NOTES ON SOUR MILK.

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I. THE DETERMINATION OF THE SPECIFIC GRAVITY OF SOUR MILK.

WEIBULL (*Chem. Zeit.*, 1893, xvii. 1679) proposed, in order to determine the specific gravity of sour milk, the addition of a known volume of ammonia; the density of the mixture, corrected for the volume of ammonia added, of which the density is known, gives the density of the milk.

It has been our practice to slightly modify this method; we add to each 100 c.c. of sour milk 5 c.c. of strong ammonia, and to the density of the mixture we apply a constant correction deduced from the change of density on adding 5 c.c. of strong ammonia to 100 c.c. of fresh milk; this correction has varied from 0.0065 to 0.0070 with different samples of strong ammonia.

We can fully confirm Weibull's statement that the density of sour milk can be accurately determined by this means, and have used the method extensively.

De Koningh (*ANALYST*, xxiv. 142) has substituted a solution of caustic soda (specific gravity 1.030) for the strong ammonia; he finds that a constant correction of 0.0008 must be added to the results, this being the mean figure by which the density is lowered on mixing 5 c.c. of soda solution with 100 c.c. of milk. He accounts for the lowering of density by the assumption that calcium phosphate is rendered insoluble; we neither entirely confirm his figure nor are we satisfied with his explanation; as we propose to show, the correction is not constant, but varies

with the "acidity" of the milk, and can be, we think, explained when the action of acids on alkalis is considered.

In Table I. we give the densities of solutions of caustic soda, ammonia, and sulphuric and hydrochloric acids, and the salts resulting from their mutual action.

The figures are obtained by interpolation from well-known tables, which we have taken from the *Chemiker Kalender*, and are expressed in terms of $\frac{N}{5}$, $\frac{N}{10}$, and $\frac{N}{20}$ solutions; they are probably correct to the fourth place of decimals.

TABLE I.

Densities of Solutions of Bases, Acids, and Salts.

Substance.	$\frac{N}{5}$.	$\frac{N}{10}$.	$\frac{N}{20}$.	Authority
NaOH ...	1.00920	1.00465	1.00235	Lunge.
NH ₃ ...	0.99856	0.99928	0.99964	Carius.
HCl ...	1.00372	1.00186	1.00093	Lunge and Marchlewski.
H ₂ SO ₄ ...	1.00774	1.00387	1.00193	Lunge and Isler.
NaCl ...	1.00846	1.00423	1.00212	Gerlach.
NH ₄ Cl ...	1.00338	1.00169	1.00085	"
Na ₂ SO ₄ ...	1.01266	1.00639	1.00322	"
(NH ₄) ₂ SO ₄ ...	1.00752	1.00376	1.00188	Schiff.

If a salt in solution had a density which was the sum of the densities of the acid and base of which it was composed, the density of a solution of a salt should be equal to the sum of the densities of corresponding strengths of its acid and its base, less 1. This is not, however, the case, and the difference between the sum of the densities of the acid and base (less 1), and the density of the salt is given in Table II. :

$\frac{N}{5}$ $\frac{N}{10}$ $\frac{N}{20}$	NaOH.		NH ₃ .	
	HCl.	H ₂ SO ₄ .	HCl.	H ₂ SO ₄ .
	...	-0.00446	+0.00110	+0.00122
	...	-0.00228	+0.00055	+0.00061
	...	-0.00116	+0.00028	+0.00031

As the acidity of fresh milk is about $\frac{N}{50}$, and that of sour milk about $\frac{N}{20}$ to $\frac{N}{10}$, it is seen that the change of density on neutralization will go a long way towards explaining the change noticed by De Koningh.

Thinking that it would not be quite correct to compare the acids such as sulphuric and hydrochloric, with milk, we have made a few experiments with weaker acids.

We prepared a solution of caustic soda of density 1.032, which was 0.666 N., and determined the densities of various dilutions of this with water. There were :

No. of c.c. diluted to 100 c.c. = x.	Density found.	Density calculated.
4.10	1.00138	1.00138
7.96	1.00266	1.00265
11.81	1.00388	1.00391

$$\text{Formula : Density} = 1 + 0.000339x - 0.0000007x^2.$$

The determinations show that when 5 to 20 c.c. of this solution are added to 100 c.c. the density is about 0.0001 higher than that calculated proportionally.

A solution of citric acid, 1·0064 N., was prepared; the densities of various dilutions were determined:

No. of c.c. diluted to 100 c.c. = x .	Density found.	Density calculated.
2·39	1·00059	1·00064
4·80	1·00135	1·00130
7·47	1·00200	1·00201

Formula: Density = $1 + 0·0002688x$.

