6. If no flaws are discovered, plunge the "gelatinized frame" into ice-cold formalin (40 per cent.) and transfer the formalin to an ice-chest where it should remain until, by the action of the formalin, the gelatin is rendered completely insoluble in boiling water. Two weeks' time has proven entirely satisfactory for this purpose. Perhaps less time may be found sufficient.

7. Wash the now completed membrane in running water, or boil it, or steam it in free steam or in steam under pressure, to get rid of the free formalin adherent to the gelatin.

If at step 5, flaws are found (there should be none, however, if ordinary care be taken), it is best to return the "frame" to the hot gelatin solution and leave it there for ten or fifteen minutes more, at or near 100° C., to remelt the gelatin already cooled upon it, and reimpregnate the whole silk tissue. Patching flaws found in cold gelatin films with hot gelatin is not likely to yield satisfactory results.

The essentials of success are, the writer believes, first, fine, rigid meshes in the "frame;" second, close, firm, rigid seams, if the "frame" is sewed at any point; third, thorough impregnation with hot gelatin; fourth, ice-cold temperatures for the water used in setting and testing and for the formalin used in hardening; fifth, sufficient time for the formalin to act thoroughly on the gelatin. The writer has used Gold Label Brand gelatin, Comte Fils, Magdebourg, but any good sheet gelatin would probably be satisfactory.

BOARD OF HEALTH, BACTERIOLOGICAL LABORATORY, BOSTON.

[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES, COLUMBIA UNIVERSITY, NO. 108.]

COMPARATIVE EXPERIMENTS UPON CHEMICAL PRESERVATIVES IN MILK.

BY H. C. SHERMAN, A. W. HAHN, AND A. J. METTLER.

Received July 16, 1905.

IN CONNECTION with other studies concerning the composition and analysis of milk, experiments upon the detection and determination, efficiency, and mode of action of chemical preservatives have been undertaken in this laboratory. The destruction of lactose and the development of acidity under the influence of sodium fluoride, sodium salicylate, and a mixture of boric acid and borax, together with notes on the determination of these com-

pounds and preliminary results obtained with hydrogen peroxide as a milk preservative are given in this paper.

For the sake of brevity the results are tabulated without detailed descriptions of individual experiments and without regard to the chronological order in which they were obtained.

DESTRUCTION OF LACTOSE AND FORMATION OF ACIDS.

The milk used in each experiment of the first series was a portion of the mixed product of about 500 cows, handled under good sanitary conditions, mixed, divided, and treated with the preservatives in previously sterilized bottles within two hours after being drawn from the udder. Each bottle was then tightly stoppered and kept at room temperature (20 to 25°). In this series each sample was

TABLE A.—DESTRUCTION OF LACTOSE AND FORMATION OF ACIDS IN SAMPLES KEPT STOPPERED.

	Lactose.		Acidity calculated as lactic acid.			
	Remaining.	Destroyed (*a*).	Total (*b*).		After boiling (*c*).	
Description of sample.	Per ct.	Per ct.	Per ct.	*a*:*b*::100:	Per ct.	*a*:*c*::100:
First experiment, 4 days:						
Milk with no preservative.....	3.43	1.28	0.864	67.5	0.66	51.5
Same (second bottle).....	3.43	1.28	0.86	67.0	0.65	51.0
Same milk +0.1 per cent. sodium fluoride.....	3.68	1.03	0.65	63.0	0.43	42.0
Same milk +0.1 per cent. sodium salicylate.....	3.71	1.00	0.71	71.0	0.51	51.0
Same milk +0.1 per cent. of boric acid and borax (1:1)..	3.43	1.28	0.864	67.5	0.74	58.0
Second experiment, 4 weeks:						
Milk with no preservative.....	2.93	1.80	1.24	69.0	0.99	55.0
Same (second bottle).....	2.91	1.82	1.23	67.5	0.92	51.0
Same (third bottle).....	3.02	1.71	1.25	73.1	1.03	60.0
Same milk +0.1 per cent. sodium fluoride.....	3.26	1.47	0.87	59.0	0.72	42.0
Same milk +0.1 per cent. sodium salicylate.....	3.43	1.30	1.00	77.0	0.87	67.0
Same milk +0.1 per cent. of boric acid and borax (1:1)..	3.19	1.54	0.77	77.0	1.05	68.0
Third experiment, 8 weeks:						
Milk with no preservative.....	2.52	2.25	1.59	70.0	1.39	62.0
Same (second bottle)	2.57	2.20	1.63	74.0	1.44	65.0
Same milk +0.1 per cent. sodium fluoride..	3.19	1.58	1.11	70.0	0.82	52.0
Same milk +0.1 per cent. sodium salicylate.....	3.35	1.42	1.19	84.0	0.92	65.0
Same milk +0.1 per cent. of boric acid and borax (1:1)..	2.65	2.12	1.52	72.0	1.35	64.0

