number, and even such material as manufacturer's catalogues and illustrated price lists will be found to contain information of much value upon subjects relating to the supplies needed in all factories.

In conclusion it may be acknowledged that this plan of instruction is essentially a utilitarian one. This is in accordance with the general drift of the scientific education of to-day. To use Lord Bacon's expression the aim is to gather fruit, not flowers. The training of the mind will incidentally be one of the results of such a system. But the main object will be the imparting of actual knowledge and the teaching of specific facts. The manufacturing chemist who has to meet the difficulties that constantly arise in the practice of his profession is like a man who is lost in the Alps. What he wants, is not to improve his mind, but to find the way. The present system of instruction does not meet the requirements made upon it. It teaches the principles of the science but does not go far enough. As Macaulay said of the ancient philosophers, every trace of intellectual cultivation is there except a harvest. The subject of industrial chemistry evidently has not received the attention it deserves in our schools of science.

PHILADELPHIA, PA.

THE DETERMINATION OF CASEIN IN COWS' MILK.

BY L. L. VAN SLYKE.

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THE methods originally proposed by Hoppe-Seyler and Ritthausen have been commonly employed for the separation and determination of casein in cow's milk. In both methods, the milk is diluted with water and a small amount of acetic acid is added. The precipitation is rendered complete, in one case, by raising the temperature to 40° C., and, in the other case, by passing a current of carbon dioxide through the mixture at ordinary temperatures. The precipitate is filtered, washed first with water, and then with ether to remove fat and is finally dried and weighed on the filter. The absence of specific directions touching several steps of the operation led me to investigate some of the conditions pertaining to the determination of casein, among which were the following:

1. Comparison of the two methods.
2. Influence of amount of acid used in precipitating casein.
3. Use of different acids in precipitating casein.
4. Influence of temperature and time of digestion on precipitation of casein.
5. The precipitation of casein in fresh and in old milk.
6. The use of preservatives in keeping milk, and influence on the determination of casein.

The tedious method involved in washing the precipitated casein free from fat by ether and in subsequent drying and weighing on the filter paper was entirely discarded. The precipitated casein was washed by decantation and on the filter two or three times. The filter and contents were then treated by the ordinary Kjeldahl method for the determination of nitrogen, the factor 6.25 being used to convert the amount of nitrogen into an equivalent of casein, when this was desired. This use of the Kjeldahl method in determining the amount of casein was suggested and employed sometime since by several chemists and is now very generally used in the analysis of dairy-products. The advantages of its use over the old method in point of accuracy and simplicity are too evident and too well known to deserve further mention. Much credit is due to Mr. A. L. Knisely who has done valuable work in carrying out the analytical details of the investigation.

(*1*) *Comparison of Results Obtained by Precipitating Casein in Milk at a Temperature of 40° C. without Carbon Dioxide and with Carbon Dioxide at Ordinary Temperatures.*—The method employed in making the comparison was, briefly, as follows: About ten grams of milk were used in each case. This amount of milk was diluted with water at 40°–42° C. to 100 cc. and then 1.5 cc. of a solution containing ten per cent. of acetic acid were added and the solution was carefully stirred with a glass rod. The resulting precipitate was washed two or three times by decantation and on the filter; finally, the filter-paper and contents were digested according to the Kjeldahl method for determining nitrogen. In the other case, the milk was diluted to 100 cc. with water of the temperature of the room, then 1.5 cc. of the same dilute acetic acid as that employed above were added,

after which a stream of carbon dioxide was passed through the solution, until the liquid above the precipitate was clear or very nearly so. The subsequent filtration, washing, and determination of nitrogen were made as above.