Solutions were now made up containing varying quantities of citric acid and a slight excess of soda solution:

No. of c.c. of Citric Acid in 100 c.c.	No. of c.c. of Soda in 100 c.c.	Density found.	Density calculated.
2·41	4·04	1·00174	1·00200
4·82	8·48	1·00361	1·00412
7·41	11·99	1·00524	1·00595

The calculated density was obtained by adding together the figures obtained by calculating the densities due to the citric acid and soda by the formulæ given above, and subtracting 1.

Fifty c.c. of a solution of lactic acid diluted to 100 c.c. (making 0·0256N) had a density of 1·00060; 50 c.c. of this solution + 4·76 c.c. solution of caustic soda, diluted to 100 c.c., had a density of 1·00195. The calculated density was 1·00220.

Calculated to a strength of $\frac{N}{10}$, the differences are for citric acid 0·00105, 0·00104, and 0·00095, or a mean of 0·00100, and for lactic acid 0·00098, figures which are less than half that given by the stronger acids. As citric acid, in addition to being a weak acid, is a tribasic acid, we thought that the lower figure obtained might be partly due to the three hydrogen atoms not having the same value. It was convenient to test this question in the first instance with oxalic acid on account of the ease with which normal and acid salts could be prepared.

We made a quantity of each salt, the purity of which is shown by the following figures:

	Normal Salt $\text{Na}_2\text{C}_2\text{O}_4$		Acid Salt $\text{NaHC}_2\text{O}_4\cdot\text{OH}_2$	
	Found	Theory	Found	Theory
Total C_2O_4	65·55, 65·48	65·67	67·58, 67·82	67·69
C_2O_4 (as acid)	none	none	33·61	33·85
Loss in water-bath	none	none	13·92	13·85
Further loss at 105° to 110°	none	none	none	none

The following densities were determined:

(i.)	Oxalic acid 0·0406 N.	...	...	...	...	1·00082
	„ „ 0·10 N.	...	...	...	...	1·00217
	„ „ 0·2597 N.	...	...	...	...	1·00563
(ii.)	* $\text{Na}_2\text{C}_2\text{O}_4$ 0·10 N.	...	...	...	...	1·00556
(iii.)	* NaHC_2O_4 0·20 N.	...	...	...	...	1·00720
(iv.)	Oxalic acid 0·10 N., to which 14·43 c.c. soda solution per 100 c.c. had been added = 0·0961 N.	...	...	...	...	1·00572

* These two solutions contained the same amount of sodium.

From the above figures we calculate the following losses of density for $\frac{N}{10}$ solutions :

When the total acidity is neutralized	from i., ii., and density of NaOH	0·00126
” ” ” ”	from i. and iv.	0·00124
When the first hydrogen atom is neutralized	from i., iii., and density of NaOH	0·00179
When the second hydrogen atom is neutralized	from i., ii., and iii.	0·00053
” ” ” ” ”	from i., iii., and iv.	0·00068
” ” ” ” ”	from ii., iii., and density of NaOH	0·00073
” ” ” ” ”	from i., ii., iii., and iv.	0·00071

From the above results it appears that the first hydrogen atom in oxalic acid is the more powerful.

It is seen from the above that although neutralization of an acid by soda always produces a loss of density, the figure varies not only with the acid, but also with the hydrogen atom neutralized in a polybasic acid. For this reason it is useless to apply any theoretical correction for milk, and we have made some experiments to see if we can find a value which may be used in milks.

To milk of density 1·0319 increasing quantities of soda solution were added and the densities taken. Three c.c. of the solution rendered this milk exactly neutral to phenolphthalein.

Quantity added.	Density.
1 c.c. to 100 c.c.	1·03175
2 ” ”	1·0316
3 ” ”	1·0316
4 ” ”	1·0316

Five c.c. of soda solution was added to 100 c.c. of different milks, and the densities determined.

Original	...	1·0325	1·0327	1·0319	1·0328	1·0339 } 1·0338 }	1·0309	1·0328
Alkaline	...	1·0322	1·0321	1·0316	1·0324	1·0334 } 1·0335 }	1·0306	1·0325

The third milk in the above table was allowed to get sour ; when the acidity was equal to 9 c.c. of the soda solution for 100 c.c., it still was liquid, and had a density of 1·0319. On the addition of 5 c.c. soda solution the density fell to 1·0316, and to 1·0310 by the addition of 10 c.c. ; when 15·1 c.c. were required to neutralize it, the addition of 20 c.c. of soda dissolved the precipitated curd, and the density was 1·0298. In each case Weibull's method, applying the correction found for fresh milk, gave a density of 1·0320.

