Issued April 8, 1913.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 162.
A. D. MELVIN, CHIEF OF BUREAU.

FACTORS INFLUENCING THE CHANGE IN FLAVOR IN STORAGE BUTTER.

BY

L. A. ROGERS, *Bacteriologist;* W. N. BERG, *Chemist;*
C. R. POTTEIGER, *Assistant Chemist,*
AND
B. J. DAVIS, *Assistant.*

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., October 8, 1912.

Sir: I have the honor to transmit herewith for publication in the bulletin series of this bureau a manuscript entitled "Factors Influencing the Change in Flavor in Storage Butter," by Messrs. L. A. Rogers, W. N. Berg, C. R. Potteiger, and B. J. Davis, of the Dairy Division.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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INTRODUCTION.

The economic conditions in this country which have made it necessary to hold butter in storage for long periods have increased the importance of the changes that take place in butter on standing. A change that passes unnoticed in butter that is used when a week or two old may become a serious defect after three or four months in storage. The great variation and complexity of the changes in flavor indicate a corresponding complexity in the chemical alteration in the butter, and while it is true that some of the modifications are well known it is becoming evident that the various flavors are produced by changes too small to be measured by the ordinary methods of the laboratory. Under certain circumstances free fatty acids may be formed, a condition usually associated with a rancid flavor. However, it is evident that the fatty acids alone are not the cause of the rancid flavor, since, in the process of renovating, the rancid flavor is removed while a large part of the acid remains.

It is possible that the flavor-giving substances are produced in very small quantities and that their formation is not necessarily connected with or in proportion to the grosser changes measurable by the ordinary analytical methods. There are several substances in butter that are more or less unstable under ordinary circumstances, i. e., the proteins of milk in their hydrated condition, lecithin, citric acid, lactic acid, and other products of bacterial action. But little work has been done in which the storage flavor was shown to be related to chemical changes involving any of these substances. This is probably due to the fact that while butter fat is easily handled for analytical purposes, it is difficult to separate from the butter fat the other fatlike substances, such as lecithin. The remaining part of the butter, which will be called the butter curd solution, is of such a physical consistency that it can not very well be used directly for quantitative analytical work.

In considering the problem of storage flavor, its causes, and the methods of studying the problem, it is well to bear in mind one or two of the facts involved in the physiology of the senses of taste and smell. It is well known that several different substances may taste alike: Thus sugar, saccharin, lead acetate, glycerin, and perhaps still other substances, all taste sweet. Chemically they are not at

all similar. While trimethylamin may be the specific cause of fishy flavor in herring brine, it is not necessarily the cause of fishy flavor in butter. It is possible, reasoning by analogy, that many different substances may cause "fishy" flavor.

The sense of smell is very delicate and can detect astonishingly small amounts of material, so small that the most delicate balances could not weigh them. A flavor is a mixed sensation in which the sense of taste and smell take a leading part. Howell [1] states that 0.00005 grams of quinine in 100 cubic centimeters of water is detectible on the root of the tongue. "It is recognized in chemical work, for instance, that traces of known substances too small to give the ordinary chemical reactions may be detected easily by the sense of smell. According to the experiments of Fischer and Penzoldt, mercaptan may be detected in a dilution of $\frac{1}{460,000,000}$ of a milligram in 50 cubic centimeters of air." [1]

While the off flavors of butter may not be caused by the formation of such inconceivably small amounts of odoriferous substances, yet such data are of practical significance in so far as they indicate that the analytical method of studying storage flavors may be wholly inadequate. There may be many substances [2] whose isolation or detection in butter might be very difficult if not impossible by present methods, and which would still impart to the butter sufficient odor and taste to be distinctly perceptible.

POSSIBLE CAUSES OF CHANGE.

The marked influence of bacteria on the flavor of milk, cheese, and other dairy products naturally leads to the conclusion that the same organisms would be an important if not the only factor concerned in the changes in butter. It has been demonstrated, particularly by the work of Jensen,[3] that under certain conditions bacteria multiply in butter and have a direct influence on the flavor of the product.

It should be remembered, however, that the butter on which Jensen and other European investigators worked differs in one very essential particular from the ordinary American butter. While the salt content of most European butter is low enough to permit the growth of bacteria, American butter contains sufficient salt to bring its concentration in the water of the butter to 18 per cent or more. It is to be expected that bacteria would not grow—or at least would

[1] Howell, William H. Textbook of Physiology. Philadelphia, 1906. See pp. 275–280.

[2] Zwaardemaker, H. Geruch. Ergebnisse der Physiologie. Abteilung 2, vol. 1, pp. 896–909. Wiesbaden, 1902.

[3] Jensen, Orla. Bakteriologische Studien über dänische Butter. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Abteilung 2, vol. 29, no. 23/25, pp. 610–616. Jena, Apr. 8, 1911.
Jensen, Orla. Studien über das Ranzigwerden der Butter. Centralblatt für Bakteriologie. Parasitenkunde und Infektionskrankheiten. Abteilung 2, vol. 8, no. 1, pp. 11–16, Jan. 4; no. 5, pp. 140–144, Feb. 5; no. 6, pp. 171–174, Feb. 10; no. 7, pp. 211–216, Feb. 17; no. 8, pp. 248–252, Feb. 24; no. 9, pp. 278–281, Mar. 4; no. 10, pp. 309–312, Mar. 8; no. 11, pp. 342–346, Mar. 13; no. 12, pp. 367–369, Mar. 15; no. 13, pp. 406–409, Mar. 24. Jena, 1902.

grow only very sparsely—under these conditions, and the investigations in this country confirm this supposition. Rahn, Brown, and Smith[1] found in some samples of butter a torula able to grow very slowly in salt solutions at low temperatures, but this occurred in such small numbers that it could not account for much of the deterioration of the butter. In our own work we have found no evidence of bacterial growth under normal conditions, with the exception of a small multiplication of torula at high storage temperatures. In these cases there was no apparent relation between the growth of torula and change in flavor. Moreover, the same changes took place in duplicate lots of butter held at temperatures so far below the freezing point that there could be no possibility of growth. Any flavors that appear in butter may be found in butter held at the commercial storage temperature of zero or below (Fahrenheit), and any explanation of the cause of these changes which does not take this fact into consideration is obviously fallacious, or at best valid for certain conditions only.

In some of our earlier work[2] the possible influence of lipolytic enzyms was suggested, but it was soon found that in many cases butter showed a marked change in flavor without any appreciable hydrolysis of the fat. This observation is confirmed by the work of Rahn, Brown, and Smith cited above. The action of other enzyms, as, for instance, the proteolytic enzym of the milk or those secreted by bacteria, is not necessarily excluded.

The influence of the acidity of the cream on the flavor of butter has already been pointed out.[3] It has also been suggested [4] that a slow oxidation may take place in the interior of a package of butter, due to the numerous small bubbles of air inclosed in the butter. Even a superficial examination of the work already done shows that the question is a very complicated one and that the difficulties in the way of a solution are many. In studying the ripening of cheese pronounced chemical changes are available for measuring the progress of the ripening. In butter the changes are scarcely appreciable. The investigator is thus forced to rely on the sense of taste and smell for a measure of the change. Some butter judges have developed marked ability in detecting and estimating the intensity of various flavors, but at best the sense of taste is uncertain, and any numerical scale based on this faculty is necessarily an arbitrary one and subject to fluctuation in its value. Two butter judges can not be expected

[1] Rahn, Otto, Brown, C. W., and Smith, L. M. Keeping qualities of butter. Michigan Agricultural College Experiment Station, Technical Bulletin 2. East Lansing, September, 1909.

[2] Rogers, Lore A. Studies upon the keeping quality of butter. United States Department of Agriculture, Bureau of Animal Industry, Bulletin 57, Washington, 1904.

[3] Rogers, L. A., and Gray, C. E. The influence of acidity of cream on the flavor of butter. United States Department of Agriculture, Bureau of Animal Industry, Bulletin 114, Washington, 1909.

[4] Rogers, L. A. Fishy flavor in butter. United States Department of Agriculture, Bureau of Animal Industry, Circular 146, Washington, 1909.

always to agree, because the definitions of flavors can not be reduced to exact terms and the amount of deduction on the numerical scale for various flavors can not be fixed.

The most serious difficulty in experimental work on butter is in controlling the conditions under which the butter is made. So many apparently unimportant factors have an influence on the flavor that it is nearly impossible to make butter with a normal flavor and have only one varying factor. The work is further complicated by the sequence of flavors that frequently occurs in butter held in storage. It is evident that the usual off flavors are in many cases a combination of flavors and that the flavors themselves are caused by a combination of circumstances and not by a single cause. It is probable also that identical flavors may be caused by different factors.

In the work reported in this paper we have attempted to determine the part played by certain factors in the general change in flavor in storage butter without directing the investigation toward any particular flavor or attempting to cover all of the causes of deterioration.

In this we have been guided by the previous work, which has indicated certain points at which the problem could be attacked with some promise of positive results. It has been observed, for instance, that when a lot of sweet cream is divided, one half churned at once and the other half pasteurized and churned, the butter from the unpasteurized half deteriorates very quickly, while the pasteurized-cream butter has exceptionally good keeping qualities. What has been removed by the pasteurization that has such a marked influence on the butter? The enzyms of the milk are partly or entirely destroyed and a large proportion of the bacteria are killed. Are the proteolytic enzyms of the milk able to work under the conditions existing in butter and have they any influence on the flavor of the butter? Is there any appreciable proteolysis in butter even under favorable conditions? If the two lots of cream are ripened, the keeping quality of the butter from the unpasteurized cream is increased, while that from the pasteurized cream is decreased. In the process of ripening the bacterial growth is confined almost entirely to one variety, but it does not necessarily follow that these bacteria have any direct deleterious action. The growth of the bacteria produces a considerable quantity of acid, and the chemical instability of the product is increased accordingly.

Does the air which, as has been shown, is inclosed in the butter effect an appreciable oxidation? Milk and cream is handled in containers in which it may be exposed to tin, iron, or copper. Under these conditions it is reasonable to suppose that small amounts of the metals, especially the iron and copper, will be dissolved and carried into the butter. Do the salts formed by the metals with organic acids of the cream affect the flavor of the butter?

PROTEOLYSIS IN BUTTER.

It has long been known that butter differs from milk in its composition only in the relative amounts of the constituents present in the two. Among these constituents which early attracted attention as possible causes of storage flavor because of their chemical instability were the proteins, mainly casein. Proteins in the hydrated or moist condition in the presence of water are known to be unstable, and it is but natural that these substances, wherever they may occur in food, should be looked upon as possible sources of off flavor. It is almost certain that in butter containing no salt, or salt in an insufficient amount, or in butter that is not stored at sufficiently low temperature, the proteins present do undergo hydrolysis and perhaps putrefaction and other obscure changes as well. But the present work does not concern itself with such material. The problem is: If storage flavor develops in butter properly made and properly stored, do the proteins contribute in any way toward this off flavor?

Certain conditions in butter favor proteolytic changes, namely, the presence of water, bacteria, and of the proteolytic enzym known as galactase, which occurs normally in milk. Other conditions, as low temperature, the presence of sodium chlorid, the partial inactivation of the galactase by pasteurization, tend to prevent or retard proteolytic changes.

It has already been shown by other investigators that under conditions of comparatively high temperature and low salt the butter proteins will undergo changes. In the present work an attempt was made to determine whether the galactase present in butter made from pasteurized or from unpasteurized cream can digest casein in spite of the retarding influence of low temperature and high salt concentration.

PREVIOUS WORK.

Analytical difficulties.—When butter is melted and allowed to stand, the water present, containing the salt and casein in solution and in suspension, will settle to the bottom of the container, leaving the supernatant fat clear. The fat may be poured off and filtered if desired and at once used for quantitative work. However, all of the fat can not be poured off, because part of it is thoroughly mixed with the particles of curd, so that after the most careful removal of fat by decantation a considerable amount is still left. Some of this residual fat can be removed by the addition of ether. This will dissolve the fat on the upper surface of the curd solution and permit more fat to rise; but even three or four such washings with ether still leaves in the curd solution a considerable quantity of fat, probably 20 grams of fat in 100 cubic centimeters of curd solution. It is obvious that such a mixture of fat, sodium chlorid solution, and

casein suspension is not very well adapted to quantitative work. The material will not filter, nor can small samples of uniform composition be easily withdrawn from it.

In order to study the possible changes in the proteins of butter, this is the material to be used. In principle, the method of testing for the presence of active proteolytic enzyms in this material is no different from that used for other purposes, as, for instance, in tracing the proteolytic changes in ripening cheese or in animal tissue undergoing autolysis. At first it would seem as if there should be no difficulty in making the usual nitrogen partition in this curd solution just as it is made on other viscous mixtures that are equally difficult to filter and sample.

Evidently it was at the first step in the nitrogen partition that the difficulties began, for, to the best of our knowledge, none of the previous investigators succeeded in precipitating the casein in the curd solution, filtering and determining nitrogen in the filtrate or the precipitate, in such a manner as to enable the investigator to draw safe conclusions from the analytic data thus obtained. To this statement there are apparent exceptions. On adding acetic acid in usual amounts to some of the curd solution as if it were so much milk for the purpose of flocculating the casein and filtering no flocculation is seen to occur and the mixture will filter so slowly as to make quantitative work unreliable for obvious reasons. If the curd solution be diluted with water until the casein can be flocculated by acetic acid in usual amounts, filtration is then rapid and the filtrate can then be used for nitrogen determinations. The nitrogenous substances in butter, however, are about 75 per cent casein, so that on removal of the casein there is so little nitrogen left in the filtrate from the diluted curd solution that the unavoidable errors in such work are very large when compared with the analytic data obtained. Still less certain are the results obtained on the nitrogen partition in such a filtrate, because the total nitrogen is too small for even that determination.

In order to avoid the introduction of comparatively large errors, we made many attempts to increase the amount of curd solution used and to reduce the dilution before adding the precipitant. Acetic or other acids evidently were not previously used in quantities sufficient to flocculate the casein. Other precipitants, such as ferric chlorid, phosphotungstic acid, tannic acid, copper sulfate, etc., were tried. When added to curd solutions diluted with but two volumes of water, these precipitants will thoroughly flocculate the protein and give a filtrate that is clear, comes through rapidly, and can be used for quantitative work. Unfortunately, these precipitants remove from the curd solution practically all of the nitrogen, leaving too little in the filtrate. The water-soluble nitrogen in good butter is approximately one-fifth to one-tenth of the total, and in so far as the total

nitrogen is represented by 1 per cent of curd, or about 0.1 to 0.2 per cent of nitrogen, it is necessary to use large amounts of curd solution for these precipitations in order that the filtrates may contain sufficient nitrogen for accurate determinations.

Results by Gray and others.—Several years ago (1906) Mr. C. E. Gray, then connected with the Dairy Division, began a study of the possible proteolytic changes in storage butter and their relation to the change in flavor. Following is his method of making the partition of nitrogen in butter:

Total nitrogen.—Introduce 10 grams of butter into a Kjeldahl flask, digest, and distill as usual.

To obtain nitrogen in other forms: Melt 2 kilos of butter in a hot-water jacketed funnel, temperature about 80° C. The melted butter is allowed to run into a cream separator with a special bowl having a capacity of 700 cubic centimeters without milk outlets. This was just large enough to hold all of the curd solution plus a small amount of fat. As the butter was fed in the bowl soon became filled and the excess of butter fat ran out, leaving the curd solution in the bowl. The larger part of the fat was washed out by feeding gasoline into the bowl. The last portion of gasoline was removed by feeding in water. The addition of water was stopped as soon as the outflowing liquid carried particles of casein. In this way the curd solution in 2,000 grams of butter was separated from most of the fat. The contents of the bowl were transferred to a 1-liter flask, 25 cubic centimeters of 10 per cent ferric chlorid solution were added, and the total volume made up to the mark. The mixture was filtered on a 32-centimeter filter and the faintly colored filtrate used in the following determinations. Although but 600 to 700 cubic centimeters of filtrate were obtained, the results on aliquot portions were always calculated to 1,000 cubic centimeters.

It is obvious that these filtrates contained only nitrogen not precipitated by ferric chlorid; that is, nitrogen largely in the form of amino acids and ammonia. Certain peptones are precipitated by ferric chlorid.[1]

Total soluble nitrogen.—Transfer 50 cubic centimeter portions of the filtrate (corresponding to 100 grams of butter) to Kjeldahl flasks and determine total nitrogen.

"*Amino and ammonia nitrogen.*"—Transfer a 200 cubic centimeter portion of the ferric chlorid filtrate to a 300 cubic centimeter volumetric flask. Add 1 gram of sodium chlorid and sufficient 12 per cent tannic acid solution for maximal precipitation. Three or four cubic centimeters were usually required. Make up to the mark with distilled water, filter, and determine total nitrogen in 100 cubic centimeter portions of the filtrate, each of which corresponds to 133⅓ grams of butter.

"*Ammonia nitrogen.*"—The method described by Van Slyke and Hart [2] was used. Transfer 100 cubic centimeters of the ferric-chlorid filtrate (corresponding to 200 grams of butter) to a Kjeldahl flask, add 2 grams of magnesium oxid, and boil for about 1½ hours, catching the distillate in N/20 acid. The excess of acid was titrated in the usual way.

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[1] Siegfried, M. Zur Kenntniss der Phosphorfleischsäure. Zeitschrift für Physiologische Chemie, vol. 21, no. 5/6, pp. 360–379. Strassburg, Apr. 2, 1896.

Siegfried, M. Ueber Antipepton. Zeitschrift für Physiologische Chemie, vol. 27, no. 4/5, pp. 335–347. Strassburg, June 24, 1889. See p. 342.

[2] Van Slyke, L. L., and Hart, E. B. Methods for the estimation of the proteolytic compounds contained in cheese and milk. New York Agricultural Experiment Station, Bulletin 215, Geneva, September, 1902.

Amino nitrogen is the difference between the sum of the amino and ammonia nitrogen and the ammonia nitrogen.

Proteose and peptone nitrogen is the difference between the total soluble nitrogen and the sum of the amino and ammonia nitrogen.

This method of studying the distribution of nitrogen in butter was used by Gray from 1906 to 1907 on a very large number of samples of butter churned from ripened, unripened, pasteurized, and unpasteurized cream and stored at various temperatures. The plan of the investigation was very comprehensive. It aimed to ascertain the best conditions for the production of butter of best keeping quality and the chemical changes causing the off flavors of storage butter. This method was also used by us on one series of 24 samples in the spring of 1908.

Gray's method of removing most of the fat from the butter by the use of the centrifuge was an improvement, without doubt. But for reasons to be made apparent presently the analytic data obtained by this method were not regarded as conclusive. More accurate data, it is believed, were later obtained by a method that is free from some of the objections that might be made to the method as originally devised by Gray.

In Table 1 are some results obtained by Gray. The butter was obtained from one lot of cream which was divided into eight portions from which eight separate churnings were made. The eight lots of butter were packed in 20-pound tubs and stored soon after churning, at −10° F. (−23° C.). Analyses were made on the fresh butter, representing the condition of the nitrogen in the butter before storage. The two following series of analyses were made on the butter after different periods in storage. Two similar series of results were obtained by Gray on portions of the same lots of butter, stored at 10° F. (−12° C.) and at 32° F. (0° C.). The figures are not given here, but are in general similar to those in Table 1.

From the results obtained by Gray it would seem that in the samples of butter examined slow proteolytic changes took place during storage. At least this is the inference to be drawn on the assumption that the method of obtaining the chemical data was free from avoidable errors.

