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The Relation of Acid Fermentation to Butter Flavor and Aroma.

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The Relation of Acid Fermentation to Butter Flavor and Aroma.

(By C. H. Eckles.)

The market value of butter is determined largely by its flavor. Imperfect mechanical conditions and their accompanying results are overlooked sooner than defects in flavor. Its importance is justly recognized in the score cards of the various Dairy Associations, which usually assign 45 to 50 out of a 100 points to flavor. The dairy industry has now assumed such vast proportions that a slight increase in value of each pound of butter produced would amount to an immense sum, even with the production of Iowa alone. Everyone who understands the conditions under which much of the butter of the country is produced is aware, that the possibilities of improvement are great, especially during the winter season. The markets are constantly demanding an improvement in this line, and the increased competition requires that the producers regard this demand.

Every intelligent butter-maker is fully aware of the uncertainty and the difficulty of producing a uniform high flavor. Experience has taught that when certain processes are followed, the resulting product is ordinarily of at least fair quality. But even under the best conditions, and the greatest cleanliness, the product is often strikingly variable in flavor from day to day.

The causes of these variations and difficulties opens an important field for investigation. The subject is one of great scientific interest, as well as one of immense practical value. Milk-fat itself is supposed to have little or no taste, at least not the flavor of butter, so it appears butter flavor is due to something other than the fat. The flavor substances are possibly absorbed by the fat, or are associated with the other constituents which compose a portion of the butter. The chemical nature of the substances which give the delicate flavor and aroma is not known. It is generally accepted now that the flavor substances, whatever they may be, are products resulting from a breaking down of the milk solids, and it was shown by Storch that they are the result of the
growth of bacteria. Storch (1) held that the flavor of good butter comes from the decomposition of the milk sugar, while Conn (2) thinks that the nitrogenous material in the milk, as the casein and albumen, furnish the products which give the flavor.

FERMENTATION AND BUTTER FLAVOR.

Although the close relation which the fermentation, or ripening of cream, as it is popularly called, bears to the flavor of the butter has been known now for several years, the facts are still not fully appreciated as they should be.

The feed of the animals furnishing milk undoubtedly has a certain influence as well as on the color and hardness of butter, but in general this influence is much less than the effect of the fermentations which have taken place in the milk and cream. In cases where feeds such as turnips, carrots, or certain wild plants and weeds are consumed by cows, the flavor may be imparted directly to the milk and hence to the butter. An undesirable fermentation, however, may easily produce a flavor much worse than any of these. The difference between the flavor of winter and summer butter is probably partly due to the difference of feed, but much more to the differences in kinds of fermentations, which are in the milk, as is shown later on in this article.

The effect of different kinds of fermentations is easily shown by isolating various species of bacteria common to milk, and using them in pure cultures for ripening cream. This has been done by various investigators and the effects of several species determined. There are numerous species of bacteria found more or less common in milk that give very bad flavor when used as a culture for ripening cream. Most of these belong to the putrefactive group,—those which cause ordinary decay.

EXPERIMENTS WITH BACILLUS SUBTILIS.

Among those bacteria of decomposition which produce bad flavors, the organisms belonging to the hay bacillus group are always numerous. The peculiar effect of this group of bacteria is not nearly as marked as that of some others common to filth and dirt. But on account of its widespread distribution it is taken as an example of an undesirable fermentation. As its common name indicates, the hay bacillus is found common on hay, and the dirt from hay and straw is a common source of contamination of milk in the ordinary barn. It is one of the numerous species which have the power of causing "sweet

1. Nogle Unders. over Floed. Syrning. 1890.
2. Conn, Report Storrs Station (Conn.) 1898, p. 66.
This coagulation is brought about by the action of rennet-like products known as enzymes or chemical ferments, which result from the growth of these bacteria.