The following additional experiments were performed :

Original Density of Milk.	Acidity.	Soda added.	Density of Alkaline Milk.
1·0331	136°	21 c.c.	1·0300
1·0326	124°	19 c.c.	1·0302

Fresh milk has, according to our experience, an average acidity of 20°, and we assumed that all the milks tested in a fresh condition had this acidity.

Tabulating the acidities and the lowering of density, we get :

Original Density.	Acidity Neutralized.	Lowering of Density.	Calculated Original Density.
1·0319	6·7°	0·00015	1·0319
„	13·3°	0·0003	1·0319
„	20·0°	0·0003	1·0320
1·0325	20·0°	0·0003	1·0326
1·0327	20·0°	0·0006	1·0325
1·0319	20·0°	0·0003	1·0320
1·0328	20·0°	0·0004	1·0328
1·03385	20·0°	0·0004	1·03385
1·0309	20·0°	0·0003	1·0310
1·0328	20·0°	0·0003	1·0329
1·0319	33·3°	0·0003	1·0323
„	60·0°	0·0009	1·0321
„	101·0°	0·0021	1·0318
1·0326	124·0°	0·0024	1·0327
1·0331	136·0°	0·0031	1·0327

The figures calculated are obtained on the assumption that each degree of acidity causes a lowering of the density of 0·00002, and with this correction the results are fairly accurate.

On referring to Table II. we see that with strong acids the change of density on neutralizing with ammonia is very much smaller than with soda, and in the opposite direction, and our results with milk indicate that it may practically be neglected. This is due partly to the fact that dilution of strong ammonia produces a small change in density in the direction opposite to that due to neutralization, and partly to the fact that there may be a small loss in solids when milk becomes sour, and the small errors in the one case tend to neutralize the small errors in the other.

With De Koningh's method the errors are larger, and tend in the same direction, and though an approximate correction depending on the acidity of the milk can be worked out, it appears preferable to use Weibull's method.

II. THE POINT AT WHICH MILK MAY BE CONSIDERED SOUR, AND THE RATE OF SOURING IN THE PRESENCE OR ABSENCE OF PRESERVATIVES.

There is considerable divergence of opinion as to the degree of acidity at which milk may be considered sour. Thus Stokes (*ANALYST*, xvi., 122) states that milk which has not yet reached an acidity of 0·3 per cent. lactic acid (33·3°), but is near it, will coagulate on boiling; he records, however, the fact that three samples did not coagulate when the acidity had reached 0·54 per cent. lactic acid (60°). He also gives figures which show that milk tastes sour at an average acidity of 0·396 per cent. lactic acid (44°). Thorner (*Chem. Zeit.*, 1891, 1108) gives 23° as the acidity at which milk curdles on boiling, and gives the normal acidity of milk as 12° to 16°.

Rideal and Foulerton (*Public Health*, May, 1899) say that milk turns sour at an acidity of 0·5 per cent. lactic acid (55·6°), but Rideal (*Lancet*, January 27, 1900, 228) finds that milk curdles on boiling at 28° acidity, while with other samples slight coagulation was noticed at 25·6° and 21·6°.

We have made a series of experiments, and our figures confirm almost absolutely

the figures of Stokes. On the average we find that milk tastes sour at 45°, though the variations are fairly wide. The following experiments show the point at which milk curdles on boiling :

		Acidity.		Remarks.
i.	...	33·3°	...	Faintly curdles on boiling.
ii.	...	28·0°	...	Does not curdle on boiling.
iii.	...	52·0°	...	Curdles on warming.
iv.	...	32·7°	...	Curdles on boiling.
v.	...	{ 33·3°	...	Faintly curdles on boiling.
		{ 34·0°	...	Curdles on boiling.
vi.	...	24·7°	...	Does not curdle on boiling.
vii.	...	37·8°	...	Curdles on boiling.
viii.	...	34·9°	...	Just curdles on boiling.
ix.	...	32·5°	...	Just curdles on boiling.

