carbon dioxide was expelled. It was found that prolonged heating did not drive off any hydrochloric acid, providing the solution was not boiled but was kept at such a temperature that only an occasional bubble was formed. The solution was then cooled and titrated back with $N/10$ sodium hydroxide. One-tenth the number of cc. $N/10$ acid used gives the per cent. of malic acid.

The method is also applicable to fresh and boiled ciders and to cider vinegars.

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[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, NO. 155.]

THE EFFECT OF PASTEURIZATION UPON THE DEVELOPMENT OF AMMONIA IN MILK.

BY W. G. WHITMAN AND H. C. SHERMAN.

Received June 9, 1908.

A method for the determination of ammonia in milk and the results of examination of a number of samples, fresh, stale and preserved by means of antiseptics, have been described in previous papers from this laboratory.[1] These determinations as well as the colorimetric tests for ammonia in milk recorded by Trillat and Sauton[2] appeared to warrant a further study of the ammonia content of milk as a measure of protein decomposition and the effect of pasteurization upon the development of ammonia and upon the acid: ammonia ratio. An especial study of the effect of pasteurization was suggested by the well-known bacteriological observation that milk may contain peptonizing or putrefactive bacteria capable of withstanding pasteurization and developing with increased vigor after the destruction of the acid-forming organisms. If this is commonly the case, it would seem that there should be chemical evidences of an increased breaking down of proteins in the pasteurized, over that in the corresponding raw milk.

The method used in the present investigation was the same as has been described by Berg and Sherman (*loc. cit.*) and its accuracy was again verified by blank tests and check determinations with known amounts of ammonia salts. The essential feature of this method, which is an adaptation of that of Boussingault[3] and Shaffer,[4] is that the milk is diluted with an equal volume of methyl alcohol, made alkaline with sodium carbonate and distilled under diminished pressure. Under these

[1] Berg and Sherman, THIS JOURNAL, **27**, 124 (1905); Sherman, Berg, Cohen and Whitman, *J. Biol. Chem.*, **3**, 171 (1907).

[2] *Bull. soc. chim.*, **33**, 719 (1905).

[3] *Ann. chim. phys.*, **29**, 472 (1850); *J. prakt. Chem.*, **51**, 281 (1850).

[4] *Am. J. Physiol.*, **8**, 330 (1903).

conditions, the addition of salt before distillation, diminishes the amount of ammonia obtained, doubtless because in the absence of the salt there is some cleavage of ammonia from organic nitrogen compounds present in the milk. As the amount of ammonia thus yielded by cleavage was found to vary considerably in different samples, its determination has been included in the examination of many of the samples here recorded with a view to ascertaining whether the percentage of this "cleavage ammonia" is of practical importance from a sanitary standpoint.

Table I shows the percentage of ammonia and of the so-called cleavage ammonia in samples of milk obtained from retail dealers in the upper part of New York City, and examined while fresh.

TABLE I.—AMMONIA AND "CLEAVAGE AMMONIA" IN FRESH MARKET MILK.

Sample.	Description.	Date.	Ammonia. present.	"Cleavage ammonia."
C	Bottled milk, T. F.	June 23	0.00034	0.00017
F	" " "	" 30	0.00000	0.00051
G	" " B.	" 30	0.00000	0.00000
2	" " W. C. D.	Sept. 19	0.00100	0.00070
3	" " "	" 20	0.00034	0.00017
5	" " "	" 22	0.00016	0.00035
6	" " "	" 24	0.00016	0.00035
7	" " "	" 28	0.00034	....
8	" " "	Oct. 4	0.00016	0.00035
9	" " E. F.	Nov. 1	0.00000	0.00051
10	" " "	" 7	0.00102	0.00000
11	" " "	" 14	0.00068	0.00016
12	" " "	" 24	0.00068	0.00050
13	" " "	" 30	0.00084	0.00018
14	" " W. C. D.	Dec. 1	0.00068	0.00034
15	" " E. F.	" 21	0.00000	0.00042
16	" " W. C. D.	" 22	0.00017	0.00051
17	" " "	" 29	0.00034	0.00034
18	"Loose" milk, E.	Jan. 19	0.00051	0.00033
19	" " E.	" 26	0.00068	0.00016
20	" " A.	Feb. 9	0.00016	0.00052
Average			0.00039	0.00033

It will be seen that these samples contain at most about 0.001 per cent. and average only 0.00039 per cent. of actual ammonia. With two exceptions, the samples also show measurable amounts of the so-called cleavage ammonia (indicative of readily decomposable nitrogenous organic matter), but as these figures are obtained by difference, they are subject to greater analytical errors than are the ammonia determinations.

