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THE PROTEIDS OF BUTTER IN RELATION TO MOTTLED BUTTER.

L. L. VAN SLYKE AND E. B. HART.

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BULLETIN No. 263.

THE PROTEIDS OF BUTTER IN RELATION TO MOTTLED BUTTER.

L. L. VAN SLYKE AND E. B. HART.

SUMMARY.

1. Points investigated. The questions studied in this bulletin are: (1) The relation of casein compounds to cream-ripening; (2) casein compounds present in butter and buttermilk; (3) the relation of casein compounds to mottled butter.

2. Casein compounds in ripened cream. In ordinary methods of cream-ripening, neither calcium casein nor free casein is present, but only casein lactate, when the lactic acid is allowed to exceed 0.5 per ct. Casein lactate is the substance most familiar as curdled sour milk.

3. Casein compounds in butter and buttermilk. When the amount of lactic acid in cream exceeds .5 per ct., the casein in the butter and buttermilk is present as casein lactate. In butter and buttermilk made from so-called sweet cream, we usually find calcium casein and some free casein, and on standing for some weeks these may be changed in the butter into a mixture of free casein and casein lactate or wholly into casein lactate.

4. Views commonly held in respect to cause of mottled butter. It has been quite universally believed that the light spots or streaks in butter, known as mottles, are caused by the uneven distribution of salt, the more concentrated brine deepening the yellow color of the fat, and the lighter portions being the unsalted or lightly salted areas.

5. Points studied in relation to mottled butter. The

investigation covered the following conditions in relation to the mottling of butter: (1) Richness of cream, (2) degree of ripeness of cream, (3) temperature of churning, (4) size of butter-granules, (5) temperature of wash-water, (6) working of butter.

6. Results of investigation of preceding conditions. When the churning was managed so as to make the butter-granules of the size of rice-grains and these were carefully washed twice with water below 45° F., removing most of the buttermilk adhering to the outer surface of the granules, no mottles were obtained, however conditions were varied in other respects. Mottles were always found when the buttermilk was not sufficiently removed.

7. Relation of proteids to mottled butter. The amount of proteid (casein lactate) in mottled butter is greater in the light portions than in the darker portions, and is the cause of the lighter color of the mottles.

8. Relation of salt to mottles. (1) Salt brine does not change in any way the color of butter-fat. (2) Salt brine, as it commonly occurs in butter, has the power of hardening and localizing the proteid particles, the action requiring several hours for completion. (3) Butter, free from buttermilk adhering to the outer surface of the granules, does not produce mottles when salted, whether the salt is evenly or unevenly distributed. (4) Mottles do not occur in unsalted butter. (5) In mottled butter, the light portions usually contain less salt than the darker portions.

9. Cause of mottled butter. Mottles in butter are due, primarily, to the presence and uneven distribution of buttermilk adhering to the outer surface of the small granules; and, secondarily, to the hardening and localizing effect of salt brine upon the proteid of the buttermilk thus retained in butter. The light portions of mottled butter owe their lighter color to the presence of localized proteid (usually casein lactate).

The yellow or clear portions occur where the spaces between the butter-granules are filled with clear brine and are comparatively free from casein compounds. Several hours are required to complete the action of the brine upon the proteid of the butter.

10. Prevention of mottles in butter. Mottles in butter can be prevented by avoiding those conditions that retain buttermilk in the butter and observing those conditions that favor the removal of buttermilk from butter-granules before salting. The butter-granules should be about the size of rice-grains and should be washed twice with water at a temperature of 35° to 45° F.

INTRODUCTION.

In connection with our study of the action of acids upon milk-casein (calcium casein), it occurred to us that the results might have an application in some of the stages of butter-making. We have clearly established the fact that two distinct substances are formed in succession when calcium casein is brought into contact with an acid, whether it be the lactic acid produced in milk by the fermentation of milk-sugar, or some other acid such as acetic, hydrochloric or sulphuric. When a small amount of acid is added to milk or to a preparation of calcium casein, a precipitated substance unlike calcium casein is formed, this substance being soluble in warm 5 per ct. solution of sodium chloride and also in hot 50 per ct. alcohol, and possessing characteristic properties of plasticity and ductility. This substance we were at first led to regard as a compound formed by direct combination of casein and acid and we called it a casein mono-salt of the acid used as precipitant; but we have recently shown (Bulletin No. 261) that the compound is base-free casein.

When this body is treated with an additional amount of acid a substance is formed which is insoluble in warm 5 per ct. salt solution and nearly so in hot 50 per ct. alcohol and which has lost the plastic and ductile properties that characterise free casein.

This substance we formerly regarded as a casein di-salt of the acid used to form it, in the belief that there were two series of precipitated salts formed by casein with an acid. Since our later work shows that there is only one series, this substance we now regard as simply a casein salt of the acid used as precipitant

The compound we formerly designated as casein monolactate is free casein or simply casein, and the body we previously called casein dilactate is casein lactate.

