}_4$  prior to pasteurization.

Storage :		Levels of Phosphate Added					
Periods :		(Grams/100 Grams Skimmilk )					
in Hours :		Control	.04	.08	.12	.16	.20
.42	(1)	1710	1765	1809	1837	1912	2000
	(2)	1737	1833	1886	1917	2031	2096
	(3)	1690	1719	1758	1803	1883	1916
		1712	1774	1818	1852	1942	2004
5.6	(1)	1789	1822	1866	1913	1996	2058
	(2)	1792	1872	1930	1978	2092	2158
	(3)	1741	1802	1849	1974	1990	2041
		1774	1832	1882	1955	2026	2086
24.6	(1)	1825	1869	1926	2001	2094	2169
	(2)	1836	1934	1969	2033	2191	2173
	(3)	1788	1853	1965	1984	2072	2131
		1816	1885	1953	2006	2119	2158
48.6	(1)	1827	1889	1962	2043	2112	2188
	(2)	1832	1911	1979	2050	2152	2211
	(3)	1824	1882	1967	2021	2107	2164
		1828	1894	1969	2038	2124	2188
97.2	(1)	1849	1922	1980	2050	2125	2188
	(2)	1930	1998	2056	2083	2211	2221
	(3)	1845	1913	2008	2063	2147	2203
		1875	1944	2015	2065	2161	2204
144.8	(1)	1886	1965	2012	2077	2166	2222
	(2)	1874	1990	2074	2101	2280	2236
	(3)	1861	1941	2033	2074	2186	2281
		1874	1965	2040	2084	2211	2246

- (1) Trial 1, June 12, 1956  
 (2) Trial 2, June 14, 1956  
 (3) Trial 3, June 19, 1956



Table 23. Analyses of variance. Data compiled from Table 22.

Source of Variation:	Degree of Freedom :	Mean Square:	F
<u>Treatments</u>	5	290,663.0	167.67***
Linear	1	1,445,293.9	833.74***
Quadratic	1	332.5	n.s.
Cubic	1	228.8	n.s.
Quartic	1	4,626.4	2.67 n.s.
Quintic	1	2,833.4	n.s.
<u>Storage Periods</u>	5	118,092.4	68.12***
Linear	1	447,961.0	258.41***
Deviations from linear	4	35,625.3	20.55***
Treatments and Storage Periods	25	690.6	.40
Error	<u>73</u>	1,733.5	
Total	108		

\*\*\*  $P < .001$

Table 24. Individual and mean values of viscosity (Poises  $\times 10^5$ ) for three trials. Phosphate added as  $\text{Na}_2\text{HPO}_4$  prior to pasteurization at  $71^\circ\text{C}$ .

Storage Periods in Hours :		Levels of Phosphate Added (Grams/100 Grams Skimmilk)					
	Control	.04	.08	.12	.16	.20	
.34	(1)	1714	1746	1879	1948	1970	2026
	(2)	1756	1823	1881	1940	2036	2053
	(3)	1722	1784	1831	1885	1961	2029
		1731	1784	1864	1924	1989	2036
5.4	(1)	1795	1856	1917	1989	2078	2113
	(2)	1814	1883	1947	2012	2104	2167
	(3)	1803	1866	1935	2000	2066	2147
		1804	1868	1933	2000	2083	2142
24.4	(1)	1836	1917	1978	2063	2151	2214
	(2)	1869	1946	2039	2099	2178	2235
	(3)	1852	1924	1999	2075	2171	2223
		1852	1929	2005	2079	2167	2224
48.6	(1)	1888	1946	2013	2091	2184	2242
	(2)	1890	1965	2043	2135	2214	2269
	(3)	1887	1962	2056	2104	2183	2250
		1888	1958	2037	2110	2194	2254
96.6	(1)	1898	1977	2049	2145	2198	2266
	(2)	1921	2022	2122	2190	2240	2314
	(3)	1917	1993	2084	2142	2214	2277
		1912	1997	2085	2159	2217	2286
144.5	(1)	1906	2004	2069	2170	2226	2302
	(2)	1968	2056	2163	2202	2259	2350
	(3)	1951	2036	2114	2170	2244	2300
		1942	2032	2115	2181	2243	2317

- (1) Trial 1, August 7, 1956  
 (2) Trial 2, July 12, 1956  
 (3) Trial 3, August 14, 1956

Table 25. Analyses of variance. Data compiled from Table 24.

