The Limitations of Significance of Some of the Methods of Analyzing Ice Cream¹

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THIS PAPER consists primarily of a plea for the use of common sense in the interpretation of the results of laboratory analyses of dairy products. There is an ever increasing emphasis being placed on the necessity for quality control of ice cream. The rapidly increasing number of public health regulations affecting the manufacture of ice cream, such as pasteurization requirements, bacterial standards, weight standards, stipulations of the quality of ingredients, composition of the mix, health certificates for employees, etc., should serve as warning to the ice cream manufacturer that he will soon be operating under as strict regulation as the market milk producer. In fact, the time may not be so far ahead when ice cream will be graded as market milk is now graded. This situation suggests that the ice cream manufacturer should become familiar with the significance and limitations of the various laboratory methods employed in the analysis of his product.

Any method of analysis has certain inherent limitations of greater or lesser magnitude. These limitations may consist of wide variations in the results of replicate analyses or the presentation of an indirect or perhaps only a partial index to the thing to be measured. It does not necessarily follow that a method is rendered valueless because of such limitations, but it does follow that intelligent interpretation of the significance of results obtained presupposes due recognition of the limitations imposed by the procedure employed.

The purpose in presenting a frank discussion of the limitations of some of these methods is not in the least to destroy confidence in them but,

¹Presented before the International Association of Ice Cream Manufacturers, Oct. 16, 1935. St. Louis, Mo. Contribution No. 167 from the Department of Bacteriology, Kansas Agricultural Experiment Station, Manhattan, Kansas.

quite on the contrary, to increase confidence in them. Much of the dissatisfaction encountered with laboratory analyses can be traced to disappointments and embarrassments resulting from misinterpretation of their significance. It is always a safe practice, though it may not be as satisfying, to avoid speculations by confining conclusions to the data at hand.

The basis of the program for sanitary control should be inspection, and the effectiveness of that inspection should be enlarged by laboratory analysis. If one strips the sanitary control program of its technicalities, he finds that the central objective is to provide and insure a clean, wholesome, safe product. This objective can be attained most effectively by a suitable balance between inspection and laboratory analysis. Overemphasis of one or the other of these two factors is a common mistake; but, of the two, it is more common to find the sanitary control program resolving itself into a routine laboratory examination of samples collected periodically. Too frequently the plant is adjudged "clean" or "dirty," and the product regarded as "safe" or "dangerous" on a basis of laboratory findings not confirmed by inspection. Such a system totally ignores the fact that there are factors other than cleanliness which affect laboratory findings and which may have little if any very direct relation to the safety of the product. As valuable and indispensable as laboratory analyses are to a well-rounded sanitary control program, they cannot be used as a sole means of inspection.

STANDARD PLATE COUNT

The standard plate count reveals the number of organisms in a sample (of ice cream) capable of growing under rigidly prescribed conditions which are favorable for only a certain, and perhaps uncertain, percentage of the total bacterial flora of the sample. It is assumed that about the same percentage of the total number of bacteria will grow if all samples are plated under these strictly standardized conditions. Logic and experience justify the general applicability of this assumption, but likewise both logic and experience expose exceptions to the general hypothesis. This is not a serious limitation but it should not be entirely neglected.

Another limitation of the plate count as applied to ice cream is of interest perhaps only to the laboratory technician. For some reason, not entirely clear, ice cream frequently contains rather large numbers of saccharophilic, heat-resistant organisms which will not grow on plain agar, but will grow if a very small amount of sugar is added to the medium. Even the small amount of sugar carried over from the sample in the lowdilution plates is frequently enough to support growth, whereas the high-dilution plates in the same series will not show the expected number of colonies. It is not uncommon to find low-dilution plates containing so many colonies that it is impossible to count them, whereas the plates next higher in dilution are apparently sterile. Other samples of ice cream are commonly encountered which contain organisms manifesting this same saccharophilic tendency in a slightly different way. If some plain agar plates, which at first appear to be practically sterile, are examined with a strong lens, literally myriads of very small colonies may be discerned. Unlike the colonies just described, they are present in the expected numbers in the series of dilutions but are too small to count without special lens equipment. If ice cream samples containing either of these types of organisms are replated on the same agar to which one percent of dextrose has been added, the colonies of the first type appear in the expected numbers in all dilutions; and the colonies of the second type develop sufficiently large to be counted without the aid of magnification.

