AIR-BORNE MICROORGANISM POPULATIONS IN
FOOD PACKAGING AREAS

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SUMMARY

A slit air-sampler was used to ascertain the numbers of air-borne bacteria, yeasts and molds in dairy product packaging areas. The mean air-borne bacteria count for all food packaging areas investigated was below 6 per ft$^3$. The highest mean count for a daily sampling period was just over 10 per ft$^3$. The mean count for air-borne yeasts was 2 per ft$^3$ for all samples collected. About 17% of all counts were less than 1 yeast per 5 ft$^3$. The mean air-borne mold count for all samples was over 12 per ft$^3$ with nearly 10% of the counts over 20 per ft$^3$. A mean count in cottage cheese packaging was over 22 mold per ft$^3$ while mean counts for individual sampling days revealed populations as high as 67 mold per ft$^3$. There were significant day to day variations in all areas and standard deviations of daily sampling periods indicated significant variations within 4-hr test periods. Some degree of correlation between worker activity and air-borne counts was indicated by the results. However, it is evident that other factors also contribute to the air-borne population.

The trend toward sterile processing of milk and other food products introduces new problems associated with the packaging of such products. One of the problems involved is that of preventing contamination of the product from air-borne microorganisms. However, before this type of contamination can be controlled, the microorganism populations associated with air in food packaging areas must be investigated.

Contamination of milk and food products from air has been given very little consideration in the past since the effect on shelf life was not appreciable. In products with high heat treatments and low microorganism populations, contamination from air-borne microorganisms can greatly reduce the shelf-life.

The objective of the investigation reported in this paper was (a) to determine microorganism populations of the air associated with various food packaging areas under normal operating conditions, and (b) to determine factors which contribute to air-borne contamination.

LITERATURE REVIEW

Quantitative evaluation of air-borne microbiological particles was first introduced by Wells (9), who used centrifugal force to collect the particles on a solid surface. Since this initial development, many other methods and improvements have been introduced. Among the more effective of these methods are the slit samplers (2), filtration samplers (8), electrostatic precipitation samplers (1) and thermal precipitation samplers (5).

Any of the above methods will provide information on air-borne microorganism populations. Information on these populations in dairy and food plants is very limited. Labs (6) and Cerna (3) have reported on air-borne bacteria counts in dairy plants in foreign countries. Labs (6) used a slit air sampler and reported counts of 18 colonies per liter. In addition, the same worker reported counts of 300 colonies per petri dish per minute when using the sedimentation technique. Using the same technique, Cerna (3) revealed that counts ranged from 1 to 550 colonies per petri dish per 10 min of exposure. It was found that counts were effected by: (a) the presence of workers in the given area, (b) the number of workers in the area, and (c) the activity of the workers.

PROCEDURE

All phases of the reported investigation were conducted in food packaging areas of the Michigan State University dairy plant. The study extended over a 7-month period from January to August, 1963. In all cases, experiments were conducted in a manner which would create the least interference with normal operating conditions.

Equipment.

A Casella slit sampler was used to quantitatively sample the air in various food packaging areas. This sampler operates on a solid impaction technique, using a solidified agar medium as a collection surface. A vacuum source is used to draw air through a narrow slit, which is calibrated to provide an airflow rate of 1 ft$^3$ per min. The amount of air sampled can be carried by changing duration of the sampling time to 0.5, 2 or 5 min. The number of microorganisms collected on the agar surface was determined by counting the colonies after suitable incubation periods.
Sampling locations.

Air samples were collected adjacent to milk, cottage cheese, and butter packaging locations. Milk packaging is located in a large room which also contains the milk and ice cream processing areas. The room has 16 doors or openings leading to other areas of the plant. These openings provide access for air mixing from area to area. Additional air movement is created by a central ventilation system. The sampling point at the milk packaging location was next to the paper filling operation and as near as possible to the point where air and milk contact occurs.

The cottage cheese and butter packaging locations are in a room separate from the rest of the plant, with only two doors leading into it. Two air conditioning units and the plant ventilation system provide a nearly consistent air movement except for that created by opening and closing of the doors.

The sampling point in the cottage cheese area was next to the manufacturing and packaging operations. Samples in the butter area were collected at a point next to the butter printing operation.

Sampling methods.

At each sampling location, samples were collected at 15 min intervals throughout a test period of 4 hr or more according to the procedure of Greene, et al. (4). These tests were conducted on 6 or more days at each location, and samples were collected at a level approximately 4 ft above the floor. In every case, precautions were taken to prevent contamination of the area by the operator of the sampler. Sampler parts were sterilized with 95% ethyl alcohol before each 4-hr test to prevent contamination from this source. A tryptone glucose yeast extract agar medium was used in the standard plate count determination. In
addition, potato dextrose agar adjusted to pH 3.5, by addition of a tartaric acid solution, was used to evaluate the number of air-borne yeast and mold.