The acidity at which milk curdles on boiling is about 33°, and as we find that milk, when fresh, has an acidity of 20°, this corresponds to an increase of 13°. We have tried to ascertain the amount of acid added to milk which will curdle it on boiling ; aqueous solutions of various acids were added little by little with constant shaking, till a faint coagulation on boiling was observed. The results were :

Acid.					Volume necessary expressed as $\frac{N}{10}$ c.c. to 100 c.c.
Sulphuric	...	...	...	...	8·8
Hydrochloric	...	...	...	...	8·6
Oxalic	...	...	...	...	29 and 28
Lactic	...	...	...	...	9·7

We find that milk curdles at about 85°, or an increase of 65°, without boiling ; this was the figure found at 17° to 35°, and between these limits does not appear to vary appreciably ; it is difficult to determine the point with accuracy.

It is quite certain that the " acidity " of milk is not wholly due to lactic acid ; indeed, the " acidity " of fresh milk is due to the mono- and di-basic phosphates, and not to free acid at all. Seeing that 9·7 c.c. $\frac{N}{10}$ lactic acid will curdle milk on boiling, while it requires a development of about 13° " acidity," it is highly probable that another acid very much weaker than lactic is produced, and we venture to think that carbonic acid is responsible for a portion of the acidity of sour milk ; we know that carbonic acid is produced, and we have found that when milk is sufficiently sour to develop gas about half the acidity, as indicated by phenolphthalein, is shown to litmus (to which both milk and carbonic acid are approximately neutral).

We have based a hypothesis on the facts that different acids do not give the same result, that salts of polybasic acids are present in milk, that both casein and albumin have acidic functions, and that the coagulation of milk at temperatures between 17° and 35° does not appreciably vary with the temperature ; it appears to us that curdling of milk is due to an amount of acid being present to set up an equilibrium between the acids and bases present, such that certain acids, *e.g.*, casein and albumin, are liberated. At a boiling temperature we are inclined to think that the curdling is determined by the coagulation of the albumin, the equilibrium being destroyed by the removal of one acid (albumin) from solution, and fresh amounts of albumin, and finally perhaps casein, are liberated.

When milk tastes sour, it would appear that the equilibrium is such that a sour-tasting free acid exists in solution; while when milk curdles spontaneously the equilibrium is such that the insoluble acid casein is produced.

We have mentioned this hypothesis to emphasize the fact that the point at which milk must be considered sour is a purely arbitrary one; the three points we have mentioned are determined by the coagulation of albumin, the production of a sour-tasting acid, and the formation of insoluble casein respectively, and others could doubtless be found.

We have made some experiments to determine the rate of souring of milks with and without preservatives. The preservatives used were a mixture of borax and boric acid, in the proportion of one part borax to three parts boric acid, from which sufficient water had been driven off to raise the percentage of B_2O_3 to 56.3 per cent. (this is a mixture largely sold under fancy names), and a solution of formaldehyde.

Our results are, with boric preservative (the figures represent degrees of acidity):

Temp. 17.5° C.	Milk alone.	Milk + 0.05 per Cent.	Milk + 0.10 per Cent.
52 hours	69.9°	31.3°	35.3°
76 „	95.0°	67.3°	52.6°
Temp. 24.5° C.			
24 hours	32.6°	31.3°	35.0°
48 „	99.2°	97.1°	82.6°
Temp. 34.5° C.			
22.5 hours	93.6°	71.3°	45.3°
46 „	116.6°	114.8°	108.2°

The acidity of the milk when fresh was:

Milk alone	...	...	...	...	19.7°
„ + 0.05 per cent.	...	...	...	...	25.3°
„ + 0.10 „	...	...	...	...	31.3°

With formaldehyde:

Temp. 17.5° C.	Milk alone.	Milk + 0.0023 per Cent.	Milk + 0.0047 per Cent.	Milk + 0.0093 per Cent.
24 hours	22.9°	—	—	—
48 „	72.7°	29.3°	23.3°	22.0°
72 „	93.6°	99.2°	33.3°*	24.0°
Temp. 27° C.				
24 hours	58.7°	26.7°	23.3°	—
48 „	106.3°	115.3°	81.3°*	27.3°
72 „	118.0°	120.8°	123.3°	56.7°
Temp. 36° C.				
24 hours	83.3°	80.0°	28.0°	22.7°
48 „	116.7°	141.6°	129.2°	52.0°*
72 „	158.3°	152.4°	139.2°	132.0°

The acidity of the fresh milk was in all cases 20.7°.

In the samples marked with an asterisk we could only obtain a very slight formaldehyde reaction with Hehner's tests. All the samples with an acidity below 30° gave a strong reaction, and the others a most indefinite one.

