moderate speeds in unbaffled vessels is that due to low turbulence there is virtually no tendency to foam. Foaming is commonly observed in aeration systems which use baffled or indented flasks and depend for their efficacy on a high degree of turbulence and on the entrainment of air bubbles. Foam production interferes with aeration and has other undesirable effects. It may be reduced by chemical additives, but these additives themselves interfere with aeration. Corman *et al.* (1957) have shown that anti-foam agents may reduce the oxygen absorption rate drastically, in one case from 455 to 54 ml oxygen per 100 ml per hr. It is likely, therefore, that rolling aeration is more efficient than aeration by shaking with certain types of culture media, since rolling aeration does not depend on bubble entrainment, does not generate foam, and eliminates the need for anti-foam agents.

ACKNOWLEDGMENT

This work was facilitated by a grant from the Temple University Committee on Research.

SUMMARY

For the aeration of liquid cultures of microorganisms in test tubes and larger vessels up to perhaps 1-L load, rolling has certain advantages. First, rolling of test tubes is more effective than shaking. For Erlenmeyer flasks rolling and shaking are equally effective unless baffling is used. The installation of baffles increases aeration about two-fold on the roller, but 10- to 20-fold on a high speed rotary shaker. Second, the absence of foaming is a distinct advantage of rolling aeration. Third, because of the low rotation speed, centrifugal stresses are neglibible and power requirements are moderate. Consequently, roller equipment is considerably simpler to design and construct than rotary shakers.

REFERENCES

CHAIN, E. B. AND GUALANDI, G. 1954 Aeration studies. Rend. inst. Super. sanità (Eng. Ed.), **17**, 5–60.

COOPER, C. M., FERNSTROM, G. A., AND MILLER, S. A. 1944 Performance of agitated gas-liquid contactors. Ind. Eng. Chem., **36**, 504–509.

CORMAN, J., ISUCHIYA, H. M., KOEPSELL, H. J., BENEDICT, R. G., KELLEY, S. E., FEGER, V. H., DWORSCHACK, R. G., AND JACKSON, R. W. 1957 Oxygen absorption rates in laboratory and pilot plant equipment. Appl. Microbiol., **5**, 313–318.

MONOD, J. 1950 La technique de culture continue theorie et applications. Ann. inst. Pasteur, **79**, 390–410.

PAUL, J. 1959 *Cell and tissue culture.* The Williams & Wilkins Co., Baltimore, Maryland.

STEEL, R., ed. 1958 *Biochemical engineering.* The Macmillan Co., New York, New York.

WHITE, P. R. 1954 *The cultivation of animal and plant cells.* Ronald Press Co., New York, New York.

Sampling Procedures for Bacterial Analysis of Prepared Frozen Foods[1]

PAUL A. HARTMAN AND DAVID V. HUNTSBERGER

Departments of Bacteriology and Dairy and Food Industries and Department of Statistics, Iowa State University, Ames, Iowa

Received for publication March 7, 1960

Various investigators have reported that large differences exist in the numbers of microorganisms associated with various types of frozen foods (*cf.* Borgstrom, 1955; Canale-Parola and Ordal, 1957; Huber *et al.*, 1958; Ross and Thatcher, 1958). However, little practical information is available regarding fluctuations of bacterial numbers within a single brand and type of product. Sampling procedures which are of utmost import to processors and to others interested in the microbiology of frozen foods have been in need of thorough examination. The following studies were made to determine the impact of seasonal, day-to-day, diurnal, and sample-to-sample variations on the total counts to be expected in a prepared frozen food.

The data to be presented are exemplary of certain sampling problems which are frequently encountered in the microbiological enumeration of microorganisms in prepared frozen foods. Obviously, conditions of production will vary between processing plants, between processors, and even within the same plant from year to year as improvements are made. Bacterial counts will be affected by processing technique, yet the general considerations presented here should remain relatively unchanged.

[1] Journal paper no. J-3733 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project no. 1379. This investigation was supported, in part, by research grant no. E-1141 from the Institute of Allergy and Infectious Diseases of the National Institute of Health, U. S. Public Health Service.

Methods and Materials

Frozen chicken pot pies served as the experimental material for the major portions of these studies. As suggested by Gunderson (1957) and Fanelli and Ayres (1959), all samples were obtained directly from the processor so that the history of production, treatment, and storage would be known. Immediately upon removal from the processing line, samples were blast frozen for several hours, then were stored at −10 C or lower prior to assay. Pies were selected from two processors designated A and B. Pertinent details of sampling procedure are given in appropriate places of this report.

