Studies on Dry Yeast (VI)

Change of Dry Yeast in the Fermenting Activity and the MB-stainability during Storage at 0° and 30°C

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Received May 25, 1959

When the sample was taken from the fermenting dry bakers' yeast suspension, the ratio of yeast cells negative to methylene blue staining (MBN) was calculated easily and exactly. MBN decreased more slowly than any of three factors of fermenting power, which were shown with three quotients (Q_{CO_2} (20'~40'), ΔQ_{CO_2} , Q_{CO_2} (max)), during the storage of the dry yeast. ΔQ_{CO_2} was the most inconstant of three quotients and the inconstancy was particularly clear in storage at 0°C, where others were almost constant.

When bakers' yeasts are dried to a certain moisture content, the rate of the decrease in the fermenting activity varies with the fresh yeast used as the material: the activity in one material decreases to 60% but in another material to 40%, even if their activities were almost the same in the fresh condition¹⁻³⁾. Such a relation is recognized also when dry yeasts are stored^{2,3)}. In the dry yeasts which have the same fermenting activity soon after dehydration, one loses much fermenting activity in only a few days but another does not lose so much even after a few months. The essential and sufficient qualities which the bakers' yeast must have in order to be made into the dry yeast have not yet been found. The content of cell components, e.g., total carbohydrate and total nitrogen, has been hitherto taken as a criterion in preparing the dry yeast⁴⁻⁶⁾. But how such components protect the fermenting

power from its decrease has been studied very little⁷⁾. As these components were found to change very slightly in the author's experiment⁸⁾, the yeast cells which have the optimum content of these components might be given a physiological condition suitable to the dry yeast.

But, before looking into such a physiological condition, it might be necessary to examine again whether the fermenting power of the dry bakers' yeast may be taken not as a matter of free enzymes such as "zymase", but as that of yeast cells. Whether or not the fermenting power of dry yeast depends upon the living cells was formerly a matter of debate^{9,10)}. At present, the power is regarded as that of the living cell¹¹⁾, but the relation between the fermenting power and the ratio of the number of living cells to total cells (the ratio of living cells) has not yet been studied in detail. Fink devised the methylene blue staining procedure to distinguish dead cells from living cells¹²⁾ and this procedure has been utilized as a simplified

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method to determine the ratio of living cells¹³. So, in this report, the ratio of yeast cells negative to methylene blue staining (MBN) which was determined according to the Fink's method was taken as a value to suggest the ratio of living cells. The relation between MBN and the fermenting power was looked into statistically, and it was confirmed that a high positive correlation exists between these values. Consequently, we should be aware of the most suitable physiological conditions of cells for preparing the dry bakers' yeast.

For ascertaining such physiological conditions, it might be useful to examine how yeast cells change generally in the process of dehydration or storage after dehydration. Formerly, the author found that the fermenting power of the dry bakers' yeast can be represented by three factors¹³: the initial fermenting activity; the power which recovers fermenting activity; the maximum fermenting activity to be recovered. Therefore, in this report, the retentions of MBN and these three activities were examined during the storage of dry yeast, and the relations between them were also examined.

MATERIALS AND METHODS

The preparation and the storage of dry yeast were carried out in the same way as in the author's previous report², i.e., air-dried at 30°~35°C and stored at 30°C, except the samples described particularly. The determination of the fermenting power was carried out also according to the same report and the fermenting power was shown with three quotients¹³: Q_{CO_2} (20'~45') (the initial fermenting activity); ΔQ_{CO_2} (the power which recovers fermenting activity); Q_{CO_2} (max) (the maximum fermenting activity to be recovered). The moisture content was measured through drying 1 g of dry yeast at 100°~105°C till its constant weight was attained. The measurement of the number of living cells was carried out according to the Fink's method, where the cell which is not stained with methylene blue (0.01%) in the phosphate buffer of pH 4.6 is regarded as a living cell. But the yeast cells used in this measurement were sampled from the suspension after the determination of the fermenting activity. The suspension had been shaken as usual till the evolution of the maximum volume of

carbon dioxide, or for exactly eighty minutes. Besides, in this report, the yeast cells which were not stained with methylene blue and had a clear outline of the cell membrane were regarded to be MB-negative, and the ratio of these MB-negative cells to the total cells (usually 500 cells) was shown with percentage. This value is represented by MBN. The error in the measurement was 1.0~2.7% (Table I) and little change was seen till the evolution of the maximum carbon dioxide as shown in Fig. 1. K. Shinma et al. found that the measurement in the Fink's method should be carried out

