THE PROTEIDS OF CREAM, BUTTER AND BUTTERMILK IN RELATION TO MOTTLED BUTTER.

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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 a warm 5 per cent. solution of sodium chloride and also in hot 50 per cent. 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 shown¹ 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 cent. salt solution, and nearly so in hot 50 per cent. alcohol and which has lost the plastic and ductile properties that characterize 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

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might have to the ripening of cream, preliminary to butter-making. (2) Which of these compounds was commonly present in butter and buttermilk? (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. Hours.	Amount of milk-sugar in cream. Per cent.	Amount of milk-sugar changed. Per cent.	Amount of lactic acid formed. Per cent.	Total amount of nitrogen in cream. Per cent.	Nitrogen in cream in form of casein and its compounds. Per cent.	Nitrogen in cream in form of casein lactate. Per cent.
1	Fresh	3.64		0.15	0.39	0.32	0.00
	8	3.58	0.06	0.18	0.39	0.31	
"	24	2.92	0.72	0.60	0.38	0.33	0.33
"	32	2.90	0.74	0.61	0.39	0.30	0.30
"	48	2.88	0.76	0.62	0.39	0.32	0.32
"	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
"	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 calcium 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 twenty-four 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 sour-

ing. 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 cent., 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 cent. in twenty-four hours. In the first experiment, which was continued seventy-two hours, the amount of milk-sugar decreased very little after twenty-four 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 cent., which increased in twenty-four hours to 0.6 to 0.7 per cent. In the first experiment, which was continued seventy-two hours, the acid increased very little after twenty-four hours.

In connection with these experiments it may be mentioned that, in several lots of cream ripened so as to show an acidity of 0.38 to 0.43 per cent., 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 an 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. While results vary with individual cows, the average amount of acidity of fresh milk is about 0.08 per cent., 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, 0.1 per cent. 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 cent. In butter made from cream ripened so as to contain less than 0.5 per cent. 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 following compounds of casein: (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 gradually be 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 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 0.5 or 0.6 per cent., 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 the Proteids of Butter to the Mottling of Butter.—One of the common defects of butter is more or less marked variation in color, or what is technically called "mottles," showing in the form of white streaks, waves, spots or blotches. It has been universally believed that these light-colored portions are caused by 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.

We have studied the following conditions of manufacture in their relation to mottled 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, and (6) working of butter. The results of this work indicate that when the churning is managed so as to make the butter-granules of the size of rice-grains and these are carefully washed twice with water below 7° C., removing most of the buttermilk adhering to the outer surface of the granules, no mottles appear, however conditions are varied in other respects. Mottles are always found when the buttermilk is not sufficiently removed.

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 the question experimentally 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 cent. 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 and 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 cent. In another unmottled butter the salt varied from 4.6 to 5.3 per cent., 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 where 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 twenty-four 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 cent. 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 the 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 same butters referred to in the preceding paragraph. The results are as follows:

	24 hours.	48 hours.	5 days. Salt. Per cent.
Butter 1, light portion.....	0.91	1.79	1.40
" 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 twenty-four 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.	Proteid in light portion of butters. Per cent.	Proteid in dark portion of butters. Per cent.
1.....	1.30	0.76
2.....	0.76	0.14
3.....	0.76	0.36
4.....	0.63	0.25
5.....	0.86	0.31
Average.....	0.86	0.36

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:

Water in mottled butter. Per cent.	Water in unmottled butter. Per cent.
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 cent. 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 after about twenty-four 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 complete until twenty-four 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:

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

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

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

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

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

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

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

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

(9) 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.

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

(11) Salt brine, as it usually occurs in butter, has the power of hardening and localizing 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 uniformly distributed. In general, those conditions that favor the elimination of excess of buttermilk or its even distribution do not favor mottles, while those conditions that favor the retention and uneven distribution of buttermilk in butter favor the formation of 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.

SUMMARY.

(1) *Points Investigated.*—The questions studied in this paper are: (a) The relation of casein compounds to cream-ripening; (b) casein compounds present in butter and buttermilk; (c) 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 cent. 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 0.5 per cent. 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: (a) *Richness of cream*, (b) degree of ripeness of cream, (c) temperature of churning, (d) size of butter-granules, (e) temperature of wash-water, (f) 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*.—(a) Salt brine does not change in any way the color of butter-fat. (b) 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. (c) 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. (d) Mottles do not occur in unsalted butter. (e) 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.