always allowed to remain entirely undisturbed until the day upon which the analysis was to be made, when it was well shaken, opened, and examined for percentage of lactose, total acidity, and acidity after diluting with water and boiling gently for ten minutes to expel carbonic acid.

This series includes three distinct experiments made during April to June. The original percentages of lactose found for the three lots of milk were 4.71, 4.73, and 4.77 respectively. The results obtained in these three experiments are given in Table A.

The results given in Table A show that the milk, when treated with 0.1 per cent. of sodium fluoride or sodium salicylate and then kept in stoppered bottles, lost less lactose and developed less acidity than when kept under the same conditions without preservative or with 0.1 per cent. of a mixture of boric acid and borax. In these experiments, the shortest of which was four days long, the preservative effect of the boric acid and borax was scarcely perceptible. The influence of this preservative is shown in later experiments in which the acidity was determined daily (Table C). The results of two experiments, comparing the effect of fluoride and salicylate in milk not kept closed throughout the experimental period, are given in Table B.

These results show less uniformity than those obtained in the experiments in which the bottles were kept tightly stoppered throughout. While both fluoride and salicylate evidently retard lactic acid fermentation to a considerable extent, it is probable that other acid-forming organisms, entering the milk from the air or from the apparatus used in mixing and in withdrawing portions for analysis, were affected differently in the different samples; both directly by the kind and amount of preservative present, and indirectly through the influence of the preservative in retarding the growth of the bacteria originally contained in the milk.

In the samples kept stoppered, equal weights of fluoride and salicylate were about equally efficient in retarding the destruction of lactose and the development of acidity, but the nature of the fermentation, as indicated by the ratio of lactose used to acid formed, was affected differently in the two cases. Using as a basis for comparison the samples to which no preservative was added, it appears that the fluoride so altered the fermentation that a smaller amount of total acid was formed in the destruction of a given amount of lactose, while the salicylate had the opposite

TABLE B.—DESTRUCTION OF LACTOSE AND FORMATION OF ACIDS IN OPENED SAMPLES.

Description of sample.	Lactose. Remaining. Per ct.	Lactose. Destroyed (*a*). Per ct.	Acidity calculated as lactic acid. Total (*b*). Per ct.	Total (*b*). *a*:*b* 100.	After boiling (*c*). Per ct.	After boiling (*c*). *a*:*c* 100.
First experiment, 3–4 weeks:						
Milk[1] with no preservative when first opened	.....	.....	0.90	...	0.74	...
Four days later	2.85	1.96	1.03	53	0.81	41
Same milk +0.1 per cent. sodium fluoride when first opened	.....	.....	0.68	...	0.50	...
Four days later	3.26	1.55	0.89	57	0.63	41
Same milk +0.1 per cent. sodium salicylate when first opened	.....	.....	0.76	...	0.70	...
Four days later	3.04	1.77	0.76	43	0.72	41
Second experiment, 4 weeks: Samples opened frequently.						
Milk[2] with no preservative	2.83	1.92[2]	1.01	53[2]	0.81	42[2]
Same (second bottle)	2.85	1.90	0.95	50	0.80	42
Same milk with sodium fluoride, 0.1 per cent	2.95	1.80	0.68	38	0.57	32
Same milk with sodium fluoride, 0.02 per cent	2 30	2.45	1.54	63	1.20	49
Same milk with sodium fluoride, 0.005 per cent	3.00	1.75	0.81	46	0.63	36
Same milk with sodium salicylate, 0.1 per cent	2.83	1.92	0.86	45	0.71	37
Same milk with sodium salicylate, 0.02 per cent	2.30	2.45	1.11	45	0.83	34
Same milk with sodium salicylate, 0.005 per cent ...	2.06	2.69	1.25	46	0.96	36

effect. In the samples which were exposed to the air, these relations are sometimes reversed. The irregularities are especially marked in the samples containing only small amounts of preservatives.

