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Cultured Buttermilk from Non-Fat Dry Milk Solids

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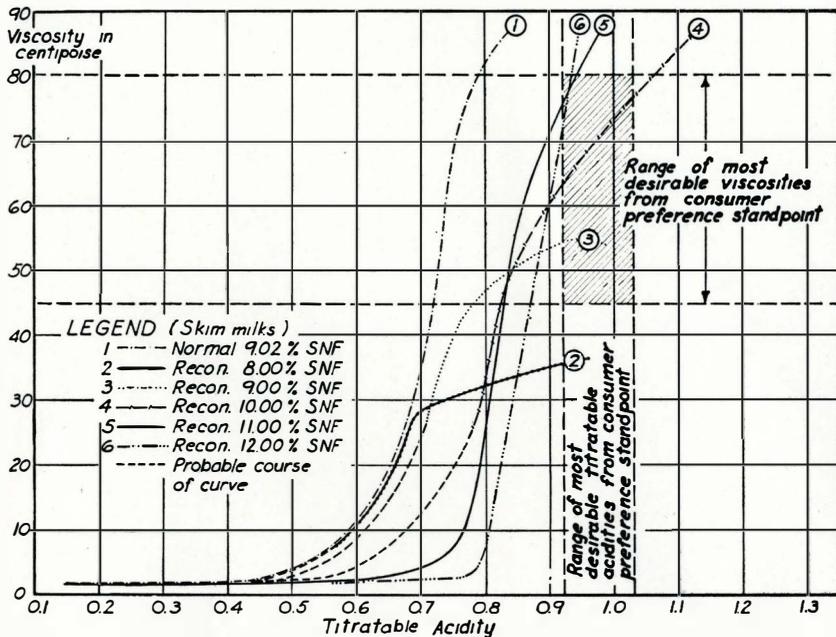
Cultured Buttermilk

from

Non-Fat Dry Milk Solids

by

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Cultured Buttermilk*

From Non-Fat Dry Milk Solids

ALVIN A. SCHOCK *and* D. F. BREAZEAL

Introduction

During the late summer and fall months, milk production in South Dakota and areas of neighboring states is considerably below the seasonal peak reached in the April-June period. A survey by D. L. R. Hansen, Assistant Extension Dairyman of South Dakota State College, in 1947 showed that milk production in South Dakota was 40 per cent less during the short season than during the summer flush period. This extreme reduction in milk production during the late summer and fall months has necessitated the importation of considerable quantities of milk into this area from areas of greater and more uniform milk production.

Since fluid milk receives first sales priority, there is little surplus milk available for the manufacture of by-products such as cultured buttermilk. This results in considerable loss of business volume to milk plant operators. More serious, however, is the fact that this highly nutritious product is unavailable to the consumer. Consumers, especially older people, desire cultured buttermilk for healthful consumption in the fluid state. In addition, it is used in the preparation of delicious food products like griddle cakes and pastry products.

The preparation of cultured buttermilk on a seasonal basis is very unsatis-

factory, and for the most part, an unprofitable practice. Unless consumers can obtain this product regularly, sales suffer and its merits as a money maker are overlooked. Thus, if the sale of cultured buttermilk is to prove profitable for the dairy plant, it must be made available to the consumer at all times. This is not possible in many cases in this area if the dairy plant depends entirely upon the local milk supply.

During the recent war, many dairy drying plants were established to produce concentrated milk products such as non-fat dry milk solids (NFMS). The demand for the latter product out-distanced the supply during the war and still continues good. However, with the expected lessening demand abroad, the creation of new outlets for NFMS is important.

The manufacture of cultured buttermilk from NFMS not only creates an outlet for this product, but also offers a solution to the dairy plant which cannot manufacture cultured buttermilk for lack of sufficient milk obtainable from local producers.

*The name "Cultured Buttermilk" is a misnomer. Practically all cultured buttermilk marketed today is made from a skim milk base with or without added butterfat. The name "Cultured Buttermilk" is used in this discussion to avoid confusion which might arise by the use of another name. More nearly correct names would include "Cultured Milk," "Cultured Skim Milk," or "Cultured Dairy Drink."

Literature Review

Larsen and White (16), in a limited study on the use of milk powder for starter making in creameries, reported as early as 1910 that milk powder was a suitable substitute for natural skim milk in creameries where skim milk was not easily obtainable. In 1930 Reid and

Welch (22) in a study of the factors influencing the properties of cultured buttermilk made from reconstituted milk made the following observations: (1) a fermented milk of desirable quality and characterized by a body and texture that was consistently very smooth could be

made from reconstructed skim milk; (2) a pasteurization temperature above 180° F. for longer than 30 minutes decreased the flavor score by causing a powder taste and odor to become apparent; (3) increasing the quantity of starter inoculum up to 10 per cent gave the cultured buttermilk a more desirable flavor, aroma, body, and texture; (4) the addition of normal skim milk to reconstructed milk increased the desirability of the fermented milk at the time of breaking the curd; (5) the addition of 0.35 per cent gelatin improved the body and texture of the fermented milk; and (6) storage of the fermented milk made from reconstituted skim milk at a temperature of 39° F. caused no deterioration in the quality of the product in five days.

In 1933, Alldredge and Burke (1) studied the use of NFMS in the manufacture of cultured buttermilk. Among the observations made by these workers were the following: (1) a total solids content in the reconstructed skim milk of 10.0 per cent (± 0.2 per cent) was found most desirable from the standpoint of producing a desirable flavor, body, and texture; (2) the buttermilk should be cooled to 50° F. or lower prior to breaking; (3) an acidity of 0.95 to 1.00 per cent in the finished buttermilk was most satisfactory from the standpoint of flavor; (4) the flavor improved and the slightly heated taste noticeable in the freshly made product practically disappeared upon holding in cold storage for 24 hours; (5) the incorporation of air by vigorous agitation was responsible for many major defects of cultured buttermilk, especially wheying-off; (6) the addition of small quantities of NFMS to normal skim milk prior to fermentation increased the viscosity and improved the flavor and consistency of the resulting cultured buttermilk; and (7) cultured buttermilk of a quality practically equal

to and in some respects superior to that prepared from normal skim milk could be made by using NFMS.

Although the work of the above mentioned research workers indicated that cultured buttermilk of relatively good flavor, body and texture could be made using NFMS, the use of NFMS in the preparation of this product has not been as widely adopted by the industry as was expected. To a large extent this probably can be attributed to the fact that the NFMS until recent years were of inferior quality as compared to those currently available. As recently as 1941 Burke (4) stated in effect that if he were called upon to make a choice as to the type of milk to use in preparing cultured buttermilk he would list them in the following order:

1. Whole milk containing about 4 per cent fat.
2. Part skim milk of 1.5 to 2 per cent fat.
3. Skim milk to which cream is later added.
4. Reconstructed skim milk.
5. Condensed milk.

In the present study, several factors deemed important to the production of cultured buttermilk from NFMS were studied. Primary emphasis was placed on the development of a product possessing a pleasing flavor and aroma and desirable body and texture qualities. Circumstances necessitated the termination of this project at an earlier date than had been planned originally and consequently some phases are not as complete as might be desired. On the basis of the research findings recorded in this publication, the College Creamery has successfully marketed, for a period of over six months, cultured buttermilk prepared from NFMS. During this period its sales have increased steadily and not a single consumer complaint has been received.

Flavor Studies

Michaelian et al. (17) showed that considerable acetylmethylcarbinol plus diacetyl ($amc + ac_2$) was present in butter cultures possessing satisfactory flavors and aromas and that only small amounts (or none) were present in cultures lacking in flavor and aroma. Satisfactory cultures yielded 10.0 to 39.5 mg. of nickel dimethylglyoxime equivalent to the $amc + ac_2$ in a 200 gm. sample. The maximum for cultures lacking in flavor and aroma was 7.4 mg. The observations of Michaelian et al have been repeatedly confirmed by other research workers and in plant practice. Recently, however, Rodenkircken (24) made the observation that the flavor quality of cultured milk did not run parallel with the $amc + ac_2$ content.

Procedure

Reconstituted skim milks of varying percentages of solids-not-fat (SNF) content were prepared by reconstituting in distilled water. Reconstitution was effected by adding the NFMS to the water at 70°—80° F. and dispersing at high speed (approx. 600—700 r.p.m.) agitation using an electrically driven stirrer. All samples were then pasteurized along with normal skim milk "controls" at

180° F. for 30 minutes in a laboratory pasteurizer. Following cooling to 70° F. the samples were inoculated with 1 per cent of active starter culture and incubated from 14 to 16 hours at 70° F.

Immediately following incubation, all samples were rapidly cooled to below 40° F. At selected intervals the $amc + ac_2$ was determined by the steam distillation method employed by Michaelian and Hammer (18). All results are expressed as the weight in milligrams of nickel dimethylglyoxime (NDMG) equivalent to the $amc + ac_2$ per 200 gm. of cultured buttermilk.

Titratable acidity determinations were made using 0.1 N sodium hydroxide and four drops of one per cent alcoholic solution of phenolphthalein indicator in a nine gm. sample. All acidities are expressed as per cent lactic acid. The total solids content of the skim milks was determined by the method of Mojonier (19).

Results

Several trials were made using reconstituted skim milks of varying SNF content and representing several different lots of NFMS from two large Minnesota drying plants.

