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A NEW METHOD FOR ESTIMATING THE TRUE FAT CONTENT OF BUTTERMILK[1]

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An accurate method for estimating fat in buttermilk has been a need of the dairy industry for some time. The fundamental reason for testing buttermilk is to determine the exhaustiveness of churning. This has been impossible with present methods.

Considerable discrepancy has been revealed in the past in comparing the different methods of testing buttermilk. Meyers (1891) apparently was the first to show that the Babcock method gives results considerably lower than ether-extraction methods. Chemists in the laboratory of the American Association of Creamery Buttermakers concluded that the Babcock method gave results too low for buttermilk as compared to the official Roese-Gottlieb method. In 1921 they contributed a modified Babcock method, which was named the American Association test and later known as the Normal Butyl Alcohol test. This test was recommended as a result of numerous favorable comparisons with the official ether-extraction method and was adopted by some experiment stations and commercial plants. Hunziker (1927) recommends the modified Babcock or ether-extraction methods, while McKay and Larsen (1922) favor the American Association test. The lack of uniformity of results in estimating fat by the different methods indicated that further research was necessary to determine which method could be relied upon.

Petersen observed that when powdered buttermilk was extracted thoroly with alcohol and ether, large quantities of phospholipoids were removed. A review of the literature substantiated these findings. Dornic and Daire (1910) state that buttermilk contains a higher percentage of lecithin than any other milk product, with the possible exception of cream. As a result of this preliminary work, experimental work was initiated to determine the status of the problem.

The results obtained by Thurston and Petersen (1928) and later confirmed by Chapman (1928) showed that the Roese-Gottlieb, the Mojonnier (a commercial adaptation of the Roese-Gottlieb method), and the normal butyl alcohol methods do not give an accurate estimate of the true fat content of buttermilk. This discrepancy was shown to be due to the fact that buttermilk contains relatively large amounts of ether- and alcohol-soluble phospholipoids of which lecithin and cephalin are the principal constituents.

Having definitely established the status of the problem, it became evident that a test was needed whereby the true fat content of butter-

[1] Patent applied for.

milk could be accurately estimated. The researches here reported have revealed such a method, which employs alkaline reagents and the Babcock apparatus.

REVIEW OF THE LITERATURE

The use of alkaline reagents is not a new discovery. Recently Overman and Garret (1928) employed an alkaline solution to estimate the fat content of ice cream. Between 1904 and 1909, considerable discussion for and against the use of alkaline reagents is evident in German literature. The literature is too voluminous for a comprehensive review. It will suffice to mention a few of the first papers dealing with alkaline reagents and their application to the estimation of butterfat in dairy products. Those interested in a summary of the earlier literature are referred to a paper by Windisch (1909).

Owing to patented formulas and trade names, little information can be obtained relative to the composition of the first alkaline reagents made by the German chemists. However, Sichler (1904) was granted four patents on alkaline solutions to which the term "sinacid" was applied. The original "sinacid" reagents consisted of two salt compounds. One was tri-sodium phosphate; the other consisted of tri-sodium citrate and ammonium tri-borate. Amyl or isobutyl alcohol was added separately when making the test. This formula was later modified several times.

Gerber's (1906) "sal" method consisted of a sodium tartrate solution made alkaline with sodium hydroxide plus sodium chloride. The literature reveals considerable contradictory evidence as to the accuracy of Gerber's "sal" and Sichler's "sinacid" methods of estimating the fat content of dairy products as compared to the Roese-Gottlieb method. Two investigators, du Roi and Kohler (1904), showed that with cream the Sichler test checked very closely with the Roese-Gottlieb test, but was a trifle lower than the Gerber "sal" method. They also found the Sichler test to check with the Gerber method on milk testing 3.3 to 3.6 per cent, but the Sichler to be high for milks above 3.3 per cent and low for those below 2.3 per cent fat. For buttermilk, the Sichler test was lower than the Gerber, while for skimmilk the Sichler gave no reading when the Gerber was 0.1 per cent or less.

