A Comparison of Media for Determining the Total Bacterial Count of Butter

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VARIOUS media have been used in bacteriological studies of butter according to the specific investigation under consideration and the types of organisms to be determined. The increasing interest in the microbiological content of butter as a means of sanitary control made it desirable to study different media used for bacteriological analysis with a view to the standardization of media, as well as other procedures, in order that the results obtained in different laboratories would be more comparable.

With this aim in view, the committee appointed by the American Dairy Science Association to study bacteriological methods of analyzing dairy products, through the subcommittee on butter, published a preliminary report (1) in September, 1930, on bacteriological methods of analyzing butter. In this report three media were suggested for making total bacterial counts of butter, namely: (1) Bacto peptonized-milk agar, (2) whey agar, and (3) beef-infusion agar containing 1 percent lactose.

REVIEW OF LITERATURE

Hammer (2) states that whey agar is satisfactory for plating fresh butter, especially when made with a starter, but that beef-infusion agar is likely to give higher counts on old butter in which many of the original organisms have died. He also concludes that beef extract agar gives a poor growth of butter organisms. Hammer and Hussong (3) used beef-infusion agar in studying changes in the bacterial content of butter during holding, while Derby and Hammer (4) used beef-infusion agar for determining total counts on surface taint butter.

Grimes (5) used whey agar for starter butter but beef-infusion agar when plating butter made from raw and pasteurized sweet cream because it gave higher counts. Grimes (6), in a study of the relation of the micro-

biological content of butter to the flavor score, used nutrient agar with the addition of 1 percent lactose. In commenting (7) on the report of the committee of the American Dairy Science Association, he recommends this medium for making total counts of butter.

Myers (8) states that Bacto nutrient caseinate agar was satisfactory for making total counts on butter; he found it comparable to peptonized milk agar for this purpose.

North and Reddish (9) in a bacteriological study of high grade experimental butter used whey agar for determining total bacterial counts, while Macy (10) used whey agar in determining the quantitative changes in the microflora of butter during storage.

Brown and Peiser (11) used litmus lactose agar (Standard nutrient agar + 1 percent lactose + .003 percent azolitmin) and casein agar for making total counts on butter and for the differentiation of bacteria. They found that litmus lactose agar gave slightly higher counts than casein agar when plating fresh butter made from cream ripened with a starter. After the butter was 30 days old, the counts were closer and the average counts for 12 samples showed very little difference for the two media.

Cullen (12) used nutrient agar and gelatin for making total counts on butter made from sweet cream in a milk receiving factory and found that in some cases the count on gelatin was higher than on the nutrient agar.

Sadler and Vollum (13) used several media for making quantitative and qualitative bacterial studies on butter. In nearly every case they found that the total count on beef-peptone-lactose agar was considerably higher than the total count on beef-peptone agar.

Hood and White (14) used Bacto nutrient agar for making total bacterial counts on surface taint butter and obtained high counts on most samples of the defective butter.

As no data could be found giving comparative counts on butter using the media suggested by the butter committee of the American Dairy Science Association, it appeared desirable to gather data on the total bacterial counts by employing the media suggested by the committee as well as the media recommended or suggested by other workers.

Since this study was completed, the subcommittee on butter has published a revised report (15) in which beef-infusion agar made from fresh beef or Bacto beef plus 1 percent lactose is the only medium recommended for determining total bacterial counts on butter.

METHODS

The experimental platings of butter were run in two series. In the first series comparative counts were obtained on Bacto peptonized-milk agar, Bacto whey agar, and two beef-infusion agars. One beef-infusion agar was prepared from fresh lean beef infused in tap water for 20 hours in a refrigerator at 40° F. with further procedures as recommended in the

report of the Butter Committee (1), and the other agar was made from dehydrated Bacto beef using 50 grams to 1,000 cc. of water. The latter medium is only about half strength of the former, but it is recommended by the manufacturer for making satisfactory culture medium. One percent of peptone, 0.5 percent of sodium chloride and 1 percent of lactose were added to both beef-infusion agars which were standardized to a pH of 6.8 by means of a Hellige comparator, standard model.

In the second series of samples, five media were compared, namely: Bacto peptonized-milk agar, Bacto whey agar, Bacto nutritive caseinate agar, Bacto nutrient agar ("Standard Methods" formula) + 1 percent lactose, and beef-infusion agar prepared from Bacto beef plus 1 percent lactose as in the first series.