Since the presence of one or both of these compounds is necessarily involved in all operations where milk undergoes the change of ordinary souring, it seemed desirable to make a special study of the following points:—(1) What, if any, relation these compounds might have to the ripening of cream, preliminary to

butter-making; (2) which of these compounds is commonly present in butter and buttermilk; and (3) whether the occurrence of what is known as "mottling" in butter has any relation to either of these casein bodies.

THE RELATION OF CASEIN AND CASEIN LACTATE TO CREAM-RIPENING.

We wished first to ascertain what form of casein compound was obtained in the ripening of cream, when the cream showed different percentages of acid. Three series of experiments bearing on this point were made. Some fresh, pasteurized cream was treated with a carefully prepared starter in erlenmeyer flasks, stoppered with cotton. These were allowed to stand at room temperature, samples being withdrawn for examination from time to time. The results of these experiments are given in the following table:

TABLE I.—SHOWING CHEMICAL CHANGES IN CREAM-RIPENING.

No. of experiment.	Age of cream when analyzed.	Amount of milk-sugar in cream.	Amount of milk-sugar changed.	Amount of lactic acid formed.	Total amount of nitrogen in cream.	Nitrogen in cream in form of casein and its compounds.	Nitrogen in cream in form of casein lactate.
	Hours.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	Fresh	3.64	——	0.15	0.39	0.32	0.00
1	8	3.58	0.06	0.18	0.39	0.31	——
1	24	2.92	0.72	0.60	0.38	0.33	0.33
1	32	2.90	0.74	0.61	0.39	0.30	0.30
1	48	2.88	0.76	0.62	0.39	0.32	0.32
1	72	2.90	0.74	0.63	0.38	0.34	0.34
2	Fresh	3.84	——	0.14	0.41	0.34	0.00
2	24	3.04	0.80	0.64	0.40	0.35	0.35
3	Fresh	3.81	——	0.14	0.41	0.35	0.00
3	24	2.93	0.88	0.70	0.40	0.36	0.36

The following statements are suggested by the data contained in Table I:

(1) *Nitrogen compounds in ripened cream.*—When the cream was fresh and when it was eight hours old, there was only calci-

um casein (milk-casein) present; that is, neither of the compounds (free casein and casein lactate), formed from calcium casein by treatment with lactic acid, was present. In 24 hours the only casein compound present in the cream was casein lactate, the compound insoluble in warm dilute salt solution; this is the compound commonly observed in milk curdled by ordinary souring. In each of these experiments the cream had curdled completely to a definite coagulum. It would therefore appear that in ordinary methods of cream-ripening, when the lactic acid is allowed to reach 0.6 per ct., neither calcium casein nor free casein is present, but only casein lactate, the substance most commonly observed as curdled sour milk.

(2) *Decrease of milk-sugar in cream-ripening.*—In the three experiments the milk-sugar decreased to an extent varying from 0.72 to 0.88 per ct. in 24 hours. In the first experiment, which was continued 72 hours, the amount of milk-sugar decreased very little after 24 hours.

(3) *Formation of lactic acid in cream-ripening.*—When the cream was fresh, the apparent amount of lactic acid was only 0.14 or 0.15 per ct., which increased in 24 hours to 0.6 to 0.7 per ct. In the first experiment, which was continued 72 hours, the acid increased very little after 24 hours.

In connection with these experiments it may be mentioned that, in several lots of cream ripened so as to show an acidity of .38 to .43 per ct., we found much free casein and little casein lactate.

THE ACIDITY OF MILK AND CREAM.

It may be well in this connection to call attention to the inaccuracy involved in attributing the acidity of milk or cream to lactic acid alone. The usual method of determining acidity in milk or cream is to titrate a given amount of milk with a standardized solution of fixed alkali, using phenolphthalein as indicator, the alkali being added to the milk until a fairly permanent pink color appears. Lactic acid is not the only compound in milk that neutralizes alkali. It is well known that strictly fresh milk which contains no lactic acid, neutralizes an appreciable amount of alkali. The compounds in fresh milk or cream that have the power of neutralizing alkali are the following: (1)

Calcium casein (milk-casein), (2) acid phosphates and citrates, and (3) carbon dioxide. Of these, the calcium casein and acid phosphates appear to be most prominent in neutralizing alkali. While results vary with individual cows, the average amount of acidity of fresh milk is about 0.08 per ct., calculated as equivalent to lactic acid. If one desires to estimate more closely the amount of real lactic acid in milk, it is necessary only to subtract from the results commonly found .1 per ct. Just how much deduction should be made in case of cream, it is more difficult to say. For ordinary work in creameries, such distinctions are not essential.

Therefore, in the results given in Table I, we have not stated the absolute amount of lactic acid present in the cream; but the results, in the form given, are more readily comparable with the results obtained in creamery work.

THE RELATION OF CALCIUM CASEIN, CASEIN AND CASEIN LACTATE TO THE PROTEIDS OF BUTTER AND BUTTERMILK.