Bacterial counts were made utilizing methods similar to those which have appeared in the literature (Zaborowski *et al.*, 1958). Samples for analysis (100 g) were taken from a representative portion of the contents of the pie (Hartman, 1960). The majority of the total counts were made using trypticase soy agar, although tryptone glucose extract agar was used upon occasion. Fanelli and Ayres (1959) observed no differences in counts obtained on the above two media. An incubation period of 48 hr at 32 C was used.

Results and Discussion

Seasonal variation in total counts. Nineteen lots of 24 samples each were taken from a commercial processing line on the first day of the work week during the period from March 31, 1958, through January 6, 1959. With few exceptions, sampling was bi-weekly (figure 1). Each lot usually consisted of six groups of four pies each, taken at half-hour intervals. Samples were usually plated on the 2nd day following processing, although in several instances the storage period was extended to 3 days.

Some increases in total count were evident during the warm months (figure 1). A mean total count of 33,000 per g was obtained on 240 samples examined during the cool months, March 31 through May 19, and October 27 through January 6. The mean total count of 216 samples taken during the warmer June 2 through October 13 period was 58,000 per g.

Seasonal fluctuations in total count appeared to be associated with the degree of control each plant operator exerted over processing techniques. Results of analysis of 606 random in-plant samples are shown in figure 2. No two samples came from any one lot of pies. When total counts were controlled satisfactorily during the winter, little increase in count was observed during the spring or summer (chicken *A*, figure 2). When very low counts were not maintained during the winter months, a 10-fold rise in mean counts was observed when the ambient temperatures increased in April and May (chicken *B*, figure 2). Bacterial contamination did not change greatly with season in one product (beef *B*, figure 2), yet large changes were observed in another product processed in the same plant (chicken *B*, figure 2). In addition, bacterial counts in one set of samples were similar (chicken and beef *A*, figure 2), yet the chicken and beef samples from processor *B* (figure 2) differed considerably in bacterial content.

Several generalizations might be made on the basis of the above information. First, when very low counts were constantly maintained, seasonal influences were minimized. If rigid control was not constantly exercised, samples which possessed very high counts appeared, especially during the warmer months, which resulted in considerable increases in mean bacterial counts. Secondly, an in-plant quality control program apparently must include simultaneous observation of all products; the use of only one product as an "indicator" of plant control may lead to erroneous conclusions.

Day-to-day variation in total count. A study of day-to-day variation in bacterial counts of chicken and

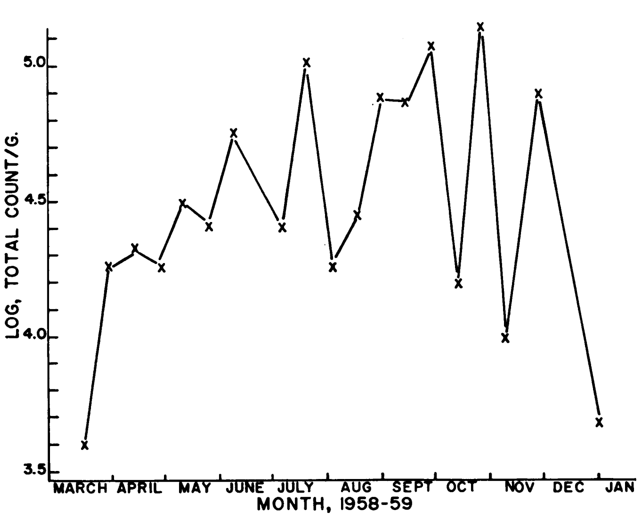

Figure 1. Seasonal fluctuations in total counts of 456 frozen chicken pies.

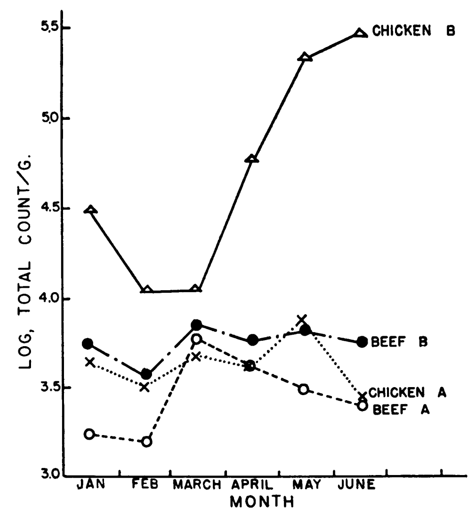

Figure 2. Seasonal fluctuations in total counts of 606 frozen chicken and beef pies obtained from two processors.