The butter samples were plated using dilutions of 1:100 to 1:1,000,000. All dilutions were poured in duplicate with each medium, and the sets of plates were made from the same dilution blanks. The plates were prepared and poured in rotation to compensate for any difference in the time of holding the samples in the dilution water.

The plates were incubated for five days at 25° C. since a controlled temperature of 21° C., recommended in the report of the Butter Committee (1), was not available. Counting was done with the aid of a lens; the usual procedure followed was to count and mark all colonies visible to the naked eye and then to go over the plates with the lens. The results are expressed as the average count of the duplicate plates for each medium. As far as possible, plates of the same dilution were counted for each medium, but in a few cases this was impractical.

TYPES OF BUTTER ANALYZED

The samples of butter that were plated represented, for the most part, first and second grades according to Canadian standards of quality and were taken from both freshly made and stored butters two to three months old. Several of the samples were from high-scoring exhibition butters which had been in storage for six to eight weeks. Practically all lots of butter were manufactured from pasteurized cream and, as far as is known, all of the butter was made without starter. The counts obtained were not influenced, therefore, by the addition of starter organisms.

THE pH OF THE MEDIA

As all media were prepared from dehydrated products of the Digestive Ferments Company, no attempt was made to standardize the pH, with the exception of the Bacto beef-infusion agar, which was standardized before sterilization to 6.8 ± .2. Nevertheless, the pH of several lots of the various media were checked colorimetrically by means of a Hellige comparator. The pH of each medium was found to be as follows:

Medium	pH
Bacto whey agar	6.6
Bacto peptonized-milk agar	6.4
Bacto nutritive caseinate agar	6.7
Bacto nutrient agar + 1% lactose	6.8
Bacto beef-infusion agar + 1% lactose	6.8

EXPERIMENTAL RESULTS

RESULTS OF THE FIRST SERIES OF PLATINGS

The comparative data secured from plating 16 samples of butter on the media used in series I are given in table 1, the samples having been plated between April and August, 1931. All of the samples employed had higher counts on the beef-infusion agars than on either whey or peptonized-milk agars with the exception of sample 2, which had a slightly higher count on whey agar than on the beef-infusion agars, and sample 4, which showed a slightly higher count on both whey and peptonized-milk agars than on the Bacto beef-infusion agar. In 11 of the 16 samples plated, the highest counts obtained on beef-infusion agars were from 40 to over 100 percent greater than the lowest counts obtained on peptonized-milk or whey agars. In eight trials the highest counts were obtained on fresh beef-infusion agar, in six trials with Bacto beef-infusion, and in one trial with whey agar; one trial had the same high count on both beef-infusion agars.

TABLE 1. *Comparative total bacteria counts of butter on four different media*

Sample No.	Bacteria per cc. of butter on			
	Peptonized milk agar	Whey agar	Fresh beef-infusion agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
1	13,250	13,650	19,500	20,350
2	10,000	18,000	17,500	17,500
3	5,850	5,500	6,850	6,850
4	46,500	45,000	67,000	43,500
5	46,000	49,000	60,500	57,500
6	405,000	385,000	505,000	540,000
7	410,000	445,000	900,000	845,000
8	144,000	161,500	242,000	265,500
9	253,500	277,000	337,000	305,000
10	16,200	19,850	25,700	26,000
11	270,500	300,000	346,500	339,000
12	61,000	61,000	78,000	73,500
13	647,500	689,000	793,000	777,000
14	5,400	5,400	8,800	6,700
15	4,150	5,350	9,600	9,700
16	37,000	49,500	60,000	67,000
Ave.	148,490	158,112	217,309	212,506

Although the differences in the counts on whey and peptonized-milk agar were not great, 11 samples had higher counts on whey agar, while 3 samples showed higher counts on peptonized milk, and 2 samples had the same count. In comparing the two beef-infusion agars the counts obtained show only slight differences except for sample 4, where the fresh beef-infusion agar gave a count over 50 percent greater than the Bacto beef-infusion agar.

The average counts for the different media given in table 1 show that the peptonized-milk agar regularly gave the lowest count, and the fresh beef-infusion agar gave the highest count. There was no significant difference in the average counts on peptonized-milk and whey agars, or in the average counts on the two beef-infusion agars. There was, however, an appreciable increase in the counts on the beef-infusion agars over those obtained on either peptonized-milk or whey agar.