We have made butter at various times from pasteurized cream, ripened with the hay bacillus, as an illustration for class instruction in Dairy Bacteriology. For this purpose a second lot of the same cream is ripened with a culture of acid bacteria, and the results compared. In order to determine the actual difference in market value of butter made from cream ripened with these two cultures, two lots, made as noted above, were sent to A. H. Barber & Co., of Chicago, and were scored by them and sold for what they would bring in the market. The results were as follows:

<table>
<thead>
<tr>
<th>No.</th>
<th>Species used for Starter.</th>
<th>Score on Flavor, (45 perfect).</th>
<th>Selling Price per Pound.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacterium lactarii</td>
<td>39</td>
<td>$.20</td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus subtilis</td>
<td>31</td>
<td>.14</td>
</tr>
</tbody>
</table>

Of No. 2, the hay bacillus butter, the judge said: “Package No. 2 is old and is evidently an imitation or process butter.”

If the average butter-maker had examined the two lots he would have been surprised that even more difference was not made in the score. In eight or ten lots made at various times before under the same conditions, exactly the same flavor was present in the butter from the B. subtilis. So this was not an exceptional case. The score on the butter from the B. lactarii is one of the lowest we have had on butter from that culture.

The difference in market value of the two lots is noticed at once, the B. subtilis’ butter selling for 6 cents per pound less than the other—a difference of nearly one-third in value—due to the character of the fermentation which had taken place in the cream. This comparison shows the great influence the character of the fermentations has on the product. When we consider that the kind of fermentation which takes place in cream is often, or probably we might say usually, a matter of chance, we can easily understand the cause of much lack of uniformity, and the poor flavor of butter.

THE RIPENING OF CREAM.

The term ripening or souring is applied to the change or fermentation which takes place in cream before it is considered in proper condition for churning. As superficially observed, it consists in the production of a more or less acid taste, and a slight granular appearance. In natural ripening the fermentation is due to the growth of a variety of organisms that gain entrance to the milk and cream from the time the milk is drawn
until it is churned. When the cream is ripened by the aid of starters, an attempt is made to control the fermentation by adding a large number of bacteria of a kind expected to give favorable results.

Bacteriologists differ somewhat regarding the production of the normal high quality of flavor and aroma of butter. Conn (1) found that most bacteria present in the cream are either harmless or beneficial, and under good conditions, the beneficial are usually sufficient to give a fair produce. Practical experience has indicated that the development of a good acid taste in the cream, produced if necessary by the use of a starter with the same acid taste, is the proper condition for good results.

Storch (2) came to the same conclusions from experiments, and, following him, the Danish butter, is mostly made from pasteurized cream ripened with pure cultures of lactic acid producing bacteria. Others maintain that the acid species alone are not sufficient to develop the best flavor and aroma (3), and that the best results are produced by a variety of species working together, and giving high aroma and flavor as a joint product of their growth. It is evident that ordinary flavor is the joint product of all the species present that have any power of producing flavor, but this does not exclude the possibility of one class of organisms being normally the cause of good flavor, and the good or bad qualities depending upon the proportion of this class present. It is known that lactic acid itself will not produce the fine flavor or aroma desired. Conn found that some bacteria other than the acid produces give a fine flavor to butter, others a desired aroma. This led him to believe that flavor, aroma, and acid production are not associated and not produced by the same class of bacteria. We do not doubt that species can probably be found which will give one of these qualities without the others.

Our experiments, however, indicate that the chief agents of the best natural cream ripening are the typical lactic acid producing group, and that apparently equally good results can be had when they are the only specie present.

THE BEST NORMAL RIPENING A DEVELOPMENT OF ACID BACTERIA.

A series of tests was made at irregular intervals covering several months time, to determine approximately the ratio of

1. Report Storrs Station (Conn.) 1896, p. 26

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acid to non-acid producing species in naturally ripened cream when ready for churning. The system used to determine the number of acid germs were neutral litmus-agar containing 4 per cent of Lactose. This was supplemented by numerous inoculation into sterile milk.