TABLE I. CRITERION ON STAINING WITH METHYLENE BLUE

Name of Test	Number of Sampling	Not Stained		
		%	average	error*
1-A	1	33	34	1.0
	2	34		
	3	35		
1-B	1	32	34	1.6
	2	35		
	3	34		
2	1	49	48	1.2
	2	47		
	3	47		
3	1	33	35	2.7
	2	33		
	3	37		
	4	38		
	5	32		

* Error is shown by standard deviation.

Note: The sample in 3 is a dry baker's yeast stored for 500 days at 0~5°C. 1-A, 1-B were taken from the same sample which were shaken in different vessels.

FIG. 1. Relation Between MBN and Fermenting Activity in Process of Fermentation
↓ shows the time when sample was taken and MBN measured.

within three minutes after mixing yeast suspension and methylene blue solution¹⁴, but, in this method, no dif-

13) M. Takakuwa, This Bulletin, 23, 316 (1959).

14) K. Shima, T. Kada and M. Ikeda, unpublished data

FIG. 2. Correlation Between MBN and Q_{CO_2} (20'~40')

A size of ■ shows frequency.

ference was found between the value measured within five minutes and that within sixty minutes.

RESULTS

1) **The relation between MBN and Q_{CO_2} (20'~40')**
The relation was studied with 390 examples, which consisted of all values measured soon after dehydration and during storage not only in the air-dried yeast but also in the dry yeast prepared with various methods⁹⁾. The distribution is shown in Fig. 2. It was deduced that the positive correlation ($r=0.839$) exists between them. But each variance of MBN or Q_{CO_2} (20'~40') in a certain Q_{CO_2} (20'~40') or MBN was of considerable extent as seen on this figure.

2) **The relation between the moisture content and the retention of MBN during storage at the various temperature.** As shown in Fig. 3, the retention of MBN during storage at various temperatures (0°~5°C, 20°C, 30°C, 37°C) was examined with the dry yeasts of various moisture contents. In this case, the state of the retention was observed

FIG. 3. Relation Between Moisture Content and MBN

□---□ soon after drying; — after 30 days of storage in various temperatures; ○—○ 0°~5°C, ×—× 15°~20°C, ●—● 30°C, △—△ 37°C
No cell of 15.2% sample was not stained after 30 days in 30°C or 37°C.

with each of the samples which were air-dried. There was seen a tendency similar to the retention of fermenting activity, which was reported previously²³. That is to say, the lesser the moisture content of the dry yeast, the smaller was the value of MBN when measured soon after drying, but, the lesser the content the larger was the retention of MBN after the storage of the dry yeast. The relation between the moisture content and MBN after the storage was opposite to that soon after dehydration; i.e., the lesser the moisture content of a sample, the lesser was the influence of the storage temperature. In other words, the sample of the less moisture content retained MBN during the storage at the higher temperature.

The retention ratios of Q_{CO_2} (20'~40') and MBN during storage were almost the same at 0°~5°C as shown in Fig. 4, but, when the temperature became high, the ratio of Q_{CO_2} (20'~40') decreased sooner than that of MBN. Even at the low temperature, the moisture content had influence on both ratios after the long period of storage. Both ratios were almost the same in the sample of 7.6% of the moisture

FIG. 4. Retention Ratios of MBN and Q_{CO_2} (20'~40') in Storage at Various Temperatures

— RMBN, ---- RQ_{CO_2} (20'~40')
○ 0°~5°C, × 20°C, ● 30°C, △ 37°C

content, but became very different in the sample of 10.0%. Hereafter the retention ratios are abbreviated to RMBN and RQ_{CO_2} (20'~40'), respectively.