Parallel bacterial counts, using standard agar and 1 percent dextrose agar, have been made on 271 samples of ice cream collected over a two-year period from practically every manufacturer in the state of Kansas. In approximately 6 percent of these samples the use of dextrose agar materially increased the count by enabling colonies to grow which either failed completely to grow on plain agar or were so extremely small that it was practically impossible to count them. With such samples the use of standard agar was entirely unsatisfactory and the use of dextrose agar completely solved the difficulty. With an additional 50 percent of the samples, the use of dextrose agar proved to be distinctly advantageous in counting, although the differences between the dextrose agar and plain agar counts were within the limits of normal variation. With the remaining 44 percent of the samples dextrose agar proved to be of no advantage or disadvantage. Before standard procedures for the plating of ice cream have become too firmly established to be changed, it would be well to investigate the advantages presented by dextrose agar.

Perhaps the most common objection to the plate count on milk or ice cream is the persistent tendency, even on the part of some inspectors, to misinterpret the significance of the results. This is attributable perhaps to a lack of familiarity with the limitations of the method and to the popular tendency to regard bacteria, dirt and disease as inseparable associates.

We must not conclude that all samples of ice cream with high bacterial counts have necessarily been produced in dirty plants and that they are absolutely dangerous to the consumer. We may sanely conclude that there exists in that plant a faulty practice which must be identified and corrected. A high plate count on a sample of finished ice cream may result from such things as the use of poor ingredients, ineffective pasteurization, inadequate refrigeration of the mix during aging or improperly sterilized utensils. These conditions are, of course, undesirable and should be corrected; but further inspection only will reveal which one or which combination of these factors is out of control.

Similarly, we must not conclude that all samples of ice cream with low bacterial counts have necessarily been produced in clean plants. The demand for cleanliness of the plant can best be enforced by frequent and rigid inspection. The real value and place of bacterial counts in the program of sanitary control are to insure the inspector or the plant manager that previous inspections have been thorough, that instructions are being carried out and that the raw ice cream mix of unquestionable quality is being properly pasteurized, aged and frozen in clean equipment. These are indeed important factors in the safety and cleanliness of the final product, but they constitute only a part of complete sanitary control. Until we realize that bacteriological analyses alone do not constitute a complete sanitary control system, and learn to confine the interpretation of bacterial counts to their logical limitations, we cannot approach intelligently the problem of producing a clean, wholesome, safe product.

BACTERIAL STANDARDS

Bacterial standards for ice cream are extremely useful, providing they are properly interpreted. When municipal or state authorities set a bacterial standard of, let us assume, 100,000 per cubic centimeter by the plate count, that is a brief method of saying that in their opinion any ice cream containing more than the specified number has been improperly processed and the plant should receive the immediate attention of a qualified inspector. It does not say that the ice cream is unclean or unsafe or that the plant is dirty. Inspection may reveal the specific cause, and directions may be given for immediate correction.

The standard serves merely as an arbitrary basis of judgment, and excessive counts call the attention of the plant operator and the inspector to the immediate need for co-operative, corrective effort. The use of the bacterial standard beyond this interpretation is difficult to defend. Continued failure on the part of the manufacturer to correct the defects in his processing reflects his incapacity to handle human food properly, and his prompt removal is in the interest of public welfare. In the rare cases when prosecution of a manufacturer is justified, it should be based on his inability to handle human food or persistent unwillingness to co-operate with public health officials, never upon the flimsy pretext of an excessive bacterial count. The latter is merely evidence of the former. It is to the interest of the ice cream industry as a whole, as well as of the general public health, that persistently dirty ice cream plants be closed regardless of the bacterial count.

