

Chemistry of Butter and Butter Making

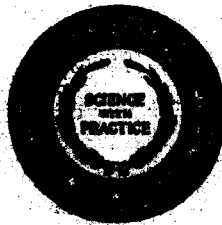
IV. The Relationships Among the Cream Acidity, the Churning Loss and the Churning Time

BY E. W. BIRD, D. F. BREAZEALE AND E. R. BARTLE

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R. E. BUCHANAN, Director

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SUMMARY AND CONCLUSIONS

1. The influence of acidity, developed in cream that had been pasteurized while sweet and subsequently ripened, was studied over a pH range (in the buttermilk) from 4.5 to 7.0.

2. Three series of creams, viz., 20, 30 and 37.5 percent fat, were investigated.

3. The losses (calculated as percentage of the total fat) for all three series varied little in the pH range 7.0 to 5.5. In this region the least variation was encountered with 30 percent cream; such tendency in loss changes as was exhibited by 30 percent cream was toward a decreasing loss with decreasing pH, while the 37.5 percent cream losses tended to pass through a minimum at pH 6.3 to 6.4. From pH 5.5 to pH 4.8 or 4.9 the losses rose to maxima (at 4.8 to 4.9) with 20 and 37.5 percent cream; a slight rise with no definite maximum at pH 4.8 to 4.9 occurred with 30 percent cream. With all three creams a marked change of function in the curves (loss vs. pH of buttermilk) occurred at pH 4.8 to 4.9; the loss dropped sharply and in practically linear fashion from that point to pH 4.5.

4. The above facts (especially the maximum at pH 4.8 to 4.9) were interpreted as indicating that casein plays an important role in the protection of the fat globules in cream, if the churning loss is taken as a measure of protective action.

5. The churning loss data correlated very well with electrokinetic potentials of the fat globules, determined by Sommer and North and re-presented here.

6. Churning times show closer correlation with pH of buttermilk the lower the fat test of the cream. Other factors such as change in protein to fat ratio, increased viscosity, greater ease of whipping, lower specific gravity, etc., may be involved in affecting the churning times of the richer creams.

7. Churning time data in this and the third bulletin of this series indicate that, if the fat and serum in cream are in proper physical state and chemical equilibrium, no hard and fast rule can be drawn that long or short churning times must be associated with high losses.

8. Data show that the fat test of the buttermilk in low fat (18 to 20 percent), highly ripened creams (pH 4.5 to 4.6) is considerably lower than those for high fat (30 to 37.5 percent), sweet cream (pH 6.5). Calculated as the percentage of the total fat churned, however, the low fat, highly ripened cream losses are approximately equivalent to those for 30 percent sweet

cream and are slightly higher than those for 37.5 percent sweet cream. This shows that the American, Australian and New Zealand churning losses compare very favorably with those obtained in Denmark, Germany and Holland.

9. Based on the data presented and others from the literature it was hypothesized that the protective action at the fat globule interface was caused by two types of protective materials—one labile and one non-labile. The latter is closely associated with the fat, presumably on the fat side of the interface, and consists of a protein-phospholipin complex. The former is oriented from the water side of the interface and is composed of all the surface tension lowering constituents of the serum. Of the serum constituents casein probably plays the most important protective role as indicated by certain dairy phenomena.

10. If the validity of the hypothesis presented is assumed, the following explanation of the churning process seems logical: Utilization of the labile protective materials, to stabilize foam interfaces, decreases their concentration at the fat-serum interface. When the labile to non-labile protective material ratio is sufficiently small that the fat globules are in an unstable state, they merge and lose their identity. This merger weakens the forces at the force centers of the fat globules to such an extent that the non-labile materials are released from the fat globule surfaces and are incorporated in the buttermilk, while the fat unites to form butter.

ERRATA

P. 188—Fig. 2 is at bottom of page with legend for Fig. 3, and Fig. 3 at top of page with legend for Fig. 2.

P. 197—Lines 1 and 3, figure 5.9 should be 4.9.

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IV. The Relationships Among the Cream Acidity, the Churning Loss and the Churning Time¹

BY E. W. BIRD, D. F. BREAZEALE AND E. R. BARTLE²

A number of contributions relative to the relationship between the acidity of cream and the churning loss have been published. With two exceptions, these studies deal with (a) comparisons between "sweet" and "sour" cream, (b) narrow ranges of acidity on the acid side of the isoelectric point of casein, or (c) have been discussions in which no data were presented. The two exceptions mentioned deal with cream systems treated in such fashion that normal churning conditions were undoubtedly not represented.

At the present time cream with low acidity is churned in New Zealand and Australia; cream with low or slightly developed acidities is churned in this country, while highly ripened cream is churned in certain European countries. To date, no comprehensive study that permits comparison of churning losses among these different methods of manufacture has been presented.

The possibility that a fairly complete study, conducted under factory conditions, would yield data that might throw considerable light on the relationships among the milk components, is one that deserves consideration.

With the above facts in mind, this problem was undertaken. Three series of churnings were run: (a) With 20 percent cream, (b) with 30 percent cream and (c) with 37.5 percent cream. These three cream richnesses were chosen because (a) 30 percent cream is common in many Iowa creameries, (b) it was shown (8) that in the range 20 to 40 percent fat the lowest losses occurred in the vicinity of 37.5 percent cream and (c) a comparison of the 20 percent series with the other two

¹ Project 107 of the Iowa Agricultural Experiment Station.

² The authors are indebted to Profs. F. E. Brown and E. I. Fulmer, of the Department of Chemistry, Iowa State College, and to Prof. M. W. Lisse, of the Department of Biochemistry, Pennsylvania State College, for having read and commented on the manuscript of this bulletin, and to Prof. L. S. Palmer, of the Department of Biochemistry, University of Minnesota, for having read and commented on that portion of the manuscript concerning the hypothesis relative to the nature of the protective action at the fat globule interface (pages 205-211).

would show similarities and differences that exist between the European and the New Zealand, Australian and American churning systems. Each series ranged in pH (of the buttermilk) from approximately 4.4 to 7.1.

The objectives of the project were: (a) To determine the relationships among the cream acidity, the churning loss and the churning time, with the hope that information that might be employed to reduce the churning loss would be obtained; (b) to determine the relationships among the various commercial churning practices and (c) to obtain information that would help clarify the physical chemical processes involved in churning.

LITERATURE REVIEW

Literature relative to this problem may be divided into three sections: (a) that comparing the churning of sweet and sour cream, (b) that dealing with the pasteurization of sweet and sour cream and (c) that comparing the churning time and churning loss with the pH of the cream.

SWEET vs. SOUR CREAM LOSSES

Myers (67) considered that if sweet cream were churned, the resulting buttermilk skimmed and the cream obtained from the buttermilk churned, sweet cream churning losses would be very low. McKay (56) indicated that high acid cream tended to yield high churning losses. From a survey of Minnesota creameries, Combs (16, 17) presented an average value for sweet cream losses of 0.64 percent and for sour cream losses of 0.79 percent. Combs (18) showed that a comparison of losses for sweet and sour cream churnings obtained in two surveys of Minnesota creameries yielded the following averages: 1925-26, sweet cream buttermilk—0.64 percent, sour cream buttermilk—0.79 percent; 1929-30, sweet cream buttermilk—0.65 percent, sour cream buttermilk—0.74 percent. Combs and Coulter (19) again presented the values for sweet and sour cream buttermilks, from a survey of Minnesota creameries, as 0.64 and 0.79 percent, respectively.

Schmöger (87) obtained a greater butter yield from sour than from sweet cream. This he attributed partly to a more exhaustive churning of sour cream and partly to a higher moisture content in the sour cream butter. Babcock (5) concluded that ripening cream increased the butter yield 15-20 percent. Mixed sweet and sour cream gave the same yields as though each were churned separately. He likewise stated that the addition of acid to sweet cream just before churning increased the yield of butter somewhat.

Babcock's results indicate that the acidity has little effect on

the churning time if the same procedures are employed in both the sweet and sour cream churnings. Penny's data (79) indicate a greater churning efficiency with sour than with sweet cream. A similar indication is shown by one set of buttermilk analyses presented by Patrick (75).

Robertson (84, 85) churned sweet, mixed sweet and sour, and sour cream. The loss was least with sour, greatest with sweet, and intermediate with mixed cream. The churning time was longest with the sweet cream. Dean (24) showed a higher buttermilk test and a longer churning time with sweet than with sour cream.

Patrick, Leighton and Bisbee's (76) results indicate (a) that the churning, washing and working losses were less with sour than with sweet cream, (b) that the yield of butter was higher, but the fat content of the butter was lower from sour than from sweet cream and (c) that the churning time was shorter for the sour cream. Results of Adametz and Wilkins (2) show a lower buttermilk test with sour cream. Similar results were reported by Wallace (108) and by Gilchrist (32).

Dean (25) reported higher buttermilk tests for sweet cream with added culture than for ripened cream, and for sweet cream without culture than for sweet cream with culture. A later report (26) confirmed the above and showed in addition that the buttermilk test was lower the greater the amount of culture added to the sweet cream.

Tiemann (103) reported that butter yields were higher and buttermilk tests lower for sour cream or cream pasteurized and then ripened with culture, than were those from pasteurized or raw sweet cream.

Burr (13) stated that sour cream losses are less than those of sweet cream. He thought that the lower loss resulted from shrinkage of casein in the presence of high acid; this lessened the protective action of the casein and permitted greater ease of coalescence of fat globules. Burr thought there was a narrow range of low loss and that a change in acidity in either direction from this range caused the fat percentage of the buttermilk to rise. In certain cases in which long ripening periods were encountered, excessive losses and high degrees of foaming existed. These phenomena were attributed to high protective action of the proteins, which Burr considered, resulted from proteolysis caused by a high ratio of proteolytic to acid-forming bacteria in cultures which ripened cream slowly.

van Dam and Holwerda (23) consider that if the fat is in a state of equilibrium at the churning temperature and if the acidity is above a limit at which the casein is sufficiently separated, the fat loss is less the greater the degree of acidity.

Ruehe and Stiritz (86) studied the effect of the addition of acids and of sodium chloride on the churning loss. HCl or NaCl or both were added to the cream after neutralization. Each reagent or combination of reagents yielded lower Mojonnier tests of the buttermilk than the controls; HCl was more effective in this respect than NaCl, and a combination of the two was more effective than either alone.

Ladd (48) considered that if churning conditions (chiefly temperature) were properly controlled the loss with sweet cream would be no greater than that with sour cream. Hills' (40) data indicate that changes in acidity have little effect on the churning loss. Douglas (31) concurs with Ladd in that at low temperature the loss with sweet cream is no greater than that with sour cream, and Mitchell (58) concluded that there was no advantage in ripening cream above 0.4 to 0.5 percent acid to reduce losses because higher acidities lowered the butter quality.