Of course, on account of the enormous number of bacteria present and the very great dilution necessary in making plates it is not to be supposed that the results are more than approximately correct. Although possibly as near correct as is usually obtained by the present bacteriological methods. The determinations were made from cream when just ready for churning. Several samples of cream were usually separated from the milk of different patrons bringing milk to the College Creamery, and the sample developing the finest flavor was selected for the test. The amount of acid developed was as near .6 of 1 per cent as possible. The location of the laboratory in the dairy building was of great advantage in carrying out the practical part of the experiments.

The percentage of acid bacteria was estimated to be between 90 and 98 in the best cream. The number of species present in ripened cream is smaller than the number present before souring. The cream from which the butter was made that scored second on flavor among the 500 entries at the National Creamery Butter-makers Association, Topeka, Kansas, 1898, was among the lots examined. This cream, however, was ripened with the aid of a starter, and was the only lot examined in which a starter was used. It showed 92 per cent of acid bacteria.

The following table gives a summary of the results of these determinations:

<table>
<thead>
<tr>
<th>Date</th>
<th>Quality of Cream</th>
<th>No. per C.C.</th>
<th>No. Acid Class per C. C.</th>
<th>per cent Acid Class</th>
<th>No. non-acid Class per C. C.</th>
<th>Per cent non-acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 11</td>
<td>Fine</td>
<td>280,000,000</td>
<td>257,600,000</td>
<td>92</td>
<td>22,400,000</td>
<td>8</td>
</tr>
<tr>
<td>July 18</td>
<td>Poor</td>
<td></td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 30</td>
<td>Excel.</td>
<td>3,002,000,000</td>
<td>2,851,190,000</td>
<td>95</td>
<td>150,810,000</td>
<td>5</td>
</tr>
<tr>
<td>Aug. 11</td>
<td>Good</td>
<td>1,107,000,000</td>
<td>1,012,072,200</td>
<td>91.5</td>
<td>94,928,800</td>
<td>8.5</td>
</tr>
<tr>
<td>Sept. 3</td>
<td>Fair</td>
<td>1,027,000,000</td>
<td>955,110,000</td>
<td>93</td>
<td>71,890,000</td>
<td>7</td>
</tr>
<tr>
<td>Sept. 5</td>
<td>Good</td>
<td>2,007,958,000</td>
<td>1,827,370,000</td>
<td>91</td>
<td>180,588,000</td>
<td>9</td>
</tr>
<tr>
<td>Oct. 28</td>
<td>Good</td>
<td>392,958,000</td>
<td>385,098,840</td>
<td>98</td>
<td>7,859,160</td>
<td>2</td>
</tr>
<tr>
<td>Oct. 30</td>
<td>Good</td>
<td>393,700,000</td>
<td>381,889,000</td>
<td>97</td>
<td>11,811,000</td>
<td>3</td>
</tr>
</tbody>
</table>
This table indicates that the acid group constitutes the greater part of the bacterial flora of the ripened cream. Although the flavor of butter appears to be closely connected with this group of bacteria, those not belonging to the acid group are present in sufficient number to aid in making the flavor if their products are necessary. But when the facts brought out by the table are considered in connection with the fact that pasteurized cream, ripened with a pure culture of the acid class of bacteria produces butter of a quality corresponding with that from of normally ripened cream of a high grade, and apparently with the same flavor, the relation of the two is apparent.

COMPARISON BETWEEN THE RELATIVE NUMBER OF BACTERIA IN UNRIPENED AND RIPENED CREAM AND MILK.

If a gelatin plate culture is made from milk before fermentation has progressed very far, quite a variety of species is usually seen at once. If a plate culture of the same is made after normal acid coagulation has taken place, the results are quite different. The greater part of the germ content is now the same, while some species recognized in the first culture have entirely disappeared. It is known that the products of growth of one species may retard the growth of other species as well as interfere with its own development. This is true of the acid products and accounts for the elimination and suppression of many species. The acid species seem to be thoroughly at home in milk, and under ordinary conditions gain the ascendancy. Sweet milk which has been kept under good condition usually has quite a large per cent of acid bacteria. We have made no systematic investigation of this, but in the few examinations made, the mixed milk of 40 or 50 patrons of the creamery contained from 65 to 85 per cent of acid germs. A sample of cream was taken on one occasion as separated from the mixed milk and tested with the following result: Number per cubic centimeter, 1,597,320; per cent acid germs, 67. The same cream ripened to .61 of one per cent acid and tested again; the number per cubic centimeter was then 392,985,000, and 98 per cent of the total number belonged to the acid class.