It is believed that much of the misinterpretation, misuse and dissatisfaction with bacterial standards are due to the phraseology with which they are incorporated in state and municipal regulations. The following is quoted from a milk ordinance:

"Milk containing in excess of 100,000 bacteria per cubic centimeter shall be regarded as unsafe and dangerous to the consumer, and shall not be offered for sale." This clause implies a more imminent danger to the consumer of such products than experience would justify. In order to give the bacterial standard a legal status without erroneous implication, would it not be better to state that, "Bacterial counts in excess of 100,000 per cubic centimeter shall be officially regarded as excessive"? After all, that is essentially what the bacterial standard is—an official interpretation of an excessive count and not a line of demarcation between safety and danger.

DIRECT MICROSCOPIC METHOD

The direct microscopic examination of ice cream consists, briefly, of staining a known volume of the product which has been spread over a known area and counting the organisms in a few representative fields of a standardized microscope.

The principal limitation of this method as applied to ice cream is that the organisms killed by pasteurization are not distinguishable from the survivors, and all are counted. If one is interested in determining what the bacterial content of the mix was before pasteurization, this limitation becomes an asset to the method.

An important limitation of the microscopic method of analysis is the inevitably small sample ultimately employed for judging the sanitary quality. In the ordinary examination one actually observes only about 0.0001 cc. of mix or melted ice cream, but upon this small sample he passes judgment and perhaps condemns a large volume. The smallness of the sample may be forcibly illustrated by comparing the 0.0001 cc. sample used in judging the quality of a 300-gallon volume of mix to a proportionately small sample of wheat. By suitable calculation it can be shown that the analysis for protein of only one grain of wheat from 14.19 carloads would be comparable to judging a 300-gallon vat of mix on a basis of a 0.0001 cc. sample. If the illustration be based on the examination of 0.0001 cc. from a 10-gallon can of mix, it is comparable to using the grain of wheat as representative of 473 bushels, or approximately 10 wagonloads. It is needless to emphasize that due recognition should be given to the smallness of the sample when interpreting the results of the microscopic method of analyzing ice cream.

COLON TEST

The determination of the numbers of colon types of organisms plays such a very important part in judging the extent of fecal pollution of water, that dairy bacteriologists have attempted to apply the test to dairy products. Unfortunately, however, practically all, if not all, milk contains organisms of this type and these organisms grow rapidly at ordinary temperatures. Although the numbers found in a perfectly fresh sample of milk may reveal the extent of fecal pollution, the growth of these organisms while the milk or cream is reaching the market obscures the index to fecal pollution and reflects primarily the temperature of handling.

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Since many strains of the colon types of organisms are destroyed by pasteurization, the test has been used as an index to ineffectively pasteurized milk and ice cream mix. More careful investigation reveals, however, that the original hypothesis is subject to criticism and that many colon types do survive pasteurization at 145° F. for 30 minutes. When higher temperatures are employed for a 30-minute period, the colon index may reveal more effectively contamination subsequent to pasteurization.

The value of the colon test on dairy products is essentially limited to the analysis of perfectly fresh milk or milk which has been so effectively refrigerated that its original bacterial flora has not changed materially, or, in the case of ice cream mix pasteurized at 155° F. or higher for 30 minutes or more, to the detection of contamination after the pasteurization process.

THE METHYLENE BLUE REDUCTION TEST

Although the methylene blue reduction is not frequently applied directly to ice cream, it is discussed here because of its wide use in many ice cream plants for judging the quality of milk used in compounding the mix. Its popularity may be due to its simplicity, its practicability and, supposedly, its accuracy. The test consists of adding a standard concentration of methylene blue to milk and incubating it at 37° C. until the blue color disappears.

This test is based upon the assumption that bacteria induce changes in milk which cause the oxidation-reduction potential to fall and, as a result, the dye to lose its color. The larger the original bacterial population, the shorter is the time required to bring about this visible change in the indicator. This assumption, however, overlooks three important considerations: (1) The necessary reducing intensity is much more effectively produced by some organisms than by others; (2) it may be induced altogether independently from bacterial action by the organic constituents of the milk; or (3) it may be induced by leucocytes in freshly drawn milk.