Table 1. Production of Acetylmethylcarbinol plus Diacetyl in Normal and Reconstituted Skim Milks of Different SNF Content. (Culture No. 1 used)

Description of sample (skim milks)	Acidity as % lactic acid		Mg. of nickel dimethylglyoxime equivalent to $amc + ac_2$ per 200 gm. of culture			
	Initial	After 14 hours	Hours following end of incubation*			
			0	48	72	96
Normal 8.98% SNF	0.160	0.840	19.60	33.10	-----	17.80
Reconstituted 8.98% SNF	0.150	0.800	12.50	24.50	-----	17.00
Reconstituted 8.5% SNF	0.140	0.770	5.80	15.20	9.50	-----
Reconstituted 9.0% SNF	0.145	0.760	5.30	23.50	-----	15.00
Reconstituted 9.5% SNF	0.160	0.840	5.90	12.90	8.80	-----
Reconstituted 10.0% SNF	0.170	0.870	4.40	20.80	-----	14.10
Reconstituted 10.5% SNF	0.175	0.900	6.70	10.40	8.60	-----
Reconstituted 11.0% SNF	0.190	0.920	8.40	23.30	-----	13.20
Reconstituted 11.5% SNF	0.200	0.940	7.00	13.50	6.60	-----
Half normal, Half reconstitut. 8.98% SNF	0.160	0.830	12.00	25.40	-----	12.30

*Following incubation cultures were cooled rapidly in ice water and stored in refrigerator at approximately 35° F.

Trial 1. The results obtained in trial 1 as shown in Table 1 were somewhat inconsistent and for the most part discouraging. The culture made from normal skim milk had a pleasing flavor and aroma following storage in the refrigerator for 24 and 48 hours after incubation. To a slightly lesser degree this also was true of the culture prepared from half normal and half reconstituted skim milk (8.98 per cent final SNF concentration). All of the cultures prepared from reconstituted skim milks possessed definite powder and tallowy type off-flavors regardless of the SNF concentration or $\text{amc} + \text{ac}_2$ content. This flavor defect was so pronounced that all of the cultures had to be considered unfit for consumption.

During the course of trial 1 it was observed that ice cream made from mix containing this same lot of NFMS also possessed a rather distinct powder and tallowy type off-flavor. Upon checking the powder it was discovered that it had a stale, tallowy off-flavor. An analysis of the powder revealed that it had a moisture content of 2.80 per cent, a solubility index of 0.15, and a fat content of 3.03 per cent. Obviously the high fat content was the reason for the flavor deterioration of this lot of powder.

Mention is made of the above experience to emphasize the need for good quality NFMS in the preparation of cul-

tured buttermilk. In purchasing NFMS **extra grade** should be specified on the purchase order.

The results in Table 1 failed to show a direct relationship between the flavor and aroma and the $\text{amc} + \text{ac}_2$ content of the cultures. This is in accord with the findings of Rodenkircken (24), however, Hammer and Babel (9) have observed that in the presence of distinct off-flavors the delicate starter aroma imparted by diacetyl may be so completely masked as to be imperceptible.

The production of $\text{amc} + \text{ac}_2$ in the reconstituted skim milks did not equal the amounts produced in normal skim milk or in the culture prepared from equal amounts or normal and reconstituted skim milk. This is a rather interesting observation and in general was borne out in all succeeding trials but generally to a lesser degree. No work was done to secure an explanation for these observations. It was observed, however, in later trials that the addition of citric acid or sodium citrate to the reconstituted skim milk permitted the development of increased quantities of $\text{amc} + \text{ac}_2$ equalling or exceeding amounts present in cultures prepared from normal skim milk.

Trial 2. In this trial the procedure was the same as in trial 1 with the exception that a new lot of powder was obtained

Table 2. Production of Acetylmethylcarbinol plus Diacetyl in Normal and Reconstituted Skim Milks of Different SNF Content. (Culture No. 1 used)

Description of Sample (skim milks)	Acidity % lactic acid Initial	After 14 hours	Mg. of nickel dimethylglyoxime equivalent to $\text{amc} + \text{ac}_2$ per 200 gm. of culture		
			Hours following end of incubation* 0	48	96
Normal 8.76% SNF	0.165	0.825	22.20	24.30	21.00
Reconstituted 8.50% SNF	0.120	0.810	12.40	16.60	14.60
Reconstituted 9.00% SNF	0.140	0.850	11.10	16.10	15.60
Reconstituted 10.00% SNF	0.150	0.900	8.40	15.00	13.20
Reconstituted 11.00% SNF	0.170	0.960	11.90	14.30	14.00
Half normal, Half reconstituted 8.76% SNF	0.140	0.830	13.70	19.70	17.90
Reconstituted 9% SNF + 0.15% sodium citrate	0.125	0.825	19.00	23.40	17.00

*Following incubation cultures were cooled rapidly in ice water and stored in refrigerator at approximately 35° F.

which was of excellent quality. The results are recorded in Table 2.

The results in Table 2 show that in all cases the $\text{amc} + \text{ac}_2$ content of the cultures made from reconstituted skim milks was higher than those secured in trial 1 but in no case was it as high as in the culture made from normal skim milk. Some of the increase in $\text{amc} + \text{ac}_2$ over the cultures in trial 1 was undoubtedly due to the slightly higher acidities at the close of the incubation period (see page 6).

The $\text{amc} + \text{ac}_2$ following storage of the ripened cultures made from reconstituted skim milk for 48 and 96 hours in the refrigerator paralleled closely the values obtained with the poor quality NFMS powder used in trial 1. The flavor and aroma of all of the cultures was, however, considerably improved, but still

possessed slight to distinct (most distinct in cultures containing 8.5 and 11 per cent SNF) powder type flavors at the end of the incubation period. These defects largely disappeared on storage for 24 to 48 hours in a refrigerator and there was marked improvement in the flavor and aroma of all cultures. The improvement in flavor appeared to parallel the increased $\text{amc} + \text{ac}_2$ content of the cultures. This is in accord with the observations of Michaelian et al. (17) and Hammer and Babel (9).

The addition of 0.15 per cent sodium citrate to the reconstituted skim milk containing 9 per cent SNF resulted in a marked increase in the quantity of $\text{amc} + \text{ac}_2$ produced. The flavor was also considerably improved. These observations agreed with those made by Ritter and Christen (23) Ruehe (25), Prill and Hammer (21), and Nelson (20).

Effect of Addition of Sodium Citrate on Flavor and Aroma of Cultures Made from Reconstituted Skim Milk

To further ascertain the beneficial effects of added citrates, several experimental trials were conducted. Typical of the results obtained are those listed in Table 3.

The results in Table 3 indicate rather

conclusively that citrates, either in the form of citric acid or sodium citrate, are highly effective in increasing the amounts of $\text{amc} + \text{ac}_2$ produced in the cultures made from reconstituted skim milk. The increased quantities of $\text{amc} +$

Table 3. Production of Acetylmethylcarbinol plus Diacetyl in Normal and Reconstituted Skim Milks Containing Added Citrates

Sample number and description (skim milks)	Acidity as % lactic acid		Mg. of nickel dimethylglyoxime equivalent to $\text{amc} + \text{ac}_2$ per 200 gm. of culture		
	Initial	After 16 hours	0	48	96
No. 1 Normal 8.82% SNF	0.165	0.845	21.80	25.10	21.80
No. 2 Reconstituted 10.0% SNF	0.170	0.930	9.40	21.70	12.90
No. 3 Reconstituted 10% SNF plus 0.15% citric acid	0.220	0.920	14.50	31.30	25.80
No. 4 Reconstituted 10% SNF plus 0.15% sodium citrate	0.170	0.900	15.40	30.20	19.60
No. 5 Normal 8.82% SNF	0.160	0.900	15.00	17.70	14.00
No. 6 Reconstituted 10.0% SNF	0.170	1.040	14.70	15.20	3.00
No. 7 Reconstituted 10% SNF plus 0.15% citric acid	0.220	1.010	11.50	20.30	15.60
No. 8 Reconstituted 10% SNF plus 0.15% sodium citrate	0.170	1.040	15.00	18.20	14.20

*Following incubation, cultures were cooled rapidly in ice water and stored in refrigerator at approximately 35° F. Samples 1-4 inclusive were inoculated with Culture No. 1. Samples 5-8 inclusive were inoculated with Culture No. 2.

ac_2 were manifested in improved flavor and aromas, especially following storage for 24 to 48 hours after ripening. Seldom, however, was a typical powder flavor entirely absent.

Mother culture No. 2, a commercial lactic flavor culture used in inoculating samples 5-8, inclusive, was not as satisfactory from the standpoint of producing pleasing flavors and aromas as was mother culture No. 1. Presumably this can be attributed to its inability to produce amounts of $amc + ac_2$ equalling those of the latter.

All cultures prepared from reconstituted skim milks and containing added citrates produced considerable quantities of carbon dioxide. The carbon dioxide produced gave the ripened cultures a desirable flavor and tended to aid in covering the powder flavor initially present. This could be demonstrated readily by placing a quantity of the culture in a suction flask and removing the dissolved gases by use of a vacuum pump. The degassed cultures had a distinctly flat flavor and the powder flavor was again more noticeable.

Effect of Addition of Butterfat on Flavor and Aroma of Cultured Buttermilk from Reconstituted Skim Milk

The presence of butterfat in cultured buttermilk made from normal skim milk is desirable from the standpoint of improved flavor and aroma of the finished product. Several trials in the present

study indicated that this was also true when cultured buttermilk was prepared from reconstituted skim milk. Typical of the results obtained are those in Table 4.