Patrick (74) and Hesselberg (39) consider that if the sweet cream buttermilk is separated and this cream is churned the losses for sweet and sour cream churnings are practically identical.

Patrick, Leighton and Heileman (77) and Shutt and Charon (91) present loss data that do not particularly favor either sweet or sour cream.

Anthony (3) studied the manufacture of farm butter. He reported buttermilk tests of 0.52, 0.23, 0.20, 0.25 and 0.40 percent for creams with acidities of 0.2, 0.3, 0.4, 0.5 and 0.85 percent, respectively. Sebelien (89) likewise believes that there is a decrease in loss followed by an increase in loss as the acidity progressively increases; the buttermilk test decreases as the acidity increases up to a certain point (approximately 40 ml. N/10 alkali per 50 ml. cream). Beyond this point the test does not drop with an increase in acidity but, on the contrary, shows a tendency to increase. Sebelien's findings agree rather well with those of Burr, previously reviewed.

PASTEURIZATION OF SWEET vs. SOUR CREAM

Jones (43) considers that pasteurization of mixed sweet and sour cream, without adequate mixing and holding to effectively distribute the acid throughout the system, increases the churning loss. This can be lowered somewhat by neutralization. Mortensen, Gaessler and Cooper (65) showed that pasteurized sour cream churning losses were higher than raw sour cream losses. Vat pasteurization caused higher churning losses than did continuous pasteurization. Bouska (10) stated that pasteurization of sour cream increased the churning loss, but that this

loss (9) could be reduced by neutralizing the cream before pasteurization.

Sproule and Grimes (95) consider that the higher the acidity at the time of pasteurization, the greater the loss. This may be reduced as much as 50 percent in some cases by partial neutralization before pasteurization. Dean (29, 27) considers that the higher the pasteurization temperature of sour cream the greater the loss. The reduction of the acidity to 0.3 percent before pasteurization lowers the loss (29). In another report, Dean (28) found no higher losses from the pasteurization of sour than of sweet cream. Thomsen (102) states that pasteurization of sour cream or the churning of sweet and sour cream, mixed just before churning, increases the churning loss.

CHURNING TIME AND LOSS AS FUNCTIONS OF pH

Guthrie and Sharp (33) churned small quantities of cream (approximately 1,500 gm.) of which 375 ml. were water, HCl solution, NaOH solution or the acid or base plus salt to bring the cream to the desired pH. The pH range studied was approximately 1 to 13. These authors were concerned only with churning times. They encountered maxima for "unwashed" creams in the vicinities of pH 3.3 to 3.5 and pH 10. The churning times approached infinity above pH 13. These authors state, "The isoelectric point of casein is near a pH of 4.7, in which region the casein is not in solution and the churning time is at a minimum." Their general results indicate that casein plays an important part in determining the churning time for the greater the casein dispersion the greater the churning time and vice-versa.

Sommer and North (93) studied the relationships among the electrokinetic potential (set up when the serum obtained by centrifuging cream was forced through a "butteroil capillary"), the churning time and the churning loss. The cream was processed as follows:

"In all cases sweet cream from the University Creamery was used that had been standardized to 35% and aged overnight at 42° F. The increase in hydrogen ion concentration was accomplished by means of increasing additions of hydrochloric acid. The pH measurements were made using the quinhydrone electrode. Various salts were added in M/4 concentrations and enough distilled water to make a total of 100 cc. of solution to 1,400 cc. of cream. After the additions of this solution the cream was shaken and allowed to remain in a water bath of 14° C. for one-half hour. All the churnings in this investigation were made at a temperature of 14° C. A small portion was saved, centrifuged and the serum used in the electrokinetic potential determinations."

TABLE 1. RELATION OF pH TO CHURNING.

[Data of Sommer and North (93)]

No.	pH	Time ratio	Fat loss ratio	Electrokinetic potential
1*	6.59	1.00	1.00	6.92
2	5.94	.91	.90	6.71
3	5.50	.93	.76	6.60
4	5.27	.92	.70	5.74
5	5.03	.90	.58	5.55
6	4.43	.80	.52	1.51
7	4.01	.94	.56	1.43
8	3.43	1.88	.47	4.02
9	3.37	1.51	.46	—**
10	2.67	1.75	.56	—
11	1.85	.97	.70	—

*Average of six churnings.

**Conductivity too high for measurement.

Churning time and fat loss data were expressed as ratios with the control in each series as unity. The data concerning pH, churning time, churning loss and electrokinetic potential (as originally presented by Sommer and North) are in table 1.

Sommer and North interpret these data as follows: "As will be noted, the isoelectric point" (of the fat globule) "was found to be at a pH of 4.2. This is in agreement with the work of Mommsen and also of Prieger.

"The churning time, in relation to pH, was found to form a curve similar to that previously found by Guthrie and Sharp. The curve denoting the fat content of the buttermilk showed its minimum at a slightly lower pH than did the churning time and potential curves. As the charge decreased down to the isoelectric point, the mutual electrostatic repulsion was progressively less, and should function as a favorable factor in the churning process. Then as the charge passed through the isoelectric point and became progressively more positive we would expect the churnability to become more difficult, requiring greater time and having an increased fat loss in the buttermilk. In general, as can be seen from the table and graphs, this was found to be true. However, in churning over such a wide range of pH values, other factors should also be considered in any complete picture of the churning process."

EXPERIMENTAL

METHODS

PROCESSING CREAM

STANDARDIZATION TO DEFINITE FAT PERCENTAGES

Twenty percent cream was prepared from approximately 30 percent cream and skim milk. Thirty percent cream was ob-

tained either by skimming part of the cream (if it were slightly less than 30 percent fat) and standardizing the original cream to 30 percent with this richer fraction or by diluting a cream somewhat richer than the desired percentage with skim milk. Thirty-seven and one-half percent cream was standardized by skimming cream of about 30 percent fat to a richness greater than that desired and diluting the rich cream with its own skim milk.

The standardizations indicated above were made in such fashion that, when the cream had been standardized, pasteurized, cooled to a low temperature and held near this temperature overnight, the fat percentage would be 20, 30 or 37.5 after 8 percent culture had been added. The culture was to ripen the cream.

PROCESSING THE CREAM PRIOR TO CHURNING

When it was necessary to separate the cream it was first pasteurized at 150° F. for 30 minutes, cooled to 90° F., separated, standardized, cooled to 36° F. and held overnight at 35 to 40° F. When skimming was not necessary the cream was standardized, pasteurized, cooled to 36° F. and held overnight as above.

On the following morning 8 percent of butter culture were added. The fat percentage was then approximately 20, 30 or 37.5 percent. The cream was ripened at temperatures varying from 60 to 85° F. The temperature chosen depended on the activity of the culture and the highest acidity that was desired in any particular run. As the cream acidities rose, lots of cream sufficient for one churning each were drawn from the large horizontal coil vat in which the cream had been processed and were placed in 50-gallon horizontal coil vats.

Each lot was separately cooled to 40° F. or lower in the small vat, placed in 10-gallon cans and held in the cooler at temperatures between 35 and 40° F. No attempt was made to have definite acidity points coincident among the various runs but rather to have the points so distributed over the entire range of acidity that there would be no question of the trends of the curves over the entire acidity range.

It will be noted later that certain churnings had buttermilk pH values as high as 7.1 to 7.2. These churnings were removed from the large vat of cream after standardization and addition of culture but before any ripening occurred; the acidity was reduced to the desired point by adding an aqueous solution of a "soda neutralizer" (C.A.S.). The neutralizer was added at the ripening temperature, allowed to act and then the cream was cooled to 40° F., placed in 10-gallon cans and stored in the cooler as were the companion, ripened samples.

CHURNING PROCEDURE

The churnings were made during the winters of 1931-1932 and 1932-1933. Churning temperatures were the same as those which yielded butter of firm body in approximately 45 minutes for the non-experimental churnings in the Iowa State College creamery. The creams were warmed to the churning temperature and held at that temperature 10 to 15 minutes before churning.

One hundred ninety pounds of each cream churned were weighed into the churn. This amount filled the churn to approximately one-third its cubical capacity.

Churning time was considered the time elapsed from the starting of the churns to the clearing of the glass. The buttermilk was drawn when the butter granules were between wheat and corn kernel sizes.

TYPES OF CHURNS EMPLOYED

The two Cherry Junior single roll, Model 2B, churns used in the preceding study (8) were employed here. These churns had been shown to yield like results and results representative of factory conditions.

CONTROL OF VARIABLES

The same control measures were employed here as were used in the previous study (8), including the churning of "winter-fat" cream. It is considered, therefore, that in each series differences that occur are functions of the acidity only.

ANALYTICAL METHODS

FAT ANALYSIS OF CREAM

The cream was tested in accordance with the official Babcock method (4).

FAT ANALYSIS OF BUTTERMILK

The Mojonnier method, as previously described (7), was employed.

pH DETERMINATIONS

The pH determinations were measured with a modified quinhydrone electrode and a calomel half-cell of the Schollenberger (88) type. The quinhydrone electrode was made from a section of test tube about 2 inches long. A gold flag approximately 25 square millimeters in area was welded to a length of platinum wire, the end of which was gold plated where it joined the gold foil or came in contact with the solution of which the pH was to be measured. The wire was so fused into the closed end of the test tube that only a loop of platinum wire sufficiently large to make contact with the wire from the potentiometer was ex-

posed outside the tube. On the inside only the gold foil and gold plated wire were exposed.

The sample and quinhydrone were mixed (by shaking) in the tube; the tip of the calomel cell was immersed in the sample in the test tube electrode and the voltage was read. Temperatures of the contents of the electrode were measured immediately after the reading was taken. The pH values calculated were corrected for temperature. Duplicates checked within 0.02 pH unit.

The pH values were determined for the buttermilk rather than for the cream. The reasons for this procedure were that (a) the cream viscosities often made the measurement difficult to obtain (especially at the higher acidities); (b) incorporation of air into cream of high viscosities seemed at times to invalidate the readings, and (c) should any change have occurred in the acidity of the cream the buttermilk pH would probably more nearly represent the condition of the cream at the time the butter "broke" than would that of the cream.

ACIDITY DETERMINATIONS

The cream samples (9 gm.) were weighed on a butter moisture balance; the buttermilk samples were pipetted with a 17.6 cc. milk pipette and the readings were halved. All samples were titrated without heating with N/10 NaOH. Ten drops of a saturated solution of phenolphthalein in 50 percent alcohol were employed as an indicator.

PREPARATION OF BUTTERMILK SAMPLES

The first and last gallon of buttermilk from each churning was discarded. Aliquots were taken from time to time as the rest of the buttermilk ran from the churn, so that a full pint sample (undiluted) was available for the acidity, fat and pH determinations.