3) As the fermentation of dry yeast depends not only upon Q_{CO_2} (20'~40') but also upon ΔQ_{CO_2} and Q_{CO_2} (max), their retention ratios were calculated also and are shown as $R\Delta Q_{CO_2}$ and RQ_{CO_2} (max), respectively. Which factor has more connection with MBN during the storage at 30°C became the next subject for study.

(i) There was a positive correlation between RQ_{CO_2} (20'~40') and RMBN ($r=0.741$): Each RMBN was usually larger than its corresponding RQ_{CO_2} (20'~40') as shown in Fig. 5. This might support certainly the above findings that Q_{CO_2} (20'~40') decreases sooner than MBN during storage.

(ii) There was a very high positive correlation between RQ_{CO_2} (20'~40') and RQ_{CO_2} (max) ($r=0.970$): Like RMBN RQ_{CO_2} (max) was larger than the corresponding RQ_{CO_2} (20'~40') as shown in Fig. 6. This might suggest that Q_{CO_2} (max) does not decrease sooner than Q_{CO_2} (20'~40') in the storage at 30°C. Moreover, when the values of A and B were calculated from the following equation: $A=RMBN-RQ_{CO_2}$ (max), $B=RMBN-RQ_{CO_2}$ (20'~40'), the relation of $A>B$ existed in 3 out of 23 examples, $A=B$ in 4, and $A<B$ in 16. So MBN might be deduced to have more connection with Q_{CO_2} (max) than with Q_{CO_2} (20'~40').

(iii) r between RMBN and $R\Delta Q_{CO_2}$ was -0.039 in 43 examples. So the existence of the correlation between them was not regarded as of any significance.

Therefore, considering the results of (i), (ii) and (iii), MBN might have more connection with Q_{CO_2} (max) than Q_{CO_2} (20'~40') or ΔQ_{CO_2} , in three quotients.

4) The positive correlation existed between RQ_{CO_2} (20'~40') and $R\Delta Q_{CO_2}$ as shown in Fig. 7 ($r=0.611$). The examples on this figure were limited to those of $R\Delta Q_{CO_2}$ smaller than 180, but the

FIG. 5. Correlation between Retention Ratios of MBN and Q_{CO_2} (20'~40')

ΔQ_{CO_2} was sometimes so small (0 or near to 0) that some $R\Delta Q_{CO_2}$ were ∞ or very large. Zero of ΔQ_{CO_2} measured soon after drying was seen in the dry yeasts prepared from 8 out of 28 materials of the fresh yeast, where Q_{CO_2} (max) was equal to Q_{CO_2} (20'~40'). This might mean that the value of Q_{CO_2} (max) is often near to that of Q_{CO_2} (20'~40') when they are measured soon after drying. As shown in the figure, moreover, when Q_{CO_2} (20'~40') decreased much, the degree of the decrease was larger in ΔQ_{CO_2} than in Q_{CO_2} (20'~40'), while, when Q_{CO_2} (20'~40') decreased only a little as in an initial period of the storage, ΔQ_{CO_2} decreased less than Q_{CO_2} (20'~40'), or ΔQ_{CO_2} increased. It might be deduced from these results that the initial fermenting activity decreases in the initial period of storage and the induction period appears and

then the decrease of ΔQ_{CO_2} (the increase of the induction period) goes on more rapidly than that of Q_{CO_2} (20'~40').