Table 4. Acetylmethylcarbinol plus Diacetyl and Flavor Scores of Cultured Buttermilks made from Normal and Reconstituted Skim Milks Containing Added Citrates and Butterfat (Culture No. 1 used)*

Sample Number and description	Acidity as % lactic acid		Flavor score†				Mg. of nickel dimethylglyoxime equivalent to $amc + ac_2$ per 200 gm. of culture			
	Initial	After 16 hrs.	0	Hours after incubation			0	Hours after incubation		
				24	48	72		24	72	92
No. 1 Normal skim 9.1% SNF	0.155	0.780	35	37	36	35	10.60	27.00	18.40	18.00
No. 2 Normal skim 9.1% SNF and 2% fat	0.155	0.790	36	38	38	38	7.90	24.30	21.10	18.70
No. 3 Normal skim 9.1% SNF and 2% fat plus 0.15% sodium citrate	0.150	0.810	37	38	39	39	17.00	31.90	34.80	35.70
No. 4 Reconstituted 10% SNF	0.170	0.820	34	34	33	30	4.40	10.20	9.30	9.30
No. 5 Reconstituted 10% SNF and 2% fat	0.170	0.920	38	40	37	37	9.80	13.70	14.10	6.30
No. 6 Reconstituted 10% SNF and 2% fat plus 0.15% sodium citrate	0.165	0.940	40	41	39	37	17.40	32.40	34.00	13.60

*Butterfat was added in form of freshly separated sweet cream before pasteurization.

†Perfect score 45. A score of 40 represents a culture which is pleasing, mildly acid, smooth and full of delicate starter aroma. Values given represent average score of three competent judges.

The data in Table 4 clearly demonstrate the beneficial effect that the added butterfat had on the flavor scores of the cultures made from both normal and reconstituted skim milks. This effect was accentuated when the cultures contained

added sodium citrate. None of the judges was able to detect a powder flavor in sample No. 6, the cultured buttermilk made from 10 per cent SNF reconstituted skim milk containing two per cent butterfat and 0.15 per cent of added

sodium citrate. This is a rather interesting observation and in general was confirmed in subsequent experimental trials.

Samples No. 1 and No. 2 were criticized for lacking in acidity and also slightly in flavor. Sample No. 4 did not develop normally. It lacked in acidity

and possessed a very definite and objectionable powder flavor. As will be pointed out below, the development of proper amounts of acidity is extremely important to the production of cultured buttermilk possessing a pleasing flavor and aroma from reconstituted skim milk.

Titrateable Acidity and Flavor Production

Early in the present experiment it was observed that the cultures made from reconstituted skim milk possessing the most pleasing flavors and aromas had moderately high acidities. This observation was in accord with that made by Allredge and Burke (1). In search for an explanation for this observation an ex-

periment was set up to ascertain whether there existed a relationship between the titrateable acidity and the production of $amc + ac_2$. The results of this experiment are summarized in Table 5.

The results in Table 5 show that low titrateable acidities are not conducive to the production of large quantities of

Table 5. Relationship Between Production of Acetylmethylcarbinol plus Diacetyl and Titrateable Acidity in Cultured Buttermilks Prepared from Normal and Reconstituted Skim Milks

Sample number and description	Incubation time hours	Acidity as % lactic acid	Mg. of nickel dimethylglyoxime equivalent to $amc + ac_2$ per 200 gm. of culture		
			Hours after incubation		
			0	48	72
No. 1 Normal 8.86% SNF	14	0.73	0	5.1	9.5
No. 2 Normal 8.86% SNF	15	0.79	3.1	14.9	16.7
No. 3 Normal 8.86% SNF	16	0.85	21.8	25.1	21.8
No. 4 Reconstituted 10% SNF	14	0.90	8.4	15.0	13.2
No. 5 Reconstituted 10% SNF	15	0.94	9.4	21.7	12.9
No. 6 Reconstituted 10% SNF	16	1.05	14.7	25.2	13.0

$amc + ac_2$. Ripening the cultures to moderately high acidities resulted in a marked increase in the quantity of $amc + ac_2$ produced. This was true for cultures made either from normal or reconstituted skim milks. There was, however, one important difference. The marked increase in $amc + ac_2$ observed in the cultures made from reconstituted skim milk occurred at higher titrateable acidities than was true in case of the cultures made from normal skim milk.

The above observations on the relationship between titrateable acidity and the production of $amc + ac_2$ in cultures

made from reconstituted skim milks parallel similar observations of cultures made from normal skim milk (17, 2) Barnicoat (2) reported that with a commercial culture in normal skim milk the amount of $amc + ac_2$ at 0.75 per cent acidity was only about one-third that at 0.81 per cent.

In view of the current findings it must be concluded that the beneficial flavor effects of moderately high acidities in cultured buttermilk made from reconstituted skim milks were due to the increased quantities of $amc + ac_2$ and carbon dioxide produced at these higher acidities.

Relationship Between Titratable Acidity of Cultured Buttermilk and Consumer Preference

In an attempt to determine the amount of acidity in cultured buttermilk most acceptable from the consumer preference standpoint, 15 lots of cultured buttermilk were made from reconstituted skim milk containing 10 per cent SNF. All cultures were incubated for varying periods of time at 70° F. to obtain considerable variation in titratable acidities. Following incubation all cultures were placed in the refrigerator for 24 hours prior to scoring and making $\text{amc} + \text{ac}_2$ determinations. Five judges, members of the dairy department, were asked to select the cultures possessing the most desirable flavor and aroma and to rate the cultures as acceptable or unacceptable. The

cultures uniformly acceptable to all the judges had titratable acidities ranging from 0.92 to 1.03 per cent. The two cultures considered best had titratable acidities of 1.00 and 1.03 per cent. The $\text{amc} + \text{ac}_2$ content of these two cultures at the time of scoring was 30.2 mg. and 27.8 mg., respectively. All cultures having acidities in excess of 1.05 or below 0.90 per cent were for the most part criticized as possessing either too much or insufficient acid.

The relationships between titratable acidity, viscosity and consumer preference of cultured buttermilks made from normal and reconstituted skim milks is further illustrated in Figure 3, page 21.

Rate of Acid Development by Cultures Carried in Normal and Reconstituted Skim Milks

Knudsen and Sorensen (13) made the observation that lactic acid bacteria grew better, the higher the milk had been heated. They offered the explanation that heating at low temperatures resulted in the destruction of bactericidal substances only, while at higher temperatures products were formed which were more easily utilized by the bacteria. Sommer (26), however, states "A more correct explanation probably is that higher heat treatment lowers the oxidation-reduction potential of the milk to a point where prompt growth of the bacteria is possible."

The following experiment was designed to determine rates of acid development in normal and reconstituted skim milks.

Procedure

A duplicate series of reconstituted skim milks ranging from 8 to 12 per cent SNF were prepared using distilled water. In each series a normal skim milk

sample was included to serve as a control. One series was pasteurized at 180° F. for 30 minutes and the second series was sterilized by heating to 248° F. (15 pounds of steam pressure) in an autoclave for 30 minutes. Following cooling to 70° F. all samples received a 1 per cent inoculum of mother culture and were incubated at 70° F. At selected intervals, portions of the cultures were removed aseptically and titratable acidities determined. Results are recorded in Table 6, and in Figs. 1 and 2.

A study of Figure 1 shows that there is a direct relationship between the rate of acid development, the amount of acid ultimately produced, and the per cent SNF of the skim milk. With the exception of the 8 per cent SNF reconstituted skim milk, the rate of acid development was initially greater in all of the cultures carried in reconstituted skim milks than it was in the culture carried in normal skim milk. With the exceptions of the 8

Table 6. Rate of Acid Development in Cultures Carried in Normal and Reconstituted Skim Milks Pasteurized at 180° F. for 30 Minutes and Sterilized at 248° F. for 30 Minutes*

Sample number and description	Titratable acidity as per cent lactic acid							
	0 hrs.	4 hrs.	7 hrs.	9 hrs.	11 hrs.	13 hrs.	16 hrs.	28 hrs.
Normal skim milk (8.84% SNF) pasteurized 180° F. for 30 minutes	0.160	0.190	0.270	0.445	0.725	0.835	0.930	1.000
NS as above but sterilized at 248° F. for 30 minutes	0.160	0.225	0.315	0.430	0.680	0.805	0.895	1.025
8 Reconstituted skim milk (8.00% SNF) pasteurized 180° F. for 30 minutes	0.150	0.175	0.260	0.390	0.610	0.700	0.775	0.815
8S as above but sterilized at 248° F. for 30 minutes	0.150	0.185	0.270	0.380	0.560	0.680	0.765	0.850
9 Reconstituted skim milk (9% SNF) pasteurized 180° F. for 30 minutes	0.170	0.200	0.285	0.410	0.705	0.790	0.860	0.940
9S as above but sterilized at 248° F. for 30 minutes	0.170	0.225	0.320	0.450	0.610	0.760	0.835	0.910
10 Reconstituted skim milk (10% SNF) pasteurized 180° F. for 30 minutes	0.190	0.225	0.320	0.460	0.770	0.855	0.940	1.050
10S as above but sterilized at 248° F. for 30 minutes	0.190	0.270	0.360	0.480	0.620	0.820	0.925	1.020
11 Reconstituted skim milk (11% SNF) pasteurized 180° F. for 30 minutes	0.210	0.250	0.345	0.520	0.800	0.910	1.035	1.155
11S as above but sterilized at 248° F. for 30 minutes	0.210	0.290	0.385	0.490	0.640	0.850	0.980	1.100
12 Reconstituted skim milk (12% SNF) pasteurized 180° F. for 30 minutes	0.230	0.270	0.365	0.540	0.830	0.995	1.130	1.230
12S as above but sterilized at 248° F. for 30 minutes	0.230	0.295	0.410	0.530	0.695	0.890	1.055	1.190

*All samples received 1 per cent inoculum of culture A and were incubated at 70° F.

and 9 per cent SNF reconstituted skim milks, all of the cultures carried in reconstituted skim milks produced ultimately larger amounts of acid than did the culture carried in normal skim milk. The amount of acid produced increased with increasing SNF content of the reconstituted skim milks.

