is used approximately one-third should be added to the indicated quantities. Or, to use the same table, the titration can be considered as one-third more and the figures read direct from the table. For example, if the defecator contained 400 gallons of juice and if the titration showed 18 cc. of $N/10$ potassium hydroxide solution used, instead of reading the lime as 42 oz., it would be very simple to run over to the column headed 24 cc. and read the lime required as 72 oz. The indicated amount of lime should be weighed out, slaked and added as milk of lime.

SUMMARY

1—The acidity of the juice of sorghum cane decreases during defecation and increases during evaporation, with the lime and phosphate methods. The acidity varies inversely with the amount of lime used.

2—The volume of the lead subacetate precipitate is not a good indication of the efficiency of defecation. With high acidity there is a large precipitate.

3—The ash content increases after lime has been added. In the phosphate method there is a decrease in the amount of ash.

4—Calcium oxide increases during defecation and decreases during evaporation.

5—During defecation there is an apparent increase in sucrose and decrease in reducing sugars.

6—The nitrogen content decreases during defecation. In cane which has stood after being cut the nitrogen is changed to a non-precipitable form which is not removed by defecation.

7—Solids-not-sugar decrease during defecation and evaporation, the larger decrease being during the latter process.

8—The color of the juice is not darkened materially until more than the theoretical amount of lime has been added. The amount of lime to produce alkalinity is 2.2 times the theoretical amount.

9—Acidity in syrups varies inversely with the amount of lime used.

10—In syrups the total ash, insoluble ash and calcium oxide increase with the amount of lime added.

11—Sucrose increases and dextrose decreases with the amount of lime used.

12—Crystallization is due to a high percentage of dry matter, a relatively high sucrose content and a juice relatively free from "gummy materials." It occurs most frequently in samples where lime was used in defecation.

13—The darkness of color of a syrup varies directly with the amount of lime used in defecation and with the time required for evaporation.

14—The phosphate and lime methods give the best tasting syrups. For economic reasons the lime method is considered the better to use.

15—The theoretical amount of lime gives proper defecation with the minimum darkening of the juice. The titration of the juice is an efficient method of factory control.

DIVISION OF AGRICULTURAL BIOCHEMISTRY
AGRICULTURAL EXPERIMENT STATION
ST. PAUL, MINNESOTA

THE CORRECTION REQUIRED IN APPLYING THE BABCOCK FORMULA TO THE ESTIMATION OF TOTAL SOLIDS IN EVAPORATED MILK¹

By O. L. EVENSON

Received March 13, 1917

The Babcock formula,²

$$T = \frac{L}{4} + 1.2 \times \text{Per cent Fat},$$

where T = total solids and L = Quevenne Lactometer reading,³ or $1000 \times \text{sp. gr.}^3 - 1000$, might not be expected to yield as accurate results when applied to evaporated milk as when applied to whole, fresh milk because when milk is concentrated the specific gravity and total solids do not increase at the same rate. Babcock's² complete formula

$$\text{Solids-not-fat} = \frac{(100s - sf)}{(100 - 1.0753sf)} (100 - f) \quad 2.5,$$

where s = specific gravity and f = per cent fat, gives still less satisfactory results than the short formula. While Richmond's⁴ formula,

$$T = 0.2625 \frac{G}{D} + 1.2 \times \text{Per cent Fat},$$

where T = total solids, $G = 1000 \times \text{sp. gr.}^3 - 1000$ and D = specific gravity³, will give more accurate results than either, it is not as simple as the short formula of Babcock and is not directly applicable without a small correction. Bigelow and Fitzgerald⁵ showed that more accurate results could be obtained with the Babcock formula on the original evaporated milk than on the same milk diluted with an equal weight of water. No mention, however, is made of the temperature at which the specific gravity was determined or at which the evaporated milk had been kept preceding the determination of the specific gravity. That the specific gravity of freshly drawn milk, when measured at a definite temperature, $\frac{15^\circ}{15^\circ}$ or

$\frac{15.5^\circ}{15.5^\circ}$ C., gradually increases on standing was, according to Fleischmann,⁶ first observed by Quevenne, but is generally known as Recknagel's⁷ phenomenon. It has been shown by Fleischmann⁸ to be due to a solidification or change in the physical state of the fat. When milk is cooled below the solidification point of butter-fat, the latter begins to solidify and in this way increases the specific gravity. Conversely when milk which has been cooled to its maximum specific gravity, is heated, its specific gravity decreases until the fat is again melted, when no further change takes place. The same phenomenon has been observed with an emulsion of butter-fat and water. This change in the specific gravity increases with the percentage of fat, skimmed milk showing little or no change.