RESULTS OF THE SECOND SERIES OF PLATINGS

After data had been secured on 16 samples of butter, copies of written comments by Myers (8) and Grimes (7) on the report of bacteriological analytical methods were received by the Division of Dairy Research at Ottawa, which made it advisable to include the media recommended by these workers, namely, Bacto nutritive caseinate agar and Standard nutrient agar plus 1 percent lactose. Since in the first series there was no significant difference in the results obtained when fresh lean beef or Bacto beef agars were used, only the Bacto beef-infusion medium was employed in the second series.

The data obtained from comparative platings of 55 samples of butter on 5 different media are presented in table 2. In this second series beef-infusion agar again showed the highest counts for the majority of samples and, in addition, had the highest average count, since in 32 trials the counts were highest on beef-infusion agar. Nutrient agar plus lactose gave the highest counts in 17 comparisons, whey agar in 5 and nutritive caseinate agar in 1. In no case did peptonized-milk agar have the highest count when the five media were compared; but it did show higher counts for some samples when compared with the other media separately, although the differences in the counts were not significant. In two comparisons the counts on peptonized-milk agar were higher than on beef-infusion agar and nutrient agar plus lactose, while in two other trials the counts were higher on the peptonized-milk agar than on nutritive caseinate or whey agar.

The lowest counts for the 55 trials on the different media were distributed as follows: peptonized-milk agar, 46; whey agar, 4; nutritive caseinate, 2; and beef-infusion agar and nutrient agar plus lactose, 1 each. In one comparison the low count was the same on peptonized-milk and whey media.

TABLE 2. *Comparative total bacteria counts of butter on five different media*

Sample No.	Bacteria per cc. of butter on				
	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
1	28,500	32,500	50,500	87,500	116,500
2	1,500	3,650	5,100	6,500	7,200
3	8,750	15,350	13,650	16,950	67,000
4	13,150	169,500	178,500	188,500	219,000
5	26,300	151,000	162,000	179,000	174,500
6	33,750	162,500	139,500	180,500	198,500
7	13,900	44,000	58,500	66,000	65,000
8	340,000	2,750,000	4,400,000	3,960,000	4,275,000
9	24,000	207,000	51,000	131,000	690,000
10	3,400	4,550	6,050	6,750	5,900
11	67,500	76,000	85,500	87,500	94,500
12	76,000	106,500	142,000	168,000	188,000
13	9,550	19,300	17,500	17,700	18,400
14	815,000	1,015,000	1,270,000	1,460,000	1,465,000
15	64,000	88,500	259,000	720,000	815,000
16	1,655,000	1,660,000	2,280,000	5,675,000	8,595,000
17	95,000	115,000	125,000	145,000	335,000
18	83,000	172,000	142,500	237,500	288,000
19	7,750	8,200	9,700	14,350	15,500
20	13,750	17,500	20,750	22,700	23,150
21	7,900	9,700	11,300	12,950	12,600
22	11,500	13,200	19,100	20,650	19,600
23	3,400	4,350	6,800	10,500	10,250
24	333,000	397,000	407,000	463,500	388,000
25	77,500	78,500	96,000	119,000	109,500
26	80,000	100,500	90,500	144,500	155,000
27	11,700	14,850	18,450	20,050	19,850
28	34,000	31,150	32,800	34,750	34,350
29	27,500	31,000	50,000	64,000	79,500
30	11,300	13,950	13,700	17,000	19,650
31	439,000	386,000	532,500	648,000	655,000
32	156,000	167,500	175,000	223,500	228,500
33	523,000	524,000	752,500	844,000	941,500
34	1,740,000	3,790,000	2,770,000	3,070,000	5,370,000
35	276,500	317,500	280,500	364,500	357,000
37	365,000	470,000	1,425,000	1,280,000	1,855,000
38	2,490,000	2,470,000	2,795,000	2,860,000	3,205,000
39	11,375,000	13,650,000	13,600,000	12,950,000	13,680,000
40	18,450,000	23,100,000	20,300,000	17,400,000	16,250,000
41	168,500	211,000	258,500	260,500	265,500
42	1,090,000	1,595,000	1,695,000	1,860,000	1,840,000
43	2,805,000	2,785,000	3,210,000	3,880,000	4,475,000
44	4,970,000	5,820,000	5,395,000	4,710,000	4,395,000
45	11,450	12,900	13,800	14,050	16,500
46	11,750	13,350	14,050	20,700	16,800
47	12,900	14,150	17,350	25,100	22,100
48	11,000	13,850	16,750	32,750	25,300
49	70,000	125,000	85,000	110,000	120,000

TABLE 2. (Continued)