Sixteen of the samples, representing the milk of four different dealers, were examined at irregular intervals after standing at a room temperature averaging about 20° for two days or longer. With one exception, the amount of ammonia was considerably increased over that found when fresh. Of three samples examined at 2–3 days old and again 2 days later, two showed an increase and one a decrease of ammonia during this second two-day period. Of three examined at 2–3 days and again 4–8 days later, two increased and one decreased in ammonia content during the second period. Of two examined at 4-5 days and again 2–6 days later, one showed a decrease and one showed no change in the second period. These results show that samples of milk kept at room temperature do not always increase continually in ammonia content but may show considerable fluctuation when examined at intervals of a few days. In the earlier work in this laboratory where the intervals between examinations were usually longer, such fluctuations had not been noticed, the later examinations having always shown an equal or greater amount of ammonia than the earlier examinations of the sample. Most of the samples represented in Table I were heated to 65° or 85° and then allowed to stand and subsequently examined to ascertain the influence of pasteurization, but since the examinations were not made at regular intervals and the ammonia content of the same milk when kept under the same conditions without pasteurization was found to fluctuate, we have not attempted to draw any conclusions regarding pasteurization from the first series of observations.

Second Series.—Samples Examined at Regular Intervals.—Ten samples (nine of bottled and one of "loose" milk) were taken at intervals during a period of two months and representing the milk of six different dealers. Each sample was mixed and divided into three portions, one of which was kept raw, one pasteurized[1] at 65° and one at 85°, and all were examined regularly after standing at a temperature of 15° to 20° for 2, 4 and 7 days. The percentages of ammonia found in these samples are shown in Table II.

Table II. Influence of Pasteurization on Ammonia Content.

Sample.		Ammonia found after standing. 2 days. Per cent.	4 days. Per cent.	7 days. Per cent.
21	Raw	0.0078	0.0094	0.0036
	Pasteurized at 65°	0.0022	0.0107	0.0107
	Pasteurized at 85°	0.0022	0.0099	0.0111
22	Raw	0.0070	0.0056	0.0026
	Pasteurized at 65°	0.0012	0.0075	0.0094
	Pasteurized at 85°	0.0014	0.0065	0.0111
23	Raw	0.0005	0.0058	0.0058
	Pasteurized at 65°	0.0009	0.0019	0.0024

[1] In pasteurizing, the milk was poured into sterile flasks, stoppered with cotton, placed in a water bath, heated gradually to the desired temperature, maintained at this point for 15 minutes and then cooled quickly to room temperature, usually about 15°.

TABLE II (*Continued*).

Sample.	Ammonia found after standing. 2 days. Per cent.	4 days. Per cent.	7 days. Per cent.
Pasteurized at 85°	0.0007	0.0044	0.0124
24 Raw	0.0104	0.0105	0.0097
Pasteurized at 65°	0.0012	0.0121	0.0107
Pasteurized at 85°	0.0007	0.0022	0.0023
25 Raw	0.0070	0.0085	0.0126
Pasteurized at 65°	0.0019	0.0077	....
Pasteurized at 85°	....	0.0060	0.0226
26 Raw	0.0075	0.0071	0.0044
Pasteurized at 65°	0.0024	0.0058	0.0082
Pasteurized at 85°	....	0.0044	0.0082
27 Raw	0.0104	0.0102	0.0077
Pasteurized at 65°	....	0.0041	0.0041
Pasteurized at 85°	0.0012	0.0027	0.0054
28 Raw	0.0005	0.0049	0.0048
Pasteurized at 65°	0.0003	0.0031	0.0131
Pasteurized at 85°	0.0005	0.0036	0.0126
29 Raw	0.0049	0.0104	0.0085
Pasteurized at 65°	0.0003	0.0010	0.0022
Pasteurized at 85°	0.0003	0.0019	0.0148
30 Raw	0.0007	0.0088	0.0070
Pasteurized at 65°	0.0003	0.0015	0.0019
Pasteurized at 85°	....	....	....
Average Raw	0.0056	0.0081	0.0065
Pasteurized at 65°	0.0011	0.0057	0.0065
Pasteurized at 85°	0.0010	0.0047	0.0116
Average (21, 22, 23, 24, 28, 29[1]) Raw	0.0052	0.0078	0.0058
Pasteurized at 65°	0.0010	0.0061	0.0081
Pasteurized at 85°	0.0010	0.0047	0.0107

From these results it appears that the raw milk increased rapidly in ammonia content during the first period after purchase, continued to increase, but more slowly, during the second period and decreased during the third period, always, however, remaining very greatly in excess of the amount in milk of the same quality when fresh. In the milk pasteurized at 65°, the development of ammonia was slow during the first period, rapid during the second, somewhat less rapid during the third; in that pasteurized at 85° the ammonia content increased slowly during the first period, rapidly during the second and third. The higher temperature of pasteurization resulted in greater irregularities of ammonia content and much higher final figures than in either the raw milk or that pasteurized at a low temperature. In every case of milk pasteurized at 85°, there was continuous increase of ammonia throughout the 7 days of the experiment. The agencies which produced a decrease of