¹ Read at the 53rd Meeting of the American Chemical Society in New York City, September 25-30, 1916. Published by permission of the Secretary of Agriculture.

² Twelfth Ann. Report, Wis. Agr. Expt. Sta., 1895, 120-126, Madison, Wis.

³ At 15.56° C. (60° F.).

⁴ Richmond's "Dairy Chemistry," 2nd Edition, p. 69 (1914).

⁵ Bull. 5 (1915), Research Laboratory, Nat'l Canners' Assn.

⁶ J. Landw., 1913, 282.

⁷ Milchzeitung., 12 (1883), 419.

⁸ Loc. cit.; also 1902, 33.

The purpose of this investigation was to determine the error of the Babcock short formula when applied to evaporated milk so that an average correction might be made and more accurate results obtained when the formula is applied to estimate total solids in milk at the temperatures of 5° and 37° to 40°.

For the work here recorded, 16-oz. samples of commercial evaporated milk were obtained as required, so that the samples were always in good condition.

The total solids were calculated from the specific gravity and percentage of fat by the formula

$$T = \frac{L}{4} + 1.2 \times \text{Per cent Fat},$$

and the results obtained compared with those obtained gravimetrically. The specific gravity of the milk was determined at 15.56° C. by means of a pycnometer.

Almost invariably duplicate results did not differ from each other by more than 0.0001. The Röse-Gottlieb¹ Method was used for the determination of fat and the Official Method,² using sand, was employed in determining solids.

In Table I is shown the change in specific gravity

TABLE I—THE CHANGE IN SPECIFIC GRAVITY OF SEVERAL COMMERCIAL EVAPORATED MILKS KEPT AT DIFFERENT TEMPERATURES

No.	Per cent Fat	Specific gravity at 15.56°/15.56° C.			Difference in Sp. Gr. of milk held at 5° C. and at 37-40° C.
		Held 24 hours at 5° C.	20-24° C.	1.5 hrs. at 37-40° C.	
1.....	8.06	1.0672	1.0666	1.0658	0.0014
2.....	8.28	1.0735	1.0724	1.0719	0.0016
3.....	8.24	1.0677	1.0668	1.0659	0.0018
4.....	7.50	1.0711	1.0705	1.0701	0.0010
Average.....					0.0014

of several commercial evaporated milks kept for certain intervals at different temperatures. In each case the milk was brought to 15.56° C. immediately after the holding period and the specific gravity taken without delay. It will be observed that a considerable difference in specific gravity may be obtained by holding the milk at different temperatures. The average difference obtained in the specific gravity of the milk when held at 5° C. and when held at 37-40° C. is 0.0014. This would make a difference of 0.35 per cent in the calculated solids. This does not apply to skimmed evaporated milk from which the fat has been largely removed.

In Table II is shown the application of the Babcock formula to evaporated milk which has been kept at 5° C. for 24 hours; also, to the same milk diluted with an equal weight of cold, recently boiled distilled water. It is evident that the specific gravity does not vary directly as the percentage of total solids when the milk is concentrated but increases at a more rapid rate. As a consequence, the calculated results are high. In normal evaporated milk which has been cooled so that no further increase in specific gravity occurs, the figure +0.46 given in Table II is believed to represent the average difference between the calculated and gravimetrically determined total solids when using Babcock's short formula. Consequently, when cor-