We would draw attention to the fact that when a milk treated with formaldehyde

begins to develop acidity, the rate of souring is greater than that of untreated milk, and this is noticed also, to a smaller extent, with boric preservative.

By drawing a curve including the results at 17.5° , we get an expression of the rate of souring, and we find that the same curve with a different time factor applies without great error to the other temperatures, and to the results with boric preservative; a second slightly differing curve applies to the formaldehyde results.

By means of these curves we have constructed tables of the time which milk takes to arrive at the three points of "sourness"—*i.e.*, an increase of 13° , 25° , and 65° acidity. These tables are, of course, only approximate, but we do not think that the deviation exceeds 10 per cent. of the values stated.

Table of the Time in Hours taken by Milks to reach 13° Additional Acidity.

Temp. ° F.	Milk alone.	Boric Preservative.		Formaldehyde.		
		Milk + 0.05 per Cent.	Milk + 0.10 per Cent.	Milk + 0.0023 per Cent.	Milk + 0.0047 per Cent.	Milk + 0.0093 per Cent.
60	42	71	93	51	85	119
70	29	36	46	34	49	78
80	19	22	31	25	34	56
90	13	15	23	15	26	44
100	7.5	10	20	9	23	37

Time in Hours to reach 25° Additional Acidity.

Temp. ° F.	Milk alone.	Boric Preservative.		Formaldehyde.		
		Milk + 0.05 per Cent.	Milk + 0.10 per Cent.	Milk + 0.0023 per Cent.	Milk + 0.0047 per Cent.	Milk + 0.0093 per Cent.
60	50	84	110	60	100	140
70	34	43	54	40	58	92
80	22	26	36	29	40	66
90	15	18	27	18	31	52
100	9	12	23	11	27	44

Time in Hours to reach 65° Additional Acidity.

Temp. ° F.	Milk alone.	Boric Preservative.		Formaldehyde.		
		Milk + 0.05 per Cent.	Milk + 0.10 per Cent.	Milk + 0.0023 per Cent.	Milk + 0.0047 per Cent.	Milk + 0.0093 per Cent.
60	75	126	165	84	140	192
70	51	64	81	56	81	129
80	33	39	54	41	56	92
90	22	27	40	25	43	73
100	12.5	18	34	15	38	62

The only results we have been able to find which are comparable with ours are those of Rideal (*loc. cit.*). Applying the same methods to his results, we obtain practically the same figures as those given above.*

We would draw attention to the fact that at high temperatures (say 80° hot summer weather) preservatives are comparatively useless unless added in relatively

* We assume that as Rideal does not notice the increased acidity when boric acid is added to milk, he has subtracted a constant amount for this throughout his series.

large quantities; the minimum quantities used by us, and also by Rideal, only increase the life of milk a few hours, and are equivalent only to a lowering of temperature of about 5° F. Unless milk can be made to keep at least twelve hours longer than without preservatives, we do not think much is gained by their use, and to do this in summer we think that the minimum amounts are 0.09 per cent. boric preservative and 0.004 per cent. formaldehyde.

We would also draw attention to the increased rate of souring as time goes on, when preservatives are added. This indicates a possible danger in using preservatives in milk, as it seems far from improbable that succeeding generations of micro-organisms become in the presence of preservatives more active and more virulent, and if the use of preservatives were universal, there is a probability that they would cease to act.

The work of Effront on yeast grown in the presence of sodium fluoride shows that this view is not a mere hypothesis.

DISCUSSION.

The CHAIRMAN (Mr. Allen) inquired whether there was any reason to suppose that milk contained carbonic acid or carbonates. If this were the case, the reliability of titrations with phenol-phthalein would be seriously affected.

Mr. RICHMOND said that a portion of the acidity of sour milk was undoubtedly due to carbonic acid, and the question had been considered in connection with this paper. The figures, however, were not given as representing the acidity absolutely, but were only intended for comparative purposes. The question of carbonic acid would not affect the immediate object of the determinations, which was to ascertain the time required for the acidity to reach a certain point. If different samples contained different proportions of carbonic acid, there would, of course, be a distinct error; but these samples had been treated as nearly as possible under the same conditions, and the ratio of carbonic acid to other acids was probably not sufficiently divergent to affect the conclusions.

Mr. CHAPMAN asked whether the experiments took place in the winter or summer months. The effect of a temperature of 80° F. in the summer and in the winter would probably not be the same.

Mr. RICHMOND said that the experiments were conducted during the winter. If any difference occurred, it would be rather in the autumn months—September and October—when an increase in the rate of souring might be expected. He did not think there would be any increase in the summer.