5) **The dry yeast of a moderate moisture content retains an almost constant Q_{CO_2} (20'~40') even after about one and a half years in the storage at 0°C:** MBN also was retained almost constant during the storage at the low temperature. When a sample which had been stored previously at 0°C was stored at 30°C, however, both Q_{CO_2} (20'~40') and MBN decreased more rapidly than those of the sample which was stored at 30°C soon after drying (Fig. 8). This fact might suggest that some change which is not recognized from values of Q_{CO_2} (20'~40') and MBN had occurred in the storage at 0°C. So RQ_{CO_2} (20'~40') and RQ_{CO_2} (max) in the storage at 0°C were compared with each other and it was

FIG. 6. Correlation between Retention Ratios of Q_{CO_2} (20'~40') and Q_{CO_2} (max)

FIG. 7. Correlation between Retention Ratios of Q_{CO_2} (20'~40') and ΔQ_{CO_2}

FIG. 8. Effect of Previous Storage at 0°C to Storage at 30°C

— soon after drying, ---- stored previously at 0°C for 460 days. ○ MBN, × Q_{CO_2} (20'~40'). ● and △ show MBN and Q_{CO_2} (20'~40') respectively in the sample stored for 410 days at 0°C after 50 days of storage at 30°C.

FIG. 9. Correlation between Retention Ratios of Q_{CO_2} (20'~40') and Q_{CO_2} (max)

found that Q_{CO_2} (20'~40') did not decrease more rapidly than Q_{CO_2} (max) in the retention ratio over 95% (Fig. 9); unlike the fact in the storage at 30°C. Also in the sample after the

storage at 0°C, the relation between RMBN and RQ_{CO_2} (20'~40') was $RMBN > RQ_{CO_2}$ (20'~40') during the storage at 30°C like the one stored at 30°C soon after drying (Fig. 8). The retentions of ΔQ_{CO_2} and Q_{CO_2} (20'~40') in the storage at various temperatures varied especially in the low temperature, as shown in Fig. 10. That is to say, at 0°~5°C, Q_{CO_2} (20'~40') was almost constant but ΔQ_{CO_2} remarkably inconstant. Besides, when the sample stored for four hundred days at 0°C after fifty days of the storage at 30°C was compared with the sample stored for fifty days at 30°C after four hundred days at 0°C, the former was much larger than the latter in both MBN and Q_{CO_2} (20'~40') (Fig. 8). The sum of the above results is that, in storage at a low temperature (0°~5°C), the fermenting activity and MBN hardly changed but became labile to increase of temperature, and that, at 0°C, only ΔQ_{CO_2} was the most inconstant in three quotients of fermenting power of dry yeast.

FIG. 10. Retention Ratios of ΔQ_{CO_2} and Q_{CO_2} (20'~40') in Storage of Various Temperatures

— ΔQ_{CO_2} , ---- Q_{CO_2} (20'~40')
○ 0°~5°C, × 20°C, ● 30°C, △ 37°C

DISCUSSION

Fink counted the number of cells stained deep blue with methylene blue as that of dead cells to calculate the ratio of living cells¹²⁾, but Shinma et al. modified this method and counted the number of cells not stained instead of the ones stained deep blue¹⁴⁾. Shinma et al. also found that the ratio of unstained cells varies with various conditions and decided definitely the time for suspending cells with shaking and other conditions.

But it is affirmed in this paper that, when the sample was taken from the yeast suspension after measuring the fermenting activity, the difference between stained cells and unstained ones was clearer and the method did not need such strict conditions as the time of measurement. It may be convenient also for the sufficient dispersion of cells to take the sample from the yeast suspension which was fermented in a shaking apparatus. It is difficult to distinguish the unstained cells from the partly stained cells or the lightly-stained cells. Perhaps such weakened cells as the latter die out and other quite living cells strengthen the living force, when the cells are allowed free action in controlled situations, the difference between the stained and the unstained ones becomes clear. Strengthening the living force is supposed easily from the fact that the fermenting activity of dry yeast is usually recovered in the process of fermentation. Besides, the fact that little increase in the ratio of unstained cells was recognized in the process of fermentation suggests probably that ΔQ_{CO_2} does not depend upon the increase of the number of the yeast cells.

The fact that out of the three quotients of fermenting power during the storage at 30°C RMBN had the most connection with RQ_{CO_2} (max) may suggest that the value of each Q_{CO_2} (max) can be estimated from the value of each MBN in comparing several samples, even if the measurement is not continued for several hours till the appearance of Q_{CO_2} (max). The fact that the relation between MBN and the mois-

ture content was similar to that in Q_{CO_2} (20'~40') during the storage at various temperatures might suggest that even a little difference in moisture content is closely connected with the life of cells even after the cells have been dried till within the bound water.