Source of Variation :	Degree of Freedom:	Mean Square:	F
<u>Treatments</u>	5	323295.4000	568.45***
Linear	1	1614580.00	2838.89***
Quadratic	1	1447.00	2.54 n.s.
Cubic	1	203.00	.36 n.s.
Quartic	1	1.00	.00 n.s.
Quintic	1	245.00	.43 n.s.
<u>Storage Periods</u>	5	155966.6000	274.23***
Linear	1	592598.00	1041.96***
Deviations from linear	4	46808.75	82.30***
Treatment and Storage Periods	25	383.4800	.68 n.s.
Error	<u>72</u>	568.7361	
Total	107		

\*\*\*  $P < .001$

Table 26. Individual and mean values of viscosity (Poises  $\times 10^5$ ) of three trials. Citrate added as  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  prior to pasteurization.

Storage :	Levels of Citrate Added						
Periods :	(Grams / 100 Grams Skimmilk)						
in Hours:	Control :	.04 :	.08 :	.12 :	.16 :	.20	
.54	(1)	1728	1766	1901	1951	1970	2050
	(2)	1742	1764	1865	1950	2039	2065
	(3)	1727	1748	1823	1915	1998	2044
		1732	1759	1863	1939	2002	2053
5.6	(1)	1767	1827	1922	2017	2094	2171
	(2)	1764	1844	1938	2042	2103	2195
	(3)	1757	1833	1934	2021	2111	2196
		1763	1835	1931	2027	2103	2187
24.6	(1)	1776	1890	2000	2076	2191	2245
	(2)	1786	1915	1997	2088	2202	2267
	(3)	1798	1907	2025	2113	2187	2262
		1787	1904	2007	2092	2193	2258
48.6	(1)	1823	1928	2042	2155	2217	2272
	(2)	1851	1946	2048	2139	2218	2294
	(3)	1818	1931	2055	2130	2196	2260
		1831	1935	2048	2141	2210	2275
96.5	(1)	1851	1968	2071	2165	2294	2288
	(2)	1862	1960	2071	2175	2241	2291
	(3)	1838	1968	2090	2160	2236	2256
		1850	1965	2077	2167	2257	2278
144.5	(1)	1867	1990	2100	2183	2242	2284
	(2)	1874	1994	2105	2208	2277	2290
	(3)	1861	2009	2129	2194	2261	2284
		1867	1998	2111	2192	2260	2286

(1) Trial 1, August 7, 1956

(2) Trial 2, August 9, 1956

(3) Trial 3, August 14, 1956

Table 27. Analyses of variance. Data compiled from Table 26.

Source of Variation:	Degree of Freedom:	Mean Square:	F
<u>Treatments</u>	5	465617.40	1904.15***
Linear	1	2301703.00	9412.83***
Quadratic	1	23312.00	95.33***
Cubic	1	2537.00	10.38**
Quartic	1	185.00	.76 n.s.
Quintic	1	351.00	1.44 n.s.
<u>Storage Periods</u>	5	133401.20	545.55***
Linear	1	475658.00	1945.21***
Deviations from linear	4	47837.00	195.63***
Treatments and Storage Periods	25	1536.80	6.28***
Error	<u>72</u>	244.5278	
Total	107		

\*\*\* P &lt; .001

\*\* P &lt; .05

Table 28. Individual and mean values of viscosity (Poises  $\times 10^5$ ) for three trials. Calcium removed.

