Evaluation of Yeast Activity and Its Application to Our Industrial Brewing

By N. Fukui, A. Kogin, S. Furukubo, H. Kondo, H. Yomo and Y. Kakimi

ABSTRACT

It is very important to use active yeast for brewing high quality beer. Many papers have already been published proposing various evaluation methods for yeast activity. However, most of these evaluation methods were aimed more at the fundamental yeast characteristics such as yeast growth and fermentation intensity, rather than at the other fermentation performances related with beer quality. Therefore, we have developed the evaluation method for yeast activity from the viewpoint of the intracellular conditions of yeast. This explains the total fermentation characteristics which affect beer quality. Our new evaluation method consists of:

1. intracellular pH
2. leakage of protease
3. vacuole condition

Using our evaluation method, we could improve the yeast handling and succeeded in the improvement of beer quality.

INTRODUCTION

It is very important to use active yeast for brewing high quality beer. In many breweries, several trials have been carried out in order to gain highly active yeast.\(^1\) For this purpose, an accurate and sensitive evaluation method for yeast activity is indispensable. Many papers have already been published proposing various evaluation methods for yeast activity, such as dead cell ratio with methylene blue staining,\(^2\) budding ability with slide culture,\(^3\) fermentation intensity with acidification power,\(^4\) intracellular glycogen,\(^5\) to mention a few. Most of these evaluation methods were aimed at the fundamental yeast characteristics such as yeast growth and fermentation intensity, rather than directly at the quality of the final beer. Therefore, we developed an evaluation method for yeast activity so that allows us to predict yeast characteristics and fermentation performances which affect beer quality.

In general, good flavor balance, easiness to drink and good foam adhesion are important characteristics of a high quality beer. The yeast is considered to play an important role in brewing such a beer. Therefore, it is necessary to obtain highly active yeast not only for stable yeast growth and good fermentation intensity but also for the following fermentation performances.

1. The stable assimilation of amino acids, that affects foam adhesion and production of fusel alcohols.
2. The vigorous froth separation of hydrophobic compounds, that affects the content of harsh bitter substances in final beer.
3. The complete removal of off-flavor.
4. The minimum decomposition of foam protein, that affects foam adhesion and its stability on shelf.

We have developed several methods to evaluate yeast activity on these points, improving yeast handling and consequently succeeded in the improvement of beer quality for use in our industrial brewing.

EXPERIMENTAL

Fermentation conditions

Fermentations were carried out using 300 KL scale industrial tanks and the temperature was kept constant at 10°C. Wort gravity was adjusted to 11.7°P.
Analytical methods

"Intracellular pH" was measured by NMR analysis(8). It was calculated by measuring the chemical shift value of $^{31}$P phosphate in the cytosol, which had good correlation with the cytosolic pH, namely, intracellular pH. "Leakage of protease" was estimated by measuring the protease activity leaked from the yeast in the young beer(9). Protease activity was obtained by measuring the fluorescence emitted by the degradation of the specific fluorescent substance after incubation of the beer sample with the substrate. "Vacuole condition" was measured by the count of vacuole appearance ratio using morphological observation with a phase contrast microscope(10). Foam adhesion of beer was measured by the SHV method which had been developed in our laboratory(11). The other analyses were carried out according to ASBC method(12).

RESULTS AND DISCUSSION

The preparation of the yeasts for evaluation

The yeasts evaluated by our methods were collected on the 6th and 13th day of fermentation. On the 6th day, the assimilable sugars had almost been consumed. On the 13th day, the reduction of VDK had been completed and the cooling of the young beer had already finished. The suspending yeast with centrifuge NA, which were decided by comparing the actual values with the desirable values.

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The evaluation of yeast activity by various methods

Table 1 shows the comparison of evaluated yeast activities by methylene blue staining method, intracellular pH, leakage of protease and vacuole condition. The results were shown as both the actual values and the evaluation symbols such as VA, A and NA, which were decided by comparing the actual values with the desirable values.

By methylene blue staining method, yeast A, B and C were evaluated as "active" and only yeast D was evaluated as "not active." The staining ratio of yeast C was distinctly different from yeast A, Band C.

Table 1.

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Methylene blue staining (%)</th>
<th>Intracellular pH</th>
<th>Protease leaking activity ($\times 10^{-3}$ ng/ml/cell)</th>
<th>Vacuole appearance ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Sus 6</td>
<td>6.9 (A)</td>
<td>6.83 (VA)</td>
<td>35 (VA)</td>
<td>20 (VA)</td>
</tr>
<tr>
<td>B-Sus 13</td>
<td>7.2 (A)</td>
<td>6.46 (NA)</td>
<td>65 (A)</td>
<td>60 (A)</td>
</tr>
<tr>
<td>C-Sed 6</td>
<td>8.0 (A)</td>
<td>6.64 (A)</td>
<td>51 (A)</td>
<td>50 (A)</td>
</tr>
<tr>
<td>D-Sed 13</td>
<td>22.1 (NA)</td>
<td>6.44 (NA)</td>
<td>107 (NA)</td>
<td>95 (NA)</td>
</tr>
</tbody>
</table>

Desirable value

- Below 10 (VA) Very active
- Above 6.50 (A) Active
- Below 45 (NA) Not active

By intracellular pH, yeast D was evaluated as "not active" as in the case of methylene blue staining method. However, this method clarified some differences among yeast A, B and C. The intracellular pH value of yeast A was sufficiently high and the value of yeast C was at least higher than the desirable value to evaluate it as "active." Being different from the results by methylene blue staining method, yeast B was evaluated as "not active" by this method, because of the lower than the desirable value.