The curd present in butter is casein lactate, when the amount of lactic acid formed in cream ripening exceeds 0.5 per ct. In butter made from cream ripened so as to contain less than 0.5 per ct. of acid, the same compound of casein is apt to be present ultimately in the butter, especially if buttermilk is left in the butter to any extent; because the milk-sugar, present in the buttermilk remaining in the butter, is changed in time to lactic acid, which acts upon any calcium casein or free casein in the butter, producing finally the compound usually present in butter made from well-ripened cream.

In butter made from sweet cream, we find in the butter essentially calcium casein with, perhaps, some free casein. With sufficient milk-sugar incorporated in such butter through the presence of buttermilk, we may have at different times any one, or a mixture, of the compounds of casein, as follows:—(1) calcium casein, (2) calcium casein and free casein, (3) free casein and casein lactate, and (4) casein lactate. It is hardly probable that we should often find calcium casein alone in sweet-cream butter, as it is commonly made; though it would be possible to

make the butter so that it would contain only calcium casein. In ordinary sweet-cream butter, when fresh, we commonly find a mixture of the two forms, calcium casein and free casein. Later, after the formation of more lactic acid, we may have free casein alone, which with increasing amounts of lactic acid will be gradually changed into casein lactate, the form commonly present in commercial butter made from ripened cream.

The amount of milk-albumin in normal butter is very minute under any conditions.

The curd of different butters and of the same butters at different ages presents quite different appearances. For special examination of its properties, the curd of butter is obtained by dissolving the fat in ether, or by melting the butter at as low a temperature as practicable and allowing the curd and brine to settle beneath the layer of butter-fat. The curd prepared from fresh butter, made from cream having an acidity of .5 or .6 per ct., is gelatinous in appearance and does not readily separate into small particles on standing. The curd of butter that is a few weeks old often appears granular. This is due, as we shall point out later, to the action of salt upon the physical properties of the casein lactate.

THE RELATIONS OF PROTEIDS OF BUTTER TO THE MOTTLING OF BUTTER.

The subject of mottled butter has attracted more or less attention and has been much discussed in dairy papers. However, the literature relating to actual investigations of mottled butter is very meagre. The only available work done in this country is described in Bulletin No. 64, Maryland Agricultural Experiment Station, by C. F. Doane, on "A Study of the Cause of Mottled Butter;" and in Bulletin No. 80 of the Iowa Agricultural Experiment Station, by G. L. McKay and C. Larsen, there is a brief treatment of "Gritty Salt as a Cause of Mottles." In order to ascertain the consensus of dairy authorities on this subject, the following inquiries were sent to the most prominent teachers of dairying in our country:

1. Does mottling of butter occur at one particular time of year more than another? If so, when? Why?

2. What different kinds of mottling do you recognize?
3. What do you regard as the causes that produce mottling?
4. Has ripening of cream anything to do with mottling? If so, how?
5. Does the temperature of churning influence mottling?
6. Does the temperature of the wash-water influence mottling?
7. Does the size of granules influence mottling?
8. Does salt influence mottling?
9. Has the butter color used anything to do with mottling?
10. Can you control effectively the mottling of butter? If so, how?
11. What is your theory in regard to the mottling of butter; that is, the causes producing it and the manner in which they produce it?
12. Are butter-makers in your section troubled with mottling so far as your observation goes?

It is a matter of much interest to consider the replies received in answer to these questions.

1. *Does mottling of butter occur at one particular time of year more than another? If so, when? Why?*

In most cases it is reported that mottling occurs more frequently in cold weather, because it is more difficult to distribute the salt evenly through the butter, owing to the lower temperature of the wash-water and the greater firmness of the butter.

2. *What different kinds of mottling do you recognize?*

There is considerable variation in the replies made to this question. Summarizing all the different statements, the question is answered as follows: (1) An uneven distribution of color showing in small patches, (2) streaking or wavy mottles, (3) white specks, and (4) dapples. In most cases the expression indicates only one kind of mottling but varying in form and showing as streaks, waves or cloudiness.

3. *What do you regard as the cause of mottling?*

Every reply indicated the uneven distribution of salt in butter as the chief or sole cause of mottling. Additional or contributory causes are given as follows: (1) Uneven cream-ripening, (2) pasteurizing sour cream, (3) insufficient working, (4) chilled granules worked too soon.

4. *Has ripening of cream anything to do with mottling? If so, how?* The general opinion is that cream-ripening has no relation to the mottling of butter, except that white specks in butter are often caused by over-ripe cream.

5. *Does the temperature of churning influence mottling?*

The general belief expressed is that churning has little or nothing to do with mottling. The following special suggestions are made: (1) Some temperature conditions of churning make difficult the even distribution of salt and so contribute to mottling. (2) Too low or too high churning temperature may interfere with proper working of butter and consequent distribution of salt, thus favoring the formation of mottles. (3) If butter is made too hard the salt cannot be easily distributed uniformly. (4) Over-ripe cream churned at too high temperature produces mottles.