TABLE 1.—*Changes in the distribution of nitrogen in butter during cold storage (−10° F., −23° C.).*

Butter No.	Age of sample.	Total nitrogen.	Total soluble nitrogen.	Proteose and peptone nitrogen.	Amino nitrogen.	Ammonia nitrogen.
	Days.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10.311	0	0.136	0.0039	0.0013	0.0019	0.00074
	206	.....	.0070	.0032	.0028	.00095
	298	.....	.0083	.0043	.0030	.00105
10.312	0	.139	.0041	.0026	.0006	.00091
	206	.....	.0065	.0035	.0021	.00095
	298	.....	.0074	.0026	.0039	.00095
10.313	0	.139	.0053	.0003	.0037	.00130
	206	.....	.0067	.0032	.0022	.00134
	298	.....	.0071	.0041	.0017	.00131
10.314	0	.143	.0045	.0005	.0028	.00115
	206	.....	.0061	.0023	.0025	.00132
	298	.....	.0090	.0046	.0031	.00128
10.321	0	.140	.0020	.....	.0018	.00058
	206	.....	.0037	.0014	.0016	.00066
	298	.....	.0073	.0052	.0014	.00070
10.322	0	.138	.0030	.....	.0030	.00053
	206	.....	.0053	.0021	.0036	.00061
	298	.....	.0035	.0007	.0022	.00062
10.323	0	.139	.0037	.0003	.0029	.00049
	206	.....	.0047	.....	.0050	.00066
	298	.....	.0061	.0034	.0030	.00062
10.324	0	.141	.0065	.0007	.0049	.00087
	206	.....	.0081	.0024	.0048	.00088
	298	.....	.0087	.0068	.0010	.00086

The method of Gray in a slightly modified form was used in the summer of 1908 and the spring of 1909. The results obtained were, in general, similar to those obtained by Gray. They seemed to indicate that slow proteolysis was taking place.

After using the method for a short time several improvements suggested themselves. It will be noticed in Table 1 that the largest amount of nitrogen estimated in a ferric-chlorid filtrate (column headed "total soluble nitrogen") was equivalent to 0.009 per cent of nitrogen in the butter, or to 6.3 cubic centimeters N/10 nitrogen. This is not a large amount. The largest difference between total soluble nitrogen before and after storage in Table 1 is that for sample 10.321, and is equivalent to not quite 4 cubic centimeters N/10 nitrogen. It seemed desirable so to change the method as to increase the amounts of nitrogen actually estimated. Whether proteolysis did or did not take place would then be decided with the aid of figures that are not so small that the unavoidable errors in such work are comparatively large. Filtration was so slow that evaporation undoubtedly took place to an unnecessarily large extent. The analyst could not be certain that 100 cubic centimeters of a ferric-chlorid filtrate obtained after storage corresponded to exactly the same weight of butter as an equal volume of filtrate obtained before storage. Either filtration must be so rapid that evaporation may be disregarded because of its slight extent, or if filtration must be slow the volumes of filtrates should be care-

fully measured, so that the weight of butter corresponding to any volume of filtrate can be definitely ascertained.

Objections to ferric chlorid and tannic acid as protein precipitants.—Ferric chlorid as a protein precipitant was not wholly desirable because, as here used, it precipitated not alone the casein and other undigested proteins, but their immediate digestion products down to the peptone stage. The "ferric-chlorid filtrate" probably does not contain proteoses. The use of the term "total soluble nitrogen" in this connection will lead to no confusion if it be borne in mind that it means nitrogen not precipitated by ferric chlorid. The figures for "proteose and peptone nitrogen" in Table 1 probably represent only some of the simpler peptones. The amount of nitrogen left in the ferric-chlorid filtrate is small, at least, when used for the separation of the different forms of nitrogen in butter-curd solution.

Tannic acid as a protein precipitant is perhaps still more objectionable than ferric chlorid. It is well known that the precipitation limits of tannic acid may be varied by the presence of salts, etc. Two results obtained with the aid of tannic acids are comparable only when the precipitant has been used in both cases under conditions that are exactly alike as regards the concentration of sodium chlorid, the amount of protein to be precipitated, the precipitating power of the samples of tannic acid used, etc.[1] The greatest care must be taken in the use of the reagent to insure absolute uniformity in procedure. This is shown by the voluminous literature on the use of this reagent, in which the numerous difficulties and necessary modifications are pointed out.

In addition to the difficulties just mentioned are those resulting from differences of opinion among investigators as to the best method of using the reagent. Van Slyke and Hart,[2] in their determinations of peptones in cheese are extremely careful to avoid an excess of tannic acid, probably because of the alleged solubility of the precipitate in excess of the precipitant.

According to Bigelow and Cook[3]—

* * * a considerable excess of tannin may be employed without any tendency of the reagent to dissolve the precipitate formed in excess. * * *

Gray used tannic acid as directed by Van Slyke and Hart.

It is not surprising that certain workers should advocate the discontinuance of the use of tannic acid as a reagent for the determination of amino acid nitrogen.[4]

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[1] Bigelow, W. D., and Cook, F. C. The separation of proteoses and peptones from the simpler amino bodies. Journal of the American Chemical Society, vol. 28, no. 10, pp. 1485-1499. Easton, October, 1906.

[2] Loc. cit.

[3] Loc. cit., p. 1493.

[4] Proceedings of the Twenty-sixth annual convention of the Association of Official Agricultural Chemists, United States Department of Agriculture, Bureau of Chemistry, Bulletin 132. Washington, 1910. See p. 156.

The results obtained by Gray for amino nitrogen in Table 1 are not concordant, probably due to difficulties inherent in the use of tannic acid. Our own results are likewise difficult to interpret. In a certain series of analyses (butter No. 13.5) less amino nitrogen was found in the butter after storage than before. The figures are not given here, as they are essentially similar to those of Gray.

After obtaining a considerable number of results on the distribution of nitrogen in butter before and after storage, with the aid of ferric chlorid and tannic acid, we were unable to conclude that the results proved anything. It seemed more and more desirable to perfect a method that would permit the precipitation of the casein, then the estimation of proteoses by zinc sulfate, peptones by tannic acid, and ammonia by any of the methods that did not give too high results.

In order to obtain a filtrate containing sufficient nitrogen for analytic work, the curd solution can not be diluted very much. In the undiluted condition it is a thick, viscous suspension of casein containing a variable amount of fat to which acetic acid may be added without any apparent effect. No flocculation can be observed. The mixture would filter extremely slowly. We made many attempts to find out why filtration was so slow. At first it was thought that fat particles clogged the filter paper. The first attempts were centered on the more thorough separation of the butter fat from the remainder of the butter, which in this paper is referred to as butter curd solution. It is obvious that unless the same amount of fat is removed in comparative analyses of butter before and after storage, an error will be introduced because the fat remains in the precipitate, giving a smaller volume of more concentrated filtrate. The error from this source is probably much larger than anyone might suppose. The amount of fat present in the butter curd solution used for analytical purposes should be estimated so that corrections can be made if necessary.

The butter fat may be separated from the remainder of the butter in more than one way. But obviously, when the curd solution and not the fat is wanted for quantitative work, the separation must consist of something more than a mere decantation of the melted fat. The butter fat and curd are so thoroughly mixed in the butter that when the butter is melted and allowed to stand, the separation between fat and curd solution is not complete. Most of the fat can be decanted, but very appreciable amounts still remain in the curd. Although Gray attempted to wash out the fat with the aid of gasoline, it is certain that very much fat was still present in the material used for analysis. We tried to remove the fat with ether, but without success. The fat particles are embedded in curd and in this condition ether can not reach them. Besides, the ether could only be poured on top of the curd solution. Thorough mixing was inadvisable because of

the possibility of forming emulsions that could not be separated. The use of ether was soon discontinued because of the possible dehydrating action of the ether upon the protein material and the subsequent obscuring of the results for nitrogen. The complete separation of fat from curd solution was then abandoned with the intention of estimating the amounts of fat present in portions used for analysis. In their work on storage butter, Rahn, Brown, and Smith [1] did not record a quantitative separation of butter fat from curd solution. Instead, they proceeded as follows:

> Into a weighed 2-quart [fruit] jar about 500 grams of butter were poured and weighed to the 0.5 of a gram. Then 2 grams of hot water (70° C. to 75° C.) for every gram of butter was poured into the jar and the water and butter stirred occasionally for about an hour. The cover to which an arrangement for letting in air and drawing off water had been attached was put on and the jar inverted. After 15 or 20 minutes the water which had been separated from the fat was drawn off. Aliquot parts were taken for analysis.

One objection to such a procedure lies in the fact that the water so separated from the fat contains too little nitrogen. The analytic data of Rahn, Brown, and Smith on nitrogen partition in butter are open to the same criticism as are Gray's data in Table 1. Their results differ little from those of Gray. Their work did not assist in explaining why acetic acid will flocculate casein in milk, but not in butter curd solution. This problem had been evaded by practically all who studied butter chemistry.

In a recent investigation on the influence of preservatives on the keeping qualities and composition of butter and oleomargarin, Fischer and Gruenert [2] used the methods of Rahn, Brown, and Smith in their studies on proteolytic changes. They state that the addition of 3 per cent of salt to butter greatly retards, but does not entirely prevent, proteolytic and other changes in butter stored in a cool cellar.

NEW METHOD FOR DETECTING PROTEOLYSIS IN BUTTER.

The influence of sodium chlorid on the precipitability of casein by acetic acid.—Butter curd solution differs from milk in many respects; one of them is that butter curd solution may contain sodium chlorid in amounts ranging from nothing up to saturation (over 30 per cent), depending upon the moisture and salt content of the butter. Perhaps the presence of the salt prevented flocculation of casein.

During a previous investigation on the temperature of pasteurization for butter making [3] the following method of precipitating casein

[1] Loc. cit., p. 14.

[2] Fischer, K., and Gruenert, O. Über den einfluss einiger Konservierungsmittel auf Haltbarkeit und Zusammensetzung von Butter und Margarine. Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, vol. 22, no. 10, pp. 553-582, Berlin, Nov. 15, 1911.

[3] Rogers, L. A., Berg, W. N., and Davis, Brooke J. The temperature of pasteurization for buttermaking. United States Department of Agriculture, Bureau of Animal Industry, Circular 189, Washington, 1912. See p. 317.

from buttermilk, milk, or skim milk was used. The object was to obtain a filtrate that was as concentrated in nitrogen as possible, and from which the casein had been quantitatively separated:

Transfer 200 cubic centimeters of buttermilk to a 500 cubic centimeter volumetric flask. Add distilled water to about 450 cubic centimeters. Add one-fifth normal acetic acid (1.2 per cent) slowly until the casein separates completely in large flocculi leaving the supernatant liquid practically water-clear. In practically every case 44 cubic centimeters of N/5 acetic acid was used and found sufficient for the purpose. After diluting to the mark and filtering, nitrogen determinations were made on the filtrate.

The questions to be studied now were: Would the presence of salt in milk prevent the flocculation of the casein and the subsequent attempt at filtration?

In a sample of skim milk from which the casein can be easily flocculated and filtered would the presence of added fat interfere with filtration?

Results were almost immediately obtained which threw a great deal of light on the difficulty. The following experiment is typical:

Two hundred cubic centimeters of buttermilk obtained from a churning of pasteurized cream were transferred to a 500 cubic centimeter volumetric flask. Water was added to about 400 cubic centimeters. On slowly adding 85 cubic centimeters N/10 acetic acid (0.6 per cent) the casein was completely flocculated. To a second 500 cubic centimeter volumetric flask 200 cubic centimeters of the same sample of buttermilk was transferred. Thirty-six grams of sodium chlorid were added. This concentration of approximately 18 per cent is the concentration of salt in butter curd solution when the butter contains 16 per cent moisture and 3 per cent salt. Water was added, as before, to about 400 cubic centimeters. The addition of 85 cubic centimeters N/10 acetic acid as before did not flocculate the casein.

This experiment showed that the presence of salt very materially affected the precipitability of the casein. This was clearly a case where the physical condition of a colloid was so altered by the presence of large amounts of electrolytes that it did not react toward a precipitant as it usually does. The acetic acid added certainly was not in excess. The slow addition of the acid without at any time resulting in even a partial precipitation of casein indicated that more acid was required. After adding 40 cubic centimeters more of N/10 acetic acid, without any signs of flocculation, 10 per cent acetic acid (5/3 normal) was carefully added instead of the weaker N/10 acid. After adding 11 cubic centimeters of 10 per cent acetic acid, the casein was completely flocculated, and the mixture could be filtered rapidly, yielding a perfectly clear filtrate.

In the presence of salt 300 cubic centimeters N/10 acetic acid were required for the precipitation of casein, which in the absence of salt would have been precipitated by 85 cubic centimeters of N/10 acetic acid. It is probable that observations of a somewhat similar nature were simultaneously made by other investigators, as is evident from

the following quotation from a paper by Schryver [1] which appeared a few months after the above-described experiment was made:

> During the course of some investigations on the action of formaldehyde on the proteins, the observation was made that this aldehyde, when added to an aqueous solution of Witte's peptone, produces a precipitate, and that the reaction could be either partially or completely inhibited by the presence of neutral salts. This phenomenon was also noticed some years ago by T. Sollman (American Journal of Physiology, 1902, vol. 7, p. 220). * * *

Besides being extremely interesting theoretically, the observation just made on the effect of salt on the precipitation of casein was of interest practically because of its possible application to the separation of casein from butter curd solution. The theoretical side of the phenomenon is discussed by Schryver in the above-mentioned paper.

Method for the estimation of water-soluble nitrogen in butter.—After numerous experiments the following procedure was adopted for the separation of the fat from the butter curd solution to be used for nitrogen determinations. As the method was new, many precautions were taken, some of which were later found to be unnecessary. This method of separating fat and casein from butter yields a filtrate that is well adapted, not alone to a study of the nitrogenous constituents of the filtrate, but to many other purposes as well. The filtrate, for example, contains the peroxidase which is practically always present in butter.

To two or three large beakers, capacity 2 to 2½ liters, transfer 5 to 6 kilograms of the sample of butter to be studied. Two samples (one from raw, one from pasteurized cream, for example) may be worked with at one time. The larger the amount of butter taken the better. Place the beakers, properly marked, in a hot-air bath maintained at about 45° C. If the butter to be stored is packed in cans, five or six 2-pound cans are placed in the hot-air bath to be melted. In the work here described the cans were taken out of the ice box late in the afternoon, allowed to warm up during the night at room temperature, and placed the next morning in the air bath, the temperature of which varied usually 2° above and below 45° C. The temperature should not be permitted to rise much beyond 45° C., because of the danger of coagulating some of the protein present. The high temperature at which Gray and Rahn, Brown and Smith (see pp. 11, 16) melted their samples of butter undoubtedly removed, by coagulation, comparatively large amounts of nitrogen from their filtrates.

At this temperature from six to eight hours will be required for the complete melting of the butter and the settling of the curd solution. Stirring does little good. As fast as the butter fat forms a clear layer on top of the butter it may be poured off, care being taken that none of the curd solution is lost at any time. The loss of a few small particles of solid protein is not material. This long heating at a tem-

[1] Schryver, S. B. Some investigations dealing with the state of aggregation of matter. Proceedings of the Royal Society, London, series B, vol. 83, no. B562, pp. 96-123. London, Dec. 19, 1910. See p. 96.

perature that is perhaps best for proteolytic action is an objection to the method. It will be apparent, however, that the error introduced in this way is inappreciable. (See p. 20.)

The melted butter fat is decanted until no more can be so removed without danger of losing some of the curd solution. With the aid of a 100 cubic centimeter pipette having its lower tip cut off to permit more rapid flow of viscous materials, remove the curd solution from the bottom of the beaker or can and transfer it to a dry, clean, 500 cubic centimeter volumetric flask. Considerable fat will, of course be mixed with the curd solution, but by taking only part from the bottom of each vessel sufficient curd solution will be obtained from 5 kilograms of butter to fill two 500 cubic centimeter flasks. Let stand till the next morning at room temperature. With the aid of a rapid-flow 100 cubic centimeter pipette, remove from the bottom of each of these flasks between 200 and 250 cubic centimeters of curd solution and transfer this to a clean, dry, 500 cubic centimeter volumetric flask. From the original 5 kilograms of butter there have now been separated not quite 500 cubic centimeters of curd solution containing approximately 10 per cent of fat. The rest of the curd solution containing much larger proportions of fat may be rejected.

In a separate portion of the original sample of butter, determine moisture, curd, and salt. For this purpose the methods described in Bulletin 107 (revised edition), Bureau of Chemistry, page 123, were used. From these figures the weight of butter corresponding to a given volume of curd solution may be calculated if desired, but for the present work such calculation was not necessary.

The curd solution should be well mixed, and then with the aid of a rapid-flow pipette a portion is transferred to a pycnometer (50 cubic centimeters capacity) and the weight ascertained. The object of this determination is to make certain that the curd solution used for analysis before and after storage of butter is practically the same so far as the density of the material is concerned. It is obvious that if the proportion of fat and salt solution differs very much in two samples of curd solution obtained from the same lot of butter before and after storage the specific gravity will be different. This determination, when repeated on the same portion of curd solution, will show that it is possible to withdraw samples of uniform composition from the flask if care be taken to mix the contents well and to withdraw the sample rapidly before the fat rises to any appreciable extent.

Portions of this curd solution may now be withdrawn for nitrogen determinations. To several clean, dry, 500 cubic centimeter volumetric flasks transfer 100 cubic centimeter portions of curd solution, using a rapid-flow pipette and sampling quickly. This is the amount found most convenient when acetic acid is to be used as a precipitant. When other precipitants, such as ferric chlorid, are to be used, a larger volume may be taken.

In our work we generally made three and sometimes four determinations on a single sample of butter, although two are perhaps sufficient. Each 100 cubic centimeter portion was used for one determination, so that as many such portions are to be pipetted into 500 cubic centimeter flasks as determinations are wanted. (See Table 2.) In the remainder of the curd solution we determined fat by removing 10 cubic centimeter portions, transferring to absorbent paper, drying at the temperature of boiling water, and extracting the fat in a Soxhlet apparatus, as usual. These figures for fat are for the purpose of control only, and indicate how much of the volume of the portion of curd solution is taken up by fat.

The undiluted curd solution may be allowed to remain in the stoppered flasks for 24 or 48 hours at room temperature without any apparent change in the results. In several cases even a week's standing was without effect. The concentration of salt is usually high enough to make the addition of other preservatives unnecessary. Under these conditions the proteolytic enzym present, galactase, is not very active; at least, on standing several days practically the same results are obtained as on freshly prepared curd solution.

The curd solution is diluted to about 450 cubic centimeters with distilled water, mixed well and then 10 per cent acetic acid is added slowly, with constant mixing of the contents of the flask. It is obvious that sufficient acetic acid must be added to completely precipitate the casein in the flocculent condition. This will occur generally when 18 to 22 cubic centimeters have been added. The curd solutions from pasteurized cream butter generally require the larger amount for flocculation. In Table 2 the amounts of acetic acid used are indicated. It seems that the more fat present the more slowly does the casein flocculate. On standing about 15 minutes the casein will be seen to have flocculated. The amount of acetic acid added should be recorded so that that same amount can be used after storage. In fact, all data should be recorded that might be necessary for the purpose of duplicating the determination exactly after storage. Dilute with distilled water to the mark and filter on a 32 centimeter folded filter (S. & S. No. 588 or 595) into a clean, dry, 500 cubic centimeter volumetric flask. The curd solutions should not be allowed to stand long after the addition of water. The filter stands, clean funnels, and flasks were in readiness before the addition of water and acid to the curd solutions, so that filtration was begun within a very few minutes. The entire contents of the flask were transferred to the filter paper. If the filtrate at first was cloudy, it was returned to the filter as often as necessary. Usually the first portions of filtrate, about 50 cubic centimeters each, were returned to the filter two or three times. In no case was a filtrate used for nitrogen determinations that was so cloudy as to indicate the presence of unprecipitated casein. Usually the filtration was begun in the afternoon

and allowed to go on till the next morning. The funnel was covered with a well-fitting watch glass to minimize evaporation.

On several occasions the precipitate on the filter paper was examined at the end of the filtration for peptonizing bacteria. Such small numbers were found that their effect was inappreciable. It is obvious that the digestion of protein on the filter paper by bacteria or their enzymes might vitiate the results. We have no reason to believe that in any of the results appreciable errors were introduced in this way.