By leakage of protease, yeast D was evaluated as "not active" as same as the former two cases. Among yeast A, B and C, yeast A was different from the others and was evaluated as "very active," because of its extremely low amount of leaked protease. Yeast B and C were evaluated as "active," because their values were close to the desirable value.

By vacuole condition, yeast A was evaluated as "very active," yeast B and C were "active," and yeast D was "not active," almost as same as the evaluation by leakage of protease.

From these results, yeast D, which was collected from the bottom of the fermentation tank on the 13th day of fermentation, could be classified into "not active" by all methods.

On the other hand, although there was not any large difference among yeast A, B and C, as "active," by methylene blue staining method, these three yeasts were clearly distinguished by our new methods. It was suggested that the order of yeast activities by using our new methods was as the following.

Yeast A > Yeast C > Yeast B > Yeast D

Yeast characteristics and fermentation performances using each yeast

Table 2 shows yeast characteristics and fermentation performances using each yeast in an industrial fermentation trial. The following indices were applied for this evaluation.

The assimilated amount of free amino nitrogen during fermentation was applied as the index for the assimilation of amino acids by yeast.

The separation ratio of the bitterness units against ones of wort was applied as the index for the degree of the froth separation of hydrophobic compounds during fermentation.

The half time of reduction of total VDK was applied as the index for the degree of removal of off-flavor.

The leaked protease activity in the final beer was applied as the index for the protease leaking property of yeast during fermentation which should affect the decomposition of foam protein, resulting the deterioration of foam on shelf.

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Assimilated amount of FAN (mg/100ml)</th>
<th>Separation ratio of bitterness units (%)</th>
<th>Half time of reduction of T-VDK (hr)</th>
<th>Leaked protease activity in final beer (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Sus 6</td>
<td>12.7</td>
<td>30.0</td>
<td>38.4</td>
<td>1.9</td>
</tr>
<tr>
<td>B-Sus 13</td>
<td>11.5</td>
<td>28.5</td>
<td>40.1</td>
<td>3.6</td>
</tr>
<tr>
<td>C-Sed 6</td>
<td>12.1</td>
<td>30.0</td>
<td>40.1</td>
<td>2.8</td>
</tr>
<tr>
<td>D-Sed 13</td>
<td>9.8</td>
<td>27.0</td>
<td>40.9</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Desirable value

- Above 12
- Above 29
- Below 40
- Below 2.5

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The half time of reduction of total VDK was applied as the index for the degree of removal of off-flavor.

The leaked protease activity in the final beer was applied as the index for the protease leaking property of yeast during fermentation which should affect the decomposition of foam protein, resulting the deterioration of foam on shelf.
Yeast C-Sed 6 also had enough ability to reduce off-flavor, the smallest effect. A-Sus 6 and B-Sus 13 beer qualities produced by using each yeast in industrial fermentation.

Table 3 shows the results of yeast growth and fermentation intensity. Although yeast A, B and C showed satisfactory values, yeast D showed insufficient results on both indices even in this evaluation. From the results shown in Table 1, 2 and 3, it was suggested that the yeast activities evaluated by our new methods exactly corresponded with the order of yeast activity as evaluated by our new method.

The application in industrial brewing
On the basis of the results of evaluated yeast activity, we decided to use yeast A, which was evaluated as the most active yeast, for industrial brewing. In order to obtain this kind of yeast, we carried out the dropping on the 6th day of fermentation when assimilable sugars were almost consumed, and collected the suspending yeast with centrifuge. After the dropping, the fermenting wort in which 20% of the total yeast remained was kept at the same temperature (10°C) in the 2nd fermentation tank for a few days, and was cooled down after the enough reduction of the off-flavor.

We also made the yeast handling procedure, such as yeast recovery and storage, more suitable by using our evaluation methods. Consequently we could obtain yeast which had the satisfactory fermentation performances, and the beer quality was much improved.

CONCLUSIONS

Through our studies on yeast activity, the following conclusions were made:

1. Our new evaluation methods were effective for predicting yeast characteristics and fermentation performances which should affect beer quality.

2. Intracellular pH above 6.5, protease leaking property below 45 and vacuole appearance ratio below 40 were indispensable for brewing high quality beer.

3. We refined yeast handling using our new evaluation method and succeeded in improving beer quality.
REFERENCES


QUESTIONS & ANSWERS

Q. 1: Have you observed significant differences between different yeast strains in terms of their propensity to leak intracellular protease?
A. 1: We have observed small differences between yeast strains in the case of bottom fermentation yeasts and large differences in the case of top fermentation yeasts. Top fermentation yeasts have propensities for leaking more intracellular protease than bottom fermentation yeasts.

Q. 2: Could you give us expected reason for less activity in yeast C than yeast A?
A. 2: In the case of the semi-flocculent yeast, we think that the less active yeast doesn’t have enough ability to suspend in the fermenting wort and sediments into the bottom of fermentation tank during fermentation. Therefore, the sedimented yeast like yeast C is considered to be less active than the suspending yeast like yeast A.

Q. 3: What do you do with the “beer” that results after yeast removal on the 6th day of fermentation?
A. 3: We collected 80% of the total suspending yeast with centrifuge on the 6th day of fermentation. Attenuation and reduction of off-flavor were well done because the fermenting wort in which 20% of the total yeast remained was kept at the same temperature (10°C) in the 2nd fermentation tank for a few days.