TABLE II—APPLICATION OF THE BABCOCK FORMULA TO EVAPORATED MILK HELD AT 5° C. FOR ABOUT 24 HOURS

No.	Per cent Fat	UNDILUTED			DILUTED 1 : 1			
		Specific Gravity	Per cent Solids (Dry- ing) (Calc.)	Diff.	Specific Gravity	Per cent Solids (Calc.)	Diff.	
1	7.87	1.0656	25.52	25.84	+0.32	1.0318	25.34	-0.18
2	7.99	1.0658	25.67	26.04	+0.37	1.0318	25.49	-0.18
3	8.10	1.0652	25.74	26.02	+0.28	1.0318	25.62	-0.12
4	7.84	1.0673	25.76	26.23	+0.47	1.0327	25.76	0.00
5	7.90	1.0675	25.97	26.36	+0.39	1.0328	25.88	-0.09
6	8.04	1.0673	26.07	26.47	+0.40	1.0327	26.00	-0.07
7	7.86	1.0685	26.10	26.56	+0.46	1.0334	26.13	+0.03
8	7.74	1.0692	26.13	26.59	+0.46	1.0337	26.14	+0.01
9	8.02	1.0674	26.14	26.47	+0.33	1.0327	25.97	-0.17
10	7.47	1.0708	26.17	26.66	+0.49	1.0343	26.11	-0.06
11	7.50	1.0711	26.22	26.77	+0.55	1.0344	26.20	-0.02
12	8.26	1.0672	26.30	26.71	+0.41	1.0328	26.31	+0.01
13	8.24	1.0677	26.31	26.81	+0.50	1.0329	26.34	+0.03
14	8.08	1.0681	26.46	26.72	+0.26	1.0331	26.25	-0.21
15	8.28	1.0684	26.60	27.04	+0.44	1.0333	26.59	-0.01
16	8.38	1.0688	26.70	27.26	+0.56	1.0333	26.71	+0.01
17	8.44	1.0692	26.95	27.43	+0.48	1.0336	26.93	-0.02
18	8.03	1.0722	27.04	27.68	+0.64	1.0350	27.13	+0.09
19	8.04	1.0721	27.10	27.67	+0.57	1.0351	27.20	+0.10
20	8.10	1.0730	27.35	27.97	+0.62	1.0354	27.42	+0.07
21	8.15	1.0727	27.42	27.95	+0.53	1.0353	27.43	+0.01
22	8.28	1.0735	27.65	28.31	+0.66	1.0358	27.84	+0.19
23	8.23	1.0768	28.60	29.08	+0.48	1.0373	28.53	-0.07
Average.....					+0.46			-0.03

rected for this difference the Babcock formula becomes

$$T = \frac{L - 1.8}{4} + 1.2 \times \text{Per cent Fat}^1$$

if applied to the cooled undiluted evaporated milk.

In Table III is shown the application of the Babcock formula to some milks kept at 37-40° C. for one and a half hours, and also to the same milks diluted with an equal weight of water at about the same temperature. As mentioned above, the milk must be brought to 15.56° C. and the specific gravity taken without delay.

TABLE III—SHOWING THE APPLICATION OF THE BABCOCK FORMULA TO EVAPORATED MILK HELD AT 37-40° C. FOR 1½ HOURS

No.	Per cent Fat	UNDILUTED			DILUTED 1 : 1			
		Specific Gravity	(Dry-Ing)	(Calc.)	Diff.	Specific Gravity	Solids (Calc.)	Diff.
1	8.06	1.0659	26.06	26.15	+0.09	1.0321	25.72	-0.34
2	7.86	1.0671	26.10	26.21	+0.11	1.0328	25.83	-0.27
3	7.84	1.0664	26.12	26.01	-0.11	1.0323	25.56	-0.56
4	7.47	1.0696	26.17	26.36	+0.19	1.0338	25.86	-0.31
5	7.50	1.0701	26.22	26.52	+0.30			
6	8.24	1.0659	26.31	26.36	+0.05			
7	8.08	1.0669	26.46	26.42	-0.04	1.0325	25.95	-0.51
8	8.28	1.0719	27.65	27.91	+0.26	1.0350	27.44	-0.21
9	8.23	1.0757	28.60	28.80	+0.20			
Average.....					+0.12			-0.37

As may be seen from Table III, the average difference is +0.12 when the formula is applied to milk held at 37-40° C. Therefore, the correction for milk at 37-40° C. is 0.5 and the formula to be used for this milk then becomes

$$T = \frac{L - 0.5}{4} + 1.2 \times \text{Per cent Fat}.$$

Lower results were obtained when the milk was diluted.

SUMMARY

It is clear that when exact percentages of total solids are demanded the Babcock formula cannot be applied directly to the determination of total solids in evaporated milk held either at 5° C. or at 37-40° C. But results, the accuracy of which are comparable with those obtained with the Babcock formula on whole milk, may be obtained if the formula

$$T = \frac{L - 1.8}{4} + 1.2 \times \text{Per cent Fat}$$

is applied to the undiluted evaporated milk cooled to its maximum specific gravity and if the formula

¹ In the formula, a difference of 0.46 per cent in solids is equivalent to 1.84 in the lactometer reading or 0.00184 in specific gravity (4 × 0.46 = 1.84).

² U. S. Dept. Agr., Bureau of Chemistry, *Cir.* 66 (1911).

³ U. S. Dept. Agr., Bureau of Chemistry, *Bull.* (Revised), 107 (1903).

$$T = \frac{L - 0.5}{4} + 1.2 \times \text{Per cent Fat}$$

is applied to milk kept at 37–40° C. for one and a half hours or until no further change in specific gravity occurs.

DEPARTMENT OF AGRICULTURE
BUREAU OF CHEMISTRY
WASHINGTON, D. C.

IMPROVEMENTS IN THE COPPER METHOD FOR ESTIMATING AMINO ACIDS

By PHILIP ADOLPH KOBER

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For sometime we have been trying to develop further, methods for the study and analysis of proteins. In connection with this work, which will involve the scheme of Van Slyke, we have first attempted to improve and standardize some parts of the copper method¹ for estimating amino acids.