METHODS OF CALCULATION

Buttermilk tests are all expressed as Mojonnier analyses. From these the losses, as percentages of the total fat placed in the churn, were calculated, assuming a 20 percent overrun at the time the buttermilk was drawn (8). It was impossible to standardize all lots of cream exactly to the percentage fat desired. Such differences as existed were taken into account when the fat remaining in the buttermilk was calculated as the percentage loss of the total fat placed in the churn.

CHECK ON EXPERIMENTAL METHODS

In the preceding bulletin of this series (8) it was shown that the churns employed in this study checked each other and that the results obtained were representative of large churning.

Before proceeding with the experimental work herein reported, it was considered advisable to again check the churns in order to be certain that they gave similar results with the same cream. For this reason four runs were made, two with 37.5 percent cream and two with 30 percent cream. Six churnings (three with each churn) were made in each run. Data are presented in table 2.

These figures indicate that the variations in losses among churnings of the same cream are not significant. It is considered that the churns employed yield results that are not invalidated by variations between churn 1 and churn 2.

It was considered that the pH of the buttermilk and the acidity of the cream should yield a smooth curve unless, because of long churning time or other factors, an appreciable change in pH occurred between the time the churn was started and the time the buttermilk was drawn. Figure 1 presents such curves for creams of 20, 30 and 37.5 percent fat content. The graphs show that the points fall rather closely to a line drawn through them. In addition to their lending assurance that no appreciable changes in pH occurred during churning, they likewise permit the conversion of buttermilk pH values to titratable acidities for each richness of cream.

TABLE 2. VARIATIONS BETWEEN CHURNS AND AMONG CHURNINGS WITH THE SAME CREAM.

Run number	Churn number	Fat in cream, percent	Acid in cream, percent	Acid in buttermilk, percent	pH of buttermilk	Total fat lost, percent
1	1	37.5	0.23	0.33	5.65	1.16
	2		0.23	0.33	5.67	1.23
	1		0.23	0.34	5.62	1.17
	2		0.24	0.34	5.61	1.28
	1		0.24	0.34	5.63	1.25
	2		0.24	0.34	5.63	1.25
Average			0.235	0.337	5.635	1.223
2	1	37.5	0.21	0.27	5.78	1.11
	2		0.21	0.27	5.82	1.10
	1		0.21	0.27	5.80	1.11
	2		0.21	0.27	5.80	1.12
	1		0.21	0.27	5.79	1.11
	2		0.21	0.27	5.80	1.15
Average			0.210	0.270	5.798	1.117
3	1	30.0	0.23	0.30	5.82	1.24
	2		0.23	0.30	5.83	1.25
	1		0.23	0.30	5.81	1.26
	2		0.23	0.30	5.82	1.27
	1		0.23	0.30	5.82	1.23
	2		0.23	0.30	5.82	1.24
Average			0.230	0.300	5.820	1.248
4	1	30.0	0.26	0.33	5.64	1.42
	2		0.26	0.33	5.63	1.38
	1		0.26	0.33	5.71	1.42
	2		0.26	0.33	5.70	1.41
	1		0.26	0.33	5.71	1.40
	2		0.26	0.33	5.72	1.42
Average			0.260	0.330	5.685	1.408

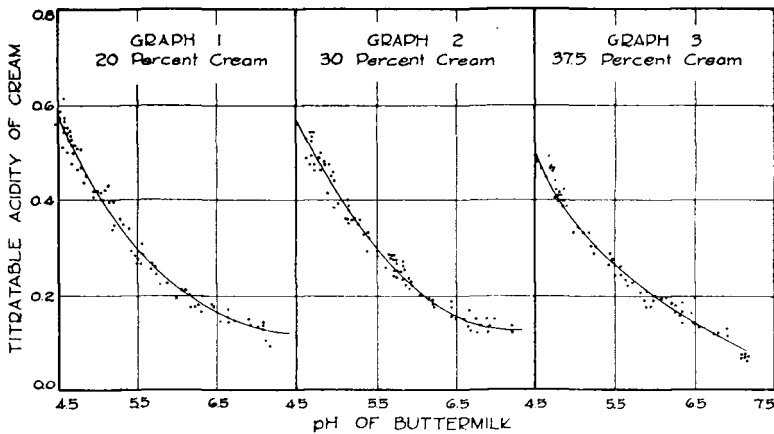


Fig. 1. Variation of titratable acidity in 20, 30 and 37.5 percent cream with change in pH of the buttermilk from these creams.

RESULTS OF CHURNING EXPERIMENTS

RELATIONSHIP BETWEEN THE MOJONNIER TEST AND THE BUTTERMILK PH

Figure 2 indicates that all of the curves presented have one feature in common, viz., a marked change in function at a buttermilk pH of 4.8 to 4.9. With the curves for 20 and 37.5 percent cream there is a maximum, as well as a change in function, at this pH value. In all three graphs the curves appear to be linear and have very steep slopes in the region more acid than pH 4.8 to 4.9. All of the curves rise in the less acid ranges (pH's 6.2 to 7.3) and have low sections varying from pH 5.9 to 6.1. These graphs likewise correlate very well in the sweet cream section with the data previously presented (8), in that the Mojonnier tests for 20 and 30 percent sweet cream buttermilk lie rather closely together, while those for 37.5 percent sweet cream buttermilk are higher than those of either of the other two richnesses of cream.

RELATIONSHIP BETWEEN THE PERCENTAGE OF THE TOTAL FAT CHURNED THAT IS LOST IN THE BUTTERMILK AND THE BUTTERMILK PH

Figure 3 presents these data. They show as did the Mojonnier test curves (fig. 2) that a change of function occurs at pH 4.8 to 4.9. Again differences exist among the graphs. The effect of increased acidity on the acid side of pH 4.8 to 4.9 is considerably more marked for the 20 percent cream curve than it is for either of the other two, and the change is more abrupt for

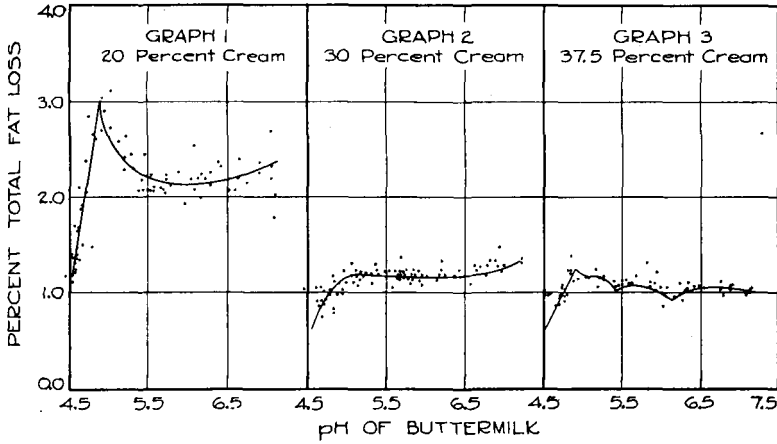


Fig. 2. Variation of the Mojonnier test for fat of buttermilk with change in pH of the buttermilks from creams containing 20, 30 and 37.5 percent fat.

the 20 and 37.5 percent cream graphs than for the 30 percent cream curve.

In the region more alkaline than pH 4.8 to 4.9 the curve (for 20 percent cream) drops gradually from a 3 percent loss (pH 4.8 to 4.9), to a low point of approximately 2.1 percent (pH 5.9 to 6.0) and then rises to approximately 2.35 percent at pH 7.0. The 37.5 percent curve shows poorly defined minima at pH

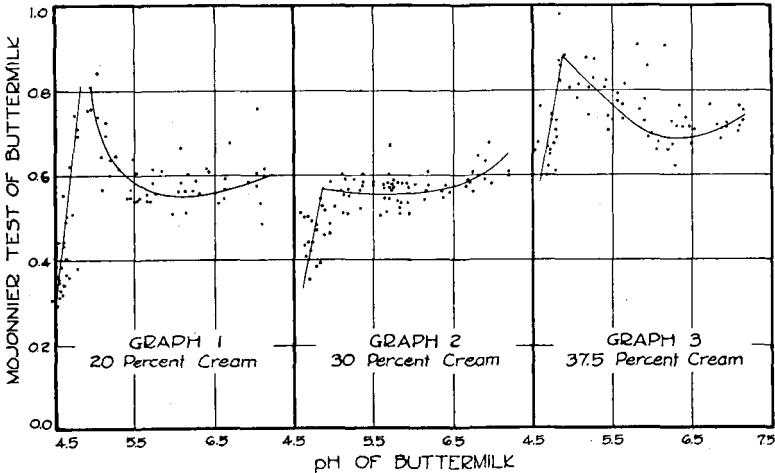


Fig. 3. Variation of the churning loss (as percentage of the total fat churned) with change in pH of the buttermilks from creams containing 20, 30 and 37.5 percent fat.

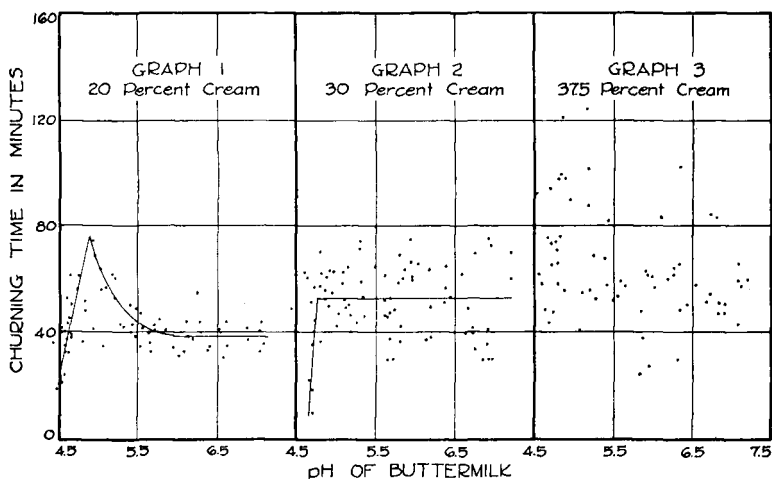


Fig. 4. Variation in churning time with the pH of the buttermilks from creams containing 20, 30 and 37.5 percent fat.

5.45 and at pH 6.2; it flattens in the more alkaline range. The portion of the 30 percent curve more alkaline than pH 5.0 is reasonably flat to a pH of 6.5 and then rises slightly to pH 7.2.

RELATIONSHIP BETWEEN THE CHURNING TIME AND THE BUTTERMILK PH

Charts in fig. 4 indicate that only with the low fat cream is there very marked variation of churning time with buttermilk pH. The churning time of the sweet cream portions of the 30 and 37.5 percent cream charts fall chiefly between 40 and 60 minutes. It would appear that as the ratio of fat to protein increases, accompanied as it is by greater ease of whipping, more resistant foams that "ride" in the churn and increased viscosity, the churning time is no longer a specific function of the pH of the buttermilk (or cream).

