EFFECT OF TIME AND TEMPERATURE OF INCUBATION ON THE PLATE COUNT OF DRY MILKS

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ABSTRACT

Bacterial counts of nonfat dry milk of various heat types and of dry buttermilk were determined by the agar plate method using three incubation conditions, 35°C for 48 hours, 32°C for 48 hours, and 32°C for 72 hours. Generally higher plate counts were obtained when incubating samples at 32°C for 72 hours. No outstanding differences were found when samples were incubated at 35°C for 48 hours, as compared to incubation at 32°C for 48 hours. There was no relation between the direct microscopic clump count and differences in plate counts due to varying incubation conditions. Results clearly demonstrate that only a very small percentage of samples were affected in their grade classification as a result of extending the incubation time to 72 hours.

In the 11th edition of "Standard Methods for the Examination of Dairy Products" (1), the incubation conditions for colony counts as determined by the agar plate method for dry milks were changed from 48 ± 3 hr at either 32°C or 35°C to 3 days at 32°C. The incubation temperature of 35°C, according to "Standard Methods" is no longer optional. These changes apply only to dry milks. The agar plate method for other dairy products, including raw and pasteurized fluid milk, still allows incubation of the plates for 48 ± 3 hr at either 32°C or 35°C.

The present American Dry Milk Institute procedure, as described in Bulletin 916 "Standards for Grades for the Dry Milk Industry, including Methods of Analysis", (2) specifies incubation at 35°C for 48 ± 3 hr.

It is the purpose of this work to determine the effect of these specific changes of incubation conditions in the bacterial estimate of dry milks and to evaluate the results of such changes.

EXPERIMENTAL PROCEDURE

Samples of nonfat dry milk (NDM) from a number of manufacturers and processed under various heat treatments, as well as of dry buttermilk, representing production from different manufacturers, were analyzed in duplicate for bacterial estimate using three different sets of incubation conditions, namely, 35°C, 48 hr; 32°C, 48 hr; and 32°C, 72 hr.

Buffered distilled water tempered to 45°C was used for reconstituting the samples. Plates for the three sets of incubation conditions were prepared from the same dilution bottles. The media used in the preparation of the plates was standard plate count media, as specified in "Standard Methods" (1). Only the incubation conditions were varied in the experiments.

Undenatured whey protein nitrogen (WPN) according to the Harland-Ashworth (2) method was determined in a number of samples of nonfat dry milk in order to classify them into low, medium, and high heat and thus observe the influence of the heat treatment as evaluated by the WPN values on the bacterial counts obtained. WPN was also run on some of the dry buttermilk samples investigated.

The Direct Microscopic Clump Count (DMCC) was determined in some of the nonfat dry milk samples analyzed to illustrate any possible relationship of this value and the bacterial counts obtained at different incubation conditions.

RESULTS

Table 1 presents a summary of all the plate counts obtained using the three sets of incubation conditions under study. The data are presented graphically in Figure 1. When 57 samples of low heat NDM were incubated at 32°C for 48 hr, there was a 13.6% in-
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Figure 1. Effect of varying conditions of incubation on the plate count of dry buttermilk and nonfat dry milk.

Increase in counts over results obtained at 35°C for 48 hr. When samples were incubated at 32°C for 72 hr, there was a 39.3% increase in counts over those incubated at 32°C for 48 hr and a 58.3% increase over those incubated at 35°C for 48 hr. The WPN in these samples ranged from 6.0 to 7.9 mg per g. The DMCC on 32 of the samples tested ranged from 6 million to 72 million per g. There was no relation between the DMCC and differences in plate counts due to varying incubation conditions. The plate counts at 35°C for 48 hr ranged from 200 to 930,000; those obtained at 32°C for 48 hr ranged from 100 to 1,100,000; and those obtained at 32°C for 72 hr ranged from 410 to 930,000 per g of dry milk.

When the 19 medium heat samples of NDM were incubated at 32°C for 48 hr, there was a 9.0% decrease when compared to the count obtained at 35°C for 48 hr. Samples incubated at 32°C for 72 hr showed a 99% and 81% increase in counts over those obtained at 32°C for 48 hr, and 35°C for 48 hr, respectively. The DMCC of 14 of the samples ranged from 12 to 440 million per g and again there was no relation between the DMCC and the differences in plate counts due to different incubation conditions. The plate counts obtained ranged from 210 to 95,000, 300 to 82,000 and 300 to 120,000 per g when incubation was carried at 35°C for 48 hr, 32°C for 48 hr and 32°C for 72 hr, respectively. The WPN ranged from 1.7 to 5.9 mg per g on the 19 samples of medium heat NDM.