Our improvements are: (I) A simpler method for making standard copper solutions; (II) a method for making a stock suspension of cupric hydroxide which keeps for months, and (III) a method for making and keeping iodometric reagents; *i. e.*, saturated potassium iodide solutions, containing starch and acetic acid.

I—THE METHOD FOR MAKING STANDARD COPPER SOLUTIONS

In the original paper² no specific directions were given for making an iodometric standard, the directions in any standard text-book having been considered sufficient. None of the methods given in these books, however, are very convenient nor are the solutions stable. Standardization of copper solutions by electrolysis, which is of course most accurate, is decidedly unsuitable for biological laboratories, nor is it a particularly short method unless special apparatus involving rotating cathodes is used.

The weighing-out of a suitable amount of a pure copper salt was thought to be the simplest procedure. Cupric sulfate is the purest and cheapest source of copper on the market and the only disadvantage encountered in using it for standard solutions is its uncertain water content and, therefore, unknown copper content. This disadvantage may be practically overcome by dehydrating the salt, as shown below, in a very simple and inexpensive apparatus.³

About 1 lb. of "parowax" is melted in a liter beaker or other suitable container and used in a hood as an oil bath at 245° to 260° C. A test tube (18 mm. inside diameter, length 155 mm.) is cleaned and dried at 105 to 110° C., and weighed with a paraffined cork stopper. Allowing the stopper to remain in the desiccator, 1.77 g. of monohydrate cupric sulfate⁴ are put into the test-tube and connected with the aerating tubes as shown in Fig. 1. Upon heating for 2 or 3 hrs. with

¹ Kober and Sugiura, *J. Am. Chem. Soc.*, **35** (1913), 1546; applied to soils by Potter and Snyder, *Ibid.*, **37** (1915), 2219; *THIS JOURNAL*, **7** (1916), 1049; applied to study of Lecithin, by MacArthur, *J. Am. Chem. Soc.*, **36** (1915), 2397.

² *J. Am. Chem. Soc.*, **35** (1913), 1546.

³ For working out this method and for all the analytical work, I am indebted to Mr. Walther Eberlein of this laboratory.

⁴ $\text{CuSO}_4 + 5\text{H}_2\text{O}$ heated to 105 to 110° C. for several hours is converted to monohydrate $\text{CuSO}_4 + \text{H}_2\text{O}$, having only a light blue tint.

shaking every 15 min. the cupric sulfate is practically dehydrated and is obtained perfectly white; the apparatus is then removed from the oil bath without interrupting the aeration and the oil, while the tube is still hot, carefully and completely wiped off with a towel. Then, when cool, the paraffined stopper is quickly inserted and the tube placed in the desiccator until it is ready for weighing. By using apparatus with ground glass joints, which was not available to us for this investigation, more accurate results can undoubtedly be obtained. If care is taken the tube may be heated without an oil bath, by putting it over a Bunsen burner, so as to maintain it just below visible red heat at about 400° C.

The following results were obtained:

G. CuSO_4 Taken	G. Cu (Electrolysis)	G. Cu Expected	Ratio Cu Found to Theory (%)
1.1846	0.4706	0.4720	99.7
1.0515	0.4173	0.4190	99.6
1.1044	0.4374	0.4390	99.6
1.0695	0.4247	0.4250	99.7

This method of dehydrating cupric sulfate, as the work of T. W. Richards¹ indicates, may not give suffi-

FIG. 1

ciently accurate results for the purpose of calculating atomic weights, but the shaking from time to time in an atmosphere of dry air, may make the results slightly higher than the lowest given by Richards at 99.9 per cent. However, with the apparatus and equipment at our disposal, we were not able to test this out.

DIRECTIONS—After weighing, the contents of the tube are dissolved, avoiding loss due to spattering by keeping the tube covered or in a horizontal position: 1.77 grams of the monohydrate cupric sulfate will give sufficient copper when dissolved in 250 cc. of water to make a $\frac{1}{25}$ molar solution, which if carefully stoppered can be used for standardizing stock solutions almost indefinitely.

To guard against the formation of basic salt, due to hydrolysis and any alkalinity of the glass, it is well to add 1 or 2 drops of concentrated sulfuric acid.

II—A METHOD FOR MAKING A PERMANENT SUSPENSION OF CUPRIC HYDROXIDE

In the original directions, cupric hydroxide had to be made fresh every day. Its preparation, because it required chopped ice, neutralization, filtration and

¹ *Z. anorg. Chem.*, **1** (1892), 179.