The following results were obtained by the two methods with two different samples of milk, triplicate determinations being made in each case :

Method A, precipitation at 40° C., sample No. 1.	Method B, precipitation with aid of carbon dioxide, sample No. 1.
(a) 0.475 .... per cent. nitrogen	(a) 0.486 .... per cent. nitrogen
(b) 0.474 .... " " "	(b) 0.480 .... " " "
(c) 0.469 .... " " "	(c) 0.461 .... " " "
Average 0.473	Average 0.476
Sample No. 2.	Sample No. 2.
(a) 0.458 .... per cent. nitrogen	(a) 0.454 .... per cent. nitrogen
(b) 0.458 .... " " "	(b) 0.444 .... " " "
(c) 0.449 .... " " "	(c) 0.455 .... " " "
Average 0.455	Average 0.451

Statement of Results.—The above results indicate: First, that essentially the same results are obtained whether we precipitate the casein in cows' milk at a temperature of 40° C. without carbon dioxide, or at ordinary temperatures with the aid of carbon dioxide, other conditions being uniform. In sample No. 1, the results differed by 0.003 per cent. of nitrogen; in sample No. 2, by 0.004 per cent. of nitrogen, the excess being first with one method and then with the other.

Second, that the individual results obtained by several determinations of the same milk agree closely by either method, somewhat closer concordance being given by precipitation at 40° C. Thus, in sample No. 1, the greatest difference in three determinations was 0.006 per cent. of nitrogen at 40° C., and 0.025 per cent. nitrogen with the other method. In sample No. 2, the greatest difference was 0.009 per cent. nitrogen at 40° C. in three determinations, and 0.011 per cent. nitrogen with the other method.

(2) *Influence of Amount of Acid Used in Precipitating Casein.*—The acid used was a solution containing ten per cent. by weight of acetic acid. This dilute acetic acid was used in amounts

varying from 0.5 to 5 cc. To about ten grams of milk, sufficient water at 40°–42° C. was added to dilute to 100 cc. and the acetic acid was then added. The operation was completed in the manner described above.

Below we present the analytical results tabulated:

	Amount of acid used.	Character of filtrate.	Per cent. of nitrogen found in the precipitate.
Sample No. 1	0.5 cc.	slightly cloudy	0.458
	1.0 "	clear	0.458
	2.0 "	clear	0.449
	3.0 "	clear	0.441
Sample No. 2	1.0 cc.	clear	0.475
	1.5 "	clear	0.474
	2.0 "	clear	0.469
	3.0 "	slightly cloudy	0.450
Sample No. 3	0.5 cc.	milky	0.117
	1.0 "	quite cloudy	0.471
	1.5 "	clear	0.545
	2.0 "	clear	0.550
	2.5 "	clear	0.545
	3.0 "	clear	0.537
	4.0 "	slightly cloudy	0.527
	5.0 "	milky	0.340
Sample No. 4	0.5 cc.	milky	0.059
	1.0 "	slightly cloudy	0.551
	1.5 "	clear	0.550

Statement of Results.—The data presented in the preceding table indicate:

First, that in one sample 0.5 cc. of acid gave a maximum amount of nitrogen in the precipitate, while in two other samples, the amount of nitrogen obtained in the precipitate was very low. In each instance where the lowest results were given, the filtrate was milky in appearance.

Second, that in three samples out of four, the use of 1.0 cc. of acid gave highest results, the filtrate being clear or only faintly cloudy.

Third, that, in most instances, the use of 1.5 cc. of acid gave the highest results, with a clear filtrate in every case.

Fourth, that the use of two cc. of acid gave very nearly the same results as the use of 1.5 cc. of acid in most cases, the filtrate being clear.

Fifth, that the use of 2.5 cc. or more of acid gave lower results, the decrease becoming greater with increase of acid and the character of the filtrate becoming more turbid in appearance.

Sixth, that, with fresh milk, the best results can be secured in determining casein by using from one to two cc. or, in general, 1.5 cc. of a ten per cent. solution of acetic acid.

Seventh, that the completeness of precipitation is quite fairly indicated by the character of the filtrate. When the precipitation is incomplete as a result of using too little acid, the filtrate will be more or less turbid, varying from opaque milkiness to barely perceptible cloudiness. If, on the other hand, an excess of acid is used, thereby causing more or less casein to remain in solution, the filtrate will be more or less turbid according to the amount of casein in solution. In some cases, a filter paper may allow some precipitated casein to pass through and render the filtrate turbid. In such cases, two or three repeated filtrations generally serve to remove the casein and to give a clear filtrate.