The amount of acetic acid was varied a little when precipitating the casein from different portions of the same curd solution in order to find out whether, under the conditions of the work, small variations in the amount of acetic acid would give rise to undesirably large variations in the results. Of course, if insufficient acetic acid is added all of the casein will not be precipitated and the mixture will filter so very slowly that that alone will indicate incomplete precipitation. The more completely the casein is precipitated the more rapid is the filtration. Slight excesses of acetic acid apparently have an inappreciably small solvent action on the precipitate. It is true that in precipitating casein from diluted milk an excess of even very dilute acetic acid is undesirable. In the presence of the sodium chlorid, however, conditions are so altered that the solvent action of the acetic acid is apparently very much diminished. In case of doubt, more rather than less acetic acid was used.

The clear, slightly opalescent filtrate may be tested for completeness of precipitation by the addition of more acid or of alkali. In no case did the addition of small amounts of acid or of alkali (N/10) to the filtrate result in the precipitation of protein. If sufficient alkali was added to make the filtrate alkaline to phenolphthalein, a precipitate was obtained which was probably calcium phosphate containing adsorbed protein. In appearance it resembled some protein precipitates. The appearance of such a precipitate in an alkaline filtrate may, of course, be disregarded. Another way to test for completeness of precipitation is to use slightly different amounts of the precipitant. The results tabulated in Table 2 show that the amounts of acetic acid used were sufficient and that slight variations in the strength of the acid made no difference in the results. For the sake of certainty, the 10 per cent acetic acid solutions were titrated against standard alkali before being used as precipitants. This is especially desirable where the first determination is made in one laboratory and the second after cold storage in another.

In a few instances ferric chlorid was used as a precipitant for the purpose of comparing the results with those obtained with acetic acid.

Since evaporation can not be altogether prevented during the long filtration, it is necessary to be certain that equal volumes of filtrates obtained from the same lot of butter before and after storage corre-

spond to exactly equal weights of butter, or, if through any considerable difference in evaporation the two filtrates are unequally concentrated, the difference in concentration must be ascertainable.

After filtration is nearly complete—that is, after obtaining a little over 400 cubic centimeters of filtrate—its specific gravity is determined. A 50 cubic centimeter pycnometer was used. The same pycnometer filled a second time with some of the same filtrate will differ from its first weight by only 1 milligram. A little calculation will show that before the nitrogen content of the filtrate can be appreciably varied through evaporation, the specific gravity will be varied so much more that its detection will be easy and require no fine weighings of the pycnometer. The pycnometer full of filtrate was always quickly dried and weighed, and the weight recorded. The weight of a known volume of the clear filtrate is the best of the control figures, and together with the others should in every case show whether or not two filtrates of supposedly equal concentration really were so. The container in which the butter is stored might leak. There would result, not alone a loss in moisture, but in salt and nitrogen as well. Or if the container did not altogether prevent evaporation of water and a subsequent concentration of salt and nitrogen resulted, the pycnometer weighings will probably indicate the source of variation. When 100 cubic centimeters of curd solution were used, the filtrate contains so much sodium chlorid that considerable variation in specific gravity is possible. The amount of curd solution used corresponded to nearly 700 grams of butter.

After weighing the pycnometer full of the clear filtrate, 400 cubic centimeters of it were transferred to a Kjeldahl flask and total nitrogen was determined in the usual way. The remainder of the filtrate was measured and the total volume of filtrate obtained was recorded. If filtration was very slow, sometimes less than 400 cubic centimeters were used. The results were calculated to 400 cubic centimeters. The weight of the precipitate and inclosed filtrate was ascertained to the nearest gram on a torsion balance, and recorded. The 400 cubic centimeters of filtrate actually kjeldahled and titrated corresponded to 560 grams of butter. Rahn, Brown, and Smith [1] determined nitrogen in butter not precipitated by acetic acid. They washed butter with water in the proportion of 1 gram of butter to 2 cubic centimeters wash water, transferred 100 cubic centimeters of these washings to a flask, added acetic acid, filtered off the precipitated protein, and determined total nitrogen in 25 cubic centimeters of filtrate, which corresponded to not quite 12½ grams of butter. Apparently they obtained very few results with acetic acid and did not use them in drawing their conclusions. It was pointed out before (see p. 10) that when butter curd solution is diluted with sufficient water it may be treated with acetic acid as if it were so

[1] Loc. cit., pp. 14–15.

much diluted milk and the casein will be flocculated, permitting very satisfactory filtration. The filtrate, however, contains an undesirably small amount of nitrogen.

The figure for total nitrogen in the filtrate taken for analysis was multiplied by 5/4, and the result recorded as the number of cubic centimeters of N/5 nitrogen in 100 cubic centimeters butter curd solution. (See Table 2.) From the data it is obvious that the unavoidable errors of ordinary nitrogen determinations are very small when compared with the amount of nitrogen determined.

A separation of the various forms of nitrogen in the filtrate from the casein precipitation could easily have been made. But it was considered desirable first to find out whether this filtrate contained any more nitrogen after storage than before. If it did, indicating that proteolysis was taking place, then a more detailed examination of the filtrate would have been made. But the filtrates differed very little in their total nitrogen content before and after storage, indicating that proteolytic changes were not taking place to any great extent, and other lines of work were begun.

The above-described method for the estimation of water-soluble nitrogen in butter was used in the summer of 1910 and in the spring of 1911. (See Table 2.)

Description of samples.—Samples Nos. 42 and 40 were churned from the same lot of sweet cream, half of which was churned unpasteurized (butter No. 42) and half of which was churned after pasteurization (butter No. 40). Samples 52 and 50, and samples 65 and 62 were likewise obtained from churnings of two lots of sweet cream, part of which was pasteurized, part of which was not, before churning. (See Table 2.) The expectation was that if galactase is active in butter during cold storage, the figures for water-soluble nitrogen in samples 42, 52, and 65 would become larger, for in these samples of butter from unpasteurized cream the conditions for proteolysis were as favorable as they ordinarily can be. No great changes were expected in the water-soluble nitrogen in the controls Nos. 40, 50, and 62 because at the temperature of pasteurization used, 75° C. (167° F.) in a "flash" pasteurizer, the galactase ordinarily present in butter is strongly inactivated or partly destroyed.

Samples 511 and 523 were churned from the same lot of pasteurized cream. Sample No. 511 contained a proteolytic enzym preparation obtained from cultures of acid-forming bacteria which also liquefied gelatin. Twelve grams of dry enzym powder were worked into about 30 pounds of butter with the salt. The control lot of butter No. 523 was made in the same way, except that a similar amount of enzym preparation was added after it was first boiled in water. Similarly, sample No. 466 was churned from pasteurized cream and contained an added proteolytic enzym preparation, while its control, No. 478, contained an equal amount of the enzym that had

been boiled before being worked into the butter. The object of studying these samples was to ascertain whether the proteolytic enzym secreted by bacteria often present in cream can digest any of the butter proteins, under storage conditions.

All of the samples in Table 2 were churned in the experimental creamery in Albert Lea, Minn., in the summer of 1910. They were stored a short time in the creamery cooler and then shipped to cold storage at 10° F. (minus 12° C.) in Chicago. Naturally, care was taken to move the material into cold storage as soon after churning as possible. When samples were shipped from Chicago to Washington for analysis, they were removed from the railroad station as soon after their arrival as possible and placed in a refrigerator in the laboratory maintained at a few degrees below 0° C.

At appropriate times samples of the butter were sent to competent judges for scoring. The scores are indicated in their appropriate places in Table 2.

TABLE 2.—*Analytic data and scores—Water-soluble nitrogen* [1] *in sweet-cream butter before and after storage. 10° F. (−12° C.).*

Treatment of cream.	Butter sample No.	N/5 water-soluble nitrogen in 100 c. c. butter curd solution.			Age of sample.	Butter scores.		Volume of butter curd solution used for analysis.	Precipitant, 10 per cent acetic acid.
		Before storage.	After storage.	Difference.		Before storage.	After storage.		
		C. c.	*C. c.*	*C. c.*	*Days.*			*C. c.*	*C. c.*
Unpasteurized	42	32.8	28.9	− 3.9	265	85	85	50	7
		34.0	28.7	− 5.3				50	7
		19.9	14.0	− 5.9				100	[2]
	52	30.3	31.9	1.6	251	87	85	100	10
		28.9	30.3	1.4				100	14
		28.7	29.0	0.3				100	16
	65	26.1	21.2	− 4.9	250	90	.....	100	16
		25.1	21.2	− 3.9				100	20
		26.0	21.2	− 4.8				100	22
Pasteurized	40	23.4	24.6	1.2	265	91	90	50	7
		23.4	23.6	0.2				50	7
		13.4	16.0	2.6				100	[2]
	50	19.4	20.7	1.3	251	93	91	100	16
		19.0	19.0	0.0				100	18
		18.7	19.4	0.7				100	20
	62	22.8	17.6	− 5.2	250	92	.....	100	16
		22.3	17.6	− 4.7				100	20
		22.7	16.8	− 5.9				100	22
Pasteurized. Dry proteolytic enzym added [3]	511	31.8	26.6	− 5.2	250	92	92	100	18
		32.6	26.5	− 6.1				100	20
		35.1	27.1	− 8.0				100	12
	466	[4] 32.7	72.9	40.2	296	92½	93	[5]	[6]
		[4] 86.1	86.6	0.5				[7]	9
		[4] 117.9	84.1	−33.8				[7]	5
Pateurized. Heated bacterial proteolytic enzym added	523	22.4	23.5	1.1	250	93	93	100	18
		22.8	23.5	0.7				100	20
		23.6	24.4	0.8				100	12
	478	[4] 22.0	.....	.....	296	93½	93½	[5]	[2]
		[4] 23.9	55.0	31.1				[5]	[2]
		[4] 75.0	68.7	− 6.3				[7]	9
		[4] 112.8	72.3	−40.5				[7]	5

[1] Calculated total nitrogen in 100 cc butter curd solution equivalent to nearly 700 grams butter=350 cc N/5 nitrogen.
[2] Eight c. c. 10 per cent ferric chlorid solution.
[3] Analytical work, June, 1911, on butter, buttermilk, etc., by R. P. Norton.
[4] N/5 water-soluble nitrogen in 1,000 grams butter.
[5] Equivalent of 800 grams of butter.
[6] Ten c. c. 10 per cent ferric chlorid solution.
[7] Equivalent of 400 grams of butter.

Discussion of results, Table 2.—In general, fresh butter made from unpasteurized cream (No. 42, for example) has a little more water soluble nitrogen than the corresponding sample (No. 40) churned from some of the same lot of cream after pasteurization. Buttermilk from raw-cream butter contains more water-soluble nitrogen than the corresponding sample of buttermilk from pasteurized cream. (Compare samples 13 and 14, Table 3.) In sterilized skim milk the soluble nitrogen content is still lower. (Compare samples 20, 22, and 24 with 14, 16, and 18, Tables 3 and 4.) These differences between pasteurized and unpasteurized samples are very likely due to the partial or entire coagulation of the milk albumin, and its removal from the water-soluble condition.

The coagulation of water-soluble nitrogenous substances in buttermilk due to high pasteurizing temperatures was shown in a previous publication from the Dairy Division laboratories.[1]

It is believed that samples 42, 52, and 65 contained more water-soluble nitrogen than their controls Nos. 40, 50, and 62, because there was a partial precipitation of protein during the pasteurization of the cream from which the latter were made and not because the galactase undoubtedly present in Nos. 42, 52, and 65 was active. If it were, there should have been an increase in the amount of water-soluble nitrogen after storage. In so far as there was none, it is inferred that the activity of the galactase was inhibited by the combined effect of the salt and cold storage.

The differences between the amounts of water-soluble nitrogen in the different samples of butter before and after storage are not very large, except in samples 466 and 478, and they represent in all probability the unavoidable error in such work. It is to be borne in mind that the first analysis was made in Albert Lea, Minn., and the second on a different lot of cans in Washington, D. C. Under such circumstances the differences are not considered large. In samples 466 and 478 it is probable that the figures obtained for nitrogen are erroneous.

CONCLUSIONS.

From the data obtained it is evident that proteolysis did not take place to any appreciable extent in the samples studied. Nor was there any simple or obvious relation between the figures for nitrogen and the butter scores.

As the following calculations show, the method for detecting proteolytic action in butter is quite delicate and should lead to the detection of proteolysis were it appreciable. In a determination of water-soluble nitrogen in butter 100 cubic centimeters of curd solution were used. This is equivalent to a little over 700 grams of butter

[1] Rogers, L. A., Berg, W. N., and Davis, Brooke J. Loc. cit., p. 317.

containing 13 per cent of moisture. A nitrogen determination was made in 400 cubic centimeters of the filtrate from the acetic acid precipitation, equivalent to 560 grams of butter. The butter used contained an average of 0.9 per cent of curd. The 700 grams of butter contained, therefore, $700 \times 0.009 \times 0.1567 = 0.9872$ grams of nitrogen, equivalent to 352 cubic centimeters N/5 nitrogen.

Suppose that during the cold-storage period 5 per cent of the casein was slightly proteolyzed and became water soluble. It should be borne in mind that the method of detecting proteolytic action here described will detect it in its first stages. In this respect the method possesses undoubted advantages over others in which results are obtained for variations in amounts of proteoses, amino acids or ammonia, which correspond to later and later stages in the digestive process. It is here supposed that the first step in the digestion of 5 per cent of the casein has taken place, the rest of the protein remaining unchanged. Allowing for 30 cubic centimeters N/5 nitrogen already present in the 100 cubic centimeters of curd solution as water-soluble nitrogen, there would be formed by the proteolysis 0.05×320 cc $= 16$ cubic centimeters N/5 nitrogen in the water soluble form in addition to the 30 cubic centimeters originally present. The titrations made after storage would then show $16 \times 4/5$ or 12 cubic centimeters more of N/5 nitrogen than before storage. From the results obtained it is probable that this increase, had it taken place, would have been detected.

A protein, such as casein, can of course undergo more than one kind of chemical change. These changes may be hydrolytic, oxidative, or putrefactive. It is obvious that the methods used in this work would detect the hydrolytic change only. Some work on the possibility of oxidative changes in the protein in butter is described on page 64 et seq.

PROTEOLYSIS IN MILK.

POSSIBLE OBJECTION TO THE NEW METHOD FOR DETECTING PROTEOLYSIS IN BUTTER.

An apparent objection to the method of studying possible proteolytic changes in butter just described lies in the fact that a long time (five days) may elapse between the beginning and end of the determinations of nitrogen, during which time the galactase or bacterial proteolytic enzyms present in butter may be active. The results would not represent proteolysis during cold storage but proteolysis during the determinations of nitrogen. For this reason the following experiments were made.

The objects of these were two-fold: First, to ascertain by a method that was free from the objections to the method for butter whether

galactase could digest protein in an 18 per cent sodium chlorid solution. Second, whether the sodium chlorid usually present in butter curd solutions can inhibit the proteolytic action, not alone of the small quantity of galactase ordinarily present in butter, but also the action of larger amounts of proteolytic enzyms that might find their way into butter in any one of several ways, as, for instance, the proteolytic bacteria that may grow in the milk and cream.

THE INHIBITING EFFECT OF SODIUM CHLORID AND COLD STORAGE UPON THE ACTIVITY OF GALACTASE IN BUTTERMILK.

Six lots of buttermilk of about eight liters each were obtained from three churnings of unpasteurized sweet cream and from three churnings of pasteurized sweet cream. In Table 3, page 29, is indicated the number of the lot of butter corresponding to each lot of buttermilk. Buttermilk samples 13 and 14 were obtained from the churning of one lot of sweet cream, half of which was churned unpasteurized (butter No. 42, buttermilk No. 13) and half of which was pasteurized before churning (butter No. 40, buttermilk No. 14).

To each liter of buttermilk 5 cubic centimeters of chloroform and 180 grams of sodium chlorid were added. These samples were intended to represent approximately butter minus butterfat. In such material a study of possible proteolytic action could be made in a comparatively short time, since no time is necessary for the melting of the butter or the separation of the fat.

The buttermilk was then sealed in cans, each containing 600 cubic centimeters of the sample. These cans, which were also used for butter, were of heavy tin, thoroughly lacquered. The smaller part of these samples remained in the creamery cooler; the rest were shipped to cold storage in Chicago. The samples were not removed from the cooler for analysis until it was certain that sufficient time had passed to permit the transportation, by refrigerator freight, of the samples from Albert Lea, Minn., to Chicago, and the placing of the samples in the cold-storage rooms. It was intended that the first analysis of these samples should show, as closely as possible, the amount of water-soluble nitrogen present in the material as it went into cold storage, and not as it left the churn. It is highly probable that galactase, even in the presence of 18 per cent sodium chlorid, can slowly digest protein material, if the temperature is above that of cold storage as it is ordinarily practiced. It seems reasonable to suppose that the proteolysis in butter observed by Rahn, Brown, and Smith [1] was due, in part at least, to the comparatively high temperatures at which their butter was stored. But in so far as this investigation is concerned with possible chemical

[1] Loc. cit.

changes that may take place in butter while in cold storage and not at higher temperatures, the times at which analyses were made were always chosen so as to give results as nearly representative of the condition of the material immediately before and after cold storage as possible.

Method of measuring the activity of galactase in buttermilk.—When it was reasonably certain that the other samples of buttermilk had reached the cold storage, samples were removed from the creamery cooler and water-soluble nitrogen was determined in them by practically the same method as was used for butter. The buttermilk was treated as if it were so much butter curd solution entirely freed from fat. All the precautions that were taken in the work on butter were taken here also. The method is described on page 18.

The first analyses were made in September and October, 1910. For the analyses made in December, 1910, and in June, 1911, samples were shipped from cold storage in Chicago by express to Washington. Upon their arrival the material was at once transferred to the refrigerator in the Dairy Division laboratories, where it remained till used for analysis. The total time during which the samples were out of cold storage was as short as possible.

Results.—From the results obtained on samples 13, 15, and 17, summarized in Table 3, it is evident that in buttermilk obtained from raw-cream butter the activity of galactase is practically entirely inhibited by the presence of 18 per cent of sodium chlorid and by the low temperature, 0° F. (−18° C.) of the cold storage. When some of this same material is allowed to remain for a long time at room temperature, the galactase apparently becomes much more active, because the casein is seen to clot and the mixture assumes the appearance of one in which digestion is going on.

Samples 14, 16, and 18 were to serve as controls on those of the other three. Although galactase in cream is not entirely destroyed by ordinary pasteurization, it is partly inactivated.[1] It was expected that the figures for water-soluble nitrogen in samples 14, 16, and 18 would change little during storage, thereby affording a check on the correctness of the work. The figures for water-soluble nitrogen in samples 13, 15, and 17, had they increased during storage, could then have been considered as obtained by a method that showed no change where none is to be expected.

[1] Rogers, L. A., Berg, W. N., and Davis, Brooke J. Loc. cit., p. 318.

TABLE 3.—*Effect of sodium chlorid and cold storage (0° F., −18° C.) upon the activity of galactase in buttermilk.*

Buttermilk obtained from churnings of unpasteurized sweet-cream butter.					Buttermilk obtained from churnings of pasteurized sweet-cream butter.				
Buttermilk, lot No. 16.612, sample No.	Butter, lot No. 10.311, sample No.	N/5 water-soluble nitrogen in 100 c. c. buttermilk.		10 per cent acetic acid used as precipitant for 200 c. c. buttermilk.	Buttermilk, lot No. 10.622, sample No.	Butter, lot No. 10.321, sample No.	N/5 water-soluble nitrogen in 100 c. c. buttermilk.		10 per cent acetic acid used as precipitant for 200 c. c. buttermilk.
		Age.	N/5 N.				Age.	N/5 N.	
		Days.	*C. c.*	*C. c.*			*Days.*	*C. c.*	*C. c.*
13	42	25	39.2	20	14	40	25	36.1	18
			36.8	18				38.2	12
		116	40.7	20			116	39.1	12
			41.0	18			301	36.3	18
		301	37.2	20				38.3	12
			37.4	18	16	50	10	31.1	20
15	52	10	42.1	16				30.3	24
			40.9	20			124	31.9	20
		124	43.4	16				30.3	24
			42.6	20			297	30.1	20
		297	39.5	16				28.6	24
			40.0	20	18	62	10	35.0	22
17	65	10	42.6	14				36.3	18
			45.3	12			101	35.2	22
		101	45.1	14				36.3	18
			46.3	12			286	33.6	22
		286	41.1	14				34.8	18
			38.7	12					

179 cubic centimeters N/5 nitrogen=average total nitrogen in 100 cubic centimeters buttermilk.

