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A comparative study of the bacterial content of vanilla and chocolate ice cream from two Indianapolis producers

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During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner , included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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A COMPARATIVE STUDY OF THE BACTERIAL CONTENT OF VANILLA AND CHOCOLATE ICE CREAM FROM TWO INDIANAPOLIS PRODUCERS

By LEANDER C. PARKER

The wide use of ice cream and its high susceptibility to bacterial contamination have stimulated many investigations to determine the extent, the nature and the cause of such contamination and the means of preventing or minimizing it. Inasmuch as the types of organisms found in ice cream are generally the same as those found in milk or cream, the methods of investigation are basically alike. These methods involve the use of agar plate cultures, direct microscopic observations and biochemical tests. The first two of these techniques were used in this study.

Breed and Brew (2) developed the direct microscopic method of counting bacteria in milk. Fabien (8) adapted this method to the microscopic examination of ice cream. Due to the high viscosity and fat content of ice cream, the film has been found to be too thick for practical use. Fay (6) described a modification of this method. He used 0.1 cc of melted ice cream together with 2 to 4 drops of water. These were mixed thoroughly and spread over the entire surface of the standard slide. This was dried and the film was then treated as described for milk.

Practically all of the work reported on the bacterial content of ice cream is based on agar plate counts. Hammer and Goss (9), in a study of the bacterial content of 54 samples of ice cream after one day's storage, obtained plate counts of from 30,000 to 190 million per cc. Fabian (4), in an analysis of 1,110 samples of ice cream from 36 plants in Michigan, obtained plate counts ranging from 1,000 to 300 million bacteria per gram. Yale (14) found an average plate count of 58,800 for store samples of ice cream from 12 producers in small cities in New York. Brown (3), in analyses of 570 samples of commercial ice cream from several Indiana cities, obtained plate counts below 30,000 in 66% of the samples and above 100,000 in 20% of the samples. Martin, Nelson and Caulfield (11) obtained plate counts of 100,000 or less in 60% of the samples from

over 300 Kansas ice cream manufacturers. Kamplain (10), using the direct microscopic method along with the plate method, made a study to determine the sources of bacterial contamination as well as the extent of contamination from three outlets in Indianapolis. His results indicate that poor dispensing methods account for a considerable portion of the bacterial content of ice cream.

Work previously done has been confined chiefly to plain ice cream and sherberts and to use of the agar plate technique. The wide variation in counts which have been noted suggest that some producers are either careless or unscrupulous in their manufacturing procedures. At one time, ice cream was widely considered to be a dumping ground for undesirable milk. The manufacturers who engaged in this questionable practice depended on the power of flavoring materials to conceal the evidence of the presence of undesirable ingredients. Chocolate ice cream, because of its color and intense flavoring, is an ideal medium for concealing such adulterants.

In the light of these conditions, this study was undertaken to determine whether the bacterial content on the products of either of two Indianapolis ice cream producers offers evidence of such careless or unscrupulous practices.

PROCEDURE

Samples of vanilla ice cream and chocolate ice cream from each of two producers were collected each week in sterilized half-pint jars. These were brought to the laboratory and placed in a water bath at 45° C. for melting. The samples were agitated several times as they were being melted to facilitate the removal of air. The material was then plated in Bacto-trypto extract skim milk agar at 37° C. for 48 hours. The colonies which developed were counted under 7.5X magnification. Counts were recorded as number of organisms per cc. Fay (7) has shown that the variations inherent in the plate method overshadow any variations introduced by the air incorporated in volumetric samples. Consequently, it makes little difference whether volumetric or gravimetric samples were used.

Slides for direct microscopic counting were prepared by a slight modification of the method described by Fay (6). Three drops of sterile water and 0.05 cc of melted ice cream were placed on a clean 75 x 25 mm slide. The water and melted ice cream were mixed thoroughly, spread evenly over the entire surface of the slide and dried on a warm level surface. The dried film was immersed in

xylol for 20 minutes, removed and dried of xylol. The film was then fixed by immersion in 95% alcohol for 5 minutes. After evaporation of the alcohol from the slide, it was stained in aqueous-alcoholic methylene blue, prepared as described in "Standard Methods" (1).