An explanation of the results shown in Figure 1 is not readily apparent. Previous heat treatment of the NFMS powder undoubtedly had some effect on the initial rate of acid development (see Figure 2). It is, however, doubtful that it has a marked bearing on the amount of acid ultimately produced. It is more probable that the larger quantities of acid produced by the cultures carried in reconstituted skim milks of increasing SNF contents were the result of the better buffering capacities present in such milk systems. The more rapid decrease in pH and lower pH readings at equivalent titratable acidities in cultures carried in 8 per cent SNF reconstituted skim milk compared to those carried in reconstituted skim milks of higher SNF lends support to this supposition (see Table 9 page 20).

A study of Table 6 and the graph of Figure 2 shows that the initial rate of acid development was greater in all cultures carried in the sterilized skim milks than in those carried in skim milks pasteurized at 180° F. for 30 min. Further study reveals that the rate of acid development gradually declined in the former until it equaled the rate of the latter; thereafter, the rate of acid development varied with the SNF content.

Although it is known that the high heat treatment of milk results in the formation of simpler nitrogenous compounds (5), it is improbable that the theory of Knudsen and Sorensen (13) can be applied in explanation of the present results. The latter research workers

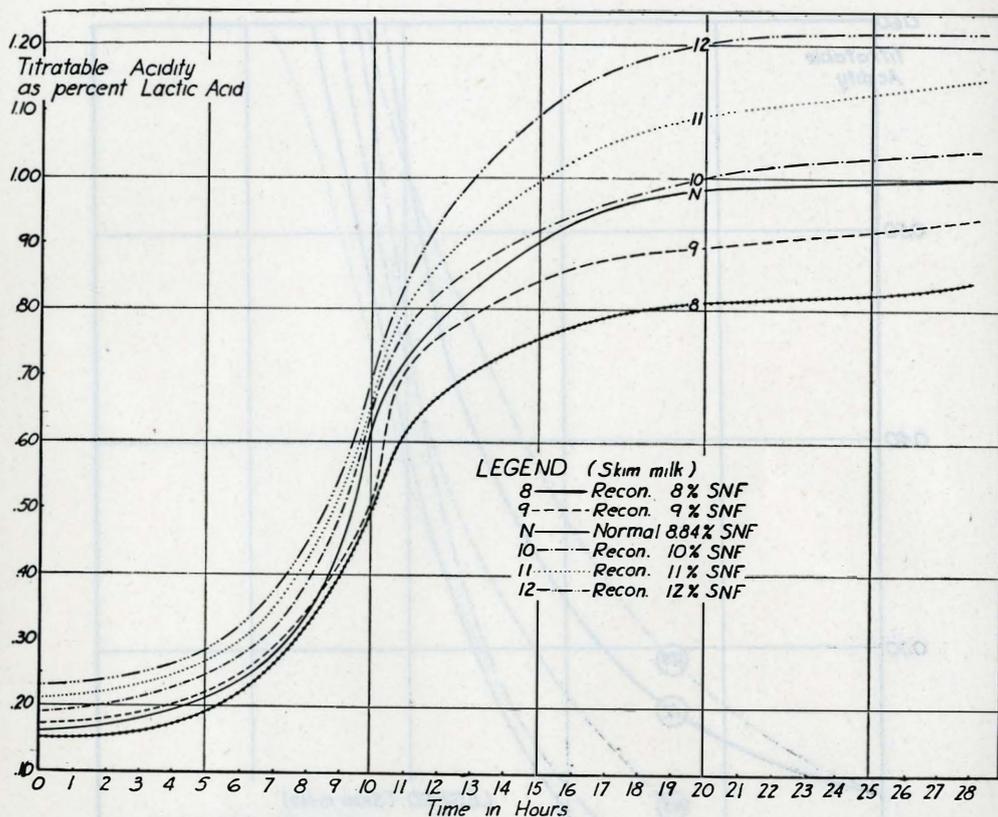


Figure 1. Rate of acid development in cultures carried in normal and reconstituted skim milks pasteurized at 180° F. for 30 minutes.

offered the explanation that heating at low temperatures resulted in the destruction of bactericidal substances only, while at high temperatures decomposition products were formed which were more easily utilized by the bacteria. If the rate of acid development in the more highly heated skim milks were dependent upon the better utilization of these decomposition products, a more consistent and uniformly greater rate of acid development could be expected. It would also be expected that the ultimate amounts of acid developed would be greater. This was not consistently true in the present study.

It is known that the high heat treatment of milk results in the formation of decomposition products containing free sulfhydryl groups and that these in turn cause a decrease in the oxidation-reduction potential (12, 7, 10). Fresh milk contains no free sulfhydryls (11) and has an O/R potential of approximately + 0.2 to + 0.3 volts. In view of all this supporting evidence, the theory of Sommer (26) (see page 11) must be considered more applicable in explaining the initially greater rate of acid development in the more highly heated skim milks. The observation that the rate of acid development of the culture carried in the skim

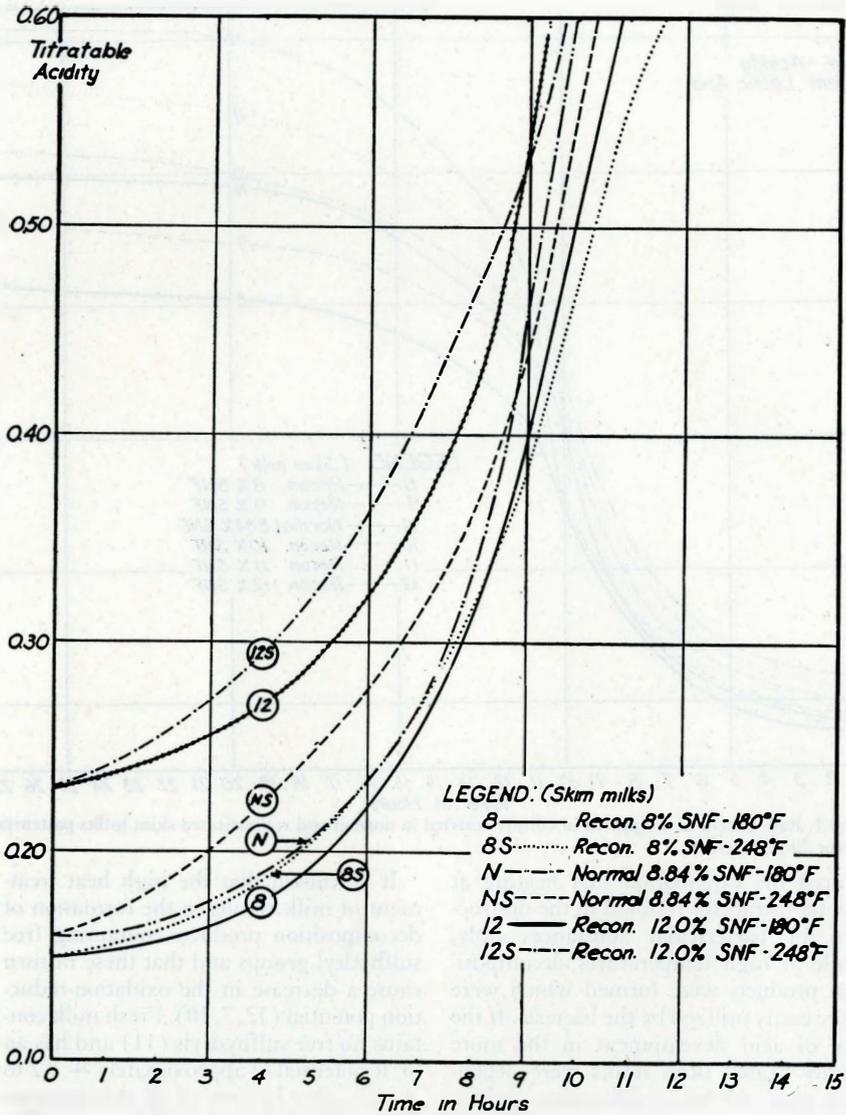


Figure 2. Rate of acid development in cultures carried in normal and reconstituted skim milks pasteurized at 180° F. for 30 minutes and sterilized at 248° F. for 30 minutes.

milks pasteurized at 180° F. gradually increased and after a short period of incubation equaled the initially greater rate of the cultures carried in sterilized milks, is explained on the basis that the

growing bacteria must first establish a certain degree of reducing intensity before rapid logarithmic growth can take place.

Relation of Method of Propagation of Mother Culture to Flavor Production in Cultured Buttermilk Made from Reconstituted Skim Milks

Citrates are frequently added to milk used for cultured buttermilk manufacture to increase the production of $amc + ac_2$; however, a search of the literature has failed to reveal any recommendation that citrates be added to the milk used in carrying the mother cultures. The following experiment was designed to determine the value of carrying the mother cultures in milk reinforced with added citrates with respect to maximum and most uniform production of $amc + ac_2$.