Sample No.	Bacteria per cc. of butter on				
	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
50	8,135,000	8,370,000	8,630,000	9,205,000	10,090,000
51	9,300,000	10,200,000	10,150,000	9,450,000	10,450,000
52	2,150,000	2,600,000	5,100,000	6,000,000	15,700,000
53	7,100,000	8,750,000	5,950,000	8,700,000	7,700,000
54	290,000	290,000	405,000	540,000	530,000
55	56,000	52,000	44,500	61,500	63,000
56	232,000	289,000	330,000	374,500	374,000
Ave.	1,385,567	1,700,518	1,711,061	1,802,935	2,139,539

The average counts as given in table 2 show that beef-infusion agar gave the highest, which was over 54 percent greater than the lowest average count given by the peptonized-milk agar. Nutrient agar plus lactose gave the second highest average count, but this was only slightly higher than those obtained on nutritive caseinate and whey agars. The average counts on nutrient agar plus lactose, nutritive caseinate and whey agars were 30.1, 23.5 and 22.7 percent higher than on peptonized-milk agar, respectively.

Table 3 shows the distribution of the high and low counts of the 55 samples for the different media and indicates the relative standing of the various media for total bacterial counts of butter.

COMPARISON OF THE MEDIA FOR PLATING LOW- AND HIGH-COUNT BUTTER

In order to determine if there was a marked variation in the relative value of the different media for low- and high-count butter, the counts for

TABLE 3. *Analysis of highest and lowest counts*

Medium	Highest counts		Lowest counts	
	No. of samples	Percent samples	No. of samples	Percent samples
Peptonized-milk agar	0	0.0	46	85.2
Whey agar	5	9.1	4	7.4
Nutritive caseinate agar	1	1.8	2	3.7
Nutrient agar + 1% lactose	17	30.9	1	1.85
Beef-infusion agar + 1% lactose	32	58.2	1	1.85
	55	100.0	54[1]	100.00

[1] One sample had the same lowest count on whey and peptonized-milk agar.

TABLE 4. *A comparison of the bacterial counts on different media of low-count butter*

Sample No.	Bacteria per cc. of butter on				
	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
2	1,500	3,650	5,100	6,500	7,200
3	8,750	15,350	13,650	16,950	67,000
7	13,900	44,000	58,500	66,000	65,000
10	3,400	4,500	6,050	6,750	5,900
11	67,500	76,000	85,500	87,500	94,500
13	9,550	19,300	17,500	17,700	18,400
19	7,750	8,200	9,700	14,350	15,500
20	13,750	17,500	20,750	22,700	23,150
21	7,900	9,700	11,300	12,950	12,600
22	11,500	13,200	19,100	20,650	19,600
23	3,400	4,350	6,800	10,500	10,250
27	11,700	14,850	18,450	20,050	19,350
28	34,000	31,150	32,800	34,750	34,350
29	27,500	31,000	50,000	64,000	79,500
30	11,300	13,950	13,700	17,000	19,650
45	11,450	12,900	13,800	14,050	16,500
46	11,750	13,350	14,050	20,700	16,800
47	12,900	14,150	17,350	25,100	22,100
48	11,000	13,850	16,750	32,750	25,300
55	56,000	52,000	44,500	61,500	63,000
Ave.	16,825	20,647	24,767	28,622	31,807

these two types of butter were grouped together. Any sample for which the maximum bacterial count on any medium was 100,000 or less was considered to be low-count butter, and all samples which had a minimum on any medium of 1,000,000 was considered to be high-count butter. The comparison of the counts obtained on different media for low-count butter is given in table 4, while the comparative counts for the high-count butter are given in table 5.

In the 20 comparisons of low-count butter, the average counts for the different media showed greater differences than the average counts of all samples, although the relative standing of the various media was not changed. Whereas the high average count for beef-infusion agar was only approximately 54 percent greater than the low average count for peptonized-milk agar with all samples, in the low-count butter the average count for beef-infusion agar was 89 percent greater than the average count for peptonized milk. With the low-count butter, the differences in the average counts on nutrient agar plus lactose and nutritive caseinate agar, as compared with those on peptonized milk were also considerably greater than for all samples. The difference in the average count for whey agar as compared with that for peptonized milk was practically the same for low-count butter as for all samples.