The stained slides were observed with a 1.8 mm oil immersion objective and 12.5X ocular. The diameter of the field was 0.146 mm. Counts were recorded as the number of organisms present in 30 fields. The factor used to convert this to the number of organisms per cc was calculated as follows:

$$F = \frac{A}{v \times 30a} = \frac{1875}{.05 \times 30 \times .0167} = 74810$$

or approximately 75,000, where "A" is area of slide in sq. mm., "a" is area of microscopic field in sq. mm., and "v" is volume of material used. This technique proved to be satisfactory when used with vanilla ice cream but was found to be wholly inadequate with chocolate ice cream. In addition to a high fat content, chocolate ice cream contains many particles which complicate the staining procedure to such an extent that methylene blue becomes useless as a stain for these slides. The similarity in size between many of these particles and bacteria eliminates the centrifuge as a means of separating them. Other simple stains reacted in the same manner as methylene blue.

The only staining procedure attempted that offered any hope of solution was one reported by Stoughton (12) in which thionin and orange G were used for the differential staining of bacteria and plant tissues. After treatment with xylol and alcohol in the usual manner, the ice cream films were (1) stained in carbol thionin for 5 minutes, (2) washed with water, (3) treated with 95% alcohol, (4) differentiated in orange G for several minutes, and (5) washed well in absolute alcohol. This procedure affected some differentiation but such bacteria as could be found on these mounts appeared to stain somewhat feebly. In addition, some particles which seemed not to be bacteria reacted with the stain.

RESULTS

Results are presented in tables I and II. They show a wide range in the number of bacteria per cc as determined by both the plate and the direct microscopic method. The lowest plate count in the case of each type of ice cream was recorded on a day which was

not during the winter, and the highest count in each case was recorded on a day which was not during the summer. The counts varied in each kind of ice cream from each company from a few thousand to hundreds of thousands per cc.

Enumeration of bacteria in vanilla ice cream by the direct microscopice method shows counts that range from a few million to hundreds of millions per cc. The lowest count for each was during the summer and the highest count during the winter. These wide variations in the bacterial content indicate that some or all of the factors which lead to bacterial contamination of ice cream are highly variable and difficult to control.

At first thought, it would be expected that highest counts should come during the summer and lowest during the winter. But, after careful consideration of all conditions, it is to be noted that the volume of ice cream sold during the winter is much less than that sold during the summer. As a consequence, the two companies considered, both of which are relatively small producers, make ice cream every day during the summer, but on only two or three days each week during the winter. Such an intermittent use of equipment in winter permits the development of those types of bacteria which are the sources of equipment and utensil contamination. Also, the longer periods of holding mix and the possible re-use of material are probably important contributing factors. This interpretation agrees with the fact that staphylococci, tetrads and sarcinae were found in large numbers in the samples. These types of bacteria commonly enter milk and milk products from fat and casein residues found in crevices and seams of utensils and machinery. Diplococci were also found in large numbers. These develop rapidly in a milk product that is held, particularly when the temperature is not low. Their presence in large numbers, especially during the winter, indicates the use of milk that is not fresh.

A comparison of vanilla ice cream from company A with that from company B reveals that the plate count, by monthly averages, of the company A product was lower than that of company B for 8 of the 10 months of observation. For the entire 10-month period the average plate count for company A was 64,525 per cc and that for company B was 179,588 per cc. Tbis is approximately a 3 : 1 difference. These results indicate that, from the standpoint of sanitation,

the conditions surrounding the production of vanilla ice cream by company A were more desirable than those surrounding company B.

A comparison of the total number of bacteria per cc, as determined by the direct microscopic counts on samples of vanilla ice cream, shows a striking resemblance in the general trend of results. These counts are very high in December and February for company B and in December and January for company A. In general, the counts are higher during winter than during any other season. As suggested before, the intermittent use of equipment and differences in the nature of raw materials used probably account for this trend.

The total number of bacteria per cc by yearly averages, as determined by the direct microscopic count of vanilla ice cream from company A, was 79,725,000 and for company B was 104,700,000 per cc. These results further support the conclusion that, in the manufacture of vanilla ice cream, company A is more careful in the selection of its raw materials and in its manufacturing procedures than company B.

The ratio, for vanilla ice cream, of the number of groups of bacteria observed in direct microscopic counts to the number of colonies as determined by the plate counts was 189 to 1 for company A and 279 to 1 for company B. The ratio of total number of bacteria (microscopic count) to the number of colonies per cc was 1,063 to 1 for company A and 1,396 to 1 for company B. These ratios indicate that company B depends on pasteurization to control the number of viable bacteria to a greater extent than does company A. It might be argued that the mix of company B contained a smaller percentage of heat resistant bacteria than that of company A, but the occurrence of bacteria according to morphological type in the products of the two companies is too similar to justify acceptance of such an explanation. This apparent dependence of company B on pasteurization, together with the larger viable bacterial content of its product, indicates that, from a standpoint of bacterial contamination, it markets ice cream of inferior quality as compared to that of company A.