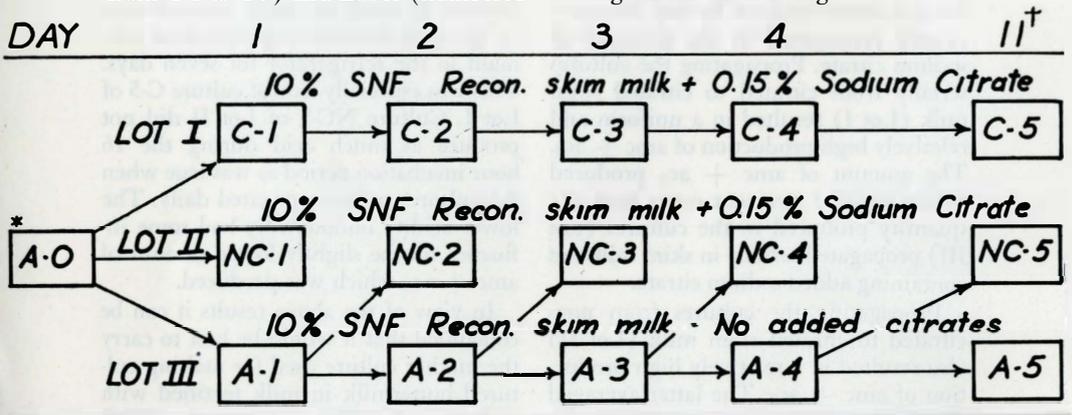
Procedure

Ten per cent SNF reconstituted skim milk was prepared in distilled water. This was divided into 3 lots; each lot consisted of 5 quart milk bottles, each containing 495 ml. of the reconstituted skim milk.

To each container in Lot I (numbered from C-1 to C-5) and Lot II (numbered

from NC-1 to NC-5) was added 0.15 per cent of sodium citrate. The containers of skim milk of Lot III (numbered A-1 to A-5) received no added sodium citrate. All of the quart containers of skim milk were fitted with protective parchment hoods and sterilized by free flowing steam in an autoclave for one hour. All samples thus prepared were then stored in a refrigerator at 35° F. until used.

Prior to carrying out the experiment, culture A-0, the mother culture used in this experiment, was propagated daily for five days in 10 per cent SNF reconstituted skim milk. It was then used to inoculate at the rate of 1% the first numbered container of skim milk in each of the three lots of milk on the first day of the experiment. Thereafter the inoculations were made as illustrated in the following schematic drawing:



*A-0 was mother culture used in the experiment. It was propagated daily for 5 days in non-citrated, 10 per cent SNF reconstituted skim milk prior to the start of the experiment.

†During the seven-day interval, the cultures were stored in the refrigerator at approximately 35° F. They were not propagated during this period.

It is evident from the above illustration that the mother culture of Lot I was transferred serially from day to day for four days and again on the 11th day in milk reinforced with 0.15 per cent sod-

ium citrate. In Lot III the mother culture was also transferred as in Lot I, but in skim milk containing no added citrates. It, in turn, was used to inoculate the containers of skim milk of Lot II as illustrat-

ed. The mother culture carried in Lot II was thus inoculated into milk containing added sodium citrate without having previously been propagated in milk containing added sodium citrate.

All of the cultures were incubated at 70° F. for 16 hours. Titratable acidity

determinations were made at the end of the incubation period. The cultures were then rapidly cooled in ice water and stored in the refrigerator for 24 hours. At the end of the 24-hour holding period the $amc + ac_2$ was determined in the usual manner. Results are shown in Table 7.

Table 7. Relation of Method of Propagation of Mother Culture to Flavor Production in Cultured Buttermilk Made from Reconstituted Skim Milk

Lot I sample number	Acidity %	Days	Amc + ac ₂	Lot II sample No.	Acidity %	Days	Amc + ac ₂	Lot III sample No.	Acidity %	Days	Amc + ac ₂
								A-0	0.91	0	21.7
C-1	0.95	1	36.5	NC-1	0.93	1	36.1	A-1	0.94	1	23.9
C-2	0.95	2	36.0	NC-2	0.96	2	42.8	A-2	0.95	2	26.9
C-3	0.93	3	39.6	NC-3	0.93	3	33.2	A-3	0.93	3	21.8
C-4	0.91	4	35.1	NC-4	0.91	4	29.4	A-4	0.92	4	17.8
	Average		36.80		Average		35.38		Average		22.42
C-5	0.92	11	37.40	NC-5	0.89	11	32.00	A-5	0.89	11	22.00

Amc + ac₂ = mg. of nickel dimethylglyoxime equivalent to acetylmethylcarbinol plus diacetyl in 200 gm. of culture.

Lot I—mother culture propagated serially from citrated to citrated milk.

Lot II—mother culture inoculated into milk containing added sodium citrate without having previously been propagated in milk containing added sodium citrate.

Lot III—mother culture propagated serially in milk not containing added sodium citrate.

The results in Table 7 show that flavor producing cultures readily utilized added sodium citrate without having been previously propagated in the presence of sodium citrate. Propagating the cultures serially from citrated to citrated skim milk (Lot I) resulted in a uniform and relatively high production of $amc + ac_2$. The amount of $amc + ac_2$ produced averaged 64.13 per cent more than the quantity produced in the cultures (Lot III) propagated serially in skim milk not containing added sodium citrate.

Propagating the cultures from non-citrated to citrated skim milk (Lot II) also resulted in a relatively high production of $amc + ac_2$. The latter averaged 57.81 per cent more than that of the cultures of Lot III which were propagated in skim milk not containing added sodium citrate. This amount of increased production of $amc + ac_2$ was only slightly less than the amount produced in the cultures of Lot I. It was, however, not produced in as uniform quantities as in the cultures of Lot I.

The results in Table 7 show also that flavor-producing bacteria were able to produce as much or nearly as much $amc + ac_2$ even after having been held dormant in the refrigerator for seven days. This was especially true of culture C-5 of Lot I. Culture NC-5 of Lot II did not produce as much acid during the 16 hour incubation period as was true when the cultures were propagated daily. The lower acidity undoubtedly had some influence on the slightly lower amount of $amc + ac_2$ which was produced.

In view of the above results it can be concluded that it would be best to carry the mother culture used for making cultured buttermilk in milk fortified with added citrates. This procedure has yielded slightly greater and most nearly uniform response with respect to the production of $amc + ac_2$ when the culture was subsequently used in the preparation of cultured buttermilk from milk containing added citrate.

From the standpoint of the amounts of flavor substances produced by the cul-

tures after having been held dormant for seven days, the need for making daily transfer of the mother culture is not so obvious. The practice of many small dairy plants of transferring mother cultures only once or twice per week has, for the most part, been condemned by dairy bacteriologists. In view of the results of the present experiment the justification of this condemnation may be questioned. For the small dairy plant making only one or two small batches of cultured buttermilk per week such a practice is not only most convenient, but is also saving of considerable amounts of labor. At the

College Creamery the practice of transferring the mother culture once per week has proved to be both a convenient and satisfactory practice for a period of over one year. In following such a practice it is highly essential that the milk be properly sterilized. Sterilization under pressure is best. It is also essential that contamination be avoided in making the inoculations and that the cultures be kept as cold as possible in the refrigerator during the weekly storage interval. When these precautions are observed, the activity of the cultures is not greatly reduced.

Viscosity Studies

The viscosity of cultured buttermilk made from normal skim milk is dependent upon several interrelated factors. In general, a weak or thin body may be attributed to the development of insufficient quantities of acid or low total solids in the skim milk. It may also be due to the agitation or disturbance of the culture during the ripening period (3).

Kosikowsky and Brueckner (14) observed that the viscosity of cultured buttermilk was directly related to the acidity at the time of breaking the curd. Increased acidities resulted in an increase of the viscosity of the cultured buttermilk. They observed further that overripening the cultured buttermilk when incubated at 72° F. did not cause whey separation, but that on the contrary the increase in acidity caused disappearance of whey in cultured buttermilk made from milk that had been subjected to high pasteurization temperatures. In connection with these observations, the following remarks of Sommer (26) are of interest. "Overripening of the cultured buttermilk is sometimes mentioned as a probable cause for whey separation, but this is incorrect. At the curdling point of milk the casein is at its isoelectric point, which also is the minimum point in hydration. Souring beyond this point increases rather than decreases the hydration (resulting in an increase in viscosity) so that improvement might be expected. However, actual observations show little relation between acidity and whey separation in cultured buttermilk."

The heat treatment given the milk used in the preparation of cultured buttermilk has a pronounced effect on the viscosity of the resulting culture. Kosikowsky and Brueckner (14) made the observation that a pasteurization temperature of 150° F. for 30 min. produced the lowest viscosity studied while pas-

teurization at 185° F. for 30 minutes produced the highest viscosity. Pasteurization temperatures of 205° F. and 170° F. produced a viscosity intermediate between 185° F. and 150° F. They observed further that a pasteurization temperature of 185° F. for 30 min. produced the minimum of whey while a pasteurization temperature of 150° F. for 30 min. produced a maximum amount of whey separation. Holding periods for 60 min. were not effective in changing the viscosity or improving whey-retaining properties of cultured buttermilk as compared to holding for 30 min.

Procedure

Many different methods and types of apparatus have been used to determine the viscosities of dairy products such as cultured buttermilk. This has resulted in a considerable amount of confusion and has made it difficult for different research workers to compare their findings. There exists an urgent need for standardization of the methods to be followed in making viscosity determinations of dairy products possessing relatively high viscosities.

Early in the experimental procedure a Saybolt viscosimeter was used as it was the only one available. For the most part it proved unsatisfactory. A great variation in results was obtained even on identically prepared and treated cultures. In general, the same difficulties were encountered with a calibrated viscosity pipette.

In search of a viscosimeter which would give more uniform and consistent results a Hoeppler* viscosimeter was obtained. By use of this instrument the viscosity is measured by the length of time required for a ball of known density and diameter to fall through the sample.

*Obtainable from the Fish-Schurman Corporation, 250 East 45th Street, New York 17, N. Y.

The latter is confined in an accurately calibrated glass tube surrounded by a water jacket. The temperature in the water jacket is kept constant by pumping water through the jacket from a constant temperature bath.