Storage	Levels of Calcium Removed					
Periods	(Percent Removed)					
in Hours	Control	92.2	69.1	46.1	23	
.46	(1)	1752	1773	1817	1845	1882
	(2)	1735	1777	1813	1857	1861
	(3)	1738	1771	1813	1859	1860
		1742	1774	1814	1854	1881
5.4	(1)	1813	1858	1907	1938	1985
	(2)	1828	1859	1905	1958	1991
	(3)	1818	1861	1906	1945	1982
		1820	1859	1906	1947	1986
24.4	(1)	1885	1930	1995	2028	2049
	(2)	1868	1918	1985	2030	2069
	(3)	1884	1941	1987	2034	2090
		1879	1930	1989	2031	2069
48.5	(1)	1911	1937	2006	2063	2108
	(2)	1913	1942	2000	2056	2109
	(3)	1909	1949	2013	2075	2129
		1911	1943	2006	2065	2115
96.5	(1)	1926	1960	2038	2084	2152
	(2)	1916	1951	2038	2090	2149
	(3)	1924	1957	2029	2081	2142
		1922	1956	2035	2085	2148
144.4	(1)	1950	1990	2069	2124	2178
	(2)	1936	1990	2078	2122	2180
	(3)	1935	1981	2062	2121	2181
		1940	1987	2070	2122	2180

- (1) Trial 1, September 4, 1956  
 (2) Trial 2, September 6, 1956  
 (3) Trial 3, September 8, 1956

Table 29. Analyses of variance. Data compiled from Table 28.

Source of Variation:		Degree of Freedom:	Mean Square:	F
<u>Treatments</u>	4		111859.00	2071.00***
Linear	1		4431.00	82.00**
Quadratic	1		198975.00	3685.00***
Cubic	1		29358.00	544.00***
Treated vs. others	1		214671.00	3975.00***
<u>Storage Periods</u>	5		126220.00	2337.00***
Linear	1		440912.00	8165.00***
Deviations from linear	4		47547.00	880.00***
<u>Treatments and Storage Periods</u>	20		781.00	14.00***
Error	<u>60</u>		54.00	
Total	89			

\*\*\*  $P < .001$

Table 30. Individual and mean values of viscosity (Poises  $\times 10^5$ ) for three trials. Lecithin addition.

Storage Periods In Hours :		Levels of Lecithin Added (Grams/100 Grams Skimmilk)					
		Control	.20	.40	.60	.80	1.00
.42	(1)	1744	1778	1795	1827	1873	1848
	(2)	1745	1786	1828	1838	1850	1860
	(3)	1747	1775	1781	1804	1808	1863
		1745	1780	1801	1823	1844	1857
5.4	(1)	1828	1871	1891	1926	1996	2112
	(2)	1819	1856	1902	1902	1934	1940
	(3)	1824	1861	1862	1894	1894	1924
		1824	1863	1885	1907	1941	1959
24.4	(1)	1886	1927	1948	1980	2080	2074
	(2)	1859	1901	1943	1964	1999	2010
	(3)	1884	1921	1941	1949	1966	1999
		1876	1916	1944	1964	2008	2027
48.5	(1)	1899	1962	1972	2025	2086	2106
	(2)	1885	1931	1971	1997	2035	2053
	(3)	1895	1944	1948	1992	1994	2033
		1893	1946	1964	2005	2038	2064
96.5	(1)	1922	1990	2003	2046	2119	2136
	(2)	1934	1973	2029	2050	2082	2105
	(3)	1924	1974	1982	2012	2023	2060
		1927	1978	2005	2036	2075	2100
144.5	(1)	1939	2007	2034	2071	2154	2169
	(2)	1928	1967	2018	2045	2079	2111
	(3)	1944	2027	2041	2092	2106	2134
		1937	2000	2031	2069	2113	2138

- (1) Trial 1, August 21, 1956  
 (2) Trial 2, August 28, 1956  
 (3) Trial 3, August 30, 1956



Table 31. Analyses of variance. Data compiled from Table 30.