(3) *The Use of Different Acids in Precipitating Casein.*—In order to ascertain whether some other acid could be used to precipitate casein more effectively than acetic acid, comparative trials were made with solutions containing ten per cent. of the following acids: (1) acetic, (2) lactic, (3) sulphuric, (4) hydrochloric. Below are given the results of the comparative trials:

Kind of acid used.	Amount of acid used.	Character of filtrate.	Character of precipitate.	Per cent. of nitrogen in precipitate.
Acetic acid	1.5 cc.	clear	flocculent	0.395
Lactic acid	0.5 "	milky	"	0.074
" "	0.75 "	slightly cloudy	"	0.396
" "	1.0 "	clear	"	0.394
" "	1.5 "	milky	"	0.268
Acetic acid	1.5 cc.	clear	flocculent	0.465
Sulphuric acid	0.5 "	clear	s'ewh't gelatinous	0.470
" "	0.75 "	clear	" "	0.457
" "	1.0 "	cloudy	" "	0.417
Acetic acid	1.5 cc.	clear	flocculent	0.464
Hydrochloric acid	0.3 "	cloudy	gelatinous	0.410
" "	0.5 "	cloudy	"	0.404
" "	0.75 "	milky	"	0.199

Statement of Results.—The results contained in the foregoing table show:

First, that lactic acid gives results closely agreeing with those obtained by the use of acetic acid, when about one cc. of a ten

per cent. solution of lactic acid is used, but if 0.5 cc. more or less than one cc. of lactic acid are used, the results are very low. The precipitate was flocculent and filtered readily.

Second, that a ten per cent. solution of sulphuric acid when used to the extent of 0.5 cc. for ten grams of milk gave results closely agreeing with those given by the use of acetic acid, but that when more than 0.5 cc. were used, the results became lower. The filtrate was clear, but the casein did not separate in flocculent condition, having a tendency to become gelatinous. Several filtrations were required to get a clear filtrate, and filtration was slow.

Third, that very unsatisfactory results were given by the use of hydrochloric acid, whatever the amount used. The filtrate was cloudy or milky, the precipitate gelatinous, the filtration extremely slow, and the results low.

Fourth, for the most satisfactory results, for the flocculent character of the precipitate, for rapidity of precipitation, filtration, etc., the use of acetic acid had a great advantage over the other acids tried.

(4) *Influence of Temperature and Time of Digestion on Precipitation of Casein.*—In making the experiments, the results of which are tabulated below, the temperature and time of digestion were made to vary, all the other conditions being kept uniform.

Temperature of digestion.	Time of digestion.	Character of filtrate.	Per cent. of nitrogen in precipitate.
30 C.	5 minutes	clear	0.448
40 "	0 "	"	0.475
40 "	1 "	"	0.479
40 "	2 "	"	0.481
40 "	3 "	"	0.471
40 "	10 "	"	0.477
40 "	20 "	"	0.484
45 "	0 "	"	0.468
45 "	2 "	"	0.476
45 "	5 "	"	0.483

The data presented in the foregoing table show:

First, that, when the precipitate was digested at 30° C. for five minutes, the results were lowest.

Second, that, when the precipitate was digested at 40° C.

without standing or with standing one, two, three, ten, or twenty minutes, the results were practically uniform. The greatest difference obtained by digesting different periods of time did not exceed 0.01 per cent. nitrogen.

Third, that, when the precipitate was digested at 45° C. for periods of time varying from a few seconds to five minutes, the results were fairly uniform, the greatest difference being 0.015 per cent. nitrogen. As compared with digestion at 40° C., digestion at 45° C. gave essentially the same results.

Fourth, it would, therefore, seem that a variation of temperature two or three degrees above or below 40° C. and variation of time of digestion after the addition of acetic acid exercises comparatively little influence upon the results. However, in practice, I think it is desirable to adhere quite closely to uniformity of temperature.