Summarizing these statements, it is the prevailing belief that the temperature of churning may affect the mottling of butter, when the uniform distribution of salt is made difficult, especially churning at low temperature.

6. *Does the temperature of the wash-water influence mottling?*

Some state that the temperature of the wash-water has no influence upon the mottling of butter. Several hold that the use of water so cold as to harden the butter considerably would cause mottling by preventing the uniform distribution of salt. One states that mottles are produced whenever the wash-water is colder than the butter. Another claims that mottles are unusual when the wash-water, salt and butter are of the same temperature.

7. *Does the size of granules influence mottling?*

The general belief expressed is that there is no relation between the size of butter-granules and mottling. One states that large granules, when hard, favor mottling; but, when the butter is soft, the size of granules makes no difference. Two state that small granules are preferable since large granules favor mottling.

8. *Does salt influence mottling?*

The expression is unanimous that the uneven distribution of salt is the chief, if not the sole, cause of mottling.

9. *Has the butter color used anything to do with mottling?*

All state that butter color properly used is not, in their experience, ever a cause of mottling.

10. *Can you effectively control the mottling of butter? If so, how?*

Summarized in the briefest expression, the belief of all is that

mottles can be prevented by proper working of butter, that is, by effecting a complete and uniform distribution of salt through the mass of butter. Described in more detail, the even distribution of salt and the resulting control of mottles can be accomplished by churning between 52° and 55° F. to granules the size of wheat grains, drawing off buttermilk, washing once with water below 50° F., draining, salting and allowing to stand 2 to 4 hours before working.

11. What is your theory in regard to the mottling of butter, that is, the causes producing it and the manner in which they produce it?

All answers agree in assigning as the main cause of mottles in butter the uneven distribution of salt or brine as the result of insufficient or inefficient working. The manner of production is generally explained by the statement that salt or brine intensifies or deepens and so changes the color of butter, the light parts containing less salt or brine than the darker portions. One holds the belief that there is more moisture in those portions of butter containing more salt and this produces change of color.

12. Are butter-makers in your section troubled with mottling so far as your observation goes? There is more or less trouble but less than formerly, owing to the better education of butter-makers.

The general teaching of today about the mottling of butter is well summarized by Mr. H. Hayward in Circular No. 56 of the U. S. Department of Agriculture, Bureau of Animal Industry, entitled "Facts concerning the history, commerce, and manufacture of butter;" and we quote from this circular, pp. 185–6:—"One of the serious defects often found in butter is lack of uniformity of color, or what is commonly known as 'mottles.' This defect is seen in white streaks, spots or blotches, which are most pronounced when a lump of butter is cut so as to show a broad, smooth surface. If this cut surface is held at a proper angle to the light, any lack of uniformity in color will be plainly noticed. So serious is this defect considered that butter otherwise perfect, but mottled, is graded as second class in the large markets. The causes to which this fault can be attributed are, first, particles of curd, differing in size, incorporated in the but-

ter, and, second, an uneven distribution of salt. Mottles in creamery butter are seldom caused by specks of curd, but in the poorer classes of dairy butter this kind of mottles is not infrequently seen. They are most likely to occur when the cream from which the butter is made is thin and allowed to ripen without being stirred, or when it is over-ripened without being strained. The cream being churned under these conditions, lumps of coagulated cream are incorporated in the butter, and as the casein does not take the butter color the result is a product full of white specks. When the trouble is caused in this way it can be obviated by washing the butter twice in a weak brine after the buttermilk is thoroughly drained off. After the last washing, instead of draining the brine from the butter, as is usually done, the butter should be dipped out of the brine with a hair or wire sieve ; the specks of curd, being heavier than butter or water, will have sunk to the bottom of the churn.

" Most of the mottles found in butter, however, are caused by an unequal distribution of salt. When the wash-water is considerably colder than the butter-granules the exterior of the latter become harder than the interior ; this prevents an equal absorption of the salt when the butter is salted and worked, and mottles result. Also, when thin cream is churned at a low temperature the butter usually comes in round, shot-like granules ; on account of being round and quite firm it is with difficulty that the salt is equally distributed and, unless great care is exercised, the finished butter is mottled. Of course, if under the most favorable conditions the butter is not worked enough to distribute the salt equally, mottles will be noticed in the finished product.

" Mottles may be prevented, then, by avoiding high temperatures in ripening cream, by frequent stirring during ripening, by straining the ripened cream into the churn, by avoiding exposure of the butter to temperatures too low while in granular form (which causes a difference between the interior and exterior of the butter-granules) and by working the butter sufficiently to cause an equal distribution of the salt."

It is very noticeable that in all the dairy literature which has come to our hand there is no suggestion that the proteids present in the cream have any connection with mottles in butter,

except that single form which comes from curdled casein specks or particles ; but this form is of so infrequent occurrence, compared with the other form, that many teachers do not apply the term mottles to the discoloration caused by curd particles. It is to the other form of mottles that we have given our attention, that is, the form characterized by light-colored streaks, waves or large patches. It should be stated that Doane in the bulletin already referred to considered the relation of casein (casein lactate) to mottled butter but concluded that this proteid had no relation to the light-colored streaks of mottled butter.