Gordon (1904) states that for testing buttermilk, skimmilk, cream, whole milk, and cheese by the Sichler "sinacid" method, the test bottles should be heated on a water bath at 65-90° C. He observed that when the test was completed, a dark gummy smudge settled on the bottom of the test bottle. According to his observations, if the test bottles were heated for two hours the fat would separate without centrifuging.

Apparently the interest in testing dairy products with alkaline reagents moderated considerably between 1905 and 1920, owing perhaps

to the universal adoption of the Babcock test. However, Höyberg (1921) formulated a new reagent, which he patented, for estimating fat. His approximate formula contained 50-70 grams of sodium hydroxide and 70-130 grams of potassium sodium tartrate per liter. A different mixture of these salts was required for milk and for cream. Orla-Jensen (1923) modified the Höyberg reagent so that the same alkaline solution and mixture of alcohols could be used for milk and cream. He compared this modified reagent with the Roese-Gottlieb and Gerber methods on milk and on cream and found close agreement with Roese-Gottlieb; the Gerber method gave slightly higher results. Orla-Jensen believed that the modified Höyberg method would be universally adopted because of its accuracy and ease and simplicity of operation in that no centrifuge is required.

The Höyberg method was further improved by Spur (1926) who combined the solutions into one reagent, consisting of a mixture of higher alcohols in 4.5 per cent sodium hydroxide. Later (1926) he reduced the alkalinity of the reagent and also reduced the temperature of the water bath to 50° C. This modified Höyberg method checked to the accuracy of 0.1 per cent for milk and 0.5 per cent for cream as compared to the Gerber method. For milk containing less than 0.5 per cent fat and for cheese, a different alkaline reagent and the centrifuge are recommended. Van Woerden (1928) states that the Höyberg method is efficient and accurate and gives an average value only slightly higher than the Gerber, the difference on milk being generally less than 0.05 per cent.

Magliano and Porzio (1927) reported unsatisfactory results with the Höyberg method, and they devised what is essentially a modification. Their alkaline solution is Fehlings B, consisting of 60 grams sodium hydroxide and 173 grams potassium sodium tartrate and the volume made up to 500 cc. with distilled water. The alcohol mixture consists of 45 parts methyl and 55 parts isobutyl alcohol. To estimate fat in milk, the test is manipulated as follows: 10 cc. of milk, 3 cc. of the alkaline reagent, and one cc. of the alcohol mixture are added to a Gerber test bottle and the contents well mixed. The test bottles are next placed in a water bath at 82° C. for about five minutes and are shaken several times during this interval. When the contents of the bottle have taken on an orange color and a layer of fat has separated on the surface, the percentage of fat is read directly after standing three to five minutes. The authors submit data on 56 samples of milk and, as compared to the Gerber method, the results check to within plus or minus 0.07 to 0.1 per cent.

Kreis and Studinger (1927) describe the Neusal method for estimating fat in milk and cream. The alkaline solution consists of sodium citrate and sodium salicylate. Evidently this method is not standard-

ized, because the authors recommend the addition of isobutyl alcohol until the variations, as compared to the Gerber method, are less than 0.05 per cent.

The most recent alkaline method is the one devised for ice cream by Overman and Garret (1928). Their procedure is given in detail in another section.

The review of literature revealed that the principal reason for the large amount of research with alkaline reagents was an endeavor to develop a method that would dispense with the use of acids. No differentiation was made between true fat and the phospholipoid content of dairy products. In fact, the problem of testing buttermilk was not considered.

EXPERIMENTAL

Preliminary experimental work indicated that the alkaline reagents, specified for ice cream by Overman and Garret (1928) and known as the Garret-Overman method, do not include lecithin with the fat in testing buttermilk, using the Babcock equipment. This reagent is composed of 200 grams of tri-sodium phosphate and 200 grams of sodium salicylate made up to one liter with a mixture of three volumes of ammonium hydroxide and seven volumes of water. The procedure used consisted of measuring 9 grams of buttermilk into a Babcock skimmilk test bottle and adding 0.8 cc. of normal butyl alcohol. The contents were mixed well and 9 cc. of the alkaline reagent was added. The test bottles were next placed in a water bath at 71-82° C. for about ten minutes and shaken several times during the heating process. The rest of the procedure was the same as for the regular Babcock method.