(5) *The Precipitation of Casein in Fresh and in Old Milk.*— The fact is well known that milk undergoes rapid and complex changes when left to itself at ordinary temperatures. In the course of our work, it occurred to the writer that, as a result of these changes, the casein might be so changed that the method used in determining casein in fresh milk might not be entirely applicable to milk that had undergone more or less change; or that the formation of lactic acid might make necessary the use of less acetic acid than is used in precipitating the casein of fresh milk. It was generally found that, after milk had stood at a temperature of 17° to 22° C. for twenty-four hours or more, the use of 1.5 cc. of a ten per cent. solution of acetic acid gave low results, the filtrate being decidedly turbid. In some cases the casein was almost entirely rendered soluble and passed into the filtrate. It was found that the only method of handling milk under such circumstances was to add the acetic acid to the milk, diluted with water at 40°–42° C., in small portions of a few drops at a time, stirring after each addition and continuing the additions until the liquid above the precipitate became clear or nearly so. But, even with this treatment, there was found to be more or less loss. The terms "fresh" and "old" milk are, of necessity, rather vague. Much depends upon the conditions under which milk is kept. The extent of

change rather then the actual age of the milk should form the basis of distinction. Coagulated milk gave low results.

	Age of milk.	Per cent. of nitrogen in precipitate.	Amount of loss.
Sample No. 1	Fresh	0.429	....
	7 days	0.378	0.051
Sample No. 2	Fresh	0.447	....
	1 day	0.427	0.020
	3 days	0.428	0.019
	7 days	0.428	0.019
Sample No. 3	Fresh	0.428	....
	1 day	0.410	0.018
Sample No. 4	Fresh	0.438	....
	1 day	0.415	0.023
Sample No. 5	Fresh	0.624	....
	1 day	0.617	0.007
Sample No. 6	Fresh	0.397	....
	1 day	0.397	0.000
Sample No. 7	Fresh	0.466	....
	1 day	0.445	0.021

The author has in progress an investigation relating to the detailed changes that take place in the nitrogen compounds of milk under various conditions.

Statement of Results.—First, when milk was kept at a temperature of 17° to 22° C. for twenty-four hours, the amount of nitrogen as casein was found, in most cases, to be nearly 0.02 per cent. less than in fresh milk.

Second, in case of one milk kept seven days, the amount of nitrogen in the casein was 0.051 per cent. less than in fresh milk, while, in another case, it was 0.019 per cent. less.

Third, in general, it was found necessary, in order to get results that were at all satisfactory, to use less than 1.5 cc. of dilute acetic acid to precipitate the casein in milk that had been standing long enough to undergo any marked change.

Fourth, while good results may, in exceptional cases, be secured in determining casein in milk that has undergone noticeable change without any modification of the method given for the determination of casein in fresh milk, the accuracy of such determinations must be regarded as uncertain in regular work.

(6) *The Influence of Mercuric Chloride Used as a Preservative on the Determination of Casein in Milk.*—In view of what has

just preceded, can we be certain of our results when we determine casein in milk that is not fresh? The thought suggested itself to the author that if some germicide could be added to fresh milk to prevent or retard the changes that affect the casein, then we might be able to determine the casein in milk that had stood indefinitely. In order to test the question, finely powdered mercuric chloride was used in proportion of about one part of mercuric chloride to two thousand parts of milk by weight. A sample of milk was analyzed when fresh and portions were set aside for varying periods of time, some with and some without the addition of mercuric chloride. Determinations of casein were made from time to time, using less than 1.5 cc. dilute acetic acid. The results were as follows:

Age of milk.	Per cent. of nitrogen in precipitate when no mercuric chloride was used.	Amount of loss.	Per cent. of nitrogen in precipitate when mercuric chloride was used.	Amount of loss.
Fresh	0.447	...	0.447	....
1 day.....	0.427	0.20	0.444	0.003
3 days.....	0.428	0.19	0.444	0.003

Statement of Results.—The foregoing results appear to indicate:

First, that the use of mercuric chloride in fresh milk largely prevents or retards the occurrence of those changes that effect the determination of casein.

Second, that even when mercuric chloride is used in milk, it is generally desirable to use less than 1.5 cc. of dilute acetic acid in precipitating the casein.