Storch[1] appears to attribute the formation of mottles to an excessively large number of minute liquid drops of what he calls "watery mucoid substance," the difference in the size and number of these liquid drops being caused by the action of special micro-organisms during the ripening of cream. This theory lacks satisfactory demonstration and has not been received with favor.

GENERAL METHOD USED IN MAKING BUTTER IN EXPERIMENTS.

Before giving the detailed results of our work, we will give an outline of the method employed in making the butter used in our experiments. In carrying on some of the practical details of this work, we are indebted for coöperation to Mr. Geo. A. Smith, Dairy Expert. The butter-making was carried on as follows, except when otherwise specified in individual experiments :— The cream was pasteurized and contained 28 to 30 per ct. of fat. A carefully prepared starter was used in ripening the cream, the degree of acidity being made to vary in different experiments, but in most cases amounting to 0.6 to 0.7 per ct. The temperature of churning was commonly at 50° to 55° F. The churning was stopped when the granules were about the size of grains of rice, except in those cases where the churning was purposely carried beyond this point. After the buttermilk was removed from the granules, they were treated twice with water at 40° to 45° F., being allowed to drain well after each washing. We were careful to keep the temperature of the granules down during the washing

[1] *The Analyst*, **22** : 206–7 (1897).

so that they would not adhere and form lumps but retain their individuality. The completeness of washing the granules can be effected by adding the water in the churn and giving the churn a few revolutions. For conditions of normal work we took pains to remove the buttermilk as completely as possible from the granules. The butter was then salted at the rate of one ounce of salt for one pound of butter, and worked at once in some cases or allowed in others to stand a varying length of time before working, from a few minutes to one or two hours, according to convenience.

RELATION OF RICHNESS OF CREAM TO MOTTLED BUTTER.

Cream was separated so as to contain 40 per ct. of fat, and from a portion of this we made cream containing 20 per ct. of fat by adding skim-milk. The creams were handled under the same uniform conditions. The churning temperature was 50° to 52° F.; the granules were treated twice with wash-water at 45° F.; salt was added at the rate of one ounce to a pound of butter. When the butter-granules were about the size of grains of rice, there were no mottles in either case but when the butter was over-churned and gathered in chunks, mottling took place. In the case of the rich cream, when over-churned, the butter was pasty and the mottles occurred in large patches, the whole mass of butter being rather light-colored from the presence of an excess of buttermilk. The richness of cream does not appear to have any influence in causing mottles in butter provided it is handled in a normal manner during the process of butter-making. More care needs to be exercised in churning rich cream to prevent over-churning than in case of cream poorer in fat.

RELATION OF RIPENESS OF CREAM TO MOTTLED BUTTER.

We churned creams having acidity of .22 and .25 per ct. and also some of the same creams ripened to acidity of .4 and .65 per ct. These were all churned at 55° F. and the granules washed twice with water at 40° F. The sweet cream was churned so as to come in fine granules and in lumps as large as hickory nuts or larger. The ripened cream was similarly treated. The butters were salted in the usual way. In each case, mottles were obtained in the butter in the case of the over-

churned cream and no mottles in the butter made from granules of the usual size. In numerous other experiments, we have varied the acidity of the ripened cream from .38 to .78 per ct. and obtained butter without mottles except as the result of some condition other than that of the acidity of the cream used.

In one case, we added a starter to some sweet cream and churned at once, churning at 52° F. and washing the butter-granules twice with water at 45° F. Salt was added in the usual amount. In one case, the granules were fine and produced butter without mottles, while in another case the granules were over-churned into lumps and mottled butter resulted.

The matter of white specks of curd in butter, as the result of over-ripened cream, we have not touched at all in our work, since all the facts in connection with this trouble have been well known for some years.

RELATION OF TEMPERATURE OF CHURNING TO MOTTLED BUTTER.

The temperature of churning was usually kept between 50° and 55° F. but variations were made in special experiments, ranging from 43° to 61° F. Churning at higher temperatures favors the production of mottles to some extent, since overchurning takes place more readily at higher temperatures and the butter-granules aggregate into larger masses more easily.

RELATION OF SIZE OF BUTTER-GRANULES TO MOTTLED BUTTER.

We controlled the operation of churning so as to produce butter-granules and aggregations of these varying in size. In our work we have designated the size of the granules as follows: Rice-grain size, pea-size (about a quarter inch in diameter), peanut size (about half an inch in diameter), hickory-nut size (about three-fourths inch in diameter) and walnut size (an inch or more in diameter).