This method gave clear fat columns that could be easily read and the duplicates checked. Lecithin added to the extent of 0.3 per cent did not increase the test of buttermilk of known fat content. However, the alkaline solution has several disadvantages. The tri-sodium phosphate crystallizes on cooling, which necessitates heating the reagent before using. The presence of ammonia makes it objectionable to handle, and experience indicated that the solution deteriorates on standing.

A number of modifications of the Garret-Overman formula were tried by varying the concentration of the salts and the amount of normal butyl alcohol, but the results did not check with those obtained with the recommended reagent. One modification whereby dry tri-sodium phosphate was added to dry test bottles, followed by the buttermilk and the other reagents, gave results comparable with the standard formula and obviated the difficulty of crystallization.

The favorable results obtained with the Garret-Overman reagent encouraged further research, which led to the development of a practical test for buttermilk, using alkaline reagents and the Babcock apparatus. Space will not permit publication in detail of the formulas of forty-six different combinations tried experimentally in this laboratory. Several combinations were used that gave excellent results on buttermilk, but the reagent selected showed possibilities of application to other dairy products. This reagent consists of 110 grams of sodium carbonate and 200 grams of sodium salicylate dissolved in water and the volume made up to 1,000 cc. To this solution is added 30 cc. of 50 per cent sodium hydroxide and 100 cc. of normal butyl alcohol. The name given to this solution is "The Minnesota Babcock Test Reagent."

The method used for testing buttermilk employing this reagent is as follows:

1. Nine grams of buttermilk are placed in a skimmilk test bottle.
2. Ten cc. of the Minnesota reagent is added and the contents of the bottle are well mixed.
3. The test bottles are placed in a water bath at 71-82° C. for six to seven minutes, and shaken several times during this interval.
4. The bottles are centrifuged for five minutes at a speed of 800 revolutions per minute in an 18 inch centrifuge.
5. Warm water is added to the base of the neck of the test bottles and they are centrifuged for two minutes.
6. Sufficient warm water is added to bring the fat into the graduated neck of the test bottles and the centrifuge is operated for another minute.
7. The test bottles are placed in a water bath at 57-60° C. for five minutes and the fat reading taken is multiplied by 2 because a 9-gram sample of buttermilk is used.

This method gave excellent results. The contents of the test bottle after completing a buttermilk fat determination were only slightly opaque, indicating that the solids-not-fat were highly dispersed in the alkaline solution.

Having developed a method that showed promise of giving a true estimate of the fat content in buttermilk, it became evident that further research was necessary to determine its accuracy and applicability under practical laboratory conditions.

Natural buttermilk was used. The advisability of using synthetic milk was considered but not adopted, owing to the impossibility of duplicating the physical properties of natural buttermilk. Additions of fat and of lecithin were made by weighing definite amounts into a known weight of buttermilk. All weighings were made on an analytical

balance. It is obviously wrong to make additions of fat or lecithin to a given volume of buttermilk and assume such additions to be on a percentage basis.

The lecithin used in this experimental work was originally prepared from dried egg yolks according to Maclean's (1918) method, and was granular and light yellow in color. However, the purity of lecithin prepared by this method is questionable. Owing to the solubility of fat in acetone, which is a phospholipoid precipitant, it is reasonable to expect that some fat would be enclosed within the lecithin particles during the purification process. Fortunately the literature revealed a method whereby fat-free lecithin could be prepared.

Gies (1912) and his associates, in their diffusion studies, found that fat could be separated from lecithin by dialysis. According to this method lecithin-fat mixtures are dissolved in ether and the contents poured into a thin rubber container surrounded by ether. The fat diffuses through, leaving the lecithin behind.