The results on these samples of buttermilk confirm the results obtained on the corresponding samples of butter. It is practically certain, for example, that butter sample No. 42 and the buttermilk obtained from it both contained galactase, though in different amounts. Under the conditions of the experiments proteolytic action was uniformly inhibited, both in the butter and in the buttermilk.

As the following calculations show, the method used for the detection of proteolytic action in buttermilk (skim milk) is quite delicate and should lead to the detection of proteolytic action were it appreciable. In the determination of water-soluble nitrogen in buttermilk 200 cubic centimeters were taken, which contained very close to 360 cubic centimeters N/5 total nitrogen. Let it be assumed that during the cold storage period 5 per cent of the casein became water soluble. Allowing an average of 80 cubic centimeters N/5 nitrogen in water-soluble form originally present in the 200 cubic centimeters of buttermilk, there would be formed by the proteolysis $0.05 \times 280 = 14$ cubic centimeters N/5 water-soluble nitrogen. In the actual determination 200 cubic centimeters of buttermilk were diluted to 500 cubic centi meters precipitated with acetic acid, and two 200 cubic centimeter portions of the filtrate were used for nitrogen determinations. Therefore if 5 per cent of the casein had become hydrolized the titrations after cold storage would have been 5.6 cubic centimeters higher than the corresponding titrations before storage. It is probable that this increase would have been detected.

THE INHIBITING EFFECT OF SODIUM CHLORID AND COLD STORAGE UPON THE ACTIVITIES OF PROTEOLYTIC ENZYMS IN STERILIZED SKIM MILK.

Description of samples.—Several 5-liter flasks full of skim milk were kept in a steam sterilizer for about two hours at a temperature varying between 94° and 99° C.

Three 3-liter portions were measured out roughly and rapidly while the skim milk was hot, into bottles, samples No. 20, 22, and 24. A weighed quantity of the enzym preparation was then added. The amounts are given in Table 4, page 30.

After cooling, 540 grams of sodium chlorid was added to each sample. The bacterial enzym was prepared from cultures of an acid-forming bacterium that secreted a proteolytic enzym. The usual method of precipitating with alcohol, etc., was used. The dry enzym preparation was tested before it was used in the experiments and found to be proteolytically active. The other enzym preparations were the ordinary commercial ones.

Three-liter portions of the skim milk were quickly cooled to 35° C. To each was added 540 grams of sodium chlorid, to which there had been previously added the amount of enzym indicated in the table. Obviously, samples 20, 22, and 24 were controls on Nos. 19, 21, and 23.

These samples were canned as before. Most of these were shipped to cold storage in Chicago, where they were maintained at a temperature of 20° F. (− 7° C.). Determinations of water-soluble nitrogen were made by the method already described in a previous publication [1] and in this paper page 18.

TABLE 4.—*Effect of sodium chlorid and cold storage upon the activities of proteolytic enzymes in sterilized skim milk stored at 20° F. (−7° C.).*

Skim milk, lot No. 10.612, sample No.	Composition of mixtures.	N/5 water-soluble nitrogen [2] in 100 c.c. skim milk after 75 days' storage.	10 per cent acetic acid used as precipitant for 200 c.c. skim milk.	Skim milk, lot No. 10.622, sample No.	Composition of control mixtures.	N/5 water-soluble nitrogen [2] in 100 c.c. skim milk after 77 days' storage.	10 per cent acetic acid used as precipitant for 200 c.c. skim milk.
		C. c.	*C. c.*			*C. c.*	*C. c.*
19	Skim milk, 3 liters	44.9	20	20	Skim milk, 3 liters	28.1	20
	Dairy salt, 540 grams.	48.1	10		Dairy salt, 540 grams.	33.1	10
	Bacterial				Bacterial		
	Enzym (dry), 15 grams.				Enzym (boiled), 15 grams.		
21	Skim milk, 3 liters	118.2	20	22	Skim milk, 3 liters	22.8	20
	Dairy salt, 540 grams.	121.0	10		Dairy salt, 540 grams.	28.5	10
	Pancreatin, 3 grams, dry (U. S. P.).				Pancreatin (U. S. P.), boiled, 3 grams.		
23	Skim milk, 3 liters	54.1	20	24	Skim milk, 3 liters	19.8	20
	Dairy salt, 540 grams.	65.5	10		Dairy salt, 540 grams.	24.2	10
	Pepsin (U. S. P.), dry, 3 grams.				Pepsin (U. S. P.), boiled, 3 grams.		

[1] Rogers, L. A., Berg, W. N., and Davis, Brooke J. Loc. cit., p. 315.
[2] 198 c. c. N/5 nitrogen=average total nitrogen in 100 c. c. skim milk.

Results.—In sample No. 19 there was present a large quantity of a proteolytic enzym of bacterial origin that was known to be active on gelatin. It is highly probable that much more enzym was present here than there is ever present in butter, and the figures indicate plainly that the salt strongly inhibited its action during the period under observation. It is, of course, possible that, given a period of time greatly exceeding ordinary storage periods, further proteolysis in this sample might have been observed.

In samples 21 and 23 digestion took place to a large extent. In No. 21 approximately two-thirds of the total protein had become water soluble.

On samples 20, 22, and 24 practically identical results were obtained before and after storage, as would be expected. This indicated that in the controls no proteolytic changes were detected.

CONCLUSIONS.

It is evident from these results that in the presence of very large amounts of strongly active proteolytic enzyms, proteins will be hydrolized even in cold storage (or in transit) and in strong salt solutions. But there is no reason to suppose that at any time such amounts of enzyms are ever found in butter.

It is very probable that samples 21 and 23 contained several thousand times as much proteolytic enzym as is present in ordinary butter of any kind.

However, it must be borne in mind that the claim is not made that sodium chlorid does not exert an inhibiting influence on proteolytic action. Whether it does or does not depends upon the conditions of the experiment. It is comparatively easy to show that the action of pepsin-acid can be inhibited very strongly by large amounts of sodium chlorid. In the spring of 1909 some experiments were made in which the speed of digestion of casein in several pepsin-acid solutions was compared with that in the same solutions to which 20 grams of sodium chlorid to 100 cubic centimeters of acid solution had been added. The presence of the salt almost completely inhibited the action of the pepsin-acid during the time of the experiment, 40 minutes' digestion, but it is, of course, possible that proteolytic action would have taken place had the digestion period been several months. The method of comparing speeds of digestion was that described by Gies.[1] In general, the statement that sodium chlorid does inhibit proteolysis is true, therefore, at low temperatures, when the amount of enzym is small and the digestion period (storage period) is long or when the amount of enzym is large and the digestion period is short, as in ordinary digestion experiments.

[1] Berg, William N., and Gies, William J. Studies of the effects of ions on catalysis, with particular reference to peptolysis and tryptolysis. Journal of Biological Chemistry, vol. 2, no. 6, pp. 489–546. New York, March, 1907.

The statement that sodium chlorid does not inhibit proteolytic action is true at comparatively high temperatures when the amount of enzym is very large and the digestion period very long. The apparently contradictory statements are the results of testing the action of the sodium chlorid over a wide range of enzym concentration and over widely varying digestion periods.

Several investigators have studied the action of sodium chlorid on the tryptic digestion of casein. Their conflicting statements are, of course, easily accounted for by the fact that the experiments were made under conditions that were not uniform with regard to the concentration of sodium chlorid, the relative proportions of alkali, trypsin, and casein, etc. Their work is summarized by Robertson.[2]

THE INDIRECT ACTION OF BACTERIA.

The improbability that the proteolytic enzyms are responsible for the difference in keeping quality between unpasteurized and pasteurized sweet-cream butter is shown by the work given in detail in the preceding pages. The bacteria are another possible factor removed by pasteurization. While it is certain that they do not grow at the low temperature of commercial storage, it is possible that the presence of a large number of living cells, or various active enzyms which may possibly be liberated by the death of the bacteria, may have an influence on the flavor of the butter. If the bacteria destroyed by the pasteurization could be replaced in the cream their influence on the flavor should be shown in a comparison of the butter made from this reinoculated cream and that made from a part of the same pasteurized cream, but without the addition of bacteria. Before this could be done intelligently it was necessary to obtain a general knowledge of the bacteriological content of the raw cream. The normal bacteria of the cream from one skimming station was determined by sampling every day and plating on lactose agar. After 7 days' incubation at about 30° C. the plates were counted and all of the colonies on a representative plate were transferred to litmus milk tubes. These were incubated and examined after 2, 5, and 14 days. This enabled a separation into high acid forms which curdled the milk in less than 2 days; low acid forms forming acid but curdling the milk slowly or not at all; alkali formers, peptonizers, and those that produce no visible change in the milk. These groups were calculated as percentages of the total bacteria, and the results are given in Table 5.

[2] Robertson, T. Brailsford. On some chemical properties of casein and their possible relation to the chemical behavior of other protein bodies, with especial reference to hydrolysis of casein by trypsin. Journal of Biological Chemistry, vol. 2, no. 4, pp. 317–383. New York, January, 1907. See p. 355.

TABLE 5.—*Numbers of bacteria with distribution in different groups in cream.*

No.	Bacteria per cubic centimeter.	High acid.	Low acid.	Alkali.	Peptonizers.	No change.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	116,000,000	25.8	11.3	1.0	1.0	60.8
2	61,000,000	41.6	16.6	6.7	10.0	25.0
3	23,500,000	21.2	7.7	1.0	7.3	62.1
4	40,000,000	48.8	2.4	.....	12.2	36.6
5	99,000,000	14.1	9.9	1.1	25.3	50.5
6	34,000,000	36.1	8.4	.....	.....	55.6
7	11,800,000	16.3	12.1	1.7	12.9	55.9
8	33,500,000	49.0	23.0	.....	3.0	25.7
9	51,000,000	27.1	14.6	.....	4.2	54.2
10	40,000,000	9.3	2.3	.....	7.2	80.0
11	8,000,000	27.9	8.9	6.3	1.3	55.7
12	21,800,000	20.4	3.3	.....	6.4	70.3
13	37,000,000	27.8	.....	2.8	8.3	61.1
14	101,000,000	68.7	3.6	4.4	4.4	18.7
15	147,000,000	60.2	8.0	5.3	1.8	24.7
16	54,500,000	36.0	6.0	6.0	6.0	46.0
17	69,500,000	29.5	11.5	8.2	3.2	47.5
18	30,000,000	51.7	17.2	3.4	3.4	24.1
19	89,000,000	42.9	7.7	2.2	1.1	46.2
20	98,000,000	15.3	.....	.....	.....	84.7
21	110,000,000	15.0	7.0	.....	.....	78.0
22	45,000,000	12.6	18.9	.....	.....	68.5
23	52,000,000	30.0	12.0	.....	12.0	46.0
24	87,500,000	6.8	5.7	.....	.....	87.5
25	39,500,000	7.5	7.5	.....	5.0	80.0
26	5,700,000	33.3	1.8	.....	.....	64.9

It will be noticed that the number of bacteria in the cream was high and that there was great variation in the proportion of the groups from day to day.

REINOCULATION OF CREAM.

Typical cultures were saved from each group and used to inoculate one-half of a lot of pasteurized cream. Sufficient amounts of milk cultures were added to the pasteurized cream to bring the bacterial count at the end of about 24 hours to numbers and proportions approximately those of the cream before pasteurization. This was of course very difficult to control, but the results given in Table 2 show that this was attained within reasonable limits. Two experiments of this kind were made, each consisting of three lots of butter. One-third of a lot of sweet cream was cooled and churned; two-thirds was pasteurized by holding at 145° F. for 20 minutes; one-half of the pasteurized cream was cooled and churned, and the remaining portion inoculated with the milk cultures and held about 24 hours to allow bacteria to develop. There was no appreciable increase in acidity in this period. The cream was then cooled and churned as before. It is probable that the lipase was destroyed and the galactase weakened. Tests showed that catalase was present in the raw cream, absent in the pasteurized cream, and present again in the inoculated cream, while all three lots gave a test for peroxidase.

The score of the butter given in Table 7 shows that while there was a marked difference in the rate of deterioration in the raw-cream butter and the pasteurized-cream butter, the reinoculation of the cream with bacteria had little or no effect on the keeping quality of the butter. In one case the inoculated-cream butter changed more than the uninoculated, while in the other lot the reverse was true.

TABLE 6.—*Bacteria in cream used for making butter.*

Treatment of cream.	Bacteria per cubic centimeter.	High acid.	Low acid.	Alkali.	Peptonizers.	No change.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
No. 1.						
Raw	48,000,000	13.1	10.9	0.0	17.4	58.6
Pasteurized	53,000					
Pasteurized reinoculated	20,000,000	18.7	6.6	0.0	2.5	72.2
No. 2.						
Raw	47,000,000	46.0	6.3	0.0	6.3	41.4
Pasteurized	23,400					
Pasteurized reinoculated	217,000,000	26.8	.5	1.0	1.0	70.7

TABLE 7.—*Scores of butter—raw, pasteurized, and reinoculated cream.*

Treatment of cream.	First score.			Second score.		
	Age, days.	Total score.	Comments.	Age, days.	Total score.	Comments.
Raw	6	86	Very unclean, somewhat rancid.	38	82	Rank, woody, rancid.
Pasteurized	6	92	Slightly woody	38	87	Very woody.
Pasteurized and inoculated.	6	91	Woody	38	91	Somewhat woody.
Raw	4	86	Unclean, rancid	36	82	Rancid, woody.
Pasteurized	4	92	Slightly woody	36	90	Slightly unclean, woody.
Pasteurized and inoculated.	4	91	Woody	36	89	Woody.

It is perhaps significant that raw, sweet-cream butter always becomes rancid, a flavor usually associated with the liberation of fatty acid. It is not improbable that the lipase of the milk is able to act under these conditions, but that the ripening of raw cream, which usually prevents the development of this flavor, inhibits the action of this enzym.

THE POSSIBLE OXIDATION OF BUTTER BY INCLOSED AIR.

The possible oxidation of butter by finely divided air globules inclosed in it, as previously pointed out, made it desirable to find out how much air was present in butter exclusive of large pockets, and second, whether the air present in butter underwent a change during storage through the transfer of oxygen from the air to some

oxidizable substance in the butter. The work was done in the field laboratory of the Dairy Division, Troy, Pa.

Method of gas analysis.—The following diagram shows the apparatus used for obtaining the gas from a can of butter. The determination of the amount of air (or gas) in a can of butter was made as follows: The can of butter was removed from the refrigerator late in the afternoon and allowed to remain at room temperature over night. The next morning the can was placed in water at 45° C. for about an hour until the contents of the can were melted. The flasks, previously connected, and the Toepler pump,[1] were then evacuated by the Geryk pump through the tube *d*. Tube *f* could be evacuated by opening the stopcock toward the Toepler pump. The Geryk pump was worked until the McLeod gauge on the Toepler pump read about 0.3 millimeter. The flask *i* and tube *j* were then evac-

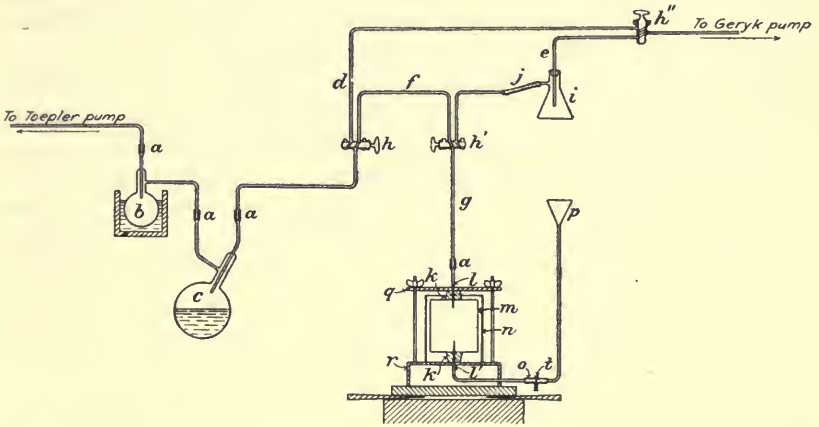

FIG. 1.—Apparatus used for obtaining gas from a can of butter.

uated. The can punch was then brought under tube *g* and the can of butter placed inside of *n*. The brass plate *q*, carrying the upper needle, was placed over the can. The plate is held over the can by two brass guide rods, threaded at their upper ends and screwed into *r*, the brass base. *q* was then screwed down a short distance, so that the upper end of the upper needle *l* could be brought under the lower end of tube *g*. *l* and *g* were connected by the usual rubber tube and rubber nipple mercury seal. Water at 60° C. was poured into the container until the can was completely submerged. The water was then colored by adding a few cubic centimeters of alcoholic solution of methylene blue. Previous to putting the can of butter into *n*, the funnel *p* was filled with a three-fourths saturated sodium chlorid solution at 50° C. and air bubbles removed from *o*, partly by squeezing *o* and forcing the air bubbles up to *p*,

[1] We used an improved form of Toepler pump designed by Dr. Clark, of this laboratory.

and then by opening the screw clamp at *t* and allowing the solution to flow through *l'* into *n*. The two needles were forced into the can by screwing down *q*. In order not to open the mercury seal at the upper end of *l*, the entire punch was raised as fast as *l* was lowered, by means of several thin wooden strips and a wedge on both sides. When it was apparent that the rubber stoppers *k* were pressing very tightly against the can, and very soon the needles would punch the can, *l* and *g* were evacuated by opening the stopcock toward *i*. The entire system was now empty, from *l* to *g*, *f*, *c*, *b*, and the Toepler pump. The Geryk pump was then cut off altogether from the rest of the system. *q* was still further forced down until the lower and then the upper needles pierced the can. When the upper cover was pierced, butter fat would fill tube *g* to stopcock *h'*. This was then opened and butter fat was slowly passed through *f* into *c*, where it remained. The inclosed gases passed on through *b* to the Toepler pump. The screw clamp *t* was removed soon after the butter began to flow out of the can, permitting salt solution to enter the can as fast as butter left it. During the passage of butter fat, and later of butter curd solution, the entire can was covered with water. In only one experiment did any colored solution find its way into the tube *g*. Stopcock *h'* shut off the connection between *g* and *f* when salt solution began to come through *g*. This usually happened when most of the butter fat and part of the curd solution had passed into *c*. Knowing the weight of the can of butter, the empty can, the weight of *c* empty and when filled with butter from the can, the amount of butter passed into *c* could be ascertained approximately. In general between seven and eight tenths of the contents of the can were passed into *c* during the experiment. The gas in the Toepler pump and flasks was then pumped out in the usual way and measured. Part of it, 50 to 60 cubic centimeters, was used for the determination of carbon dioxid and of oxygen. The usual apparatus was used. The determinations were made according to the methods described in Hempel's Methods of Gas Analysis, 1906, pages 149 and 201.

One blank on air was made before the results were obtained, one after, and two between results, so that the reagents were certainly in good condition.

In the third determination on 2A a few drops of colored water were seen to pass up the tube *g*. No more butter (butter fat) was allowed to pass into *c* and the estimation of the quantity and composition of the gas was made as usual. It is probable that the apparently high carbon dioxid was due to greater volatilization of acid-reacting material. In this experiment flask *c* contained 525 grams of butter instead of the usual 700–800 grams.

