THE CORRELATION BETWEEN THE NUMBERS OF LIVING BACTERIA AND THE MONTHS ELAPSED SINCE MANUFACTURE IN POWDERED MILK II.

STUDIES ON SPRAY-DRIED MODIFIED MILK POWDER*

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Some investigations of the changes in number of living bacteria during storage of milk products, especially of milk powder have been reported by HIGGINBOTTOM (1944), FINDLAY et al. (1945) and several other workers. SUPPLEE & ASHBAUGH (1922) and HIGGINBOTTOM (1953) have also investigated the variations of number of bacteria in the milk powder stored under different relative humidities. However, in these cases, few statistical tests on the changes of number of bacteria were made.

In the writers' previous report, it was stated that the numbers of living bacteria in spray-dried skim milk powder of 26 samples preserved at room temperature tend to decrease with the lapse of time; the results obtained were analysed statistically. But, as the data used in the first report were taken from materials which are respectively different in respect to storage period after manufacture, it is possible that this factor influenced the results. To avoid such influences, for this study samples of the same lot number spray-dried modified milk powder were employed. There was no alternative but it was necessary to employ the modified milk powder as no suitable material of skim milk powder could be obtained.

MATERIALS AND METHODS

For the present experiment, three cans each 450 g net weight of modified milk powder were supplied from a certain Dairy Products Co., Ltd. They had been processed on Aug. 4th, 1956: their numbers of living bacteria were found to be respectively 26, 29 and 64 thousand per g of milk powder at the preliminary examination on 10th Oct. On 16th Oct.,

* So-called modified milk powder in Japan is one fortified with vitamins, minerals, β-lactose and so on for nursing babies specially.

all milk powder in these 3 cans was aseptically mixed in one after which it was again redivided and sealed into 18 poly-ethylen bags pasteurized by ultra violet ray for 20~30 minutes; each weighed about 50 g. The numbers of bacteria of all of them were again simultaneously counted on 13th Nov. and then after resealing they were kept at room temperature. Taking out one of them at random every two weeks from the 14th Nov., the writers made enumeration of number of bacteria. The last sample was taken out at the 38th week of the experiment—on Aug. 7th, 1957, just one year after manufacture.

As reported in the previous paper, the count of living bacteria was made using the standard agar plate counts method described in the "Manual of Food Hygienic Inspection" compiled by the Ministry of Health. However, as it seemed inaccurate to enumerate the numbers of bacteria on only 2 agar plates, 10 plates were used in this experiment. From the results of the preliminary experiment, the solution of sample to pour in dish was found preferably to be 1 ml of hundredfold diluted original material. So, this technique was employed through this experiment. The agar used was the same lot one made by the

TABLE 1. *Changes and Survival Rates of Number of Living Bacteria in Spray-Dried Modified Milk Powder Preserved at Room Temperature*

NUMBER OF LIVING BACTERIA/g			SURVIVAL RATES b/a (%)	LOGARITHMIC SURVIVAL RATES
Before Storage a	After Storage			
	Weeks	b		
21,090	0	21,050	99.81	2.00
23,060	2	22,330	96.83	1.99
26,190	4	19,130	73.04	1.86
24,290	6	15,350	63.19	1.80
33,270	8	10,600	31.86	1.50
25,310	10	13,190	52.11	1.72
23,430	12	11,880	50.70	1.71
21,970	14	7,320	33.32	1.52
22,440	16	8,810	39.26	1.59
29,670	18	10,270	34.61	1.54
30,290	20	7,060	23.31	1.37
30,200	22	14,680	48.61	1.69
24,540	24	7,440	30.32	1.48
23,180	26	6,180	26.66	1.43
22,330	28	10,240	45.86	1.66
23,130	30	9,340	40.38	1.61
·	32	·	·	1.48*
33,020	34	7,350	22.26	1.35
·	36	·	·	1.22*
34,490	38	4,270	12.38	1.09

· Data missing
* Estimates by calculation

Nihon Eiyo Kagaku Co., Ltd. for the routine examination of dairy products.