DIFFERENCE BETWEEN WINTER AND SUMMER FLAVOR.

The flavor of the regular market supply of butter is at its best during the summer months, and especially during the months of May and June. For this reason the June flavor is often taken as a standard which should be approached as nearly as possible throughout the year.

The excellence of the June flavor has been accounted for in
different ways. In the popular mind the superiority is generally associated with the fine condition of the grass common at that time of the season.

Conn (1) thinks the difference is largely the result of the difference in fermentations. He found a larger variety of species of bacteria present during the months of May and June, and gives this as a possible explanation of the good flavor. The results of our observations also indicates that the principal cause of the difference in flavor is the fermentations. The fermentations which take place during the summer months appear to result from the growth of a larger per cent of acid producing or souring bacteria, than during the winter months, with correspondingly smaller per cent of putrefactive bacteria. During the winter of 1897-98 the markets reported the flavor of the butter poorer than usual, while the scores at the National Convention averaged lower than ever before. (2)

During this period it was observed that the milk soured much slower than usual. The acid fermentation did not seem to be present in normal numbers. In some factories the cream was reported to become bitter and disagreeable, and the butter made from it very poor in quality.

The cream separated at the College Creamery contained sweet curdling and other undesirable fermentations to such an extent that cream ripened without a starter, as a test, gave butter ranked as third class by a competent judge; while with a heavy acid starter gave a product scored 97 by the same judge. It was almost impossible to secure a natural skim milk starter fit for use during these months.

On nine successive days in February milk was selected from that delivered by the best patrons of the creamery and allowed to undergo natural fermentation. In but one case did the milk sour in normal condition. In the others, some were sweet-curdling, curd-dissolving fermentations; others developed strong, bitter, or putrefactive flavors in connection with more or less acid. This milk had been handled better than the average creamery supply. The milk furnished by the College Herd at the same time coagulated without acid repeatedly, although all the conditions demanded by modern sanitary measures were fully complied with. After these fact were observed, fermentation tests of the milk supplied by certain patrons of the College Dairy were made at intervals from March to July. As soon as the summer conditions began, the per

(1.) Report Storrs Ag. Exp. Station (Conn.) 1896. p. 31.
(2.) Chicago Produce, Feb. 26 and March 5, 1898.
percentage of normal souring milk increased, until during June and July nearly all fermentations were acid with a clean acid taste. A result of a portion of these tests are given in the following table:

<table>
<thead>
<tr>
<th>Date</th>
<th>Pure Acid Flavor</th>
<th>Impure Acid Flavor</th>
<th>Rapid Decomposition of Curd.</th>
<th>Gasey</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 5</td>
<td>12.5%</td>
<td>48 %</td>
<td>39.5%</td>
<td></td>
</tr>
<tr>
<td>April 8</td>
<td>50 %</td>
<td>36.7%</td>
<td>13.3%</td>
<td></td>
</tr>
<tr>
<td>May 10</td>
<td>90 %</td>
<td>10 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 13</td>
<td>90 %</td>
<td>10 %</td>
<td></td>
<td>30%</td>
</tr>
</tbody>
</table>

Results of this kind, of course, can not be expressed accurately by percentages, for there is no sharp line of division as expressed in the table. The results, however, show the general change in the fermentation which corresponded with the natural ripening of the cream at the same time. It is not surprising that a large part of the bacterial flora of milk, under winter conditions, is injurious. During this season the cows are in and around the barn a large part of the time, and, especially where the conditions are not good, the milk becomes easily contaminated from the hay dust and the manure on the bodies of the animals or in the air. The typical lactic acid group of bacteria are not usually inhabitants of the filth and contamination from the barn conditions is not such as to give a large number of them access to the milk. So while the number of germs in the milk during the winter season may be sufficient to cause rapid fermentation, the products are often not desirable, and the effect on the butter more or less injurious. This explains why the use of a starter of good quality is especially valuable during the winter season. A good natural starter is almost a pure culture of acid producing bacteria, as is shown later on in this article.