A comparison of figs. 3 and 4 shows that, with the richer creams, a high loss does not necessarily accompany a long or a short churning time when the cream has been properly processed prior to churning.

DISCUSSION OF RESULTS

PRACTICAL CONSIDERATIONS

These data, together with those in the third bulletin in this series, permit a comparison of various commercial churning practices. The data of fig. 2 show why an unfavorable comparison is obtained when the fat tests of the buttermilk re-

sulting from the conditions extant in Denmark, Holland and Germany are compared with those in common practice in Australia, New Zealand and the United States.

Sweet or slightly ripened creams of moderately high fat content show high buttermilk tests, while highly ripened, low fat content creams have relatively low tests. In this connection a 37.5 percent sweet cream will yield a buttermilk with a Mojonnier test of 0.7 percent fat, a 30 percent sweet cream, one of approximately 0.6 percent fat, whereas a highly ripened, 20 percent cream will yield a buttermilk with from 0.3 to 0.4 percent fat.

The actual losses (as percentages of the total fat churned) present an entirely different picture. These percentages are: 37.5 percent sweet cream, 1.0 to 1.1 percent; 30 percent sweet cream, 1.15 to 1.25 percent, and 20 percent highly ripened cream, 1.1 to 1.5 percent. The loss with sweet 20 percent cream is much higher than that for the same cream highly ripened, viz., 2.1 to 2.2 percent of the total fat. The sweet cream figures here presented agree with those previously obtained (8). Data, presented in this and the other study referred to, have been obtained under conditions that are representative of factory practice and are not, therefore, to be classed as laboratory experiments. Data are sufficient in number and cover a sufficiently wide range that they are adaptable for predicting results over wide ranges of conditions.

Figures 1 and 3 show that for *cream pasteurized in the sweet condition and then ripened with culture to different acid levels*, the development of acidity has relatively little effect on the percentage total fat lost in 37.5 percent cream within the acidity range 0.10 to 0.30 percent, in 30 percent cream within the acidity range 0.12 to 0.38 percent and in 20 percent cream within the range 0.15 to 0.25 percent. The changes that occur in the vicinity of the isoelectric point of casein are pronounced with all three cream richnesses: Maxima occur with 20 and with 37.5 percent creams; there is but a change of function with the 30 percent cream.

These graphs (fig. 3) substantiate the contentions previously made (8) that: (a) A change in the nature of the phase relationships among the colloidal systems in cream seems to occur from 30 to 35 percent fat, with the probable point in the vicinity of 32.5 percent, (b) casein plays a more important role in the churning process than any of the other protein constituents of cream serum, and (c) all losses should be compared as percentages of the total fat churned.

The churning times (fig. 4) show that there is considerably

less variation in churning time (at any definite pH) the lower the fat content of the cream. They likewise indicate that high losses associate to a greater extent with long churning times with 20 percent cream than with the richer creams; with 20 percent cream this association is more noticeable with ripened than with sweet cream.

It was pointed out previously (8) that with 30 to 40 percent sweet creams the association of high losses with long and with short churning times was not borne out experimentally if the fat were in the proper physical state; these data corroborate those previously presented. They are likewise in agreement with the former data in that the richer the cream the greater the possibility for marked fluctuations in churning time in the sweet cream range without having these fluctuations reflected in the churning loss to any marked degree.

It would appear that certain reasons for long or short churning times need not necessarily be the same as those causing high or low losses (when the physical condition of fat is correct) and for this reason it would appear that an attempt to correlate them in a cause-effect fashion might lead to erroneous conclusions.

In richer creams other factors, as greater ease of whipping, formation of more resistant foams that "ride" more easily in the churn, increased viscosity, increased fat to serum ratio and lowered specific gravity, may cause the churning time to be less a direct function of pH of the cream than is true at the lower fat levels. Changes in the fat to serum ratios, although they change the types of fat-loss curves, do not cause the wide random variations that occur among the churning times; if anything the points fall more closely on the loss curves with the richer creams than with 20 percent cream.

The portions of the curves on the acid side of pH 4.8 to 4.9 in figs. 2 and 3 agree with the findings of Burr (13), van Dam and Holwerda (23) and Sebelien (89) in that they show that there is a narrow acidity range (at high cream acidities) in which the losses vary greatly, the higher losses occurring at the lower acidities. These data agree with the fact that fat losses decrease with increased casein content of the cream when the pH values of the cream approximate 4.5 (22). These data confirm the statement of Guthrie and Sharp (33) that casein plays an important role in the churning process. They do not agree with the losses and churning times presented by Sommer and North (93), a fact which it will be shown later results, very probably, from the widely different conditions under which the churnings of these authors and the churnings herein reported were conducted.

THEORETICAL CONSIDERATIONS

From a theoretical viewpoint the data presented in this publication appear to correlate with and in some instances clarify the work done on (a) isoelectric points of casein and of milk fat globules; (b) the question of the molecular homogeneity or heterogeneity of casein; (c) the variations in the electrokinetic potential of the fat globules of milk with variation in pH and (d) the nature of the so-called "membrane surrounding the fat globules of milk."

ISOELECTRIC POINTS OF CASEIN, MILK FAT GLOBULES AND THE MOLECULAR STATE OF CASEIN

The isoelectric point of casein has been variously valued; its magnitude lies chiefly within the pH range 4.5 to 5.0. Buchanan and Peterson (12) considered that "casein was precipitated in the range 4.5 to 5.0, usually at about pH 4.7." Lebermann (53) states that the isoelectric point of natural casein is 4.89, that of isolated casein 4.6 to 4.7; Csonka, Murphy and Jones (21) give the value as 4.85, and the value as nearly as it can be read from the pH surface tension curves of Mohr and Moos (61) seems to be 4.8. Although there are no data in the region pH 4.9 to 5.1, when the surface tension data of Mohr and Brockman (59) for skimmilk are plotted they indicate strongly that a definite maximum exists in the neighborhood pH 4.9 to 5.0.

Similarly the point of minimum solubility of acid casein in HCl and NaOH solutions presented by Sharp and McInerney (90) seems to be at approximately pH 4.8 to 4.9. Michaelis and Pechstein (57) give values for the C_{H+} of the isoelectric point of casein equivalent to pH values of 4.60 and 4.62. Moir (62) gives the value as 4.5 to 4.7; later he (63) states that caseins precipitated at pH values of 4.2 and 4.6 are identical in so far as the formol titration and alkaline hypobromite oxidation methods are concerned and suggests, therefore, that casein must be homo-molecular and should be defined as the milk protein with isoelectric point at pH 4.6.

St. Johnston (96) using surface tension methods gives the value 4.62. Kondo and Tomiyama (47) present the value 4.70 to 4.71 for goats' milk casein; they state that this value is influenced by the casein concentration and that the association power of casein at its isoelectric point is influenced by metallic ions.

In connection with the conflicting statements of Moir (63) and of Kondo and Tomiyama (47) in the preceding paragraphs it is of interest to consider certain statements relative to the constancy of the isoelectric point. Kondo and Hayashi (45)

believe it is impossible to determine the isoelectric points of difficultly soluble proteins such as casein and rice-glutelin and therefore term their points of maximum flocculation as "apparent" isoelectric points. Kondo, Hayashi and Matsushita (46) consider that the optimum flocculation point of rice-glutelin is shifted toward the acid side by salts; variations resulting from varying concentrations of sodium acetate were: 0.05 N—pH range 5.46-5.99, 0.10 N—pH range 5.08-5.38, 0.20 N—pH range 4.57-4.69 and 0.30 N—pH range 4.51 to 4.68.

La Rotonda (52) precipitated casein with various acids and concluded that casein either has no definite isoelectric point or that this point is not coincident with the value 4.6 to 4.7. Several acids were employed in the study; in the two cases in which definite isoelectric points seemed to occur the pH values were: hydrochloric acid—4.6; oxalic acid—4.7 to 4.8.

Vonk (106) states that the point of minimum swelling of fibrin is a function of the buffer system as well as of the pH; values for different systems were: phthalate+HCl or NaOH, 3.63; citrate+HCl, 4.22; acetic acid-sodium acetate, 5.3; phosphate, 5.84 and HCl+NaOH, 6.90. Sørensen and Sladek (94) show that the solubility curves of casein (on both sides of the isoelectric point) shift to the acid side as the concentration of NaCl (in low concentrations) is increased. This is equivalent to saying that the isoelectric point is shifted toward the acid side by increasing the NaCl concentration.

Closely allied with the isoelectric points of the proteins are those of the fat globules of milk. The isoelectric points of the fat globules are rather definitely determined by the adsorbed materials. Nugent (69) recently has reviewed some of the literature relative to this fact. He gives the isoelectric point of the milk fat globule as pH 4.1 (determined by the Mudd technique).

Mommsen (64) determined the isoelectric points in acetate buffers by cataphoretic methods. His values are: Normal cows' milk—4.11, homogenized cows' milk—4.22, dry milk dispersed in acetate buffers—4.21 and cream (1 : 10 dilution with buffer)—4.17. These values were all changed toward the acid side by Ringer's solution. Sodium chloride changed the pH values of the isoelectric points toward the acid side—cf. N/10, 4.0; N/5, 3.8; N/1, 2.9; while calcium chloride shifted them toward the alkaline side—cf. N/10, 4.1; N/5, 4.85; and N/1, >4.85. Mohr and Brockman (60) gave values (obtained cataphoretically) from pH 4.25 to 4.30 depending on the treatment of the milk; values of 2.96, 4.34 and 4.44 were given respectively for fat-water emulsions, diluted milk and fat-water-casein emulsions.

Similarly Jack and Dahle (41) report the isoelectric points

of butterfat globules to be: 3.2 for fat-water emulsions, 4.6 for fat emulsified in casein sol to which phospholipin, lactose and milk salts were subsequently added and pH 4.3 for fat globules emulsified in a phospholipin sol to which later casein, milk salts and lactose were added. Prieger (80) considers that the presence of the protective materials at the interface of the fat globule of milk causes its isoelectric point to occur in a much more alkaline range than that of naked, fat-water emulsions. North and Sommer (68) place the value of the isoelectric point of the fat-serum interface at pH 4.2 as a result of their electrokinetic potential measurements.

The values of Mohr and Brockman (60) indicate that casein may influence the magnitude of the isoelectric point of the fat globule to a considerable extent and Moir's (63) statements indicate his belief in homomolecular casein which possesses a definite isoelectric point. It is interesting to correlate the supposition of a constant isoelectric point with the fact that the formol titration and the alkaline hypobromite oxidation values were identical for casein precipitated at pH 4.2 and at 4.6.