Results.—The butter used had been churned from pasteurized ripened cream, packed carefully in 2-pound tin cans, and sealed by a

sealing machine. Determinations were made before and after storage. Part of the butter was worked normally in the churn (samples marked "A"), part was overworked (samples marked "B"). Samples 1A and 1B were obtained from the same lot of butter and samples 2A and 2B were from another lot.

TABLE 8.—*Quantity and composition of gas in pasteurized ripened-cream butter.*

BEFORE STORAGE.

Lot No. 19.4.	Volume of gas obtained from 1 can (capacity approximately 1 liter) of butter reduced to 0° C. and 760 mm. of mercury.	Composition of gas.	
		Carbon dioxid.	Oxygen.
Sample No.—	*c.*	*Per cent.*	*Per cent.*
1A (normal)	99.6		
1B (overworked)	101.4		
1A	107.7	31.3	19.1
1B	98.8	29.7	20.3
2A	141.5	36.8	19.1
2B	104.6	37.8	19.5
2B	101.4	37.8	18.6
Blank on air		.8	21.87

AFTER STORAGE.

Sample No.—			
1A	94.1	34.2	13.3
1B	76.9	32.5	11.0
2A	139.0	31.5	13.1
2A	112.0	39.3	12.1
2A	71.0	52.8	10.0
2B	117.5	33.6	13.3
Blanks on air		.38	22.1
		.39	21.5
		.39	20.77
		.66	21.82

The results (see table 8) before storage were obtained in November and December, 1911. All of the results for carbon dioxid were obtained on the same pipetteful of potassium hydroxid solution and all of those for oxygen on the same pipetteful of sodium pyrogallate. The blank was made after all the other results had been obtained. Two separate determinations on two cans of 2B showed that the same result could be obtained on two cans of the same lot of butter.

On the assumption that the gas in butter is a mixture of carbon dioxid and air, the oxygen content found is apparently high.

If the percentage of carbon dioxid be subtracted from 100, leaving, for example, 70 per cent of the gas supposedly air, the percentage of oxygen (20) forms too large a part of the total volume of gas from which carbon dioxid has been removed.

The butter was stored at 0° F. until the following March, 1912, during which month the results after storage were obtained.

The results are difficult to interpret, partly because very few of them have as yet been obtained and partly because certain check

experiments not yet made will be necessary. There seems to be no great variation in carbon dioxid content, but a decided lowering of the oxygen content as if part of this gas had been removed. It is possible that further work will show that the oxygen in these experiments was actually transferred to some oxidizable substance in the butter. It would perhaps be better to defer conclusions until further work shows that the oxygen, if it was indeed removed, went to some butter constituent and was not combined with metal or diffused out through the seals. It seems highly desirable to ascertain definitely whether cans sealed by a machine without the use of solder are gas tight, measuring gas tightness for a period of several months.

The cans used were heavily tinned and well lacquered on the inside. The rims of the covers were provided with a layer of rubber cement, which was forced into the seal by the sealing machine. Cans sealed in this way are air tight when tested under 15 pounds' pressure for a short time.

The figures for the composition of the gases in butter obtained after storage show that the gas is apparently a mixture of carbon dioxid and air. At present any conclusion regarding the nature of the gas is premature. It seems almost certain that the gas in these samples was not a mixture of carbon dioxid and air only, but that some volatile substance was mixed with it. The gas obtained from butter had an intensely "buttery" odor. Perhaps a more detailed analysis of the gas will make the results more intelligible. Under the circumstances it is not safe to assume that the figures for carbon dioxid represent carbon dioxid alone, for any substance having an acid character and volatile under the conditions of the experiment would probably be included with the carbon dioxid in the potassium hydroxid absorption. These results show that butter contains about 10 per cent by volume of gases.

Overworked butter did not contain any more air than that which had been normally worked. For obvious reasons these results are not comparable with those obtained by overworking small amounts of butter with a spatula.[1] It may, however, be significant that the decrease in oxygen as shown in Table 8 was about 50 per cent greater in the overworked sample, 1B, than in any other.

THE EFFECT OF METALS ON BUTTER.

EARLIER INVESTIGATIONS.

The influence of metals on the changes in butter has received some attention, although most of the experiments along this line have not included storage butters. In 1902 Henzold[2] found butter with

[1] Rogers, L. A. Fishy flavor in butter. United States Department of Agriculture, Bureau of Animal Industry, Circular 146. Washington, 1909.

[2] Henzold, Ottomar. Bittere Butter. Milch-Zeitung, vol. 31, no. 52, pp. 822–823, Leipzig, Dec. 27, 1902.

a bitter astringent taste, which he concluded was caused by iron in the salt. He made butter from pasteurized cream, to which salt containing 0.05 to 0.1 per cent iron oxid was added. The butter made, using this salt, had a decidedly bitter taste. By elimination and control of conditions, the iron was found to be the factor influencing this taste.

L. Marcas [1] showed the effect of holding milk and cream in rusted cans for from 2 to 46 hours and making butter from the cream treated in this manner. He determined the amount of iron in the milk, skim milk, cream, butter, and buttermilk, holding part in a clean can and the other in a rusted can for comparison. He found in all cases a bitter, astringent taste and bad odor in butter made from milk held in rusted cans, while the butter made from the milk held in clean cans was of good quality. He concluded that the milk coming in contact with the iron rust forms iron lactate from the iron oxid and that it is the lactate which causes the bitter taste. This solvent action he found to be especially active in cream, owing to its acidity. He found that cream with a normal iron content of 0.005 parts per 1,000 would increase to 0.240 by 22 hours' contact with a rusted can and to 0.270 by 46 hours' contact. Butter made from the cream containing 0.240 parts per 1,000 contained 0.080 parts of iron per 1,000, while butter made from cream containing 0.270 parts per 1,000, contained 0.134 parts of iron per 1,000.

Höft [2] in 1909 added iron salts (ferrous ammonium sulphate and iron lactate) to cream, allowed the cream to stand up to 22 hours, made butter, scored it for physical changes, and made qualitative tests for iron in the butter. He added iron in quantities ranging from 2 parts per 1,000,000 to 33 parts per 1,000,000. He published results of only eight tests, which showed in most cases an oily metallic taste in the butter and in which the presence of iron in the curd solution was determined with potassium sulphocyanid. He, however, cautioned against definite conclusions, as he had not decided that the change was due to iron unconditionally. He found that small amounts of iron acting for a long time caused more effect than large quantities of iron for a short time.

In 1911 the Molkerei-Zeitung [3] published some work on the effect on butter of washing it with water containing 9 to 15 milligrams iron per liter. This caused the butter to have a metallic, oily, tallowy taste. The butter when washed with water from which the iron had been removed by oxidation and filtration did not show the faults

[1] Marcas, L., and Huyge, C. Influence de la rouille sur la qualité du beurre. L'Industrie Laitière, vol. 30, no. 16, pp. 187–188. Paris, Apr. 16, 1905.

[2] Höft, Dr. Kann man aus dem chemischen Nachweis von Eisen in der Butter auf eine Qualitätsverminderung der Butter durch das Eisen schliessen? Milchwirtschaftliches Centralblatt, vol. 5, no. 6, pp 250–252. Leipzig, June, 1909.

[3] Enteisenungsanlage für Molkereiwasser. Molkerei-Zeitung, vol. 25, no. 58, pp. 1095–1096. Hildesheim, July 28, 1911.

mentioned. It was also found that holding cream in rusted vessels caused this oily, metallic taste.

Kooper,[1] in 1911 did some work to show that washing butter with water containing pure metallic iron would not, in quantities up to 36 milligrams per liter, cause any noticeable changes in the quality of the butter, but that the changes that took place were caused by other substances in the water together with the iron. He is of the opinion that water containing a high percentage of iron is very likely to contain H_2S or nitrous acid, which would be more likely to cause the changes and defects in the butter than the iron itself. Kooper used a saccharated iron carbonate in his work. He says, however, that a change of the iron to lactate is caused by contact of high acid cream with iron, and this will produce the oily and metallic flavors. According to Kooper the iron taken up depends on the length of time of contact and the acidity of the cream. By adding rusted nails or pulverized iron rust to the cream before ripening and allowing the cream to ripen in contact with the iron rust he found the cream to be changed to a grayish color and showing defects in odor and taste. He also showed that the washing tended to take some of the iron from the butter.

A summary of the preceding work, then, shows that some investigators think the iron in wash water (9 to 15 milligrams per liter) is responsible for the changes in butter, while others do not think the iron alone (up to 36 milligrams per liter) will cause the changes. All seem to agree that the contact of acid cream will take up iron from rusted containers, change it to a lactate, and produce butter of poor flavor.

In order to see whether butter containing iron would change more in storage than clean butter, it became necessary to make butter which contained no impurity or foreign matter other than iron and also to make a control butter containing no foreign material and free from iron.

Anyone familiar with creamery methods will readily see that to make butter free from contact with iron would require careful supervision of the cream from the time of milking to the making of the butter. In order to do this, the cream was all selected from farmers who were careful in the handling of the cream and whose cans were free from rust, as this is the first opportunity for contact with iron. The cream was, in most cases, pasteurized in a Jensen flash pasteurizer at 75° C. This was carefully cleaned. The cream was cooled, weighed, and then ripened in enamel-lined tanks free from iron. These tanks were made especially for this work to eliminate any chance for contact with iron during ripening, at which time, owing

[1] Kooper, W. D. Ist der Eisengehalt des Wassers von Einfluss auf die Qualität der Butter? Milch-Zeitung, vol. 40, no. 29, pp. 285–287. Leipzig, July 22, 1911.

to the acidity of the cream, the cream would be most likely to attack and dissolve the iron. A pure culture starter was used, the same precautions in regard to metals being taken in the preparation of the starter. After pasteurizing, the cream was divided into two portions, each being put into an enamel-lined tank for ripening. The cream was cooled by running brine through tinned copper coils so suspended in the vats that they could also be used for stirring and mixing. The cream in one vat was held under normal conditions and free from iron. To the cream in the second vat there were added known amounts of iron. Both ferrous sulfate and ferrous lactate were used in this work. This iron was added in amounts varying from 1 to 500 parts of iron per million of cream (or 1 to 500 milligrams of iron in a kilo of cream). The two vats of cream were then ripened under like conditions and churned at the same time in the Disbrow combined churns and workers (B2, of 50 gallons capacity). These churns were shipped to Troy, Pa., from Albert Lea, Minn., in the spring of 1911 and were exposed for some time. Before using at Troy they were taken apart, scraped, sandpapered, and thoroughly cleaned, though in spite of this one iron plate at the side showed rust which was exposed to the cream. The butters were worked the same amount, salted the same, and washed with the same amount of water and the same number of revolutions in wash water. This was considered an essential point, as Kooper found that butter that was washed and worked held less iron than that which was worked without washing. The butters were then carefully packed in glass jars, with glass tops, to avoid any contact with metals, and placed in storage. The butters made at Albert Lea, Minn., were scored about a week after making and again after three months' storage at 10° F. This butter was scored by J. B. Neumann, J. C. Joslin, P. H. Keiffer, and the Fox River Butter Co., none of whom were familiar with the history of the butter. As these butters were shipped to several cities, it was impossible to get scores on the day the butters were made, the time usually approximating one week.

The butter made at Troy, Pa., was scored by Mr. Fryhofer and Mr. Smarzo, of New York City, after from two to four days, again after about a month, and again after about three months' storage at from 6° to 10° F. By comparison of the scores of the control butters and those to which iron had been added at the different intervals the effect of the iron on the quality of the butter was determined by the score for flavor, aroma, and body.

In order to determine the relation between the amount of iron present in the butter and the deterioration of the butter, it became necessary to know the amount of iron present, as well as the changes shown by the scores.

The very small amount of iron present in normal butter (averaging 1.33 milligrams per kilo for 10 samples from Troy Creamery Co.) necessitated the use of a very delicate method. The colorimetric method for iron with potassium sulphocyanid is, according to Neumann,[1] so delicate as to show 1 part of iron in 1,600,000 parts of water. This method was employed for the determination of the iron.

METHOD OF ANALYSIS.

The method of analysis used is as follows: About 500 grams of butter are melted and the clear supernatant fat separated from the curd solution. At first the fat was disregarded and only the curd solution used for analysis. Later the fat, too, was analyzed and found to take up approximately 10 per cent of the total iron in the butter, so that in all later work the fat and curd were both analyzed for iron content. The curd solution is evaporated almost to dryness, the remaining fat burned off, and then ashed at a low heat. The fat, by heating to its ignition point, will ignite and burn; the residue containing some small pieces of curd which remained in the fat is ashed. This ash, when total iron is wanted, is added to the ashed curd and the whole extracted with hot dilute hydrochloric acid and filtered into a graduated 300 cubic centimeter flask. It may be necessary to extract several times, and perhaps even burn the filter and residue and again extract in order to be sure to get out all the iron. In order to avoid any contamination with iron, this process of ashing is carried out in platinum dishes and a platinum spatula used in stirring or scraping the ash from the dish. These separate extracts and washings are all filtered into the same volumetric flask and diluted to 300 cubic centimeters with iron-free distilled water. This solution is then oxidized to have all iron in the ferric condition and an aliquot portion (from 0.5 to 10 cubic centimeters, depending on the iron present in the solution) transferred to a 50 cubic centimeter Nessler tube. Then 5 cubic centimeters of a 10 per cent solution of potassium sulphocyanid are added and the whole diluted to 50 cubic centimeters with distilled water. These samples are then compared with a set of standards made by using known amounts of a solution containing 0.0001 gram iron per cubic centimeter and made up to 50 cubic centimeters in the same way as the unknown. These standards fade on standing, so they should be compared shortly after the standards and unknowns are made. The results are calculated to parts per million (milligrams per kilo).

By such an analysis the actual amount of iron in the butter samples is determined. In most cases the iron was added to the cream, and

[1] Neumann, B. Die Grenzen der Empfindlichkeit verschiedener Reactionen auf Metalle. Chemiker-Zeitung, vol. 20, no. 79, pp. 763–764. Cothen, Sept. 30, 1896.

although the conditions were kept as nearly alike as possible, still a difference in the consistency of the butter when washed, or the thoroughness and length of the draining and washing would make a difference in the amount of iron retained in the butter.

Notwithstanding the fact that care was taken to get the best possible cream, the iron content can not be expected to be so low as in cases where the milk is drawn into glass vessels and that portion used for analysis. The normal creams for about 34 samples had an average iron content of 1.53 milligrams per kilo, with a maximum of 3.8 and a minimum of 0.45. Only 6 samples showed over 2 milligrams per kilo. These samples were taken, some at Albert Lea, Minn., some at Troy, Pa., and some at Washington, D. C.

Relation of iron in butter to iron in the cream.—Kooper,[1] in his investigations found that by washing and working butter it would lose some of its iron content. In this work, although care was taken to add the same relative amount of wash water and to work and wash the butter with the same number of revolutions, the iron found in the butter did not show any definite relation to the amount of iron added to the cream. In the first series of experiments analyses were made of the cream, butter, buttermilk, and wash water in an effort to determine whether there was any definite relation between the amount of metal added to the cream and that found in the butter, buttermilk, and wash water, but, as will be seen by the following table, there was no uniformity in the results. The analyses were made on samples of about 500 grams and calculated to milligrams per kilo. The total amount of butter, buttermilk, and wash water were not weighed accurately, and so the total weights of iron in the table are only approximated. In the following results the total approximated milligrams of iron in butter, buttermilk, and wash water have been shown, as well as the percentage return of iron in each of these. A percentage comparison, however, unless based on the same amount of cream (of same fat content), butter, buttermilk, and wash water, and to the cream of which the same amount of iron has been added in each case, really does not enable us to draw any definite conclusions. If the amount of iron added were small, a small variation in the amount returned would make a very much larger percentage difference than if the same variation were shown on a larger addition of iron. This would be especially marked on controls, the cream of which would have from 1 to 2 milligrams of iron per kilo, while the butter in controls, however carefully made, would show as much or in most cases more than the cream, to say nothing of the amount of iron found in the buttermilk and wash water. The possible error of

[1] Kooper, W. D. Loc. cit.

sampling when working on these small amounts also tends to minimize the value of the percentage relations.

Following are some of the results as found, the milligrams per kilo being accurate but the total weights of iron only approximated:

TABLE 9.—*Relation between iron added to cream and the iron found in butter, buttermilk, and wash water.*

Butter No.	Cream.		Butter.			Buttermilk.			Wash water.		
	Iron added.	Total iron added.	Iron found.	Total iron.	Total iron found.	Percentage of added iron.	Total iron.	Percentage of added iron.	Iron found.	Total iron.	Percentage of added iron.
	Mgs. per kilo.	*Mgs.*	*Mgs. per kilo.*	*Mgs.*	*Per cent.*	*Mgs. per kilo.*	*Mgs.*	*Per cent.*	*Mgs. per kilo.*	*Mgs.*	*Per cent.*
8	1,000	2,736.0	180.90	172	6.3	1,189.00	2,310.0	84.40	17.70	35	1.30
13	500	1,310.0	84.00	77	5.9	510.20	962.0	73.40	36.40	73	5.60
19	200	9,613.0	29.40	402	4.2	69.60	2,554.0	26.57	92.60	4,222	42.92
27	100	4,808.0	11.40	140	2.9	61.20	2,252.0	46.85	2.90	137	2.90
38	50	2,296.0	4.29	66	2.8	24.10	914.0	39.80	15.60	711	30.97
81	50	2,269.0	15.60	212	9.3	39.40	1,253.0	55.22	1.20	27	1.20
51	20	862.0	7.78	85	9.9	8.89	304.0	35.27	.40	10	1.20
[1] 55	20	862.0	8.60	112	13.0	18.90	605.0	70.20	7.20	164	23.95
43	[2]	40.0	3.03	33	82.5	2.17	58.7	146.30	1.44	33	82.50
85	[3]	103.2	2.22	39	37.8	1.73	75.0	72.70	.97	22	21.30

[1] Iron in contact twenty minutes. [2] Control. Found 1 milligram per kilo.
[3] Control. Found 1.98 milligrams per kilo.

Although from these results no definite relationship can be stated between the amount of iron added to the cream and the amount of iron found in the butter, buttermilk, and wash water, yet in a general way it might be stated that a relatively small part of the iron goes into the butter as compared with the buttermilk, which seems to take most of the iron, and in which the presence of a flavor due to the iron was most noticeable.

Distribution of iron between fat and curd solution.—When this work was first started it was thought that the amounts of iron taken up by the fat of the butter were negligible, and so in the analyses of butter the curd solution only was used for analysis. Later analyses were made of the fat as well as the curd solution on 22 samples of butter made at Troy, Pa., during the summer of 1911, with the following results, basing the fat content in butter at 80 per cent, since all the fat was not separated from the curd solution and the fat contained small particles of curd. The fat was not filtered, and so in some instances contained small particles of curd, which may account for a variance in iron content of the fat.

TABLE 10.—*Iron content of butter, curd, and fat.*

Butter No.	Iron added to cream.	Total iron found in butter.	Percentage of total iron in fat.	Percentage of total iron in curd.	Iron in fat.	Iron in curd.
	Mgs. per kilo.	*Mgs. per kilo.*	*Per cent.*	*Per cent.*	*Mgs. per kilo.*	*Mgs. per kilo.*
120	0	6.64	7.90	92.10	0.66	30.59
122	20	25.60	22.70	87.30	7.27	98.80
125	0	6.92	9.40	90.60	.82	31.32
127	10	10.99	3.25	96.75	.45	53.18
132	0	9.11	4.20	95.80	.48	43.64
134	5	7.20	10.50	89.50	.95	32.25
140	0	8.24	8.40	91.60	.87	41.38
142	2	7.40	12.00	88.00	1.11	32.55
148	0	7.56	12.00	88.00	1.13	33.27
150	1	8.79	10.70	89.30	1.18	39.28
157	0	5.45	15.50	84.50	1.06	23.01
159	0	5.09	32.40	67.60	2.06	17.21
164	0	4.62	27.50	72.50	1.59	16.75
166	0	5.71	17.90	82.10	1.28	23.43
171	0	4.04	19.60	80.40	.99	16.22
173	0	7.76	13.10	86.90	1.27	33.70
193	0	4.78	9.39	90.61	.56	21.73
194	(1)	6.33	7.44	92.56	.59	29.29
201	0	6.77	8.03	91.97	.68	31.11
203	(2)	6.93	10.00	90.00	.89	31.21
205	0	5.18	8.91	91.19	.58	23.61
207	(2)	9.11	7.69	92.31	.88	42.07

[1] Rusted can. [2] Iron strip.