beef pies was conducted using randomly selected pies on each day of the work week. Sample sizes varied from 16 to 43 pies. The results were subjected to analyses of variance (Snedecor, 1956) on the basis of log counts (table 1). In all cases the linear and quadratic component of daily trend in mean log count was highly significant while higher order components were not. In each case a second degree polynomial was fitted to the data to estimate the relationship between log count and days (see figure 3). The general form of the fitted relation is $\hat{y} = b_0 + b_1X + b_2X^2$, where $\hat{y}$ is the estimated mean log count, b is the regression coefficient (b_0 = intercept), and X is the coded day ($X = 1, 2, 3, 4, 5$).

In three of the series (beef *A*, beef *B*, and chicken *B*) the mean count showed a decrease from Monday to the middle of the week, and then increased as the end of the week approached. One series (chicken *B*) also had a strong second degree trend, but unlike the others, showed a peak mean count between the beginning and end of the week. It appears that a certain degree of contamination was obtained on the initial day of the work week, possibly from equipment which had not been adequately cleaned prior to the start of processing operations on Monday. Increases in counts during the latter days of the work week probably were due to microbial build-up in certain areas of the plant.

Diurnal variation in total count. The effect of time of sampling is shown in figure 4. Moderate or low total counts were obtained on 12 of 19 processing days studied. Four examples are plotted in part *A* of figure 4. Dates when similar curves were obtained are listed in the key (within parentheses) following the appropriate examples. On 9 of the 12 days shown (curves *a*, *b*, and *d*, part *A*, figure 4) samples taken at any time during the examination period were indicative of the general status of all pies made on those days. Nevertheless, there were 3 days on which moderate or low counts were obtained (curve *c*, part *A*, figure 4) when hourly sampling would have been necessary to detect considerable changes in the total counts. Frequency of sampling became of greater import when mean counts of over 80,000 per g were considered (part *B*, figure 4). In one run the high count appeared on the last sampling period. In five of the six remaining runs, samples taken during at least two adjacent sampling periods yielded mean counts which exceeded 80,000 per g. In only one of the six instances examined, however, did the high count remain for more than two consecutive sampling periods. These results indicate that hourly sampling would have detected the majority of high count product, but a large portion of the high counts would have been missed if samples had been taken at less than hourly intervals.

As would be expected, high count pies appeared much less frequently when a very low count was normally observed. For example, of 105 samples examined to obtain the data plotted in one curve of figure 2 (chicken *A*), only one sample had a total count which exceeded 80,000 per g.

Sample-to-sample variation in total count. One major problem of in-plant sampling is defining what constitutes an adequate sample. Ideally, 28 to 35 pot pie

TABLE 1

*Day-to-day variation of bacterial count in frozen foods: analyses of variance**

Sample	Source of Variation	Degrees of Freedom		Mean Square
Chicken A	Among days	4		1.08056
	Linear and quadratic		2	3.10315
	Within days	103		0.28280
	Total	107		
Beef A	Among days	4		0.57423
	Linear and quadratic		2	1.84612
	Within days	97		0.22220
	Total	101		
Chicken B	Among days	4		0.93303
	Linear and quadratic		2	2.79164
	Within days	195		0.34138
	Total	199		
Beef B	Among days	4		0.33552
	Linear and quadratic		2	1.36608
	Within days	191		0.17391
	Total	195		

* Log counts by days.

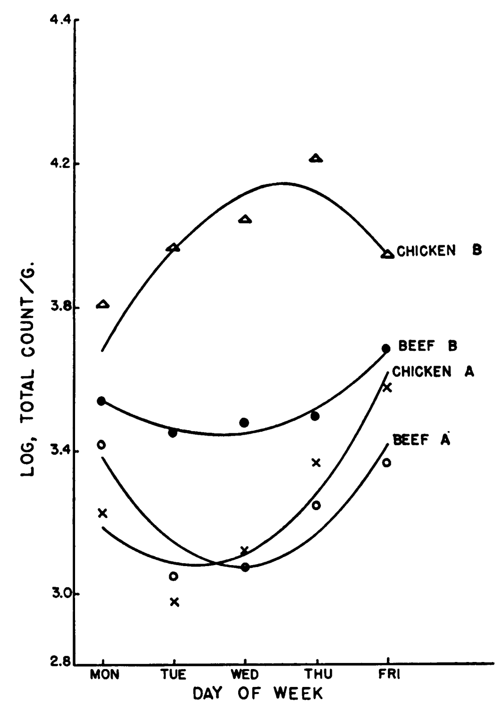

Figure 3. Log, bacterial count, as affected by the day of the week.