In general, when the butter-granules were the size of rice grains at the close of churning, it was easy to make butter free from mottles by washing the granules twice with water at a temperature below 50° F., preferably at 40° F. But in several trials

when the churning was made at 55° to 60° F. and the granules (rice-fine) were washed with water at 60° F., the butter was badly mottled since the granules adhered and formed lumps at that temperature before the washing could be completed. In several cases, we obtained very slight mottles when the temperature of the wash-water was 50° to 52° F., the granules being rice-fine.

When the granules of butter were about the size of peas, there was no difficulty in making unmottled butter, provided the temperature of the wash-water was kept down to 40° F. When the wash-water was at 52° F., we had traces of mottles. When the butter-granules were allowed to get as large as hickory nuts, mottled butter resulted in nearly every case, even when all other conditions were favorable to the production of unmottled butter. If, however, the buttermilk was once well removed, we found that aggregation of granules did not produce mottles. In several experiments we churned well-washed butter-granules in water at a temperature of 60° F. and allowed them to aggregate into lumps the size of walnuts and no mottles were found in the finished butter as a result of this treatment.

It would, therefore, appear that any condition favoring the production of large aggregations of granules of butter *before removal of buttermilk*, such as overchurning, churning at higher temperatures, using wash-water at 60° or above, will favor mottling, In other words, failure to remove buttermilk from the butter-granules favors the production of mottles.

In this connection, it may be well to consider the manner in which buttermilk is retained in butter. We may for practical purposes regard buttermilk in butter as being present in two different relations. In the first relation the buttermilk is held enclosed in every individual granule of butter, and in the second relation it adheres mechanically to the outer surfaces of each granule. The buttermilk held within the minute granules can not readily be removed, while that adhering to the outer surfaces can be practically all removed by proper treatment of the granules with cold water. It is the buttermilk adhering to the outer surface of the small granules that we must consider in relation to mottles.

RELATION OF TEMPERATURE OF WASH-WATER TO MOTTLED BUTTER.

Best results were obtained by washing the butter-granules with water at about 40° F. or even lower. Water at a temperature of 60° F. or above tends to make the granules adhere to one another and form larger masses, thus tending to prevent a sufficiently complete removal of buttermilk. It is important that the wash water be cold enough to keep the granules separate during the treatment with water.

RELATION OF WORKING BUTTER TO MOTTLED BUTTER.

When butter came in rice-fine granules and these were properly washed with cold water, working had no influence in producing mottles. When the butter was overchurned, or allowed to form large aggregations before removal of buttermilk, mottles were present whether the butter was worked or not. In the case of the unworked butter, the mottled patches were much larger than in the worked. In one case, a badly-mottled butter was worked 12 times in an effort to remove the mottles. The result was simply to destroy the grain of the butter and reduce the mottles to a smaller size, distributing them more uniformly thoroughout the mass of butter but not removing them. In an another case the granules were divided, one portion being worked only the usual amount, with mottles resulting. The other portion was worked several additional times, still preserving the grain, and the appearance of mottles was lessened, but the white portions were simply distributed more uniformly and the whole butter appeared lighter in color than normal.

RELATION OF SALT TO MOTTLES.

When the butter was not salted, there were no mottles, even when butter was overchurned and the granules were aggregated into lumps as large as walnuts ; but in such cases the butter contained an excess of buttermilk and was much lighter in appearance than normal butter or than butter from the same cream would be when properly made and salted.

When the butter-granules were obtained in size of rice-grains and these were washed free from adhering buttermilk, mottles were not produced by the addition of salt even in very unequal

proportions through the mass of butter. For example, some well-washed granules were placed in a butter-mold and then a layer of salt sprinkled on the layer of granules and then alternately layers of granules and salt. The cake of butter produced by this extreme treatment showed no mottles.

In one experiment, some unsalted butter, entirely free from any appearance of mottles, was cut into chunks and these were partly submerged in saturated salt solution for some hours. The portions of butter not covered by the brine were unchanged, while there was a very marked appearance of mottling on the surface of the submerged portion of butter. The appearance of mottles did not extend into the mass of butter but was confined to the surface in contact with the brine.

THE CAUSE OF MOTTLES IN BUTTER.

The fact that we have no mottles in butter when we have no salt has led to the belief that the salt is the sole cause of mottles and produces mottles as the result of its uneven distribution. It is held that the salt deepens the color of the butter-fat, the lighter portions containing little or no salt, the darker portions containing salt.

Effect of salt on color of butter-fat.—We desired to test experimentally the question whether salt brine has any influence on the color of butter-fat. We took some fresh, colored, unsalted butter, melted and filtered it, in order to separate the butter-fat from the other constituents of the butter. The filtered portion was allowed to harden and then cut into cubes and immersed in 30 per ct. brine in such a way that a part of the cube was in brine and a part out. There was not the slightest increase of depth of color or any other change in color noticeable.

In another experiment, some butter-fat was stirred with salt. The mixing produced a variegated color, not a true mottling, due to the grains of undissolved salt. The same effect is produced by mixing any white substance, sugar for instance, with butter-fat.

It would, therefore, appear that the mottling of butter is not produced by salt in the way generally held, viz., by affecting the color of the butter-fat itself.