Van Slyke and Bosworth (105) conclude that casein exists in milk as a calcium salt such that eight valences per molecule are satisfied by calcium, which would point toward molecular homogeneity of casein. More recent work indicates definitely the heteromolecularity of casein. Linderström-Lang (54) considers that casein is a heteromolecular mixture, the components of which can be reversibly fractionated and are so closely associated that they are co-reactive and form co-precipitation mixtures; this accounts for casein's having been considered homomolecular.

More convincing is the work of Svedberg, Carpenter and Carpenter. These investigators (99) show that the "apparent" diffusion constants of Hammarsten casein varied from 7.03 to 25.53, which fact indicates its heteromolecularity. They conclude that at least 33 percent of the preparation was soluble in 70 percent alcohol. This fraction consisted, apparently, of a monomolecular species with a molecular weight of $375,000 \pm 11,000$. Later, they studied (100) casein prepared by the Van Slyke and Baker method. It was in no way more nearly homomolecular than was the Hammarsten preparation; the molecular weight of the bulk of the preparation was calculated to lie between 75,000 and 100,000. If this preparation were dispersed in buffer solutions for 1 hour at 40° C. the range in molecular weights was found to be 188,000 to 370,000 as compared to those above when dispersed in cold buffer. Apparently heating in the buffer caused association.

These authors commented further that different samples of casein by the same method of preparation were not found to

yield like molecular mixtures. The investigations of Svedberg, Carpenter and Carpenter were conducted with casein "preparations." It would be interesting could these experiments have been conducted with the natural product since Sjögren and Svedberg (92) using similar sedimentation methods conclude that the materials from which "purified" lactalbumin preparations were formed had molecular weights not to exceed 1,000, whereas the purified material had molecular weights ranging from 12,000 to 25,000. The isoelectric point of the material was given as approximately 5.2.

Later Pedersen (78) using similar methods stated that casein was present in milk as a coarse, poly disperse suspension, although his interest was chiefly in the proteins remaining in skim milk serum after the casein had been removed by centrifugal force.

The reviews presented indicate that: (a) Either the isoelectric point of casein is variable or that the variations in isoelectric point (pH 4.5 to 5.0) result from the use of different buffers, of different concentrations of the same buffer in the determination of the values presented or of different casein concentrations; (b) rather good agreement exists among the various values for the isoelectric point of the fat globules, and (c) "casein" must be made up of several molecular species which combine, in the "methods of preparation" of casein, in such fashion that casein functions in certain reactions as a homomolecular material.

Data presented in figs. 2 and 3 indicate that at pH 4.8 to 4.9 a region of minimum protective action of the aggregate colloidal systems in cream serum occurs. This point is considered the mean isoelectric point of the colloidal systems, cream serum. At this point, because of the minimum protective action, a maximum fat loss occurs (with 20 and 37.5 percent cream), while with 30 percent cream a change of function exists. On the acid side of pH 4.8 to 4.9 a very rapid drop in losses that is apparently linear occurs.

This is a region in which the negative potential of the fat globule decreases progressively; very probably some of these globules become positively charged. The workers who have determined the isoelectric points of the fat globules have shown that the anodic and cathodic migrations are approximately equal at that point. It would be expected then that as greater and greater numbers become positive the churning loss drops until the isoelectric point or point at which more or less equal numbers of positively and negatively charged globules exist is reached; at this point the minimum loss occurs. Such an explanation is wholly in accord with the results of Burr (13), van Dam and Holwerda (23) and Sebelien (89) and with the postu-

lations of Sommer and North (93) quoted in the literature review.

If it be considered that the charges on the fat globules are determined largely by the protective materials associated with them, the values of Mohr and Brockman (60) and Jack and Dahle (41) strongly indicate that casein is of extreme importance in determining the milk fat globule isoelectric point. From the fact that the region of change of potential of the fat globule, as represented by the fat losses and by the electrokinetic potentials (fig. 5), is in the region of what is generally accepted as the isoelectric point of casein, it appears that the churning data agree with the following statements: (a) That natural casein is heteromolecular and exhibits an "isoelectric point range," and (b) that it functions as the chief protective agent affecting changes in churning losses with changes in pH of the cream.

ELECTROKINETIC POTENTIAL AND CHURNING LOSS

Data presented in fig. 3 agree exceptionally well with the variations in electrokinetic potential as presented by Sommer and North (93) (see table 1). They do not agree, however, with the loss data presented by these authors. Their data have been plotted by the authors of this bulletin and are presented in figs. 5 and 6. It will be noted that (figs. 5 and 6) the maximum rate of change of fat loss which Sommer and North obtained is in the region in which the electrokinetic potential

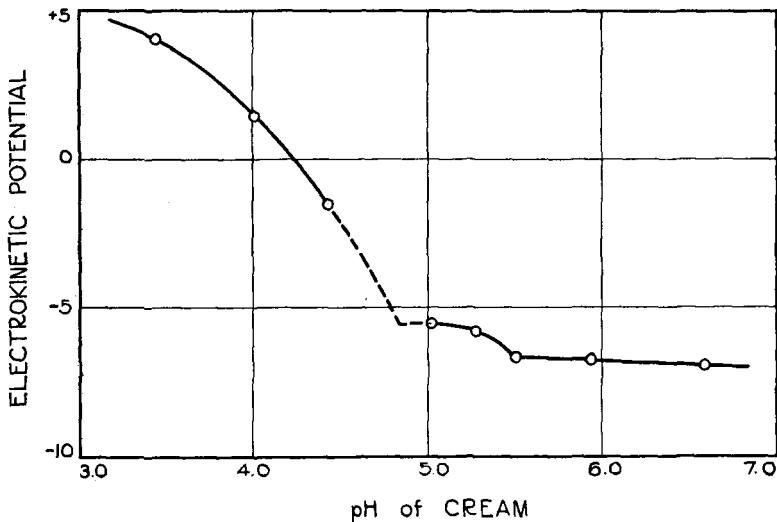


Fig. 5. Graph, prepared from the data of Sommer and North (93), showing variation of the electrokinetic potential (when a stream of cream serum was forced through a "butter oil capillary") with pH of the cream.

varied least (pH range 7.0 to 5.9), whereas in the region in which the electrokinetic potential changed very rapidly (pH 5.9 to 3.0) practically no change in loss ratios occurred. Moreover, the data concerning losses show no minimum loss at the isoelectric point of the fat globule (pH 4.2) which is not in agreement with the interpretation (of Sommer and North) of the variation of loss which should occur with change in electrokinetic potential (see literature review).

A comparison of the variation in losses of fig. 3 and the variation in electrokinetic potential (fig. 5) shows that excellent correlation is obtained. The variations in electrokinetic potential are slight from pH 6.8 to 5.5; the losses vary but little in the same region. At a pH of approximately 5.5 the electrokinetic potential becomes a different function of pH and continues so to a pH in the neighborhood of 4.8 to 4.9; similar changes are noted in the loss data especially of 20 and 37.5 percent creams. At pH 4.8 to 4.9 the electrokinetic potential again becomes a different function of pH and in the region pH 4.9 to pH 4.2 increases from approximately -5.1 to ± 0.0 in value. In approximately the same region (pH 4.9 to pH 4.5) the losses are practically a linear function of pH becoming smaller (with increasing acidity) at a very rapid rate in this narrow pH range.³

³The authors of this bulletin assume responsibility for the method of graphing of the Sommer and North data as presented and likewise for the interpretation of these data that is presented, since it is somewhat different from that of these authors.

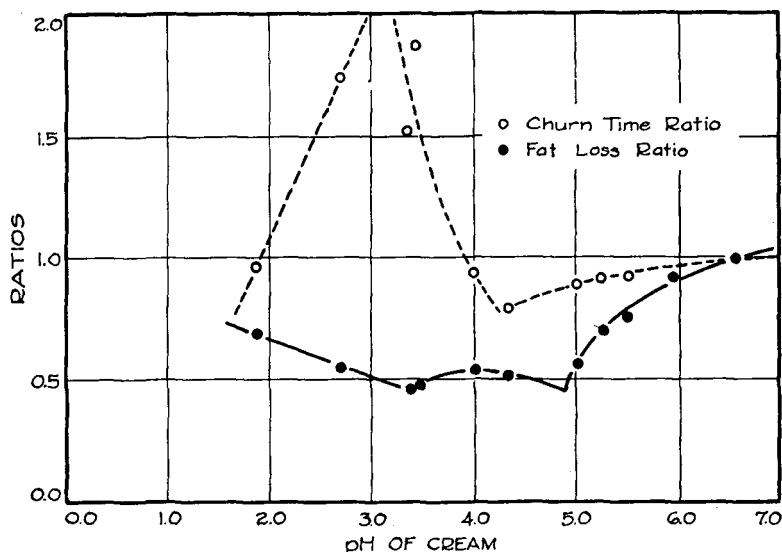


Fig. 6. Graph, prepared from the data of Sommer and North (93), showing variation of the churning time and fat loss ratio with pH of cream.

It is considered that an assumption of an "isoelectric condition" of the aggregate systems, cream serum at pH 4.8 to 4.9, is in agreement with the electrokinetic potential data in fig. 5. It would appear that these systems (cream serum) begin to change at pH 5.5 and attain a "mean isoelectric condition" at pH 4.8 to 4.9. As the potential of the colloidally dispersed materials become less the total aggregate systems, cream, progressively approach a zero potential which is considered as a "mean isoelectric point" of the entire set of colloidal systems in cream.

The reason for the differences that exist between the loss data of Sommer and North (fig. 4) and those presented in this bulletin (fig. 3) result, very probably, from two differences in experimental procedure. One of these is the method of acidification, that of Sommer and North having been the addition of hydrochloric acid, while that used in these studies was the ripening of cream with a normal butter culture of associated organisms. The second is the method of churning the cream. Sommer and North used 1,500-ml. volumes of cream in an "improved 'Kangaroo' type" of churn while the data herein presented were obtained with small churns constructed and operated in the same manner as the usual commercial churns; the amount of cream used was 190 pounds.

It might be possible that in the Sommer and North experiments some local action with casein was obtained to cause the rate of change of loss to be greatest in the region of minimum rate of change in electrokinetic potential. It is considered, however, that the method of churning was the important factor. When the data presented in the third bulletin of this series were obtained, the work was started on a laboratory scale with 1,500-gm. charges of cream and a laboratory "jig" churn.

When the loss data were plotted against fat percentage of the cream, a minimum loss was obtained at 30 percent fat and the curve rose rather steeply on either side of this low point. It was considered necessary to check this on a semi-plant scale with small churns and 190-pound charges of cream. Only one or two runs were needed to show that (a) the rate of change of loss was great in thin creams; (b) no minimum occurred at 30 percent fat; (c) the minimum loss point was at approximately 37.5 percent fat, and (d) the rate of increase in loss for high fat creams (up to 40 percent) was not rapid as the small churnings indicated. The same explanation should suffice for the differences between the two sets of data relative to churning time.