A comparison of the bacterial count of vanilla ice cream of each company with that of good market milk (table II) reveals that ice cream, as produced by each of these companies, is decidedly inferior

to market milk in all points of comparison. This is especially noticeable in the case of staphylococci, a form which originates largely from unsterile utensils and equipment.

A comparison of the viable bacterial content of the chocolate ice cream of company A with that of company B, as shown by the monthly averages of plate counts (table I), reveals no marked difference in the two products. Although considerable differences are shown in some of the individual months covered, the samples from company A had higher counts for six of these months and those from company B had higher counts for the other six months. The average count for the entire year was 162,563 in the product of company A and 175,764 in that of company B. The difference is insufficient to justify any conclusion regarding the superiority of one product as compared with the other.

The viable bacterial content as shown by plate counts, (table I) of vanilla ice cream from company A is generally much lower than those of chocolate ice cream from the same company. Contamination from utensils, equipment and handling probably would be approximately the same for the two flavors of ice cream from the same company. The most likely cause for such a significant difference in the viable bacterial count of the two ice creams from the same company would be the condition of the materials used. It thus seems probable that company A selected its materials, particularly milk and milk derivatives, for vanilla ice cream with more care than it did those for chocolate ice cream. This difference could be due to a more intense pasteurization of vanilla ice cream mix than of chocolate ice cream mix.

For company B these counts are generally slightly lower for chocolate ice cream than for vanilla. The small average difference in these counts could be due to variations which might be expected in the technique of counting, or in manufacturing procedures. It thus appears evident that company B does not select fresher materials for the production of vanilla ice cream than for the production of chocolate ice cream. This interpretation overlooks the possibility that company B might use milk for chocolate ice cream inferior in quality and sterility to that used for vanilla ice cream and then subject the material to a more vigorous pasteurization procedure. In such a case advantage would be taken of the intense flavoring

power of chocolate powder to cover any off-flavor occasioned by such treatment. A reduction of these counts to simple proportion follows:

Company A vanilla to company A chocolate, 1 : 3
Company A chocolate to company B chocolate, 3 : 3
Company B vanilla to company B chocolate, 3 : 3

These results, which reveal a lower ratio of the vanilla to chocolate count for company A than for company B, support the argument that Company A uses ingredients of lower bacterial content in the manufacture of vanilla ice cream than in the manufacture of chocolate ice cream. The principle difference probably is due to the use of fresher milk and cream for vanilla ice cream mix and the older milk and cream for the chocolate ice cream mix. The results indicate that such procedures are not used by company B. The fact that company A produces milk and prepares its own ice cream mix, whereas company B buys its ice cream mix from another concern, helps to account for such differences of procedure of the two producers. Under the circumstances, company A might have left-over milk and mix and might make such use of them.

Table I also shows results of the direct microscopic counts of bacteria in samples of chocolate ice cream from each company over a seven-week period. Each of these counts indicate an extremely high degree of bacterial contamination of the samples. For company A the total number of bacteria, as shown by these counts, ranges from a low of 1,647 million to a high of 8,635 million per cc. The range for company B is practically the same, viz., 1,502 million to 8,389 million per cc. For vanilla ice cream these ranges are, respectively, 5 million to 280 million and 7 million to 340 million, for the two companies. If these counts could be shown to be reliable, they would serve as definite evidence of either careless or unscrupulous practices in the manufacture of chocolate ice cream by each of these concerns. The reliability of these counts, however, is open to serious question.

Most of the particles of solid matter in chocolate powder stain to the same extent with methylene blue as bacteria. Further, large numbers of these particles are approximately the same size as bacteria. A study of the microscopy of chocolate powder, as described by Winton (13), reveals that these particles consist of aleurone grains, cell inclusions from pigment cells, and fine particles of

cellulose. In the examination of chocolate ice cream by the direct microscopic method, it was ever difficult and often impossible to distinguish these particles from bacteria. The differential staining of this material within thionin and orange G eliminated some of the difficulties due to cellulose, but lessened the intensity of staining most of the particles which appeared to be bacteria. The use of this differential stain did not reduce the uncertainties of the identification of the particles sufficiently to justify confidence in the results obtained. These counts, therefore, are probably considerably higher than those which the actual number of bacteria present would justify. Consequently, any conclusions made therefrom must be taken with reserve.

