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Bacteriology of Butter

I. Influence of the Distribution of the Non-Fatty Constituents on the Changes in Bacterial Content During Holding

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SUMMARY AND CONCLUSIONS

1. Unsalted butter held at 21° C. showed a rapid increase in the number of bacteria it contained, whether it was held in the normal physical condition or with the serum largely separated. In general, the multiplication of bacteria was much more rapid when the serum was separated than when it was not.

2. Unsalted butter held at 7° C. also showed an increase in the number of bacteria contained, whether it was held in the normal physical condition or with the serum largely separated, but the increase was much less rapid than at 21° C. The multiplication was, in general, considerably more rapid when the serum was separated than when it was not.

3. Salted butter held at 21° C. tended to decrease in bacterial content, whether it was held in the normal physical condition or with the serum largely separated. In general, it appeared that the separation of the serum increased the rate of destruction of the organisms.

4. Salted butter held at 7° C. also tended to decrease in bacterial content, whether it was held in the normal physical condition or with the serum largely separated. At this temperature there was less of a tendency for the separation of the serum to increase the rate of destruction of the organisms than at 21° C.

BACTERIOLOGY OF BUTTER

I. Influence of the Distribution of the Non-Fatty Constituents on the Changes in Bacterial Content During Holding

By B. W. Hammer and R. V. Hussong

Various types of bacterial deterioration of butter have been under investigation at the Iowa Agricultural Experiment Station for several years. In studies on unsalted butter, it was noted that objectionable flavors and odors commonly developed more quickly when the serum was largely separated, as a result of the butter having been melted, than when the serum was distributed normally. This suggested that such factors as the species of microorganisms present, the holding temperature and the percentage of salt are not the only factors influencing the changes in the bacterial content of butter during holding and that the physical condition of the butter is also important. Trials accordingly were carried out to determine whether or not the distribution of the non-fatty constituents in butter has an influence on the changes in the numbers of bacteria during holding; the bacterial counts were made by the plate method.

GENERAL PROCEDURE

The general procedure used was as follows: A lot of butter was divided into three portions (A, B and C). At the first examination a bacterial count was made on Portion A only; at the second examination counts were made on Portions A and B, Portion A having had the serum largely separated since the first examination (as a result of the melting incident to the making of the bacterial count) while Portion B was in the normal physical condition; at the third examination counts were made on Portions A, B and C, Portions A and B having had the serum separated for different periods, while Portion C was in the normal physical condition.

The data secured made possible a comparison of the changes in the numbers of bacteria in butter held in a normal physical condition with the changes in material held with the non-fatty constituents largely separated from the fat.

METHODS

As soon as the lot of butter which was to be used in a trial was taken from the churn, it was shaped into an approximately rectangular parallelepiped having two dimensions from 1½ to 2 inches, while the third was considerably greater. The shaping was done in a sterile parchment paper to prevent contamination, and this paper was also used to wrap the butter. After the butter was firmed in the cooler, slices from ⅜ to ½ inch thick were cut from the block with a sterile spatula and each put into a sterile petri dish; these slices represented the different portions of a lot.

The bacterial counts were made on the cubic centimeter basis by the plate method. Beef infusion agar was used, and incubation was for 4 days at 21°C. The water blanks were warmed to about 40° C. in a water bath, and each pipette was warmed in the flame just before use; the plates were used at room temperature.

When a count was to be made, all the butter in a portion was melted slowly over a flame with considerable rotation of the unmelted material. After thoro mixing of the completely melted butter, 1 cubic centimeter was pipetted into a water blank, the butter being rinsed out with the warm water. The desired dilutions were then made, care being taken thoroly to distribute the material in each blank before any of it was removed. The agar to be used for a set of plates was melted and cooled before the butter was melted so that it could be added very soon after the dilution water was distributed to the plates.

With the butter which was held after being melted, the curd that settled out sometimes stuck so firmly to the bottom of the dish that a sterile bent rod was used to distribute it thruout the fat just before removing the cubic centimeter to be plated.

EXPERIMENTAL

Trials were carried out with both unsalted and salted butter, and two holding temperatures—21°C. and 7°C.—were used. In three instances a small lot of cream was churned in a glass jar agitated in a shaking machine and the butter worked with a paddle, but most of the butter studied came from regular commercial churnings. With a commercial churning the unsalted lot of butter was taken at the time the preliminary moisture determination was made, which means that the butter had received comparatively little working and was low in moisture; the salted lot was secured when the butter was ready to be taken from the churn.