By use of the Hoesppler viscosimeter reproducible results were possible through the curdling point. Past the curdling point and as the viscosity increased it was generally impossible to obtain close checks when more than one viscosity reading was taken on the same

sample. The length of time required for the ball to fall through the sample decreased with each successive reading (see Table 8). The decrease undoubtedly can be attributed to a gradual disintegration of the coagulated casein particles.

In view of the difficulties encountered in determining the viscosity of the coagulated cultures and the inadvisability of using the average of several readings, the following procedure was adopted. Samples of normal and reconstituted skim milks were prepared as outlined on page

Table 8. Hoesppler Viscosimeter Readings of Uncoagulated and Coagulated Cultured Buttermilk* at 68° F.

Reading	Fresh skim milk acidity = 0.155% Ball F6-seconds	Inoculated and incubated skim milk not coagulated acidity = 0.55% Ball F6-seconds	Coagulated† culture acidity = 0.92% Ball H8-seconds
1	23.6	22.8	48.0
2	23.7	22.8	45.2
3	23.7	22.9	44.4
4	23.7	22.7	43.6
5	23.6	22.9	43.0

*Made from 10% SNF reconstituted skim milk.

†100 ml. of inoculated culture was allowed to coagulate in a 500 ml. of titration flask. Curd was broken by rotating flask 30 times in a period of 15 seconds through the circumference of a circle approximately one foot in diameter.

6. After the samples were inoculated (one per cent inoculum) 100 ml. of the sample were distributed into sterile 500 ml. Erlenmeyer flasks and incubated at 70° F. At selected intervals flasks of the culture were removed for titratable acidity, pH, and viscosity determinations. Each flask was rotated 30 times in a period of 15 seconds through the circumference of a circle approximately one foot in diameter. A variable speed wrist action shaker was originally used but this proved unsatisfactory largely because it resulted in the incorporation of air into the cultures which tended to alter the viscosity readings. Single viscosity readings were made at 20° C. (68° F.). Results are expressed in terms of absolute units for viscosity, the centipoise.*

*The absolute viscosity of water at 20° C. is 1.008 centipoise. A fluid having a viscosity of 50.4 centipoises at the same temperature is 50 times more viscous than water.

Results

The relationship between titratable acidity, pH and viscosity of cultured buttermilks made from normal and reconstituted skim milks are shown in Table 9. In Figure 3 are shown the relationships among titratable acidity, viscosity, and consumer preference of cultured buttermilks made from normal and reconstituted skim milks of varying SNF content.

The data recorded in Table 9 and Figure 3 are typical of the results secured in several similar trials using different lots of powder, but the same culture. When different cultures were used, there was marked variation in maximum viscosities produced. One culture studied, for example, lacked the ability to produce a viscosity greater than 45 centipoises in 10 per cent SNF reconstituted skim milk ripened to an acidity of 0.93 per cent. The latter was the maximum acidity attained

Table 9. The Relationship between Titratable Acidity, pH, and Viscosity of Cultured Buttermilks Made from Normal and Reconstituted Skim Milks† of Varying SNF Content. (Culture No. 1)

Description of sample	Acidity %	pH	Viscosity (centipoise)	Description of sample	Acidity %	pH	Viscosity (centipoise)
Normal skim milk	0.155*	6.64*	1.94*	Reconstituted skim milk	0.190	6.52	2.07
	0.200	6.32	1.89		0.230	6.30	1.92
	0.210	6.31	1.84		0.270	6.08	1.91
	0.300	5.85	1.84		0.330	5.81	1.88
	0.400	5.50	2.09		0.410	5.47	1.94
9.02% SNF	0.560	5.00	7.42	10.0% SNF	0.780	4.70	30.49
	0.680	4.88	26.73	0.835	4.68	48.04	
	0.760	4.62	70.90	0.970	4.60	69.53	
	0.930	4.50	99.33	1.050	4.41	78.53	
	1.030	4.38	116.54	1.140	4.42	88.29	
Reconstituted skim milk	0.145	6.65	1.81	Reconstituted skim milk	0.210	6.51	2.27
	0.180	6.31	1.74		0.250	6.28	2.19
	0.225	6.00	1.71		0.280	6.15	2.17
	0.320	5.58	1.72		0.530	5.30	2.17
	0.420	5.31	1.75		0.750	4.83	7.23
8.0% SNF	0.650	4.62	18.84	11.0% SNF	0.850	4.70	57.79
	0.690	4.63	27.54	0.920	4.70	70.00	
	0.790	4.50	31.53	1.080	4.60	106.63	
	0.820	4.40	-----	1.130	4.43	132.68	
	0.960	4.38	36.60	1.210	4.44	119.65	
Reconstituted skim milk	0.160	6.52	1.93	Reconstituted skim milk	0.230	6.50	2.43
	0.200	6.28	1.84		0.265	6.26	2.37
	0.240	6.15	1.85		0.370	5.85	2.26
	0.350	5.50	1.85		0.560	5.30	2.21
	0.410	5.38	1.90		0.780	4.87	3.30
9.0% SNF	0.710	4.68	26.96	12.0% SNF	0.950	4.70	87.91
	0.760	4.68	41.63	1.020	4.70	96.05	
	0.880	4.55	49.71	1.150	4.60	141.64	
	0.940	4.41	54.66	1.250	4.45	167.68	
	0.980	4.39	53.93	1.310	4.46	155.47	

*First readings in each series represent those made on the freshly pasteurized milk samples. Last readings in each series were made after cultures had been incubated at 70° F. for 72 hours. All viscosity readings were made at 20° C.

†Non-fat dry milk powder contained 2.40% moisture, 0.95% fat and had solubility index of 0.15 cc.

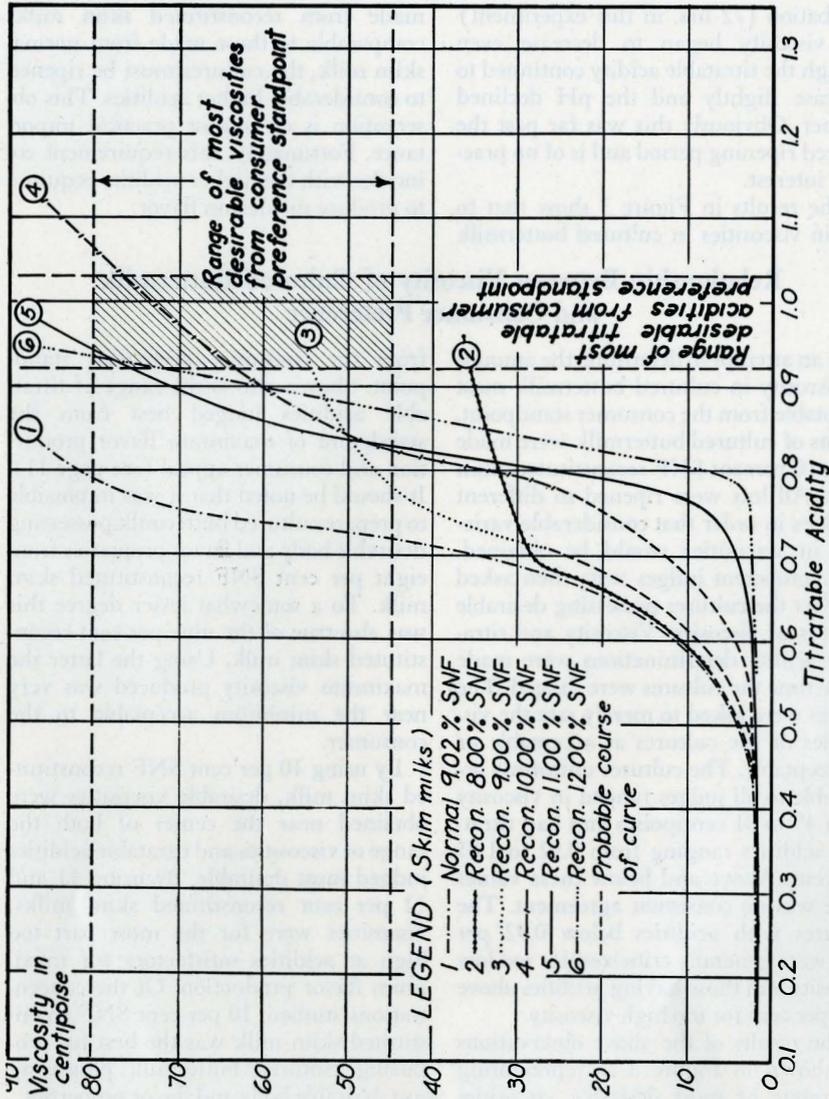


Figure 3. Relationships among titratable acidity, viscosity and consumer preference of cultured buttermilks made from normal and reconstituted skim milks of varying SNF content.

by this culture during incubation for 24 hours at 70° F.

A study of Table 9 and Figure 3 shows that there was a direct relationship between the titratable acidity, pH and viscosity. Early in the ripening period there was a slight decrease in the viscosity with

increasing titratable acidity and decreasing pH. This was followed by a gradual increase in viscosity past the curdling point; thereafter there was a marked increase in the viscosity with a comparatively slight increase in titratable acidity and slight decline in pH. After prolonged

incubation (72 hrs. in this experiment) the viscosity began to decrease even though the titratable acidity continued to increase slightly and the pH declined further. Obviously this was far past the desired ripening period and is of no practical interest.

The results in Figure 3 show that to obtain viscosities in cultured buttermilk

made from reconstituted skim milks comparable to those made from normal skim milk, the cultures must be ripened to considerably higher acidities. This observation is of utmost practical importance. Fortunately, this requirement coincides with the higher acidities required to produce maximum flavor.