INFLUENCE OF AMOUNT OF PRESERVATIVE UPON THE DEVELOPMENT OF ACIDITY.

Two series of experiments with different amounts of preserva-

[1] From same source, and treated in same manner to time of first opening as the milk used in the preceding experiments. Lactose originally present 4.81 per cent.

[2] This milk was from a different source, was inferior to that previously used, and was between one and two days old when the experiment was begun, having been obtained from a restaurant. As the determination of lactose in the original sample was accidently omitted, it is assumed that 4.75 per cent. was present.

tives were carried out, the first comparing sodium fluoride and sodium salicylate, the second comparing hydrogen peroxide and the mixture of equal parts borax and boric acid. In each case the milk used was obtained in a restaurant and was probably 1 to 2 days old at the beginning of the experiment.

In determining total acidity, 10 cc. of milk were diluted to 100 cc. and titrated with tenth-normal sodium hydroxide, using phenolphthalein as indicator. For acidity after boiling, 10 cc. were diluted in the same way and the mixture boiled gently for ten minutes in a beaker loosely covered with a watch-glass, cooled to room temperature, and titrated exactly as in the determination of total acidity. Table C gives the results of these determinations, the actual volumes of alkali used in each titration being given under the heading "degree of acidity." While the arrangement of the experiments was similar for the two series, they were carried out independently upon different lots of milk.

As would be expected in view of the fact that these samples were opened frequently to remove portions for analysis, the results are somewhat irregular, especially in those cases in which small amounts of the preservatives were used. In some of these samples there is at first a retardation, but afterward a rapid increase in acidity to an amount greater than that found where no preservative was added. In all cases where 0.1 per cent. of preservative was added, its retarding effect upon the development of acidity was permanent throughout the continuance of the experiment.

The results obtained in the absence of preservatives agree with those of Van Slyke and Hart[1] in showing a sudden check to the acid fermentation after four to six days at room temperature, when a degree of acidity equivalent to 0.7 to 1.0 per cent. of lactic acid had been reached, and about 28 per cent. of the original lactose had disappeared. Beyond this point the fermentation proceeded slowly, samples kept stoppered for eight weeks showing acidity equivalent to 1.6 per cent. of lactic acid and a loss of 47 per cent. of the lactose originally present. Both of the latter figures were exceeded in some of the samples which received very small amounts of preservatives and were frequently opened for analysis.

[1] *Am. Chem. J.*, **32**, 145.

TABLE C.—INFLUENCE OF THE KIND AND AMOUNT OF PRESERVATIVE UPON THE DEVELOPMENT OF ACIDITY IN MILK.

"Degrees of Acidity"—Cubic centimeters of tenth-normal alkali to neutralize 10 cc. of milk.