[1] These are the samples for which the analytical data are complete throughout so that the averages are strictly comparable.

ammonia in raw milk during the third period of observation were evidently much weakened by heating at 65° and destroyed by heating to 85°. This would indicate that the change is not due simply to inorganic agencies, but whether it is due to the activity of organisms or enzymes and into what substance the ammonia is transformed, was not determined.

"Cleavage Ammonia."

Some of the samples in each of the above series were examined at intervals for "cleavage ammonia" with the results shown in Table III. All figures given are averages, the number in parenthesis indicating the number of samples examined in each case.

TABLE III.—AVERAGE PERCENTAGES OF "CLEAVAGE AMMONIA."

	Examined after standing for:			
Description.	2-3 days.	4-5 days.	6-11 days.	12-21 days.
Raw	(12) 0.0003	(5) 0.0004	(6) 0.0006	(2) 0.0006
Pasteurized at 65°	(4) 0.0004	(4) 0.0005	(7) 0.0005	(2) 0.0015
Pasteurized at 85°	......	(4) 0.0004	(9) 0.0005	(3) 0.0053

While the figures in the table are not strictly comparable, they are sufficient to indicate that under the conditions of these experiments the development of the so-called "cleavage ammonia" was slow during the first ten days, whether the milk was pasteurized or kept raw; that during the second ten days the raw milk showed little if any increase while the increase was considerable in the milk which had been pasteurized at 65° and very large in that which had been pasteurized at 85°.

The Acid: Ammonia Ratio.

Since there is no necessary relation between the development of acidity and ammonia and since the souring of milk is the principal factor determining its rejection under ordinary market conditions, it seemed important to ascertain the effect of pasteurization upon the development of ammonia not only in percentage as expressed above, but also in its relation to the development of acidity. Hence, most of the determinations of ammonia in the samples above described were accompanied by titrations of the total acidity of the samples. For the sake of brevity, the data of individual determinations are here omitted, but the average acidities and acid: ammonia ratios for nine samples of the second series which were examined at regular intervals, and the average ratios for twenty other samples examined at irregular intervals, are shown in Table IV. By "acidity" is here meant the number of cc. of tenth-normal sodium hydroxide required to neutralize 10 cc. of the milk, using phenolphthalein as indicator and titrating at room temperature. The acid: ammonia ratio is obtained by calculating the acidity as lactic acid and determining the parts of ammonia present per 100 parts of lactic acid; *i. e.*,

$$\text{Total acid as lactic} : \text{ammonia} :: 100 : x$$

TABLE IV.—ACIDITIES AND ACID : AMMONIA RATIOS.

	Results of examination after standing		
	2 days.	4 days.	7 days.
Acidity.			
Average of samples 21–29.			
Raw	8.3	10.1	10.1
Pasteurized at 65°	2.3	6.4	8.0
Pasteurized at 85°	2.0	5.1	7.7
Acid: Ammonia Ratio (Total acid as lactic . ammonia :: 100 : x).			
Average of samples 21–29.			
Raw	0.77	0.87	0.80
Pasteurized at 65°	0.53	1.36	1.22
Pasteurized at 85°	0.56	1.34	1.83
Average or irregular samples.			
Raw	0.64	0.75	0.56
Pasteurized at 65°	0.51	1.53	1.11
Pasteurized at 85°	..	1.28	1.99

These results indicate that the average market milk had nearly the same acid: ammonia ratio after 2, 4 or 7 days' standing; in other words, that from the second day onward to at least the end of the first week the percentages of acid and of ammonia increased in about the same relative proportion. Pasteurization of such milk checked both the acid and ammonia development in about the same ratio for 2 days, after which both acid and ammonia increased at a fairly rapid rate, but the development of ammonia was relatively much more rapid than that of acid and this was true to a greater extent of the milk pasteurized at the higher temperature.

Odor and Taste.

At the time of each determination of ammonia the odor and taste of the milk sample were observed and recorded.

Raw milk after two days' standing was regularly found curdled, with a clean, sour taste and a sour odor with occasionally a slight mustiness; when older the musty odor, if present, becomes more distinct and the sour taste much sharper.

Milk pasteurized at 65° was not curdled after two days' standing; its taste was variously recorded as "sweet," "flat," or "chalky;" its odor as "old," "musty," or "slightly sour."