Butter culture was not used in any of the churnings because it seemed desirable with most of the lots of butter investigated to have (1) the initial bacterial content comparatively low and (2) the initial flora varied, rather than dominated by *Streptococcus lactis,* which makes up such a large percentage of the organisms present in a butter culture.

The histories of the lots of butter used are presented in table I, together with the partial composition, as given by the butter plant records, of some of the samples.

TABLE I. HISTORIES OF THE LOTS OF BUTTER USED, INCLUDING PARTIAL COMPOSITION OF SOME OF THE LOTS.

Butter no.	Type of churning	Cream	Unsalted butter	Salted butter	
			% Moistrue	% Moisture	% Salt
1	Laboratory	Sour, raw			
2	Laboratory	Sweet, pasteurized, inoc. with pure culture of a fat-splitting organism			
3	Laboratory	Sweet, pasteurized			
4	Commercial	Sour, neutralized and pasteurized			
5	Commercial	Sweet, pasteurized			
6	Commercial	Sweet, pasteurized			2.5
7	Commercial	Sour, neutralized and pasteurized			2.8
8	Commercial	Sweet, pasteurized			2.6
9	Commercial	Sweet, pasteurized	12.7	15.9	2.7
10	Commercial	Sweet, pasteurized	12.2	15.8	2.6
11	Commercial	Sweet, pasteurized	12.6	16.1	2.5
12	Commercial	Sweet, pasteurized	13.2	15.5	2.9
13	Commercial	Sour, neutralized and pasteurized	12.1	15.8	2.7

UNSALTED BUTTER HELD AT 21° C.

The results obtained on unsalted butter held at 21°C. are given in tables II and III. The data show that organisms multiplied rapidly at 21°C., whether the butter was held in its normal physical condition or with the non-fatty constituents largely separated from the fat. The count obtained on Portion A after the serum had been separated one day was always much higher than the original count on Portion B, which was also made after one day. With 11 of the 13 lots of butter the count obtained on Portion A when the serum had been separated more than one day was higher than the original count on Portion C which was made at the same time. With the two exceptions (Nos. 1 and 2), the original count was very high and, following a great increase in the bacterial count during the first day Portion A was held with the serum separated, there was a pronounced decrease indicating that conditions had become unfavorable for the bacterial types present. After Portion B was held with the serum separated, the count was much higher than the original count on Portion C with 11 of the lots. The exceptions (Nos. 1 and 2) are the 2 lots

TABLE II. CHANGES IN BACTERIAL CONTENT OF UNSALTED BUTTER HELD AT 21° C. (THREE-DAY SERIES)

Butter No.	Portion A			Portion B		Portion C
	Original butter 0 days old	Serum separated 1 day	Serum separated 3 days	Original butter 1 day old	Serum separated 2 days	Original butter 3 days old
1	19,415,000	555,000,000	235,000,000	162,500,000	180,000,000	340,000,000
2	3,500,000	680,000,000	138,000,000	237,000,000	127,000,000	275,000,000
3	5,700	363,000	239,000,000	57,000	59,000,000	2,915,000

noted above as showing irregularities which may have been due to the high initial count; with one of these (No. 1) there was comparatively little increase in bacterial content as a result of the serum being separated two days, while with the other (No. 2) there was a large decrease.

The data presented in tables II and III show, in general, that in unsalted butter held at 21°C. the multiplication of bacteria was much more rapid when the serum was separated than when it was not.

Many of the bacterial counts obtained, especially on the material held after the non-fatty constituents had separated, were surprisingly high. The highest count secured on butter in its normal physical condition was 340 million per cc. With butter held after the separation of the serum the highest count obtained was 1,260 million per cc., and a number of counts were over 500 million per cc.

TABLE III. CHANGES IN BACTERIAL CONTENT OF UNSALTED BUTTER HELD AT 21° C. (TWO-DAY SERIES)

Butter No.	Portion A			Portion B		Portion C
	Original butter 0 days old	Serum separated 1 day	Serum separated 2 days	Original butter 1 day old	Serum separated 1 day	Original butter 2 days old
4	163,500	32,500,000	570,000,000	8,900,000	405,000,000	70,000,000
5	10,000	258,000,000	840,000,000	63,000,000	440,000,000	134,000,000
6	17,100	66,500,000	210,000,000	13,750,000	158,000,000	58,000,000
7	116,500	77,000,000	350,000,000*	12,250,000	189,000,000**	52,000,000†
8	17,250	62,000,000	350,000,000	6,700,000	174,000,000	80,500,000
9	25,100	15,300,000	460,000,000	2,800,000	163,000,000	29,000,000
10	61,000	98,000,000	370,000,000	16,100,000	106,000,000	37,500,000
11	21,100	10,800,000	650,000,000	600,000	270,000,000	29,750,000
12	52,000	82,500,000	1,260,000,000	4,450,000	820,000,000	29,500,000
13	22,200	29,000,000	665,000,000	10,800,000	435,000,000	64,000,000

*Held 4 days with serum separated.
**Held 3 days with serum separated.
†Original butter 4 days old.