Description of sample.	After 1 day.		After 2 days.		After 4 days.		After 6 days.		After 8 days.		After 11 days.		After 13 days.		After 20 days.		Remarks.
	Total.	After boiling.	Total.	After boiling.	Total.	After boiling.	Total.	After boiling.	Total.	After boiling.	Total.	After boiling.	Total.	After boiling.	Total.	After boiling.	
First Series (A. W. Hahn):																	
Milk with no preservative	2.0	1.45	6.35	5.7	10.1	8.1	10.8	8.20	10.7	9.0	11.2	8.9	11.0	9.0	11.2	9.0	Curdled within 2 days.
Same (second bottle)...	2.0	1.45	6.25	5.5	10.5	7.8	10.9	8.35	10.7	9.3	10.5	9.3	10.7	9.3	10.5	8.9	" " 2 "
Same milk + sodium fluoride:																	
0.1 per cent (1:1000)	1.65	1.20	5.7	4.9	6.9	4.9	7.6	5.5	7.7	5.5	7.5	6.7	7.5	6.3	9.1	6.75	" " 2 "
0.02 " (1:5000)	1.9	1.45	6.0	4.6	9.3	7.3	9.5	6.9	10.6	8.6	13.1	10.8	14.2	12.6	17.1	13.3	" " 2 "
0.005 " (1:20,000)	2.0	1.4	6.0	4.4	8.5	6.5	9.0	6.5	8.8	7.2	8.9	7.1	8.7	7.8	9.0	7.0	" " 2 "
Same milk + sodium salicylate:																	
0.1 per cent (1:1000)	1.6	1.1	2.9	2.2	6.2	4.1	8.0	5.1	7.9	5.9	8.7	8.4	9.0	7.1	9.5	7.9	Curdled after 6 days.
0.02 " (1:5000)	1.9	1.25	5.8	3.95	9.7	6.5	10.8	7.9	10.7	8.6	10.7	9.1	11.5	9.5	12.3	9.2	" within 2 days.
0.005 " (1:20,000)	1.85	1.25	6.0	3.9	13.1	7.8	11.3	8.1	10.6	9.4	10.2	8.5	10.7	9.0	11.6	8.2	" " 2 "
Second Series (A. J. Mettler):					After 3 days.		After 4 days.		After 5 days.		After 7 days.		After 9 days.				
Milk with no preservative	2.15	1.8	5.0	3.5	6.6	5.6	7.9	5.9	8.4	6.5	8.55	6.2	8.5	6.3			Curdled in 2 to 3 days.
Same (second bottle)...	2.2	1.8	4.95	3.45	6.6	5.6	7.8	5.9	8.3	6.1	8.5	6.15	8.3	6.2			" " 2 " 3 "
Same milk + boric acid and borax:																	
0.1 per cent (1:1000)	1.9	1.5	2.85	2.4	4.4	3.1	6.8	4.85	7.1	5.9	7.7	6.2	7.9	6.2			" " 4 " 5 "
0.02 " (1:5000)	1.95	1.6	4.5	3.05	7.25	5.7	8.0	6.2	8.3	6.4	8.1	6.1	8.4	6.25			" " 2 " 3 "
0.005 " (1:20,000)	2.15	1.75	4.9	3.25	7.0	5.4	7.6	5.5	8.1	6.0	8.3	6.1	8.4	6.2			" " 2 " 3 "
Same milk + hydrogen peroxide:																	
0.1 per cent (1:1000)	1.5	1.1	1.85	1.45	3.25	2.65	5.2	4.1	6.8	4.9	7.3	5.6	7.7	5.8			" " 7 days.
0.02 " (1:5000)	1.6	1.25	2.9	2.4	5.5	3.65	7.3	6.0	7.8	5.4	8.25	6.1	8.0	6.1			" " 4 "
0.005 " (1:20,000)	1.8	1.4	4.2	3.0	6.4	4.4	7.5	6.1	8.25	6.8	8.3	6.0	8.4	6.1			" " 3 to 4 days.

DETERMINATION OF PRESERVATIVES.

Boric Acid.—Thompson's method[1] was used for the determination of boric acid in milk to which a mixture of crystallized boric acid and borax had been added with the following results (A. J. M.):

	I. Per cent.	II. Per cent.
Total boric acid added	0.1116	0.0104
Total boric acid found.....	0.1101	0.0099
	0.1095	0.0097

Sodium Fluoride.—Good results were obtained when 100 cc. of milk were mixed with 1 gram of sodium carbonate, evaporated, burned to ash, leached thoroughly with hot water, nearly neutralized with sulphuric acid, and the determination completed by Rose's method as modified by Treadwell and Koch.[2] In a sample containing 0.1 per cent. of fluoride, 94 per cent. of the amount added was found. When the addition of sodium carbonate before ignition was omitted only 2 per cent. of the fluoride originally present was recovered in the ash (A. W. H.).

Sodium Salicylate.—Attempts to recover the salicylic acid after precipitation of proteids by mercuric nitrate gave very low results, and distillation with steam is much too slow for a practical method. The following modification of Remont's method was found fairly satisfactory. To 20 cc. of milk in a separatory funnel or cylinder, add five drops of 50 per cent. sulphuric acid and shake well to break up the curd, add 25 cc. of ether, mix thoroughly, and allow to stand until the layers separate. Withdraw 5 cc. of the ether solution, evaporate in a test-tube, and boil the residue with 10 cc. of 40 per cent. alcohol, allow to cool, adjust the volume to 10 cc., and filter. To 5 cc. of the filtrate, corresponding to 2 cc. of milk, add 2 cc. of a 2 per cent. solution of ferric chloride, dilute the solution if necessary, and match the color against that developed in a solution containing a known amount of salicylic acid. After practice, it was found possible to determine salicylic acid (present as sodium salicylate, 0.1 to 0.2 per cent.) with a probable error of about 5 per cent. (A. W. H.)