Relationship Between Viscosity of Cultured Buttermilk and Consumer Preference

In an attempt to determine the amount of viscosity in cultured buttermilk most acceptable from the consumer standpoint, 13 lots of cultured buttermilk were made from 10 percent SNF reconstituted skim milk. All lots were ripened to different acidities in order that considerable variation in viscosities would be obtained. Five competent judges were then asked to select the cultures possessing desirable degrees of viscosity. Viscosity and titratable acidity determinations were made at the time the cultures were judged. The judges were asked to merely rate the viscosities of the cultures as acceptable or unacceptable. The cultures uniformly acceptable to all judges ranged in viscosity from 45 to 81 centipoises and had titratable acidities ranging from 0.92 to 1.05 per cent. Above and below these values there was no consistent agreement. The cultures with acidities below 0.92 per cent were generally criticized for too low viscosity and those having acidities above 1.05 per cent for too high viscosity.

The results of the above observations are shown in Figure 3 as representing the range of most desirable viscosities

from the consumer preference standpoint. Shown also is the range of titratable acidities judged best from the standpoint of maximum flavor production and consumer appeal (see page 11). It should be noted that it was impossible to prepare cultured buttermilk possessing desirable body and flavor properties from eight per cent SNF reconstituted skim milk. To a somewhat lesser degree this was also true of the nine per cent reconstituted skim milk. Using the latter the maximum viscosity produced was very near the minimum acceptable to the consumer.

By using 10 per cent SNF reconstituted skim milk, desirable viscosities were obtained near the center of both the range of viscosities and titratable acidities judged most desirable. By using 11 and 12 per cent reconstituted skim milks, viscosities were for the most part too high at acidities satisfactory for maximum flavor production. Of the concentrations studied, 10 per cent SNF reconstituted skim milk was the best for producing cultured buttermilk possessing most desirable body and flavor properties.

Whey Separation in Cultured Buttermilk Made from Reconstituted Skim Milk

A cross-section survey of 45 dairy plants throughout the United States by Kosikowsky (15) in 1944 revealed that wheying-off was deemed one of the greatest problems in the manufacture of cultured buttermilk.

Kosikowsky and Brueckner (14) made the following observations on whey separation in cultured buttermilk made from normal skim milk: (1) Underripening at the time the curd was broken was directly related to whey separation until an acidity of 0.725 to 0.750 per cent had been reached; (2) overripening of cultured skim milk when incubated at 72° F. did not cause whey separation. On the contrary, as the acidity increased, whey disappeared in the cultured skim milk that had been subjected to high pasteurization temperature; and (3) the amount of whey separation in the low range of acidity was directly related to the length of the storage period. Storage at 38° F. was more effective in preventing the separation of whey for a period of one to three days than a higher storage temperature. After this period a low storage temperature had no apparent effect on prevention of whey separation.

Glazier and Lindquist (6), in a study of some of the factors affecting wheying-off of cultured buttermilk made from normal skim milk and whole milk, made the following observations: (1) The higher the developed acidity, the less was the whey separation during storage. At acidities of 0.68 per cent wheying-off occurred freely, it was less at 0.80, but at 0.87 to 0.93 per cent it occurred only after long periods of storage; (2) skim milk gave less desirable results than whole milk; (3) a pasteurization temperature of 200° F. gave more desirable

results from the standpoint of body and texture than did a pasteurization at 180° F.; (4) storage temperatures as high as 50° F. were unsatisfactory with most desirable results being obtained at 33° F.; and (5) the turnover of cultured buttermilk should be as rapid as possible if wheying-off is to be a minor problem even under best production.

Allredge and Burke (1), in a study of the manufacture of cultured buttermilk from non-fat dry milk solids, made the following observations with respect to whey separation: (1) breaking the curd prior to cooling caused much separation of whey; (2) vigorous agitation of curd particularly to the point of slight foaminess was responsible for wheying-off; and (3) the incorporation of excessive air into the buttermilk by pumping was responsible for a porous, broken, and gassy appearance of the curd.

Procedure

Lots of reconstituted milk of varying percentages of SNF were prepared in distilled water. All lots were pasteurized at 180° F. for 30 minutes in a laboratory pasteurizer, cooled to 70° F., inoculated with one per cent of mother culture, and incubated 14 to 16 hours so as to obtain a variation in acidity at the end of the incubation period.

At the end of the incubation period the cultures were rapidly cooled in ice water prior to breaking the curd. Titratable acidity determinations were then made and 100 ml. of each culture were placed in a 100 ml. stoppered graduated cylinder. The cylinders were stored in a refrigerator at 35° F. and observed for whey separation at the end of 24, 48, 72, and 96 hours.

Table 10. The Relation of Whey Separation to the Percent SNF and Titratable Acidity of Cultured Buttermilk Made from Normal and Reconstituted Skim Milks

Description of sample	Acidity %	Whey separation — ml. per 100 ml. sample			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
Normal skim 8.76% SNF	0.83	0.00	0.00	0.00	0.00
Reconstituted skim 8.5% SNF	0.77	5.00	6.00	7.00	8.00
Reconstituted skim 8.5% SNF	0.81	0.00	0.00	0.50	1.00
Reconstituted skim 9.0% SNF	0.76	1.00	2.00	3.00	5.00
Reconstituted skim 9.0% SNF	0.85	0.00	0.00	0.00	0.50
Reconstituted skim 10.0% SNF	0.82	2.00	3.00	5.00	10.00
Reconstituted skim 10.0% SNF	0.87	0.50	1.50	2.50	3.50
Reconstituted skim 10.0% SNF	0.90	0.00	0.00	0.00	0.50
Reconstituted skim 10% SNF plus 2% fat	0.92	0.00	0.00	0.00	0.00
Reconstituted skim 10% SNF plus 2% fat + 0.15% sodium citrate	0.94	0.00	0.00	2.00	3.00

Results

In Table 10 are listed typical results obtained in four trials using three different lots of powder.

The results in Table 10 show that there is a direct relationship between titratable acidity, per cent SNF and the rate and amount of whey separation during storage of the cultures. Acidities below 0.85 per cent caused rapid and large amounts of whey to separate in those cultures containing nine per cent

or less of SNF. Titratable acidities below 0.87 per cent favored whey formation in cultures made from 10 per cent SNF reconstituted skim milk. In those cultures with titratable acidities of 0.90 per cent or above there occurred only negligible amounts of whey separation even after 96 hours of storage. The presence of two per cent fat in the cultures made from the 10 per cent SNF reconstituted milk did not appear essential for preventing wheying-off at titratable acidities above 0.90 per cent.

Whey Separation As Related to Method of Breaking and Cooling the Curd Prior to Bottling the Cultured Buttermilk

From practical experience in preparing commercial quantities of cultured buttermilk from 10 per cent SNF reconstituted skim milk, it was observed that the following procedure of breaking and cooling the curd was most satisfactory from the standpoint of preventing whey separation during storage. Immediately following the incubation period the curd should be broken to a smooth consistency and cooled to 40° F. or lower as rapidly as possible and with a minimum of agitation. During the cooling period intermittent but gentle agitation is necessary to increase the rate of cooling. Immediately following cooling, the cultured buttermilk should

be held for 3 to 4 hours at 40° F. or lower without further agitation.

The effect of different methods of breaking and cooling the curd prior to bottling on the curd character of cultured buttermilk made from normal and reconstituted skim milk is shown in Figures 4 and 5. The samples in Figure 4 are cultured buttermilks made from normal skim milk containing two per cent fat and the samples in Figure 5 are cultured buttermilks made from 10 per cent SNF reconstituted skim milk containing two per cent fat.

A study of Samples 1 and 4 of Figures 4 and 5 shows that breaking and bottling the cultured buttermilk immediately fol-

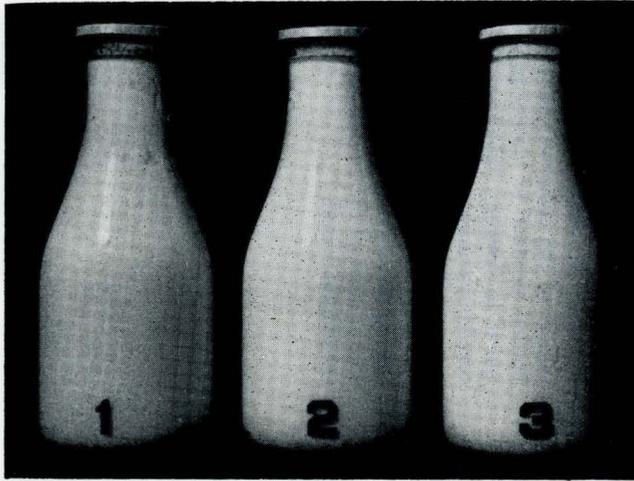


Figure 4. Effect of Different Methods of Breaking and Cooling Curd Prior to Bottling on Curd Character of Cultured Buttermilk Made from Normal Skim Milk Containing 2 per cent Fat:

Sample 1. Curd broken at 70° F. and bottled immediately.

Sample 2. Curd broken at 70° F. and cooled with intermittent agitation to 40° F. and bottled immediately.

Sample 3. Curd broken and cooled as in Sample 2 but allowed to remain in quiescent state for four hours at 40° F. prior to bottling.