Third, that the use of mercuric chloride in the proportions stated did not precipitate in appreciable quantity any nitrogen compounds such as albumen.

(7) *General Summary.*—We may summarize the results of our work as follows:

First, precipitation of casein in cows' milk gives essentially the same results whether made at 40° C. without carbon dioxide or at ordinary temperatures with the aid of carbon dioxide, other conditions being uniform.

Second, between one and two cc. of a ten per cent. solution of acetic acid, generally about 1.5 cc., gave the best results.

Third, the use of lactic acid, sulphuric acid, and hydrochloric

acid gave results much less satisfactory than the use of acetic acid.

Fourth, a variation of a few degrees from 40° C. and a variation of time of digestion after the addition of acetic acid exercised comparatively little influence on the results.

Fifth, the precipitation of casein in milk that had undergone noticeable change was generally found to give lower results than in case of the same milk when fresh, even when less than 1.5 cc. of acetic acid were used. Coagulated milk always gave low results compared with fresh milk.

Sixth, the use of mercuric chloride in fresh milk, in the proportion of one part of the former to two thousand parts of milk, largely prevented or retarded the occurrence of those changes that affect the determination of casein in old milk.

Seventh, in a general way, the character of the filtrate serves as a fairly reliable guide in regard to the completeness of the precipitation of casein from milk. When the filtrate is clear or only perceptibly cloudy, the precipitation is generally complete; but, when the filtrate is decidedly turbid or milky and two or three repeated filtrations do not remove the turbidity, the precipitation will generally be found incomplete and such a determination, if carried out, will give low results.

(*8*) *Detailed Description of Method Used in Determining the Casein of Cows' Milk.*—I. In Fresh Milk: Weigh out about ten grams of milk, dilute in a beaker with about ninety cc. of water at 40°–42° C., and add at once 1.5 cc. of a solution containing ten per cent. of acetic acid by weight. Stir with a glass rod and let stand three to five minutes or longer. Then decant on filter, wash two or three times with cold water by decantation, and then transfer precipitate completely to filter. Wash once or twice on filter. The filtrate should be clear or very nearly so. If the filtrate is not clear when it first runs through, it can generally be made so by two or three repeated filtrations, after which the washing of the precipitate can be completed. The washed precipitate and filter paper are then digested as in the regular Kjeldahl method for the determination of nitrogen and the determination of nitrogen is completed as usual. To calculate the nitrogen into an equivalent amount of casein, multiply the

amount of nitrogen found by the factor 6.25. Ordinarily, for this purpose, milk can be regarded as "fresh," when it does not show marked development of lactic acid.

II. In Old Milk: When milk has undergone such change as to show marked development of lactic acid, the method above given cannot generally be relied upon to give accurate results in determining casein. So far as our results go, they indicate that we cannot with positive accuracy determine casein in such milk by any method now known, and results obtained with changed milk must be regarded as only approximate. If, however, one part of finely powdered mercuric chloride is added to two thousand parts of milk, when fresh, the changes, which would otherwise take place, are prevented or greatly retarded, so that milk treated in this manner may be used after standing some days for the determination of its casein. In such cases, the method given above for fresh milk may be followed, except that the acetic acid should be added in small portions, a few drops at a time, stirring after each addition, and continuing the addition of acetic acid until the liquid above the precipitate becomes clear or very nearly so.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION, GENEVA, N. Y.,
AUGUST, 1893.

SOME POINTS RELATING TO THE COMPOSITION OF COWS' MILK.[1]

BY L. L. VAN SLYKE, CHEMIST OF N. Y. AGR. EXP. STA.

IT is my purpose to call attention to some general results secured in our work at the New York State Agricultural Experiment Station at Geneva in the course of an extended examination of the milk of cows in that State. Our results are based upon the analysis of lots of milk aggregating over 200,000 pounds and representing the product of not less than 1,500 individual animals, and 20,000 separate milkings extending over a period of some six months.

The points to which I wish to call especial attention are the following:

1. The relation of casein to albumen in normal milk.

[1] Read before the World's Congress of Chemists, August 24, 1893.