Under any circumstances bacterial reduction depends upon two important variables—the number of organisms and the kind of organisms. If the same kind of organism always dominated the bacterial flora of all samples of milk, then the only variable to be considered in the examination of several samples of milk would be the original number of bacteria in each of the samples. Unfortunately, the domination of some one type cannot be depended upon in fresh milk; but a dominant type is likely to occur when the milk is older.

By the time the bacterial populations of a number of samples of milk have reached 500,000 or 1,000,000 per cc., the odds are much in favor of the assumption that the dominating organism in each sample will probably be *S. lactis.* To the extent that this assumption is justified, the kind of organisms in relatively poor milks may be considered to be fairly uniform. In such samples practically the only variable then would be the number of organisms; if this is so, the reduction time would reflect the original bacterial population. Under these conditions, the relatively small influence exerted by the reducing capacity of the milk constituents and by the leucocytes will be so greatly overshadowed by the reduction intensity induced by the large bacterial population that they will be unimportant. On the other hand, if the milk is of high sanitary quality and contains relatively few organisms, the dominance of a uniform type is less likely to have been established, and the reducing capacity of the milk constituents and the leucocytes plays a proportionately larger part in the reduction time. Under these conditions there are so many variables that the reduction time no longer can be relied upon to reflect the original bacterial population.

It is customary to recognize as Class I any milk in which the reduction time exceeds 5.5 hours; such milk, it is assumed, usually contains less than approximately 500,000 bacteria per cc. In the absence of an understanding of the fundamental principles of this test, there has been a tendency on the part of some inspectors to assume that if a 5.5-hour reduction time indicates a count of 500,000, reduction times of 10, 16 or 24 hours indicate correspondingly fewer organisms in direct order. Experiments which have been carried on at the Kansas Experiment Station indicate the fallacy of this assumption. A series of samples of sterile milk and sterile ice cream mix were inoculated with serially increasing numbers of a pure culture of S. lactis, adjusted to give reduction times varying from 5 to as high as 24 hours. Since the kind of organism introduced was uniform for each sample, the only variable was the number and, as might be expected, even the reduction periods of 24 hours reflected accurately the relative original bacterial populations. When this experiment was repeated, however, using similar serial inoculations of a mixed culture of common milk types of organisms, the reduction periods did not reflect the original populations. Such results tend effectively to defend the statement that the reduction period of milk depends upon the kind as well as the number of organisms; and, unless the kind of organisms in several samples of milk is uniform, their respective reduction periods cannot be relied upon as an index to their relative bacterial populations. This relative uniformity of flora cannot be relied upon until the count reaches the arbitrary minimum of approximately 500,000 or a reduction time of 5.5 hours.

This imposes a serious limitation on the methylene blue reduction test which should not be overlooked. Bacterial counts for milk of recognized quality in the United States are of the order of magnitude of 10,000 to perhaps 200,000 per cc. By the time a sample of milk reaches the more or less arbitrary lower limit of 500,000, where the methylene blue reduction test comes into play, our interest in this milk has diminished to the vanishing point. In other words, the methylene blue test begins to measure relative bacterial numbers in milk effectively only after it is too late.

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SUMMARY

Again it should be emphasized that this discussion of the shortcomings of the various methods of milk analysis is not intended as destructive criticism, but to increase their utility by avoiding the pitfalls of misinterpretations. These are the best methods available; and, until better methods are provided, they must be used. The important part these bacteriological methods have played in the improvement of the milk supply of the United States during the past quarter of a century is in itself adequate recommendation for their continued use despite their limitations. The limitations of a method need not be a serious handicap so long as the limitations are recognized and not completely ignored. As stated in the beginning, most of the dissatisfaction experienced in the past with these methods is traceable to failure to recognize the limitations imposed by the procedure employed. For emphasis may it be repeated that intelligent interpretations of the significance of the results of any method presupposes an understanding of the limitations of the data.