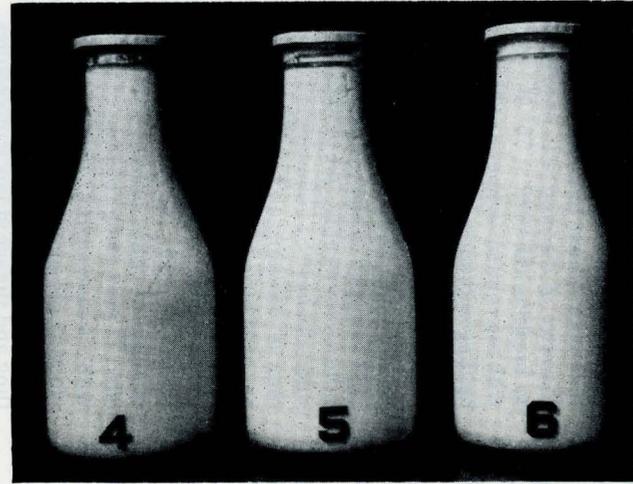


Figure 5. Effect of Different Methods of Breaking and Cooling Curd Prior to Bottling on Curd Character of Cultured Buttermilk Made from 10 per cent SNF Reconstituted Skim Milk Containing 2 per cent Fat.

Sample 4. Curd broken at 70° F. and bottled immediately.

Sample 5. Curd broken at 70° F. and cooled with intermittent agitation to 40° F. and bottled immediately.

Sample 6. Curd broken and cooled as in Sample 5 but allowed to remain quiescent for four hours at 40° F. prior to bottling.

NOTE: Pictures taken following storage of all samples for 48 hours at 40° F.

lowing the incubation period and without cooling has marked detrimental effects on the character of the curd during storage. The curd is porous and gas bubbles and furrows are very prominent. Whey separation is also beginning to show. These defects are more obvious in the cultured buttermilk made from reconstituted skim milk than in the cultured buttermilk made from normal skim milk.

Breaking the curd at 70° F. and cooling with intermittent agitation to 40° F. prior to bottling (Samples 2 and 5) resulted in greater stability of the curd during storage; however, the curd could not be considered entirely acceptable. The curd showed a definite tendency toward breaking up and gas bubbles, furrows and some whey separation were visible. This was especially true in cultured buttermilk made from the reconstituted skim milk.

Miscellaneous Factors Influencing Whey Separation

The following factors were studied in addition to those already discussed and were observed to favor whey separation in cultured buttermilk made from 10 per cent SNF reconstituted skim milk:

1. Incorporation of air by too vigorous agitation of the curd prior to bottling.
2. Storage at temperatures above 40° F.
3. Failure to refrigerate the cultured buttermilk while being carried in the delivery vehicle. This is especially serious during hot weather.

A study of the photographs of Samples 3 and 6 shows that most satisfactory results with respect to the stability of the character of the curd was possible only by breaking the curd at 70° F., cooling with intermittent agitation to 40° F. and holding without agitation for four hours at 40° F. prior to bottling. Equally good results were obtained regardless of whether normal or reconstituted skim milk was used in the preparation of the cultured buttermilk. The satisfactory results obtained by following this procedure of breaking and cooling the curd are explained on the basis, that at low temperatures the growth activity of the microorganisms and perhaps also the activity of their secretory enzymes diminishes or ceases entirely. This has the effect of preventing resetting of the curd with accompanying defects such as wheying-off and the development of gas bubbles and furrows subsequent to bottling and storage at low temperatures.

4. Allowing the temperature to fluctuate widely in the cooler or allowing the bottled product to warm up subsequent to bottling. Holding the cultured buttermilk at room temperature for periods of 15-20 minutes shortens the time for whey separation to occur during subsequent storage.
5. Disturbance of the culture during the incubation period.

Summary

1. Cultured buttermilk of excellent quality was prepared from *extra grade* non-fat dry milk solids (NFMS), but inferior quality NFMS were unsatisfactory.
2. The production of acetylmethylcarbinol plus diacetyl ($\text{amc} + \text{ac}_2$) in reconstituted skim milk by flavor producing cultures did not equal the amounts produced by these cultures in normal skim milk.
3. The addition of sodium citrate or citric acid to reconstituted skim milk permitted the development of increased quantities of $\text{amc} + \text{ac}_2$ equalling or exceeding amounts present in cultured buttermilk made from normal skim milk not containing added citrates.
4. The $\text{amc} + \text{ac}_2$ increased upon storage of cultured buttermilk at refrigerator temperatures for 24 to 48 hours. There was a corresponding improvement in the flavor and aroma with a marked decrease in the powder flavor initially present.
5. The addition of sodium citrate caused an increase in the production of carbon dioxide which gave the cultured buttermilk a desirable carbonated flavor.
6. The presence of 2 per cent butterfat in the reconstituted skim milk was found essential for the production of most satisfactory flavor and aroma.
7. From the standpoint of maximum flavor production and consumer preference, titratable acidities ranging from 0.92 to 1.03 per cent in cultured buttermilk made from NFMS were best.
8. In reconstituted skim milks of varying SNF, the rate of acid development increased with an increase in the concentration of SNF.
9. The rate of acid development was initially greater in normal and reconstituted skim milks heated at 248° F. for 30 minutes than in the same skim milks heated at 180° F. for 30 minutes. An explanation for this observation is offered.
10. Flavor producing cultures readily utilized added sodium citrate without having been previously propagated in the presence of it, but slightly greater and more nearly uniform production of $\text{amc} + \text{ac}_2$ was obtained when the mother cultures were propagated in milk fortified with added citrates.
11. Mother cultures propagated in sterile milk and held dormant at low temperatures for seven days produced nearly as much $\text{amc} + \text{ac}_2$ as when the cultures were propagated daily. The practical application of this observation is discussed.
12. The viscosities of cultured buttermilks made from normal and reconstituted skim milks were found to increase to certain maximum values with increases in SNF and titratable acidities. Viscosities in the range of 45 to 81 centipoises were found most acceptable to the consumer.
13. In order to obtain desirable viscosities and whey retaining properties, cultured buttermilk made from 10 per cent reconstituted skim milk should be ripened to an acidity of 0.90 to 1.05 per cent.
14. Miscellaneous factors influencing whey separation are discussed.
15. A detailed procedure for making cultured buttermilk from reconstituted skim milk is given.

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**Recommended Procedures
For Making Cultured Buttermilk
From Reconstituted Skim Milk**

Recommended Procedures

The Mother Starter Culture

1. Obtain a flavor producing lactic type culture from a reliable firm supplying butter and cheese cultures.
2. Propagate mother cultures preferably daily (but see also discussion on page 16) in sterilized skim milk to which has been added 0.15 percent of sodium citrate. If more detailed information is desired in regard to the method of propagating the mother starter culture, see reference (8) or (27).

Reconstituting Non-fat Dry Milk Solids

1. Obtain *extra grade* non-fat dry milk solids. Specify this on your purchase order.
2. Prepare the reconstituted skim milk by dispersing the correctly calculated and weighed quantity of NFMS in the proper amount of water.

Example Problem:

Five hundred pounds of reconstituted skim milk containing 10 per cent SNF and 2 per cent fat are desired. Cream with 30 per cent fat is available.

$$500 \times 0.10 = 50 \text{ pounds NFMS}$$

$$\frac{500 \times 0.02}{0.30} = 33.3 \text{ pounds of 30 per cent cream}$$

$$500 - (50 + 33.3) = 416.7 \text{ pounds of water}$$

The above calculations are sufficient for practical purposes; however, it is obvious that two compensating errors do exist. These are the SNF in the cream (about 6.2 per cent) and the moisture in the NFMS (about 3 per cent).

In reconstituting the NFMS place the correct quantity of water in a stainless steel vat (or stainless steel can if a small quantity is prepared). The temperature of the water, at least insofar as present available information is concerned, is not critical. As satisfactory results may be secured at 70° F. as at 120° F. This is, however, true only if the NFMS are of *extra grade* and contain only a small percentage of fat. It was observed in one instance during the course of this experiment that a poor grade of powder containing 3.03 per cent fat could not be completely dispersed in water having a temperature below 120° F.

Having placed the water in the vat, add the NFMS in several small installments, agitating vigorously between additions and continuing the agitation until complete dispersion is obtained. Prompt and vigorous agitation is essential. After the NFMS are completely dispersed, the cream and 0.15 per cent sodium citrate are added.

Pasteurizing the Reconstituted Milk

1. Pasteurize at 180°—190° F. for 30 minutes with constant agitation. The latter is especially important if the creaming properties of the added fat are to be destroyed.
2. Cool to setting temperature (68°—72° F.).

Inoculating and Incubating the Reconstituted Milk

1. Inoculate with 1 per cent of mother culture. Avoid all possible sources of contamination. Thoroughly mix the inoculum with the milk.
2. Incubate the inoculated milk at 68°—72° F. until an acidity of 0.90 to 1.00 per cent is reached (approx. 16 hours).

Breaking and Cooling the Curd

1. At the end of the incubation period break the curd to a smooth consistency with a minimum of agitation. Cool rapidly with intermittent agitation to below 40° F. and hold at this temperature for 3 to 4 hours without agitation.

Bottling the Cultured Buttermilk

1. Transfer the cultured buttermilk to the bottle filler by direct gravity flow. Avoid pumping.
2. Set the bottler at slow speed to allow sufficient time for all bottles to fill.
3. Place the filled bottles in the refrigerator immediately after bottling. Keep temperature in the refrigerator at 35° F. or lower and avoid wide temperature fluctuations.

Marketing the Cultured Buttermilk

1. The cultured buttermilk will possess its best flavor 24 to 48 hours after bottling. It should be marketed so as to reach the consumer when it is approximately 1 to 2 days old.
2. Whenever possible, keep the cultured buttermilk refrigerated while enroute to the consumer.
3. Encourage the consumer to place the cultured buttermilk in the refrigerator as promptly after delivery as possible.