Milk pasteurized at 85° often had after two days' standing, a sickish, sweet, cooked odor and taste, sometimes an offensive odor and flat, insipid taste. It sometimes curdled within four to six days without the development of sufficient acid to make it taste sour.

An offensive or putrid odor was not found in any of the samples of raw milk examined after 2, 4 and 7 days' standing; in the pasteurized milk, it was sometimes found after 2 days and was very common after 7 days.

Bitter tastes were found in none of the samples of unpasteurized milk, in some of those pasteurized at 65°, and in many of the samples which had been pasteurized at 85°.

Pasteurization followed by long storage at 15° to 20°, evidently favored the development of putrid odor and bitter taste, the more so when the pasteurization was conducted at the higher temperature. Such conditions also resulted in a high development of ammonia. The odor and taste appear to be of equal sanitary significance with the ammonia determination.

Summary.

The ammonia content of unpasteurized milk kept at 15° to 20° usually increased rather rapidly for 2 days, more slowly during the third and fourth days, and then decreased somewhat for the few days following, increasing again later if the observations were sufficiently prolonged. A similar slight decrease of ammonia toward the end of the first week was sometimes, but not usually, observed in milk pasteurized at 65°; never in milk pasteurized at 85°, which always showed a continuous increase in ammonia content. It is therefore inferred that the pasteurization did not entirely destroy the bacteria which attack the proteins with production of ammonia, but did destroy (sometimes at 65° and always at 85°) the bacteria or enzymes which caused the decrease of ammonia in raw milk.

The determination of ammonia as a measure of the decomposition of proteins in milk appears to be especially useful in samples which have been pasteurized at high temperatures and in which the development of ammonia is continuous; in samples which have been pasteurized at low temperatures or not at all, the sanitary significance of the ammonia determination is less certain, since the ammonia content at any given time cannot be assumed to be proportional to the extent to which protein decomposition has taken place.

In milk obtained under ordinary market conditions in New York City, and thereafter kept at 15° to 20° pasteurization retarded the development of both acid and ammonia to about the same extent during the first two days, after which the development of ammonia was relatively more rapid than that of acid. In general, therefore, the pasteurization was less efficient in checking the development of ammonia than in retarding the production of acid and this was especially true of the milk pasteurized at the higher temperature (85°) which before becoming sour often showed an amount of ammonia considerably in excess of raw milk of the same age and origin.

The samples kept raw always developed within a few days a sour taste and an odor which was always sour, sometimes "musty" or "cheesy," but never suggestive of putrefaction. Pasteurization greatly retarded

souring but favored the development of an offensive, putrid odor, and a bitter taste. This effect was much less noticeable in the samples pasteurized at 65° than in those of the same origin pasteurized at 85°, the same precautions having been taken to prevent subsequent contamination in either case.

NEW YORK CITY, June, 1908.

THE ISOLATION OF PICOLINE CARBOXYLIC ACID FROM SOILS AND ITS RELATION TO SOIL FERTILITY.[1]

BY OSWALD SCHREINER AND EDMUND C. SHOREY.

Received June 4, 1908.

Recent investigations have shown, contrary to the generally accepted belief since Liebig's time, that lack of productivity in soils is not always due to the lack of nutrient substances, but even with an abundance of these, lack of productivity may be produced by the presence of substances actually deleterious to plant growth. The presence of toxic substances has been virtually established by the properties which the soil and soil extracts possess. When an unproductive soil is extracted with water, the aqueous solution has been found to possess the properties of the soil itself so far as plant growth is concerned. When a good soil is extracted, a good soil extract is obtained, and a poor soil yields a poor soil extract, although the extract may have been made from adjoining fields of the same soil type and be practically identical in mineral composition. Such poor soil extracts produce in many instances plants which are far less developed than similar plants grown in pure distilled water under identical conditions. It has been found that these toxic properties can be removed from such a soil extract by a number of quite simple operations, such as treatment with carbon black, ferric hydroxide, and other absorbent agents, whereby the toxic material is apparently removed from the solution. This action seems to be quite common for all toxic soils studied although in other respects the properties of toxic soil extracts differ appreciably. In some cases the toxicity can be destroyed by merely boiling the extract and this is due in some cases to a destruction and in others to a volatilization. In the latter case the toxic properties can be found in the distillate. The toxicity can also be much weakened by dilution with pure distilled water so that in the weaker solution there may even result a stimulation in growth such as is commonly observed with a large number of poisons in weak solutions. These toxic properties are also overcome in many instances by the addition of comparatively minute amounts of pyrogallol, naphthylamine, and other compounds, a phenomenon which can be explained only by assuming that these compounds have acted upon the toxic substance, or have so altered the plant by stimulation

[1] Published by permission of the Secretary of Agriculture.