Hydrogen Peroxide.—This was determined as follows:[3] To 40 cc.

[1] *Analyst*, **21**, 64 (1896). The tenth-normal alkali used for titration was freshly standardized against crystallized boric acid.

[2] *Z. anal. Chem.*, **43**, 469 (1904).

[3] Modification of the method used by Chick: *Centrbl. Bacteriol. u. Parasitenk.*, II Abth. 1901, I, 705.

of water, 0.5 gram of potassium iodide, and 10 cc. of 12 per cent. sulphuric acid in a flask, having projecting lip and ground glass stopper, add 10 cc. of the milk, stopper, fill the gutter formed by the projecting lip with a solution of potassium iodide to prevent any possible loss of iodine, and allow to stand in a cool dark place for two and one-half hours. Finally titrate the iodine (set free according to the reaction $2KI + H_2O_2 + H_2SO_4 = K_2SO_4 + I_2 + H_2O$) with fiftieth-normal sodium thiosulphate. It is unnecessary to use starch as indicator, since the disappearance of the yellow color produced by the action of the iodine upon the proteids affords a satisfactory end-point. Test analyses gave results about 3 per cent. too low, doubtless because of the absorption of iodine by the milk fat. Error from this cause could be avoided by curdling the milk and working upon a measured amount of filtrate, but this seems inadvisable in view of the fact that some of the peroxide might be decomposed during filtration. On account of the rapid decomposition of hydrogen peroxide in milk the amount found by analysis will always be less than that added.

Thus in an experiment in which 1 : 1000 was added, 93 per cent. of this amount disappeared within twenty-four hours (A. J. M.).

We hope to study further the determination and rate of disappearance of hydrogen peroxide in milk.

SUMMARY.

Milk kept at 20 to 25° without preservative showed a rapid increase in acidity and decrease in percentage of milk-sugar during the first three to six days, after which the acid fermentation proceeded much more slowly, but neither the destruction of lactose nor the formation of acid had ceased entirely after four weeks.

Hydrogen peroxide, sodium fluoride, sodium salicylate, and a mixture of equal parts boric acid and borax were each found to diminish distinctly the development of acidity in milk, when added in the proportion of 1 : 1000.

When present in about this proportion, any one of the preservatives can be determined quantitatively with a probable error of 1 to 6 per cent. of the amount in the milk. In the case of hydrogen peroxide, however, the preservative disappears so rapidly that the amount found present gives little indication of the quantity originally added.

In the presence of fluoride or salicylate the fermentation is

changed qualitatively as shown by the ratio of acid formed to lactose destroyed. With these preservatives it was found that occasional exposure of the samples to air, especially when the quantity of preservative present was small, led to irregular results, the final acidity sometimes exceeding that in the control samples to which no preservative had been added.

In the experiment with samples preserved by boric acid and borax or by hydrogen peroxide such irregularities did not appear. The boron preservative had no apparent influence upon the nature of the acid fermentation. The experiments with hydrogen peroxide are only preliminary.

Experiments upon the development of ammonia in milk under the influence of these preservatives and a further study of hydrogen peroxide are being undertaken in this laboratory. Discussion of the effects of preservatives upon the wholesomeness of milk will be deferred until the completion of these and other experiments.

NEW YORK, July, 1905.

TESTING WHEAT FLOUR FOR COMMERCIAL PURPOSES.

BY HARRY SNYDER.

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AT PRESENT no standards have been adopted by either commercial bodies or chemists for the purpose of determining the commercial value of wheat flour. The United States Department of Agriculture, however, has adopted standards as to purity and for the purpose of detecting adulterations. Various attempts have been made to adapt chemical and allied tests to purposes of determining the commercial value of flour, but, owing to the complex nature of wheat flour, none of these tests have been found to be generally applicable.

When wheat is subjected to the roller process of milling, different grades of flour are secured, as first and second patents, straight grade, and first and second clear, also "Red dog," which is sometimes used as human food but is more extensively used for animal-feeding purposes.[1]

As is well known, different kinds of wheat make distinct types of flour, which have different values for bread-making purposes, and wheat grown in the same locality during different years may,

[1] U. S. Dept. Agr., Office of Experiment Stations, Bull. 101, pp. 7-8.