Exposed plates for the standard plate count were incubated and counted after 48 hr at 35 C. However, to obtain maximum growth and a better indication of the total number of microorganisms, the plates were given additional incubation at 37 C for 48 hr and 40 F for 7 days. Exposed plates for the yeast and mold count were incubated for 7 days at 70 F.

Worker activity evaluation.

During each 4-hr test, an evaluation of worker activity, within the test area, was made. This evaluation was based on factors of the following type: (a) activities which cause air movement within the test area, (b) activities which may cause the worker to contribute microorganisms to the air, and (c) activities which bring contaminated air directly into the air sampler. In order to set some quantitative value on the factors mentioned, the following worker activity factors were considered: 

\[ \text{WAL} = a + b + 2c \]

This relationship was established in the following manner. Although the first factor (a) probably results in a continuous contribution of microorganisms to air in the test area, the second factor (b) results in movement of these air-borne microorganisms to the sampling point. Using this assumption, these factors appear to be relatively equal in degree of contribution to the activity level. However, the third factor (c) not only causes movement of air to the sampling point, but in addition, the worker may contribute additional microorganisms at the...

FIG. 2 VARIATIONS IN AIR-BORNE YEAST AND MOLD COUNTS IN THE MILK PACKAGING AREA.
TABLE 1. NORMALIZED DISTRIBUTIONS OF DAILY AIR-BORNE BACTERIA COUNTS AND CORRESPONDING WORKER ACTIVITIES IN THE MILK PACKAGING AREAS

<table>
<thead>
<tr>
<th>Sampling day</th>
<th>No. of samples</th>
<th>Normalized mean - x</th>
<th>SD</th>
<th>Mean WAL*</th>
<th>WAL SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(per 5 ft³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>8.3</td>
<td>4.1</td>
<td>7.2</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>30.6</td>
<td>8.4</td>
<td>8.3</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>41.3</td>
<td>19.8</td>
<td>13.1</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>25.3</td>
<td>7.4</td>
<td>16.3</td>
<td>7.3</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>12.8</td>
<td>6.9</td>
<td>11.7</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>23.4</td>
<td>4.9</td>
<td>13.6</td>
<td>3.8</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>21.0</td>
<td>13.5</td>
<td>12.6</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>45.2</td>
<td>29.9</td>
<td>13.7</td>
<td>5.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>122</td>
<td>26.0</td>
<td>-</td>
<td>12.1</td>
<td>-</td>
</tr>
</tbody>
</table>

*Worker activity level.

Results

An initial survey of air-borne bacteria counts, conducted by collecting air samples at random points throughout the plant, reveal a mean count of 1 colony per 5 ft³ of air for 53 samples collected under non-operating conditions (no workers present). This level compared to a mean count of 22 colonies per 5 ft³ for 100 samples collected at random points under normal operating conditions, with large variations among counts. However, the random sampling approach was not designed to provide an indication of the factors causing: (a) differences between operating and non-operating conditions or (b) variations during operating conditions. Therefore, the air-sampling approach described in the procedure was adopted.

Figure 1 illustrates typical variations in air-borne bacteria counts within a 4-hr test period during normal milk packaging operations. These variations are also typical of results obtained in the butter and cottage cheese packaging areas. Of significance in Figure 1 are the changes in air-borne count which can occur in a 15 min interval. In the illustration, the count changes from a low of 19 colonies per 5 ft³ to a high of 117 per 5 ft³ within 15 min. These changes result in a standard deviation of 26 from a mean of 47 colonies per 5 ft³.

The changes in worker activity level (WAL) and machinery (Pure-Pak filler) activity are presented in Figure 1. In this case, the variations in air-borne bacteria count appear to be related to variations in worker activity level. However, this relationship did not appear in all test periods, indicating that other factors were contributing to the variations. The effects of machine activity on air-borne count are not evident; however, other factors such as air movement or worker activity could overshadow these effects. Process activities such as operation of the butter printer in the butter area or draining or whey in cottage cheese area resulted in increased air-borne counts.

Figure 2 illustrates the variations in air-borne yeast and mold counts within a 4-hr test period in the milk packaging area. These data are typical of results obtained in the butter and cottage cheese packaging areas, also. Again significant variations are evident with yeast counts varying from 0 to 61 per 5 ft³ and mold counts from 16 to 42 per 5 ft³ within this test period.

Possible correlation between the WAL and air-borne yeast and mold counts (Figure 2) is less evident than with air-borne bacteria. In general,
results indicated somewhat closer relationships between air-borne mold counts and worker activity than between air-borne yeast counts and worker activity. There is little evidence of a direct effect of machine activity on these air-borne counts.