In this table, as in any table of this kind, the percentage relation is relatively unimportant. The question of the actual amount of iron found in the fat is of much greater importance. An average of the control butters, of which there are 14 in the above table, will show a content of 1 milligram of iron per kilo of butter fat, a very small amount that could be disregarded in most cases. In normal butters made under proper conditions where the total iron content is about 1.3 milligrams per kilo, even a difference of 0.5 milligram of iron in a pound of butter would be noticeable on a comparative basis between the fat and curd content, but when total iron is being considered so small an amount as 0.5 milligram of iron could easily be discarded in the calculation.

THE INFLUENCE OF IRON ON FLAVOR.

The ideal condition for the solution of this problem would of course be to have butter made absolutely free from iron while known amounts of iron, or any other metal as the case might be, could be added to a portion of the cream used in making this metal-free butter. This could be done only by drawing the milk samples directly into glass vessels, skimming by hand, and ripening and churning in small quantities in glass. This method of procedure seemed impracticable, since enough butter could not be made to satisfy all the requirements of the experiment. The first few samples were, however, churned in a tall glass jar by shaking by hand, but proved unsatisfactory in that only a small amount could be

made and the butter was not very good. The best thing to be done under the circumstances was to control conditions as carefully as possible and avoid any undue contact with iron during the whole process of butter making. The method of butter making has been described. The butters were scored for the first time in most cases very shortly after making, being kept in cold storage until scored. It will be noticed that the butters made at Albert Lea, Minn., were scored by the Fox River Butter Co. and those made at Troy, Pa., or Washington, D. C., were scored by Mr. Fryhofer and Mr. Smarzo, of New York City. For this reason these scores are not comparable, as in our experience there is considerable variance between the scores and methods of scoring even among professional butter scorers. Following is a table showing the butter scores. In every case the butter to which metal has been added is followed by a control butter, made at the same time under the same conditions.

TABLE 11.—*Influence of iron on flavor of butter.*

Butter No.	Data on ripened, pasteurized cream.					Butter.		
	Iron content normal in cream.	Iron added as ferrous sulphate.	Duration of contact.	Acidity at time of churning.	Iron found in butter.	Age of butter when scored.	Score.	Remarks.
	Mgs. per kilo.	*Mgs. per kilo.*	*Hours.*	*Per cent of lactic acid.*	*Mgs. per kilo.*	*Days.*		
[1] 19	.....	200	22	.....	29.4	7	85.0	Very fishy.
						187	85.0	Do.
[1] 23	10.90	0	.....	0.53	8.3	7	92.5	Coarse, unclean.
						187	85.0	Very fishy.
27	3.84	100	23	.77	11.4	11	92.0	Oily.
						184	85.0	Very fishy.
31	3.84	0	.....	.65	2.59	11	93.5	
						184	85.0	Do.
39	.40	50	23	.73	4.29	14	86.0	Fishy.
						179	85.0	Very fishy.
[1] 35	.40	0	.....	.73	1.70	14	87.0	Fishy.
						179	85.0	Very fishy.
[1] 47	.99	25	23	.53	26.5	9	87.0	Slightly fishy.
						175	85.0	Very fishy.
[1] 43	1.00	0	.....	.51	3.03	9	92.0	Coarse.
						175	86.0	Very fishy.
[1] 81	2.76	50	22	.60	15.6	16	93.5	Clean.
						160	88.0	Oily, unclean.
[1] 77	2.76	0	.....	.62	2.49	16	94.0	
						160	88.0	Fishy.
122	2.93	20	21	.57	25.6	3	86.0	Very oily; very fishy.
						44	85.0	Do.
						116	82.0	Rank, oily, and fishy.
120	2.90	0	.....	.58	6.64	3	93.0	Clean.
						44	84.5	Very fishy.
						116	80.0	High acid, clean, rank fishy.
127	2.11	10	23	.49	10.99	5	84.0	Oily, metallic, unclean, rancid.
						42	85.0	Oily, metallic, very salvy.
						114	84.0	Very oily, metallic.
125	2.48	0	.....	.48	6.92	5	88.0	Oily, slightly metallic, unclean.
						42	85.0	Oily, metallic.
						114	84.0	Very oily, metallic.
134	1.64	5	21½	.59	7.20	3	91.5	Metallic, unclean.
						39	86.5	Metallic, stale.
						111	83.0	Very fishy.
132	1.64	0	.....	.55	9.11	3	94.0	Very clean.
						39	85.5	Fishy.
						111	82.0	Very high acid, clean, very fishy.

[1] Butter made at Albert Lea, Minn. Scored by Fox River Butter Co. All other butters made at Troy, Pa.

TABLE 11.—*Influence of iron on flavor of butter*—Continued.

Butter No.	Data on ripened, pasteurized cream.					Butter.		
	Iron content normal in cream.	Iron added as ferrous sulphate.	Duration of contac.	Acidity at time of churning.	Iron found in butter.	Age of butter when scored.	Score.	Remarks.
	Mgs. per kilo.	*Mgs. per kilo.*	*Hours.*	*Per cent of lactic acid.*	*Mgs. per kilo.*	*Days.*		
142	1.17	2	17	.59	7.40	2	91.0	Slightly metallic.
						47	87.0	Very metallic.
						105	88.0	Oily.
140	1.17	0	.....	.60	8.24	2	94.5	Slightly metallic aroma, clean.
						47	88.0	Metallic.
						105	83.0	Very fishy, clean, low acid.
150	.45	1	20	.60	8.79	6	86.0	Very oily and fishy.
						45	86.0	Oily and fishy.
						103	85.0	Do.
148	.74	0	.....	.60	7.56	6	89.0	Milky
						45	87.0	Slightly fishy.
						103	86.0	Fishy, clean, high acid.
[1] 93	.....	20	.....	.....	5.80	11	86.0	Oily, very fishy.
						128	86.0	Fishy.
[1] 92	.....	0	.....	.....	3.41	11	91.5	
						128	91.0	
223	.....	[2] 10	20½	.668	7.37	4	84.0	Unclean, stale, oily.
						29	84.0	Unclean, metallic, gritty.
						61	84.0	Rank, metallic.
222	.....	0	.....	.552	3.39	4	86.0	Stale, oily.
						29	86.0	Very oily, metallic.
						61	86.0	Oily, metallic.
225	.....	[2] 5	21	.66	5.4	4	88.0	Do.
						27	86.0	Very oily.
						59	84.0	Very metallic and fishy.
224	.....	0	.....	.55	2.15	4	90.0	Slightly metallic.
						27	87.0	Unclean, oily.
						59	87.0	Oily, metallic.
227	.....	[2] 2.5	21	.60	3.59	4	87.0	Tainted, very oily.
						25	87.0	Oily.
						57	85.0	Very oily, metallic.
226	.....	0	.....	.54	2.85	4	93.0	Good butter.
						25	93.0	
						57	89.0	Old flavor.
229	.....	[2] 2	19½	.44	1.61	7	88.0	Very metallic.
						22	87.0	Oily, metallic.
						54	85.0	Very metallic.
228	.....	0	.....	.42	1.89	7	92.0	Somewhat oily, coarse.
						22	92.0	
						54	88.0	Oily, trifle fishy.
231	2.99	[2] 1	20¾	.46	1.63	5	94.0	Good, clean, creamy.
						20	93.0	
						52	86.0	Very fishy.
230	2.73	0	.....	.52	2.16	5	92.0	Somewhat oily, coarse.
						20	92.0	
						52	88.0	Little fishy.

[1] Butter made at Albert Lea, Minn. Scored by Fox River Butter Co. All other butters made at Troy, Pa.
[2] Iron added as lactate.

In Table 11 it will be noticed that in every instance on the first scoring the butters to which iron had been added scored lower than their controls. This holds in most cases on the second and third scoring, the most noticeable feature being that the butters to which iron has been added show the deterioration much faster than the control butters. After the butters have deteriorated to a score of 85 or lower, the butter is so poor that a difference of a point or two in the score really does not indicate a very great difference in the quality of the butter. A great many of the butters became fishy, and where both were not scored fishy at the same time it will be noticed that the control butter was the last to become "fishy," though

in one or two instances the control was scored "fishy," while the other butter was not marked fishy at all. A very noticeable feature about these butters is the production of a very oily flavor. This was present in most amples that were marked fishy and seems to be a stepping stone to the fishy flavor.

THE INFLUENCE OF COPPER ON FLAVOR.

In the work on copper only the physical changes will be considered, as considerable difficulty was experienced in making accurate determinations of the very small amounts of copper present in the butter.

These butters were made in exactly the same way as the butters showing the influence of iron. The cream was all very carefully handled to avoid contact with copper, and divided into two portions, which were treated in exactly the same manner, excepting that to one-half of the cream, copper in the form of a solution of copper sulfate or lactate was added in amounts varying from one-half to 20 milligrams per kilo of cream. The cream was ripened and churned and the butter stored in glass jars without metal parts, or in ash tubs, in 1912, and scored at intervals. The changes in the butter are shown in the following tables:

TABLE 12.—*Influence of copper on butter.*

Butter No.	Data on ripened and pasteurized cream.			Butter.		
	Copper added as sulfate.	Duration of contact.	Acidity at churning.	Age on scoring.	Score.	Remarks.
	Mgs. per kilo.	*Hours.*	*Per cent of lactic acid.*	*Days.*		
[1] 89	20	19	0.51	14	90.0	Very fishy.
				158	85.0	Do.
[1] 85	0	.....	.60	14	92.5	Oily.
				158	86.0	Very fishy.
[2] 173	4	22	.60	5	85.0	Oily, fishy, unclean.
				38	85.0	Oily, fishy.
				96	80.0	Rank, fishy, clean, high acid.
[2] 171	0	.....	.60	5	91.0	Oily.
				38	90.0	Slight metallic.
				96	86.0	Fishy, clean, low acid.
[2] 166	2	.....	.56	2	87.0	Oily, unclean, rancid.
				40	84.0	Very oily, fishy.
				98	80.0	Rank, oily and fishy, unclean, low acid.
[2] 164	0	.....	.52	2	93.0	Slight metallic.
				40	87.0	Oily, metallic.
				98	87.0	Oily.
[2] 159	1	.....	.51	4	85.0	Oily, metallic, stale.
				42	84.0	Very oily and fishy.
				100	80.0	Rank, fishy, unclean, low acid.
[2] 157	0	.....	.50	4	91.0	Slight oily.
				42	86.0	Very oily, fishy.
				100	82.0	Very oily, fishy, clean, high acid.
[3] 235	[4] 5.0	19	.36	3	86.0	Very oily and metallic.
				18	84.0	Oily and fishy.
				50	84.0	Rank, fishy.
[3] 234	0	.....	.385	3	92.0	Somewhat oily and coarse.
				18	89.0	Oily.
				50	89.0	Old flavor.
[3] 237	[4] 2.5	19	.415	2	88.0	Oily and metallic.
				15	87.0	Unclean, oily.
				47	84.0	Rank, fishy.

[1] Butter made at Albert Lea, Minn., 1910.
[2] Butter made at Troy, Pa., 1911.
[3] Butter made at Troy, Pa., 1912.
[4] Copper added in form of copper lactate.

TABLE 12.—*Influence of copper on butter*—Continued.

Butter No.	Data on ripened and pasteurized cream.			Butter.		
	Copper added as sulfate.	Duration of contact.	Acidity at churning.	Age on scoring.	Score.	Remarks.
	Mgs. per kilo.	*Hours.*	*Per cent of lactic acid.*	*Days.*		
[1] 236	0	.....	.58	2	92.0	Trifle coarse, slightly tainted.
				15	91.0	Slight unclean.
				47	86.0	Tallowy flavor.
[1] 239	[2] 1.5	18½	.376	2	92.0	Slight oily and metallic.
				13	92.0	Slight oily.
				45	86.0	Metallic and fishy.
[1] 238	0	.....	.355	2	94.0	Good and clean.
				13	94.0	
				45	93.0	Clean.
[1] 241	[2] 1.0	21	.40	3	88.0	Very oily and metallic.
				43	87.0	Metallic.
[1] 240	0	.....	.34	3	91.0	Trifle metallic.
				43	91.0	Old flavor.

[1] Butter made at Troy, Pa., 1912. [2] Copper added in form of copper lactate.

From Table 12 it will be seen that in every instance the scores on the control butters were better than the scores on the butter made from cream to which copper had been added, even in the small amount of 1 milligram of copper per kilo of cream.

Unfortunately, the "Remarks" on the butter do not show any definite characteristics that can be attributed to the copper. The control butters, too, show deterioration in storage, though they seem to keep better than the butters made from cream to which the copper was added. On the second scoring after 40 days in storage most of the butter to which copper had been added showed a fishy flavor and after three months a very decided rank, fishy flavor that was unmistakable, and was called by scorers decided mackerel flavor. These butters were all made during the summer months.

An experiment to show the effect of having the cream stand in contact with a small surface of copper for a long time was made by having a vat of cream ripened in contact with two sheets of bright copper each 2 by 6 inches. The sheets were placed on edge in the bottom of the vat during the process of ripening. The result of this is shown in the following table:

TABLE 13.—*Effect of copper on flavor of butter.*

Butter No.	Cream.		Duration of contact.	Age.	Score.	Remarks.
	Normal acidity.	Churning acidity.				
	Per cent.	*Per cent.*	*Hours.*	*Days.*		
256	0.17	0.355	.....	1	93	
				31	91	Trifle old flavor.
257	.17	.37	21	1	91	Trifle oily.
				31	88	Metallic.

The difference in flavor is marked in the fresh butter but more marked in storage butter.

Another experiment to show the effect on the flavor of the butter, by having the cream come in contact with a large surface of copper for a short time, was made by pasteurizing the cream in two parts. One part was pasteurized in a No. 3 Peerless pasteurizer, the copper lining being completely covered with tin. The second part was pasteurized in a No. 5 Peerless pasteurizer, the tin coating of which had been worn off by continued usage, leaving the cream exposed to a surface of copper during the process of pasteurization. The duration of contact with the copper was only a few seconds, though the surface was quite large. The temperature of pasteurization was about 60° C.

The results of this experiment are found in the following table:

TABLE 14.—*Comparison of the effect of tin and copper on the flavor of butter.*

Butter No.	Pasteurizer.	Acidity of cream before pasteurization.	Temperature of pasteurization.	Acidity at churning.	Age.	Score.	Remarks.
		Per cent.	° *C.*	*Per cent.*	*Days.*		
245	Tin.....	0.18	60	0.55	2	91	Slight oily.
					39	92	Good.
246	Copper..	.18	60	.50	2	87	Oily, stale, metallic.
					39	85	Very fishy.
252	Tin.....	.19	60	.52	1	92	Slight oily.
					33	89	Oily.
253	Copper..	.19	60	.47	1	89	Do.
					33	84	Very fishy.

These two experiments show very plainly the deteriorating effect of poorly tinned pasteurizers, for aside from this all other conditions were exactly alike during the complete process of butter manufacture. Considering the short duration of contact, the change in the flavor of the butter even when fresh is very marked. The effect of copper even in small amounts seems to cause more marked changes in butter flavor than iron, with a marked tendency toward a fishy flavor in storage.

CONTAMINATION OF CREAM WITH IRON FROM CONTAINERS.

For this experiment a lot of cream was divided into two portions, one portion being held in a clean can and the other in a can which showed many rust spots. This was the most rusted of all the cans that were used for the handling of cream at the Troy Creamery Co., of Troy, Pa. However, this showed only small spots of rust on the bottom of the can. This raw cream was ripened without the addition of a starter at room temperature. The temperature, acidity, and iron content are as follows:

TABLE 15.—*The absorption of iron from rusty can.*

Duration of contact.	Clean can.			Rusted can.		
	Temperature.	Acidity.	Iron found.	Temperature.	Acidity.	Iron found.
Hours.	° *F.*	*Per cent.*	*Mgs. p. kilo.*	° *F.*	*Per cent.*	*Mgs. p. kilo.*
0	66	0.114	1.045	66	0.114	1.045
2	67	.101	1.031	67	.120	1.132
4	67	.146	1.539	67	.158	1.772
6	67	.177	1.028	67	.176	1.265
8	68	.202	1.677	68	.212	1.129
14	66	.412	.792	66	.422	1.627
21	66	.561	1.082	66	.561	1.459
26	64	.581	.951	56	.568	1.187
32	52	.634	1.082	57	.624	1.257
48	44	.632	1.185	57	.638	2.381
	Butter made from above cream 4.78.			Butter made from above cream 6.33.		

Another experiment on the same basis but without the acidity of the cream at the various intervals gave the following results, from a cream having a normal iron content of 0.9 milligram per kilo and an acidity of 0.16 per cent lactic acid.

TABLE 16.—*Effect of rusty can on the flavor of butter.*

Butter. No.	Iron in butter.	Cream contact in cans.	Iron content in ripened cream.	Churning acidity.	Butter.		Remarks.
					Age.	Score.	
	Mgs. p. kilo.	*Hours.*	*Mgs. p. kilo.*	*Per cent.*	*Days.*		
259	1.37	15	4.31	0.51	19	92	Slight oily.
					29	89	Trifle metallic, oily.
260	1.60	15	4.35	.49	19	88	Oily, metallic.
					29	86	Unclean, oily.

From Table 15, it is seen, as would be expected, that the acidity of cream increases with the length of time it is held, though there is no material difference in the rate of increase between the two samples. The cream in the rusted can does not show any marked increase in iron content over that in the clean can. The difficulty in getting an accurate sample of the cream after standing long enough to thicken may account for the variation from what might have been expected. At the end of 48 hours the cream from the rusted can showed an iron content of 1.2 parts per 1,000,000 more than the cream from the clean can. The butters made from these creams checked very closely, as the butter made from the cream held 48 hours in the rusted can showed 1.55 parts per 1,000,000 more than the butter made from the cream held 48 hours in a clean can.

In another instance to find whether cream on standing in contact with iron rust would take up iron, a lot of cream was divided into four parts, each placed in a clean can, and a strip of rusted iron tape one-half inch wide and 24 inches long (giving a surface of 24 square

inches) was placed in two of these cans. Two cans, one with and one without the iron, were ripened at room temperature for 21 hours, and two cans, one with and one without iron, were held in cold storage at 32° F. for 21 hours. Butter was made from each of these four samples of cream. There was no starter added to this cream. The cream from which these four lots were taken had acidity of .223 and iron content of 1.25 milligrams per kilo.

TABLE 17.—*Data on cream ripened in contact with rusty iron.*

No.	Cream.			Butter.		
	Temperature.	Acidity.	Iron content.	Butter No.	Iron content.	Score.
	° F.		*Mgs. per kilo.*		*Mgs. per kilo.*	
1	32	0.385	1.53	1	6.77	94
[1]2	32	.368	1.81	2	6.93	91
3	66	.655	.90	3	5.18	94
[1]4	66	.670	11.32	4	9.11	91

[1] In contact with iron tape.

From this table, although it does not show the acidity and iron content at intervals as in Table 15, still it shows here that with the development of acidity the iron is taken up by the cream. This is very marked in cream and butter No. 4, the iron content in both being very high as compared with their respective controls. The effect of the iron is also very marked in the butter scores, the controls (Nos. 1 and 3) scoring three points higher than the ones to which the iron had been added.