UNSALTED BUTTER HELD AT 7° C.

The data secured on unsalted butter held at 7°C. are presented in table IV. From these results it is evident that there was a definite multiplication of bacteria, whether the butter was held in a normal condition or with the serum largely sep-

arated. A comparison with the data reported in table III, which includes counts on the lots of butter covered by table IV, shows that the growth of the bacteria was much slower

TABLE IV. CHANGES IN BACTERIAL CONTENT OF UNSALTED BUTTER HELD AT 7° C.

Butter No.	Portion A			Portion B		Portion C
	Original butter 0 days old	Serum separated 2 days	Serum separated 4 days	Original butter 2 days old	Serum separated 2 days	Original butter 4 days old
4	163,500	170,000	580,000	212,000	890,000	860,000
6	17,100	485,000	71,500,000	110,500	47,300,000	15,210,000
7	116,500	46,000,000	231,000,000	5,600,000	207,000,000	35,000,000
8	17,250	62,000	14,700,000	37,000	1,660,000	335,000
9	25,100	85,000	12,900,000	54,000	5,600,000	1,000,000
10	61,000	870,000	33,000,000	258,000	38,000,000	11,400,000
11	21,100	26,000	1,840,000	28,500	3,600,000	122,500
12	52,000	285,000	154,000,000*	75,000	36,400,000**	11,550,000†
13	22,200	63,000	43,000,000	67,000	37,100,000	3,450,000

*Held 5 days with serum separated.
**Held 3 days with serum separated.
†Original butter 5 days old.

at 7°C. than at 21°C. At 7°C. butter No. 7 showed an outstandingly rapid development of bacteria, while at 21°C. the development of organisms in it was quite comparable with that in a number of other lots; presumably the flora of this butter was one well adapted to growth at 7°C. The count obtained on Portion A after the serum had been separated two days was higher than the original count on the two-day-old Portion B with six of the nine lots of butter and lower with the other three; it should be noted that when it was lower the difference was comparatively small so that in these instances the melting of the butter cannot be assumed to have inhibited growth. The count obtained on Portion A when the serum had been separated more than two days was much higher than the original count on Portion C with all the lots except one (No. 4), and with it the difference was so small that it is of little significance. The count obtained on Portion B after being held with the serum separated was always higher than the original count on Portion C, and with all the lots except one (again No. 4) it was much higher.

The results given in table IV show that with unsalted butter held at 7°C. bacterial development was more rapid when the serum was separated than when it was not.

The highest count obtained on butter in its normal physical condition was 35 million per cc., while with the butter held after the separation of the serum the highest count secured was 231 million per cc. Presumably, with at least some of the lots of butter, the count would have gone higher with a longer holding period. Of the three lots of butter (Nos. 4, 8

and 11) which gave comparatively little growth when they were in the normal physical condition, two (Nos. 8 and 11) showed a definitely accelerated growth when the serum was separated, while one (No. 4) did not.

SALTED BUTTER HELD AT 21° C.

The counts secured on the salted butter held at 21°C. are given in table V. The salt very effectively prevented an in-

TABLE V. CHANGES IN BACTERIAL CONTENT OF SALTED BUTTER HELD AT 21° C.