BACTERIAL COUNT AND SURVIVAL RATE

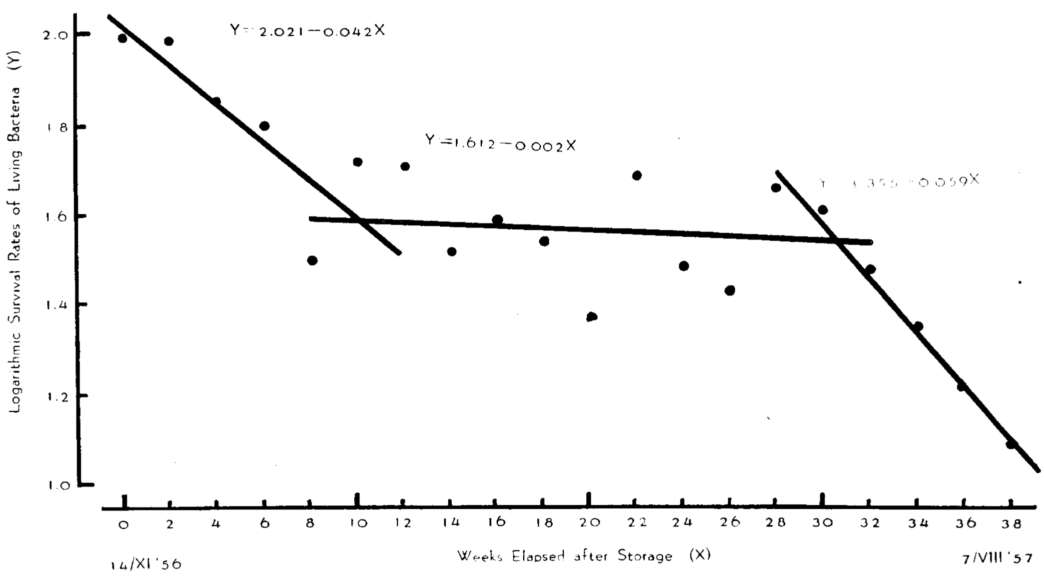
Respective numbers of living bacteria obtained in the 18 samples are shown in table 1. Despite complete mixture of milk powder in 3 cans, the numbers of bacteria in the redivided 18 samples on 13th Nov., ranged from 34,490 to 21,090 per g, but these numbers of bacteria belong to the same population ($p<0.01$). The survival rate of the number of living bacteria in each sample is respectively presented by the ratio of the number of bacteria estimated after storage to the number before storage. On the whole, with the lapse of time the survival rates of number of bacteria decrease from 99.81% (the first examination at 0 week) to 12.38% (the last examination at the 38 th week).

On the other hand, in order to ascertain the bacteria species in the samples, 0.1 ml of a hundredfold diluted solution of each sample was cultivated on sheep blood agar. After 24~48 hours' cultivation at 37°C, large, flat, dried, greyish-yellow colonies and large, flat, moist, yellow ones were most frequently seen. These colonies consist of gram-positive cocci in tetrad which at 60°C survive for 15 minutes but not for 30 minutes. *Staphylococcus aureus*, *B. subtilis*-like spore-forming bacillus and others were not observed in any great number.

STATISTICAL ANALYSIS

When the logarithmic survival rates (Y) are plotted against the weeks elapsed (X), the regressions appear to be nearly linear in three different groups (Fig. 1), which consist of the survival rate between 0~10th week (A group), between 8~30th (B group) and between 28~38th (C group). As in the previous study, by the calculation of a regression

FIG. 1. *Correlation between the Weeks Elapsed after Storage and Logarithmic Survival Rates of Living Bacteria in Spray-Dried Modified Milk Powder*



coefficient for each group the authors can obtain the following results and establish three regression equations.