The lecithin used in the experiments herein reported was purified according to this method. Dialysis was continued for four days. The diffusate were then removed and a fresh supply of ether added and dialysis continued for another two days. The amount of fat in the diffusate was not determined quantitatively, but upon evaporation of the ether a residue of fat-like material was obtained. This residue, when dissolved in ether and acetone added in excess, did not yield a precipitate, indicating that it was an ether- and acetone-soluble substance, probably mostly fat. The nitrogen and phosphorus content of the lecithin, 1.925 and 4.220 per cent, respectively, is higher than figures previously reported in the literature. This is considered sufficient evidence that the lecithin used in this experimental work was as pure as it is possible to obtain with present methods.

The butterfat used was rendered and clarified on a water bath funnel, filtered and subjected to high centrifugal force. The filtering and centrifuging processes were repeated three times. A portion of the purified butterfat was weighed into an aluminum evaporating dish, placed in a vacuum oven at 135° C. for five minutes, cooled, and on reweighing showed no loss in weight, indicating that the butterfat was moisture-free.

The data reported in the following section are a summary of experiments conducted to determine the accuracy of the Minnesota Babcock test reagent in comparison with the other methods of estimating fat in buttermilk.

EFFECT OF ADDING LECITHIN TO BUTTERMILK ON FAT DETERMINATIONS BY VARIOUS METHODS

The results recorded in Table I substantiate the findings of Thurston and Petersen (1928) and Chapman (1928) in that the Mojonnier and normal butyl alcohol methods for estimating fat include lecithin. However, results obtained with the Babcock tests are at variance with those obtained by Chapman. He was able to recover an average of 71 per cent of the lecithin added, while in the experiments here reported only slight increases in the fat tests were obtained by the Babcock method that could be attributed to added lecithin.

When testing buttermilk to which lecithin was added, by the Babcock method, it was observed that partially hydrolyzed material, presumably lecithin, aggregated at the base of the neck of the test bottles. This obstruction probably prevented the rising of fat globules into the neck of the test bottle, and accounts for the low results obtained with the Babcock method. It was found possible to force some of the hydrolyzed material into the small neck of the test bottle by increasing the speed of the centrifuge.

TABLE I

EFFECT OF ADDING LECITHIN TO BUTTERMILK ON FAT DETERMINATIONS BY VARIOUS METHODS

Sample No.	Added lecithin per cent	Methods used and fat percentages									
		Mojonnier		Babcock		Normal butyl alcohol		Garret-Overman reagents		Minnesota Babcock test reagent	
A 1.....	0.0	0.5601	0.5623	0.01	0.01	0.48	0.48	0.32	0.28		
A 2.....	0.1	0.6863	0.6488	0.01	0.01	0.52	0.72	0.30	0.30		
A 3.....	0.2	0.7557	0.7434	0.03	0.02	0.60	0.56	0.30	0.28		
A 4.....	0.3	0.8389	0.8445	0.06	0.14	0.64	0.66	0.28	0.26		
B 1.....	0.0	0.5930	0.5920	0.05	0.05	0.42	0.40	0.30	0.30	0.30	0.30
B 2.....	0.1	0.6740	0.6760	0.04	0.05	0.44	0.48	0.31	0.30	0.32	0.30
B 3.....	0.2	0.7470	0.7420	0.10	0.08	...	0.52	0.26	0.29	0.30	0.29
B 4.....	0.3	0.8540	0.8310	0.08	0.14	0.78	0.66	0.28	0.30	0.30.	0.30
C 1.....	0.0	0.5500	0.5450	0.08	0.07	0.54	0.56	0.28	0.30	0.32	0.30
C 2.....	0.1032	0.6512	0.6449	0.08	0.10	0.52	0.57	0.29	0.24	0.28	0.30
C 3.....	0.2042	0.7503	0.7360	0.22	0.20	0.65	0.68	0.24	0.26	0.29	0.28
C 4.....	0.3074	0.8419	0.8500	0.18	0.18	0.60	0.62	0.30	0.29	0.30	0.27
D 1.....	0.0	0.5995	0.5994	0.16	0.14	0.60	0.62	...	...	0.52	0.54
D 2.....	0.1066	0.6855	0.7182	0.20	0.22	0.70	0.71	...	...	0.54	0.54
D 3.....	0.2027	0.8086	0.8092	0.40	0.31	0.74	0.70	...	...	0.50	0.48

In no case did added lecithin increase the fat tests when the Minnesota and the Garret-Overman methods were used. This is considered conclusive evidence that these reagents, using the Babcock apparatus, do not include lecithin in estimating the fat content of buttermilk.