Butter No.	Portion A			Portion B		Portion C
	Original butter 0 days old	Serum separated 1 day	Serum separated 2 days	Original butter 1 day old	Serum separated 1 day	Original butter 2 days old
6	29,400	10,000	8,800	16,000	11,500	17,200
7	86,000	33,500*	11,300**	49,500†	11,800††	31,000‡
8	21,300	14,300	8,700	19,800	12,100	9,950
9	45,000	25,500	20,200	43,500	25,200	28,900
10	58,500	36,000	13,600	34,000	13,800	12,900
11	14,850	9,500	10,000	13,600	6,050	10,000
12	31,500	11,600	15,900	23,500	16,100	18,200
13	30,500	32,000	17,900	34,500	19,250	24,700

*Held 4 days with serum separated.
**Held 7 days with serum separated.
†Original butter 4 days old.
††Held 3 days with serum separated.
‡Original butter 7 days old.

crease in the number of organisms, and, in general, there was a decrease as the butter was held, whether with the serum separated or in the original physical condition. This is strikingly shown by the fact that with seven of the eight lots of butter the highest count was obtained in the original examination of Portion A; with the variant lot (No. 13) the increase in the count was so small that no significance can be attached to it. A comparison of the original counts on Portions A, B and C shows that those of Portion B were lower than those of Portion A with seven of the lots of butter, and those of Portion C were lower than those of Portion B with seven of the lots; the variations from the general trend are again too small to be significant. The count obtained on Portion A after the serum had been separated one day was lower than the original count on Portion B with seven of the lots, and the count on Portion A after the serum had been separated more than one day was lower than the original count on Portion C, or equal to it, with seven of the lots. The count secured on Portion B after the serum had been separated was lower than the original count on Portion C with six of the lots.

It appears that there was a general tendency for the sepa-

ration of the serum to increase the rate of destruction of the organisms. This trend in the changes in the numbers of organisms is best illustrated with a single lot of butter by No. 7, since the differences between the counts obtained following the separation of the serum and those obtained on the corresponding butter in the original physical condition are larger than with the other lots; with butter No. 7 the holding periods were unusually long and the initial count was comparatively high.

SALTED BUTTER HELD AT 7° C.

Table VI presents the data secured on salted butter held at 7°C. As at 21°C., the salt very effectively prevented an increase in the bacterial content of the butter, either with or

TABLE VI. CHANGES IN BACTERIAL CONTENT OF SALTED BUTTER HELD AT 7° C.

Butter No.	Portion A			Portion B		Portion C
	Original butter 0 days old	Serum separated 2 days	Serum separated 4 days	Original butter 2 days old	Serum separated 2 days	Original butter 4 days old
6	29,400	17,150	11,850	15,700	10,050	15,650
7	86,000	27,000*	35,500**	28,000†	27,500††	35,500‡
8	21,300	11,250	15,000	11,800	11,800	11,900
9	45,000	47,500	30,500	41,500	24,000	23,800
10	58,500	16,600	27,600	19,700	27,800	33,400
11	14,850	10,900	11,500	13,400	13,500	15,000
12	31,500	21,100	5,600′	19,300	5,600††	8,900″
13	30,500	29,800	23,700	28,900	16,700	21,300

*Held 4 days with serum separated.
**Held 7 days with serum separated.
†Original butter 4 days old.
††Held 3 days with serum separated.
‡Original butter 7 days old.
′Held 5 days with serum separated.
″Original butter 5 days old.

without the serum being separated. The highest count for a lot of butter was obtained in the original examination of Portion A with seven of the eight lots; the variations from the general trend are entirely too small to indicate an increase in bacteria. The original count on Portion B was lower than that on Portion A in all cases, and the original count on Portion C was lower than that on Portion B in four cases and higher in four, with the increases much too small to be significant. The first count obtained on Portion A after holding with the serum separated was lower than the original count on Portion B with four lots of butter and higher with four, while the second count on Portion A after holding with the serum separated was lower than the original count on Portion C with four lots, higher with three and the same with one. The count secured on Portion B after holding with the serum

separated was lower than the original count on portion C with seven lots and higher with one. In general, the differences between comparable counts are too small to be significant.

With the butter held at 7°C. there was less tendency than with the butter held at 21°C. for the material having the serum separated to be lower in bacterial content than the corresponding butter held in the original physical condition.

DISCUSSION OF RESULTS

The results obtained on unsalted butter indicate that, when the serum of butter was separated, the conditions at either 21°C. or 7°C. were more favorable for the development of bacteria than when the product remained in the normal physical condition. Altho the butter held with the serum separated was exposed to a higher temperature than the unseparated product during the melting incident to making the initial bacterial count and for the time required to cool to the holding temperature, it would not appear that this temperature variation could be an important cause of the difference in bacterial growth; the availability of the moisture and the materials in solution in it seems to be the logical explanation. The results secured are in agreement with the ideas of Rahn and Boysen[1] who believe that a considerable portion of the moisture of cream becomes sterile during churning by being divided into very small droplets. These investigators point out that, "The amount of moisture shut off from bacteria depends upon two factors: i.e., the number of bacteria in the cream at the moment of churning and the degree of dispersion of the moisture in butter." The unsalted butter from commercial churnings used for the trials herein reported was worked comparatively little because salted butter was to be manufactured, and it was necessary to take the samples before the salt was worked into the product. With more working the growth of bacteria in the butter held without the separation of the serum might have been slower than that shown by the counts made.