PROTECTIVE MATERIALS ASSOCIATED WITH THE
MILK FAT GLOBULE

A variety of opinions concerning the "fat globule membrane" have been offered. Storch (97, 98) considers that approximately 27 percent of the globule is membrane material, mucoid in nature. Bredenberg (11) believes the chief portion of the membrane is composed of calcium salts of fatty acids associated with some protein material. Degli Atti (30) postulates a film of calcium soaps of all the butter acids with the soaps of the insoluble acids predominating. The other contributions that will be reviewed fall chiefly in three classes: (a) Those which definitely indicate that the membrane material is casein; (b) those which indicate that it is casein associated with other milk constituents, and (c) those which indicate it either as an entirely new protein or a new protein combined or associated with phospholipin.

Müller (66) states that casein plays an important role in the fat globule membrane, Wiegner (109), as a result of homogenization studies, considers casein its chief constituent and Titus, Sommer and Hart (104) believe that the membrane is identical with casein, barring slight impurities. Zsigmondy and Spear (112) state that the membrane is composed of casein and albumin. Abderhalden and Völtz (1) think that casein is mixed with other proteins in the protective material which is variable in composition. They believe the protective material is not a wholly different substance.

Palmer (70) referring to Wiegner's work states that not only should Wiegner have considered 2 percent of the casein in milk to be adsorbed on the fat globule surfaces but lactalbumin and dicalcium phosphate as well; these materials, he believes, are present in proportion to their ability to lower the surface tension.

Bauer (6), as a result of some staining experiments, considers that (a) the fat globule membrane is continuous; (b) it contains some fat; (c) it reacts differently when milk is soured naturally than when soured artificially with lactic acid; (d) it changes during the souring of cream, and (e) the membranes are not all alike. He questions the presence of casein in the membrane. Hattori (37) conducted one experiment to determine whether or not casein was the membrane material, because casein carries the fat with it when precipitated from milk. Milk was treated with several casein precipitants and, with the exception of the trial in which copper sulfate was employed, he was able to wash the greater part of the fat from the coagulum. He concludes, therefore, that casein is not the membrane material.

Völtz (107) believes that no solid or continuous membrane exists around the fat globule. It is composed of organic and inorganic materials; the former are both nitrogenous and non-nitrogenous. The membrane material varies greatly in composition and is very labile.

Palmer and Samuelson (71) believe that the ultimate hull substance, remaining when cream globules are washed until the wash waters are negative to biuret and Fehling tests, is composed of a single globulin-like protein and a mixture of unidentified phospholipins. The latter comprised the greater portion of the material, the former never more than 15 percent.

Hattori (36) reported that the protective material was a definite, new protein yielding all protein tests except the furfural and Lieberman reactions. The ash material was chiefly calcium and phosphorus. The new protein was called haptin and was considered not contaminated with casein in the membrane. Later Hattori and Ogimura (38) precipitated the casein of cream with acid or rennet, washed out the fat globules with ether-saturated water and studied the protective materials surrounding the fat globules obtained. This material was identical with that previously reported by Hattori although the method of preparation was different. Wiese and Palmer (110) prepared pseudo-creams in which calcium caseinate, lactalbumin, globulin and phospholipins were employed as single stabilizers.

After these authors compared the appearance, separatability and churnability with normal cream, "washed" creams and emulsions of butterfat in buttermilk, they concluded that no one of the artificial emulsifiers constituted the sole emulsifying agent of cows' milk.

Palmer and Wiese (73) reported the material as a protein-phospholipin complex on the basis of the fact that its isoelectric point was pH 3.9 to 4.0. The complex contained dializable calcium and non-dializable phosphorus. The authors considered it was not lactalbumin because it would not coagulate with heat and not casein because it would not coagulate with rennet. The protein and phospholipins did not always exist in the same ratios in the complex. These authors concluded (111) that the analyses of the protein portion of the complex indicated that it was the same protein described by Hattori, barring variations caused by different impurities resulting from the widely different methods of preparation.

Rimpila and Palmer (83) investigated a number of "washed" creams and emulsions of butterfat in whey, skim milk, buttermilk and casein. When "water-washed" creams were washed in whey and then were subsequently washed with water the phospholipin was more readily removed by water washing than it was before the whey treatment. They interpret this be-

havior to indicate that the phospholipin "cannot be regarded as a chemical component of the 'membrane' protein molecule." The composition of the natural membrane varies from one cream to another and is effective in its protective action through many (10 to 12) washings; it is different in composition from the washed cream membranes of the other fat emulsions studied. With subsequent washings the ratio of protein to phospholipin in the membrane becomes smaller.

Palmer and Tarassuk (72) state that "Palmer and Samuelson . . . and Palmer and Wiese . . . have shown that the natural fat globule 'membrane' of cows' milk which is released during the churning of cream, is a phospholipide-protein complex which is not coagulated by rennet."

At first glance the variability of the results reviewed above leaves one with the feeling that the viewpoints and results presented are so at variance with one another that reconciliation among them to formulate a workable hypothesis compatible with both theoretical concepts of surface phenomena and the actual findings among plant practices is impossible. In actuality a tenable hypothesis can be evolved when (a) differences of experimental procedure are taken into account; (b) certain concepts of colloidal chemistry are applied, and (c) data of both theoretical and practical significance are mutually interpreted.

Experimental results of Völtz, Abderhalden and Völtz and Titus, Sommer and Hart were obtained with creams the fat globules of which had been allowed to rise through long columns of water; it was assumed that by this treatment the globules were freed from cream serum but retained the protective materials adsorbed at the fat interfaces. These investigators all consider casein the whole or the principal constituent of the globule membrane.

Palmer and his associates, on the other hand, diluted cream with water and re-separated the diluted cream; they continued these treatments until the "skimmilk" from the "washed" creams gave negative biuret and Fehling reactions. In some instances this washing was repeated as many as 10 to 12 times although this number was not necessary to yield the negative reactions.

Hattori and his collaborators employed two procedures: In the first the natural fat emulsions were treated with chloroform saturated water, while in the second the fat was washed from an acid or rennet casein coagulum with ether saturated water. In both cases the organic solvent permeated the fat globule, increasing its size. The chloroform increased its density and the ether decreased it; either change in density aided in separating the globules from the aqueous layer.

Palmer's and Hattori's proteins were, apparently, the same substance. Palmer emphasizes the association of phospholipin with the protein while Hattori does not. It may be that the chloroform and ether that Hattori used contained sufficient alcohol to cause a decrease in the loose chemical affinity that binds protein and phospholipin in these complexes and in this way caused the phospholipin to associate with the fat rather than the protein in the subsequent separation of fat and protein material.

It appears that two distinctive types of materials exist at the fat globule interface: (a) Those which can be removed more or less readily and (b) those which resist such removal to a very marked degree. Indeed North and Sommer (68) state that two types of adsorption exist at the fat globule interface—one reversible and one irreversible. They state that the former "must be the net result of the adsorption of the various ions, the adsorption of each ion reaching equilibrium at a definite concentration of that ion in the serum proper (reversible adsorption)," while the irreversible film is composed of milk colloids.

The authors of this bulletin are in agreement with North and Sommer that a reversible and an irreversible adsorption occurs at the fat globule interface but consider its mechanism and the type of compounds in the two protective materials to be different from those visualized by North and Sommer. Jack and Dahle (41) state that their data "indicate that the inner layer of the fat globule membrane is phospholipin and that the outer surface is composed chiefly of protein." This view approximates closely the concept which the authors of this bulletin have developed.

Clayton (20) has presented an admirable review of modern adsorption theories so that none will be presented here. Brief reference to a few articles seems necessary in explanation of the hypothesis that is to be proposed for the nature and mechanism of the protective action at the fat globule interface.

In 1916 Langmuir (49) presented the first article directly establishing our modern ideas of the mechanism of adsorption. It is sufficient for the purpose at hand to state that (a) the theoretical concept was stimulated by Bragg's work on crystal structure; (b) Langmuir considered it unnecessary to divide interatomic or intermolecular forces into physical and chemical categories; (c) he considered them all chemical; (d) he classified adsorption, evaporation, surface tension, etc., as chemical phenomena, and (e) he considered that adsorbed substances may be held at surfaces by either primary or secondary valences both of which valence types he considered chemical.

In an abstract of a paper read before the American Chemical

Society, Langmuir (50) extends his concept to include liquids, particularly with regard to surface tension. In this and a later paper (51) it was pointed out that the least active groups of molecules of liquids are directed toward the surfaces of the liquids; active groups are directed toward the center. Films of organic liquids on water orient themselves in such manner that active (polar) groups are attracted to and dissolve in water while the less active (non-polar) portions of the molecules are oriented away from the water unless these groups are so small that they are carried within the water by the active group. Typical active groups are $-\text{COOH}$, $-\text{NH}_2$, $-\text{C} \begin{smallmatrix} \text{=O} \\ \text{—H} \end{smallmatrix}$, $-\text{OH}$, $-\text{CN}$, $>\text{C}=\text{C}<$, etc.

In molecules such as ethyl alcohol and acetic acid the active group causes complete solution of the molecule; with oleic acid the inactive group is sufficiently large and its attraction to itself is so much greater than its affinity for water that it remains as a layer on the water surface. The orienting group (as carboxyl) determines the cross-sectional area of the material at a water interface so that monohydroxy alcoholic esters and saturated fatty acids have like molecular cross-sectional areas, glycerol esters three times the cross-sectional area of the monohydroxy alcoholic esters, while unsaturated fatty acids have larger cross-sectional areas than saturated acids because of the affinity of the unsaturated linkage for water.

When acids are packed closely on a water surface the aliphatic chains stand perpendicularly to the water surface, if further force is applied stress areas form and, Langmuir believes, at a critical pressure a second layer of molecules forms, but not until the single closely packed layer is completed. Such acids closely packed under constant force appear to change phase from liquid to solid. It appears that adsorbed materials may exist in states corresponding to solids, liquids or gases; in a state corresponding to gaseous the dissolved materials obey laws analogous to gas laws.

During the interval between the second and third Langmuir papers, Harkins, Brown and Davies (34) and Harkins, Davies and Clark (35) presented a closely analogous concept of liquid surface structures, surface energy relationships, solubility, adsorption, emulsification, etc., based on surface tension phenomena; an example of one of those coincidences not unusual in chemistry in which simultaneously and from different angles of approach different workers have arrived at the same fundamental concept.