Source of Variation:		Degree of Freedom:	Mean Square:	F
<u>Treatments</u>		5	61040.00	86.09***
Linear		1	302002.00	425.95***
Quadratic		1	1496.00	2.11 n.s.
Cubic		1	194.00	.27 n.s.
Quartic		1	1509.00	2.13 n.s.
Quintic		1	0.00	0.00 n.s.
 <u>Storage Periods</u>		5	139325.00	196.51***
Linear		1	524564.00	739.86***
Deviations from linear		4	43015.00	60.67***
 <u>Treatments and Storage Periods</u>		25	420.00	.59 n.s.
 <u>Error</u>		72	709.00	
Total		107		

\*\*\*  $P < .001$

VISCOSITY OF SKIMMILK AS AFFECTED  
BY ION CONCENTRATION

by

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AN ABSTRACT OF A THESIS

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To date little research has been done on the effect of the addition of ions on the viscosity of skimmilk. Since ions possessed remarkable stabilizing properties on the proteins in whole milk, it was decided to determine the affects of ions on the viscosity of skimmilk.

The purposes of this study were (a) to determine the effects of certain cations and anions on the viscosity of skimmilk, (b) to determine the effects of added ions on the relative particle size of the casein molecule in relation to hydration and viscosity of skimmilk, and (c) to determine the effects of the addition of certain ions on the palatability of skimmilk.

Before any results were reported in this study, an analysis of variance was run on each study to determine the number of trials needed to report valid results, and to state the response curves between the variables being studied. It was established that three trials within a study were sufficient to report valid results.

The first phase of this study dealt with the addition of calcium as calcium chloride on calcium acetate to skimmilk in amounts equivalent to 0.02, 0.03, 0.04, 0.05, and/or 0.06 g. per 100 g. of skimmilk. After the addition of ions was made, the samples were pasteurized at 61°C. for 30 minutes, cooled to 4°C., and the viscosity determined at 4°C.  $\pm$  0.01°C. by a modified Ostwald viscometer. Viscosity values were reported as poises  $\times 10^5$  as calculated by the equation:

$$\eta \text{ at } 4^{\circ}\text{C.} = \frac{\eta_m}{\eta_w} = \frac{F_m}{F_w} \cdot \frac{d_m}{d_w}$$

The addition of calcium ions, at all levels, decreased the viscosity of skimmilk, and it was concluded from previous literature and the results of this study, that the particle size of casein was increased and protein hydration decreased by the addition of calcium.

In another cationic study calcium was removed from skimmilk by an ion exchange resin (Amberlite IR-120) generated in the sodium cycle. The results of this study showed that the removal of various percentages of calcium increased the viscosity of skimmilk above the control.

The addition of calcium in various forms tended to impart an off-flavor to the skimmilk which was best described as "chalky", while the removal of calcium did not impart any objectionable off-flavor to the product.

The second phase of this study was concerned with the addition of phosphates or citrates to skimmilk as disodium hydrogen phosphate or sodium citrate in levels of 0.04, 0.08, 0.12, 0.16, and 0.20 g. per 100 g. skimmilk. Viscosity values showed that the addition of these ions increased the viscosity of skimmilk above the control. In addition, it was concluded from previous literature, and the findings of this study, that the addition of anions decreased viscosity of skimmilk by decreasing the particle size of casein which resulted in an increase in protein hydration.

The addition of lecithin produced similar results to those

found in the above anionic additions.

Phosphates, citrates, and lecithin imparted "chemical" off-flavors to the skimmilk which markedly affected palatability although viscosity was increased. Added phosphate least affected flavor.

These studies have merely introduced a large area of study on the effects of ions on the viscosity of skimmilk. However, these results have shown that it is possible to increase viscosity of skimmilk, and it is probable that some technique of processing may increase the viscosity of skimmilk without affecting flavor, consequently increasing its palatability. The results of this study also substantiate previous literature in regard to the affects of certain ions on the particle size of casein, which is of fundamental interest to the dairy chemist. The academic value of these findings is also of concern.