In the first experiment Mr. Larson, superintendent of the Troy Creamery Co., said he could taste the iron in the cream after 32 hours' contact, and in the second experiment the cream in contact with iron ripened at room temperature showed a bitter metallic taste.

In every case the butter made from cream which had stood in contact with iron rust showed a peculiar taste and was easily picked out from a lot of samples. The taste was, however, most noticeable in the buttermilk, to which it gave a decided metallic taste.

In Table 15 the difference in the amounts of iron found in the cream are very small, in only one case being more than 1 milligram of iron per kilo of cream. Taking into consideration the possibility of error in sampling a very thick cream, these small differences do not seem to be enough to warrant any definite conclusions as to the absorption of iron by the cream. It will be noticed in Table 11 that all the control butters made by us at Troy, Pa., have a very high iron content, as compared with control butters made by us at Albert

Lea or made by the Troy Creamery Co. at Troy. As the wash water was tested and the cream did not show a high iron content, the churns are the only means whereby iron might be introduced into the butter. Although these churns were taken apart and scraped, sandpapered, and thoroughly cleaned before being used at Troy, it is possible that there were some rust spots on the iron bolt heads and plates coming in contact with the cream, and that the iron was attacked at these points by the high acid cream and the iron taken up by the butter. Unfortunately the butter samples were not analyzed at the time the butter was made, otherwise this error would have been noticed in the chemical results and corrected before continuing the work. The high iron content of the butters made at Troy, Pa., seemed to show conclusively that there was an error by contamination at some step in the process of butter making. The only check possible on the butter itself was by analysis of the butter made by the Troy Creamery Co. in their large churn. In most cases the cream for the experimental butter was selected from the best cream brought to the creamery, while the cream used in the creamery proper was the regular run of cream as brought in by the farmers and from the several skimming stations. In this way the butter from the creamery proper, if the fault were in the cream, should show a higher iron content than the experimental butter. The cream used in the experimental creamery was analyzed for iron (in most cases) at three stages; first, raw sweet cream; second, immediately after pasteurizing (to determine whether there was any iron exposed in the pasteurizer or cooler), and, third, after ripening in the ripening vats to determine whether there was any iron taken up in the vats. The butter was then analyzed, thus giving the last stage in the process of butter making.

The butter made in 1912 at Troy, Pa., was churned in new No. 5 Bestov box churns, and worked on a table worker, eliminating this contamination by iron in the churn. The butter for scoring was packed in 10-pound ash butter tubs instead of glass jars in order to avoid injuring the body of the butter in packing.

TABLE 18.—*Iron content of cream and controls.*

[Milligrams per kilo.]

Butter.	Raw cream.	Pasteurized cream.	Raw ripened cream.	Pasteurized ripened cream.	Curd as 100 per cent of butter.	Curd as 20 per cent of butter.	Fat as 80 per cent of butter.
120 E	.....	.....	.....	2.93	6.12	30.59	0.66
125 E	2.11	2.48	.....	2.57	6.26	31.32	.82
132 E	1.89	1.49	.....	1.64	8.73	43.64	.48
140 E	1.19	1.12	.....	1.17	7.54	41.38	.87
148 E	.45	.55	.....	.74	6.65	33.27	1.13
157 E	1.47	.....	.....	1.59	4.60	23.01	1.06
164 E	1.46	.....	.....	1.63	3.35	16.75	1.59
171 E	1.94	.....	.....	1.44	3.24	16.22	.99
201 E	1.25	.....	1.53	.....	6.22	31.11	.68
205 E	1.75	.....	.90	.....	4.72	23.61	.58
135 T	.....	.....	.....	.....	1.12	5.59	.....
136 T	.....	.....	.....	.....	1.16	5.81	.....
143 T	.....	.....	.....	.....	1.25	6.26	.....
151 T	.....	.....	.....	.....	.96	4.81	.....
152 T	.....	.....	.....	.....	1.19	5.97	.....
160 T	.....	.....	.....	.....	.89	4.47	.....
161 T	.....	.....	.....	.....	1.21	6.05	.....
167 T	.....	.....	.....	.....	[1] 1.55	[1] 7.77	.....
168 T	.....	.....	.....	.....	[1] 1.31	[1] 6.52	.....
195 T	.....	.....	.....	.....	[1] 1.50	[1] 7.51	.....
196 T	.....	.....	.....	.....	[1] 1.79	[1] 8.96	.....
197 T	.....	.....	.....	.....	[1] .99	[1] 4.96	.....
198 T	.....	.....	.....	.....	[1] 2.35	[1] 11.74	.....
[2] 224	.....	.....	.....	.....	2.15	.....	.....
[2] 226	.....	.....	.....	.....	2.85	.....	.....
[2] 228	.....	.....	.....	.....	1.89	.....	.....
[2] 230	.....	.....	.....	2.73	2.16	.....	.....
[2] 234	.....	.....	.....	.....	2.04	.....	.....
[2] 236	.....	.....	.....	.....	3.16	.....	.....
[2] 238	.....	.....	.....	.....	3.50	.....	.....
[2] 240	.....	.....	.....	.....	3.59	.....	.....
[2] 246	.94	2.04	.....	.....	1.95	.....	.....
[2] 247	.....	.....	.....	.....	1.36	.....	.....
[2] 248	.....	.....	.....	.....	1.14	.....	.....
[2] 252	.....	2.01	.....	.....	1.41	.....	.....
[2] 253	.....	1.14	.....	.....	1.41	.....	.....
[2] 256	1.67	.....	.....	.....	3.40	.....	.....
[2] 257	1.67	1.10	.....	.....	6.63	.....	.....
[2] 259	.....	.9	.....	.....	1.37	.....	.....
[2] 263	.....	.....	.....	4.31	2.69	.....	.....
[2] 264	.....	.....	.....	4.35	1.45	.....	.....

E Butter made in experimental creamery, Troy, Pa., 1911.
T Butter made by Troy Creamery Co., 1911.
[1] Curd burned in porcelain.
[2] Butter made in experimental creamery, Troy, Pa., 1912.

Table 18 is arranged to show the iron content found in the cream and butter made in the experimental creamery and also, for comparison, the iron content of the butters made by the Troy Creamery Co. In these analyses the fat was disregarded and the iron found in the curd solution used as the total iron in the butter. It will be noticed in the column, "Fat as 80 per cent of the butter" that the iron content averages 0.89 milligram per kilo. As the usual charge of butter was 500 grams, the total iron in fat would be less than 0.4 milligram of iron, which is a very small and almost negligible quantity.

Ten samples of control butter made in the experimental churns gave an average iron content of 5.74 milligrams of iron per kilo of butter using the curd solution only and calculating as butter, while 13 samples of butter made by the Troy Creamery Co. in their large churn

during the same period showed an average iron content of 1.33 milligrams of iron per kilo of butter, using curd solution only and calculating as butter. The same cream, with a possible advantage in favor of the experimental cream, the same salt, and the wash water from the same source, were used in the manufacture of both the experimental and the Troy Creamery Co.'s butter, so that the only point of entry for the increased iron content in the experimental butters was in the churns. This might possibly account for the deterioration in the control butters made in the experimental creamery in 1911, and although these results detract from the results in the other experiments, still the value of the evidence to show that iron may be taken up in the churn is of great importance to the butter maker. Rusty bolt-heads, plates, or other castings should be carefully guarded against by the butter maker.

THEORETICAL CONSIDERATIONS.

Having ascertained definitely, partly from the empirical observations of others, and partly from the experimental data obtained on butter containing added iron, that iron itself lowers the keeping quality of butter, it was desirable to find out how iron affects butter.

That the iron acts catalytically in an oxidative reaction at once suggests itself. As has been shown, butter made by the usual methods contains gases in the proportion of approximately 10 cubic centimeters in 100 grams of butter. It seems possible that a few parts of iron per million parts of butter, present in a finely divided, colloidal condition might be able to transfer slowly some of the inclosed oxygen to any one of the oxidizable substances present. It is of course well known that in the presence of a peroxid such as hydrogen peroxid the iron will rapidly transfer oxygen from the peroxid to an oxidizable substance. A transfer of oxygen such as this is often referred to as peroxidase action and the substance through which the transfer is brought about, the colloidal iron compound in this case, is called a peroxidase. It is also well known, at least the statement is often seen in the literature, that peroxidases can transfer oxygen only from peroxids and hence are inactive in the absence of peroxids. Kastle,[1] in discussing the mode of action of peroxidase, states: "According to Bach and Chodat [2] the peroxidases exert not the slightest oxidizing action in the absence of the peroxid." It is perhaps to be regretted that this statement was not accompanied with another to the effect that this conclusion follows from an experi-

[1] Kastle, J. H. The oxidases and other oxygen-catalysts concerned in biological oxidations. United States Treasury Department, Public Health and Marine-Hospital Service, Hygienic Laboratory, Bulletin 59. Washington, 1910. See p. 117.

[2] Bach, A., and Chodat, R. Ueber Peroxydase. Berichte der Deutschen Chemischen Gesellschaft, vol. 36, no. 3, pp. 600–605. Berlin, Feb. 21, 1903.

ment in which Bach and Chodat [1] made observations on oxygen absorption for a period of 24 hours. Bach and Chodat,[2] in support of their contention that peroxidases are without action in the absence of peroxids, quote the work of Linossier [3] which was likewise a series of observations on peroxidase action covering only very short periods of time.[4]

It may be true that peroxidases in the presence of air are inactive when their activities are measured for intervals of 24 hours. But in the case of cold-storage butter, in which iron and air may interact for several months, the possibility that oxidative action may take place is not excluded, in spite of the fact that there is at present no reason to suppose that peroxids are present in butter.

The experiments here described on the oxidation of lactose in milk through the action of iron salts are in many respects similar to those made in recent years by several investigators whose work throws some light on the subject.

Löb and Pulvermacher [5] studied the action of gaseous oxygen and of hydrogen peroxid on dextrose and sucrose. They used as an oxidative agent a preparation made by extracting pig pancreas with alcohol and adding iron to the alcohol filtrate. The precipitate of iron-pancreatic material was dried and used in the experiments. In 24 to 48 hours this substance could oxidize dextrose in aqueous solution either in the presence of hydrogen peroxid or by the aid of a stream of air or oxygen. Sucrose was also oxidized by hydrogen peroxid and iron-pancreas powder, but to a much less extent. They suggest the necessity of the inversion of the sugar before oxidation can take place.

Battelli and Stern [6] and Harden and MacLean [7] studied oxidation in isolated, hashed animal tissues. These investigators used such material as hashed muscle, suspended in water. The suspensions were placed in a flask filled with gaseous oxygen. The substance to be oxidized was added to the suspension and the flasks were connected with an apparatus for measuring the amount of oxygen absorbed.

According to Battelli and Stern, succinic acid is very easily oxidized in a very few hours under the conditions of their experiment. Harden and MacLean repeated some of the experiments of Battelli and Stern

[1] Bach, A., and Chodat, R. Loc. cit. See pp. 604-605.

[2] Bach, A., and Chodat, R. Loc. cit. See p. 605.

[3] Linossier, G. Contribution á l'étude des ferments oxydants. Comptes Rendus Hebdomadaires des Séances de la Société de Biologie, vol. 50, no. 12, pp. 373-375. Paris, Apr. 1, 1898.

[4] The reference to Linossier given by Bach and Chodat is incorrect; the reference by Kastle is correct.

[5] Löb, Walther, and Pulvermacher, Georg. Über die oxydative Zuckerzerstörung unter der Einwirkung von Organpräparaten. Biochemische Zeitschrift, vol. 29, no. 4/5, pp. 316-346. Berlin, Nov. 22, 1910.

[6] Battelli, F., and Stern, L. Die oxydation der Bernsteinsäure durch Tiergewebe. Biochemische Zeitschrift, vol. 30, no. 1/2, pp. 172-194. Berlin, Dec. 23, 1910.

[7] Harden, Arthur, and MacLean, Hugh. The oxidation of isolated animal tissues. Journal of Physiology, vol. 43, no. 1, pp. 34-45, Sept. 11, 1911.

and found that the oxidation of succinic acid was not as vigorous as Battelli and Stern had found.

There are several reasons why the results on peroxidase action obtained by one investigator might not be the same as those obtained by another on material intended to represent exactly the material previously used. The activity of peroxidases is influenced by so many conditions that an exact reproduction of any particular mixture is perhaps more difficult than might at first be supposed. Furthermore, according to Wolff,[1] the iron peroxidase is very specific in its action, its specificity being determined by the other substances which may be present. Certain iron salts or combinations of such may oxidize one phenol and be incapable of oxidizing any other.

The results obtained in experiments on the oxidation of such substances as dextrose must be interpreted carefully, as Levene and Meyer [2] have pointed out. They showed that in a sugar solution the reducing power of which had been lowered by the combined action of muscle plasma and pancreatic extract the reducing power was restored to its original height by boiling under a return condenser for two hours in the presence of 1 per cent hydrochloric acid and that a substance having the properties of a biosazone could be obtained from the above described solution. So that loss of reducing power does not necessarily imply destruction of sugar; it may mean a simple polymerization.

The results of the above-mentioned investigations were used in planning the experiments that follow:

THE OXIDATION OF LACTOSE IN BUTTER.

For the purpose of ascertaining whether lactose which is ordinarily present in butter to the extent of about 0.1 to 0.2 per cent is being oxidized in cold-storage butter, with the production of substances having a disagreeable taste or smell, any one of several methods suggest themselves as possible. The lactose present in a lot of butter before and after storage may be estimated by any one of the well-known methods, or the oxidation products of lactose may be looked for in storage butter.

But the possible oxidation of lactose with the formation of off flavors might be brought about with such little change in the lactose content that the ordinary methods of analysis might be inadequate for the detection of the change. It was pointed out before (p. 6) that very small amounts of some substances are easily detected by the senses of taste and smell and that these amounts are smaller than are

[1] Wolff, J. Relations entre les phénomènes oxydasiques naturels et artificiels. Annales de l'Institut Pasteur, vol. 24, no. 10, pp. 789-797. Paris, Oct. 25, 1910.

[2] Levene, P. A., and Meyer, G. M. On the combined action of muscle plasma and pancreas extract on glucose and maltose. Journal of Biological Chemistry, vol. 9, no. 2, pp. 97-107. Baltimore, April, 1911.

detectable by the best analytic methods of the present time. It was also pointed out (p. 15) that the separation of fat quantitatively from butter is difficult. This introduces a difficulty in the direct estimation of lactose in the butter curd solution.

For these and still other reasons it was considered advisable to avoid attempting to detect directly very minute changes in the lactose content of storage butter. The problem was approached indirectly.

On account of the presence of sodium chlorid in butter in amounts varying from about 12 to over 30 per cent in the curd solution, care must be taken in applying the results of other investigators on peroxidases to this problem. It may be that the sodium chlorid is without effect. Obviously, only experimental data in which the peroxidase action in the presence of sodium chlorid is studied are directly applicable here.

One method used in studying (sample No. 25) the utilization of oxygen by iron in butter was similar in some respects to that used by Horbaczewski [1] and others in their studies of the utilization of atmospheric oxygen by oxidases of animal tissues.

Description of samples.—Several gallons of raw milk were obtained from a dealer and separated in the cream separator in the laboratory. To 8 liters of skim milk sodium chlorid was added, in the proportion of 180 grams of sodium chlorid to 1 liter of skim milk. Two and one-half liters of this sample (milk No. 25) were transferred to an 8-liter flask provided with a rubber stopper through which an inlet and outlet glass tube passed to permit the passage of the oxygen gas through the sample. Part of the sample was set aside. The milk in the flask was the material on which the experiment was made.

Methods and experimental procedure.—Before beginning the experiment the lactose content of the sample was determined by its reducing power and with the polariscope. In so far as no very great change in this quantity was expected, the greatest care was taken to obtain accurate results and uniformity of procedure.

For the gravimetric estimation of lactose the method described on pages 42, 48, and 119, Bulletin 107 (revised), of the Bureau of Chemistry was used. It is of course questionable whether the amount of copper reduced by a given weight of lactose in milk will be the same as that reduced by the same weight of lactose in milk containing 18 per cent of sodium chlorid. But what was sought was not the absolute amount of lactose present, but rather the difference, if any, between the amount of lactose present before and after a certain treatment of the sample.

[1] Horbaczewski, J. Untersuchungen über die entstehung der Harnsäure im Säugethier organismus. Monatshefte für Chemie, vol. 10, pp. 624–641. Vienna, 1889.

Six Gooch crucibles were prepared and used as described by Kendall.[1]

The weights of the Gooch crucibles after a determination were less than their weights before by an amount that varied between 0.4 and 1.2 milligrams. The average loss in weight for 19 determinations was 0.8 milligram. Only once did a crucible show a gain in weight, 0.3 milligram.

Although reagents (copper sulfate, sodium potassium tartrate, sodium hydroxid) of the highest purity were used, a slight reduction was always obtained in blank experiments made on an 18 per cent aqueous solution of sodium chlorid. The weight of cuprous oxid obtained in the blank determinations (9) varied from 3.6 to 9.1 milligrams, average 5.9 milligrams.

The reducing power of sample No. 25 was determined six times in duplicate during the course of the experiment. The duplicates differed by the following weights of cuprous oxid: 4.3, 0.4, 2.1, 0.2, 0.4, 0.8 milligrams. Very nearly 378 milligrams of cuprous oxid were always obtained in a determination, from which amount the amount of the blank determination was subtracted.

For the determination of lactose by the polariscope, the method given in Bulletin 107 (revised), Bureau of Chemistry, pages 118–119, was used: Eight cubic centimeters of acid mercuric nitrate was used as the precipitant for 131.6 grams of the sample in a 200 cubic centimeter flask. The filtrate was polarized in a 400-millimeter tube in a Ventzke polariscope. The readings divided by 4 give per cent of lactose, if it be assumed that the sodium chlorid was without effect on the rotatory power of the lactose. The results are collected in Table 19. Lactose containing one molecule of water of crystallization calculated from the polariscope readings was present to the extent of 4.75 per cent. The average weight of cuprous oxid found, 0.371 gram, corresponds to 0.2577 gram of lactose or 5.19 grams of lactose in 100 cubic centimeters sample No. 25.

The filtrate from the mercuric nitrate precipitation, although perfectly clear at first, soon becomes cloudy and unfit for reading in the polariscope. However, readings can be taken without interference by cloudiness if they are taken within one-half hour after filtering. The polariscope readings of the filtrates were found to undergo no change even after being allowed to remain in the laboratory for two weeks. After adding the precipitant and diluting to the mark the mixture was allowed to stand one hour. It was then filtered and the filtrate immediately polarized. Several days after, the filtrate was again filtered, a precipitate having formed in the meantime, and polarized. In this way several filtrates were repeatedly polarized at

[1] Kendall, Edward Calvin. A quantitative study of the action of pancreatic amylase. Columbia University, Dissertation, 1910. See p. 10.

intervals of a few days without in any case detecting an appreciable variation in polariscope reading.

After having determined the lactose in the sample No. 25, its specific gravity (in a 25 cubic centimeter pycnometer) was determined. Oxygen was then passed through the sample for 72 hours. The oxygen was passed from the oxygen tank into an 8-liter flask containing 3 liters of 18 per cent sodium chlorid solution and then through the milk. This was done so that the gas as it slowly bubbled through the milk would alter the concentration of the sample as little as possible. While the contact between gas and liquid was poor, it is almost certain that the liquid was saturated with the gas. About 25 gallons (not quite 100 liters) of oxygen passed through the sample in the 72 hours. Before each determination of lactose the specific gravity of the sample was determined and recorded. No significant variations were observed.