In the former paper (34) it was stated that the occurrence of polar groups ($-\text{OH}$, $-\text{COOH}$, $-\text{C} \begin{smallmatrix} \text{=O} \\ \text{—H} \end{smallmatrix}$, $-\text{NH}_2$, etc.) in organic

molecules increases the solubility greatly when the non-polar radicals are small, and the solubility of these molecules decreases as the length of the organic radical lengthens; the solubility, then, would be the resultant of these opposing factors. The free energy between water and the organic molecule is reduced to a minimum because of the action of the polar group; in opposition to the foregoing, the free energy decrease is less the longer the carbon chain up to a definite length beyond which no further change in free energy occurs although the solubility continues to decrease with increased length of chain.

The free energy decrease as two liquids (one water) approach each other is determined primarily by the polar group and to a lesser degree by the shape and size of the molecule. In the second paper (35) Harkins, Davies and Clark outline certain concepts which apply here. Molecular orientation in liquid surfaces is summarized as follows: "The general law for surfaces seems to be as follows: *If we suppose the structure of the surface of a liquid to be at first the same as that of the interior of the liquid, then the actual surface is always formed by the orientation of the least active portion of the molecule toward the vapor phase, and at any surface or interface the change which occurs is such as to make the transition to the adjacent phase less abrupt.*"

The first layer of molecules at the interface between the water and paraffin derivatives has its polar groups such as $-\text{COOH}$, $-\text{COOM}$, $-\text{COOR}$, $-\text{NH}_2$, $-\text{OH}$ and $>\text{C}=\text{C}<$ oriented toward and dissolved in the water (with the possible exception of $>\text{C}=\text{C}<$ in closely packed chains). In other words if this concept is applied to emulsions, the stability of the dispersed medium is brought about by orientation of molecules of hydrophilic colloids at the interface between the dispersed and dispersing media.

According to the above concepts the surface of the fat globule (in milk) must consist of orientated glycerated ends of fatty acids and free carboxyl groups of fatty acids dissolved in the fat. Whether or not all the hydroxyl groups of all the glyceryl radicals that extend into the water at the interface are satisfied by fatty acid radicals cannot be stated; the theoretical possibility of mono and diglycerides exists although the actual quantities of them is probably small.

This orientation is undoubtedly distorted to some extent by the unsaturated acids since their cross sectional areas are greater than those of the saturated acids because of the attraction of the unsaturated linkage for water. The surface of the fat globule will, therefore, be rough and uneven, presenting a chestnut-burr-like appearance, for the degree to which free fatty acids and glycerides of different acid composition will

extend into the aqueous phase will vary considerably, especially when the types of acids are as diverse in molecular weight as are those of butterfat.

Protective materials will be held by chemical forces of varying degrees of strength at the fat aggregate interface, according to the concept of Langmuir. These are no doubt present as force centers. The nature of such union would, probably, be similar to that postulated by Zsigmondy and Thiessen (113) and by Joël (42), for protective materials much smaller in size than the aggregate which is protected. The protective materials need not be considered symmetrically spaced over the fat globule surface because there is no reason to visualize the chemical force centers evenly distributed, by virtue of the heterogeneity of the composition and form of the fat globule, and the variety of groupings that may be oriented at its surface.

The following hypothesis is presented to explain the mechanism of the protective action at the fat globule interface and to clarify the nature of the materials concerned in the protective action.

Protective materials do not form a continuous membrane at the globule interface, but are held at force centers. Two distinct protective materials exist. One is a phospholipin-protein complex, oriented at the fat side of the interface; it is non-labile because of the affinity of its fatty acid groupings for the fat of the globule and resists removal from the fat-water interface.

The second is oriented in the water side of the interface. It is composed of all the surface tension lowering constituents of the serum but functions in such manner as to indicate that casein is its most important constituent. This material is labile, passing readily from serum to interface and vice versa and is much more easily removed from the fat globule interface than the non-labile material. Of all the labile protective materials, casein functions as the chief protective agent as indicated by certain dairy phenomena. A schematic presentation of this concept is shown in fig. 7.

Figure 7 attempts to portray that (a) the surface of the fat globule is uneven; (b) some groups of the fat globule extend farther into the fat-serum interface or serum than others; (c) the protein-phospholipin may be held by forces which either cause a certain portion of its fatty acid chains to be soluble in the fat surface or else are sufficiently strong to prevent easy removal of the complex from the globule surface despite the fact that they are not strong enough to incorporate it in the fat of butter, and (d) the serum constituents are oriented more or less irregularly at force centers among the hills and valleys

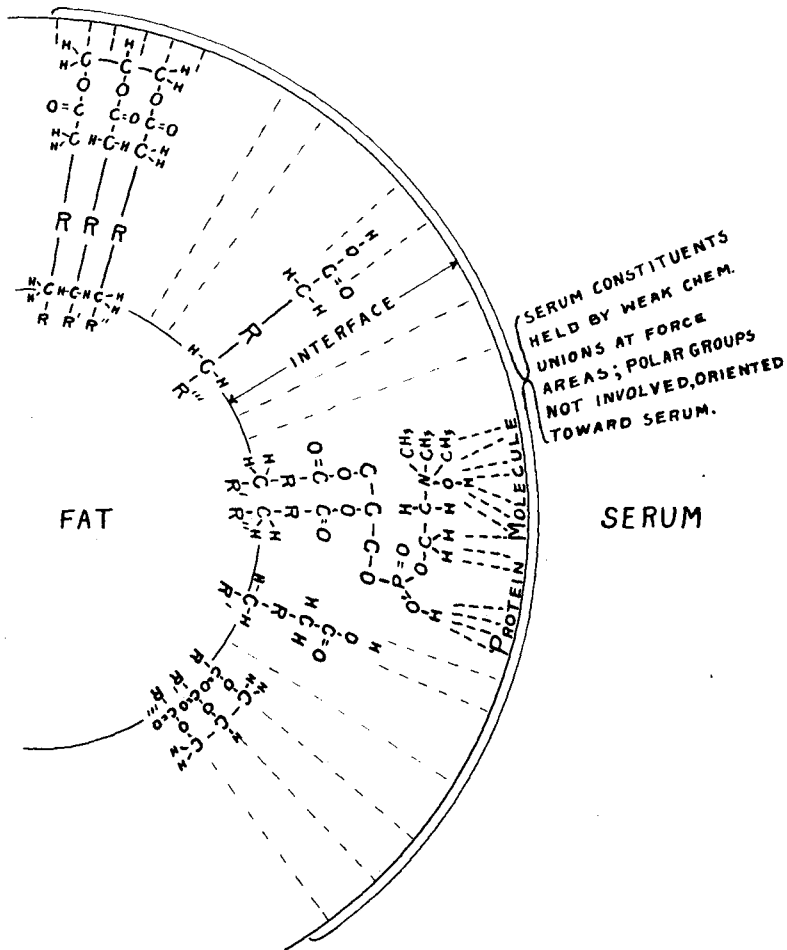


Fig. 7. Concept of the orientation of protective materials in the butterfat globule-serum interface.

around the globule on the water side of the interface. Such a concept is difficult to depict graphically but it is hoped that fig. 7 will aid in clarifying it.

PROTECTIVE MATERIALS ARE NOT IN THE FORM OF A MEMBRANE

If it were considered that a definite protein membrane or a phospholipin-protein membrane existed as an envelope surrounding the fat globule, it would seem that it would be necessary to attribute to this membrane certain of the properties of a cell wall. Were this done it would necessitate considering such

membranes semi-permeable. Semi-permeability would make it difficult to visualize attack on the fat of the fat globule by lipolytic and oxidative enzymes because the generally accepted properties of enzymes would indicate that most of them are not capable of passing through such semi-permeable membranes.

As colloids, enzymes could be a part of a heterogeneous protective system and could readily act on the fat globule. In addition such a non-membraneous concept of the protective materials affords a logical explanation for the protective action at the greatly increased surfaces that are presented when milk or its products are homogenized; it eliminates the necessity of considering that such "membranes" have sufficient elasticity to permit covering the considerably larger surfaces exposed after homogenization by the same quantity of protective material that was associated with the fat surfaces before homogenization.

NON-LABILE PROTECTIVE MATERIAL ORIENTED ON THE FAT SIDE
OF THE INTERFACE

Storch (98) showed that cream treated with water and then separated retained protein materials at the fat interfaces after four or five such treatments. Hattori (36) has shown that when cream was treated with chloroform saturated water, a procedure which increased the globule density and facilitated its separation, a protein remained associated with the fat globules. Hattori and Ogimura (38) washed the fat globules from the acid or rennet casein coagulum of milk with ether saturated water and showed that the protein previously reported was associated with the fat globules.

Palmer and Samuelson (71) used separation methods analogous to Storch's and found that a material composed of protein and phospholipin remained associated with the fat. Palmer and Wiese (73) used the same technique and found that the material, which could not be removed from the globule by repeated dilution with water followed by separation, was a protein-phospholipin complex with an isoelectric point of pH 3.9 to 4.0. Rimpila and Palmer (83) question the possibility of a chemical union between protein and phospholipin in the complex.

Josephson and Dahle (44) have shown that ice cream mixes made from washed creams had whipping times equivalent to those from normal cream; mixes from butter emulsified with washed cream membrane suspension whipped comparably to those prepared with cream, but butter mixes to which the same amount of membrane suspension (as was emulsified with the butter in the previous experiment) was added, had longer whipping times than did those made with

cream. Similarly, butter mixes in which butter was first emulsified with buttermilk or skimmilk had longer whipping times than normal cream mixes; butter oil, emulsified with egg yolk before incorporation in the mix, whipped normally whereas butter oil emulsified with egg or milk phospholipins before incorporation in the mix had longer whipping times.

The above résumé indicates that a protective material is practically irreversibly associated with the fat globule interfaces, that this material is a protein-phospholipin complex and that to function normally the phospholipin-protein complex must associate itself with the newly formed fat surface before the fat globule comes in contact with the milk serum. This would suggest the association of cell protein-phospholipin complexes with the "nascent" fat globules at the time of their formation. Were this type of association assumed it would explain the fact that the protein in the complex has been reported by Palmer and his associates and by Hattori as unlike any in milk. Such an association of cell phospholipin-protein complexes is logical since it is known that these are definitely a part of the cell structure, persisting to death in cases of emaciation resulting from starvation.

A phospholipin alone associated on the fat side of the interface should be included in the fat phase of butter. It has already been pointed out (7) that (a) despite the existence of phospholipins in butter, the data in the literature show that it is not found in filtered fat from butter, and (b) the phospholipins of milk seem to be in combination with protein. Moreover, Macheboeuf and Sandor (55) indicate that an alcohol treatment is necessary in the extraction of phospholipins to free them from proteins to which they are weakly bound chemically. The combination functions as a highly hydrophilic body insoluble in ether. These factors together with the isoelectric point data of Palmer and Wiese and the whipping effects of mixes made from butter that had been emulsified with milk or egg phospholipins before having been incorporated into the mix, indicate that this non-labile material is a protein-phospholipin complex.