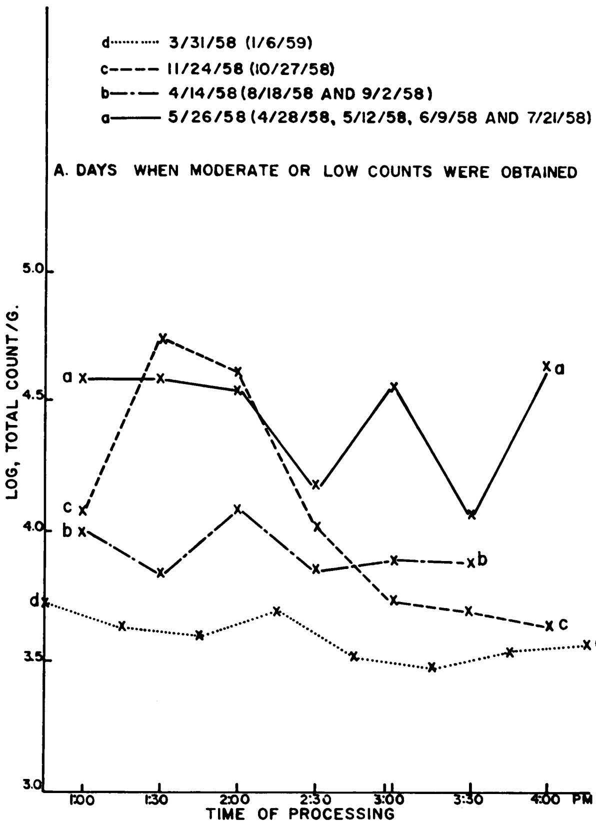

Figure 4A

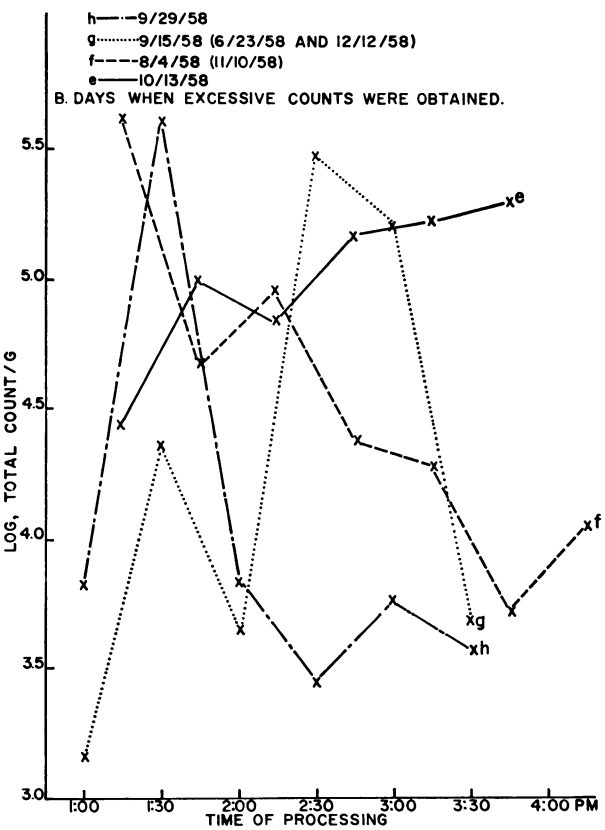

Figure 4B

Figure 4. Diurnal variation of total counts in frozen chicken pies. (*A*) Days when moderate or low counts were obtained; (*B*) days when excessive counts were obtained.

samples per line per hr should be examined (Gunderson, 1957). Practically, a much smaller number of samples must suffice for routine purposes.

To obtain a quantitative measure of the agreement, or lack of agreement, among the total counts obtained from samples taken simultaneously from the processing line, 117 groups of pies (three to 12 samples per group) were examined. An analysis was performed on the log counts (Snedecor, 1956), and the total variation was separated into variation among the groups and variation within groups by means of the analysis of variance techniques as shown in table 2.

The mean squares among pies within groups are the estimates for the variance of log counts of samples taken at the same time. The corresponding standard deviation was $S = 0.28699$, and the coefficient of variation was $C = 6.8$ per cent.

The variation among groups of pies is considerably greater than that within groups, therefore, if a fixed number of samples is to be taken during the working day. Taking only one or two pies at frequent intervals would yield more information and give a better indication of the bacterial quality of the product than would taking larger numbers of samples at wider intervals.