Results in Table 1 represent air-borne counts and worker activity levels from 8 different sampling days at the same point in the milk packaging area. In each case, the mean and standard deviation have been obtained for a normalized distribution of the values from each sampling day. Normalization was performed by plotting the experimental values on probability paper. This transformation allows a somewhat more detailed statistical analysis of day to day variations in the air-borne counts.

In general, the results (Table 1) indicate considerable variations in the means of daily air-borne counts, with a range from 8.3 colonies per 5 ft³ on sampling day no. 1 to 45.2 per 5 ft³ on sampling day no. 8.

Since the standard deviations within sampling periods are as high as ± 66%, it is evident that the variations of air-borne counts within periods are considerable, also. In addition, the results reveal that standard deviations of daily air-borne counts vary on a day to day basis, indicating that the amount of variation within sampling periods changes from day to day.

In order to establish some quantitative magnitude for the day to day variations, tests were conducted comparing the mean air-borne counts of 2 different sampling days. For the case of homogeneous variances (sampling days no. 1 and no. 6), the means were different at 0.1 level of significance. When the variances are non-homogeneous as is the case for sampling days no. 1 and no. 8, the means are significantly different at a significance level less than 0.01.

The results indicate that significant variations between air-borne count means do not exist for all sampling days. For example, a test comparing the means from sampling days no. 4 and no. 6 reveals that the difference is not significant even at a significance level of 0.4. However, the tests which have been conducted indicate that significant day-to-day variations do exist in most cases.

Table 2 provides a summary of air-borne bacteria counts for 315 air samples collected in three food packaging areas. The mean count for all samples was 27 colonies per 5 ft³ with mean counts for individual sampling days ranging from 9 to 53 per 5 ft³. Over one-half (56.1%) of the counts were below 30 per 5 ft³, but 14.3% were over 50 per 5 ft³.

A summary of air-borne yeast counts for 282 air samples collected in the milk, butter, and cottage cheese packaging areas is presented in Table 3. The mean count for all samples was 10 yeasts per 5 ft³ of air, with daily means ranging from 1 to 30 per 5 ft³. Only 26.6% of the counts were greater than 10 per 5 ft³ and 17.3% were less than 1 per 5 ft³.

Air-borne mold counts for 282 air samples collected in three dairy food packaging areas are summarized in Table 4. The mean count for all samples was 68 molds per 5 ft³ with a range from 7 to 334 per 5 ft³ for means of daily sampling periods. Nearly 10% (9.2%) of the counts were over 100 per 5 ft³ with 57.1% being less than 30 per 5 ft³.

The air-borne counts for the three packaging areas were all relatively close to the overall mean, except for the air-borne mold counts. Although the mean air-borne mold count for the milk packaging area was relatively low, the mean counts for the butter and cottage cheese packaging areas were much higher in comparison.

The results in Table 1 also reveal rather good agreement between mean daily air-borne bacteria counts and mean daily WAL for the milk packaging area. A calculated correlation coefficient for these values is 0.417, indicating some degree of agreement between worker activity and air-borne counts. Although this does not represent a high degree of correlation, the coefficient does indicate the influence of air-borne counts on worker activity.
air-borne microorganisms

Table 4. Air-Borne Mold Counts in Food Packaging Areas

<table>
<thead>
<tr>
<th>Area</th>
<th>Samples</th>
<th>Mean count</th>
<th>SD</th>
<th>No. of sampling days</th>
<th>Range of daily mean counts</th>
<th>Range of daily SD</th>
<th>Percentage distribution of counts/5 ft³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>89</td>
<td>21</td>
<td>9.0</td>
<td>6</td>
<td>7 - 42</td>
<td>2.6 - 13.7</td>
<td>29.2 (%) &lt;30 (%) &lt;30 (%) &lt;10 (%)</td>
</tr>
<tr>
<td>Butter</td>
<td>98</td>
<td>69</td>
<td>272.0</td>
<td>6</td>
<td>17 - 276</td>
<td>6.5 - 690.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Cottage Cheese</td>
<td>98</td>
<td>111</td>
<td>332.0</td>
<td>6</td>
<td>19 - 334</td>
<td>6.7 - 831.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Total</td>
<td>282</td>
<td>68</td>
<td>249.5</td>
<td>6</td>
<td>7 - 334</td>
<td>2.6 - 831.0</td>
<td>11.4</td>
</tr>
</tbody>
</table>

*Per 5 ft³.

of worker activity on the air-borne counts. However, this correlation also reveals that factors other than worker activity must contribute to air-borne counts.

A comparison of standard deviations of air-borne bacteria counts and standard deviations of WAL obtained in the cottage cheese packaging area is presented in Table 5. The correlation coefficient for the values obtained on six different sampling days is 0.825. This coefficient reveals very good agreement between variations in air-borne bacteria counts and standard deviations of WAL. In other areas, this correlation also reflects the degree of correlation was not evident in other areas indicating that factors other than worker activity are involved in air-borne count variations, also.