EFFECT OF ADDING FAT TO BUTTERMILK ON FAT DETERMINATIONS BY VARIOUS METHODS

The results obtained by the addition of lecithin to buttermilk showed that the Minnesota and the Garret-Overman methods do not estimate lecithin. However, the question arose as to whether these reagents would remove all the fat. Therefore it was thought advisable to determine the effect of added fat, and in Table II are recorded the results.

TABLE II

EFFECT OF ADDING FAT TO BUTTERMILK ON FAT DETERMINATIONS BY VARIOUS METHODS

Sample No.	Added fat per cent	Methods used and fat percentages									
		Mojonnier		Babcock		Normal butyl alcohol		Garret-Overman reagents		Minnesota Babcock test reagent	
E 1.....	0.0	0.5601	0.5623	0.01	0.01	0.48	0.48	0.32	0.28		
E 5.....	0.1	0.6714	0.7025	0.08	0.07	0.65	0.68	0.40	0.40		
E 6.....	0.2	0.7350	0.7344	0.14	0.15	0.74	0.70	0.50	0.50		
E 7.....	0.3	0.8420	0.8260	0.28	0.28	0.84	0.83	0.60	0.64		
F 1.....	0.0	0.5930	0.5920	0.05	0.05	0.40	0.42	0.30	0.30	0.30	0.30
F 2*.....	0.1	.....	.....	...	...	...	...	...	...	...	...
F 3.....	0.2	0.7880	0.7710	0.18	0.22	0.76	0.64	...	0.48	0.50	0.50
F 4.....	0.3	0.8260	0.8450	0.28	0.28	0.76	0.72	0.60	0.60	0.60	0.58
G 1.....	0.0	0.5500	0.5450	0.08	0.07	0.54	0.56	0.28	0.30	0.32	0.30
G 2.....	0.1025	0.6250	0.6270	0.06	0.06	0.58	0.60	0.28	0.29	0.39	0.39
G 3.....	0.2043	0.7283	0.7369	0.10	0.07	0.64	0.64	0.36	0.44	0.50	0.49
G 4*.....	0.3021	.....	.....	...	...	...	...	...	...	...	...

* This sample was accidentally lost.

It is evident that added fat was not quantitatively recovered, tho the Minnesota and the Garret-Overman methods gave the best results. The failure of the Mojonnier method to recover the added fat was difficult to explain, but on emptying the flasks containing the buttermilk a layer of fat was observed on the inside of the container. This source of error and the fact that the Mojonnier fat determinations were made after all the other tests were completed, undoubtedly accounts for the incomplete recovery of fat. With the exception of buttermilks Nos. G 1 and G 2, the normal butyl alcohol tests are increased with each addition of butterfat, but the results with the Babcock tests show little uniformity.

EFFECT OF ADDITION OF BOTH LECITHIN AND FAT TO BUTTERMILK ON FAT DETERMINATIONS BY VARIOUS METHODS

Lecithin is known to be a good emulsifier and if present in sufficient quantities in buttermilk would act as a fat stabilizer. This being true, a relatively high lecithin content in buttermilk would change the surface and interfacial tension relationships existing between the fat

and the lecithin phase. Because these physical properties are important in testing buttermilk, it was thought advisable to prepare several buttermilks in which the fat or the lecithin or both are varied in concentration and note particularly the recovery of fat by the Minnesota Babcock method in the presence of relatively large amounts of lecithin.