The total counts are grouped in table 3 according to the per cent distribution of the counts observed in samples which were taken simultaneously from the processing line. Each sample was compared with each of the other samples in the sample group, then the paired combinations were tabulated on the basis of the lower count *vs.* the higher count of the pair. As shown in

TABLE 2

Analysis of variance for log total count of 453 groups of pies

Source of Variation	Degrees of Freedom	Mean Square
Among groups	116	1.107313
Within groups	336	0.082364
Total	452	

TABLE 3

Per cent distribution of total counts in frozen chicken pies

Lowest Total Count/g, Comparison Samples, × 10³	Highest Total Count/g, Comparison Samples, × 10³							Total No. of Comparisons
	<5	5–10	10–20	20–40	40–80	80–160	>160	
<5	40	43	10	6	1	0	0	129
5–10		23	47	24	4	1	1	152
10–20			32	47	9	5	7	194
20–40				64	21	4	11	160
40–80					44	15	41	34
80–160						32	68	22
>160							100	19

table 3, when one sample of any group had a count of less than 5000 per g, all other samples of the groups had sufficiently low counts to meet an arbitrary maximum of 80,000 per g. In groups in which the minimum counts were in the 5000 to 10,000 per g range, about 2 per cent of the other pies tested had counts exceeding 80,000 per g. In like manner, when the lowest counts within any sample group ranged from 10,000 to 20,000 per g, 12 per cent of the other samples had counts exceeding 80,000 per g. These results show that bacterial counts must be maintained at a level very much lower than any proposed standard, or considerable loss of product would result. In addition, the data suggest that in any testing program the number of samples examined could be based on minimum, mean, and median counts, as well as maximum counts obtained on any lot of samples.

Relative influence of plating errors. Although there was rather close agreement in total counts obtained on different samples which had been taken simultaneously, sample-to-sample variations were much greater than are most plating errors (Jennison and Wadsworth, 1940). The use of duplicate plates for each dilution of the sample was probably unnecessary for the routine estimation of the bacterial content of most prepared frozen foods. One plate each of the 1:2.5 or 1:5, and 1:25 or 1:50 dilutions would appear to suffice. A plate of the 1:250 or 1:500 dilution could be included when circumstances indicate.

Acknowledgments

The excellent technical assistance of Mr. Robert D. Olson and Mr. Thomas M. Moore is gratefully acknowledged.

Summary

A study was made of seasonal, day-to-day, diurnal, and sample-to-sample variations in bacterial counts obtained from commercially prepared frozen chicken and beef pies. Total counts were higher during the warm months than during the cool months; however, seasonal influences were minimized when very low counts were constantly maintained. Samples obtained on Mondays and on the latter days of the work week were generally higher in count than samples obtained on Tuesdays or Wednesdays. Sampling at hourly intervals during processing was necessary to detect the majority of high count product. Bacterial counts obtained on one product were not necessarily indicative of the level of bacterial contamination to be expected in another product produced in the same plant.

REFERENCES

Borgstrom, G. 1955 Microbiological problems of frozen food products. Advances in Food Research, **6**, 163–230.

Canale-Parola, E. and Ordal, Z. J. 1957 A survey of the bacteriological quality of frozen poultry pies. Food Technol., **11**, 578–582.

Fanelli, M. J. and Ayres, J. C. 1959 Methods of detection and effect of freezing on the microflora of chicken pies. Food Technol., **13**, 294–300.

Gunderson, M. F. 1957 Sampling problems in foods. Bacteriol. Revs., **21**, 241–244.

Hartman, P. A. 1960 Further studies on the selectivity of autoclave-sterilized violet red bile agar. J. Milk and Food Technol., **23**, 45–48.

Huber, D. A., Zaborowski, H., and Rayman, M. M. 1958 Studies on the microbiological quality of precooked frozen meals. Food Technol., **12**, 190–194.

Jennison, M. W. and Wadsworth, G. P. 1940 Evaluation of the errors involved in estimating bacterial numbers by the plating method. J. Bacteriol., **39**, 389–397.

Ross, A. D. and Thatcher, F. S. 1958 Bacteriological content of marketed precooked frozen foods in relation to public health. Food Technol., **12**, 369–371.

Snedecor, G. 1956 *Statistical methods*. Ed. 5. Iowa State University Press, Ames, Iowa.

Zaborowski, H., Huber, D. A., and Rayman, M. M. 1958 Evaluation of microbiological methods used for the examination of precooked frozen foods. Appl. Microbiol., **6**, 97–104.