Discussion

The data provide an indication of air-borne microorganism populations in food packaging areas of a dairy plant. These data are not expected to apply to food packaging area of all plants; however, this information provides a general view of the air conditions in such areas. Furthermore, the information presented should provide an indication of the factors which contribute to air-borne microorganism populations and must be controlled in order to maintain desired low counts.

All air-borne bacteria counts reported (28 per 5 ft³ in milk packaging; 31 per 5 ft³ in butter packaging; 22 per 5 ft³ in cottage cheese packaging) are 6 bacteria per ft³ or less. These are low counts when compared to the only other reported value for a dairy plant by Labots (8) of 18 bacteria per liter or approximately 510 bacteria per ft³. Also, they are very low when compared to mean counts of 22.3 and 13.2 colonies per ft³ for 3079 and 934 samples, respectively, collected in many areas of two different hospitals as reported by Greene, et al. (4). The air-borne bacteria counts in these areas compares favorably with recent reports for hospital operating rooms and industrial white rooms by Michaelsen and Vesley (7). Populations in hospital rooms ranged from 1 to 81 bacteria per ft³ with a mean of 10.5 per ft³ for 795 samples, while counts in the industrial white rooms ranged from 1 to 10 colonies per ft³ with a mean of 3.32 per ft³ for 76 samples. However, counts in food packaging areas may depend on outside air counts which may be as low as 2 colonies per ft³ or as high as 28 per ft³ (10) for a particular geographical location. Approximately 10% of the counts reported in the milk and cottage cheese packaging areas and 25% of the counts in the butter packaging area were greater than 50 colonies per 5 ft³ or greater than 10 per ft³ indicating the potential of higher counts.

The air-borne yeast counts encountered (highest was 121 yeast per 5 ft³ in butter packaging area) were all relatively low. Air-borne mold counts, such as 111.3 per 5 ft³ in the cottage cheese packaging area, do seem significantly high and could be detrimental to the shelf-life of a product such as cottage cheese.

Theoretically, it would be desirable to maintain the air-borne population in food packaging areas at zero to prevent any degree of contamination. However, under operating conditions it is impossible to
avoid all contamination due to the presence of workers and the many other factors which contribute to the over-all count. On the other hand, methods available for removing microorganisms from air provide a means of limiting the population at least in an isolated area in which the factors contributing to the count can be controlled.

Significant day to day variations in all types of air-borne counts were found. The day to day variation in air-borne bacteria count could be related to a corresponding day to day variation in worker activity. In many cases, it was evident that worker activity was not the only contributing factor. Day to day variations in outside air-borne bacteria counts and variations in operation activities also could contribute to the day to day variations.

Significant variations in air-borne microorganism counts within 4-hr test periods are also evident in data reported. In many cases these variations were related to variations in worker activity. In other cases, variations in air-borne counts occurred without corresponding variations in worker activity. These results indicate that although worker activity is a contributing factor to air-borne microorganism population, other factors must be involved.

Additional exceptions to the relationship between worker activity and air-borne microorganism count are air-borne yeast and mold counts. All results obtained indicate a lack of agreement between worker activity and air-borne yeast counts. There was a closer relationship between worker activity and air-borne mold counts; but the degree of correlation was less than that for air-borne bacteria.

From observations during test periods and results obtained, many specific factors which contribute to air-borne microorganism counts are apparent. These contributing factors can be divided into two areas: (a) the factors which cause air movement and (b) the sources of the air-borne microorganisms.

Within a food packaging area, several factors may contribute or cause air movement: (a) workers or people in the area, (b) moving parts of the packaging machine or related operations, (c) ventilation systems, (d) movement of materials, and (e) opening and closing of doors. Workers and material will cause air movement to different extents depending on activities, while movement created by machines and ventilation systems are relatively consistent. However, all factors may be contributing simultaneously.

Without some major sources of air-borne microorganisms, air movement would not be an important factor. In a food packaging area, there are sufficient number of contributing sources such as workers, dust particles, outside air, and drains. Workers may act as a source of air-borne microorganisms by talking, coughing, or even natural breathing. In addition, workers may carry dust particles and associated microorganisms from area to area within a food or dairy plant. Materials being moved into the packaging area may also carry dust particles, thereby acting as a source of dust. Outside air acts as a source of air-borne microorganisms in the food packaging area by movement from outside into the plant. This movement occurs by opening and closing of doors between areas. Drains also appear to be a definite source of air-borne microorganisms. Because of the availability of nutrients in food packaging areas, drains provide excellent locations for multiplication of microorganisms. When drains become flooded with liquids, it appears that a certain portion of the microorganisms become airborne and are picked-up by passing air currents.

REFERENCES