A group $0 \sim 10$th week

$r = -0.837$

$F_s = 9.32$

$P_r.\{F>6.61\} = 5\%$, $P_r.\{F>16.26\} = 1\%$

$\therefore P_r.\{F>F_s\} < 5\%$

$Y = 2.021 - 0.042X$

B group $8 \sim 30$th week

$r = -0.151$

$F_s = 0.23$

$P_r.\{F>4.84\} = 5\%$

$\therefore P_r.\{F>F_s\} > 5\%$

$Y = 1.612 - 0.002X$

C group $28 \sim 38$th week

$r = -0.995$

$F_s = 321.16$

$P_r.\{F>16.26\} = 1\%$

$\therefore P_r.\{F>F_s\} < 1\%$

$Y = 3.355 - 0.059X$

As mentioned above, in all the groups, A, B and C, the coefficients of correlation are negative, in other words, the number of bacteria in the milk powder decreases with the lapse of storage period. In group B the numbers of bacteria decrease very slowly, while in both group A and group C each coefficient of correlation is rather great; the second power of their regression equations is not significant ($p<0.05$), that is, the numbers of bacteria decrease linearly in each group. It follows that the survival rates of living bacteria decrease in proportion as time passes.

DISCUSSION

It was reported in the previous work that the logarithmic values of the numbers of living bacteria in preserved spray-dried skim milk powder would decrease linearly with the lengthening of storage period.

According to the report of SUPPLEE & ASHBAUGH, the numbers of living bacteria in the roller-dried milk powder containing more than 10 thousand living bacteria per g, decreased remarkably for several months immediately after manufacture, but they became almost unvarying from 6 months after manufacture. HIGGINBOTTOM[3] also reported that many samples of spray-dried milk powder showed little alteration in their numbers of living bacteria after 6 months or more after manufacture.

In the present experiment, as shown in figure 1, in group A the numbers of bacteria decrease rapidly, but in group B slowly. The fact of the rapid or slow decrease of the numbers of living bacteria in A and B groups is in accord with the above mentioned reporters' results. This is true because the present experiment was begun with the 3rd month after manufacture and the final count in A group was over at the 10th week from the beginning of the experiment, in other words at about the 6th month after manufacture; on the other hand, the first count in B group was made at about 6 months after manufacture. However, it is noticeable that the decrease of the numbers of bacteria in group C was again remarkable; moreover it decreased more rapidly than in group A. For explanation of this phenomenon, such factors as the difference of bacteria species in the milk powder, the containers of the samples, the conditions of storage, the quality and the quantity of milk powder, etc. should be considered. But additional and more precise experiments are needed to clarify the reason of decrease in numbers of bacteria in group C.

As it was presumed that the bacterial count made on only 2 plates as provided by the Manual would cause large error in observation of changes of number of living bacteria, in other words as it was desirable to get higher precison for the enumerations of number of bacteria, 10 plates were used for every diluted sample solution.

The variance resultant from use of 10 plates for each of samples was small and the significant test of these variances showed no significance ($p<0.05$).

On the other hand, YAMADA (1947) has pointed out that the dissociation of bacteria clumps in cultures of raw milk would cause the mean value to divide into 2 groups, one of which consists of 14 plates distributed around coordinate A (66.8) and the other of 6 plates around B (271.3), even though use was made of 20 plates. However, in this present work, the dissociation of bacteria clumps was not observed as it was by YAMADA in one diluted solution.

SUMMARY

In order to know how the number of living bacteria in spray-dried modified milk powder changes with the lapse of time after manufacture, a series of observations of the bacterial count in milk powder preserved till 1 year after manufacture was designed. The results were subjected to statistical analysis. The bacterial counts were calculated in 18 samples originated from 3 cans with the same lot number. In advance all the milk powder of 3 cans was aseptically well mixed and redivided into many small bags; then these bags were kept at room temperature. The number of living bacteria in each of bags selected at random was counted at two week intervals from 14th to 52nd week after manufacture.

The result of this observation shows that there is a negative correlation

between the logarithmic survival rates of living bacteria and the weeks elapsed after storage.

When the test period was divided into 3 groups, A (0~10th week after storage), B (8~30th) and C (28~38th), the coefficients and regression equations for each group were obtained as follows:

A group $r = -0.837$, $Y = 2.021 - 0.042X$
B group $r = -0.151$, $Y = 1.612 - 0.002X$
C group $r = -0.995$, $Y = 3.355 - 0.059X$

From these equations, it was shown that the survival rate of living bacteria in modified milk powder tends to decrease remarkably in the first group A, slightly only in B group and rapidly again in C group (Fig. 1).

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