After the 72 hours' passage of the oxygen gas the lactose content of the sample was determined and found to have undergone no change. This showed, at least in this particular case, that the naturally occurring peroxidase in milk could not utilize molecular oxygen for the oxidation of lactose. From time to time tests for peroxidase were made, using tincture of guaiac and a few drops of a dilute solution of hydrogen peroxid. Peroxidase was present in the material throughout the experiment. Although always looked for, oxidase was not found in the several tests, except once, and that was probably due to some unaccountable error. The reagent, tincture of guaiac, was not the cause of the unusual positive test.

To sample No. 25 there was then added 8 grams of ferrous sulfate containing 7 molecules of water of crystallization ($FeSO_4 7H_2O$). One gram of metallic iron is present in 4.978 grams of this salt. On adding ferrous sulfate to the milk a very strong disagreeable odor was produced, suggesting putrefying protein. The odor was undoubtedly produced by the action of the iron on the milk, as the sample was odorless before the addition. The odor of the material throughout the experiment was always carefully noticed. (See p. 64.) The quantity of ferrous sulfate added was calculated to make one part of metallic iron present in 1,000 parts of milk. This is undoubtedly much more than is ever present in milk or butter, excluding the case where butter is in contact with iron rust, which has gone into solution but has not diffused very far into the butter mass. But the amount of iron was purposely made very high because the experimental time was to be much shorter than the storage period. The milk was allowed to stand one day after the addition of the iron, and the lactose determined. No change was noticed in this quantity nor was any change found after blowing oxygen through the sample for a second period of 72 hours. The acidity of the sample to phenolphthalein did not change during this time, nor were bacteria present during the experi-

ment in numbers sufficient to affect the results. Oxygen was passed through the sample for 17 days longer with a decided drop in reducing power and polarization at the end of that period. This was due to bacterial action, as was shown by a suitable examination.

TABLE 19.—*Action of iron and oxygen on lactose in milk No. 25.*

No.	Treatment.	Reduction.		Polarization.	
		Date of determination.	Weight of Cu_2O.	Readings on Ventzke scale.	Date of readings.
			Grams.		
25	Original sample, containing sodium chlorid; age, 7 days	Mar. 28, 1911	0.3721	+19.0	Mar. 28
25	Repetition on same sample	Mar. 30, 1911	.3720	19.0	Mar. 30
25.1	After blowing oxygen through for 72 hours and letting stand 1 day	Apr. 4, 1911	.3703	18.9	Apr. 3
25.2a	Added ferrous sulfate and let stand 1 day	do	.3721	18.8	Apr. 5
25.2a	Repetition on part of the sample set aside until	Apr. 7, 1911	.3716		
25.2b	After blowing oxygen through sample for second period of 72 hours, a total of 144	do	.3692	18.6	Apr. 8
25.3	After 10 days slow and 7 days rapid blowing of oxygen through sample	Apr. 25, 1911	.2084	17.7 13.2	Apr. 17 Apr. 25

During the summer of 1910 three experiments, samples Nos. 1, 2, and 3, essentially similar to that on milk No. 25, were made. Air instead of oxygen was used. The results are not appreciably different from those in Table 19. Sample No. 1 was milk, samples 2 and 3 were skim milk; all three were soured before use in the experiments. Only the reduction was determined in these samples which, because of the formation of lactic acid, was lower than the reduction in sample No. 25. But the reduction did not change appreciably by the action of the air blown through the samples. The results were not as uniform as in Table 19, very likely because it is more difficult to sample accurately the sour milk containing particles of casein. After a few trials during the summer of 1910 on the best way of obtaining a uniform sample for reduction, it was found best to transfer the portion of the sample in the pycnometer (25 cubic centimeters) to the volumetric flask for clarification. In this way both the volume and weight of the portion used was known.

THE POSSIBLE OXIDATION OF LACTOSE IN STORAGE BUTTER BY A PEROXID.

It was stated before (p. 56) that there are at present no reasons for supposing that peroxids are present to any appreciable extent in butter. This statement is not free from assumption, for there is a possibility of the slow formation of an organic peroxid in butter. A review of the literature on the subject and an interesting discussion of the formation of organic peroxids by direct combination of the compound with molecular oxygen has been made by Kastle.[1] The

[1] Loc. cit.

detection of an organic peroxid in butter at any particular time might be practically impossible because of its almost immediate decomposition either by the catalase naturally present in unpasteurized cream butter or by iron almost always present, even in butter most carefully churned, to the extent of three or four parts per million of butter (see p. 54 for amounts of iron in butter) and yet the oxidative process may be taking place. Perhaps a careful analysis of the gas in butter will show whether any of the oxygen is being removed in this way. The work on the analysis of the gases in butter has been begun and some of the analyses are given on page 37.

For the reasons just mentioned the fact that no oxidation of lactose was detected in skim milk containing iron and through which oxygen was passed does not exclude the possibility of the slow formation of organic peroxids in butter and their subsequent oxidative action.

On the assumption that organic peroxids might be slowly formed in butter, and that such peroxids might be used by the peroxidase present for oxidation, a few experiments were made in which the polarization of skim milk was observed before and after the addition of hydrogen peroxid, with and without iron. The data are summarized in Table 20. One liter of each mixture was prepared from which portions were transferred to a 200 cubic centimeter flask for clarification and polarization.

It is evident that in these mixtures containing hydrogen peroxid and iron (Nos. 18, 19, and 23) there was a very appreciable lowering in the polarization. This lowering probably was not due to the easy reducibility of the mercury by the lactose, because in those filtrates containing hydrogen peroxid but no iron (Nos. 17 and 21) there was no lowering, although these filtrates contained unprecipitated mercury as well as the others. While there may be other possibilities, the most reasonable tentative conclusion to be drawn from the results of Table 20 is that the lactose was oxidized by the action of the peroxid and iron, and if there were peroxid formation in butter such oxidation of lactose might take place there. The experiments detailed in Table 20 were not expected to be conclusive; others are undoubtedly necessary.

TABLE 20.—*Oxidation of lactose by a peroxid and iron.*

Composition of mixtures.													
Sample No.	Skim milk unpasteurized.	Hydrogen peroxid 3 per cent solution.	Water.	10 per cent ferric chlorid solution.	Toluol.	Sodium chlorid.	Precipitated and filtered.....Jan. 26 Let stand still.....Jan. 29 Polarized.....Jan. 29	Jan. 31 Feb. 3 ...do.....	Feb. 14 Feb. 17 ...do.....	.... Feb. 20	.... Feb. 23	.... Mar. 4
	C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*Grams.*	Sample—					
16	500	None.	490	None.	10	None.	No. 16..... +10.3	10.2	9.8	9.7	9.8	9.8
17	500	400	90	None.	10	None.	No. 17..... 10.3	10.3	10.1	10.1	9.6	9.6
18	500	400	65	25	10	None.	No. 18..... 3.3	6.7	5.5	3.6	3.6	3.5
19	500	300	65	25	10	180	No. 19..... 4.7	5.3	Sample lost.			

Composition of mixtures.												
Sample No.	Skim milk unpasteurized.	Hydrogen peroxid 3 per cent solution.	Water.	Sodium chlorid.	Ferric acetate powdered.	Precipitated and filtered.....Feb. 2 Let stand still.....Feb. 4 Refiltered and polarized.....Feb. 4	.... Feb. 6	Feb. 6 Feb. 9 ...do.....	.... Feb. 13	Feb. 14 Feb. 17 ...do.....	.... Feb. 17	.... Feb. 20	.... Feb. 23	.... Mar. 4
	C. c.	*C. c.*	*C. c.*	*Grams.*	*Grams.*	Sample—								
20	500	None.	500	180	None.	No. 20..... +9.2	9.4	9.0	9.0	8.9	9.1	9.2	9.2	9.2
21	500	500	None.	180	None.	No. 21..... 9.4	9.6	9.2	9.1	9.0	9.0	9.0	9.1	9.0
22	500	None.	500	180	[1] 4	No. 22..... 9.4	9.4	9.5	9.4	9.3	9.3	9.3	9.2	9.3
23	500	500	None.	180	4	No. 23..... 8.4	6.3	6.2	2.7	2.3	5.9	4.0	3.1	2.9

[1] Equivalent to 1 gram Fe per liter.

NOTE.—Readings on Ventzke scale, divided by 4, give per cent of lactose in mixture.

Löb and Pulvermacher's observation (p. 56) that dextrose is much more easily oxidized than sucrose, and that the sucrose apparently can oxidize only as fast as it is first inverted, suggests the desirability of further experiment along the lines of the work on sample No. 25, but in which the sample is soured before being used in the experiment. In the presence of lactic acid (or its combination with casein) it is very probable that the lactose present would be inverted, even if very slowly. Then the iron and oxygen would have the entire storage period of several months to bring about the slight chemical changes presumably sufficient to give butter an "off flavor."

ODORS PRODUCED IN MILK BY THE ADDITION OF IRON SALTS.

In our experiments the production of substances having a disagreeable odor and taste was the most important part of the work. No chemical change, however pronounced, that presumably did not affect the flavor of butter was of more than incidental interest. For this reason the odor of the experimental material was always carefully noted.

It was stated before (p. 60) that on adding ferrous sulfate to milk containing salt, a very strong, nauseating odor was produced. This observation had been made before in mixtures of milk, hydrogen peroxid and ferric chlorid, but the odor was not produced every time that ferric chlorid and hydrogen peroxid were added to milk. It was obviously desirable to know whether iron salts could produce undesirable odors in milk and whether the experimental conditions under which such odors were produced were in any way similar to the conditions in cold storage butter.

After several trials it was found that ferrous salts added to milk in the proportion of 1 part of metallic iron to 1,000 parts of milk would result in the production of a very powerful odor. The method of the experiment was very simple as the following example shows:

Several liters of raw milk as obtained fresh from the dealer were separated. Sodium chlorid was added to the skim milk, anywhere from 18 per cent to saturation, or the sodium chlorid may be omitted altogether. The sodium chlorid serves both as a preservative and as a normal constituent of butter curd solution. Skim milk was used because by eliminating the fat and fat soluble substances the substances causing the odor could be better determined. The sample of salted skim milk could be used at once or after several days' standing at room temperature. Portions of 300 cubic centimeters each were transferred to 1-liter Erlenmeyer flasks. To each flask there was then added a calculated quantity of the metal salt. The following salts were used in the several experiments; some of them were used very many times: Ferrous sulfate, ferrous ammonium sulfate,

ferrous lactate, ferric acetate, ferric chlorid, and to a lesser extent some salts of the following metals: Copper, manganese, aluminium, and lead.

In no case was any odor produced by any metal salt other than iron. Ferrous salts almost always, ferric salts very seldom, produced an odor in the milk. The quantities used were calculated to make 1 part of metal present in 1,000 parts of milk. When this quantity of a ferrous salt is used the odor becomes very powerful in a few minutes. However, 1 part of ferrous iron in 50,000 parts of milk could easily be shown to produce an odor plainly perceptible by several persons to whom no information had been previously given regarding the nature of the samples to be smelled. The odor develops slowly when the amount of iron is small. For 1 part of iron to 50,000 of milk an hour should be allowed.

It seems that the odor is developed at that time when the color of the mixture changes to the more highly colored ferric salt.

Although the odor strongly suggests putrefying protein or hydrogen sulfid, tests made for hydrogen sulfid were negative. The tests were made by passing a current of air through milk in which an odor had been developed by the addition of ferrous iron, and then through an alkaline solution of lead acetate.

It is doubtful whether the odor came from the fat, because skim milk was used, and it was doubtful whether lactose was the cause. It seemed probable that protein was being acted upon by the iron. In one experiment in which solutions of egg-white and of egg-yolk were used the same results were obtained as before, i. e., the addition of iron salts (ferrous sulfate and ferric chlorid) resulted in the production of a strong odor. Whether protein was acted upon in the experiments or not, is difficult to say.

On looking through the literature on the oxidation of proteins, very little was found that would throw light on the oxidation of protein by means of comparatively mild oxidizing agents. In most of the researches the protein was broken down completely by the reagents used, and the work was done for the purpose of studying either the products of the oxidation or the various oxidation stages through which the protein goes during the course of metabolism. No records were found in which the protein oxidation was studied for the particular purpose of observing whether odoriferous substances were formed. The oxidation of protein in storage butter (if it occurs at all) is probably very mild. There seems to be no apparent change in the quantity of protein in butter before and after storage.

However, two researches were found in the literature which were highly instructive.

Neuberg and Blumenthal [1] studied the oxidation products of gelatin, using ferrous sulfate and hydrogen peroxid. In the distillates from 2 kilograms of gelatin they isolated and identified isovaleraldehyde. Other volatile products were also formed. Orgler [2] repeated some of the work of Neuberg and Blumenthal, using crystallized egg albumin, copper sulfate, and hydrogen peroxid. Acetone was detected and identified in the distillates from such a mixture, which distillate, according to Orgler, had a strong fruity odor.

It follows that if the ferrous salts used in the production of an odor in milk act on some milk protein with the formation of aldehyde or ketone substances, it ought to be possible to take milk (containing salt), add a ferrous salt, and obtain from it by distillation some of the substances mentioned by Neuberg and Blumenthal and by Orgler. Two experiments were made which, while not so conclusive as to make further experiment unnecessary, gave results so much in accord with expectations that there is little doubt but what the ferrous sulfate used in the experiments caused the formation of substances which gave the iodoform test. Beyond this their chemical nature was not investigated.

THE PRODUCTION OF IODOFORM-REACTING SUBSTANCES IN MILK BY FERROUS IRON.

To an ordinary 2-liter side-arm distillation flask 1 liter of fresh skim milk was transferred (sample 42.2). The sample contained 200 grams of sodium chlorid to 1 liter of skim milk. It was quickly brought to a boil and 10 cubic centimeters distilled over. This was tested with sodium hydroxid solution (1:3) and iodin solution without heating for substances giving the iodoform test.[3]

The test was positive. The above procedure was repeated on some of the same sample of milk under the same conditions, except that 5 grams of the crystallized ferrous sulfate (Fe 1 : 1 000) was added just before distilling. The distillate gave a much stronger iodoform test; that is, there was a very appreciable immediate precipitate of iodoform. A second sample of skim milk was distilled as before. This sample (No. 37x) was obtained from the same dealer as the previous sample (sample 42), and after being skimmed they probably did not differ very materially in their composition. At the time of the experiment this sample (37x) was raw skim milk containing 30 per cent of sodium chlorid (300 grams of salt to 1 liter of milk) and 1 part of iron as ferrous sulfate to 5 000 parts of milk.

[1] Neuberg, C., and Blumenthal, F. Über die Bildung von Isovaleraldehyd und Aceton aus Gelatine. Beiträge zur Chemischen Physiologie und Pathologie, vol. 2, no. 5–6, pp. 238–250. Braunschweig, May, 1902.

[2] Orgler, Arnold. Über die Entstehung von Aceton aus krystallisiertem Oralbumin. Beiträge zur Chemischen Physiologie und Pathologie, vol. 1, no. 10–12, pp. 583. Braunschweig, January, 1902.

[3] Mulliken, Samuel Parsons. Identification of Pure Organic Compounds. See vol. 1, p. 166.

The salt and iron were added on the same day the sample was received. After remaining at room temperature in the laboratory for 12 days, during which time several liters were used for other purposes, 3 liters of this sample were transferred to a 7-liter bottle and oxygen was blown through it slowly for 4 days (96 hours). One liter of this was distilled. The distillate (10 c. c.) yielded more iodoform than either of the other two. Judging by inspection, on distilling fresh skim milk a very small but distinctly perceptible quantity of iodoform-reacting substance was obtained; fresh skim milk and iron yielded more, and skim milk and iron first saturated with oxygen yielded most. There could be little doubt about the relative amounts of iodoform, but the experiment was repeated on portions of the same samples as before for the purpose of estimating quantitatively the iodoform obtained.

Each distillation was continued until six 10 cubic centimeter portions of distillate were obtained. Iodoform tests on these were made by adding to each portion contained in a test tube 10 drops of sodium hydroxid solution (1 : 3) and sufficient iodin solution to insure a slight excess. In every case precipitates of iodoform were obtained almost immediately and without the aid of heat. Very little iodoform, if any, was obtained in the last 10 cubic centimeters of distillate.

The iodoform was washed first by decantation, then transferred to filter papers and washed till the filtrates gave only a very faint cloud with silver nitrate solution. Some of the iodoform was, of course, lost through solution in the wash water, but the relative amounts in this case rather than the absolute were just as desirable. The amount lost in this way probably was small compared with the amounts present. The blank determination (No. 42.3) was lost. It contained a distinctly perceptible precipitate, but too small in amount to compare with either of the other two. For the estimation of the iodoform a method given in the Pharmaceutical Journal, page 555, volume 82, 1909, was used. The iodoform on the filter papers was dissolved in alcohol and ether and the solutions received in 300 cubic centimeter Erlenmeyer flasks. To these flasks and to controls 1 cubic centimeter nitrous acid (fuming nitric acid) and 50 cubic centimeters approximately tenth normal silver nitrate solution were added. The mixtures were heated on the steam bath over night. The silver still remaining in solution was estimated with standard ammonium sulfocyanid solution, using ferric ammonium sulfate as indicator.

The distillate from the fresh milk containing ferrous sulfate (No. 42.4, Fe 1 : 1,000) yielded 15.4 milligrams of iodoform; the distillate from the 12 days' old milk containing ferrous sulfate (No. 37x, Fe 1 : 5,000) and saturated with oxygen gave 54.7 milligrams of iodoform.

These amounts of iodoform correspond in the titrations to 1.37 cubic centimeters in the first and to 4.88 cubic centimeters of silver nitrate solution (N/11.7) in the second determination.

While these results do not prove that the iodoform was obtained from oxidation products of milk protein, they do prove the possibility of such oxidation. By distilling such mixtures under low pressure and at low temperature to remove the possible objection that the temperature of distillation is not the temperature at which chemical changes take place in storage butter, the identity of the iodoform-reacting substances could without doubt be ascertained.

Whether the small amounts of iron ordinarily present in butter (see p. 54) can slowly bring about the same kind of a change that larger amounts of iron bring about in milk in a very much shorter time is to be determined by future investigation.

SUMMARY.

The failure of previous investigators to find evidences of proteolysis in cold-storage butter may have been due to difficulty in obtaining proper precipitations in the curd solution.

Methods of analysis have been perfected which permit the use of large samples and show the first stages in the proteolysis. This method gave no evidence of an increase in soluble nitrogen in butter on long standing at 0° F., even when the conditions of the manufacture were most favorable to such changes.

Buttermilk from sweet unpasteurized cream and from sweet pasteurized cream when preserved with 18 per cent sodium chlorid to correspond to butter-curd solution showed no proteolysis during a long period in cold storage.

Bacterial enzym held in cold storage in milk containing 18 per cent of sodium chlorid gave some evidence of proteolysis. The action of pepsin and trypsin under similar conditions was not completely inhibited.

Butter made from sweet pasteurized cream keeps much better than butter made from similar cream without pasteurization, but the changes in the unpasteurized cream butter can not be reproduced by reinoculating the pasteurized cream with the bacteria of the cream before pasteurization.

By means of specially designed apparatus exact analysis was made of the gases contained in butter. About 10 per cent, by volume, of fresh butter is gas consisting approximately of nitrogen (by difference) 33 per cent, oxygen 20 per cent, and the remainder of gases absorbable by sodium hydroxid. The oxygen was materially less after storage.

The addition of iron to the cream even in as small an amount as one or two parts per million parts of cream has an influence on the

flavor of the butter. This work gives nothing to show that the nature of the flavor is appreciably changed, but the rate of development is accelerated.

The cream may take up iron in quantities sufficient to affect the flavor from rusty cans or even from the exposed boltheads or other metal parts of the churn.

The action of copper is similar but perhaps more intense.

It was found that in milk to which 18 per cent sodium chlorid had been added there was no change in the lactose when iron was added and a current of oxygen passed through the milk for 72 hours.

A strong odor may be produced in milk by the addition of small amounts of iron salts. The ferrous salts are more active than the ferric salts.

The iodoform test is much stronger in distillates from milk containing ferrous sulfate.

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