The variations in the rate of growth in the different lots of butter were probably due, in part at least, to variations in the flora, altho differences in the composition of the serum may also have been a factor. The fact that the ratio between the increases at the two temperatures varied from one lot of butter to another suggests that the flora is an important factor in determining the bacterial increases.

The more rapid growth of the bacteria in the unsalted but

[1]Rahn, Otto, and H. H. Boysen. Distribution and Growth of Bacteria in Butter. Jour. Dairy Sci. 11:446. 1928.

ter held with the serum separated than in that held in the normal condition undoubtedly explains the comparatively rapid development of off flavors and odors in many of the lots of butter held after being melted. In studying bacterial deterioration of butter there appears to be a distinct advantage, if results are desired quickly, in making some of the trials with butter which has been melted. It should be recognized, however, that with the serum separated the comparative growth of the various species of bacteria present may be different than in butter held in the normal physical state so that results obtained under the former condition are not always applicable to the latter.

The considerably slower growth of bacteria in the unsalted butter, either with or without the serum separated, at 7°C. than at 21°C. shows the importance of temperature in controlling bacterial development in the unsalted product. The counts secured indicate that comparatively short holding periods at relatively high temperatures may result in great increases in the numbers of bacteria, a condition which is objectionable from the standpoint of a high quality product.

The data presented clearly show the action of salt in restraining bacterial development in butter, either in its normal physical state or with the serum separated. The effect of salt in inhibiting the development of bacteria in butter in its usual condition is evident from the results of various investigators. Macy[2] has recently reported that with 483 samples of salted butter and 123 samples of unsalted butter studied, there was a general tendency for the mold, yeast and bacterial counts to decrease during the storage of the salted lots and an equally significant tendency for the counts to increase with the unsalted lots.

The series of counts on the salted butter cannot be interpreted as indicating that growth never occurs in this type of product. With longer holding periods the development of certain salt-tolerant types might be expected, and some of the lots of butter studied showed definite bacterial increases with considerably longer holding periods than those recorded in the tables. Moreover, bacteria exist which can very quickly develop in salted butter, as is evident from the bacterial deterioration that occasionally occurs with this type of product. The division into salted and unsalted butter is by no means a sharp one, due to the great variations in the salt content, and results that are applicable to a product having a high salt content may need to be modified with one of a low salt content.

The general tendency for the separation of the serum to increase the rate of destruction of organisms in salted butter at

[2]Macy, H. Quantitative Changes in the Microflora of Butter During Storage. Jour. Dairy Sci. 13:266. 1930.

21° C. might be expected from the effect of the separation of the serum on the growth of organisms in unsalted butter. If the readily usable nutrients are made more available to the organisms in the unsalted butter when the serum separates, the materials inhibiting or destroying bacteria would probably come into more definite contact with the organisms in the salted product under similar conditions. The lessened tendency for the separation of the serum to lower the bacterial content of salted butter at 7° C. than at 21° C. may be a general temperature effect which is in agreement with the effect of temperature on various agents restraining or destroying bacteria.

The influence of salt in controlling bacterial development should be definitely recognized in the manufacture of unsalted butter. The demand for this product has resulted in its manufacture by many plants formerly making salted butter, and it should be appreciated that methods which have proven satisfactory in preventing serious bacterial deterioration in the salted material may fail with the unsalted because of the more favorable conditions for the growth of microorganisms. It appears that with unsalted butter the principal factor controlling bacterial development is the holding temperature, while with the salted product an important additional factor is the salt.

The data presented show that salted butter from a churning may be held without a bacterial increase, while unsalted butter from the same churning may undergo rapid bacterial development. In the manufacture of unsalted butter, it is even more desirable that microorganisms, other than the normal butter culture types, be kept out as completely as possible than in the manufacture of salted butter and this demands very careful methods for the handling of equipment and the carrying out of the plant procedures. Undoubtedly many species of organisms can develop in butter until enormous numbers are present without seriously affecting the flavor and aroma, but certain types are very definitely objectionable and comparatively small numbers can bring about a conspicuous deterioration.

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