TABLE III

EFFECT OF ADDING BOTH LECITHIN AND FAT TO BUTTERMILK ON FAT DETERMINATIONS BY VARIOUS METHODS

Sample No.	Added fat, per cent	Added lecithin, per cent	Methods used and fat precentages							
			Mojonnier		Babcock		Normal butyl alcohol		Minnesota Babcock test reagent	
II 1.....	0.0	0.0	0.5879	0.5797	0.12	0.14	0.60	0.60	0.44	0.46
II 2.....	0.2103	0.1031	0.8983	0.8973	...	0.30	0.64	0.64	0.63	0.65
II 3.....	0.2065	0.2056	0.9805	0.9688	0.28	0.30	0.76	0.76	0.62	0.62
II 4.....	0.1054	0.2033	0.8898	0.8854	0.18	0.20	0.68	0.70	0.56	0.56

The results are summarized in Table III. It is evident that the addition of lecithin did not inhibit the complete recovery of fat. Therefore it is safe to assume from these results that an abnormally high phospholipoid content in buttermilk will not interfere with the estimation of fat by the Minnesota method.

QUANTITATIVE RECOVERY OF LECITHIN AND FAT BY THE MOJONNIER METHOD FOR DETERMINING FAT

Contrary to the results obtained in this laboratory by Thurston and Petersen (1928), Chapman (1928) showed that lecithin added to buttermilk was not recovered quantitatively by the Mojonnier method. Because of these contradictory results, we are submitting additional evidence to clarify the situation.

TABLE IV

QUANTITATIVE RECOVERY OF LECITHIN AND FAT BY THE MOJONNIER METHOD FOR DETERMINING FAT

Sample No.	Added fat, per cent	Added lecithin, per cent	Ether extract by Mojonnier method, per cent		Average, per cent	Recovery of fat and lecithin, per cent
I 1.....	0.0	0.0	0.5879	0.5797	0.5838	.....
I 2.....	0.2103	0.1031	0.8983	0.8973	0.8978	100.00
I 3.....	0.2065	0.2056	0.9805	0.9688	0.9746	97.86
I 4.....	0.1054	0.2033	0.8898	0.8854	0.8876	99.40
J 1.....	0.0	0.0	0.5995	0.5994	0.5994	.....
J 2.....	0.0	0.1066	0.6855	0.7182	0.7018	99.40
J 3.....	0.0	0.2027	0.8086	0.8092	0.8089	100.80

While the recovery of fat and of lecithin, as shown in Table IV, is not exactly within the realm of quantitative accuracy, yet all sources of error considered, the figures show that pure lecithin can be recovered quantitatively by the Mojonnier method. These data further

emphasize the inaccuracies incurred when the fat content of buttermilk is estimated by the Roese-Gottlieb and the Mojonnier methods.

RELATION OF THE FAT CONTENT OF THE CREAM TO THE PHOSPHOLIPOID CONTENT OF THE BUTTERMILK

We have shown that the Mojonnier method of fat extraction includes lecithin. Then it can be assumed that the other alcohol- and ether-soluble phospholipoids are also extracted. We have further shown that the Minnesota method does not estimate lecithin. With these facts at hand we can calculate the phospholipoid content of buttermilk as the difference between the fat determinations according to the Mojonnier and the Minnesota tests.

TABLE V

FAT PERCENTAGE OF THE CREAM IN RELATION TO THE PHOSPHOLIPOID CONTENT OF THE BUTTERMILK

Measured by the difference between the fat determination according to the Mojonnier and the Minnesota methods

Churning No.	Fat content of cream, per cent	Fat in buttermilk				Calculated phospholipoids, per cent
		Mojonnier, per cent		Minn.-Babcock test reagent, per cent		
A 1	38.5	1.5020	1.5152	1.00	1.00	0.5086
A 2	21.0	1.2017	1.2382	0.90	0.90	0.3199
B 1	35.5	1.9830	1.9522	1.50	1.55*	0.4426
B 2	18.5	0.6593	0.6466	0.40	0.40	0.2529
C 1	38.5	1.7985	1.8110	1.35	1.35*	0.4547
C 2	19.0	0.7637	0.7304	0.44	0.44	0.3070
D 1	34.0	1.8900	1.8780	1.55	1.55*	0.3340
D 2	19.5	1.2439	1.2429	1.10	1.05	0.1684
E 1	39.5	3.0339	3.0292	2.50	2.50*	0.5315
E 2	19.0	1.3531	1.3760	1.10	1.10	0.2645
F 1	38.5	3.2650	3.2921	2.90	2.85*	0.4035
F 2	20.0	0.9347	0.9503	0.72	0.74	0.2125
G 1	36.0	2.4268	2.4319	2.10	2.15*	0.3043
G 2	18.5	0.9652	0.9548	0.70	0.71	0.2550

* Fat estimated in whole-milk test bottle, and a small amount of glymol placed on the surface of the fat to remove the meniscus.