**EXAMINATION OF NATURAL STARTERS.**

Practical experience has taught that an addition of well soured milk or of good flavored butter-milk to cream has a favorable influence on the development of a desirable and uniform flavor in butter. In considering the question of butter flavor it is important to determine from a bacteriological standpoint, what kind of a fermentation experience has taught us to use. Mr. Campbell (1), in speaking of natural starters, says

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(1) Transactions of Highland and Agricultural Society of Scotland, 1808, p. 230
that after several transfers, and several days' growth, a home­
made starter is nothing but a pure culture of the bacterium of
sour milk or whey. Our examinations of natural skim milk
starters, although but two in number, indicate the same to be
true here. Skim milk was taken from milk of good quality and
allowed to stand at warm temperature until coagulated into the
usual solid mass. The two samples selected for examination
had the qualities desired in a good starter. The results were as
follows:

<table>
<thead>
<tr>
<th>No.</th>
<th>No. per C. C.</th>
<th>No. Acid Class per C. C.</th>
<th>Per cent Acid Class</th>
<th>No. non-acid Class</th>
<th>Per cent non-acid class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,000,000,000</td>
<td>1,880,000,000</td>
<td>94</td>
<td>120,000,000</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>839,160,000</td>
<td>772,027,000</td>
<td>92</td>
<td>67,133,000</td>
<td>8</td>
</tr>
</tbody>
</table>

These samples were the first cultivation, and not the result of
several transfers and several days' growth, as in the case men­
tioned by Campbell, and this probably accounts for the number
of non-acid bacteria present being more than he found in his
investigations.

**BUTTER FLAVOR FROM PASTEURIZED CREAM RIPENED WITH ACID
PRODUCING BACTERIA.**

The close relation which the acid fermentation bears to a good
quality of ripened cream and consequently to the production of
butter flavor and aroma has been indicated in the preceding
page. The evidence is not complete, however, without a study of
the flavor produced by acid bacteria when not interfered with
by other species. In another article a detailed account will be
given of the experiments made with several species of bacteria,
including several of the acid class.

In the spring of 1897, experiments were begun with different
species of bacteria. Two species of the acid class had previously
been isolated from ripened cream and grown in pure cultures.
When pasteurized cream was ripened with a culture of either
one of these species, butter was produced with a flavor appar­
tently the same as that of the regular creamery make, which has
always ranged among the best.

Later two other species of acid bacteria were isolated and
their power of producing flavor tried. One of these (Bacterium
lactarii) (1) is the most common bacteria causing ordinary
souring of milk in this locality. This species gave the most
satisfactory results of all the species tried as a starter for cream

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(1) Dinwiddie, Bul. 45, Arkansas Exp. Station.
ripening. Its rapid growth causes the cream to sour rapidly and the flavor of the butter is first class.

Experiments were first carried on with small quantities of cream pasteurized by placing a can in hot water, or by heating in the Russell Pasteurizer. Pasteurization is about the only practical way to free cream from the numerous bacteria it ordinarily contains. When large quantities of milk or cream are to be pasteurized, what is called the continuous flow machines are probably the most practical. The per cent of the bacteria destroyed is not as high as in the intermittent machines, but the pasteurization is probably thorough enough for practical work.

When favorable results were obtained from using the acid cultures for ripening small lots of cream, two of the same species were used in the entire lot of cream daily separated in the creamery. The amount of butter made per day at times reached 600 pounds. In some cases the milk was pasteurized and separated at the same temperature. In others the cream alone was pasteurized in the machines. The machine used was made by A. H. Reid, of Philadelphia, and was loaned to the College.