CONCLUSIONS

1. Both plate and direct microscopic counts on ice cream were generally higher during winter than in other seasons. This was possibly due to the intermittent use of equipment and longer periods of holding in winter.

2. The viable and total bacterial content of vanilla ice cream from company A is lower than that from company B. The vanilla ice cream from company A had lower ratios of (1) the number of groups and (2) the total number of bacteria to the number of agar plate colonies. This indicates that company B used milk products of higher bacterial content and pasteurized the product to a greater extent than did company A.

3. Results of plate counts indicate that company A which produces vanilla ice cream of lower bacterial content than its chocolate ice cream, selects milk and cream of better quality for the production of vanilla ice cream than it does for chocolate ice cream.

4. There is some indication that the total bacterial content of chocolate ice cream from each company is much higher than that of its vanilla ice cream. Conclusions on this point await the development of a staining procedure which will clearly differentiate bacteria from other particles of similar size and staining properties in chocolate ice cream.

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TABLE I

Results of plate and direct microscopic counts on vanilla and chocolate ice cream, given as monthly averages of numbers of baeteria per cc of material.

Date	Co.	Kind	Direct counts: baeteria per 1/75,000 cc								Total in millions per cc	Plate count thousands per cc
			Diplococci	Short Strep.	Long Strep.	Staph.	Isol. cocci	Rods	Sarcinae	No. gps.		
June	A	V	13	3	4	20	5	1	24	14	5.2	19
		C										249
	B	V	42	2	0	8	5	3	32	29	6.8	79
		C										160
July	A	V	115	21	2	39	19	5	22	29	16.7	46
		C										200
	B	V	59	16	4	27	7	6	50	50	10.8	52
		C										48
Aug.	A	V	298	59	55	84	66	3	64	255	52.8	61
		C										134
	B	V	362	84	91	423	24	2	39	269	71.1	249
		C										209
Sept.	A	V	172	12	35	87	22	1	97	136	30.4	122
		C										26
	B	V	216	76	43	131	24	13	19	169	36.3	113
		C										211
Oct.	A	V	104	17	0	136	4	2	52	74	23.5	133
		C										55
	B	V	157	54	7	348	5	4	200	133	57.3	274
		C										156

TABLE I—(Continued)

Date	Co.	Kind	Direct counts: bacteria per 1/75,000 cc								Total in millions per cc	Plate count thousands per cc
			Diplococci	Short Strep.	Long Strep.	Staph.	Isol. cocci	Rods	Sarcinae	No. gps.		
Nov.	A	V	94	24	110	1124	0	1	228	124	118.6	22
		C										324
	B	V	90	25	72	1184	2	2	232	115	122.4	220
		C										188
Dec.	A	V	285	121	41	1276	5	1	418	276	181.1	114
		C	5598	4935	896	9360	1941	145	23268		3458.1	269
	B	V	259	75	17	1149	3	2	977	304	185.4	638
		C	6000	1840	3368	22812	2842	245	6248		3216.6	176
Jan.	A	V	524	31	40	691	3	1	2069	552	290.4	116
		C	4020	675	2205	14400	1575	200	22124		5973.7	83
	B	V	144	5	45	462	3	1	496	153	100.8	74
		C	18720	1060	2655	13575	3825	180	44640		6352.8	61
Feb.	A	V	180	18	21	497	6	2	447	175	87.8	56
		C										243
	B	V	1232	914	68	781	2	1	3540	1417	325.1	351
		C										219
Mar.	A	V	97	24	20	443	7	1	349	196	71.5	86
		C										41
	B	V	115	21	66	1103	5	1	226	148	115.1	102
		C										312
Average	A	V									79.7	64.5
		C									4715.9	162.5
	B	V									104.7	179.6
		C									4784.7	175.7

TABLE II

Comparison of bacterial count of vanilla ice cream with that of average milk. Results are given for 1/75,000 cc in each case.

Kind of Organism	Average milk	Ice Cream	
		Company A	Company B
Diplococci	25-50	188	388
Short Streptococci	0-6	33	127
Long Streptococci	0-3	33	41
Staphylococci	0-6	435	562
Isolated rods	0-1	1.8	3.5
Total number of bacteria	25-125	1063	1396
Number of groups	6-40	189	279
Average number bacteria per group	1.5-3	5.6	5