Dornic and Daire (1910) state that lecithin is concentrated on the surface of the fat globules and is detached during the churning process. Palmer and Samuelson's (1924) work supports this explanation. If this is true, buttermilk from high testing cream should contain relatively larger amounts of phospholipoids.

To study the relative phospholipoid content of buttermilk from creams of high and of low fat content, a series of seven comparisons was made, a total of fourteen churnings. In each case a cream of

high fat content was obtained. A part of it was churned and the remainder was standardized with skimmilk. Five pounds of cream was churned in Dazey churns and temperatures were carefully controlled. The figures in Table V show that the calculated phospholipoid content is higher for buttermilk from creams of high fat content. With the exception of churnings 13 and 14 the difference as compared to buttermilk from low-testing cream is more than 0.1 per cent. These figures are only approximate, because the limit of accuracy in reading a Babcock whole-milk test bottle is 0.1 per cent. With more finely calibrated test bottles, our method of calculations should give a true estimate of the phospholipoid content of buttermilk.

PURITY OF FAT AS EXTRACTED BY THE MINNESOTA METHOD

The experimental evidence in this paper indicates that the Minnesota method is free from the objectionable features of the normal butyl alcohol and the Mojonnier methods in that the alcohol- and ether-soluble phospholipoids are not extracted and estimated as true fat. However, the question was raised as to the purity of the fat estimated by the Minnesota method. This was in part proved by the results recorded in Table II where added fat was recovered quantitatively, but did not preclude the possibility of the fat being contaminated with alcohol from the alkaline reagent, an observation reported by earlier workers (Sichler, 1904).

We proceeded to determine the purity of the fat by estimating the fat content of buttermilk with a type of skimmilk test bottle having a removable neck (known in the trade as "Russian"). A 9-gram sample of buttermilk was used. The fat in the removable neck was washed with ether into a weighed Mojonnier evaporating dish. The dish was placed in the Mojonnier vacuum oven at 135° C. for five minutes, cooled and weighed. From the net weight of the dried residue and the weight of the buttermilk sample, the fat percentages were recalculated and compared with the original volumetric readings. It is evident from the results recorded in Table VI that the fat was not contaminated with any appreciable quantity of foreign volatile material.

TABLE VI

PURITY OF FAT AS EXTRACTED BY THE MINNESOTA METHOD
Determined by drying in a vacuum oven

Sample No.	Fat content as read from test bottle	Recalculated fat content*
	per cent	per cent
1	0.40	0.394
2	0.39	0.375
3	0.38	0.365
4	0.34	0.293

* As determined from weight of dried fat residue and weight of buttermilk sample.

SOME FUNDAMENTAL CONSIDERATIONS OF THE MINNESOTA BABCOCK TEST REAGENT

The separation of the fat from the other ingredients is affected by a dispersion of the non-fatty materials of milk and a change in the interfacial tension between the fat particles and the solution. The interfacial tension is so affected by the alcohol that the fat particles rapidly coalesce and separate as a clear liquid layer. The alcohols are readily soluble in the alkaline solution, where they remain and do not contaminate the supernatant fat.