PRESENCE OF PROTECTIVE MATERIALS IN ADDITION TO THE PROTEIN-PHOSPHOLIPIN COMPLEX

It has been pointed out that the isoelectric point of an emulsoid is largely determined by the nature of the protective materials at the interfaces between the dispersed and dispersing media. Mohr and Brockman (60) have shown that butterfat-water emulsions are isoelectric at pH 2.9; in diluted milk the fat globule was isoelectric at pH 4.34 and in water-butterfat-casein emulsions at pH 4.44. Jack and Dahle (41) give values

for butterfat isoelectric points as: pH 3.2 in butteroil-water emulsions; pH 4.7, in butteroil-3 percent casein sol emulsions; pH 4.6, in butteroil-casein sol emulsions to which phospholipins, lactose and milk salts were added, pH 4.3 in butteroil-phospholipin sol emulsions to which casein, lactose and milk salts were added and pH 2.0 for butteroil phospholipin sols.

Mommsen (64) showed that the isoelectric point of the fat globule was changed from pH 4.11 to 4.22 by homogenization which indicates that an increase in the ratio of labile and non-labile protective materials causes the isoelectric point to shift to the alkaline side. The isoelectric point of the protein-phospholipin complex is at pH 3.9 to 4.0 (73) so that were it the only protective agent the isoelectric point of the fat globule should be at or below this value. It would appear, therefore, that there is little doubt but that proteins other than those in the protein-phospholipin complex are involved.

In this connection, Palmer and Wiese (73) attribute the higher value obtained for the fat globule isoelectric point, than for the isolated protein-phospholipin complex, to be caused "either by the presence of divalent cations or to the presence of other milk proteins in the outer shell of interfacial material." The trends of the churning losses presented in this paper are in agreement with the idea that protective materials other than the protein-phospholipin complex play an important role in the protective action at the fat globule-serum interface.

POSSIBILITY OF CASEIN AS AN IMPORTANT PROTECTIVE MATERIAL AT THE MILKFAT GLOBULE INTERFACE

The studies of Völtz (107) and of Abderhalden and Völtz (1) show that milk serum constituents are sufficiently closely associated with the fat globule interface to rise with the globules through long water columns. They considered the material to be casein with other serum components and thought that it was labile and variable in composition from one globule to another. Titus, Sommer and Hart (104) reported material similarly obtained to be casein. Their studies indicate that casein and perhaps other constituents of the serum are present in the interface and probably exercise protective action.

It has been pointed out in the preceding section that serum constituents undoubtedly affect the fat globule isoelectric point. The magnitudes of the values reported by several workers (pH 4.10 to 4.34) are sufficiently close to the value for casein-milkfat-water emulsions (pH 4.44; pH 4.7) that it seems logical that casein plays an important part in determining these values.

Hattori (37) precipitated casein with acetic acid, rennet, copper sulfate "nach Ritthausen," mercuric chloride and hydro-

chloric acid, calcium chloride and potassium alum. In all cases except that of copper sulfate he was able to wash from 70 to 88 percent of the fat from the curd while with copper sulfate very little of the fat could be removed.

In this connection data (as yet unpublished) obtained in these laboratories show that a zero fat test is obtained for the glacial-acetic-sulfuric acid method for ice cream analysis with samples preserved with formalin; the necks of the bottles contain what appears to be a curd layer of the approximate depth that the fat column should be. It is impossible to state whether, in both instances cited, the casein at the globule surface is irreversibly denaturated, so that in one case it will not free the fat globules from the coagulum, while in the other the acetic and sulfuric acids cannot decompose it (under the conditions of the test). However, the data do point in this direction.

Guthrie and Sharp (33) show that the nature of the churning time curves for "twice washed" and "unwashed" creams are not at all similar in the experimental set-up which they employed. Their washed cream curve shows that the churning time ratio increases steadily from a pH approximating 7 to a pH about 3.8 to 4.0 (cf. isoelectric point of phospholipin-protein complex) and then decreases steadily to a pH about 1.0. The unwashed cream curve, on the contrary, shows a decreasing churning time ratio from about pH 6.6 to pH 4.0 to 4.2 (cf. isoelectric point of fat globule); it then rises until a pH 3.3 to 3.4 is attained, after which it drops to a pH of 1.0 and becomes asymptotic to the abscissa in this region. Unfortunately there are no data points between pH 4.5 to 4.6 and pH 5.2.

St. Johnston's (96) surface tension curves⁴ for casein show maxima near pH 4.62 and pH 3.4 and a minimum near 3.8-3.9 with HCl-NaOH-water-casein systems. With H_3PO_4 -water casein systems a maximum occurs near pH 3.3 to 3.4 and a minimum near pH 3.9; this graph was not extended into the alkaline region beyond pH 4.1 to 4.2. These data correlate well with those of Guthrie and Sharp for unwashed cream in that the points of change in function for the surface tension curves of casein and the loss-ratio curves for unwashed cream agree rather well.

Data presented in this bulletin indicate that casein is of considerable importance in the churning process. They show that the losses (calculated as percentage of the total fat) are relatively constant from pH 6.5 to pH 5.5. At this point (5.5) there is either a tendency or a definite trend for them to increase as the pH decreases to pH 4.8 to 4.9 at which point (within the casein isoelectric point range) a very marked and abrupt

⁴ Figure 1, p. 1,318, of this article.

change of function in the curves occurs followed by a linear drop in loss that is rather great in a narrow pH range.

These data (fig. 3) agree very well with the electrokinetic potentials of Sommer and North (fig. 5) as regards points of inflection. In the region from pH 5.5 to 4.8 it might be expected that the losses should drop as the electrokinetic potential increases, whereas they increase, just as the casein, surface-tension curves of St. Johnston (96) do in the same region. The marked linear drop on the acid side of the maximum at pH 4.8 to 4.9 accompanies a sharp rise in interfacial potential; this must result in large measure from change in casein potential, for van Dam and Holwerda (22) have shown that in the neighborhood of pH 4.5 to 4.6 an increase in the quantity of suspended casein is accompanied by a decrease in the fat loss.

It is likewise of interest that the viscosity curves of Tapernoux and Vuillaume (101) show the viscosity of skim-milk to be practically constant in the acidity range 17° to 60° Dornic (1.7 to 6.0 gm. lactic acid per liter); in the vicinity 60° to 65° Dornic the viscosity started to rise and became infinite at the coagulation point, which was approximately 70° when the milk was soured with culture. The region of lack of change and of subsequent rise to the isoelectric point agrees well with the loss curves (fig. 3); there is good correlation among the ranges of little change of the losses, the electrokinetic potentials and the skim-milk viscosities.

It would appear that casein is not altered sufficiently to affect its protective action until the medium becomes more acid than a pH of approximately 5.5. In this connection Button and Levowitz (15) studied interfacial tensions between casein and Nujol. The casein concentrations ranged from 0.00317 to 4.00 percent and the pH range was from 6.0 to 7.8. Any concentration of casein above 0.00625 percent exerted a "very definite diminution of interfacial tension." Increased casein concentrations permitted more rapid establishment of equilibrium conditions, but the lowering was independent of the concentration above 0.00625 percent. The pH had no effect on the equilibrium in the range studied.

CHURNING PROCESS IN THE LIGHT OF THE FOREGOING HYPOTHESIS

Foaming or whipping of cream is associated with the churning process. In fact, Rahn (81, 82) has evolved a churning theory based on the fact that a special protein causes cream to foam and that the fat globules migrate into the foam interfaces, lie closely together because of surface tension forces and gather in small butter clumps. The foam membranes become solid, the

foam breaks and the particles of fat gather as butter. Button (14) believes that casein is the important constituent in foam formation in obtaining overrun in ice cream. Although this does not agree with the ideas advanced by Josephson and Dahle (44) it is not illogical when the persistent foams of skim milk are considered.

It was pointed out (8) that when creams varying from 20 to 40 percent fat were churned, the losses (calculated as percentage of the total fat) indicate changes in phase relationships among the colloidal systems of cream; at least a marked change in function occurs between 30 and 35 percent fat in the cream when the losses are plotted against the percentage fat in the cream. Josephson and Dahle (44) show that as the cream used in preparing mixes increased in fat content from 37 to 80 percent the whipping time of mixes increased. The cc. "oiling-off" per 50 cc. of mix was zero from 37 to and including 55 percent cream; above this point the "oiling off" of the mixes increased from 4.3 cc. at 65 percent cream to 7.0 cc. at 80 percent cream. This oiling off apparently results from a destabilization of the richer creams.

If a change in the relationships among the colloidal systems of cream does exist, it is logical to assume that the ratio of labile to non-labile materials can become small enough to decrease the stability of cream to a marked extent. The data of Josephson and Dahle indicate that there is a possibility that a critical point above which stability decreases with rise in fat percentage and below which creams are relatively stable may occur between 55 and 65 percent fat in the cream. Moreover, Jack and Dahle (41) state that "the values found by analysis and synthetic studies suggested that 60-65 percent cream is composed of the fat globule plus its entire absorbed membrane."

With such an assumption it is possible to consider that as cream foams, greater portions of the labile protective materials serve to stabilize the foams thus decreasing the ratio labile to non-labile protective materials at the fat globule interface. The fat globules packed in the foam lamellae will gradually increase the fat percentage of these films. The ease of coalition of the globules should increase with decreased labile to non-labile protective material ratio, with increased numbers of globules per unit volume of water and with closer packing of globules.

When the globules are sufficiently closely packed and the labile materials are reduced to a point at which a condition analagous to creams richer than 60 or 65 percent exists, the attraction of the portions of the globules at which the non-labile

protective materials are not attached and from which the labile materials have migrated assists the forces of churning in combining the fat globules.

Because no dilution of serum proteins other than that caused by migration into foam lamellae has occurred, the non-labile materials no doubt retain their full quotas of hydrophilic (protein especially) groups. The attraction of these aggregates for water together with a rearrangement of the chemical forces (which have held them to the fat globules), when these globules coalesce and lose their identity, releases the non-labile materials from the fat and causes them to become a part of the serum.

Whether this hypothesis is a precise explanation of the protective mechanism of the milkfat globule, time and further experimental work must prove. The materials presented from ~~the researches of other workers do correlate with the results~~ presented in this bulletin to the extent of indicating that there is a possibility that it may be the correct explanation. If it is, then the visualized picture of the churning process may likewise be valid.

The authors are in agreement with Josephson and Dahle in that a precise understanding of the mechanism of the protective action at the milkfat globule interface would be a great help in solving certain of the problems of the dairy industry. It is hoped that, even though this explanation may not be the correct one, it may serve as a stimulus toward the complete understanding of the protective action in milk.

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