The plate count obtained on 18 samples of high heat NDM at 32°C for 48 hr was 10.3% less than the count obtained at 35°C for 48 hr. The count obtained at 32°C for 72 hr was 38.5% higher than the count obtained at 32°C for 48 hr, and 24.1% higher than the resulting count at 35°C for 48 hr. The WPN of these 18 samples ranged from 0.3 to 1.5 mg per g. The DMCC of 11 of these samples ranged from 4 to 167 million per g and again there was no relation between the DMCC and the differences in counts due to different incubation conditions. The plate counts obtained ranged from 300 to 100,000, 370 to 80,000 and 300 to 98,000 per g when samples were incubated at 35°C for 48 hr, 32°C for 48 hr and 32°C for 72 hr, respectively.

It was also observed on the samples of NDM studied that there was no relation between the DMCC and the plate counts obtained using any of the three sets of incubation conditions above mentioned. The relation between the DMCC and the differences in plate counts obtained at 32°C for 48 hr and 32°C for 72 hr is shown in Figure 2 for data described in the preceding paragraphs.

When 73 samples of NDM of unknown heat treatment were plated at 32°C for 48 hr, the plate count was 5.7% higher than the count obtained by incubating at 35°C for 48 hr. Samples incubated at 32°C for 72 hr were 60.3% and 69.3% higher than those obtained by incubating at 32°C for 48 hr and 35°C for 48 hr, respectively. The plate counts obtained at 35°C for 48 hr ranged from 190 to 44,000; those obtained at 32°C for 48 hr ranged from 210 to 44,000; and those obtained at 32°C for 72 hr ranged from 300 to 75,000 per g. The DMCC and WPN tests were not run on these samples.

The results obtained by incubating 54 samples of dried buttermilk at 32°C for 48 hr were 20.8% lower.
TABLE 2. GRADE CHANGES AS A RESULT OF VARYING INCUBATION CONDITIONS FROM 35 C FOR 48 HR TO 32 C FOR 72 HR

<table>
<thead>
<tr>
<th>Product</th>
<th>Total</th>
<th>Extra to below grade</th>
<th>Extra to standard</th>
<th>Standard to below grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low heat NDM</td>
<td>57</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Medium heat NDM</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>High heat NDM</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NDM - heat treat-</td>
<td>73</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ment unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buttermilk</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Total            | 221   | 7 (3.1%)              | 1 (0.45%)         | 5 (2.2%)                |

than the counts obtained by incubating at 35 C for 48 hr. There was a 27.4% increase in the counts obtained at 32 C for 72 hr compared with those obtained at 32 C for 48 hr. The average counts for the 32 C 72 hr, and the 35 C 48 hr incubation, were practically similar. The plate count yielded results ranging from 320 to 54,000, 280 to 29,000, and 280 to 45,000 per g for samples incubated at 35 C 48 hr; 32 C 48 hr; and 32 C 72 hr, respectively. The WFN analyses performed in some of these samples yielded results ranging from 0.4 to 2.1 mg per g.

The mean plate count of all of the above samples, a total of 221, obtained at 32 C for 48 hr was 4.3% higher than that obtained at 35 C for 48 hr. The mean plate count of all samples incubated at 32 C for 72 hr was 43.4% higher than that obtained at 32 C for 48 hr. The mean plate count obtained at 32 C for 72 hr was 49.6% higher than that obtained at 35 C for 48 hr.

Since the plate count obtained on dry milks is part of a grading specification, it is of value to determine the effect of the various incubation conditions on the grade assigned. "Extra" grade of nonfat dry milk and dry buttermilk respectively, should have a bacterial estimate no greater than 50,000 per g. The plate count of "Standard" grade must not be greater than 100,000 and 200,000 per g for nonfat dry milk and dry buttermilk, respectively, and if this is exceeded, no grade may be assigned.

Table 2 presents the grade changes in the various types of dry milk products as a result of varying the incubation conditions. It will be noted that most changes occurred in the low heat type of NDM. For the 221 samples tested when the incubation conditions were changed from 35 C for 48 hr to 32 C for 72 hr, 3.1% of the samples were changed from "Extra" grade to "Standard" grade, 0.45% were changed from "Extra" grade to below grade and 2.2% were changed from "Standard" to below grade. Out of a total of 221 samples, 13 or 5.9% were affected by the change in incubation conditions.

DISCUSSION AND CONCLUSIONS

The data presented in this paper indicates that higher plate counts were obtained at 32 C when the incubation time was increased from 48 hr to 72 hr.