As the fermentation of the dry yeast depends upon the living cells, it may be supposed naturally that the fermentation of dry yeast is controlled by more varied factors than that of zymase. On the other hand, the fermentation of dry yeast is also different from that of fresh yeast. Besides, the state of the former varies according to the method of preparing or storing dry yeast. These facts might show that some change is made in the enzyme systems of yeast cells when yeast cells suffer from any damage, and that the change of function of the enzymes varies with the state of the damage of the yeast cells.

As the Q_{CO_2} (20'~40') is near to the Q_{CO_2} (max) generally, ΔQ_{CO_2} increases apparently in the initial period of storage. But perhaps ΔQ_{CO_2} does not increase in reality. It looks probably so because Q_{CO_2} (20'~40') is very near Q_{CO_2} before storage. And since the dry yeast cell soon after dehydration holds a very strong power in recovering the fermenting activity, such a cell shows almost the same value in Q_{CO_2} (20'~40') as in Q_{CO_2} (max), which shows that this cell is very similar to a fresh cell. Therefore the appearance of the induction period might depend on the decrease of ΔQ_{CO_2} as well as that of the initial fermenting activity.

When the cell stored previously at 0°C was stored at 30°C, the decrease of MBN and Q_{CO_2} (20'~40') was more rapid than when the cell was stored at 30°C without the previous storage at 0°C. This might mean that the change in the storage at 0°C made the cell labile to the increase of the storage temperature.

SUMMARY

When the sample in the Fink's method of staining yeast cells with methylene blue was taken from the yeast suspension after deter-

mining the fermenting activity, the measurement was carried out more easily and exactly. Next the existence of the positive correlation ($r=0.839$) was confirmed between the ratio of yeast cells negative to methylene blue staining (MBN) and Q_{CO_2} (20'~40'). So the change of dry yeast during storage was examined concerning MBN and three quotients of fermenting power (Q_{CO_2} (20'~40'), ΔQ_{CO_2} , Q_{CO_2} (max)). Besides, the interrelation of these quotients was discussed in connection with the influence of the storage temperature.

1) The relation between moisture content and MBN retention of dry yeast in the storage at various temperatures (0°~5°, 20°, 30°, 37°C) was similar to that reported formerly about Q_{CO_2} (20'~40'). The lesser the moisture content of dry yeast, the smaller was the value of MBN but the value retained during the period of storage was larger. Besides, the lesser the moisture content, the lesser was the influence of the storage temperature. But such a tendency in the storage was seen more clearly in Q_{CO_2} (20'~40') than in MBN.

2) In the storage at 30°C, the relation between the retention ratios of MBN and above three quotients was examined. In the initial

period of storage, Q_{CO_2} (20'~40') decreased and the induction period appeared. Afterwards, the decrease of ΔQ_{CO_2} (the increase of the induction period) went on more rapidly than that of Q_{CO_2} (20'~40'). Of the three quotients Q_{CO_2} (max) decreased most slowly. MBN decreased more slowly than Q_{CO_2} (max), and had more connection with Q_{CO_2} (max) than with Q_{CO_2} (20'~40') or ΔQ_{CO_2} , in the three quotients.

3) The decrease of MBN was slowest also in the storage at 0°C. Q_{CO_2} (20'~40') also was almost constant in the case of dry yeast of a moderate moisture content, but only ΔQ_{CO_2} was clearly inconstant.

4) In the samples stored at 30°C after the storage at 0°C, the decrease of MBN and Q_{CO_2} (20'~40') was more remarkable than in those stored at 30°C soon after drying. On the contrary, both MBN and Q_{CO_2} (20'~40') decreased only a little when the same samples were stored at 0°C after the storage at 30°C.

Acknowledgment. The author wishes to express his sincere thanks to Prof. I. Yamasaki of Kyushu University for his guidance in this work.