TABLE 5. *A comparison of the bacterial counts on different media of high-count butter*

Sample No.	Bacteria per cc. of butter on: Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
8	340,000	2,750,000	4,400,000	3,960,000	4,275,000
14	815,000	1,015,000	1,270,000	1,460,000	1,465,000
16	1,655,000	1,660,000	2,280,000	5,675,000	8,595,000
34	1,740,000	3,790,000	2,770,000	3,070,000	5,370,000
37	365,000	470,000	1,425,000	1,280,000	1,855,000
38	2,490,000	2,470,000	2,795,000	2,860,000	3,205,000
39	11,375,000	13,650,000	13,600,000	12,950,000	13,680,000
40	18,450,000	23,100,000	20,300,000	17,400,000	16,250,000
42	1,090,000	1,595,000	1,695,000	1,860,000	1,840,000
43	2,805,000	2,785,000	3,210,000	3,880,000	4,475,000
44	4,970,000	5,820,000	5,395,000	4,710,000	4,395,000
50	8,135,000	8,370,000	8,630,000	9,205,000	10,090,000
51	9,300,000	10,200,000	10,150,000	9,450,000	10,450,000
52	2,150,000	2,600,000	5,100,000	6,000,000	15,700,000
53	7,100,000	8,750,000	5,950,000	8,700,000	7,700,000
Ave.	4,852,000	5,935,000	5,931,333	6,164,000	7,290,333

In the 15 trials using high-count butter, the percentage differences in the average counts were slightly less than those obtained on all samples. Beef-infusion agar gave an average count that was approximately 50 percent greater than the average count on peptonized milk for high-count butter as compared with a difference of 54 percent for all samples. The differences in the average counts with the other media as compared with peptonized milk were also slightly less for the high-count butter than for all the samples. The only variation in the relative value of the different media as indicated by the average counts was for whey and nutritive caseinate agars. For all samples, the average count of the nutritive caseinate agar was higher than for whey agar, while for the high-count butter the average count on whey was very slightly higher than the average count on nutritive caseinate agar.

TABLE 6. *The comparative values of the average counts on different media for all samples and for low- and high-count butter*

Butter	No. of samples	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
All samples	55	100	122.7	123.5	130.1	154.4
Low-count butter	20	100	122.7	147.2	170.1	189.0
High-count butter	15	100	122.3	122.2	127.0	150.3

The percentage differences in the average counts with the different media were much greater for the low-count butter than for the high-count butter when compared with the differences in the average counts of the various media for all samples. This is shown clearly in table 6, wherein the comparative values of the average counts for all samples and for both high- and low-count butter are tabulated. A value of 100 was given to the low average count obtained on peptonized milk, and the average counts for the other media were valued accordingly.

OBSERVATIONS ON THE SIZE AND TYPES OF COLONIES

In both series the colonies on the beef-infusion agars appeared to be slightly larger than on the other media employed, although in some cases the surface colonies on peptonized milk were as large as on the beef-infusion agars. Colonies on the other media were much the same size. Most of the colonies on all media were visible to the naked eye, but there were a few which could only be detected with the aid of a lens.

While no attempt was made to classify the types of colonies present, it was observed that chromogenic colonies were frequently present in considerable numbers on all media except peptonized-milk agar; the yellow colonies on the latter were generally few in number and often failed to show up at all, either being absent altogether or failing to produce color.

When spreading colonies appeared on the plates they seemed to grow profusely on the peptonized-milk agar and very little on the whey agar.

CONCLUSIONS

Comparative total bacterial counts on sweet cream butter made without starter, using whey, peptonized-milk, nutritive caseinate, nutrient plus 1 percent lactose and beef-infusion plus 1 percent lactose agars, showed that there was a better development of bacterial colonies both as to numbers and size on beef-infusion agar than on the other media. Comparative counts on beef-infusion agars made from fresh beef and from Bacto dehydrated beef did not show any significant differences.

The counts on peptonized-milk agar were commonly lower than those obtained on the other media employed. There were no significant differences among the counts obtained on whey, nutritive caseinate or nutrient lactose agars.

The percentage differences among the average counts on the various media, with the exception of whey agar, were comparatively higher for low-count butter than for high-count butter.

Chromogenic colonies of yellow organisms often failed to appear on peptonized-milk agar but they were present on other media in considerable numbers. Spreader colonies grew profusely on peptonized-milk agar.

While it is recognized that a medium giving the highest total counts is not necessarily the most suitable for making routine analyses and that

factors of cost, ease of preparation and availability must be considered, the use of beef-infusion agar plus 1 percent lactose as a medium for routine analyses of butter does not offer any serious disadvantages as to the cost or the preparation.

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