The alkaline solution disperses the milk solids-not-fat, including the phospholipoids. The soluble alkaline salts act as buffers, which eliminates the danger of fat saponification. Subjecting samples of buttermilk mixed with the reagent to a temperature of 77-82° C. for thirty minutes did not affect the fat reading. A one per cent aqueous lecithin emulsion was exposed to the action of the reagent for five minutes at 82° C. and, when acetone was added, a precipitate of apparently normal lecithin was obtained. The presence of choline in the molecule, which is strongly alkaline, probably accounts for the stability of lecithin toward alkalies, whereas lactose is readily oxidized because of the instability of sugars in alkaline solutions. Furthermore, the soluble salts in the reagent do not crystallize on standing at room temperature, 21° C. At lower temperatures crystallization takes place and it becomes necessary to heat the reagent slightly before using. Creamery operators have used this reagent for the last five months for testing buttermilk and report excellent results.

SUMMARY

1. Further evidence is presented to show that the phospholipoids are a factor in estimating the fat content of buttermilk.

2. A method is described for determining the true fat content of buttermilk.

3. Preliminary work indicates that the Minnesota Babcock test reagent may be used to estimate the fat content of other dairy products, including ice cream and condensed milk. However, more extended studies are necessary before definite recommendations can be made.

LITERATURE CITED

Chapman, O. W.
1928 The effect of lecithin in dairy products upon butter fat determinations. Jour. Dairy Sci. 11:429-435.

Dornic, M. M., and Daire
1910 La Contribution a' la Étude de la Lecithin du Lait. Ann. des Falsif. 3:533.

du Roi, Dir., u Kohler, Dr.
1904 Versuche uber die Brauchbarkeit der Sinacid-Butyrometrie des Chemikers und Apothekers. A. Sichler, Leipzig. Milch Ztg. 33: 787-790.

Gerber, N.
1906 Milch Ztg. 35:37.

Gies, W. J.
1912 Studies of diffusion through rubber membranes. Biochem. Bull. 2:55.

Gordon, P.
1904 Versuche mit Sichlers Sinacid—Butyrometrie. Milch Ztg. 33:755.

Höyberg, H. M.
1921 Testing cream for fat. Chem. Abs. 15:79.

Hunziker, O. F.
1927 The Butter Industry. Ed. 2, p. 307. O. F. Hunziker, La Grange, Ill.

Kreis, H. and Studinger, J.
1927 The Neusal method. Mitt. Lebensmtl. Untersuch. u. Hyg., Eidg. Gsndhtsamt. 18:333.

Maclean, H.
1918 Lecithin and Allied Substances: The Lipins. Longmans, Green, and Company, New York.

Magliano, A., and Porzio, J.
1927 Méthode pratique pour le dosage de la matière grasse du lait. Lait 7:713-728.

McKay, G. L., and Larsen, C.
1922 Principles and Practices of Buttermaking. Ed. 3, p. 101. Wiley and Sons, New York.

Meyers, J. A.
1891 Methods of testing milk. W. Va. Expt. Sta., 3rd Ann. Rept., pp 68-87.

Orla-Jensen
1923 Le procédé Höyberg pour la détermination de la matière grasse dans le lait et la crême. Lait 3:177-187.

Overman, O. R., and Garret, O. F.
1928 The Garret-Overman fat test on ice cream. The Ice Cream Trade Jour. 24:51.

Palmer, L. S., and Samuelson, E.
1924 The nature of the substances adsorbed on the surface of the fat globules in cow's milk. Proc. Soc. Expt. Biol. Med. 21:537.

Sichler, A.
1904 Milch Ztg. 33:417, 436, 790.

Spur, B.
1926 Modification et simplification du procédé proposé par M. le Dr. Höyberg pour la détermination de la matière grasse dans le lait et la créme. Lait 6:518-533.

Spur, B., Continued
1926 Modification et simplification du procédé proposé par M. le Dr. Höyberg pour la détermination de la matière grasse dans le lait et la crême. Lait 6:639-655.

Thurston, L. M., and Petersen, W. E.
1928 Lipins and sterols as sources of error in the estimation of fat in buttermilk by ether extraction methods. Jour. Dairy Sci. 11:270-283.

Van Woerden, J.
1928 Le procédé Höyberg pour le dosage de la matière grasse du lait. Lait 8:678-683.

Windisch, K.
1909 Ueber das Sinacidverfahren und das Salverfahren Zur Fettbestimmung in der Milch. Milchw. Zentbl. 5:344-352.