Very slight differences in counts were obtained when plates were incubated at 32 C for 48 hr, as compared to plates incubated at 35 C for 48 hr, although the colonies were commonly larger and consequently easier to count at the higher temperature.

While higher plate counts were obtained by using the 72-hr incubation period, still higher counts may result by incubating for 5 or 7 days at lower temperatures as shown by Thomas, et al. (3). A method to be included in "Standard Methods" (1) must be practical. Therefore, considerations other than the one criterion of obtaining the highest possible count must be evaluated. As generally understood, there are four major criteria for judging the practicability of a method: accuracy, precision, economy and safety. With the exception of safety, which is not relevant in the evaluation of the plate count, the other factors will be mentioned in this discussion.

Of the above criteria, the only one usually considered in evaluating standard plate count procedures is that of accuracy. The term accuracy, may be considered the degree to which the results of the method approach the true or absolute value of the quantity present of the substance or character measured in the sample. Accuracy also depends upon the fundamental knowledge of the basis for the method, so that the more empirical a method is, the less accurate it is likely to be. The standard plate count is an empirical method, since the results obtained by its use depend upon certain arbitrary conditions.

An assumption has been made that those techniques which result in the highest total count are those which achieve the greatest accuracy, yet it seems that this simple assumption has not been carefully explored. It is generally recognized that dairy products contain a variety of organisms, some of which necessitate special techniques for their detection. It would appear difficult to devise any single method capable of detecting at one time all viable organisms, due to the numerous specific conditions which the different bacteria present in the product require for growth. Consequently, it may be seen that the absolute accuracy of the standard plate count, sought by some investigators, will not be readily attained.

Another factor which should be considered in evaluating the standard plate count method is that of precision. The term precision, according to the
technical usage of the word, means the degree to which the results obtained by a given method are reproducible. A method may be precise, yet lack accuracy. Precision assumes a great deal of importance when a method is used to determine the compliance of a product with a standard. Under these circumstances a laboratory must be capable of duplicating its own results, as well as those obtained by other laboratories. The complexity of a method or the ease of its performance, influences its precision.

Precision of the standard plate count also leaves much to be desired, since the method is highly empirical. Close adherence to the prescribed method is essential until all the variables have been investigated from the standpoint of their effect upon precision. The consideration of precision, however, does assume importance from the standpoint of the use to which the results will be put. Nonfat dry milk, for example, is purchased on the basis of grade, one of the requirements of which is the standard plate count. The standard plate count requirement for "Extra" grade instant nonfat dry milk is a maximum of 35,000 per g and for "Extra" grade nonfat dry milk, a maximum of 50,000 per g. These values are 3,500 and 5,000 per ml, respectively, on a reconstituted basis. Compared with existing standard for fluid milk on the order of 30,000 per ml, these standards for nonfat dry milk are, in fact, exceptionally rigid, yet a majority of the samples tested complied with these specifications, most of them being well below the maximum. The precision of the method, then, is not of great importance, since in many cases even doubling the standard plate count would not change the grade.

The level of the standard, however, is low enough to usually ascertain and prevent improper processing methods or any possible health considerations. It does not seem reasonable, therefore, from either an accuracy or a precision standpoint that there is an advantage in using a 3-day incubation period over the 2-day.

It would seem that the criterion of economy should carry some weight in considering the relative merit of the 2-day and 3-day incubation period. Speed or time required, is a factor which affects economy. The speed of analysis may be of considerable importance, particularly in routine control tests. Where products are graded before use, the time involved in grading influences the amount of product which must be held in inventory until the product is released. Furthermore, in testing for quality control, rapid methods may result in more adequate control since the knowledge obtained from the results may be used by the plant operator to alter the process favorably.

In most drying plants today, grade analyses are made continuously with the manufacture of the products. Since the present edition of "Standard Methods" (1) requires a 3-day incubation period instead of a 2-day, as previously specified, additional storage space for dry milks is required and a larger inventory maintained before the product can be released for shipment. In the case of Government purchase or the testing of preshipment sample by a customer, the 3-day incubation period again results in one more day's delay in the release of the product by the Government or customer's laboratory. In addition, a 3-day incubation period requires 50% more incubator space and petri dishes.

It would appear that certain thermoduric bacteria surviving the heat treatment as may be used during processing, are impaired to an extent and require a slightly longer time for their development during incubation. These organisms, however, are apparently not of wide importance since only approximately 6% of the samples tested in this survey were affected by the longer incubation period. It is possible, however, that such organisms could be of importance to some users of dry milk and in this case special specifications may be formulated for these purposes by the parties concerned thus allowing the large majority of the users to save one day's time in the grading of dry milk products.

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**References**