The starter was made by growing a pure culture of the acid bacteria under trial in skim milk pasteurized by heating to 160 degrees and cooling at once. The starter was added to the cream at the rate of from 8 to 20 per cent.

The butter made from these cultures had the typical butter flavor and aroma and was of such quality that butter judges ranked it equal to good raw cream butter, both in score and in value. In eight lots made where part of the cream was pasteurized and ripened with this culture (B. lactarrii) and the other part ripened raw, the average score on the flavor of the butter was close. The average score of the acid culture being .2 of one point higher. In this series, which was scored by Mr. Healy of New York, the highest score on the acid culture was 96. Other judges have scored butter from this acid culture 95 and above many times, several times 97 and once 98.

In the October butter contest at the Trans-Mississippi Exposition, Omaha, the third place was given to a tub of butter made from pasteurized cream ripened with a pure culture of this acid bacteria. The score was 97, and the total number of entries about 110. The scoring was done by W. D. Collyer of Chicago.

A considerable portion of the butter which the College has shipped to England during the season of 1898, under contract with the U. S. Department of Agriculture, was made from cream ripened with an acid culture. The same was used in both raw
and pasteurized cream. The idea has been quite commonly held that the acid fermentation alone produces a butter of good, but mild flavor, and one especially deficient in aroma. We have found the flavor and aroma of butter made from acid cultures in pasteurized cream to be usually slightly milder than that of butter made from the same cream ripened without pasteurization.

Whether the process of heating or the difference in fermentation is responsible for the slight difference in flavor and aroma is an interesting question not yet settled. This difference is very little when both kinds of cream have been ripened properly, and only a good judge can distinguish it at all.

At one time a lot of cream was divided into two parts. One was ripened with a heavy starter of acid bacteria; the other was pasteurized and ripened with a natural starter, one-half skim milk and one-half buttermilk. This latter starter should contain the bacteria present originally in the milk, and it would appear the resulting flavor should be that of cream ripened raw.

But the pasteurized butter under these conditions had a slightly milder, or “cleaner” taste, as the judge called it; so a judge familiar with pasteurized butter was able to select the lot which was from pasteurized cream. Contrary to the somewhat common opinion, we find the aroma from acid fermentation alone equally good and practically the same as that from raw cream, except as mentioned before, at times it is not quite so pronounced. In other cases it has been higher and better than that from the same cream ripened raw. Proper control of the state to which ripening is allowed to go, is an important factor in getting the aroma. When ripening is not carried far enough the aroma is not pronounced, while if the process is carried too far the aroma and flavor are both injured.

CONCLUSIONS.

Butter flavor is produced mostly by the bacterial fermentations which have taken place in the milk and cream. The kind of flavor produced depends upon the class of bacteria causing the fermentation. Cream ripened with common bacteria found in hay dust (Bacillus subtilis) gives a very undesirable flavor to butter.

The general superiority of butter flavor in the summer season is mainly due to the difference in the fermentations that are in the milk.

This difference is due to the greater number of bacteria of the acid class found in the milk during the summer season.
The ripening of a good quality of natural cream is mostly a development of acid bacteria. When good flavored cream is ready for churning, the number of bacteria per cubic centimeter varies from 280 million to 3 billion. Of this number the acid producing bacteria constitute from 91 to 98 per cent. As the process of ripening advances, the relative per cent of acid bacteria greatly increases. As this proceeds, some species disappear; others are prevented from increasing in numbers.

A good natural skim milk starter is practically a pure culture of acid producing bacteria.

The flavor producing power of four species of acid producing bacteria was tried by using them to ripen pasteurized cream. Any one of these gave the butter the typical flavor and aroma produced in natural ripening.

The most common milk souring organism (Bacterium lactarium), all things considered, gives the most satisfactory results of any of the species tried as a culture for ripening cream.

Practical experience and experimental evidence both indicate that the most important factors in cream ripening are the development of the typical acid fermentations and the elimination or suppression of other and injurious types of fermentations.
Eckles: The relation of acid fermentation to butter flavor and aroma.