Amount of salt in mottled aud unmottled butter.—We took samples of butter by means of a trier, taking a plug through the ends of the prints examined. In one sample of unmottled butter, the amount of salt at opposite ends of a pound print was 3.65 and 2.61 per ct. In another unmottled butter, the salt varied from 4.6 to 5.3 per ct. ; and in still another, from 1.86 to 2.59. In some badly mottled butter, the salt at different ends of some prints varied from 5.13 to 5.19 and 4.66 to 4.73, which showed very even distribution of salt. We may, then, have mottles where the salt is evenly distributed, and no mottles when the salt is unevenly distributed. Samples taken in the manner indicated by a trier give only the average of the mass but do not enable one to distinguish the amounts of salt in the lighter and darker portions separately.

Amount of salt in light and dark portions of butter.—It is a matter of common experience that the lighter portions of mottled butter appear to the taste to contain less salt than the darker portions. We examined two samples of mottled butter, determining the amount of salt in the different colored portions when the butter was 24 hours old. The butter was somewhat overchurned purposely to produce conditions for mottling. The washing, salting and working were done in the usual way. The two butters gave the following results :

Butter 1	Light	portion	0.91	per ct.	of salt.
" 1	Dark	"	5 45	"	"
" 2	Light	"	0.70	"	"
" 2	Dark	"	6.74	"	"

These results agree with the sense of taste in showing that the lighter portions contain less salt than the darker portions of butter.

Distribution of brine in butter on standing.—In order to learn to what extent the salt of butter becomes more evenly distributed on standing, we determined at intervals the salt in the different colored parts of some butters referred to in the preceding paragraph. The results are as follows :

			24 hours.	48 hours.	5 days.		
Butter 1	Light	portion	0.91	1 79	1.40	per ct.	salt.
" 1	Dark	"	5.45	5.94	6.22	"	"
" 2	Light	"	0.70	1.69	1.69	"	"
" 2	Dark	"	6.74	6.04	6.22	"	"

These results indicate that after 24 hours, the tendency of the salt to distribute itself more uniformly through the mass of butter is not very marked. The differences indicated may come mostly from variation in the samples examined.

The amount of proteid in mottled and unmottled portions of butter.—The amount of proteid was determined in mottled and unmottled portions of several butters and the results are as follows:

No. of experiment.	Per ct. proteid in light portion of butters.	Per ct. of proteid in dark portion of butters.
1	1.30	.76
2	.76	.14
3	.76	.36
4	.63	.25
5	.86	.31
Average...	.86	.36

These results indicate that the light portions of butter contain more casein lactate than the deeper-colored portions. This casein lactate comes from retention of buttermilk. It is generally noticed that in working butter the liquid coming from the worker is turbid and milky when the buttermilk has not been removed, while it is more or less clear when the buttermilk has been properly washed out of the granules before salting and working.

The amount of water in mottled and unmottled butters.—The determination of water in mottled and unmottled butters gave the following results:

	Per ct. of water in mottled butter.	Per ct. of water in unmottled butter.
	13.03	12.87
	13.00	10.52
	11.97	10.50
	15.35	12.00
	15.50	13.30
Average.....	13.77	11.84

The mottled butters vary greatly among themselves as well as the unmottled in respect to content of water. The larger amount of water in these mottled butters is the result of the retention of more buttermilk.

Properties of proteid in butter and buttermilk.—The chief proteid of butter made from ripened cream is casein lactate. In fresh, sweet-cream butter, the chief proteid is calcium casein (milk-casein) with more or less free casein. On standing, these constituents may change, forming more largely a mixture of free casein and casein lactate and, if milk-sugar is present to form sufficient acid, casein lactate entirely. The proteids of fresh buttermilk are the same as those contained in the butter, and the exact kind and amount depend upon the amount of acid formed. In ordinary buttermilk, in which the proteid is casein lactate, we notice that the proteid exists in fine particles in suspension. When buttermilk is treated with salt so as to form a brine about equal to that of normal butter, the salt causes the proteid to condense or concentrate in a more or less solid mass. This effect of salt is readily shown in the accompanying illustration. In the samples containing 20 per ct. or more of salt, the proteid has separated in a marked manner. This same action takes place whether the proteid is calcium casein, casein or casein lactate. This action appears to be a purely physical one; the brine seems to harden the particles of proteid and cause the proteid mass to contract into less space in the tubes.

Relation of time to formation of mottles.—It is well known that the light-colored portions do not appear in butter at once after adding salt and working the butter, but several hours are required to develop them and the maximum development occurs in about 24 hours in our experience. In the experiments in which we treated buttermilk with salt, the separation or condensation of the proteid by the brine was not at its greatest until 24 hours had elapsed.

RELATION OF PROTEIDS OF BUTTER TO MOTTLES.

The facts presented in the foregoing pages appear to us to furnish a satisfactory explanation of the causes of mottles in butter. Reviewing these facts, we have seen that:

(1st) Salt brine does not change in any way the color of butter-fat.

(2d) The amount of salt may vary considerably in different portions of butter that is not mottled.

PLATE I.—ACTION OF SALT SOLUTIONS OF DIFFERENT STRENGTH ON THE PROTEIDS OF BUTTERMILK.

(3d) In different portions of badly mottled butter, the distribution of salt may be very uniform throughout the mass of butter as a whole.

(4th) In mottled butter, the light portions usually contain less salt than the darker portions.

(5th) Mottles proper do not occur in unsalted butter.

(6th) The amount of proteid in mottled butter is greater in the light portions than in the portions of normal color.

(7th) Unsalted butter, containing buttermilk adhering to the outer surface of the granules, mottles on the surface when submerged in concentrated salt brine.

(8th) Mottling does not occur in butter when the buttermilk, adhering to the outer surface of the small granules, is mostly removed.

(9th) Small butter-granules (rice-size) washed with water at low temperature lose most of the adhering buttermilk and no mottles appear in the finished butter in the presence of salt.

(10th) Large granules or chunks favor the retention and uneven distribution of buttermilk and we get mottles in the finished butter.

(11th) Salt brine, as it usually occurs in butter, has the power of hardening, condensing and localizing in space the proteid (usually casein lactate) of butter. The action requires several hours.

From these facts, it would appear that mottles in butter are due, primarily, to the presence and uneven distribution of buttermilk adhering to the outer surface of the small granules; and, secondarily, to the effect of salt brine upon the proteid of the buttermilk thus retained in butter. In the absence of either salt or excess of buttermilk, we have no mottling. Mottling occurs most frequently as the result of an uneven distribution of buttermilk in the presence of salt distributed either evenly or unevenly. Mottling may be produced by an uneven distribution of salt in the presence of an excess of buttermilk even when uniformily distributed. In general, those conditions that favor the elimination of excess of buttermilk or its even distribution tend to prevent mottles; while those conditions that favor the retention and uneven distribution of buttermilk in butter tend to form mottles in the presence of salt.

THEORETICAL CONSIDERATIONS RELATING TO THE FORMATION OF MOTTLES IN BUTTER.

We have presented the facts showing that the formation of mottles in butter is due primarily to the presence and uneven distribution of an excess of buttermilk adhering to the outer surface of the small butter-granules, and, secondarily, to the action of salt brine upon the proteid of the buttermilk thus retained in butter. We desire to consider a little more fully how the action takes place.

A mass of butter must be regarded as an aggregation of butter-granules more or less loosely compacted. There is a variable amount of space between the granules and in these interstices we find the salt brine and also the buttermilk that is left adhering to the outer surface of the granules. There exist, therefore, more or less extensive channels through which movement of brine or buttermilk may take place to a limited extent, when conditions favor any movement. The brine present in freshly packed butter does not necessarily remain, each particle, in the exact position in which it is left when packed and not subject to any further mechanical manipulation. The conditions that favor movement or limited circulation of the brine in butter are an unequal distribution of salt brine and buttermilk, producing a variation in the specific gravity which tends thus to become uniform within limited areas. When there is an imperfect removal of the buttermilk adhering to the outer surface of the small butter-granules-the buttermilk is unevenly distributed, being more or less localized in different portions of the mass of butter. When the salt brine comes into contact with these masses of buttermilk, the casein lactate is slowly acted upon by the brine, being hardened and remaining localized. The yellow or clear portions occur where the spaces between the butter-granules are filled with clear brine and are comparatively free from casein compounds. The fact that time is required to produce mottles is explained, first, by the time required for the movement or circulation of brine to take place and come in contact everywhere with the casein compounds, assuming that this has not occurred in the working of the butter, and second, by the amount of time required for brine to act upon the casein compounds of butter.

The discussion presented in the foregoing paragraph is, apart from the facts that have been clearly established, intended as a theoretical explanation or suggestion of what takes place when mottles form in butter. While the data presented point to the fact that there is some movement of brine in packed butter, the matter has not been studied in sufficient detail to justify any specific statements. Such a study is attended with serious difficulties, but we plan to make further investigation of this phase of the subject.

PREVENTION OF MOTTLES IN BUTTER.

Since the presence of mottles in butter is primarily due to an excess of buttermilk in the mass of butter-granules, the most effective method of preventing mottled butter is to free the butter-granules as completely as practicable from the buttermilk adhering to the small granules. In order to accomplish this, the churning should be stopped when the granules are about the size of rice-grains, preferably at a temperature of 50° to 55° F. After the buttermilk has been drawn from the granules, they are treated with an amount of water at 35° to 45° F., about equal to the buttermilk drawn off, the churn being rotated a few times to insure complete contact, after which the water is drawn off and the granules are similarly treated a second time. The granules are then allowed to drain; the final drainage water from the granules should be clear. After this the salting and working are carried out in the usual way.

In working with large quantities of butter, it is obvious that somewhat more care will need to be used to make the washing effective than in the case of smaller amounts.